ISSN: 2448-8461

FRONTERA BIOTECNOLÓGICA

Revista Digital del IPN, CIBA Tlaxcala - No. 11 septiembre-diciembre 2018

Memorias/Proceedings

2nd Biotechnology World Symposium y 11° Encuentro Nacional de Biotecnología del IPN

"Biotecnología e Innovación, Ciencia con Impacto"

16-20 de octubre del 2018,
San José del Cabo, Baja California Sur
México

ISSN: **2448-8461**

Directorio Institucional

Cintillo Legal

FRONTERA BIOTECNOLÓGICA, año 6, número 11, septiembre - diciembre 2018, es una publicación cuatrimestral editada por el Instituto Politécnico Nacional a través del Centro de Investigación en Biotecnología Aplicada. Ex-Hacienda San Juan Molino Carretera Estatal Tecuexcomac - Tepetitla Km 1.5, Tlaxcala C.P. 90700, México. Tels.: 01-248-48707-65 y 66 Conmutador IPN: 57296000, Ext. 87816. http://www.revistafronterabiotecnologica.cibatlaxcala.ipn.mx, Editor responsable: Dra. Martha Dolores Bibbins Martínez. Reserva de Derechos al Uso Exclusivo del Título No. 04-2015-120313501700-203, ISSN: 2448-8461, ambos otorgados por el Instituto Nacional del Derecho de Autor (INDAUTOR). Responsable de la última actualización de este número, Centro de Investigación en Biotecnología Aplicada. Dra. Martha Dolores Bibbins Martínez., Ex-Hacienda San Juan Molino Carretera Estatal Tecuexcomac - Tepetitla Km 1.5, Tlaxcala C.P. 90700, fecha de última modificación, 31 de octubre de 2018.

ISSN: **2448-8461**

Contenido

ISSN: **2448-8461**

Mensaje Editorial

Octubre del 2018

Estimados lectores,

En esta edición especial de **FRONTERA BIOTECNOLÓGICA** presentamos las memorias del 2**nd Biotechnology World Symposium y 11° Encuentro Nacional de Biotecnología del IPN.**

El simposium se tituló **"BIOTECNOLOGÍA E INNOVACIÓN, CIENCIA CON IMPACTO"** y fue un foro para hablar de ciencia, innovación y tecnología, teniendo un programa de actividades académicas distribuido en el periodo del 16 al 20 de octubre del 2018, con sede en el Hotel Holiday Inn, San José del Cabo, BCS, México.
Los avances en investigación y desarrollos biotecnológicos presentados representan los esfuerzos que la investigación biotecnológica a nivel mundial, dirige a la salud humana, seguridad alimentaria y protección de recursos bióticos y abióticos, así como la importancia socioeconómica y de las políticas de apoyo a la innovación y desarrollo de tecnología, de esta importante área de estudio. Todos estos aspectos fueron abordados a través de las actividades que conformaron el programa de este evento, las cuales incluyeron:

1. CONFERENCIAS PLENARIAS dictadas por renombrados investigadores extranjeros y mexicanos, los cuales nos hablaron de sus principales logros científicos y de sus desarrollos tecnológicos, así como de su gran trayectoria en el desarrollo de empresas de base biotecnológica.

2. MESAS DE DISCUSIÓN con especialistas que hablaron del futuro y perspectivas de la biotecnología, innovación y desarrollo, patentamiento y del camino seguido por jóvenes emprendedores en el establecimiento de empresas biotecnológicas.

3. SESIONES ORALES Y DE CARTELES las cuales tuvieron como principal objetivo el promover el acercamiento entre pares, con investigadores y alumnos asistentes, un espacio que permitió compartir experiencias relevantes e innovadoras.

4. EXPO-INDUSTRIAL donde proveedores de diversas ramas tuvieron un espacio para presentar los equipos, instrumental y enseres que facilitan y mejoran la investigación.

Es indudable que a través de las actividades del **2nd Biotechnology World Symposium y 11° Encuentro Nacional de Biotecnología del IPN** se logró transmitir la gran importancia de la investigación **BIOTECNOLÓGICA** para resolver problemáticas actuales, satisfacer las necesidades humanas esenciales, crear crecimiento económico y dirigirnos hacia un desarrollo sustentable.
Los invitamos a leer y a compartir con otros investigadores, estudiantes, trabajadores y público en general, esta edición tan relevante de **FRONTERA BIOTECNOLÓGICA.**

"LA TÉCNICA AL SERVICIO DE LA PATRIA".

DRA. MARTHA BIBBINS MARTÍNEZ
EDITOR EN JEFE

ISSN: 2448-8461

2nd Biotechnology World Symposium
11° Encuentro Nacional de Biotecnología

San José del Cabo, BCS, México
October 16-20, 2018

Coordinadora general/ Chair Organizing Committee

Claudia Judith Hernández Guerrero

Comité Editorial del libro de memorias/ Editorial Committee of abstracts book:

Yoloxochitl Elizabeth Rodríguez Montesinos
Claudia Judith Hernández Guerrero
Dora Luz Arvizu Higuera
Mauricio Muñoz Ochoa
Ruth Noemí Aguila Ramírez

Comité Técnico/ Technical Committee:

Claudia Judith Hernández Guerrero, Ruth Noemí Aguila Ramírez, Sergio Francisco Martínez Díaz, Bárbara González Acosta.

ISSN: 2448-8461

"Biotecnología e innovación, ciencia con impacto"
"Biotechnology and innovation, science with impact"

2nd Biotechnology Word Symposium
11° Encuentro Nacional de Biotecnología

San José del Cabo, BCS, México
Octubre 16-20, 2018

El Arco, Cabo San Lucas, BCS, México. Foto tomada de/ Picture taken from: http://tendenciaelartedeviajar.com/2017/06/gastronomia/cabo-san-lucas/

Organized by
Red de Biotecnología del Instituto Politécnico Nacional
Centro Interdisciplinario de Ciencias Marinas

DIRECTORIO / DIRECTORY

Dr. Mario Alberto Rodríguez Casas
Director General del IPN

Dr. Juan Silvestre Aranda Barradas
Secretario de Investigación y Posgrado del IPN

Dra. Laura Arreola Mendoza
Directora de Investigación del IPN

Dr. Luis Cuauhtémoc Gil Cisneros
Director de Posgrado del IPN

Dr. Hugo Necoechea Mondragón
Coordinador de las Redes de Investigación

Dr. Hervey Rodríguez González
Coordinador de la Red de Biotecnología

COMITÉ ORGANIZADOR
ORGANIZING COMMITTEE

Centro Interdisciplinario de Ciencias Marinas

Dr. Sergio Hernández Trujillo
Director del CICIMAR

Dr. Silverio López López
Subdirector Académico y de Investigación

M.C. Felipe Neri Melo Barrera
Subdirector de Servicios Educativos e Integración Social

Lic. Luz de La Paz Pinales Soria
Subdirectora Administrativa

Dra. Claudia Judith Hernández Guerrero
Presidente del Comité Organizador

Dra. Ruth Noemí Aguila Ramírez
Secretario

Dr. Sergio Francisco Martínez Díaz
Vocal Técnico

MC. Dora Luz Arvizu Higuera
MC. Yoloxochitl Elizabeth Rodríguez Montesinos
Dra. Claudia Judith Hernández Guerrero
Dr. Mauricio Muñoz Ochoa
Dra. Ruth Noemí Aguila Ramírez
Comité Científico y Editorial

Dra. Bárbara González Acosta
Tesorera

Dr. César Salvador Cardona Félix
Dr. Gustavo Hernández Carmona
Dr. Jesús Iván Murillo Álvarez
Apoyo a la Difusión

MC. José Roberto Aguilera Angulo
MC. Gonzalo Pérez Araiza
Diseño y Soporte Sitio Web

Lic. Gabriela Faviola García Gallegos
María Fernanda Hernández Lucio
Diseño Gráfico

COMITÉ EVALUADOR / REVIEWERS

Dra. Ruth Noemí Aguila Ramírez	CICIMAR
Dra. Dioselina Álvarez Bernal	CIIDIR-Michoacán
MC. Dora Luz Arvizu Higuera	CICIMAR
Dra. Martha Dolores Bibbins Martínez	CIBA-Tlaxcala
Dr. César Salvador Cardona Félix	CICIMAR
Dra. Aracely Evangelina Chávez Piña	ENMH
MC. María Isabel Cortés Vázquez	CEPROBI
Dra. María Del Carmen Cruz López	CIBA-Tlaxcala
Dr. Raúl Jacobo Delgado Macuil	CIBA-Tlaxcala
Dr. Bárbara González Acosta	CICIMAR
Dr. Juan Manuel González Prieto	CBG
Dr. Gustavo Hernández Carmona	CICIMAR
Dra. Regina Hernández Gama	CICATA-Querétaro
Dra. Claudia Judith Hernández Guerrero	CICIMAR
Dra. Yolanda Herrera Arrieta	CIIDIR-Durango
Dra. Luicita Lagunez Rivera	CIDIR Oaxaca
Dra. Alma Leticia Martínez Ayala	CEPROBI
Dr. Sergio Francisco Martínez Díaz	CICIMAR
Dr. Jorge Montiel Montoya	CIIDIR-Sinaloa
Dr. Eduardo Morales Sánchez	CICATA-Querétaro
Dr. Mauricio Muñoz Ochoa	CICIMAR
Dr. Jesús Iván Murillo Álvarez	CICIMAR
Dra. Sandra Pérez Álvarez	CIIDIR-Sinaloa
Dra. Erika Teresa Quintana Cano	ENCB
Dr. Francisco Roberto Quiroz Figueroa	CIIDIR-Sinaloa
Dr. Miguel Ángel Reyes López	CBG
Dr. Mario Rodríguez Monroy	CEPROBI
MC. Yoloxochitl Elizabeth Rodríguez Montesinos	CICIMAR
MC. Minerva Rosas Morales	CIBA-Tlaxcala
Dra. María Elena Sánchez Pardo	ENCB
Dra. Gabriela Sepúlveda Jiménez	CEPROBI
Dra. María Myrna Solís Oba	CIBA-Tlaxcala
Dr. Christopher René Torres San Miguel	ESIME-Zacatenco

STAFF

CICIMAR

Laura Angélica Ibeth Álvarez Lee

Jessica Dolores Arrona Ortiz

Liliana Edith Couttolenc Lyle

Dania Andrea Di Filippo Herrera

Priscilla Casandra Gutiérrez Gálvez

Gabriela Margarita García Zamora

Ana Laura González Castro

Naylin Herrera Hernández

Zulema Juárez Cortés

Estrella Marrón Grijalva

José Manuel Mendoza Alcalá

Yanelis Quevedo Morales

Erika Guadalupe Rico Virgen

Clemente Robles Geraldo

Kimberly Ruiz Rodríguez

Diana Elizabeth Sánchez Rodríguez

Miguel Ángel Villa Arce

Valeria Alexandra Villegas Silva

Lina Angélica Zermeño Cervantes

CONTENIDO / CONTENT

ISSN: 2448-8461

BIENVENIDA

“Biotecnología e innovación, ciencia con impacto”

Estimados colegas e invitados

Nos complace darles la más cordial bienvenida al **2nd Biotechnology World Symposium y 11° Encuentro Nacional de Biotecnología del IPN**. En esta ocasión la organización de este evento corresponde al Centro Interdisciplinario de Ciencias Marinas, que tiene como sede San José del Cabo en Baja California Sur, México.

Este evento está dirigido a profesores, profesionales y estudiantes interesados en adquirir conocimientos y compartir experiencias en el área de la Biotecnología. Por lo que el objetivo final es integrar un foro donde la comunidad de expertos en biotecnología tanto nacionales como internacionales, pueda fomentar el debate crítico, compartir hallazgos, conocimientos, ideas y experiencias; así como el análisis de nuevas tendencias, avances e innovaciones en las áreas de la biotecnología ambiental, acuática y animal, médica y farmacéutica, vegetal alimentaria, y en el desarrollo de procesos biotecnológicos. Además de favorecer el establecimiento de redes profesionales y grupos de colaboración que intensifiquen la capacidad para aportar soluciones y hacer frente a los retos biotecnológicos del futuro.

Este simposium les ofrece la oportunidad de intercambiar ideas, hacer nuevas colaboraciones y sembrar la semilla de la innovación en todos nuestros quehaceres. De tal forma que contaremos con conferencias magistrales de expertos en diferentes áreas de la biotecnología, como son el Dr. Channapatna S. Prakash, Dr. Stephen Mayfield, Dra. Raquel Lía Chan, Dr. William Fenical, Dr. Albert Tacon, Dra. Claire Hellio, Dra. Luisa Mannina, Dr. Gerardo Toledo, Dr. Aristóbulo Loaiza, Dr. Octavio García, Dr. Daniel Jacobo Velázquez y el Ing. Scott Munguía. También tendremos mesas de discusión en diferentes aspectos de la biotecnología, como son, el desarrollo de patentes, la innovación tecnológica y promover el interés de los jóvenes por la innovación. Además, se presentarán los últimos avances científicos en biotecnología de diferentes instituciones nacionales e internacionales, con la presentación de más de 250 trabajos libres en modalidad oral y póster. Así como pláticas, presentaciones de equipos y desarrollos tecnológicos.

Estamos muy complacidos de recibir a investigadores de Universidades y Centros de Investigación del país, así como a colegas de instituciones de investigación del extranjero. Como comité organizador, esperamos que disfruten su estancia en San José del Cabo.

Dra. Claudia Judith Hernández Guerrero

Presidente del Comité Organizador del 2nd BWS, 11°ENB

ISSN: 2448-8461

WELCOME

"Biotechnology and innovation, science with impact"

Dear colleagues and guests

We are pleased to welcome you to the **2nd World Biotechnology Symposium and 11° Encuentro Nacional de Biotecnología del IPN**. This time the organization of this event corresponds to the Centro Interdisciplinario de Ciencias Marinas (CICIMAR-La Paz, BCS), that has as venue San Jose del Cabo in Baja California Sur, Mexico.

This event is aimed at professors, professionals and students interested in acquiring knowledge and sharing experiences in the area of Biotechnology. So, the ultimate goal is to integrate a forum where the community of biotechnology experts, both national and international, can stimulate the critical debate, share findings, knowledge, ideas and experiences; as well as the analysis of new trends, advances and innovations in the areas of environmental biotechnology, aquatic and livestock biotechnology, medical and pharmaceutical biotechnology, plant biotechnology, food biotechnology and in the development of biotechnological processes. In addition to promoting the establishment of professional networks and collaboration groups, that intensify the capacity to provide solutions and face the biotechnological challenges of the future.

This symposium offers you the opportunity to exchange ideas, to make new collaborations, and sow the seed of innovation in all our tasks. We will have expert lectures in different areas of biotechnology, such as PhD. Channapatna S. Prakash, PhD. Stephen Mayfield, PhD. Raquel Lía Chan, PhD. William Fenical, PhD. Albert Tacon, PhD. Claire Hellio, PhD. Luisa Mannina, PhD. Gerardo Toledo, PhD. Aristóbulo Loaiza, PhD. Octavio García, PhD. Daniel Jacobo Velázquez and Eng. Scott Munguía. Discussion panels on different aspects of biotechnology, as developing of invention and patents and technological innovation. We aim to promote the interest of young people for innovation. In addition, the latest scientific advances in biotechnology from different national and international institutions will be presented in more than 250 papers in oral and poster modality. We will also have the presentations of scientific equipment and technological developments.

We are very happy to receive researchers from universities and research centers from México, as well as colleagues from research institutions abroad. As an organizing committee, we hope you enjoy your stay in San José del Cabo.

PhD. Claudia Judith Hernández Guerrero

Chair, Organizing Committee

ISSN: 2448-8461

PATROCINADORES / SYMPOSIUM SPONSORS

ISSN: 2448-8461

PROGRAMA GENERAL / GENERAL PROGRAM
2nd BIOTECHNOLOGY WORLD SYMPOSIUM, 11° ENCUENTRO NACIONAL DE BIOTECNOLOGÍA DEL IPN
16 al 20 de octubre 2018 / October 2018

Martes / Tuesday 16

11:00-13:30	Registro de participantes / Registration *Salón Los Cabos AB*
13:30-15:00	LUNCH
15:00-17:30	Registro de participantes/ Registration *Salón Los Cabos AB*
17:30- 18:30	Inauguración del evento / Opening Ceremony *Salón Los Cabos AB*
18:30-20:00	Conferencia Magistral / Keynote Dr. C. Prakash Enhancing the public acceptance and understanding of GMO´s and gene-edited crops – What can plant scientists do? *Salón Los Cabos AB*
20:00-22:00	Coctel de Bienvenida / Icebreaker

Miércoles / Wednesday 17

8:00-9:00	Registro de participantes / Registration
9:00-10:00	Conferencia Magistral / Keynote Dr. Albert Tacon Aquaculture and the role of biotechnology in improving nutrition and global food supply *Salón Los Cabos AB*
10:00-11:00	Conferencia Magistral / Keynote Dra. Claire Hellio From chemical ecology to marine biotechnology biomimetism approaches *Salón Los Cabos AB*

11:00-11:30	ISSN: 2448-8461 COFFEE BREAK	
	Conferencias Tecnológicas / Technology Conferences (*Salón Los Cabos AB*)	
11:30-12:00	Dr. Octavio García "Desarrollando biotecnología en México: crónica de un fracaso anunciado" Empresa: T4 Oligo	
12:00-12:30	MC. Leonardo García "Aceleración en el desarrollo de anticuerpos" Empresa: Sartorius	
12:30-13:00	MC. Manuel Mondragón Olguín "Análisis metabolómico en Tequila utilizando cromatografía de líquidos y espectrometría de masas de alta resolución" Empresa: Agilent	
13:00-13:30	MC. José Manuel Carranza Flores "Nuevas tendencias en la detección de patógenos vivos: PCR de viabilidad" Empresa: QIAGEN	
13:30-15:00	LUNCH	
15:00-16:30	Mesa de discusión de biotecnología (**Patentes**) / Discussion panel of biotechnology (**Patents**) Dra. Raquel Lía Chan, Dr. Stephen Mayfield, Dr. William Fenical *Salón Los Cabos AB*	
16:30-17:00	COFFEE BREAK	
	Ponencias orales / Oral Presentations *Salón Los Cabos A* Biotecnología Ambiental / Environmental Biotechnology (**AM**)	**Ponencias orales / Oral Presentations** *Salón Los Cabos B* Biotecnología Acuática y Animal / Aquatic and Livestock Biotechnology (**AP**)
17:00-17:15		AP 6
17:15-17:30	AM 6	AP 8
17:30-17:45	AM 8	AP 13
17:45-18:00	AM 11	AP 14
18:00-18:15	AM 12	AP 15
18:15-18:30	AM 19	AP 23
18:30-20:00	**SESIÓN DE PÓSTERS I / POSTER SESSION I** **(*Terraza*)** Biotecnología Ambiental / Environmental Biotechnology (**AM**) Biotecnología Acuática y Animal / Aquatic and Livestock Biotechnology (**AP**) Desarrollo de Procesos Biotecnológicos / Development of Biotechnological Process (**PB**)	

ISSN: 2448-8461

Jueves / Thursday 18

8:00-9:00	Registro de participantes / Registration	
9:00-10:00	Conferencia Magistral / Keynote Dr. William Fenical Present and future perspectives on the research of marine natural products *Salón Los Cabos AB*	
10:00-11:00	Conferencia Magistral / Keynote Dr. Gerardo Toledo Microbial discovery and the prospect of new product development *Salón Los Cabos AB*	
11:00-11:30	COFFEE BREAK	
	Ponencias orales / Oral Presentations. *Salón Los Cabos A* Biotecnología Ambiental / Environmental Biotechnology (**AM**)	Ponencias orales / Oral Presentations *Salón Los Cabos B* Biotecnología Médica y Farmacéutica / Medicinal and Pharmaceutical Biotechnology (**MF**)
11:30-11:45	AM 24	MF 2
11:45-12:00	AM 25	MF 7
12:00-12:15	AM 27	MF 10
12:15-12:30	AM 33	MF 34
12:30-12:45	AM 40	MF 37
12:45-13:00	AM 42	MF 39
13:00-13:15		MF 40
13:15-13:30		
13:30-15:00	LUNCH	
15:00-16:30	Mesa de discusión de Biotecnología (Innovación Biotecnológica) Discussion panel of Biotechnology (Innovations in Biotechnology) Dr. Octavio García, Dr. Aristóbulo Loaiza, Dr. Gerardo Toledo *Salón Los Cabos AB*	

16:30-17:00	ISSN: 2448-8461 COFFEE BREAK	
	Ponencias orales / Oral Presentations *Salón Los Cabos AB* Biotecnología Vegetal / Plant Biotechnology (**VE**)	**Ponencias orales / Oral Presentations** *Salón Napa* Desarrollo de Procesos Biotecnológicos / Development of Biotechnological Process (**PB**)
17:00-17:15	VE 4	PB 4
17:15-17:30	VE 11	PB 6
17:30-17:45	VE 15	PB 14
17:45-18:00	VE 19	PB 18
18:00-18:15	VE 29	PB 23
18:15-18:30		
18:30-20:00	**SESIÓN DE PÓSTERS II / POSTER SESSION II** **(*Terraza*)** Biotecnología Médica y Farmacéutica / Medicinal and Pharmaceutical Biotechnology (**MF**) Biotecnología Vegetal / Plant Biotechnology (**VE**) Biotecnología Alimentaría / Food Biotechnology (**AL**)	
20:00	Evento Social / Social Event	

Viernes / Friday 19

8:00-9:00	Registro de participantes / Registration
9:00-10:00	Conferencia Magistral / Keynote Dra. Raquel Lía Chan Éxitos y fracasos en el desarrollo de herramientas biotecnológicas para el mejoramiento vegetal. El largo camino desde el laboratorio al campo y desde el modelo al cultivo. *Salón Los Cabos AB*
10:00-11:00	Conferencia Magistral / Keynote Dra. Luisa Mannina NMR methodologies in food science. *Salón Los Cabos AB*

ISSN: 2448-8461

11:00-11:30	COFFEE BREAK	
	Ponencias orales / Oral Presentations *Salón Los Cabos AB* Biotecnología Vegetal / Plant Biotechnology (**VE**)	Ponencias orales / Oral Presentations *Salón Napa* Biotecnología Alimentaria / Food Biotechnology (**AL**)
11:30-11:45	VE 35	AL2
11:45-12:00	VE 36	AL8
12:00-12:15	VE 42	AL16
12:15-12:30	VE 49	AL22
12:30-12:45	VE 50	AL24
12:45-13:00	VE 51	AL25
13:00-13:15		AL31
13:15-13:30		AL6
13:30-15:00	LUNCH	
15:00-16:30	Mesas de discusión de Biotecnología (**Jóvenes emprendedores**) Discussion panel of Biotechnology (**Young Entrepreneurs**) Dr. Daniel Jacobo, Ing. Scott Munguía *Salón Los Cabos AB*	
16:30-17:00	COFFEE BREAK	
17:00-18:00	Conferencia Magistral / Keynote Dr. Stephen Mayfield Photosynthetic bio-Manufacturing in green algae- Food and fuel for the 21st century *Salón Los Cabos AB*	
18:00-18:40	Ceremonia de clausura / Closing Ceremony *Salón Los Cabos AB*	

Sábado / Saturday 20

LIBRE / FREE DAY

ISSN: 2448-8461

PROGRAMA DIARIO / DAILY PROGRAMS

SESIÓN ORAL / ORAL SESSION

Martes / Tuesday, 16 October

Salón Los Cabos AB

11:00-13:30	Registro de participantes / Registration
13:30-15:00	LUNCH
15:00-17:30	Registro de participantes / Registration
17:30-18:30	Inauguración del evento / Opening Ceremony
	Conferencia Magistral / Keynote *Moderador / Moderator: Dr. Carlos Ligne Calderón Vázquez*
18:30-20:00	Dr. Channapatna S. Prakash Enhancing the public acceptance and understanding of GMO´s and gene-edited crops – What can plant scientists do?
20:00-22:00	Coctel de Bienvenida / Icebreaker

Miércoles / Wednesday, 17 October

Salón Los Cabos AB

8:00-9:00	Registro de participantes / Registration
	Conferencia Magistral / Keynote *Moderador / Moderator: Dr. César Escobedo Bonilla*
9:00-10:00	Dr. Albert Tacon Aquaculture and the role of biotechnology in improving nutrition and global food supply
10:00-11:00	Dra. Claire Hellio From chemical ecology to marine biotechnology biomimetism approaches
11:00-11.30	COFFEE BREAK
	Conferencias Tecnológicas / Technology Conferences *Moderador / Moderator: Dr. Gustavo Hernández Carmona*

11:30-12:00	Dr. Octavío García "Desarrollando biotecnología en México: crónica de un fracaso anunciado" Empresa: T4 Oligo
12:00-12:30	M.C. Leonardo García "Aceleración en el desarrollo de anticuerpos" Empresa: Sartorius
12:30-13:00	MC. Manuel Mondragón Olguín "Análisis metabolómico en Tequila utilizando cromatografía de líquidos y espectrometría de masas de alta resolución" Empresa: Agilent
13:00-13:30	MC. José Manuel Carranza Flores "Nuevas tendencias en la detección de patógenos vivos: PCR de viabilidad" Empresa: Qiagen
13:30-15:00	LUNCH
	Mesa de discusión de Biotecnología (**Patentes**) Discussion panel of Biotechnology (**Patents**) *Moderador / Moderator: Dr. Gustavo Hernández Carmona*
15:00-16:30	Dra. Raquel Lía Chan, Dr. Stephen Mayfield, Dr. William Fenical
16:30-17:00	COFFEE BREAK

Salón Los Cabos A

Biotecnología Ambiental / Environmental Biotechnology (AM)

Moderador / Moderator: Dra. María Myrna Solís Oba

Salón Los Cabos B

Biotecnología Acuática y Animal / Aquatic and Livestock Biotechnology (AP)

Jueves / Thursday, 18 October

Salón Los Cabos AB

8:00-9:00	Registro de participantes / Registration
	Conferencia Magistral / Keynote
	Moderador / Moderator: Dra. Aracely Evangelina Chávez Piña
9:00-10:00	Dr. William Fenical
	Present and future perspectives on the research of marine natural products
10:00-11:00	Dr. Gerardo V Toledo
	Microbial discovery and the prospect of new product development
11:00-11:30	COFFEE BREAK

Salón Los Cabos A

Biotecnología Ambiental / Environmental Biotechnology (AM)

Moderador / Moderator: Dra. Diana Verónica Córtes Espinosa

AM42 12:45-13:00	Nanotechnology for soil and water remediation, and for growing vegetables: does it really work? Fabián Fernández-Luqueño & Fernando López-Valdez	104
13:30-15:00	LUNCH	

Salón Los Cabos B

Biotecnología Médica y Farmacéutica / Medicinal and Pharmaceutical Biotechnology (MF)

Moderador / Moderator: Dr. Juan Santiago Salas Benito

		Page
MF2 11:30-11:45	New regulatory drugs of the cholecystokinin hormones for the treatment of overweight and obesity Vique-Sánchez J.L., Caro-Gómez L., Cruz-Aguirre A.S., Benítez-Cardoza C.G.	181
MF7 11:45-12:00	Identification and production of lunamycin, a novel lassopeptide from *Streptomyces scabrisporus* NF3 Stefany Daniela Rodríguez Luna, Corina Diana Ceapă, Melissa Vázquez Hernández, Carlos Naranjo Castañeda, Sergio Sánchez	185
MF10 12:00-12:15	Genome mining in action – natural product discovery and expression from endophyte *Actinomycetes* Corina Diana Ceapă, Karol Rodríguez Peña, Melissa Vázquez Hernández, Omar Jiménez Rodríguez, María Paula Gómez Román, Stefany Daniela Rodríguez Luna, Carlos Naranjo Castañeda, Sebastián de la Rosa Hernández Garibay, Dulce Ramirez, Sergio Sánchez	188
MF34 12:15-12:30	Encapsulation effect of PLGA-lupeol-manguiferin nanocomposites on the topoisomerase inhibition Fabián Razura-Carmona, Alicia P. Cárdenas-Castro, Jorge A. Sánchez-Burgos, Marco V. Ramírez-Marez	211
MF37 12:30-12:45	Identification of apoptotic molecular mechanisms induced in human HepG2 cells and dermal human fibroblast adults in response to maize (*Zea mays* L.) zein-derived peptides Plinio A. Trinidad-Calderón, Rodrigo B. Muñoz-Soto; Fabiola Castorena-Torres; Silverio García Lara	214
MF39 12:45-13:00	Bioavailability of sulforaphane (1-isothiocyanate-4-(methylsulfinyl)-butane) from broccoli seed, in microplate Pedro Alberto Duarte Portillo, Olga Nydia Campas Baypoli, Dalia Isabel Sanchez Machado, Jaime Lopez Cervantes, Rafael Canett Romero	216

MF40 13:00-13:15	Establishment of hairy roots system in *Phyllantus acuminatus* to production of anticancer compounds Giovanni Garro-Monge, Karol Jiménez-Quesada	217
13:30-15:00	LUNCH	

Salón Los Cabos AB

	Mesa de Discusión de Biotecnología (Innovación Biotecnológica) Discussion Panel of Biotechnology (Innovations in Biotechnology) *Moderador / Moderator: Dr. Sergio Francisco Martínez Díaz*
15:00-16:30	Dr. Octavio García, Dr. Aristóbulo Loaiza, Dr. Gerardo Toledo
16:30-17:00	COFFEE BREAK

Biotecnología Vegetal / Plant Biotechnology (VE)

Moderador / Moderator: Dra. Erika T. Quintana Cano		Page
VE4 17:00-17:15	Bioinsecticida microencapsulado a base de hongos entomopatógenos para el control de *Heliothis virescens* (Fabricius) Yareli Padilla-Mendoza, Cipriano García-Gutiérrez	223
VE11 17:15-17:30	Response of *Fouquieria splendens* explants co-cultivated with endophyte bacteria Verónica Azeret Salinas-Patiño, Marcial García-Pineda, Maria del Rosario Espinoza Mellado and Angélica Rodríguez-Dorantes	230
VE15 17:30-17:45	The endophytic actinobacterium *Streptomyces scabrisporus* NF3 compensates for growth hormone deficiency in *Arabidopsis thaliana* Corina Diana Ceapă, Angélica Patricia Cruz Vázquez, Verónica Jiménez Suárez, Elena R. Alvarez-Buylla, Sebastián de la Rosa Hernández Garibay, Sergio Sanchez	234
VE19 17:45-18:00	Endophytic bacteria of *Stevia rebaudiana* Bertoni with growth promoting activity Alejandra María Montes-Salazar, Ignacio Eduardo Maldonado-Mendoza, Mario Rodríguez-Monroy	238

VE29 18:00-18:15	Compatibility and biocontrol effect of yeast mixtures for control of *Colletotrichum gloeosporioides, Fusarium* sp. and *Penicillium digitatum* Rose Meena A. Edward, Erika A. De la Cruz-Arguijo, José A. Narváez-Zapata, Claudia Patricia Larralde-Corona	248

Salón Napa

Desarrollo de Procesos Biotecnológicos / Development of Biotechnological Process (PB)

Moderador / Moderator: Dra. Angélica Ruíz Font		Page
PB4 17:00-17:15	Metagenomic insights into the microbial dark matter for the search of biotechnological applications: the bacteriocins example Alejandra Escobar-Zepeda, Alfredo Esquivel, Alejandro Sanchez-Flores, Maricarmen Quirasco	138
PB6 17:15-17:30	Spores production of *Trichoderma asperellum* Tc74 in bioreactor Violeta Balene Ramírez Hernández, César Guigón López, Leticia Bravo Luna, Mario Rodríguez Monroy	140
PB14 17:30-17:45	Optimization of the acid hydrolysis process in lignocellulosic materials to obtain xylose and second-generation bioethanol López-Zamora L., Morales-Martínez J.L., Aguilar-Uscanga M.G.	148
PB18 17:45-18:00	Endemic fungal cellulases producers from the Jalisco's ecosystems Neydeli Ayala-Mendivil, Iliana Barrera Martínez, Georgina Sandoval	152
PB23 18:00-18:15	Engineering *Yarrowia lipolytica* to enhance lipid production from lignocellulosic materials Xochitl Niehus, Leticia Casas-Godoy, Neydeli Ayala-Mendivil, Anne-Marie Crutz-Le Coq, Georgina Sandoval, Jean-Marc Nicaud and Rodrigo Ledesma-Amaro	157
20:00-22:00	Evento social / Social Event	

Viernes / Friday, 19 October

Salón Los Cabos AB

8:00-9:00	Registro de participantes / Registration
	Conferencia Magistral / Keynote *Moderador / Moderator: Dra. Claudia Patricia Larralde Corona*

9:00-10:00	Dra. Chan Raquel Lía Éxitos y fracasos en el desarrollo de herramientas biotecnológicas para el mejoramiento vegetal. El largo camino desde el laboratorio al campo y desde el modelo al cultivo
10:00-11:00	Dra. Luisa Mannina NMR methodologies in food science
11:00-11:30	COFFEE BREAK

Salón Los Cabos AB

Biotecnología Vegetal / Plant Biotechnology (VE)

Moderador / Moderator: Dra. Alma Leticia Martínez Ayala		Page
VE35 11:30-11:45	*In vitro* selection of mutant plants of blackberry (*Rubus fruticosus* Cv. Tupi) tolerant to *Botrytis cinerea* Huerta-Olalde Ana Maria, Hernández-García Alejandra, López-Gómez Rodolfo, Zavala-Páramo María Guadalupe, Salgado-Garciglia Rafael	254
VE36 11:45-12:00	Micropropagation of *Hedeoma piperita* (Lamiaceae) an aromatic medicinal herb of Michoacan, Mexico Torrez-Sosa S., Huerta Olalde A.M., Hernández-García A., Salgado Garciglia Rafael	255
VE42 12:00-12:15	Efficient *in vitro* micropropagation protocols for cactus and succulents Estrella Karina Hernández-Vázquez, Hye Hyeong Kim, Gee Young Lee, Youn Hee Kim, María de la Luz Guerrero-González, Jae Hong Lee, Jae Hong Jung, Sang Dug Lee, Pablo Delgado-Sánchez.	261
VE49 12:15-12:30	Development of a platform for recombinant protein expression in tobacco cell suspensions using biolistic Lourdes J. Germán-Báez, Claudia D. Norzagaray-Valenzuela, Angel Valdez-Ortiz	268
VE50 12:30-12:45	Development of a selectable marker based on phosphite for chloroplast transformation in *Chlamydomonas reinhardtii* José Sandoval-Vargas, Noé V. Durán-Figueroa, Jesús A. Badillo-Corona	269
VE51 12:45-13:00	Seaweed as potential plant growth stimulants for agriculture in Mexico Rosalba Mireya Hernández-Herrera, Fernando Santacruz-Ruvalcaba, Diego Ramón Briceño-Domínguez, Dania Andrea Di Filippo-Herrera and Gustavo Hernández-Carmona	270
13:30-15:00	LUNCH	

Salón Napa

Biotecnología Alimentaria / Food Biotechnology (AL)

Salón Los Cabos AB

	Mesas de Discusión de Biotecnología (Jóvenes emprendedores) Discussion Panel of Biotechnology (Young Entrepreneurs) *Moderador / Moderator: Dr. César Salvador Cardona Félix*
15:00-16:30	Dr. Daniel Jacobo, Ing. Scott Munguía
16:30-17:00	COFFEE BREAK
	Conferencia Magistral / Keynote *Moderador / Moderator: Dra. Martha Dolores Bibbins Martínez*
17:00-18:00	Dr. Stephen P. Mayfield Photosynthetic bio-manufacturing in green algae- Food and fuel for the 21st century
18:00-18:40	Ceremonia de clausura / Closing Ceremony

SESIÓN DE PÓSTERS I / POSTER SESSION I

Miércoles / Wednesday, 17 October

18:30 – 20:00 Terraza

Biotecnología Ambiental / Environmental Biotechnology (AM)

Biotecnología Acuática y Animal / Aquatic and Livestock Biotechnology (AP)

Desarrollo de Procesos Biotecnológicos / Development of Biotechnological Process (PB)

SESIÓN DE PÓSTERS II / POSTER SESSION II

Jueves / Thursday, 18 October

18:30 – 20:00 Terraza

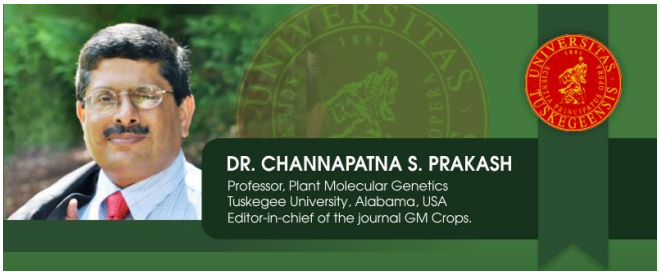
Biotecnología Médica y Farmacéutica / Medicinal and Pharmaceutical Biotechnology (MF)

Biotecnología Vegetal / Plant Biotechnology (VE)

Biotecnología Alimentaria / Food Biotechnology (AL)

ISSN: **2448-8461**

Resúmenes de Conferencias Magistrales / Keynote Abstracts



Actividades científicas: Área de investigación en genómica de cultivos, genética, desarrollo de marcadores moleculares, mapeo genético, bioinformática y biotecnología.

Reconocimientos: Referente mundial en materia de biotecnología y una eminencia en los temas de seguridad alimentaria. El Consejo de Información Biotecnológica lo nominó dentro de los 12 "pioneros, visionarios e innovadores detrás del progreso y la promesa de la biotecnología vegetal". Participó en el Comité de Consulta de Biotecnología Agrícola del USDA y en el Comité de Consulta para el Departamento de Biotecnología del Gobierno de la India. Editor en jefe de la revista GM Crops & Foods.

Publicaciones: Más de 100 artículos científicos en revistas indexadas y cientos de presentaciones científicas en conferencias nacionales e internacionales.

Scientific Activities: Current research focuses on crop genomics, genetic, development of molecular markers, genetic mapping bioinformatics and biotechnology.

Awards/Honors/Leadership: He was named one of a dozen 'pioneers, visionaries and innovators behind the progress and promise of plant biotechnology' by the Council for Biotechnology Information. He was chosen by his peers as among the "100 Top Living Contributors to Biotechnology" while the prestigious 'Nature' magazine readers' short listed him for "Who's who in biotech - some of biotech's most remarkable and influential personalities from the past 10 years. Editor in chief of the journal GM Crops& Foods.

Publications: Over 100 scientific papers in peer-reviewed journals and delivered hundreds of scientific presentations at national and international conferences.

ENHANCING THE PUBLIC ACCEPTANCE AND UNDERSTANDING OF GMO´S AND GENE-EDITED CROPS – WHAT CAN PLANT SCIENTISTS DO?

Channapatna S. Prakash

College of Arts & Sciences, Tuskegee University. Alabama, USA.

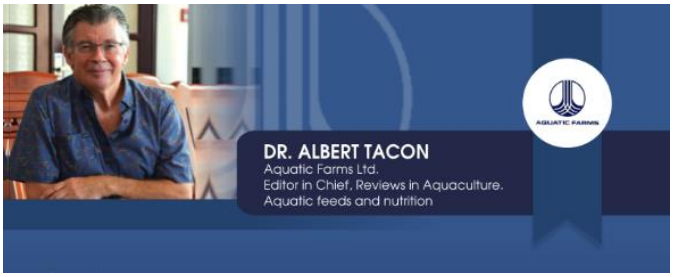
Genetically modified (GM) or bioengineered crops were first planted widely in 1996. Last year, nearly 19million farmers planted GM crops on 195million hectares - roughly one-tenth of the arable land across the globe in almost 30 countries so far.

Many experts believe that the broader adoption of molecular breeding tools including gene transfer, gene editing and genomics in agricultural research can foster greater food security. Agricultural biotechnology has the potential to develop crops with broad adaption and improved productivity. Potential benefits include developing climate-resilient; smaller environmental footprint of farming (through reduced consumption of pesticides, fertilizers and fuel); mitigating global warming through reduced emission of greenhouse gases; conserving biodiversity; make food more affordable; developing greener energy alternatives; nutritionally enhanced food products including foods with improved flavor, better taste and longer shelf life; and, developing hypoallergenic foods.

There are many reasons why there is a limited application of biotechnology in agricultural research in

the developing world: financial, technical, political, intellectual-property, biosafety regulation, public policy, and trade-related issues. An essential first step to moving forward with this technology is to ensure that policy makers, media and the public understand and appreciate the benefits from this technology while recognizing that any risks addressed through meaningful scientific regulation.

Increased societal understanding of the benefits and safety of gene-modified and gene-edited crops is critical to ensure their acceptance. The scientific community can help foster this through proactive knowledge sharing with various stakeholders, increased interaction with the media and impacting policymakers through science-based information on the food and environmental safety aspects of this technology. The scientific community must also make use of the innovative tools in information and communications technology especially social media to enhance greater societal understanding and acceptance of crops modified through new breeding techniques.



Actividades científicas: Experto en investigación y desarrollo en acuicultura, con especial énfasis en nutrición acuícola. Sus estudios han permitido un aumento de la contribución de la acuicultura en la seguridad alimentaria mundial y la mitigación de la pobreza.

Reconocimientos:14 años de experiencia con la Organización de las Naciones Unidas para la Agricultura, Alimentación y Nutrición (FAO), trabajando en proyectos nacionales, regionales e interregionales de desarrollo de acuicultura y dentro del Programa regular de la FAO en Roma. Editor en jefe de la revista Aquaculture, miembro del consejo editorial de Aquaculture Nutrition y Aquaculture Research; editor asociado de la revista International Aquafeed.

Publicaciones and Patentes: 228 publicaciones científicas relacionadas con la acuicultura y 1 patente de tecnología acuícola.

Scientific Activities: Aquaculture research and development, with a specialty focus on aquatic feeds and nutrition, and increasing the contribution of aquaculture to global food security and poverty alleviation.

Awards/Honors/Leadership:14 years of in-house experience with the Food and Agriculture Organization of the United Nations (FAO) working within National, Regional, and Inter-regional Aquaculture Development Projects, and within the Regular Program of FAO in Rome, Italy.Editor in Chief of Reviews in Aquaculture, Member, Editorial Board, Aquaculture Nutrition, Aquaculture Research and Associate Editor, International Aquafeed Magazine, Perendale Publishers.

Publications and Patents: 228 aquaculture related publications and one patent on aquaculture technology.

AQUACULTURE AND THE ROLE OF BIOTECHNOLOGY IN IMPROVING NUTRITION AND GLOBAL FOOD SUPPLY

Albert Tacon

Aquatic Farms Ltd. Kaneohe, HI 96734 USA. agjtacon@aquahana.com

Aquaculture has been the world's most rapidly growing food sector for over a quarter of century, with total global production (includes all farmed aquatic plants and animals) increasing over nine-fold from 10.2 million tonnes in 1984 to a new record high of 110.21 million tonnes in 2016. According to FAO agricultural biotechnology includes "Any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use". The paper presents examples of microbially produced feed additives commonly used within commercially formulated aquaculture feeds (total global aquafeed production estimated at about 43.5 million tonnes in 2016), including microbially produced dietary essential amino acids, dietary enzymes, vitamins, trace minerals, carotenoid pigments, nucleotides and immune enhancers, organic acids, and probiotics. In addition to feed additives, agricultural biotechnologies have recently placed particular effort to the mass production of microbial biomass for use as dietary fishmeal replacers and/or as a source of long-chain polyunsaturated fatty acids. Finally, particular emphasis was given to the fact that aquatic food products represent one of the world's most nutritious and healthy food sources.

Actividades científicas: Investigación de productos naturales marinos con actividad biológica, ecología química, enzimas marinas y estudios de compuestos con actividad antifouling.

Reconocimientos: Vicepresidente de la ESMB (European Society of Marine Biotechnology). Miembro del consejo editorial de International Journal of Molecular Sciences. Miembro del Advisory Board of "Challenges". Miembro del comité de dirección del grupo de trabajo Blue Biotech Europabio, el cual se centra en el desarrollo de vínculos entre la academia y las industrias en la Unión Europea y miembro del grupo de interés en Biotecnología Marina.

Publicaciones: 54 artículos científicos y 1 libro en el desarrollo de nuevos compuestos con actividad antifouling.

Scientific Activities: research of marine natural products with biological activity, chemical ecology, marine enzymes and anti-fouling studies.

Awards/Honors/Leadership: Vice-President of the ESMB (European Society for Marine Biotechnology). Member of the Editorial Board for IJMS, Challenges and Marine Biotechnology. Member of the steering committee of the Blue Biotech Europabio Working Group, focuses on the development of links between academia and industries in EU and member of Marine Interest Group.

Publications: 54 research articles (in the field of marine biochemistry, phycology and invertebrates biology) and one book on new anti-fouling development.

FROM CHEMICAL ECOLOGY TO MARINE BIOTECHNOLOGY BIOMIMETISM APPROACHES

Claire Hellio

Université de Bretagne Occidentale LEMAR

The growing demand to develop a novel, environmentally friendly antifouling (AF) or bioadhesive material is ever increasing. Bioinspiration is an attractive alternative in developing such a material, learning from nature's own designs and solutions and transferring them to solve particular problems. In order to achieve this goal, the actual mechanisms and strategies that evolution has produced needs to be elucidated from the subject species. The work presented in this talk, regarding new antifouling product, has investigated successfully chemical ecology (macroalgae and sponges), the role of microflora in the production of defense molecules, seasonality of the production of defences molecules, and defences synergy. The role of surface topography and chemistry combined in a single material, a property that exists naturally in some common macroalgae, has been as well investigated and led to promising results. The second part of this talk will focus on bioadhesion strategies used by marine organisms and how from fundamental studies, we have develop a new bioassay for testing the activity of compounds in inhibition or promotion of algal adhesion.

Actividades científicas: Su investigación involucra el aislamiento e identificación de compuestos químicos de microorganismos, plantas y animales marinos que pueden tener usos farmacéuticos o agrícolas potenciales.

Reconocimientos: Pionero en el campo de la química de productos naturales marinos. Sus contribuciones han sido consideradas como ejemplos de investigaciones científicas innovadoras y de valor biomédico. Lo que le ha merecido ser galardonado con diferentes distinciones, premio en "Química de Productos Naturales" por la "American Chemical Society". Premio al mérito del "National Cancer Institute" por su descubrimiento de nuevos antibióticos y agentes antitumorales. Medalla de plata por la "International Chemical Ecology Society".

Publicaciones and patentes: Más de 400 artículos científicos en investigaciones relacionadas con la química marina y 23 patentes.

Scientific Activities: His research involves the isolation and identification of chemical materials from marine plants, animals from marine plants, animals, and microorganisms that may have potential pharmaceutical or agricultural uses.

Awards/Honors/Leadership: He is listed in American Men in Science and Who's Who in the West. Win Ernest Guenther Award in the Chemistry of Natural Products from American Chemical Society. Natural Cancer Institute's highly respected Merit Award for his discovery of new antibiotics and antitumor agents. Win Silver Medal Award from International Society of Chemical Ecology, Senior Queens Fellowship and Pauley Fund Award. He founded the Consortium for Marine Biotechnology in San Diego. He is member of the American Chemical Society. American Society of Pharmacognosy, American Society of Chemical Ecology, and Western Society of Naturalists.

Publications and patents: More than 400 scientific articles on marine chemistry research and 23 patents.

PRESENT AND FUTURE PERSPECTIVES ON THE RESEARCH OF MARINE NATURAL PRODUCTS

William Fenical

Center for Marine Biotechnology and Biomedicine. Scripps Institution of Oceanography. University of California, San Diego. La Jolla, CA 92093

The field of marine natural products began in the late 1960's with studies of the pigments of marine invertebrates. In the 1970's the field enlarged to include marine plants and a broader cross section of complex metabolites. In these early days, an emphasis was on exploration with the goal of understanding where secondary metabolites were being produced and whether they were fundamentally different from those produced on land. It soon became clear that life in the sea had evolved under different conditions and that unique biosynthetic processes had evolved to produce structurally diverse compounds not observed previously. In the late 1980's and early 1990's the potential of marine sources to yield life-saving drugs was realized and many researchers turned their efforts toward the major diseases such as cancer. Since those days numerous drugs for cancer, pain and nutrition have been developed. Currently, marine natural products chemistry is a world-wide activity leading to major understanding of marine ecosystems. In this presentation, the brief history of marine natural products will be presented along with a summary of the exciting areas that still are unexplored.

Actividades científicas: Estudió microbiología ambiental y su aplicación para soluciones comerciales en empresas de biotecnología al aprovechar el alcance de la diversidad microbiana y su relevancia para las especificaciones del producto. Estos incluyen biomas marinos y terrestres colonizados por bacterias, hongos, algas, plantas, animales y conjuntos de ellos caracterizados tanto por la implementación de nuevos métodos de cultivo y aislamiento microbiano y la secuenciación del ADN. La exploración y el descubrimiento microbiano comercial (bioprospección) en programas industriales dieron como resultado lanzamientos de productos en aceites nutricionales, soluciones agrícolas y diagnósticos.

Premios / Honores / Liderazgo: Miembro inicial de 4 compañías nuevas enfocadas en aplicaciones de microbioma. Ex Vicepresidente Discovery en Indigo Ag, Director Sr. Microbial Discovery en Synthetic Genomics and Scientist II en Diversa Corp. Recientemente creado, lanzado y recaudado fondos para Solarea Bio, Inc. en Cambridge MA para desarrollar terapias microbianas para tratar trastornos metabólicos y musculoesqueléticos. Solarea incubó en Illumina Accelerator en San Francisco durante la etapa de descubrimiento para la compañía. Ha diseñado, implementado y progresado programas de descubrimiento actualmente en etapa comercial.

Publicaciones: 12 artículos científicos.

Scientific Activities: Studied environmental microbiology and its application for commercial solutions in biotech companies by leveraging the extent of microbial diversity and its relevance for product specs. These included marine and terrestrial biomes colonized by bacteria, fungi, algae, plants, animals and assemblages of them characterized both by implementing novel microbial isolation and cultivation methods and DNA sequencing. The exploration and commercial microbial discovery (bioprospecting) on industrial programs resulted in product launches in nutritional oils, agricultural solutions and diagnostics.

Awards/Honors/Leadership: Early member of 4 startup companies focused in microbiome applications. Former Vice President Discovery at Indigo Ag, Sr. Director Microbial Discovery at Synthetic Genomics and Scientist II at Diversa Corp. Recently created, launched and fundraised for Solarea Bio, Inc. in Cambridge MA to develop microbial therapeutics to treat metabolic and musculoskeletal disorders. Solarea incubated at the Illumina Accelerator in San Francisco during the Discovery stage for the company. He has designed, implemented and progressed discovery programs currently in commercial stages.

Publications: 12 scientific publications.

MICROBIAL DISCOVERY AND THE PROSPECT OF NEW PRODUCT DEVELOPMENT

Gerardo V Toledo

Biotlan/Microbiome

Our understanding about the extent of biological diversity is constantly expanding by surveys using molecular markers revealing greater than 8.7 million species (mostly microbial) that coexist and interact to create complex assemblages shaping ecosystems. This functional and genetic diversity creates a vast reservoir for commercial products derived from algae and other microbiota in multiple industries. To study, collect or profit from these genetic resources there is an international legal framework through the Conventional of Biological Diversity and agreements including the Nagoya protocol, which establishes the guidelines for, benefit sharing. The development of products such as DHA from algae, fluorescent proteins from marine invertebrates for diagnostics or biofertilizers for row crops from endophytes has been enabled by a clear definition of product specs and the survey of natural habitats where these environmental conditions promote such adaptations. Recognizing strong biological signals in nature and establishing stringent assays to select for strong lead candidates reduces the screening space and shortens the discovery stage. An understanding of the incumbent technology, benchmarks and products to be replaced by the inventions is essential to access the addressable markets or to create a new market. Beyond the technical aspects of production, scale up and manufacturing it is necessary to have access to distribution channels to provide a solution delighting costumers and growing the demand for the product and upgrades. The new industry created by the microbiome sciences motivated by the health benefits conferred and regulated by microorganisms present in digestive tracts of humans and animals will expand and accelerate discoveries. These approaches to commercialize microbial-derived products enabled launches in relatively rapid cycles that can be replicated and adapted to the current.

Actividades científicas: Dedicada a trabajos de Fotosíntesis y Enzimología Vegetal. En Argentina comenzó un proyecto para comprender cómo las plantas responden a las condiciones ambientales, investigando los factores de transcripción implicados en tales respuestas y las vías de transducción de señales en las que participan estos factores de transcripción. También está interesada en desarrollar herramientas moleculares para mejorar las defensas de las plantas contra el estrés abiótico.

Reconocimientos: Nombrada una de las 10 científicas más destacados de América Latina por parte de la BBC. Directora del Instituto de Agrobiotecnología de Santa Fe (IAL) en Argentina.

Publicaciones y patentes: 62 artículos en revistas especializadas y 7 patentes licenciadas a empresas de biotecnología.

Scientific Activities: Research was devoted to photosynthesis and Plant Enzymology. In Argentina started a project aiming to understand how plants respond to environmental conditions. She investigated the transcription factors involved in such responses and the signal transduction pathways in which these transcription factors participate. She is also interested in developing molecular tools to improve plant defenses to abiotic stress.

Awards/Honors/Leadership: Named one of the ten most outstanding scientists in Latin America by the BBC. Director of Agrobiotechnology Institute of Santa Fe (IAL).

Publications and patents: 62 papers in specialized journals and 7 biotechnological patents licensed to biotechnology companies.

ÉXITOS Y FRACASOS EN EL DESARROLLO DE HERRAMIENTAS BIOTECNOLÓGICAS PARA EL MEJORAMIENTO VEGETAL. EL LARGO CAMINO DESDE EL LABORATORIO AL CAMPO Y DESDE EL MODELO AL CULTIVO.

Raquel Lía Chan

Instituto de Agrobiotecnología del Litoral (UNL-CONICET) y FBCB (UNL). E-mail: rchan@fbcb.unl.edu.ar

La investigación en Biología Molecular Vegetal se ha llevado a cabo mayoritariamente utilizando especies modelo. Sin embargo, cuando el objetivo es mejorar cultivos de interés agronómico y el conocimiento adquirido deriva del estudio en estos modelos, se presenta un largo camino plagado de obstáculos. Las características analizadas no siempre se conservan entre especies y lo que ocurre en un campo experimental dista mucho de los ensayos en cámaras de cultivo controladas.

HaHB4 es un factor de transcripción de girasol que al ser introducido en Arabidopsis confirió características de tolerancia a estrés hídrico y salinidad. Si bien no con la misma intensidad, en plantas de soja y trigo evaluadas en cámara de cultivo, invernadero y finalmente a campo en diferentes ambientes, el transgén incrementó el rendimiento en especial en situaciones estresantes. Por ejemplo, el trigo HaHB4 rindió entre 0 y 95% más que su control dependiendo del año y ambiente. Durante la última campaña la soja HaHB4 registró un incremento muy significativo que varió en diferentes localidades. Esta tecnología ha sido aprobada por las oficinas regulatorias correspondientes en

Argentina y EEUU, sin embargo, todavía no es un producto comercial ya que distintos tratados internacionales frenan su liberación. HaHB11, un gen emparentado con HaHB4, fue introducido en maíz y arroz con resultados prometedores que se discutirán.

Luego de transitar el largo camino con diferentes tecnologías podemos concluir que todo resultado obtenido en un modelo debe ser considerado preliminar hasta no ser validado en cultivos y en condiciones reales de campo.

Actividades científicas: Estudio de matrices alimentarias realizadas mediante espectroscopia de Resonancia Magnética Nuclear (RMN). Ha desarrollado un protocolo analítico innovador que incluye la elaboración estadística de los datos de RMN y el análisis Multivariado para el estudio del perfil metabólico de alimentos.
Reconocimientos: Editor asociado del Journal of Integrated Omics. Miembro de GIDRM (Italian Magnetic Resonance Group). Miembro del comité científico y técnico de NMR "Retelab Involving". Miembro del comité científico de la revista Italiana delle Sostanze Grasse.

Publicaciones: Más de150 publicaciones en revistas especializadas.

Scientific Activities: Study of food matrices performed by means of Nuclear Magnetic Resonance (NMR) spectroscopy. She has developed a protocol that includes the study of the foodstuffs metabolic profile and according to the specific problem the statistical elaboration of the NMR data.

Awards/Honors/Leadership: Associated Editor of the Journal of Integrated Omics. Board member of GIDRM (Italian Magnetic Resonance Group). Member of Scientific and Technical Committee of the NMR Retelab involving. Member of the Scientific Committee of La Rivista Italiane delle Sostanze Grasse.

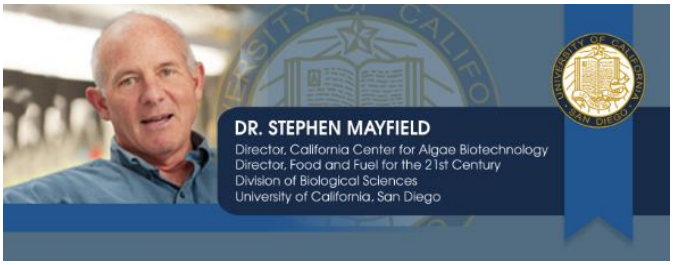
Publications: More than 150 papers on International and National journals.

THE NMR METHODOLOGIES IN FOOD SCIENCE

Luisa Mannina

Sapienza Universita di Roma. Dipartimento di Chimica e Tecnologie del Farmaco, Piazzale Aldo Moro 5, 00185 Rome, Italy

High field NMR spectroscopy, as powerful tool to solve spectra of complex mixtures and to recognize and quantify each component without chemical separation, has found a constantly increasing application in metabolomics and food chemistry. ^{1}H high field NMR spectroscopy has shown to be a valuable tool for the qualitative and quantitative analysis of the metabolic profiling of food stuff such as olive oils, sea bass, truffles, lettuce, tomatoes, beers, fruits etc. (1-6). The quantitative analysis of the metabolic profiling along with the application of a suitable statistical analysis has allowed food characterization in terms of geographical origin, genetic origin and farming. The potential of NMR spectroscopy to detect food adulterations has been also demonstrated. Here, the NMR methodology used to study foodstuffs is discussed reporting some significant examples.



Actividades científicas: Su investigación se centra en la genética molecular en algas verdes y en la producción de proteínas terapéuticas y moléculas biocombustibles, utilizando algas como plataforma de producción. Ha desarrollado una serie de vectores de expresión que permiten una máxima acumulación de proteínas complejas de mamíferos, incluidos anticuerpos monoclonales, factores de crecimiento y una variedad de enzimas industriales potenciales.

Reconocimientos: Reconocido con el premio "Dean's Leadership Council–Biology". Fundador del Centro de Biotecnología Algal en San Diego (SDCAB), así comoTriton Algae Innovations, Verdant Therapeutics Inc. y Algenesis Materials. Editor Asociado de las revistas Biofuels, ACS Synthetic Biology, Algae Research, y editor invitado en Current Opinions of Chemical Biology.

Publicaciones y Patentes: Alrededor de 100 artículos científicos en revistas indexadas y 13 patentes.

Scientific Activities: His research focuses on gene regulation in eukaryotic algae and the use of this alga for the production of human therapeutic proteins and as a platform for biofuel production. He is developing genetic and synthetic biology tools algae, expression of therapeutic proteins in eukaryotic algae, production of biofuels in micro algae, nutraceutical production and gene regulation in green algae.

Awards/Honors/Leadership: Recognized with Dean's Leadership Council–Biology award. He was founded the San Diego Center for Algal Biotechnology (SDCAB), Triton Algae Innovations, Verdant Therapeutics Inc. and Algenesis Materials. Associate Editor of Biofuels, ACS Synthetic Biology, Algae Research, and Guest Editor in Current Opinions of Chemical Biology.

Publications and Patents: Around 100 scientific papers in peer-reviewed journals, and 13 patents

PHOTOSYNTHETIC BIO-MANUFACTURING IN GREEN ALGAE – FOOD AND FUEL FOR THE 21ST CENTURY

Stephen P. Mayfield

University of California San Diego

Eukaryotic algae offer tremendous potential for the large scale production of bio-products as algae require only sunlight as an energy source and sequester CO2 during the production of biomass. Algae are also much more efficient then terrestrial plants in fixing CO2 and producing biomass, and algae can be grown using non-potable water on non-arable land. Using "designed for purpose" photosynthetic microorganisms we have the opportunity to develop production platforms for food, fuel, and biomaterials that have unmatched efficiencies and productivities. In order to fully exploit the biomass productivities of algae we need to develop the genetic tools and production processes that will enable algae to become a commercially viable bio-products platform. We are developing these genetic and synthetic biology tools to enable the production of high value products, including recombinant proteins used as nutritional supplements, as well as precursors for renewable polymers. The challenges, potential, and some early successes of engineered algae for the production of high value products will be discussed.

Mesa de discusión en Biotecnología / Discussion panel of Biotechnology

Patentes / Patents

Expertos en biotecnología con amplia experiencia en el desarrollo de patentes biotecnológicas contarán sus experiencias y la importancia de patentar un desarrollo tecnológico.

Dra. Raquel Lía Chan, Dr. Stephen Mayfield, Dr. William Fenical

Innovación Tecnológica / Innovations in Biotechnology

Se abordarán aspectos del desarrollo de nuevos productos, servicios, procesos o fuentes de abastecimiento dirigido hacía el sector social, educativo y empresarial.

Dr. Gerardo Toledo, Dr. Aristóbulo Loaiza, Dr. Octavio García

Jóvenes Innovadores / Young Entrepreneurs

Ideas de jóvenes que han logrado el desarrollo de nuevos procesos o productos biotecnológicos que han impactado a la sociedad mexicana.

Dr. Daniel A. Jacobo Velázquez, I.Q. Scott Munguía

Líder natural que aprovecha los sistemas inteligentes y las redes para impulsar los resultados comerciales. Amplia capacitación en productos químicos, biotecnología y comercial, con un profundo conocimiento y una red sólida en varias cadenas comerciales como Biotech, Ag, Nutrition and Food Safety.

Natural leader that leverages systems thinking and networking to drive business results. Extensive chemicals, biotechnology and commercial training, with deep knowledge and a solid network in various value chains including Biotech, Ag, Nutrition and Food Safety.

Actividades científicas: Doctor en Epidemiología Molecular y Biología de la infección por el Instituto Karolinska de Suecia. Biólogo egresado de la FES Iztacala, UNAM. Fungió como investigador en la UNAM, Escuela Superior de Medicina IPN y del Centro CONACYT CIATEJ A.C. en Guadalajara, Jalisco.

Reconocimientos: Asesor en sanidad para la unión europea durante 2009-2011, asesor de la embajadora de Suecia en México en materia de bioseguridad desde 2008 hasta 2010. Formó parte del grupo de diagnóstico de Influenza AH1N1 formado en por el Gobierno de la Ciudad de México durante la contingencia sanitaria de 2009.

Publicaciones y Patentes: 19 artículos publicados en revistas científicas internacionales en el área de virología y microbiología, y 3 patentes internacionales.

Scientific Activities: PhD. in Molecular Epidemiology and Biology of the infection by Karolinska Institute of Sweden. Biologist graduated from FES-Iztacala, UNAM. He worked as a researcher at the UNAM, School of Medicine of Instituto Politécnico Nacional and CIATEJ A.C. (CONACYT) in Guadalajara Jalisco.

Awards/Honors/Leadership: Advisor in health for the European Union (2009-2011). Adviser to Swedish ambassador in Mexico on biosecurity matters (2008-2010). He was part of the diagnosis group of Influenza AH1N1 formed by the Government of Mexico City during the health contingency of 2009.

Publications: 19 articles published in international scientific journals in the area of virology and microbiology and 3 international patents.

Actividades científicas: Ha desarrollado la tecnología de un sistema alternativo, efectivo y simple que desencadena la activación del metabolismo de las plantas, que permite la sobreproducción de biomoléculas nutritivas y medicinales. Como el ácido shikímico (utilizado en la producción del Tamiflu antiviral) o el resveratrol (compuesto con posibles efectos beneficiosos en la prevención de enfermedades cardiovasculares), de plantas que no pasan los estándares de calidad para el consumo humano.

Reconocimientos: Es parte de los 10 Innovadores menores de 35 años de México, de acuerdo a los premios MIT Technology Review. Miembro del Sistema Nacional de Investigadores.

Publicaciones y Patentes: 53 publicaciones científicas. 1 patente registrada que protege su tecnología de sobreproducción de ácido shikímico y compuestos fenólicos en cultivos hortofrutícolas en México y EEUU.

Scientific Activities: Technological development is based in an alternative system, which is effective and simple that triggers the activation of plant metabolism, leading the overproduction of nutritive and medicinal biomolecules. Like shikimic acid (used in the production of the antiviral Tamiflu) or resveratrol (compound with potential beneficial effects in the prevention of cardiovascular diseases) of plants that do not pass quality standards for human consumption.

Awards/Honors/Leadership: part of the 10 Innovators under 35 years of age in Mexico, according to the MIT Technology Review Awards. SNI I.

Publications and Patents: 53 scientific publications, one patent that protects his technology of overproduction of shikimic acid and phenolic compounds in horticultural crops in Mexico and the USA.

Actividades científicas: Joven emprendedor mexicano, que desarrolló una tecnología para la obtención de un plástico biodegradable a partir de la semilla del aguacate, material que se degrada mucho más rápido que los plásticos hechos a base de hidrocarburos. Fundó su propia empresa Biofase en el año 2012.

Reconocimientos: Nombrado como parte de los 10 Innovadores menores de 35 años de México, de acuerdo a los premios MIT Technology Review. Ha recibido más de 11 premios entre los que destacan, el primer lugar en el concurso internacional de emprendedores del Foro "Red Emprendia Spin 2012" en Madrid. Innovación Tecnológica Clean Tech Challenge México 2012, PREMIO FRISA de Desarrollo Empresarial, y "RECICLA 2012". Es socio fundador de la Red Mexicana de Innovadores y la Comisión Nacional de Bioplásticos en México.

Patentes: 1 patente mexicana y patente internacional pendiente.

Scientific Activities: Chemical Engineer ITESM graduated, is a young Mexican entrepreneur who has created a bioplastic from avocado seeds, a material that degrades much faster than hydrocarbon-based plastics. He founded his own company Biofase in 2012.

Awards/Honors/Leadership: Named as part of the 10 Innovators under 35 years of Mexico, according to the MIT Technology Review Awards. He has received more than 11 awards among which stand out, the first place in the international entrepreneurship contest of the "Red Emprendia Spin 2012" Forum held in the city of Madrid. Technological Innovation Clean Tech Challenge Mexico 2012, FRISA PRIZE for Entrepreneurial Development, and "RECICLA 2012".Further is founding partner of the Mexican Network of Innovators and National Bioplastic Commission in Mexico.

Patents: One Mexican patent, although an international patent is already pending.

ISSN: **2448-8461**

Resúmenes / Abstracts

ISSN: 2448-8461

AM1

PHYTOREMEDIATION OF SALINE SOILS THROUGH THE USE OF *BACOPA MONNIERI* PENELL

Salvador Moreno García, Dioselina Álvarez Bernal, Marina Olivia Franco Hernández, Héctor René Buelna Osben, Instituto Politécnico Nacional, CIDIIR Michoacán, Jiquilpan, 59510. Email: chavo.mo.17@gmail.com, dalvarezb@ipn.mx

Key words: Salinity, Phytoremediation, Halophytes

Introduction. Soil salinity is a worldwide problem. It is estimated that of the 1470 million hectares dedicated to agriculture worldwide, 76.6 million of these present salinity (1) and in Mexico, of the 27 million hectares for agricultural use, about one million also present it (2); To remedy this problem there are different methods, both physical-chemical and biological (3). For the present work a method of biological remediation (phytoremediation) is carried out. *B. monnieri* is a halophyte to which benefits have been attributed as a remedy for contaminated water and soil.

The objective of this work is to evaluate the phytoremediation potential of *B. monnieri* in saline soils.

Methods. Two SN and SNm saline soils were characterized to evaluate the phytoremediatory potential of *B. monnieri*. The experimental design consists of 4 treatments for each soil with ten repetitions: 1) soil alone (control soil); 2) soil plus corn; 3) soil plus *B. monnieri* plant plus corn and 4) soil plus *B. monnieri* plant. The experiment lasted 120 days, after which electrical conductivity (EC), pH, cations and soluble anions were measured. For the plant, the same analyzes were made and biometric measurements were made. Soil analysis is based on the NOM-021-SEMARNAT-2000 (4), for the analysis of the vegetable tissue Sadzawka, (2007) (5) and the experiment in Rabhi, (2010) (6).

Results. Characterization of soil shows that is saline type, the salts that were found are carbonates, bicarbonates, and cations such K+, Mg++, Na+ and Ca ++. The results of the experiment 1 show a decrease of the electrical conductivity (EC) of 6.22 to 2.34 and 2.99 dS/cm for the treatments that used *B. monnieri* in the soil SNm. For the soil SN (experiment 1b, Table 2) no favorable results were obtained since there was no development of the plant, and the salts that were in the plant were deposited in the pots, for that reason the CE was elevated.

Table 1. EXP 1a. pH 8.45 and CE 6.22 ǂinitial soil SNm

TRATAMIENTO	PH	CE dS/m
SNm+B	7.74 b	2.34 b
SNm+B+M	7.97 b	2.99 b
SNm+M	7.66 b	5.12 a
SNm CONTROL	7.95 b	5.46a

Table 2. EXP 1b. pH 8.58 and CE 22.73 ǂinitial soil SN

TRATAMIENTO	PH	CE dS/m
SN+M	8.67 a	29.06 d
SN+B	8.5 bc	44.13 a
SN Control	8.48 bc	40.34 b
SN+M+B	8.4 c	37.15 c

Conclusions. The characterization of the soils of the region with salinity problems, confirmed that they are of the saline type, finding salts such as carbonates, bicarbonates and cations such as K+, Mg++, Na+ and Ca ++, these last in greater concentration than the rest. *B. monnieri* has phytoremediation potential of saline soils with electrical conductivities less than 7 dS / m.

Acknowledgements. We thank the National Council of Science and Technology (CONACYT) for funding the project 2015-1-1165 and SIP20180085.

References.

1. Cuero, R. (2012). Hacia un sistema complementario de producción más limpia en suelos degradados por salinidad. Ambiente y Sostenibilidad, 2(1), 59-68.
2. Martínez, N., López, C. V., Basurto, M. & Pérez, R. (2011). Efectos por salinidad en el desarrollo vegetativo. Tecnociencia Chihuahua, 5(3), 156-161.
3. Ortiz, B. I., Sanz, J., Dorado, M., &Villar, S. (2007). Técnicas de recuperación de suelos contaminados. Informe de Vigilancia Tecnológica. Universidad de Alcalá. Dirección General de Universidades e Investigación. España.
4. Norma Oficial Mexicana NOM-021-SEMARNAT-2000. *Que establece las especifica ciones de fe rtilidad, sa linidad y clasificación de suelos, estudio, muestreo y análisis: México*, *31*, 85.
5. Sadzawka, A., Carrasco, M. A., Demanet, R., Flores, H., Grez, R., Mora, M. L., &Neaman, A. (2007). Métodos de análisis de tejidos vegetales. Segunda Edición. Instituto de Investigaciones Agropecuarias. *Serie Actas INIA*, (40).
6. Rabhi, M., Talbi, O., Atia, A., Abdelly, C., & Smaoui, A. (2008). Selection of a halophyte that could be used in the bioreclamation of salt-affected soils in arid and semi-arid regions. In *Biosaline Agriculture and High Salinity Tolerance*(pp. 241-246). Birkhäuser Basel.

AM2

RESPONSE OF THE BACTERIOPLANCKTON COMMUNITY TO A SIMULATED OIL SPILL IN MESOCOSMS EXPERIMENTS

Sonia Valencia-Agami, Sébastien Putzeys, Daniel Cerqueda-García, Ulises García-Cruz, Abril Gamboa, Rosa Canul, Oswaldo González, Víctor Ceja, Flor Árcega, Jorge Herrera, Daniel Pech, Leopoldina Aguirre-Macedo, José Q. García-Maldonado

Centro de Investigación y Estudios Avanzados del IPN, Unidad Mérida. Yucatán; C.P. 97310

Laboratorios de: Patología Acuática; Geoquímica; Producción Primaria; sonia.valencia@cinvestav.mx

Key words: Mesocosms, 16S ARNr, Yucatán sea platform

Introduction. Several studies conducted on the Deep-Water Horizon oil spill have shown that bacterioplankton rapidly responds to hydrocarbon inputs[1]. Enclosed experimental ecosystems, namely mesocosms, are essential research tools to evaluate the destination and effects of xenobiotic chemicals (*e.g.,* crude oil), parties (individuals, populations, communities) and whole environments (ecosystems) simultaneously[2]. The employment of mesocosms for environmental pollution simulation studies has never been undertaken in tropical conditions (e.g. oligotrophic, high evaporation) such as the conditions in Yucatan sea platform, Mexico.

This study aimed to assess the response of the native microbial community (NMC) of the water column of the Southern Gulf of Mexico (SGM) during a simulated oil spill experiment at mesocosm scale.

Methods. A mesocosm experiment was carried out over a period of 17 days in a 2500 L tank filled with marine water from Progreso harbor on the Yucatan Peninsula and was supplemented with 250 mL of light crude oil from "Pozo Pol" (35 API) (80 ppm). The concentration of total petroleum hydrocarbons (TPH) in the water column was measured by GC-MASS and GC-FID. Culturable hydrocarbonoclastic bacteria (CHB) were grown in Broth (Bushnell-has) and quantified by the most probable number method (MPN). Bacterial community was analyzed by Illumina sequencing of the 16S rRNA gene. Bioinformatic analyses were conducted following the QUIIME2 pipeline. Statistical analyses were performed on R Software.

Results. The highest concentration of available TPH in the water column became present on the 6th day (6503.86 µL/L), and it showed a relationship with the maximum number CHB (24,000 UFC/mL). The microbial diversity, measured through Shannon index (H'), presented a significant decrease on the 4th day (from 5.2 to 4). However, over the following days the diversity increased, reaching a similar value to the initial time (5.13). Bacterioplankton composition previous to the crude oil contamination was dominated by members of the classes Gamma-, Alphaproteobacteria, Cyanobacteria, Flavobacteria, and Verrumicrobiae. However, the PCoA analysis showed that community structure was distinct among all the samples, where the final sample time was the most dissimilar to the initial structure. PERMANOVA analysis suggested that these changes were related to the aromatic compounds concentration, chlorophyll *a* content and total carbon percentage (21, 18 and 16% contribution respectively). Finally, thanks to a search of shared OTUs we found that genera *Luminiphilus*, *Phaeodactylibacter, Roseibacillus*, an uncultured genus belonging to the family Surface 1 and, an unassigned genera of the Family OM182 were present throughout the experiment.

Conclusions. The results of this experiment showed that the bacterioplankton diversity from SGM had a decrease after 2 days of crude oil pollution. However, final bacterial community displayed a diversity increase but, with differences in bacterioplankton composition compared to initial time. Also, 5 bacterial genera (7 OTUs) were found among all samples; we can infer that these are capable of persisting crude oil impact. Finally, based on the quantifications of CHB and TPH the results suggested that the NMC from the water column of SGM were capable of performing hydrocarbons degradation 4 days after the oil contamination event.

Acknowledgments. "Research funded by National Council of Sciences and Technology (CONACyT) of Mexico-Mexican Ministry of Energy (SENER)- Hydrocarbon Trust, project 201441. The present work is a contribution of the Gulf of Mexico Research Consortium (CIGoM)"

References.

1. King G., Smith C., Tolar B and Hollibaugh J. 2013. *Analysis of Composition and Structure of Coastal to Mesopelagic Bacterioplankton Communities in the Northern Gulf of Mexico. Front. Microbiol*. Vol 3. 438

2. Cappello S. & Yakimov M. 2010. *Mesocosms for Oil Spill Simulation. Handbook of Hydrocarbon and Lipid Microbiology*. 3514-3521.

ISSN: 2448-8461

AM3

EFFECT OF HUMIC ACID ON DEVELOPMENT OF *Capsicum annuum* L. var. *glabriusculum* (Dunal) Heiser & Pickersgill

Dionisia Lara-Arredondo[1], Rey David Ruelas-Ayala[1], Adolfo Dagoberto Armenta-Bojórquez[2], Estela Sañudo-Ayala[1], Jaime Alberto Félix Herrán[1]

[1]Universidad Autónoma Intercultural de Sinaloa, Department of Engineering and Technologies, Mochicahui, El Fuerte, Sinaloa C.P. 81890, jfelixherran@hotmail.com

[2]Researcher of the Interdisciplinary Research Center for Integral Regional Development of Instituto Politécnico Nacional (CIIDIR-IPN, Unidad Sinaloa).

Key words: plant quality, sustainability, soil organic matter

Introduction. Humic acids are the most widely studied among the humic substances group. Humic acids improve soil physical and chemical properties, in addition to its physiological function in the development of plant to promote its growth, cell division and elongation of roots, as well as their participation in the plant nutrition with results similar to those obtained with phytohormones.
The role attributed to the humic acids in plant development the objective of the present study is to evaluate the effect of humic acids from composts on seed germination and development of Chiltepin.

Methods. Was collected organic solid waste of: sawdust, sorghum, maize, market waste and garden grass pruning, which were mixed with cow manure in ratio 1:3 and the mixture were composted. The composting process lasted 120 days, at the end was sampled 1 kg of each compost, for its physicoquemical characterization and was determined the HA content. The HA were extracted, and 5% per Liter of each simple of HA were prepared. The irrigation with the solution of HA started 15 days after the seeds germinate. The irrigation was weekly, applying 4 mL of solution per cavity, for a month. At the end of the month, the following variables were measured: height (mm), stem diameter (mm) and length of the root (mm), was also weighed the foliar and root biomass.

Results. The humic acids promoted higher height and root length in the chiltepin seedlings (Table 1) compare to the reference treatment. The difference was statistically significative. The dry weight of root was not different statistically significative, but there was statistically significative difference in the foliar dry weight.

Table 1. Mean comparison of the evaluation of the effect on the development and growth of Chiltepin seedlings.

Compost	Diameter (mm)	Root (mm)	Height (mm)
P	0.4235ab* ±0.028	52.590ab ±0.24	44.147a ±0.15
A	0.4340ab ±0.022	47.345b ±0.27	42.020a ±0.18
RM	0.4560a ±0.024	46.576b ±0.29	42.972a ±0.21
M	0.3355b ±0.018	54.128ab ±0.32	40.483a ±0.15
S	0.4193ab ±0.025	60.929a ±0.38	43.270a ±0.15
T	0.5150a ±0.028	54.356ab ±0.34	42.657a ±0.12

*Different letters in columns show significative differences, according to Tukey test ($p < 0.05$). The data after ± is the mean estandar error.

Conclusions. The humic acids improve the development of chiltepin seedlings, which were higher and with longer roots, this could help plants to have higher possibilities to survive in field.

Acknowledgements. This project was supported by PRODEP, with the Project Aprovechamiento de los residuos orgánicos del Norte de Sinaloa: Influencia en la Producción de Sustancias Húmicas y su efecto en el crecimiento de plantas de interés forestal de la región.

References.

1. Tan, K.H. 2014. Humic matter in soil and the environment, Principles and Controversies. 2nd edition, CRC Press Taylor and Francis Group, Boca Raton, Florida, US. p. 441

2. Rodríguez del Bosque L. A., M. Ramírez y O. Pozo C. 2004. Tecnología de producción de chile piquín en el noroeste de México. Editado por el INIFAP, Tamaulipas, México. 43 p.

3. Paul E. A. 2015. Soil microbiology, ecology and biochemistry. 4th edition, Elsevier Inc., Oxford, United Kingdom. p. 598

4. Peña-Méndez, E. M., J. Havel y J. Patočka. 2005. Humic substances: compounds of still unknown structure: applications in agriculture, industry, environment, and biomedicine. Journal of applied biomedicine. 3: 13 – 24.

ISSN: 2448-8461

AM4

EVALUATION OF INCUBATION PARAMETERS FOR POLY-3-HYDROXYBUTYRATE (P3HB) PRODUCTION BY *BACILLUS* SP.

Enrique Martínez, Verónica Almaguer, Guadalupe Rojas, Isela Quintero, Lucia Palacios, Elizabeth Alemán.
Instituto de Biotecnología, Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León. Av. Pedro de Alba y Manuel L. Barragán s/n. Ciudad Universitaria. C.P. 66455 San Nicolás de los Garza, Nuevo León, México.
Enrique Martínez: enq2802@gmail.com

Keywords: Biopolymers, Poly-3-hydroxybutyrate, Bacillus.

Introduction. Poly-3-hydroxybutyrate (P3HB), is the most abundant and characterized type of Polyhydroxyalkanoate found in bacteria (1). It is an interesting biomaterial due to its biodegradable, biocompatible, thermo-resistant, moldable and innocuous properties, which is why it is considered as a substitute for polypropylene (2). Some of the most common applications of this biopolymer in the medical area are the manufacture of suture threads and nerve guides for bone marrow (3). In the industrial area, the P3HB it's used for food packing and the generation of biofuels additives (4). And in agriculture, this biopolymer is used for the design of formulations of long-acting pesticides (5).
Currently, research around P3HB is based on improving the production of this biopolymer. That is why the objective of the present work was to study the temperature (T°), pH, and agitation speed (rpm) of a *Bacillus* strain in a nutrient-rich culture medium.

Methods. The following incubation parameters were evaluated: temperature (20º, 30º, and 40º C), pH (6, 7, and 8) and agitation speed (100, 150, and 200 rpm) under a factorial design 3 x 3. We started with a 100 mL pre-inoculum of nutrient-rich culture medium, which was incubated for 24 h. Subsequently, 2% (v / v) of the pre-inoculum was inoculated in 200 mL of the culture medium and incubated under the parameters to be evaluated for 48 h. The extraction of the biopolymer was carried out by digestion with NaOCl and suspension in chloroform. The evaluated variables were the amount of cell dry weight (g / L), biopolymer extracted (g / L) and the biopolymer accumulation percentage (% P3HB).

Results. The *Bacillus* sp. strain was able to grow and generate the biopolymer under all the evaluated parameters, presenting the highest generation of cellular biomass, biopolymer production and accumulation percentage at the incubation conditions of 30º C, pH 6 and 150 rpm (**Fig. 1**).

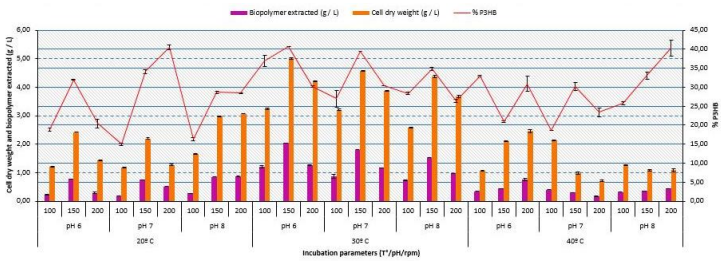


Fig.1 Evaluation in the P3HB production by *Bacillus* sp. under the effect of different incubation parameters (T°, pH, and rpm).

The bacterial strain generated up to 5 g / L of cellular biomass and 2 g/L of polymeric extract with characteristics of P3HB type polymers, as well as a 40% accumulation (dry cell biomass).

Conclusions. The bacterial strain of the *Bacillus* genus produced up to 2 g / L of P3HB-type biopolymers under the incubation parameters of 30° C, pH 6 and agitation speed of 150 rpm in a nutrient-rich culture medium.

Acknowledgments. We thank the Consejo Nacional de Ciencia y Tecnología (CONACyT) for the support given as a Ph.D. grant (grant No. 468278).

References.

1. Berekaa M, Al Issa A. (2016). *J. Microbiol. Biotechnol. Food Sci*. 5: 606–611.
2. Masood F, Hasan F, Ahmed S, Hameed A. (2012). *Ann. Microbiol.* 62: 1377–1384.
3. Bugnicourt E, Cinelli P, Lazzeri A, Alvarez V. (2014). *Express Polym. Lett.* 8: 791–808.
4. Koller M. (2017). *Bioengineering*. 4: 1-7.
5. Voinova O, Kalacheva G, Grodnitskaya I, Volova T. (2009). *Appl. Biochem. Microbiol.* 45: 384–388.

ISSN: 2448-8461

Biochemical characterization of the purified chitinases of *Bacillus cereus sensu lato* strain B25 and evaluation *in vitro* of antagonism against *Fusarium verticillioides*

Priego-Rivera R.[1] Figueroa-López A. M.[2] Cazares-Alvarez J. E.[1] Maldonado-Mendoza I. E.[1 1] Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional Unidad Sinaloa, Instituto Politécnico Nacional, Departmento de Biotecnología Agrícola Guasave, Sin. imaldona@ipn.mx

Key words: chitinases, characterization, antagonism

Introduction. Chitinases are enzymes that degrade chitin (1). These enzymes are produced by a large number of organisms, including bacteria from the genus *Bacillus* (2). *Bacillus cereus sensu lato* B25 is an endemic bacterium from Northern Sinaloa that was selected as an effective antagonist against *Fusarium verticillioides* both *in vitro* and in field trials. *B*25 is capable of producing two chitinases (ChiA and ChiB), which we propose may be involved in the antagonistic mechanism (3).

In the present work, we purified the recombinant B25 chitinases and characterized them biochemically, as well as we performed *in vitro* antagonistic assays to elucidate the role that *B*25 chiA and chiB play in the biological control against *Fusarium verticillioides*.

Methods. Recombinant versions of ChiA and ChiB containing a 6X-His tag located at both C- and N- ends of the proteins and expressed in *E. coli* BL21 Star™ (DE3) were used (4). Induction of the recombinant proteins was achieved by adding L-arabinose (0.4%) for 4 hours. Both were purified using Ni-NTA Spin columns under native conditions. Once the purified proteins were obtained, the type of activity they possessed and their optimal activity conditions were determined. This allowed us to determine the affinity for substrate using the Michaelis-Menten constant. *In vitro* antagonism tests will be carried out to observe the effect of the addition of the purified proteins against the fungus.

Results. ChiA6xHis and ChiB6xHis, presented a higher activity against three different substrate (GlcNAc 1, 2 and 3) that suggest they can act as both exo and endochitinases. Both chitinases present greater activity at acidic pH and at temperatures close to 50 °C, we are currently working on characterizing the different kinetic parameters such as V_{max} and K_m. Results on the *in vitro* antagonistic tests with the purified enzymes will be showed and discussed relating to B25 biocontrol activity on *F. verticillioides*.

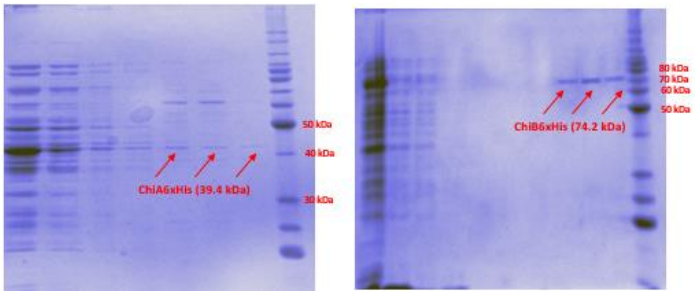


Fig.1 Purification of ChiA6xHis and ChiB6xHis under native conditions using Ni-NTA spin columns followed by visualization using SDS-PAGE.

Table 1. Biochemically characterization of chitinases from *Bacillus cereus sensu lato* B25

Chitinase	Type of Activity	Optimal conditions
ChiA6xHis	Exo and Endo	pH 3, 40-60 °C
ChiB6xHis	Exo and Endo	pH 3-4, 60 °C

Conclusions. ChiA6xHis and ChiB6xHis presented both types of activity, endo and exochitinase, ChiA6xHis presented its highest activity at pH 3 and 40-60 °C; ChiB6xHis presented its highest activity at pH 3-4 and at 60 °C.

Acknowledgements. This work has been financially supported by CONACyT through the Fronteras de la ciencia funding program, grant number 2016-01-2510.

References.

1. Jalil S. U., Mishra M. and Ansari M. I. (2015). *Trends in Biosciences*, 8(24), 6733-6743.
2. Senol M., Nadaroglu H., Dikbas N. and Kotan R. (2014). *Annals of Clinical Microbiology and Antimicrobials*, 13, 35-42.
3. Figueroa-López A. M., Leyva-Madrigal K. Y., Cervantes-Gámez R. G., Beltrán-Arredondo L. I., Douriet-Gámez N. R., Castro-Martínez C. and Maldonado-Mendoza I. E. (2017). *Romanian Biotechnological Letters*, 22(4), 12722-12731.
4. Figueroa-López A. M. (2017). Tesis doctoral. Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional Unidad Sinaloa, Guasave, Sinaloa.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
AM6

TRATAMIENTO DE UN EFLUENTE INDUSTRIAL FENÓLICO MEDIANTE UN PROCESO SIMULTÁNEO AEROBIO-ANAERÓBIO EN UN REACTOR DE FLUJO ASCENDENTE A BAJAS TASAS DE OXÍGENO DISUELTO

J. Terreros-Mecalco[1,2*], C. Muro-Urista[1], S. C. Manzur-Quiroga[2], F. J. Castillo-Hernández[2]

[1]Instituto Tecnológico de Toluca División de Estudios de Posgrado e Investigación. Avenida Tecnológico s/n, colonia ExRancho La Virgen 52140 Metepec, Estado de México, México Teléfonos: +52 (457) 2239 49813 y +52 (722) 2087 224

[2]Universidad Politécnica del Valle de Toluca. División de Ingeniería en Biotecnología y Licenciatura en Negocios Internacionales. KM. 5.6 carretera Toluca-Almoloya de Juárez, Santiaguito Tlalcilalcali, C. P. 50904, Almoloya de Juárez, Estado de México. Tels.: 722 276 60 60

[*]E mail: jesusterr@yahoo.com.mx

***Palabras clave**: tratamiento biológico, efluente industrial, fenol, reactor hibrido, anaerobio, aerobio*

Introducción.- El fenol, es uno de los contaminantes que se encuentra con mayor frecuencia en efluentes industriales. Debido a su origen bencénico, es altamente tóxico y recalcitrante (1). El objetivo de este trabajo fue estudiar una nueva configuración de reactor aerobio-anaerobio tipo UASB a bajas tasas de oxígeno disuelto, para su tratamiento.

Métodos. Para el desarrollo de los experimentos y caracterización del agua residual a tratar (tabla 1). Se determinó la Demanda Química de Oxígeno (DQO), basado en un método colorimétrico, Sólidos Totales (ST) y Sólidos Volátiles (SV), de acuerdo al métodos estándar APHA, (2005), pH por potenciometría y fenoles totales, por colorimetría segun la norma NMX-AA-050-SCFI-2001.

Tabla 1. Características del influente (ARF) alimentada al reactor.

Parámetro/Experimento	1	2	3	4
Carga orgánica (Kg/DQO·m^3/día)	3.2	13.9	33.4	33.6
pH	7.02±0.1	6.94±0.1	6.81±0.2	6.82±0.1
DQO (g/L)	1.43±0.05	7.3±0.13	17.7±0.3	17.6±0.3
Fenol (g/L)	0.48±0.03	1.47±0.04	3.37±0.03	3.37±0.02
ST (g/L)	0.12±0.07	0.51±0.6	0.95±0.12	0.73±0.3
SV (g/L)	0.03±0.01	0.27±0.3	0.66±0.2	0.6±0.2

Resultados. La figura 1, muestra la tasa de remoción (η) de DQO de un 64 y 29% respectivamente, durante los experimentos 1 y 2. Y se observa que a mayor tasa de carga orgánica, la η pasó de un 58 a un 56% (experimentos III y IV). La literatura menciona una tasa de remoción similar de DQO (2). Por su parte la figura 2, muestra la eficiencia de remoción de fenol (η_f). Alcanzando un 74% con una carga orgánica de 3.2 KgDQO/m^3·d. Sin embargo, al incrementar la tasa de carga orgánica de 13.9 a 33.4 KgDQO/m^3·d, la remoción disminuyó pasando de un 54% hasta un 20%.

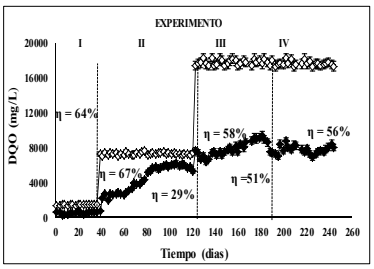


Figura 1. Remoción de DQO. Influente (◇), efluente (◆).

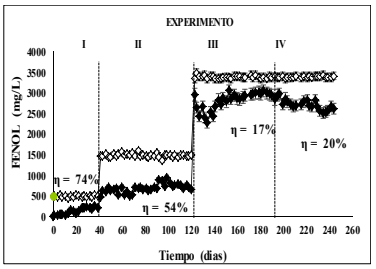


Figura 2. Eliminación de Fenol. Influente (◇), efluente (◆).

Conclusiones. El empleo de un sistema secuencial aerobio-anaerobio a bajas tasas de oxígeno disuelto, sin una fuente alterna de co-sustrato permitió la mineralización de fenol hasta CH_4 y CO_2.

Agradecimientos. Este trabajo fue financiado por el CONACYT para la realización de una estancia postdoctoral en el Instituto Tecnólogico de Toluca, así como a la Universidad Politecnica del Valle de Toluca, por sus instalaciones otorgadas para la formación de recursos humanos de alto nivel.

Referencias.

1. Ortega-Méndez, J.A., Herrera Melián, J.A., Araña, J., Doña Rodríguez, J.M., González Díaz, O. y Pérez-Peña, J. (2015). Detoxification of waters contaminated with phenol, formaldehydeand phenol–formaldehyde mixtures using a combination of biologicaltreatments and advanced oxidation techniques. *Appl. Catal., B. Environ.* vol (163): 63–73.
2. Almasi, A., Pirsaheb, M. y Dargahi, A. (2012). The efficiency of anaerobic Wastewater stabilization pond in removing phenol from Kermanshah Oil Refinery Wastewater. *Iran J Environ Healt J.* (1): 41–50.

ISSN: 2448-8461

AM7

PRETREATMENT AND SACCHARIFICATION OF CORN STOVER FOR PRODUCTION OF SECOND GENERATION BIOETHANOL.

Dulce P. Beltrán-Orduño; Laura I. Beltrán-Arredondo; Lelie D. Castro-Ochoa; Ignacio E. Maldonado-Mendoza; Claudia Castro-Martínez; Instituto Politécnico Nacional, CIIDIR-Sinaloa, Departamento de Biotecnología Agrícola, Guasave, Sinaloa CP 81100; claudiacm30@hotmail.com

Key words: Pretreatment, saccharification, corn stover.

Introduction. Excessive use of fossil fuels has contributed to increased pollution levels in the last decades (1). Lignocellulosic biomass is abundant and available worldwide for biofuel production. It is composed mainly of cellulose hemicellulose and lignin (2). Due to the strong bond of the hemicellulose cellulose and lignin matrix, a pretreatment is necessary to make the cellulose available to enzymatic hydrolysis (3).
The aim of this study was to find the best conditions for pretreatment and saccharification of corn stover for second generation bioethanol production.

Methods. A combined pretreatment with H_2SO_4 2% and H_2O_2 at different concentrations was designed (1%, 3 % and 5%) and different reaction times were tested (20, 30 and 40 hours), the response variable was % of lignin removal. An enzymatic extract was produced using native fungi (*Penicillium* sp. and *Cladosporium* sp.) and corn stover as a carbon source; these extracts were applied during the saccharification stage, using a solid-liquid ratio of 1:20, 50 mM sodium acetate buffer, a *Trichoderma viride* cellulase (10 mg= 40 U), and 300 mg of an enzyme extract from *Penicillium* sp. and *Cladosporium* sp. A factorial design 2^3 with 4 central points was employed to analyze the effect of temperature, pH and agitation rate as study factors.

Results. The highest percentage (80%) of lignin removal was obtained in corn stover pretreated with H_2SO_4 (2%) and H_2O_2 (5%) after 20 hours of reaction time (Fig. 1). This indicates that alkaline solutions efficiently remove lignin by breaking ester bonds and increasing the porosity of biomass (4).

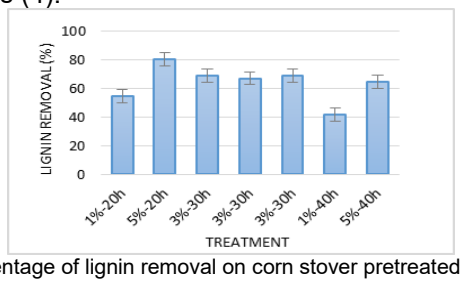


Fig.1 Percentage of lignin removal on corn stover pretreated with H_2SO_4 and H_2O_2.

T. viride showed the highest percentage of saccharification at 45°C, pH 4.5 and 100 rpm, while the enzymatic extract from *Cladosporium* sp. and *Penicillium* sp showed the highest percentage of saccharification at 30°C, pH 6, 200 rpm and 45°C, pH 4.5, 100 rpm, respectively (Table 1).

Table 1. Percentage of saccharification of corn stover pretreated with H_2SO_4 and H_2O_2.

Factorial design				% Saccharification		
No.	T(°C)	pH	rpm	*Penicillium sp.*	*Cladosporium* sp.	*T. viride*
1	30	3	0	5.163	4.644	5.293
2	60	3	0	1.956	0.837	0.752
3	30	6	0	5.12	11.103	9.326
4	60	6	0	7.506	1.471	0.376
5	30	3	200	4.529	2.442	2.192
6	60	3	200	2.278	0.216	0.316
7	30	6	200	20.205	**20.765**	1.385
8	60	6	200	5.552	8.506	0.336
9	45	4.5	100	20.525	8.197	21.485
10	45	4.5	100	**21.582**	8.317	**22.446**
11	45	4.5	100	21.149	8.293	21.293
12	45	4.5	100	21.485	8.245	21.678

Conclusions. Acid/alkaline pretreatment allowed to obtain efficient lignin removal for second generation bioethanol production using corn stover. Cellulase from *T. viride* showed a similar percentage (21.3%) in saccharification against enzymatic extracts used. Enzymatic extracts have a potential application in bioethanol production for economically viable processes.

Acknowledgements. The authors acknowledge the financial support of SAGARPA CONACYT Grant No. 291143 and SIP 20180302.

References.

1. Kumari D., Singh R. (2018). Renew. Sust. Energ. Rev. Vol. (90): 877-891.
2. Haghighi S., Hossein A., Tabatabaei M., Salehi G., Hassan G., Gholami M., Ardjmand M. (2013). Renew. Sust. Energ. Rev. Vol. (27): 77-93.
3. Behera S., Arora R., Nandhagopal N., Kumar S. (2014). Renew. Sust. Energ. Rev. Vol. (36): 91-106.
4. Kim J. S., Le Y. Y., Kim. T. H. (2016). Bioresour. Technol. Vol. (199): 42-48.

ISSN: 2448-8461

AM8

BIODEGRADACIÓN DE FENOL A ALTAS TASAS DE CARGA ORGÁNICA EN UN REACTOR DE MEZCLA COMPLETA DE LODOS ACTIVADOS

Jesús Terreros Mecalco, Silvia Cristina Manzur Quiroga, Felipe de Jesús Castillo Hernández, Itzel Stefany Morales González, Diana Laura Carbajal Aguilar y Daniela Loera Torres.

Universidad Politécnica del Valle de Toluca División de Ingeniería en Biotecnología.
Carretera a Almoloya de Juárez km 14 Santiaguito Tlalcilalcalli, Almoloya de Juárez, Estado de México, México
Teléfonos: +52 (457) 2239 49813 y +52 (722) 2087 224
Email: jesusterr@yahoo.com.mx

Palabras clave: fenol, aguas residuales, lodos activados, toxicidad, biodegradación.

Introducción. Las descargas de efluentes industriales de resinas poliméricas a base de fenol y formaldehido, llegan a presentar concentraciones de fenol entre 35 y 400 mg/L. Los métodos utilizados para su tratamiento incluyen: degradación, eliminación y recuperación. El tratamiento biológico para este efecto, es complicado debido que a concentraciones de 100 mg/L provoca inhibición bacteriana. En el presente trabajo se estudió el efecto de la carga orgánica sobre la tasa de biodegradación del fenol, utilizando un reactor de lodos activados, para su eliminación.

Métodos. Para evaluar el desempeño del reactor, se analizó el pH, DQO, sólidos totales (ST), sólidos volátiles (SV) por el Estándar Methods (1). Para la determinación de fenol, se realizó mediante el método colorimétrico de la 4-aminoantipirina según la NMX-AA-050-SCFI-2001, en el influente y efluente del sistema estudiado. Se utilizó como inóculo, lodo activado de la planta de tratamiento de aguas residuales municipales "Cerro de la Estrella" de la Ciudad de México, el cual presentó una concentración de 12.6 g/L de ST y 9.6 g/L de SV respectivamente. Alimentando al reactor de lodos activados con un efluente fenólico pre-tratado, cuyas características se presentan en la siguiente tabla.

Tabla 1. Características generales del influente.

Parámetro	Experimento		
	I	II	III
DQO (g/L)	19.73±3.36	14.04±1.9	9.27±1.7
Fenol (g/L)	2.81±0.35	3.21±0.7	3.18±0.3
pH	6.56±0.19	6.65±0.14	6.66±0.12
ST (g/L)	0.32±0.2	0.25±0.1	0.26±0.1
SV (g/L)	0.19±0.1	0.19±0.08	0.17±0.1

X: promedio aritmético, S: desviación estándar

Resultados. En la tabla 2 se muestra los promedios de los principales parámetros evaluados en el efluente del sistema estudiado a lo largo de 170 días de operación, en la que se puede apreciar una disminución en la concentración de DQO y de fenol con respecto a la inicial.

Tabla 2. Características generales del efluente tratado.

Parámetro	Experimento		
	I	II	III
DQO (g/L)	13.65±3.16	6.24±1.6	6.08±0.9
Fenol (g/L)	1.67±0.4	2.01±0.37	2.1±0.27
pH	6.31±1.06	6.68±0.28	6.54±0.25
ST (g/L)	1.07±0.5	0.39±0.1	0.52±0.1
SV (g/L)	0.72±0.3	0.28±0.1	0.4±0.1

X: promedio aritmético, S: desviación estándar

La figura 1, muestra una eliminación de DQO del 21% a una carga orgánica de 11.2±0.3 kg de DQO/m^3·d durante los primeros 73 días. Con la aclimatación de la biomasa, su eliminación mejora en un 51%. Notando que a menor tasa de carga orgánica, mayor eficiencia de eliminación de DQO.

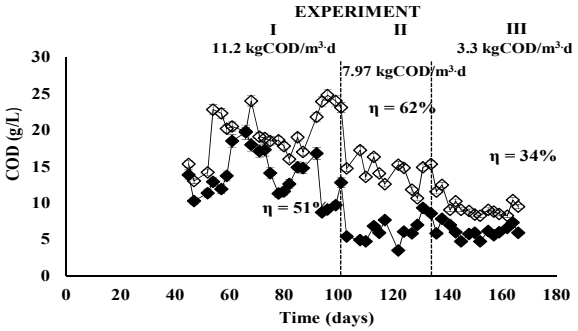


Figura 1. Variación de la concentración de DQO con el tiempo.

Conclusiones. La aclimatación de la biomasa del reactor de lodos activados a la presencia de fenol, juega un papel medular sobre la eliminación de este tipo de compuestos tóxicos.

Agradecimientos. Este trabajo se realizó en colaboración con el Instituto Tecnológico de Toluca y la Universidad Politécnica del Valle de Toluca.

Referencias.

1.- APHA., AWWA., WPFC, 2016, Standard Methods for the Examination of Water and Wastewater. 22nd edition. American Public health Association, Washington, D.C., U.S.A.

ISSN: 2448-8461

AM9

WHOLE GENOME SEQUENCE ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBON (PAH) MULTITOLERANT *STENOTROPHOMONAS* SPECIES ASS1

Temidayo Oluyomi Elufisan[1], Isabel Cristina Rodríguez-Luna[1], Alejandro Sánchez-Varela[1], José Antonio Vilchis-Carmona[1], Patricia Bustos-Arcos[2], Víctor González[2], Luis Lozano[2], Miguel Ángel Villalobos-López[3] and Xianwu Guo[1]

[1]Laboratorio de Biotecnología Genómica, Centro de Biotecnología Genómica, Instituto Politécnico Nacional, Cd. Reynosa, Tamaulipas 88710, México.

[2]Centro de Ciencias Genómicas, Universidad Nacional Autónoma de México, Cuernavaca, Morelos 62210, México.

[3]Centro de Investigación en Biotecnología Aplicada, Instituto Politécnico Nacional, Tlaxcala 90700, México.

Corresponding author: asanchezv@ipn.mx

Key words: Polycyclic Aromatic Hydrocarbon, Stenotrophomonas, Naphthalene Genomic Sequencing

Introduction. *Stenotrophomonas* are ubiquitous versatile non-fermentative, Gram-negative, rods that occupy wide range of habitat. The ubiquitous nature of these bacteria manifest by their presence in extreme environments such as geothermal vent, acidic lake and hot sulfur spring [1, 2, 3]. The complex nature of *S.* spp. have enable them to use a wide variety of compound for their growth, among which are PAH. Although, many studies have employed *S.* spp. for the degradation of PAH, an in depth understanding of the mechanism employed by *S.* for the degradation of PAH and other hydrocarbons is still restricted to the amplification of genes associated with the degradation such as the alkane 1-monooxygenase gene and catechol 1,2-dioxygenase [4]. In this study we analyzed the potential of *S.* species isolated from Crude Oil contaminated soil recovered from Tabasco, Mexico to tolerate 5 PAHs (biphenyl, naphthalene, anthracene, anthraquinone and xylene). We did a complete genome sequence analysis to understand the pathway involved in the degradation of PAH tested.

Methods. The bacterium was isolated using the standard microbiological technique as described by Mahdi, O. *et al*., 2014 which involved the use of Selective medium StenoVIA. Isolates were identified using the conventional biochemical technique. MALDI-TOF and by amplifying and sequencing the 16S rRNA fragment of the bacterial genome. Hydrocarbon tolerance test and analysis was carried out as described by Hassanshahian, M. *et al*., 2013. DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega) according to the manufacturer's instruction. The genome was sequenced with Illumina MiSeq® and quality of raw reads were checked with fastqc program and de novo assembly was done with Spades. Genome annotation was achieved with *Prokka pipeline* and *Prodigal* was used for gene calling while *RNAmmer* predict the RNA present in the genome. Functional annotation and COG analysis was done with Online based *WebMGA server*. Biosynthentic gene clusters were predicted with *antiSMA SH* while *PHASTER* was employed for the prediction of the possible presence of prophage sequences in the genome. Horizontally acquired genes were predicted with *Island viewer* which searches the genomic Island.

Results. The three methods employed for identification confirmed that bacterium ASS1 is a *S.* species. The biodegradation assay via emulsification of PAH revealed that ASS1 can employ this method for the degradation of PAHs (Figure 1). Six biosynthentic clusters two of which are related to hydrocarbon degradation were identified in the genome of ASS1. Similarly, a complete prophage with many oxido-reductases was found in the genome of ASS1.

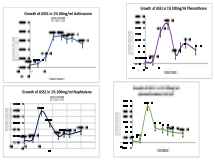


Fig. 1. Growth of ASS1 in different PAH tested.

Conclusion. The complete genome sequence analysis revealed its potential for survival in the presence of PAHs and another hydrocarbon. Hence may be the reason for its isolation from a crude oil contaminated soil. This potential suggests the possibility of using ASS1 as a bioremediating agent for Oil polluted sites.

Acknowledgements. We are grateful to CONACyT for providing Temidayo O. Elufisan with the opportunity to conduct his Ph.D studies in Mexico. SIP del Instituto Politécnico Nacional (No. SIP20171762 and SIP20171793) for financial support for the study and the COFAA-IPN for providing the professors scholarship.

References.

1. Wilson M., Siering P., White C., Hauser M., & Bartles A. (2008). Novel archaea and bacteria dominate stable microbial communities in North America's Largest Hot Spring. *Microbial ecology*, *56*(2), 292-305.

2. Tobler D. & Benning L. (2011). Bacterial diversity in five Icelandic geothermal waters: temperature and sinter growth rate effects. *Extremophiles*, *15*(4), 473.

3. Arulazhagan P., Al-Shekri K., Huda Q., Godon J., Basahi J. & Jeyakumar D. (2017). Biodegradation of polycyclic aromatic hydrocarbons by an acidophilic S. maltophilia strain AJH1 isolated from a mineral mining site in Saudi Arabia. *Extremophiles*, *21*(1), 163-174.

4. Urszula G., Izabela G., Danuta W. & Sylwia Ł. (2009). Isolation and characterization of a novel strain of Stenotrophomonas maltophilia possessing various dioxygenases for monocyclic hydrocarbon degradation. *Brazilian Journal of Microbiology*, *40*(2), 285-291.

5. Mahdi O., Eklund B. & Fisher N. (2014). Laboratory culture and maintenance of Stenotrophomonas maltophilia. *Current protocols in microbiology*, 6F-1.

6. Hassanshahian M., Tebyanian H. & Kariminik A. (2013). Hexadecane-degradation by Teskumurella and Stenotrophomonas strains isolated from hydrocarbon contaminated soils. *Jundishapur Journal of Microbiology*, *6*(7), 1-7.

7. Singh, V. (2012). Biosurfactant–Isolation, production, purification & significance. *J. Sci. Ind. Res*, *2*, 1-4.

ISSN: 2448-8461

11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México

AM10

IS THE HOST INTERACTION LOCUS A MAJOR DETERMINANT OF *BDELLOVIBRIO* PREDATION?

Leonardo Iván Aranda-Vivas, Temidayo Oluyomi Elufisan, Isabel Cristina Rodríguez-Luna, Alejandro Sánchez-Varela and Xianwu Guo

Laboratorio de Biotecnología Genómica, Centro de Biotecnología Genómica, Instituto Politécnico Nacional, Cd. Reynosa, Tamaulipas 88710, México.

Corresponding author: asanchezv@ipn.mx

Key words: Bdellovibrio, HIT-locus predatory, Host, Prey-range.

Introduction. *Bdellovibrio* are important deltaproteobacteria with the ability to prey on a wide variety of pathogenic Gram-negative bacteria and are being considered as one of the solution to the continuously emerging drug resistance in bacteria. The ability of *Bdellovibrio* to live as free living non-predatory bacterium has been reported to be associated with the alteration in the host interaction locus. However, recent studies by Oyedara, O. *et al*., 2018 revealed a novel strain of *Bdellovibrio* with an altered host interaction locus. In a bid to understand the event taking place, we have decided to carry out prey range analysis with the said strains and other strains in our laboratory with host interaction locus. This is aim at confirming the significance of the host interaction locus on the predatory activities of *B.* species.

Methods. *Bdellovibrio* species were isolated using the double layer agar plating technique as described by Jurkevitch, E., 2012 and Oyedara, O. *et al*., 2016. *Bdellovibrio* was identified by the sequencing of the 16S rRNA fragment of the *Bdellovibrio* genome using specific primer described by Van Essche, M. *et al*., 2009 (BbsF216:BbsR707). The bacterial DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega, Ref. A1120) according to the manufacturer's instruction. The Prey range analysis for the isolated *Bdellovibrio* strains was tested as described by Oyedara, O. *et al*., 2016 and the host interaction locus was amplified with the primers (BdsHITF:BdsHITR). The prey range activities of 10 *Bdellovibrio* strains were evaluated on microtiter plate and the decrease in turbidity of the medium with respect to reduction in the recorded optical density were taken for the *Bdellovibrio'* s ability to prey on their hosts. This was corroborated with the molecular determination of the host interaction locus (HIT) via amplification by PCR. Sixteen bacteria including Gram-negative and Gram-positive as the prey in this study.

Results. Ten bacteria isolates were confirmed as *Bdellovibrio* by the amplifying the 16S rRNA region of their genome (Figure1).

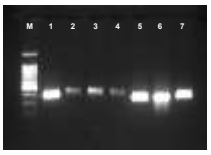


Fig. 1. Isolations of *Bdellovibrio* species from soils and sewage from Baja California, Coahuila and Tamaulipas. Agarose gel 2.0% with SYBR® Gold, 80 V for 1 h. (M) Molecular Weight Marker 100bp DNA Ladder ™, (1 to 7) *Bdellovibrio* sp.

Conclusions. We isolated ten *Bdellovibrio* species from soils and sewage from Baja California, Coahuila and Tamaulipas, México. Only two of the isolates showed the presence of HIT locus in their genome. The presence of the HIT locus in isolate did not have a positive effect on the predative activities of the isolates which possess this region. The isolates with the best predatory activities in this study did not show the presence of an intact HIT locus. It may therefore be concluded that the presence of a HIT locus in the genome of *Bdellovibrio* may not be the only factor that is important for their predatory life.

Acknowledgements. We are grateful to CONACyT for providing Temidayo O. Elufisan with the opportunity to conduct his Ph.D studies in Mexico. Secretaría de Investigación y Posgrado del Instituto Politécnico Nacional (No. SIP20180654 and SIP20180816) for financial support for the study and the COFAA-IPN for providing the professors scholarship.

References.

1. Oyedara O., Segura A., Guo X., Elufisan T., Cantú R. & Rodríguez M. (2018). Whole-Genome Sequencing and Comparative Genome Analysis Provided Insight into the Predatory Features and Genetic Diversity of Two Bdellovibrio Species Isolated from Soil. *International journal of genomics*, *2018*.

2. Jurkevitch E. (2012). Isolation and classification of Bdellovibrio and like organisms. *Current protocols in microbiology*, *26*(1), 7B-1.

3. Oyedara O., Luna E., Olguin O., Guo X., Mendoza M. & Rodriguez M. (2016). Isolation of Bdellovibrio sp. from soil samples in Mexico and their potential applications in control of pathogens. *MicrobiologyOpen*, *5*(6), 992-1002.

4. Van Essche M., Sliepen I., Loozen G., Van Eldere J., Quirynen M., Davidov Y. & Teughels W. (2009). Development and performance of a quantitative PCR for the enumeration of Bdellovibrionaceae. *Environmental microbiology reports*, *1*(4), 228-233.

ISSN: 2448-8461

ELECTROACTIVE BACTERIA EXTRACTED FROM MANGROVE SEDIMENT MICROBIAL FUEL CELLS

Adeline Laure[(1)], Adèle Silbande[(2)], Florent Robert[(1)], Juliette Smith-Ravin[(2)], Christophe Roos[(1)], Paule Salvin[*(1)]; [(1)]L3MA laboratory, University of French West Indies, F-97233 Shoelcher (Martinique), [(2)]BIOSPHERES laboratory, University of French West Indies, F-97233 Shoelcher (Martinique); paule.salvin@univ-antilles.fr.

Key words: electroactive bacteria, mangrove sediments, microbial fuel cell

Introduction. In the current context of climate change, renewable energies have been booming since the early 1990s, notably through the production of electricity from wind and solar power. It is within this dynamic of increasing the use of clean and renewable resources that the Microbial Fuel Cell (MFC) is part of. The main advantages of this generator lie in the catalysis of electrochemical reactions by bacterial consortia and the use of organic materials as fuel. Currently, several sources of electroactive bacteria (EAB) capable of exchanging electrons with conducting materials, have already been discovered: compost, brewery wastewater, seabed… In particular, in the tropics, mangroves have been presented in precedent studies as an adequate environment to investigate EAB. Indeed, these environments have the physicochemical characteristics required to constitute potential sources of EAB: sedimentary soils, neutral pH, high temperature (26-30 °C), and high salinity (marine or brackish). However, there is little knowledge of the microbial communities present in this type of environment, even less of the EAB from mangrove SMFC.
The objective of the present study was to improve knowledge on EAB formed from natural bacterial consortia from mangrove areas.

Methods. Single-compartment with air-cathode mangrove SMFCs were loaded with a 1-kohm resistor each (triplicate). The cells were connected to a potentiometer (VMP-3, Bio-Logic, USA) and the values were taken every 5min using the software EC-Lab (v11.10, Bio-Logic). The bacterial community formed on the anode was compared to the microbial ecosystems present at initial and final times in sediment and water using a Next-Generation Sequencing platform (Illumina MiSeq). At the end of the experiment, 26 bacterial strains were isolated and characterized (Gram, catalase, oxidase). The electroactive capacity of 4 strains were tested in duplicate in 3-electrodes experimental devices.

Results. The maximal power of the SMFCs, measured in a stable plate of electrical activity following an acetate addition as substrate, was 450 mW/m^2 of projected anode surface. The anodic potential switched from 400 mV to -400 mV during period without acetate and with acetate, respectively.

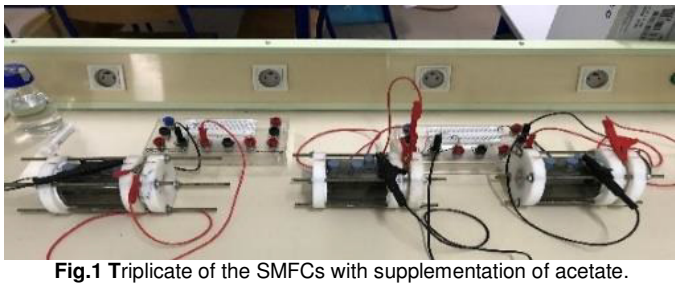


Fig.1 Triplicate of the SMFCs with supplementation of acetate.

The formation of the anodic biofilm was essentially influenced by the sediment with a similar diversity of bacteria. The proteobacteria phylum dominated the ecosystem and was mostly composed by two genera: *Sulfurimonas* sp. (34.0 ± 18.3%) and *Desulfobacter* sp. (14.2 ± 1.8%). Other bacteria were also represented (>1%): *Arcobacter* sp., *Spirochaeta* sp., *Flavobacterium* sp., *Desulfocapsa* sp., *Thiomicrospira* sp. and *Pseudomonas* sp. Moreover, the sequencing results made it possible to measure the influence of the acetate and of the polarization on the microbial communities.
Two of the isolated strains showed interesting electron transfer capacities and reached a maximum current density between 366 and 865 mA/m^2.

Conclusions. This experiment showed interesting electrochemical and microbiological results that conducted to a better understanding of the electroactivity performance of tropical microbial fuel cells.

Acknowledgements. The project was supported by the French Ministry of Overseas.

References.

1. Doyle L., Yung P., Mitra S., Wuertz S., Williams R., Lauro F., Marsili E. (2017). *J. Power Sources*.356:539-548.
2. Salvin P., Roos C., Robert F. (2012). *Bioresour. Technol*.120:45-51.
3. Rivalland C., Madhkour S., Salvin P., Robert F. (2015). *Bioelectrochemistry*.106(Pt A):125-132

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
AM12

EFFECT OF INHIBITORY COMPOUNDS AT CONCENTRATIONS FOUND IN TEQUILA VINASSES IN FERMENTATIVE PROCESS OF *Clostridium acetobutylicum* ATCC 824

Sara Gisela Sánchez Ureña[1a], Ofelia Yadira Lugo Melchor[1c], Jorge Bravo Madrigal[1b], Luis Manuel Rosales Colunga[2], Erika Nahomy Marino-Marmolejo[1b]

[1a]Unidad de Tecnología Ambiental, [1b]Unidad de Biotecnología Médica y Farmacéutica, [1c]Unidad de Servicios Analíticos y Metrología; Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco A.C., Guadalajara, JAL, México CP 44270;
[2]Universidad Autónoma de San Luis Potosí, Facultad de Ingeniería, San Luis Potosí, SLP, México CP78290. (emarino@ciatej.mx)

KEYWORDS: Clostridium acetobutylicum, inhibitory compounds, hydrogen production

Introduction. Hydrogen is considered as a clean biofuel because its combustion produces H_2O instead of CO_2. In order to produce Hydrogen, and not risking food security, novel substrates have been tested, like wastes from agroindustries. One of these wastes is Tequila vinasses, a wastewater from Tequila distillation, which has a high DQO[1]. Unfortunately, Tequila vinasses contents furfural and acid compounds, which may cause inhibition of fermentative process[2].In this work, we tested hydrogen production of *Clostridium acetobutylicum* ATCC 824 with the adding furfural and acid compounds at concentrations found in some Tequila vinasses[2], to asses if those compounds, at that concentration, could cause an effect in the fermentative process, and thus, in hydrogen production.

Methods. Previous to inoculate bottle reactors (400ml work volume), *C. acetobutylicum* ATCC 824 was cultivated from a criopreserved strain (acquired by ATCC, reactivated and storaged at -80°C). We used Clostridial Reinforced Media (DIFCO), with MES (SIGMA) 10mM as buffer solution; pH was adjusted to 6.8 (Media pH) after addition of inhibitors. Concentrations for Hydroxymethyl-furfural, Furfural, Acetic acid and Butyric acid was selected from Vinasses characterization of workgroup (Chart 1). Hydrogen production kinetics was followed with an Automatic Methane Potential Test System (AMPTS) II, Bioprocess Control[3], and periodically samples was taken from each reactor to measure pH. Hydrogen production was determinated using Gompertz modified ecuation[4]. At the end of fermentation, Volatile Fatty Acids were measured from each reactor using HPLC with RID[5]. Analysis of variance (ANOVA) was determinated with Statgraphics Centurion XV software.

Chart 1. Inhibitor concentration, according vinasses characterization.

Inhibitor	Conc (mg*l^{-1})
Hydroxymethyl-furfural	347.61
Furfural	53.57
Acetic Acid	182.00
Butyric Acid	38.86

Results. In Figure 1, shown Fermetative process (A), and Volatile Fatty Acids (VFA) production (B). There was not significant difference between groups respect a control, only in total production of Hydrogen, where inhibition of butyric acid was significantly lower than the others ($P<0.05$). However, VFA profile was not significantly different. Butyrate/Acetate ratio show a typical acidogenic fermentation.

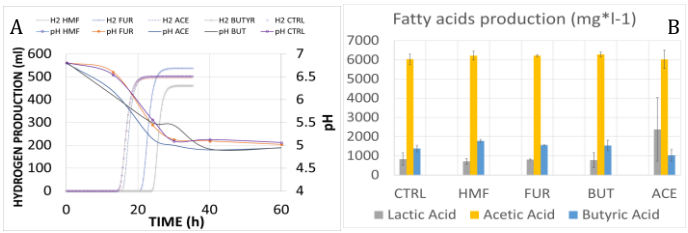


Fig.1 Fermentative process with *C. acetobutylicum*. ATCC 824 (A). VFA profile at the end of fermentation (B).

Conclusions. At vinasses concentration only one inhibitor shows a negative effect in hydrogen production. However, vinasses concentration for the other compounds is low than inhibitory, but after removal of compounds, such as HMF or Furfural, a higher Hydrogen production was observed. This could be happening because of a synergic effect between inhibitory compounds.This experiment will be evaluated by mixing compounds, and increasing concentration to determinate synergic efects and inhibition concentration for hydrogen production.

Acknowledgements. This work was made in CIATEJ-Guadalajara facility, with a CONACYT scholarship 539317/298655.

References.

1. López-López, A., Davila-Vazquez, G., León-Becerril, E., Villegas-García, E. & Gallardo-Valdez, J. Tequila vinasses: generation and full scale treatment processes. *Reviews in Environmental Science and Bio/Technology* **9**, 109-116 (2010).
2. Rodríguez-Félix, E., Contreras-Ramos, S.M., Davila-Vazquez, G., Rodríguez-Campos, J. & Marino-Marmolejo, E.N. Identification and quantification of volatile compounds found in vinasses from two different processes of tequila production. *Energies* **11**, 490 (2018).
3. Badshah, M., Lam, D.M., Liu, J. & Mattiasson, B. Use of an Automatic Methane Potential Test System for evaluating the biomethane potential of sugarcane bagasse after different treatments. *Bioresource Technology* **114**, 262-269 (2012).
4. Kumari, S. & Das, D. Improvement of biohydrogen production using acidogenic culture. *International Journal of Hydrogen Energy* **42**, 4083-4094 (2017).
5. de Sa, L.R.V., de Oliveira, M.A.L., Cammarota, M.C., Matos, A. & Ferreira-Leitao, V.S. Simultaneous analysis of carbohydrates and volatile fatty acids by HPLC for monitoring fermentative biohydrogen production. *International Journal of Hydrogen Energy* **36**, 15177-15186 (2011).

ISSN: 2448-8461

AM13

ISOLATION AND CHARACTERIZATION OF NATIVE ENTOMOPATHOGENIC NEMATODES FOR THE CONTROL OF THE FALL ARMY WORM *SPODOPTERA FRUGIPERDA* (J.E. SMITH)

Héctor Alejandro Leyva-Hernández[1], Jaime Ruíz-Vega[2], Carlos Ligne Calderón-Vázquez[1], Gabriela Lizbeth Flores-Zamora[1], Cipriano García-Gutiérrez[1]. [1]Instituto Politécnico Nacional, Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional (CIIDIR-IPN, Unidad Sinaloa) Guasave, Sinaloa. CP: 81101. [1]Instituto Politécnico Nacional, Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional (CIIDIR-IPN, Unidad Oaxaca) Santa Cruz Xoxocotlán, Oaxaca. CP: 71230. halexleyva@hotmail.com

Key words: Rhabditis blumi, Acrobeloides camberenensis, entomopathogenic.

Introduction. Corn (*Zea mays* L.) is the main crop in Mexico; however, its production is at risk due to various diseases and pests, as the fall armyworm (FAW) *Spodoptera frugiperda* (J.E. Smith), being the most important economic pest [1]. Chemical insecticides have been used intensively generating various adverse environmental phenomena such as the resistance of the insect to the pesticide, which increases the dependence on chemical pesticides. An alternative to the use of these insecticides is the biological control, whose effect is specific on the insect pest and friendly to the environment. The entomopathogenic nematodes (EN) have been shown to be a viable option as biological control agents.

Methods. Emtomopathogenic nematodes were isolated from soils in the valley of Guasave. In addition, the isolates were characterized morphologically and molecularly. An axenic breeding of *S. frugiperda* was maintained to test the pathogenicity and virulence of EN in 3rd instar larvae for bioassays. Mortality was determined, lethal time 50 LT_{50} and lethal dose LD_{50} at laboratory level. The experiments were performed under a CRD and a Tukey comparison test ($\alpha = 0.05$).

Results. Entomopathogenic nematodes were found in two locations, which proved to be pathogenic for the fall armyworm [2] (Fig. 1).

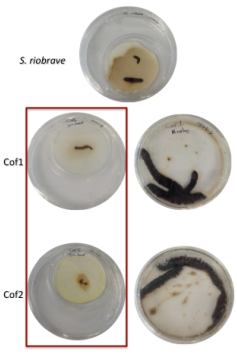


Fig.1 Pathogenicity bioassay of native EN on FAW larvae.

According to the molecular characterization, isolate 1 had a high identity with the entomopathogenic species *Rhabditis blumi*. The second isolate showed identity with the nematode *Acrobeloides camberenensis* (Fig. 2).

Description	Max score	Total score	Query cover	E value	Ident	Accession
Rhabditis blumi strain DF5010 28S ribosomal RNA gene, partial sequence	532	532	100%	3e-147	83%	EU195965.1

Description	Max score	Total score	Query cover	E value	Ident	Accession
Acrobeloides camberenensis large subunit ribosomal RNA gene, partial sequence	769	769	100%	0.0	91%	AF147069.1

Fig.2 Nucleotide sequencing comparison results of isolates 1 and 2 with Gene Bank database.

The morphological identification confirmed that both nematodes correspond to the genera indicated by the molecular identification [3;4].

Conclusions. Isolated entomopathogenic nematodes belong to the species *Rhabditis blumi* and *Acrobeloides camberenensis* and caused mortality in *S. frugiperda* larvae, so they have potential to be used as bioinsecticide agents.

Acknowledgements. We thank CONACYT for the scholarship provided, the Instituto Politecnico Nacional and the SIP 2018316 project for the financial support in this research project.

References.

1. García, C; González, M; Bautista, N. 2011. Pathogenicity of isolates of entomopathogenic fungi against *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and *Epilachna varivestis* (Coleoptera: Coccinellidae). *Rev. Colomb. Entomol.* 37 (2): 217-222.
2. Leyva-Hernández, H. A., García-Gutiérrez, C., Ruiz-Vega, J., Calderón-Vázques, C.L., Luna-González, A., García-Salas, S. 2018. Evaluation of the Virulence of *Steinernema riobrave* and *Rhabditis blumi* against Third Instar Larvae of *Spodoptera frugiperda.* SOUTHWESTERN ENTOMOLOGIST. VOL. 43, NO. 1, 189-197.
3. Carta, L. K. y Osbrink, W. 2005. Rhabditis rainai n. sp. (Nematoda:Rhabditida) associted with Formosan subterranean termite, *Coptotermes formosanus* (Isoptera:Rhinotermitidae). Nematology. Vol. 7(6), 863-879.
4. Holovachov, O., De Ley, I. T., Mundo-Ocampo, M., De Ley, P. 2009. Identification of Cephaloboidea (Nematoda). Nematology, UC Riverside. 86 pp.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
AM14

STUDY OF THE STRUCTURE AND DIVERSITY OF MICROBIAL COMMUNITIES FROM THE ANAEROBIC DIGESTION OF MEZCAL WASTE FROM *Agave angustifolia* Haw

Agustin Vidal Gómez-Guerrero[1], Magdaleno Caballero Caballero[1], Fernando Chiñas Castillo[2], Idania Valdez Vazquez[3]
[1]Instituto Politécnico Nacional, CIIDIR Unidad Oaxaca, Hornos N°. 1003, Col. Noche Buena, Santa Cruz Xoxocotlán, Oaxaca, C.P. 71230, México. [2] Departamento de Ingeniería Mecánica, Instituto Tecnológico de Oaxaca Calz. Tecnológico No. 125, Oaxaca, Oax. C.P. 68030, Mexico. [3]Unidad Académica Juriquilla, Instituto de Ingeniería, Universidad Nacional Autónoma de México, Blvd. Juriquilla N° 3001, C.P. 76230 Querétaro, México.
E mail: agomezg1500@alumno.ipn.mx
Key words: Agave angustifolia Haw*, Biogas, Microbial community.*

Introduction. In mezcal production process 3,237x10^6 leaves from *Agave angustifolia* Haw are discarded in land crops [1], 4,807x10^6 t of bagasse [2] and 43,500x10^6 L of vinasse are generated annually [3]. Those waste represents a constant source of pollutants, so it is necessary to apply processes that take advantage of them and thus mitigate the harmful effects. Anaerobic digestion is a process that can be used for this purpose, although it can be influenced by different factors, being the substrate-inoculum ratio (S/I) a key factor for the process optimization [4], also study the structure and diversity of microbial communities will allow identifying high-efficiency microorganisms to increase methane production.
This study evaluated the effect of the S/I ratio and the diversity of microbial communities present in treatments with best performance using as a substrates waste from mezcal industry.

Methods. Pig manure (PM), activated sludge (AS), and a 1:1 mixture (PM:AS) served as source de inocula. PM was collected from a municipal slaughterhouse and AS was obtained from a wastewater treatment plant both located in Oaxaca City, Mexico and stored at 4 °C until its use. The substrate consisted in a mixture as follows: 75% bagasse, 15% vinasse and 10% leaves dry-base. Batch reactors consisted in 120 mL serological bottles and coupled to a syringe to measure the biogas by displacement of acidified brine. Biogas composition was analyzed using gas chromatography. Metagenomic DNA extraction was performed from essays that showed the highest methane production, as well as their respective controls. The process was carried out using the protocol recommended by *PowerSoil*® DNA extraction kit. The DNA obtained was sequenced using the 16S rDNA gene using the Illumina MiSeq platform, for bacteria a set primers 28F was used and for archaea Arch517F.

Results. The S/I ratio that showed the highest methane yield was AS-0.25 with 33 NmLCH4gSV^{-1} compared to that obtained with PM-0.25 and M-0.25 ratio of 13 and 12 NmLCH4gSV^{-1}, respectively. There was a difference between bacterial communities and methanogenic archaea as show Fig. 1, being AS-0.25 treatments with the highest Shannon index values, with a greater presence of beneficial microorganisms for the methanogenic stage.

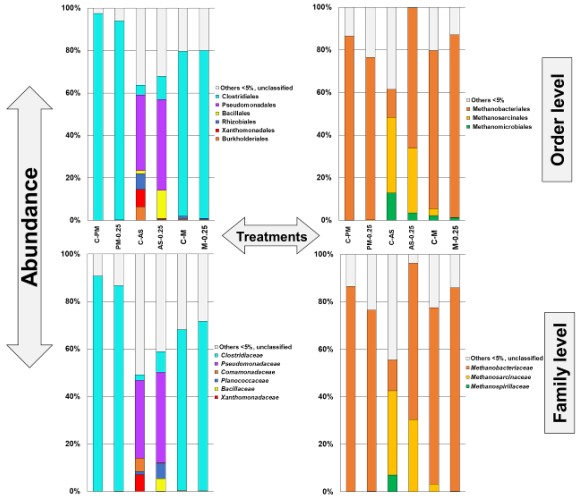


Fig.1 Taxonomic classification and abundance of bacteria - archaea for essays with best performance.

Conclusions. The S/I ratio had a significant effect on the waste methanisation. In general, for the three types of inoculum were determined that S/I ratios of less than 1 allow a best performance. In AS treatments the concentration of archaea from the genus *Methanosarcina* contributed positively reaching a potential methane production of 498 NmL CH_4.

Acknowledgements. To the Instituto Politécnico Nacional, CIIDIR-Oaxaca. This work was partially financed by the Energy Sustainability Fund (CONACYT-SENER), Mexican Center for Bioenergy Innovation, Gaseous Biofuels Cluster (247006).

References.

1. López-Hernández, I. (2009). Pruebas de resistencia a la tensión en fibras de las hojas del *Agave angustifolia* Haw para determinar su comportamiento mecánico. Instituto Politecnico Nacional. Santa Cruz Xoxocotlán, Diciembre 2008, 4-5
2. SIAP-SAGARPA, (2016). Servicio de Informacion Agroalimentaria y Pesquera, Anuario agricola 2000-2015. México, http://www.siap.sagarpa.gob.mx/..
3. Robles-González , V., et al. (2012) Treatment of mezcal vinasses: A review. *Journal of Biotechnology*,. 157(2): 524–546.
4. Iván, V.-R., et al. (2015). Potencial de generación de biogás y energía eléctrica. Parte II: residuos sólidos urbanos. Ingeniería, *Investigación y Tecnología*,. 16(3): 471-478.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
AM15

ANALYSIS AND CHARACTERIZATION OF COMPOUNDS WITH ANTIOXIDANT CAPACITY BY INFRARED SPECTROSCOPY IN Sargassum spp.

Abdú Orduña; Raúl Delgado; Marlon Rojas, Janet Morales; Rafael Salinas; Laura García
Centro de Investigación en Biotecnología Aplicada del Instituto Politécnico Nacional (CIBA-IPN), Tlaxcala 72197, México.

Key words: Sagrsassum spp, FTIR, Antioxidant

Introduction. The seaweeds were the main source of phycocolloids, carrageenan and algin, which were extensively used in various food, confectionary, textiles, pharmaceuticals, dairy and paper industries [1]. Seaweed contains more than 60 trace elements at a much higher concentration than in terrestrial plants. They are an excellent source of components such as polysaccharides, tannins, flavonoids, phenolic acids, bromophenols and carotenoids that have exhibited different biological activities [2]. The algal genus Sargassum spp constitutes an important ecological habitat in many tropical and temperate coastal regions in the north of México. FTIR spectroscopy can be used for the qualitative determination of organic constituents and functional groups present in plants and marine algae species.

In this project, the analysis of compounds with the possible antioxidant activity of the Sargassum spp was carried out using the Fourier Transform Infrared Spectroscopy (FTIR) technique.

Methods.

To obtain information on different bonding in the structures, the algae sargassum were examined by Fourier Transform Infrared Spectroscopy (Vertex 70 Bruker, spectrometer), using the Attenuated Total Reflectance mode (ATR) in the middle region from 400 to 4000 cm^{-1}. Three samples were analyzed: Sargassum spp, sargassum horridum, and its ethanol extract.

Results.

The Figure 1 shows a FTIR-ATR spectrum in the middle region of Sargassum spp. The absorption band located in the 1045 cm^{-1} region corresponds to the C-H flexion or the C-O or C-C vibrations of the carbohydrates and polysaccharides present. The observed band around 817 cm^{-1} is due to the vibration outside the C-H plane of an aromatic compound. The weak absorption band centered at 648 cm^{-1} can be attributed to the C-H flexion vibration, which also confirms the presence of carbohydrates. The existence of chlorophyll groups is confirmed by the C-H stretching vibrations at 2879, 2932 and 2969 cm^{-1}. The absorption band centered at 1655 cm^{-1} is due to the C-O and N-O asymmetric stretching of the ester group. The bands around 1400 and 1440 cm^{-1} are due to the C-C stretch vibration of the aromatic ring compound. The mean absorption band at 1273 cm^{-1} indicates the C-N stretch vibration of chlorophyll.

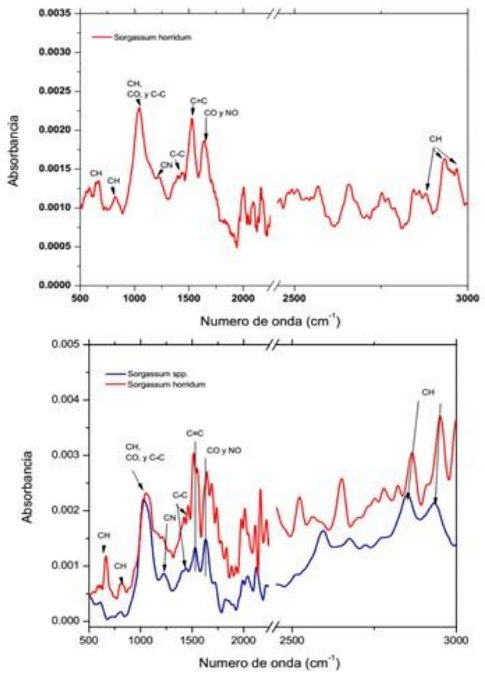


Fig.1 a) Spectrum FTIR-ATR of algae sargassum horridum, b) Comparison of dry algae species of sargassum.

Conclusions.

The characterization of functional groups such as carboxyls, sulfhydryls, hydroxyls belonging to constituent groups of algae, such as; carbohydrates, aromatics, polysaccharides and phenols were identified.

Acknowledgements. PhD. Gustavo Hernández Carmona and Centro Interdiciplinario de Ciencias Marinas IPN. To proyect SIP 20180461 and Ciencia Básica [grant number 256513] and R. Salinas by scholarship awarded.

References.

1. S.S. Sumayya and K. Murugan. 2017 J. of Pharmacognosy and phytochemistry. Vol. 6. Pp. 325-330.
2. de Quiros R.-B., A, Lage-Yusty MA. and Lopez Hernandez J., 2010. Food Chem., vol. 121, pp. 634-638.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
AM16

DETECTION AND QUANTIFICATION OF ENTEROBACTERIA BY PCR TECHNIQUES AND CHROMOGENIC CULTURES IN SEWAGE SLUDGE

Nicolás Fonseca[1], Fernando Lopez[1], Fabian Fernandez[2], Aline Lopez-Lopez[3]; [1]Instituto Politécnico Nacional, Centro de Investigación en Biotecnología Aplicada (CIBA), Ex-Hacienda San Juan Molino Carretera Estatal Tecuexcomac-Tepetitla Km 1.5, C.P. 90700, Tlaxcala, México;[3]Catedrática CONACYT-UATx, Centro de Investigación en Genética y Ambiente, Universidad Autónoma de Tlaxcala, Km. 10.5 Autopista Tlaxcala-Texmelucan Ixtacuixtla, Tlaxcala. C.P. 92120;[2]Cinvestav, Laboratorio de Ecología del Suelo, Av. Instituto Politécnico Nacional 2508, San Pedro Zacatenco, C.P. 07360, Ciudad de México, México niesfope@hotmail.com

Key words: sewage sludge, Escherichia coli, Salmonella sp, endophyte, Phaseolus vulgaris, colonization

Introduction. The sewage sludge has been reported as a fertilizer in some commercial interesting crops, due to its high content of organic matter, micro and macronutrients. Nevertheless, the content of microorganisms (1), mostly enteric pathogens, represent a risk group in public health, Fecal coliform (*Escherichia coli*) and *Salmonella* sp. In function of their cellular enumeration, USEPA (2,3) allows the sewage sludge application in crops but the interaction and their behavior as pathogen or endophyte is not well understand and have a few information about, for these reasons the aim of this work was the identification of *E.coli* and *Salmonella* sp. isolated from sewage sludge and the quantification of both bacteria in *Phaseolus vulgaris* tissues by infection *in vitro* assay to elucidate if they represents a risk for human health.

Methods.

***E. coli* detection and quantification**

- The methods tested was 1682 (2), pre-enrichment in lauryl tryptose and EC broth and plate count was in VRBG and EMB agar (37°C/24h)
- Lacz3 and yaiO primers (4) were used to PCR identification and qPCR quantification.

***Salmonella* sp detection and quantification**

- The method tested for quantification was method 1680 (3), to detect was pre-enrichment in tripticase soy broth and plate in XLD agar and MSRV semisolid medium (37°C/24h all of them)
- ST-11 and ST-5 primers were used to PCR identification and qPCR quantification

Results From sewage sludge *E. coli* and *Salmonella* sp were able to be isolated using chromogenic media for rapid identification (Fig. 1) and their identity were confirmed with16 S rRNA sequencing (data not shown). Pre-enrichment in lauryl tryptose broth shown the best recuperation compared with EC broth, using VRBG and EMB media detection of *E.coli* was possible and cellular viable count was 10^9 and 10^{10} . However, *Salmonella* sp detection was possible only with XLD and MSRV media, but even with pre-enrichment the quantification of this microorganism was not done.

Lacz and *YaiO* genes were amplified for *E.coli* detection, the expecting fragments (283pb, 113pb) were observed only for *E.coli* and negative for plant and *Salmonella* sp DNA (Fig. 2)

Fig.1 Detection and isolate of *Salmonella* sp and *E.coli* in chromogenic mediums.

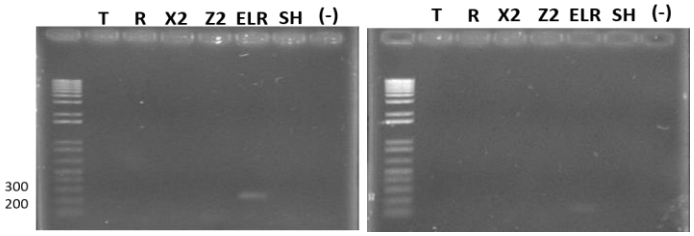


Fig.2. At the left observe the amplified product of PCR corresponding to *LacZ* (283bp), at the right the amplified product of PCR corresponding to *YaiO*: stem (T), root (R), X2 (*Salmonella* fenotype), Z2 (*Salmonella* fenotype), ELR (*E. coli* from sewage sludge), SH (*Salmonella* sp from sewage sludge) and (-) (negative control).

Conclusions

- The pre-enrichment methods are important for the quantification of viable cells in chromogenic culture mediums.
- By microbiological techniques is possible the isolation and detection of *E.coli* and *Salmonella* sp and quantification of *E.coli* from sewage sludge.
- By molecular methods is possible the identification of *E.coli* and *Salmonella* and quantification of *E.coli* isolated from sewage sludge.

References

1. López-Valdez, F, Fernandez-Luqueño, F, Luna-Suarez, L. (2011) *Science of the Total Environment* (412-413): 257-264.
2. USEPA. (2010). *Method 1680: fecal coliforms in sewage sludge (biosolids) by multiple-tube fermentation using lauryl tryptose broth and EC medium.* Pennsylvania, United States of America
3. USEPA. (2006). *Method 1682: Salmonella in sewage sludge (biosolids) by modifies semisolid Rappaport-vassiliadis (MSRV) medium.* Pennsylvania, United States of America
4. Molina, F., López-Acedo, E, Tabla, R, Roa, I, Gomez, A, Rebollo, J. (2015) *BMC Biotechnology* (15): 1-9.

ISSN: 2448-8461

AM17

IN VITRO BIOCONTROL OF *Penicillium digitatum* BY COMBINING ANTAGONISTIC YEASTS

Karem Frinette Rodriguez-Castillejos, María del Socorro Ramírez-González, Aharhel Rodríguez de Lara, José A. Narváez-Zapata, Claudia Patricia Larralde-Corona. Instituto Politécnico Nacional, Centro de Biotecnología Genómica, Laboratorio de Biotecnología Industrial, Reynosa (Tam) México, C.P. 88710. E-mail: frinettecastillejos@hotmail.com

Key words clave: *Biocontrol*; *Yeasts Saccharomyces* and *non-Saccharomyces; Phytopathogenic Fungi*.

Introduction. Mexico is currently 5th place worldwide for citrus production; however, postharvest diseases, caused mainly by fungi *Penicillium digitatum* and *Penicillium italicum*, provoke important losses during storage and fruit transport. An environmentally friendly alternative is the use of biocontrol agents (BCA) and as such, yeasts have many biological and technological advantages, such as the easiness of production and good stress resistance[1]. Some authors have explored the use of monocultures of yeasts as BCAs, but there is little information about the use of combinations of yeasts to enhance their biological effectivity.

The aim of the present work was to assess the *in vitro* biocontrol efficacy of different mixtures of four yeast strains (*Saccharomyces* and non-*Saccharomyces*) against *P. digitatum*, and to verify if any extracellular compound (antibiosis, parasitism) was involved in the biocontrol activity displayed by such yeast mixtures.

Methods. All yeast and fungal strains belong to the Laboratorio de Biotecnología Industrial (LBI-CBG) and were originally isolated from citrus fruits (*Citrus limon* var Eureka) and from agave musts, and had been previously characterized in our lab. Two *Saccharomyces cerevisiae* strains (Sc4Y3 and Sc3D6) and two non-*Saccharomyces* strains (*Meyerozyma guilliermondii* and *Pseudozyma* sp) were tested against green rot fungus *Penicillium digitatum*. Yeasts were either, inoculated together, or separated by a dialysis membrane (100 and 50 kDa), and the effect of such interaction was confronted with *P. digitatum* on 50% PDA plates, to evaluate their biocontrol performance and sporulation level, where a value of 0 was no sporulation and a value of 3 meant that it was the same as the one observed in the fungal control.

Results

The effect of interaction of yeasts (directly inoculated and both alive, one yeast alive and the supernatant of the other, or after grown together but mediated by a membrane) on biocontrol activity against *P. digitatum* radial growth rate as well as the sporulation level can be observed in Table 1.

Table 1. Performance of the yeast combination for the *in vitro* biocontrol of radial growth rate and sporulation of *P. digitatum*.

Interaction	Treatment	Yeast combination	V_r inhibition (%)	Sporulation level
Direct	Mixed	*M. guilliermondii* - *Sc 3D6*	**22**	1
		Sc 4Y3 - *M. guilliermondii*	**54**	1
	Yeast 1 + Supernatant yeast 2	*M. guilliermondii* - Sup. *Pseudozyma sp.*	**75**	1
		Pseudozyma sp. – Sup. *Sc 4Y3*	**76**	0
Membrane-mediated	100 kD	*Sc 3D6* - *M. guilliermondii*	**84**	1
		M. guilliermondii – *Sc 3D6*	**67**	1
		Sc 3D6 - *M. guilliermondii*	**79**	0
		Sc 4Y3 - *M. guilliermondii*	**70**	0
		M. guilliermondii - *Sc 4Y3*	**82**	0
	50 kD	*M. guilliermondii* - *Sc 4Y3*	**58**	0
		Sc 4Y3 - *M. guilliermondii*	**72**	0

It is clear that the best inhibition was obtained when *M. guilliermondii* was grown together with *S. cerevisiae* mediated by a 100 kDa membrane, that is, when there is one yeast (*M. guilliermondii*) alive and the extracellular products of the *S. cerevisiae* strains.

Conclusions. The best biocontrol performance in vitro was observed for a non-*Saccharomyces* strains mixed with the extracellular products of a *S. cerevisiae* strain, which could indicate a combination of biocontrol mechanisms, specifically competition of nutrients and space and antibiosis.

Acknowledgments. We thank the finantial support of projects CONACYT Ciencia Básica 2013-221289 and SIP2018-1748 and SIP2018-0983 (Instituto Politécnico Nacional), as well as support BEIFI-IPN given to KFRC and ARL.

1. Droby, S.; Chalutz, E.; Wilson, C. L.; Wisniewski, M. E. Phytoparasitica 1992, 20, S149-S153.

ISSN: 2448-8461

AM18

BIOMASS N RELATED TO DYNAMICS OF RESPIRATION, N AND P IN VILLAMAR MICHOACÁN SOIL

M..A. Gisell Flores Nolasco[a], D. Zahory Montiel García[a], Dioselina Álvarez Bernal[b], Marina Olivia Franco Hernández[a]

[a]*Instituto Politécnico NacionalUnidad Profesional Interdisciplinaria de Biotecnología. Av. Acueducto S/N Col. Barrio La Laguna Ticomán. Ciudad de México. CP07340 Email:mofrancoh@hotmail.com*

[b]*Instituto Politécnico Nacional, CIIDIR Unidad Michoacán. Justo Sierra No. 28 Jiquilpan, Michoacán, México. C.P. 59510*

Key words: saline soil, metals, carbon dioxide

Introduction. Microbial biomass is constituted by bacteria, fungi, yeasts and actinomycetes in the soil, represents the labile fraction, and therefore responds quickly to the effect of soil disturbance or recovery.
In the present investigation biomass-N was evaluated in a saline soil. On the other hand, the carbon dynamics through respiration and nitrogen were evaluated.

Methods. pH, soluble phosphorus, and CO_2 carbon were evaluated in a 42-day kinetics at microcosm level. It was incubated under greenhouse conditions and samples were taken at 0, 3, 7, 14, 28 and 42 days with 40% CRA (NOM-021-RECNAT -2000). Biomass nitrogen was made through the Fumigation-Extraction technique.

Results. The soil presented clay-silty texture, pH was 8.3, and electrical conductivity of 10.5 $dS*m^{-1}$, this value is considered strongly alkaline according to the NOM021 RECNAT 2000 and very similar to the one mentioned by Liu and Col. (2017), the percentage of organic matter (8.2%) is considered as a medium, even though the concentration of nutrients is low. The concentration of Chlorides of 521.6 mg of Cl^- /Kg of soil. On the other hand, the most abundant metal was Aluminum with 9432.50 mg de Al/Kg of soil. In the dynamics, soluble phosphorus showed an initial concentration of 9.18 $mgKg^{-1}$, but on day 4 and 7 it increased to 30.89 $mgKg^{-1}$, subsequently decreasing to 13.90 $mgKg^{-1}$.

Table 1. Metal content in Villamar, Mich. soil.

Metal	Concentration, mg/kg
Al	9432.5
Fe	5968.6
Mg	3668.1
Ca	1564.7

Biomass increased to 400 mg Nkg^{-1} soil on day 4 and remained constant until day 21, then decreased to 30 mg
The reported in bibliography by Zagal and collaborators, 2003, present in volcanic soils is 123.06 mg N-Biomass / Kg of soil, while Huang Zhao, 2017, reports a nitrogen biomass of 60 mg of N-Biomass*Kg^{-1} of soil, and in this work, it is presented 3.25 times more regarding the work of Zagal and 6.6 times with respect to Zhao.
With this we can say that there is great microbial activity, despite the fact that the soil is highly saline, and with respect to the bibliographies, there is a greater amount of biomass Nitrogen

Conclusions. Soils of Villamar Michoacán have a great microbial activity, despite the fact that the soil is highly saline, there is a greater amount of biomass Nitrogen
Concentration of salts, as well as the pH of the soil do not affect the availability of nutrients; however, they affect the availability of water for plants.

Acknowledgements. We thanks to SIP 20180132.

References.

1. Liu, S., Meng, J., Jiang, L., y col. App Soil Ecol. (2017). 116:12-22
2. Zagal, E., Rodríguez, N., Vidal, I., Maureira, M. (2003). Chile. Ciencias del Suelo y Nutrición vegetal. 3:1 17-28
3. Zhao, Huan; Sun, Jian; Xu, Xingliang; Qin, Xiaojing. (2017). Europ J. Soil Biol. 83:1-8
4. SEMARNAT (2000). NOM-021-SEMARNAT-2000. Establece las especificaciones de fertilidad, salinidad y clasificación de suelos. Estudios, muestreo y análisis.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
AM19

EFFECT OF FERTILIZATION AND CUT-OFF INTERVALS ON YIELD AND PROTEIN CONTENT IN GRASS (*Lolium perenne*).

Hassam Montalvo, Myrna Solís, Laura García; Instituto Politécnico nacional, Centro de Investigación de Biotecnología Aplicada del Instituto Politécnico Nacional, Tlaxcala, México; persianraccoon@hotmail.com

Key words: perennial ryegrass, digestate, cutting frequencies.

Introduction. Several authors state that knowing the growth curve of each forage species and applying adequate cut-off intervals, is essential to carry out an adequate agronomic management that increases the yield and the nutritional composition of the forage, looking for the best moment of harvest or grazing (1). On the other hand, other authors mention that the digestate contains large amount of macro and micronutrients, organic matter and phytoregulators, and it can be used as an organic fertilizer to increase the yield and nutritional composition of in agriculture, reducing the environmental impact as in the case of chemical fertilizers (2) (3).
The aim of this work was to evaluate cutting frequencies, application frequencies and digestate concentrations, on the yield and protein content of ryegrass (*Lolium perenne*), during three seasons (summer, autumn and winter).

Methods. The experimental design was a completely randomized 3x3x2 factorial arrangement. Three concentrations of digestate (20, 40, 60%), three frequencies of defoliation (4, 5, 6 weeks) and two frequencies of application (15, 30 days), plus 6 controls. The dry mater (DM) was calculated in every cut (1). Protein percentage per gram of leaf DM was obtained with the FLASH 2000 SERIES Organic Elemental Analyzer and the CHNS analytical method.

Results. Figure 1 shows protein content of leaf DM

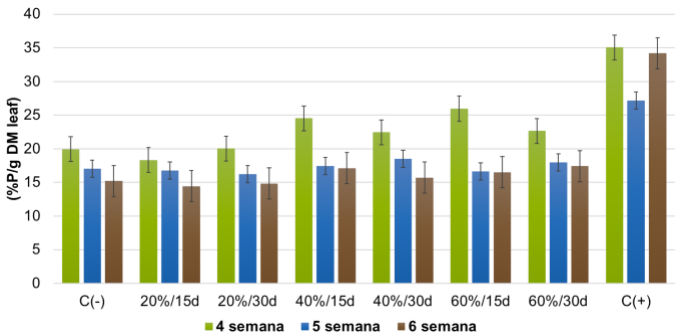


Fig 1. Protein content (%P/g DM leaf) of eight fertilizations with three cutting frequencies in perennial ryegrass.

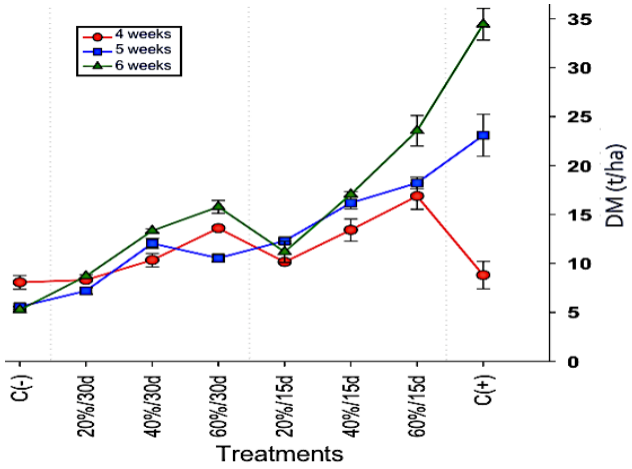


Fig 2. Seasonal and total DM yield (t/ha) of eight fertilizations with three cutting frequencies in perennial ryegrass.

The digestate-fertilized treatment that showed the best nutritional quality was 60%/15d/4sem and the treatment that showed the best yield of DM was 60%/15d/6sem as shown in figure 2. The concentration and cut frequency factors influenced significantly ($P<0.05$) on the yield of DM, however, only the cut frequency influenced ($P<0.05$) on the protein content.

Conclusions. The concentration of protein decreases, as the cut-off interval is longer. In an agronomic management, the cutting frequency is a very important factor, which helps to determine the production of forage of better nutritional quality for grazing or one of higher yield in DM for silage.

Acknowledgements. The authors would like to thank CONACYT for the scholarship CVU 789678 and SIP-IPN for the financial support received for this work, project 20170379.

References.

1. Velasco-Zebadúa, M. E.; Hernández-Garay, A.; González-Hernández, V. A. (2007). *Rev. Fitotec. Mex.* vol. 30 (1): 79 – 87.
2. Walsh, J. J.; Jones, D. L.; Edwards-Jones, G.; Williams, A. P. (2012). *J. Plant Nutr. Soil Sci.* (175): 840–845.
3. Ramos, D. A.; Terry E. A. (2014). *Cultivos Tropicales*. vol. 35 (4): 52-59.

ISSN: 2448-8461

AM20

BIODEGRADATION OF NAPROXEN BY *AMYCOLATOPSIS* SP. POZ 14

Blanca Alanís[a], Juan C. Cancino-Diaz[a], César M. Flores[b], Juan A. Cruz-Maya[c], Janet Jan-Roblero[a]*
[a] Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional (IPN), Ciudad de México, 11340, México.
[b] UBIPRO, FES Iztacala, Universidad Nacional Autónoma de México, Tlalnepantla, Estado de México. 54090. México.
[c] Unidad Profesional Interdisciplinaria en Ingeniería y Tecnologías Avanzadas, IPN, Ciudad de México, 07340, México.
* corresponding author: jjan_r@yahoo.com.mx

Key words: Naproxen, biodegradation, Amycolatopsis.

Introduction. Recently, nonsteroidal anti-inflammatory drugs have been detected in the environment, mostly in surface, drinking and ground water. Naproxen (NPX) is one of the most widely used analgesics. Its incidence in aquatic environments could become a risk for non-target organisms causing health problems, such as development defects in common carp and impairing of the lipid peroxidation system of bivalves. Degradation by microorganisms could be an ecological alternative for the removal of these drugs and their metabolites.
The aim of this work was to determinate the ability of *Amycolatopsis* sp. Poz 14 to degrade NPX as a sole carbon source and with the supplementation of yeast extract (YE) and naphthalene.

Methods. To evaluate NPX degradation by *Amycolatopsis* sp. Poz 14, 21-days removal assays were carried out. The experiments were performed in flasks with 30 mL of mineral medium (MM), 100 µL of biomass adjusted at 0.5 in McFarland standard and NPX at 50 mg/L. The analysis with additional carbon sources included naphthalene or YE at 50 mg/L. The controls were NPX-MM without biomass (abiotic control) and NPX-MM and biomass inactivated by sterilization (biomass inactivated control). The growth was determined by cell count of colony forming units and the concentration of NPX by HPLC/MS.

Results. The best growth rate of *Amycolatopsis* was in NPX-MM supplemented with YE (Fig. 1A-C). However, the growth rate was very similar in the conditions of NPX-MM and NPX-MM with naphthalene (Fig. 1A-B). The complete removal of the NPX was performed in the three-kinetics assayed; in the case of NPX-MM was at the 12th day; with NPX-MM supplemented with naphthalene, at the 15th day; and NPX-MM supplemented with YE, at the 6th day. The abiotic control exhibited approximately a 68% NPX decrease; probably due to photolysis or oxidation. The concentration of NPX in the inactivated biomass control was maintained during–the kinetics.

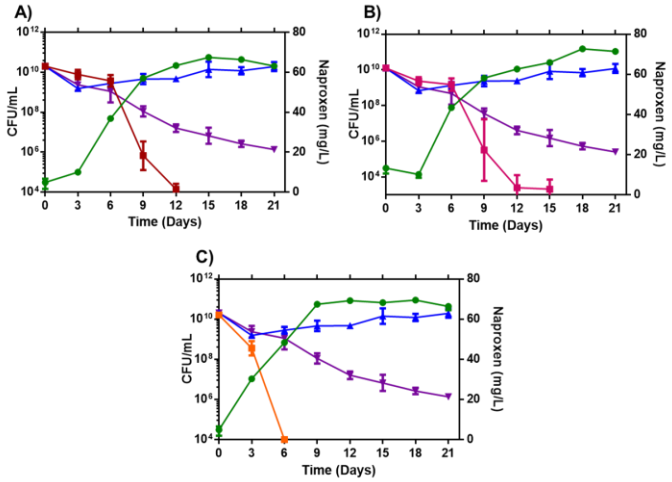


Fig.1 Degradation kinetics of Naproxen. A) Naproxen kinetics at 50 mg/L. B) Naproxen with naphthalene supplementation at 50 mg/L .C) Naproxen with YE supplementation at 50 mg/L. (●) Growth. (■) Naproxen. (▲) Abiotic control. (▼) Inactivated biomass control.

Conclusions. *Amycolatopsis* sp. Poz 14 exhibited a complete NPX removal in the three treatments. Nevertheless, the YE supplementation, as an additional carbon source, increased the rate of NPX removal and shortened kinetics time. This microorganism demonstrated that it could be used as a potential approach for NPX degradation in water.

Acknowledgements. This work was supported by grant SIP20180359 from the IPN.

References.

1. Domaradzka, D., Guzik, U., Wojcieszyńska, D. (2015). *Rev Environ Sci Biotechnol. 14*(2), 229-239.
2. Sehonova P., Plhalova L., Blahova J., Doubkova V., Prokes M., Tichy F., Fiorino E., Faggio C., Svobodova Z. (2017). *Chemosphere. 188*, 414-423.
3. Gagné F, Blaise C., Fournier M., Hansen PD. (2006). *Comp Biochem Physiol C Toxicol Pharmacol. 143*(2):179-86.
4. Ortega-González, D., Martínez-González, G., Flores, C., Zaragoza, D., Cancino-Diaz, J., Cruz-Maya, J., Jan-Roblero, J. (2015). *Int Biodeterior Biodegradation. 99*, 165-173.
5. Wojcieszynska, D., Domaradzka, D., Hupert-Kocurek, K., Guzik, U. (2016). *Pol J Microbiol. 65*(2), 177-182.

ISSN: 2448-8461

USO POTENCIAL DE *Bacillus velezensis* COMO AGENTE DE CONTROL BIOLÓGICO DE HONGOS FITOPATÓGENOS EN FRUTOS TROPICALES

Manuela Reyes-Estebanez[1], Benjamín O Ortega Morales[1], Juan C Camacho-Chab[1], Manuel Chan Bacab[1], Susana De la Rosa-García[2], Patricia Sanmartín[3], Erika De la Rosa-Escalante[1], Ruth N Aguila Ramírez[4], Claus Härti[5], Jorge L Arteaga-Garma[1].

[1]Universidad Autónoma de Campeche. Depto. Microbiología Ambiental y Biotecnología. Campeche, Campeche. CP. 24039. [2]Lab. de Microbiología, Div. Académica de Ciencias Biológicas, Universidad Juárez Autónoma de Tabasco, Carr. Villahermosa-Cárdenas Km. 0.5 s/n. C.P. 86150, Villahermosa, Tabasco. Depto de Edafoloxía e Química Agrícola. Facultade de Farmacia. Universidade de Santiago de Compostela, 15782 Santiago de Compostela, España. [4]Centro Interdisciplinario de Ciencias Marinas IPN, Av. Instituto Politécnico Nacional s/n. Col. Playa Palo de Santa Rita, C.P. 23096. La Paz, BCS. Department of Environmental Microbiology Helmholtz Centre for Environmental Research-UFZ Permoserstraβe. 15 04318 Leipzig, Germany.

Manuela Reyes Estebanez mamreyes@uacam.mx
Palabras clave: bacterias nativa, antagonism,

Introducción. Las frutas tropicales son recursos de importancia para la economía Mexicana y figuran en el mercado internacional el mango y la papaya[1]. Las enfermedades causadas por hongos como *Colletotrichum gloeosporioides* durante su almacenamiento, representan pérdidas importantes para la industria agrícola[2,]. Para contrarrestar estos efectos y evitar la aplicación intensiva de fungicidas sintéticos, que cada vez esta más regulada por la aparición de resistencia en los patógenos y a problemas de salud humana y ambiental. Permite que el control biológico sea alternativa para disminuir las pérdidas por hongos en postcosecha y el uso de bacterias antagónicas epifíticas se traduce como una alternativa prometedora a los fungicidas químicos[4,].

El objetivo de este trabajo fue aislar y evaluar *in vitro* la capacidad antagonica de bacterias epifiticas de a la filósfera de mango.

Metodología. Se aislaron y seleccionaron bacterias antagónicas de la filósfera de mango considerando caracerísticas necesarias para considerarse antagonistas potenciales como la producción de biomasa. Los aislados con estas caracteristicas se les evaluó su capacidad antagónica *in vitro* contra *Colletotrichum gloeosporioides* var. *minor* ATTC 42374. En el aislado más prometedor se comparó el efecto antagónico con otros métodos de control utilizados para este patógeno; un bioproducto comercial (quitosano), dos fungicidas químicos Benomyl 50® y Prochloraz, bicarbonato de sodio, un tratamiento térmico y como control la bacteria *Bacillus subtillis* ATCC 55614. El aislado más prometedor se caracterizó bioquímica y molecularmente y finalmente se evaluó en un ensayo *in vitro* contra otros patógenos aislados de frutos tropicales de la región.

Resultados. Se aislaron 178 bacterianos de la filósfera de Mango Tommy Atkins en huertos del estado de Campeche. Los aislados seleccionados por su actividad antagónica *in vitro* contra *C. gloeosporioides* ATTC 42374, fueron cuatro TS3B-44, TS3B-45, TS3B-92 y TS3B-183. El aislado con la mejor actividad antagónica fue TS3B-45, el cual se comparó con otros métodos de control empleados para el control del patógeno. El mejor efecto inhibidor se logró con el fungicida sintético procloraz (84%), seguido del aislado TS3B-45 (80 %) y *B. subtilis* ATCC 55614 (77%). El aislado TS3B-45 se probó adicionalmente contra otros patógenos fúngicos (*Colletotrichum gloeosporioides* nativo, *Curvularia clavata, Fusarium nivale, F. solani y Pestalotiopsis maculans*) con resultados positivos en el ensayo de antagonismo i*n vitro*. Las características bioquímicas y la identificación molecular determinaron como *Bacillus velezensis* el aislado bacteriano más prometedor. Este estudio demostró que esta bacteria es un candidato para futuras evaluaciones contra *C. gloesporioides* y otros patógenos en condiciones semicomerciales de poscosecha.

Conclusión. *Bacillus velezensis* es una bacteria con potencial antagóico efectivo contra *C. gloeosporioides* y otros patógenos de importancia económica en zonas tropicales de México.

Agradecimientos.Fondo Mixto CONACYT-Gobierno del Edo de Campeche (BOOM 146245). Patricia Sanmartín financiamiento “Plan para la Investigación, Innovación y Crecimeinto de Galicia” Plan I2C, Modalidad B (2016).

Referencias.

1. Hui Y, (2006). *Major Tropical Fruits and Products: Banana, Mangoes, and Pineapple Handbook of Food Science*, Technology and Engineering. Taylor & Francis.

2. Janisiewicz W, Korsten L. (2002). Biological control of postharvest diseases of fruit, *Ann Rev Phytopatho*. **40:**411–41.

3. Govender V, Korsten L, Sivakumar V. (2005). Semi-commercial evaluation of Bacillus licheniformis to control mangoes postharvest diseases in South Africa. *Postharvest Biol. Technol. 38*:57-65.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
AM22

ELABORATING FILMS OF CASTOR BEAN (*Ricinus communis* L.) PROTEIN ISOLATES

Juan Gustavo Mulato-Crispin[1, 2], Alma Angélica Del Villar-Martínez[1], María Asunción Bravo-Díaz[1], Enrique Vladimir Macedo-Orellana[1], Silvia Liliana Bucio[2], Pablo Emilio Vanegas-Espinoza[1]
[1]Departamento de Biotecnología, Centro de Desarrollo de Productos Bióticos-Instituto Politécnico Nacional, Yautepec, Mor., México. C.P. 62731.
[2]Biotecnología, Universidad Tecnológica de Morelia, Morelia, Mich., México C.P. 58200. gusmulato@gmail.com

Key words: biopolymer, physical properties, protein extraction

Introduction. Worldwide consumption of synthetic plastics is about 200 million tons/year. Most of them are persistent in the environment synthetic polymers, so they are a dangerous source of pollution (1). An alternative to this problem is the production of bioplastics, which are favorable for the environment due to its easy degradability. One of the main raw materials to produce these biofilms, are proteins, which may be obtained from different, sources, such as plants or animals (2). *Ricinus communis* L. is a plant used to produce biodiesel, once the oil has been extracted from seeds, a protein-rich byproduct is produced (3).

The aim of this study was to obtain protein isolates from castor bean (*Ricinus communis* L.), use them for elaborating biofilms and analyze some physical properties.

Methods. Protein isolates were obtained, from defatted cake flour, by alkaline solubilization (NaOH 1 N, pH 10.5) and acid protein precipitation (HCl 1 N, pH 4.5) at 50° C, isolates were lyophilized and stored until samples analysis (4). Films were produced by casting process; protein isolates were dissolved in distilled water at room temperature. Three concentrations of glycerol (5, 9 and 15%) were tested as plasticizing agent, filmogenic solutions were poured into plates (5). Films were physically characterized (color, humidity, water solubility, and thickness). Films were analyzed by electronic scanning environmental microscopy (ESEM).

Results. Protein content increased from 47.19% in defatted flour, to 78.40% in protein isolates, Films were accurately obtained with the three tested glycerol concentrations. Table 1 shows some physical characteristics of the elaborated biofilms. Color parameters evaluated did not show significantly differences (data not shown), thus all films are similar in this characteristic.

Microscopy evaluations showed the increase in the structural compaction according to the increase in the concentration of the plasticizing agent used (Figure 1).

Table 1. Physical characterization of films elaborated from castor bean protein isolates, with different glycerol concentrations as plasticizing agent.

Glycerol (%)	Humidity (%)	Solubility (%)	Thickness (µm)
5	31.94±3.69^{b}	61.93±2.54^{c}	94±0.008^{a}
9	26.00±2.28^{a}	52.84±2.03^{b}	117±0.003^{b}
15	25.28±2.46^{a}	43.99±1.53^{a}	119±0.006^{c}

Average values ± standard deviation. Same letters in columns indicate no significant differences (P<0.05).

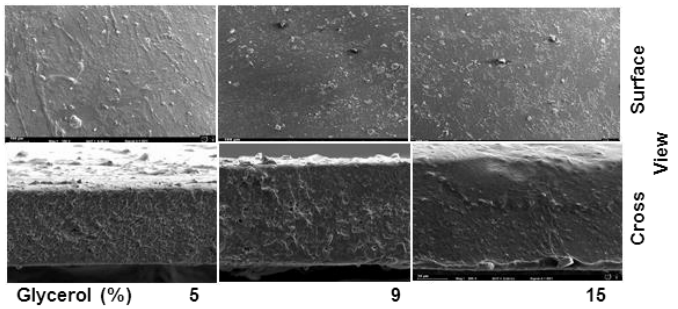


Figure 1. Electronic scanning environmental microscopy (ESEM) observations of films elaborated from castor bean protein isolates, with different glycerol concentrations as plasticizing agent.

Conclusions. Proteins isolates from castor bean (*Ricinus communis* L.) were able to form a structural matrix with proper physical features to form a film with potential uses as biodegradable material.

Acknowledgements. SIP-IPN by the financial support SIP 20180334.

References.

1. Zhang, H., Mittal, G. 2010. *Environ. Prog. Sust.* 23: 203-220.
2. Lacerda, R.S., Makishib, G.L., Chambib, H.N., Monica, A., Bittanteb, Q.B., Gomidea, C.A., Sobralb, P.J. (2014). *Chem. Eng. Sci*. *37*.
3. Gutarra, M.L.E., Cavalcanti, E.D.C., Castilho, L.R., Freire, D.M.G., Sant'Anna Jr., G.L. 2005. *Appl. Biochem. Biotech.* 105–116.
4. Peralta-Flores, L., Gallegos-Tintoré, S., Solorza-Feria, J., Dávila-Ortiz, G., Chel-Guerrero, L., Martínez-Ayala, A. (2012).*Grasas Aceites*. 63(3): 253-259.
5. Chambi, H.N., Lacerda, R.S., Makishi, G.L., Bittante, A.M.Q., Gomide, C.A., Sobral, P.J. (2014). *Ind. Crop. Prod*. 61: 217-224.

ISSN: 2448-8461

AM23

NEW GENES FOR MONITORING OF *IN SITU* REMEDIATION OF AROMATIC HYDROCARBONS

Sofía Guadalupe Zavala Meneses[1,2] (szavala.meneses@cas.cz), Maria V. Brennerova[1]; Institute of Microbiology, Laboratory of Molecular Genetics of Bacteria, Czech Academy of Science[1], Prague 14220, Czech Republic; Charles University, Faculty of Science[2], Prague 12843, Czech Republic.

Key words: polycyclic aromatic hydrocarbons, dioxygenases, bioremediation

Introduction. Adaptability and versatility of microbial populations are pivotal for bioremediation of soils, contaminated with polycyclic aromatic hydrocarbons. Microorganisms have developed specialized pathways to use aromatic compounds as their energy source converging usually in the production of dihydroxylated central intermediates. These compounds are substrates for ring-cleavage extradiol dioxygenases (EXDO), key enzymes in aromatic compounds degradation (1). New dioxygenases, similar to DbtC of the naphthalene degradating *Bulkholderia* sp. DBT1 were isolated (2).
Our research is using high-throughput sequencing and analysis of the metagenomic organization of new EXDO genes. The environmental importance of the isolated genes is confirmed by transcriptional study, mapping of their geographical distribution, and profiling changes in microbial populations in highly contaminated environment.

Methods. Mesocosm system was established for 60 days using soil polluted with crude oil from Moravia, Czech Republic. Concentration of TPH was 20600 mg/kg dw. Soil was used for CFU counts, DNA, RNA extractions and chemical analysis. DNA was isolated as described by Praveckova et al., 2016 (2). RNA was extracted using a commercial kit (Qiagen) and cDNA was obtained from the isolated transcripts. Microbial enrichments and cultivations were conducted in order to identify EXDO genes using MM and R2A medium with 300 mg phenanthrene/ml. Sequencing was carried out using Illumina MiSeq platform.

Results. High-throughput sequencing revealed a different from the so far known archetypal organization (Fig.1).

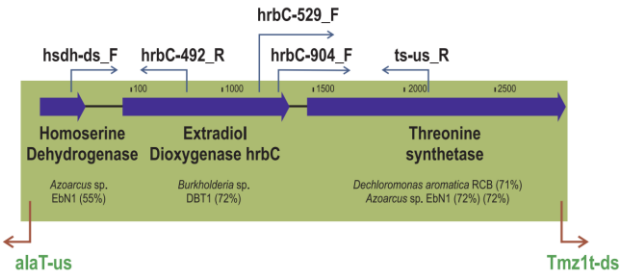


Fig.1 Gene organization. *hrbC* encoding for catechol and 2,3-dihydroxybiphenyl dioxygenase showed a solitary localization.

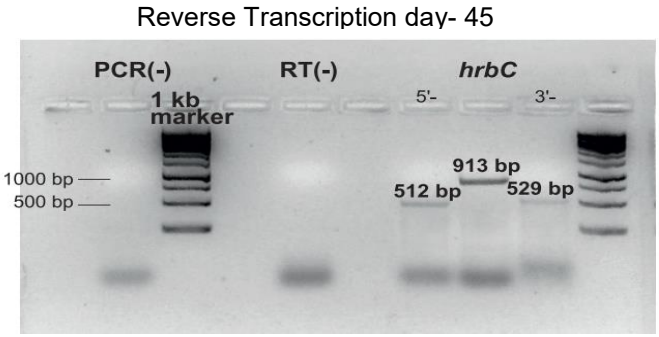


Fig.2 Identification of *hrbC* expression in polluted soil.

Bacterial diversity decreased after increasing the concentration of total TPH from 9800 to 20600 mg/kg dw and the number of soil bacteria grew, which correlated with faster degradation of phenanthrene and fluorene. Transcription analysis revealed expression of *hrbC* as a polycistronic mRNA (Fig. 2), thus, utilizing the high speed transcription of the vicinal housekeeping genes.

Conclusions. The new genes identified belong to the uncultivable soil bacterial population and count for over 55% of the extradiol ring-cleavage dioxygenases detected. A remedial response of the microbial community was detected by the increase of both bacterial and catabolic gene abundance. It corresponded to a faster reduction of the PAH compounds when compared to the control. The lower GC content, compared to the adjacent metagenomic DNA, indicates its acquisition by horizontal transfer. We assume that *hrb*C-type of genes, having broad substrate specificity to dihydroxylated degradation products of aromatic compounds may provide a survival advantage to the biodegrading community.

Acknowledgements. This research is supported by the Czech Science Foundation (grant No. 17-23794S).

References.

1. Vilchez-Vargas, R., Junca, H., & Pieper, D. H. (2010). *Environ. Microbiol*, 12(12), 3089–3104.
2. Praveckova, M., Brennerova, M. V., Holliger, C., De Alencastro, F., Rossi, P. (2016) Frontiers in Microbiology. Jun 16;7:933.

ISSN: 2448-8461

AM24

Optimization of *Chlorella vulgaris* biomass recuperation by Coagulation-Flocculation

Guerrero Carreño C.A.[1], De la Peña Arellano L.A.[1], Rodríguez Rosales M.D.J.[1], Perales Vela H.V.[2], Martínez Roldan A.J.[1,3]
[1]Maestría en Sistemas Ambientales. Tecnológico Nacional de México/I.T. Durango. México. [2]Laboratorio de Bioquímica. Unidad de Morfología y Función. FESI–UNAM. México. [3]CONACyT-Tecnológico Nacional de México/I.T. Durango. México.
alfredo.martinez@itdurango.edu.mx; adjmartinezro@conacyt.mx
Key words: Chlorella vulgaris, Flocculation, Ferric Chloride.

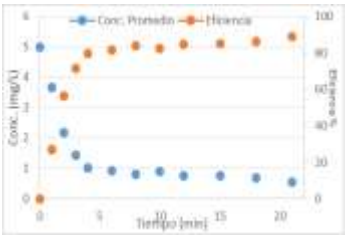
Introduction. Microalgae have the potential to be used as a source of different high-value products, but for its obtaining, some steps have to be optimized, One of them is the biomass separation because the traditional methods (as centrifugation) consume high amounts of energy and are very expensive. (1-3). This work had as objective the study of the coagulation-flocculation process for the recovery of Chlorella biomass, by the employment of a Central Composite Design (CCD) and its analysis by the software JMP®.

Methods. The culture used was *Chlorella vulgaris* growth in Basal Bold medium, and the coagulation-flocculation process was done using 2-L acrylic jars. The agitation (300 rpm) was applied at the beginning until the complete dissolution of the coagulant (FeCl3), after that the stirrer was stopped to favor the generation of flocs. The experiments were 21 min long with a sampling frequency of 3 min carrying out determinations of Total Chlorophyll (TC) (3). The CCD included variation in initial biomass and coagulant concentration as Table 1 showed.

Table 1. Levels of the DCC and values of the efficiency reached.

Treatment	Biomass Concentration	Coagulant concentration	Recuperation Efficiency (%)
++	15	1000	82
+-	15	300	84
-+	5	1000	93.3
--	5	300	81.3
0a	10	206.5	62.6
a0	3.66	650	82.3
0A	10	1093.4	92
A0	16.3	650	95.7
00	10	650	96.4

Results. The figure 1A shows the behavior of the TC concentration in one of the experimental condition assayed. The biomass concentration diminishes from the 1 min to the end of the experiment when the TC concentration reached 0.55 mg/L; this represents a biomass recuperation efficiency close to 95%. The majority of the experimental conditions employed in the CCD reached final efficiencies close to the 90%; because that the time required to reach the 90% of efficiency was selected as response. The response surface of the CCD (Fig. 1B) presented an optimal point (local) close to the central point; this means 10 mg/L of chlorophyll concentration and 650 mg/L of ferric chloride with a biomass recovery of 96.5%.



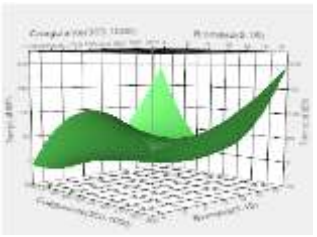


Fig.1a) Chlorophyll concentration and biomass recovery in the coagulation-flocculation process. **Fig.1b)** Response surface of the CCD.

Conclusions. In the CCD both, the chlorophyll concentration and the coagulant concentration have a direct effect of the biomass recovery; nevertheless, the effect of the TC concentration (biomass concentration) was almost despicable. The coagulation-flocculation process resulted in an efficient and low-cost strategy to recover microalgal biomass.

Acknowledgements. Guerrero-Carreño received a graduate scholarship awarded by the CONACyT for Master studies, and all the authors thank to the Tecnológico Nacional de México/I.T. Durango for all the resources used in the realization of this project.

References.

1. Abdel-Raouf, N., Al-Homaidan, A. A., Ibraheem, I.B.M. (2012). *Saudi J Biol Sci*, 19(3), 257–275.
2. Papazi, A., Makridis, P., Divanach, P. J. Appl. Phycol. 22(3). 350-355
3. Arias-Peñaranda, M.T., Martínez-Roldán, A.J., Cañizares-Villanueva, R.O. (2013). *Acta Biológica Colombiana*. 18(1), 43-68.
4. Wellburn, A.R. (1994). *J Plant Physol* 144(3), 307–313.

ISSN: 2448-8461

EMPLOYMENT OF THE EFFLUENT FROM THE SAND TRAP TO PRODUCE MICROALGAL BIOMASS

Villanueva-García Rebeca[1], Rodríguez-Rosales Josefina[1], De la Peña-Arellano Armando[1], Perales-Vela Hugo[2], Martínez-Roldán Alfredo[1,3]

[1]Maestría en Sistemas Ambientales. Tecnológico Nacional de México/I.T. Durango. México. [2]Laboratorio de Bioquímica. Unidad de Morfología y Función. FESI-UNAM. México. [3]CONACyT-Tecnológico Nacional de México/I.T. Durango.
alfredo.martinez@itdurango.edu.mx; adjmartinezro@conacyt.mx

Key words: Microalgae, Photobioreactor, Nutrients removal

Introduction. Industrialization and population growth have a huge effect on the quality and quantity of the fresh water; also, cause an increase in the volume of wastewater to treat (1). The wastewaters (WW) contain nutrients as nitrogen, phosphorus, organic matter (as DQO), etc. and these can cause eutrophication in water bodies. Nevertheless, these nutrients can be removed in a treatment step based on microalgae, this because these microorganisms can remove them very quickly (2).
This work had the objective to evaluate the growth, biomass productivity and nutrients consumption (N and P) by two native microalgal strains *Synechocytis aquatilis* Saivageau (Cyanophyceae) y *Stigeoclonium nanum* (Chlorophyceae).

Methods. Both microalgae grown in filtered WW from the san-filter in the Wastewater Treatment Plant from Durango city in an airlift photobioreactor (3) at 23±2ºC, an insufflation of 2.1 L/min and irradiation of 130 µE/(m^2s). The monitoring of the cultures was done by six days by dry weight (2), Chlorophyll (4), P-PO_4, N-NO_3 y N-NH_3 (5) concentrations. For the WW, initial and final determinations of pH, alkalinity, total coliforms bacteria (CB), and chemical oxygen demand (COD) (6).

Results. *S. aquatilis* initiated with a biomass concentration of 0.14±0.01 g/L reaching 0.44 ± 0.06 g/L at the 6th day; the chlorophyll presented a similar behavior reaching a maximal concentration of 7.8±0.25 mg/L. For *S. nanum* the biomass concentration finalized at 0.28±0.01 g/L while the chlorophyll concentration at the 6th day was 6.9±1.17 mg/L (Fig. 1A and 1B). *S. aquatilis* reduced the P-PO_4 close to 79%, while for *S. nanum* was 63%. For N-NO_3 (Fig. 2B) and N-NH_3 (Fig. 2C), both strains consumed the nitrate preferably this was caused because the amount of ammonia in me WW was very low.
The N-NO_3 in the culture of *S. aquatilis* started at 38.21±0.540 mg/L reaching 20.05±2.795 mg/L at sixth (reduction of 47%). The N-NH_3 present in the WW was eliminated. In the case of *S. nanum* had an initial N-NO3 concentration of 37.1±0.01 mg/L, and reached 25.2±3.46 mg/L at the end of the growth (a reduction of 32%), as occurred for *S. aquatilis* the N-NH3 was complete eliminated at the sixth day.
The CB, COD, and alkalinity drastically reduced its values; the total coliforms started at 2.4*10^{10} and finalized close to 1*10^3 for both strains. The COD diminished close to 50 and 70% for *S.aquatilis* y *S. nanum* respectively. The pH increase above 10.3 for both cultures and its cause was the photosynthetic activity, and the conductivity remained altos constant. The alkalinity presented a diminution close to 50% for both strains.

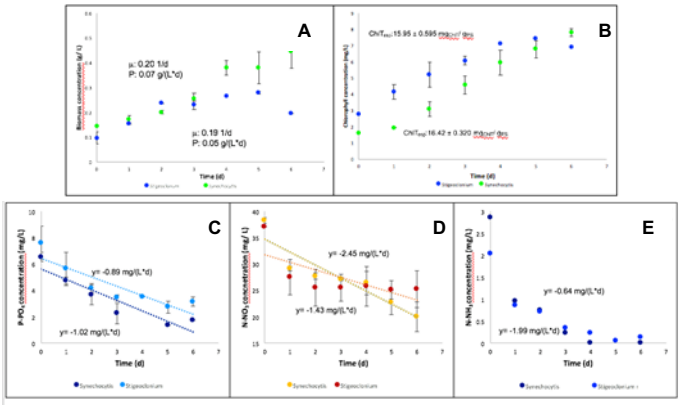


Fig. 1. Different culture parameters. (A) Dry weight. (B) Total Chlorophyll concentration. (A) Consume of P-PO_4. (B) N-NO_3. (C) N-NH_3.

Conclusions. *S. aquatilis* and *S. nanum* have potential to develop of a process to eliminate N-NO_3, P-PO_4, and N-NH_3 form WW. Nevertheless, the values of DW were low, so it is necessary optimization of its growth in the PBR employed.

Acknowledgements. Villanueva-Garcia received a graduate scholarship awarded by the CONACyT for Master studies. All the authors thank to the TecNM/I.T. Durango for all the resources used in the realization of this investigation.

References.

[1]Abdel-Raouf, N., Al-Homaidan, A.A., Ibraheem, I.B.M. (2012). *Saudi J Biol Sc*, 19, 257–275.
[2]Martínez Roldán A.J. (2013). *Tesis*. CINVESTAV-IPN.
[3]Osuna Salazar, N.K.; Pacheco Fernández, M.A.; Gómez Lozano, B.P., Martínez Roldán, A.J. (2016).*3rd Biotechnology Summit,* 436-440 pp.
[4]Wellburn. (1994). J*ournal of Plant Phisiology*. 144, 307-313.
[5]González-Moreno, S., Peñalosa-Castro, I., Perales-Vela, H., Salcedo-Alvarez M., Sánchez-Correa S., Medrano-Vazquez, J. Flores-Ortiz, C., Quintanar-Zúñiga R., Delfín-Alcalá, I., Chino-Vargas S., Puga-Ruiz P., Moreno-Colín R. (2003). ISBN: 968-36-9271-0
[6]NOM-001-ECOL-1996.

ISSN: 2448-8461

AM26

PRODUCTION OF INDOLEACETIC ACID AND GIBBERELLIC ACID BY ANAEROBIC DIGESTION OF COW MANURE

Job Castro-Ramos[1], Myrna Solís-Oba[1], Carlos Calderón-Vázquez[2], Aida Solís-Oba[3], Eduardo Gómez-Sánchez[1]; [1]Centro de Investigación en Biotecnología Aplicada, Instituto Politécnico Nacional, Tlaxcala, México CP 90700; [2]CIIDIR-IPN Unidad Sinaloa, Instituto Politécnico Nacional, Guasave, Sinaloa, México CP 81101; [3]Universidad Autónoma Metropolitana Unidad Xochimilco. Cd. México, México CP 04960; jonathan_castro@live.com.mx

Key words: Plant hormones, indoleacetic acid, gibberellic acid, anaerobic digestion

Introduction. Plant hormones (PH) are molecules that control development and response to environmental changes of plants (1). These compounds are produced by plants, some microorganisms and by chemical synthesis. PH is used in agriculture to improve crops. Among these compounds are indoleacetic acid (IAA) and gibberellic acid (GA_3). Anaerobic digestion (AD) is divided into four steps including hydrolysis, acidogenesis, acetogenesis, and methanogenesis, in which various microorganisms transform organic matter into biogas and digestate. Fertilizing properties has been attributed to digestate due to the presence of nutrients (N, P, K) and PH (2). The AD process represents low operating costs compared to aerobic processes, due to the use of residual substrates, as well as minimum energy requirements (3).
In this study, it was evaluated the use of cow manure (CM) to obtain a product of interest, such as the production of IAA and GA_3 through the AD process.

Methods. AD process was performed in serological bottles (120 ml) with 70 ml of CM adjusted to 7% of total solids (TS), maintaining the temperature at 39 °C. The pH, C/N ratio and TS of the CM were determined. IAA and GA_3 were extracted with ethyl acetate (4) and subsequently detected and quantified by HPLC with gradient elution, using water, methanol and a mixture of both as a mobile phase.

Results. The results of the characterization of CM (Table 1) fits the requirements of hydrolytic and acidogenic microorganisms (5).

Table 1. Cow manure characterization.

Compound	Value
Total solids (%)	14.23 ± 0.11
C/N ratio	18.24 ± 0.49
pH	6.54 ± 0.09

GA_3 was produced during the hydrolysis-acidogenesis stage of AD, reaching a concentration of 68.7 mg/L at 11 days. IAA production reached 3 mg/L at 5 days (Fig. 1).

The characteristic pH increase of methanogenic stage was not observed. Xin et al. (2) obtained a maximum concentration of IAA and GA_3 of 23 mg/L and 16 mg/L respectively in the AD of CM and sewage sludge at 37 °C.

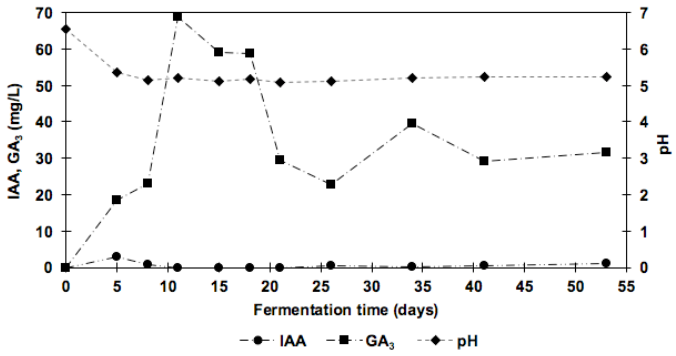


Fig.1 IAA and GA_3 production during the anaerobic digestion of cow manure at 39 ºC.

Conclusions. AD process represents an alternative for the valorization and the use of CM, waste that generates greenhouse gases and pollution problems due to its inadequate disposition. Through the AD process, important agricultural value products like GA_3 and IAA can be obtained.

Acknowledgments. To CONACyT by the grant of the scholarship 634440/337135.

References.

1. Bose, A., Shah, D., Keharia, H. (2013). Production of indole-3-acetic-acid (IAA) by the white rot fungus *Pleurotus ostreatus* under submerged condition of Jatropha seedcake. *Mycology*. 4 (2): 103-111.
2. Xin, L., Jianbin, G., Changle, P., Renjie, D. (2016). Anaerobic Digestion and Storage Influence Availability of Plant Hormones in Livestock Slurry. *ACS Sustainable Chem. Eng*. (4): 719−727.
3. Lorenzo-Acosta, Y., Obaya-Abreu, C. (2005). La digestión anaerobia. Aspectos teóricos. Parte I. *ICIDCA*. 39 (1): 35-48.
4. Ünyayar, S., Topcuoglu, S., Ünyayar, A. (1996). A modified method for extraction and identification of IAA, GA_3, ABA and zeatin. *Bulg. J. Plant Physiol.* 22 (3): 105–110.
5. Deubleien, D., Steinhauser A. (2008). Formation of biogas. In: *Biogas from waste and renewable resources.* Deubleien, D., Steinhauser A. Wiley-VCH, Germany. 87-137.

ISSN: 2448-8461

AM27

WATER QUALITY INDEX OF COASTAL SYSTEMS BASED ON MACROALGAE SPECIES

Ardila-Poveda, Leidy Solange[1]; Siqueiros-Beltrones, David A.[2]. Centro Interdisciplinario de Ciencias Marinas - Instituto Politécnico Nacional, 1) Departamento de Biotecnología; 2) Departamento de Plancton y Ecología Marina. La Paz, 23090; México. lsardilap@gmail.com, dsiquei@ipn.mx.

Keywords: Bioindicator, seaweed, pollution

Introduction. Macroalgae respond to the variation of the environment. Especially, the change in temperature produces different floristics (taxocoenoses) as has been observed over time in coastal systems of Baja California Sur. Other physicochemical changes (salinity, pH, dissolved oxygen) and concentration of nutrients (nitrate, phosphate, and silicate), as well as metals concentrated in water, influence species composition. Depending on the composition and production of polysaccharides in the intracellular matrix of each species of macroalgae, can be found to a greater or lesser minerals content. This will depend on the chemical affinity of the mineral with the polysaccharide; as well as, the chemical species of the mineral and its valence state. Other conditions intervene in this relationship, especially the pH and dissolved oxygen in the water, which can drastically affect the formation of compounds that in some cases can be highly toxic.

The objective was to create a water quality indicator for coastal systems of Baja California Sur using macroalgae species.

Methods. The distribution of 78 species of macroalgae (Guisande 2013) from a registered taxonomic list for Baja California Sur in the Global Biodiversity Information Facility database (Siqueiros-Beltrones et al. 2017) was studied. The average values and standard error (range) of each of the physicochemical characteristics pH, salinity (unitless) dissolved oxygen (ml/L) and nutrient concentration (µmol/L of NO_3, $S0_4$ and PO_4) of seawater from 2005 to 2012 of the National Oceanic Atmospheric Administration NOAA (Garcia et al. 2013a b; Zweng et al. 2013) were gathered, and were compared between species, in function on the increase of the variable in the water.

Results. For the water quality index, species with wide tolerance intervals were chosen to establish them as "passive monitors" of the mineral content, as well as species that occur at narrow intervals of variation, which would indicate a certain state of the quality of the water. It also proposes possible taxocoenoses that can occur in Baja California Sur warning on specific water conditions and would indicate the quality status. The brown algae of BCS studied were shown to be not very tolerant, especially with temperature increase, which is why they develop mainly in temperate waters. The changes in dissolved oxygen, pH, dissolved organic matter and silicates in the water were the variables with the greatest contribution to the association of species by habitat similarity.

Conclusions. The variation of water quality in terms of physico-chemical characteristics and nutrient concentration causes distinct macroalgal taxocoenoses in coastal systems, the specific composition can at the same time determine the water quality status. It can be observed that the possible associations reflect situations found in the theory of intermediate disturbance (Roxburgh et al. 2004) where the greatest diversity of species were found within average values of nutrient concentration. As well as the smallest number of species, there will be coastal waters with very low degrees of disturbance or with very high values, given that few species have tolerances in extreme values. A larger number of species survive at lower concentrations of salinity and nutrients than at higher concentrations, so the established upper limits are usually critical for most of the macroalgae species studied in Baja California Sur.

Acknowledgments. The first author received scholarship funds from Consejo Nacional de Ciencia y Tecnología Conacyt, for her post-graduate studies in CICIMAR.

References

Guisande C (2013) *ModestR Software User's Manual*. 36:1202–1207.

Siqueiros-Beltrones D, Ramos Rivera P, Careaga-Olvera S (2017) *Macroalgas marinas de Baja California Sur Parte I: Bahía de la Paz y Bahía de la Ventana*. GBIF. Data occurrence. Accessed 20 Mar 2018

Garcia H, Locarnini T, Boyer J, Antonov O, Baranova M, Zweng J, Reagan D, Johnson R, 2014. World Ocean Atlas 2013, Volume 4: Dissolved Inorganic Nutrients (phosphate, nitrate, silicate). S. Levitus, Ed., A. Mishonov Technical Ed.; *NOAA Atlas NESDIS 76*, 25 pp.

Garcia. H, Locarnini T, Boyer J, Antonov O, Baranova M, Zweng J, Reagan, D and Johnson R. 2014. World Ocean Atlas 2013, Volume 3: Dissolved Oxygen, Apparent Oxygen Utilization, and Oxygen Saturation. S. Levitus, Ed., A. Mishonov Technical Ed.; *NOAA Atlas NESDIS 75*, 27 pp.

Zweng M, Reagan J, Antonov J, et al (2013) Volume 2: Salinity. World Ocean Atlas 2013, *NOAA Atlas NESDIS 74*, p 27.

Roxburgh S, Shea K, Wilson B (2004) The intermediate disturbance hypothesis: patch dynamics and mechanisms of species coexistence. *Ecol.* 85:359–371.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
AM28

PHYLOGENETIC ANALYSIS BY DNA BARCODE OF SEA TURTLES

Fátima Y. Camacho-Sánchez[1], Hervey Rodríguez-González[3], Alan A. Zavala-Norzagaray[4], José A. Narváez-Zapata[2], Héctor H. Acosta-Acosta[5], Miguel Angel Reyes-López[1]

[1]Conservation Medicine Lab., [2]Biotecnología Industrial, Centro de Biotecnología Genómica-Instituto Politécnico Nacional, Reynosa, Tamaulipas. MEX., C. P. 88700. [3]Lab. Nutrición Acuícola, Depto. de Acuacultura, [4]Lab. Vida Silvestre, Depto. Medio Ambiente CIIDIR Sinaloa-Instituto Politécnico Nacional, Guasave, Sinaloa. MEX., C. P. 81000. [5]Comisión Nacional de Áreas Naturales Protegidas, Cd. Victoria, Tamps., MEX., C. P. 87000.

Key words: Barcode, COI, Lepidochelys kempii

Introduction. DNA barcoding is a system that uses short sequence instead of whole genome; therefore, it makes ecological or biological system more accessible. Cytochrome c oxidase subunit I gene, offers the solution to quickly distinguish among species, providing information on species diversification and molecular evolution (1). Sea turtles occupied several ecosystems through their highly migratory and threatened worldwide; we should take conservation measures for their study such as molecular tools by DNA barcoding (2). Six out of 7 worldwide sea turtles nest in Mexico, all of them are grouped in 2 known families, Dermochelidae (*Dermochelys coriacea*) and Chelonidae (*Lepidochelys kempii, L. olivacea*, *Caretta caretta*, *Eretmochelys imbricata*, *Chelonia mydas*) (3).

The objective of this work is to analyze reported and generated sequences from COI gene to make the first described database about it and to compare genetic variability among sea turtles worldwide.

Methods. One hundred and fifty-six sea turtle sequences were compared in total, 143 were download from BOLD and 13 sequences obtained *in vitro* were compared; afterwards, those ones assigned to similar groups and transformed into FASTA format, then a nucleotide alignment was made by ClustalW to obtain haplotype networks by Network 5 and finally a Neighbor joining tree was constructed by MEGA 7 with 10,000 replicates, using nucleotide substitution model of K2p (4).

Results. Thirty-five haplotypes were separated in different networks for each species, due to the variation between nucleotides and to 670 mutations found in total for all the sequences (Fig. 1). The average distance was 8.2% and the average distance between the families Dermochelidae and Cheloniidae was 8.1%. The average distance between Cheloniidae was 6.6% (Table 1).

Table 1. Mean divergence of the sequences (K2p) within the species (underlined number) and between pairs of marine turtle species.

	Lk	*Cc*	*Cm*	*Dc*	*Ei*	*Lo*	*Nd*
Lepidochelys kempii (Lk)	0.00						
Caretta caretta (Cc)	0.06	0.01					
Chelonia mydas (Cm)	0.08	0.09	0.00				
Dermochelys coriacea (Dc)	0.08	0.12	0.14	0.00			
Eretmochelys imbricata (Ei)	0.08	0.09	0.09	0.03	0.03		
Lepidochelys olivacea (Lo)	0.02	0.07	0.08	0.09	0.09	0.00	
Natator depressus (Nd)	0.09	0.09	0.07	0.10	0.10	0.06	0.00

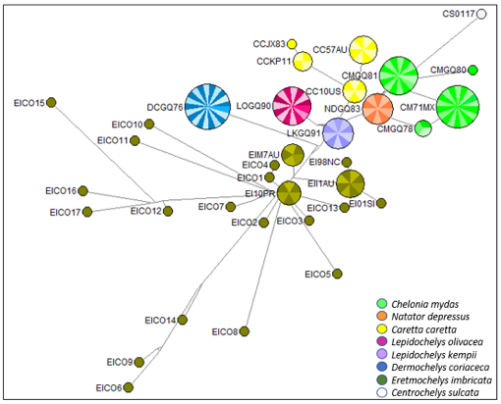


Fig.1. Haplotypes Network of the COI gene (mtDNA) for sea turtles. Each of the colors represents each species of sea turtles.

Conclusions. The COI fragment is variable in sea turtle taxa. In general, haplotypes were not interspecifically shared in all samples analyzed. The DNA barcode based on the COI gene identifies each species of sea turtles and to become a useful tool for determining intra and inter species variability of the world's marine turtles. Particularly, the nucleotide sequences reported in BOLD from *L. kempii* (Kemp's ridley) were compared with 3 sequences obtained in vitro, 100% all ones were aligned and clustered in the same haplotype. In the end, this work set up the first database for sea turtles worldwide by the study of mitochondrial COI gene.

Acknowledgements. To CONACYT and BEIFI-IPN for the scholarships granted, as well as IPN for the support in the SIP project: 20161179 and 20171851. Thank you to all the people from Rancho Nuevo, Tam., and CONANP-SEMARNAT

References.

1.- Hebert, P. D. N., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings. Biological Sciences / The Royal Society*, *270*(1512), 313–21.

2.- Naro-Maciel, E., Reid, B., Fitzsimmons, N. N., Le, M., Desalle, R. & Amato, G. (2010). DNA barcodes for globally threatened marine turtles: A registry approach to documenting biodiversity. *Molecular Ecology Resources*, *10*(2), 252–263.

3.- Vargas, S. M., Araujo, F. C. F., & Santos, F. R. (2009). DNA barcoding of Brazilian sea turtles (Testudines) AN - prod.academic_MSTAR_21050128; 11319878. *Genetics and Molecular Biology*, *32*, 608–612.

4.- Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution*, *33*(7), 1870–1874.

ISSN: 2448-8461
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
AM29

EVALUATION OF THE ANTAGONIC EFFECT OF *Oxyporus latemarginatus* ON GROWTH, PRODUCTION OF AFLATOXIN B1 (AFB1) AND DIFFERENTIAL EXPRESSION OF *aflR*, *brlA* AND *laeA* GENES OF *Aspergillus flavus*

Iván González Caloch, Soley Berenice Nava Galicia, Martha Dolores Bibbins Martínez
CIBA-Instituto Politécnico Nacional, Tepetitla de Lardizábal, Tlaxcala CP. 90700
marthadbm1104@yahoo.com

Key words: AFB_1, Aflatoxinas, Aspergillus flavus, Oxyporus latemarginatus

Introduction. The loss of crops and contamination of grains of economic and food importance by toxin producing pathogenic fungi, is an economic and health problem that has great relevance worldwide. In Mexico, the invasion of maize by *Aspergillus flavus* and its subsequent aflatoxin contamination occurs during cultivation, harvest, postharvest and during grain processing. Therefore, its presence in food represents a potential risk to the health of humans and animals. The degradation of aflatoxins such as AFB1 by white rot fungi has been previously investigated (1). Therefore, it is important to study and develop new biological methods that help to degrade or inhibit the production of aflatoxins, as well as the growth of *A. flavus*.
The objective of this work was to evaluate the antagonistic effect of the basidiomycete fungus *O. latemarginatus* on the growth, production of AFB1 of *A. flavus* and the differential expression of the genes that code for the *aflR*, *brlA* and *laeA* master regulators involved in the aflatoxin biosynthesis, sporulation and secondary metabolism, respectively.

Methods. The confrontation of the fungi was carried out in Petri dishes with PDA medium supplemented with 10% wheat straw extract. A 6 mm disc of mycelium from *O. latermaginatus* was placed at 3 cm distance of a filter paper disk containing 10 µL of a suspension of $1x10^6$ sp/mL of *A. flavus*. The plates were then incubated at 25 °C with a photoperiod of 12:12, a photographic record was made every 24 h. The concentration of AFB1 was monitored by fluorescence spectroscopy. Also, RT-qPCR was performed to determine the expression profile of the *aflR*, *brlA* and *laeA* genes.

Results. Figure 1 shows the representative days of the antagonism of *O. latemarginatus* vs *A. flavus*, as well as the degradation of AFB1. It was observed that *O. latemarginatus* grew faster than *A. flavus* in the whole degradation kinetics. The maximum and minimum concentration of AFB1 was 540 and 20 ppb on days 13 and 23 respectively.

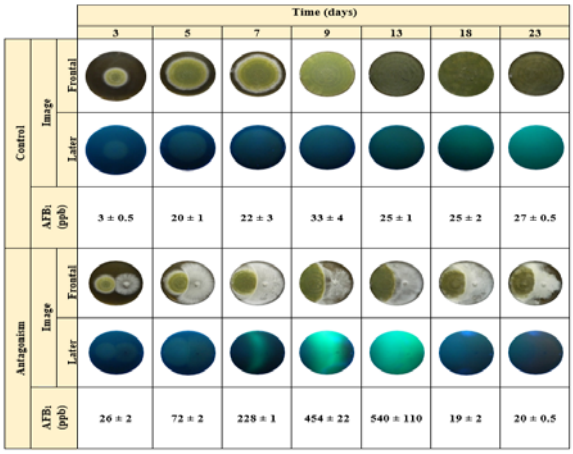


Figure 1. *O. latemarginatus* vs *A. flavus* antagonistic assay and degradation kinetics of AFB1.

Figure 2 shows the expression profiles of *aflR*, *brlA* and *laeA*. It was observed that only on day seven the three genes showed induction, contrary to the rest of the days where repression was observed.

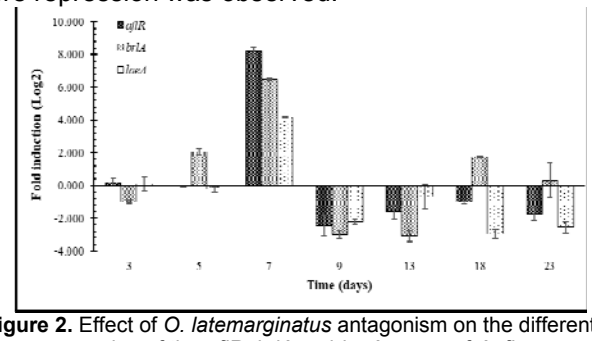


Figure 2. Effect of *O. latemarginatus* antagonism on the differential expression of the *aflR, brlA* and *laeA* genes of *A. flavus*.

Conclusions. *Oxyporus latemarginatus* inhibited the production and/or synthesis of aflatoxins and the development of *Aspergillus flavus.* The expression profile of the *aflR*, *brlA* and *laeA* master regulators showed a differential regulation during antagonism kinetics.

Acknowledgements. Project SIP20181110 and CONACyT scholarship 599292.

References

1. Cohen, R., Persky, L., & Hadar, Y. (2002) AMB, vol. 58 (5), 582-594.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México

AM30

EVALUATION OF THE BIODEGRADATION OF PHENOL BY *Fusarium petroliphillium* IN SUBMERGED FERMENTATION

Guadalupe Pérez Alonzo, Martha Dolores Bibbins Martínez and Soley Berenice Nava Galicia*. Centro de Investigación en Biotecnología Aplicada del Instituto Politécnico Nacional, Carretera Estatal Santa Inés Tecuexcomac-Tepetitla, km 1.5, Tepetitla de Lardizábal, CP 90700 Tlaxcala, México. *snava@ipn.mx

Palabras clave: biodegradation, phenol, catechol

Introduction. Industrial activities cause the accidental or deliberate introduction into the biosphere of a large number of recalcitrant chemical compounds. The chemical, textile and paper industries, among others, use large amounts of water, generating effluents with high concentrations of toxic pollutants, which, when discarded without proper treatment, generate serious environmental contamination problems and high toxicological risk to both humans and animals. There are several remediation technologies of recalcitrant compounds which are mainly based on chemical, physical or a combination of several methods, whose main disadvantages are the high cost and the low removal efficiency (Castillo Rodriguez et al., 2005). The use of fungi as a biological treatment is one of the best options, since their ability to reduce emissions is related to the formation of exoenzimas such as peroxidases and catechol oxidases (Dos Santos et al., 2007). *Fusarium sp* . is a fungus that has the ability to degrade several xenobiotic compounds improving the industrial wastewater treatment. The objective of this work was to evaluate the oxidases activities that are produced by *Fusarium petroliphillium* during the process of phenol degradation.

Methods. The fermentations were performed in 125 ml Erlenmeyer flasks containing 50 ml of basal medium (BM) (Téllez-Téllez, et al., 2008). The fermentations of *F petroliphillium* grown in basal medium (BMF) and in the presence of 1000 ppm of phenol. Each flask was inoculated with three mycelial plugs taken from the periphery of *F. petroliphillium* colonies grown for 10 d at 25 °C in Petri dishes containing potato dextrose agar. The cultures were incubated at 25 °C for 23 days on a rotary shaker at 120 rpm. The growth kinetics and enzyme activity were measured during the fermentation process (Cai, 2007).

Results. The presence of phenol in submerged fermentation modified the growth kinetic parameters of *F. petroliphillium* (Fig. 1). The main enzyme activities detected were manganese peroxidase (MnP) with the maximum activity of 2,103 UI/L at 168 h and the minimum of 120 UI/L at 96 h, and the catechol 1,2-dioxygenase with a maximum activity of 621.25 U/L at 48 h. These results, may indicate the participation of MnP and catechol dioxygenase in phenol degradation.

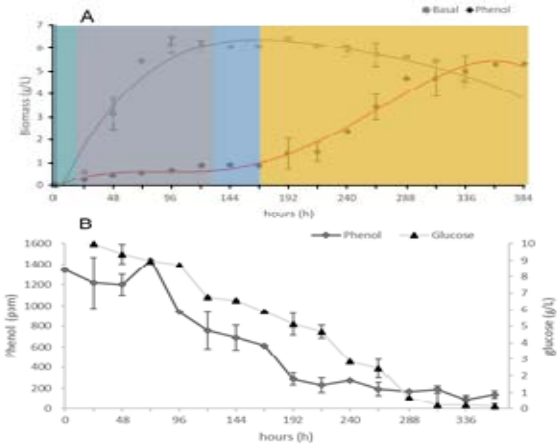


Fig. 1 A) Fermentations of *F. petroliphillium* grown in basal medium and presence of 1000 ppm of phenol. B) Glucose and phenol consumption

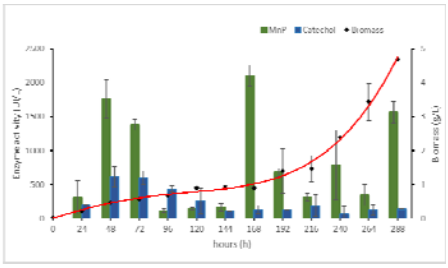


Fig. 2 *F. pe troliphillium* growth kinetics and MnP and Catechol 1,2-dioxynase activity, during phenol submerged fermentation

Conclusions. *F. petroliphillium* had the ability to grow on a submerged fermentation supplemented with 1000 ppm of phenol. MnP and catechol dioxygenase may be the main enzymes participating in phenol degradation.

Acknowledgements. This work supported by the project No. SIP20182087.

References.

1. Castillo Rodríguez et al., 2005 Enviroment Biotechnol (8): 1254-1262.2. Dos Santos et al., (2007). Bioresource technology (16) 2369-2385 3. 3. Téllez-Téllez, et al., (2008). Biotechnologically relevant enzymes and prote (4) 675-679
4. Cai et al., (2007) Journal of Hazardous Materials (4) 38-42

ISSN: 2448-8461

AM31

ASOCIACIÓN ENTRE HIDROCARBUROS AROMÁTICOS POLICÍCLICOS EN PARTÍCULAS AMBIENTALES Y LOS NIVELES DE 1-OHP URINARIO DE PERSONAS EXPUESTAS A EMISIONES DE COCEDORES DE LADRILLO

José Martinez[1], Julio Grijalva[1], Miguel Soto[1], Sandra Martínez[1], Jaime Herrera[1], Laura Paez[1], Cinthia Nuñez[1], Ignacio Villanueva[2], Verónica Loera[2], Isaías Chairez[2], Ismael Lares[2].

Estudiante del Doctorado en Ciencias en Biotecnologia[1], Docente de la Maestría en Ciencias en Gestión Ambiental[2] del Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, del Instituto Politécnico Nacional. Victoria de Durango, lapaz_isra@hotmail.com

Palabras clave. HAPs, 1-hidroxipireno, Partículas.

Introducción. Dentro de los contaminantes atmosféricos clasificados como orgánicos encontramos los Hidrocarburos Aromáticos Policíclicos (HAPs) que son de especial preocupación para la salud humana, debido a que muchos de ellos poseen propiedades carcinogénicas y son persistentes en el ambiente[1]. Se forman por pirolisis de materia orgánica, pueden condensarse para formar partículas y/o depositarse sobre otras ya formadas[2].
Diversos estudios indican que el aumento a la exposición a partículas atmosféricas provoca un aumento en la morbilidad y mortalidad de las personas[3].
Objetivo general: Fue asociar los niveles de HAPs encontrados en partículas ambientales con los niveles de 1-HOP urinario en personas expuestas a emisiones de cocedores de ladrillo.
Secundarios:
1.-Fue medir los niveles de 1-HOP en personas expuestas a emisiones de cocedores de ladrillo.
2.-Fue identificar los niveles de HAPs en partículas ambientales mediante muestreos de aire.

Métodos. Se establecieron dos zonas de muestreo, una donde había cocedores de ladrillo activos(expuesta) y otra alejada de los mismos (control). Se realizaron muestreos de aire de partículas menores a 10µm (PM_{10}) con una posterior extracción asistida con ultrasonido y cuantificación por HPLC. De cada zona se reclutaron 30 voluntarios y se recolectaron muestras de orina para determinar niveles de 1-hidroxipireno (1-HOP); un biomarcador de exposición a HAPs[4]. Este estudio contó con la aprobación de un comité de ética y se clasifico en la categoría de riesgo mínimo.

Resultados. Participaron 60 voluntarios (previo consentimiento informado) y se realizaron muestreos de aire semanales.
Para los muestreos se obtuvieron los promedios: 151 y 61.9 ($\mu g/m^3$) esto en muestreos de PM_{10}. Para la cuantificación de HAPs totales se obtuvo un promedio de 189 y 3.13 (ng/m3) (zona expuesta y control respectivamente) y para el 1-HOP se cuantificaron los valores de 3.77 y 1.76 (µgmol 1-OHP/mol creatinina) todos los anteriores de la zona expuesta y zona control respectivamente.

Conclusiones. Este estudio confirma que los niveles de HAPs en las partículas están asociados con el 1-OHP en la orina. La zona expuesta presenta mayores niveles de partículas y HAPs lo que indica su grado de contaminación. Si bien estos niveles son muy inferiores en la zona control, la presencia de 1-HOP en los voluntarios controles indica la presencia de un factor externo que eleva los niveles cuantificados en su orina, además de la variación interindividual reportada por otros autores[5].

Reconocimientos. Al Consejo Nacional de Ciencia y Tecnología (Conacyt) por su apoyo con el programa de becas para posgrados. También agradecer su apoyo al Consejo de Ciencia y Tecnología del Estado de Durango (Cocyted).

Referencias.

1.Vázquez-Gómez, G., Rubio-Lightbourn, J., & Espinosa-Aguirre, J. J. (2016). *TIP*, 19(1), 54-67.
2. Mastandrea, C., Chichizola, C., Ludueña, B., Sánchez, H., Álvarez, H., & Gutiérrez, A. (2005). *Acta bioquímica clínica latinoamericana*, 39, 27-36.
3. Stellman, J., & McCan, M. (1998). Hidrocarburos poliaromáticos. En: *Enciclopedia de Salud y Seguridad en el Trabajo.* Chantal Dufresne. Madrid, 310-319.
4. Jongeneelen, F. J., Anzion, R. B., Leijdekkers, C. M., Bos, R. P., & Henderson, P. T. (1985). 1-hydroxypyrene in human urine after exposure to coal tar and a coal tar derived product. [Research Support, Non-U.S. Gov't]. International Archives Occupational and Environmental Health, 57(1), 47-55.
5. Jacob, J., & Seidel, A. (2002). [Review]. *J Chromatography B Analyt Technol Biomed Life Sci*, 778(1-2), 31-47.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
AM32

OPTIMIZATION AND KINETIC STUDY OF REMOVAL OF METHYLENE BLUE FROM SHRIMP WASTE *(Litopeneus spp)*

Enrique Martínez-Herrera, Estefanía Jiménez-Manzano, Elizabeth Alemán-Huerta, Ileana Castro-González, Verónica Almaguer-Cantú
Laboratorio 10 Instituto de Biotecnología, Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León. Cd. Universitaria, Pedro de Alba s/n CP 66450. San Nicolás de los Garza, Nuevo León, México
veronica.almaguerct@uanl.edu.mx
Key words: response surface, methylene blue, optimization

Introduction. The *Penaeidae* family constitutes an economic importance in the food industry; however, it represents an environmental problem due to the discarded shrimp exoskeleton creating millions of tons of garbage worldwide.[1, 2] These wastes have been used as bio-absorbent through various treatments for the removal of different pollutants. In this study, the adsorption capacity of methylene blue in a queous solution was determined using the exoskeleton of *Litopeneus* spp. as an adsorbent, without previous treatment. Response surface was used to optimize the process through the variables: contact time (min), pH, dose (g/L) and initial concentration of methylene blue dye (mg/L).[3, 4]

Methods. Shrimp exoskeleton was obtained from La Pesca, Tamaulipas (23°46′05″N 98°12′27″O), was washed and dried in desiccator at 30° - 40°C for 48 h then was powdered and pestle. A central composite design combined with response surface methodology was employed to determinate the effects of the main independent variables, namely, contact time, solution pH, adsorbent dosage and MBD initial concentration. Kinetic study was performed under optimized conditions to evaluate the removal mechanism and to determine the kinetic parameters, 0.25 g of adsorbent was used in solutions with initial MBD concentration of 25, 50, 75 and 100 mg/L at pH 7.

Results. The fig. 1 shows the combined effect of pH and adsorbent dosage (g/L) on MBD removal at constant values of contact time (97.5 min) and initial dye concentration of 100 mg/L. That both independents variables positively influenced the removal of Methylene blue dye in values of pH 7 and adsorbent dosage of 2.5 g/L.
The values of kinetic parameters estimated of dye adsorption capacity of shrimp waste along the time by the selected kinetic model are listed in table 1.

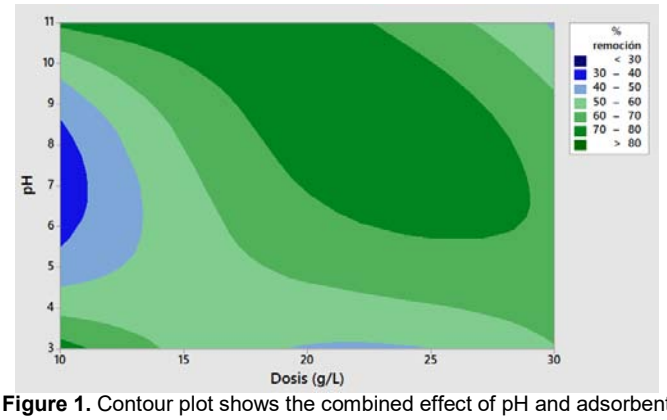


Figure 1. Contour plot shows the combined effect of pH and adsorbent dosage (g/L).

	Pseudo-second order model			
[MBD] (mg /L)	$q_{e\ exp}$ (mg/g)	q_e (mg/g)	K_2 (mg/g/min)	R^2
25	16.3510	16.5567	0.0284	0.9995
50	35.5989	36.3232	0.0067	0.9975
75	52.3216	53.0149	0.0086	0.9990
100	68.6382	80.0679	0.007	0.9930

Table 1. Kinetics constans of MBD adsorption onto shrimp exoskeleton at different initial MBD concnentrations (pH 7, adsorbent dosage 2.5g/L)

Conclusions. The response surface indicated the optimum conditions (97.5 min, pH 7, dose 20 g/L and 100 mg/L). The adsorption kinetic data was adjusted to the pseudo-second order model with a determination coefficient greater than 0.99, achieving about 80% of the total load in equilibrium at 30 minutes.

References.

(1)Auta, M., & Hameed, B. H. (2014). *Chemical Engineering Journal*, *237*.
(2) Bulut, Y., & Aydin, H. (2006). *Desalination*, *194*(1–3), 259–267.
(3) Dehghani, M. H., Dehghan, A., Alidadi, H., Dolatabadi, M., Mehrabpour, M., & Converti, A. (2017). *Korean Journal of Chemical Engineering*, *34*(6), 1699–1707.
(4) Tan, I. A. W., Ahmad, A. L., & Hameed, B. H. (2008). *Desalination*, *225*(1–3), 13–28

ISSN: 2448-8461

AM33

EFFECT OF ESSENTIAL OILS IN *SPODOPTERA EXIGUA* LARVAE DEPENDING ON THE MODE OF APPLICATION

Ninfa M. Rosas-García, Jesús M. Villegas-Mendoza, and Maribel Mireles-Martínez
Centro de Biotecnología Genómica-Instituto Politécnico Nacional, Reynosa, Mexico. Email: nrosas@ipn.mx
Key words: beet army worm, bioassay, insect control

Introduction. Essential oils are secondary, odorous metabolites derived from aromatic plants which have been used since middle ages because of their toxic and medicinal properties and more recently are used in the cosmetic, food and agricultural industries [1, 3]. Various essential oils are toxic to insects and they have shown inter-specific differences in susceptibility and dissuasive effects of the feeding in different species. Essential oils become a suitable alternative to control *Spodoptera exigua*, a generalist pest that has demonstrated natural resistance to many other insecticidal products [2].
The objective is to determine the effects caused by an essential oil in *S. exigua* larvae depending on the mode of application used.

Methods. Experiments were conducted with 15 essential oils, eucalyptus (2), cypress (3), rosemary (4), lime (5), peppermint (6), mint (7), chocolate (8), oregano (9), pine (10), cinnamon (11), clove (12), lavender (13), geranium (14), tangerine (15) and citronella (16) and one control (1). Three different bioassays were conducted and different concentrations of each essential oil were used: vapor phase (500 µg/L, 50 µg/L)[5], contact (500 µg/cm^2) and ingestion (500 µg/cm^2,50 µg/cm^2) [4]. In all cases neonate larvae were used.

Results. Almost all essential oils were toxic at the highest concentration in vapor phase bioassays with the exception of chocolate, cinnamon, clove and geranium. No essential oil caused mortality in contact bioassays except oregano. In ingestion bioassays only cinnamon, geranium and citronella caused mortality around 40%.

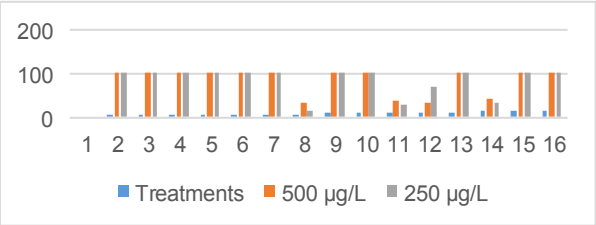


Fig. 1 Vapor-phase assay

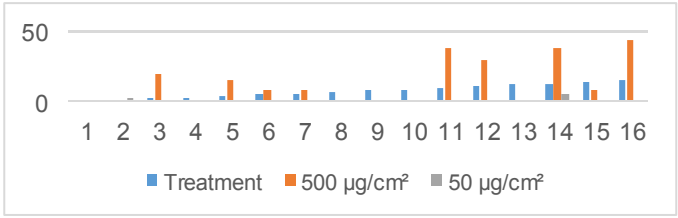


Fig.2 Ingestion assay

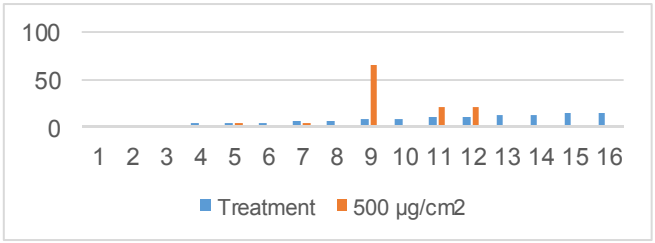


Fig. 3 Contact assay

Conclusions. Essential oils can be offered as a good alternative for the control of *S. exigua*. The best mode of action could be the vapor phase bioassay and oregano oil, however more studies are necessary to determine the best mode of application and the most toxic essential oil toward this important pest to be successful in pest control.

Acknowledgements. Authors are deeply grateful to Secretaría de Investigación y Posgrado, Instituto Politécnico Nacional, project no. 20180519 for providing financial support.

References.

1.Bakkali, F., S. Averbeck, D. Averbeck, and M. Idaomar. 2008. Biological effects of essential oils--a review. Food Chem Toxicol 46 (2):446-475.
2.Garza-Urbina, E., Terán-Vargas, A.P. . 2007. El gusano soldado Spodoptera exigua y su manejo en la planicie Huasteca. San Luis Potosí, México: Instituto Nacional de Investigaciones forestales, acuícolas y pecuarias.
3.Isman, M. B. 2000. Plant essential oils for pest and disease management. Crop Protection 19:603-608.
4.Regnault-Rogers, C., C. Vincent, and J. T. Arnason. 2012. Essential Oils in Insect Control: Low-Risk Products in a High-Stakes World. Annual Review of Entomology 57:405-424.
5.Sim, M.-J., D.-R. Choi, and Y.-J. Ahn. 2006. Vapor phase toxicity of plant essential oils to Cadra cautella (Lepidoptera: Pyralidae). Journal of Economic Entomology 99 (2):593-598

ISSN: 2448-8461

AM34

BIODEGRADATION OF AZO DYES UNDER HIGH ALKALINITY AND SALINITY CONDITIONS IN PACKED BED REACTOR

Brenda Montañez- Barragán, Blanca E. Barragán-Huerta*. Department of Environmental Systems Engineering. National School of Biological Sciences. Instituto Politécnico Nacional., México City, 07738 Mexico. bbarragan@ipn.mx

Key words: azo dyes, Halomonas sp., salinity, alkalinity

Introduction. The main pollutants of these effluents are dyes (mainly azo dyes) which are very recalcitrant to biodegradation [1], the high salinity and alkalinity conditions of these effluents hinders biodegradation even more [2][3]. The use of extremophile microorganisms is a valuable alternative for removing such dyes, furthermore, immobilization of microorganisms is an interesting technique as it favors both aerobic and microaerophilic conditions necessary for the degradation of azo dyes. In this study, a newly isolated strain of *Halomonas* sp. was immobilized in a packed bed reactor and thoroughly studied according to its capacity to decolorize three azo dyes Reactive Black 5 (RB5), Remazol Brilliant Violet 5R (RV5), and Reactive Orange 16 (RO16), under different operating conditions.

Methods. In the system shown in Fig. 1, the medium with the azo dye was introduced to the reactor from a closed flask using a peristaltic pump; air was supplied through a compressor and passed through a water container to introduce a humidity saturated air flow which becomes the exhaust air flow.

Fig. 1 Packed bed reactor

After has percolated through the packed bed, the effluent is collected and analyzed by centrifuging and later measuring the supernatant at the λmax of each dye, using UV-Vis spectrophotometer.
The performance of the reactor was evaluated over a period of 72 days analyzing the effect of nutrients' concentration, HRT, type of dye, dye concentration and salinity in the decolorization efficiency.

Results. The performance of the reactor demonstrates that the strain can decolorate the three azo dyes under several operating conditions. It was observed that the decolorization efficiency remained up to 80% when reducing nutrients from casein peptone 5 to 1.66 mgL^{-1} and beef extract from 1.5 to 0.5mg/L^{-1}. The maximum percentages of removal were 81% for RB5, 79.5% for RV5 and 95% for RO16 with a maximum HRT of 11.8 hours. Decrease in the decolorization efficiency was mainly observed when decreasing the HRT, and increasing the dye concentration. The strain quickly adapted to the exchange in the type of dye.

Conclusions. By immobilizing the microorganism, the amount of nutrients could be reduced 6 fold, with a maximum dye decolorization from 79.5 to 95, with a HRT of 11.8 h. Moreover, the strain rapidly adapted to the dye exchange and increase in dye concentration.

Acknowledgements. This research was financially supported by the Instituto Politécnico Nacional (Project SIP-20180898). We would like to thank Jennifer Pérez and Laura Capula (Instituto Politécnico Nacional, Mexico), for their technical help

References.

[1] Barragán B, Costa C, Márquez C (2007) Biodegradation of azo dyes by bacteria inoculated on solid media. *Dyes Pigm*, 75(1):73-81.
[2] Lalnunhlimi S, Krishnaswamy, V (2016) Decolorization of azo dyes (Direct Blue 151 and Direct Red 31) by moderately alkaliphilic bacterial consortium. *Braz J Microbiol* 47(1): 39-46.
[3] Tan L, He M, Song L, Fu X, Shi S (2016) Aerobic decolorization, degradation and detoxification of azo dyes by a newly isolated salt-tolerant yeast *Scheffersomyces spartinae* TLHS-SF1. *Bioresour Technol* 203: 287-294.

ISSN: 2448-8461

AM36

ISOLATION AND SELECTION OF MICROORGANISMS CAPABLE OF TOLERATING DIFFERENT PESTICIDES FOR THEIR APPLICATION IN SOILS BIOREMEDIATION BY BIOAUGMENTATION

Rosa Arenas Saldaña[1], Ruth Noemí Aguila[2], Ángel E. Absalón[1] and Diana V. Cortés-Espinosa[1*]

[1]Instituto Politécnico Nacional, Centro de Investigación en Biotecnología Aplicada, Tlaxcala, México, CP 90700.
[2]Instituto Politécnico Nacional, Centro Interdisciplinario de Ciencias Marinas, La Paz, Baja California Sur, CP. 23096.

*Corresponding author: dcortes@ipn.mx

Keywords: pesticide mixture, contaminated agricultural soil, mixed microbial consortium, Bioremediation

Introduction. The need to produce enough food to supply a growing world population has led to an extensive use of pesticides, making them a key element in agriculture. However, regardless of their benefits, they are highly toxic substances whose characteristics such as mobility and persistence allow entry into the food chain and create a significant impact on ecosystems and human health. Numerous strategies to eliminate these pesticides from the environment have been investigated (1). Several works have used the native organisms of the soil as well as their metabolic and enzymatic mechanisms to treat these contaminants and promote their complete degradation to innocuous products (2).
Thus, the aim of this work was to isolate, identify, and select tolerant bacterial to high concentrations of pesticides, in order to build a microbial consortium able to degrade different groups of pesticides for bioremediation of agricultural soils. For this purpose, 102 bacteria were isolated from different agricultural soils highly contaminated with pesticides.

Methods. Six soils contaminated with pesticides, collected from Puebla and Tlaxcala were used in this study. Soil samples were dried, homogenized, sieved with a 2-mm test sieve, and conserved at 4 °C until physicochemical analyses were conducted.
Bacterial populations were isolated from the different contaminated soils by inoculate plates of basal saline medium (BSM) containing different pesticides. For the selection, the isolated microorganisms were subjected to Tolerance test to several doses of a mixture of pesticides by inhibition surface assays using 6 mm diameter polyvinylidene fluoride discs (Millipore, USA), each one impregnated with a pesticide mixture of carbofuran, endosulfan, methyl paration and paraquat (1:1:1:1) containing final concentrations of 50, 100, 150, 200, 250, 300, 350, 400, 450 and 500 mg l^{-1}.

Results. A total of 102 bacterial strains were isolated, based on their ability to tolerate pesticides such as Paraquat, Methyl Paration, Carbofuran and Endosulfan. The concentrations used were 10 times higher than the LD50 based on data from Cofepris. 61 bacterial strains tolerated the mixture of pesticides and only 9 were able to grow in 500 ppm. Microscopic and macroscopic analyzes, reflected typical characteristics of *Pseudomonas, Enterobacteria, Bacillus* among others.

Fig.1 Halos of inhibition formed by bacteria at different concentrations of a mixture of pesticides (0-500 mg l^{-1}).

Conclusions.
This is the first study of selection of microorganisms capable of tolerating extreme concentrations of a mixture of different groups of pesticides, this rigorous selection will allow obtaining a mixed microbial consortium applicable in the bioremediation by bioaugmentation of agricultural soils contaminated with different types of pesticides.

Acknowledgements.
This work was supported by Instituto Politécnico Nacional project SIP20180442 and grand BEIFI: 022-220914-08201820180442, Consejo Nacional de Ciencia y Tecnología by Grant: 819175

References.
1. Maqbool, Z. et al (2016). Perspectives of using fungi as bioresource for bioremediation of pesticides in the environment: a critical review. Environ. Sci. Pollut. Res. 23, 16,904–16,925.
2. Kirubakaran, R. et al (2017). Emergence of multi drug resistance among soil bacteria exposing to insecticides, Microb. Pathog. 105 (2017) 153–165

ISSN: 2448-8461

AM37

SELECTION OF FUNGI AND BACTERIA STRAINS TOLERANT TO BISPHENOL TO APPLICATION IN BIORREMEDIATION PROCESSES

Gerardo López[1], Angel E. Absalón[1] and Diana V. Cortes-Espinosa[1]
[1]Instituto Politécnico Nacional, Centro de Investigación en Biotecnología Aplicada, Tlaxcala, México, C.P. 90700
dcortes@ipn.mx
Key words: Bisphenol A, bacteria, fungi, tolerance

Introduction. Bisphenol A (BFA), an emerging contaminant, is used in the production of polycarbonate and epoxy resins. The improper disposal of waste, deficiencies in BFA removal techniques in wastewater and synthesis processes, have promoted their accumulation in water, air and soil, being considered a ubiquitous pollutant. BFA is considered an endocrine disruptor and has been associated with conditions in the male reproductive system as female and various investigations associate it with thyroid conditions, inflammation of the digestive tract, diabetes, eating disorders and cardiovascular diseases [1].
The aim for this study was to select tolerant microbial strains to BFA to conformation a microbial consortium for their application in the bioremediation of contaminated soils.

Methods. Fungal and bacterial strains were taken from a collection of the Bioremediation research group from CIBA-IPN. The tolerance test for fungi was carried on plates with Czapek medium and Toyama's medium plus the addition of different concentration of BFA (0, 50, 100, 250, 500 and 750 ppm), 10 µL of a suspension of $1x10^6$ spores ml^{-1} were inoculated, all plates were incubated at 30°C. For bacteria were performed in plates with BSM medium, the strains were inoculated by massive seeding (OD=0.14 to 600nm), after 9 discs with different concentrations of BFA (0, 50, 100, 250, 350,500, 650, 800 and 1000 ppm) were placed on surface of solid medium and the plates were incubated at 30°C[2]. The radial growth rate for fungi was measured, and a micro and macroscopic analysis was made, the formation of inhibition halos was measured in the bacterial cultures.

Results. Twenty-nine fungi and 12 bacteria strains were evaluated, strains of *Aspergillus niger* and H20R showed tolerance up to 750 ppm of BFA in both media. *Aspergillus terreus* tolerated 500 ppm in Czapek and Toyama's medium up to 750 ppm. *Aspergillus flavus*, *Aspergillus nomius* and H13R tolerated up to 500 ppm of BFA in both media (Figure 1a). Phenotypic changes were observed in some fungi strains as the concentration of BFA was increased, this effect could be cause in response to the stress occasioned by the presence of BFA as was observed in *A. niger* strain. In *A. terreus* strain, the toxic effect of BFA was more clearly observed when increasing the concentrations (Figure 1c), this could be linked to survival, where the strain grew on itself to be able to complete the life cycle and sporulate to survive. In Toyama's medium was observed that the strains tolerated higher concentrations and reached a higher growth compared with the Czapek medium, these results showed that when the media nutrients are more available, the fungi showed greater tolerance to BFA. The bacterial strains evaluated did not present any inhibition halos (Figure 1b).

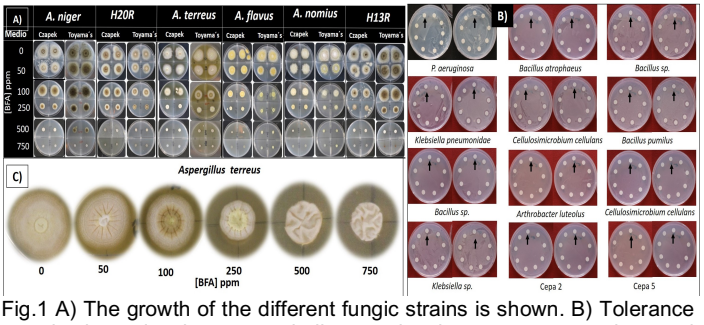


Fig.1 A) The growth of the different fungic strains is shown. B) Tolerance test in bacteria the arrow indicates the lowest concentration and increases in a clockwise direction, no inhibition zones are observed. C) Morphological changes in fungal strains by increasing the concentration of BFA.

Conclusions. The Toyama's medium favored the tolerance of fungal strains. The genus *Aspergillus* has a greater tolerance to BFA. Bacterial strains tolerated up to 1000 ppm of BFA. The toxicity induces marked morphological changes in fungal strains as the concentration of BFA increases.

Acknowledgements: The Instituto Politécnico Nacional by support project SIP20180442 and the Conacyt for grant number 482630.

References.

[1] Reddy, P. V. L., Kim, K. H., Kavitha, B., Kumar, V., Raza, N., & Kalagara, S. (2018). Photocatalytic degradation of bisphenol A in aqueous media: A review. *Journal of Environmental Management*, *213*, 189–205.

[2] Zafra, G., Absalón, Á. E., Cuevas, M. D. C., & Cortés-Espinosa, D. V. (2014). Isolation and selection of a highly tolerant microbial consortium with potential for PAH biodegradation from heavy crude oil-contaminated soils. *Water, Air, and Soil Pollution*, *225*(2)

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
AM38

EVALUATION OF DIFFERENT SOURCES OF CARBON AND NITROGEN DURING A BIOREMEDIATION OF SOIL CONTAMINATED WITH CRUDE OIL BY A CONSORTIUM IMMOBILIZED IN CORN STOVER.

Rita Pacheco and Diana V. Cortés-Espinosa,
Instituto Politécnico Nacional, Centro de Investigación en Biotecnología Aplicada Tlaxcala, México. CP. 90700
rkpacheco@hotmail.com

Key words: Bioremediation, Consortium, TPH, inmovilization, corn stover

Introduction. Crude oil is considered as one of the main sources of energy in the world, and growth in population and industries increases the demand for more crude oil extraction, this can cause intensive damage to living organisms and result in changes in the environment. Bioremediation of oil-contaminated soil has been a hot issue in environmental research due to their efficiency and low costs.[1] However, these techniques have shown some disadvantages, one of this is the lack of a balanced C:N:P (carbon: nitrogen: phosphorus) ratio for the stimulation of growth of the native soil-microbiota capable to degrading of hydrocarbon from oil or for the bioaugmentation of exogenous microorganisms to accelerate the biodegradation process.[2]
In this work, we evaluated the degradation capacity of total petroleum hydrocarbons (TPHs) in soil by a mixed microbial consortium immobilized in corn stover using 2 different carbon sources and 2 different nitrogen sources.

Methods. Microbial consortium was formed by strains of *Aspergillus flavus, Aspergillus nomius, Thrichoderma sperellum, Bacillus cerus, Pseudomonas aeruginosa, Klebsiella pneumonia, Kelebsiela sp.* and *Stenothrophomonas maltophilia*; these strains were immobilized by adsorption technique in corn stover. The treatments were performed with sterile and non-sterile oil-contaminated soil:

Treatment	Soil	C source	N_2 source
a)	Sterile	Molasses	Urea
b)	Non-Sterile	Molasses	Urea
c)	Sterile	Sugar	Urea
d)	Non-Sterile	Sugar	Urea
e)	Sterile	Molasses	$(NH_4)_2SO_4$
f)	Non-Sterile	Molasses	$(NH_4)_2SO_4$
g)	Sterile	Sugar	$(NH_4)_2SO_4$
h)	Non-Sterile	Sugar	$(NH_4)_2SO_4$

The humidity was adjusted at 30% and soil was texturized with corn stover in a relation 95:3.5:1.5 (soil: sterile corn waste: immobilized consortium) and all treatmets were incubed to 30ºC for 30 d. The measurement parameters were: heterotrophic activity (CO_2), colony forming units (CFU) and degradation of total petroleum hydrocarbon (TPH).

Results. CO_2 measurements show that treatments with molasses as a C source showed higher accumulated CO_2, however, there is no significant difference with the other treatments. This agrees with the CFU analysis, where all treatments were in order of magnitude of 10^7 and did not show significant differences between them. However, during the degradation a significant difference is shown between the treatments $(NH_4)_2S0_4$-sterile and non-sterile with molasses.

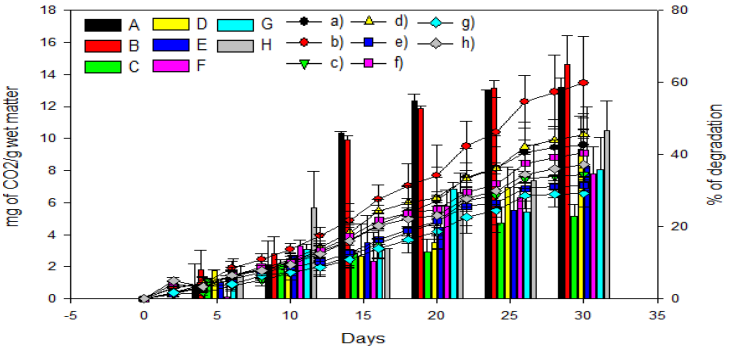


Fig 1. Degradation of THPs after 30 d of incubation by a mixed microbial consortium in soil. Bars (% degradation) and lines (accumulated CO_2)

Conclusions. The medium formulated with $(NH_4)_2SO_4$ and molasses increased the degradation of TPHs in soils contaminated with crude oil by the mixed microbial consortium immobilized in corn stover (58.6% in sterile treatments and 64.8% degradation in the non-sterile treatments) in 30 d, which corroborates that the native microbiota also contributes to the degradation.

Acknowledgements. This research was supported by project SIP20180442 of Instituto Politécnico Nacional and CONACYT grant: 598787.

References.

[1]Abbasian, F., Lockington, R., Megharaj, M., & Naidu, R. (2016). The Biodiversity Changes in the Microbial Population of Soils Contaminated with Crude Oil. *Current Microbiology*, *72*(6), 663-670.
[2]Xu, Y., & Lu, M. (2010). Bioremediation of crude oil-contaminated soil: Comparison of different biostimulation and bioaugmentation treatments. *Journal of Hazardous Materials*, *183*(1-3), 395-401.

ISSN: 2448-8461

AM39

EFFECT OF CO_2 CONCENTRATION ON *Chlorella sorokiniana* GROWTH AND LIPIDS' ACCUMULATION

Adriana Marín[1], Juan Carlos Sigala[2], Marcia Morales Ibarria[2]
[1] Posgrade in Natural Sciences and Engineering, Universidad Autónoma Metropolitana Cuajimalpa. [2]Department of Processes and Technology, Universidad Autónoma Metropolitana-Cuajimalpa, Mexico City, Zip code 05348;
azuriana1717@gmail.com ; mmorales@correo.cua.uam.mx

Key words: Lipid accumulation, Microalgae, N-deplete condition.

Introduction. Global demand for energy is increasing rapidly. Fossil fuels combustion and related CO_2 emissions have led to global environmental problems. Therefore, it is necessary to look for renewable energy alternatives and CO_2 capture. Microalgae provide options for both aspects[1]. In order to use microalgae at large scale, it is important that strains exhibit tolerance to a wide range of temperatures, irradiances and CO_2 concentrations. On the other hand, lipid accumulation required for biodiesel production must be triggered under N-deplete condition. The objective of the study was to determine the CO_2 tolerance and the lipid accumulation of *Chlorella sorokiniana*.

Methods. *Chlorella sorokiniana* was cultivated in BG-11 medium. The effect of temperature and irradiance on photosynthetic activity was previously done in a mini-photobioreactor equipped with a dissolved oxygen sensor and control of temperature and light [2]. It was proven that *Chlorella sorokiniana* is a thermophilic organism and light saturation is reached above 200 μmol m^{-2} s^{-1} [3]. Subsequently, experiments at different concentrations of CO_2 (15%,10%,5% and air-0.04%) were performed under conditions in a 2.5 L bubble column photobioreactors, placed in a chamber at constant 39.5 ° C and continuous 164.15 μmol m^{-2} s^{-1} under both N-replete and N-deplete conditions. The biomass was determined by dry weight, elemental analysis CHONS was performed and lipid content was determined with the SPV method. N-NO_3 in medium was determined by spectrophotometry at 220 nm

Results. Based on elemental analysis of the biomass produced at different conditions, the general formulas for the biomass composition were determined (see Table 1) As it can be appreciated, more carbon was accumulated by the microalgal biomass under growth conditions using N-complete in the BG11 medium and in the presence of CO_2.
Table 2 shows the growth parameters for the different conditions evaluated.

Table 1. Elemental analysis of *C. sorokiniana*

C. sorokiniana biomass growht in	Elemental Analysis (%,w/w) C	H	O	N	S	Empirical formula of algal biomass	G.H.V. (MJ/kg)	N.H.V. (MJ/kg)	H.H.V. (MJ/Kg)
5% CO_2, with N	55.4	7.73	28.44	7.63	0.8	$CH_{1.663}O_{0.389}N_{0.118}S_{0.005}$	30.22	28.56	24.76
5% CO_2, without N	50.23	7.44	39.58	2.29	0.45	$CH_{1.766}O_{0.591}N_{0.039}S_{0.003}$	28	26.4	20.59
Air, without N	51.8	7.96	38.48	1.75	0	$CH_{1.832}O_{0.558}N_{0.029}$	29.23	27.52	22.01
Air, with N	53.46	7.74	30.86	7.94	0	$CH_{1.726}O_{0.433}N_{0.127}$	29.48	27.82	23.61

G.H.V (Gross Heat Value); N.H.V. (Net Heat Value) and H.H.V. (Higher Heating Value).

The highest CO_2 capture and biomass productivity were observed under N- replete conditions and 15% of CO_2. Finally, the strain exhibited a good lipid accumulation, 40%, under N-deplete condition in the experiment fed with air (0.04% of CO_2).

Table 2. Growth parameters of *C. sorokiniana* under different conditions.

C.sorokiniana biomass growht in	X_{max} (g L^{-1})	μ_{max} (d^{-1})	P_{max} (g L^{-1} d^{-1})	R_{CO2} (mg L^{-1} d^{-1})	Lipids (%)
Air	0.79	0.488	0.186	230.364	15.79
Air without N	0.89	1.401	0.394	341.880	39.75
5% CO_2	1.71	1.48	0.371	489.535	26.64
5% CO2 without N	1.24	2.23	0.394	445.220	29.07
10 % CO_2	1.48	1.440	0.474	729.248	N.R.
15% CO_2	1.72	1.647	0.615	836.909	N. R.

N.R.; No reported.

Conclusions. The present study showed the tolerance of the *Chlorella sorokiniana* to high CO_2 concentrations and lipid accumulation under N-deplete condition. Because of the tolerance to a wide range of temperatures and CO_2 concentrations, this strain is considered to have a great potential to mitigate combustion gases and to obtain lipid for biodiesel.

Acknowledgements. We thank the UAM-C, and the CONACyT for the support to this study and the financing by project 247006 of the FSE-SENER CONACyT.

References.

1. Hartwig J., Schneider L., Vieira J A. 2016. Bior Tech 214: 159–16
2. Cabello J., Toledo A., Sánchez L., Revah S, Morales M. 2015. Bioresour. Technol. 181: 128–135.
3. Marín A., Sigala JC., Morales M. 2017. VII Congreso Nacional de Biotecnología y Bioingeniería. 25-30 de Junio de 2017. Puerto Vallarta Jalisco

ISSN: 2448-8461

AM40

EFFECT OF A FLOCCULANT ON THE RHEOLOGY OF A RESIDUAL SLUDGE DURING A THREE PHASE ANAEROBIC DIGESTION

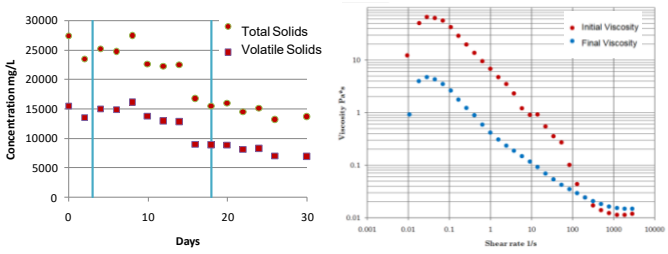
Harim Castorena-Quintanar, Josefina Rodríguez-Rosales, Walfred Rosas-Flores, Cuauhtémoc Moreno-Medina, Roberto Valencia-Vázquez; Tecnológico Nacional de México/I.T Durango, Departamento de Ingeniería Química y Bioquímica, Maestría en Sistemas Ambientales, Durango, Dgo., México, 34080.
mdjoserr@itdurango.edu.mx

Key words: Anaerobic, Sludge, Rheology

Introduction. Wastewater treatment plants are primarily needed around the world due to the negative impact that an untreated wastewater can cause to water bodies. Sludge byproducts from these wastewater treatment plants are a source for the production of energy and bio fertilizers. Sludge characteristics determine its rheological properties; at low tensions, a viscoelastic behavior can be observed (1).Solid concentration and temperature have a significant effect in the sludge rheology (2). After anaerobic digestion, the fluidity of the sludge increases (3).
This study has the objective to evaluate the effect of a flocculant on the rheological properties of sludge in athree phase anaerobic digestion.

Methods. The anaerobic digestion processwas conducted inthree stainless steel reactors with a capacity of 14 L and provided witha heating and stirring automated system. Experiments were runwith activated sludge with presence and absence of a synthetic organic flocculant, coming from the wastewater treatment of a beverageplant. The operating conditions were: organic loadings of 0.6 and 1.2 kg VS/m^3*d, and retention times of 25 and 30 days.The determination of total solids and total volatile solids were analyzed by themethod APHA 2540 (1998). Gas productionmeasurementwasmadeby volumetric displacement anda meter Landtec Biogas 2000 was used to determinethe percentage of methane produced during the digestion process. Rheological analyzes were determine by a rheometer Discovery HR-3 of TA Instruments.

Results. The biodigesters produced biogas (methane)with concentrationsabove60-70%. At high organic loadings and with the presence of a flocculant, the production of methane and volume produced were higher. During the thermophilic phase there was an increase in both the volume and concentration of methane.The reduction of total volatile solids was 18.4% at low organic loadings with absence of flocculant, and 42% at high organic loadings with the presence of flocculant. Fig 1 a) shows the behavior of total solids and total volatile solids of a sludge with flocculantatthe organic loadingof 0.6 kg VS/m^3*d during a digestion with 30 days of retention time. The rheogram of the sludge samples showed a reduction of the viscosity in the sludge after the anaerobic digestion. Sludge samples with presence of a flocculant showed a highviscosity.Fig 1 b) shows a rheogram of a sample with an organic loading of 0.6 kg VS/m^3*d at30days of retention time, in which it is shown that the viscosity was decreasing from the beginning to the end of the anaerobic digestion process.



a) b)

Fig.1a)Total and volatile solids of sludge with presence of a flocculant. b)Sludge Rheogrambehavior.

Conclusions. The flocculant has an effect in the viscosity of the sludge. The sludge showed an improvement of its rheological properties after the anaerobic digestion.The concentration of total solids is related to the fluidity of the sludge.Anaerobic digestion with a flocculant in sludge and athigh volatile solids concentration,showed an improvement in gas production, as well as, abetter reduction of solids.

Acknowledgements.To the TecnológicoNacional de México/I.T.Durango for the opportunity to be in the Environmental Systems Graduate Program.ToCONACyT for the scholarship granted to Castorena-Quintanar.

References.

1. Baudez J. C. (2011). The rheological behavior of anaerobic digested sludge. *Water Research*, Vol. 45, (17):5675-5680.
2. SaeidB. (2013). Rheology of a primary and secondary sewage sludge mixture: Dependency on temperature and solid concentration. *Bioresource Technology*, Vol. (140):227-233.
3. Xiaohu D. (2014).Rheology evolution of sludge through high-solid anaerobic digestion.*Bioresource Technology*, Vol. (174): 6-10.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México

AM41

BIOPROSPECTING RUDERALS & SOIL SEED BANK ASSOCIATED MICROBIOME

Flor Melo Perez, Roberto Rosales-Fuentes y **Angelica Ruiz-Font**.
Center for Applied Biotechnology in National Polytechnic Institute, Tlaxcala, MEXICO, & Graduate Program Productive Biotechnology, IPN. (email: convenios22@hotmail.com)

Key words: microbial, forest, restoration

Introduction. We investigated the potential application of microbiome of the persistent soil seed bank (SSB) in post-disturbance restoration of the herb layer in a seven different type of vegetation. Seed persistence in the soil under field conditions is an important issue for the maintenance of local plant populations and the restoration of plant communities, increasingly so in the light of rapidly changing land use and climate change. Reduction of seed-bank persistence is an important goal for weed management systems. Recent interest in more biological-based weed management strategies has led to closer examination of the role of soil microorganisms. Incidences of seed decay with certain weed species occur in the laboratory; however, their persistence in soil indicates the presence of yet-unknown factors in natural systems that regulate biological mechanisms of seed antagonism by soil microorganisms (3).

Methods. The samples of the BSS were taken from 9 different sites (1), which were taken to the laboratory where the soil was sown and the seeds were separated manually, for BSA they were taken directly from the plants having a total of 6 species, once the seeds were obtained they were disinfected superficially and isolated the endophytic batteries, where the number of CFU was obtained, later the different bacterial morphotypes were identified and purified, to finally perform 5 tests of biological functionality, nitrogen fixation, solubilization of phosphates, production of indolácetico acid, cellulase and pectinase (2).

Results. You can see, at figure 1 shows the amount of CFU in each seed bank, having the highest amount of CFUs in Izotal and Urban seeds. At the aerial seed bank, we found important data about germination in antibiotic treatment (fungicide and bacterial substances). The figure 2 we show the response of two kind of Amaranth. When we put bactericide, the germination decrease.
In total we isolate 81 bacterial morphotypes, a total of 65 were obtained and the BSAs a total of 16 morphotypes Functionality tests indicate that most of the aerial seed bank bacteria exhibit nitrogen-fixing activity.

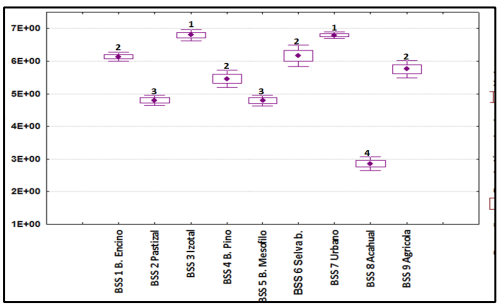


Fig.1 UFC/gr soil from each Soil Seed Bank

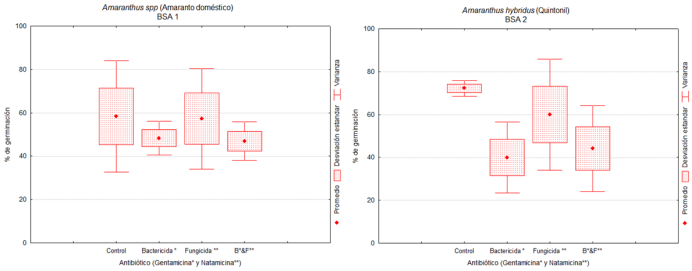


Fig.2 Pine response to addition of bacterial formula

Conclusions. A total of 81 endophytic bacterial morphotypes were obtained from seed banks, the most important biliary functional activity in BSS was the degradation of cellulases

References.

1. Ferrandis P., Herranz J., Martínez J., y Martínez J. 2001. Response to fire of a predominantly transient seed bank in a Mediterranean weedy pasture (eastern-central Spain). Ecoscience, 8(2), 211-219.
2. Loredo C., López L., y Espinosa D. 2004. Bacterias promotoras del crecimiento vegetal asociadas con gramíneas: Una revisión.Terra Latinoamericana, 22(2), 225-239.
3. Roberts, H.A., 1981. Seed banks in soils. In: Coaker, T.H. (ed.).
Gyaneshwar, P.; Naresh,G.; Parekh, J. and Poole, P. 2002. Role of soil microorganisms in improving P nutrition of plants. Plant and Soil.245: 83–93.

ISSN: 2448-8461

AM42

NANOTECHNOLOGY FOR SOIL AND WATER REMEDIATION, AND FOR GROWING VEGETABLES: DOES IT REALLY WORK?

Fabián Fernández-Luqueño[1] & Fernando López-Valdez[2]
[1] Sustainability of Natural Resources and Energy Programs, Cinvestav-Saltillo, Coahuila de Zaragoza. C.P. 25900, Mexico.
[2] Instituto Politécnico Nacional, CIBA-IPN, Tepetitla de Lardizábal, C.P. 90700, Tlaxcala. México.
[1]E-Mail: cinves.cp.cha.luqueno@gmail.com

Key words: Agronanobiotechnology, environmental pollution, crop production

Introduction. During the last years, nanotechnology (NT) has been the center of attention of scientists and technologists worldwide because it has been able to synthesize and produce materials or devices with specific characteristic never known before (Leon-Silva *et al*. 2016). These scientific advances have used the cutting-edge knowledge of different areas, but also these new developments have driven the emergence of new knowledge areas such as nanotoxicology. Now the population can use and enjoy new fashion cosmetics, innovative sunscreens, high-tech devices, controlled-release medication, and even technologies to clean quickly, safely and cheaply a considerable amount of the pollution released around the world. Even more, the production of healthy food in less time and at a lower cost with NT has also been speculated. However, is there scientific evidence regarding how many energy, water, or scarce and dangerous chemical elements are required to synthesize these nano-size materials? Is there a protocol regarding the handle, byproducts or final disposition of products containing nano-sized components that fulfilled their function for which they were created?
The objective of this research is identifying the cutting-edge knowledge regarding the use of NT to remediate soil and water and to produce vegetables without risks for people.

Methods. Several experiments were carried out at a plant growing chamber and greenhouse level while a drinking-water filter with nanoparticles (NP) and waste organic and inorganic materials was designed, built, and evaluated (Fernández-Luqueño *et al*. 2008; Medina-Pérez *et al.* 2018a, 2018b). Also, we exhaustively cover all the studies reporting NP until the beginning of 2018, which were regarding crop production and remediation.

Results. We found the effects of NP on crop production and remediation technologies depend on the type, doses, physical or chemical characteristic, and exposure time-span of the NP. Also, the crop, contact form, and other biotic or abiotic factors could also change the global effect of NT.
Environmental remediation and agriculture require the services of living organisms. Soil, water, and plants coexist with billions of microorganisms, and none of them, nor the set of them are NP; this is an essential and easy consideration that has to be taking into account when talking about soil or water nanoremediation or nanoagriculture. Otherwise, mistakes in the design of experiments and the interpretation of results will arise. Will have hundreds of scientific articles published, but the real solution will be hidden behind the experimental mistakes? Should NT developers use tools and protocols that employ the nanotoxicology before releasing their products? Are we finding new options to shape sustainable development or are we only producing a few scientific articles?

Conclusions. The cutting-edge knowledge regarding the use of NP to decontaminate soils or to growing crops has to move forward, but the environmental quality, the human health, and the social welfare should also be ensured. Otherwise, these patents regarding modern nanomaterials might jeopardize the sustainability.

Acknowledgements. 'Ciencia Básica SEP-CONACyT' projects 151881 and 287225, and the Sustainability of Natural Resources and Energy Programs (Cinvestav-Saltillo).

References.

1. Fernández-Luqueño, F., L. Dendooven, A. Munive, L. Corlay-Chee, L. M. Serrano-Covarruvias and D. Espinoza-Victoria. 2008. Micro-morphology of common bean (*Phaseolus vulgaris* L.) nodules undergoing senescence. Acta Physiol. Plant. 30: 545-552.
2. Medina-Pérez, G., F. Fernández-Luqueño, E. Vazquez-Nuñez, F. López-Valdez, J. Prieto-Mendez, A. Madariaga-Navarrete and M. Miranda-Arámbula. 2018a. Remediation of polluted soils using Nanotechnologies: Environmental benefits and risks. Pol. J. Environ. Stud. IN PRESS.
3. Medina-Pérez, G., F. Fernández-Luqueño, L.I. Trejo-Tellez, F. Lopez-Valdez and L. Pampillón-González. 2018b. Growth and development of common bean (*Phaseolus vulgaris* L.) var. Pinto Saltillo exposed to iron, titanium, and zinc oxide nanoparticles in an agricultural soil. Appl. Ecol. Environ. Res. 16, 1883-1897
4. León-Silva, S., F. Fernández-Luqueño and F. López-Valdez. 2016. Silver nanoparticles (AgNP) in the environment: a review of potential risks on human and environmental health. Water Air Soil Pollut. 227(9). Art. Number 306.

ISSN: 2448-8461

AM43

ANTIBACTERIAL EFFECT OF METALLIC NANOPARTICLES (TITANIUM DIOXIDE DOPED WITH SILVER NITRATE) IN WASTEWATER

Esquivel Barajas Gabriela Guadalupe[1], Flores Magallón Rebeca[1], Bretado Aragón Luis. Aalberto[2], Álvarez Bernal Dioselina[1], Silva García Teodoro[1]., and Nuñez Minerva[1].

[1] Instituto Politécnico Nacional - Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional Unidad Michoacán. Justo Sierra Ote. 28, Centro 1, CP 59510 Jiquilpan de Juárez, Michoacán, Mexico.

[2] Universidad de La Ciénega del Estado de Michoacán de Ocampo. Avenida Universidad Sur 3000, Lomas de Universidad, CP 59518 Michoacán, MEXICO.

gabyesba01@gmail.com, Phone: 3531153787.

Key words: Titanium, silver, bactericide.

Introduction.

The objective of this research is the development of a new technique to disinfect and purify wastewater from nanomaterials, as the first place is to obtain nanoparticles of TiO_2 / $AgNO_3$ by the sol-gel technique, in second place, to develop comparative using different concentrations of the material exposed to ultraviolet light and in lack thereof, at different time intervals and in this way determine both the minimum inhibitory and minimum bactericidal concentrations.

Methods.

For the development of the research, it is divided into 2 phases:

a) Synthesis of the material.

The sol-gel technique was used, the process was carried out following the methodology proposed by (Navarro M., 2016). For the characterization was used: MEB (Scanning Electron Microscope JEOL ISO 9000). b) Evaluation of $TiO_2/AgNO_3$ with pathogenic microorganisms.The micro dilution method was used for bactericidal tests NCCLS-CLSI N7 A7, with the purpose of identifying the minimum inhibitory concentration and the minimum bactericidal concentration of the metal nanoparticles of the composition.

Results.

In relation to the morphology a rough surface with a particular irregular size can be seen, due to its heterogeneous particle size. At the same time, the elemental chemical analysis (ESD) was carried out in which the intensities of the present elements were shown, which showed a greater incidence attributed to their electronic configuration. The thermal treatment favors the particle size that oscillates between: 70-123nm for the case of (Amarjargal A., *et al* 2012) and 0.2-2µm for the case of (Esparza P., *et al* 2010).

An initial count of colony forming units (CFU) was initiated from: $8.3x10^7$, you can observe a decrease both in exposure and without exposure, the best result was the 5 and 10% treatments, given that an inhibition of between 5 and 7 logarithms was manifested. For *Salmonella* the initial account was: $6.2x10^7$, at zero time there was even a proliferation of the pathogen, but with the passage of time the best results were those of the treatments at 10w/p, because the inhibition at 60 minutes was up to 6 logarithms. For the evaluation before *Streptococcus sp* the initial account was: $1.5x10^9$, at time zero there was a desceso of a logarithm, but with the passage of time the best results were those of the treatments at 10w / p at minute 60 because the proliferation of microorganisms was only 2 logarithms.

Conclusions.

$TiO_2/AgNO_3$ nanoparticles are synthesized by the Sol-Gel technique, between 100-500nm. Samples of residual water, a favorable tendency to the 45-minute exposure was observed, attributed mainly to the fact that it is the precise moment when the material can release its maximum inhibitory action.

References.

1. Amarjargal, A.; Tijing, L.D.; Yu, M.-H.; Kim, C.-H.; Park, C.-H.; Kim, D.-W.; Kim, C.-S. (2012) "*Characterization And Photocatalytic Efficiency Of TiO_2/Ti Beads Fabricated By Simple Heat-Treatment*" J. Mater. Sci. Technol., P.p. 184–192. 54.
2. Navarro Segura María Edith (2016) "*Assessment Antibacterial Behavior Tio2-Ag Composite Using Staphylococcus aureus And Escherichia coli*" Universidad de La Ciénega Estado de Michoacán de Ocampo. P.p. 50-55.

ISSN: 2448-8461

AM44

REVIEW ABOUT CONSOLIDATION AND MANAGEMENT OF EXPIRED DRUGS

García-Morales Marco Antonio[a,b] , Pedro Antonio Martínez-Arteaga[a], Contreras-Rodríguez Araceli[c], Hernández-Gama Regina[a], Garzón-Zúñiga Marco Antonio[d] , Morales-García Rosario[a*]

[a] Instituto Politécnico Nacional. Centro de Investigación en Ciencia Aplicada y Tecnología Avanzada. Departamento de Investigación. Cerro Blanco 141. Col. Colinas de Cimatario C.P 76090. Querétaro, Qro.
[b] Universidad Autónoma de Querétaro. Facultad de Ingeniería. Cerro de las Campanas s/n C.P, 76090. Querétaro, Qro.
[c] Instituto Politécnico Nacional. Escuela Nacional de Ciencias Biológicas. Departamento de Microbiología. Prolongación de Carpio y Plan de San Luis s/n. Col. Santo Tomás. C.P 11340. Ciudad de México, México.
[d] Instituto Politécnico Nacional. Centro Interdisciplinario de Investigación para el Desarrollo Integral y Regional. Unidad Durango. Departamento de Investigación. Calle Sigma 119, 20 de Noviembre II, C.P 34220. Durango, Dgo.
* mdmoralesg@ipn.mx; mdrmorales1013@gmail.com

Key words: waste, pharmaceuticals, medicines, toxicity

Introduction Pharmaceutical products for their compounds have adverse effects on ecosystems and human health when they are discarded to the environment. In Mexico, the main and almost only way to handle pharmaceutical waste is through disposal in landfills or drainage systems.

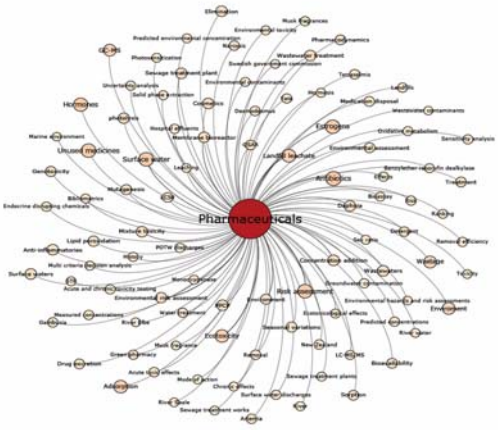
OBJECTIVE.

Establish the development how to dispose of obsolete or obsolete drugs has been investigated, in the different social sectors in which they are used, in different places of our planet compared with what has been done in our country.

Methods. Bibliographic research, carried out through the page of the National Consortium of Scientific and Technological Information Resources (www.conricyt.gob.mx) and the Google Scholar search engine (Google Scholar). The search for information required the use of the following keywords: pharmaceutical expired, drug waste, medicine, landfill, groundwater, contamination, water, self-medication, cost, open dumps site, unused medication, Mexico.

Results. The Graphs of nodes with different filters were elaborated. In the first, all the criteria were included regardless of their frequency (raw data) see graphica 1. The size of the nodes was determined based on the number of connections with other nodes and the frequency of mention in each of the articles.

Conclusions. In our country, the action of properly disposing of expired medicines is imperative; we do not know the level of toxicity that their chemical components have caused in our aquifers, floors of garbage dumps and even in the air. It is necessary to emphasize the formalization of a procedure for the final disposal of non-useful medicines (expired or waste) generated by all the population of our country.



Graphica.1 Most relevant trends "trending topics" regarding the criteria found in the bibliographic compilation.

Acknowledgements. The present work was carried out in the Center for Research in Applied Science and Advanced Technology of the Querétaro Unit of the National Polytechnic Institute (IPN), under the direction of Dra. María del Rosario Jovita Morales García. Supported by SIP 20161168, 20161242, 20171847and 20180432 of the IPN.

References.

Diniz Chaves, G. de L. (2015). Descarte de medicamentos vencidos e em desuso: um levantamento do comportamento dos consumidores em São Mateus / ES Disposal of expired and unused medications : a consumer behavior survey in São Mateus / *ES. Revista Eletrônica Em Gestão, Educação E Tecnologia Ambiental, 1083–1096.*

ISSN: 2448-8461

AM45

EVALUATION OF ANTIFOULING ACTIVITY OF MARINE BACTERIA SUPERNATANTS IN FIELD EXPERIMENTS

Monserrat Alemán-Vega*[1,2], Claudia J. Hernández-Guerrero[1], Ilse Sánchez-Lozano[1], Ángelo Santana-del Pino[2], Claire Hellio[3]

[1]Instituto Politécnico Nacional-CICIMAR, La Paz, B.C.S., MEXICO. [2]Facultad Ciencias del Mar, Universidad Las Palmas de Gran Canaria, SPAIN.[3]Université de Bretagne Occidentale, FRANCE. *cguerrer@ipn.mx;monsealeman93@gmail.com*

Key words: Antifouling, Bacteria, Paint

Introduction. The biofouling causes problems and economic losses to marine and shipping industries. Actually, to retard or inhibit fouling, toxic substances are applied, but have several consequences. Tend to accumulate in the food chain, are related to the death of sea mammals and can declines the aquaculture systems(1). Microorganisms are a source of new compounds, and from a biotechnological perspective this compound can be use in antifouling strategies (2). For this reason, the objective of this research was to investigate the antifouling potential of marine bacteria supernatant in a paint matrix in the field.

Methods. The marine bacteria used for this study were isolated from sponge M ycales p. (My30 *Bacillus liqueniformis*) and sediment (S69 *Vibrio harveyi*, S74 *Halobacillus* sp.). For the isolation of bacterial supernatant, the antagonist bacteria were standardized in saline solution, inoculate in marine broth cultured. It was centrifuged at 4000 rpm for 20 min (3). The supernatants toxicity was test with nauplii of *Artemia salina* model. A matrix antifouling paint was prepared (4). And the supernatants were added to the paint in a concentration of 40 µL mL^{-1}. Sand blasted acrylic plates were cover with the different paints and submerged in La Marina dock of La Paz for 130 days. All the treatments were performed in triplicate. The coverage percentages for each group of organisms settled in panels were estimated and all the organisms of the plates were dried. The significant difference between treatments was tested.

Results. The supernatants showed low levels of toxicity. The field test showed a temporally coverage percentage evolution. For 28 days , the differences plates show non-significant differences between them with A NOVA. For 130 days , post-hoc Tukey test showed non-significance difference between the paint plate with the *B. licheniformis* supernatant (My30) and the antifouling commercial paint platebut had significant differences (p-value < 0.01) with the Control.

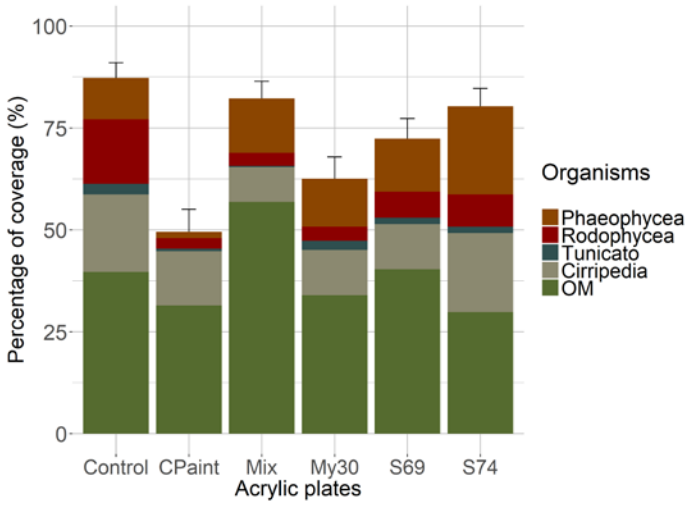


Figure 1. Percentages of coverage of epibiont organisms in different treatments: My30 (Paint + *B. licheniformis*), S69 (Paint +*V. harveyi*), S74 (Paint + *Halobacillus* sp.) Control (experimental paint without supernatant), CPaint (commercial antifouling paint), after 130 days of exposure in the La Paz marina, B.C.S.

Conclusions. Marine bacteria *Bacillus liqueniformis* present antifouling potential similar to the commercial paint but with low toxicity.

Acknowledgements. Instituto Politécnico Nacional Projects (SIP20170434 and SIP20181803). CJHG thank COFAA and EDI.

References.

(1)Hellio, C ., De La Broise, D ., Dufosse, L ., Le Gal, Y., and Bourgougnon, N. (2001).*Marine Environmental research*, 52(3), 231-247.

(2) Qian, P.Y., Xu, Y. and Fusetani (2010). *Mar. Biotechnol*., 9: 399-410

(3) Sayem, S. A ., Manzo, E ., Ciavatta, L. , Tramice, A ., Cordone, A ., Zanfardino, A., De Felice, M., and Varcamonti, M. (2011). *Microbial Cell Factories*, 10(1), 74.

(4) Acevedo, M. S., Puentes, C., Carreño, K., León, J. G., Stupak, M., García, M ., Pérez, M ., and Blustein, G . (2013). *International Biodeterioration & Biodegradation*, 83, 97-104.

ISSN: 2448-8461

AM46

MEXICAN MARINE SEDIMENTS AS A SOURCE OF SALINOSPORAE

Erika T. Quintana[1], Luis Contreras-Castro[1], Jair A. Acevedo-Becerra[1], Carlos Vargas-Corona[1], Carla R. Martínez-Rodríguez[1], Jaqueline A. Silva-González[1], Luis A. Maldonado[2], Claudia J. Hernández-Guerrero[3], Sergio F. Martínez Díaz[3]
[1]Instituto Politécnico Nacional, Escuela Nacional de Ciencias Biológicas, Departamento de Microbiología, Ciudad de México, Mexico, C.P. 11340. [2]Universidad Nacional Autónoma de México, Facultad de Química, Ciudad Universitaria, Ciudad de México, Mexico, CP 45000.[3]Instituto Politécnico Nacional, Centro Interdisciplinario de Ciencias Marinas, Av. Instituto Politécnico Nacional s/n Col. Playa Palo de Santa Rita. Código Postal 23096 La Paz, B.C.S., México.
erika_quintana@hotmail.com

Key words: Salinisporae, Mexican marine sediments, Punta Arena de la Ventana.

Introduction. The class *Actinobacteria* (1) contains almost the most diverse and complex group of organisms in the planet. The genus *Salinispora* (2) is the only marine obligate microbe within this class. The distribution of salinisporae is worldwide however they have been isolated in specific sites and sediments (3, 4). The genus encompasses three validly described species: *Salinispora arenicola*, *S. pacifica* and *S. tropica* and all of them produce natural products with biological activity. For example, *S. tropica* produces a novel and potent anti-cancer compound, *S. pacifica* produces enterocine and *S. arenicola* produces rifamycina-like compounds. Salinisporae is a model organism with a clear bioprospective potential.
The objective of the present work was to isolate and identify salinisporae from Mexican marine sediments.

Methods. A selective isolation strategy was developed and carried out using marine sediment collected in Punta Arena de la Ventana (10 m depth). Typical colonies of salinisporae where observed on the selective isolation plates after six weeks of incubation. Serial dilutions (a) and stamp methods (b) were used and compared. A quick test using media with and without marine salt was performed and selected obligate marine microbes. A MLSA analysis was designed using five house-keeping genes.

Results. Sixty six colonies were purified by method (a) and ten from (b). The corresponding MLSA analysis showed that the strains belong to the genus *Salinispora.* Five of them may be novel species. All the isolates were obligate marine microbes. Some the isolates showed microbial and enzyme activities.

Conclusions. Dilutions method is more effective than stamp method in isolating salinispoare. All the isolates were marine obligate microbes. Punta Arena de la Ventana is an important source from sediments to selective isolate salinisporae.

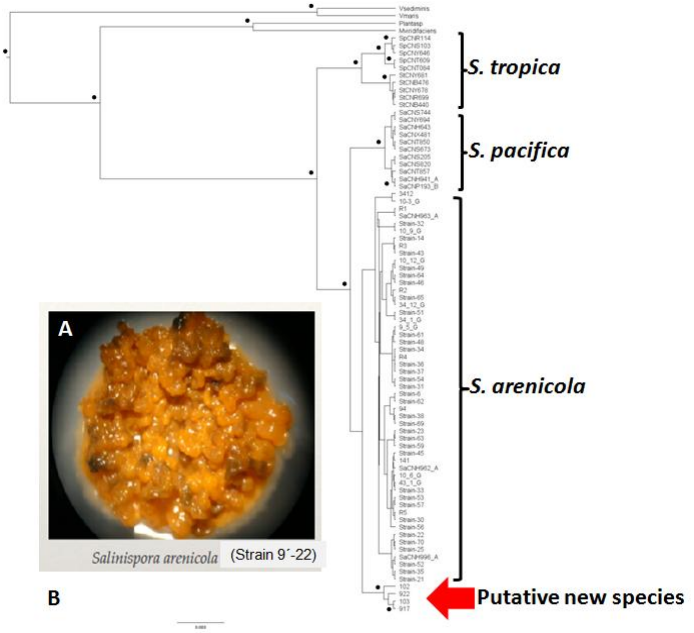


Fig.1 A) typical morphology of salinisporae like-microbes growing in GYM media with marine salt and b) filogenetic tree based on the MLSA.

Acknowledgements. Authors acknowledgement Grants numbers SIP20170432 and SIP20181528.

References.

1. Stackebrandt, E., Rainey, F. A. & Ward-Rainey, N. L. (1997). Proposal for a New Hierarchic Classification System, Actinobacteria classis nov. Int J Syst Bacteriol 47, 479–491.
2. Maldonado, L. A., Fenical, W., Jensen, P. R., Kauffman, C. A., Mincer, T. J., Ward, A. C., Bull, A. T. & Goodfellow, M. (2005). Salinispora arenicola gen. nov., sp. nov. and Salinispora tropica sp. nov., obligate marine actinomycetes belonging to the family Micromonosporaceae. Int J Syst Evol Microbiol 55, 1759–1766.
3. Jensen, P. R., Gontang, E., Mafnas, C., Mincer, T. J. & Fenical, W. (2005). Culturable marine actinomycete diversity from tropical Pacific Ocean sediments. Environ Microbiol 7, 1039–1048.
4. Maldonado, L. A., Fragoso-Yáñez, D., Pérez-García, A., Rosellón-Druker, J. & Quintana, E. T. (2009). Actinobacterial diversity from marine sediments collected in Mexico. Antonie van Leeuwenhoek, 95, 111–120.

ISSN: 2448-8461

AM47

WASTEWATER TREATMENT AND REUSE IN A PRIMARY SCHOOL, USING MODULAR CONSTRUCTED WETLANDS

Mónica Rodríguez-Estrada*; Alfredo González-Medina*; Ernesto Carlos Reyes-Alvarado**; Ulises Rojas-Zamora***; Víctor Manuel Luna-Pabello**; Sergio Aburto-Castañeda**; Cecilia Téllez-Quintanar*; Ana Gabriela Ortega-Alvarado**; Alejandra Arreola-Franco**, Andrés Cicerón-Monroy**; José Rodrigo Espino-Chávez**.

* Grupo Multidisciplinario Integral GMI SC, Mexico City 09890, Mexico, (E-mail: mlrestrada@gmintegral.com)
** Grupo Académico Interdisciplinario Ambiental. Faculty of Chemistry, National Autonomous University of Mexico, Mexico City 04510, Mexico, (E-mail: lpvictor@unam.mx, victormlp@yahoo.com)
*** Department of Biotechnology, Metropolitan Autonomous University – Iztapalapa, Mexico City 09340, Mexico, (E-mail: ulises.rojaz@gmail.com)
Key words: constructed wetlands, wastewater treatment, water reuse.

Introduction. Constructed wetlands are biological systems, which are an alternative for treating different kinds of wastewater. This systems, require low level energy, maintenance and non-specialized labour (Ramprasad *et al*. 2017). A modular constructed wetland (MCW) was designed to treat a mixture of yellow water (urine from men urinals) and gray water (from sinks and cleaning activities), in a primary school located in a zone with lack of tap water in Mexico City. The school was carefully chosen using indicators such as: social vulnerability (margination, human development index and social backwardness); building typology and basic services; urban, territorial and technic aspects like available space, topography and street illumination. The MCW has an innovative hexagonal configuration in which it is possible to assemble units as needed in order to adjust the treatment capacity, depending on space and the geometry of the site. The MCW is a horizontal subsurface flow wetland, followed by a free water surface one. The support materials are gravel and limestone; the vegetation is *Phragmites australis, Arundo donax, Cyperus haspan* y *Equisetum arvense*; the aquatic wetland vegetation is *Lemna gibba, Ceratophyllum demersum, Sagittaria macrophylla, and Hydrocotyle ranunculoides.* The MCW was inoculated with nitrifying bacteria. The effluent's quality must achieve mexican's regulation for water reuse with direct contact with people, so that it can be used for WC and for garden irrigation. Two environmental education workshops where implemented as a social strategy for the school population. This system can be adapted in other places where water can be treated *in situ*, mainly in ones with lack of tap water.

The objective of the project was to design, construct and evaluate a modular constructed wetland, for wastewater treatment and reuse of a mixture of yellow and gray water, in a primary school, located in a zone with lack of water in Mexico City.

Methods. The MCW was built with 6 modules connected in series; the support system of limestone and rhyolite, allows a stable pH in the system; the diameters used where o 0.5, 0.75 and 1 inch, at 40 cm and 37 cm of water level; the modules where built out of wood panel, and covered with 1 mm HDPE geomembrane. The system can treat 400 L/day, in 6.8 m^2, plus 3 m^2 of free flow wetland. The analyzed parameters where pH, temperature, electric conductivity, dissolved oxygen, chemical oxygen demand (DQO), ammonia, nitrate, nitrite, fecal coliforms, phosphates and surfactants.

Results. The results of the first sixteen weeks of operation, in which water was recirculated and rainwater was also added, show that nitrogen in its multiple species, was transformed through nitrification.

Conclusions. The MCW system treats near 400 L/day, its materials and operation are low cost, and it was constructed on the surface of a garden. The results of water analysis show nitrification activity, and bacterial establishment on the gravels. Denitrification is expected to occur with the free flow wetland, and with disinfection water will be able to be used in WC; this will reduce the daily volume of tap water used in the restrooms.

Acknowledgements. To the Science, Technology and Innovation Secretary of Mexico City, for the financial support (SECITI/082/2017); to the Experimental Microbiology Laboratory, Faculty of Chemistry, National Autonomous University of Mexico; and to the Department of Biotechnology, Metropolitan Autonomous University; both of them for their help in laboratory analysis.

References.

1. Ramprasad, C., Smith, C.S., Memon, F.A., Philip, L. 2017 Removal of chemical and microbial contaminants from greywater using a novel constructed wetland: GROW. *Ecological Engineering* (106):55–65.

ISSN: 2448-8461

AP1 #

CLORINATION OF WATER, PERFORMANCE OF Litopenaeus vannamei AND ITS RESISTANCE TO EARLY MORTALITY SYNDROME

Héctor M. Esparza-Leal, Ely S. López-Álvarez, Nadia Vazquez-Montoya, Mariel López-Espinoza, Wenceslao Valenzuela-Quiñónez

Instituto Politécnico Nacional-Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional Unidad Sinaloa, Boulevard Juan de Dios Bátiz Paredes # 250, Guasave, Sinaloa 81101, Mexico. Héctor M. Esparza-Leal, e-mail: hesparza@ipn.mx

Key words: Litopenaeus vannamei, performance, clorination of water

Introduction.
One of the previous practices to realize a culture with biofloc is to chlorinate the water, in order to eliminate organisms that can have a negative effect in the crop. However, it is unknown if this practice eliminates organisms that may also have a positive effect on shrimp, either as nutrients or by stimulating the immune system.
The present proposal was carried out in low salinity (≈ 12 mg / l) in order to determine if the chlorination of the water has a negative effect on the biofloc formation time and its quality, determine if shrimp growth is affected and, as well as to know if the Litopenaeus vannamei resistance to the syndrome of early mortality (EMS) are related to the chlorination of water.

Methods. Four treatments were designed (T1 = Chlorination + molasses, T2 = Chlorination + without molasses, T3 = without chlorination + with molasses and T4 = without chlorination + without molasses). Also, after the stage of culture, the organisms of each treatment (except those of T3, whose mortality was 100% before concluding this stage) were subjected to a challenge with a strain of the bacterium Vibrio parahaemolyticus that cause the syndrome of early mortality (EMS).

Results. The results of the study showed significant differences between some treatments, observing a lower final average weight (1.31 ± 0.23 g) and specific growth rate (SGR, 2.0 ± 0.6%/d) where it was grown without chlorination and without molasses (T4), with a lower survival (32.9 ± 2.8%) and biomass (3.4 ± 0.0 g/m2) in the treatment where it was cultivated with chlorination and without molasses (T2). In the challenge, the results showed only significant differences in cumulative mortality (%) in the treatment where it was grown without chlorination and without molasses (T4), where after 72 hours post-infection there was a lower accumulated mortality that remained until 84 hours post-infection.

During the culture, the physico-chemical variables did not present significant differences between the treatments, although there was a trend of lower pH where it was cultivated with chlorination and without molasses (T2).

Conclusions. A lower final average weight and specific growth rate were observed where it was cultivated without chlorination and without molasses, with a lower survival and biomass where it was cultivated with chlorination and without molasses. Where it was grown without chlorination and with molasses, 100% mortality was presented before the end of the experiment. In the challenges with Vibrio parahaemolyticus strain that cause the early mortality syndrome (EMS), there were only significant differences in cumulative mortality (%) in the treatment where it was grown without chlorination and without molasses, from the 72 hours post infection, where there was a lower accumulated mortality that remained until 84 hours post infection.

Acknowledgements. This study is part of the project funded for the Instituto Politécnico Nacional (SIP-20160057).

References.

1. Cuadros, W., E. Beltrame. 1998. Nuevas técnicas de alimentación con bandejas en el Brasil. Panorama Acuícola 3:8-9.
2. Strikland, J.D.H., T.H. Parsons. 1972. A practical handbook of seawater analysis. Fish Research Board of Canada Bulletin, Ottawa, Canada.
3. APHA (American Public Health Association). 1998. Standard methods for the examination of water and wastewater. 20st edition, Washington, DC.
4. Gomez, K.A., A.A. Gomez. 1984. Statistical procedures for agricultural research, 2nd edition. John Wiley and Sons, New York.
5. Gómez-Gil, B. 2006. Manual de bacteriología. CIAD-Mazatlán, Sinaloa, México.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
AP2

CONCENTRACIÓN DE METALES PESADOS EN EL OSTIÓN JAPONÉS *CRASSOSTREA GIGAS* CULTIVADO EN EL ESTERO LA PITAHAYA, GUASAVE, SINALOA

Manuel García Ulloa, Andrés Góngora, Brenda Villanueva, Lizeth Villanueva, Isabel Sotelo, Carlos Sepúlveda. Instituto Politécnico Nacional. Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional. Departamento de Acuacultura, Blvd. Juan de Dios Bátiz Paredes # 250, Guasave, Sinaloa, México. C.P. 81101. Tel/Fax (687)8729626 y 29625. turbotuag@hotmail.com

Key words: Acuacultura, ostión del Pacífico, Contaminación.

Introducción. Los ecosistemas costeros en los que se practica la ostricultura, están expuestos a cargas de metales pesados provenientes de arrastres pluviales, ríos, canales de riego, o derivados de actividades industriales y urbanas que se desarrollan en las cercanías o alrededor de ellos. En ocasiones, los niveles de metales rebasan el límite permitido por la normatividad. Por ser sésiles y filtradores, los moluscos están expuestos a la acumulación de este tipo de contaminantes (1, 2).
El objetivo de la siguiente investigación fue determinar la concentración de metales pesados: Cadmio (Cd), Cobre (Cu), Níquel (Ni), Cromo (Cr), Mercurio (Hg), Arsénico (As), Zinc (Zn) y Plomo (Pb), en el ostión japonés *Crassostrea gigas* cultivado en el estero La Pitahaya, Guasave, Sinaloa.

Métodos. El estudio se realizó de marzo a diciembre de 2011. Del tejido suave de ostiones muestreados cada mes, se analizaron Cu, Cd, Cr, Ni, Pb, Zn, As y Hg; las muestras fueron secadas a 65°C/72 h. Posteriormente, se maceraron, pesaron (1.5 g /peso seco) y digirieron con 3 ml de NHO_3 al 70% y 0.5 ml 30% H_2O_2, usando un digestor de microondas (300 W/5 min y 600 W/10 min). Los extractos se enfriaron a temperatura ambiente (20 min), y diluidos a 10 ml con agua de-ionizada. Las digestiones fueron almacenadas a 0-5°C y finalmente, analizados por espectrofotometría de absorción atómica de flama (Cu, Cr, Cd, Ni, Pb y Zn), generación hídrica (As) y medio de vapor frío (Hg).

Resultados. Los niveles promedio de metales fueron: Cd=13.84±4.22, Cu=51.42±25.92, Ni=10.26±12.18, Cr=24.97±32.38, Hg=0.02±0.01, As=0.37±0.08, Zn=267.42±92.29 y Pb=2.18±1.28 µg g^{-1}(peso seco). El orden decreciente de la carga de metales fue: Zn>Cu>Cr>Cd>Ni>Pb>As>Hg. Los niveles de Cu, Cr, Cd y Pb (µg g^{-1}, peso húmedo) rebasaron los límites máximos permitidos por la normatividades. Las mayores concentraciones se registraron en lluvias (julio-octubre) y no presentaron correlación (P > 0.05) con las biometrías del ostión.

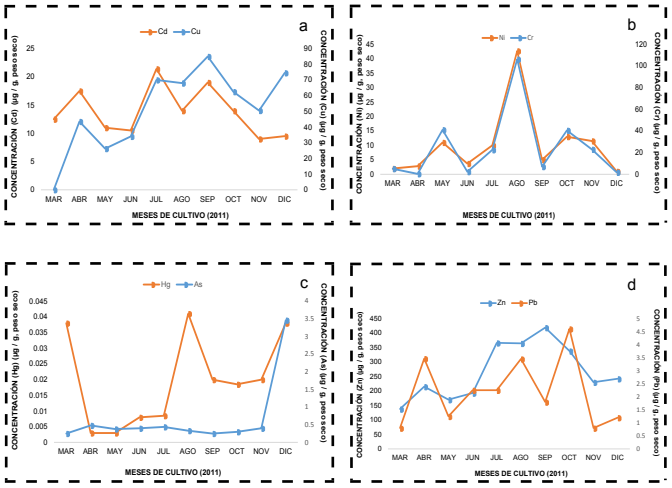


Fig.1 a) Concentración de Cd y Cu. **b)** Concentración de Ni y Cr. **c)** Concentración de Hg y As. **d)** Concentración de Zn y Pb.

Conclusiones. Las concentraciones de metales variaron con el tiempo. Los resultados obtenidos sugieren que la carga de metales pudo haber sido influenciada por actividades antropogénicas desarrolladas en los alrededores del área de cultivo, como agricultura y acuacultura. Debido a su consumo crudo, se recomienda monitorear los metales en ostión, agua y suelo con frecuencia e informar acerca de posibles riesgos de contaminación.

Referencias.

1. Cuong D.T., Bayen, S., Wurl, O., Subramanian, K., Shing Wong, K. K., Sibasoti, N. & Obard, J.P. (2005). Heavy metal contamination in mangrove habitats of Singapore, Marine Pollution Bulletin, 50: 1732 - 1738.
2. Vane C.H., Harrison, I., Kim, A.W., Moss - Hayes, V., Vickers, B.P. & Hong K. (2009). Organic and metal contamination in surface mangrove sediments of south China. Marine Pollution Bulletin. 58: 134 - 144.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México

AP3

PREVALENCIA DE *PERKISNUS* SP. (PROTOZOA) EN LA ALMEJA CHOCOLATA *MEGAPITARIA SQUALIDA* DEL ESTERO BACOREHUIS, AHOME, SINALOA

Manuel García Ulloa, Andrés Góngora, Juan Hernández, Ana Domínguez. Instituto Politécnico Nacional. Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional. Departamento de Acuacultura, Blvd. Juan de Dios Bátiz Paredes # 250, Guasave, Sinaloa, México. C.P. 81101. Tel/Fax (687)8729626 y 29625. turbotuag@hotmail.com

Key words: Perkinsosis; Megapitaria squalida; fluid thioglycolate medium

Introducción. Los moluscos bivalvos marinos de la Familia Veneridae forman parte del recurso "almeja" y se explotan principalmente para consumo humano (1). Debido principalmente a su demanda, diversificación de cultivo y la globalización en su producción, el riesgo por la dispersión de agentes patógenos entre moluscos (incluyendo la almeja chocolata *Megapitaria squalida*), ha ocasionado un impacto negativo y pérdidas en la industria (2). Uno de los parásitos de mayor injerencia es el protozoario *Perkinsus* sp., el cual se ha dispersado entre diferentes especies de bivalvos comerciales, por el transporte de ejemplares vivos entre localidades y países, provocando brotes de enfermedades y epizootias (3).
El objetivo de la siguiente investigación fue determinar la prevalencia anual de *Perkinsus* sp., en la población silvestre de *M. squalida* del estero Bacorehuis, Ahome, Sinaloa.

Métodos. Se colectaron mediante buceo libre, 30 ejemplares de *M. squalida* durante 12 meses (Figura 1a). En cada muestreo, se tomaron los parámetros fisicoquímicos del agua (temperatura, oxígeno disuelto, salinidad, pH, profundidad y transparencia). Una vez en el laboratorio, las almejas se pesaron (g) y midieron (longitud, alto y ancho de la concha, mm). Después, se obtuvo una biopsia de 5 gramos de tejido de cada ejemplar, incluyendo branquia, manto, músculo aductor, parte gonadal y digestiva (2). Después se maceró el tejido con una navaja de bisturí y se sembró en un tubo con Medio Fluido de Tioglicolato (MFT). Las muestras se incubaron en oscuridad a una temperatura de 22-25°C durante 4 a 7 días (4). Después de la incubación, se estimó la carga parasitaria (hipnosporas/gramo de tejido analizad) de acuerdo a los lineamientos de la OIE (2009) (2). Mediante la observación de las muestras bajo el microscopio, se determinó la cantidad de esporas de *Perkinsus* sp. por gramo de tejido y la prevalencia de la infección.

Resultados. Se detectó la presencia de presuntas hipnosporas del protozoario *Perkinsus* sp. en las almejas analizadas (Figura 2c), las cuales, se caracterizan por presentar forma esférica de color azul oscuro (2). La prevalencia de *Perkinsus* sp. fluctuó desde 0% (diciembre 2013) hasta 70% registrada en mayo (2013), con un promedio anual de 31.38%. La carga parasitaria anual fue baja (11.74 células/gramo de tejido analizado). No se encontró correlación ($P > 0.05$) entre las variables ambientales y biométricas con la intensidad del parásito.

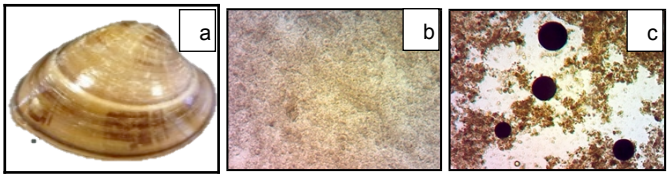


Fig.1 a) Almeja chocolata *M. squalida*. b) Muestra libre de *Perkinsus* sp. y c) Hipnosporas de *Perkinsus* sp. observadas a 100X.

Conclusiones. Los niveles de prevalencia e intensidad de infección del parásito en este trabajo, sugieren que la almeja es poco susceptible a ser infectada. El presente representa el primer registro de la presencia del protozoario *Perkinsus* sp. en la almeja *M. squalida.*

Referencias.

1. FAO. (2000). Anuario Estadístico de Pesca Capturas (1988). Vol. 86/1. Pesca No. 54, Estadística No. 152. Colección FAO (Organización de las Naciones Unidas para la Agricultura y la Alimentación). Roma: 206 p.
2. OIE. (2009). Infection with *Perkinsus marinus.* Manual of Diagnostic Test for Aquatic Animals. Chapter 2.4.5. 342-253 p.
3. Cáceres J, Vásquez R, Padilla G, Del Río M. (2008). *Perkinsus marinus* in pleasure oyster *Crassostrea corteziensis* from Nayarit, Pacific coast of México. *Journal of Invertebrate Pathology,* Vol. 99, Issue 1, September 2008, Pages 66-73.
4. Villanueva L. (2012). Detección de los protozoarios patógenos *Perkinsus* sp. y *Haplosporidium* sp. y su relación con los parámetros ambientales en un cultivo experimental del ostión japonés *Crassostrea gigas* (Thunberg, 1851) en el estero La Pitahaya, Guasave, Sinaloa. Tesis de Maestría. CIIDIR Sinaloa. IPN. 60 p.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
AP4

DISEÑO DE UN ROBOT PLEGABLE PARA RESCATE BIOINSPIRADO EN LOS INSECTOS HEXÁPODOS

Cruz-López, Salvador. Urriolagoitia-Calderón, Guillermo Manuel. Romero-Ángeles, Beatriz. Urriolagoitia-Sosa, Guillermo.
Instituto Politécnico Nacional, Escuela Superior de Ingeniería Mecánica y Eléctrica, Sección de Estudios de Posgrado e Investigación, Unidad Profesional Adolfo López Mateos "Zacatenco", Edificio 5, 2° piso, Col Lindavista, Delegación Gustavo A. Madero, C.P. 07320, México, Ciudad de México.
salvadorcruzlopezim@gmail.com

Palabras Clave: Diseño, Rescate, Robot.

Introducción. En la actualidad, existen trabajos o procesos potencialmente peligrosos para el ser humano. Al presentarse un desastre se debe afrontar de forma segura y adversidades del entorno como un ambiente puede desorientar al rescatista. Lo cual provoca que el lugar de exploración no sea reconocible [1]. Por otra parte, el uso de los robots se aplica a tareas sucias, aburridas, peligrosas o difíciles. Para el proceso de exploración de terrenos hostiles, robots de locomoción ya sea rueda u oruga, no son capaces de sobrepasar obstrucciones u obstáculos. Es por esto por lo que el uso y diseño de robots zoomórficos podrá reducir el porcentaje de fracaso para la inspección en estructuras, reconocimiento de zonas peligrosas, búsqueda de caminos a través de escombros y localización de víctimas. Lo anterior se fundamenta en la exitosa adaptabilidad de entes orgánicos al terreno.

Método. Se realizará el diseño mecánico a partir, de un estudio de movimientos de los insectos hexápodos empleando teoría de análisis y síntesis de mecanismos. Segundo, las patas deben de cumplir tres movimientos, 1) alzar/bajar, 2) avanzar/retroceder, 3) enrollamiento. Para obtener las ecuaciones de posición, velocidades, aceleraciones, así como torque que generen las extremidades se empleará cinemática inversa y dinámica inversa, respectivamente.

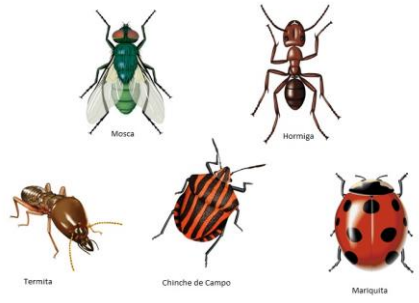


Fig.1 Insectos Hexápodos para el estudio de movimiento motriz.

Teniendo esto, como tercer proceso, se propondrá la manufactura y los componentes mecánicos del autómata. Realizar un análisis numérico y analítico de la estructura del robot.

Resultados. Se espera que, con su estructura, el modelo propuesto pueda sobrepasar los obstáculos existentes en ambientes hostiles presentes en siniestros. Que la manufactura de este sea sencillo, portable y de bajo costo para el uso de los rescatistas. Que la estructura pueda soportar los esfuerzos producidos por su propio peso y evitar que colapse. Este proyecto pretende que sirva como base en el análisis y estudios futuros de robots zoomórficos para investigadores dedicados a esta rama.

Conclusiones. Al realizar todos los estudios correspondientes, se compararán los análisis numéricos con los analíticos. La estructura mecánica y la dinámica de insectos hexápodos son clave para el diseño de robots especializados en la exploración y adaptabilidad a terrenos desconocidos. El análisis de movimiento se comprobará mediante simulación.

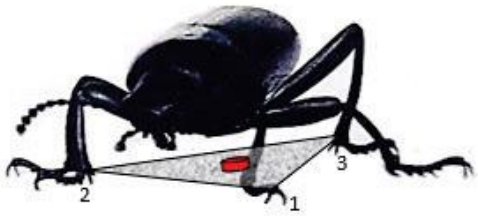


Fig.2 Representación del polígono de apoyo Insectos Hexápodos [2].

Agradecimientos. Los autores agradecen al Instituto Politécnico Nacional y al Consejo Nacional de Ciencia y Tecnología por el apoyo brindado, en la elaboración de este trabajo.

Referencias.

1. Rivas, J. (2015). *Definición y Análisis de los modos de marcha de un robot hexápodo para tareas de búsqueda y rescate.* Politécnica, M., España. Pp.63-74.
2. Rodríguez, J. P. Ramos M. F. Peña, M. V. (2015). *Simulación de un Robot Hexápodo Bioinspirado en el Tenebrio.* Lámpsakos, Colombia. Vol. 14: Pp. 33-39.
3. Kumar, S. (2008). *Reglas generales para la decisión sobre el uso de un robot*. Roig P. México. Pp 9-13.

ISSN: 2448-8461

AP5

PATHOGEN SURVEY IN FARMED SHRIMP FROM THE PACIFIC COAST OF COSTA RICA

Cesar Marcial Escobedo-Bonilla[1], Nelson Peña-Navarro[2], Guillermo Hurtado Cam[2]
[1]Instituto Politécnico Nacional-CIIDIR Sinaloa, Aquaculture Dept. Blvd. Juan de Dios Batiz Paredes 250, Col. San Joachin, Guasave Sinaloa, C.P. 81101. Email: cesar_escobedomx@yahoo.com
[2]Universidad Técnica Nacional, Sede Pacífico, Subdirección de Investigación, Ingenieria en Acuicultura. Edificio Tobías Vargas en la Antigua Aduana, 100 metros al oeste del Muelle de Cruceros sobre el Paseo de los Turistas. Puntarenas, Costa Rica. Codigo Postal 60101.

Key words: Shrimp aquaculture, Pathogens, Costa Rica.

Introduction. Shrimp aquaculture is an important activity in many tropical countries. Nonetheless, the impact of infectious diseases on health status is largely unknown in various Central American countries (1). Costa Rica is one of the main shrimp farming countries in the region and they are threatened by the occurrence of infectious agents such as viruses and bacteria from Mexico and South America (2).
This study aims to survey the presence of viral and/or bacterial agents in farms along the Pacific coast, which is the main shrimp farming region in Costa Rica.

Methods. Farmed shrimp were sampled in 14 farms located in the Pacific Coast of Costa Rica through the rainy and dry seasons in 2017-2018. Tissues (foregut, hepatopancreas and pleopods) were collected and stored in 70% ethanol for PCR analyses using specific primers against viruses (white spot syndrome virus [WSSV], infectious hypodermal and haematopoietic necrosis virus [IHHNV]) and/or bacteria (*Vibrio parahaemolyticus*) causing acute hepatopancreas necrosis disease (AHPND). Presence of pathogens was recorded and the season when they occurred as well.

Results. Pathogens affecting semi-intensive culture systems were vibrios causing AHPND and IHHNV. The pathogen WSSV was not detected in any of the samples (Table 1).
The bacterium causing AHPND triggered moderate to high mortalities. The pathogen occurred at the end of the rainy season (November) in the Central Pacific region and at the end of the dry season (april) also in the south.
In contrast, the virus IHHN was found in four farms: three located in the southern part of Costa Rica and one located in the Gulf of Nicoya. The frequence of IHHNV in the south was high in the dry season.
It is possible that the origin of the larvae may determine the presence of pathogens, since all the larvae is imported from neighbor countries such as Guatemala, Honduras, Nicaragua, El Salvador and even from South America (Ecuador). This makes it difficult to avoid entrance of viral and/or bacterial pathogens to the country, since scarce monitoring is done for sanitary diagnostics in aquatic organisms. Therefore, it is necessary to promote technical and physical conditions to follow the sanitary status of larvae and shrimp entering the country.
The risk to introduce shrimp larvae infected with emerging pathogens, both viral and bacterial is high. Therefore, it is very important to develop sanitary measures and diagnostic facilities in order to cope with disease threats.

Table 1. Pathogens surveyed and found in the study.

Pathogen	Farms sampled	Farms found
V. parahaemolyticus	14	5
WSSV	14	0
IHHNV	14	4

Conclusions. Shrimp farming in Costa Rica is an increasingly important activity, which is threatened by the presence of infectious diseases such as AHPND causing important economic losses. It is necessary the continuous monitoring of larvae stocks coming from neighbor countries in order to determine the health status of cultured shrimp in Costa Rica and to reduce the risk of emerging diseases entering the country.

Acknowledgements. This study was funded by the projects FITTACORI F33-17 and SIP20180486.

References.

1. Cabrera, C., Gómez, R., Zúñiga, A. (2007). La resistencia de bacterias a antibióticos, antisépticos y desinfectantes: una manifestación de los mecanismos de supervivencia y adaptación. *Colombia Médica*. 38(2): 149–158.
2. Varela-Mejias, A., Peña-Navarro, N., Aranguren-Caro, L. (2017) Necrosis aguda del hepatopáncreas: una revisión de la enfermedad en *Penaeus vannamei*. Agron. Mesoam 28(3): 735-745. [online]. Available from: <http://www.scielo.sa.cr/scielo.php?script=sci_arttext&pid=S1659-13212017000300735&lng=en&nrm=iso>. ISSN 1021-7444. http://dx.doi.org/10.15517/ma.v28i3.27788.

ISSN: 2448-8461

AP6

Extracción química de beta glucanos de levaduras de origen marino

Norma A. Ochoa-Álvarez,[2,1] Ramón Casillas-Hernandez.[2] Francisco. Magallón-Barajas.[1]
1 Centro de Investigaciones Biológicas del Noroeste (CIBNOR), Instituto Politécnico Nacional Nº195. Col. Playa Palo de Santa Rita, La Paz, BCS, 23096, México
[2] Instituto Tecnológico de Sonora., Departamento de Ciencias Agronómicas y Veterinarias, Área de Acuicultura. 5 de febrero 818 sur Cd. Obregón, Sonora México.
nochoa04@cibnor.mx Norma Ochoa Álvarez
Palabras claves: Extracción química, biomasa, beta-glucanos

Introducción. Levaduras de origen marino han sido reportadas como fuente excelente de beta glucanos (inmunoestimulantes) ya que se ha demostrado que confieren protección a camarones peneidos en algunos casos de vibriosis o contra el virus de la mancha blanca (WSSV) (1 2, 3,4,).
El presente trabajo se concentró, en la producción de biomasa, así como la extracción de beta glucanos insolubles con métodos químicos (álcali-acido) con la finalidad de contribuir significativamente tanto al conocimiento de metodologías para combatir afectaciones por virus en el camarón, así como usarlo como opción preventiva contra patógenos. (5).

Métodos. Se empleó un tubo- tratamiento de cada cepa con un promedio 1 x 10^{11} UFC / ml y se inició con el escalamiento y obtención de biomasa. Los glucanos fueron extraídos de biomasa seca de levadura siguiendo el método Sukumaran y colaboradores (3) con modificaciones por métodos químicos y secados por liofilización.

Resultados.
Los resultados mostraron que la cepa de levadura con mayor rendimiento en la producción de biomasa (tabla 1) fue del género *Debaryomyces* (4.37 g/l en peso seco) y la de menor rendimiento fue para el genero Cándida (2.07 g/l). En relación a la extracción de glucanos las máximas cantidades extraídas fueron en la cepa *Debaryomyces* (0.87 g/ 2 g de biomasa seca) y la de mínima extracción fueron para las cepas Wickerhamomyces (0.05 g/ 2g de biomasa seca. (Tabla 2)

Clave	Nombre de genero	Volumen Empleado (litro)	Peso seco (g)
Pk	*Pichia*	1	3.99
Wa	*Wickerhamomyces*	1	2.28
42 A	*Cándida*	1	2.89
64	*Debaryomyces*	1	4.37
9 A	*Cándida*	1	2.07
N-4	*Cándida*	1	3.31
Sc	*Saccharomyces*	1	2.61

Tabla 1. Peso seco de la biomasa obtenida de las cepas de levaduras marinas y terrestres

Clave	Nombre de genero	Peso seco de biomasa (g)	Beta glucanos secado en liofilizadora
42 A	*Cándida*	2	490 mg
64	*Debaryomyces*	2	870 mg
9 A	*Cándida*	2	185 mg
N-4	*Cándida*	2	106 mg
Pk	*Pichia*	2	140 mg
Sc	*Saccharomyces*	2	211 mg
Wa	*Wickerhamyces*	2	50 mg

Tabla 2: Peso seco de los glucanos extraídos de las levaduras marinas y terrestres

Conclusiones. Considerando la diversidad de géneros que se está trabajando, los aislados variaron en su crecimiento y contenido de glucanos y en este trabajo se observa que se obtiene una mayor producción de glucanos en función de la biomasa.

Referencias.

1) Bai, N., Gu, M., Wenbing, Z., Xu, W. and Mai, K., 2014. Effects of β-glucan derivatives on the immunity of white shrimp *Litopenaeus vannamei* and its resistance against white spot syndrome virus infection. Aquaculture, 426–427, 66–73.

2) Deng, D., Mei, C., Mai, K., Tan, B.-P., Ai, Q. and Ma, H. (2013), Effects of a yeast-based additive on growth and immune responses of white shrimp, *Litopenaeus vannamei* (Boone, 1931), Aqu Resc (44): 1348–1357

3) Sukumaran, V., Douglas, W.L., Sajeevan, T.P. and Philip, R., (2010). Marine yeast glucans con4er better protection than that of baker's yeast in Penaeus monodon against white spot syndrome virus infection. Aqu Resc, (41), 1799-1805.

4) Wilson, W., Lowman, D., Swapna P. A., Puthumana, J., Bright Singh, I.S., Philip, R., (2015). Immune gene expression profile of *Penaeus monodon* in response to marine yeast glucan application and white spot syndrome virus challenge. F & S I, Volume 43, Issue 2, Pages 346-356.

5) Sarlin, P.J. y Philip, R., (2011). Efficacy of marine yeasts and baker's yeast as immunostimulants in Fenneropenaeus indicus: a comparative study, Aq, 321, 173-178.

ISSN: 2448-8461

AP7

BIOSORCIÓN DE CADMIO POR BIOPOLÍMEROS SINTETIZADOS POR BACTERIAS DE BIOPELÍCULAS INTERMAREALES

Juan Carlos Camacho-Chab[1], Benjamín Ortega-Morales[1], María Castañeda-Chávez[2], Manuel Chan-Bacab[1], Ruth Aguila-Ramírez[3], Itzel Galaviz-Villa[2], Pascual Bartolo-Pérez[4],Fabiola Lango-Reynoso[2] y Carolina Tabasco-Novelo[4]. [1.-] Universidad Autónoma de Campeche, Departamento de Microbiología Ambiental y Biotecnología (DEMAB). San Francisco de Campeche, Campeche 24039. [2.-] Instituto Tecnológico de Boca del Río, Laboratorio de Investigación en Recursos Acuáticos (LIRA). Boca del Río, Veracruz 94290. [3.-]Centro Interdisciplinario de Ciencias Marinas (CICIMAR), Laboratorio de Microbiología y Biología Molecular. La Paz, Baja California Sur 23096. 4.- Centro de Investigación y de Estudios Avanzados (CINVESTAV), Departamento de Física Aplicada. Mérida, Yucatán 97310.
*juanccam@uacam.mx

Palabras clave: bioremediación, biopolímeros, biopelículas, contaminación marina

Introducción. El cadmio es de los metales pesados con mayor presencia e impacto en el ambiente acuático (1). Uno de los métodos biológicos más eficientes para la remediación de metales pesados ha sido la biosorción. La biosorción es un proceso físico-químico por el cual una substancia se adhiere a un compuesto biológico, implica la adherencia física o unión de iones y moléculas sobre la superficie de otra molécula (2). Debido a su diversidad química, los biopolímeros microbianos son considerados una fuente importante de biomateriales con gran potencial en la biosorción de metales pesados (3).

Por lo anterior, en el presente trabajo se llevó a cabo la evaluación del potencial de adsorción de cadmio por dos biopolímeros microbianos nombrados Microbactano y MC3B-22, y su posible aplicación como un producto biotecnológico en la remediación metálica de agua de mar.

Métodos. Microbactano y MC3B-22 fueron caracterizados por su contenido de carbohidratos y proteínas por espectrofotometría de UV-visible. Al finalizar los ensayos de biosorción EPS-cadmio, las concentraciones de cadmio residual fueron determinadas por espectrometría de emisión óptica con plasma de acoplamiento inductivo (ICP-OES) y la capacidad máxima de adsorción fue determinada a través del modelo de equilibrio de isotermas de Langmuir. La composición y el estado de oxidación de los elementos de la superficie de ambos biopolímeros fueron determinados por espectroscopía de fotoelectrones de rayos X (XPS) antes y después del proceso de biosorción.

Resultados. Los resultados de adsorción de cadmio demostrarón que ambos biopolímeros presentaron actividad adsorbente de cadmio. El modelo de equilibrio de Langmuir permitió determinar que la capacidad máxima de adsorción de cadmio en agua fue de 97 mg g^{-1} para Microbactano y de 141 mg g^{-1} para MC3B-22 (Tabla 1).

Tabla 1. Parámetros de Langmuir (Q*max* y K) de la capacidad de biosorción de cadmio por ambos biopolímeros.

EPS	Q_{max} (mg · g^{-1})	*K* (L · mg^{-1})	R^2
Microbactan	97.12	0.254	0.954
MC3B-22	141.10	0.064	0.990

El análisis de XPS confirmó la actividad adsorbente de cadmio por ambos biopolímeros y reveló que la fracción correspondiente a la proteína es la que interviene en la remoción metálica (Figura 1).

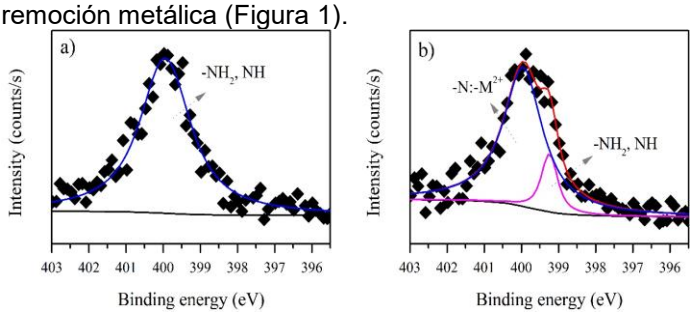


Fig.1 Espectro N1s XPS de Microbactano (a) antes y (b) después del proceso de biosorción.

Conclusión. Microbactano y MC3B-22 presentaron actividad biosorbente a cadmio con valores superiores reportados en otros biosorbentes.

Referencias.

1.- Wu, X.Y., Yang, Y.F. (2011). Heavy metal (Pb, Co, Cd, Cr, Cu, Fe, Mn and Zn) concentrations in harvest-size white shrimp *Litopenaeus vannamei* tissues from aquaculture and wild source. *J. Food Compost Anal.* 24, 62-65.

2.-Fomina, M., Gadd, G.M. (2014). Biosorption: current perspectives on concept, definition and application. *Bioresour. Technol.* 160, 3-14.

3.- Li W.W., Yu H.Q. (2014). Insight into the roles of microbial extracellular polymer substances in metal biosorption. *Bioresour. Technol.* 160, 15-23.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México

AP8

IDENTIFICATION OF SEXUAL DIFFERENTIATION GENES IN *HEMICHROMIS GUTTATUS*.

Jaqueline Flores Salinas[1], Karen Garza Cuéllar[2], Claudia J. Aguilar Díaz de León[2], Carlos J. Aguilera González[3], Roberto E. Mendoza Alfaro[3] and Dvorak Montiel Condado[2]. [1] Universidad de Monterrey. División de Extensión, Consultoría e Investigación. Dirección de Investigación. San Pedro Garza García, 66238. jaqueline.flores@udem.edu; [2] Universidad Autónoma de Nuevo León. Facultad de Ciencias Biológicas. Laboratorio de Ciencias Genómicas. [3]Laboratorio de Ecofisiología. San Nicolás de los Garza, 66455.

Key words: Cichlidae, Sexual determination, Sexual differentiation

Introduction. *Hemichromis guttatus*, an invasive introduced species in the Natural Protected Area of Cuatrociénegas, Coahuila, in Mexico, represents an evident threat to the local biodiversity as a result of its high reproduction rate, its aggressive behavior and its ability to outcompete endemic species. Thus, the employment of genetic biocontrol methods represents a crucial step in the eradication strategy.
Genes involved specifically in female gonadal development in the cichlid *Hemichromis guttatus* were analyzed in order to identify sexual determination mechanisms for the mentioned species.

Methods.
6 wild *H. guttatus* adults were caught in Cuatrociénegas. To obtain genomic DNA (gDNA) a traditional phenol:chloroform extraction was performed (*Chen, et al.*, 2007), and for total RNA extraction a modified version of trizol method (*Sambrook and Russell,* 2006) was employed. cDNA was prepared with the Accuscript (Qiagen) protocol and employing Omniscript Kit (Qiagen) reagents. The obtained genetic material was used in PCR reactions using oligonucleotides taken from *Böhne et al.*, (2013). Amplified gene fragments were purified, cloned and a Sanger automatic sequencing protocol was requested to obtain the genetic sequences.

Results. Amplification of *Ctnnb1b*, *Figla*, *Wnt4a* and *Foxl2a* gene fragments was achieved and, based on their known functions in other vertebrates, the following image shows the sexual determination mechanism proposed.

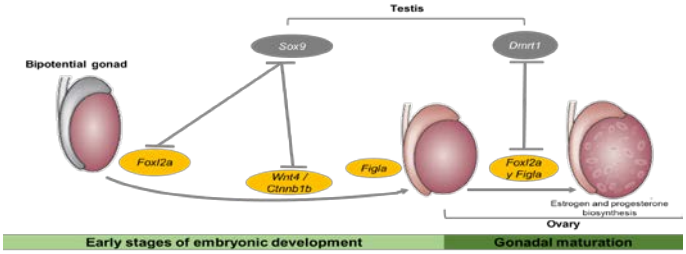


Figure 1. Female sexual determination mechanism in *H. guttatus*. *Foxl2a* and *Wnt4a*/*Ctnnb1b* pathways directly inhibit the activity of *Sox9*. Once inhibited, and during adulthood, *Figla* and *Foxl2a* are responsible for the formation of the ovarian follicle and inhibit *Dmrt1* activity.

Once the genetic sequences were obtained, several *in silico* analysis were performed between known orthologue sequences and *H. guttatus* sequence fragments. Results are summarized in the table below.

Table 1. Results from the *in silico* comparative analysis of the 4 gene fragment sequences of *H. guttatus*.

Gene	Size (pb)	Query cover	Similarity	Base change
Figla	70	98%	99%	21 C/T
Foxl2a	59	100%	97%	21 G/A 30 C/T
Wnt4a	75	100%	100%	NA
Ctnb1b	200	100%	98%	25 G/A 67 C/T 76 T/C 125 A/G

Conclusions. Amplification of transcript fragments from genes involved in the formation and maturation of ovary (*Figla*, 70pb), the production of estrogen (*Foxl2a*, 59pb), and in the process of blocking the expression of genes that promote male sexual differentiation (*Wnt4a*, 75pb-*Ctnnb1b*, 200pb), was achieved. Their identification and their sequence similarity with orthologue species, supports the suggested female sexual determination mechanism. Nowadays, we aimed to perform RT q-PCR experiments to determine the tissues and developmental stages where the genes are expressed.

Acknowledgements. We want to thank Elías Lozano, Sergio Luna and Carlos Barriga who participated in the capture, maintenance and reproduction of the specimens. We want to especially acknowledge CONABIO for the financial support given to the FCB, UANL, project LI003.

References.
1. Área de Protección de Flora y Fauna Cuatrociénegas. (2008). Comisión Nacional de Áreas Naturales Protegidas. Disponible en internet desde:http://www.conanp.gob.mx/acciones/fichas/cuatrocienegas/info/info.pdf [accessed: 10 December 2015].
2. Böhne, A., Heule, C., Boileau, N., & Salzburger, W. (2013). *Mol. Biol. and Evol.*, vol. 30(10): 2268–2285.
3. Chen, S., Li, J., Deng, S., Tian, Y., Wang, Q., Zhuang, Z., et al. (2007). *Marine Biotech.,* vol. (9): 273–280.
4. Sambrook, J., & Russell, D. (2006). *CSH Protoc.* prot4455. 10.1101/pdb.prot4455.

ISSN: 2448-8461

AP9

BACTERIOPHAGE *in vitro* EFFICACY AGAINST *Staphylococcus aureus* MULTI-RESISTANCE ISOLATED FROM BOVINE MASTITIS

Abner Gutiérrez-Chávez[1], Jaquelina Guzmán-Rodríguez[1], Iván Medina-Estrada[2], Fabiola León-Galván[1], Pedro Loeza-Lara[2], Eleazar Barboza-Corona[1], Karla Ramírez-Urbina[3], Arianna Lechuga-Arana[1], Mauricio Valencia-Posadas[1], Diana Ramírez-Sáenz[3]. [1]Universidad de Guanajuato, División de Ciencias de la Vida, Irapuato, Guanajuato, México, 36500; [2]Universidad de la Ciénega del Estado de Michoacán de Ocampo, México, 60154; [3]Agrobiotecnología del Bajío SA de CV, León, Guanajuato, México, 37390. Email: guca731023@hotmail.com; ajgutierrez@ugto.mxd

Key words: subclinical mastitis, phage-therapy, milk quality

Introduction. The most common method of treatment of bovine mastitis is the administration of antibiotics. However, this kind of strategy has shown some disadvantages including: low cure rate, increasing occurrence of resistance, and the presence of antibiotics residues in the milk (Gomes and Henriques 2016). Other problem of humanity faces is the rapid increase of antibiotic resistance seen amongst pathogenic microorganisms (Michael et al. 2014), which is increased by the marked reduction in the development of novel antibiotics (Norrby et al. 2005). The necessity of identifying novel methods to combat infections caused by antibiotic resistant bacteria is increasing each year. Because of the concern in the treatment of diseases caused by pathogens with multiple resistance to antibiotics, it has revived the interest in the development and use of the bacteriophage therapy to treat diseases in both animals and humans. Bacteriophages (phages) are viruses able to infect highly specifically and kill the bacterial species targeted but not eukaryotic cells (Haq et al., 2012).

The objective was to determine the bacteriophage in vitro efficacy against *S. aureus* multi-resistance isolated from bovine mastitis cases.

Methods. Somatic cell count of the milk samples was measured using a DeLaval Cell Counter DCC™. Seventy-two *S. aureus* isolated from subclinical quarters were sent to microbiological analysis. Bacterial cultures and susceptibility analysis were realized by mean Mueller-Hinton agar (Difco, BD, Sparks, MD) and standard disk-diffusion assays for Gram-positive bacteria (Bio-Rad, Hercules, CA, USA). The phage-susceptibility was evaluated by Spot Test Method (Armon and Kott 1993).

Results. All tested isolates exhibited a perceptible degree of resistance to antibiotics. Thirty-six *S. aureus* isolated of this study (50%) showed resistance from six to nine antimicrobial agents, mostly to penicillin, dicloxacillin, cefotaxime, ampicillin and cephalothin while resistance to ten or more antimicrobial agents was found in the rest of 36 isolates (50%) (Figure 1). Partial results (36/72 isolates) were observed for the effect of phage infection on isolates from SCM cases was observed in the 100% of *S. aureus* samples by mean display of clear zone of inhibition.

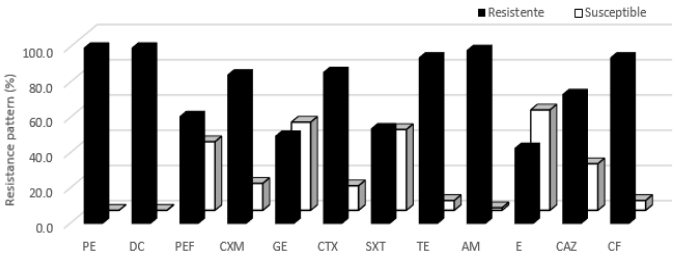


Fig.1 Percentage of sensitivity *in vitro* by standard disk diffusion (BioRad®) of different antibiotics against *S. aureus* isolates from bovine mastitis. Graphic bars represent the percentage of sensitive (white), intermediate (grey), or resistant (black). Penicillin (PE), dicloxacillin (DC), pefloxacin (PEF), cefuroxime (CXM), gentamicin (GE), cefotaxime (CTX), sulfamethoxazole-trimethoprim (SXT), tetracycline (TE), ampicillin (AM), erythromycin (E), ceftazidime (CAZ), cephalothin (CF).

Conclusions. Alternative approaches based on bacteriophages has proved to be useful as potential tool to eliminate the multi-resistance *S. aureus* isolated from subclinical bovine mastitis.

Acknowledgements. This research was supported by Grants from FINNOVATEG (CFINN0525) and Agrobiotecnología del Bajío SA de CV.

References.

1. Gomes F, Henriques M (2016) *Curr Microbiol* 72:377–382.
2. Michael C, Dominey-Howes D, Labbate M (2014). *Front Public Health* 2:145.
3. Norrby S, Nord C, Finch R (2005). *Lancet Infect Dis* 5:115-119.
4. Haq I, Chaudhry W, Akhtar M, Andleeb S, Qadri I (2012). *Virol J* 9:9.
5. Armon R, Kott Y (1993). *J Appl Bact* 74:490-496.

ISSN: 2448-8461

AP10

"EFFECT OF ENRICHMENT WITH ADDITIVES TO COMMERCIAL DIETS ON PRODUCTIVE AND PHYSIOLOGICAL VARIABLES OF WHITE SHRIMP *Litopenaeus vannamei*"

Ana Karen Gastélum-Contreras, Miguel Ángel Reyes-López, Manuel García-Ulloa Gómez, Carlos Ligne Calderón-Vázquez, Hervey Rodríguez-González, Departamento de Acuacultura, Centro Interdisciplinario de Investigaciones para el Desarrollo Integral Regional, Unidad Sinaloa, Guasave, Sinaloa, C.P. 81000; ana.karen.92@hotmail.com

Key words: white shrimp, prebiotics, digestibility

Introduction.

Pacific white shrimp, *Litopenaeus vannamei*, is increasingly important for aquaculture, a production with more than 3 million tons per year (1). However, intensification in cultivation techniques has led to a significant increase in the existence or spread of infectious diseases (2), which cause losses of billions of dollars to the shrimp-growing industry (3). Because of this research has focused on the establishment of alternatives for prevention, treatment and control of pathologies, so it is necessary to know the defense system of the culture organisms and understand how to nurture and modulate the different components (4). Among the alternatives used are different additives, which can have the activity of probiotics, immunostimulants, peptides antimicrobial or prebiotics. Because of that, it has been shown to positively affect the host by stimulating the growth and activity of certain species of beneficial bacteria in the gastrointestinal tract, which can improve the efficacy of growth and resistance to diseases of the host organism (5). Among the additives with prebiotic potential that have shown to exert a positive effect in terms of shrimp productivity is ß-glucan, Inulin and spirulina.

Objective.

To evaluate productive and physiological variables of *Litopeneus vannamei* white shrimp fed with commercial feed enriched with β-glucan, inulin and spirulina.

Materials and methods.

A bioassay was developed in humid laboratory of the aquaculture Department of the CIIDIR Sinaloa. 12 juvenile shrimps of *L. vannamei* from 0.75 ± 0.25 g were introduced in 24 experimental units with a capacity of 40 L. Feeding was 3 times a day (9:00, 13:00 and 17; 00 h), for 60 days.

Eight experimental diets were elaborated: Diet 1: commercial food (food control); Diet 2: commercial food + inulin (1g/Kg); Diet 3: commercial food + ß-glucan (0.6g/Kg); Diet 4: commercial food + Spirulina (5g/Kg); Diet 5: commercial food + inulin (0.8 g/Kg) + ß-glucan (0.6 g/Kg); Diet 6: commercial food + ß-glucan (0.6 g/Kg) + Spirulina (5g/Kg), Diet 7: commercial food + inulin (0.8 g/Kg) + Spirulina (5g/Kg); Diet 8: commercial food + inulin (0.8 g/Kg) + ß-glucan (0.6 g/Kg) + Spirulina (5g/Kg). During Bioassay temperature, salinity and pH were daily evaluated; every two weeks nitrite, ammonium, growth of organisms were evaluated, too, and at the end of the experiment, FCA (food conversion Factor), survival and digestibility were determined. The data were processed with the Statistical software

Results and conclusion.

The physicochemical parameters evaluated are within the limits established for the cultivation of shrimp, no significant difference between the treatments ($P > 0.05$). The final growth after 60 days was between 7.09 – 8.35 g. Organisms fed with diet 6 or 7 showed significantly higher growth ($P < 0.05$). Diets including spirulina and inulin increase growth in *Litopenaeus vannamei* shrimp.

References.

1 - Zeng, S., Huang, Z., Hou, D., Liu, J., Weng, S., & He, J. (2017). Composition, diversity and function of intestinal microbiota in pacific white shrimp (Litopenaeus vannamei) at different culture stages. *PeerJ*, *5*, e3986.
2 - Lightner, D. V. (2005). Biosecurity in shrimp farming: pathogen exclusion through use of SPF stock and routine surveillance. *Journal of the World Aquaculture Society*, *36*(3), 229-248.
3 - Huang, Z., Li, X., Wang, L., & Shao, Z. (2016). Changes in the intestinal bacterial community during the growth of white shrimp, Litopenaeus vannamei. Aquaculture research, 47(6), 1737-1746.
4 - Pohlenz, C and D. Gatlin. 2013. Nourishing the immune system of fish. Department of Wildlife and Fisheries Sciences Texas A&M University College Station, TX 77843, USA.
5 - Burr, G., Gatlin, D., & Ricke, S. (2005). Microbial ecology of the gastrointestinal fish tract and the possible application of prebiotics and probiotics in fish aquaculture. Journal of the World Aquaculture Society, 36 (4), 425-436.

ISSN: 2448-8461

"EFECTO DE LA CONCENTRACIÓN Y FRECUENCIA DEL β-GLUCANO EN EL CULTIVO DE CAMARÓN BLANCO *L. vannamei*"

Griselda Karina Gonzalez Felix; Dr. Hervey Rodríguez González, Dr. Víctor Manuel Peinado Guevara, Manuel García Ulloa Gómez, Sergio Medina Godoy, Carlos Rubén Sosa Aguirre; Departamento de Acuacultura, CIIDIR, Unidad Sinaloa, Guasave, Sinaloa, C.P. 81000; grisk_07@hotmail.com

Palabras clave: camarón blanco, β-glucano, concentración y frecuencia"

Introducción. La camaronicultura es una actividad económica de gran importancia, debido a la alta demanda del camarón como alimento, y a que representa una importante fuente de proteína de alta calidad disponible en gran parte del mundo (1).
La camaronícultura en México se encuentra en una etapa de busqueda de innovaciones en los alimentos y aditivos, después de haber decaído casi a la mitad en su producción registrada en los últimos años ocasionado principalmente, por enfermedades, las cuales, han tenido repercusiones no sólo de control biológico, sino económicas y sociales (2).
Por lo tanto, debido a que las tendencias dentro de esta actividad se inclinan hacia la utilización de alimentos con características óptimas para un sano crecimiento del camarón, pero que al mismo tiempo, asegure el consumo del alimento. Dentro de los últimos desarrollos científicos alimenticios, se ha incluido la aplicación de aditivos nutricionales ideales para evitar lixiviación y combatir enfermedades (3).
Los β-glucanos son elementos que se encuentran disponibles en la pared celular de una gran variedad de plantas, y una de las principales características por las que este compuesto ha generado interés dentro del área de nutrición acuícola, es por la función que desempeña en el sistema inmune de los crustáceos (4).

Objetivo. Determinar la factibilidad técnico-económica por la inclusión del aditivo β-glucano en la dieta de camarón *L. vannamei,* mediante la obtención de indicadores biológicos, productivos y comerciales en la operatividad de un sistema de producción semi-intensivo.

Metodología. Se llevó a cabo un bioensayo en laboratorio, alimentando a juveniles de camarón (0.35 ± 0.04 g), durante 60 días, con una densidad de siembra de 10 camarones/unidad experimental. Se aplicaron tres concentraciones del aditivo (β-glucano) en alimento comercial (0.2%, 0.4% y 0.8%), con dos frecuencias diferentes (diario y cada tercer día). Cada tratamiento fue por triplicado, y el tratamiento control fue un alimento comercial (Purina ®).

Una vez definido la concentración y frecuencia optima de β-glucano, se realizó un bioensayo en una granja comercial para determinar el efecto en variables productivas de *L. vannamei* (tasa de crecimiento específico, peso ganado, sobrevivencia, producción de biomasa y factor de conversión alimenticia). Así como, determinar el análisis de sensibilidad financiera e índices de rentabilidad (Tasa Interna de Retorno, Valor Actual Neto y la relación Costo-Beneficio) con y sin la aplicación del aditivo.

Resultados. Los resultados y discusión de este trabajo serán presentados, el día de la exposición en el mismo evento.

Agradecimientos. Se agradece al Instituto Politécnico Nacional por su apoyo.

Referencias.

1. Ayisi, C. L., Hua, X., Apraku, A., Afriyie, G., & Kyei, B. A. (2017). Recent Studies Toward the Development of Practical Diets for Shrimp and Their Nutritional Requirements. HAYATI *Journal of Biosciences*. doi: https://doi.org/10.1016/j.hjb.2017.09.004
2. Servicio de Información Agroalimentaria y Pesquera, S. (2018). *Avance mensual de la producción pesquera 2017 (Camarón)*. México: Retrieved from: http://pesca.siap.gob.mx/produccion/rep-mensual_est_opc_gobmx.php?tipo=0&anioRep=2017&entidadFed=90.
3. Martínez-Córdova, L. R., Martínez, P., & Cortés-Jacinto, E. (2009). Mexican and world shrimp aquaculture: sustainable activity or contaminant industry*? Revista internacional de contaminación ambiental,* 25(3), 181-196.
4. Vargas-Albores, F., & Yepiz-Plascencia, G. (2000). Beta glucan binding protein and its role in shrimp immune response. *Aquaculture, 191*(1-3), 13-21.
4. Servicio de Información Agroalimentaria y Pesquera, S. (2018). *Avance mensual de la producción pesquera 2017 (Camarón)*. México: Retrieved from: http://pesca.siap.gob.mx/produccion/rep-mensual_est_opc_gobmx.php?tipo=0&anioRep=2017&entidadFed=90.

ISSN: 2448-8461

AP12

ANTIBIOFILM POTENTIAL OF SHALLOW HYDROTHERMAL VENTS-ASSOCIATED MARINE BACTERIA

Gutiérrez-Almada Karla M., Aguila-Ramírez Ruth N., González-Acosta Bárbara.
IPN-CICIMAR, Microbiology and Molecular Biology Lab., La Paz, B.C.S. Zip Code 23096
karla.gtz.al@gmail.com

Key words: Quorum sensing, Pathogens, Biofouling

Introduction. Marine bacteria associated to shallow hydrothermal vents are becoming increasingly attractive for biotechnology because they have adapted to survive extreme physicochemical conditions, reflected in the compounds them produce.
In this work we analyze the potential of the culturable bacterial fraction as a producer of compounds with biofilm inhibitory activity for future biomedical applications and biofouling prevention.

Methods. Water and sediments samples were collected from three shallow hydrothermal vents in B.C.S. Bacterial strains were isolated and purified on marine agar at 35 and 60°C. We carried out a degrading assay of autoinducers AHL type (C6HSL) involved in the quorum sensing process by preparing sterile filtered supernatants from cultures and testing the samples with *Chromobacterium violaceum* CV026 as a biosensor, bioassay was realized using well diffusion technique. The qualitative assay for biofilm formation was performed from organic extracts obtained with ethyl acetate from the active strains and they were tested in 96-well microplates. Three pathogenic strains: *Pseudomonas aeruginosa* PAO1, *Aeromonas caviae* and *Vibrio parahaemolyticus* M8, and two strains identified as biofilm producers on submerged surfaces (*Virgibacillus* sp. C29 and *Vibrio alginolyticus* C96) were used to evaluated the microplate test. The absence/presence of the biofilm was detected with violet crystal staining in the previous 96-well microplates. Finally, a molecular identification of the active strains was carried out.

Results. The 14% of the isolated strains showed degrading activity of C6HSL molecules (10 mesophiles and 13 thermotolerant strains). Twenty-three organic extracts were obtained. Some thermotolerant strains, mostly *Bacillus*, produce compounds that inhibit bacterial biofilms (*B. licheniformis, B. paralicheniformis, B. firmus, B. oceanisedimenis, B. aerius and B. sonorensis*). As well as other bacteria such as *Vibrio alginolyticus* and *Brevibacillus thermoruber* showed activity.

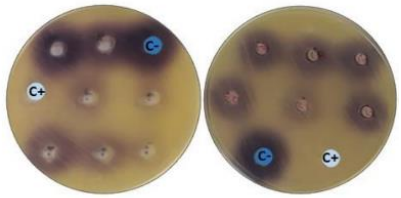


Fig.1 Some results of the isolated bacteria supernatants that degrading AHLS molecules from *C. violaceum* CV026.

Conclusions. Thermotolerant strains showed higher antibiofilm activity than mesophiles probably because at these temperatures they synthesize more active compounds. We concluded that these environments are an accessible source of culturable microorganisms that synthesize biofilms inhibitors compounds and therefore they have biotechnological potential.

Acknowledgements. CONACYT, IPN, BEIFI, CICIMAR-Microbiology and Molecular Biology Lab.

References.

1. Nithya, C., Begum, M. F., & Pandian, S. K. (2010). Marine bacterial isolates inhibit biofilm formation and disrupt mature biofilms of Pseudomonas aeruginosa PAO1. *Appl Microbiol Biotechnol*, 88(1), 341-358.
2. Stepanović, S., Vuković, D., Dakić, I., Savić, B., & Švabić-Vlahović, M. (2000). A modified microtiter-plate test for quantification of staphylococcal biofilm formation. *J Microbiol Methods*, 40(2).
3. McClean, K., Winson, M., Fish, L., Taylor, A., Chhabra, S., Camara, M., Daykin, M., Lamb, J., Swift, S., Bycroft, B., Stewart, G., & Williams, P. (1997). Quorum sensing and *Chromobacterium violaceum*: exploitation of violacein production and inhibition for the detection of N-acylhomoserine lactones. *Microbiology*, 143(12), 3703-3711.

ISSN: 2448-8461

AP13

COMPUTATIONAL ANALYSIS OF THE EFFECT OF nsSNP´s ON *SNCA Bos taurus* PROTEIN

F.A. Paredes-Sánchez[1], A. M. Sifuentes-Rincón[1], E Lara[2]
[1]CBG, Instituto Politécnico Nacional, Reynosa, Tamps 88710, México; [2] UIBMZ, Instituto Mexicano del Seguro Social, Zacatecas, Zac 98000, México

Key words: Bioinformatics, Cattle temperament, nsSNP´s

Introduction. Cattle temperament, defined as the animal's response to handling, has been studied in different breeds and has been shown to affect growth, health, and carcass quality [1]. A GWAS analysis identified *SNCA* as a gene associated with beef cattle temperament. According to Gene Bank, *SNCA* has 5 exons that code for a protein of 140 aa. In the coding regions there are 16 SNPs, of which the markers rs876209903 and rs876349718 of exon 1 cause a change of Alaline by Proline (A> P) and Arginine by Lysine (R> K) respectively; whereas the markers rs876537338 and rs876598068 of exon 3 cause a change of Histidine by Glutamine (H>Q) and Methionine by Leucine (M> L). There is no information in cattle about the potential effect of these mutations, here we focused to explore it, using bioinformatic tools.

Methods. A functional and structural analysis was achieved using a 3D homology model for the bovine wild-type *SNCA* (wtSNCA) protein constructed with SWISS-MODEL. The aa changes produced by each nsSNPs were inserted in the wtSNCA protein (i.e., A30P, R32K, H99Q, M100L and its haplotypes AR3032PK, HM99100QL, ARHM303299100PKQL), and seven mutant SNCA models (mutSNCA) were generated. The RMSD values were calculated by superimposition of the modelled structures. We performed binding site predictions for the bovine SNCA protein. To conduct a protein-protein docking study of the wtSNCA and mutSNCA and its interacting partner protein bovine synphilin-1 (SNCAIP), the ClusPro serve was used.

Results. The RMSD calculations indicate that there were slight deviations between the wtSNCA structure and the mutSNCA structures, which in turn change their functional activity.

Additionally the results of binding site prediction revealed a ligand binding site involving amino acids E28, G31 and R32 (p-value 2.28 E-07). The mutation R32K directly affects this identified binding site.

Analysis of polar interactions between mutSNCA proteins with the coiled-coil domain of its interacting SNCAIP showed that the variations H99Q, M100L and MH99100QL conserve a similar interaction pattern compared to that of the wtSNCA.
Interestingly, the haplotype AR3032PK causes a complete change in the interaction pattern compared to that of the wtSNCA-SNCAIP docking complex, both in the number and identity of the interacting amino acids (Fig. 1).

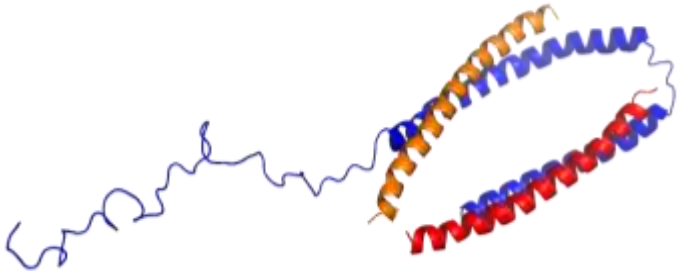


Fig.1 . Interaction pattern of complex wtSNCA-SNCAIP (blue α-helices and red α-helices respectively). Interaction pattern of the complex AR3032PK -SNCAIP (blue α-helix and orange α-helix respectively).

This haplotype causes the interaction pattern of wtSNCA and SNCAIP to change from an N-terminal A2 lipid-binding alpha helix domain to a non-amyloid β component (NAC) domain.

Conclusions. The non-synonymous mutations A30P and R32K reported for the SNCA gene flank the binding site and change the interaction patterns with the SNCAIP interacting protein.

Acknowledgements. This investigation was funded by a IPN project (SIP 20171674).

References.

1 Hulsman L. L, Garrick H. J, Gill C. A, Herring H. D, Riggs P. K, Miller R. K, Sanders J. O, Riley D. G. 2016. Genome-wide association study of temperament and tenderness using different Bayesian approaches in a Nellore–Angus crossbred population. Livestock Science. 161: 17–27.

ISSN: 2448-8461

AP14

PEARLIN, A PROTEIN INVOLVED IN MOLLUSKS BIOMINERALIZATION WITH POTENTIAL IN BIOMATERIAL SYNTHESIS

Raquel G. Arroyo, Crisalejandra Rivera, Norma Y. Hernández; Centro de Investigaciones Biológicas del Noroeste (Department of Fisheries Ecology), La Paz (612); rarroyo@pg.cibnor.mx

Key words: pearlin, biomineralization, biomaterials

Introduction. Shell formation in mollusks is the biomineralization of calcium carbonate, mainly in two polymorphs: aragonite and calcite. It is controlled by specialized epithelial cells of the mantle, which is the secretory organ of all the necessary compounds for crystal formation known as organic shell matrix[1]. This matrix is formed by polysaccharides, glycoproteins, β-chitin and proteins; some molecules of the matrix are incorporated into the inorganic (intracrystalline) phase, while others form a base around the crystals (organic matrix)[3]. The organic matrix has proteins known as Shell Matrix Proteins (SMPs) that assure the interaction between macromolecules and minerals, as well as modulate the arrangement, nucleation and growth of aragonite and calcite crystals for shell nacre formation. The study of the SMPs and their biochemical properties, as well as their role in the formation of nacre and, therefore, of calcium carbonate polymorphs, is important at a biological and biotechnological level. On one hand, it allows us to know the first line of defense of mollusks and, on the other hand, its potential use in the production of biomimetic materials for the medical industry[4], since it has been reported that these proteins are biocompatible to osteoblast cells, which form the human bone[2].
The main objective of this work is to characterize the pearlin of two model organisms which produce nacre, a bivalve (*Pteria sterna*) and a gastropod (*Haliotis fulgens*).

Methods. Proteins from the shell of *P. sterna* and *H. fulgens* were obtained by the acetic acid method, soluble and insoluble proteins were analyzed by several stain methods after electrophoresis (CBB, PAS, Stains All, silver nitrate). To identify the native pearlin from the soluble and insoluble fractions, a specific antibody anti-pearlin was tested by western blot in both species[5]. The identified pearlin was purified by preparative electrophoresis and characterized by the previous described stains. The identity of the pearlins will be evaluated by Mass Spectrometry.

Results. Shell matrix proteins, soluble (ASM, acid acetic soluble matrix) and insoluble proteins (AIM, acid acetic insoluble matrix), were obtained from the shell of *P. sterna* and *H. fulgens* after 10% acetic acid extraction. The ASM and AIM fractions were evaluated by CBB and silver nitrate stains to visualize all the proteins present. Two bands were identified, one of 16 kDa (*P. sterna*) and another of 15 kDa (*H. fulgens*) in the AIM (but not in the ASM). The identified bands were recognized by a Western Blot with a specific anti-pearlin antibody. The pearlin corresponding to the AIM from *P. sterna* displayed glycosylations in the PAS stain; however, with Stains All stain (which recognizes calcium binding proteins by staining them blue) indicated no Ca^{2+} binding. For pearlin from *H. fulgens*, showed a molecular weight of 15 kDa band, which didn't present glycosylations, but it possessed calcium binding interactions.

Conclusions. The pearlins from *P. sterna* and *H. fulgens*, although having a similar molecular weight, they present different biochemical characteristics: *P. sterna* pearlin is glycosylated while the one from *H. fulgens* is not, however, the last shows calcium binding capacity, while the first does not. These differences can indicate they are regulated by different mechanisms, having different post-translational modifications which can affect their folding, their location in the shell matrix, and even their function in shell formation. Further analysis needs to be done to corroborate these characteristics, as it is shown by some previous work that stains and assays results can differ[1] and to evaluate if both pearlins have the same function in shell formation.

Acknowledgements. To CONACyT for the granted scholarship (358437/242891) and to the Project 253040 PEI-CONACyT.

References.

1. Bahn, S. Y., Jo, B. H., Hwang, B. H., Choi, Y. S., & Cha, H. J. (2015). *Cryst Growth Des*, *15*(8), 3666–3673.
2. Huang, G., Bi, X., Zhang, B., Qu, T., Liu, B., Fan, S., & Yu, D. (2017). *J Shellfish. Res*, *36*(2), 373-377.
3. Levi-Kalisman, Y., Falini, G., Addadi, L., & Weiner, S. (2001). *J Struct Biol*, *135*(1), 8-17.
4. Ma, J.Y., Wong, K.L., Xu, Z.Y., Au, K.Y., Lee, N.L., Su, C.,Shaw, P.C. (2016). *J Nat Prod*, *79*(1), 204-212.
5. Montagnani, C., Marie, B., Marin, F., Belliard, C., Riquet, F., Tayalé, A., Cochennec-Laureau, N. (2011). ChemBioChem, 12(13), 2033-2043.

ISSN: 2448-8461

AP15

AN EFFICIENT PROTOCOL FOR THE *AGROBACTERIUM*-MEDIATED GENETIC TRANSFORMATION OF THE GREEN MICROALGAE *DUNALIELLA TERTIOLECTA*

Claudia D. Norzagaray-Valenzuela, Angel Valdez-Ortiz, Lourdes J. Germán-Báez*; Facultad de Ciencias Químico-Biológicas, Universidad Autónoma de Sinaloa, Culiacán, Sinaloa, México, Cp. 80030. janeth_882@hotmail.com

Key words: Dunaliella tertiolecta, Genetic transformation, Agrobacterium tumefaciens

Introduction. Microalgae are photosynthetic microorganisms widely used for the production of highly valued compounds with diverse applications in food, cosmetics and pharmaceuticals [1,2]. Recently they have been shown to be promising as a system for the heterologous expression of proteins. This study aimed to find an optimized genetic transformation method for *Dunaliella tertiolecta* using the *A. tumefaciens* system by optimizing the transformation conditions: infection medium (liquid and solid), *Agrobacterium* concentration (OD_{600} = 0.5, 1.0 and 1.5) and co-culture times (24, 48 and 72 h).

Methods. The microalgae *D. tertiolecta* (strain DUT2) was infected by *A. tumefaciens* strain EHA105::pCAMBIA1304. Through the combination of 3 critical parameters, 18 independent experiments were designed, which were compared through the Transformation Efficiency Index (TEI) [3], using multifactorial analysis of variance and means were compared using the Fisher's test ($p<0.5$) using Statgraphics Centurion XV software.

Results. Transformed cells were stained blue, whilst untransformed cells did not retain any staining (Fig. 1). Statistical analysis of TEI values showed five treatments as the best transformation conditions (Fig. 2); these were used to repeat the genetic transformation procedure, and each treatment was driven until molecular analysis colonies. PCR screening of hygromycin-resistant colonies is shown in Table 1. The tx 12 (solid infection medium, OD_{600}=0.5 and 72 h of co-culture) showed the highest percentage of transformation efficiency (~20%).

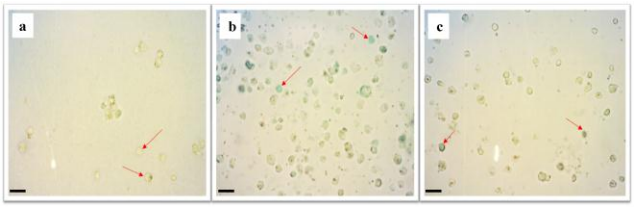


Fig.1 Biochemical assay of *gusA* expression. a) *D. tertiolecta* non-infected cells used as negative control; b) and c), *Agrobacterium*-infected *Dunaliella* cells from solid and liquid medium respectively. Bars represent 20 μm.

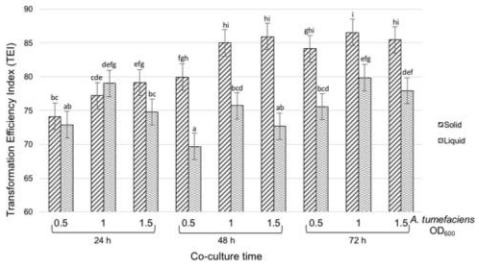


Fig.2 TEI of 18 independent experiments tested for transformation of *D. tertiolecta*. Each value is the mean of three independent experiments. Errors bars indicate the LSD value (3.88)

Table 1. Stable transformation efficiency in *Dunaliella tertiolecta* cells

Treatment	No. analyzed colonies	*gusA* (PCR+)	% Transformation efficiency
tx 12 (solid infection medium, OD_{600}=0.5, 72 h)	26	5	19.23
tx 14 (solid infection medium, OD_{600}=1.0, 48 h)	32	1	3.13
tx 15 (solid infection medium, OD_{600}=1.0, 72 h)	23	2	8.70
tx 17 (solid infection medium, OD_{600}=1.5, 48 h)	31	1	3.23
tx 18 (solid infection medium, OD_{600}=1.5, 72 h)	35	1	2.86

Conclusions. An optimized method for genetic transformation of *D. tertiolecta* by *A. tumefaciens* has been developed. The protocol is based on the use of Agro-infection, OD_{600}=0.5, in a solid medium and 72 h of co-ture; opening the possibility for further genetic manipulation of this commercially-important microalgae for biotechnological applications.

Acknowledgements. This project was supported by grants from Universidad Autonoma de Sinaloa (PROFAPI2015/113) and Consejo Nacional de Ciencia y Tecnología-México (Programa Ciencia Básica de CONACyT-2015, 255198).

References.

1.Santos-Ballardo DU, Rossi S, Reyes-Moreno C y Valdez-Ortiz A (2016) *Reviews in Environmental Science and Bio/Technology* 15(2): 243-264.

2.Norzagaray-Valenzuela C, Valdez-Ortiz A, Shelton L, Jiménez-Edeza E, Rivera-López J, Valdez-Flores M, Germán-Báez L (2017) *J Appl Phycol* 29:189-198.

3.Norzagaray-Valenzuela C, Germán-Báez L, Valdez-Flores M, Hernández-Verdugo S, Shelton L, Valdez-Ortiz A (2018) *J Microbiol Methods* 150:9-17.

ISSN: 2448-8461

AP16

ISOLATION AND CHARACTERIZATION OF POTENTIAL PROBIOTIC BACILLI FROM CRAB *CALLINECTES BELLICOSUS*

Paulina Serrano-Espinosa; Antonio Luna-González, Genaro Diarte-Plata, Ruth Escamilla-Montes, Arturo Fierro-Coronado, Karime Valdez-Chávez; Instituto Politécnico Nacional CIIDIR-Sinaloa, Guasave, Sinaloa C.P. 81049, paumserrano@hotmail.com

Key words: probiotics, crab, Callinectes bellicosus

Introduction. In Sinaloa and Sonora, the commercial fishing of crabs of the genus *Callinectes* has caused a decrease in their populations (1). For that reason, research is needed on the cultivation of these crabs, including environmentally friendly additives (such as probiotics) against bacterial and viral diseases (2).
In this work, bacilli with probiotic potential were isolated and characterized from wild organisms and tested in shrimp against a bacterial disease.

Methods. Presumptive bacilli were isolated from crab. Each intestine was homogenized in sterile saline and heated at 80°C for 10 min. The isolation was done on plates with tryptic soy agar medium (TSA) by cross streak method. The resulting isolates were characterized through a series of tests (Table 1). As there was no crab larvae, a bioassay (12 d) of protection against *Vibrio parahaemolyticus* was performed on white shrimp postlarvae (200 ± 10 mg) cultured in glass tanks with 4 L of seawater and constant aeration. Strains (10^6 CFU/mL) were inoculated in water every 3 d. Five treatments were tested in triplicate (Fig. 1). The cleaning and replacement of water was done every 3 d. Survival results were analyzed by one-way ANOVA and Tukey ($p < 0.05$).

Results. Two strains of presumptive bacilli, with potential as probiotics, were isolated mainly by having gamma hemolysis (Table 1). Shrimp survival was significantly different ($p<0.05$) in treatments IV and V as compared with positive control (Fig. 1).

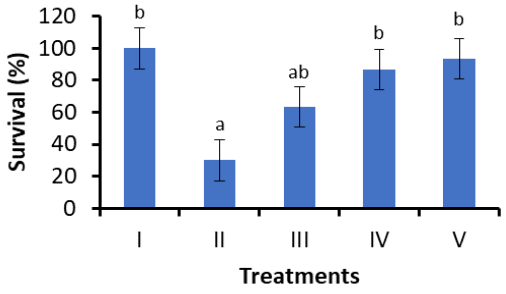


Figure 1. Survival of *L. vannamei* challenged with *V. parahaemolyticus*. Treatments: I) negative control; II) positive control (*Vibrio* CL_{50}); III) *B. licheniformis* BCR 4-3; IV) strain 3; V) strain 4.

Table 1. Characterization of isolates 3 and 4.

Characterization	Strain 3	Strain 4
Gram stain	+	-
Shape	bacilli	bacilli
Hemolysis	γ	γ
Hydrophobicity (%)		
p-xylene	16.18	11.88
Autoaggregation (%)	83.33	18.18
Biofilm		
24 h	0.036±0.019	0.046±0.012
48 h	0.046±0.016	0.016±0.010
Bacterial growth kinetic		
Lag phase	0 - 6	0 - 9
Log phase	6 - 48	9 - 24
Stationary	48 - 144	24 - 96
CFU/mL	206,500,000	3,000,000
Salinity tolerance		
05 – 2	+	+
3 – 5	+	-
6 – 8	+	-
9 – 12	+	-
pH tolerance		
4	-	-
5 – 7	+	+
8 – 10	+	-
Extracellular enzymatic activity		
Protease (casein)	1.5	-
Protease (grenetin)	-	-
Lipase (Tween 80)	1	-

Conclusions. Strains 3 and 4 showed a probiotic effect in white shrimp by increasing survival when they were challenged with *V. parahaemolyticus*, the causative agent of AHPND.

Acknowledgements. We recognize the financial support of Instituto Politécnico Nacional and CONACYT.

References.

1 Cisneros MMA, Ramírez FE, García BJA, Fernández LVC, Labastida CA, Gómez RC & Madrid VJ. 2014. Pesca de jaiba en el litoral del Pacífico Mexicano. Instituto Nacional de Pesca, p.86
2 Seenivasan C, Radhakrishnan S, Muralisankar T & Saravana Bhavan P. 2014. Proc. Zool. Soc. 69(1): 52-60.

ISSN: 2448-8461

BASIC CONSIDERATIONS IN THE STUDY OF RECOMBINANT MUREIN HYDROLASES TO AVOID ARTIFACTS

Lina Zermeño-Cervantes, Sergio Martínez-Díaz, César Cardona-Félix
Instituto Politécnico Nacional (IPN), Centro Interdisciplinario de Ciencias Marinas (CICIMAR), Departamento de Desarrollo de Tecnologías, La Paz, México; violinrojo_17@hotmail.com.

Key words: murein hydrolases, autolysins, zymograms, negative control of protein purification.

Introduction. Recently, the study and characterization of murein hydrolases to combat bacterial pathogens has increased. Its great structural and biochemical diversity offers the potential to apply them against a broad strains spectrum in multiple conditions (1). However, for their study, they are obtained mainly via recombinant, using bacterial expression systems in most cases (e.g. *Escherichia coli*) (2). *E. coli* naturally produce a large number of these enzymes, so-called autolysins, that accomplish multiple functions, as bacterial cell wall growth, division, turnover among others (3). Nevertheless, in these studies, the visualization of a single band in acrylamide gels is taken as indicative of purity.

The aim of this study was to demonstrate the importance of including a negative control of the purification, and running zymograms using renaturation conditions of the *E. coli* autolysins, to verify that the recorded activity is only of the recombinant protein. A practice that should be usual in the study of these enzymes, which however was not evident in an exhaustive bibliographic search.

Methods. The purification protocol established to obtain a phage murein hydrolase was used to obtain the elution from *E. coli* Rosetta 2 strain without transformation. The enzymatic activity of elution was evaluated by turbidity reduction assay and zymography.

Results. The purification protocol used for recombinant murein hydrolase, co-purifies an autolysin from *E. coli* that is not visualized in acrylamide gels stained with Coomassie blue. However, its activity was evidenced in the elution obtained from non-transformed *E. coli* (Fig 1b, RMH). In addition, the zymogram revealed that autolysin has a molecular weight of ≈25 KDa (Fig. 1b, E-R2), which is present in the recombinant murein hydrolase elution (Fig. 1a, RMH), and coincides with one band of the lytic extract obtained from *E. coli* (Fig. 1b, R2 aut).

Conclusions. When working with catalytic recombinant proteins, which are also encoded by the expression system used, it is necessary to include these controls once a purification protocol has been established, and the activity was verifies, in order to rule out artifacts due to the activity of some protein produced by *E. coli* with equivalent activity to the interest protein, and that could be eluted with the established purification conditions. In this study, an autolysin contributes to the activity recorded in the recombinant murein hydrolase elution.

Acknowledgements. Funding was provided by Consejo Nacional de Ciencia y Tecnología of Mexico (CONACYT grants 247842 and 222100). LAZC acknowledge Doctoral fellowship provided by CONACYT No. 349005/ 239000.

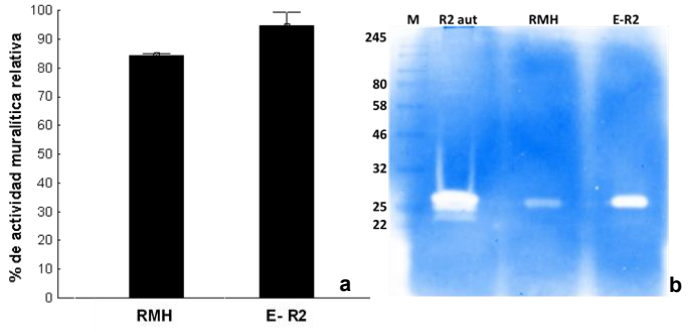


Figure 1. a) Enzymatic activity test of the recombinant murein hydrolase purified from Rosetta 2 (RMH) and of elution obtained from non-transformed Rosetta 2 purification (E-R2, negative control of purification). Relative muralytic activity is measured as the slope of the OD600 nm min^{-1} curve, given in percentage as compared to the treatment with the highest activity recorded (the highest slope is 100% relative activity). Average, and standard error are shown (n=3). **b)** Zymogram at 10% acrylamide. Renaturation buffer for *E. coli* autolisyns (PB 20 mM, 10 mM $MgCl_2$, pH 7.0, 0.1 % Triton X-100). M, molecular weight marker; R2 aut, Rosetta 2 autolysin extract; RMH, elution of recombinant protein; E-R2, elution from non-transformed Rosetta 2 purification. Clear bands indicate lytic activity of renatured proteins with a molecular mass of ≈ 25 KDa.

References.

1. Gräslund, S., Nordlund, P., Weigelt, J., Hallberg, B. M., Bray, J., Gileadi, O… & Ming, J. (2008). *Nature methods*, *5*(2), 135.
2. Schmelcher, M., Donovan, D. M., & Loessner, M. J. (2012). *Future microbiology*, *7*(10), 1147-1171.
3. Vollmer, W., Joris, B., Charlier, P., & Foster, S. (2008). *FEMS microbiology reviews*, *32* (2), 259-286.

ISSN: 2448-8461

AP18

RATIONAL DESIGN OF CHIMERIC ENDOLYSIN AGAINST *Vibrio parahaemolyticus*

Zulema Juárez, César Cardona, Bárbara González & Lina Zermeño; Centro Interdisciplinario de Ciencias Marinas; Technology Development Department; La Paz, México; C.P. 23096; marizujuarez@yahoo.com.mx

Key words: Vibrio, chimeric protein, rational design

Introduction. Bacterial resistance is a particularly critical problem for pathogenic Gram-negative bacteria due to its outer membrane (1). Therefore, the rational design of chimeric proteins to generate antimicrobials with potentiated activity (2) and a lower probability of generating said resistance has been proposed as an alternative (3). In this work we carried out the rational design of a new chimeric endolysin (called LysVPMS1-PCNP), from the structural analysis of the wild-type endolysin (LysVPMS1) from the phage VPMS1, which we know exerts lytic activity on sensitized cells of the genus *Vibrio* (4).

The aim of this study was potentiate the muralytic activity against *Vibrio parahemolyticus*, due to the serious economic losses caused by this bacterium in the aquaculture industry.

Methods. The *ab initio* three-dimensional structure of wild and chimeric endolysin was predicted using Quark online software. Molecular coupling analysis (AutoDock vina 1.11.2) was performed using cell wall monomer as a ligand and then a Gram-negative cell wall fragment. The recombinant protein was obtained and finally the bacteriolytic activity was evaluated in sensitized *Vibrio parahaemolyticus* cells.

Results. *In silico* analysis determined the presence of Glutamic 135 amino acid in LysVPMS1 catalytic sites. Molecular coupling in this site was -5.3 kcal/mol. The best coupling in LysVPMS1-PCNP was Glutamic 144 (-5.44 kcal/mol). Furthermore, the N-terminal domain exposed in three-dimensional structure of wild-type enzyme was observed, as well as fusion peptide in chimera. The relative muralytic activity of chimera endolysin was 5.8 times higher than wild endolysin (**Fig.1**).

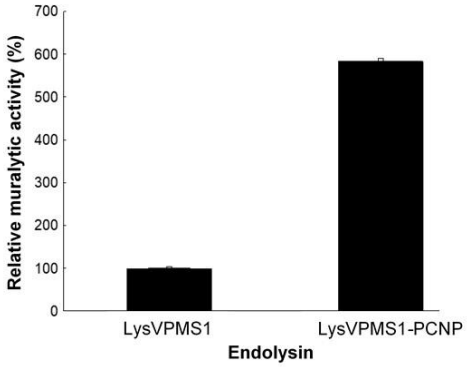


Fig. 1 Comparison of relative muralytic activity of wild and chimera endolysin.

Conclusions. Chimera endolysin design was experimentally verified throw successful expression and activity verification. Both stability indicators, since proteins are active only when their structural conformation is adequate.
The increase in muralytic activity is attributed to fused peptide as it causes an increase in protein concentration in substrate cell wall by interactions of peptide positive charges with polyanionic cell wall surface.

Acknowledgements. To CONACyT for financing Project Number 247842: "*Endolisinas fágicas recombinantes: Nueva estrategia para el control de bacterias patógenas en cultivos de importancia acuícola en México*", and Master's scholarship (738218). To Microbiology and Molecular Biology laboratory in CICIMAR-IPN for all facilities to carry out this study.

References.

1. Rodríguez-Noriega, E., León-Garnica, G., Petersen-Morfín, S., Pérez-Gómez, H., González-Díaz, E. &Morfín-Otero, R. (2014). *Biomedica, 34: 181-190.*
2. Schmidt, S. (2009). *Curr Opin Drug Discov Devel*, 12(2): 284-295.
3. Briers, Y., Walmagh, M., Puyenbroeck, V., Cornelissen, A., Cenens, W., Aertsen, A., Verween G., Pirnay, P., Miller, S., Volckaert, G. & Lavigne, R. (2014). *mBio 5(4): 1–10.*
4. Zermeño-Cervantes, L. (2018). *Aquacult Res 49:1723-1726.*

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México

AP19

ADVANCES IN THE REPRODUCTIVE CYCLE OF ECHINOMETRA VANBRUNTI (ECHINODERMATA:ECHINOIDEA) IN BAHIA ESTELA, SONORA

Naomi Yerith Reyes Jiménez, Ana Gloria Villalba Villalba, Amir Maldonado Arce, Reina Castro Longoria, Christian Minjarez Osorio; Universidad de Sonora, Department of Scientific and Technological Research, Department of Physics; Hermosillo, Sonora 83000; naomiyreyesj@gmail.com

Key words: Reproductive cycle, sea urchin, Sonora

Introduction. The sea urchins are a group of marine invertebrates that play an important role in ecosystems. Besides the ecological importance, sea urchins are highly valued in the market for their gonads, used for human consumption (1) and are valuable sources of bioactive compounds.

The objective of this work is to describe the annual reproductive cycle of the *Echinometra vanbrunti* in Bahia Estela, Sonora in the August 2017-August 2018 period.

Methods. Organisms were collected in Bahía Estela, Sonora, by diving, at a depth of 1-2 m. The length and weight of the organisms was recorded. The gonadosomatic index was determined based on the total weight of each organism and the fresh weight of the gonads. The histology of the gonads was performed, and water parameters were determined at the sampling point: temperature, dissolved oxygen, pH and salinity.

Results. The average length test was 6.56 ± 6.68 cm, 6.55 ± 1.05 cm, 7.0 ± 0.54 cm, 6.4 ± 0.92 cm, 6.5 ± 0.60 cm y 6.6 ± 0.21 cm in August to January, respectively.

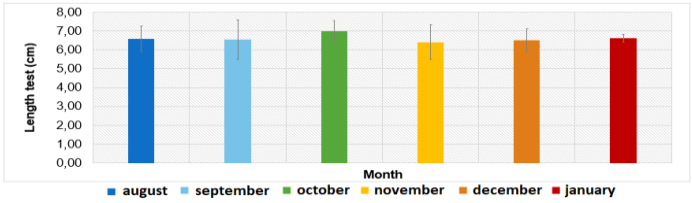


Fig.1 Average length test (cm) of *Echinometra vanbrunti* in the period August 2017- January 2018.

The average total weight was 144.25 ± 48.25 gr, 139.73 ±61.06 gr, 159.88 ±34.50 gr, 121.13 ± 45.74 gr, 148 ± 37.37 y 154.80 ± 47.50 in August to January, respectively.

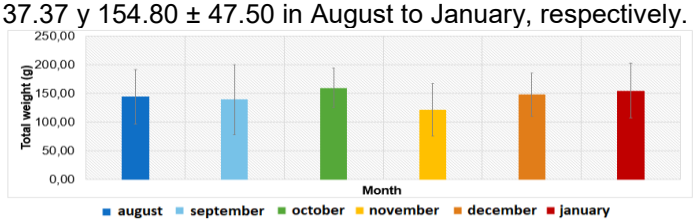


Fig.1 Average total weight (gr) of *Echinometra vanbrunti* in the period August 2017- January 2018.

The gonadosomatic index was 17.3 ± 0.04 %, 8 ± 0.06 %, 9.23 ± 3.29 %, 8.71 ± 3.95 %, 10.2 ± 5.56 % y 13 ± 3.79 % from August to January, respectively.

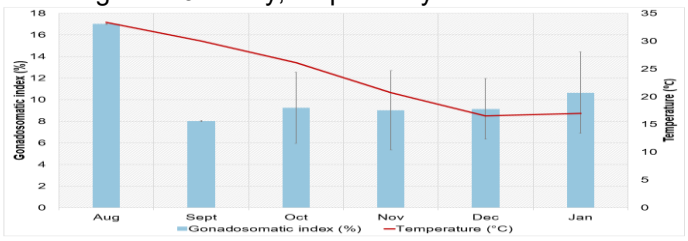


Fig. 3 Gonadosomatic index of *Echinometra vanbrunti* and Temperature in the period August 2017- August 2018

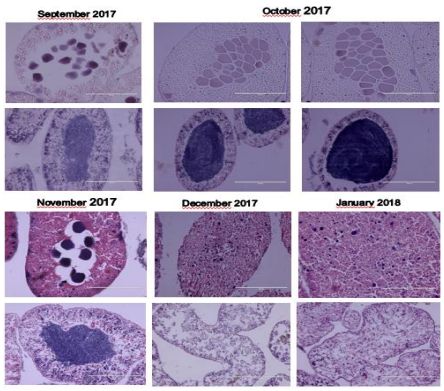


Fig. 4 Female and male *Echinometra vanbrunti* gonad histology observed in 200 um.

Conclusions. In conclusion, with the data obtained so far, it could be inferred that the peak of maturity is related to temperature, since in August the highest gonadosomatic index and a higher temperature were observed. The work is in process to obtain the peak of sexual maturity of this species during the year.

Acknowledgements. Darana Gutièrrez, Department of Physics and Department of Scientific and Technological Research of the University of Sonora.

References.

1. Olguín-Espinoza, I. 2000. Investigación Regional La pesquería de erizo en México. Situación actual y perspectivas de desarrollo. 4 (10) 5- 6

ISSN: 2448-8461

AP20

SELECTION OF A BACTERIAL CONSORTIA FOR SYMBIOTIC AMMONIA BIOREMEDIATION DURING THE INTENSIVE SHRIMP AQUACULTURE

Salvador González Rubio Gavarain; Eduardo-Quiroz Guzmán, Sergio F. Martínez Díaz. Centro Interdisciplinario de Ciencias Marinas (Biotecnología Marina), La Paz 3003;
sergiofranciscomartinez@gmail.com Sergio Francisco Martínez Díaz

Key words: Bioremediation, microbial fermentation, water quality.

Introduction. Water quality deterioration by accumulation of nitrogenous waste products is a main concern in the intensive aquaculture production. Naturally, these compounds are derived from the metabolism of cultured organisms and from the decomposition of the uneaten food. These products induce intestinal damage, growth rate reduction, stress and mortality under extreme condition (Gan Quin *et al.,* 2005). Bioremediation is an alternative to mitigate its negative effects in which living organisms; use, transform and/or eliminate the harmful compounds (Foght *et al.,* 1999). Some aquatic bacteria assimilate ammonia as unique nitrogen source and improve the culture conditions; this ability is apparently affected by the kind and amount of carbohydrates available in the system. The ability to regulate the composition of the microbiota by prebiotic dietary substances and probiotic microorganisms is an interesting approach to improve the microbial functions in the aquaculture systems.

The aim of this study was to select a consortium of not-harmful bacteria with high ability to assimilate ammonia and evaluate different carbon sources to stimulate its bioremediation ability.

Methods. The strains used in this study were previously isolated from different sources, including marine sediments and fish guts. The ability to assimilate ammonia was corroborated under in vitro conditions using ammonia as the sole nitrogen source. The assimilation rate was evaluated under different environmental conditions and with different carbon sources. The most effective strains were incorporated as a consortium during fermentation of different inexpensive carbon sources and the ability of the fermented product to remove ammonia was evaluated under shrimp culture conditions.

Results. Currently, a consortium of seven different strains belong to *Bacillus* genus is the most promising combination to bioremediate the water during intensive shrimp production, based on its ability to promptly assimilate ammonia under different conditions and by its tolerance to different environmental conditions. The consortia shown the ability to reduce the ammonia from 10 ppm to less than 0.5 ppm during 24 h at 30˚C using glucose as the sole carbon source.

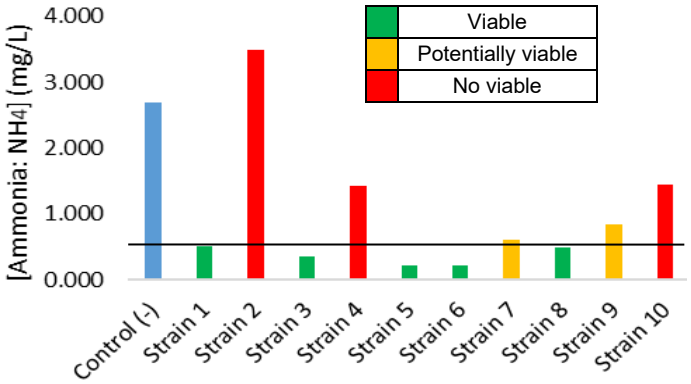


Fig. 1 Selection of strains (*Bacillus* sp.) Based on ammonium consumption (collection: CICIMAR-IPN).

The incorporation of unexpensive carbon sources together with these bioremediation consortia will result in an increase in the ability to improve the water quality and reduce the risk during intensive or super intensive aquaculture.

References.

Foght, J., Semple, K., Westlake, D., Blenkinsopp, S., Sergy, G., Wang, Z., & Fingas, M. (1998). Development of a standard bacterial consortium for laboratory efficacy testing of commercial freshwater oil spill bioremediation agents. Journal of Industrial Microbiology & Biotechnology(21), 322–330.

Gan Quin, G., Moncura, J., Liu, N., & Richman , H. (2005). Aquaculture wastewater treatment and reuse by wind-driven reverse osmosis membrane technology: a pilot study on Coconut Island, Hawaii. : . Aquacultural Engineering(32), 365–378.

ISSN: 2448-8461

AP21

USE OF NOVEL CELLULASES TO SACHARIFICATION OF DIFFERENT AGROINDUSTRIAL WASTES

Adrián O. Máynez-Pérez[1], Martha Arevalos-Sanchez[1], Monserrath Felix-Portillo[1], Oscar Ruiz-Barrera[1] and Agustín Corral-Luna[1]

1 Facultad de Zootecnia y Ecología, Universidad Autónoma de Chihuahua. Periférico Francisco R. Almada km 1, Chihuahua, Chihuahua, 31453, México.

Key words: Cellulase, Agricultural waste, animal feed

Introduction. Cellulose is the most abundant renewable energy source in the earth. However, this energy is not available for all mammalian animals due they lack the enzymatic complex necessary to depolymerize this carbohydrate. It seems to be an exclusive ability of some microorganisms that produce these cellulases. On the other hand, the most of agriculture and wood industry wastes are characterized by a high cellulose content. On the aim to increase their utilization as potential animal feed or biofuel precursors, many strategies to breakdown the cellulose are studied. However, the results still being variable, probably due the enzymes used.
The objective of the present study was to evaluate the synergistic effect of novel cellulases in the sacharification of different agroindustrial wastes.

Methods. Enzymes EGL-FZYE and CEL-FZYE were overexpressed in *E. coli* strain BL21. The enzymatic activity was measured by DNS method according Miller, (1959). Corn and sorghum stover and pine sawdust were used as substrates to evaluate the ability of both enzymes alone or in combination, to release glucose. All the reactions were carried out by triplicate and also Carboxymethyl cellulose and Avicel were used as reference substrates. One unit of enzymatic activity (U) was defined as the amount (mg) of enzyme that release a µM of glucose per minute. Specific activity was defined as the number of U mg^{-1} of enzyme. All the data were analyzed by ANOVA through PROC GLM. Also a mean comparison through student t was applied for all the different substrates.

Results. Figure 1 shows a greater degradation of substrates by EGL-FZYE (endoglucanase), being an enzyme that acts in the first stage of cellulose degradation by hydrolyzing β -1,4 bonds, unlike CEL-FZYE (β glucosidase) that intervenes in the final stage breaking glucosil units from non-reducing endings of celo-oligascarides [1]. When both enzymes were used together, an synergistic effect was observed for all substrates, being the ratios 1.63, 2.16 and 1.74 for corn stover, pine sawdust and sorghum stover respectively. These results looks very similar with those observed for specific substrates such as CMC and avicel (1.59 and 1.40 respectively), and also with those obtained by Ng et al. [2] who obtained ratios of 1.69 and 1.34 for cellulase isolated from *Trichoderma reesei* and combined with β glycosidase.

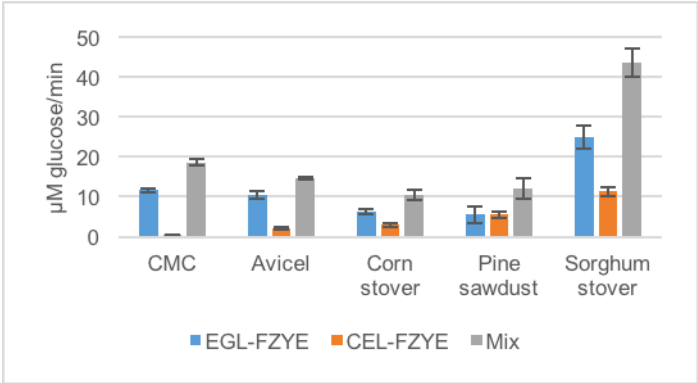


Fig.1. Glucose released from different substrates by two novel cellulases isolated from *Trabulsiella odontotermitis*.

Conclusions. Both cellulases exhibit activity on agro industrial wastes, however, their effect was improved when they were used as mix. More research should be conducted in order to determine their potential use as pre treatment for the sacharification of agroindustrial wastes used as feed for ruminants and biofuel production industry.

Acknowledgements. The authors thank the National Science and Technology Council (CONACyT) for the founding through the CB-239593 grant.

References.

1. Zhang Y, Himmel M, Mielenz J. (2006). *Biotech Adv.* (24): 452-481.
2. Ng I, Tsai S, Ju Y. (2011). *Bioresou Technol.* 102.(10):6073-6081.

ISSN: 2448-8461

AP22

PRODUCTION OF SEAWEED DETRITUS BY ENZYMATIC HYDROLYSIS AND FERMENTATION AS INGREDIENT FOR SHRIMP FEED

Alexia Omont, Regina Elizondo-González, Cristina Escobedo-Fregoso, Dariel Tovar-Ramírez, Eduardo Quiroz-Guzman, Alberto Peña-Rodríguez; Centro de Investigaciones Biológicas del Noroeste (CIBNOR), La Paz, B.C.S., 23090, México; email: apena@cibnor.mx

Key words: Single cell detritus, Seaweed, Shrimp feed

Introduction. Different extraction methods on seaweed have been investigated to improve the nutritional value and to eliminate antinutritional factors that limit high inclusion in aquafeed. Most of them are time consuming, require large amount of solvents and the efficiency of extraction is limited.[1] Nevertheless, enzymatic hydrolysis of seaweed cell-wall and fermentation process improve protein digestibility by removing anti-nutritional factors and help animals to absorb substantial amounts of nitrogen directly from the plant material[2]. The objective of this work was to evaluate the efficiency of enzymatic hydrolysis and fermentation to produce seaweed detritus and use it as a valuable ingredient for a partial substitution of fish meal in shrimp feed.

Methods. Marine single cells detritus were produced from three seaweeds: *Ulva lactuca, Ulva clathrata* and *Eisenia sp.* The method consisted in a cellulase hydrolysis for one hour following by a 48 hours fermentation using *Lactobacillus plantarum* and *Saccharomyces cerevisiae.* Detritus were dried 12 hours at 60°C. Whole meal and detritus proximal composition (crude protein, lipids, ash, crude fiber and nitrogen-free extract (NFE)) of each alga was analysed.
Seaweed detritus were included in shrimp diets at 10 and 20%, in partial substitution of fish meal. A 30 days feeding assay was performed on shrimp *Litopenaeus vannamei* (Initial weight 0.177g ± 0.01). Feed treatments consisted in a commercial feed and diets containing 0, 10 and 20% of seaweed detritus inclusion, in controlled conditions and per triplicate (10 shrimps per replicate). Growth parameters (final weight, gain of weight, specific growth rate, feed intake and feed conversion rate) were calculated.

Results. Enzymatic hydrolysis and fermentation of seaweed meals permitted to improve protein and lipid content and reduce crude fiber and nitrogen-free extract in detritus products (Table 1).

Table 1. Proximal composition of *U. lactuca, U. clathrata* and *Eisenia sp.* whole meal (WM) and detritus (D).

	Ulva lactuca		*Ulva clathrata*		*Eisenia sp.*	
	WM	**D**	**WM**	**D**	**WM**	**D**
Protein	15.5	26.5	15.4	25.2	9.3	23.9
Lipids	0.33	7.70	0.23	6.68	0.33	7.56
Ash	35.2	31.2	33.3	47.5	47.9	28.1
Crude fiber	2.10	0.80	1.60	0.56	2.20	1.21
NFE	47.2	33.8	49.5	20.1	40.3	39.3

The inclusion at 10 and 20% of detritus in *L. vannamei* feed showed no significative difference in gain of weight, except for *Ulva lactuca* included at 20%.

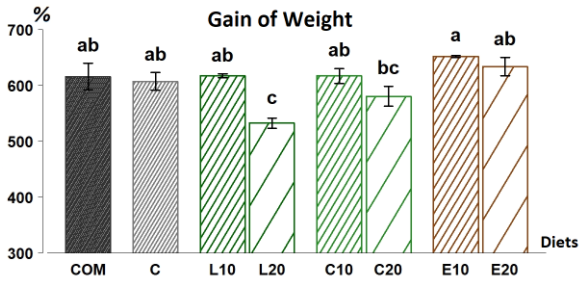


Fig.1 *L. vannamei* weight gain (%) after 30 days fed commercial feed (COM) and diets with 0% (C), 10 and 20% of *U. lactuca* (L10 and L20), of *U. clathrata* (C10 and C20) and of *Eisenia sp.* (E10 and E20)

Conclusions. The seaweed detritus production permitted to improve protein and lipid content of seaweed meal in an economic and time saving process. This method allowed to include seaweed at a rate of 20% as a partial fish meal substitute in feed without negative effect on shrimp growth compared to a commercial feed and 0% detritus inclusion.

Acknowledgements. This work was founded by CONACYT Mexico (Project PDCPN 2015-887).

References.

1. Kadam SU, Álvarez C, Tiwari BK, O'Donnell CP. (2017). *Food Research International*, 99, 1021-1027.
2. Felix S, Pradeepa P. (2012). *Tamilnadu J. Veterinary and Animal Sciences*, 8(2), 76-81.

ISSN: 2448-8461

AP23

SEASONAL CHANGES OF THE BIOCHEMICAL PROFILES OF BARRED SAND BASS *Paralabrax nebulifer* (TELEOSTEI: SERRANIDAE)

Ysla-Guzmán J. A. [1], J. L. Ortíz-Galindo [1], M. O. Rosales-Velázquez [1], T. Grayeb del Álamo [1], V. Carrasco-Chávez [1], J. Sánchez Gallegos [1] & L. Carreón-Palau [2]
alejandroysla@gmail.com

1 Laboratorio de Biología Experimental, Centro Interdisciplinario de Ciencias Marinas - Instituto Politécnico Nacional, 23096, La Paz, Baja California Sur, México.
2 Laboratorio de Biotecnología de las Microalgas, Centro de Investigaciones Biológicas del Noroeste, 23090, La Paz, Baja California Sur, México.

Key words: Proximate analysis, histology, diet composition

Introduction. The reproductive control of fish subjected to captive conditions is essential to sustain commercial aquaculture by producing quality embryos, which is obtained with the feeding of broodstock based on knowledge of their dietary requirements (1). Barred sand bass is an economic importance marine fish in Baja California Sur, Mexico (2), which was reproduced continuously under controlled conditions.
The aim of this study was to compare the food categories, proximal analysis of the whole fish and lipid composition of liver, muscle and gonads from wild fish to gain information on broodstock dietary requeriments and improve spawns quality.

Methods. For the present study, fish samplings during two annual cycles were carried out in the field, in order to know their biochemical composition (3). Proximal analyzes of the whole fish were made; while of the tissues: Liver, muscle and gonad; analyzes of fatty acids, total lipids and carotenoids were performed (4). Histological studies of the gonads were carried out in order to determine the state of maturity of the fish. The stomach contents were examined. The indice relative importance (%IRI) was calculated to define the main food categories (5).

Results. 56 specimens of barred sand bass were captured in Bahia Magdalena: 38 in the reproductive season (RS) and 18 in the non-reproductive season (NRS), which presented in both seasons an average length and weight of 25.2 cm and 371 g, respectively. Through histological analysis of the gonads confirmed the vitellogenic stages corresponding to each stage of maturity and by reviewing the stomach, it was confirmed that fish were the most important food component of the RS; while the crustaceans for the NRS. Bromatological tests showed that the RS samples presented the highest percentage of lipids and less protein (23.3 and 58.8%) and the other way around for specimens of the NRS (13.6 and 67.6%). The muscle, gonad and liver tissues of these fish, were dissected and their total lipid and the highly unsaturated fatty acid (HUFA) levels were analyzed; the greatest amount of total lipids was presented in the liver and HUFA (20: 4 n-6; 20: 5 n-3; and 22: 6 n-3) in the gonad of both seasons, however, there were differences between the three tissues of both seasons.

Conclusions. *P. nebulifer* is a species that is completely defined with the lipids in its reproductive season and with the protein in non-reproductive season. In the other hand, this species show the major amount of lipids and HUFA on its reproductive season, nevertheless the liver and the gonad always were superior in the same levels in both seasons.

Acknowledgements. The authors thanks to Instituto Politécnico Nacional and CONACYT for the financial support.

References.

1. Izquierdo, M., Fernández-Palacios, H., Tacon, A. (2001). *Aquaculture*. 197: 25-42.
2. DOF. (2010). *Diario Oficial de la Federación*, México, 318 p.
3. Yanes-Roca, C., Rhody, N., Nystrom, M., Main, K. (2009). *Aquaculture*. 287: 335-340.
4. Hauville, M., Rhody, N., Resley, J., Bell J., Main K., Migaud, H. (2015). *Aquaculture*. 446: 227-235.
5. Liao, H., Pierce, C.L., Larscheid, J.G. (2001). *T Am Fish Soc* 130. (4): 583–591.

ISSN: 2448-8461

AP24

PRODUCTION OF ANTIMICROBIAL METABOLITES BY *Salinispora arenicola* IMMOBILIZED IN ALGINATE BEADS

Naylin Herrera Hernández, Claudia J. Hernández Guerrero, Sergio F. Martínez Díaz, Mauricio Muñoz-Ochoa, Erika T. Quintana Cano. Instituto Politécnico Nacional – Centro Interdisciplinario de Ciencias Marinas Department of Technology Development, La Paz, Baja California Sur, Mexico; corresponding author: naylinherrera.92@gmail.com; sdiaz@gmail.com

Keywords: actinobacteria, immobilization, antimicrobials

Introduction. *Salinispora* is actinobacteria associated with marine sediments and benthic organisms; they produce multiple active metabolites, including strong antimicrobial compounds [(1)]. Under in vitro conditions, specific strains of *Salinispora arenicola* from the Gulf of California; inhibit the proliferation of strains of *Vibrio parahaemolyticus* (VP) involved in the acute hepatopancreatic necrosis in shrimp. Consequently, could be an agent for biologic control, which will be directed to colonize the sediments and to regulate the proliferation of VP. In that case, the immobilization may be a strategy to improve the survival of *S. arenicola* in sediments [(2)], however, as apparently the ability of *S. arenicola* to produce active metabolites depends on the availability of nutrients and of the stage of development, it is unknown whether incorporation into an artificial matrix can modify its development and affect its ability to produce active metabolites. The present study was designed to verify the capacity of one of the strains of *S. arenicola* isolated from the Gulf of California, immobilized in alginate for the production of antibiotics.

Methods. *Salinispora arenicola* strain 60-S was cultured during 21 days. The biomass at 7, 14 and 21 days was harvested by centrifugation and immobilized in a sodium alginate matrix. The immobilized cells were maintained under culture conditions in marine broth media and they ability to produce antimicrobial metabolites at 7, 14 and 21 days post-immobilized was evaluated by agar diffusion on petri dishes and by co-culture.

Results. In this study, 3-mm sodium alginate beads with 6% of *S. arenicola* were obtained. The immobilized cells showed the highest inhibitory effect against *V. parahaemolyticus* at 14 and 21 days (Fig. 1). Apparently, the increase in the number of beads during the assay did not improve the apparent antimicrobial activity. In the co-culture assay, *S. arenicola* at 7, 14 and 21 days post immobilization were confronted with *V. parahaemolyticus*. The growth of *V. parahaemolyticus* was significantly reduced with encapsulations performed at 21 days of culture and from 14 days post-encapsulation (Table 1).

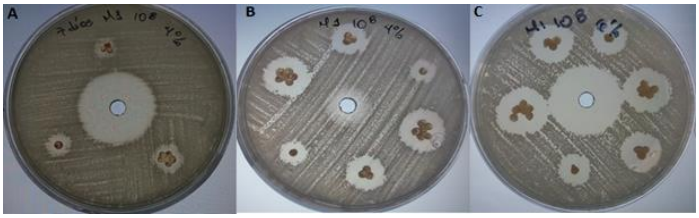


Figure 1. Activity of the cells of *S. arenicola* (A) immobilized in sodium alginate at 7 days of culture (B) 14 days of culture (C) 21 days of culture, against *V. parahaemolyticus* during an agar diffusion assay.

Table 1. Antibacterial activity of the immobilized cells of *S. arenicola* at 21 days against *V. parahaemolyticus* during a co-culture assay.

Days post-encapsulation	Control +	19 beads	38 beads	p
0	0.37±0.01^{a}	0.40±0.02^{a}	0.36±0.01^{a}	>0.05
7	0.51±0.06^{a}	0.27±0.05^{a}	0.14±0.07^{b}	<0.05
14	0.71±0.03^{a}	0.02±0.01^{b}	0.001±0.001^{b}	<0.01
21	0.86±0.07^{a}	0.07±0.03^{b}	0.01±0.002^{b}	<0.000

Conclusions. *Salinispora arenicola* keep their ability to produce antimicrobial compounds when is immobilized in a matrix of sodium alginate. The antimicrobial activity against *Vibrio parahaemolyticus* could be recorded on solid or in liquid environments which support the idea to use it, as biocontrol in the shrimp ponds.

Acknowledgments. Multidisciplinary Project IPN SIP-20181803, to CONACYT and BEIFI for granted scholarships.

References.

1.Jensen P. R. & Mafnas C. (2006) *Environ. Microbiol.* 8(11):1881-1888.

2.Fajardo-Ochoa R., Osuna-Castro J., Velázquez-Mendoza C. V., Escalante-Minakata P. y Ibarra-Junquera V. (2011) *Revista Científica de la Universidad Autónoma de Coahuila.* 3(6): 42-56.

ISSN: 2448-8461

AP25

ANTIBACTERIAL ACTIVITY OF NANOPARTICLES SUSPENSIONS AGAINST *Vibrio parahaemolyticus*

Diana Elizabeth Sánchez Rodríguez[1], Erika Guadalupe Rico Virgen[1], Marissa Pérez Alvarez[2], Gregorio Cadenas Pliego[3], Ruth Noemí Aguila Ramírez[1]

[1] Instituto Politécnico Nacional, Centro Interdisciplinario de Ciencias Marinas, 23098, La Paz, B.C.S.; [2] Instituto Mexicano del Petróleo, 07730, Ciudad de México, México; [3] Centro de Investigación en Química Aplicada, 25294 Saltillo Coahuila, México
Email: sanchez.rodriguez08@gmail.com

Key words: Shrimp, aquaculture, bacteria

Introduction. In recent years, aquaculture has been affected by the impact of diseases on various aquatic organisms that halt their development and production levels. *Vibrio parahaemolyticus* is an important pathogen causing significant economic problems within the aquaculture industry worldwide, especially in shrimps. The wide and frequent use of antibiotics in aquaculture has resulted in the development and spread of resistance. Because recent implementation of stricter regulations on the prophylactic use of antibiotics and the presence of antibiotic residues in aquaculture products, novel strategies to control bacterial infections are needed (1).

With the aim of exploring alternatives that deal with this problem, the application of copper and silver nanoparticles was evaluated for its antibacterial activity, as well as whether the presence and concentration of these substances are toxic to the environment.

Methods. Different methods of dispersion of Ag and Cu nanoparticles (NP) in solution were performed to evaluate their antimicrobial activity by well diffusion against *Vibrio parahaemolyticus*. These tests were repeated after three months to evaluate if the activity of the solution was maintained. The active nanoparticles were subjected to toxicity tests with *Artemia franciscana*.

The effect of nanoparticules on the growth of *V. parahaemolyticus* was evaluated.

Results. To identify the agents with potential antibacterial effects, five metal oxide nanoparticles (nCu, CuAg-1, Ag, CuO, AgCu-4) were selected. The best dispersion method was temperature and sonication. Among the NP tested, only Ag and AgCu-4 showed growth inhibition (Fig. 1), However, when evaluating the nanoparticles after a month of dispersing them, the AgCu-4 lost their activity so only the copper ones were considered for the next stage.

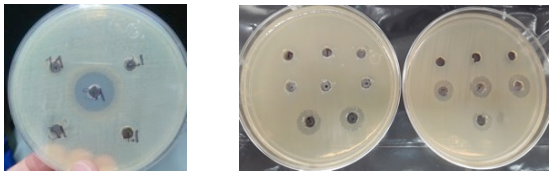


Fig.1 Antimicrobial activity against *Vibrio parahaemolyticus*

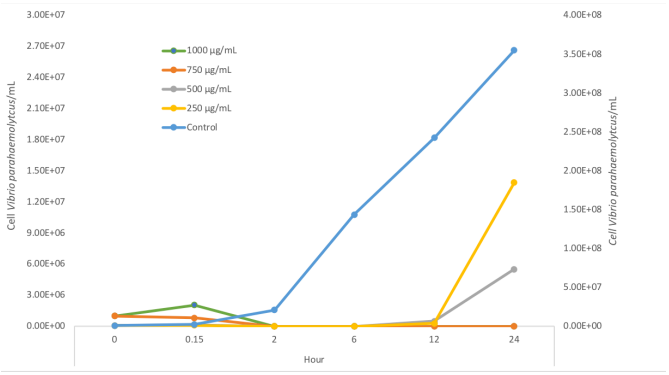


Fig. 2 Effect of nanoparticles against *V. parahaemolyticus* viable cells

The best concentrations of silver nanoparticles that inhibited the growth of *V. parahaemolyticus* was 750 and 1000 µg/mL concentrations after 2h. Viables cells of *V. parahaemolyticus* growth after 12h in the 250 y 500 µg/mL concentrations despite lethargic growth (Fig. 2). CL_{50} of the silver nanoparticles against *A. franciscana* was higher than 750 µg/mL concentration. Therefore, the best concentration to inhibit *Vibrio* without being considered as toxic is 750 µg/mL.

Conclusions. The data suggest that Ag nanoparticles is not toxic at low concentrations and have a potential application as a bacteriostatic agent against *Vibrio parahaemolyticus*.

References.

1. Defoirdt, T., Sorgeloos, P., & Bossier, P. (2011). Alternatives to antibiotics for the control of bacterial disease in aquaculture. *Current opinion in microbiology*, 14(3), 251-258.

ISSN: 2448-8461

AP26

EFFECT OF EXTRUDED CHICKPEA FLOUR AND WORM (*Eisenia foetida*) FLOUR ADDITION IN DIETS FORMULATION ON THE GROWTH OF WHITELEG SHRIMP (*Litopenaeus vannamei).*

José Pedro Tejeda-Miramontes*, Hervey Rodríguez-González, Gerardo Rodríguez-Quiroz. Instituto Politécnico Nacional. CIIDIR-Sinaloa. Guasave, Sin., México. CP 81000. jp-tejedamx@hotmail.com

Key words: Chickpea flour extruded, worm flour, Litopenaeus vannamei.

Introduction. Aquaculture has become an alternative for the production of species of great importance for the World population. Considering that shrimp consumption has increased in recent years, it is relevant to intensify its cultivation and production using alternative sources of protein in their diets (1). The most critical challenge for diets is the reduction of fishmeal, which is the main source of protein and represents the most expensive ingredient (2).

The present project objective was to evaluate the use of vegetable protein (extruded chickpea) and animal protein (earthworm) as an alternative to fishmeal ingredient in shrimp diets.

Methods. The effect of the extruded chickpea flour and earthworm (*Eisenia foetida*) on the growth of white shrimp (*Litopenaeus vannamei*) was evaluated. Six animals were introduced per treatment with three replicates, for 75 days each. Water exchanges were carried out every 15 days (3). In the first bioassay, four diets were elaborated with a substitution of 15, 30, 45 and 60% of flour of extruded chickpea in isoproteic diets (40% of crude protein) and a control (100% fishmeal). In the second growth bioassay, the best diet formulation obtained from the first bioassay was added with earthworm flour from 5 to 20% and compared to a control (without earthworm meal).

Results. Evaluating the growth of white shrimp in the first bioassay, it was possible to substitute up to 60% fishmeal for extruded chickpea flour (Figure 1) without affecting growth and survival. Besides the incorporation of earthworm flour in the diets added with extruded chickpea flour (Figure 2) resulted on a lower growth compared to the control diet.

Conclusions. The results obtained show that extruded chickpea flour can be used as an adequate substitute of fishmeal protein in shrimp diets. On the other hand, earthworm flour (*E. foetida*) addition has no marked advantages to replace the use of fishmeal.

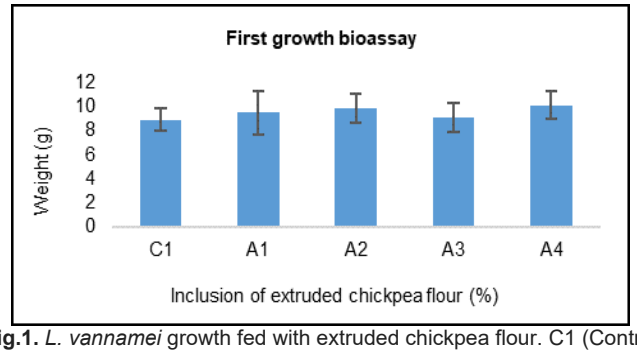


Fig.1. *L. vannamei* growth fed with extruded chickpea flour. C1 (Control 1) = 100% HP (fish meal). A1 - A4 Treatments including extruded chickpea flour (15, 30, 45 and 60%).

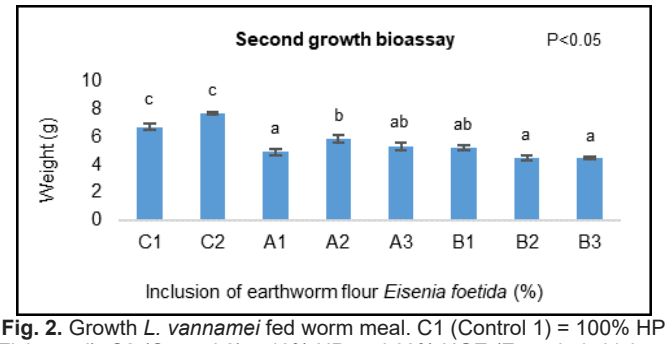


Fig. 2. Growth *L. vannamei* fed worm meal. C1 (Control 1) = 100% HP (Fish meal); C2 (Control 2) = 40% HP and 60% HGE (Extruded chickpea flour); A1-A3 = Diets with 5, 10 and 20% inclusion of worm-compost meal; B1-B3 = Diets with 5, 10 and 20% inclusion of worm-compost flour.

Acknowledgements. The present work was supported economically through the project SIP-IPN 20130709.

References.

1. Juárez, P.J.R. (2015). Mexican fishing development 2012-2015. México, Secretaría de Pesca. L II: 219-232
2. Molina C., Cadena E. and Orellana F. (2013). Memories of the V International Aquaculture Nutrition Symposium. 19-22 Nov. Mérida, Yucatán, México.
3. Block R.J. and Mitchell H.H. (1998). Fisheries systems for intensive use, 16: 249-278

ISSN: 2448-8461

PB2

CELLULASE PRODUCTION FROM ORANGE, APPLE, AND HAWTHORN WASTES THROUGH SUBMERGED FERMENTATION

Hernandez-Teyssier, Eva[1]; Ramirez-Castillo, María Leticia[2]
[1]Universidad Interserrana del Estado de Puebla-Ahuacatlán (Biotechnology Faculty), Ahuacatlán, 73330;
[2]Universidad Politécnica de Puebla (Graduate department), Juan C. Bonilla, 72640;
[1]eva.hernandez@uiepa.edu.mx;
[2]letyram@unam.mx

Key words: cellulase production, submerged fermentation, fruit wastes

Introduction. Cellulase is a generic name of an enzymatic complex that act synergy to hydrolyzed cellulose to monomer (glucose), the complex are form of three enzymes named according to action site on substrate: β-glucosidase, exoglucanase and endoglucanase, the last one was determined in this job. This enzyme cuts unions of cellulose fiber generating oligosaccharides of various lengths [1].
In this work was studied cellulase production capacity from agroindustrial waste: peel orange (PO), apple pomace (AP) and hawthorn fruit (HF) on submerged fermentation (SF)

Methods. SF was performance on bioreactor with waste (50g/L), mineral solution (1L) [2] and *Penicillium* spp (CPHD13); operation initial conditions was 100 rpm, 0.3 **vvm** and 28 °C. Biomass, enzyme extract and extracellular protein was separed and recolected by centrifuged; biomass was quantified indirectly [3] enzymatic activity were determined used CMC 1% [4], and extracellular protein by Biuret technique.

Results. In this work were used kinetic parameter (Table 1) to modeled biomass (Logistic Model), substrate (Aborhey-Williamson Model) and enzyme (Luedekin-Piret Model)

Table 1. Kinetic parameter used to modeled values, substrate and enzyme yields.

Kinetic parameters	PO	AP	HF
Xo	1.42	2.66	2.50
Xmax	65.54	18.66	13.24
So	0.26	0.33	0.40
μmax	2.25	0.15	0.51
Ks	7.2×10^{-03}	3.2×10^{-03}	2.3×10^{-04}
m	1.5×10^{-04}	6.3×10^{-04}	4.5×10^{-04}
Yxs	760.84	241.84	14.82
Ypx	7.410	7.831	3.757
Cellulase máx.	11.41	5.35	**12.20**

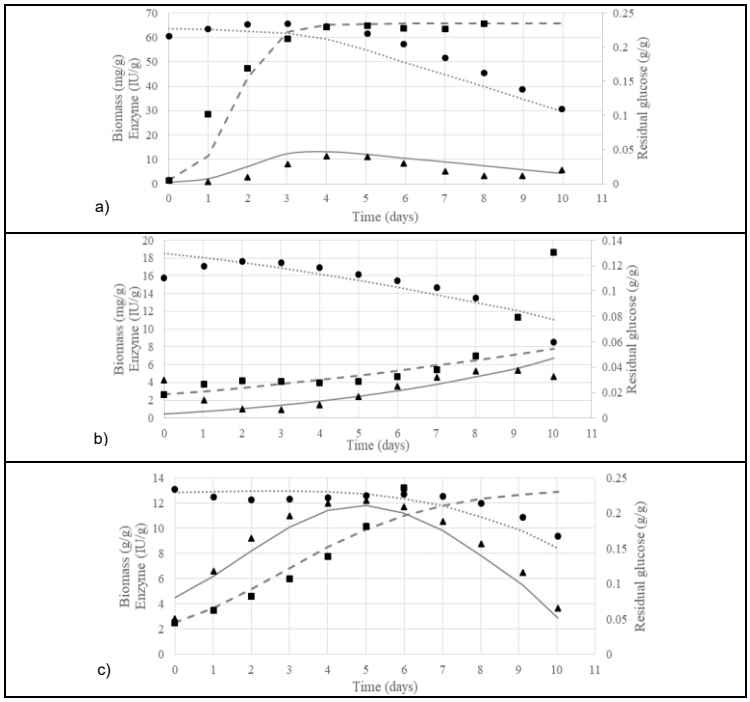


Fig. 1 Cellulase production on a) peel orange, b) apple pomace and c) hawthorn fruit. Experimental data (symbol) and modeled values (line) of biomass (■,- - -), substrate (●, …) and enzyme (▲, ─).

Conclusions. Hawthorn fruit is the best waste to produce cellulase on a submerged fermentation.

Acknowledgements. The authors are sincerely thankful to CONACyT for financial support.

References.

[1] Escudero, A. J., Daza, M. Z. T., Gil, Z. N. J., Mora, M. O. Y. (2013). *Rev Col Biotecnol.*15(1):108-117.
[2] Stoilova, I., Krastanov, A. (2008). *Appl Biochem Biotechnol.*149:45-51.
[3] Córdova-López, J., Gutiérrez-Rojas, M., Huerta, S., Saucedo-Castañeda, G., Favela-Torres, E. (1996). *Biotechnol Tech.*10(1):1-6.
[4] Márquez-Araque, A. T., Mendoza, M. G. D., González, M. S. S., Buntinx, D. S. E., Loera, C. O. (2007). *Interciencia*.32(11):780-785.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México

PB3

PLACA DE ACERO 316L PARA TRATAR FRACTURAS DE RADIO DISTAL TIPO 2B

Islas-Jiménez, D.I., Urriolagoitia-Sosa, G., Romero-Ángeles, B., Vázquez-Feijoo, J.A. & Urriolagoitia-Calderón, G.M.
Instituto Politécnico Nacional, Escuela Superior de Ingeniería Mecánica y Eléctrica, Sección de Estudios de Posgrado e Investigación, Unidad Profesional Adolfo López Mateos "Zacatenco" Avenida Instituto Politécnico Nacional s/n, Edificio 5, 2° piso. Col. Lindavista, C.P. 07320, Ciudad de México.
islas-jimenez_17@hotmail.com

Palabras clave: fracturas, análisis numérico, biocompatibilidad.

Introducción

Desde la sociedad contemporánea hasta nuestra actualidad, se ha ido trabajando en buscar las mejores herramientas para tratar fracturas de antebrazo. Con el paso de los años los avances tecnológicos y científicos se ven reflejados en los tratamientos de fracturas en el miembro superior con los que se cuenta actualmente. Dichos tratamientos son realizados con Titanio AL6-V4 este material es el más utilizado para implantes ortopédicos debido a las propiedades físicas, químicas y su grado de biocompatibilidad con el cuerpo humano. El principal problema de estos implantes es su elevado costo de adquisición, lo que afecta en su mayoría a las personas con fracturas. En este trabajo se realiza un modelo computacional para tratar fracturas de radio distal tipo 2B según la (AO) [1]. Dicho modelo está diseñado con Acero 316L debido a la similitud en las propiedades anteriormente enunciadas para el Titanio.

Desarrollo

Para este trabajo se propone el desarrollo de un diseño de una placa de Acero 316L como se muestra en la Figura 1, para fracturas de radio distal exclusivamente. Con la finalidad de reducir el precio en comparación con una de Titanio.

Figura 1.- Diseño de la placa

El Acero 316L cuenta con características físicas, químicas altamente aceptables y su grado de biocompatibilidad con el cuerpo humano es alto lo cual es indispensable para este tipo de implantes ortopédicos. Principalmente se realiza un análisis numérico de dicha placa mediante el programa de elemento finito comúnmente conocido con el nombre de *ANSYS*® [2]. Para realizar dicho estudio, primero se debe contar con el modelo de la placa dentro del programa, además de todos los parámetros de análisis como el tipo de elemento, discretizado y finalmente se introducen los agentes externos, siendo los apoyos y fuerza que intervienen directamente.

Resultados

Con dicho análisis numérico se podrá observar el desplazamiento total (Figura 2a), deformación unitaria (***strain)*** (Figura 2b) y finalmente el esfuerzo de Von-Mises (Figura 2c). Los resultados obtenidos se podrán comparar con las características que se requieren para el diseño de dichos implantes. El objetivo a futuro es poder manufacturar dichas piezas de una manera más económica para que la sociedad afectada pueda adquirir un tratamiento que le ayude a su pronta recuperación y se integre a su vida cotidiana.

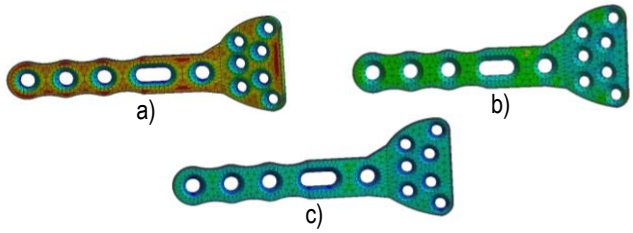


Figura 2.- Solución numérica en ANSYS®.

Conclusiones

Mediante la elaboración e innovación de material ortopédico las personas afectadas podrán gozar de un tratamiento quirúrgico de calidad lo que ayuda a su pronta recuperación y volver a sus actividades cotidianas de una manera más rápida.

Agradecimientos

Los autores agradecen al Instituto Politécnico Nacional y al Consejo Nacional de Ciencia y Tecnología por el apoyo brindado, en la elaboración de este trabajo.

Referencias

1.-Graff, S. y Jupiter, J., Fracture of the distal radius; Classification of treatment and indications for external fixation, Injury, Vol. S-D, No. 25, pp 14-25, 1994.
2.- Pavlou, D. G., Essentials of the Finite Element Method; For Mechanical and Structural Engineers, Ed. Academic Press, pp 1-17, 2015.

ISSN: 2448-8461

PB4

METAGENOMIC INSIGHTS INTO THE MICROBIAL DARK MATTER FOR THE SEARCH OF BIOTECHNOLOGICAL APPLICATIONS: THE BACTERIOCINS EXAMPLE

Alejandra Escobar-Zepeda1, Alfredo Esquivel2, Alejandro Sanchez-Flores1, Maricarmen Quirasco2
1) Unidad de Secuenciación Masiva y Bioinformática, Instituto de Biotecnología, UNAM, Cuernavaca, Morelos, 62210
2) Departamento de Alimentos y Biotecnología, Facultad de Química, UNAM, Ciudad de México, 04510
ales@ibt.unam.mx

Key words: Food metagenomics, metabolic potential prediction, novel bacteriocins

Introduction. Fermented foods are a reservoir of genes never studied before (named as dark matter) with potential biotechnological application in areas as important as food safety. Nowadays, it is possible to exploit this novel genetic pool thanks to the advances in sequencing technologies and to the development of bioinformatic tools designed *ad hoc* for this purpose. Commonly, metagenomic projects remain at the descriptive level of both, the population structure of the organisms in the fermented product and their metabolic potential. In this work, we show a success case of *in silico* predicted functional activities that have been confirmed *in vitro* and, we show their potential for a real application in the food area.
The aim of this work was to assess the antilisterial activity of new bacteriocins identified bioinformatically from the metagenomic analysis of the Cotija cheese microbiota.

Methods. A 15-g sample of a Cotija cheese was used for metagenomic DNA extraction. Purified DNA was used for whole metagenome shotgun sequencing. Illumina technology was used in a paired-end format of 200 cycles. The bioinformatic analysis included taxonomic annotation of phylogenetic marker genes, as well as the metabolic potential reconstruction according to the following pipeline: metagenomic assembly, open reading frame prediction, functional annotation by sequence similarity using blastp against the SwissProt database, and functional domains prediction by Hmmer v3.1b1 versus the Pfam-A database (1). A functional analysis was made for the detection of new bacteriocins, i.e, genes with bacteriocin domains and absent blastp annotation. Two contigs, with adjacent bacteriocin/immunity putative genes were synthesized, inserted in pET28a(+) vectors and cloned in *E. coli* BL 21. The activity of the expressed bacteriocins was tested against *Listeria monocytogenes* by agar diffusion tests and the minimum inhibitory concentration (MIC) was assessed, as well.

Results. The microbial community of the Cotija cheese is composed mainly by lactic acid bacteria and three species were dominant: *Lactobacillus plantarum, Weissella paramesenteroides and Leuconostoc mesenteroides*.
The functional analysis allowed the detection of a total of 134 contigs containing clusters of genes with domain annotation of bacteriocin and/or immunity proteins. Of those, 17 had both genes in the same genomic context. An unexpected amount of immunity genes was observed with respect to the number of bacteriocin ones, suggesting that the immune mimicry (2) is a key strategy of the strains survival in this system. Selected fragments for cloning and expression in a heterologous system (contigs 295 and 14178), included a gene with domain for a Lactococcin type protein family (PF04369) and an adjacent gene with an immunity protein domain for enterocin type A (PF08951). After their heterologous expression, the putative bacteriocins in both contigs were active vs. *L. monocytogenes*, as predicted, with an activity 4 times higher than the commercial bacteriocin (Table 1).

Table 1. Antilisterial activity (against *L. monocytogenes CFQ-B103*) of selected metagenomic gene clusters and its comparison with Nisaplin®, as a reference.

Contig ID	MIC (µg/mL)
295	78
14178	78
Nisaplin®	312

Conclusions. To our knowledge, this is the first work in which novel antilisterial bacteriocins had been *in silico* predicted, cloned and expressed, showing a higher activity than the only bacteriocin commercially available. This suggest that these bacteriocins could be used in as an additive in dairy food or in the industrial equipment sanitization.

Acknowledgements. AEZ and AE thank CONACyT for their PhD and Masters scholarships, respectively. PAPIIT-DGAPA-UNAM IN222115 and IN222717.

References.

1. Escobar-Zepeda A, Sanchez-Flores A, Quirasco M. (2016). *Food Microbiol*.57:116-127.
2. Draper L, Grainger K, Deegan L, Cotter P, Hill C, Ross R. (2009). *Microbiol*.71:1043-1054.

ISSN: 2448-8461

PB5

Implementación de la aleación Ti-6Al-4V para manufactura de prótesis para pie diabético.

Hernández-Ramírez, Aldo, Urriolagoitia-Sosa, Guillermo, Romero-Ángeles, Beatriz,
Martínez, Reyes, Jacobo, Urriolagoitia-Calderon, Guillermo, Manuel
Instituto Politécnico Nacional
Escuela Superior de Ingeniería Mecánica y Eléctrica
Sección de Estudios de Posgrado e Investigación
Unidad Profesional "Adolfo López Mateos" Zacatenco
Edificio 5 Segundo Piso, Colonia Lindavista, Delegación Gustavo A. Madero C.P. 07738,
Ciudad de México, México
alher9194@gmail.com

Palabras clave: Prótesis, Titanio, Biocompatibilidad.

Introducción. La implementación de la aleación Ti-6Al-4V para la manufactura de prótesis de pie diabético tiene una gran importancia debido a que se trata de un material utilizado para aplicaciones biomédicas, incluyendo la fabricación de instrumentos quirúrgicos [1].
En el presente trabajo se realiza la investigación sobre la biocompatibilidad del material en contacto directo con el tejido vivo en pacientes diabéticos que han sufrido amputación en cualquiera de sus pies.

Método. Se establece el diseño para obtener una aleación de Ti-6Al-4V en relación a las proporciones de mezcla, todo en función de los porcentajes de concentración para posteriormente analizar la biocompatibilidad del material en contacto directo con el tejido vivo de un paciente diabético que haya sufrido amputación en cualquiera de sus pies. Una vez obtenida la aleación se puede diseñar y manufacturar una prótesis funcional que parte de la articulación de chopart hasta las falanges distales de la planta del pie como se muestra en la figura 1.

Fig.1 Articulacion Chopart.

Resultados. La aleación a entrar en contacto directo con el medio vivo sobre su superficie se forma una capa de óxido que impide la propagación de la corrosión al resto del material, de esta forma no se liberan iones metálicos al dentro del cuerpo humano [2]. La prótesis al ser manufacturada de titanio ofrece mayor tiempo de vida útil al usuario y hay menor probabilidad de que la estructura sufra alguna deformación o fractura por fatiga a causa de las constantes cargas a las que puede ser sometida durante la marcha, las cuales pueden ser fuerzas de carga vertical y fuerzas de reacción del suelo (FRS).

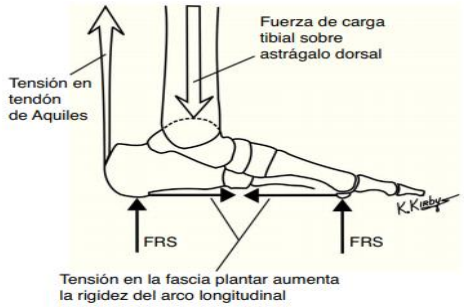


Fig.2 Cargas que actúan sobre el pie durante la marcha.

Conclusiones. La manufactura de exoprótesis para pacientes con pie diabético que han sufrido amputación a causa de esta enfermedad resulta una opción muy viable para ayudar a las personas a volver a caminar retomando sus actividades diarias con la mayor naturalidad posible, por otra parte, se busca que las personas tengan acceso a este tipo de prótesis reduciendo los costos del producto finalizado.

Agradecimientos. Los autores agradecen al Instituto Politécnico Nacional y al Consejo Nacional de Ciencia y Tecnología por su apoyo en la elaboración de este trabajo.

Referencias.

1. Shetty R.H., Mechanical and corrosion properties of nitrogen diffusion hardened Ti6Al4V alloy. Medical applications of titanium and its alloys ASM-STP 1272, ISBN 0-8031-2010-9 pp 240-251 (1996).
2. R. Zwicker, K. Buehler, R. Mueller et al., Mechanical properties and tissue reactions of a titanium alloy for implant material, titanium '80, Science and Technology Proe. 4th Int. Conf. On Titanium, Kyoto, May t980. IVlet. Soc. AIME (ISBN-No. 89520-370- 7), pp. 505-514.

ISSN: 2448-8461

PB6

SPORES PRODUCTION OF *Trichoderma asperellum* Tc74 IN BIOREACTOR

Violeta Balene Ramírez Hernández[a], César Guigón López[b], Leticia Bravo Luna[a], Mario Rodríguez Monroy[a]; a. Instituto Politécnico Nacional, Centro de Desarrollo de Productos Bióticos, Yautepec, Morelos, 62731. b. Centro de Investigación para los Recursos Naturales México.; mariorodriguezmonroy@gmail.com

Key words: Trichoderma asperellum, Bioreactor, antagonistic activity

Introduction. *Trichoderma asperellum* Tc74 is an isolated strain of a chili crop in the state of Chihuahua, Mexico and has activity against phytopathogenic fungi with agricultural importance. At present, this fungus is grown in microbiological culture media in stirred flask, however, it is desirable to obtain its culture in bioreactor with a non-microbiological medium.
The objective of the work was to establish a culture of *T. asperellum* Tc74 in a culture medium of defined composition, which allows its growth and sporulation in agitated tank type bioreactor, as well as to preserve its antagonistic activity against *Fusarium oxysporum*.

Methods. The production of spores of *T. asperellum* Tc74 was evaluated in liquid culture medium selective for Trichoderma (SST) (1) and potato dextrose broth (PDB); 250 mL Erlenmeyer flasks and a 1 L stirred tank bioreactor were used. The cultures in flasks were incubated in an orbital shaker at 30 °C and 400 rpm. While for the cultures in bioreactor, an Applikon equipment of 1 L was used, agitation at 400 rpm with a Rushton impeller, aeration of 0.5 vvm and 30 °C. Growth kinetics were performed with both systems and the production and productivity of spores/mL, its viability was determined. The antagonistic activity against *F. oxysporum* was determined in dual culture tests (3).

Results. The biomass yield of *T. asperellum* Tc74 in the TSM medium reached only 15% of that obtained in PDA. However, the spore yield was 10.8 and 56.7 times higher in flasks and bioreactor, respectively. This may be due to the fact that *T. asperellum* during its cultivation in TMS medium, produces compounds that lower the pH of the medium to values of 2.89, while in the PDB medium the pH remains at a value of 7.03. Then the change of pH, can induce the process of sporulation of the fungi. The viability of the spores in the TMS medium was 96%. At the flask level, the antagonistic activity of *T. asperellum* against *F. oxysporum* was the same in the TSM medium as with the PDA medium. However, *T. asperellum* spores produced in flask inhibited growth of *F. oxysporum* 1.17 times more than spores produced in bioreactor. Therefore, future work is aimed at defining the culture conditions in the bioreactor that allow obtaining *T. asperellum* spores that preserve their antagonistic activity against *F. oxysporum.*

Table 1. Kinetic parameters of *T. asperellum* TC74 cultures developed in shake flask and bioreactor; using PDA and TSM medium. Antagonic and antibiotic activity against *F. oxysporum*.

Parameter	Erlenmeyer flask		Stirred tank bioreactor
	PDB	TSM	TSM
Biomass Yield (g dw/L)	5.79	0.88	0.55
Spore Yield (Spores x10^8/g dw)	1.20	13.00	68.10
Viability (%)		96.75	96.00
pH	7.03	2.89	3.55
Dual Culture			
Micelial Growth	26.31	26.44	20.10
Micelial Growth Inhibition (%)	23.99	23.62	14.99

Conclusions. TSM media can be used to growth *T. asperellum* Tc74 in a stirred tank bioreactor, the spores produced maintain its viability and preserve its antagonistic activity against *Fusarium oxysporum.*

Acknowledgements. VBRH to CONACYT and SIP-IPN (BEIFI) for the scholarship. The work was conducted with support of SIP-IPN (20180427 and 20180307).

References.

1. Elad Y, Chet I, Henis Y, 1981. A selective medium for improving quantitative isolation of *Trichoderma* sp. from soil. *Phytoparasitica* 9(1): 59-67.
3. Rajendrian R, Jegadeeshkumar D, Sureshkumar BT, Nisha T. 2010. *In vitro* assessment of antagonistic activity of *Trichoderma viridae* against post harvest pathogens *Agric Technol* 6(1):31-3

ISSN: 2448-8461

AT-LINE METHOD FOR QUANTIFICATION OF ALCOHOLS IN ACETONE-BUTANOL-ETHANOL (ABE) FERMENTATION

Jesus Vega-Estrada, Laura J Noriega-Medrano, Fernando Grijalva-Hernández, Monserrat Escobar-Rosales, MC Montes-Horcasitas; Departamento de Biotecnología y Bioingeniería, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV), CDMX, CP 07360, Mexico; vegaj@cinvestav.mx

Key words: ABE fermentation; alcohols quantification; butanol; Clostridium acetobutilicum; non-chromatographic method

Introduction. Alcohols produced in ABE fermentation are commonly quantified by gas chromatography (GC) or high performance liquid chromatography (HPLC). Recently, non-chromatographic methods for estimation and detection of butanol has been developed (1,2). One method is based on alcohols separation by "salting out extraction" followed by reaction with diquat reagent and measured spectrophotometrically (1); in the other method, alcohols separation was carried out by "microdiffusion principle" in a type Conway closed chamber using acid-dichromate as trapper and oxidant agent, the reduction of dichromate was proportional at alcohols present in ABE fermentation samples (2). The goal of this work is development a rapid method for alcohols quantification in *C. acetobutilicum*-ABE fermentation samples.

Methods.
In this method, alcohols separation by gas stripping and acid-dichromate as trapper and oxidant agent were used. Simultaneously, a stream of air is bubbled into a liquid (containing fermentation sample) and, the exhaust gas carrying alcohol was bubbled into acid-dichromate solution, where the reduction of the dichromate was proportional to alcohols concentration in the sample. This method was adapted varying air flow, reaction time and butanol concentration and, validated for ABE fermentation samples.

Results. Fig. 1 shows gas-stripping system used. With 5 ml reaction volume containing sample and bubbled air at 179 mL/min during 10 min The validation revealed a linearity (0.9916), limit of detection (LOD), and a limit of quantification (LOQ) of 0.021, and 0.061 g/L, respectively. The acceptance criterion was a coefficient of variation (CV) ≤ 5% for repeatability and 10% for reproducibility. Butanol estimation in ABE fermentation with this method was above 95 % measured by GC.

Conclusions. The butanol concentration in ABE fermentation samples was estimated semiquantitative and rapid using this method economical and easy.

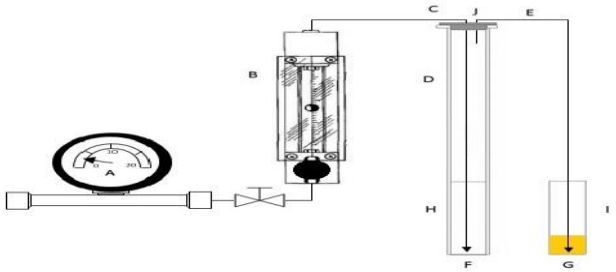


Fig. 1 Gas-stripping system: A) air pressure regulator (1 kg/cm^2); B) rotameter; C) silicone tubing (14 cm); D) stainless steel needle (14 cm); E) PTFE tubing; F) glass tube (16 ml); G) Collector; H) 5 mL containing properly diluted sample; I) acid dichromate; J) rubber plug.

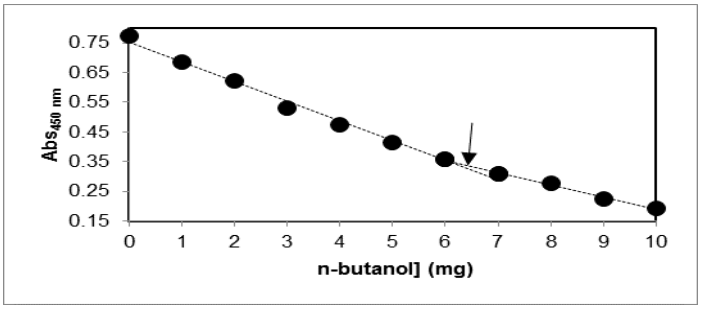


Fig. 2 Calibration curve for alcohol determination with at-line method. Arrow indicates linearity end

Acknowledgements.
CINVESTAV –IPN by financial support and, Carmen Fontaine Sánchez for technical assistance.

References.
1. Maiti S, Sarma SJ, Brar SK, Bihan YL, Drogui P, Buelna G, Verma M, Soccol CR. (2015). *Talanta.* 15(21):116-21
2. Noriega-Medrano LJ, Vega-Estrada J, Ortega-López J, Ruiz-Medrano R, Cristiani-Urbina E, Montes-Horcasitas MC. (2016). *J Microbiol Methods* 126: 48-53

ISSN: 2448-8461

PB8

BIOCHEMICAL CHARACTERIZATION OF TA0338, A PLANT-TYPE ASPARAGINASE FROM *THERMOPLASMA ACIDOPHILUM* WITH BIOMEDICAL AND BIOTECHNOLOGICAL RELEVANCE

Juan Carlos González-Zamora, Diana Elizabeth Aguilar-Estrada, Karla Grisel Calderón-González, Leticia Santos; Division of Molecular Biology, Instituto Potosino de Investigación Científica y Tecnológica, A.C. (IPICYT); 78216 San Luis Potosi, SLP;
Leticia Santos: lsantos@ipicyt.edu.mx

Key words: plant-type L-asparaginase, Thermoplasma acidophilum, characterization

Introduction. L-asparaginases (ASNases EC 3.5.1.1) are hydrolytic enzymes that process the no essential amino acid asparagine (ASN) into aspartate and ammonium. This mechanism of action has been key for 1) the treatment of the acute lymphoblastic leukemia (ALL) in the depletion of ASN from the blood and 2) acrylamide content diminution in a wide range of foods containing high content of reducing sugars. *Thermoplasma acidophilum* Ta0338, is a plant-type L-ASNase classified into the N-terminal nucleophile (Ntn)-hydrolase superfamily (1). We have demonstrated that Ta0338 ASNase sequence is closer to human AGAs, which makes it desirable for therapy.

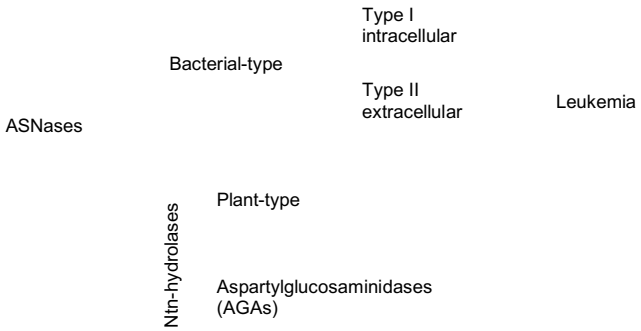


Fig.1 Classification of ASNases according to their enzymatic activity.

The objective of this work was to evaluate the enzymatic activity of this ASNase and identify its stability against commercial ASNAses.

Methods. Biochemical characterization was performed by direct nesslerization technique in the presence of Nessler reagent to detect the ammonium production at 450 nm for the quantification of L-asparaginase activity (2). The effect of temperature, pH, metal ions, and time resistance was evaluated as well as the kinetic parameters Km, Vmax, and Kcat. The activity of the enzyme is reported as specific activity in μmol NH_3/mg ASNase/min. All experiments were performed in triplicates.

Results. Ta0338 ASNase showed maximum specific activity at 70ªC, pH between 4.5-5.5, in the presence of 50 mM $MgCl_2$ (Table 1).The specific activity found was 13 μmol/min/mg. Kinetic parameters showed a Km of 19.3 mM, Vmax 16.42 U/mg and Kcat/Km 0.23 mM/s (Table 2).

Table 1. Specific activity of Ta0338 ASNase against other L-ASNases

Source	Specific activity (μmol/min/mg)	Maximum Temp. (°C)	Opt. pH	Reference
T. acidophilum	13	70	5.5	This work
E. coli	200	37	7-8	3
E. chrysanthemi	118.7	37	8	4
T. kodakaraensis	2350	85	9.5	5

Table 2. Kinetic parameters of Ta0338 ASNase against other L-ASNases

Source	Km (mM)	Kcat (s^{-1})	Kcat/Km ($Mm^{-1}s^{-1}$)	Reference
T. acidophilum	19.37	4.52	0.23	This work
E. coli	0.015	24	1600	3
E. chrysanthemi	0.058	2.4E+04	4.12E+05	4
T. kodakaraensis	5.5	1397	254	5

Conclusions. Plant-type ASNase Ta0338 from *Thermoplasma acidophilum* displayed L-ASNase activity. Our results shows Ta0338 is active at broader range of temperatures and pH, this reflects the potential of recombinant Ta0338 ASNase in the food processing industry and possibly, in leukemia therapy. Moreover, the thermal stability of the protein may help its usage, storage, and transportation.

Acknowledgements. Division of Molecular Biology, IPICYT.

References.

1. Guzman-Rodríguez M, Serna-Domínguez, MG, Santos L. (2014). *Biocat & Biotrans.* Vol. (32):295-301.
2. Imada A, Igarasi S, Nakahama K, Isono M. (1973). *J Gen Microbiol.* Vol. 76(1):85-99.
3. Derst C, Henseling J, Röhm KH. (2000). *Protein Sci.* Vol. 9(10):2009-17.
4. Kotzia GA, Labrou NE. (2007). *J Biotechnol.* Vol. 20;127(4):657-69.
5. Hong SJ, Lee YH, Khan AR, Ullah I, Lee C, Park CK, Shin JH. (2014). *J Basic Microbiol.* Vol. 54(6):500-8.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México

PB9

COMPARATIVE STUDY OF THE SPECTRAL ABSORBANCE OF TWO MUCILAGES INTENDED FOR THE ELABORATION OF ORGANIC SUNSCREENS

Juan Carlos Ramírez-Granados, Blanca E. Gómez-Luna, Rafael A. Veloz-García, César Díaz-Pérez, Milagros López L. and Paola F. Magdaleno M., University of Guanajuato, Department of Agro-industrial Engineering, Celaya, Gto. 38060
jcramirez@ugto.mx

Key words: sunscreen, mucilage, absorbance

Introduction. The solar radiation impinging upon the Earth´s surface is essential for life. People, animals, plants, and other microorganisms depend directly or indirectly on the sun´s energy. The solar light is useful in many human activities and, it provides benefits such as the stimulation of D-vitamin production; nevertheless, the direct and long-lasting exposure of the skin to the sunlight may induce severe lesions including melanoma and carcinoma skin cancers (1). For this reason, dermatologists currently recommend the daily use of topical sunscreens and continuous reapplications. However, most of the commercial sunscreens are chemical-based. Although chemical sunscreens are considered innocuous, their low toxicity may cause undesired long-term effects to consumers (2). Hence, it would be desirable to have safer and natural photoprotection alternatives (3).
In this research work, the absorbances of the mucilages of *Opuntia* spp. (nopal) and *Salvia hispanica* L. (chia) are compared to evaluate their capacity to absorb ultraviolet (UV), visible and near-infrared radiation, and to analyze the feasibility of using these natural materials as active ingredients in organic sunscreens for the skin.

Methods. Two previously reported methods were implemented to extract, to purify, and to dry the mucilages (4,5). After obtaining the powdered mucilages, each of them was individually dissolved in distilled water to obtain two saturated solutions. The solution with mucilage of *Opuntia* spp. had a concentration of 5.5 g/L; while the solution with mucilage of *Salvia hispanica* L. had a concentration of 12.2 g/L. Then, the spectral absorbances of these saturated solutions were measured in the UV, visible, and near-infrared bands.

Results. Fig. 1 shows the mucilages extracted from the cladodes of *Opuntia* spp. (A) and from the seeds of *Salvia hispanica* L. (B). The spectral absorbances of the solutions saturated with these mucilages were measured with a Jenway 7305 spectrophotometer from 200 to 1000 nm. Fig. 2 presents the absorbance spectra of the solutions with mucilages of *Opuntia* spp. (A) and *Salvia hispanica* L. (B).

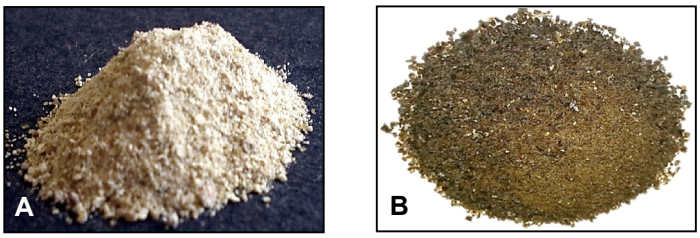


Fig. 1 Purified and dehydrated mucilages of *Opuntia* spp. (A) and *Salvia hispanica* L. (B).

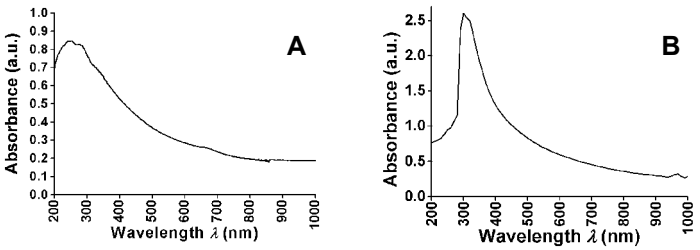


Fig. 2 Absorbance spectra of solutions saturated with mucilages of *Opuntia* spp. (A) and *Salvia hispanica* L. (B).

These spectra demonstrate that both mucilages have strong absorption of UVB/UVA (290-400 nm) radiation.

Conclusions. The mucilages of *Opuntia* spp. and *Salvia hispanica* L. have a strong absorbance in the UVB and UVA spectral bands. Consequently, they may be employed in the production of sunscreens. The mucilage of *Salvia hispanica* L. has a more intense absorbance in the UV region and therefore, it is more suitable for use as active ingredient in organic sunscreens for the skin.

Acknowledgements. The authors express thanks to the Depts. of Agro-industrial Engineering, and Support to Professors, the DCSI and the DAIP of the University of Guanajuato for supporting this research.

References.

1. Latha M. S., *et* al. (2013). *J Clin Aesthet Dermatol* (6): 16–26.
2. Diffey B. L. (2011). *J Cosmet Dermatol* (10): 245–250.
3. Saewan N., Jimtaisong A. (2013). *J Appl Pharm Sci* (3): 129–141.
4. Domínguez V., *et* al. (2011). *Desplegable informativa* INIFAP (21): 1-6.
5. Capitani M. I. (2013). Doctoral dissertation, Universidad Nacional de la Plata, Buenos Aires, Argentina, 99-100.

ISSN: 2448-8461

PB10

MÁQUINA PARA EVALUAR DESGASTE EN INSERTOS DE PRÓTESIS DE TOBILLO BAJO LA ACCIÓN DE MARCHA

González-Uribe, I., Urriolagoitia-Sosa, G., Romero-Ángeles, B., Martínez-Rodríguez, R., Urriolagoitia-Calderon, G. M.
Instituto Politécnico Nacional, Escuela Superior de Ingeniería Mecánica y Eléctrica, Sección de Estudios de Posgrado e Investigación, Unidad Profesional Adolfo López Mateos "Zacatenco" Avenida Instituto Politécnico Nacional s/n, Edificio 5, 2° piso. Col. Lindavista, C.P. 07320, Ciudad de México.
ing.mec.igu@hotmail.com

Palabras clave: Prótesis, desgaste, máquina, inserto

Introducción

Desde que el ser humano ha existido se encuentra expuesto a sufrir lesiones en menor grado, desde torceduras hasta llegar a amputaciones. Para cualquiera de los casos la persona afectada se ven en la necesidad de remplazar la parte afectada por una funcional. Este tipo de acción se hace mediante prótesis [1]. El principal problema en el uso de las prótesis es la vida útil que presentan, debido a lo anterior, el ser humano ha buscado formas de realizar pruebas de desgaste a los distintos materiales, con el fin de aumentar su vida. Por lo cual, el objetivo de este trabajo es visualizar la funcionalidad de una máquina que permita realizar pruebas de desgaste en los insertos de las prótesis para la articulación de tobillo [2].

Desarrollo

En este trabajo se analiza el desgaste producido en el inserto de prótesis de tobillo con ayuda de la máquina diseñada para realizar pruebas de desgaste dentro del Instituto Politécnico Nacional (IPN) la cual se muestra en la Figura 1 la cual realiza los movimientos de Flexión-Extensión, Inversión-Eversión, Aducción-Abducción.

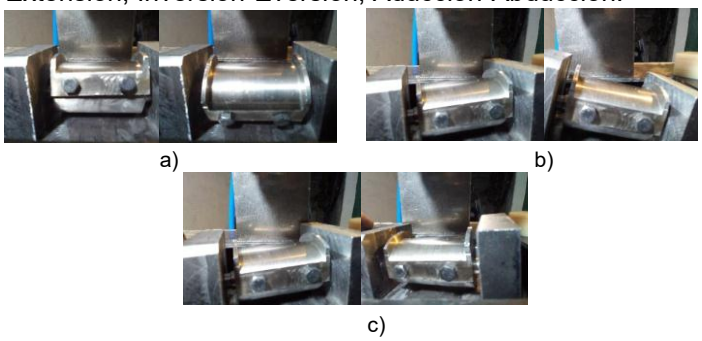


Figura 1.- Movimientos de la máquina a) Flexión-Extensión, b) Inversión-Eversión c) Abducción-Aducción

El primer paso consiste en colocar el inserto en la máquina el cual es fijado en dos soportes que sustituyen las piezas superior e inferior de una prótesis. Posteriormente programar la máquina para realizar el movimiento de flexión extensión para observar el desgaste que se produce. Finalmente al introducir todos los parámetros como son la forma de aplicación de la carga, la velocidad de la marcha y el número de ciclos.

Resultados

Las pruebas de desgaste obtenidas en los insertos mediante el experimento manifiestan drásticamente una reducción de volumen de estas piezas (Figura 2).

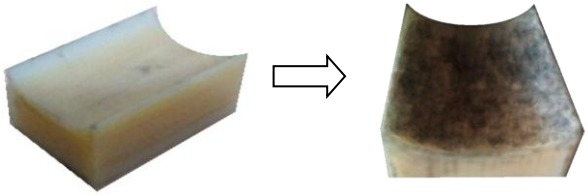


Figura 2.- Desgaste en los insertos

Conclusiones

El realizar pruebas de desgaste a los insertos de las prótesis permite, no solo determinar la vida útil de estas, sino seleccionar distintos materiales biocompatibles con el cuerpo humano para fabricar prótesis con materiales de alta calidad que ayuden en la calidad de vida de los personas que utilicen estos implantes médicos y no tener que remplazar las prótesis en un corto plazo así disminuyendo el daño ocasionado en los huesos.

Agradecimientos

Los autores agradecen al Instituto Politécnico Nacional y al Consejo Nacional de Ciencia y Tecnología por el apoyo brindado, en la elaboración de este trabajo.

Referencias

1.- López E. A., Lesso A. R., Esquivel G. J. Desarrollo de Bancos de Pruebas para Desgaste Usando Metodologías KANO- TRIZ. Memorias del XVIII Congreso Internacional Anual de la SOMIN., del 19 al 21 de Septiembre 2012. Salamanca Guanajuato, México.

2.-. González U. I. (Tesis de Maestría). "Diseño y construcción de una máquina para análisis de desgaste de prótesis para la articulación de tobillo". Instituto Politécnico Nacional. Ciudad de México. pp. 10-18.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
PB11

IDENTIFICATION AND QUANTIFICATION OF PHENOLIC COMPOUNDS IN PEEL AND SEED OF REGIONAL AVOCADO FROM SINALOA, MÉXICO

Melina R. Baldenebro-Cervantes[1], Sandy R. Hernández-Leyva[1], Carlos L. Calderón-Vázquez[1], Claudia Castro-Martínez[1].
1. – Departamento de Biotecnología Agricola, Instituto Politécnico Nacional-CIIDIR Unidad Sinaloa, C.P. 81101. Guasave, Sin. Claudiacm30@hotmail.com, melina.balcer@gmail.com
Key words: Phenolics compounds, avocado peel and seed

Introduction. The avocado waste (peel and seed) can be processed to yield value added products for which promising and significant applications in the food and related industry exists. Currently, there is a growing interest in finding phytochemicals as alternatives to synthetic substances commonly used in the food, pharmaceutical and cosmetic industries. Some phytochemical studies on avocado seeds have identified saponin, phytosterols, triterpenes, fatty acids, furanoic acids, flavonols and proanthocyanidins. Phenolic compounds are relevant because retarding oxidative degradation and thus provide quality and nutritional value to foods, this antioxidant activity is associated with its role against cardiovascular diseases, cancer and aging processes (1). Thus, the objective of this work is to identify and quantify phenolic compounds in peel and seed of regional avocado of Sinaloa, México, for its possible use in the industry.

Methods. Peel and seed were obtained from local orchards located in Sinaloa, México. They were first physically treated with low heat and a mechanical grinding to reduce the size of the samples. An extraction was necessary in order to obtain free and bound phytochemical compounds from the dry samples according to the method described by Adom and Liu (2002) (2). Then, identification and quantification of phenolic compounds by HPLC-DAD was performed, according to Dueñas *et al*., (2009) (3).

Results. Nine and six phenolic compounds in peel and seed respectively, were identified. Epicatechin is the compound with the highest concentration compared to other compounds in both peel and seed, (Table 1).

Table 1. Concentration of phenolic compounds found in the peel and seed of regional avocado from Sinaloa, México.

COMPOUND	PEEL µg/g of dry sample	SEED µg/g of dry sample
Epicatechin	517.61 ± 2.1	120.77 ± 1.75
Caffeic acid	98.42 ± 2.51	40.25 ± 0.48
Quercetin	93.45 ± 1.25	29.13 ± 0.32
Isorhamnetin	74.17 ± 1.07	ND
Ferulic acid	64.38 ± 1.02	15.69 ±0.23
p-cumaric	60.9 ± 0.31	1.96 ± 0.98
Catechin	40.15 ± 1.22	ND
o-cumaric	22.69 ± 0.11	0.83 ± 0.41
Kaempferol	2.68 ± 0.06	ND

ND: not detected

The peel contained 517 µg/g, and the seed contained 120 µg/g of epicatechin. Moreover, caffeic acid, quercetin, isorhamnetin, ferulic acid, p-cumaric, catechin, o-cumaric and kaempferol were found in peel, while in seed the same were found except isorhamentin, catechin and kaempferol. Melgar *et al*., 2018 (4) and Claderón-Oliver *et al*., 2016 (5), showed similar results, peel had the major concentration of phenolic compounds and epicatechin was the more abundant phenolic compound in the peel of Hass avocados, these authors also found the compounds mentioned above when analyzed Hass avocado byproducts.

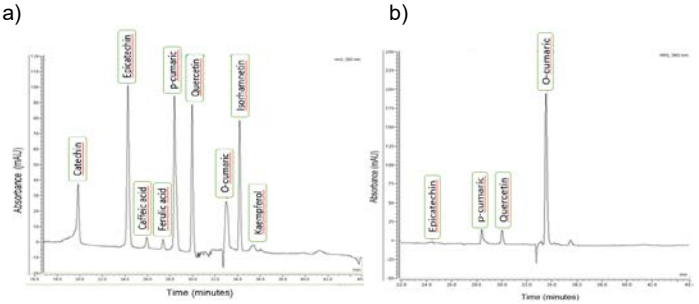


Fig.1 Chromatogram of phenolic compounds found in regional avocado a) peel at 260 nm, were found nine compounds, and b) seed at 360 nm, were found six compounds at different time.

Conclusions. Avocado peel contained nine phenolic compounds and the seed six, like epicatechin with the highest concentration. Caffeic acid, quercetin, isorhamentin, ferulic acid, p-cumaric, catechin, o-cumaric and kaempferol were also found and these could be used in pharmaceutical and food industries.

Acknowledgements. The authors gratefully acknowledge the financial support of SAGARPA CONACYT No. 291143, project SIP20180302 and SIP20180446.

References.

1. Barbosa-Martín, E., Chel-Guerrero, L., Gonzalez-Mondraagon, L., Betancur-Ancona, D., (2016). *Food Bioprod. Process*. (100); 457-463.
2. Adom, K. K., y Liu, R. H. 2002. J. Agric. Food Chem (21): 6182-6187
3. Dueñas, M., Hernández, T., Estrella, I., Fernández, D., 2009. *Food Chem*., (117): 599-607
4. Melgar, B., Dias, M.I., Ciric, A., Sokovic, M., Garcia-Castello, E.M., Rodríguez-López, A.D., Barros, L., Ferreira, I., (2018). *Ind. Crops. Prod*. (111): 212-218
5. Calderón-Oliver, M., Escalona-Buendía,H.B., Medina-Campos, O.N., Pedraza-Chaverri,J., Pedroza-Islas, R., Ponce-Alquicira, E., (2016). *Food Sci. Technol.* (65); 46-52.

ISSN: 2448-8461

PB12

EFFECT OF ACID AND ALKALINE PRETREATMENT IN FERMENTABLE SUGARS PRODUCTION FROM WHEAT STRAW FOR 2G BIOETHANOL

Laura Ivonne Beltrán-Arredondo[1], Ángel Manuel Guerra-Martínez[2], Grethel Priscila Gaytán-Pinzón[3], Xiomara Machado-Velarde[1], Claudia Castro-Martínez[3]. [1]Facultad de Ciencias Químico Biológicas· Universidad Autónoma de Sinaloa, Culiacán, Sin. C.P. 80013. [2]Instituto Tecnológico Superior de Guasave, Guasave, Sin. C.P. 81148. [2]Depto. de Biotecnología Agrícola, CIIDIR-IPN Unidad Sinaloa, Guasave. Sin. C.P. 81101. laura_beltran_a@hotmail.com

Key words: Agricultural residues, enzymatic hydrolysis, 2G bioethanol

Introduction. The northwest region of Mexico generates a large amount of residues from crops that have the potential of becoming a central focus for bioethanol production (1). Second generation bioethanol is produced from lignocellulosic biomass like stems, leaves and straw of agricultural crops. Bioethanol production involves three main steps: pretreatment, enzymatic hydrolysis and fermentation. One of the crucial steps in biomass degradation is enzymatic hydrolysis; however, the lack of an effective pretreatment can negatively affect the efficiency of saccharification (2).
This work aimed to evaluate acid and alkaline pretreatment of wheat straw and to compare the efficiency of its enzymatic hydrolysis through the release of fermentable sugars.

Methods. Wheat straw obtained from the northern region of Sinaloa, Mexico was used for all experiments. Sulfuric acid was employed for acid pretreatment. Hemicellulose content was measured by HPLC in the liquid fraction after pretreatment. For alkaline pretreatment, hydrogen peroxide was used. The percent of lignin removal was calculated after pretreatment using the procedures provided by NREL (3). A 2^3 factorial design with concentration, time and solid-liquid ratio (SLR) as dependant variables was used to determine the optimal conditions for each pretreatment. Then, enzymatic hydrolysis was carried out using the pretreated wheat straw with a commercial cellulase. Efficiency was evaluated measuring the release of the total reducing sugars (4).

Results. For acid pretreatment, H_2SO_4 concentration (1 and 3% v/v), time (15 and 45 min) and SLR (1:8 and 1:12) were evaluated to determine the amount of hemicellulose removal after wheat straw pretreatment. The highest amount of hemicellulose in the liquid fraction (16.4%) was reached (Fig.1A) with 1% (v/v) of H_2SO_4, 45 min and SLR 1:12 as optimal conditions. For alkaline pretreatment, H_2O_2 concentration (2 and 6% v/v), time (30 and 70 h) and SLR (1:10 and 1:20) were evaluated to determine the amount of lignin removal after wheat straw pretreatment in the solid fraction. The optimal conditions were 6% (v/v) of H_2O_2, 70 h and SLR 1:20, with 68.62% of removed lignin. Fig. 1B shows that a higher concentration, time and SLR, resulted in a greater quantity of lignin removed from wheat straw.

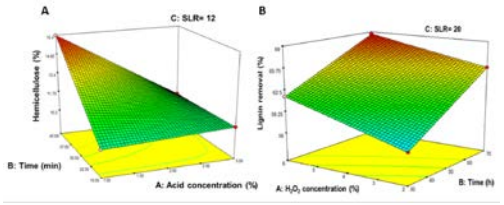


Fig.1 (A) Effect of acid concentration, time and SLR on the hemicellulose content (%) during acid pretreatment and (B) Effect of hydrogen peroxide concentration, time and SLR on lignin removal (%) during alkaline pretreatment in wheat straw.

Enzymatic hydrolysis of acid and alkaline pretreated wheat straw in the optimal conditions was carried out with a commercial cellulase of *T. reesei* for 28 h (Fig.2). Alkaline pretreated straw reached the major amount of reducing sugars (219.20 mg/g dry biomass), doubling the sugars released by acid pretreated straw (102.69 mg/g dry biomass).

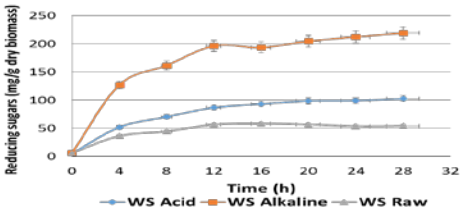


Fig.2 Enzymatic hydrolysis of raw, acid and alkaline pretreated wheat straw.

Conclusions. Results revealed that alkaline pretreatment has a great effect in the reducing sugars recovered from wheat straw, lignin removal and improvement of cellulose digestion as observed in the enzymatic hydrolysis assays. Overall, alkaline pretreatment demonstrated to be the best experimental strategy for 2G bioethanol production.

Acknowledgements. The authors gratefully acknowledge the financial support of SAGARPA-CONACYT No 291143 and project SIP20180302.

References.

1. Rios, M., Kaltschmitt, M. (2013). *Biomass Conv. Bioref.* vol (3): 239–254.
2. Prasad, D., Singla A., Negi S. (2015). *Biotech.* vol. (5): 597–609.
3. Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D. (2008) *NREL/TP-510-42618.*
4. Miller, G .L., Blum, R., Glennon, W.E., Burton, A .L. (1959). Anal Biochem. vol (2): 127–132.

ISSN: 2448-8461

OPTIMIZATION OF SACCHARIFICATION OF WHEAT STRAW USING *Cladosporium* sp. ENZYMATIC EXTRACT BY THE BOX-BEHNKEN METHOD

Sandy R. Hernández-Leyva.[1,] Lelie D. Castro-Ochoa[1,] Itzel G. López-Zamora[2], María G. Aguilar-Uscanga[3], Claudia Castro-Martínez[1]. [1] IPN, CIIDIR Unidad Sinaloa. Dpto. de Biotecnología Agrícola. Blvd. Juan de Dios Bátiz Paredes, C.P. 81101. Guasave, Sinaloa, México. [2] UAdeO, Dpto. de ingeniería y Tecnología, Los Mochis, Sinaloa. [3]TNM, Instituto Tecnológico de Veracruz, H. Veracruz, Veracruz, México. Email. sandy.rocio@hotmail.com claudiacm30@hotmail.com

Key words: Saccharification, wheat straw, cellulases

Introduction. The lignocellulosic biomass is the largest source of carbohydrate, it consists of lignins chains, cellulose, and hemicellulose, which are intertwined and chemically linked by noncovalent forces and by covalent crossed connections (1). Wheat straw (WS) is one of the major agricultural residues, and it is frequently studied as a lignocellulosic raw material for bioethanol production. The NaOH-delignification is an alkaline pretreatment where the major effect are the cleavage of ester bonds between lignin and hemicellulose and the swelling of the cellulose fibers, resulting in better enzymatic hydrolysis (2).
The objective of this work is optimization of saccharification process of what starw using *Cladosporium* sp. enzymatic extract by the box-behnken method.

Methods. Cellulase were produced by *Cladosporium* sp. (native strain) grown in the mineral medium with stover of corn as source carbon at 30°C, 150 rpm for 5 days. To the residue of the wheat straw an alkaline pretreatment was previously applied under the following conditions, 6% H_2O_2 at 30 ° C for 72 h. For saccharification 1g of wheat straw was placed in a 50 ml Erlenmeyer flask, sodium acetate buffer (pH 5, 50 mM) was added. The reducing sugars obtained from hydrolysis were measured by DNS method (3). Optimization studies of hydrolysis process parameters were carried out maximize the reducing sugar yield using the Box- Behnken method. Liquid- solid ratio, stirring rate and enzyme loading are the variables that affect the saccharification yield. The experimental variables were studies at two levels (-1, 1) and three central points.

Results. The statistical treatment combination of test variables along with the measured response value, expressed as hydrolysis yield corresponding to each combination, are summarized in Table 1. Data obtained from response to surface methodology was validated using ANOVA. The coefficient of determination (R^2) was calculated for 0.99 for sugar yield, indicating that the statistical model can explain 99% of the variability in the response.

Table 1. Box-Behnken design matrix for optimization of variables and response value for of saccharification.

Run no.	RPM	Enzyme (mg/g)	LSR	Response Saccharification %
1	50	50	12.5	10.81
2	300	50	12.5	11.12
3	50	400	12.5	40.57
4	300	400	12.5	39.75
5	50	225	5	18.92
6	300	225	5	18.01
7	50	225	20	22.54
8	300	225	20	23.14
9	175	50	5	5.44
10	175	400	5	39.37
11	175	50	20	8.65
12	175	400	20	33
13	175	225	12.5	24.57
14	175	225	12.5	10.81
15	175	225	12.5	24.12

The effects of enzyme loading and liquid-solid ratio on the hydrolysis yield of WS are show in Figure 1. When enzyme loading was at low level, hydrolysis yield was low. Enzyme loading is considered to be one of the major factors affecting the conversion rate of enzymatic hydrolysis of cellulose. The stirring rate had not significant effect.

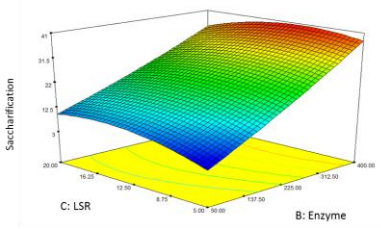


Fig.1. Response surface plot of the combined effects of enzyme loading and liquid-solid ratio (LSR).

Conclusions. Optimization of the hydrolysis process was carried out using Box-Behnken desing. Significant improvement in the hydrolysis yield could be obtained by increasing the amount of enzyme loading to some extent.

Acknowledgements. The authors gratefully acknowledge the financial support of the projects SIP-20180302 and SAGARPA- CONACyT No. 291143.

References.

(1).Sartori, T., Tibolla, H., Prigol, E., Colla, L.M., Costa, J., and Bertolin, T. (2015). BioMed Research International, vol. 2015, pp 9.
(2).Prasanna, H. N., Ramanjaneyulu, G., & Rajasekhar Reddy, B. (2016). *3 Biotech*, 6(2), 162.
(3). Miller, G.L. (1959). Anal. Chem. 31:426-428.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
PB14

Optimization of the acid hydrolysis process in lignocellulosic materials to obtain xylose and second-generation bioethanol

López-Zamora L., Morales-Martínez J.L., Aguilar-Uscanga M.G.; Tecnológico Nacional de México/Instituto Tecnológico de Orizaba; llopezz02@yahoo.com.mx

Key words: xylose, hemicellulose, optimization

Introduction. The use of crop residues has directed many investigations to utilization of glucose and xylose as a carbon source from lignocellulosic material, this residue has been explored for biotechnological of applications, as production of alcohol, xylitol and lactic acid among others, through different forms of operation [1], however, due to the lignocellulosic structure, a pretreatment process is necessary to maximize the production of fermentable sugars; this has created a transformation alternative and lignocellulosic waste management [2].

Methods. The optimization and comparison of the acid hydrolysis (AH) in process of coffee and wood waste, it started with the evaluation of parameters that are shown in Table 1, using a hybrid optimization design (HOD), serving as an explorer design, this process was carried out at 15 psia with diluted acids. The minimization of hemicellulose composition and the production of xylose by HPLC was evaluated as a response variable.

Table 1. Exploratory factors and levels of operation of AH in DHO.

	Coffee/Wood		
Level	X_{h1}; Time (min)	X_{h2}; $[H_2SO_4]$ (v/v)	X_{h3}; (v/w)
-1	15	1	5:1
0	30	2	7:1
1	45	3	9:1

Results. The HOD reached concentrations of xylose for coffee and wood of 19.03 g/L and 27.68 g/L respectively, mathematically no stationary point was found, however, through a statistical analysis, the factors: liquid/solid ratio (1:6) and time (30 min) were discarded and fixed, because they are not significant in the process, this was proven by an F_{ctr}I=6.39, therefore a two factor DCC was used (Table 2).

The optimization of the acid process can be verified in Figure 1, while canonical modeling is described in Figure 2, this analysis generated the stationary points shown in Table 2, the conditions found generate xylose concentrations of 18.19 g/L and 24.85 g/L to coffee and wood, also final concentrations of hemicellulose were obtained of 6.31% and 14.56% respectively.

Table 2. Factors and levels of operation of DCC for optimization of AH in lignocellulosic residues and stationary points of the process.

	Coffee		Wood	
Level	X_{opC1};Time (min)	X_{opC2};$[H_2SO_4]$ (v/v)	X_{opM1} (v/g)	X_{opM2};$[H_2SO_4]$ (v/v)
-1	35	3	2.5:1	2
0	45	4	3.5:1	3
1	55	5	4.5:1	4
X_0	[45.1218	4.2371]	[3.3652	3.4566]

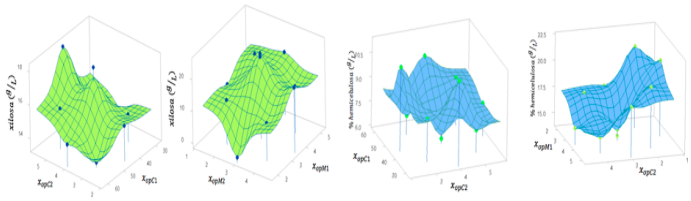


Fig 1. Response surfaces; maximization of xylose concentration and minimization of hemicellulose content.

Therefore, during the optimization process the ideal conditions were located, to remove the most hemicellulose, as the first stage and conditioning of the second-generation ethanol generation process.

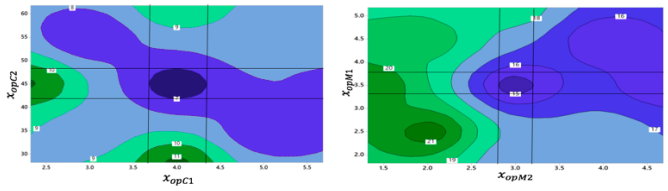


Fig 2. Contours of optimal zones of AH; Coffee characterized by the function; $\%Hem_C=68.07+1.04W_1^2+0.01W_2^2$ and wood characterized by the function; $\%Hem_M=48.09+1.36W_1^2+1.25W_2^2$.

Conclusions. Through the use of diluted acids, the pretreatment process of lignocellulosic materials was optimized; removing 75.48% and 43.48% of hemicellulose and obtaining xylose with concentrations of 18.19 g/L and 24.85 g/L for coffee and wood, respectively.

References.

1. Castañon-Rodriguez J.F., Dominguez-González J.M., Ortiz-Muñiz B., Torrestiana-Sanchez B., Ramirez-de León J.A., Aguilar-Uscanga M.G., 2015. *Engineering in Life Sciences.* 15(1):96-107.
2. Chandler C., Villalobos N., González E., Arenas E., Marmol Z., Rios J., Aiello C. 2012. Multiciencias. 12(3):245-253.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
PB15

OPTIMIZATION OF ENZYMATIC SACCHARIFICATION OF WHEAT STRAW USING RESPONSE SURFACE METHODOLOGY

Lelie D. Castro-Ochoa[1], Sandy R. Hernández-Leyva[1], Itzel G. López-Zamora[2], María G. Aguilar-Uscanga[3], Claudia Castro-Martínez[1]. [1]IPN, CIIDIR Unidad Sinaloa. Depto. de Biotecnología Agrícola. Blvd. Juan de Dios Bátiz Paredes, C.P. 81101. Guasave, Sinaloa, México. [2]UAdeO, Depto. de Ingeniería y Tecnología, Los Mochis, Sinaloa. TNM, Instituto Tecnológico de Veracruz, H. Veracruz, Veracruz, México. E-mail: lelie_denise@yahoo.com, claudiacm30@hotmail.com

Key words: Optimization, Saccharification, Wheat straw

Introduction. Wheat is one of the major crops grown around the world. The conversion of lignocellulosic biomass from wheat straw (WS) to bioethanol could be a promising technology though; the process has several challenges and limitations. The enzymatic hydrolysis ratio of cellulose in WS is relatively low due to the presence of lignin and hemicellulose (1). Proper pretreatment methods can increase the concentration of fermentable sugars after enzymatic saccharification, thereby improving the efficiency of the whole process (2).
The aim of this study was to evaluate the potential of cellulolytic extract produced by *Penicillium* sp. in obtaining maximum saccharification yield of WS using three factor-three level Box–Behnken design.

Methods. Cellulases were produced by *Penicillium* sp. cultivated in submerged fermentation with corn stover as carbon source. Pretreatment of WS was carried out using 1% H_2S0_4, with a solid to liquid ration of 1:11 at 121ºC for 45 min. For saccharification, the pretreated substrate (1g) was placed in 50 mL Erlenmeyer flasks. Buffer (50 mM, pH 5.0) and enzymatic extract was added according to the experimental Box-Behnken design (BBD). The reaction was incubated for 24 h at 50ºC, and the reducing sugars obtained were determinate by DNS method (3).

Results. Table 1 shows the levels of selected variables for the BBD along with the percent of saccharification yield obtained from 15 experimental runs. Data obtained from response to surface methodology was validated using ANOVA. The model *F*-value of 57.55 and a low probability value ($P< 0.002$) showed that the model terms were significant. Two process parameters: substrate concentration and enzyme loading had significant effect on saccharification yield (Fig 1). However, saccharification yield was mainly enhanced by enzyme loading**.** Agitation speed was not significant (0.4255). Response analysis predicted maximum yields (19.99%) obtained at 195 rpm, substrate loading 8% and enzyme loading of 398 mg/g.

Table 1. Three level Box-Behnken design and the experimental response of dependent variable (% saccharification)

Run No.	Agitation (rpm)	Enzyme (mg/g)	Substrate (% w/v)	Saccharification (%)
1	50	50	12.5	5.81
2	300	50	12.5	5.18
3	50	400	12.5	14.26
4	300	400	12.5	18.36
5	50	225	5	15.10
6	300	225	5	10.21
7	50	225	20	6.52
8	300	225	20	10.29
9	175	50	5	1.66
10	175	400	5	19.40
11	175	50	20	4.24
12	175	400	20	10.81
13	175	225	12.5	16.44
14	175	225	12.5	16.55
15	175	225	12.5	16.25

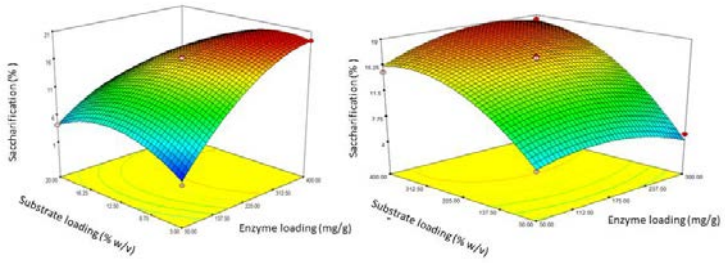


Fig.1. 3-D Response surface plots showing effects of various parameters on enzyme saccharification of pretreated wheat straw.

Conclusions. Saccharification yield of pretreated wheat straw was improved by optimizing a series of parameters. The cellulolytic extract from *Penicillium* sp. has great potential for application in biomass hydrolysis processes.

Acknowledgements. The authors very much appreciate the financial support by the projects SIP-20180302, SIP-20170347 and SAGARPA-CONACYT No. 291143.

References.

1. Zheng, Q., Zhou, T., Wang, Y., Cao, X., Wu, S., Zhao, M., Wang, H., Xu, M., Zheng, B., Zheng, J., Guan, X. (2018. *Sci. Reports*. 8:1321.
2. Maurya, D.P., Singla, A., Negi, S. (2015). *3 Biotech*. *5*(5):597–609.
3. Miller, G.L. (1959). *Anal. Chem.* 31:426–428.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
PB16

ANÁLISIS DE LA MANUFACTURA DE UNA PRÓTESIS DE MIEMBRO SUPERIOR

J. R. Sánchez-González[1], C. R. Torres-San-Miguel[1], J. A. Leal-Naranjo[2] , G. Urriolagoitia-Sosa[1], B. Romero-Ángeles[1];
[1]Instituto Politécnico Nacional, ESIME, SEPI, Zacatenco, Edificio 5, 2do. Piso, C.P. 07300, CDMX
[2]Departamento de Ingeniería Electrónica, DICIS, Universidad de Guanajuato, Carr. Salamanca-Valle de Santiago Km. 3.5 + 1.8 Km., 36885 Salamanca, GTO
e-mail: jesus_jrsg@outlook.com / ctorress@ipn.mx / lealnaranjo@gmail.com

Palabras clave: manufactura de extremidad superior, análisis de manufactura, optimización de prótesis

Introducción. Diversas literaturas consideran a la extremidad superior como una herramienta mecánica sensitiva de manipulación física gracias a su precisión y tacto al realizar movimientos y manipular objetos [1]. De acuerdo al Instituto Nacional de Estadística Geográfica e Informática el 5.3% de la población en México sufre alguna limitación motriz [2]. En la actualidad los tratamientos de un amputado buscan la restauración de una función desarrollada por el aparato locomotor mediante la protetización, la biomecánica del cuerpo humano ha sido factor en el desarrollo de prótesis; sin embargo, el avance en el manejo de los materiales empleados en el desarrollo con el fin de mejorar o remplazar una función ha estado ligada directamente con el avance de técnicas para manufacturar así como métodos que permitan estimar el tiempo de vida útil de una prótesis [3].

Métodos. Debido a que la principal variable que se busca optimizar es el peso de la prótesis, se establece una etapa con los requerimientos necesarios. Se empleará el método de las *"propiedades ponderadas"* en la selección de material, el cual consiste en asignar un peso a una pieza según el grado de importancia en servicio y así calcular un índice de rendimiento, aquellos con el más dominante serán considerados para la fabricación [4]. En la ***Tabla 1*** se muestran los materiales posibles para la manufactura de prótesis.

Tabla 1 Propiedades de los materiales candidatos.

Material	Densidad (Kg/m^3)	Cedencia (MPa)	Elasticidad (GPa)	Límite de fatiga (MPa)	Maquinabilidad
Aluminio	2700	425	68.9	96.5	Muy buena
Acero	7870	370	205	275	Regular
Polietileno	962	26.3	0.882	N/A	Muy buena
Titanio	4430	790	114	590	Pobre

Resultados. La mayoría de las prótesis solo buscan cumplir un patrón de movimientos sin considerar a profundidad el tema del peso, razón por la cual se espera la optimización a través de la manufactura mediante la selección adecuada del material así como las técnicas de manufactura a emplear y validar con estudios analíticos en aras de conseguir un modelo tangible del prototipo mostrado en la ***Fig 1.***

Fig.1 Prótesis con 7 grados de libertad.

Conclusiones. Un producto comprende la preparación de modelos físicos para determinar fuerzas, esfuerzos, deflexiones y una forma óptima. En la actualidad la construcción de modelos se simplifica altamente con el uso de la manufactura asistida por computadora.

Agradecimientos. Los autores agradecen al Instituto Politécnico Nacional, a la Sección de estudios de Estudios de Posgrado e Investigación de la Escuela Superior de Ingeniería Mecánica y Eléctrica Unidad Zacatenco y al Consejo Nacional de Ciencia y Tecnología de la Ciudad de México por el apoyo brindado.

Referencias.

1. L. Loaiza, J., & Arzola, N. (2011). Evolución y tendencias en el desarrollo de prótesis de mano. DYNA, 78, 191-200.
2. INEGI, «Instituto Nacional de Estadística Geográfica e Informática,» 24 Mayo 2013. [En línea]. Disponible: http://www.beta.inegi.org.mx/proyectos/ccpv/2010/default.html. [Último acceso: 22 Diciembre 2017].
3. J. M. Dorador González, P. Ríos Murillo, I. Flores Luna y A. Juárez Mendoza, «Robótica y prótesis inteligentes,» Revista Digital Universitaria, vol. 6, nº 1, pp. 1-15, 2004. 4. L. Loaiza, J., & Arzola, N. (2011). Evolución y tendencias en el desarrollo de prótesis de mano. DYNA, 78, 191-200.
4. M. Kutz, Handbook of Materials Selection, Nueva York: John Willey & Sons, Inc., 2002.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
PB17

CHARACTERIZATION OF IMMOBILIZATION SUPPORTS MATERIALS FOR LIPASES

Leticia Casas-Godoy[1], Xochitl Niehus[2], Marco Guadarrama[3], Gabriel Landazuri[4] and Georgina Sandoval[2].
[1]Cátedras CONACYT-CIATEJ, Guadalajara, 44270, Mexico. [2]CIATEJ, Guadalajara, 44270, Jalisco, Mexico. [3] Ingeniería de Proyectos, UDG, Guadalajara, 44430, Jalisco, Mexico. [4] Ingeniería Química, UDG, Guadalajara, 44430, Jalisco, Mexico.
Presenting author: lcasas@ciatej.mx

Key words: Immobilization, support, lipase.

Introduction. Lipases are enzymes that naturally catalyze the hydrolysis of the ester bond of lipids into fatty acids and glycerol. However, under thermodynamically favorable conditions they can catalyze synthesis reactions: esterification and transesterification (1). Lipases can be used in a wide variety of applications including food industry, production of fine chemicals, energy industry and bioremediation. The main disadvantage of an industrial lipase process is the enzyme cost. This problem can be overcome using immobilized lipases, which would allow reutilization of the catalyst and its use in continuous bioreactors.
The objective of this work was the characterization of different supports for the adsorption immobilization of an in-house produced lipase with many industrial applications.

Methods. The following analysis were performed to the different supports: granulometry by sieving, fracture test (ARG2, TA instruments), optical microscopy (Axio Imager M2, Carl Zeiss), X ray diffraction and scanning electron microscopy (SEM, TESCAN XFlash 6130). Lipase was immobilized by adsorption.

Results. The results obtained from the granulometry and fracture test are presented in Table 1. The fracture test showed that the most fragile support tested was S6, while the support S7 showed no breaking point up to 30N.

Table 1. Granulometry and resistance of the supports studied.

Material	Granulometry	Resistance (N)	
		Min.	Max.
S1	85%>0.40mm<1.19 mm	3	9
S2	99.9%>0.40mm<1.19 mm	8.2	15
S3	99.8%>0.40mm<1.19 mm	4.5	7.6
S4	89.1%>0.40mm<1.19 mm	4	4
S5	93.2%>0.40mm<1.19 mm	8	30
S6	99.9%<0.25 mm	1	2
S7	75%>1.19 mm<1.5 mm	ND	ND

ND: Not detected.

Results from de X ray diffraction showed that all the supports presented an amorphous structure except for S7, support that presented a semi-crystalline structure (Fig.1).

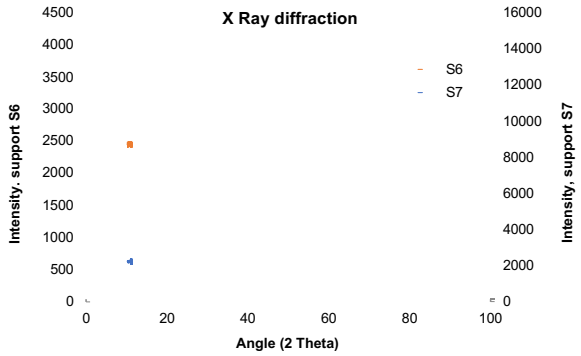


Fig.1 X Ray diffraction of supports S6 and S7.

The SEM analysis of the immobilized lipase in supports S6 and S7 are shown in Fig. 2. The highly porous structure of support S7 allowed a good immobilization of the enzyme.

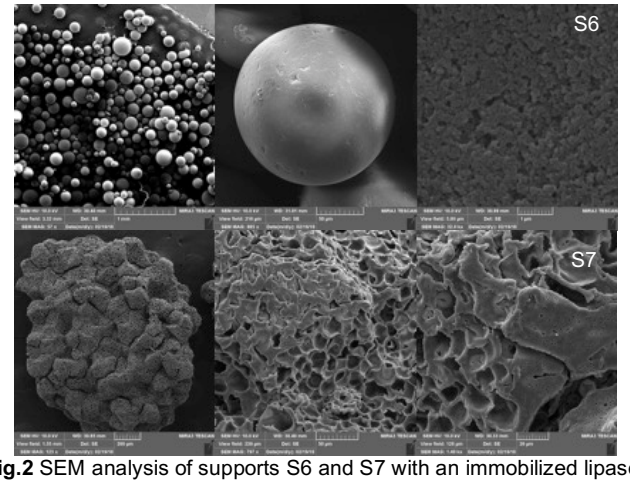


Fig.2 SEM analysis of supports S6 and S7 with an immobilized lipase.

Conclusions. The characteristics of the materials for lipase immobilization are important for their application in industries. The high porosity and resistance of the support S7 makes it a good candidate for lipase immobilization and catalyzed production at an industrial scale.

Acknowledgements. CONACYT-SENER-FSE-250014.

References. 1. Casas-Godoy L, Gasteazoro F, Duquesne S, Bordes F, Sandoval G, Marty A. (2018). Lipases: An Overview. In: *Lipases and Phospholipases: Methods and Protocols*. Sandoval G. Springer.

ISSN: 2448-8461

PB18

ENDEMIC FUNGAL CELLULASES PRODUCERS FROM THE JALISCO'S ECOSYSTEMS

Neydeli Ayala-Mendivil[1], Iliana Barrera Martínez[2], Georgina Sandoval[1]. [1]Industrial Biotechnology Department, Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco A.C. (CIATEJ), Guadalajara, 44270, México; [2]Cátedras CONACYT-CIATEJ Guadalajara, 44270, México. ibarrera@ciatej.mx

Key words: screening, cellulases, fungi

Introduction. Biotechnological conversion of cellulosic biomass is stepping up to develop novel bioprocesses and products. Microbial cellulases have become important commercial enzymes due their several industrial applications in diverse fields (1). Numerous microorganisms that can produce cellulases have been isolated and identified. However, many studies have focused on filamentous fungi due their easy cultivation, and high production of extracellular enzymes (2). In Mexico, there is a high biodiversity of fungi that have not been studied yet and can potentially be used in enzyme production.

The objective of this work is to find a fungal strain, from different ecosystems of Jalisco, able to produce cellulases by submerged and solid-state fermentation.

Methods. Fifty fungal strains were isolated from lignocellulosic and forest residues. The screening of fungi that produce cellulases was carried out in carboxymethyl-cellulose (CMC) plates. Cellulase activity was indicated as clear halos after staining with 0.1% Congo red followed by 1M NaCl (3). The culture media to produce cellulase by Submerged Fermentation (SmF) using 4 different carbon sources (CS) at 10 g/L (see table 1). Solid-state fermentation (SSF), was carried out in alkali-pretreated sugar cane bagasse (PSCB) as support and inducer. Cellulase activity was measured with azo-dyed CMC, using the protocol of the supplier (Megazyme), but miniaturized.

Results. Of the 50 strains collected, 3 were selected as potential producers of cellulases due they showed the highest halos in CMC-Congo red plates (Figure 1).

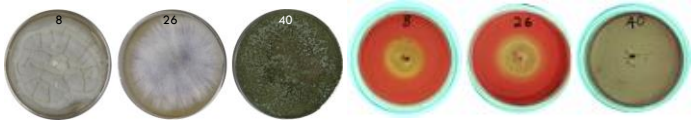


Fig.1 Selected strains for cellulase production in PDA plates (Left) and CMC-Congo red plates after 48 hours (Right).

The strains 8, 26 and 40 were tested to produce cellulases under SmF and SSF using different CS and the results obtained are showed in table 1.

Table 1. Cellulase activity (U/ml) in the supernatant of SmF after 72 hours of incubation and SSF after 120h.

Fermentation	Carbon source	Fungal strain number		
		8	26	40
SmF	PSCB	0.55	1.88 ± 0.11	1.81 ± 0.11
	Cellobiose	0.61	N. D.	0.57
	Cellulose	0.55	0.65 ± 0.07	0.55 ± 0.01
	Sucrose	0.55	N. D.	0.54
SSF	PSCB	1.45	0.74 ± 0.02	0.72

Among them, strain 8 showed activity in the 4 CS tested, however those with the highest activity in SmF were 26 and 40. The strain with the best activity in SSF was 8.
On the other hand, the cellulase production yield was calculated for the 3 strains with respect to PSCB used, in SSF and SmF. The yield is higher in the SmF with the three strains. This result gives an idea of which is the most appropriate option for the valorization of a PSCB to produce enzymes, under the scheme proposed in this work.

Table 2. Cellulase production yield (U/g of PSCB)

Fermentation	Fungal strain number		
	8	26	40
SmF	55	188	181
SSF	17.83	12.5	9.67

Conclusions. Three strains with metabolic potential to produce cellulases were found. All were collected in the Jalisco regions. The objective of this work was achieved, nevertheless the adequate conditions must be found to increase the enzymatic activity in SSF and SmF with the 3 selected strains (8, 26 and 40).

Acknowledgements. To CONACYT-SENER for project FSE-250014. N. Ayala-Mendivil received a Ph.D. scholarship from CONACYT.

References.

1. Ayala-Mendivil N.A., Calixto-Romo A., Amaya-Delgado L., Casas-Godoy L., Sandoval G., (2106). Comb Chem High Throughput Screen. 19(8): 627-635.
2. Payne, C.M., Knott, B.C., Mayes, H.B., Hansson, H., Himmel, M.E., Sandgren, M., Ståhlberg, J., Beckham, G.T., (2015). *Chem. Rev*. 115, 1308–1448.
3. Kasana R. C., Salwan H. D., Dutt S., Gulati A., (2008). *Curr Microbiol*. 57: 503-507.

ISSN: 2448-8461

ACID LACTIC PRODUCTION FROM BACTERIAL FERMENTATION OF AN AGROINDUSTRIAL CELLULOSIC WASTE

M.G. Valeriano-Martínez, M.E. Macías-Rodríguez, R.I. Corona-González
Departamento de Ingeniería Química, CUCEI-Universidad de Guadalajara, Blvd. M. García Barragán 1451, C.P. 44430 Guadalajara, Jal., México
Corresponding author M.G. Valeriano-Martínez, E-mail address: maria.valeriano@alumnos.udg.mx.

Key words: Lactic acid, fermentation, Agave tequilana leaf juice.

Introduction. Lactic acid (LA) is an organic acid used in pharmaceutical, chemical, cosmetic and food. Its production could be by chemical synthesis or fermentative routes nevertheless, chemical production generated a racemic mixture of DL-lactic acid, while fermentative process produces an optically pure L (+)- or D (-)-lactic acid using cheap renewable sources as substrates (5). Most of the world´s commercial lactic acid is produced by fermentation of glucose, sucrose, lactose and starch/maltose by homolactic bacteria; it depends on its price, availability and the cost of its recovery and purification and the substrate plays a vital role in the improvement of the process (2). Several studies have focused on reducing the cost of producing this input through the use of lignocellulosic and agroindustrial waste. Literature reported the used of cane juice and sweet sorghum for its production, so it is not discharged that the juice from another agroindustrial source can be used for the same purpose (4). In Mexico 382 thousand tons per year of *Agave tequilana* Weber var. Azul (*A. tequilana*) leaf coming of manufacturing process of tequila are generated, which represents an opportunity to use it resource in fermentation processes (1,3).

We propose to explore the use of *A. tequilana* leaf juice in batch fermentation to produce lactic acid with *Enterococcus hirae* BT2 inv strain (*E. hirae*), isolated from the tomato surface (*Solanum lycopersicum*).

Methods. It was established the optimum concentration to work of hexoses in the *A. tequilana* juice by analyzing the behavior of *E. hirae* in synthetic MRS medium at various glucose concentrations (20, 40, 60, 80 and 100 g L^{-1}), by extracting sample of the batch fermentor at 0, 2, 4, 6, 8, 10, 12, 16, 24 and 36 h, the operational conditions was a temperature of 30 °C, 120 rmp and pH 6.0.

The clean *A. tequilana* leaves were pass through a sugar cane mill for extracting the juice, which was centrifuged (4,000 rmp, 10 min) and filtered (PVDF 022 microns) to the fermentation process. Quantification of glucose, fructose and LA was performed by liquid chromatography techniques and the fermenter process was in the same conditions of MRS medium. We compared yield, productivity and LA concentration in each substrate (*A. tequilana* leaf juice and MRS).

Results.

Substratum	Yield ($g\ g^{-1}$)	Productivity ($g\ L^{-1}\ h^{-1}$)	LA ($g\ L^{-1}$)
MRS, 20 g L-1 glucose	0.84	0.83	16.85
MRS, 40 g L-1 glucose	0.75	2.61	30.08
MRS, 60 g L-1 glucose	0.73	2.45	44.06
MRS, 80 g L-1 glucose	0.60	2.53	48.11
MRS, 100 g L-1 glucose	0.47	1.42	47.29

Table 1. Comparative kinetic results between substrate concentrations in MRS.

A. tequilana fermentation is in process and the results will be ready in July 2018. The in vitro preliminars showed the adequate growth of the *E. hirae* in the analyzed substrate (*A. tequilana* leaf juice).

Conclusions. Table 1 shows that the concentration of 40 g L^{-1} of glucose in the MRS synthetic medium results in the optimum metabolite productivity; therefore, it is decided to carry out the experiment with *A. tequilana* leaf juice under this concentration of hexoses. We consider that the experimental substrate will produce comparable yields of the LA metabolite in reference to the synthetic medium.

Acknowledgements. This work was supported by CONACyT. Valeriano-Martínez thanks CONACyT for financial support.

References.

1. CRT (2017) *Consumo de Agave para tequila y tequila 100% de Agave.* Available at: https://www.crt.org.mx/EstadisticasCRTweb/ (Accessed: 16 October 2016).
2. Ghaffar, T., Irshad, M., Anwar, Z., Aqil, T., Zulifqar, Z., Tariq, A., Kamran, M., Ehsan, N. and Mehmood, S. (2014) 'Recent trends in lactic acid biotechnology: A brief review on production to purification', *Journal of Radiation Research and Applied Sciences*. Elsevier Ltd, 7(2), pp. 222–229. doi: 10.1016/j.jrras.2014.03.002.
3. SAGARPA (2017) 'Biomasa, creación ecológica de energía'.
4. Vandenberghe, L. P. S., Rodrigues, C. and Carvalho, J. C. De (2017) 'Production and Application of Lactic Acid', *Current Developments in Biotechnology and Bioengineering*, pp. 543–556. doi: 10.1016/B978-0-444-63662-1.00024-5.
5. Wang, Y., Tashiro, Y. and Sonomoto, K. (2015) 'Fermentative production of lactic acid from renewable materials: Recent achievements, prospects, and limits', *Journal of Bioscience and Bioengineering*. Elsevier Ltd, 119(1), pp. 10–18. doi: 10.1016/j.jbiosc.2014.06.003.

ISSN: 2448-8461
16-20 Octubre/October 2018
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
San José del Cabo, B.C.S., México
PB20

Ensayos mecánicos para un clavo intramedular

Sánchez, Cruz J.I., Torres, San Miguel, Ch.R.
Instituto Politécnico Nacional, Escuela Superior de Ingeniería Mecánica y Eléctrica, Sección de Estudios de Posgrado e Investigación, Unidad "Adolfo López Mateos", Zacaténco, Col. Lindavista C.P. 07738 Ciudad de México, México, e-mail:israelsanchez0410@gmail.com

Palabras clave; Clavos intramedulares, Fases de marcha humana,

Introducción. Existen diversos implantes metálicos para la osteosíntesis, como lo puede ser la fijación externa, placas óseas y clavos intramedulares. De estos últimos se tienen clavos rígidos y clavos telescópicos, diferenciándose entre sí por la capacidad de adaptarse al crecimiento de los huesos, es decir el clavo rígido es un dispositivo que no cuenta con la capacidad de elongación, mientras que el clavo telescópico presenta la singularidad de desplazamiento longitudinal [1].

Metodos. El análisis de un clavo intramedular parte de establecer las condiciones de trabajo para el elemento en cuestión. Estas condiciones deben de asemejarse a un comportamiento del clavo ya implantado. Por lo que, al analizar la marcha humana se tiene que, esta se compone por tres fases las cuales generan comportamiento de cargas distintas, es decir, la primera fase (Doble apoyo posterior de impulso) genera una tensión en el fémur, posteriormente la segunda fase (Periodo oscilante o de elevación) es gobernada por una fuerza de rotación y finalmente, la tercera fase (Apoyo unilateral) propaga una fuerza de compresión [2]. En la figura 1 se muestra el ciclo en cuestión.

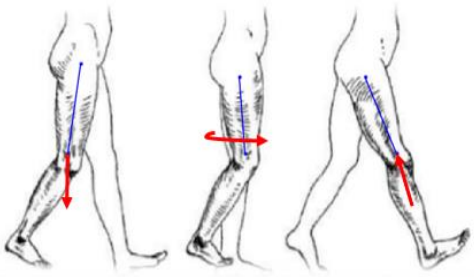


Figura 1.- Ciclo de marcha humana.

Resultados. Con base a la biomecánica del hueso en la marcha humana se tienen diferentes pruebas mecánicas para analizar estos comportamientos: Ensayo a 3 y 4 puntos figura 2a y 2b respectivamente; Ensayo a compresión figura 2c; Ensayo a tracción o tensión figura 2d; Ensayo a torsión figura 2f. Cada uno de estos ensayos están sustentados por diferentes organizaciones, como la ASTM. En su norma 1264-17 se describe de forma específica y puntual los procedimientos requeridos en la ejecución de pruebas con valides internacional sobre implantes metálicos [3].

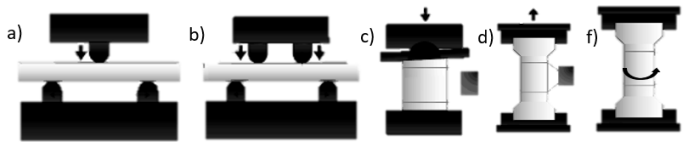


Figura 2. Ensayos mecánicos reconocidos por ASTM. a) 3 puntos. b) 4 puntos. c) Compresión. d) Tensión. f) Torsión

Conclusiones. El respaldo de diferentes organizaciones internacionales brinda un sustento de valides oficial a los ensayos clásicos de mecánica orientados a implantes intramedulares. Así mismo contribuye en el redireccionamiento del desarrollo de implantes intramedulares, de tal modo que, al apoyarse en este tipo de normativas se garantiza un funcionamiento optimo en comportamientos que contenga este tipo de agentes.

Agradecimientos. Los autores agradecen al Instituto Politécnico Nacional, a la Sección de Estudios de Posgrado e Investigación de la Escuela Superior de Ingeniería Mecánica y Eléctrica Unidad Zacatenco, al Consejo Nacional de Ciencia y Tecnología de la Ciudad de México por el apoyo brindado para el desarrollo de esta investigación.

References.

1.- Florente-Moltó, F. J., Bonete-Lluch, D. y Sotorres-Sentandreu, J., Osteogénesis Imperfecta en la infancia: Consideraciones clínicas y aspectos del tratamiento quirúrgico, *Revista española de cirugía osteoarticular*, Vol. 35, No. 202, pp 312-319, 2000

2.-Martín-Nogueras, A., Calvo-Arenillas, JL, Orejuela-Rodríguez, J, Barbero-Iglesias, F.J. y Sánchez-Sánchez, C. Fases de la marcha humana, Rev Iberoam Fisioter Kinesiol, Vol 2, pp 44-49, 1999.

3. Guede, D., González, P. y Caeiro, JR., Biomecánica y Hueso (I): Conceptos básicos y ensayos mecánicos clásicos, Rev Osteoporos Metab Miner, Vol. 5, No. 1, pp 43-50, 2013.

ISSN: 2448-8461

PB21

HETEROLOGOUS OVEREXPRESSION AND BIOCHEMICAL CHARACTERIZATION OF EGLS ENDOGLUCANASE FROM *BACILLUS SUBTILIS* RS351

Jacqueline Murillo-Guerrero, Estefania Morales Ruiz, Ignacio Maldonado Mendoza, Melina López Meyer, Claudia Castro Martínez; Instituto Politécnico Nacional, Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional (CIIDIR-IPN, Unidad Sinaloa), Guasave Sinaloa. C.P 81101; jacquelinemurillo@hotmail.com

Key words: Overexpression, heterologous, cellulases, endoglucanase.

Introduction. The lignocellulosic biomass has an enormous industrial potential, mainly in the production of second-generation biofuels (2G). Enzymes called cellulases (endoglucanase, exoglucanases and β-glucosidases) can hydrolyze the cellulose, its main component. These enzymes convert cellulose into fermentable sugars that can be used in the production of bioethanol [1,2]. We are interested in the search for cellulases produced by microorganisms isolated in the state of Sinaloa. Although we have isolated several microorganisms with the ability of cellulose degradation, higher cellulolytic activities are necessary for their biotechnology application. Therefore, the present work aims to overexpress the *eglS* gene coding for *Bacillus subtilis* RS351 endoglucanase, as well as its biochemical characterization, thus increasing its productivity without affecting its catalytic activity.

Methods. *Bacillus subtilis* RS351 was isolated from corn stubble in the state of Sinaloa. This strain showed high endoglucanase activity (validated by qualitative tests on CMC agar and Congo red staining). Its endoglucanase activity has been shown to be related to the *eglS* gene expression [3]. Then, *eglS* gene was amplify using genomic DNA from *B. subtilis* RS351 as template, and specific primers were designed to amplify the coding region and to add a 6xHis tag to its 3' end. The PCR product was cloned into the pENTR™ / DTOPO® vector and transformed into *E. coli* TOP10 competent cells. Restriction and sequencing analysis were performed to verify its directional cloning. Subsequently, a LR recombination reaction will be carried out between the generated clone and the destination vector pDEST17. Once the expression clone is generated, it will be transformed into *E coli* BL21 StarTM (DE3) competent cells and protein expression assays will be performed in the presence of L-Arabinose as inducer. Purification of the recombinant protein by affinity in nickel columns (Ni-NTA) will be carried out, its enzymatic activity will be evaluated with specific substrates and a zymogram with CMC as a carbon source. Finally, it will be characterized biochemically in terms of molecular weight, thermo stability, metal ions effects, as well as activity and stability of pH and temperature.

Results. *Bacillus subtilis* RS351 was grown at 30 °C for 18 hours in LB medium and its cellulolytic activity was confirmed by presenting hydrolysis halos of approximately 2 cm in a qualitative test on plates with CMC (Fig. 1). Using the genomic DNA from *B. subtilis* RS351 as well as specific primers, a single band with a size of 1500 bp, which corresponds to the expected weight of the gene coding for the endoglucanase EglS of *B. subtilis* RS351 [3] was obtained (Fig. 2). Currently, experiments on the cloning and overexpression of the protein in the heterologous *E. coli* system are in process.

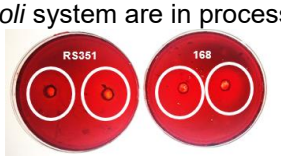


Fig.1 Cellulolytic activity test on CMC plates in the strain *B. subtilis* RS351 (left plate) and *B. subtilis* 168 as positive control (right plate), after 48 hours the hydrolysis halos are observed

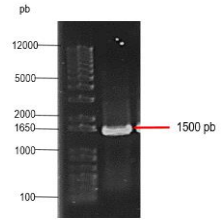


Fig. 2. Amplification of the *eglS* gene that codes for an endoglucanase of *B. subtilis* RS351 with an expected size of 1500 bp.

Conclusions. The results that will be obtained from the overexpression and characterization, will increase the production of the EglS endoglucanase of *Bacillus subtilis* RS351, for its application in the production of second-generation biofuels.

Acknowledgements. SAGARPA CONACYT No. 291143; SIP20180302.

References.

1.Savaliya M., Dhorajiya B & Dholakiya B. (2015). Recent advancement in production of liquid biofuels from renewable resources: a review. Res Chem Intermed. 41:475–509

2. Shrivastava A., Bhattacharya A & Pletschke B. (2015) Synergism of fungal and bacterial cellulases and hemicellulases: a novel perspective for enhanced bioethanol production. Biotechnol Lett 37:1117–1129

3. Jiménez M. (2015) Identificación y caracterización molecular de genes relacionados con la actividad celulolitica en bacterias del genero *Bacillus* aisladas en el estado de Sinaloa (Tesis de Maestría), CIIDIR-IPN Sinaloa.

ISSN: 2448-8461

PB22

Antimicrobial potential of food isolates for probiotic development

Daniel Guillén[1], Romina Rodríguez-Sanoja[1]; [1]Instituto de Investigaciones Biomédicas, UNAM. Departamento de Biología Molecular y Biotecnología. México, CDMX 04510.
dguillen@biomedicas.unam.mx

Key words: Fermented food, probiotics, antimicrobial activity

Introduction. At the present time, the antagonism of pathogens and spoilage microorganisms has gained increased interest in the food, biomedical and biotechnological industry. There is an active search for products or microorganisms able to inhibit another undesired microorganisms. In this regard, products supplemented with probiotics have generated great attention since they are products intended to human consumption and have active microorganisms considered not harmful and with the ability to survive under different types of stress such as antibiotic presence, low pH, lysozyme, and be able to inhibit spoilage microorganisms (1). Therefore, probiotic cultures could not only be used to improve the safety and maintain food quality, but also in other biotechnological developments with the purpose of pathogen inhibition (1).
The objective of this work is to find microorganisms who inhibit pathogen growth for its use as potential probiotics in different industries. As a first step, isolation of microorganisms and characterization of their probiotic potential is realized.

Methods. From samples taken from different foods, plate isolation was carried out in two different selective media (MRS and M17). Isolated colonies were selected randomly by its morphological characteristics and molecular distinction by random amplification of polymorphic DNA (RAPD). A screening was performed based on its ability to inhibit pathogenic microorganisms by plate antagonism assay (2). Tested pathogens include *Staphylococcus aureus*, *Streptococcus pyogenes* and *Salmonella enterica* var *typhi*. Positive isolates were tested for resistance to simulated gastric juice, tolerance to lysozyme and resistance to antibiotics. *Lactobacillus acidophilus* was used as probiotic microorganism reference.

Results. Several colonies with different macroscopic characteristics were isolated. Colonies including bacilli and cocci Gram (+) were differentiated and grouped by RAPD. Four isolates were selected for positive antimicrobial activity (id strain: T6M, T12M, K3M and T16D) and showed antibiotic resistance except for T6M (Table 1). Tolerance to lysozyme and simulated gastric juice was generally equal or better than the presented for *L. acidophilus* (Table 2).

Table 1. Antibiotic Resistance of the isolates

Id Strain	Tet (8 µg/mL)	Cam (8 µg/mL)	Ery (4 µg/mL)	Amp (4 µg/mL)
T6M	-	+	-	-
K3M	+	+	+	+
T12M	+	-	+	+
T16D	+	+	+	+

Table 2. Resistance to different conditions of the isolates

Id Strain	Lyzozyme	Simulated Gastric Fluid
T6M	99.4% ± 7.5%	40.6% ± 4.9%
K3M	99.6% ± 12.8 %	7.5% ± 1.1%
T12M	108.2% ± 6.8%	77.4 ± 6.6 %
T16D	23.93% ± 15%	70.8% ± 7.2 %
L. acidophilus	52.5% ± 3.0 %	68.4% ± 5.9%

Conclusions. The results shows microorganisms able to inhibit pathogens and with potential use as probiotics for different products development. Identification through 16S is currently being performed.

Acknowledgements. The project is realized in Instiuto de Investigaciones Biomédicas,UNAM.

References.

1. Iqbal U., Qadir, M.Z., Hussain, M.I., Janbaz, T., Khan, K.H. and Ahmad, B. (2014) Probiotics and their beneficial effects against various diseases. *Pakis J of Pharm Sci,* 27(2).
2. Alvarado, C., García, A. B., Martin, S. E., & Regalado, C. (2006). Food-associated lactic acid bacteria with antimicrobial potential from traditional Mexican foods. *Rev Latinoam Microbio*l, 48(3-4), 260-268

ISSN: 2448-8461

PB23

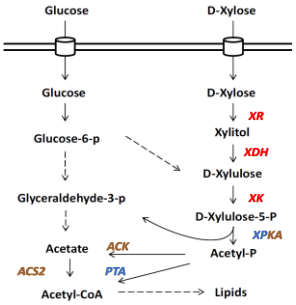
ENGINEERING *Yarrowia lipolytica* TO ENHANCE LIPID PRODUCTION FROM LIGNOCELLULOSIC MATERIALS

Xochitl Niehus[1], Leticia Casas-Godoy[2], Neydeli Ayala-Mendivil[1], Anne-Marie Crutz-Le Coq[3], Georgina Sandoval[1], Jean-Marc Nicaud[3], and Rodrigo Ledesma-Amaro[3,4]. [1] CIATEJ, Guadalajara, 44270, Jalisco, Mexico. [2] Cátedras CONACYT-CIATEJ, Guadalajara, 44270, Mexico. [3] Micalis Institute, INRA, AgroParisTech, Université Paris-Saclay, 78350 Jouy-en-Josas, France. [4] Department of Bioengineering, Imperial College London, London SW7 2AZ, UK.
Presenting author: xniehuspdoc@ciatej.mx

Key words: Yarrowia lipolytica, Xylose utilization, Acetyl-CoA, Microbial lipids, Metabolic engineering, Synthetic biology

Introduction. *Yarrowia lipolytica* is a common biotechnological chassis to produce lipids, which are the preferred feedstock for the production of fuels [1]. To reduce the cost of microbial lipid production, inexpensive carbon sources must be used, such as lignocellulosics. Unfortunately, lignocellulosic materials often contain toxic compounds, such as furfural and a large amount of xylose, which cannot be consumed by wild strains of *Y. lipolytica*. In this work, we engineered this yeast to efficiently use xylose as carbon source for the production of lipids. The manuscript is already available [2].

Methods. Detailed methodology can be found in [2]. *Y. lipolytica* was engineered by overexpressing native genes. Lipid content was increased by overexpressing heterologous genes to facilitate the conversion of xylose-derived metabolites into lipid precursors (Fig.1).



XR xylose reductase; XDH xylitol dehydrogenase; and XK xylulose kinase represent the xylose consumption pathway (in red). XPKA phosphoketolase, ACK acetate kinase, and ACS2 acetyl-CoA synthase represent the XA pathway (in brown), while XPKA phosphoketolase and PTA phosphotransacetylase are the XP pathway (in blue). Dashed lines represent multiple steps.

Fig.1 Representation of pathways leading to xylose consumption.

Results. In *Y. lipolytica* Po1d, homologous genes to consume xylose were overexpressed (ylXYL+). In parallel, same modifications were performed in a previously engineered strain [3] in which lipid production was maximized (ylXYL+Obese) Lipid production was evaluated (Fig.2).
Furthermore, XA and XP pathways were overexpressed in strain ylXYL+Obese. Since lipids and biomass increased with the XA pathway overexpression (strain ylXYL+Obese-XA), the strain was cultured with agave bagasse hydrolysate in bioreactors (Fig. 3). The modified strain was able to grow and produce lipids in a very high yield (67% of lipids, which corresponds to 16.5 g/L and yield 3.44 g/g sugars) in spite of toxic compounds.

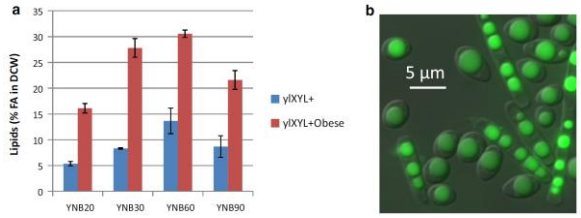


Fig.2 a: Lipid production in in varying concentrations of xylose (C/N 20=YNB20, C/N 30=YNB30, C/N 60=YNB60, and C/N 90=YNB90). **b**: Fluorescence microscopy of strain ylXYL+Obese in medium YNB60.

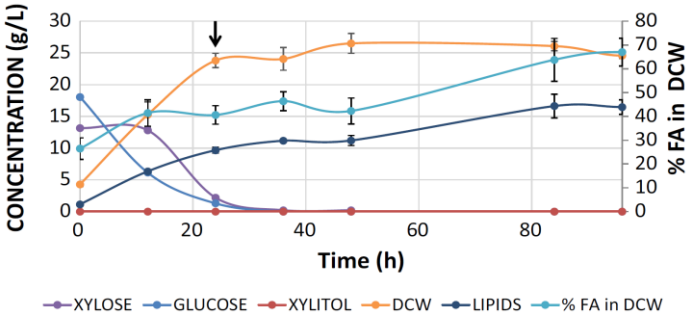


Fig.3 Lipid production in fed-batch bioreactors using lignocellulosic hydrolysate in the engineered strain ylXYL+Obese-XA.

Conclusions. This work demonstrates the potential of metabolic engineering to increase feasibility of lipid production from inexpensive substrates as source of fuels and chemicals.

Acknowledgements. ANR France project ANR-11-BTBR-0003 and CONACYT-SENER Mexico project FSE-250014 and FSE-248090. R. Ledesma-Amaro received financial support from the European Union through the Marie-Curie FP7 COFUND and from Imperial College London. X. Niehus received a Ph.D. scholarship from CONACYT.

References.

1. Ledesma-Amaro R, Nicaud J-M (2016) Prog Lipid Res. 61:40-50.
2. Niehus X, Crutz-Le Coq AM, Sandoval G, Nicaud JM, Ledesma-Amaro R (2018). *Biotechnol Biofuels* 11:11. http://rdcu.be/Ffx2.
3. Lazar Z, Dulermo T, Neuveglise C, Crutz-Le Coq AM, Nicaud JM. (2014). Metab Eng. 26C:89–99.

ISSN: 2448-8461

PB24

POLYUNSATURATED FATTY ACIDS OF THE SEED OIL OF *Rhus microphylla*, "LIMILLA"

Rafael Ortiz-Alvarado[1], Lucia Matilde Nava Barrios[1], César Rogelio Solorio-Alvarado[2]. 1.- Faculty Químico Farmacobiología, Universidad Michoacana de San Nicolás de Hidalgo, Morelia, Michoacán, street Tzintzuntzan No.173, Col. Matamoros, C.P.5800. 2.- Universidad de Guanajuato, Departamento de Química. División de Ciencias Naturales y Exactas, Campus Guanajuato. Tel. +52 443 3142152 ext. 208, rortizalvarado@gmail.com

Key words: Polyunsaturated fatty acids, *Rhus microphylla,*Extractive methods of fatty acids.

Introduction. Mexico offers a diversity of climatic regions, allowing the development of biological, endemic systems, which are exploited in a non-systematic way and have not been fully characterized. As the so-called "limilla" (*Rhus microphylla*), it is a fruit that is produced in the municipalities of Bajio in the State of Michoacán, Guanajuato, San Luis Potosi. The objective of this work is to purify and chemically characterize *R. microphylla* seed oil, by analytical methods and to offer an alternative for the obtaining of food with nutritional value. The seed of *R. microphylla* was trittled and dried, to later extract by organic solvents, the oil which was analyzed by gas chromatography, finding a concentration of 85% of polyunsaturated fatty acids, which represent an important source of essential fatty acids, for human consumption, and in this way to enhance the production and sustainable exploitation of endemic products in Mexico.

Methods. The biological material was collected from the municipalities of Villa Morelos and Puruandiro Michoacán de Ocampo, where this type of product is endemic and consumed habitually. For which it was later transported to the Faculty of Chemistry Pharmacobiology of the Michoacana University of San Nicolás de Hidalgo, where the exocarp and mesocarp (husk and pulp) of the seed were separated, which was handled as solid waste, which was recovered and subjected for a space of 24 hours to a thermal treatment, by direct light, of an incandescent lamp of 150 watts of power, on a convection surface, food-grade steel of 3 mm thickness. At the end a solid residue was obtained which was sieved, releasing the seeds of the limy fruit. The limilla seeds were subjected to a grind in mortar, for the reduction of the particle size, for the extraction by solvents in Soxhlet equipment. The oil was purified and characterized by gas chromatography to determine the profile of fatty acids.

Results. The seeds of the fruits of *R. microphylla* from solid waste, are a source of fatty acids of type, C: 18 polyunsaturated, such as linoleic and linolenic acid, using microwave treatment in five different extractions, a yield of 16.83% and a standard deviation of ± 1.58, five different extractions for ethyl ether yield an average yield of 15.18% and ± 1.44 standard deviation and 12.40% with a standard deviation of ± 1.08 with pentane in five different extractions (Table 1), and the composition of polyunsaturated fatty acids is as follows: cis-oleic 23.83%, fatty acid linolenic 7.73%, and fatty acid linoleic 57.85%.

Table 1. Percentage of yield in the extraction of the oil of the seed of *Rhus microphylla*.

Treatment	Microwaves	Ethyl eter	Pentane
Dry weigth	16.83%	15.18%	12.40%
Standard desviation	± 1.43	± 1.20	± 0.8

Conclusions. In this work it is concluded that R. microphylla fruit seed is a major nutrient important raw material for the potential obtaining of type C: 18 polyunsaturated fatty acids, which could give an alternative to obtain polyunsaturated fatty acids necessary for food and / or cosmetic industry.

Acknowledgements. We are grateful financial support from Consejo de Investigación Científica, Universidad Michoacana de San Nicolás de Hidalgo, Grant 2018–2019.

References.

Akanda M.J., Sarker M.Z., Ferdosh S., Manap M.Y., Ab Rahman N.N., Ab Kadir M.O. (2012). Applications of supercritical fluid extraction (SFE) of palm oil and oil from natural sources. *Molecules*. 10;17 (2):1764-94.

Boeing J.S., Barizão E.O., Silva B.C., Montanher P.F., de Cinque Almeida V., [3] Visentainer J.V. (2014). Evaluation of solvent effect on the extraction of phenolic compounds and antioxidant capacities from the berries: application of principal component analysis. *Chem. Cent J.* 22;8(1):48.

Bushman B.S., Phillips B., Isbell T. (2004). Chemical composition of caneberry (Rubus spp.) seeds and oils and their antioxidant potential. *J Agric Food Chem*. 52(26):7982-7.

ISSN: 2448-8461

PB25

STUDY OF THE SYNTHESIS OF POLYHYDROXYBUTYRATE (PHB) FROM *Azotobacter vinelandii* AND *Cupriavidus necator*

Mónica López; Eric López y Löpez, Raul Delgado Macuil
Instituto Politécnico Nacional, Centro de Investigación en Biotecnología Aplicada Unidad Tlaxcala, Carretera a Santa Inés Tecuexcomac, a 1.5 Km, Ex-Hacienda San Juan Molino, 90700, Tlaxcala, Tlax., México; E-mail address: monica11736@gmail.com

Key words: Polyhydroxybutyrate, A. vinelandii, C. necator.

Introduction. Polyhydroxybutyrate (PHB) is a biopolyester composed of β-hydroxybutyrate monomers that some microorganisms accumulate intracellularly as reserve material in conditions of nutritional imbalance. One organism that have been widely studied for the synthesis of PHB is the bacteria *Azotobacter vinelandii*, for their ability to accumulate up to 90 % [1].
The aimed of this study, was to develop advanced fermentation strategies to obtain a high production of this biopolymer, using the bacteria *Azotobacter vinelandii*, implementing pulses of nutrients.

Methods. Different sources of carbon (20g/L), sucrose, glucose and maltose were evaluated; and different sources of nitrogen, yeast extract, fermented corn liquor and soy peptone [2]. Each combination generated 9 media, keeping the C/N ratio constant, for the growth of A. vinelandii. Growth kinetics are adapted for 48 hours at 200 rpm in 1.0L flasks whose use volume is 250 mL. The carbohydrates were quantified by the phenol sulfuric method, cell count and biomass by dry weight [3, 4].

Results. Medium 1 was selected, which contained as sucrose and carbon source, sucrose and yeast extract, respectively. As shown in Figure 1, they showed the highest growth, compared to the rest.

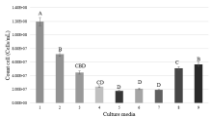


Figure 1. Evaluation of carbon and nitrogen sources for the growth of *A. vinelandii*.

Once the previous medium was selected, growth kinetics were performed, values of $5.63x10^8$ cells/ml were obtained at 36 hours for cell counting, 1.38 g/L at 20 hours for biomass, and a final concentration of sucrose. 4.5 g/L after 48 hours of culture. Finally, the bacteria is subjected to nitrogen limitation given by the variation of the C/N ratio from 7:1 to 10:1, by applying a feeding pulse at 20 hours.

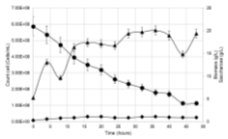


Figure 2. Growth profile of *A. vinelandii*, (▲) Cell count, (♦) Biomass, (●) Sucrose.

There was a growth of 1.7625x1011 total cells/mL at 28 hours, 1.93 g/L of total biomass at 36 hours and a final sucrose concentration of 13.423 g/L after 48 hours of culture as shown in figure 3.

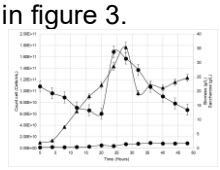


Figure 3. Growth profile of *A. vinelandii* administering a pulse of nutrients, (▲) Cell count, (♦) Biomass, (●) Sucrose.

Conclusions. The extract of yeast and sucrose, as a source of nitrogen and carbon, respectively, were of greater assimilation for *Azotobacter vinelandii* when presenting high growth compared with the other sources evaluated. The feeding pulses in the culture medium represented an adequate production strategy for the accumulation of PHB in the cell.

Acknowledgements. To CONACyT scholarship and to SIP-IPN grants.

References.

[1]. Wang *et al*., (2014). Polyhydroxyalkanoates, challenges and opportunities. In: *Current opinion in biotechnology*, China, 30, 59-65.
[2]. Chiani, *et al*., (2010). Production of desferrioxamine B (Desferal) using corn steep liquor in Streptomyces pilosus. In: *Pakistan journal of biological sciences: PJBS*, 13(23), 1151-1155.
[3]. Martínez, *et al*., (2004). Producción de polihidroxialcanoatos en bacterias diazotrofas. I. Influencia de la aeración en la sintesis de poli [beta] hidroxibutirato en *Azospirillum brasilense* CEPA 7. *Biología*, 18(1), 87-96.
[4]. López et al., (2015). Comparison of several methods for the separation of poly (3-hydroxybutyrate) from *Cupriavidus necator* H16 cultures. *Biochemical engineering journal*, 93, 250-259.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
PB26

FIRMNESS EVALUATION OF GUAVA USING LASER LIGHT SCATTERING

Adriana – Padilla Tena, Berenice – Yahuaca Juárez, Consuelo J.-Cortés Penagos, Jorge A. – Huerta Ruelas
Facultad de Químico Farmacobiología, Universidad Michoacana de San Nicolás de Hidalgo, Morelia 58240, Michoacán México. ibqadrianapad@hotmail.com

Key words: guava, firmness, non-destructive technique

Introduction. Fruit quality is related to both internal variables (firmness, sugar content, acid content) and external variables (shape, size, external defects)[(1)], but firmness is more important textural attribute of fruit quality and directly influences their shelf life and consumer acceptance[(2)]. Traditionally, it´s destructively determined by compression and penetration resistance tests, because of this the agricultural sector requires alternative non-destructive techniques to measure the maturity indices that provide global information on the quality of the fruits.

The objective of this study was to evaluate the potential of laser light scattering (LLS), as a non-destructive method to predict the firmness of the guava variety "Media china" under two storage conditions.

Methods. Guava fruits (*Psidiun guajava* L.) variety "Media china" were used. Fruits were selected in physiological maturation according to color and a quickly test of compression resistance, which were stored at a temperature of 12 and 23 °C.
The firmness was measured by the compression force test at a compression distance of 3 mm. For the optical characterization, the technique of laser light scattering was implemented used an optical system which a laser diode 650 nm (VLM-635002-LPA1) which impacts the guava fruits at an angle of 40° with respect to the normal. An array of 16 silicon photodiodes (BPW-34 OSRAM) was used as detector.

The statistical analysis was performed by means of a partial linear least squares regression model (PLS) using The Unscrambler X 10.5 software.

Results. The graphs obtained for the scattering integrated partial (PIS) profiles of a guava under temperature of 12 and 23 °C are shown in Fig.1.

The storage temperature is related with the light scattering, low temperature the scattering decreases in compared to high temperatures.

Fig.1 Partially integrated scattering (PIS) for both temperatures

Prediction of firmness

A good regression coefficient was obtained during the calibration between light scattering and the compression force. The results are shown in Table 1.

Table 1. Statistics results for firmness using partial least squares (PLS)

Temperature	Factors	Calibration		Validation	
		r	RMSEC	r	RMSECV
12 °C	4	0.73	9.0	0.66	12.43
23 °C	6	0.70	13.47	0.61	16.04

According to the literature, no studies related to this technique were found however, the comparison with Tu et al. (2000) who studied the quality in tomatoes obtaining a correlation of r = 0.78 and to predict firmness in peach fruits Lafuente et al. (2014) r = 0.77 and SECV = 0.99 N.

Conclusions. The results indicate that it is possible to use laser light scattering as a non-destructive technique for measuring guava firmness in different post-harvest conditions.

Acknowledgements. This investigation was supported by the UMSNH and CONACYT.

References.

1.García, F. J., Ortiz, J., Ruiz, M., Díez, J., Flores, L., Homer, I., & Chávez, J. M. (2003). *Journal of Food Engineering*, *58*(1), 53–57.
2. Shao, Y., He, Y., Gómez, A. H. ., Pereir, A. G. ., Qiu, Z., & Yun, (2007). *Journal of Food Composition and Analysis*, *81*, 672–678.
3. Tu, K., Jancsók, P., Nicolaï, B., & De Baerdemaeker, J. (2000). *International Journal of Food Science and Technology*, *35*(5), 503–510.
4. Lafuente, V., Herrera, L. J., Pérez, M. del M., Val, J., & Negueruela, I. (2014). *Journal of the Science of Food and Agriculture*, *95*(10), 2033–2040.

ISSN: 2448-8461

PB27

Bi_2O_3 AND ZnO NANOSTRUCTURES BIOSYNTHESIZED BY AQUEOUS EXTRACT OF *Juglans regia L.*

Maria Guadalupe Yañez Cruz, Maricela Villanueva Ibáñez*, Marco Antonio Flores González, Patricia Nayeli Olvera Venegas; Laboratory of Nanotechnology, Biological Systems and Industrial Applications, Polytechnic University of Pachuca; Zempoala Hidalgo 43830;
yanez.cruz.m.g@gmail.com; *villanueva@upp.edu.mx

Keywords: Biosynthesis, nanostructures, metal oxides

Introduction.

The nanostructures of metal oxides such as Bi_2O_3 and ZnO highlight by their electrical, photoconductive and photoluminescent properties. The traditional methods to synthesize nanostructures involve the use of solvents, expensive equipment and specific reagents that prevent the aggregation. Among biological methods, the use of plant extracts stands out because they have a wide variety of metabolites that reduce the precursor salt and stabilize the particles [1]. In this work, the walnut shell of *Juglans regia L.* is considered agro-industrial waste with potential to be used in the biosynthesis of these particles, because they contain mainly quinone, glucose, tannic acid and gallic acid that can promote the biorreduction process [2].
The aim is to propose an alternative synthesis of Bi_2O_3 and ZnO nanostructures from the aqueous extract of shell of *J. regia L.* to be studied in applications with technological impact on photocatalysis.

Methods.

The extract of *J. regia L.* was prepared by infusion and filtered with filter paper Whatman No.1 and 0.45 µm Nylon membrane to be characterized by phytochemical test and FT-IR spectroscopy. 1 and 4 mL of this extract was added drop wise separately to 5 mL of an aqueous solution of bismuth nitrate [$Bi(NO_3)_3$ $5H_2O$] (0.02 M) and 5 mL of zinc acetate [$Zn(O_2CCH_3)_2$] (0.02 M), respectively. The syntheses were carried out at pH 11, 25 °C and constant stirred for 4 h [2]. Finally, the as-obtained powders were separated by centrifugation and washed with deionized water to be characterized by complementary techniques to know structure, size and morphology of the particles.

Results.

The phytochemical screening and FT-IR spectroscopy of the extract confirmed the presence of tannins, saponins and carbohydrates, which together enable the reduction of ions and the stabilization of particles [3]. The UV-Vis absorption band was observed at 280 and 357 nm for Bi_2O_3 and ZnO, respectively (Figure 1a). The Figure 1b shows the Raman spectrum of Bi_2O_3, the peaks at 109, 298 and 461 cm^{-1} are characteristic of tetragonal β-Bi_2O_3 [3]. The spectrum of ZnO in Figure 1c presents the most intense and characteristic band at 434 cm^{-1} attributed to antisymmetric oxygen bonded with Zinc in the tetrahedral configuration [4]. Figure 2 shows the semi-spherical morphology Bi_2O_3 and ZnO nanostructures with average sizes of 2 µm and 100 nm, respectively.

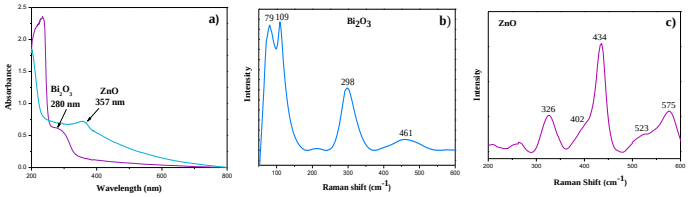


Figure 1. a) UV-Vis spectra of nanostructures synthesized by using extract of *J. regia L.*; Raman spectra of a) Bi_2O_3 and b) ZnO nanostructures.

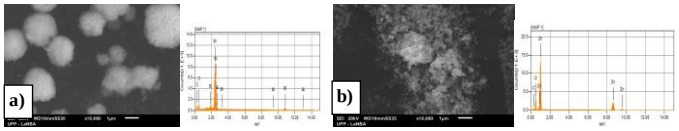


Figure 2. Micrographs and EDS of nanostructures of a) Bi_2O_3 and b) ZnO

Conclusions.

An easy synthesis route of tetragonal β-Bi_2O_3 and wurtzite-like hexagonal ZnO nanostructures was developed at 25 °C. The metabolites in the extract of *J. regia L.* were reducing agents and stabilizers of the particles.

Acknowledgments.

To CONACYT for MGYC scholarship (332012) for Ph.D. studies, and project INFRA-2018 (294909). Thanks to R. González-Montes de Oca for MEB analyses.

References.

[1] Jeevanandam, J., Chan, Y.S., Danquah, M.K. (2016). Biosynthesis of metal and metal oxide nanoparticles. ChemBioEng 3(2), 55-67.
[2] Sinner, M., Puls, J., & Dietrichs, H. H. (1979). Carbohydrate Composition of Nut Shells and Some Other Agricultural Residues. Starch/Stärke, 31(8), 267-269.
[3] Karnan, T., & Samuel, S. (2016). A novel biomimetic approach for the fabrication of Bi_2O_3 nanoflakes from rambutan (*Nephelium lappaceum L.*) peel extract and their photocatalytic activity. *Ceramics International*, 4779-4787.
[4] Londoño-Calderón, A., Jurado, J., & Vargas-Hernández, C. (2012). Estudio vibracional de nanoestructuras de ZnO sinterizadas por reaccion en estado sólido. *Revista Colombiana de Física, 44*(1), 71-75.

ISSN: 2448-8461

PB28

Vanillic acid production by Gram-negative bacteria isolated from vanilla (*Vanilla planifolia* ex. Andrews) beans

Esmeralda Escobar Muciño[a], Margarita Ma. de la Paz Arenas Hernández[a], Ma. Lorena Luna Guevara[b], Genaro Gustavo Amador Espejo[c], Ma. Elena Ramos Cassellis[b]. *[a] Centro de Investigación en Ciencias Microbiológicas, Benemérita Universidad Autónoma de Puebla, Puebla, México, [b] Facultad de Ingeniería Química, colegio de alimentos, Benemérita Universidad Autónoma de Puebla, Puebla, México, [c]CONACYT-Centro de Investigación en Biotecnología Aplicada. Instituto Politécnico Nacional (IPN), Tlaxcala C.P. 90700, México. esmeem2014@gmail.com (Esmeralda Escobar Muciño)*

Abstract

The aim of this work was to evaluate Vanillic acid (VA) production by three Gram-negative bacteria (*Ps. aeruginosa*, *E. aerogenes* and *C. freundii*) isolated from the vanilla curing process by HPLC. Under *in vitro* conditions, all the evaluated strains contribute to VA production; however, *Ps. aeruginosa* produced the highest concentration of VA [448.08 ppm] after 12 h of incubation, being the microorganism with highest potential to contribute to the aromatic profile of vanilla during the curing process.

Key words: Vanillic acid, Gram-negative bacteria, bioconversion to aromatic compounds.

Introduction. Vanillic Acid (VA) is one of the most abundant aromatic compounds in mature vanilla beans[1]. Some aromatic compounds can be produced through microbial bioconvertion[2]. Bacteria can use different sources of carbon for their growth producing aromatics compounds such as VA and alcohol, p-hydroxybenzaldehyde (HBA), and guaiacol [3, 4, 5].The aim of this study was to evaluated the VA production by three Gram-negative bacteria isolated from the Mexican vanilla curing process.

Methods. Three Gram-negative strains were isolated from the vanilla curing process identified as *Citrobacter freundii*, *Enterobacter aerogenes* and *Pseudomonas aeruginosa*. For VA production from Gram-negative bacteria, the strains were grown in 50 mL of M9 medium (ferulic acid 0.1% (w/v) and glucose 20%) and incubated in an orbital shaker at 180 rpm and 30 °C. Aliquots of culture bacterial were obtained at different time intervals (2, 4, 8, 12 and 24 h) and centrifuged at 1200 rpm (30 min 4 °C) to obtain a supernatant, which was filtered using a 0.2 µm filter paper used for HPLC quantification. The standard of VA was purchased from Sigma–Aldrich Company. Ltd., UK., and the concentration was estimated by HPLC with a chromatograph Agilent 1200, , USA using a UV index detector The injection volume was 10 µL, and the mobile phase was acetonitrile/water[5]. The VA concentration was quantified and expressed in ppm. The separations were carried out on a Supelcosil LC-18-S column (150 mm X 4.6 mm; Supelco Inc., Bellefonte, Pa.) protected with a 1-cm guard cartridge (Phase Separations Ltd., UK) a 37ºC.

Results. The three Gram-negative bacteria showed a maximum production of VA at 12 h of incubation. *Ps. aeruginosa* produced the highest VA concentration (448.08 ppm vanillic acid with a molar yield of 1.38%). In comparison with other reports, *Pseudomonas aeruginosa* produced an intermediate VA concentration[6]. Meanwhile, *E. aerogenes* produced 39.98 ppm of VA (molar yield of 0.12%). For *Enterobacter* species, only low amounts of VA [1-5 ppm] were produced at 12 h of incubation[7]. Finally, *C. freundii* produced 159.51 ppm VA (molar yield of 0.32%). The VA production has not been reported for *Citrobacter* species but the VA production in the present study was higher than *E. aerogenes*.

Conclusions. *Ps. aeruginosa* isolated from *Vanilla planifolia* was the main producer of VA and showed the greatest potential to contribute to the curing process. The results obtained are the first evidence of the study of the Mexican vanilla microbiota and its relationship with the volatile profile.

Acknowledgements.
CONACYT Doctoral Fellowship (No. 40696). Besides, the Colegío de Ingeniería de Alimentos and Instituto de Ciencias, BUAP (CICM-ICUAP) contributed with the necessary funds for the realization of the present study.

References.

1. Salazar-Rojas VM, Herrera-Cabrera BE, Delgado-Alvarado A, Soto Hernández M, González F, Cobos-Peralta M (2012). *Genet Resour Crop Evol*. 59(5):875-887.
2. Chen P, Yan L, Wu Z, Li S, Bai Z, Yan X, Wang N, Liang N, Li H (2016). *Sci Rep*. 6. 20400
3. Mishra S, Sachan A, Vidyarthi AS, Sachan SG (2014). *Biocatal Biotransformation*. 32(5-6):259-266.
4. Chen C, Pan J, Yang X,Xiao H, Zhang Y, Si M, Shen X, Wang Y (2017). *Arch Microbiol*.199(2):325-334.
5. Barghini P, Montebove F, Ruzzi M, Schiesser A (1998). *Appl Microbiol Biotechnol*. 49(3):309-314.
6. Zamzuri NA, Abd-Aziz S, Rahim RA, Phang LY, Alitheen NB, Maeda T (2014). *J Appl Microbiol*. 2014;116(4):903-910.
7. Hunter WJ, Manter DK, Van Der Lelie D (2012). *Curr Microbiol*. 65(6):752-757.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
PB29

EFFECT OF TEMPERATURE AND RELATIVE HUMIDITY IN THE PRODUCTION OF *Beauveria bassiana* SPORES ON SOLID SUBSTRATE

Yolanda Vázquez-Dávila[1], Cipriano García-Gutiérrez[2], Sergio García-Salas[1]
1. Departamento de Bioingeniería. UPIBI. Instituto Politécnico Nacional. Ciudad de México C. P. 07340
2. CIIDIR-Sinaloa. Instituto Politécnico Nacional. Guasave, Sinaloa C. P. 81049
yvazquez@ipn.mx

Key words: Beauveria bassiana, spores, solid substrate

Introduction. *Beauveria bassiana* is the entomopathogenic fungus most used as a biological control agent. Its spores are used to produce bioinsecticides that control insect pest. Spores are produced on solid substrate and temperature and relative humidity have a great effect on spore production (1-5).
The objective was to determine the optimal temperature, relative humidity and the rice concentration, to increase the spore production by means of a central composite design.

Methods. A thin substrate layer was inoculated and placed on a glass slide inside chambers with relative humidity and temperature controlled. Every experiment was done in triplicate. It was used a 2^3 + star central composite design to determine the optimal operating conditions and rice concentration for spores production Temperature, relative humidity and rice concentration was tested at two levels, three central points and α=1.681.

Results. Experiments were realized under ideal conditions because the small size of the sample favors the control of temperature and relative humidity and avoid the influence of porosity. In this way, only the influences due to temperature and relative humidity are considered.

Table 1. Central composite design and results for spores/g

Exp	RH	T	Rice	RH (%)	T (°C)	Rice (g/g)	spores/g
1	-1	-1	-1	93.5	25	0.23	6.50E+07
2	1	-1	-1	99.9	25	0.23	6.21E+07
3	-1	1	-1	84.1	35	0.23	6.89E+07
4	1	1	-1	91.1	35	0.23	7.36E+07
5	-1	-1	1	93.5	25	0.80	1.65E+08
6	1	-1	1	99.9	25	0.80	2.53E+08
7	-1	1	1	84.1	35	0.80	1.02E+08
8	1	1	1	91.1	35	0.80	6.93E+08
9	-α	0	0	84.7	30	0.51	1.68E+08
10	α	0	0	96.1	30	0.51	1.11E+08
11	0	-α	0	96.1	22	0.51	1.01E+08
12	0	α	0	85.2	38	0.51	1.83E+07
13	0	0	-α	90.2	30	0.03	2.13E+07
14	0	0	α	90.2	30	0.99	1.47E+09
15	0	0	0	90.2	30	0.51	7.15E+07
16	0	0	0	90.2	30	0.51	1.32E+08
17	0	0	0	90.2	30	0.51	1.03E+08

RH: Relative humidity, T: Temperature

The Figure 1 shows that the spore production was from 10^7 to 10^9 spores/g, being the higher $1.47x10^9$ spores/g. Except for relative humidity, the influence of temperature was at higher values compared whit that reported for other filamentous fungus. To validate that high temperature had a great influence, a validation experiment was carried out confirming a similar high production $1.96x10^9$ spores/g.

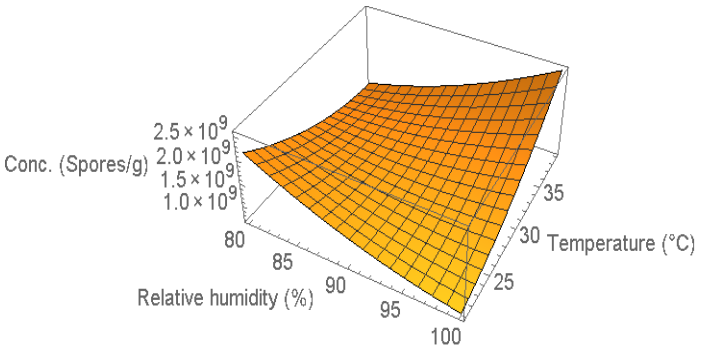


Fig.1 Response surface. Interacting effects of relative humidity and temperature on the spore production of *Beauveria bassiana*.

Conclusions. A high relative humidity (>90%) and an unusual high temperature (>35°C) produce a high concentration of *Beauveria bassiana* spores on solid substrate.

Acknowledgements. SIP 20170155 y SIP 20180327.

References.

1. Kaaya G., Hassam S. (2000). Entomogenous fungi as promising biopesticides for tick control. *Experimental and Applied Acarology, 24,* 913–926.
2. Kang S., Lee S., Yoon, C., Kim S. (2005). Conidia production by *Beauveria bassiana* (for the biocontrol of a diamondback moth) during solid-state fermentation in a packed-bed bioreactor. *Biotechnology Letters, 27,*135–139.
3. Angel A., Figueroa A., Favela E., Viniegra G., Perraud I., Loera O. (2015). Critical values of porosity in rice cultures of *Isaria fumosorosea* by adding water hyacinth: Effect on conidial yields and quality. *Appl Biochem Biotechnol, 177,* 446–457.
4. Villalba P, Grillo R., Cupull R. (2009). *Beauveria bassiana* (Bálsamo) Vuillemin espores production on powders of rice, sorghum and corn. *Centro Agrícola, 36,* 4, 25-32.
5. Mar T., Suwannarach N., Lumyong S. (2012). Isolation of entomopathogenic fungi from Northern Thailand and their production in cereal grains. *World J Microbiol Biotechnol, 28,* 3281–3291.

ISSN: 2448-8461

PB30

Effect of incubation time of Cell- free filtrate in extracellular biosynthesis of zinc oxide nanoparticles and antifungal activity against *Fusarium sp*

Ana Itsel Canales-Mendoza[1], Maricela Villanueva-Ibáñez[1,*], Maria de los Ángeles Hernández-Pérez[2]; [1]Laboratorio de Nanotecnología, sistemas Biológicos y Aplicaciones industriales- Universidad Politécnica de Pachuca, 43830 Zempoala, Hgo.; 2 Instituto Politécnico Nacional, Depto. de Ingeniería en Metalurgia y Materiales-ESIQIE, Cd. México 07738, Mexico; itselmendoza2@gmail.com, *villanueva@upp.edu.mx

Keywords: Nanoparticles, Cell-free filtrate, antifungal

Introduction. Biosynthesized metal oxide nanoparticles (NPs) have a simplicity of use and low cost compared with conventional methods. Specifically zinc oxide NPs (NPs-ZnO) exhibit antibacterial, anticorrosive, antifungal and photocatalytic properties. NPs-ZnO have been synthesized with plants and microorganisms to optimize costs, sizes and quantity (1). The use of microorganisms has a progress in the synthesis of nanoparticles; however, it is necessary to use an extracellular route to improve the process (2). As an alternative, in our working group we have investigated the use of the cell-free filtrate (C-FF) of *Mucor fragilis* isolated from a soil sample with contents of zinc, the challenge was to reduce the time of obtaining the nanomaterial in comparison with a previous work (8 days) (3). Antimicrobial properties of NPs-ZnO are well known, although the studies against phytopathogenic fungi are rare. For this reason, it is also in our interest apply the biosynthesized NPs-ZnO to a fungal pathogen of plants, such as *Fusarium* sp (4).

Methods. The synthesis of NPs-ZnO was carried out with the procedure of Marcelino (2017), modifying the incubation time of C-FF (48, 72, 96 and 120 h). For the choice of C-FF it was considered, in addition to the particle size obtained, the total protein and reducing sugars contents. A typical synthesis consists in using C-FF and $Zn(C_2H_3O_2)_2$ (70 mM), at pH 13 and constant stirring during 24h. The NPs obtained were characterized by UV-vis, Raman, XRD and MEB. The antifungal activity of NPs-ZnO was evaluated determining the minimum fungicidal concentration (MFC) against *Fusarium* sp.

Results. With the biomass of *M. fragilis* incubated for 72h, the NPs-ZnO were synthesized in 6 days in a shorter time than previously found (3). In this incubation time, the CFF presents the highest amount of total protein and reducing sugar contents, involved in the reduction of ions and stabilization of NPs. NPs-ZnO showed the UV-Vis absorption more intense and narrow at 360-370 nm. The different intensities of UV-Vis bands are related to the particle size (Fig 1a). Raman spectra (Fig 1b) show that the NPs-ZnO have a hexagonal Wurzite crystalline structure, this result was confirmed with XRD (Fig 1c). The average particle size observed in MEB was 70 nm (Fig 1d).

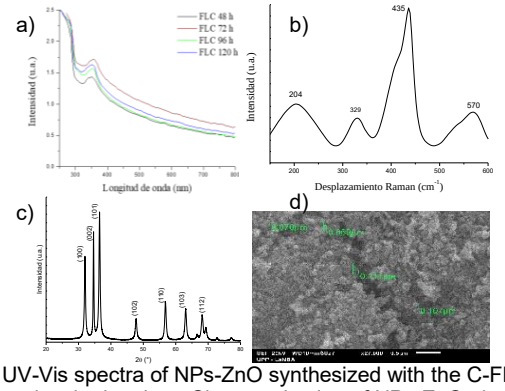


Fig.1 a) UV-Vis spectra of NPs-ZnO synthesized with the C-FF obtained in different incubation time. Characterization of NPs-ZnO obtained with C-FF (72 h): b) Raman spectrum, c) DRX and d) MEB observation.

30 mg/mL of NPs-ZnO showed antifungal effect (Table 1). A higher concentration of NPs causes more suppression of fungal growth due to the interaction with cells and generation of free radicals.

Table 1 Antifungal activity by MFC of NPs-ZnO against *Fusarium* sp.

Microorganism	Control	Concentration of NPs-ZnO (mg/mL)		
		10	20	30
Fusarium sp	+	+	+	-

Conclusions. The time of biosynthesis of NPs-ZnO was reduced when C-FF of M. *fragilis* was obtained at 72 h of incubation, at this time C-FF presented the highest concentration of proteins and reducing sugars, involved in the reduction and dispersion of NPs. *Fusarium* sp. was inhibited with NPs-ZnO at a MIC of 30 mg / mL.

Acknowledgements. To CONACYT for AICM scholarship (858302) and project INFRA-2018 (294909).

References.

1. Król, A., Pomastowski, P., Rafinska, K., Railean-Plugaru, V., Buszewski, B. (2017). *Advances in Coloid and Interface Science*. Vol. (249): 37-52.
2. Agarwal, A., Venkat, S., Rajeshkumar, S. (2017). *Resourse-Efficient Technologies*. Vol. (3): 406-413.
3. Marcelino, G. (2017). Polytechnic University of Pachuca. Mexico: 30-102.
4. Kairyte, K., Kadys, A. y Luksiene, Z. (2013). *Photochemistry and Photobiology*. Vol. (128): 78-84.

ISSN: 2448-8461

PB31

APPLICATIONS OF SELF-ASSEMBLED GOLD NANOPARTICLES IN OPTICAL BIOSENSORS

Noelia Ortega Hernández[1], Quetzali Nicte Morales Rabanales[2], Joel Díaz Reyes[1], José Francisco Ramírez Sánchez[1*]
[1]Centro de Investigación en Biotecnología Aplicada-Instituto Politécnico Nacional, Ex-Hacienda San Juan Molino Carretera Estatal Tecuexcomac-Tepetitla Km 1.5, Tlaxcala C.P. 90700, México.
[2]Unidad Profesional Interdisciplinaria en Ingeniería y Tecnologías Avanzadas, Av Instituto Politécnico Nacional 2580, La Laguna Ticoman, C.P. 07340 Ciudad de México, CDMX, México.
jfsanchez@ipn.mx

Key words: Gold Nanoclusters, Self-Assembled, Biosensor

Introduction. The self-assembly on substrate by gold nanoclusters [1] is crucial in the development of biosensors. It is well known that not only the absorbance originating from localized surface plasmon resonance (LSPR) of gold nanoclusters influenced by the dielectric properties of molecules attached to the nanoclusters also the interband absorption of the nanoclusters changes [2]. Therefore, the adsorption of chemical or biological molecules on the surface will change the absorption bands' intensity and frequencies that can be monitored by measuring the metallic nanoclusters' absorption spectra.
In this paper it is reported the self-assembly of gold nanoclusters on amine-fucionalized glass substrate, evaluating their application in the development of biosensors based on light absorption.

Methods. The synthesis of gold nanoclusters was prepared by the method described by Bastús et al, 2011 and self-assembly were studied using UV-Vis absorption spectroscopy, IR and AFM techniques. Aminopropyltrimethoxysilane (APTES) was used to obtain amine-functionalized glass substrate (AFGS). The gold nanoclusters density self-assembled on AFGS was controlled by varying the immersion time. The biosensor activity was evaluated to detect the antibody (anti-human serum albumin, Anti-HSA) – antigen (human serum albumin, HAS) recognition reaction were performed according to Frederix et al., 2003 and monitored via change of light absorption when this binding event occurs.

Results. The size distribution of the metallic colloids is presented as the inset in Figure 1a. The absorption spectrum of the colloidal metallic revealed a band with a peak near 525.1 nm (Figure 1b), which is attributed to the LSPR [3] band of monodispersed gold nanoclusters with 20 nm size. The optical absorption spectrum of AFGS after 25 h of immersion in metallic colloidal showed that the self-assembly on the glass substrates display a broadening of the band absorption and shift about 65 nm towards the high wavelengths of the LSPR. The IR absorption spectra of AFGS immersed for 25 h in the colloidal gold nanoclusters solution showed an absorption band at about 3332 cm^{-1}, which corresponds to the antisymmetric vibration frequency on the $–NH_2$[4]. The antibody-antigen recognition reaction is corroborated as a change in the interband absorption at ~278 nm and as a change in the LSPR frequency at ~620 nm. Details of these results will be published in future reports.

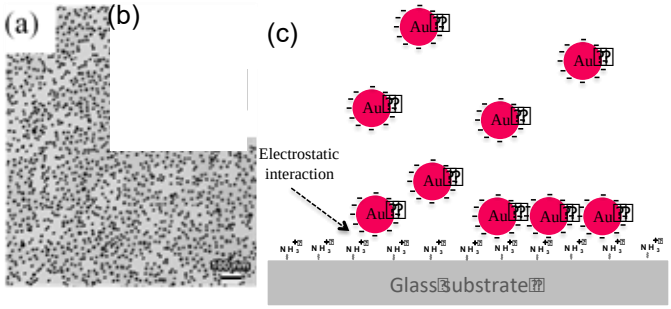


Fig.1 The typical gold nanoclusters TEM micrograph with size distribution (a), optical absorption spectrum of colloidal gold nanoclusters (b) and (c) the schematic illustration of a biosensor based on self-assembly mechanism of gold nanoclusters on amine-functionalized glass substrate.

Conclusions. Using electrostatic interaction between the ionized amine group of amine-functionalized glass substrate and the citrate anions, we could self-assemble gold nanoclusters on amine-functionalized glass substrate. Adjusting the immersion time, the amount of self-assembled in the amine-functionalized glass substrate could be controlled. The self-assembled gold nanoclusters were biofunctionalized and utilized as a biosensor based on light absorption.

Acknowledgements. This work was supported by IPN-BEIFI grant 20180870.

References.

1. Bastús N. G., Comenge J., and Puntes V., (2011). *Langmuir*, 27, 11098-11105.
2. Frederix F., Bonroy K., Laureyn W., Reekmans G., Campitelli A., Dehaen W and Maes G. (2003). *Lagmuir.* 9, 4351-4357.
3. Jain P. K., Lee K. S., El-Sayed I. H. and El-Sayed M. A., (2006). *J. Phys. Chem. B*, 110, 7238-7248
4. Ganguly P, Paranjape D. V., Sastry D. V., (1993). *J. Am. Chem. Soc.* 115, 793-794.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
PB32

Nanocomposite kaolin/ZnO obtained by a biotechnological process

Sara Ruiz-Zamora, Maricela Villanueva-Ibáñez*, Patricia Nayeli Olvera-Venegas, Marco-Antonio Flores-González; Laboratory of Nanotechnology, Biological Systems and Industrial Applications, Polytechnic University of Pachuca, Zempoala, Hidalgo, México C.P. 43830; sararuiz3112@gmail.com, *villanueva@upp.edu.mx

Keywords: nanocomposite, Cell-Free Filtrate, Mucor fragilis

Introduction. Nanocomposites can improve the properties of individual materials [1] expanding its applications in different domains. Kaolin-based nanocomposites are widely used in photocatalysis and recently explored for their antibacterial properties. Despite the extensive uses, their conventional methods of production are complex and toxic to the environment. For this reason, in this study we have proposed a simple and eco-friendly biotechnological process to obtain nanocomposites. By the cell-free filtrates (C-FFs) of *Mucor fragilis* isolated from a soil sample with zinc contents, it was possible to extracellularly obtain a kaolin-ZnO nanocomposite (NCZ) highly stable. The metabolites in C-FF [2] functioned as a reaction medium to add ZnO nanostructures onto kaolin.

Methods. C-FF of *M. fragilis* was obtained by the methodology established elsewhere [2] with minor modifications in the incubation time. The kaolin:ZnO ratios were 1:0.1 and 1:1 (named NCZ 1 and NCZ 2, respectively). NCZ was obtained by addition of the kaolin and $Zn(C_2H_3O_2)_2$ solution (70 mM final concentration) in C-FF at pH value of 13 and constant stirring at 300 rpm during 24h. NCZs obtained were characterized by UV-vis spectroscopy, Raman spectroscopy and Scanning Electron Microscopy (SEM).

Results. NCZs obtained with C-FF of *M. fragilis* were characterized by UV-vis spectroscopy, in Fig. 1a is recorded a shift to higher values of the characteristic band (355 nm) of ZnO nanoparticles (NPsZnO). This shift is related to the interaction between the materials that make up the composites [3, 4]. In Fig. 1b the Raman spectra of the NCZs present the vibrational modes corresponding to the wurtzite hexagonal structure of the NPsZnO [5] and the presence of a set of absorption bands corresponding to the kaolin [6], with the representative band at 145 cm^{-1}. The SEM analysis showed the dispersion of the NPsZnO with quasi-spherical morphology on the surface of pseudo hexagonal plates of the kaolin (Fig. 2a and 2b). The elemental analysis (EDS) shows the distribution of Zn atoms in NCZ, where it can see that the sample NCZ2 shows good homogeneity.

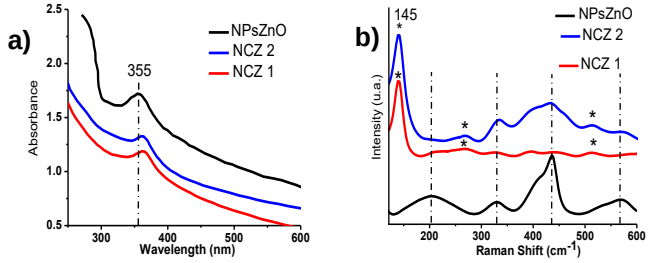


Fig. 1 Characterization of NCZ: a) UV-vis absorption spectra; b) Raman spectra, (*) kaolin.

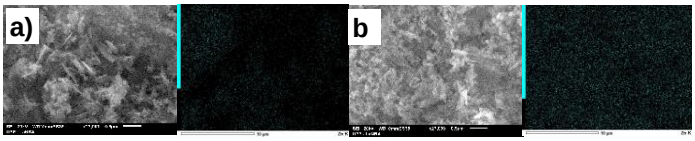


Fig. 2 Characterization of NCZ by SEM: a) NCZ 1; b) NCZ 2.

Conclusions. A protocol of extracellular synthesis of NCZ by C-FF of *M. fragilis* was established. The ratio 1:1 (kaolin:ZnO) showed a better dispersion of NPsZnO. This method provides an alternative route to obtain composites without the use of elevated temperatures or toxic reagents.

Acknowledgements. To CONACYT for SRZ scholarship (858293) and to the project INFRA 2018 (294909).

References.

1. Wan, Y., Zhu, P., Yu, S., Yang, W., Sun, R., Wong, C., y Liao, W. (2017). *Compos. Sci. Technol.* 141 (1): 48-55.
2. Marcelino, G. (2017). Polytechnic University of Pachuca. Pp. 25-33.
3. Lefatshea, K., Muiva, C. y Kebaabetswe, L. (2017). *Carbohydr. Polym.* 164 (1): 301-308.
4. Mamulova, K., Tokarsky, J., y Peikertová, P. (2015). *Appl. Catal., B.* 162 (1): 392-400.
5. Karnan, T., & Selvakumar, S. (2016). *J. Mol. Struct.* 1125 (1): 358-365.
6. Dědková, K., Janíková, J., Matějová, K., Peikertová, P., Neuwirthová, J., Holešinský, J., y Kukutschová, J. (2015). *J. Photochem. Photobiol., B* 151 (1): 256-263.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México

PB33

OPTIMIZATION OF THE ELECTROCOAGULATION PROCESS APPLIED TO THE RECUPERATION OF *CHLORELLA VULGARIS* BIOMASS

Córdova-Guerrero E.[1], Rodríguez-Rosales M.D.J.[1], Lucho-Chigo R.[1], Perales-Vela H.V.[2], Martínez-Roldan A.J.[1,3]
[1] Maestría en Sistemas Ambientales. Tecnológico Nacional de México/I.T. Durango. México. [2]Laboratorio de Bioquímica. Unidad de Morfología y Función. FESI–UNAM. México. [3] CONACyT-Tecnológico Nacional de México/I.T. Durango. México.
alfredo.martinez@itdurango.edu.mx; adjmartinezro@conacyt.mx

Key words: Electrocoagulation, Biomass recovery, Chlorella vulgaris.

Introduction. The microalgae have potential to be used as source of very different high-value compounds (1). Nevertheless, for its production, the recuperation step needs high amounts of energy and this increases the costs. Many authors proved chemical processes with the aim of reducing the energy consumption and one of them is the electrocoagulation. This technique employs electricity to produce the coagulant *in situ*, before the formation of flocs and finally its separation by sedimentation.

This work had the objective to optimize recuperation of *C. vulgaris* biomass by electrocoagulation varying the biomass concentration and voltage applied by the employment of a Central Composite Design (CCD).

Methods. The electrocoagulation assays were done with *Chlorella* biomass using a 3L-reactor, and a direct current source connected to four submerged Fe-electrodes with alternate polarities. The table 1 shows the ranges for the biomass and voltage used in the optimization. The tracing of the process was carried out by the determination of Chlorophyll (2) every 3 min while 30 min. The efficiency of the chlorophyll removal at 30 min was the response employed in the DCC, and the model was obtained using the JMP® program

Table 1. Levels of the factors employed in the DCC.

Level	Biomass concentration	Voltage
- α	2.92	3.86
- 1	5	5
0	10	7.75
+ 1	15	10.5
+ α	17.07	11.63

Results. With the efficiency values, a model with a good regression coefficient was obtained (Fig. 1). Nevertheless, only the voltage presented a significant effect ($p<0.05$). When applied high values of voltage, the effectiveness of the process was close to 90% no matter the amount of biomass used. Considering the current density, the energy wasted in the process was close to 90 W/L lower with the values reported to centrifugation.

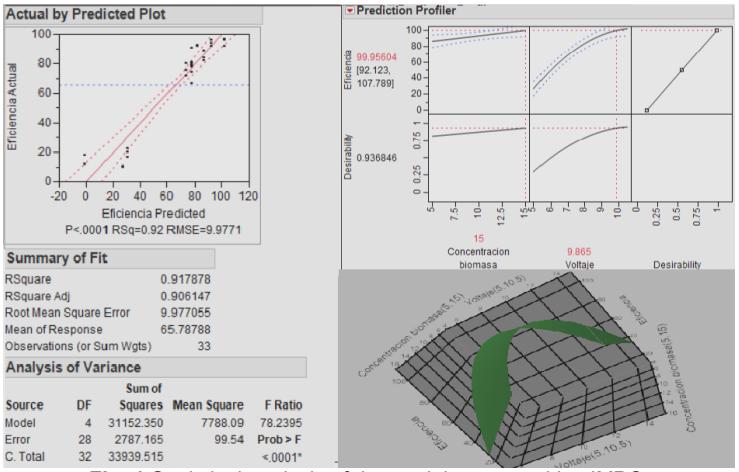


Fig. 1 Statistical analysis of the model generated by JMP®.

The values of alkalinity and conductivity slightly diminished by the action of the electricity; instead, the pH increases and this is because of the liberation of OH radicals and its reaction with the metals in the medium forming hydroxides

The process has certain flexibility with the amount of biomass that can be used without losing efficiency; nevertheless, is advisable the employment of higher biomass concentration to favor the collision of the particles and in consequence the formation of bigger flocs separates faster.

Conclusions. The electrocoagulation process has potential in the recuperation of microalgal biomass because has high efficiency and low energy consumption.

Acknowledgements. Córdova-Guerrero received a graduate scholarship awarded by the CONACyT for Master studies. All the authors thank to the Tecnológico Nacional de México/I.T. Durango for all the resources used in the realization of this project.

References.

[1]Spolaore, P. (2006). *J Biosci Bioeng*, 101(2), 87-96.
[2]Wellburn. (1994). *Journal of Plant Physiology*, 144-307-313
3. MartinezRoldan, A. de J. (2016). 3rd Biotechnology Summit 2016. 436-440 pp.

ISSN: 2448-8461

PB34

"CYTOTOXIC ACTIVITY ASSOCIATED WITH THE MICROBIOTE AND EPITHELIUM OF SPINE OF DIAMOND STINGRAY *Hypanus dipterurus* FROM LA PAZ B.C.S."

Estrella Marron Grijalba, Noemi Aguila Ramírez, Víctor Cruz Escalona, Mauricio Muñoz Ochoa, César Cardona Félix, Christine Band Schmidt.
Instituto Politécnico Nacional. Centro Interdiciplinario de Ciencias Marinas. Department: Biotechnology development.
La Paz B.C.S, 23096 email: estrelladel.marrongri@hotmail.com
Key words: cytotoxicity, stingray, spine.

Introduction. It is documented in some stingray species that the local lesion caused by their sting is due to a mechanical penetration of the spine into the tissue and subsequent release of venom. Causing a series of symptoms such as intense local pain, fever, edema, necrosis, in addition, cases of secondary infection due to the bacteria associated with the spiny apparatus of the fish, the most of them gram-negative bacteria. For freshwater species, it has been found that the extract of the tissue that covers the spine has the capacity to degrade several proteins such as fibrinogen, casein and gelatin (1). Other authors have documented the cytotoxic activity of stripe epithelium of the same genus against cancer cell lines (2), however the information for the *H. dipterurus* ray is null. The results of this study are intended to expand the knowledge about the cytotoxic effects that can be caused with the sting of the ray, also from a biotechnological point of view as a possible source of bioactive compounds.

Methods. Two samples were carried out (warm and cold season). The specimens used for this study were collected in artisanal fisheries. The epithelium and mucus used for the different bioassays were recovered from the spine of the caudal fin. In laboratory, an epithelium maceration was performed in PBS and the mucus bacteria were isolated. Hemolysis and proteolysis of casein and gelatin were performed with the macerate and the bacterial strains. In addition, a specimen was dissected to identify the presence of a possible secretion gland of cytotoxic substances. MTT cell viability assays will be developed on primary culture of human fibroblasts to determine the cytotoxicity of *Hypanus dipterurus* spine epithelium and microbiota.

Results. As preliminary results, 35 bacterial strains have been isolated, 15 cold season (CS) and 18 warm season (WS) strain, with negative cocci predominating in both seasons. We observed proteolytic activity against casein from the epithelium extract and liquefaction of the gelatin by the bacteria and the epithelium extract (Table 1). Only alpha-hemolytic activity was observed by the bacteria isolates. A gland specialized in the secretion of cytotoxic substances was not found.

Table 1. Gelatinase test results

Sample	Liquefaction
Epithelium	+
Bacterial supernatant WS	+
Bacterial supernatant CS	+
Bacterial culture WS	+/-
Bacterial culture CS	+
Blank PBS	-
Blank marine broth	-
Negative control: Grenetine	-
Positive Control: K Proteinase	+

Conclusions. As preliminary conclusions:
The spine of the stingray *H. dipterurus* has an epithelium and bacteria that hydrolyze the gelatin, because it is the main component of the collagen it could be said that the components of the spine have cytotoxic activity since they destroy it.
The epithelium of this sting also presents the ability to lyse the casein, having analogy with albumin and fibrinogen could also lyse these proteins present in human blood and trigger an inflammatory process, resulting in cellular damage by substances released in inflammatory processes.

Acknowledgements. I thank the CONACYT, for its support and sponsorship for the conduct of this investigation, SIP: 20181403

References.

1.Barbaro K., Lira M., Malta M., Soares S., Neto D., Cardoso J., (2007). "Comparative study on extracts from the tissue covering the stingers of freshwater (*Potamotrygon falkneri*) and marine (*Dasyatis guttata*) stingrays" *Toxicon*, *50*(5), 676-687.
2. Rajeshkumar K., Vennila R., Karthikeyan S., Prasad N., Arumugam M., Velpandian T., & Balasubramaniam T. (2015). "Antiproliferative activity of marine stingray *Dasyatis sephen* venom on human cervical carcinoma cell line." Journal of Venomous Animals and Toxins including Tropical Diseases, 21(1), 41.

ISSN: 2448-8461

11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México

PB35

Normalization of gene expression in larval stages of the Pacific white shrimp *Litopenaeus vannamei*

Laura Alvarez-Lee[a], Alejandra García-Gasca[b], Sergio Martínez-Díaz[a], César Cardona-Félix[a], Jesús N. Gutierrez-Rivera[c]

[a]Instituto Politécnico Nacional-Centro Interdisciplinario de Ciencias Marinas, El Conchalito, La Paz, BCS 23094, Mexico. [b]Centro de Investigación en Alimentación y Desarrollo, Avenida Sábalo Cerritos s/n, Mazatlán, Sinaloa 82110, Mexico. [c]Centro de investigaciones Biológicas del Noroeste S.C Mar Bermejo 195, Colonia Playa Palo de Santa Rita, La Paz, BCS 23090, México. Corresponding jtali04@cibnor.mx.

Key words: reference genes, shrimp larvae, Sub F0

Introduction. Larval development of the Pacific white shrimp *Litopenaeus vannamei* has become the target of molecular tests to understand physiology regulation during early stages of life[1] to improve culture conditions in farms and prevent emerging diseases. Several studies of gene expression have used non-validated reference genes affecting expression values and data interpretation. Thus, this work analyzed nine candidate reference genes through the life cycle of L. vannamei, to obtain more accurate results in gene expression.

Methods. Primers were designed from selected constitutive genes (Clathrin, Cit C, Sub F0, EF1A, b-Actin, GAPDH, bp-TATA, Pk and Ak) to evaluate their expression during larval development by qPCR[2]. GeNorm, NormFinder, and RefFinder algorithms were used to rank gene expression stability. The most stable genes were used individually to normalize and compare the expression of developmental genes Mef-2, Twist and Ubx[3]. Kruskal-Wallis and T-Student tests ($p>0.05$) were used to find statistical differences in gene expression.

Results.

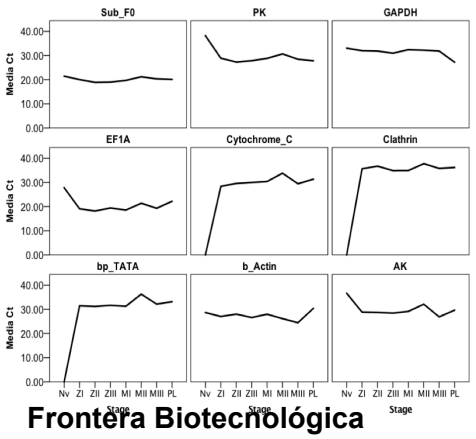


Fig.1 Expression of nine candidate reference genes in *L. vannamei* larval stages. Gene expression was measured per larval stage (n=3, pools of 50 whole organisms) by RT-qPCR.

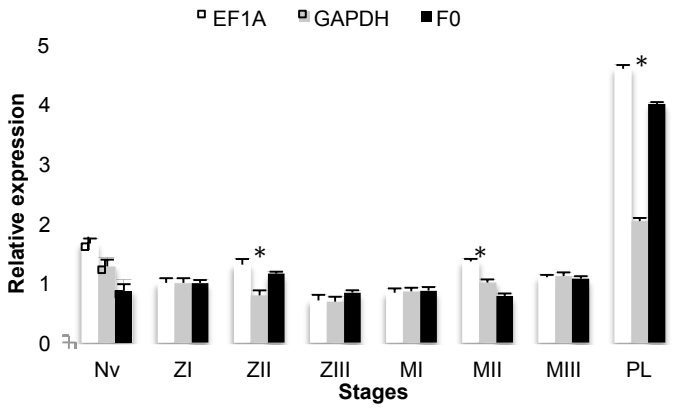


Fig 2. Relative expression of Ubx. Comparative relative expression using three different reference genes. Asterisks indicate significant mean differences according to Kruskal-Wallis test ($p<0.05$)

Conclusions. The most stable reference gene during larval development of *L. vannamei* was Sub F0. This gene alone was more stable than the geometric mean of two or three genes; this may be a methodological advantage because it is easier and less expensive to handle one gene to normalize gene expression.

Acknowledgements. The financial supported is appreciate to CONACyT project no. 247842 and no. 222100, IPN-SIP project 20172253 and ACTG Molecular Solutions S.C.

References.

1.- Jung H., Lyons R., Hurwood D. and Mather P. (2012). Genes and growth performance in crustacean species: a review of relevant genomic studies in crustaceans and other taxa. Reviews in Aquaculture 4:1–34

2-. Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25, 402–408.

3.-Wei J., Glaves R. S. E., Sellars M. J., Xiang J., Hertzler P. L. (2016) Expression of the prospective mesoderm genes twist, snail, and mef2 in penaeid shrimp Dev. Genes Evol. 226:317–324.

ISSN: 2448-8461

PB36

CHARACTERIZATION OF THE GROWTH AND THE LIPIDS CONTENT OF *STIGEOCLONIUM NANUM*

Velasco-Flores Yleanna Lizzeth[1], Rodríguez-Rosales María Dolores Josefina[1], De la Peña-Arellano Luis Armando[1], Martínez-Roldán Alfredo de Jesús[2]

[1]Maestría en Sistemas Ambientales. Tecnológico Nacional de México/I.T. Durango. México. [2]CONACyT-Tecnológico Nacional de México/I.T. Durango. alfredo.martinez@itdurango.edu.mx; adjmartinezro@conacyt.mx

Key words: Microalgae, Stigeoclonium nanum, lipids

Introduction. The microalgae are unicellular microorganisms with different types of metabolism; usually are eukaryotes, but the cyanobacteria have a prokaryotic cellular structure (1). Many microalgae have many applications and can be a source of different high-value products. *Stigeoclonium nanum* is a green microalga with filaments with ramifications and was isolated from a Municipal Wastewater Treatment Plant in Durango (Fig.1A). This work had the objective of characterizing the growth and lipid content of a native strain of *Stigeoclonium nanum.*

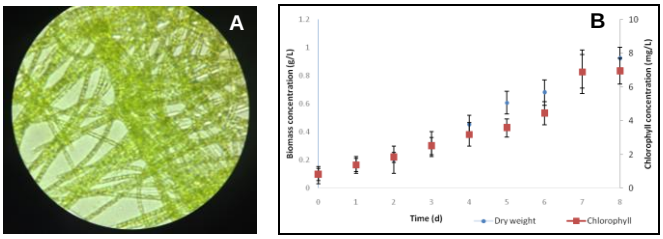


Fig.1 (A) Microphotography of *Stigeoclonium nanum* (40x) (B) Dry weight and total Chlorophyll concentration of *Stigeoclonium nanum*.

Methods. Kimax© 1-L glass bottled were used for the cultivation of *S. nanum* employing BG11 medium. The experiments had a duration of 8 d, and determinations of Dry Weight, (2), Chlorophylls, N-NO_3, P-PO_4 (3) were done daily. At the end of the experiment, the biomass was freeze-dried and the specific content of lipids was determined by a modification of the Bligh & Dyer method (4).

Results. The culture of *S. nanum* started with a biomass concentration of 0.09 ± 0.06 g/L reaching a final concentration of 0.92 ± 0.07 g/L at the 8th day. The chlorophylls presented a similar behavior with the DW and at the end of the experiment; the value was 6.93 ± 0.76 mg/L (Fig. 1B). The specific growth rate calculated using the values of the DW was 0.28 d^{-1}. The values of DW and Chlorophylls concentrations as well as nutrients consumption similar that the obtained by other author using similar culturing conditions (5).

In the case of the nutrients, the initial concentration of the P-PO_4 was 6.72 ± 1.37 mg/L, during the experiment, *S. nanum* consumed this nutrient, and the end concentration was 2.60 ± 0.69 mg/L. For the N-NO_3 the initial and final concentrations were 312.56 ± 44.89 mg/L and 144.17 ± 45.84 mg/L respectively. The rate of consumption of both nutrients was high, for N-NO_3 was 0.90 mg/ (Ld) while for P-PO_4 was 0.76 mg/(Ld) (Fig. 2). The lipid content from the freeze-dried biomass reached 16.04 ± 1.49%; this value is similar that the obtained for other green microalgae in standard culturing conditions (5).

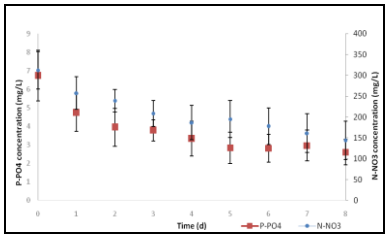


Fig.2 N-NO_3 and P-PO_4 concentration of *Stigeoclonium nanum*.

Conclusions. *S. nanum* is a potential source of lipids and probably useful to develop a process to produce biodiesel. Nevertheless, is necessary the study of the effect of different stressing conditions with the objective to increase the content of lipids in the biomass. Also is essential the optimization of its culturing conditions and medium.

Acknowledgements. Velasco-Flores received a graduate scholarship awarded by the CONACyT for Master studies. All the authors thank to the TecNM/I.T. Durango for all the resources used in the realization of this project.

References.

[1] Greenwell, H. C., Laurens, M. L., Shields, R. J., Lovitt, R. W. Flynn, K. J. (2009). *J. Roy. Soc.* 10, 703-726.

[2] Martínez-Roldán A. J. (2013). Tesis. Centro de Investigación y de Estudios Avanzados Unidad Zacatenco.

[3] González-Moreno, S., Peñalosa-Castro, I., Perales-Vela, H., Salcedo-Alvarez M., Sánchez-Correa S., Medrano-Vazquez, J., Flores-Ortiz, C., Quintanar-Zúñiga, R., Delfín-Alcalá, I., Chino-Vargas S., Puga-Ruiz P., Moreno-Colín R. (2003). *Biomoléculas: Manual de Laboratorio.* Universidad Nacional Autónoma de México. ISBN: 968-36-9271-0.

[4] Arias-Peñaranda, M.T., Cristiani-Urbina, E., Montes-Horcasitas, C., Esparza-García, F., Torzillo, G., Cañizares-Villanueva, R.O. (2013). *Bioresour. Technol.* 140, 158-164.

[5] Villanueva-García R.P. (2018) Tesis. Tecnológico Nacional de México/I.T. Durango.

ISSN: 2448-8461

PB37

Isolation, bioinformatic analysis, phylogeny and 3D modeling of a β-xylosidase gene from *Colletotrichum lindemuthianum*

Perla Jazmín Campos Díaz, Horacio Cano Camacho, Maria Guadalupe Villa Rivera, María Guadalupe Zavala Páramo; *Universidad Michoacana de San Nicolás de Hidalgo, FMVZ, CMEB, Morelia Michoacán;* jazmin_y55@hotmail.com, hcano1gz1@mac.com, gvillarivera@yahoo.es, gzavpar@hotmail.com.

Key words: *Colletotrichum lindemunthianum,* hemicellulases, β-xylosidase

Introduction. In the *Colletotrichum lindeuthianum-Phaseolus* vulgaris interaction, the mechanisms of penetration include the secretion of a group of hydrolytic enzymes that participate in the degradation process of the plant cell wall. Hemicellulose is the second most abundant and complex component of the cell wall, has a heterogeneous composition of different monosaccharides. Due to the heterogeneous nature of hemicelluloses, their complete degradation requires a complex mixture of enzymes, called hemicellulases, such as endoxylanases, β-xylosidases, endoglucanases, endomananas, mannosidases α-arabinofuranosidases and galactosidases. Many genes of cellulases and hemicellulases of fungi have been cloned and these strategies have revolutionized their use in industrial processes. In this work, isolation and bioinformatic and phylogenetic analyses, and protein 3D model of the *bxyloA* gene that encoding a β-xylosidase of *C. lindemunthianum* race 1472 was carried out.

Methods. The cDNA of *bxyloA* was isolated from mycelium of *C. lindemunthianum* race 1472 growth in culture with xylan. The PCR product was ligated to pCR™ 4-TOPO vector and cloned in *Escherichia coli* TOP10. Bioinformatic analysis of the sequence of nucleotides and deduced amino acids of *bxyloA* gene were carried out. On the other hand, phylogenetic analyses of β-xylosidase of *Colletotrichum* species were performed using Maximum likelihood and Bayesian inference methods. The 3D modeling of BXYLOAs of the genus *Colletotrichum* was carried out by homology using the software I-TASSER and Swiss-mode.

Results. The genomic sequence of the *bxiloA* gene of 1814 nucleotides showed high percentages of similarity (98-34%) and identity (97-23%) with genomic sequences of putative β-xylosidases from species of *Colletotrichum*. Two introns and three exons were identified in the nucleotide sequence. The deduced amino acid sequence of BXYLOA showed an open reading frame of 570 aa with a signal peptide, four possible N-glycosylation sites and two possible O-glycosylation sites and the characteristic domain of glycosyl hydrolases of family 43. The comparative analysis with 46 protein sequences of putative *bxyloA* genes in *Colletotrichum* species revealed few conserved domains. However, the catalytic aa residues are conserved in most of the sequences. The 3D modeling of BXYLOA showed characteristics of β-xylosidases of the GH43 family; the enzyme has a homotetramer structure, where each subunit has two domains, one known as the β-helix domain where the aa residues of the catalytic site are located, and a β-sandwich domain, whose function is to close a part of the active site to form a pocket. The consensus phylogenetic tree showed two main clades or lineages produced by events of gene duplications. One of these lineages accumulated more changes than the other, which coincides with the low percentages of identity and similarity of one group of these proteins and high percentages of another group.

Conclusions. The *bxyloA* gene of *C. lindemuthianum*, encodes to a β-xylosidase belonging to the GH43 family. BXYLOA has a high similarity and identity with some putative sequences of β-xylosidases of *Colletotrichum* species. The aa sequence of β-xylosidases showed few conserved regions, however, the aa residues of the catalytic domain are conserved in most of the proteins. The 3D modeling showed the characteristic topology of β-xylosidases GH43. Finally, in general, it can be seen that BXYLO enzymes have undergone several diversification events with several duplications within two main lineages.

Acknowledgements. The authors thank the financial support provided by UMSNH (Project CIC 2016-2017 and 2018-2019 to HCC) and CONACyT for scholarships granted to PJCD (618664).

References

1.Conejo Saucedo, U., Cano Camacho, H., López Romero, E., Lara Márquez, A., & Zavala Páramo, M. G. (2011). Hemicellulases of fungi : A vision of their function in the coordinated degradation of polysaccharides of plant cell walls. *Current Trends in Microbiology*, *7*, 1–13.
2.Cosgrove, D. J. (2005). Growth of the plant cell wall. *Nat Rev Mol Cell Biol*, *6*(11), 850–861. http://doi.org/10.1038/nrm1746

ISSN: 2448-8461

PB38

BIOREFINING FOR THE INTEGRAL USE OF TAMARIND (*Tamarindus indica L.*)

Cruz E. Cristobal, Mendoza C. Luis Alberto, Rodriguez C. Jose Amando, Sanchez A. Laura Ruth, Ramírez C. María Leticia
Biotechnology Department, Universidad Politécnica de Puebla, 3er. Carril del Ejido Serrano S/N, J.C. Bonilla, CP 72640 Puebla, México. E-mail: letyram@unam.mx

Key words: biorefinery, tamarind, proximal analysis

Introduction. The tamarind (*Tamarindus indica L.*) is a decorative fruit tree, belonging to the family of legumes; it can thrive in poor soils, with little or no irrigation and minimal care compared to other tropical fruit trees [1]. The fruit of the tamarind is a curved pod, which is harvested when it reaches its physiological maturity, which happens when it dehydrates and acquires a brown or brownish gray color with a hollow sound when it hits each other. Mexico produces approximately 29600 tons per year, the main export market is USA and Central America [2], but not everything is commercialized.

On the other hand, the term of biorefinery refers to the sustainable technological process that allows the conversion of biomass in diverse products, efficiently and at a low cost, including the processing of waste. Biomass is the raw material used in biorefining, encompassing a heterogeneous set of organic material, both by its nature or origin.

The objective of this work is to develop a biorefinery for the integral use of tamarind fruit in all its parts (pulp, seed and shell).

Methods. The proximate analysis was carried out according to Mexican standards: ash (NMX-F-066-S-1978), humidity (NMX-F-083-S-1978), ether extract (NMX-F-089-S-1978) and crude fiber (NMX-F-090-S-1978); soluble sugars (DNS) and soluble proteins (Biuret) were also determined. According to the parts that make up the tamarind fruit, the seed was used to obtain essential oil [3] and TKP (Tamarind Kernel Powder), the shell to make activated charcoal [4] and the pulp to make the Typical Mexican sweet in the form of little balls (tamarind balls).

Results. Table 1 shows the results of the proximal analysis and the soluble compounds, to separately evaluate the shell, seed and pulp. Table 2 presents the yields of the products according to the part used. TKP and activated carbon are promising products with high yields, but not the essential oil of the seed from which it was extracted very little. The TKP can be used as a rubber however studies are still needed to characterize it. The typical candy in the form of small balls has good yields given that sugar is added.

Tabla 1 Proximate analysis of the tamarind fruit.

Composition (%)	Fraction of tamarind			
	Fruit peel	Pulp	Seed	
			Testa	Cotyledon
	11.483	54.514	30.477	
Moisture	8.322 ± 0.46	24.255 ± 1.53	9.609 ± 0.43	9.41 ± 0.31
Ashes	2.479 ± 0.40	2.989 ± 0.07	0.878 ± 0.02	2.675 ± 0.77
Ether extract	10.392 ± 0.43	0.508 ± 0.035	1.460 ± 0.11	3.655 ± 0.19
Fiber	55.422 ± 0.29	22.766 ± 0.94	5.466 ± 0.25	7.418 ± 0.11
Soluble reducing sugars (g/l)		3.763 ± 0.01	2.381 ± 0.045	
Soluble proteins (g/l)	3.274 ± 0.24	5.125 ± 1.19	2.545 ± 0.13	
Pectin		1.251 ± 0.09		

Tabla 2 Performance of the products obtained.

Product	Part used	Yield on dry matter (g/g)	Yield on wet matter (g/g)
Activated carbon	shell	0.452	0.498
TKP	seed	0.854	0.943
Essential oil	seed	0.046	0.051
Typical candy	pulp	1.115	1.472

Conclusions. A detailed characterization of the tamarind was achieved, in most of the revised references only a part of the fruit is analyzed. The production of activated carbon and TKP showed high yields, being a good option to diversify the use of this fruit that is basically used to make typical drinks and sweets.

References.

1. Souza M.M.D., Alcantara B.L.R., Domelas M.S.C., Alves U.E., Pereira A.A., Cordeiro N.L. (2010) Caracterização morfológica de frutos e sementes e desenvolvimento pós-seminal de Tamaridus indica L. Revista Árbore, 34(6).
2. Silva R., Lucatero S. (2006) Diagnóstico de la cadena del sistema producto tamarino (*Tamarindus indica L.*) en el estado de Colima. Universidad de Colima.
3. Saavedra M.M.L. (2016). Extracción y caracterozación del aceite esencial de las semillas de tamarindo (*Tamarindus indica*). Tesis de Ingeniería Agroindustrial y Comercio Exterior, Universidad Señor de Sipán, Perú.
4. Fombuena M. (2009). Manual del carbón activado. Escuela Politécnica Superior, Universidad de Sevilla. Accesado Febrero 2018. Disponible: http://www.elaguapotable.com/Manual%20del%20carb%C3%B3n%20activo.

ISSN: 2448-8461

PB39

BIOREFINING FOR THE INTEGRAL USE OF MANDARIN

Ortega Dulce, Torreblanca Sergio, Toxqui Salvador, Valencia Belén, Ramírez María Leticia
Biotechnology Department, Universidad Politécnica de Puebla, 3er. Carril del Ejido Serrano S/N, J.C. Bonilla, CP 72640 Puebla, México. E-mail: letyram@unam.mx

Keywords: mandarin orange, biorefining and biotechnological products

Introduction. Mandarin orange (*Citrus reticulata*) is a poorly valued fruit compared to other citrus fruits; currently its overproduction causes the decomposition of this in the orchards [1]. It is estimated that in 2016 its production was almost 270 ton; the main producers of this fruit are the states of Veracruz, Puebla and Nuevo León. The development of a biorefining process for the use of mandarin will potentiate its production, by obtaining products of commercial interest, using all the components of the mandarin orange. The term biorefining refers to the sustainable technological process that allows the conversion of biomass in various products, efficiently and at low cost, including the processing of waste. It should be mentioned that the mandarin used in this work came from the Municipality of Calpan in the state of Puebla, where the Polytechnic University of Puebla is located, so this type of work is necessary to make the transfer of technology to producers of the region.

The objective of this work is to develop a biorefining process for the integral use of mandarin orange (*Citrus reticulata*) from the Municipality of Calpan, Puebla to obtain biotechnological products of interest.

Methods. The proximal analysis was performed according to Mexican standards for: ash (NMX-F-066-S-1978), moisture (NMX-F-083-S-1978), ether extract (NMX-F-089-S-1978) and fiber (NMX-F-090-S-1978); soluble sugars (DNS) and soluble proteins (Biuret) were also determined. The products obtained were essential oil [2], pectin [3], liquor [4] and marmalade [5] according to the references consulted.

.

Results. Table 1 shows the results of the proximate analysis and the soluble compounds, to evaluate mandarin peel and pulp separately. Table 2 presents the yields of the products according to the part used. Marmalade and liquor are promising products, while for pectin and essential oil low yields were obtained. In the case of mandarin oil a characteristic aroma was noted, for this product and for the pectin it is necessary to improve the obtaining process.

Table 1 Proximate analysis of mandarin orange.

Composition (%)	This work		Gutiérrez y Pascual (2016)
	Fruit peel	Pulp	Fruit peel
Moisture	21.550 ± 0.15	94.458 ± 0.23	12.57 ± 0.11
Ashes	0.149 ± 0.001	2.363 ± 0.001	2.73 ± 0.01
Ether extract	3.316 ± 0.04	0.550 ± 0.02	2.14 ± 0.03
Fiber	24.907 ± 3.1	22.289 ± 1.41	43.84 ± 1.14
Soluble reducing sugars	3.9 ± 0.01	4.7 ± 0.0	
Soluble proteins	1.583 ± 0.002	1.968 ± 0.003	
Pectin	0.025 ± 0.004	0.022 ± 0.009	-
Acidity		0.365 ± 0.08	

Table 2 Performance of the products obtained.

Product	Part used	Yield on dry matter (%)	Yield on wet matter (%)
Mermelada	Complete fruit		166.4
Pectin	peel	3.17 ± 0.005	
	pulp	2.6 ± 0.007	
Essential oil	peel		8 ± 0.006
Liquor	peel		98.21

Conclusions. The fruit of the mandarin was taken advantage of completely and the marmalade and the liquor were obtained as products of greater potential, the pectin and the essential oil should not be discarded, so it is necessary to improve the obtaining process. Developing a biorefining for mandarin is essential to make technology transfer to producers of Calpan in Puebla.

References.

1. Servicio de Información Agroalimentaria y Pesquera, SIAP-SAGARPA. (2016).
2. Navarrete, C., Gil, J., Durango, D., y García, C. (2009). Extracción y caracterización del aceite esencial de Mandarina obtenido de residuos agroindustriales, 77(162): 85-92.
3. Rodríguez, K. y Román, A. (2004). Extracción y evaluación de pectina a partir de la cáscara de naranja de las variedades *Citrus sinensis* y *Citrus paradisi* y propuesta de diseño de planta piloto para su producción. Tesis de licenciatura. San Salvador, El Salvador.
4. Moreno, M., Rodríguez, G., Camacho, B. (2004). Cambios fisicoquímicos en 2 aguardientes dulces aromatizados con cáscaras de mandarina y naranja. Revista de la facultad de agronomía, 21(3).
5. Guevara, A. (2015). Elaboración de pulpas, zumos, néctares, deshidratados, osmodeshidratados y fruta confitada. Facultad de industrias alimentarias. Lima, Perú.

ISSN: 2448-8461

PB40

LACCASES PRODUCTION OF *Cantharellus cibarius* GROWN IN SOLID-STATE FERMENTATION

Luna-Suárez S[1]., Grandes-Blanco A.[2], Baez-Gonzáles O. [2], Xelhuantzi-Pluma V. [2], Díaz-Godínez G[3]., Sánchez-Minutti L. [2,*]
[1]Instituto Politécnico Nacional, Centro de Investigación en Biotecnología Aplicada, Tlaxcala, México C.P. 90700, [2]Universidad Politécnica de Tlaxcala, Laboratorio de Biotecnología, Tlaxcala, México. 90180. [3]Universidad Autónoma de Tlaxcala, Laboratorio de Biotecnología, Centro de Investigación en Ciencias Biológicas. Tlaxcala, México 90000
lilia.sanchez@uptlax.edu.mx, minutti85@hotmail.com.

Key words: Laccase, Cantharellus cibarius, solid-state fermentation

Introduction. *Cantharellus cibarius* is an edible fungus with commercial, cultural and social value. This fungus presents activity of the enzyme laccase[1], which has been related to the process of sporulation, production of pigments and formation of fruiting bodies. In addition, these enzymes present several industrial applications. The potential use of the laccases of this fungus is limited when looking for its extraction from the fruiting bodies since its domestication presents difficulties, so its mycelial culture is an alternative. In this work, the laccase enzymatic activity from mycelium of *C. cibarius* grown in solid-state fermentation (SSF) was determined.

Methods. SSF was performed by triplicate in 250 ml Elermeyer flasks containing 0.5 g of polyurethane foam (0.5 cm per side) and 15 mL of modified culture medium (MTT)[2], each plate was inoculated with 3 pellets (5 mm diameter) of mycelium of *C. cibarius* grown on agar at 25 °C for 16 days. Every 24 h a sample was taken to determine the biomass by dry weight and the laccase activity using 2,6-dimethoxyphenol as substrate[2], the kinetic parameters of growth were determined by the logistic equation.

Results. The modified MTT medium favored the rapid growth of the fungus in the first 72 h of incubation (Fig. 1), with a higher specific growth rate (μ) than that reported in other fungi such as *Pleurotus mutilis* ($\mu_{máx}$= 0.007 h^{-1})[3].

The maximum laccase activity was found at 190 and 262 h (7546 and 7605 U/L, respectively) (Fig. 2), surpassing the enzyme production of *Trametes pubescen* (2278 U/L) cultivated in inert support and *T. pubescens* grown in husks of sunflower seed (7219 U/L) and *T. trogii* grown in walnut shells (2206 U/L)[5]. In this work, enzymatic activity values lower than those reported in *P. ostreatus* grown on inert support (18030 U/L) and *Pleutorus* sp. grown in coconut fiber were observed (3412500 U/L)[4].

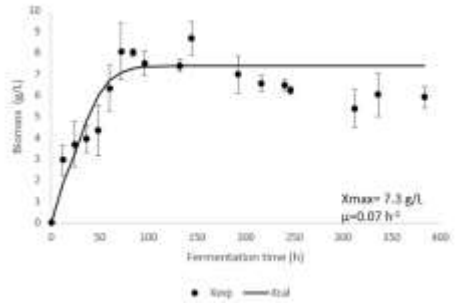


Fig.1 Biomass of *C. cibarius* grown in SSF

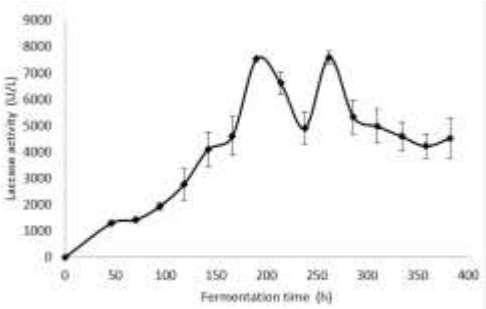


Fig. 2 Laccase activity of *C. cibarius* grown in SSF

Conclusions. The mycelium of *C. cibarius* is an alternative for the production of laccase enzyme and its subsequent use in industry.

Acknowledgements. Thanks to the Polytechnic University of Tlaxcala for providing support for the realization of the experiments.

References.

1. Ng T & Wang H. (2004). *Biochem Biophys Res Commun*. *313*(1), 37-41
2. Téllez-Téllez M., Diaz-Godinez G., Aguilar M, Sánchez C., & Fernández F. (2012). *Bioresources. 7*(2), 2038-2050.
3. Benkortbi O., Hanini S., & Bentahar F. (2007). *Biochem Eng J. 36*(1), 14-18.
4. Rodríguez-Couto S. (2018). Solid-State Fermentation for Laccases Production and Their Applications. In *Current Developments in Biotechnology and Bioengineering.* Larroche M., Sanroman M., Guocheng D. & Pandey A. Elsevier. India. 211-234.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
PB41

EFFECTS OF DIFFERENT CULTURE CONDITIONS IN THE GROWTH AND ANTIBACTERIAL ACTIVITY OF *Salinispora arenicola*

Jessica D. Arrona-Ortiz, Claudia J. Hernández-Guerrero, César S. Cardona–Félix, María M. Cueto-Prieto, Mauricio Muñoz-Ochoa, Chistine J. Band-Schmidt.
Instituto Politécnico Nacional-Centro Interdisciplinario de Ciencias Marinas, Department of Technology Development. La Paz, BCS, México; jessica_arron@hotmail.com.

Key words: secondary metabolites, salt stress, resistant strains

Introduction. From Salinispora strains a variety of compounds have been identified, including the group of potent antibiotics of the rifamycin class [(1)]. Moreover, ionic strength medium Salinispora has shown an effect on growth and production of bioactive metabolites, reporting that there is a greater production of these when subjected to salt stress [(2)]. Preliminary work with Salinispora arenicola strains isolated from marine sediment at Punta Arena de la Ventana, BCS, have shown activity against *Staphylococcus aureus* y *S. epidermidis*. The aim of the present research was established the effect of different culture conditions of *S. arenicola* (Sa-48) on the growth and antibacterial activity.

Methods. For the development of this work was evaluated the effect of two culture media that are different in their carbon and nitrogen source (GYM and SYP), different salinities (2.5, 3.5 and 4.5%) and culture times (7, 14, 21, 28 and 35 days), in the growth and antibacterial activity of *S. arenícola*. The growth of the culture was expressed as Percentage of the volume of the cellular package (VPC) [(4)]. The antibacterial test against *S. aureus* y *S. epidermidis* was realized by diffusion agar method. The extracts were evaluated in a concentration of 100 µg. For control Ampicillin was utilized (25 µg).

Results. The growth of S. arenicola varies according to the condition (Fig. 1). In the salinity of 3.5% was obtained a larger percentage of cell volume and slower growth stages with respect to 2.5 and 4.5% where a lower cell volume resulted. The evaluation of carbon and nitrogen source showed a significant change. When the strain was culture with SYP medium a greater fluctuation was observed in the stages of growth and a smaller volume of cellular package. In the antibacterial activity against *S. aureus* and *S. epidermidis* the extracts of the culture using SYP medium were more active with inhibition halos of 25.7 ± 0.5 mm and 25.3 ± 1.5 mm in a salinity from 4.5% to 28 days and a salinity of 2.5% of 21 days respectively (Table 2). The preliminary analysis of the extracts by chromatographic and spectroscopic techniques in the different days of culture showed variations in the metabolite profile. The compounds uracil and phenylacetic acid was identified as major products in the fractions of the extracts.

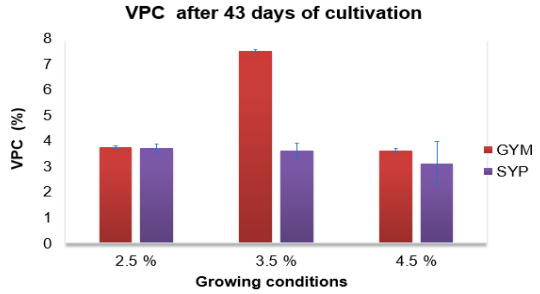


Fig.1 Growth *S. arenicola* (Sa-48) in different media culture and concentration of salt after 43 days of cultivation.

Tabla.1 Antibacterial activity against pathogenic strains.

	Inhibition halo (mm) with 100 µg of extract											
	S. aureus						*S. epidermidis*					
	GYM			SYP			GYM			SYP		
Salinity/ Culture days	2.5	3.5	4.5	2.5	3.5	4.5	2.5	3.5	4.5	2.5	3.5	4.5
7	8.3±0.5	7.0±0	11.0±1.0	16.3±1.1	15.0±0	17.7±0.5	8.0±1.0	7.0±0	9.0±1.0	17.0±0	15.8±2.2	18.0±1.
14	21.0±1.7	7.0±0	14.0±1.0	21.3±1.5	20.3±1.5	24.3±0.5	16.0±1.2	7.0±0	20.0±0.6	22.6±2.5	21.0±1.2	24.3±0.5
21	21.0 ±1.0	9.3±0.6	17.0±1.0	22.3±1.5	25.0±1.7	24.7±0.6	22.0±1.5	10.0±0	21.0±1.2	25.3±1.5	25.0±0	24.0±1.5
28	19.0 ±0.6	9.0±0	14.0±0	25.0±0	21.7±0.5	25.7±0.5	20.0 ±0	9.7±0	16.0±1.0	24.7±0	20.7±1.1	22.7±0.5
35	17.3±01.2	8.3±0.6	12.7±1.2	22.0±0	20.3±0.5	21.3±1.5	21.0 ±1	9.6±0.6	14.6±1.1	20.3±1.5	20.8±1.9	22.6±1.6
Ampicillin control 25 µg	NA											
Solvent control	NA											

Conclusions. Salt stress decreases the volume of cellular package however increases the antibacterial activity. The analysis of the extracts of all the conditions evaluated showed variations in the metabolite profile.

Acknowledgements. Multidisciplinary Project IPN SIP-20181803, to CONACYT and BEIFI for scholarships awarded.

References.

1. Bose U., Hewavitharana, A., Vidgen, M., Ng, Y. & Shaw, P. (2014). *PLoS ONE*, 9(3):1-10.
2. Ng, Y., Hodson, M., Hewavitharana, A., Bose, U., Shaw, P. & Fuerst, J. A. (2014). *Journal of applied microbiology*, 117(1):109-125.

ISSN: 2448-8461

PB42

DEVELOPMENT OF MICROBIAL-BASED FOREST RESTORATION TECHNIQUES

Elena Campos -Vazquez, Roberto Rosales-Fuentes y Angelica Ruiz-Font.
Center for Applied Biotechnology in National Polytechnic Institute, Tlaxcala, MEXICO, & Graduate Program Productive Biotechnology, IPN. (email: convenios22@hotmail.com)

Key words: microbial, forest, restoration

Introduction. This project seeks to develop a microbial consortium of plant growth promoting bacteria in order to ensure the survival of these plant species in arid lands and soils poor in nutrients.
The first source of bacterial diversity was restoration process site. We established two experimental units with 4 different nutrient treatments, We used 6 seedling pine species (*Pinus ayacahuite, P. cembroides, P. rudis, P. montezumae, P. maximartinensis, P. hartwegii*), one specie of agave (*Agave atrovirens*). We sampled bacterial diversity in treatments showed the best survival rate.
The second source of bacterial diversity was three collections of microorganism previously isolated from different pristine sources: pines forest, Chihuahua desert and agave's land.

Methods. Root-associated bacteria from six species of pine were isolated, selected and characterized for their biotechnological potential of growth promotion and biocontrol of plant pathogenic fungi. Almost one hundred strains were isolated and subjected to chemical tests. All isolates presented at least one positive feature, characterizing them as potential PGPR: Production of indole-3-acetic acid (IAA), ability to solubilize inorganic phosphate, phosphatases production, siderophores, cellulose and chitinase production. Each strain was screened for a) solubilization of phosphates in NBRIP media b) chitinase activity c) pectinase and d) cellulases were evaluated. The production of indoleacetic acid (AIA) in the presence of tryptophan was performed by the colorimetric technique using Salkowski reagent.

Results. At first source of microbial diversity 56 morphotypes were isolated, among which 26 were obtained with the capacity to solubilize phosphates, 5 strains with the ability to produce chitinase, two strains with the ability to degrade pectin (pectinase enzyme) and six strains with the ability to degrade cellulose (activity cellulase), see figure 1. We test 32 bacteria on pine platelets, the results show at figure 2. Just 22 bacteria improve the growth of mexican pines.

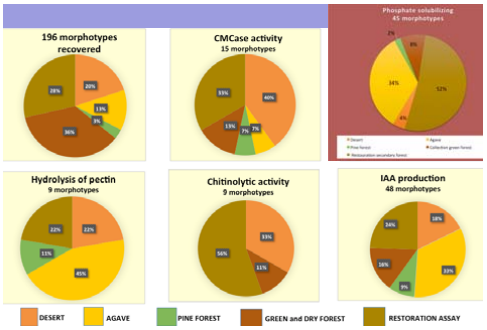


Fig.1 Screening of morphotypes for plant growth promoting activities

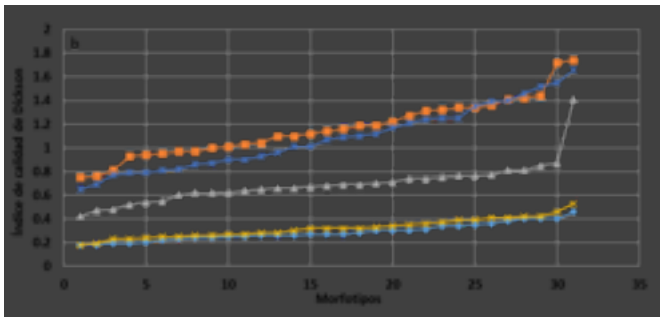


Fig.2 Pine response to addition of bacterial formula

Conclusions. We were able to identify 22 morphotypes that have significant activity on the growth of the six forest species of the bioassay

Acknowledgements.FINNOVA-NAMA program for financial support and Cox BIOPHARMA.

References.

1. Farjos, A. and Styles, B. 2004. Pinus (Pinaceae). Flora neotrópica monografia 75. New York Botanical Garden, New York.
2. Gómez, C. y Corlay, L. 2007. Microbiota edáfica y su importancia en la agricultura. In:Ferrera-Cerrato R. y Alarcón, A. pp 39-55.Microbiología Agrícola. (eds) Trillas.
Gyaneshwar, P.; Naresh,G.; Parekh, J. and Poole, P. 2002. Role of soil microorganisms in improving P nutrition of plants. Plant and Soil.245: 83–93.

ISSN: 2448-8461

PB43

IDENTIFICATION AND EXTRACTION OF HIGHLY VALUABLE FLAVONOIDS IN *AGAVE LECHUGUILLA* BY-PRODUCT: VALORIZATION OF GUISHE.

Zoé Pelletier-Morreeuw, Cristina Escobedo Fregoso, Bertha Olivia Arredondo Vega & Ana Gisela Reyes Alvarado
Centro de Investigaciones Biologicas del Noroeste, La Paz, Baja California Sur, Mexico
zpelletier@pg.cibnor.mx

Key words: Agave lechuguilla, guishe, by-product, secondary metabolite, flavonoids, bioactives, transcriptomic, supercritical fluid extraction, industrial product.

Introduction. *Agave lechuguilla* Torr. is one of the most abundant species in arid and semiarid regions of Mexico. The lechuguilla is used to extract Tampico fiber (Ixtle) through manual or mechanical shredding process mainly for export with a price of around 13 MN/kg. However, fiber recovery discarded an industrial residue, the guishe, which represent around 85% of the recollected plant material leading to environmental pollution and toxicity (1). Thus, for a sustainable development of the productive regions, valorization of the lechuguilla by-product emerged as priority through the last decade. Previous studies have considered the guishe as a source of bioethanol and bioactive compounds for application in agriculture, feed, food and cosmetology (2). Nonetheless, the flavonoid content was never be precisely investigated, eventhough they are potential bioactive compounds with high commercial value (3).
This work claims to identify and extract highly valuable flavonoids from guishe to purpose them as new natural bioactives with potential application in industrial sectors.

Methods. Flavonoids identification will be carried out by transcriptomic and biochemistry analyses trying to correlate the expression pattern of genes involved in flavonoids biosynthesis and the concentration of these metabolites observed by HPLC. Then, the identified biomolecules will be extracted using supercritical fluid as an alternative to solvent extractive method to obtain pure extracts with higher biological activities. Finally, potential bioactivities as antioxidant, antimicrobial and prebiotic will be evaluated through *in vitro* and *in vivo* assays with a focus on aquaculture system.

Results. The achievements in functional annotation of the *de novo* transcriptome of *A.lechuguilla* (255.7 Mpb) and the differential expression between the guishe and the complete leave show that genes involved in biosynthesis of secondary metabolite are overexpressed in guishe with a p-value < 0.05 and a FDR < 0.02. Moreover, within those genes, 82 were identified as coding for enzymes as chalcone synthases, hydroxycinnamoyl-transferase and glucosyltransferases from flavonoids biosynthesis pathways that suggest a production of this class of secondary metabolites in the guishe (4). In the same time, the identification of these enzymes allows prediction the structure and the physico-chemical properties of the produced metabolites which is needed for optimize the supercritical fluid extraction method.

Conclusions. Secondary metabolites of the flavonoids family are produced in the guishe accordingly to transcriptomic observations, although biochemical analyses are required to corroborate these results (5).

Acknowledgements. The authors are grateful to The national Council of Research and Technology of Mexico (CONACYT) and Biorganix Mexicana, Ramos Arizpe, Coahuila, Mexico for financial support.

References.

1. Castillo Quiroz, D., Berlanga Reyes, C., & Cano Pineda, A. (2005). Recoleccion, extraccion y uso de la fibra de lechuguilla (*Agave lechuguilla* Torr.) en el estado de Coahuila. *INIFAP-CIRNE., Publicacion Especial Num. 6*, 1-13.
2. Carmona, J., Morales-Martinez, T., Mussatto, S., Castillo-Quiroz, D., & Rios-Gonzales, L. (2017). Propriedades quimicas, estructurales y funcionales de la lechuguilla (*Agave lechuguilla* Torr.) *Revista Mexicana de Ciencias Forestales, 8*(42), 100-122.
3. Almaraz-Abarca, N., Delgado-Alvarado, E., Avila-Reyes, J., Uribe-Soto, J., & Gonzalez-Valdez, L. (2013). The phenols of the genus Agave (Agavaceae). *Journal of Biomaterial and Nanotechnology, 4*, 9-16.
4. Zhou, W., Huang, Q., Zhou, Z., Ding, M., Huang, F., Li, S., & Kai, G. (2017). Comprehensive transcriptome profiling of *Salvia miltioohiza* for discovery of genes associated with the biosynthesis of tanshinones and phenolic acids. *Nature Scientific Reports, 17*, 10554-10566.
5. Rai, A., Salto, K., & Yamakazaki, M. (2017). Integrated omics analysis of specialized metabolism in medicinal plant. *The Plant Journal*, 90, 764-787.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
PB45

ANTIBACTERIAL ACTIVITY OF *Salinispora arenicola* AGAINST PATHOGENIC *Vibrio Parahaemolyticus*

Yanelis Quevedo-Morales[1], Claudia Judith Hernández-Guerrero[1], Sergio Francisco Martínez-Díaz[1], Erika T.Quintana-Cano[2].
[1]Instituto Politécnico Nacional - Centro Interdisciplinario de Ciencias Marinas, Departamento de Desarrollo de Tecnologías. La Paz, Baja California Sur, 23096. [2]Instituto Politécnico Nacional – ENCB. Av. Carpio s/n México, D.F.
yadiris1968@gmail.com; cguerrer@ipn.mx

Palabras claves: Actinobacteria, Organic extracts, AHPND.

Introduction. *Salinispora* is a genus of actinobacteria that have been recognized as effective producers of antibacterial metabolites (1). Preliminary studies have shown that the amount and nature of active compounds produced by *Salinispora arenicola* could vary during growth and the largest amount of compounds are produced during the first days of culture (2). The aim of this study was to evaluate the ability of *Salinispora arenicola* to produce antibacterial metabolites against different strains of *Vibrio parahaemolyticus* (VP) responsible of the acute hepatopancreatic necrosis disease (AHPND) in shrimp.

Methods. Cultures of *S. arenicola* (strain S-60) in GYM broth were harvested at 7, 14, 21, 29 and 35 days; and organic extracts where obtained using ethyl acetate. The antibacterial activity of each extract was evaluated using the VP_{AHPND} strains M1, M8 and CVP2 as targets; in each case, the effects of different concentrations of the extracts (50, 100, 250 µg/µl) were recorded during a 24-hour VP kinetics. Cultures of VP without extracts and cultures of VP with chloramphenicol (at 1µg /µL) were used as blanks and controls respectively. A preliminary characterization of the extracts obtained at different days of culture of *S. arenicola* was carried out using thin-layer chromatography (TLC).

Results. At 7, 14 and 21 days, the cultures of *S. arenicola* showed the largest antibacterial activity, causing a significant reduction in the growth rate of tested VP_{AHPND} strains; which in some cases was comparable to the effect that produces the use of chloramphenicol (Figure 1). Differences in the degree of susceptibility of the evaluated VP strains were recorded and apparently the M1 strain was the more susceptible to the *S. arenicola* metabolites. Preliminary analysis of the extracts using TLC showed that there are changes in the profile of secondary metabolites produced by *S. arenicola* at different days of the culture.

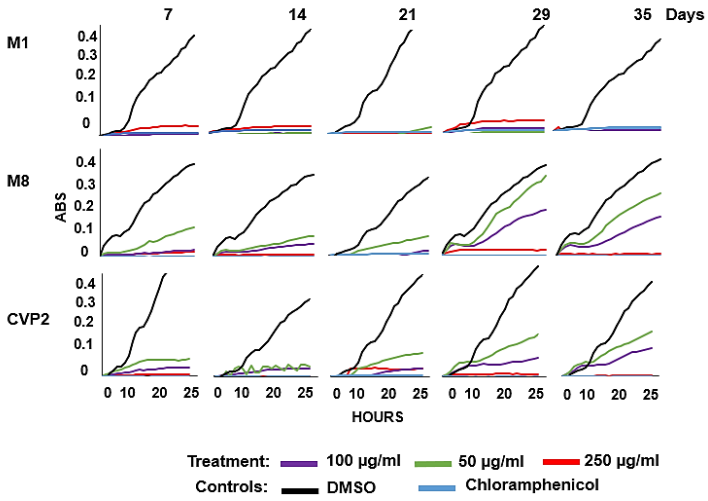


Figure 1. Effect of different doses of *S. arenicola* extracts against strains of *V. parahemolyticus* (M1, M8 and CVP2) at growth kinetics of 25 h.

Conclusions. The extracts of *S. arenicola* obtained in 7, 14 and 21 days of culture induced a significant decrease in the growth rate of different strains of VP responsible of AHPND. There are changes in the profile of secondary metabolites produced by *S. arenicola* at different stages of its culture.

Acknowledgements. This study was supported by the Instituto Politécnico Nacional Projects (SIP20170434 and SIP20181803). CJHG and SFMD thank COFAA and EDI.

References.

1. Fenical, W., & P. R Jensen. 2006. Developing a new resource for drug discovery: Marine actinomycete bacteria. Nat. Chem. Biol., 2: 666–673
2. Ng, Y. K., Hewavitharana, A. K., Webb, R., Shaw, P. N., & Fuerst, J. A. (2013). Developmental cycle and pharmaceutically relevant compounds of Salinispora actinobacteria isolated from Great Barrier eef marine sponges. *Applied Microbiology and Biotechnology*, *97*(7): 3097-3108.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
PB46

OVEREXPRESSION, PURIFICATION AND ACTIVITY EVALUATION OF TWO NOVEL CELLULASES FROM *Trabulsiella odontotermitis*

Martha Arevalos-Sanchez[1], Adrián Máynez-Pérez[1], Monserrath Felix-Portillo[1], Oscar Ruiz-Barrera[1] and Agustín Corral-Luna[1]
[1] Facultad de Zootecnia y Ecología, Universidad Autónoma de Chihuahua. Periférico Francisco R. Almada km 1, Chihuahua, Chihuahua, 31453, México.

Key words: endoglucanase, b-glucosidase, termites

Introduction.
Cellulose is considered the most abundant raw material in nature and constitutes an important source of renewable energy through its conversion to glucose [1]. Cellulose breakdown is carried out by an enzymatic complex consisting of different enzymes working in a synergistic mode, involves the action of three enzymes: endoglucanases (endo-β-1,4-glucanase, EC 3.2.1.4), cellobiohydrolases (exo-β-1,4-glucanase, EC 3.2.1.91), and β-glucosidases (EC 3.2.1.21) [2]. The use of cellulases is widespread in industries animal feeding [3] and more recently, their use in the biofuels industry is gaining interest [4]. The objective of this study was to overexpress, purify and evaluate the activity of a recombinant endoglucanase and a β-glucosidase from *T. odontotermitis* isolated from the termite gut, and to determine the synergistic effect of them on cellulose degradation.

Methods. Overexpression of endoglucanse (eg-FZYE) and β-glucosidase (cel-FZYE) enzymes were conducted in *E. coli* strain BL21 using isopropyl-β-D-1-thiogalactopyranoside (IPTG) as inductor. Cellulose degradation activity was quantified by the 3,5-dinitrosalicylic acid (DNS) method [5] using CMC (carboxymethylcellulose) for eg-FZYE and Avicel for cel-FZYE as substrates, respectively and one unit of endoglucanase activity (U) was defined as the amount of enzyme which produces 1 µmol of glucose per minute. The specific activity was expressed in U/mg of protein. Optimal temperature and pH were determined for each enzyme. The degree of synergism effect (DSE) of a binary mixture was also calculated [6]. All experiments were performed in duplicate, data was subjected to analysis of variance (ANOVA), and the mean values were compared using t-student test to determine significant differences with software SAS 9.0. Statistical significance was defined as $p < 0.05$.

Results. The enzyme eg-FZYE retained 60 % of its relative activity from 3 to 8 of pH, while cel-FZYE was more stable at acidic pH although it retained up to 60 % of its relative activity at pH from 4 to 7 (Fig 1a). Both enzymes retained up to 70% of relative activity from 37 to 42ºC (Fig 1b). The degree of synergistic effect (DSE) of the mix of the enzymes were 1.51 and 1.17 for CMC and Avicel, respectively. Results of enzymatic activity, K_M and V_{max} for both enzymes are shown in Table 1.

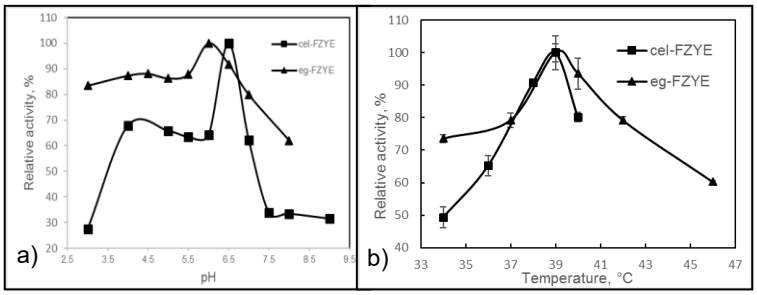


Fig.1 Effect of pH (a) and temperature (b) on the relative activity of eg-FZYE and cel-FZYE.

Table 1. Optimal activity and kinetic parameters.

Enzyme	pH	T (ºC)	Substrate	Specific activity (U/mg)	Km (mg/ml)	Vmax (U/mg)
cel-FZYE	6.5	39	Avicel	799.91± 21.69	11.49	2105.26
eg-FZYE	6.0	39	CMC	1014.17 ± 53.71	11.25	3921.57
			Avicel	566.80 ± 2.6	15.39	2314.81

Conclusions. In this study two enzymes, an endoglucanase (EC 3.2.1.4) and a β-glucosidase with cellulolytic activity isolated from *T. odontotermitis* were successfully overexpressed in *E. coli*. The purified enzymes showed high cellulolytic activity in comparison with other recombinant cellulases from similar sources and also than commercial ones from fungi. Furthermore, an important synergistic effect between them was observed.
The optimal condition of pH and temperature for both enzymes and the mixture are highly similar to the physiological conditions in a ruminal environment, which would allow their use as feed additive to improve fiber degradability. The results here obtained show the potential of eg-FZYE to be used alone or in combination with cel-FZYE to depolymerize cellulose.

Acknowledgements. The authors thank the National Science and Technology Council (CONACyT) for the founding through the CB-239593 grant. Also, we thank PRODEP for the financial support for a postdoctoral fellowship for MMAS.

References.

1. Gupta P, Samant K, Sahu A. (2012). *Intl J Microbiol*. (578925):5pp
2. Zhang Y, Himmel M, Mielenz J. (2006). *Biotechnol Adv.* (24): 452-481.
3. Kozelov L, Iliev K, Hristov A. (2008). *J Sci Food Agric* (88):2568–2575.
4. Sukumaran K, Singhania R, Pandey A. (2005). *J Scientific Ind Res*. (64):832-844.
5. Miller GL. (1959). *Anal. Chem.* (31):426-428.
6. Andersen N, Johansen KS, Michelsen M, et al. (2008). *Enzyme Microb Technol*. (42):362-370.

ISSN: 2448-8461

MF1

CORN OIL FATTY ACIDS: CREATING A POTENTIAL CHEMOTHERAPY NANOCARRIER

Gómez-Bustamante, David Emmanuel; Navarro-Tovar, Gabriela; González, Carmen; Rocha-Uribe, Alejandro; Autonomous University of San Luis Potosí, Chemical Science Faculty, San Luis Potosí, México. Zip Code 78210; egomezbustamante@outlook.com

Key words: Conjugated linoleic acid, liposomes, brain cancer

Introduction. Conjugated linoleic acid (CLA) is a group of isomers from linoleic acid (LA), one of three existing *essential fatty acids,* whose dietary consumption is vital for human health. Two main CLA isomers, *cis*-9, *trans*-11 and *trans*-10, *cis*-12, have been studied due to some interesting biologic effects: anti-inflammatory, anti-atherosclerosis, and anticarcinogenic (1).

Lipid molecules, in aqueous media, self-organize to form *liposomes*: spheres with a hollow center in which dissolved compounds could be entrapped (2).

This work focuses on the creation of nanometric CLA-based liposomes (LCLA), as promising vehicles for improved central nervous system drug delivery, reducing secondary effects from chemotherapy and maybe, increasing the survival for brain cancer patients.

Methods. A blend of the aforesaid bioactive isomers was obtained by microwave-assisted alkali isomerization of commercial corn oil according to Silva-Ramirez *et al.* (3). Liposomes assembly involved mixing different CLA and cholesterol mass-ratios in aqueous solution at biological pH (~7.3), adapting Zhang's method (4). LCLA size, stability and drug encapsulation were evaluated using DLS, ξ potential and UV–Vis spectroscopy, respectively. Cytotoxicity was evaluated via MTT colorimetric assay on *C6* glioma cells, fitting van Meerloo *et al.* protocol (5).

Results. LCLA hydrodynamic diameter and surface electrostatic charge is summed up in *table 1*; consistent values were achieved through polymeric membrane filtration. Best relationship between size and ξ potential was recorded for sets with 2:1 lipid mass ratio, so these were used in every cells viability experiment.

Table 1. *Size and surface charge, with standard deviation, from randomized LCLA batches (n=3).*

CLA:cholesterol proportion	Diameter (nm±SD)	ξ potential (mV±SD)
1:0	34.89 ± 12.35	-36 ± 12.35
1:1	54.09 ± 15.01	-39.9 ± 5.96
2:1	57.71 ± 13.64	-46.4 ± 3.6

All four LCLA tested batches displayed clear growth inhibition (*Fig. 1*) below 15 mM CLA content, confirming isomers' anticancer effect. H_2O_2 acted as positive damage control while negative control was culture media exclusively supplemented with MTT.

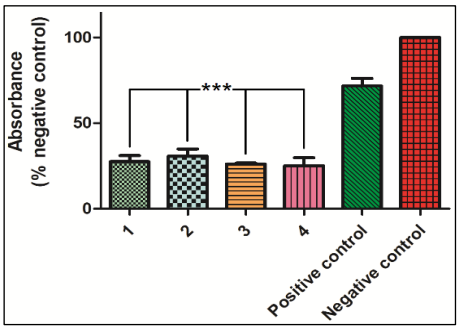


Fig. 1. *MTT assay of different LCLA batches and damage controls. ***Statistical significance (p<0.001) against both controls.*

Entrapment of a photoactive antineoplastic drug in LCLA, was monitored over time (*Fig. 2*), via UV-Vis calibration curve, until maximum absorbance stabilized.

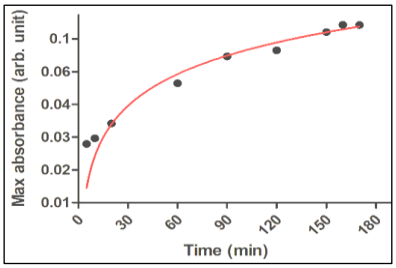


Fig. 2. *Plot of drug highest absorbance values against sampling time.*

Conclusions. LCLA diameter was below 100 nm, had excellent stability in physiological solution while lipids ratio is 2:1, and statistically substantial glioma cells viability reduction at concentrations <15 mM. Additionally, the anticancer drug entrapment efficiency found was around 90%. Very promising quantities for a simple drug delivery system against brain cancer.

References.

1. Yang, B., Chen, H., Stanton, C., Ross, R., Zhang, H., Chen, Y., Chen, W. (2015). *J Funct Foods*, (15):314-325.
2. Allen, T., Cullis, P. (2013). *Adv Drug Deliver Rev*, 65(1):36-48.
3. Silva-Ramírez, A., Rocha-Uribe, A., González-Chávez, M., González, C. (2017). *Eur J Lipid Sci Technol*, 119(4):1600079.
4. Zhang, H. (2017). Thin-film hydration followed by extrusion method for liposome preparation. In: *Liposomes. Methods and Protocols.* D'Souza, G. Humana Press, U.S.A. 17-22.
5. van Merloo, J., Kaspers, G., Cloos, J. (2011). Cell sensitivity assay: The MTT assay. In: *Cancer cell culture. Methods and Protocols.* Cree, I. Humana Press, U.S.A. 237-246.

ISSN: 2448-8461

MF2

New regulatory drugs of the cholecystokinin hormones for the treatment of overweight and obesity.

Vique-Sánchez J. L.*, Caro-Gómez L.*, Cruz-Aguirre A. S.* and Benítez-Cardoza C. G*
*Laboratorio de Investigación Bioquímica ENMH-IPN. E-mail: beni1972uk@gmail.com, jlv64@hotmail.com

Key words: new drugs, overweight, obesity, CCK regulator

Introduction. In 2016, more than 1,900 million adults, over 18 years old, were overweight. Of these, more than 650 million were obese (1). In particular, in Mexico, childhood obesity is a concern, with one third of the population aged 5 to 12 years being overweight or obese, and more than 72% of the population over 20 years of age is in the same condition (2).

Cholecystokinin (CCK) regulates the contraction of the gallbladder and the release of pancreatic enzymes. CCK is a family of peptides (CCK-58, -33, -22 and -8, according to the number of aminoacids). The function of CCK is carried out by the presence of the heptapeptide in the C-terminal position with sequence: YMGWMDF-NH2, very conserved throughout the animal kingdom (3, 4). The activity of the CCK peptides is triggered by the activation of specific CCK receptors, CCK_1 and CCK_2. The main function of the CCK family is to promote the emptying of the contents of the gall bladder and the secretion of pancreatic enzymes, for the assimilation of lipids, proteins and carbohydrates of the intake.

We want to develop a new drug that regulates the functions of CCK, describing the molecular mechanisms associated with the inhibition of the family of gastrointestinal cholecystokinin hormones, evaluating the relationship of this inhibition with overweight and obesity.

Methods.
1. Simulation of the molecular interaction with the peptides of CCK-58 and CCK-33 by Docking, about 450,000 compounds with the aminoacids of the C-terminal end of CCK (YMGWMDF) (Figure 1).
2.Overexpression and purification of CCK-58 and CCK-33 in a bacterial system and purification of peptides (*In vitro*). In our biochemical research laboratory there are constructions of the genes that code for CCK-58 and CCK-33.
3. Administration of inhibitors of CCK sulfation in a murine model of diet-induced obesity. The effect of the 16 selected compounds, as possible inhibitors of cholecystokinin, will be evaluated using administration of these compounds (mice of strain C57BL/6, *In vivo*).

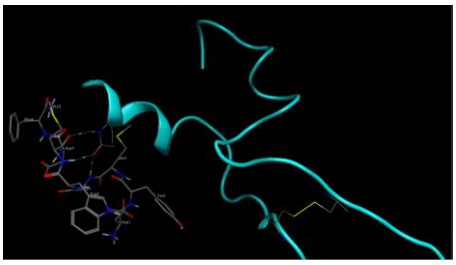


Figure 1. Receiver CCK1 (Code PDB: 1D6G.A, blue) and CCK-8 (1D6G.B)

Results. We have very favorable preliminary results, we selected 16 compounds, which we acquired, starting *the in vitro* tests and finishing the *in vivo* tests, in which we determined a compound that decreases the function of the CCK (Figure 2).

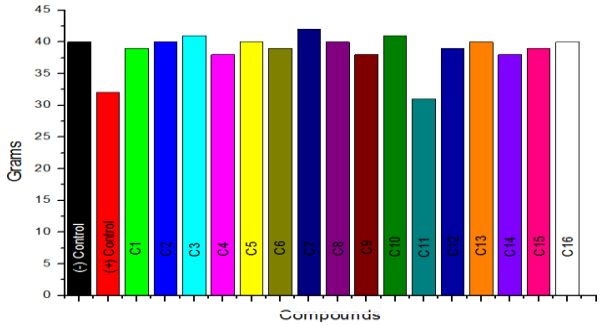


Figure 2. Weight of mice after 10 days of treatment with compounds C1 to C16. The C11 has an effect in lowering the weight as the C (+).

Conclusions. We determined that compound C11 has an effect similar to C (+) in the *in vivo* test, so we have to adjust the concentrations to determine an IC_{50} against CCK and relate the results with the *in vitro* tests that are being carried out.

Acknowledgements. Thanks to the support of SIP-IPN 20181942, 20181321 and CONACYT.

References.

1.OMS-Sobrepeso y obesidad. http://www.who.int/mediacentre/factsheets/fs311/es/.
2. ENSANUT 2016. http://ensanut.insp.mx/ensanut2016/index.php.
3. Rehfeld JF, Johnsen AH, Odum L, Bardram L, Schifter S, Scopsi L. Non-sulphated cholecystokinin in human medullary thyroid carcinomas. J Endocrinol. 1990;124(3):501-506. http://www.ncbi.nlm.nih.gov/pubmed/2332719.
4 .Bonetto V, Jörnvall H, Andersson M, Renlund S, Mutt V, Sillard R. Isolation and characterization of sulphated and nonsulphated forms of cholecystokinin-58 and their action on gallbladder contraction. Eur J Biochem. 1999;264(2):336-340. http://www.ncbi.nlm.nih.gov/pubmed/10491077

ISSN: 2448-8461
16-20 Octubre/October 2018
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
San José del Cabo, B.C.S., México
MF3

Rediseño de clavo intramedular para fracturas diafisiarias de tibia

Avalos – Flores Julio Cesar, Urriolagoitia – Sosa Guillermo, Romero – Ángeles Beatriz, Urriolagoitia – Calderón Guillermo Manuel, Rodríguez – Martínez Rafael.
Instituto Politécnico Nacional, Escuela Superior de Ingeniería Mecánica y Eléctrica, Sección de Estudios de Posgrado e Investigación, Unidad Profesional Adolfo López Mateos " Zacatenco" Avenida Instituto Politécnico Nacional s/n, Edificio 5, 2° piso. Col. Lindavista, C.P. 07320, Ciudad de Mexico.
Ingavalos05@gmail.com

Palabras clave: *Osteosíntesis, tibia, diafisis.*

Introducción. La estabilidad de diferentes tipos de miembros fracturados ha evolucionado a través de la historia de la humanidad, desde la prehistoria hasta la actualidad, teniendo un considerable desarrollo en enfrentamientos bélicos, las cuales potencializaron el desarrollo de la ortopedia y osteosíntesis mediante la transformación de diferentes dispositivos para el tratamiento de fracturas. La utilización de clavos intramedulares son una opción viable ya que representan técnicas que hacen que la alineación entre los huesos fracturados sea lo más exacta y más segura para el tratamiento de fracturas diafisiarias de tibia [1].
El rediseño del clavo intramedular para fracturas de tibia es esencial dentro del sector médico en el hospital de Xoco de la Ciudad de México. El uso en específico de dicho dispositivo es para fracturas diafisiarias de tibia.

Método.
Se realizó el rediseño Fig.1., de un clavo intramedular para fracturas de tibia existente, posteriormente se ejecutó el análisis estático analítico y numérico. Para ambos casos de estudios se establecieron las condiciones siguientes: material utilizado fue Titanio 6Al-4Av o G5, la fuerza de carga sobre el clavo, la cual es de $^5/_6$ el peso del individuo obteniendo 863.28 N sobre una sola extremidad en dirección axial de la tibia [2].

Fig.1. Clavo intramedular para fracturas diafisiarias propuesto

Dentro del análisis analítico se utilizaron ecuaciones de mecánica de materiales para obtener esfuerzos nominales, desplazamientos y deformaciones unitarias [3]. Las solicitaciones mencionadas anteriormente se ejercieron sobre los ejes "*x", "y", "z"*. Se utilizó el Método del Elemento Finito para la corroboración del estudio analítico, con el programa computacional ANSYS APDL®.

Resultados. En la Fig., 2 se muestra gráficamente el esfuerzo generado en el eje "*y"* del clavo intramedular. En la Tabla 1, se muestran los resultados obtenidos del análisis analítico y numérico solamente del eje "*y"*.

Fig. 2. Se muestra el esfuerzo axial sobre el clavo intramedular

Tabla 1. Comparativa de resultados analítico y numérico obtenidos sobre eje "*y*".

	Analitico	Numerico
Esfuerzo (MPa)	.0710	.0955
Deformacion uniaria	$4.138x10^{-7}$	$6.808x10^{-7}$
Desplazamiento mm	$1.430x10^{-4}$	$1.479x10^{-4}$

Conclusiones.
Dentro de la comparación de resultados de ambos análisis se comprobó la variabilidad, debido a que el programa computacional abarca amplios criterios de cálculo. El esfuerzo generado sobre el eje *y* se encuentra dentro de los límites generados por medio del programa del Método de Elemento Finito.

Agradecimientos Los autores agradecen al Instituto Politécnico Nacional y al Consejo Nacional de Ciencia y Tecnología por el apoyo brindado en la elaboración de este trabajo.

Referencias.
1.- Norton, K. (2007) *Un breve recorrido por la historia de la protésica*, In Motion, México. pp 5-15.
2.- McLeish, R. D. y Charnley, J. (1970) *Abduction forces in the one-legged stance, Journal of Biomechanics,* Vol. 3: pp 191-194.
3.Timoshenko, S. (1986). *Mecánica de Materiales,* Ed. Editorial Iberoamérica, México. pp 346-357.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México

MF4

DISEÑO DE UN EQUIPO DE REHABILITACIÓN LUMBAR

Calalpa-Torres, I., Torres-San Miguel, C.R., Urriolagoitia-Sosa, G., Romero-Ángeles, B., Urriolagoitia-Calderón, G.M
Instituto Politécnico Nacional, Escuela Superior de Ingeniería Mecánica y Eléctrica, Sección de Estudios de Posgrado e Investigación, Unidad Profesional "Adolfo López Mateos" Edificio 5 Segundo Piso, Zacatenco, Del. Gustavo A. Madero, C.P. 07320, Ciudad de México.
Itzia.calalpa.torres@gmail.com

Palabras clave: dolor, antigravitatorio, terapia

Introducción. El dolor lumbar es un traumatismo que se centra en el disco intervertrebal y puede incapacitar al ciudadano mexicano [1]. Es causado por procesos degenerativos debido a la edad o posturas inadecuadas [2]. De acuerdo con algunos autores, los músculos antigravitatorios encargados de oponerse constantemente a la gravedad juegan un papel importante dentro de la aparición del dolor lumbar [3]. Los tratamientos actuales se enfocan principalmente solo en la separación de los discos intervertebrales y dejan de lado, la rehabilitación de los músculos. Siendo, el objetivo principal del presente trabajo, la propuesta de diseño de un equipo que permita la rehabilitación de los músculos antigravitatorios y la descompresión intervertebral.

Métodos. Para llevar a cabo el desarrollo del proyecto se realizó una investigación sobre los antecedentes de terapias de descompresión, tracción e inversión. También, sobre las posturas adecuadas para liberar al disco intervertebral y la forma en que los músculos antigravitatorios son rehabilitados.

Resultados. El diseño del rehabilitador se divide en la parte mecánica, donde se encuentra los elementos principales como, el eje que transmitirá la potencia necesaria para rotar al equipo hacia adelante o atrás de tal forma que se rehabilite y descomprima la columna respectivamente, la base que soporta al marco del dispositivo que incluye la cama que sostendrá al paciente. Por otro lado, está la parte de automatización que mediante, un controlador lógico programable, sensores de posición y un cilindro electrónico será capaz de llevar a cabo los movimientos pertinentes para que el dolor lumbar del paciente disminuya.

La descompresión consiste en conocer el peso del paciente para determinar el grado de inclinación que el equipo debe adoptar en el proceso mencionado. Durante el tiempo que el paciente este inclinado sus rodillas deberán están ligeramente flexionadas (Fig. 1), relajando el músculo psoas y por lo tanto las cargas sobre la columna lumbar disminuyen.

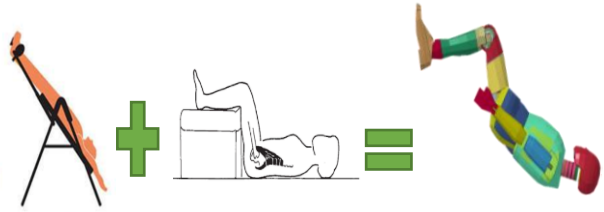


Fig. 1. Postura para la descompresión.

Para la rehabilitación, el cuerpo se inclina hacia el frente gradualmente hasta alcanzar 45° este movimiento activara las contracciones isotónicas del cuerpo que permiten que la musculatura ineficaz realice un máximo esfuerzo para la rehabilitación y ejercitación de los músculos.

Conclusiones. De acuerdo con los resultados obtenidos las posturas propuestas cumplirán con la rehabilitación y descompresión de las vértebras relevando del dolor lumbar a los afectados. Por lo que, como trabajo futuro, se considera viable realizar la manufactura y ensamble del prototipo rehabilitador lumbar (Fig. 2).

Fig. 2. Rehabilitador lumbar.

Agradecimientos. Los autores agradecen al Instituto Politécnico Nacional y al Consejo Nacional de Ciencia y Tecnología de la Ciudad de México por el apoyo brindado.

Referencias

1.- Liebenson, C. (2008). *Integración de la rehabilitación en la praxis quiropraxia.* En: *Manual de Rehabilitación de la Columna Vertebral*, Ed. Paidotribo, Barcelona, pp 33-49.
2.- Umaña-Giraldo, H.J., Henao-Zuluaga, C.D. y Castillo-Berrio, C. (2010). *Revista Médica de Risaralda*, Vol.16, No.2, pp 43-53.
3.- Aragalll, F. y Munné A. (2017). Gravitoterapia. Colgarse cabeza abajo. En: Sistema ARC.

ISSN: 2448-8461

MF5

DESING OF A SMALL INTERFERING RNA TO SILENCE ARGONAUTE 3 PROTEIN IN MOSQUITO CELLS

Juan Santiago Salas-Benito[1], Aurora Montsserrat Gozález-Flores[1], Paola Berenice Zárate-Segura[2], Fernando Guadalupe Bastida-Gonzálex[3]
[1]Escuela Nacional de Medicina y Homeopatía and [2]Escuela Superior de Medicina of Instituto Politécnico Nacional.
[3]Laboratorio Estatal de Salud Pública del Estado de México, Mexico City CP 07230; jsalas@ipn.mx.

Key words: dengue virus, viral interference, PIWI-RNA pathway

Introduction. Dengue virus is the causative agent of the most important arbovirosis worldwide and it is able to establish a life-long or persistent infection in the mosquito vector (1). The viral interference is the ability of one virus to block the replication of another virus in the same cell and this phenomenon is observed during viral persistent infections, however the mechanisms involved are not well understood (2). It has been proposed that the PIWI-RNA pathway, involved in the regulation of transposon expression, could be participate in viral interference during arbovirus infection in the mosquito and one of the key proteins of this pathway is the Argonaute 3 (Ago 3) (3,4).
The main objective of this work was design a small interfering RNA (siRNA) able to block the expression of Ago 3 in mosquito cells in order to evaluate the participation of PIWI-RNA pathway in the homologous viral interference.

Methods. VectorBase data bank and nucleotide BLAST were used to identified the putative sequences of Ago 3 protein in *Aedes albopictus* genome. With Iscore designer and Invitrogene softwares, we designed several siRNAs and two were selected according with Reynold's criteria: one for Ago 3 and one for Green Fluorescent Protein (GFP), used as control. Both siRNAs were synthetized and transfected to C6/36 cells persistently infected with dengue virus 2 (C6-L cells) using lipofectamine 3000 according with manufacturer's instructions. After 72 h of transfection, protein extracts from the cells were obtained with RIPA buffer and used to perform a Western blot assay with commercial antibodies.

Results. Two sequences that displayed 97.2 and 99.8% of homologies with Ago 3 from *Aedes aegypti*, were selected to designed a siRNA.

Table 1. Suitable sequences of siRNAs for Argonaute 3 (Ago 3) and green fluorescent protein (GFP). * indicate the siRNAs selected.

Protein	Sequence 5'-3'	Sequence 3'-5'	Score
Ago 3	ACAGAAAAUCGGUGACUAA	UGUCUUUUAGCCACUGAUU	10*
Ago 3	CGAUUUAUCCGAAGGGACU	GCUAAAUAGGCUUCCCUGA	7
GFP	CCAACACUUGUCACUACUU	AAGUAGUGACAAGUGUUGG	5*
GFP	GGUGAAGGUGAUGCAACAU	AUGUUGCAUCACCUUCACC	4.5
GFP	CCCUUUCGAAAGAUCCCAA	UUGGGAUCUUUCGAAAGGG	4.5

The sequence of two siRNAs for Ago 3 and three for GFP were obtained. One of each was selected according with its score (**Table 1**) and synthetized. siRNAs were transfected into mosquito C6-L cells and their silencing effectivity was evaluated by Western blot. As expected, the siRNA for GFP did not have any effect in the expression of Ago 3 and it will be suitable as a negative control (**Fig. 1, siGFP**).

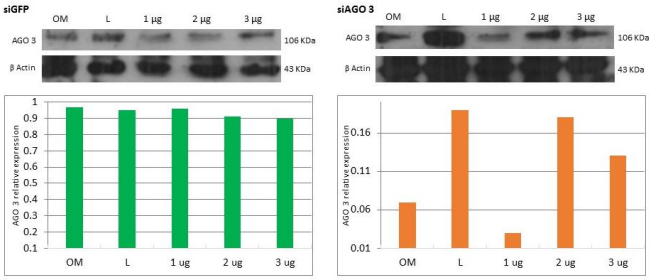


Fig.1 Western blot (upper panel) using antibodies against Ago 3 and actin proteins, and total protein extracts from C6/36 cells treated with opti-MEM (OM), Lipofectamine 3000 (L), and siRNA for GFP (left) or Ago 3 (right) at 1, 2, and 3 µg. Densitometry analysis are shown in the lower panels.

However, a reduction in the expression was observed when the siRNA for Ago 3 was used only an amount of 1 µg (**Fig. 1, siAGO 3**).

Conclusions. We designed a siRNA specific for silencing Ago 3 protein in C6/36 cells, however some other tests should be performed in order to adjust concentrations.

Acknowledgements. This proyect was supported by Secretaría de Investigación y Posgrado of Instituto Politécnico Nacional (SIP20181010).

References.

1. Goic B, Saleh MC. (2012). *Curr Opin Microbiol*.15:531-537.
2. Salas-Benito JS, De Nova-Ocampo M. (2015). *J Immunol Res*. 215:1-14.
3. Brackney ED, Scott CJ, Sagaws F, Woodward EJ, Miller AN, Schilkey DR, Mudge J, Wilusz J, Olson EK, Blair DC, Ebel DG. (2010). *Plos Negl Trop Dis*. 4 (10): 856-866.
4. Léger P, Lara E, Jagla B, Sismeiro O, Mansuroglu Z, Coppée YJ, Bonnefoy E, Bouloy M. (2013). *J Virol*. 87:1631-1648.

ISSN: 2448-8461

IDENTIFICATION AND PRODUCTION OF LUNAMYCIN, A NOVEL LASSOPEPTIDE FROM *STREPTOMYCES SCABRIPORUS* NF3

Stefany Daniela Rodríguez Luna, Corina Diana Ceapă, Melissa Vázquez Hernández, Carlos Naranjo Castañeda, Sergio Sánchez; Instituto de Investigaciones Biomédicas, Departamento de Biología molecular y Biotecnología, Universidad Nacional Autónoma de México. Ciudad de México, 04510. México. (*danielaluna227@gmail.com)

Key words: lassopeptide, Streptomyces, secondary metabolize.

Introduction. The genus *Streptomyces* is one of the most important sources of bioactive compounds in the microbiological systems. *Streptomyces* species have been found living together with plants as endophytic microbes [1]. One Actinomycete was isolated from the plant *Amphipterygium adstringens* (Rodríguez-Peña, unpublished data) and identified as *Streptomyces scabrisporus* (SSNF3). Genome mining of the SSNF3 genome identified 3 possible lassopeptides [2].Lassopeptides belong to the superfamily of RiPPs. Lassopeptides topology is similar to that of a twisted ribbon. Lassopeptides can work as inhibitors or antagonists of different receptors, and have antimicrobial activity [3].

Methods. Bagel3 [4], PRISM [5] and antiSMASH 4.0 [6], were used for mining clusters for secondary metabolites. The operon encoding the synthesis of lunamycin, (with 4045 bp), was introduced into the plasmid pET22b, cloned in *E. coli* DH5α, and expressed in *E. coli* Rosseta. The protein was expressed adding 1 mM IPTG in M9 medium supplemented with thiamin (50 mM) and biotin (50 mM) at 20°C for 3 days. The lunamycin was purified by extraction with MeOH for 1 h at RT, concentrated in a rotavapor and solubilized in 5% acetonitrile with 0,1 formic acid. Its identification was performed by SDS - PAGE and UPLC-MS.

Results. Among the 3 lassopeptide operons detected, only one presented the correct architecture (Fig. 1). The heterologous expression of the biosynthetic cluster associated to lunamycin formation was made in *E. coli* Rosseta (Figure 2) and confirmed by UPLC-MS (Figure 3).

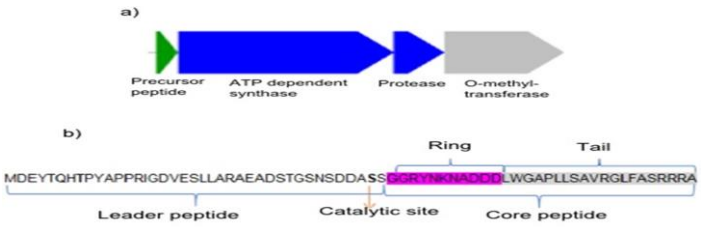


Fig.1 a) Lunamycin Biosynthetic Cluster. b) Peptidic Precursor of Lunamycin.

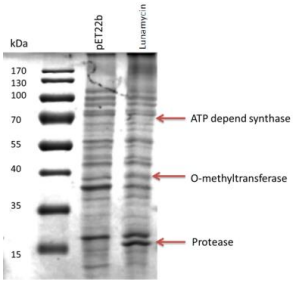


Fig 2. SDS-PAGE with the proteins associated with the cluster lunamycin stained with Coomassie blue.

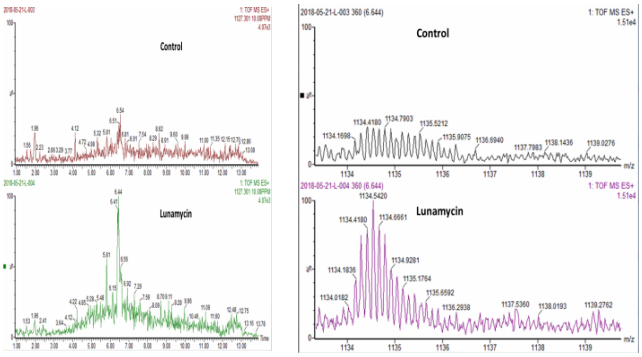


Fig 3. UPLC-MS characterization of lunamycin. A) Ultra-resolution liquid chromatography control: empty vector and lunamycin. B) Identification of the mass associated with the cyclized methylated peptide.

Conclusions. Lunamycin biosynthetic cluster was cloned and expressed. The enzymes of lunamycin formation were detected by SDS-PAGE. The cyclized methylated peptide was identified by UPLC-MS. The evaluation of the peptide bioactivity is in progress.

Acknowledgements. SDRL was supported by the CONACyT scholarsip 854897and by the PAEP stipend. Part of this work was financed by PAPIIT DGAPA, UNAM IN202216 and the NUATEI program from the Instituto de Investigaciones Biomédicas, UNAM.

References.

[1] Martínez-Klimova E, Rodríguez-Peña K and Sánchez S (2017). *Biochem Pharmacol.* 134: 1-17.
[2] Vazquez-Hernandez M, Ceapa CD, Rodríguez-Luna SD, Rodríguez-Sanoja R, Sánchez S (2017), *Genome Announc*. 5:e00267-17 .
[3] Hegemann, J. D., Zimmermann, M., Xie, X., Marahiel, M. A.(2015), *Acc. Chem. Res*, *48* (7), pp 1909–1919.
[4] Van Heel AJ, de Jong A, Montalbán-López M, Kok J and Kuipers OP (2013), *Nucleic Acids Res*. 41:1–53.
[5] Skinnider MA, Dejong CA, Rees PN, Johnston CW, Li H, Webster ALH, Wyatt MA and Magarvey NA (2015), *Nucleic Acids Res*, 43:9645-9662
[6] Weber T, et. al. (2015), Nucleic Acids Research 43: W237-243.,

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México

MF8

ANTINOCICEPTIVE EFFECT OF *SALVIA DIVINORUM* IN MICE

Myrna Déciga-Campos[1]; María Eva González-Trujano[2]; Lorenzo Leonel Tlacomulco Flores[1]
[1]Sección de Estudios de Posgrado e Investigación de la Escuela Superior de Medicina IPN, Ciudad de México. [2]Instituto Nacional de Psiquiatría Ramón de la Fuente Muñiz (INPRFM), Ciudad de México.
myrnadeciga@gmail.com

Key words: S. divinorum, antinociception, salvinorins

Introduction. *Savia divinorum*, a native plant of Oaxaca, Mexico, is widely indicated to stimulate the enteric system as an antispasmodic and analgesic for gastrointestinal disturbances such as stomach pain. Also *S. divinorum* has psychoactive properties in traditional rituals in Mexico[1]. The purpose of this study was to investigate the antinociceptive effect of an organic extract of the seeds of hydroponic *S. divinorum* and, using the writing test in mice.

Methods. The leaves of *S. divinorum* were obtained from the company Altiva Productos Horticolas S.P.R. of R. L. in Altiva Montecasino, located in Huitzilac, Morelos, Mexico (Lot INPJRF-1A / 25-05-2012) by hydroponic procedure. The air-dried aerial parts of *S. divinorum* were extracted with ethyl acetate and then concentrated in vacuo. The acetic acid-induced abdominal writhing test was performed as previously described[2-3].

Results

Figure 1 shows the antinociceptive response of *S. divinorum* extract (3-100 mg/kg, i.p.) when it was administered in mice. Tramadol (a partial opioid agonist analgesic drug) was used as a reference. The extract was fractionated by chromatography to obtain a mixture of salvinorins containing salvinorin A and B.

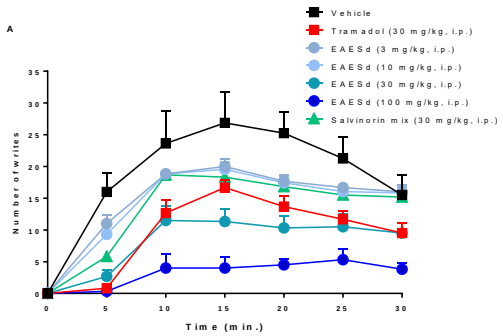


Fig.1 Time-course curves of the injection of 1% acetic acid (100 µl, i.p.) (panel A) or 1% formalin (panel B) into the right hind paw of the mice after the administration of *S. divinorum* and salvinorins mix isolated from *S. divinorum*. Data are expressed as the mean ± S.E.M. of at least six animals.

Figure 2 shows the participation of opioid receptors and serotonin 5-HT$_{1A}$ receptors as a possible mechanism of action because the antinociceptive responses were blocked in the presence of naloxone (the opioid antagonist) and WAY (serotonin 5-HT$_{1A}$) in the visceral nociception.

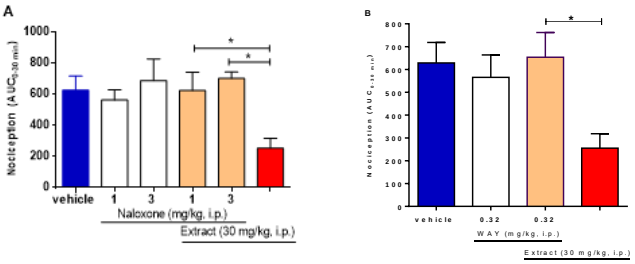


Fig.2 Antinociceptive effect of *S. divinorum* extract (30 mg/kg, i.p.) in present of naloxone, opioid antagonist (panel A) and WAY, serotonin antagonist (panel B) in 1% formalin test. Data are expressed as the mean ± S.E.M. of at least six animals. ANOVA one way, Tukey test p≤0.05.

Conclusions. The pharmacological data obtained demonstrated the use of *S. divinorum* for the treatment as an antispasmodic in stomachaches and for inflammation due to its antinociceptive properties. This study could be the basis for the search for new alternatives for the treatment of acute pain. We did not observe adverse effects in any mice, suggesting that this plant could be used safely with humans.

Acknowledgements. This work was supported by the Consejo Nacional de Ciencia y Tecnología (CONACYT NC12.3280.0)

References.

1. Giovannini P, Heinrich M. (2009). *J Ethnopharmacol* 121 (3): 383–399.
2. Déciga-Campos M, Palacios-Espinoza FJ, Reyes-Ramírez A, Mata R. (2007). J Ethnopharmacol 114:161-168.
3. Verano J, González-Trujano ME, Déciga-Campos M, Ventura-Martínez R, Pellicer F. (2013). *Pharmacol Biochem Behav* 110:255-64.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología 2nd Biotechnology World Symposium
16-20 Octubre October/2018
San José del Cabo, B.C.S., México

MF9

ASOCIACIÓN DEL POLIMORFISMO rs6214 DEL GEN *IGF1* Y EL CONSUMO DE LECHE EN EL IMC EN POBLACIÓN MEXICANA

Grijalva Avila Julio[1§], Villanueva Fierro Ignacio[1£], Rivera Sánchez Gildardo[2], Loera Castañeda Verónica[1], Chairez Hernández Isaías[1], Lares Asef Antonio[1], Martínez Estrada Sandra[1], Martínez Rivera Israel[1].
[1]Centro interdisciplinario de investigación para el desarrollo integral regional unidad Durango (CIIDIR-IPN). [2]Centro de Biotecnologia Genomica (CBG-IPN). [§]jcgrijalva69@gmail.com, [£]ifierro62@yahoo.com

Polimorfismo, obesidad, IGF-1

Introducción. La obesidad es una enfermedad crónica degenerativa que afecta al 75% de la población de México[1]. México ocupa el 4° lugar a nivel mundial en consumo de leche, la cual ha sido relacionada con una reducción del IMC, debido a sus nutrientes y proteínas[2], como la IGF-1 la cual se ve afectada en sus niveles por la presencia del polimorfismo rs6214[3]. El objetivo fue determinar la asociación entre la presencia del SNP rs6214 y el consumo de leche con el IMC en personas del estado de Durango.

Materiales. Sonda taqman Genotyping Assay 40X (C__11495137_10, Thermo Fisher Scientific).

Métodos. Se efectuó un estudio de asociación, transversal, en personas de la ciudad de Durango, de 20 a 59 años de edad, que consumieran leche y que tuvieran obesidad. Se tomaron medidas antropométricas mediante equipos de composición corporal. Los ensayos de genotipificación se realizaron por PCR-RT, mediante la metodología TaqMan® Pre-Designed SNP Genotyping Assays. Se usó el test de chi^2, odds ratio, utilizando los modelos de herencia codominante, dominante y recesivo.

Resultados. Participaron 99 pacientes previo consentimiento informado, de los cuales el 28% consumía leche y el 6% no consumía, ambos grupos en normal peso, asociando de manera significativa el consumo de leche con él IMC. En la tabla 1 se puede observar que el consumo de leche es un factor protector para la presencia de obesidad. El OR para el genotipo respecto a las medidas antropométricas mostró que es un factor protector para la presencia de IMC elevado y masa grasa. Mientras que el OR del polimorfismo respecto a masa magra, muestra que la presencia del alelo mutado nos confiere un factor de riesgo de presentar niveles de masa magra elevados.

Tabla 1. Análisis de riesgo del consumo de leche con respecto a la obesidad.

Consumo de leche	Obesidad		OR	Lc 95%
	Si	No	0.32	0.8-0.11
Si	29	33		
No	27	10		

Tabla 2. Análisis de riesgo de un genotipo en función del modelo de herencia con respecto a la masa magra

Modelo	Genotipo	↓Me	↑Me	OR	Lc 95%
CO	CC	30	18	1	
	CT	13	25	3.20	1.3-7.65
	TT	6	3	0.83	0.16-4.06
	Alelo c	73	61	1	
	Alelo T	25	31	1.48	0.79-2.76
DO	CC	30	18	1	
	CT-TT	19	28	2.45	1.08-5.53
RE	CC-CT	43	43	1	
	TT	6	3	0.5	0.11-2.11

Tabla 3. Análisis de riesgo de un genotipo en función del modelo de herencia con respecto al IMC

Modelo	Genotipo	↓Me	↑Me	OR	Lc 95%
CO	CC	24	25	1	
	CT	16	22	1.32	0.55-3.12
	TT	10	2	0.19	0.05-0.68
	Alelo C	64	72	1	
	Alelo T	36	26	0.64	0.34-1.18
DO	CC	24	25	1	
	CT-TT	26	24	0.88	0.37-2.08
RE	CC-CT	40	47	1	
	TT	10	2	0.17	0.04-0.71

Conclusiones. Se observó que existe asociación entre el consumo de leche y obesidad, por lo cual el consumo de leche proporciona un factor protector para la aparición de obesidad. La presencia del alelo mutado se asocia con un mayor índice de masa magra y un menor IMC, lo que Aunado al consumo de leche proporcionan factores que ayudan a prevenir y reducir la obesidad.

Agradecimientos: este trabajo se realizó gracias al apoyo del CONACYT y del CIIDIR-IPN.

Bibliografía

1. Kokkoris P, Pi-Sunyer FX. Obesity and endocrine disease, (2003). *Endocrinol Metab Clin North Am*.32(4):895-914.
2. Weaver J. Classical endocrine diseases causing obesity, (2008). *Obesity and Metabolism*: Karger Publishers.138:1294-301.
3. Velloso CP. Regulation of muscle mass by growth hormone and IGF-I, (2008). *British journal of pharmacology*. 154(3):557-68.

ISSN: 2448-8461

MF10

Genome mining in action – natural product discovery and expression from endophyte *Actinomycetes*

Corina Diana Ceapă, Karol Rodríguez Peña, Melissa Vázquez Hernández, Omar Jiménez Rodríguez, Maria Paula Gomez Román, Stefany Daniela Rodríguez Luna, Carlos Naranjo Castañeda, Sebastián de la Rosa Hernández Garibay, Dulce Ramirez, Sergio Sanchez; Departamento de Biología Molecular y Biotecnología, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México (UNAM), CDMX, 04510, México; corinaceapa@iibiomedicas.unam.mx

bacteriocins, antioxidants, chitinases, heterologous expression

Introduction.
The increased worldwide incidence of antibiotic resistance underscores the continuing need for new bioactive molecules discovery [1]. The phylum Actinobacteria exhibits a remarkable potential to produce secondary metabolites (SM) which encompass antibiotics, anti-tumoral, anti-viral, anti-parasitic, anti-fungal agents, insecticides, siderophores, pigments, immuno-modulators, herbicides and many others [2]. Mining unusual sources such as plant endophytes [3], implementation of new genomic technologies and the ability to analyse large sets of data facilitate the discovery of novel compounds and also understanding their mechanisms of action [4]. Symbiotic interactions could control the expression of biosynthetic gene clusters and have played a major role in the evolution of the high chemical diversity of actinomycete-produced secondary metabolites [5]. Mining of four Actinobacteria endophytes isolated from cuachalalate (*Amphyterygyum astringens*) led to the identification, production and/or characterization of several anti-tumorals, two bacteriocins, an antioxidant and various chitinases.

Methods.
Genome mining was performed after sequencing, assembly and annotation of the genomes, as a combination of AntiSMASH, PRISM, Bagel 3 and 4, and manual searches results using local databases. Expression of the SM clusters was accomplished in *E. coli* Rosetta or various *Streptomyces* hosts. Chemical characterization was carried out by spectrometry, TLC, UPLC-MS and NMR and biological assays specific to the activity were developed for each compound. Production of differential metabolites in different cultivation conditions were analyzed by UPLC-MS metabolomics.

Results.
Interpretation of genomic data to identify secondary metabolites became an almost automatic process with the development of pattern recognition algorithms combined with databases of already known clusters. Genome comparisons revealed a small degree of overlap between SM clusters present in the studied genomes. Our searches in the four genomes led to the identification of a total of more than 200 clusters for secondary metabolites including more than 80 non-ribosomal peptides and bactericins, out of which half can be considered novel. Several of the novel clusters were selected for further analysis. Anti-tumoral agents from the steffimycin family but with novel structures and properties were purified directly from *S. scabrisporus* NF3 supernatant. Their chemical characterization and tumour cell viability/ cytotoxicity were determined. Bacteriocins from *S. scabrisporus* NF3 and *S. champavatii* L06, encompassing one lasso peptide and one peptidoglycan hydrolase, respectively, were expressed in *E. coli* Rosetta and his-tag purified. Their antimicrobial activities were assayed against a panel of human and plant pathogens. The antioxidant produced by a type III polyketide synthase coded in the genome of *Actinoplanes* sp. TFC3 was expressed in *S. coelicolor* M1152 and its activity was proven by DPPH assay.
Chitinases were secreted by *S. scabrisporus* NF3 only in the presence of their plant symbiont. They show various lytic capacities in chitin degrading experiments and their anti-fungal properties are also highly compound and strain dependent.

Conclusions. The project revealed and described a diversity of molecules with medical, industrial and biocatalytic activities. The strategy can therefore be applied exhaustively towards characterization of all novel metabolites from these and other *Streptomyces* genomes. In addition, the diversity of novel operons and very little overlap confirm a role for SMs in symbiotic relationships and that endophytes from the same plant take on different roles in support of their host.

Acknowledgements.
This research was supported by a DGAPA Postdoctoral Fellowship, UNAM to C.D.C. and by PAPIIT, DGAPA, UNAM IN202216 and the NUATEI program from the Instituto de Investigaciones Biomédicas, UNAM.

References.
1. Chiang C-Y, Uzoma I, Moore RT, Gilbert M, Duplantier AJ, et al. (2018) *MBio* 9.
2. Brader G, Compant S, Mitter B, Trognitz F, Sessitsch A . (2014) *Curr Opin Biotechnol* 27: 30–37.
3. Lewis K (2017) *Biochem Pharmacol* 134: 87–98.
4. Tracanna V, de Jong A, Medema MH, Kuipers OP (2017) *FEMS Microbiol Rev* 41: 417–429.1.
5. van der Meij A, Worsley SF, Hutchings MI, van Wezel GP (2017) *FEMS Microbiol Rev* 41: 392–416.

ISSN: 2448-8461

MF11

ANTIPROLIFERATIVE ACTIVITY AND POTENTIAL PROTECTIVE EFFECT ON HUMAN ERYTHROCYTES OBTAINED FROM THE MICROALGA *Navicula incerta* EXTRACTS

González-Vega, R. I.[1]; López-Elías, J. A. [1]; Robles-Zepeda, R. E. [1]; Ruiz-Cruz, S.[2]; Perez-Perez, L. M. [1]; Cinco-Moroyoqui, F. J. [1]; Wong-Corral F.J. [1], Borboa-Flores J. [1], Rodríguez-Figueroa J.C. [1], Del-Toro-Sánchez, C. L.[1]
[1]Universidad de Sonora, Blvd Luis Encinas y Rosales SN, Centro, 83000 Hermosillo, Son., México
[2]Instituto Tecnológico de Sonora, 5 de Febrero 818 Sur, Col. Centro, Ciudad Obregón, Sonora, México.
qbc.ricardo.vega@gmail.com
Ricardo Iván González Vega

Key words: Navicula incerta, antiproliferative activity, protective effect

Introduction. *Navicula incerta* is a benthic microalga that produces secondary metabolites, which have shown positive effects on health. Numerous studies have shown that microalgae possess a wide activity against chronic-degenerative pathologies, including antihaemolytic and antiproliferative properties. Therefore, the aim of this study was to evaluate the antiproliferative activity and the protective effect on human erythrocytes of extracts obtained from the microalga *Navicula incerta*.

Methods. The studies were carried out with treatment optimized previously from the microalga *Navicula incerta*. The cell viability was evaluated by means of the MTT assay ([3- (4,5-dimethyl thiazol-2-yl) -2,5-diphenyltetrazolium]). The cell lines used were human cancer cells (A549, HeLa, LS-180 and PC-3) and a healthy human cell (ARPE-19) (2). The protective effect was carried out by the AAPH radical scavenging activity (the erythrocyte hemolysis assay) (3).

Results. The extracts from *N. incerta* inhibited the proliferation of cervical cancer (HeLa) and prostate carcinoma (PC-3) cells grown in culture (Fig. 1 and Table 1). The erythrocytes haemolysis can be inhibited by scavenging AAPH radical. The inhibition of haemolysis was concentration-dependent, with a percentage of inhibition of 98 % (Fig. 2).

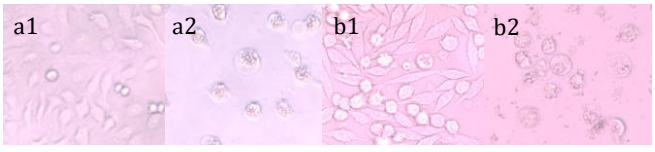


Fig.1 Effect of the different extracts obtained from *N. incerta* on HeLa and PC-3 cell lines before and after 48 h. at a concentration of 200 µg·mL-1. (a1) HeLa cell line at zero hour. (a2) HeLa cell line at 48 h. (b1) PC-3 cell line at zero hour. (b2) PC-3 cell line at 48 h.

Table 1. Antiproliferative activity of extracts obtained from *N. incerta* on HeLa and PC-3 cell lines.

Extracts	% Cell viability		IC_{50} µg·mL^{-1} ± SD	
	HeLa	PC-3	HeLa	PC-3
Acetonic	27.60^{b}± 3.91	44.43^{b} ± 2.86	75.91^{b} ± 2.47	122.58^{b} ± 2.86
Methanolic	5.87^{a} ± 0.45	26.40^{a} ± 2.89	61.93^{a} ± 3.10	107.01^{a} ± 2.49
Ethanolic	5.54^{a} ± 0.14	27.74^{a} ± 0.48	59.28^{a} ± 2.58	96.05^{a} ± 3.48

The data are shown as the mean ± SD (standard deviation) of at least three repetitions (n ≥ 3). Different letters per column represent significant differences ($p<0.05$).

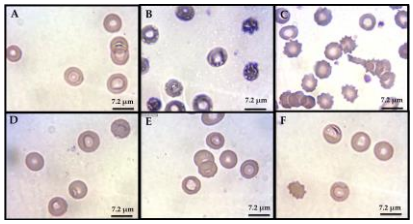


Fig. 2 Protective effect on human erythrocytes from extracts of *Navicula incerta*. (A) Control (-): erythrocytes without radical AAPH. (B) Control (+): erythrocytes with radical (haemolysis). (C) Reference control: Hemolytic anemia. (D) Acetone extract. (E) Methanolic extract. (F) Ethanolic extract.

Conclusions. The results indicate that the extracts could be useful as chemopreventive agents against cancer. In addition, the results showed that *Navicula incerta* extract could act as an effective antioxidant against oxidative stress, giving a protective effect on oxidative damage.

Acknowledgements. This study was supported by "Consejo Estatal de Ciencia y Tecnología (CONACYT)".

References.

1. Richmond, A. 2004. Handbook of microalgal culture. Biotechnology and Applied *Phycology Blackwell Science*. 147-239.
2. Adorisio, S., Fierabracci, A., Gigluiareli, G., Muscari I., Cannarile L., Liberati, A., Marcotulio, C., Ricardi, Carlo, Curini, M., Robles, R. and Delfino V. (2017). The hexane fraction of Bursera microphylla A grays induces p2l-Mediated antiproliferative and proapoptotic Effects in human cáncer-derived cell lines.
3. Cheung, P. C. K., Cheung, L. M., & Ooi, V. E. C. (2003). Antioxidant activity and total phenolics of edible mushroom extracts. *Food Chemistry*, *81*, 249–255.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México

MF12

TiO_2-ZnPc NANOPARTICLES SYNTHESIS FOR PHOTODYNAMIC THERAPY APPLICATIONS

Emma Ortiz[1], Alfredo Martinez[2,3] and Minerva Uribe[2,3]
[1]Nanotechnology Laboratory, National Institute of Neurology and Neurosurgery, México City 14269; emma170@hotmail.com.
[2]Materials Science and Engineering Program, University of California, Riverside, California 92521.
[3]College of Engineering Center for Environmental Research and Technology, University of California, Riverside, California 92507.

Key words: cancer, titania nanoparticles, photosensitizer

Introduction. The current standard therapy for treating glioblastoma (a malignant brain tumor), is a combination of radiation plus chemotherapy with temozolomide following surgery. The most significant pathological characteristic of glioblastoma is its infiltrative nature coexisting with normal brain tissue. The tumor subsequently relapses, expands, and results in the death of the patient. Therefore, new approaches to glioma cancer are necessary. One promising option is photodynamic therapy (PDT), which uses the toxicity of singlet oxygen generated by a reaction between a photosensitizer and light, at a specific wavelength to excite the photosensitizer [1]. It has been shown that photochemical reactions provide the opportunity to utilize the toxicity of singlet oxygen to damage and kill the tumor tissue. In particular, zinc phthalocyanines (ZnPc) have shown to be very promising photosensitizers due to their intense absorption in the red region of the visible spectrum. Phthalocyanines zinc (II) are photosensitizers of the second generation clinically approved, with high selectivity towards tumor cells and a greater reactivity to generate $-O_2$ [2]. TiO_2 is susceptible to photoactivation under ultraviolet light (400 nm). However, the window of irradiation approved begins at wavelengths greater than 600 nm since ultraviolet light could cause severe adverse effects to the patient.
The aim of this work is to utilize the wide range absorption and photosensitivity properties of ZnPc and the metal properties of TiO_2 to achieve a double functionality of the TiO_2-ZnPc material system.

Methods. TiO_2 and TiO_2-ZnPc nanomaterials were prepared using the modified method reported by Lopez et al. using *Zinc(II)tetranitro-phthalocyanine* as photosensitizer [3]. ZnPc was added during the titania preparation. The different obtained materials were characterized by different physicochemical techniques.

Results. The powder XRD patterns of the TiO_2-ZnPc and ZnPc samples are shown in Figure 1. The diffraction peaks of the ZnPc were broad and weak, indicating a poor crystallinity of ZnPc. The characteristic peaks of ZnPc and the anatase phase were observed in TiO_2-ZnPc sample. Furthermore, the FTIR spectra of TiO_2 and TiO_2-ZnPc showed the peaks at 1487, 1143, 1087 cm^{-1} are assignable to the skeleton stretching of ZnPc.

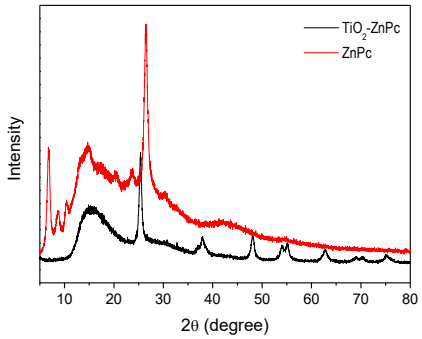


Fig.1 XRD spectra of TiO2-ZnPc and ZnPc. Characteristic Peaks of ZnPc were identified in the TiO2-ZnPc sample.

From TiO_2- ZnPc spectrum, the peaks appeared at 1522 cm^{-1} and 1335 cm^{-1} correspond to N= O stretching vibrations. The C–H stretching of benzene ring at 1082 cm^{-1}, and the Zn–ligand stretching around 900 cm^{-1} were masked by the intense Ti–O–Ti peak of TiO_2. These peaks do not appear in the spectrum corresponding to TiO_2.

Conclusions. ZnPc was identified into TiO_2 sample by XRD and FTIR spectroscopies. This work opens the possiblity of using the TiO_2-ZnPc material system as a candidate for PDT applications.

Acknowledgments. 2017 UC MEXUS-CONACYT COLLABORATIVE GRANTS (number project: CN-17-215).

References.

1. Shafirstein G, Bellnier D, Oakley E, Hamilton S, Potasek M, Beeson K, Parilov E. (2017). *Cancer*. 9(12):1-14.
2. Ogunsipe A, Nyokong T. (2004). *J. Mol. Struct*. 689: 89-97.
3. Lopez T, Ortiz E, Navarrete J, Odriozola J, Martinez-Ortega F, Páez-Mozo E, Escobar P, Espinoza K, Rivero I. (2010). *Nanomedicine*. 6: 777-785.

ISSN: 2448-8461

MF13

SCREENING OF ANTIPROTOZOAL ACTIVITY OF EXTRACTS OF FUNGI ISOLATED FROM HISTORICAL MONUMENTS

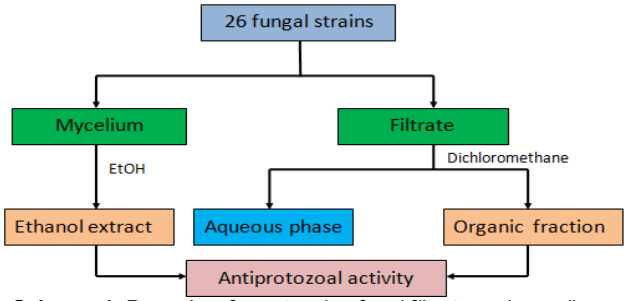
Manuel Jesús Chan-Bacab[1],Manuela Reyes-Estebanez[1], Ambre Dumontet[2], Benjamín Otto Ortega-Morales[1]. 1. Universidad Autónoma de Campeche, Departamento de Microbiología Ambiental y Biotecnología (DEMAB), Campeche, 24039; 2. Institut Universitaire de Technologie de la Rochelle, Francia. manjchan@uacam.mx

Key words: fungi, leishmanicidal, trypanocida, bioprospection, lithic environment

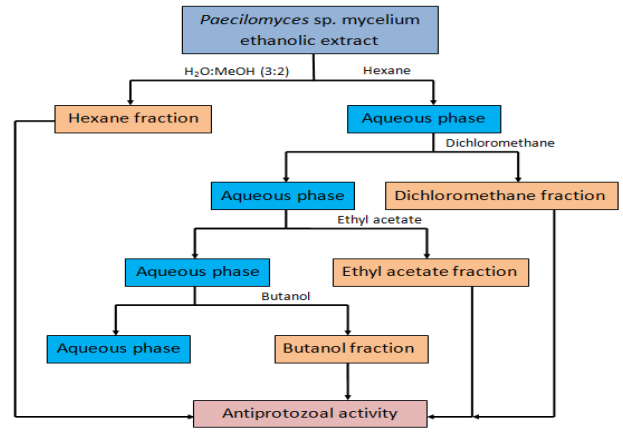
Introduction. Diseases caused by pathogenic trypanosomatids cause great suffering throughout the developing world. New drugs for these diseases are urgently needed (1). Microorganisms have contributed to the discovery of antimicrobial agents, being fungi the most promising group for the detection of novel bioactive compounds. One of the main strategies to achieve the identification of new molecules is focused on the bioprospecting of new microbial habitats (2). As far as we know, the biological activity of microorganisms isolated from lithic environments, such as the Mayan monuments has not been evaluated. These places can be considered moderate extreme environments, which can harbor a novel microbiota with diverse secondary metabolisms (3).

The objective of the present work was to evaluate the *in vitro* leishmanicidal and trypanocidal activity of extracts of fungi isolated from historical monuments.

Methods.



Scheme 1. Procedure for extracting fungi filtrate and mycelium.



Scheme 2. Partition with solvents of ascending polarity of *Paecilomyces* mycelium.

Results.

Table 1. *In vitro* antiprotozoal activity of extracts and fractions of *Paecilomyces* sp.

Extract or Fraction	*L. mexicana*		*T. cruzi*	
	100 µg/ml	50 µg/ml	100 µg/ml	50 µg/ml
Ethanol	+++	+++	+++	+++
Organic	+++	+++	++	++
Hexane	+++	++	+++	++
Dichloromethane	+++	+++	++	++
Ethyl acetate	---	---	---	---
Butanol	---	---	---	---
DMSO al 0.5%	---	---	---	---

Table 2. *In vitro* antiprotozoal and cytotoxic activity of extracts and fractions of *Paecilomyces* sp.

Extract or Fraction	IC_{50} µg/ml		
	L. mexicana	*T. cruzi*	*A. salina*
Ethanol	25.06 ± 0.49	1.83 ± 0.61	377.03 ± 13.82
Hexane	5.65 ± 0.26	0.49 ± 0.05	625.08 ± 39.51
Dichloromethane	9.09 ± 0.52	4.51 ± 0.49	266.08 ± 7.43
Miltefosine	0.83 ± 0.15	---	----
Benznidazole	---	1.81 ± 0.61	---
$CuSO_4$	---	---	15.35 ± 0.43
DMSO	>100	>100	>1000

Conclusions. Of the fungal strains evaluated *in vitro* and identified by molecular biology and classical taxonomy, *Paecilomyces* sp. inhibited the growth of both parasites. The fraction of hexane showed greater activity against both parasites. In conclusion, the extract and the fractions of *Paecilomyces* sp. have molecules with antiprotozoal activity that allow us to direct our research to the isolation and characterization of them.

Acknowledgements. This research was supported by Universidad Autónoma de Campeche.

References.

1.Barrent, M.P., Gilbert, I.H. 1999. Curr Top Med Chem. 2 (5): 471-482.
2. Bull, A.T., Ward, A.C., Goodfellow , M. 2000. Microbiol Mol Biol Rev. 64 (3): 573-606.
3. Scheerer, S., Ortega-Morales, B.O., Gaylarde, C. 2009. Adv Appl Microbiol. 66: 97-139.

ISSN: 2448-8461

ASSOCIATION OF THE POLYMORPHISMS RS12995525 AND RS2372536 OF THE ATIC GENE WITH THE RESPONSE TO METOTREXATE TREATMENT IN PATIENTS WITH RHEUMATOID ARTRITIS OF THE SINALOA STATE

Jocelyn Analy Gónzalez-Rosales[1], Karla Morales-Hernández[2], Fernando Bergez-Hernández[1], Martín Irigoyen-Arredondo[1], Alejandra Martinez-Camberos[1], Geovanni Romero-Quintana[1], Noemi García-Magallanes[2], Carolina Bojórquez-Sánchez[2], Lucila Galarza-Robles[3], Jonathan Romo-Martinez[2].
Joceglezr93@gmail.com
1. Facultad de Ciencias Químico Biológicas, Universidad Autónoma de Sinaloa, Culiacán, Sinaloa. C.P. 80013
2. Ingeniería en Biotecnología, Universidad Politécnica de Sinaloa. Mazatlán, Sinaloa. C.P.82199
3. IMSS Hospital General de Zona 3, Mazatlán, Sinaloa. C.P. 82140

Key words: Rheumatoid arthritis, rs12995526, rs2372536

Introduction. Rheumatoid arthritis (RA) is an inflammatory autoimmune disease of unknown origin (1). Methotrexate, have been shown to be effective in the treatment of the disease, however, a significant percentage of patients have an inadequate response. Unfortunately, these interindividual differences can´t be anticipated in patients, therefore markers, especially genetic polymorphisms, are necessary to individualize treatment. Pharmacogenetics focuses on the study of polymorphisms in the genes that code for proteins that transport drugs (2). One of these genes is ATIC which is located on chromosome 2 and codes for an enzyme that catalyzes the last two stages of the Novo purine biosynthesis pathway (3).
The polymorphisms rs12995526 and rs2372536 of the ATIC gene was genotyped and associated with the lack of response to treatment with MTX in patients with RA.

Methods. First, blood samples were obtained from the patients by venous puncture, in a tube with EDTA anticoagulant, after DNA were extracted through the Gustinchi method and an analysis was made by spectrophotometry of DNA, where its concentration and purity were quantified at 260 nm, 280 nm and 320nm, and its integrity was verified in 1.5% agarase gels. Genotyping was performed by Step One Plus using Applied Biosystem Taqman Probes and fluorochromes VIC and FAM. Finally, the allelic and genotypic frequencies were calculated.

Results. A total of 75 samples were collected. Of all the patients with RA, 64% did not respond to MTX and 36% of the patients did. The genotypic frequencies of the polymorphisms rs12995526 and rs2372536 were obtained. The results of the first polymorphism were that the heterozygote(C/T) frequency is higher than the frequency of homozygous (C/C) normal and homozygous mutated (C/T). For rs2372536, 37 were obtained for normal homozygote (C / C), 29 for heterozygote (C / G) and 9 for homozygous mutated (G / G). The results of the genotypic frequencies related to their response to MTX can be seen in figure 1 and 2. From the previous results, we proceeded to the Hardy-Weinberg equilibrium analysis, which indicated that our population is in equilibrium for rs12995526 (p=0.73) and for 2372536 (p= 0.31). When performing the statistical analysis, the results obtained were statistically insignificant.

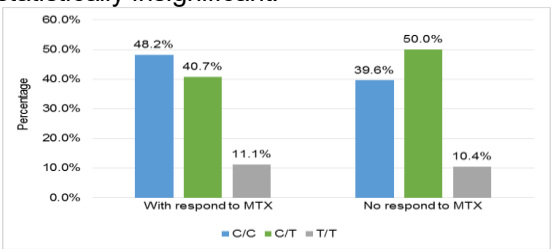


Fig. 1 Genotypic frequencies of the rs12995526 polymorphism in patients with RA

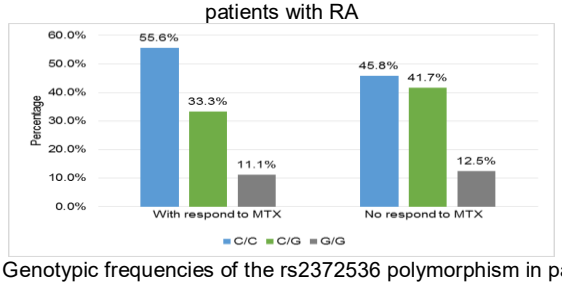


Fig. 2 Genotypic frequencies of the rs2372536 polymorphism in patients with RA

Conclusions. The project did not reveal an association of the polymorphisms rs12995526 and rs2372536 with methotrexate treatment in the Sinaloa population, however the low response to the drug indicates a pharmacogenetic relationship with it, which should continue to be evaluated.

Acknowledgements. To the Innovative Application of Knowledge (PRODEP/SES-SEP) for providing the economic financing.

References. 1. Cardiel M, Diaz A, Vazquez M, Gámez J, Barile L, Pacheco C y col. 2014. *Reumatol Clín*; 10(4): 227-240.
2. Kooloos W. M, Huizinga T. W, Guchelaar H. J, Wessels J. A. Current Pharmaceutical Design. 2010. 16(2):164-75.
3. Mena JP, Salazar M y Dávalos IP.2008. Gaceta médica de México 144(5): 446-451.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México

MF15

IN VITRO CULTURE OF *IBERVILLEA SONORAE* FOR PHENOLS AND FLAVONOIDS COMPOUNDS PRODUCTION

Alonso Gómez-Guzmán[1], María del Carmen Oliver-Salvador[2], María Guadalupe Ramírez-Sotelo[2].
[1]Instituto Politécnico Nacional, Unidad Profesional Interdisciplinaria de Biotecnología, Laboratorio de Biotecnología Molecular.
Av. Acueducto s/n Barrio La Laguna Ticomán, Ciudad de México, Del. Gustavo A. Madero. C.P. 07340.
Tel. 5296000 Ext. 56468, 56471. Agomez_guzman@hotmail.com; mgramirez@ipn.mx, oliveripn@hotmail.com

Key words: Cell culture, Flavonoids and Phenols.

Introduction.

Ibervillea sonorae (Greene) is a plant of the Curcubitaceas family, native to northern Mexico. Traditionally the root is used for the treatment of Diabetes Mellitus (DM), external dermatological damages and rheumatism(1)(2). In our work group, it has been shown that *I. sonorae* has hypoglycaemic (4) and anti-inflammatory activity (6). Due to the possible over-exploitation of the species, it is considered in danger of extinction, so the aim of the work is to use the *in vitro* culture of *I. sonorae* as an alternative to produce compounds of pharmacological interest like phenols and flavonoids compounds.

Methods.

Induction of callus and suspension culture was performed with Gamborg medium(3) supplemented with growth regulators IAA, BAP and NAA at 25 ° C, photoperiod 16/8 h light and dark; the suspension culture in flask was kept under agitation at 160 rpm. The quantification of Phenols and flavonoids was carried out by the methodologies described by García-Nava(5) and Liu (7).

Results.

The cells suspension cultures were established in B5 medium. The highest production of phenols and flavonoids compounds were obtained at 10 days of culture (figure 1).

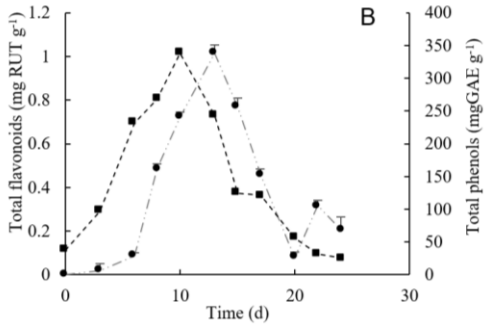


Figure 1. Production of phenos and flavonoids compounds in cell suspension culture of *I. sonorae* from stems explants.

Conclusions.

Friable cells of *I. sonorae* were obtained after 15 days in B5 medium.

The production of flavonoids and phenols in culture in suspension was 57.0.35 ± 0.35 mg GAE g^{-1} and flavonoids 1.70 ± 0.02 mg RUT g^{-1} at 10 days.

Acknowledgements.

Instituto Politécnico Nacional.[1]Becario Programa de Becas de Estímulo Institucional de Formación de Investigadores (BEIFI). [2]Proyecto SIP 20180151 y 20181435 y Becario CONACYT.

References.

1. Alarcón-Aguilar, F.J., Campos-Sepúlveda, A. E., Xolalpa-Molina, S., Hernández-Galicia, E., Ramón-Ramos, R. (2002). Hipoglyacemic activity of *Ibervillea sonorae* roots in healthy and diabetic mice and rats. Pharmaceutical Biology 40:570-575.
2. Banderas-Dorantes, T.R., Román-Ramos, R., Zamilpa, A., García-Macedo, R., Díaz, M., Campos, M.G., Tortoriello, J., Alarcón-Agilar, F.J. (2012). Influence of two hypoglucemic Curcubitaceae (Curcubitaficifolia Bouché and *Ibervillea sonorae* Greene) on ATP-sensitive potassium channels in rat aortic rings. Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas. 11(6):510-519.
3. Gamborg, O. L., Miller, R., & Ojima, K. (1968). Nutrient requirements of suspension cultures of soybean root cells. Experimental Cell Research, 50(1), 151-158.
4. García-Aranda, M. I. (2013). Identificación química y farmacológica de compuestos activos de *Ibervillea sonorae* como hiploglucemiante. Tesis, UPIBI-IPN, México. CDMX.
5. García-Nava, M. J. (2007). Cuantificación de fenoles y flavonoides totales en extractos naturales. Primer Verano de Introducción a la Investigación de la Universidad Autónoma de Querétaro.
6. Nevárez-Ramírez, A. (2014). Evaluación de la actividad anti-tumoral y anti-inflamatoria de *Ibervillea sonorae*. Tesis, UPIBI-IPN. 1-114.
7. Liu, M., Li, X. Q., Weber, C., Lee, C. Y., Brown, J., & Liu, R. H. (2002). Antioxidant and antiproliferative activities of raspberries. Journal of Agricultural and Food Chemistry, 50(10), 2926-2930.

ISSN: 2448-8461

MF17

COMPOUNDS WITH BIOLOGICAL ACTIVITY OF MARINE SEAWEEDS OF THE GENUS *Codium* (CHLOROPHYTA) OF B.C.S., MEXICO

Alejandro Marín Álvarez[1], Jesús Iván Murillo Álvarez[1], Mauricio Muñoz Ochoa[1], Juan Santiago Salas Benito[2].
[1] Instituto Politécnico Nacional-CICIMAR, Departamento de Desarrollo de Tecnologías, La Paz, BCS, CP 23096
[2] Instituto Politécnico Nacional -ENMH. Zacatenco, Cd. de México, CP 07320. E-mail: amarinal@ipn.mx

Key words: Antidengue, antituberculosis, anticancer.

Introduction. The *Codium* genus, with worldwide distribution, contains secondary metabolites with biological activity (1), such as clerosterol, alpha-tocopherol, prenylated quinones, codiosides, furaldehyde, furfuril alcohol, geraniol, syphonoxanthin, syphonein and terpinolene (2).
Antibacterial, cytotoxic and antiviral activity was assessed of natural product and extracts of *Codium amplivesiculatum*, *C. simulans*, *C. cuneatum* and *C. fragile* collected in the coast of Baja California Sur, Mexico.

Methods. Fractionation was performed ethanolic extracts of algae collected and dried in three locations in Baja California Sur, México, through chromatographic columns of silica gel. IR-ATR spectra signals were obtained from compounds. Were subjected to an antimicrobial activity test. The antituberculosis activity was determined against *Mycobacterium tuberculosis*, through the microtechnique of "Alamar Azul" (3). Prior to the antiviral assay, the cytotoxicity of the compounds isolated from C. amplivesiculatum: CC13 / F2 and CC20 / F5 was evaluated to determine the cell viability at the best dose (above or equal to 80%), using the Cytotox 96 kit Promega™. The antiviral evaluation was carried out using the pure extracts of C. amplivesiculatum, using the Platelia™ Dengue NS1 AG BioRad™ kit. The cytotoxic activity of compounds isolated from *C. amplivesiculatum*, was evaluated against tumor cell lines of prostate cancer (PC-3), colon cancer (HCT-15) and breast cancer (MCF-7), by bioassay of inhibition of cell growth with sulforhodamine B (SRB) (4).

Results. The identified compounds of the ethanolic extracts of the *Codium* algae analyzed were: 1-Octadecanol, clerosterol and diacilglucoglyceride. The determination of the minimum inhibitory concentration (MIC) of the active fractions by the broth dilution method showed that the F3 fraction of *C. amplivesiculatum* and the F11 of *C. simulans* were able to inhibit the development of *S. aureus* and *V. parahaemolyticus*, respectively at a concentration of 125 μg.mL^{-1}. The rest of the fractions showed MIC values> 250 μg.mL^{-1}. Fraction 2 had a significant effect with MIC = 100 μg.mL $^{-1}$, against *Mycobacterium tuberculosis*.

Table 1. Cytotoxicity * of clerosterol and diacylglycoglyceride, in percentage of growth inhibition on human cancer cell lines. Tested at 50 μg.mL-1.

Compound	PC-3	HCT-15	MCF-7
Diacilglucoglyceride	11.8 ±5.0	34.6	36.3 ±0.81
Clerosterol 2(*a*)	21.0 ±0.3	11.9 ±3.2	9.5 ±1.1
Clerosterol 2(a´)	0.0	7.1 ±2.0	4.3 ±4.0

* Human cancer cells PC-3: prostate; HCT-15: Colon; MCF-7: Mom.

Table 2. Antiviral activity of clerosterol and diacylglycoglyceride against dengue virus DENV2 expressed as percentage of infection.

Compound	Concentration ng.μL^{-1}		
	Control	100	150
clerosterol	99.8 ±0.47	69.8 ±0.45	59.9 ±0.33
diacilglucoglyceride	99.8 ±0.41	62.2 ±0.70	26.2 ±0.81

Conclusions. Organic extracts of *C. amplivesiculatum, C. simulans, C. cuneatum and C. fragile*, contain antibiotic substances against *Staphycoloccus aureus* (ATCC-BAA-42), *Streptococcus pyogenes* (ATCC-BAA-946), *Vibrio parahaemolyticus* (ATCC- 17802), *V. alginolyticus* (ATCC-17749) and *V. harveyi* (ATCC-14126). The fraction (CC2F2) of *C. amplivesiculatum,* inhibited *M. tuberculosis* at MIC of 100 μg.mL-1. From here, 1-octadecanol was isolated. The clerosterol) and glucoglyceride, had weak effect against human carcinoma cell lines. Glucoglyceride generated a 73.8% inhibition of infection against dengue virus (DENV2) with 150 ng.μL^{-1}, considered a good index for inhibiting infection.

Acknowledgements. Proyects:.NODE: CICIMAR, Biotechnology Network-IPN. SIP-20131081. COFFA and EDI. To SEMS-COSDAC-DGETI for the scholarship-commission granted

References.

1. Fusetani, N. 2010. Biotechnological potential of marine natural products. *Pure Appl. Chem*., 82(1):17-26.
2. Zhou J., G. Xie & X. Yan. *Encyclopedia of Traditional Chinese Medicine*., Springer-Verlag. Heidelberg Berlin. 2011.
3. Molina-Salinas, G. M., M. C. Ramos-Guerra, J. Vargas-Villarreal, B. D. Mata-Cárdenas, P. Becerril-Montes & S. Said-Fernández. 2006. Bactericidal activity of organic extracts from *Floreunsia cernua* DC against strains of *Mycobacterium tuberculosis. Arch. Med. Res.* 37(1):45-49.
4. Skehan, P., R. Storeng, D. Scudeiro, A. Monks, J. McMahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney, M.R. Boyd.1990. New colorimetric assay cytotoxicity for anticancer-drug screening. *J. Natl. Cancer Inst.*, 82:1107.

MF18

IDENTIFICATION OF CD4⁺ T LYMPHOCYTES RESTRICTED EPITOPES OF THE RECOMBINANT ELONGATION FACTOR-1α FROM *LEISHMANIA MEXICANA*: ADVANCES

Kenia López-López, Vianney Francisco Ortiz-Navarrete, Rosalío Ramos-Payán, Claudia del Rosario León-Sicairos, Evangelina Beltrán-López y Héctor Samuel López-Moreno; Red Temática y CAEC BB UAS-264, Posgrado en Biotecnología, Facultad de Ciencias Químico Biológicas, Culiacán, Sinaloa, CP. 80010, e-mail: kenia_89@hotmail.com

Key words: Epitope, Elongation Factor-1 alpha, Leishmania mexicana

Introduction. Leishmaniasis comprises a group of diseases caused by intracellular protozoan parasites of the genus *Leishmania*. There are three clinical forms, Cutaneous (CL), Mucocutaneous (ML) and Visceral (VL) (1). In Mexico, CL is mainly caused by *L. mexicana*, being endemic in several states including Sinaloa. In 2010, our research group reported five "new" antigens derived from *L. mexicana* reactive to CL confirmed patients serum (2). The most prominent antigen, p29, was identified by mass spectrometry as the Elongation Factor-1alpha (EF-1α) of *L. mexicana* (EF-Lm). Although in this species, its participation in the host-parasite relationship is unknown; in VL caused by *L. donovani*, besides its canonical function in protein synthesis, EF-1α has a non-canonical function as a virulence factor, promoting the SHP-1 phosphatase activation, which down-regulates the macrophages parasitic effector function inhibiting the NO generation, and increasing the survival of the intracellular parasite (3). Additionally, in *L. donovani* EF-1α has been studied as a potential therapeutic target for VL control. In this context, EF-1α emerging as a molecule of great relevance in host-parasite relationship, for this reason our aims were the EF-Lm expression as recombinant protein (rEF-Lm), and the identification of T-helper lymphocytes (Th) epitopes, by analyzing Th cytokine profiles related to protective or permissive mechanisms in leishmaniasis (4).
Therefore, the objective of this work is to identify Th restricted epitopes derived from rEF-Lm.

Methods. The rEF-Lm was produced as recombinant protein fused to H6-tag domain, and purified by metal affinity chromatography. BALB/c mice were immunized with rEF-Lm. Th indirect response was evidenced by specific IgG levels using an ELISA. Ths pecific response was determined by CFSE lymphoproliferation assay, using magnetic column purified CD4⁺ T lymphocytes, and activation marker CD44-PerCP, and macrophages RAW 264.7 as APC. The EF-Lm Th epitopes I-A^d (MHC-II) restricted, were identified by epitope prediction based on 4 pockets as follows: 1 (degenerate), 4 (aliphatic), 6 (A), 9 (A/S), and confirmed *in silico*. BALB/c mice were immunized with synthetic predicted epitope, and Th specific response was also evaluated by CFSE lymphoproliferation assay. Alternatively, this epitope was used as vaccine candidate, in BALB/c mice model that were challenged with 1x10^7 *L. mexicana* promastigotes. At 4 weeks post-infection, the Th response was also evaluated by lymphoproliferation assay, and serum was collected (5).

Results. rEF-Lm was purified and visualized by 12% SDS-PAGE, evidencing a band of ≈63 kDa corresponding to the fusion protein. The specific IgG levels anti-rEF-Lm increased 10 fold compared to negative control and pre-immune mice. Although the prediction showed 2 possible epitopes, only one could be synthesized by chemicals restrictions. Our I-A^d restricted epitope was designed as EFp434: SSGGKVTKAATKAAKK. By *in silico* analysis, we obtained low percentile ranges (≈2%), suggesting a higher binding affinity for I-A^d. Th specific lymphoproliferation was evidenced in both, rEF-Lm and EFp434 immunized mice. In experimental CL, we also observed an enhanced Th lymphoproliferative response, supporting a more detailed analysis as a vaccine candidate, and Th cytokines profiles.

Conclusions. rEF-Lm is a biotechnological tool that evokes both immune responses: humoral and cellular. The predicted EFp434 epitope, also evokes a potent Th specific response, which can be involved in the possible resolution of experimental CL. However, further studies are needed.

Acknowledgements. To CONACYT CB-2014 #240185, for the financing of this project.

References.

1. Ochoa-Díaz Y, López-Moreno C, Rendón-Maldonado J, López-Moreno H. (2011). *Vector Borne Zoonotic Dis*. 12 (1): 78-80.
2. Salazar-Mejía P, Tejada-Aguirre C, López-Moreno H. (2010). Salud Pública Mex. 52 (2): 165-169.
3. Nandan D, Cherkasov A, Sabouti R, Yi T, Reiner N. (2003). Biochem Biophys Res Commun. 302 (4): 646-652.
4. Hezarjaribi HZ, Ghaffarifar F, Dalimi A, Sharifi Z, Jorjani O. (2013). Exp Parasitol. 134 (1) 341–348.
5. López-Moreno HS, Correa D, Laclette JP, Ortiz-Navarrete VF. (2003). Parasite Immunol. 25 (1): 513–516.

ISSN: 2448-8461

MF19

EFFECT OF AN ELICITOR IN THE PRODUCTION OF FATTY ACIDS IN *IBERVILLEA SONORAE* CELL CULTURE

Luz Andrea García-Valencia[1], María del Carmen Oliver-Salvador[2], María Guadalupe Ramírez-Sotelo[3]; Instituto Politécnico Nacional, Unidad Profesional Interdisciplinaria de Biotecnología, Laboratorio de Biotecnología Molecular, Av. Acueducto s/n, La Laguna Ticomán, 07340 Ciudad de México, mgramirez@ipn.mx[3], luan.gv30@gmail.com[1]

Key words: cell culture, elicitors, fatty acids, Ibervillea sonorae

Introduction. *Ibervillea sonorae* (Greene) is a Mexican and medicinal plant used for the treatment of diabetes type 2. It has been documented that the fatty acids accumulated in the cells may have the anti-hyperglycemic activity [(1)]. In this study cell suspension culture of *I. sonorae* is consider as an alternative to produce compounds of pharmacological interest. The cell culture was elicited with different concentrations of yeast extract in shake flask and the fatty acids content was analyzed.
The aim of this work is to increase the production of fatty acids on *I. sonorae* cell culture by elicitation.

Methods. The callus culture was established in Gamborg medium (B5) supplemented with growth regulators (IAA, BAP and NAA) and sucrose, using as explant the stem of *I. sonorae*[(2)]. Callus of 15 days old (1 g) were used to inoculate 125 mL Erlenmeyer flask having 50 mL of Gamborg (B5) liquid medium. The cultures were maintained in rotatory shaker at 160 rpm, and a photoperiod of 16 h light and 25 °C for 20 days. The yeast extract was prepared according to Peltonen, Mannonen and Karjalainen (1997). The 11th day the elicitor was added in different concentrations (0.1, 0.58, 1.52 and 2 g/L). Each five days a sample was taken to analyzed the fatty acids content by Nile Red methodology [(4)].

Results. The callus culture was established and callus obtained were friable to obtain cells suspension culture.
The yeast extract increased significantly the accumulation of lipids in cell suspension cultures at all the concentrations of the elicitor (Figure 1). Maximum accumulation of fatty acids (245 mg/g DCW) was observed at 1.52 g/L dose of yeast extract, with 9 fold increase over the control cells at the 20th day.
The results exhibited that the cell cultures treated with yeast extract as elicitor showed maximum accumulation of fatty acids over the control cells.

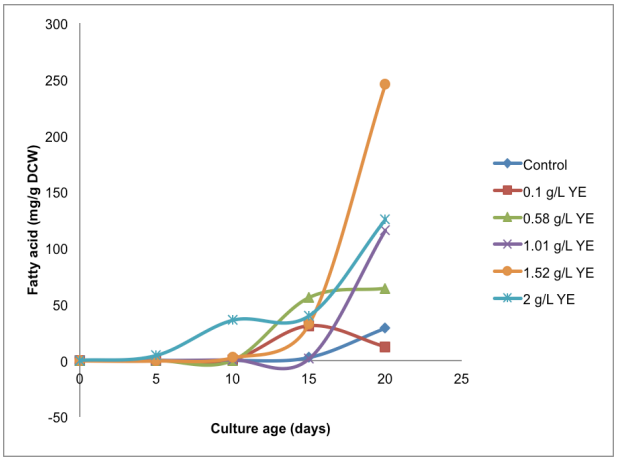


Fig.1 Effect of yeast extract (YE) elicitor at different doses (0.1-2 g/L) on the production of fatty acids (mg/g DCW).

Conclusions. *I sonorare* cells suspension culture with elicitor (1.52 g/L yeast extract) lead lipid accumulation nine-fold higher than the control cells culture.

Acknowledgements.
Instituto Politécnico Nacional.
[1]Becario CONACYT y becario del Programa de Becas de Estímulo Institucional de Formación de Investigadores (BEIFI). [2]Proyecto SIP 20181435 y SIP 20180151[3].

References.

1. Estrada-Zuñiga, M. E., Arano-Varela, H., Buendía-González, L. & Orozco-Villafuerte, J.(2012). *Rev. Mex. Ing. Química* 11: 89–96.
2. Arciniega-Carreón, I. Y., Oliver-Salvador, C., Ramírez-Sotelo, M. G. & Salas, C. E.(2017). *Hort Sci.* 52: 1000–1005.
3. Peltonen, S., Mannonen, L. & Karjalainen, R.(1997). *Plant Cell. Tissue Organ Cult.* 50: 185–193.
4. Chen, W., Zhang, C., Song, L., Sommerfeld, M. & Hu, Q.(2009). *J. Microbiol. Methods* 77: 41–47.

ISSN: 2448-8461

MF20

PREPARATION OF ERYTHROPOIETIN A THERAPEUTIC HORMONE AFFECTING THE PRODUCTION OF RED BLOOD CELLS BY EXPRESSION IN EUKARYOTIC CELL SYSTEM AND ITS FURTHER PURIFICATION

Mária Bartošová[1], Tomáš Molnár[2], Jana Adamíková[2], Monika Antošová[2], Vladimír Zelník[1], Milan Polakovič[1], Ľudovít Škultéty[1,3]

[1]Institute of Virology, Biomedical Research Center, Slovak Academy of Sciences, 845 05 Bratislava, Slovakia; [2]Department of Chemical and Biochemical Engineering, Faculty of Chemical and Food Technology, Slovak University of Technology, 812 37 Bratislava, Slovakia; [3]Institute of Microbiology, Academy of Sciences of CR, 142 20 Prague, Czech Republic.
e-mail: viruludo@savba.sk

Keywords: erythropoietin, HEK 293, multimodal resins

Introduction. Erythropoietin (EPO) is a glycoprotein hormone and essential growth factor responsible for erythroid differentiation, survival, and proliferation (1). This glycoprotein cytokine in adults is predominantly secreted by the interstitial fibroblasts of the kidney in response to cellular hypoxia (2).
Exogenous EPO, recombinant human EPO (rhEPO) is currently one of the most expensive medicinal products on the pharmaceutical market that.is used for the treatment of anemia in patients with chronic kidney disease, myelodysplasia, inflammatory bowel disease (Crohn's disease and ulcerative colitis) or cancer chemotherapy (3). Current knowledge indicated that EPO may also improve memory or influence the mood by modulating synaptic connectivity and neuronal plasticity (4).For this wide range of applications, there is an enormous demand to satisfy medical needs and introduce new variants into the market.

Methods. The rhEPO was produced by cultivation of human embryonic kidney cells 293 (HEK 293) transfected with a eukaryotic expression vector carrying the gene for EPO under the control of CMV promoter in EX-CELL 293 Serum-free production medium for HEK 293 cells (5,6). Cells were removed from the production medium by centrifugation. The supernatant was then microfiltered to remove solid particles. The filtrate was concentrated by ultrafiltration and the buffer exchanged by diafiltration. The raw product was stored in 50 mM citrate-phosphate buffer with pH 6 before further purification using a sequence formed by different types of chromatography (e.g. ion-exchange, hydrophobic, hydroxyapatite, affinity, size-exclusion and multimodal resins) (7). The total protein concentration and the content of rhEPO in chromatographic as well as preparative steps were monitored using the BCA assay and in-house prepared indirect ELISA test.

Results.
The rhEPO was produced by human embryonic kidney (HEK 293) cells and excreted to cultivation broth. By optimization of the expression system (temperature) in a serum-free medium (addition of various saccharides), up to 25 % higher production was achieved in some clones. Subsequently, three multimodal resins (Capto MMC ImpRes and Eshmuno HCX) were tested on the ultrafiltrates for rhEPO recovery. An adsorption capacity, separation selectivity, yield and purity of the product were monitored. Although the feed contained a high number of excreted proteins, we succeeded to remove up to 75% of them (contaminating proteins) at 90% of yield and a 6-fold increase in concentration (compared to the raw material) using Capto MMC. Because the purity achieved only 85%, in the consecutive step an anion bind carrier Toyopearl NH2-750F was used to increase the quality of produced rhEPO.

Conclusions. The main objective of this work was to design a novel, highly efficient eukaryotic expression system producing "native" EPO by methods of molecular cloning, and to develop an approach for its purification as a sequence of several consecutive steps primarily based on chromatography and membrane separation processes. All these steps were selected and optimized considering their potential applicability in the industry and to fulfill requirements of the current European Pharmacopoeia.

Acknowledgments. This work was supported by the grants from the Slovak Research and Development Agency (Grant number: APVV-14-0474).

References.

1.Fisher JW (2003) *Exp.Biol.Med.* 228: 1–14; 2. Moore E, Bellomo R. (2011) *Ann. Intensive Care* 1, 3; 3. Kasper C (2003) Erythropoietin. In: *The Cytokine Handbook.* (Thomson AW and Lotze MT, eds) Elsevier, London, G B. pp 149 –166; 4 . Adamcio B , S argin D , S tradomska A , Medrihan L, Gertler C, Theis F, Zhang M, Müller M, Hassouna I, Hannke K, Sperling S, Radyushkin K, El-Kordi A, Schulze L, Ronnenberg A, Wolf F, Brose N, Rhee JS, Zhang W, Ehrenreich H. (2008) *BMC Biol.* 9(6):37; 5. Inoue N, Takeuchi M, Ohashi H, Suzuki T. (1995) *Biotech. Ann. Rev.* 1: 297-313; 6. Takeuchi M, Kobata A (1991) *Glycobiology,* 1 (1991) 337-346; 7. Hu Y, Chen S, Xu M, Zhang S. (2004) *Biotech. App. Biochem,* 40: 89-94.

ISSN: 2448-8461

MF21

ANALYSIS OF MIRNA195-5P AND FRA-1 GENE EXPRESSION IN PATIENTS WITH PROSTATE CANCER

Bergez-Hernández Fernando[1], Eliakym Arámbula-Meraz[2], Alejandra Martínez-Camberos[1], Martín Irigoyen-Arredondo[1], Marco Álvarez-Arrazola[3], José G. Romero-Quintana[2], Saúl Beltrán-Ontiveros[2], José A Contreras-Gutiérrez[4], Efrén R Ríos-Burgueño[2], Noemí García-Magallanes[1].

[1]Unidad Académica de Ingeniería en Biotecnología, Universidad Politécnica de Sinaloa, Mazatlán, Sinaloa; [2]Facultad de Ciencias Químico Biológicas, Universidad Autónoma de Culiacán, Culiacán, Sinaloa; [3]Alvarez&Arraola Radiólogos, Mazatlán, Sinaloa; [4]Hosppital General de Culiacán, Culiacán, Sinaloa.

fernandobergez@gmail.com

Key words: Prostate cancer, miRNA195-5p, Fra-1.

Introduction. Prostate cancer (PCa) is the leading cause of death by cancer in Mexican men. In the state of Sinaloa has an incidence of 23.6 deaths per 100 thousand males[1]. During the progression of this disease occur molecular events as activating protooncogenes, inactivation of tumor suppressor genes and deregulation of microRNAs expression (miRNAs), among others[2]. Deregulation of miRNA 195-5p has been implicated in several cancers such as PCa. Recent studies suggest that miRNA195-5p targets a gene called Fos-linked antigen 1 (FosL1 or Fra-1), which is a proto-oncogene. Therefore, deregulation in the expression of this miRNA, can constitutively activate Fra-1, and thus promote the progression of PCa[3]. In the present study, we analyzed the expression levels of miRNA195-5p and Fra-1 in tissue of male patients, from Sinaloa, Mexico.

Methods. We obtained 19 tissue samples from prostate biopsies. Extraction of total RNA and miRNAs was performed using the miRNeasy kit (Qiagen). Subsequently, cDNA was obtained from total RNA with the ImProm-IITM Reverse Transcriptase kit (Promega) and cDNA from miRNAs was performed using the TaqMan Advanced miRNA cDNA synthesis kit. Once the cDNA was obtained, the 195-5p miRNA and Fra-1 gene were amplified using the real-time PCR technique, using taqMan probes and the miRNA 16-5p and ACTB as normalizing genes.

Results. To know if there was a difference in the expression of each of our miRNAs between patients with and without CaP, real-time PCR was performed, and the data obtained were analyzed by the ΔΔCt method. We evalatued the expression levels of microRNAs 195-5p and found no significantly difference expression between cancer and normal prostatic tissue samples (p=0.926) (Fig.1). Regarding to Fra-1 we did not find significantly difference expression between the groups (p=0.355).

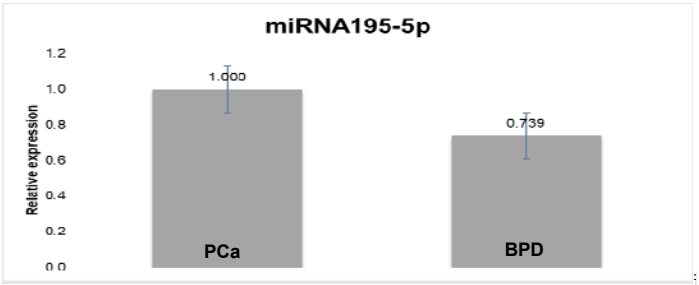


Fig.1 This figure shows the relative change of the miRNA 195-5p, which had a 0.739 decrease in PCa patients. PCa: Prostate Cáncer patients, BPD: Benign Prostate Desease.

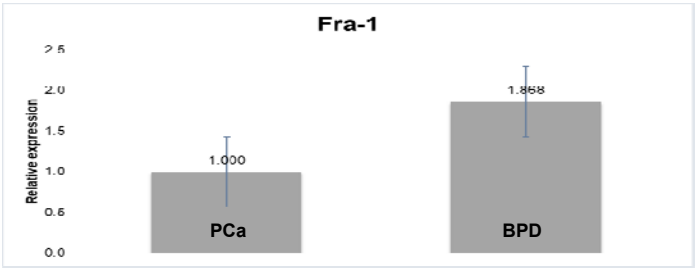


Fig.1 This figure shows the relative change of the Fra-1, which had a 1.868 increase in PCa patients. PCa: Prostate Cáncer patients, BPD: Benign Prostate Desease.

Conclusions. There was no significant difference between the expression of miRNA195-5p and the Fra-1 gene between patients with and without cancer. No correlation was observed between the expression of miRNA 195-5p and the Fra-1 gene. We suggest increasing the number of patients to confirm our results.

Acknowledgements. We thank to Universidad Politécnica de Sinaloa and Alvarez&Arrazola Radiólogos Clinic for financing the project and the contribution of the samples.

References.

1. Instituto Nacional de Estadística y Geografía. (2009). Decimotercera edición. Méxcio. *INEGI*.
2. Porkka, Kati, P., Pfeiffer, Minja, J., Waltering, Kati, K., Vessella, Robert, L., Tammela, Teuvo, L. J., Visakorpi, Tapio. (2007). *Cancer Res*. 67.(13):6130-6135.
3. Wu, Jian, Ji, Alin, Wang, Xiao, Zhu, Yi, Yu, Yasai, Lin, Yiwei, Liu, Yunfu, Li, Shiqi, Liang, Zhen, Xu, Xin, Zheng, Xiangyi, Xie, Lipinp. (2015). *J Transl Med.*(13):289.

ISSN: 2448-8461

MF22

COPY NUMBER VARIATIONS WITHIN THE BRCA1 GENE IN WOMEN WITH BREAST CANCER IN SINALOA

Alejandra Martínez-Camberos[1], Noemí García-Magallanes[1], Eunice Gárcia-Alvarez[2], Martín Irigoyen-Arredondo[1], Fernando Bergez-Hernández[1], Saúl Beltrán-Ontiveros[3], Jocelyn González[3], Enrique Jhonatan Romo-Martínez[1], Guadalupe Valdez-Zazuta[3], Eliakym Arámbula-Meraz[3]; [1]Ingeniería en Biotecnología, Universidad Politécnica de Sinaloa, Mazatlán 82199, [2]Instituto Mexicano del Seguro Social #3, Mazatlán 82149, [3]Facultad de Ciencias Químico-Biológicas, Universidad Autónoma de Sinaloa, Culiacán 80010; paolamcamberos@gmail.com

Key words: Breast cancer, heredity, BRCA1

Introduction. Breast cancer (BC) is a process of uncontrolled growth and spread of cells in the tissues of the breast; it is a multifactorial disease in which genetic and environmental factors contribute to its appearance (1). In Sinaloa it ranks fourth in the national level of incidence of 39.78 per 100 thousand women, prevailing in the first place of incidence of malignant tumors (2). BRCA1 (Breast Cancer type 1), is described as tumor suppressor gene associated with the appearance of hereditary breast and ovarian cancer, explain up to 60% of hereditary presentations of BC. It is estimated that there is a risk of BC of early onset (<50 years) of 50-80% in carriers with mutations in the BRCA1 gene. It has been shown that patients who start BC treatment in early stages have a higher survival (3, 4). Therefore, identifying women who are at high risk through genetic testing has a very important implication, however, there are no reports that indicate the prevalence of large genomic rearrangements in the BRCA1 gene in hereditary BC in our region.
The aim of this study was to identify BRCA1 rearrangements in patients with early onset or familial history of breast cancer.

Methods. Using multiplex ligation-dependent probe amplification (MLPA) for BRCA1 P002-D1 test according by manufactured instructions (MRC-Holland), we have analyzed 68 blood samples from patients with early onset or familial history of breast cancer.
The patients were divided in three groups: “Hereditary BC (HBC)” who should have the characteristics listed in the NOM-041- SSA2-2011, patients with “Sporadic BC (SBC)” who at the time of the diagnostic have 50 years or least and “Normal subjects (NS)” these people were used as the control group and must have 50 years or least.

Results. The 55.88% of the patients were diagnosed with BC, where the 53.1% were in an advanced stage with a poor prognosis. The main risk factors associated with BC in the study population are menopause (p=0.013), lactation (p=0.022), personal history of previous cancer (p=0.019), BC (p=0.001) and lung cancer (p=0.001) in the family. We identified a heterozygous deletion in exon 13 (Fig. 1) in a patient with bilateral invasive ductal carcinoma, triple negative subtype and no familial history of breast cancer.

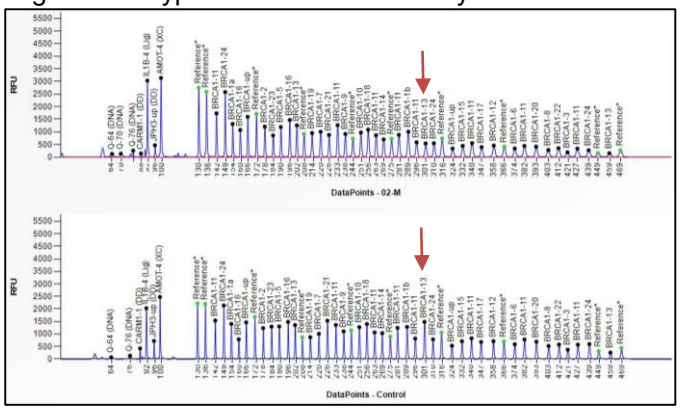


Fig.1 Heterozygous deletion of exon 13 of a patient with SBC. Deletion of exon 13 identified by fragment analysis. The numbering of exons used is the traditional exon numbering (exons 1a, 2, 3 and 5-24) in which exon 4 is not present, which differs from NCG NG_005905.2.

Conclusions. We report the first case of a heterozygous deletion in exon 13 by MLPA in Mexico, in a patient with bilateral invasive ductal carcinoma, triple negative subtype. Nonetheless it is necessary to perform analysis by sequencing to confirm the deletion detected by fragment analysis.

Acknowledgements. We wish to thank the Instituto Mexicano del Seguro Social #3 and Instituto Sinaloense de Cancerología for allowing us to conduct the research with their beneficiaries. This study was conducted with internal resources of the Universidad Politécnica de Sinaloa and the Universidad Autónoma de Sinaloa.

References.

1. Becker, S. (2015). *Int J Gynaecol Obstet*. 131:S36-S39.
2. Centro Nacional de Vigilancia Epidemiológica y Control de Enfermedades. (2017). *Secretaría de Salud.*
3. Hall, M, Reid, J, Wenstruo, R. (2010). *Cancer Prev Res*. 3(12): 1579–1585.
4. Narod, S, Rodríguez, A. (2011). *Salud Pública Méx*. 53: 420-429.

ISSN: 2448-8461

MF23

ASSOCIATION OF (TAAA)n REPETITIONS IN PCA3 GENE AND ITS EXPRESSION IN TISSUE OF PATIENTS WITH PROSTATE CANCER

Irigoyen-Arredondo Martín, Marco Álvarez-Arrazola, Eliakym Arámbula-Meraz, Fernando Bergez-Hernández, Alejandra Martínez-Camberos, Saúl Beltrán-Ontiveros, José A. Contreras-Gutiérrez, Efrén R Ríos-Burgueño, Jocelyn González-Rosales, Enrique Jhonatan Romo Martínez, Noemí García-Magallanes; Universidad Politécnica de Sinaloa, Biotechnology department, Mazatlán, Sinaloa, 82199; mirigoyen@upsin.edu.mx

Key words: Prostate cancer, PCA3, (TAAA)n

Introduction. Prostate cancer (PCa) is one of the most frequent neoplasias in men and is the second cause of cancer death worldwide. In 2015 in Mexico its mortality was 6,447, being the main cause of cancer death in men (1). Recently, a biomarker for PCa, the Prostate Cancer Associated Gene 3 (PCA3) has been identified, being the most relevant at present due to its high sensitivity and specificity, since unlike PSA, it is not influenced by the enlargement or other benign diseases of the prostate, because it is an associated gene and highly over expressed in this neoplasm (2). There are also few reported genetic studies on the PCA3 gene, especially involving the Mexican population (3). Under this approach, tandem repeats $(TAAA)_n$ have been identified that could be associated with the regulation of PCA3 expression and, in turn, be related to the development of the disease (4).
The objective of the study was determined the relation between de PCA3 expression and the genotypes of the (TAA)n repeats.

Methods. In the present study, the expression levels of the PCA3 gene were analyzed in tissue from patients with presumptive diagnosis of PCa. Four different groups of electrophoretic mobilities were established by SSCP in the patients, which were related to the expression levels of PCA3. Furthermore, the genotypes of the groups were determined by automated sequencing. Finally, the absolute expression of PCA3 was quantified by real-time PCR, and it was associated with the genotypes for the $(TAAA)_n$ repeats.

Results. A total of 19 subjects, including 13 patients with PCa (mean age at diagnosis: 67.5 years; SD: 7.9) and 6 benign pathology of the prostate (mean age at diagnosis: 66.6 years; SD: 5.08) as a control group from the "Álvarez & Arrazola Radiólogos" were analyzed for expression and polymorphism in the PCA3 gene. Differences in electrophoretic mobility between the samples were identified, with which they were grouped into four groups (I,II,III, IV). Electrophoretic mobility was observed in 15.8%, 10.50%, 5.20% and 68.40% respectively. In the present study, 3 polymorphisms were identified: 4, 5, 6 (the number represents the repeat times of TAAA in the promoter of PCA3 gene, Fig 1).

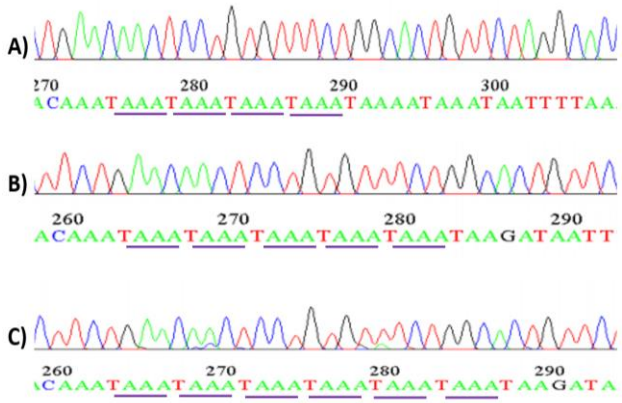


Figure 1: Representative sequencing of STR polymorphism in the promoter region of PCA3 gene. A. (TAAA)4 alleles; **B**. (TAAA)5 alleles; **C.** (TAAA)6 alleles.

Conclusions. The STR's $(TAAA)_n$ are present in the Sinaloa population and we identified 3 polymorphisms: 4, 5, 6. The electrophoretic mobilities present in groups I and II are probably related to the development of CaP and group II had the highest transcription rate for the PCA3 gene messenger. Our study has a number of important limitations, the most important of which is its small sample size, so additional larger studies are needed to provide further insight into these preliminary findings

Acknowledgements. We would like to thank Universidad Politécnica de Sinaloa and Álvarez&Arrazola Radiólogos clinic for the financing of the study

References.

1.Instituto Nacional de Estadística y Geografía. (2015). http://www.inegi.org.mx/
2.Xue, W, Ying, X, Jiang, J. (2014). J Cancer Res Ther. 10(2):218-221.
3.Gómez, L, Hérnandez, A, Blanco, A. (2012). Actas Urol Esp, 36(5):302-305.
4.Zhou, W, Chen, Z, Hu, W. (2011). PLoS ONE 6(5):1-4.

ISSN: 2448-8461

MF24

Diseño de prótesis para mano a partir de impresión 3D

Diaz León- Christian, Urriolagoitia Sosa-Guillermo, Romero Ángeles-Beatriz, Martínez Reyes-Jacobo, Vázquez Feijoo-Alejandro, Urriolagoitia Calderón-Guillermo
Instituto Politécnico Nacional, Sección de Estudios de Posgrado e Investigación,
Escuela Superior de Ingeniería Mecánica y Eléctrica, Unidad Profesional Adolfo López Mateos "Zacatenco"
Edificio 5, 2do. Piso, Col. Lindavista, C.P. 07320, Ciudad de México

Palabras clave: Prótesis de mano, Impresión 3D, Análisis cinemático

Introducción. Con el surgimiento de los homínidos las extremidades superiores se empezaron a usar como una parte del cuerpo capaz de sostener o manipular objetos. Por lo cual, el uso de esta parte del cuerpo se incrementó, originando que las manos estén expuestas a lesiones que ocasionen un daño parcial o total en estas. Causando el remplazo de la extremidad por una prótesis, sin embargo, algunos elementos protésicos llegan ser de materiales robustos o de apariencias poco estéticas.
Por lo tanto, se requiere diseñar elementos protésicos para mano a partir de fundamentos relacionados con la robótica, así como, el uso de la manufactura aditiva o impresión 3D de polímeros para la generación de prótesis para mano.

Metodología. A partir de fundamentos robóticos, la mano puede ser interpretada como un manipulador robótico, con el objetivo de desarrollar análisis cinemáticos directos e inversos con base en la metodología de *Denavit-Hartenberg* [1], sin embargo, la resolución de estos análisis estará dada en dos partes, la primera por el dedo pulgar y la segunda por el dedo índice. Posteriormente se realizó el diseño del elemento protésico (figura 1) con base en la antropometría de la población mexicana [2] y el uso de *software* de Diseño Asistido por Computadora (*CAD*).

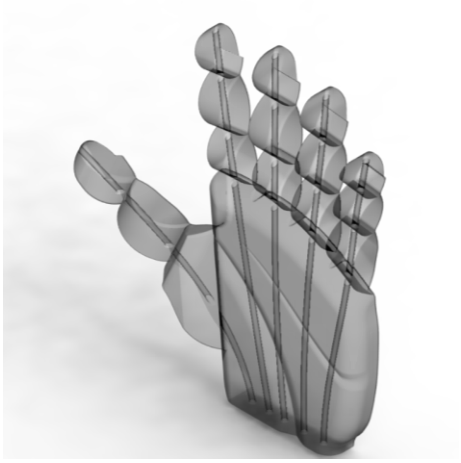


Figura 1.- Diseño final de la Prótesis

Con el diseño de la prótesis en formato *STL* se procedió a la impresión 3D de la misma a partir de un Polímero Termo Estable (*TPE*) (figura 2).

Resultados. De acuerdo a los análisis matemáticos, los cuales resultan a partir de la metodología utilizada aportan seis ecuaciones para el dedo pulgar y seis para el dedo índice. Por lo tanto, la mitad de ecuaciones de cada dedo interpretarán las posiciones finales que alcanzara cada dedo, las ecuaciones restantes representarán las posiciones angulares de cada articulación [2].

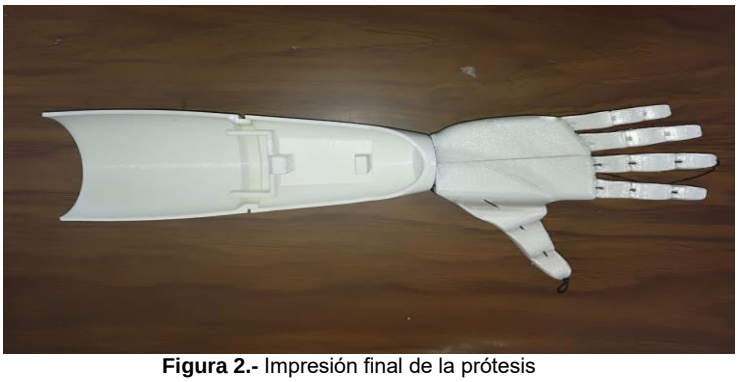


Figura 2.- Impresión final de la prótesis

Conclusiones. A partir de la impresión 3D se puede obtener elementos protésicos que ayuden a las personas a reincorporarse a sus actividades cotidianas con materiales que poseen propiedades termo-mecánicas que pueden sustituir a los convencionales.

Agradecimientos.Los autores agradecen al Instituto Politécnico Nacional y al Consejo Nacional de Ciencia y Tecnología por el apoyo brindado, en la elaboración de este trabajo.

References

1. Denevit, J. y Hartenberg, R. S., A kinematic notation for lower-pair mechanisms based on matrices, *Journal of applied mechanics*, pp 215-221, 1955
2. Diaz León, C., Tesis de Maestría, *Diseño y anañisis de una prótesis de mano con* implementación *de falanges*, México, pp 34-38, 2018

ISSN: 2448-8461

MF25

ANTI-INFLAMMATORY AND ANTIOXIDATIVE ACTION OF HYDROETHANOLIC EXTRACT IN THE GASTROPROTECTIVE EFFECT OF *Prosthechea karwinskii* IN RAT

Cristina Salinas-Nolasco[1], Gabriela Soledad Barragán-Zarate[2], Rodolfo Solano-Gómez[2], Luicita Lagunez-Rivera[2], Aracely Evangelina Chávez-Piña[1]. [1]Laboratorio de Farmacología, Sección de Estudios de Posgrado e Investigación, Esc. Nacional de Medicina y Homeopatía del Instituto Politécnico Nacional, Ciudad de México, México. [2]Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Instituto Politécnico Nacional, Unidad Oaxaca, Santa Cruz Xoxocotlán; Oaxaca, México. achavezp@ipn.mx

Key words: Prosthechea karwinskii; gastroprotective; anti-inflammatory

Introduction. *Prosthechea karwinskii* is an endemic Mexican orchid currently used as decorative element and in the traditional medicine to treat diabetes and some problems related to inflammatory processes. From this orchid, phenolic and flavonoids compounds have been reported[1]. Gastric damage induced by non-steroidal anti-inflammatory drugs is generated by cyclooxygenase inhibition, activation of oxidative stress and inflammatory processes[2]. Then, the aim of this study was to evaluate the gastroprotective effect of the hydroethanolic extract of *Prosthechea karwinskii* in a model of indomethacin-induced gastric injury in the rat.

Methods. Live specimens from *P. karwinskii* were rescued in Zaachila, Oaxaca from plants used as adornments in Easter festivity. The hydroethanolic extract of the leaves of *P. karwinskii* was obtained by ultrasonic extraction and orally administered (30-300 mg/kg, p.o.) to Wistar rats to evaluate its gastroprotective effect in an indomethacin-induced gastric injury model previously described[3]. Also, tissue samples were collected to measure LTB_4, ON, SOD and GSH[3].

Results. Figure 1 shows the gastroprotective effect of the hydroethanolic extract from *P. karwinskii* leaves (30-300 mg/kg, p.o.) in the indomethacin-induced gastric injury model in the rat. Omeprazole (O) was used as reference drug. Table 1 shows that gastric damage induced by indomethacin increases LTB_4 and nitric oxide levels while pretreatment with the hydroethanolic extract from *P. karwinskii* leaves (300 mg/kg, p.o.) significantly prevents this increment. Furthermore, *P. karwinskii* increases SOD activity and GSH levels in the same model.

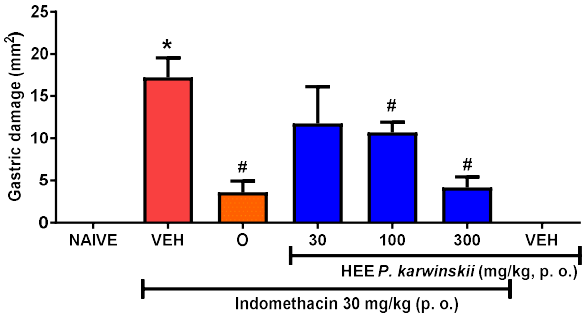


Fig.1 Gastroprotective effect of *P. karwinskii* in the indomethacin-induced gastric injury model in the rat. HEE (hydroetanolic extract), O (omeprazole). Dara are expressed as mean ± S.E.M. (n= 6-8). *P ≤ 0.05 *vs* NAIVE, #P ≤ 0.05 *vs* VEH (indomethacin-induced gastric damage).

Table 1. Anti-inflammatory and antioxidative mechanism of HEE of *P. karwinskii* during its gastroprotective effect

Group/Treatment	LTB_4 (pg/ g of tissue	NO (µM/ g of tissue)	SOD (U/ mg of protein)	GSH (nM/ g of tissue)
NAIVE	210.6 ± 9.8	37.3 ± 2.5	0.285 ± 0.04	885.9 ± 44.2
HEEPK + VEHICLE	213.2 ± 24.4	47.5 ± 4.5	1.305 ± 0.09*	1418 ± 36.6#
VEHICLE + INDO	264 ± 14.4*	61.3 ± 4.9*	0.500 ± 0.04	1037 ± 58.2*
HEEPK + INDO	179.3 ± 13.2#	45.1 ± 1.6#	0.629 ± 0.10*	1246 ± 32.7#

Dara are expressed as mean ± S.E.M. (n= 6-8). *P ≤ 0.05 *vs* NAIVE, #P ≤ 0.05 *vs* VEH (indomethacin-induced gastric damage).

Conclusions. The gastroprotective effect of HEE from *P. karwinskii* leaves has not been reported previously. Besides, its mechanism of gastroprotective effect seems to be related with its anti-inflammatory and antioxidative action in gastric tissue in the indomethacin-induced gastric injury model in the rat.

Acknowledgements. This work was supported by SIP-IPN 20180451, 20180382 and 20180435.

References.

1. Rojas-Olivas A, Solano-Gómez R, Alexander-Aguilera A, Jiménez-Estrada M, Silli-Hernández S, Lagunez-Rivera L. (2017). *Alexandria J Med* 53: 311-315.
2. Laine L, Takeuchi K, Tarnawski A. (2008). *Gastroenterology* 135: 41-60.
3. Pineda-Peña EA, Martínez-Pérez Y, Galicia-Moreno M, Navarrete A, Segovia J, Muriel P, Favari-Perozzi L, Castañeda-Hernández G, Chávez Piña A. (2018). *Eur J Pharm* 818: 585-592.

ISSN: 2448-8461

MF26

GASTROPROTECTIVE ACTIVITY OF *HYPTIS SUAVEOLENS* SEED EXTRACT

Rafael A. Veloz García, Brenda E. García Mier, Blanca E. Gomez Luna, Pablo N. Núñez-Aragón, Edith López Figueroa, **Guadalupe Piña Sáchez**; Universidad de Guanajuato, División de Ciencias de la Salud e Ingenierías, Departamento de Ingeniería Agroindustrial. Priv. de Arteaga s/n, Col. Centro, C.P. 38000 Salvatierra, Guanajuato, México.
lupiz140394@hotmail.com

Key words: gastroprotective activity, Hyptis suaveolens, indomethacin

Introduction. Peptic ulcers (PU) are frequent gastrointestinal conditions in the Mexican population, affecting 10% of the population, and being in the top 20 causes of mortality. The most common causes of PU are: *Helicobacter pylori*, alcohol and drug use (e.g. Indomethacin). In the state of Colima, the seed of *Hyptis suaveolens*, commonly known as “Chan”, is used for the preparation of the "bate", a traditional drink, considered as an agri-food handicraft, which is attributed some biological activities such as: antioxidant, gastroprotective, among others. So, the objective of the present study is to verify the gastroproctective potential of mucilaginous extract obtained from the seeds of *Hyptis suaveolens*.

Methods. The mucilaginous extracts obtained from *H. suaveolens* seeds were prepared using a sonicator (water-seed extraction ratio g/ml 1:40). To evaluate the gastroprotective acivitiy, Wistar male rats (200-250 g) were used, which remained with free access to water and feed. Prior to the experiment, the rats were fasted for 18 hours. For the induction of gastric ulcers in each rat, three doses of indomethacin (10 mg/kg) were administered orally every two hours. For the evaluation of the gastroprotective activity, the mucilage extract was administered 30 minutes before the administration of indomethacin (3 doses in total); repeating the test to evaluate doses of 1 mg/Kg. Omeprazole (8 mg/Kg) was used as a reference drug and carboxymethylcellulose (0.5%) was used as vehicle.

Results. The results show that the extract decreased the percentage of gastric damage caused by indomethacin from 2.1±0.02% to 0.7±0.06%. In addition, it presents an activity similar to that of omeoprazole (0.9±0.02%), but at a lower concentration. These results indicate that the mucilaginous extract of Chan seed presents gastroprotective activity (Table 1 and Figure 1).

Table 1. Gastric effect caused by the acute administration of *H. suaveolens* (1:40).

Treatments (Tx) / (n)	Scheme of administration	number of ulcers	Cumulative area of ulcers (mm^2)	% of damage
CMC	0.5 % (p.o. x 3)	0	0	0
Indomethacin	10 mg/kg (p.o. x 3)	19.7 ± 1.2	19.9 ± 1.6	2.1 ± 0.2
Omeprazole	8 mg/kg (p.o. x 1)	12.5 ± 2.6*	9.7 ± 2*	0.9 ± 0.2 *
H. suaveolens	1 mg/kg (p.o. x 3)	16.7 ± 2.4*	5.0 ± 0.5*	0.7 ± 0.06*

Values are mean ± SEM (n=5 rats/group), * p<0.05 vs Indomethacin.

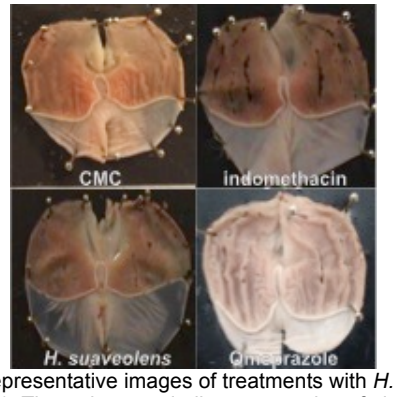


Figure 1. Representative images of treatments with *H. suaveolens* (1:40). The red arrows indicate examples of ulcers.

Conclusions. It is worth mentioning that Chan seed is a product of the Mexican countryside that is little exploited and with great agroindustrial potential that can be strengthened through the study of its nutraceutical properties.

References.

1. Ziolkovska A. (2012). *Food Hydrocoll*. Vol. (26): 197-204,
2. Gowda C (1984) *Phytochemistry*. Vol. (23):337-338.
3. Muñoz L., Cobos A., Díaz O., Aguilera J. (2012). *J. Food Eng*. Vol. (108): 216-224

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México

MF27

CICATRIZATION EFFECT OF ELABORATED GEL WITH METHANOLIC EXTRACT OF *LARREA TRIDENTATA* (GOBERNADORA)

Mayra Hidali Bautista Cruz[1], Alejandro Jonathan Hurtado Mariles[2], Yolanda de las Mercedes Gómez y Gómez[2], Marcos Ignacio Jiménez Zúñiga[2].
mjimenez.zuniga@hotmail.com

Universidad Tecnológia de Tecámac, Division Químico Biologicas[1], Tecámac Estado de México, 55740. Instituto Politécnico Nacional, Unidad Profesional Interdisciplinaria de Biotecnología, Laboratorio de Farmacología[2], Ciudad de México, 07340.

Key words: cicatrization, gel, Larrea tridentata

Introduction. The skin is a barrier that protects against external agents that are in the environment. The process of cicatrization is dynamic, interactive involving a series of extracellular soluble mediators, blood cells, cells of the tissue matrix and of parenchyma, in order to facilitate the study and understanding of the entire process of wound repair, the cicatrization process has a series of phases which occur sequentially during this process: hemostasis, inflammatory, proliferative or granulation, epithelialization, and remodeling.[3] The objective of the present work was to evaluate the cicatrizant activity.

Methods. 50 g of the dried plant was weighed in a beaker and 400 mL of methanol was placed until the sample was covered, it was passed to the sonicator for 15 minutes at room temperature, filtered and the extract was obtained. The secondary metabolites were determined and quantified as well as the antioxidant activity. Finally, a gel was elaborated as a pharmaceutical form for the extract that was tested as a healer in CD-1 mice.[1, 2, 4, 5]

Results. In table 1, we show the secondary metabolites present in the gobernadora's methanolic extract, the quantification and activity antioxidant.

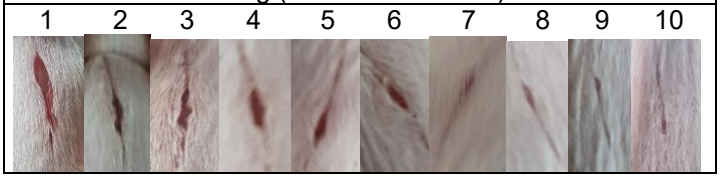
Table 1. Metabolites found in the Gobernadora extract.

Secondary metabolites	Quantification
Phenols	13.46±0.009 $C_{Phenols}$ [mg eq. of gallic acid/g of sample]
Tannins	1008.81±1.17 $C_{Tannins}$ [mg eq. of tannic acid/g of sample]
Flavonoids	2071.12±1.34 $C_{Flavonoids}$ [µg eq. of quercetin/g of sample]
Alkaloids	6.73±0.010 $C_{Alkaloids}$ [mg eq. of caffeine/g of sample]
Steroids	1578.40±1.07 $C_{Steroids}$ [mg eq. of cholesterol/g of sample]
Antioxidant activity (DPPH)	88.52±0.08 % of antioxidant inhibition
Antioxidant activity (ABTS)	98.77±0.08 % of antioxidant inhibition

Table 2. Cicatrization process

Gel of *Larrea tridentata* (Gobernadora)

Drug (silver sulfadiazine)



Conclusions. *Larrea tridentata* gel (Gobernadora) was found to have a healing effect on the seventh day of application, in comparison with the control drug that was until day ten. According to the literature, antioxidant capacity and flavonoids favor the healing process, accelerating the process in the skin and the damaged cells.

Acknowledgements. The laboratory of pharmacology of UPIBI-IPN.

References.

1. Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technology, 28(1), 25-30.
2. Chang, C., Yang, M., Wen, H., & Chern, J. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis, 10(3), 178-182.
3. Hernández, G. A. R. (2015). Fisiología de la cicatrización cutánea. RFS, 2(2), 69-78.
4. Makkar, H., Blümmel, M., Borowy, N. K., & Becker, K. (1993). Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. Journal of the Science of Food and Agriculture, 61(2), 161-165.
5. Singleton, V., & Rossi, J. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal Enology Viticulture, 16, 144-158.

ISSN: 2448-8461

MF28

HYPOGLYCEMIC EFFECT OF AQUEOUS EXTRACT OF THE ALLIUM CEPA (ONION) IN CD-1 MICE

Angelica Maria de la Luna Guzman[1], Marcos Ignacio Jimenez Zuñiga[2], Yolanda de las Mercedes Gómez y Gómez[2], Alejandro Jonathan Hurtado Mariles[2].
alejandroj_hm@hotmail.com

Universidad Tecnológia de Tecámac, Division Químico Biologicas[1], Tecámac Estado de México, 55740. Instituto Politécnico Nacional, Unidad Profesional Interdisciplinaria de Biotecnología, Laboratorio de Farmacología[2], Ciudad de México, 07340.

Key words: hypoglycemic, onion, extract

Introduction. Diabetes is a metabolic disease characterized by high levels of blood glucose, secondary to an absolute or relative alteration of insulin secretion and / or an alteration in the action of said hormone in insulin-dependent tissues. Chronic hyperglycemia is accompanied by changes in the metabolism of carbohydrates, lipids and proteins. There are three types of diabetes: type 1, type 2 and gestational diabetes. Most people with diabetes, including Latinos, have type 2. One in ten Latinos with diabetes has type 1 diabetes.[4] The aim of the present work was to evaluate the hypoglycemic power of onion in CD-1 mice.

Methods. 50 g of the dried plant was weighed in a beaker and 400 mL of purified water was placed until the sample was covered, it was passed to the sonicator for 15 minutes at room temperature, filtered and the extract was obtained. The secondary metabolites were determined and quantified as well as the antioxidant activity. Finally, the extract was dissolved in an injectable solution and subsequently, the level of blood glucose was measured in CD-1 mice.[1, 2, 3, 5]

Results. In table 1, we show the secondary metabolites present in the onion's aqueous extract, the quantification and activity antioxidant.

Table 1. Metabolites found in the onion extract.

Secondary metabolites	Quantification
Flavonoids	72.20±0.11 $C_{Flavonoids}$ [µg eq. of quercetin/g of sample]
Alkaloids	1.96±0.020 $C_{Alkaoids}$ [mg eq. De caffeine/g of sample]
Steroids	16.56±2.85 $C_{Steroids}$ [mg eq. of cholesterol/g of sample]
Antioxidant activity (DPPH)	81.70±0.085 % of antioxidant inhibition
Antioxidant activity (ABTS)	98.49±0.081 % of antioxidant inhibition

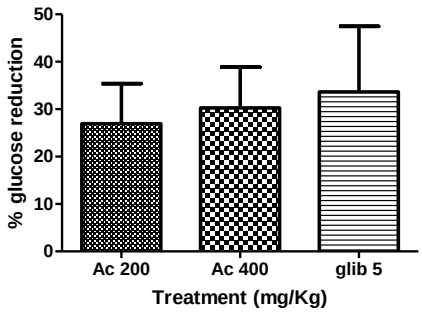


Figure 1. Blood glucose reduction percentage of aqueous extract of *Allium cepa* (Ac) at doses of 200 and 400 mg/Kg, glibenclamide (glib) 5 mg/Kg, the values indicate the mean ± SD of a n = 7 in each group with p <0.05 in relation to glib, ANOVA of a via DUNNETT

Conclusions. The aqueous extract of *Allium cepa* at doses 200 and 400 mg/Kg have a hypoglycemic activity like the glibenclamide. According to the literature, flavonoids and antioxidant capacity play an important role in the regulation of blood glucose

Acknowledgements. The laboratory of pharmacology of UPIBI-IPN.

References.

1. Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technology, 28(1), 25-30.
2. Fazel, S. H.-R. (2010). Spectrophotometric determination of total alkaloids in some iranian medicinal plants. Journal of Applied Horticulture, 12(1), 69-70.
3. Makkar, H., Blümmel, M., Borowy, N. K., & Becker, K. (1993). Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. Journal of the Science of Food and Agriculture, 61(2), 161-165.
4. Murillo, D. (2004). Guía de seguimiento farmacoterapéutico sobre diabetes. España: Universidad de Granada.
5. Singleton, V., & Rossi, J. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal Enology Viticulture, 16, 144-158.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México

MF29 #

CYTOTOXIC ACTIVITY OF THE ASCIDIAN *Distaplia stylifera*

José Manuel Mendoza Alcalá, Mauricio Muñoz-Ochoa, Raúl Flores-Mejía
Centro Interdisciplinario de Ciencias Marinas, Instituto Politécnico Nacional, Departamento de desarrollo de tecnologías
La Paz, BCS, 23096
mdozzajm@gmail.com

Key words: leukemia, didemnin, peptide

Introduction. *Distaplia stylifera* is an abundant and easy-to-collect colonial ascidian in the Bay of La Paz, BCS, México (1). According to the literature, diverse peptide compounds with cytotoxic activity have been isolated in related species (2). In some cases, they have reached advanced clinical stages for their application in humans and currently there are approved drugs to treat various types of cancer, such as Aplidine and its use to treat multiple myeloma (3). Due to this, the hypothesis of the present investigation suggests that the organism of interest contains peptides like "didemnins" with relevant anticancer and antitumor activity.

The objective of this project is to evaluate the cytotoxic activity of compounds extracted from the ascidia *D. stylifera* against three human leukemic lines.

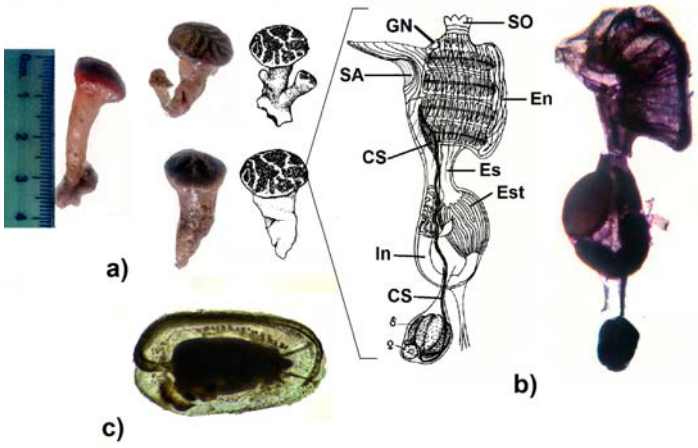


Fig. 1 *Distaplia stylifera*; a) Colonia; b) Zoide; c) Larva; SO: Sifón Oral, En: Endostilo, Es: Esófago, Est: Estómago, CS: Conducto seminal, In: Intestino, SA: Sifón atrial, GN: Gónada neural

Methods. The ethanolic extract of *D. st ylifera* was obtained and fractionated by chromatographic techniques; the fractions were evaluated for antioxidant activity (DPPH), antibacterial activity against pathogenic strains of importance in human and aquaculture health, hemolytic activity and acute toxicity in *Artemia salina* nauplii. In addition, the chemical screening was determined by autographic assay in TLC and cytotoxicity against three human leukemic lines (K562, HMC1 and THP1) will be determined by MTT assay.

Results. The chemical screening of *D. stylifera* showed a majority content of unsaturated triterpenes and phenols, followed by amino acids in the aqueous fraction. This fraction was found to be the most active, reaching a toxicity at LC_{50} of 12.6 $\mu g \cdot mL^{-1}$. In addition, the same fraction showed low activity against *Vibrio parahaemolyticus* and *Staphylococcus aureus*, as well as a slight hemolytic and antioxidant activity, with an EC_{50} of 883.7 $\mu g \cdot mL^{-1}$ against the free radical DPPH. According to the literature, compounds with CL_{50} values lower than 100 $\mu g \cdot mL^{-1}$ obtained by acute toxicity in *A. salina* nauplii have a high potential activity against cancer and tumor cell lines (4), which justifies the future isolation and purification of the compounds and their evaluation against cancer lines.

Conclusions. The biological activity shown by the fractions obtained from *D. st ylifera* suggest a high potential for the isolation of anticancer and antitumor compounds. It is likely that such compounds are selective because they do not show relevant hemolytic and antibacterial activities.

Acknowledgements. We thank the IPN for the financial support through the SIP project and COFAA exclusivity scholarship.

References.

1. Moreno, B. (2014). Taxonomía y biogeografía de ascidias del Noroeste del Pacífico Mexicano. *Universidad Autóno ma de Baja California Sur*. La Paz, B.C.S. 212 p.
2. Gogineni, V. & Hamman, M. (2016). *Biochimica et biophysica a cta*. vol. (1862): 81-196.
3. Zubia E.; Ortega M. & J Salvá. *Ciencias Marinas*. vol. (29): 251-260.
4. Peteros, N. & Uy, M. (2010). *Journal of medicinal plants research.* vol. (4): 407-414.

ISSN: 2448-8461

MF30

EVALUACIÓN E IDENTIFICACIÓN DE LOS COMPUESTOS ANTIOXIDANTES DE *HERICIUM ERINACEUS* COLECTADO EN LA REGIÓN DE EL SALTO, PUEBLO NUEVO, DURANGO, MÉXICO

Laura Anabel Páez-Olivan[1], René Torres-Ricario[2], Néstor Naranjo-Jiménez[3], Avila-Reyes Jóse[4], Jaime Herrera-Gamboa[5], Silvia Soto Ibarra[6], Chaidez-Ayala Ana[7], Miguel Soto Cardenas[8] y Imelda Rosas Medina[8,2]

[1]Centro interdisciplinario De Investigación para el Desarrollo Integral Regional-Durango, Instituto Politécnico Nacional, Calle Sigma #119, Fracc. 20 de noviembre II, Durango, Dgo, México

[2]Secretaria de investigación y posgrado- Instituto Politécnico Nacional, Av. Luis Enrique Erro S/N, Unidad Profesional Adolfo López Mateos, Zacatenco, Delegación Gustavo A. Madero, Ciudad de México, México

Correo electronico: anapaezoli@hotmail.com

Palabras clave: *metabolitos secundariosl, basidiomicetos,antioxidantes*

Introducción. *Hericium erinaceus* es considerado como un alimento funcional, productor de metabolitos secundarios. Su capacidad antioxidante contribuye al tratamiento de algunos padecimientos. El objetivo de este trabajo fue evaluar e identificar los compuestos antioxidantes de extractos etanólicos del basidioma de *Hericium erinaceus* colectados en distintas zonas de la región serrana.

Métodos. La cuantificación del contenido total fenólico se realizó siguiendo el método de Folin-Ciocalteu. La cuantificación de flavonoides totales se realizó mediante la técnica propuesta por Woisky y Salatino[1]. La capacidad antioxidante total se determinó usando el reactivo de fosfomolibdeno[2]. El poder reductor se determinó a través del método propuesto por Siddhuraju y Becker[3]. La determinación del perfil fenólico de muestras de *Hericium erinaceus*, se realizó por comparación directa de los perfiles fenólicos del cromatógrafo de líquidos de alta resolución (HPLC/DAD). Las identificaciones estructurales se hicieron de acuerdo con Campos y Markham[5].

Resultados. El contenido total fenólico presentó 369.99/20.31 mgEQ/gES y 4.25/0.6921 mgEQ/gES en los sitios El espinazo del diablo y La gallina respectivamente. El mayor contenido de flavonoides se reporta para los basidiomas de los sitios El espinazo del diablo y La gallina (4.2 y 4.0 mgEQ/gES. en cuanto a la evaluación de la capacidad antioxidante total, se registraron valores de 71.16/24.72 mgEQ/gES en comparación con los valores obtenidos de la evaluación de la reducción de fierro 0.00125/0.0019 EC50mgAA/gES, dónde el ejemplar colectado en La Peña (basidioma maduro) registró los valores más altos en comparación con el colectado en Puentecillas (basidioma joven) el cual obtuvo los valores menores. Se identificaron mediante HPLC-DAD 24 compuestos entre ellos; 11 flavonas,10 flavonoides y tres ácidos orgánicos. El ejemplar perteneciente al Espinazo del diablo (ejemplar maduro) exhibió el mayor potencial antioxidante, en todas las determinaciones. Albergó 17 de los 24 metabolitos identificados, en comparación con el ejemplar joven colectado en Los túneles que presentó 4 de los 24 compuestos. De los compuestos identificados en *Hericium erinaceus* siete ya habían sido reportados para otros basidiomicetos, pero ninguno de los 24 metabolitos se ha reportado para el hongo melena de león.

Conclusiones. En todas las especies analizadas se encontraron compuestos antioxidantes, entre ellos flavonoides, flavonas y ácidos aromáticos, que refleja gran diversidad micoquímica en estos hongos. Los compuestos evaluados presentaron actividad antioxidante sobresaliente en comparación con otros estudios realizados para esta especie.

Agradecimientos. A la Dra. Laura Guzman-Dávalos por la identificacion y resguardo de los ejemplares en Herbario Micológico del Instituto de Botánica, Jalisco, México (IBUG).

Referencias.

1.- Woisky, R. y Salatino, A. (1998). Journal of apicultural research, 37(2), 99-105.
2.- Prieto, P., M. Pineda, M. Aguilar. 1999. Analytical Biochemistry 269: 337341.
3.- Siddhuraju, P. y Becker, K. (2003). Journal of Agricultural and Food Chemistry 51: 2144–2155.
4.- Campos, M. y Markham, K. (2007). Imprensa da Universida de Coimbra/Coimbra University Press.

EFFECT OF *Prosthechea karwinskii* ON METABOLIC SYNDROME INDUCED IN WISTAR RATS

Alejandra Rojas-Olivos [1], Rodolfo Solano Gómez [1], Alfonso Alexander-Aguilera [2], Luicita Lagunez-Rivera[1].

[1] Instituto Politécnico Nacional, CIIDIR Oaxaca, Hornos 1003, Santa Cruz Xoxocotlán, Oaxaca, 71230, México.[2] Facultad de Bioanálisis, Universidad Veracruzana, Carmen Serdán s/n, Col. Flores Magón, Veracruz, Veracruz, 91700, México.
llagunez@hotmail.com

Key words: cholesterol, glucose, medicinal plants.

Introduction. Medicinal plants play an important role in the introduction of new therapeutic agents as a source of biologically active substances with antihyperlipidemic and antihyperglycemic properties, among other effects.[1] Hyperglycemia results in an increased oxidative stress due to reduced endogenous antioxidants in the body[2] and imbalance in adipose tissue, influencing lipid regulation and triggering cardiovascular disease. Metabolic syndrome (MS) is the term used to designate a set of interrelated conditions that include hyperglycemia, hyperlipidemia, obesity and hypertension.[3,4].

Furthermore, phenolic compounds can inhibit adipose tissue growth due to their antiangiogenic activity and their ability to regulate adipocyte metabolism.[5] Considering the above and the fact that this plant is widely used in the traditional medicine for the treatment of hyperglycemia, one of the conditions of metabolic syndrome, the present work was conducted to evaluate the effect of hydro-alcoholic extracts from *Prosthechea karwinskii* on characteristic parameters of MS in a rat model..

Methods. For *in vivo* assays 25 weaned male Wistar rats were divided into a control group (CG; n =5) and a Metabolic Syndrome group (MS; n=20). The rats of the latter were induced to MS with 40% sucrose in the drink water during 13 weeks. After MS induction this group was subdivided into 4 groups: MS group (n=5) received sucrose, and three groups receiving 200 mg/kg of body weight of each extract pseudobulb (P, n=5), leaf (L, n=5), and flower (F, n =5). All treatments were followed for 13 days. Blood was collected at the end of the study to measure glucose, cholesterol and triglycerides. Antioxidant activity index (AAI) were measured in the extracts by the method of DPPH. The results were analyzed using MINITAB 16.1.0, and the statistical significance was determined by ANOVA and a Tukey´s test *(P <0.05)*.

Results. Leaves (L) extract had highest values in AAI, followed by flowers (F) and pseudobulb (P) extracts (Table 1). Leaves extract had highest reducing effect on glucose level, while flower extract had highest reducing effect on the cholesterol and triglycerides levels (Table 2).

Table 1. Antioxidant capacity of *Prosthechea karwinskii* extracts

Extract	Yield w/w (%)	IC50	AAI
Pseudobulb	18.8±1.335	43.06±7.311	0.925±0.162
Leaf	16.8±2.335	6.91±0.187	5.7±0.157
Flower	33.6±2.93	30.85±2.51	1.276±0.971

Table 2. Effect of P. karwinskii on Wistar rats with metabolic syndrome induced.

Parameter (g)	GC	MS	P	L	F
Glucosa	97±10[a]	135±30.70	92±7.95[a]	64±11.4[a]	73.7±25[a]
Cholesterol	69±10.3[a]	87±7.44	76±0.32[a]	76±9.59[a]	54±10.54[a]
Triglycerides	214±18[a]	259±23.89	127±46.5[a]	109±11[a]	75±22.35[a]
ATA	12.2±1.9	16.25±5.9	9.33±3.7[a]	11.6±2.3	11.0±1.7[a]
ATE	10±2.5	15.8±6.6	6.6±2.08[a]	8.3±2.8[a]	10.3±0.5[a]
ATR	0.6±0.4	0.48±0.1	0.70±0.2	0.56±0.3	0.40±0.1
Total	22.8±4.8	32.5±12.7	16.6±6.1[a]	20.5±5.4[a]	21.7±2.4[a]

AT: adipose tissue, A :abdominal, E :epididymal, R:pericardial, GC: control group, MS: sucrose diet, P: sucrose diet and pseudobulb extract, L: sucrose diet and leaf extract, F: sucrose diet and flower extract. The values represent the mean ± SD, superscript values show statistically significant difference as revealed by the Tukey's test ([a] $P<0.05$). Adiposity index= (Total fat / final weight)*100.

Conclusions. The *P. karwinskii* extracts evaluated here reduces the glycemic and lipidemic parameters in Wistar rats with MS induced. These effects may be attributed to the high antioxidant capacity of the extracts.

References

1. Tang L-Q, Wei W, Chen L-M, Sheng L. *J Ethnopharmacol* 2006; 108: 109-115.
2. McCune L.M., Johns T. *J Ethnopharmacol* 2007; 112:461-469.
3. Alexander-Aguilera A., Hernández-Diaz G., Lara-Barcelata M., Angulo-Guerrero O., Oliart-Ros R.M.. *J Nutr Biochem* 2004; 15: 350-357.
4. Aguilar-Salinas C.A., Rojas R., Gómez-Pérez F.J., Mehta R., Franco A., Olaiz G., et al. *Arch Med Res* 2005; 36: 223-231.
5. González-Castejón M., Rodríguez-Casado A. Dietary phytochemicals and their potential effects on obesity: A review. *Pharmacol Res* 2011; 64:438-455.

EVALUATION OF EXPRESSION OF 148b-3p AND 133a-3p miRNAs IN SERUM SAMPLES AND THEIR RELATIONSHIP IN PATIENTS WITH BREAST CANCER

Saúl A. Beltrán-Ontiveros[1], Eliakym Arámbula-Meraz [1], José G. Romero-Quintana [2], Lorben López-Rodríguez [3], José A. Contreras-Gutierrez[4], Efrén R. Rios-Burgueño[4], Alejandra P-Martínez Camberos, Fernando A-Bergez Hernández Noemí García-Magallanes[5]*.

[1]Posgraduate in Biomedical Sciences, Faculty of Biological Chemistry Sciences, Autonomous University of Sinaloa. C.P 80010 Culiacán Sinaloa. [2]Laboratory of Immunology, Faculty of Biological Chemistry Sciences, Autonomous University of Sinaloa. C.P 80010 Culiacán Sinaloa. [3] Personalized Oncology Center, C.P 80040 Culiacán Sinaloa. [4]Hospital Civil de Culiacán, C.P 80030 Culiacán Sinaloa [5] Laboratory of Biomedicine Molecular Engineering in Biotechnology, Polytechnic University of Sinaloa. C.P 82199 Mazatlán Sinaloa. ngarcia@upsin.edu.mx

Key words: Breast cancer, serum, biomarkers, miRNAs.

Introduction. Breast cancer (BC), is a multifactorial disease in which genetic and environmental factors contribute to its appearance[1], it originated by the accelerated proliferation of epithelial cells lining the breast ducts or lobules[2], breast neoplasm ranks first in incidence and mortality in women worldwide[3]. We observed the potential of microRNAs (miRNAs) as biomarkers for the detection of BC since their involved in the expression of genes that play a key role in cell adhesion, proliferation, differentiation, motility, invasion and cell death, there are differences in miRNAs expression during the progression of BC, highlighting the miR-133a-3p and miR-148b-3p in patients serum with early-stage BC detection and prognosis of non-invasive BC[4-5]. The aim of this study was to analyze the expression levels of miR-148b-3p and miR-133a-3p in serum of females samples from the Sinaloa's Cancer Institute.

Methods. The study was transversal, prospective and observational. We analyzed the expression levels of 44 serum samples from female patients from the Sinaloa Cancer Institute during the period from March 2015 to March 2016 and classified into two groups: with and without breast cancer (benign tumor). We obtained different qualitative variables from a direct questionnaire and the review of their clinical file. The miRNA expression of miR-133a-3p and miR-148b-3p was determinate by qPCR using the kit TaqManTM Advanced MicroRNA Assays from biosystems by thermo Fisher Scientific specific for each miRNA and miRNA-16-5p was the normalizer, the relative miRNA expression was calculated with the 2ΔCt method. Data was analyze using Chi square and t student on the IBM SPSS v.20 software.

Results. We found that 61.36% of patients were diagnosed with BC; being contraceptive use (p=0.017) and the number of heroic deeds (p = 0.012) risk factors associated with the development of BC. A tendency to the disease with onset age of menarche (p=0.063) was found. Low expression of miR-133a-3p (p=0.298) and miR-148b-3p (p=0.053). Was observed in cancer patients compared to benign breast tumor patients. The expression levels of miR-148b-3p were not associated with clinic-pathological features; and expression levels of miR-133a-3p were associated with BC type, presenting low expression in lobular carcinoma compared with ductal carcinoma (p=0.005).

Conclusions. The majority of the patients in the population studied were in an advanced stage of the disease with a poor prognosis due to a late diagnosis. No statistically significant differences were found in the expression of hsa-mir-148b-3p and hsa-mir-133a-3p among the study groups and the main risk factors associated with BC in this population are the number of births and the use of contraceptives.

Acknowledgements.
I thank the staff of the Laboratory of Genetics and Molecular Biology of the Faculty of Biological Chemistry Sciences of the Autonomous University of Sinaloa and the personnel of the Molecular Biomedicine Laboratory of the Polytechnic University of Sinaloa. To CONACYT for the scholarship provided during the realization of this study.

References.

1. Reynoso N., & Mohar, A. (2014). El cáncer en México: propuestas para su control. *Salud Pública de México, 56*(5), 418-420.
2. NIH, N. (2016b). What is Cancer. Retrieved Enero, 2016, from http://www.cancer.gov/about-cancer/what-is-cancer
3. OMS, O. M. d. l. S. (2012). Datos y cifras. Retrieved Enero, 2016, from http://www.who.int/mediacentre/factsheets/fs297/es/
4. Jiang, X., Du, L., Wang, L., Li, J., Liu, Y., Zheng, G., Wang, C. (2015). Serum microRNA expression signatures identified from genome-wide microRNA profiling serve as novel noninvasive biomarkers for diagnosis and recurrence of bladder cancer. *Int J Cancer, 136*(4), 854-862. doi: 10.1002/ijc.29041
5. Mangolini, A., Ferracin, M., Zanzi, M. V., Saccenti, E., Ebnaof, S. O., Poma, V. V., Negrini, M. (2015). Diagnostic and prognostic microRNAs in the serum of breast cancer patients measured by droplet digital PCR. *Biomark Res, 3*, 12. doi: 10.1186/s40364-015-0037-0.

MF33

ANALYSIS OF STRUCTURAL AND FUNCTIONAL EFFECTS OF THE DEAMIDATION IN HUMAN TRIOSEPHOSPHATE ISOMERASE. AN APPROACH TO QUESTION OF DEAMIDATE.

Luis Antonio Flores López[1,2], Itzhel García Torres[2], Sergio Enríquez Flores[2], Ignacio De la Mora De la Mora[2] and Gabriel López Velázquez[2].

1.-Consejo Nacional de Ciencia y Tecnología (CONACyT). Cátedras CONACyT, Mexico City 03940.

2.-Instituto Nacional de Pediatría, Secretaría de Salud, Mexico City 04530. luisbiolexp@gmail.com

Key words: deamidation, recombinant, enzyme.

Introduction. The deamidation is considered a post-translational modification in proteins, it is the loss of the ammonium group of asparagine (Asn) or glutamine (Gln) residues to form aspartic or glutamic acid, respectively. When deamidation occurs, it introduces a negative charge and consequently the structure and function of the protein is altered (1). It has been hypothesized that Asn may participate, through deamidation, as molecular clock that stimulate the turnover of proteins (2). Although this post-translational modification is of great importance, there is not enough detailed functional and structural information to explain the effects of deamidation on the proteins. The triosephosphate isomerase (TIM) is a glycolytic enzyme that catalyzes the interconversion of the isomers dihydroxyacetone phosphate and D-glyceraldehyde 3-phosphate. Such enzyme has been well-characterized in many organisms and has been studied in the human TIM (HsTIM) the deamidation process (3). In searching new molecular targets with medical interest, we characterized the recombinant enzymes wild type (WT) HsTIM, N15D and N71D. These two latter are stable models of HsTIM deamidation because simulate the deamidation by replacing asparagine to aspartic acid amino acid residues.

Methods. The WT HsTIM gene was cloned into the pET-HisTEVP plasmid. The model of deamidation was made by site directed mutagenesis obtaining two mutants of HsTIM (N15D and N71D). The enzymes were successfully over-expressed and isolate to more than 95 % of purity. The kinetic constants were calculated obtaining the initial velocity of the enzymes at increasing GAP concentrations. For the estimation of thermal stability, protein unfolding was followed as the change in the circular dichroism signal at 222 nm ranging from 20 to 90°C (4). Finally, we analyzed the crystallographic structures of the WT and N15D deposited in the protein Data Bank (PDB code 2JK2 and 4UNK, respectively), the crystallographic structures of the WT HsTIM and N15D was analyzed (5).

Results. The kinetics parameters of N71D mutant are close to WT HsTIM (*Km* 0.79 and k_{cat}/Km 2.1) in contrast, the N15D mutant was altered such parameters (*Km* 2.16 and k_{cat}/Km 0.52). On the other hand N71D mutant resembles structurally the WT HsTIM. The thermal denaturation (Tm) for the N71D mutant closely resembles the Tm of the WT enzyme (60.3 ºC and 61.2 ºC respectively); in contrast, the Tm of N15D decreased 7°C with respect to the WT enzyme, showing that the single deamidation of N15 is enough to disrupt the thermal stability of HsTIM. The crystal structures show significant structural changes in the N15D compared with the WT, the N15D mutation lose and win molecular interactions that perturb the structure of loop 1 and loop 3, both critical components of the catalytic site and the interface of HsTIM.

Conclusions. These studies have shown that the HsTIM deamidation leads to severe alterations at the functional and the structural levels and these differences in contrast with the WT HsTIM can be considered as a therapeutic target for drug design.

Acknowledgements. This work is supported by Cátedras CONACYT (LAFL ID:72835) project number 524.

References.

1. Robinson NE, Robinson AB. (2008). Use of Merrifield solid phase peptide synthesis in investigations of biological deamidation of peptides and proteins. *Biopolymers*. 90:297–306.

2. Robinson AB. (1979). Molecular clocks, molecular profiles, and optimum diets: three approaches to the problem of aging. *Mech Ageing Dev.* 9:225–236.

3. De la Mora-de la Mora I, Torres-Larios A, Mendoza-Hernández G, Enriquez-Flores S, et al. (2013). The E104D mutation increases the susceptibility of human triosephosphate isomerase to proteolysis. *Biochim Biophys Acta.* 1834:2702–2711.

4. Gracy RW, Talent JM, Zvaigzne AI. (1998). Molecular wear and tear leads to terminal marking and the unstable isoforms of aging. *J Exp Zool.* 282:18–27.

5. Robinson NE, Robinson AB. (2001). Prediction of protein deamidation rates from primary and three-dimensional structure. *Proc Natl Acad Sci USA*. 98:4367–4372.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México

MF34

ENCAPSULATION EFFECT OF PLGA-LUPEOL-MANGUIFERIN NANOCOMPOSITES ON THE TOPOISOMERASE INHIBITION

Fabián Razura-Carmona; Alicia P. Cárdenas-Castro; Jorge A. Sánchez-Burgos; Integral Food Research Laboratory, Technological Institute of Tepic, Tepic, Mexico, 63175; Marco V. Ramírez-Marez; Chemical and Biochemical Department, Technological Institute of Morelia, Morelia, México, 58120; jsanchezb@ittepic.edu.mx

Key words: nanocomposites, topoisomerase, lupeol

Introduction. Lupeol and mangiferin are inhibitors of topoisomerases, which is why chemoprotective activity is attributed to them. The synergistic effect of these phytochemicals may favor the development of poly lactide-co-glycolide (PLGA) nanocomposites as a nutraceutical potential.

Methods. The development of the nanoparticles was carried out by the method of emulsion and evaporation of solvents, subsequently they were frozen (-80 ° C) and lyophilized. The antitopoisomerase activity was determined according to the methodology described by Jensen et al., (1), in which the inhibition of clonal expansion is evaluated, using the genetically modified yeast of *Saccharomyces cerevisiae*. For the test, JN394, JN362a and JN394$_{t-1}$ strains were used, functionalized nanocomposites (lupeol-mangiferin), lupeol, mangiferin and without phytochemicals (white), camptotecin (CPT, Topo I poison), were suspended in dimethylsulfoxide (DMSO) same that was used as a negative control.

Results. FN (functionalized nanocomposites) showed the highest inhibition JN394 (55%), compared to LN (nanocomposites of lupeol) and MN (nanocomposites of mangiferin) with 22% and 36% respectively, CPT inhibited 75% and DMSO showed no reduction in cell replication, compared to JN362a a strain competent for DNA repair, no treatment or control showed inhibition. This shows that both phytochemicals have antitopoisomerase I activity, by inhibiting the mutated strain with hypersensitivity and not causing any adverse effect on the strain with exacerbated repair mechanisms. However, NF shows greater activity since the chemical interactions formed during the formulation with the polymeric complex allow to control the release of the components, thus prolonging the activity during the test period. an inhibitory effect is shown in topo I in MG and topo II in LP previous reports discuss the antitopoisomerase II effect in lupeol (2) and Topoisomerase I in mangiferin (3, 4). These results indicate that FN inhibits topoisomerase II, which is one of the most difficult enzymes to inhibit.

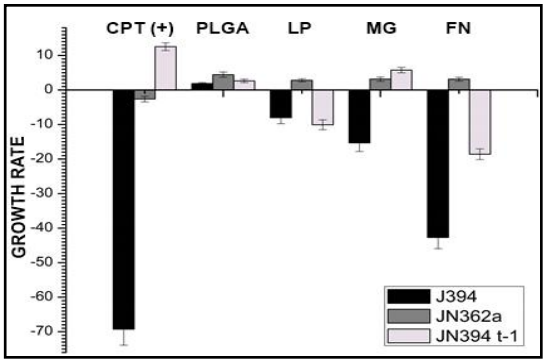


Fig.1 Growth percentage of strains JN394, JN662a and JN394t-1 by the inhibition of the above topoisomerases. The inhibitory effect of NF is observed in comparison with controls.

Conclusions. The functionalization of phytochemicals allows to potentiate the biological activity that these could present individually.

Acknowledgements. The Student Fabían Razura Carmona thanks CONACYT for the postgraduate scholarship received #7887023. Also, the TecNM for the financing of project 5857.16-P.

References.

1. Jensen, L. H., Nitiss, K. C., Rose, A., Dong, J., Zhou, J., Hu, T., Osheroff, N., Jensen, P. B., Sehested, M. y Nitiss, J. L. (2000). *The Journal of Biological Chemestry*, 273(3): 2137- 2146.
2. Wal, P., Wal, A., Sharma, G. y Rai, A. K. (2011). *Reviews in Pharmacy*, 2(2): 96-113.
3. Phoboo, S. Bhowmik, P. C., Jha, P. K. y Shetty, K. (2010). *Journal of Plant Science*, 7: 48-55.
4. Saha, P. & Das, S. (2010). *Asian Pacific Journal of Cancer Prevention*, 11(6): 1445-1449.

ISSN: 2448-8461

MF35

BIOLOGICAL ACTIVITY OF THE SEAGRASS *Phyllospadix torreyi*

Liliana Edith Couttolenc-Lyle, Mauricio Muñoz-Ochoa, Dora Luz Arvizu-Higuera, Yoloxochitl Elizabeth Rodríguez-Montesinos.
Instituto Politécnico Nacional. Centro Interdisciplinario de Ciencias Marinas. La Paz, BCS, México CP 23096.
lilicoutt@gmail,com

Key words: Secondary metabolites, antioxidant, antimicrobial, cytotoxicity, phytochemical

Introduction. Seagrasses in response to chemical defense against oxidative stress and the attack of pathogenic organisms and predators synthesize secondary metabolites such as phenols, alkaloids, terpenoides[1,2]. It has been well documented the diverse biological properties of these compounds as well as the presence in seagrasses, presenting a wide range of secondary metabolites[3,4]. The investigation of new structures in different seagrass species can provide useful information for the creation of new drugs for therapeutic purposes. So that the objective of this work was to evaluate the potential of *Phyllospadix torreyi* as a source of compounds with antioxidant, antibacterial and cytotoxic activity.

Methods. The ethanol extract of *P. torre yi* was fractionated by means of different chromatography techniques; the phytochemical analysis was determined qualitatively. The obtained fractions were subjected to an antioxidant activity assay by means of bioautography and spectrophotometric methods, using the reduction of the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). In addition, antibacterial activity was evaluated against various pathogenic strains of importance in human health, and toxicity of the brine shrimp lethality test.

Results. The ethanol extract showed a phytochemical profile composed mainly of phenols, tannins and flavonoids (Table 1), which have been reported as antioxidant compounds, while the spectrophotometric measurement of the antioxidant activity was an EC_{50} = 0.240 µg-mL^{-1}. Solid-liquid extraction resulted in 5 fractions, all of which showed an interesting sequestering activity against DPPH in the bioautography assay. The fraction extracted with MeOH was the most active. The dichloromethane and Hexane fractions, showed antimicrobial activity against *Staphylococcus au reus, Staphylococcus epidermis* and *Pseudomonas aeruginosa*, inhibiting their growth. Additionally the crude extract showed, CL_{50} = 630 µg-mL^{-1}, in the brine shrimp lethality test.

Table 1. Phytochemical profile of the ethanolic extract of *P. torreyi* .

Phytochemical	Ethanolic extract
Phenols	+
Flavonoids	+
Steroids	+
Triterpenes	+
Saponins	+
Alkaloids	–
Anthraquinones	+

Presence + absence –

Conclusions. Our results show that *P. torreyi* could be a source of bioactive compounds with potential antioxidant, antimicrobial and cytotoxic activity.

Acknowledgments. We thank the IPN for the financial support through the SIP project and COFAA exclusivity scholarship.

References.

1. Ramakrishna, A. & Ravishnkar, G. A. (2011) .*Plant Signaling & Behavior*. 6(11): 1720-1731.
2. Goyal S., Lambert C., Cluzet S., Mérillon J.M., Ramawat K.G. (2012). Secondary Metabolites and Plant Defence. In: Mérillon J., Ramawat K. (eds) Plant Defence: Biological Control. Progress in Biological Control, vol 12. Springer, Dordrecht.
3. Zidor, C. (2016). *Phytochemistry. 124, 5-28*
4. Subhashini, P.; Dilipan E.; Thangaradjou, T.; Papenbrock, J. (2013). *Natural products and bioprospecting 3(4), 129-136.*

ISSN: 2448-8461

MF36

SYNTHETIC XANTHONES BASED ON α-MANGOSTIN AND THEIR CYTOTOXIC ACTIVITY ON BREAST CANCER CELLS

María del Rosario Matamoros-Palafox[1], Aaron Mendieta-Moctezuma[1], Cynthia Ordaz-Pichardo[2], María del Carmen Cruz-López[1], Fabiola Eloísa Jiménez-Montejo[1]

[1]Centro de Investigación en Biotecnología Aplicada, Instituto Politécnico Nacional, Tepetitla, 90700, Tlaxcala. [2]Laboratorio de Biología Celular y Productos Naturales, Escuela Nacional de Medicina y Homeopatía, Instituto Politécnico Nacional, Ticomán, 07320, Ciudad de México, México. yuki_8510@hotmail.com.

Key words: Prenylated xanthones, breast cancer, MDA-MB-231

Introduction. Breast cancer is the most incident among the female population of our country, so it is necessary to development new therapeutic agents against this type cancer.[1] Xanthones constitute a class of *O*-heterocycle with a dibenzo-γ-pyrone scaffold commonly found as secondary metabolites in higher plants, fungi and lichens. Prenylated xanthones derivatives like α-mangostin have been widely reported as potential anticancer agents due to their potency and selectivity against several human tumor cell lines (Figure 1).[2-3] The present work, prenylated xanthones were synthesized and their cytotoxic activity on the MDA-MB-231 breast cancer cell line were evaluated.

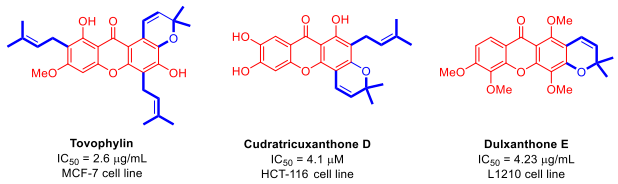
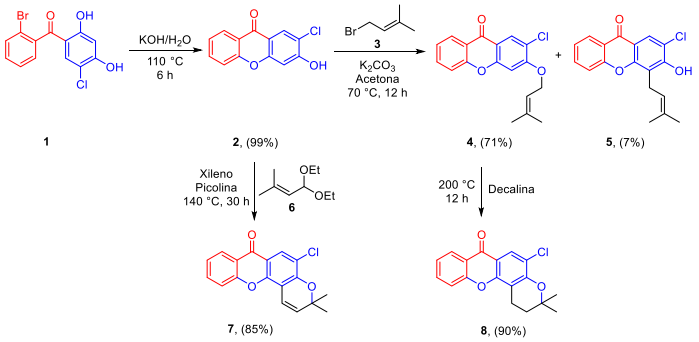


Figure 1 Prenylated xanthones with cytotoxic activity.

Methods. The xanthone **2** was obtained through the cyclization thermal of the benzophenone **1** using KOH, which was reacted with prenyl bromide to give prenylated xanthones derivatives **4** and **5**. Compound **4** was cyclized by thermal rearrangement giving rise pyranoxanthone **8**. Lastly, pyranoxanthone **7** was obtained from compound **2** by reacting with 1,1-diethoxy-3-methylbut-2-ene under heating in presence of picoline (Scheme 1). The breast cancer cell line (MDA-MB-231) was growth in a specific media supplemented. The MTT colorimetric assay was used to measure the cytotoxicity of the xanthones.[4] The concentration range of the tested samples were 0-100 µM. The cells were exposed to the compounds for 24 and incubated at 37° C in a humidified atmosphere with 5% CO_2. Paclitaxel was employed as a positive control. IC_{50} value were determined by linear regression with the GraphPad Prism v. 7 program.

Results. By analyzing Table 1, it can be observed that the compounds tested shown a more growth inhibitory effect than positive control Paclitaxel.



Scheme 1. Synthesis of prenylated xanthones.

Compared with Paclitaxel, the inhibition activity of **7** against MDA-MB-231 was increased by 550 times with IC_{50} of 0.105 µM. In appearance, the cyclization of the prenyl group enhanced the inhibitory effect.

Table 1. Growth inhibitory activity of xanthones in MDA-MB-231 cell line.

	IC_{50} (µM) ± SD			
	Paclitaxel	**5**	**7**	**8**
MDA-MB-231	5.85 ± 0.124	0.617 ± 0.097	0.0105 ± 0.0058	0.0122 ± 0.0052

Conclusions. The synthesized xanthones **5**, **7** and **8** exhibited potent growth inhibitory effect in MDA-MB-231 cell line. This suggest that MDA-MB-231 cell line seems to be sensitive to the effect of xanthones with a fused 2,2-dimethylpyran and 2,2-dimethyl-3,4-dihydropyran ring.

Acknowledgements. This work was financially supported by a project 20180968, 20181433 from SIP, IPN.

References.

1. Liu J, Zhou F, Zhang L, Wang H, Zhang J, Zhang C, Jiang Z, Li Z, Chen H. (2018). *Eur. J. Med. Chem*. 143, 1768-1778.
2. Leão M, Pereira C, Bisio A, Ciribilli Y, Paiva AM, Machado N, Palmeira A, Fernandes MX, Sousa E, Pinto M, inga A, Saraiva I. (2013). (2016). *Biochem. Pharm.* 85, 1234-1245.
3. Azevedo CMG, Afonso CMM, Soares JX, Reis S, Sousa D, Lima RT, Vasconcelos MH, Pedro M, Barbosa J, Gales L, Pinto MMM. (2013). *Eur. J. Med. Chem*. 69, 798-816.
4. Denizot F., Lang R. (1986). *J. Inmunol. Methods.* 89(2): 271-277.

ISSN: 2448-8461

MF37

Identification of apoptotic molecular mechanisms induced in human HepG2 cells and dermal human fibroblast adults in response to maize (*Zea mays* L.) zein-derived peptides

Plinio A. Trinidad-Calderón [1], Rodrigo B. Muñoz-Soto [2], Fabiola Castorena-Torres [3], Silverio García Lara [1]
[1] Escuela de Ingeniería y Ciencias, Tecnologico de Monterrey, N.L., Mexico, 64849; [2] Escuela de Medicina, Tecnologico de Monterrey, N.L., Mexico, 64710; Departamento de Bioingeniería, Tecnologico de Monterrey, CDMX, México, 14380.
A00822365@itesm.mx

Key words: apoptosis cancer peptides

Introduction. Cancer is the second cause of death worldwide [1]. In consequence, the development of novel therapeutic agents have become a global main concern [2]. Several works have found peptides with recognized anticancer activity among proteins of soybean, rice, and maize [3]. These molecules, denominated also as anticancer peptides (ACPs), have become promising drug candidate molecules due to their small size, high bio-activity, and low immunogenicity [4].

The objective of this work is to identify the molecular mechanisms displayed by maize zein-derived peptides in human HepG2 cells and dermal fibroblast adults regarding to apoptosis, and cell membrane-peptide interaction.

Methods. Zeins were extracted from 80 g of kernel by means of grinding 30 s twice. Additional extraction steps were performed according to Díaz-Gómez (2018). Zein was hydrolyzed using Alcalase® CLEA (EC 3.4.21.62) 0.0525 U/mL at 37°C during 6 h. Peptide fraction < 5KDa was separated by centrifugation at 4000 *g*. Peptides were precipitated with acetone at -20°C for 24 h. After precipitation, peptides were filtered through a PVDF filter (0.2 µm) and tested in HepG2 cells. Screenings for 6, 12, 24, and 48 h were performed, using peptide doses ranging 0-7000 ng/ml.

Results. A screening for lethal dose made in HepG2 cells showed an evident killing effect at 1500 ng/ml (Fig.1).

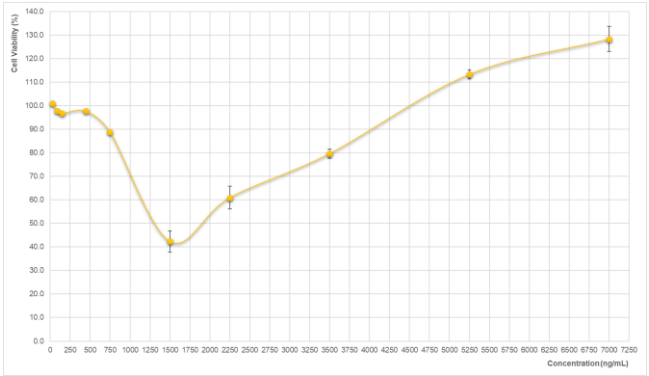


Fig. 1. Screening for lethal dosage determination in HepG2 cells at 48h. The lethal effect for these peptides was observed at 750-3750 ng/ml. The most killing lethal was achieved at 1500 ng/ml.

Permeability enhancement induced by DMSO 0.1% was also tested in HepG2 cells. DMSO addition did not enhance the lethal effect of peptides (Fig. 2). Even, an increase in cell viability has been observed between 250-3500 ng/ml of zein-derived peptides, corresponding to the resulting intake of peptides into the cytoplasm.

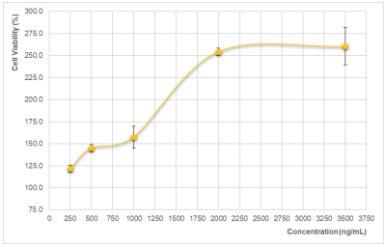


Fig. 2. Effect of permeability enhacement induced by DMSO 0.1%. Increased cell viability was observed in all doses of zein-derived peptides tested in HepG2 cells at 48 h.

In order to determine the time dependence of zein hydrolysate bioactivity, optimal lethal dose (1500 ng/ml) was tested at 6, 12, 24, and 48 h (Fig. 3a). A pronounced descendent trend was observed according time elapsing. A logistic regression analysis (XLSTAT, version 2018.1) on cell viability showed a complete fit to a time-dependent effect model ($\alpha < 0.05$) (Fig. 3b).

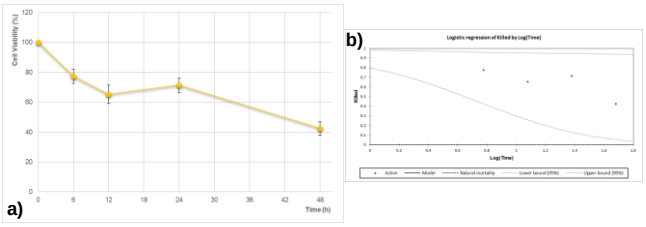


Fig. 3. Effect on cell viability by zein-based peptides at 1500 ng/ml. a) A descendent trend is observed according time elapsing. b) The regression analysis of time-cell viability results shows fit to a time-dependent effect.

Conclusions. *i)* Lethal dose of zein-derived peptides was 1500 ng/ml for HepG2 cells. *ii)* Addition of DMSO 0.1% increased cell viability in 250-3500 ng/ml. *iii)* A time-dependent effect was displayed on HepG2 cells.

Acknowledgements. Special thanks to CONACyT and Tecnologico de Monterrey for my scholarships.

References.

[1] R. L. Siegel, K. D. Miller, and A. Jemal, "Cancer statistics, 2018," *CA. Cancer J. Clin.*, 2018.
[2] C. P. and others Stewart, BWKP and Wild, "{World cancer report 2014}," *Health (Irvine. Calif).*, 2017.
[3] J. L. Díaz-Gómez, M. Ortíz-Martínez, O. Aguilar, S. García-Lara, and F. Castorena-Torres, "Antioxidant activity of Zein hydrolysates from zea species and their cytotoxic effects in a hepatic cell culture," *Molecules*, vol. 23, no. 2, 2018.
[4] K. Fosgerau and T. Hoffmann, "Peptide therapeutics: Current status and future directions," *Drug Discovery Today*, vol. 20, no. 1. 2015.

ISSN: 2448-8461

MF38

VALPROIC ACID AFFECTS DIMORPHIC TRANSITION OF *Yarrowia lipolytica*

Martha Viviana Roa Cordero[1]; Christian Alfonso Arenas[2]; María Cristina Herrera[2]; Sandra Milena Leal Pinto[1] y Juan Manuel González Prieto[3];

[1]Universidad de Santander UDES, Facultad de ciencias de la salud, Bucaramanga, Santander, Colombia; CP680003

[2]Universidad de Santander-UDES, Facultad de ciencias exactas, naturales y agropecuarias; Bucaramanga, Santander, Colombia; C.P. 680003

[3]Instituto Politécnico Nacional, Centro de Biotecnología Genómica, Laboratorio de Biotecnología Vegetal; Reynosa, Tamaulipas, México; C.P.88710 jmgonzalezp@ipn.mx

Key words: Dimorphism; Epigenenetics; Valproic acid

Introduction. Acetylation is one of the most important epigenetic mechanisms of gene expression regulation, which is mediated by histone acetyl transferases (HAT) and histone deacetylases (HDAC). The acetylation of nucleosomal histone tails is reversible and produces gene activation. Inhibitors of HDAC (HDACi) induce hyper-acetylation and therefore, gene activation. Valproic acid (VPA) is a short chain fatty acid used in pharmacological therapies due to its anticonvulsivant properties. VPA influences a broad spectrum of cellular processes in part by inhibiting class I and II HDACs (1).

Fungal dimorphism is the property of a fungi to shift from hypha to yeast depending on the environmental conditions or in response to internal stimuli (2). It is a phenomenon related to fungal pathogenesis and virulence and exist evidence that epigenetic mechanisms are involved in its regulation because their modification can reduce infectivity (3)

The objective of this work is to study the effect of histone acetylation on fungal dimorphism, taking to *Yarrowia lipolytica* P01a as study model.

Methods. We studied the kinetic growth of *Yarrowia lipolytica* P01a (C. Gallardín) in Yeast Nitrogen Base (YNB)-glucose medium with different concentrations of VPA (1.25, 2.5, 5.0, 10, 20, and 50, 70, 100 and 200 mM). The cultures were grown in flask during 40 hours with rotatory agitation at 250rpm. Cell densities were measured by cell counts stained with Trypan Blue at 8-hour intervals. Dimorphic transition was achieved as was described previously (4), Briefly, we used YNB medium supplemented withN-acetyl-D-Glucosamine (1%) instead glucose and it buffered with 100mM citric acid adjusted to pH 7.0 with saturated NaOH, both as effectors of morphogenetic switch. The VPA was added at 50mM. After 16 hours of growth, at least 200 cells were counted, discriminating between filaments and yeasts, discriminating between filaments and yeasts, after 16 hours of growth. The results were expressed as percent micelium. The data analysis was done with statistical package IBM SPSS Statistics version 25.0. We compared the average of Log_{10} Cell/mL (5 repetitions) at each point of the growth kinetics using two way ANOVA with repeated measures test. To analyze the effect of VAP on dimorphic transition independent samples T test was used (9 repetitions). In all cases, we used 99% confidence interval.

Results. We found that 50mM of VPA significantly suppressed *Yarrowia lipolytica* filamentation. In our hands, 54% of the cells germinated giving filaments compared with controls, which gave 12% ($p<0,01$). At this effective concentration, VPA reduced cell growth by 6% at 40 hours of incubation ($p<0,01$) (Fig.1).

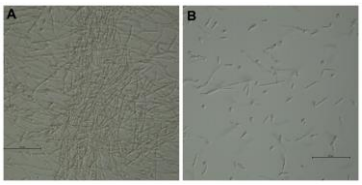


Fig.1 Yeast to hypha switch of *Y. lipolytica* under VPA effect. Negative control (A) and VPA 50mM (B)

Conclusions

Our results demonstrated that Valproic acid reduces significantly the dimorphic transition, which suggest that acetylation of histones plays a pivotal role in regulating this process in *Yarrowia lipolytica*.

Acknowledgements. This work was supported by grants from UDES (PICF0116493821748EJ) and from Instituto Politécnico Nacional (Centro de Biotecnología Genómica)

References.

1 Desfossés-Baron, K., Hammond-Martel, I., Simoneau, A., Sellam, A., Roberts, S., & Wurtele, H. (2016).Scientific Reports, 6(1). doi: 10.1038/srep36013

2. Ruiz-Herrera, J. (2012). Dubai, United Arab Emirates: Bentham eBooks.

3. González-Prieto, J., Rosas-Quijano, R., Domínguez, A., & Ruiz-Herrera, J. (2014). Fungal Genetics And Biology, 71, 86-95. doi: 10.1016/j.fgb.2014.09.002

4. Ruiz-Herrera, J., & Sentandreu, R. (2002) Arch Microbiol, 178:477–483 doi: 10.1007/s00203-002-0478-3.

ISSN: 2448-8461

MF39

BIOAVAILABILITY OF SULFORAPHANE (1-ISOTHIOCYANATE-4- (METHYLSULFINYL) -BUTANE) FROM BROCCOLI SEED, IN MICROPLATE.

Pedro Alberto Duarte Portillo, Olga Nydia Campas Baypoli, Dalia Isabel Sanchez Machado, Jaime Lopez Cervantes, Rafael Canett Romero
Centro de Investigación e Innovación Biotecnológica, Agropecuaria y Ambiental (CIIBAA), Instituto Tecnológico de Sonora. Ciudad Obregón, Sonora, C.P. 85000.
Tel: 662 345 0823. e-mail: mc_duarte_ute@hotmail.com

Keywords: Sulforaphane, Spectrophotometry, Bioavailability.

Introduction. Sulforaphane (SFN) the main bioactive compound of broccoli, has been recognized as a potent antioxidant and anticarcinogenic effect. It has been developed a method for isolate it from the broccoli seed (1). To determine bioavailability, it is necessary to perform in vivo assays. When sulforaphane is ingested and metabolized, it is conjugated with glutathione, glycine and glutamate are removed, and finally it´s acetylated to mercapturic acid, and eliminated in urine, although the isothiocyanates of these conjugates can be easily dissociated in a reversible way (2), for this reason some researchers, have validated a method for the conversion of these unstable conjugates to more stable complex to identify them more accurately by HPLC (3).

The objective of this work is to develop a method for the identification and quantification of sulforaphane metabolites through derivatization with N-terButoxy-Carbonyl-L-cysteine-methylester, for microplate, and their application in bioavailability assays.

Materials and methods. Sulforaphane extracted from broccoli seeds was used to validate the derivatization. The “In vivo” assay was done with Sprague Dawley rats, which were given a dose of sulforaphane via gastric tube. The samples were centrifuged, and 200µl of plasma and 500µl of urine were taken, and frozen. The samples were diluted and derivatizated according to Hanschen (4) and Budnowski (3), the complexes were analyzed in microplate.

Results. Figure 1 shows the spectra in the microplate that confirm the effect of the N-t-BoC-ME on sulforaphane like (3) and (4), it increases the absorbance of sulforaphane. The blood and urine average concentration of sulforaphane metabolites shows in figure 1 and 2 respectively, both show highest levels around 3-6 h, after 24 h this complex still remains.

Conclusion. N-t-BoC-ME, it´s viable to continue the bioavailability assays of isothiocyanates.

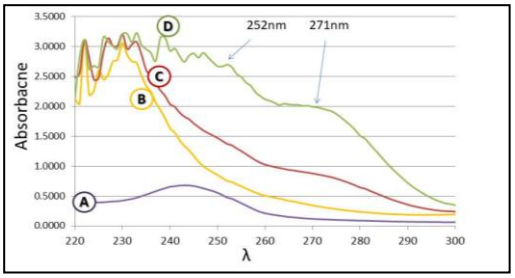


Fig.1 Effect of N-t-BoC-ME on spectra of different concentrations of sulforaphane: A) Free SFN 30µg/ml, B) N-t-BoC-ME 10mM, C) SFN 30µg/ml + N-t-BoC-ME 10mM, D) SFN 90µg/ml + N-t-BoC-ME 10mM

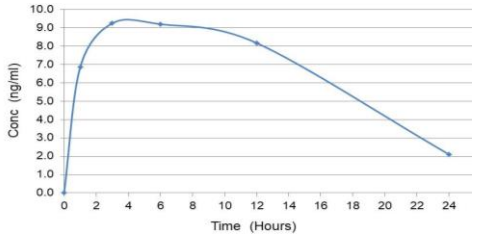


Fig.2 Blood SFN concentration, across the time

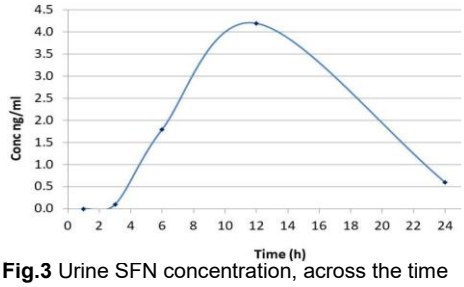


Fig.3 Urine SFN concentration, across the time

References.

1) García-Saldaña J.S.., Campas-Baypoli O.N, López-Cervantes J., Sánchez-Machado D.I., Cantú-Soto E.U., Rodríguez-Ramírez R. (2016). Food Chemistry. 201; 94-100.
2) Amin, Shankar. (2015). Life Sciences. 126; 19–27
3) Budnowski J., Hanschen F.S., Lehmann C., Haack M., Kroh L.W., Brigelius-Flohé R., Blaut M., Rohn S., Hanske L.. (2013).. Analytical Biochemistry. 441; 199–207.
4) Hanschen F.S., Bruggemann N, Brodehl-Florè R., Mewis I:, Schreiner M., Rohn S, Kroh L.W.. (2012). J. Agric. Food Chem. 60, 7735−7745.
5) Zhang Y. (2004). Mutation Research. 555. 173–19

ISSN: 2448-8461

MF40

ESTABLISHMENT OF HAIRY ROOT SYSTEM IN *PHYLLANTUS ACUMINATUS* TO PRODUCTION OF ANTICANCER COMPOUNDS

MSc. Giovanni Garro-Monge, Profesor-investigador, Centro de Investigación en Biotecnología, Instituto Tecnológico de Costa Rica. E-mail: ggarro@tec.ac.cr

Máster. Karol Jiménez-Quesada, Profesora-investigadora, Centro de Investigación en Biotecnología, Instituto Tecnológico de Costa Rica.

Key words: Phyllantus acuminatus, Hairy roots, anticancer compounds

Introduction. Plants of the genus *Phyllanthus* are currently studied for their ability to produce phenolic compounds with high antioxidant capacity, and several studies have revealed that these plants possess metabolites that among their properties can induce cellular apoptosis, which gives them a great potential for possible treatments of cancer prevention. The main objective in this work was to establishment a hairy root system of *Phyllantus acuminatus* to in vitro production of anticancer compounds (1).

Methods. *P. accuminatus* seeds were introduced *in vitro* culture of the seedlings was achieved, and the vitroplants were used in order to induce hairy roots by genetic transformation of stem segments (1-3 cm) with *Agrobacterium rhizogenes* (2) *starin* 1500 ATCC.

Results. By the methodology applied in this work, it was possible to establishment a hairy roots system of *P. acuminatus*. The best results were obtained with the treatment that included wounds in the micro-stem to facilitate the entry of the bacterium *Agrobacterium rhizogenes* strain 1500 ATCC, resulted in an average of 18.93% transformed roots. Using light microscopy, the morphological characterization of the established roots was achieved, which fully coincide with those reported in the literature through the agro-infection system. The quantification of the anticancer compounds (3) in the hairy roots was possible by using mass gas-chromatographic technique when was observed a 19.184 min retention time peak. The presence and production of bioactive compounds (Filantostatin family) in the hairy roots stablished system was very similar to obtained from the plant root tissue (3).

Fig 1. Hairy roots of *Phyllantus acuminatus* after 5 weeks of induction by *Agrobacterium rhizogenes* transformation.

Conclusions. A hairy roots system of *P. acuminatus*. Was establishment. The hairy roots grow and with morphological characteristics reported en the literature. The presence of anticancer compounds was detected by mass gas-chromatographic technique.

Acknowledgements. To Costa Rica Technological Institute for the financial support received for this work. To the LISAN Laboratories Company for the collaboration in the anticancer compounds analysis.

References.

1. Patel, R.; Tripathi, P.; Sharma, V.; Chauhan, N.; Dixit, V. 2011. *Phyllanthus amarus: Ethnomedicinal uses, phytochemistry and pharmacology: A review.* Journal of Ethnopharmcology, 138: 286-313.
2. Sevón, N., Oksman-Caldentey, K. 2002. *Agrobacterium rhizogenes-Mediated Transformation: Root cultures as a source of alkaloids*. Planta Med., 68: 859-868.
3. Esquivel, D.; Rodríguez, G.; Madrigal, S. 2013. *Evaluación de la composición química (filantósidos y lignanos) de los extractos crudos del árbol de chililllo (Phyllantus acuminatus vahl) procedente de diferentes sitios de costa Rica.* UNICIENCIA Vol. 27, No. 1, [304-318].

ISSN: 2448-8461

MF41

PRELIMINAR ANALYSIS TO OPTIMIZING THE ANTIBACTERIAL CAPACITY OF *Salinispora arenicola* BASED IN CULTURE TIME AND EXTRACTION CONDITIONS

Kimberly Ruiz-Rodríguez, Claudia J. Hernández-Guerrero, Roberto R. Navarro-Crespo, Mauricio Muñoz-Ochoa.
Instituto Politécnico Nacional-Centro Interdisciplinario de Ciencias Marinas, Departamento de Desarrollo de Tecnologías.
La Paz, BCS, México, CP 23092. kimberly_r54@hotmail.com; cguerrer@ipn.mx

Key words: Actinobacteria, Bioactivity, Extracts, Staphylococcus.

Introduction. The resistance of pathogens to antibiotics and the decrease in the efficiency of drugs are the driving force behind the development and search for new alternative antibacterial compounds(1). The secondary metabolites produced by marine bacteria have shown to be efficient against pathogens that affect human health(2, 3). Previous studies with strains of *Salinispora arenicola* at the Gulf of California have shown the presence of metabolites capable of inhibiting strains of *Staphylococcus*, which cause different diseases in humans. In the discovery of antibacterial compounds is important to develop effective strategies that involve attributes (growth, solvent extracts) that affect chemical diversity(3). The aim of this work is to evaluate different protocols of extraction at different times of the culture of *S. arenicola* to optimize the production of compounds with antibacterial activity against *Staphylococcus* pathogenic strains.

Methods. Strain of *S. arenicola* was cultured in GYM broth (Glucose, Yeast and Malt) for 35 days. Samples at the 7, 14, 21, 28 and 35 days post inoculation were taken. Subsequently, different extraction conditions were realized with three solvents, ethyl acetate (EtOAc), methanol (MeOH) and buthanol (BuOH). The antibacterial activity of the extracts against *Staphylococcus aureus* and *S. epidermidis* was performed by diffusion in agar method. Ampicilliin was used as a positive control and the negative control was the solvent of the extracts. To detect the different metabolites in the extracts the method of thin layer chromatography (TLC) was elaborated.

Results. A Venn diagram of the metabolites isolates in each solvents shows change in the compound patterns. Most of the compounds (above 66%) were isolated using EtOAc and BuOH in the extraction (Fig. 1). The culture time is another important factor in the production of the compounds. In the antibacterial activity the EtOAc extracts at 21 days post inoculation was the most active, the inhibition halos were of 29 mm against *S. epidermidis* and 26.3 mm against *S. aureus*.

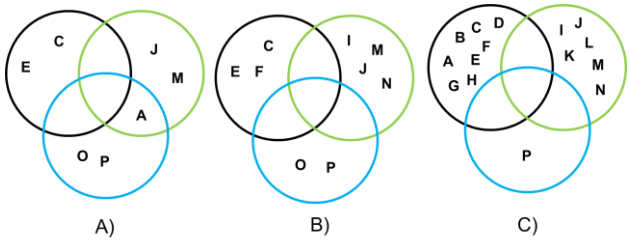


Figure1. Venn diagram for distribution of secondary metabolites signal in TLC for each solvent **EtOAc (**black) **BuOH** (green) and **MeOH** (blue). Days post inoculation A) 14, B) 21 and C) 35.

Table 1. Antibacterial activity of extracts at different culture time and extracts conditions against pathogenic strains.

	Inhibition halo (mm) with 200 µg of extract					
	Solvent					
Culture time (days)	AcOEt		MeOH		BuOH	
	S. aureus	*S. epidermidis*	*S. aureus*	*S. epidermidis*	*S. aureus*	*S. epidermidis*
7	10 ± 0	10 ± 0	13.5 ± 0.70	15.33 ± 0.57	10.5 ± 3.53	10 ± 0
14	21.66 ± 1.52	22.66 ± 1.52	10 ± 1.73	12.66 ± 1.52	13 ± 3.60	15.66 ± 1.15
21	26.33 ± 0.57	29 ± 2	11 ± 2	14.33 ± 1.15	12.33 ± 1.52	17 ± 1
28	21.33 ± 1.15	23.66 ± 1.52	9 ± 1	11.33 ± 1.52	11.33 ± 0.57	13.33 ± 1.52
35	21.33 ± 0.57	23.33 ± 2.08	10.66 ± 3.05	12.33 ± 1.52	12.66 ± 1.52	18.33 ± 1.15

Conclusions. The metabolic profile of *S. arenicola* varies according to the extraction protocol and the incubation time of the culture. Is important to use different solvents in the first extractions to ensure the greatest molecular diversity and establish the conditions to have the most metabolites with antibacterial activity.

Acknowledgements. Projects SIP-IPN 20170434 and 20181803. CJHG thanks for the support EDI and COFAA.

References.

1. Manivasagana P, Venkatesana J, Sivakumarc K and Kim S-K. (2014). *Pharmaceutically active secondary metabolites of marine actinobacteria*. *Microbiological Research.,* 169:262– 278.
2. Davies J. (2006). *Are antibiotics naturally antibiotics? J. Ind. Microbiol. Biotechnol.*, 33**:** 496-499pp.
3. Surajit-Das P, Lyla S and Ajmal-Khan S. (2006). *Marine microbial diversity and ecology: Importance and future perspectives*. *Curr. Sci*., 98: 1325-1335.
4. Crüsemann, M, O´Neill, E, Larson, C, Melnik A, Floros D, da Silva R, Jensen P, Dorrestein P, Moore B. (2016). Prioritizing natura products diversity in a collection of 146 bacterial strains base don growht and Extraction Protocols. *Natural Products*, Special Issue: 588-597.

ISSN: 2448-8461

MF42

MICROBIAL ANTAGONISM OF *PLANTACTINOSPORA* SP. BB-1 AGAINST *FUSARIUM OXYSPORUM* AND ITS BIOPROSPECTING POTENTIAL

Erika T. Quintana[1], Luis Contreras-Castro[1], Diana Itzel Vargas-Morales[1], Luis A. Maldonado[2]
[1]Instituto Politécnico Nacional, Escuela Nacional de Ciencias Biológicas, Departamento de Microbiología, Ciudad de México, Mexico, C.P. 11340. [2]Universidad Nacional Autónoma de México, Facultad de Química, Ciudad Universitaria, Ciudad de México, Mexico, CP 45000. erika_quintana@hotmail.com

Key words: Antagonism, Plantactinospora, Fusarium oxysporum.

Introduction. At present one of the main threats for biodiversity and ecosystems health are fungal infections. Emerging diseases by fungi have driven several animal species into critical risk of extinction being sea turtles one of the most endangered groups of animals. Protective microbiota have been proposed as potential conservation strategies to rescue endangered mammals and amphibians from emerging diseases. *Plantactinospora* species have been reported to be present in the microbiota in eggshells (1, 2).
The objective of the present work was to evaluate the antagonistic activity of an actinobacteria strain of the genus *Plantactinospora* isolated in sediments collected in Mexico against *Fusarium oxysporum*.

Methods. Two different methods for antagonistic activity of *Plantactinispora* against *Fusarium* oxysporum. were carried out (2). The effects of the antagonistic activity were evaluated microscopically. Foto-documentation was done in every experiment.

Results. A strong antagonistic activity of *Plantactinospora* was shown against *Fusarium* oxysporum. *Fusarium* underwent morphological changes in the presence of the actinobacteria, this is, the elongated hyphae stoped, mycelium become swollen, microconidia were minor in number and germinated conidia and macroconidia were not detected in some experiments.

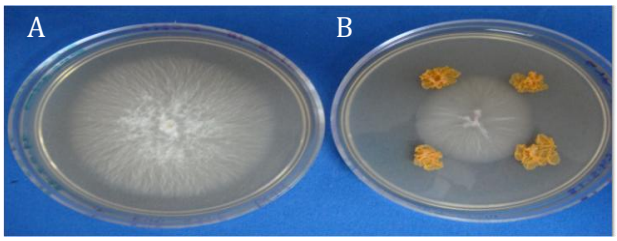
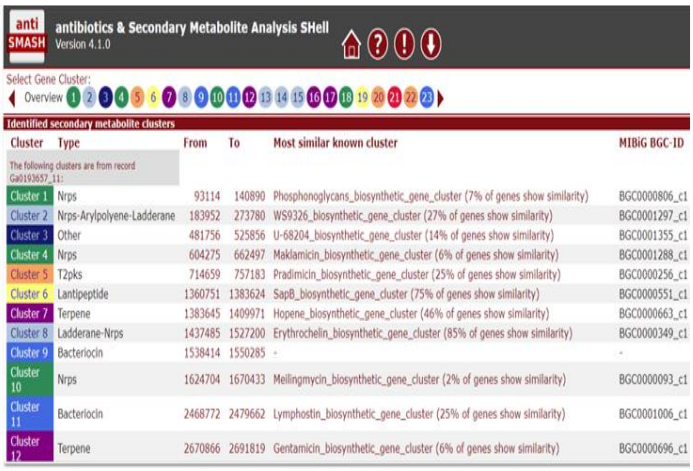


Fig.1 Microbial antagonism of *Plantactinospora* against *Fusarium oxysporum*. A) Control plate; B) bacteria against fungi.

Genome mining analysis of *Plantactinospora* genome shows the potential to produce at least two antifungal compounds.



Cluster	Type	From	To	Most similar known cluster	MIBiG BGC-ID
The following clusters are from record Ga0193657_11:					
Cluster 1	Nrps	93114	140890	Phosphonoglycans_biosynthetic_gene_cluster (7% of genes show similarity)	BGC0000806_c1
Cluster 2	Nrps-Arylpolyene-Ladderane	183952	273780	WS9326_biosynthetic_gene_cluster (27% of genes show similarity)	BGC0001297_c1
Cluster 3	Other	481756	525856	U-68204_biosynthetic_gene_cluster (14% of genes show similarity)	BGC0001355_c1
Cluster 4	Nrps	604275	662497	Maklamicin_biosynthetic_gene_cluster (6% of genes show similarity)	BGC0001288_c1
Cluster 5	T2pks	714659	757183	Pradimicin_biosynthetic_gene_cluster (25% of genes show similarity)	BGC0000256_c1
Cluster 6	Lantipeptide	1360751	1383624	SapB_biosynthetic_gene_cluster (75% of genes show similarity)	BGC0000551_c1
Cluster 7	Terpene	1383645	1409971	Hopene_biosynthetic_gene_cluster (46% of genes show similarity)	BGC0000663_c1
Cluster 8	Ladderane-Nrps	1437485	1527200	Erythrochelin_biosynthetic_gene_cluster (85% of genes show similarity)	BGC0000349_c1
Cluster 9	Bacteriocin	1538414	1550285	-	-
Cluster 10	Nrps	1624704	1670433	Meilingmycin_biosynthetic_gene_cluster (2% of genes show similarity)	BGC0000093_c1
Cluster 11	Bacteriocin	2468772	2479662	Lymphostin_biosynthetic_gene_cluster (25% of genes show similarity)	BGC0001006_c1
Cluster 12	Terpene	2670866	2691819	Gentamicin_biosynthetic_gene_cluster (6% of genes show similarity)	BGC0000696_c1

Fig.2 Clusters within the genome of *Plantactinospora* BB-1 responsible for molecules of biological activity.

Conclusions. *Plantactinospora* BB-1 showed a strong antagonistic activity against *Fusarium oxysporum* and has the genome-chemical potential to produce active antifungal compounds against it.

Acknowledgements. Authors acknowledgement Grants number SIP20170432 and SIP20181528.

References.

1. Sarmiento-Ramírez J.M, Abella-Pérez E, Phillot A.D., Slim J., van West P., Martín M.P., Marco A., Diéguez-Uribeondo J. (2014). *PLOSONE*.vol. (9): 1-9.
2. Sarmiento-Ramírez J.M., van der Voort M., Raaijmakers J.M., Diéguez-Uribeondo J. (2014). *PLOSONE*. vol. (9). 1-7.
3. Quintana E.T., Gil-Rivera D.A., Alejo-Viderique A., López-Villegas O., Maldonado L.A. (2015). *J Pharm Biomed Sci*. vol(1). 867-876.

ISSN: 2448-8461

MF43

ANTI-BACTERIAL ACTIVITY OF QUINOXALINES

Emanuel Fernández Ramírez[1]; Lucero Catalán González [1]; Alejandra Loera Piedra[1 y 2]; Gildardo Rivera[3]; Carol M. Romo-Barrera[1]; Jorge I. Castañeda-Sánchez[4]; Ma. Guadalupe Aguilera-Arreola[2]; Julieta Luna-Herrera[1].

[1]Immunology Department; [2]Microbiology Department. Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico City 11340. [3]Centro de Biotecnología Genómica, Instituto Politécnico Nacional, Reynosa City 88700. [4]Universidad Autónoma Metropolitana, Xochimilco, México City, 04960.
email address: g.u.ero69@hotmail.com

Key words: Key words: Quinoxalines, Mycobacterium, Staphylococcus aureus

Introduction. Currently, communicable diseases are responsible for high mortality and morbidity rates in the world. Despite the scientific advances, at present still around 25% of deaths worldwide are directly related to an infectious disease (1). The appearance and spread of resistance to commonly used antibiotics, such as multidrug resistance in tuberculosis (MDR, XDR), and nosocomial and community-based multi-resistant gram-positive infections, are circumstances that point to the development of new treatments for these diseases.

The goal of this study was to evaluate the activity of new Quinoxalines against *Mycobacterium tubercul osis* (MTB) and *Staphylococcus aureus* isolated from nosocomial settings from México City.

Methods. A group of quinoxaline derivatives (nearly 80) was synthetized and evaluated against the reference strain of MTB H37Rv, against some clinical isolates of MTB, and against some non-tuberculosis mycobacteria (NTM). Latter, the cytotoxicity of the most active compounds was evaluated *in vitro* in the murine macrophage cell line J774A.1 model. The IC50 (inhibitory concentration at 50%) was determined by the probit regression test and the security index (SI), was determined. The same quinoxaline derivatives were tested by the microdilution test against a group of *S. aureus* from nosocomial origin (from México City) and three *S. aureus* ATCC reference strains.

Results. The group of mycobacteria was more sensitive to quinoxaline than the *S. aureus* group. The MICs for most of the compounds were >250 μg/ml, the most active compound reached a MIC of 15.625 μg/ml (Table 1). In contrast, 18% of quinoxalines, mainly from the T group, presented antimycobacterial activity at very low concentrations ranging from 0.5-2 [μg / mL], which point them as excellent candidates for additional evaluations such as cytotoxicity (IC50%) and safety index (SI) (Table 2).

Table 1. *In vitro* activity of quinoxaline from the T series against *S. aureus* clinical isolates and reference strains.

S. aureus	Serie T (μg/mL)						
	6	18	22	69	85	88	89
IIH4	>250	>250	>250	>250	31.25	>250	>250
IIH6	>250	>250	>250	>250	>250	>250	>250
IIH8	>250	>250	>250	>250	62.5	>250	>250
IIH17	>250	250	>250	>250	15.625	>250	125
IIH20	>250	>250	>250	>250	>250	>250	>250
IIH25	>250	>250	>250	>250	125	>250	>250
IIH29	>250	>250	>250	>250	125	>250	>250
IIH30	>250	>250	>250	>250	62.5	>250	>250
IIH64	>250	>250	>250	>250	>250	250	>250
IIH119	>250	>250	>250	>250	31.25	>250	>250
IIH139	>250	>250	>250	250	15.625	>250	62.5
ATCC 12598	>250	>250	>250	>250	>250	>250	>250
ATCC 6538	>250	>250	>250	>250	>250	>250	>250
ATCC 121715	>250	>250	>250	>250	62.5	>250	>250

Tabla 2. Antimycobacterial activity of the most active quinoxaline compounds

Compuesto	MIC (μg/mL)	IC 50%	SI (IC50%/MIC)
DBCGPP-12	6.25	140.53	22.48
DBAZDHP-13	12.5	68.24	5.45
T-006	2	114.34	57.17
T-011	0.5	1.67	3.34
T-018	1	35.37	35.37
T-022	0.5	119.1	238
T-069	1	41.26	41.26
T-085	1	45.42	45.42
T-088	0.5	221.29	442.58
T-089	0.5	24.27	48.54
T-003	1	109.36	109.36
T-010	1	17.52	17.52
T-013	0.5	187.89	375.78
T-039	1	17.37	17.37
T-042	1	94.2	94.2
T-043	12.5	78.9	6.31

Conclusions. Quinoxaline oxides are excellent candidates for the development of new antimycobacterial agents, contrary to *S.aureus,* which were highly resistant.

Acknowledgements. This study received financial support from the Multidisciplinary project SIP-1829 from the Instituto Politécnico Nacional.

References.

1.-Soto-Estrada G., Moreno-Altamirano L, Pahua-Diaz D. (2013). Revista de la Facultad de Medicina de la UNAM. Vol 59, No. 6.pp 8-22

ANTI-NOCARDIA ACTIVITY OF QUINOXALINES

Susana Basurto-Hernández[1]; Gildardo Rivera[2]; Carol M. Romo-Barrera[1]; Jorge I. Castañeda-Sánchez[3]; Ma. Guadalupe Aguilera-Arreola[4]; Julieta Luna-Herrera[1].

1) Immunology Department; 4) Microbiology Department. Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico City 11340. 2) Centro de Biotecnología Genómica, Instituto Politécnico Nacional, Reynosa City 88700. 3) Universidad Autónoma Metropolitana, Xochimilco, México City, 04960.
email address: susanbash.sb@gmail.com

Key words: Key words: Quinoxalines, Microplate assay, Nocardia brasiliensis

Introduction. *Nocardia brasilensis* causes actinomycetoma, which produces mainly chronic subcutaneous lesion on the feet. Mycetoma has serious implications for human-health around the globe (1). There are few antimicrobials active against *N. brasiliensis*, so it is important to discover new compounds to improve micetoma treatment. For that a group of new compounds derived from the quinoxaline moiety, were tested in an *in vitro* microassay bases on the reduction of alamar blue.

The goals of this study were: 1) to standardize an *in vitro* assay for a simply and rapid anti-nocardia determination and 2) to test the anti-nocardia activity of a group of newly synthetized quinoxaline analogs against a reference strain of *N. brasiliensis* and several clinical isolates of *N. brasiliensis*.

Methods. A microplate assay was developed to test the susceptibility of *N. brasiliensis* against a group of quinoxaline derivatives (2). The Minimal inhibitory concentration (MIC) was established as the lowest concentration that inhibited 99% of the microbial growth. The reference *N. brasiliensis* strain CECT-3052 and seven Mexican clinical isolates of *N. brasiliensis* were used in the study.

Results. Around 80 quinoxaline derivatives were tested for their antimicrobial activity. The antimicrobial activity of some reference compounds was established also (Table 1). The most active series of quinoxaline derivatives are presented in tables 2 and 3.

Table 1. *In vitro* activity of some drugs recommended for primary testing using MABA against *Nocardia brasiliensis* strain CECT-3052.

Drug	MIC (µg/ml)
Trimethoprim/Sulfamethoxazole	0.12/1.18
Amikacin	0.25
Linezolid	0.12
Tobramycin	0.12

Table 2. *In vitro* activity of isopropyl and n-propyl quinoxaline derivatives determined by the microplate assay against *Nocardia brasiliensis*

CODE	R_1	R_2	R_3	MIC (µg/ml)						
				CECT-3052	N100	N200	N300	N400	N600	N700
T-069	CO-thienyl	CF_3	$(CH_3)_2CH$	0.25	0.5	0.25	0.25	1	0.5	0.25
T-085	$COCH(CH_3)_2$	CF_3	$(CH_3)_2CH$	0.25	1.56	1	2	1.56	2	2
T-088	$COCH_3$	CH_3	$CH_3CH_2CH_2$	1	3.12	0.25	3.12	3.12	3.12	6.25
T-089	CO-thienyl	CF_3	$CH_3CH_2CH_2$	0.25	0.5	0.5	0.5	0.25	2	0.25

Table 3. *In vitro* activity of methyl and ethyl quinoxaline derivatives determined by the microplate assay against *Nocardia brasiliensis*

CODE	R_1	R_2	R_3	MIC (µg/ml)						
				CECT-3052	N100	N200	N300	N400	N600	N700
T-003	COC_6H_5	CH_3	CH_3	6.25	50	25	25	25	12.5	ND
T-006	$COCH_3$	CF_3	CH_3CH_2	0.5	0.5	1	2	1	2	ND
T-011	CO-thienyl	CF_3	CH_3CH_2	0.062	0.25	ND	ND	0.25	0.12	ND
T-013	$COOCH_2CH_3$	CH_3	CH_3	3.125	6.25	25	6.25	50	6.25	ND
T-018	CO-phenyl	CF_3	CH_3	0.25	0.5	0.5	1	0.5	0.5	ND
T-022	$COOCH_3$	CH_3	CH_3	0.5	2	3.125	<1.56	6.25	<1.56	<1.56
T-039	$COCH(CH_3)_2$	CF_3	CH_3	0.5	1.56	1.56	1.56	2	2	ND
T-042	$COOCH_2CH_3$	C_6H_5	CH_3	6.25	12.5	6.25	3.125	12.5	3.125	6.25

ND= Not determined

Conclusions. All compounds of the methyl, ethyl, isopropyl, and n-propyl series show good anti-*N. brasiliensis* activity. Quinoxaline oxides are excellent candidates for the development of new anti-nocardia agents.

Acknowledgements. This study received financial support from the Multidisciplinary project SIP-20180411. JLH, GR, MGAA are COFAA, SNI and EDI fellows. JICS is SNI fellow. CMRB is a BEIFI fellow.

References.

1. Saubolle M.A. & Sussland, D. (2003). Nocardiosis: review of clinical and laboratory experience. *J. Clin. Microbiol.*, Vol. 41: 4497-4501

2 Woods G., et al., (2011) *Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes 2nd edition.* Clinical and Laboratory Standards Institute. Vol. 31, No. 5: 29-43.

.

ISSN: 2448-8461

VE1

MORPHOLOGICAL CHARACTERIZATION OF TWO AUTOCHTHONOUS ISOLATES OF THE ENTOMOPATHOGENIC FUNGUS *BEAUVERIA BASSIANA* FROM CUBAN FIELDS

Sandra Pérez Álvarez[1], Alianna Machín Suárez[2], Elio Minel del Pozo Núñez[2], Irma García Cruz[2], Jesús Alicia Chávez Medina[1], Marco Antonio Magallanes Tapia[1]

[1] Instituto Politécnico Nacional, CIIDIR-IPN, Unidad Sinaloa, Departamento de Biotecnología Agrícola, Blvd. Juan de Dios Batiz Paredes No 250. Guasave, Sinaloa, C.P. 81101 México, perezalvarezsandra2015@gmail.com

[2] Universidad Agraria de la Habana "Fructuoso Rodríguez Pérez", Departamento Biología y Sanidad Vegetal, Carretera Tapaste y Autopista Nacional, San José de las Lajas, Mayabeque, C. P.32700, Cuba, alianne_machin@unah.edu.cu

Key words: Agriculture, biological control, entomopathogen

Introduction. Several species of entomopathogenic fungi, especially *Beauveria bassiana* (Balsamo) Vuillemin, have a wide range of hosts, such as Coleoptera, Lepidoptera and Diptera. *B. bassiana*, like many other entomopathogenic fungi, shows a wide intraspecific variability. The knowledge of the intraspecific variability allows the fungi manipulation to obtain environmentally safe isolates and strains, to characterize the isolates, as well as to produce certain biopreparations. It is necessary basic biological research particularly in the fields of taxonomy, ecology, behavior, population dynamics, population genetics and modelling to the improvement of procedures used in biological control agent exploration (1). Taking into account the elements mentioned above, morpho-ecological, and pathogenicity criteria should be considered as a starting point towards the precise differentiation of *B. bassiana* isolates with potential for the management of pests of agricultural interest.

Methods. The B_N and B_B isolates were collected from *Atta insularis* (Güerin) (Hymenoptera: *Formicidae*) and adults of the coffee borer (*Hypotenemus ham pei* (Ferrari) (Coleoptera: *Scolytidae*) respectively, in ecosystems (municipalities) of the Cuba, Mayabeque province. The strain LBB-1 was procured by the Entomophagous and Entomopathogenic Reproduction Center. The isolates B_N and B_B and the strain LBB-1 were cultivated in the Sabouraud Dextrose Agar medium enriched with 1% of yeast extract (SDAY) and characterized macro and microscopically (2). The average and standard deviation for each isolate were calculated, expressing the results in micrometers (µm).

For the biological effectiveness of the isolates B_N, B_B and strain LBB-1 of *B. bassiana* on *Cylas formicarius* Fabricius, under laboratory conditions adult insects of *Cylas formicarius* (Fabricius) were collected in sweet potato fields (*Ipomoea batatas* (L.) Lamk) without biological or chemical treatments. Then, they were submerged for one minute into a conidial suspension with Tween 80 (0.01%) at 10^7 conidia mL^{-1} for each isolate and they were transferred into jars with a diet based in sweet potato. Since day 3 or 4 the dead insects were collected daily until the growth of the fungus appeared on the body surface. The mortality data was obtained through direct observation method at seven-day.

Results. Morphological characterization of B_N, B_B isolates and the strain LBB-1 of *B. bassiana*

Figure 1 show the macroscopic characteristics of the isolates B_N, B_B and the strain LBB-1.

Figure 1. Colonies of the isolates from *B. bassiana* after 15 days of inoculation in culture medium**. A**: B_N; **B**: B_B; **C**: LBB-1 strain.

The average size of the conidia varies from 2.7 ± 0.20 to 2.9 ± 0.40 µm long and 2.3 ± 0.4 to 2.4 ± 0.30 µm wide (Table 2). The conidia of B_N are smaller and those of B_B have a longer size.

The biological effectiveness of the isolates B_N, B_B and strain LBB-1 of *B. bassiana* on *Cylas formicarius* Fabricius under laboratory conditions demonstrated intraspecific variability, maybe because different isolates of the same entomopathogenic microorganism usually present differences in their virulence on the same insect, which is explained by the genetic diversity found in these fungi

In general, the mortality responses were different among the different isolates in this study, evidencing the great biological diversity that this entomopathogenic fungi presents.

Conclusions

In this research, all the morphological characteristics studied stands out the isolate B_N with growth speed and conidia production of 3,29 $mm.día^{-1}$ and yield greater than 10^8 conidia mL^1, respectively.

The biological effectiveness on *C. form icarius* of both isolates B_N caused a mortality higher than 60 %.

References

1- Barrat BI, Moran VC, Bigler F, Van Lenteren JC. (2018). *BioControl*. 63: 155-167.

2- Martins CC, Alves LFA, Mamprim AP, Souza LPA. (2015). *Braz J Biol.* 76 (3): 629-637.

ISSN: 2448-8461

VE4

BIOINSECTICIDA MICROENCAPSULADO A BASE DE HONGOS ENTOMOPATÓGENOS PARA EL CONTROL DE *HELIOTHIS VIRESCENS* (FABRICIUS)

Yareli Padilla-Mendoza, Cipriano García-Gutiérrez.
Laboratorio de Bioinsecticidas. CIIDIR COFAA-IPN Unidad Sinaloa. Guasave, Sinaloa, México C.P. 81049
garciaciprian@hotmail.com.

Key words: Microencapsulate, bioinsecticide, Heliothis virescens

Introduction.

En Sinaloa el gusano del fruto *Heliothis virescens* (Fab) es una plaga polífaga que daña al fruto del tomate y garbanzo (**Fig 1)**. Los hongos entomopatógenos (HE) como *Beauveria bassiana* (Vuil.) y *Metarhizium anisopliae* (Mestch) pueden ser utilizandos en el control de esta plaga (1) Acuña (2) microencapsuló *M. anisopiae* con la finalidad de extender la vida de anaquel de las esporas, propiedad deseable en el desarrollo de un bioinsecticida para el control de esta plaga, encontrando baja viabilidad de esporas del producto formulado.

Por lo anterior, el presente trabajo tuvo como objetivo elaborar un microencapsulado a base del hongo *M. anisolpiae* y evaluar su actividad insecticida sobre larvas del tercer ínstar de *H. virescens.*

Methods. Se utilizó la cepa CIDSM01 de *M. anisopliae* ($1x10^8$ conidios/mL) patogénica para larvas del tercer ínstar de *H. virescens*, la cual se uso para elaborar un microencapsulado mediante el método de secado por aspersion (3) con una temperatura de entrada/salida de 60 y 31 °C. Se evaluó la patogenicidad y tiempo de mortalidad de larvas del insecto con el microencapsulado **(tabla 1).**

Results. La prueba de patogenicidad de estos hongos contra L3 de *H. virescens* fueron diferentes al control (0.05). En el hongo sin formular la mortalidad de larvas fue de 33% a las 48 h. La mortalidad de los insectos en CIDSM01 fue de 21.14%, iniciando a las 96 h. Este resultado demostró la actividad insecticida del ingrediente activo en el microencapsulado.

Fig.1. *H. zea* en laboratorio a) dieta, b) Larva , c) Pupa y d) adulto

Table 1. Patogenicidad de HE sobre L3 de *H.virescens*

Cepa	Patogenicidad %	Tiempo H
M.anisopliae control	33	48
M.anisopliae CIDSM01 microencapsulado	21.14	96

Conclusions. Las cepas de *M.anisopliae* fueron patogénica para *H. virescens*. El método de secado por aspersión es una alternativa como método de conservación de *M. anisopliae* (CIDSM01) manteniendo actividad insecticida.

Acknowledgements. Proyecto SIP-IPN: clave 20060101

References.

1. Archuleta T.A. 2012. Tesis de Maestría: Caracterización morfológica y molecular de *Beauveria bassiana* (Balsamo Vuillemin y *Metarhizium anisopliae* (Metsch.) Sorokin de suelos cultivados y no impactados en el norte de Sinaloa. Instituto Politécnico Nacional. 83 pp

2. Acuña J. M., García G. C., Rosas G. N., López M. M. y Sainz H. J. 2015. Formulación de *Metarhizium anisopliae* (Metschnikoff) Sorokin con polímeros biodegradables y su virulencia contra *Heliothis virescens* (Fabricius). Revista Internacional Contaminación Ambiental 31 (3) 219 - 226

3. Chin-Piao L. y Shan-Da L. 2009. Low temperatura Spray Drying for the Microencapsulation of the Fungus Beauveria basssiana. Drying technology. 27 (6): 747 – 753

ISSN: 2448-8461

VE5

SCREENING OF SEAWEEDS FROM SELECTED AREAS OF VERACRUZ ROCKYSHORE, MEXICO, AS POTENTIAL SOURCES OF INHIBITORS OF DIGESTIVE HYDROLASE ENZYMES

Cristina Landa-Cansigno[1,*], Eric Edmundo Hernández-Domínguez[1], Juan Luis Monribot-Villanueva[1], Luz Elena Mateo-Cid[2], Aldo Segura-Cabrera[1], Alexei F. Licea-Navarro[3], José Antonio Guerrero-Analco[1,*]
[1]Instituto de Ecología A. C., Red de Estudios Moleculares y Avanzados, Laboratorio de Química de Productos Naturales, Xalapa, 91070; [2]Instituto Politécnico Nacional, Escuela Nacional de Ciencias Biológicas, Laboratorio de Ficología, Mexico City, C.P. 11340; [3]Centro de Investigaciones Científicas y Estudios Superiores de Ensenada, División de Biología Experimental y Aplicada, Ensenada C. P. 22860. e-mail: bio.lancan@gmail.com; joseantonio.guerrero@inecol.mx

Keywords: α-amylase inhibitor, α-glucosidase inhibitors, Marine Natural Products

Introduction. Seaweeds natural products (SNP) are sources of secondary metabolites which may act as potential α-amylase and α-glucosidase inhibitors [(1)]. These enzymes are carbohydrates hydrolyzers and play a key role in postprandial blood glucose levels regulation[(2,3)]. Nonetheless, in Mexico, seaweeds are an undervalued resources and their chemical potential to inhibit these enzymes is still unknown.
Therefore, the objective of this work was to perform a screening of Mexican seaweeds as potential sources of inhibitors of α-amylase and α-glucosidase.

Methods. Seaweeds were collected on rainy (September 2016) and dry (May 2017) seasons in selected rocky shores of Veracruz, Mexico. Fresh seaweeds were rinsed with running water and dried by lyophilization. A duplicate of algae were fixed with formaldehyde solution (0.4%) for their taxonomic determination. After total dryness, milled seaweeds were extracted by maceration with methanol (1:10, w/v). Concentrated crude extracts (CE-MeOH) were evaluated in α-glucosidase[(4)] and α-amilase[(5)] inhibitory assays; acarbose was used as drug control in both assays. For active extracts, a toxicity assay on *Artemia salina* L. and chemical profiling based on accurate mass spectrometry (LC-MS-HRMS-QTOF) were carried out.

Results. A total of 51 CE-MeOH from 33 species of Rhodophyta, Chlorophyta and Phaeophyceae were obtained. CE-MeOH bioactivities had differences among species, season and site collects (p <0.05). Four species showed significant inhibitory activity on both assays (Table 1), while others showed less than 30% of inhibition or not activity. The LD_{50} ($mg \cdot mL^{-1}$) observed in toxicity assays in decrement order were *Cladophora* sp. (0.037 ±0.001) > *Ectocarpus* sp. (1.007 ±0.001) > *Padina* sp.1 (1.57 ± 0.001) > *Padina* sp.2 (22.569 ± 0.003). Chemical profiling suggests the presence of primary and secondary metabolites in Phaeophyceae CE-MeOH (Table 2).

Table 1. Percentages of enzymatic inhibition by CE-MeOH.

Specie	α-glucosidase	α-amylase
***Cladophora* sp.**	99.87 (± 0.35)	102.76 (±1.57)
***Padina* sp. 1**	96.14 (± 0.61)	35.59 (± 0.71)
***Padina* sp. 2**	99.95 (± 0.32)	78.64 (±12.68)
***Ectocarpus* sp.**	100.1 (± 0.18)	83.87 (±0.62)
Acarbose	88.58 (± 0.25)	100.27 (±0.13)

Data are the mean of 3 replicates (± SD) expressed as a percentage (%).

Table 2. Compounds tentatively identified in CE-MeOH.

Compound	*Padina* sp.1	*Padina* sp.2	*Ectocarpus* sp.
Mannitol		+	
Fucoxanthine	+		
Cystoseirol			+
Sargachromanol			+
Linoleic acid	+		+
Nonadecanoic acid	+		+
Palmitic acid	+		+

Compounds tentatively identified based on their high resolution mass spectra (error < 5ppm) and by comparison with public databases.

Conclusions. This is the first report about Mexican seaweeds as natural sources of inhibitors of digestive hydrolase enzymes. Since crude extracts are complex mixtures, a bioassay-guided fractionation approach should be considered in order to identify the active principles and to increase the chemotaxonomic knowledge of SNP.

Acknowledgements. To CONACyT-Mexico for the PhD scholarship, INECOL, CICOLMA-INECOL & ENCB-IPN. Special grateful to M.C. Israel Bonilla Landa, and to the students from the Laboratory of QPN-INECOL.

References.

1. Sharifuddin Y, Yao-Xian C, Phaik-Eem L, Siew-Moi P. (2015). *Mar Drugs*. 13:5447-5491.
2. Bischoff H. (1994). *Eur J Clin Invest*. 24(3):3-10.
3. Mahmood N. (2016). *Comp Clim Pathol*. 25:1253-1264.
4. Kellog J, Grace M, Lila M. (2014). *Mar Drugs*. 12:5277-5294.
5. Gella F, Gubern G, Vidal R, Canalias F. (1997). *Clin Chim Acta*. 259:147-60.

ISSN: 2448-8461
16-20 Octubre/October 2018
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
San José del Cabo, B.C.S., México

VE6

ENHANCED PRODUCTION OF PODOPHYLLOTOXIN BY HAIRY ROOT CULTURES OF HYPTIS SUAVEOLENS

Crescencio Bazaldúa Muñoz[1,2], Alexandre Toshirrico Cardoso Taketa[2], María Luisa Villarreal Ortega[2]. [1]Centro de Desarrollo de Productos Bióticos–IPN, Yautepec, Mor., C.P. 62731. [2]Centro de Investigación en Biotecnología-UAEM, Cuernavaca, Mor., C.P. 62209. cbazaldua@ipn.mx

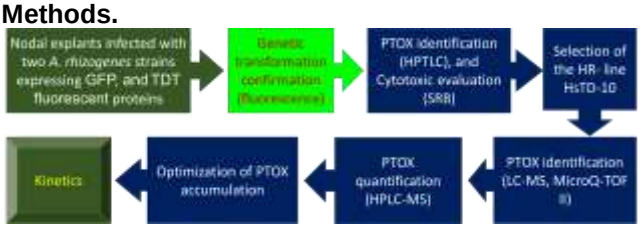
Author's names (first and last) in font Arial 10; Name of the Institution(Including department or faculty), city and area code; email of the person responsible for presenting the work (first and last name underlined) all centered

Key words: Podophyllotoxin, Hairy roots, Tiamine

Introduction. Podophyllotoxin is a natural 2,7'-cyclolignan used to obtain the semi-synthetic derivatives Etoposide®, Teniposide® and Etopophos®, medicines widely used in cancer chemotherapy. The annual PTOX production is 50-80 tons; However, its demand exceeds 100 tons per year [1]. The search for new natural sources that produce PTOX is mandatory, as well as the research of biotechnological alternatives to achieve stable and controlled production of this compound. It was recently reported that Hyptis suaveolens produces podophyllotoxin [2].

The objective of this work was to select one high PTOX-producer hairy root line of *Hyptis suaveolens* to explore the best conditions to increase the accumulation of PTOX.

Methods.



Results.

At the beginning it was achieved the establishment of ten hairy roots lines and the selection of one a highly PTOX producer HR-line. Genetic transformation confirmation was done by observing green or red fluorescence. The identification of PTOX was done by HPLC/MS.

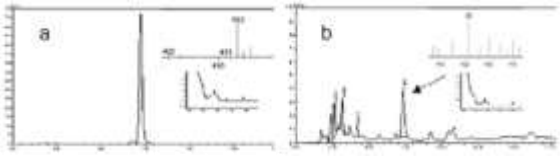
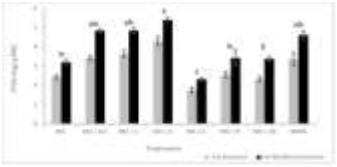


Fig 1. PTOX identification, a)HPLC chromatogram of PTOX, retention time (RT) of 7.22, as well as, molecular ion (m/z 413 negative mode, b) chromatogram from HsTD-10 HR-line, RT-peak and molecular ion (m/z 413 corresponds to PTOX

The culture medium and the method of extraction influenced the concentration of PTOX (table). It has been reported that thiamine increases the concentration of phenylpropanoids [3]. When adding thiamine to 75% MS, there was not significant difference in biomasses, but it was a difference in the PTOX concentration.

Culture medium	Concentration of PTOX (mg g^{-1} DW) Extraction method	
	Chloroform	Methanol- Dichloromethane (Sonication at 40 ± 5°C)
MS	1.52 ± 0.016	2.89 ± 0.033
MSB5	0.63 ± 0.013	4.50 ± 0.032
B5	0.58 ± 0.05	3.24 ± 0.039

Quantification of PTOX in the HsTD-10 HR-line grown on three different media at 75% of concentration. Two methods of extraction were evaluated



Influence of thiamine in the concentration of PTOX (HsTD-10 HR-line). Comparison of Koulman method of extraction [4], and its modification

During kinetic growth of the HsTD-10 HR-line suspension cultures the highest biomass was achieved at day 40 (7.2 g DW L^{-1}), and it was 9.34 times higher than the inoculum. The mean specific growth rate value (μ)= 0.11 d^{-1}. Accumulation of PTOX was growth-associated.

Conclusions. The culture medium does not affect significantly the biomass increase, but it is determinant in the accumulation of PTOX in hairy roots of *Hyptis suaveolens*. Thiamine modulates the production of PTOX. The extraction with sonication at 40 ± 5°C increases the concentration of PTOX in HR of *H. suaveolens.* The PTOX accumulation in hairy roots of *H. suaveolens* is growth-associated.

Acknowledgements. This work was supported by CONACYT (Project 222714). Crescencio Bazaldua acknowledges National Polytechnic Institute for grant of exclusivity.

References.

1. Amal A, Naik PK, Mishra GP. *Turk. J. Bot.* 2009, 33: 1-12
2. Lautié E, Quintero R, Fliniaux M-A, Villarreal M-L. *J. Ethnopharmacol*. 2008, 120: 402-412
3. Abdel-Monaim Montaser Fawzy. *Afr. J. Biotechnol*. 2011 10(53) 10842-10855
4. Koulman A, Kubbinga ME, Batterman S, et al. *Planta Med*. 2003. 69: 733-738

ISSN: 2448-8461

VE7

ARE THE PLANT SPECIES FROM MOUNTAIN CLOUD FOREST OF VERACRUZ, MEXICO, POTENTIAL SOURCES OF NOVEL ANTIFUNGAL AGENTS AGAINST AMBROSIA BEETLES' SYMBIONTS?

Freddy Tornero-Conde[1], Cristina Landa-Cansigno[1], Diana L. Murrieta-León[1], Thalía Ramírez-Reyes[1,2], Ángel S. Aguilar-Colorado[1], Djaheli Luna-Acosta[1], Laura S. Licona-Velázquez[1], Sergio Avendaño-Reyes[1], Juan L. Monribot-Villanueva[1], Óscar Carmona-Hernández[3], José A. Lozada-García[3], José A. Guerrero-Analco[1,*].
[1]Instituto de Ecología A. C., Red de Estudios Moleculares Avanzados, Laboratorio de Química de Productos Naturales, Xalapa, 91070; [2]CONACyT-Universidad Tecnológica de la Mixteca, Huajuapan de León, Oaxaca México, 69000; [3]Universidad Veracruzana, Facultad de Biología, Xalapa, 91000; *e-mail: joseantonio.guerrero@inecol.mx

Key words: Antifungal Natural Products, Mountain Cloud Forest, Fusarium Dieback

Introduction. Ambrosia beetles *Xyleborus glabratus* and *Euwallacea* sp. are invasive species in North America and vectors of Laurel wilt and Fusarium dieback diseases, respectively. Among the tree hosts are 138 plant species including avocado. Both infections are caused by the pathogenic beetles' fungal symbionts named *Raffaelea lauricola* and *Fusarium euwallaceae*, which provoke disruption of water and nutrients flows, causing death of trees in few weeks. The current detection of a closely related *Euwuallacea* species in Tijuana, Mexico, represents an urgent need to control the spread of the diseases transmitted by ambrosia beetles (1). As part of an unprecedent interinstitutional collaborative project financed by the FORDECyT-CONACyT to the Scientific and Technological BioMimic® Cluster to mitigate the impact of these pests, we have initiated a bioprospecting research program to evaluate the antifungal potential of endemic plants species from the mountain cloud forest of Veracruz (MCFV). Along with its ecological role, this ecosystem has also been considered a "hot-spot" for searching novel bioactive natural products (NP) due mainly to its high biodiversity (2,3).
Accordingly, the first objective of our program is to evaluate the antifungal potential against pathogen models in laboratory conditions and furthermore the most active species will be tested against Ambrosia beetles' symbionts for developing sustainable alternatives to control them.

Methods. Plant materials (aerial parts) were collected at the protected area "Santuario del Bosque de Niebla" of INECOL (Xalapa, Ver.) in July 2016. Herborized duplicate samples were deposited at Herbarium-XAL for their taxonomic identifications. Crude extracts (CE) were obtained by maceration in MeOH of dried milled plants (1:10 ratio w/v). All CE (2 mg/mL) were evaluated for antifungal activity against the pathogen fungi *F. solani*, *F. verticillioides* and *F. oxysporum* by the method of inhibition of mycelial growth. The identification and quantification of potential antifungal compounds were carried out by liquid chromatography and mass spectrometry with a total of 80 NP compounds database (in house analytical method).

Results. A total of 19 species from 16 genus were screened for antifungal activity. The botanical genus included species from: *Hoffmania*, *Palicourea*, *Ocimum*, *Citharexylum*, *Cestrum*, *Solanum*, *Chamaedorea*, *Cinnammun*, *Ocotea*, *Persea*, *Nectandra*, *Piper*, *Turpinia*, *Malvaviscus*, *Pavonia*, *Leandra* and *Sticherus*. The CE of the endemic *Piper* sp. showed a significant inhibition against *F. solani* (74%), *F. oxisporum* (72%) and *F. verticilloides* (67%) strains. The analysis of *Piper* sp. CE displayed a total of 12 compounds (Table 1).

Table 1. Identified compounds in the CE of endemic *Piper* sp.

Compounds		
p-coumaric acid	Caffeic acid	Protocatechuic acid
4-hidroxybenzoic acid	Chlorogenic acid	Sinapic acid
4-hidroxyphenylacetic acid	Ferulic acid	Trans-cinnamic acid
Kaempferol	Gentisic acid	Vanillic acid

Conclusions. Endemic plants from MCFV could be considered as novel sources for the development of potential antifungal agents to prevent damages from Ambrosia beetles' symbionts because they inhibit well-known pathogen growth models. Identified compounds of *Piper* sp. have not been previously described in this species and some of them may be responsible of the antifungal activity at the extract level. Interestingly, plant species from Solanaceae family, which are known to possess antimicrobial properties did not display any antifungal activity in our assays.

Acknowledgements. To FORDECyT-CONACyT grant #292399.

References.

1. Eskalen A, Gonzalez A, Wang DH, Twizeyimana M, Mayorquin JS. (2012). *Plant disease*. 96(7): 1070.
2. Pusztahelyi T, Holb IJ, Pócsi I. (2015). *Fron Plan Sci*. 6: 573.
3. Williams-Linera G. (2015). *Maderas y bosques*. 21: 51-61.

ISSN: 2448-8461
16-20 Octubre/October 2018
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
San José del Cabo, B.C.S., México
VE8

VARIATION IN PHENOLIC PROFILING OF *FOUQUIERIA SPLENDENS* ENGELM. (FOUQUIERIACEAE) ROOTS DURING DIFFERENT PHENOLOGICAL STAGES

Hugo Manuel Monreal-García*, Norma Almaraz-Abarca, José Antonio Ávila-Reyes, Rene Torres-Ricario, Marcela Verónica Gutiérrez-Velázquez, Andrés Vasavilbazo-Saucedo, Ana Isabel Chaidez-Ayala.

Instituto Politécnico Nacional, Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Unidad Durango, Sigma 119, Fraccionamiento 20 de noviembre II, 34220, Durango, Dgo., México, e-mail: hugo.monreal@gmail.com

Key words: Fouquieria splendens, phenolics, HPLC-DAD

Introduction. Plants are a significant source of bioactive compounds of pharmacological importance, such as polyphenols. Flavonoids and phenolic acids are the most important groups of polyphenols and its consumption are related to beneficial effects on human health [1]. *Fouquieria splendens* (ocotillo) is a well distributed species of the arid and semi-arid zones of Mexico and the southwestern United States, its root was used to treat wounds and painful swellings [2]. Currently, there are no reports about the phytochemical composition of *F. splendens* roots. Studies on this topic would provide support about its use in folk medicine. The aim of the present study was to analyze, by HPLC-DAD and spectrophotometric techniques, the variation of root phenolic composition of *Fouquieria splendens* subs. *splendens* across different phenological stages.

Methods. Roots of individuals from Peñón Blanco, Durango, were collected in February (A), June (B), August (C), and November (D), 2017. Ethanolic extracts (80% v/v) were prepared and fractioned with ethyl acetate; total phenolics (TFC), flavonoids (TFL), and condensed tannins (TCT) were evaluated. The phenolic profile was analyzed by HPLC-DAD according to Campos and Markham (2007) method. The data were subjected to a one-way ANOVA and a post-hoc test (Duncan's multiple range, $p \leq 0.05$).

Results. The concentration of TFC and TCT in roots of *F. splendens* were highest in November, whereas the TFC was higher in February (Table 1). These represent the coldest months in the studied area. The current results are in agreement with reports informing that environmental stresses affect the synthesis of phenolic compounds, that low temperatures may cause photo-oxidative stress, increasing TPC, as well as that low temperatures increase the phenylalanine ammonia lyase levels, enhancing the synthesis of phenolic compounds [3].

The HPLC analysis revealed similar root phenolic profiles across four different phenological stages (Fig. 1). Only variations of the relative concentrations of the individual phenolic compounds at different phenological stages were observed. The highest concentrations were displayed in February and November. A total of 11 compounds were detected, of which two were phenolic acids (1, 2), four flavones (3, 4, 10, 11), and five dihydroflavonoids (5, 6, 7, 9).

Lazarova *et al*. [4] also reported phenolic acids and flavones, besides catechines in *Asphodeline lutea* roots extracts, an edible species from Bulgaria and Turkey.

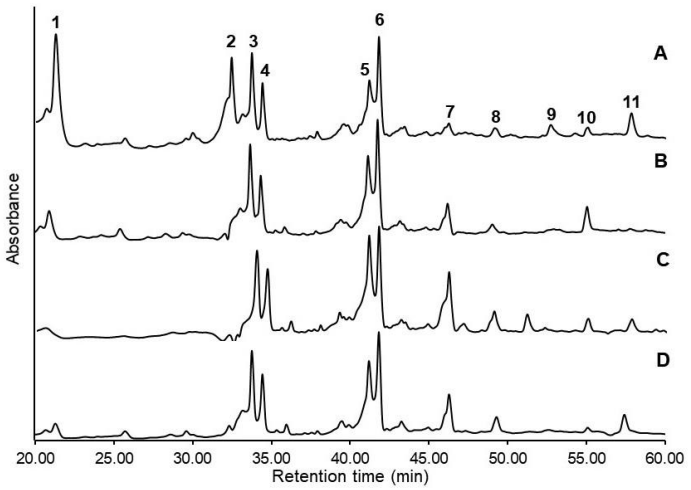


Fig.1. HPLC chromatograms (at 265 nm) of *Fouquieria splendens* roots ethanolic extracts collected in different phenological stage (A-D).

Table 1. Concentration of total phenolics, flavonoids and condensed tannins of *Fouquieria splendens* roots from Peñón Blanco, Durango.

	February	June	August	November
Phenolic content (mgGAE/gDE)	26.91±2.07^{ab}	26.40±1.67^{b}	25.11±2.11^{b}	29.67±1.21^{a}
Flavonoid content (mQE/gDE)	1.73±0.06^{a}	0.92±0.00^{c}	1.10±0.06^{d}	1.38±0.06^{b}
Condensed tannins (mgEE/gDE)	7.14±0.68^{b}	5.91±0.39^{c}	5.84±0.53^{c}	8.55±0.32^{a}

Conclusions. Significant variations in the contents of phenolic compounds take place in the roots of *F. spelendens* subsp. *splendens* across different phenological stages. The highest contents were found in the coldest months.

Acknowledgements. The authors thanks to CONACyT and COFAA-IPN for the financial support.

References.

1. Ghasemzadeh, A., Ghasemzadeh, N. 2011. *J. Med. Plants Res.* 5: 6697–6703.

2. Ladyman, A.R.J., 2005. *Fouquieria splendens* Engelm. Fouquieriaceae, in: Francis, John K. (Ed.) Wildland Shrubs of the United States and Its Territories: Thamnic Descriptions: Volume 1. Department of Agriculture, San Juan, Puerto Rico. pp. 347–349.

3. Ben Ahmed, Z., Yousfi, M., Viaene, J., Dejaegher, B., Demeyer, K., Mangelings, D., Vander Heyden, Y. 2017. *Pharm. Boil.* 55: 1185–1194.

4. Lazarova, I., Zengin, G., Aktumsek, A., Gevrenova, R., Ceylan, R., Uysal, S. 2014. *Ind. Crops Prod.* 61: 438–441.

ISSN: 2448-8461

VE9

TOOL DEVELOPMENT FOR THE STUDY OF THE *BACILLUS CEREUS B25* - *FUSARIUM VERTICILLIOIDES* - MAIZE INTERACTION

Estefanía Morales Ruiz, Astrid Ceballos López, Ignacio Eduardo Maldonado Mendoza; CIIDIR-IPN Guasave, Departamento de Biotecnología Agrícola. Guasave, Sinaloa, esmoru@gmail.com.

Key words: Bacillus cereus, Fusarium verticillioides, interaction

Introduction. *Fusarium verticillioides* (*Fv*) is a toxin producer, fungal pathogen of maize – one of the most important grains worldwide – that affects not only corn but also animal and human health[1]. The use of *Bacillus cereus* B25 has been reported as a biocontrol method to prevent *Fv* maize colonization since this bacterium produces chitinases that degrade the cell wall of the fungus[2].
The underlying molecular mechanisms responsible for the antagonism during this bacteria-fungus-plant interaction remain to be determined. In this work, we present the development of molecular tools aiming to elucidate such mechanisms.

Methods. To study the *B*25-*Fv* interaction we constructed several shuttle vectors expressing a fluorescent protein (RPG or GFP) by sub cloning the fluorescent-protein coding genes to a Gram + replicating vector. Then, the bacteria were observed under a fluorescence microscope.
In order to determine the role of the chitinases of *B. cereus B*25, by creating mutants, we designed a molecular strategy (vector) for allelic replacement of the *B*25 chitinases encoding genes [3].

Results. We constructed three shuttle vectors carrying a fluorescent protein: GFP, RFP, mCherry to visualize the *B. cereus-F. verticillioides* interaction in maize.

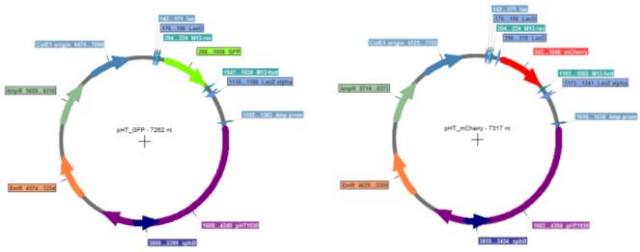


Fig.1 Schematic representation of the shuttle vectors carrying whether GFP (left) or mCherry (right).

To obtain the mutants of the chitinases genes we constructed a suicide vector with the pUC19 polylinker and an erythromycin cassette for counter selection. In each vector quitinase genes can be added with the corresponding mutations, and allelic replacement is conducted by the XXXX system from the *B*25 strain.

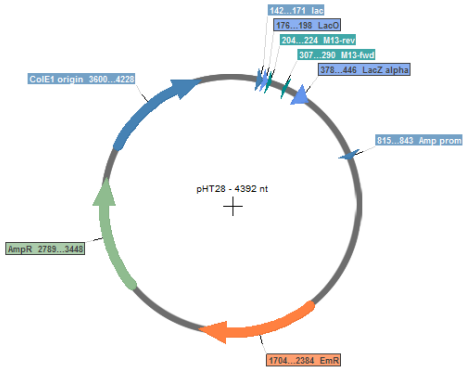


Fig.2 Schematic representation of the suicide vectors constructed for the allelic replacement.

Conclusions. These two molecular tools will allow us to deepen the study of *B. cereus-F. verticillioides* interaction to allow easy visualization of the bacteria in a tripartite *B*25-*Fv*-maize interaction and to understand the role that each one of the two different quitinase genes of *B*25 play in this interaction.

Acknowledgements. This work has been supported by a grant from CONACyT-FOINS (Fronteras de la Ciencia FC-2016 / 2510).

References.

1. Arnaud, M., Chastanet, A., & Debarbouillé, M. (2004). *Applied and Enviromental Microbiology*, 70(11), 6887–6891.
2. Duncan, K., Howard, R. (2010). *Molecular Plant-Microbe Interactions*, 23(1), 6–16.
3. Figueroa-López, A., Leyva-Madrigal, K., Cervantes-Gámez, R., Beltrán-Arredondo, L., Douriet-Gámez, N., Castro-Martínez, C., & Maldonado-Mendoza, I. E. (2017). *Romanian Biotechnological Letters*, 22(4), 12722–12731.

ISSN: 2448-8461

VE10

EDAPHIC BACTERIAL CONSORTIA AS A USEFUL BIOTECHNOLOGICAL APPROACH TO IMPROVE COCOA FARMING; A CASE STUDY IN AN EXPERIMENTAL AGROFORESTRY SYSTEM

Jorge Ricaño-Rodríguez, Enrique Hipólito-Romero, Eliezer Cocoletzi Vásquez, José María Ramos-Prado
Center of Eco-Literacy and Knowledge Dialogue. University of Veracruz, Campus USBI. Veracruz Cultures Avenue 1 Col. Emiliano Zapata, zip code 91000, Xalapa, Veracruz, México
Email of responsible author for representing this work: jricano@uv.mx

Key words: Theobroma cacao, biofertilization, agroforestry systems

Introduction. *Theobroma cacao* is one of the most consumed commodity goods because of its economical importance around the world as well as an historical context related to pre-Hispanic cultures[1,3]. This research mainly consisted in the establishment of a two block experimental farming plot using four clonal varieties of cocoa that were exposed to edaphic bacterial inoculants (*Azospirillum brasiliensis, Chromobacterium violaceum, Acinetobacter calcoaceticus*) and conventional chemical fertilizers. Under the studied conditions, different cocoa varieties subject to bacterial fertilization showed greater growth regarding height and steam radius diameter, number of branches and foliage development. Bacterial count in the soil also increased for all cases.
The aim of this work was to determine the ecomorphological response of cocoa plants under agroforestry system conditions, with the application of two different fertilization treatments.

Methods. The work was carried out in an experimental plot located in the community of Cerro Camarón, municipality of San Pedro Ixcatlán Oaxaca, Mexico. The experimental design took place in a traditional agroforestry system plot, configured by arboreous and fruit tree species in the upper canopy. The lower canopy consisted of creole varieties of cocoa, banana, palm camedora, coffee and vanilla. A randomized design was established with nine repetitions (cultivars) per fertilization treatment and block. Three different treatments were applied: a) biofertilization (bacterial consortium), b) control (water), and c) chemical fertilization (N-P-K: 20-30-10). The recorded variable responses were morphological parameters of cocoa plants: (*i.e.* height, basal diameter, number of leaves and secondary branches); physicochemical and microbiological characteristics of the soil were also determined. For those cases where the obtained results showed significant effects, a variance analysis and Tukey mean comparison ($\alpha = 0.05$) were performed.

Results. All ecophisiological responses and soils' physicochemical characteristics were recorded twice (two and 12 months after inoculation). A slight increase of pH and a low to moderate OM average values were recorded for all treatments. Total average of nitrogen content increased considerably in all treatments from two to 12 months after planting. Cultivar Inifap 8 showed a better response to the biofertilization treatment on height response. Cultivars Inifap 8 and 9 showed the highest values of basal diameter of the steam respect to the biofertilization treatment and most of the cultivars showed a high number of secondary branches. Similar to the number of branches, at two months after the inoculation most of the cultivars showed a high number of leaves with the biofertilization treatment. The largest population of mesophilic bacteria found in the soil corresponded to the control treatment in the creole cultivar ($1,45 \times 10^7$ UFC / g). The highest Phosphorous Solubilizing Bacteria count was recorded with the biofertilization treatment in Inifap 9 and control treatment showed the highest values for nitrogen fixing bacteria which results were associated with cultivars Inifap 8 and 9.

Conclusions. In addition to the implementation of a traditional agroforestry system, the results of the application of biological fertilizers suggested a magnificent biotechnologial alternative very easy to apply under experimental conditions. In this work, we have found that our bacterial consortium provides remarkable benefits[3] in cocoa plants harvested under a traditional agroforestry system regarding their ecomorphological responses.

Acknowledgements. To the General Directorate of University Improvement of the Government of Mexico through the Program for Professional Development for Higher Education (PRODEP). Thanks are also for Plan Cacao-Nestlé from Mexico.

References.

1) Graetz HA. (1997). Suelos y fertilización. In: *Manual de microbiología de suelos.* Rodríguez-Bejarano M and Flores RT. (eds.). 2nd ed. Editorial Trillas: México D.F.: 80-4.
2) Jones JD, Dangl JL. (2006). The plant immune system. *Nature*. 444: 323-9.
3) Uribe A, Méndez H, Mantilla J. (2001). Effect of balanced fertilization on cocoa yield. *Better Crops Int*. 15: 3-5.
4) Camelo-Rusinque M, Vera-Monroy SP, Bonilla-Buitrago RR. (2011). Mecanismos de acción de las rizobacterias promotoras del crecimiento vegetal. *Corpoica. Cienc. Tecnol. Agro*. 12: 159-66.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
VE11

RESPONSE OF FOUQUIERIA SPLENDENS EXPLANTS COCULTIVATED WITH ENDOPHYTE BACTERIA

Verónica Azeret Salinas-Patiño[1], Marcial García-Pineda[2], Maria del Rosario Espinoza Mellado[3] and Angélica Rodríguez-Dorantes[1]

[1]Laboratorio de Fisiología Vegetal,Departamento de Botánica, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, México City 11340, México

[2]Jardín Botánico, Facultad de Estudios Superiores, Iztacala,Universidad Autónoma de México,Los Reyes Iztacala s/n, México City 54090

[3]Central de Microscopía,Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, México City 11340, México

Key words: in vitro culture, endophytes, co-cultive

Introduction. Attempts to create functional associations between the bacteria and cultured plants under *in vitro* conditions have often been successful. Nowak (1), define the *in vitro* co-culture of tissue explants with beneficial microbes that induces developmental and metabolic changes as ‘‘biotization’’. This study analyzed the effect on development of the desert plant *Fouquieria splendens* explants, co-cultivated with isolated endophytes characterized as phytohormones producers.

Methods. Endophytes were isolated from leaves of the desert plant *F. splendens*, and characterized as indole acetic acid (IAA) producers. *In vitro* co-cultives of *F. splendens* explants and the endophytes were established according to Sharma *et al.* (2), divided in two sequential phases, in the first phase, Petri dishes containing “MS” Medium (without phytohormones), and “MSE” Medium (1mg/L NAA + 1.5mg/L KIN), supplemented with 30 g/L of sucrose and 3 g/L phytagel; were equal divided in three sections, where each endophyte strain were streaked at the left and right sides of the central division. The second phase of the established co-cultures initiated after the incubation of the endophyte strain when colonies appeared on the medium. Four pieces (16mm^2, each one) of the obtained *F. splendens* explants were deposited in the central division of the plates, with or without the endophyte bacteria. Petri dishes were incubated at 28°C with photoperiod of 16 h light /8 h dark. All the experiments were performed by triplicate and the effect of co-cultives was analyzed after 30 days.

Results. In this study, three of the seven isolates diminished their IAA production in presence of tryptophan; in three of them the IAA production was not promoted or inhibited by the amino acid and only one of the endophytes increase its production as the Trp concentration increased (Figure 1).

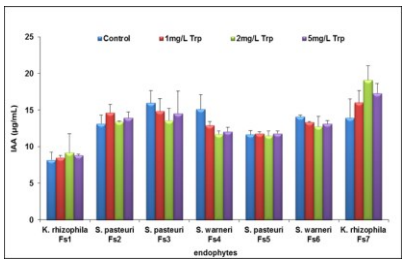


Fig.1 IAA acid production by the *Fouquieria splendens* isolated endophytes.

The effect of the endophytes in co-cultive with *F. splendens* explants, showed that only two of them promoted the growth and morphological differentiation. These isolates were also the highest producers of IAA (Figure 2).

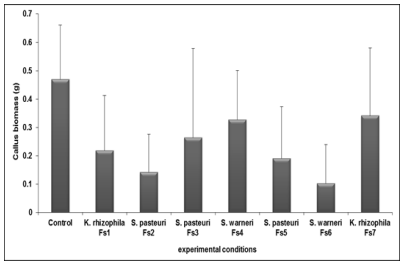


Fig. 2 *Fouquieria splendens* biomass production after co-cultivated with the isolated endophytes.

Conclusions. In this work, the established co-cultives between *Fouquieria splendens* and endophytes showed a complex network of *in vitro* growth and differentiation.

Acknowledgements. Authors are grateful to the Research Projects SIP-IPN: 20171598 and SIP-IPN: 20181504 of the Secretaría de Investigación y Posgrado del Instituto Politécnico Nacional.

References.

1. Nowak, J. (1998). Benefits of *in vitro* ‘‘biotization’’ of plant tissue cultures with microbial inoculants. *In Vitro Cell Dev. Biol. Plant.* 34: 122-130.

2. Sharma, V., Kamal, B., Srivastava, N., Negi, Y., Dobriyal, A.K., Jadon, V.S. (2015). Enhancement of *in vitro* growth of Swertia chirayita Roxb. Ex Fleming co-cultured with plant growth promoting rhizobacteria. *Plant Cell Tiss. Organ. Cult.* 121: 215-225.

ISSN: 2448-8461

VE12

INTRODUCCIÓN DE FRUTALES ALTA DENSIDAD GENERO Malus MEDIANTE AGRICULTURA PROTEGIDA.1a ETAPA EN TEPETITLA TLAXCALA

Pedro Ramírez Legorreta[1], Ada María Ríos Cortés[1], Hugo Flores Medina[2], Minerva Rosas Morales[1] y Pedro Ramírez Calva[1];

[1]Instituto Politécnico Nacional Centro de Investigación en Biotecnología Aplicada, [2]Huertos Vanguardistas S.A.

pramirezl@ipn.mx

Key words: *fruit trees, agroecosystem, orchards*

Introduction. Quantifying the needs of apple and other fruit producers in Chihuahua, the Huertos Vanguardistas company focuses on the generation of graft carriers, and the technified propagation of apple varieties in Tepetitla Tlaxcala, Mexico with the social objective of teaching and supporting producers of the region to obtain highly productive orchards within a three-year period.

Methods. It consists of an innovative action plan oriented to production processes with continuous monitoring and support in scientific research of cell propagation in laboratory and greenhouse, as well as in alternative (protected) nurseries that take into account the quality of land and water. The originality, innovation, and typicality of the products are taken care of, linked to an area of particular interest for consumers in the center of the country, investors are chosen to start with the introduction of apple and the possibility of testing the introduction of other varieties of fruit remains open plum, peach, apricot, even berries.

Results. The actions on the valorization of local products are oriented, in general, to the supply of inputs, equipment, and infrastructure; to provide the services of technological information, advice, training, professional, business and administrative training; to the promotion of production technologies (simple and interspersed gardens) and marketing processes (business plan, participation in forums, congresses, exhibitions and fairs); to market studies and food technology; to the search of sources of financing and forms of cooperation and integration of the producers, connecting the primary and secondary activities and these in turn with the market. In the development of the Scientific Project are mentioned some of the controlled conditions in water and soil. There are sustainable needs for biofertilization and the search for organic alternatives for the recovery of soil fertility, for this alternative the crops used are the ones that help nitrogen fixation and soil accumulation (white clover alfalfa), among other nutrient fixers, as well as the use of compostable materials.

Fig.1 Vegetables in development

Fig.2 Soil sampling.

Fig.3 Two-year-old apple orchards

Conclusions. The advance of the first stage shows that the cultivation of the apple tree in protected agriculture can be improved, although it requires a high degree of knowledge of the agroecosystem and constant monitoring of orchards in its development, considering the business plan and market study in high demand to the unlimited market of the center of the Republic.

Acknowledgements Avant-garde Gardens, Tepetitla City Council, Producers of Tepetitla, CIBA Tlaxcala of the National Polytechnic Institute.

References.

1. Bock S., Y., N. Pérez R., A. Argumedo M., J. Regalado L., J. I. Olvera H., y R. Mendoza R. 2004. *Proyecto de desarrollo rural en la región oriente de la Sierra Nevada (Puebla)*. Colegio de Postgraduados, Campus Puebla. 112 p.

2. Comisión Europea. 2005. Tema central: *Valorización de la producción local.* LEADER Magazine 3: 9.

3. De los Ríos C., I. (coord.). Ed. *Comunidad de Madrid*. pp: 139-174

4. Instituto Nacional de Estadística y Geografía (INEGI), Encuesta Intercensal 2015. *Varietal en manzano. Revista de Fruticultura,* 1, 13–25

5. Knudson, W. Wysocki, J. Champagne, 2004. *Entrepreneurship and innovation in the agri-food system. Amer*. J. Agr. Econ. 86: 1330-1336.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México

VE13

POTENTIAL OF TRICHODERMA ASPERELLUM TC3 IN THE CONTROL OF ONION DISEASES

AM. Luna-Vera, EF. Palacios-Pala, V. Camacho-Luna, YM. Solis Centeno, L. Bravo-Luna, M. Rodríguez-Monroy, G. Sepúlveda-Jiménez. Departamento de Biotecnología, Centro de Desarrollo de Productos Bióticos, Instituto Politécnico Nacional, Yautepec, Morelos, México. gsepulvedaj@ipn.mx

Key words: Fusarium, Alternaria porri, Stemphylium vesicarium

Introduction. The onion production in the Morelos state Mexico, is affected by diseases that reduce the bulb quality. *Fusarium oxysporum* and *Fusarium proliferatum* have been identified as the causal agents of root rot and purple color in onion bulbs. *Alternaria porri* and *Stemphylium vesicarium* cause the diseases known as purple blotch and leaf blight, respectively. The use of the *Trichoderma* fungal as biocontrol agents is an alternative to chemical control. Additionally, *Trichoderma* promotes the plant growth and induce resistance against biotic and abiotic stress. It known that the *T. asperellum* isolate TC3 obtained from roots of onion plants grown in Morelos state promotes grow of onion bulbs, modulates the content of antioxidant compounds under reduced conditions of fertilization and alleviated the copper stress in onion plants (1,2). Due the beneficial effects of TC3 in onion plants, in this work we evaluated the antagonic activity of *T. asperellum* isolate TC3 against pathogens of onion plants.

Methods. The *in vitro* antagonist activity of TC3 against *Fusarium oxysporum*, *Fusarium proliferatum*, *Alternaria porri* and *Stemphylium vesicarium* was evaluated in dual culture and antibiosis assays (n=6, three repetitions). In onion plants were applied the following treatments: 1) only *T. asperellum* TC3, 2) only *F. oxysporum*, 3) only *F. proliferatum* and 4) the mixtures of the above. The controls were onion plants without inoculate; the incidence of the disease caused by the pathogens was quantified.

Results. The results of the dual culture assay shown that *T. asperellum* TC3 inhibited 34.4, 30.8, 56 and 54.4 % the mycelial growth of *F. oxysporum, F. proliferatum, A. porri and S. vesicarium*, respectively. Similarly, the antibiosis assays indicate that TC3 inhibited 49.5, 46.6, 53 and 67% the mycelial growth of *F. oxysporum, F. proliferatum, A. porri and S. vesicarium*, respectively (Table 1). The incidence of symptoms caused by *F. oxysporum* and *F. proliferatum* was reduced 1.4 and 1.2 times in onion plants treated with *T. asperellum* TC3 (Figure. 1).

Table 1. Inhibition of mycelial growth of pathogens that affect onion plants by *Trichoderma asperellum* isolate TC3

	Dual culture	Antibiosis
Pathogen	IMG (%)	IMG (%)
F. oxysporum	34.40	49.50
F. proliferatum	30.80	46.61
A. porri	56.00	53.00*
S. vesicarium	54.40	66.90

The values correspond to the mean ± standard deviation.* Technique of antibiosis with cellophane membrane.

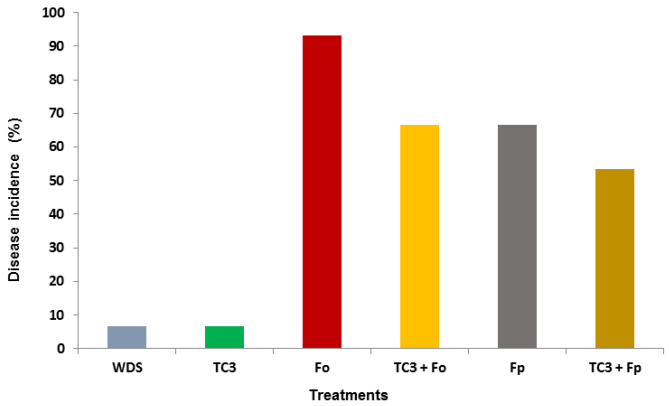


Fig.1 Disease incidence in onion plants inoculated with *T. asperellum* (TC3) and infected with *F. proliferatum* (Fp) and *F. oxysporum* (Fo). Control are plants with water distilled sterile (WDS).

Conclusions. *T. asperellum* isolate TC3 is an alternative for the biological control of *F. oxysporum, F. proliferatum, A. porri* and *S. vesicarium* that affect the onion plants.

Acknowledgements. This research was financed by SIP (20180426), Instituto Politécnico Nacional.

References.

1. Ortega G, Montes R, Rodríguez M, Ramírez J, Suárez R, Sepúlveda G. 2015. *Science Horticulturae*. 195: 8–16
2. Téllez J, Rodríguez M, López M, Montes R, Sepúlveda G. 2017. *Environmental Experimental Botany*.136: 85–93.

ISSN: 2448-8461

VE14

DEVELOPMENT OF A PRODUCT BASED ON RHIZOSPHERIC BACTERIA FOR BIOCONTROL OF *FUSARIUM OXYSPORUM* FF.SPP. *LYCOPERSICI* RACE 3 AND *RADICIS-LYCOPERSICI* IN TOMATO

Md. Masudur Rahman Khalil, Paúl A. Báez Astorga, Rosario A. Fierro Coronado and Ignacio E. Maldonado Mendoza; CIIDIR-IPN Unidad Sinaloa, Departamento de Biotechnología Agrícola. Guasave, Sinaloa; khalil.edu.bd@gmail.com.

Key words: Bacteria, Fusarium sp., antagonist

Introduction. Tomato (*Solanum lycopersicum* L.) is one of the crops with the largest production in the world. The State of Sinaloa generates 20.98% of the volume of production in tons of the national total in Mexico[1]. Tomato is affected by factors that limit its production and profitability. In Sinaloa, there are reports of the presence of both *F. oxysporum formae speciales*: Forl and Fol races 1, 2 and 3 * with the latest race being the most aggressive[2]. It is common to find co-infections of a plant by Fol and Forl in the field. As a result, the disease is even more devastating than when each fungus attacks a plant separately.

The goal of the present study was to recognize potential rhizospheric antagonistic bacteria and their plant growth promoting traits and pathogen growth inhibition potential (Fol race 3 and Forl) under greenhouse assays.

Methods. The most effective antagonistic bacterial isolates (*Bacillus methylotrophicus* (BA2B), *B. subtilis* (BA16), *B. thuringiensis* (BA26), *B. megaterium* (B2) and *Acinetobacter* sp. (BA31B)) were selected to control the tomato (PAIPAI F1) disease symptoms caused by Fol race 3 and Forl. The antagonistic bacteria were previously selected *in vitro* and *in planta*. Two of the selected bacteria (BA31A and B2) were used to treat Forl and three bacteria (BA2B, BA16 and BA26) were used to treat Fol race 3 in greenhouse assays (germination tray and pot assays). Different types of treatment (such as single, double and mixed inoculations) were applied in greenhouse assays. Antagonistic traits in these bacteria were characterized by checking siderophore production, chitinase, glucanase and protease enzymatic activity and plant growth promoting traits such as phosphate solubilization and indole-3-acetic acid production. Bio-film tests indicated that these potential antagonistic bacteria can colonize root surfaces of tomato[3].

Results. *Bacillus megaterium* and *B. subtilis* showed the most effective antagonistic and plant growth promoting effects against Forl and Fol in greenhouse assays. We will report on the growth promoting traits that each bacteria possesses and will discuss the possible mechanisms that these bacteria use for plant growth promotion and pathogen growth inhibition.

Fig.1 Effects of antagonistic bacteria in tomato plants in greenhouse pots assay against Forl (isolate B2 + Forl).

Fig.2 Effects of pathogen (Forl) in tomato plants in greenhouse pots assay

Conclusions. *Bacillus megaterium* and *Bacillus subtilis* will be used in field trial experiments in two tomato growing sessions (2018-2019 and 2019-2020).

Acknowledgements. This work has been supported by a grant from IPN (SIP20170939 and SIP20181778). MMRK has received a fellowship for doctoral studies from CONACyT (864357).

References.

1. SIAP (2016). Information Services for Agri-Food and Fisheries. National Summary on progress of plantings and harvests. Annual cycle 2016.

2. Ramirez, P. and Coha, J. M. 2003. Enzymatic degradation of cellulose for thermophilic actinomycete: isolation, characterization and cellulolytic activity determination. Rev. Peru. Biol. 10:67-77.

3. Del Re B, Sgorbati B, Miglioli M and Palenzona D 2000. Adhesion autoaggregation and hydrophobicity of strains of Bifidobacterium longum. Lett Appl Microbiol. 31: 438-442.

ISSN: 2448-8461

VE15

The endophytic Actinobacterium *Streptomyces scabrisporus* NF3 compensates for growth hormone deficiency in *Arabidopsis thaliana*

Corina Diana Ceapă[1], Angélica Patricia Cruz Vázquez[1,2], Verónica Jiménez Suárez[2], Elena R. Alvarez-Buylla[2], Sebastián de la Rosa Hernández Garibay[1], Sergio Sanchez[1]; [1]Departamento de Biología Molecular y Biotecnología, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México (UNAM), CDMX, 04510, México; [2]Laboratorio de Genética Molecular, Epigenética, Desarrollo y Evolución de Plantas, Instituto de Ecología, UNAM;
corinaceapa@iibiomedicas.unam.mx

symbiosis, chitinases, plant growth

Introduction.
One of the most important sources of bioactive compounds are microbiological systems, in particular streptomycetes. Streptomyces species have been found living together with plants as endophytic microbes [1]. In this work, one Actinomycete was isolated from the plant *Amphipterygium adstringens* (Rodríguez-Peña, unpublished data) and identified as *Streptomyces scabrisporus* (SS NF3). Genome mining of the SS NF3 genome identified various possible mechanisms by which the endophyte could influence plant life cycles, for instance predicts the ability to produce plant hormones as well as a diversity of plant-modulating secreted proteins. [2, 3].
We hypothesise that the endophyte SS NF3 is able to colonize the model plant *Arabidopsis thaliana* (AT) and to influence its growth.

Methods.
Co-colonization experiments (2 independent experiments, n>7 per group) using either wild type (Col-0) or a mutant plant (XAL2) defective in the production of indole 3 acetate (auxin) were performed both after 10 days experiments in MS plates as well as after 21 days experiments in unsterilized soil. Plant growth parameters (plant length, number of leaves, number of flowers) as well as chlorophyll production and secretion of chitinases were measured.

Results.
In axenic conditions, co-incubation of SS NF3, either with Col-0 or XAL2 led to a significant decrease in plant length and number of leaves, as well as an increase in chlorophyll levels. Plants grown in soil show decreased development for Col-0 and recovery of mutant phenotype for XAL2, and increased chlorophyll in both cases. Chitinases were secreted by *S. scabrisporus* NF3 only in the presence of their plant symbiont. They show various lytic capacities in chitin degrading experiments and their anti-fungal properties are also highly compound and strain dependent.

Conclusions.
SS NF3 is able to establish a symbiotic relationship with plants of AT and in the case of XAL2 is providing the conditions for growth and development similar to a healthy plant.
In healthy plants such as AT Col-0 this relationship may generate deficiency both in development and growth, due to an excess of molecules of interaction which cause defence mechanisms, exacerbated in axenic conditions.
SS NF3 is able to produce chitinases (in MS). In addition, the results confirm a role for these SMs in symbiotic relationships.

Perspectives
Expand the analyses to a molecular level to determine which molecules are responsible for the observed plant developmental changes.

Acknowledgements.
We thank Beatriz Ruíz and Nidia Maldonado Carmona for their support and suggestions. This research was supported by a DGAPA Postdoctoral Fellowship, UNAM to C.D.C. and by PAPIIT, DGAPA, UNAM grants IN202216, IN211516, ININ208517, IN204217 and CONACYT grants 240180, 180380, 2015-01-687. The authors thank the support of CONACYT INFR-2017.279880 that allowed the acquisition of an UPLC masses.

References.
[1] Martínez-Klimova E, Rodríguez-Peña K and Sánchez S (2017) Endophytes as sources of antibiotics. *Biochem Pharmacol.* 134: 1-17.
[2] Vazquez-Hernandez M, Ceapa CD, Rodríguez-Luna SD, Rodríguez-Sanoja R, Sánchez S (2017) Draft genome sequence of Streptomyces scabrisporus NF3, an endophyte isolated from *Amphipterygium adstringens*. *Genome Announc.* 5: e00267-17 .
[3] Ceapă CD, Vázquez-Hernández M, Rodríguez-Luna SD, Cruz Vázquez AP, Jiménez Suárez V, et al. (2018) Genome mining of *Streptomyces scabrisporus* NF3 reveals symbiotic features including genes related to plant interactions. *PLOS ONE* 13: e0192618.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
VE16

ANTI-ULCEROGENIC ACTIVITY OF *Kalanchoe gastonis-bonnieri* ETHANOLIC EXTRACTS

Abut Antonio García-Pérez[1], Pablo Emilio Vanegas-Espinoza[1], Alejandro Zamilpa-Álvarez[2], Maribel Lucila Herrera-Ruiz[2], Crescencio Bazaldúa[1], Alma Angélica Del Villar-Martínez[1]. [1]Instituto Politécnico Nacional, Centro de Desarrollo de Productos Bióticos, Departamento de Biotecnología, Yautepec, Morelos C.P. 62730, México. [2]Centro de Investigación Biomédica del Sur, Instituto Mexicano del Seguro Social, Xochitepec, Morelos C.P.62790, México. cbazaldua@ipn.mx

Key words: Ethanolic extract, Anti-ulcerogenic, Kalanchoe gastonis-bonnieri

Introduction. *Kalanchoe gastonis-bonnieri*, is a medicinal plant used in mexican traditional medicine in treatment of cancer, gastric ulcer, and skin diseases(1). There are few reports about the chemical profile and biological activity of *K. gastonis-bonnieri.* Previous studies reported the presence of phenolics, terpenes, alkaloids, saponines and fatty acids (2).
The aim of this work was to analyze the anti-ulcerogenic activity of ethanolic extract from leaves of *K. gastonis-bonnieri*.

Methods. Fresh leaves of *Kalanchoe gastonis-bonnieri* were finely cut. The extracts were obtained by maceration with ethanol (1:5 w/v) at 25° C during 24 h, then were filtered and concentrated at 40 °C. Anti-ulcerogenic activity was evaluated as described by Silva *et al* (3), three experimental groups of ICR mice were used. After 24 h of fasting, mice were treated. Group 1 received 10 ml/kg-body weight (bw) of tween 20 1.0% (vehicle), group 2 received 20 µg/kg- bw of misoprostol, and group 3 received 10 mg/kg-body weight of *K. gastonis-bonnieri*-ethanolic extract dissolved in tween-20 1.0%. Gastric lesions were induced, identified, and measured as described by Shuai *et al*. (4) data were used to calculate the ulceration index (UI), which is the damage quantification.

Results. Ethanol produced the highest gastric ulcerations mainly in the glandular segment of the stomach from mice vehicle-treated (figure 1A). While, the treatment with misoprostol, and ethanolic extract of *Kalanchoe gastonis-bonnieri* reduced gastric ulceration (figure 1B, and 1C, respectively).

Fig.1.Effect of ethanol in ulcer generation in stomachs of ICR mice treated with vehicle (A), misoprostol (B), *Kalanchoe gastonis-bonnieri* ethanol-extract (C).

In this work, the lowest induced-damage was in mice treated with ethanolic extract of *Kalanchoe gastonis-bonnieri* (UI= 5.47 ± 0.74). The highest UI was in mice vehicle-treated (20.68 ± 1.42), followed by mice misoprostol-treated (10.83 ±1.08) (figure 2).

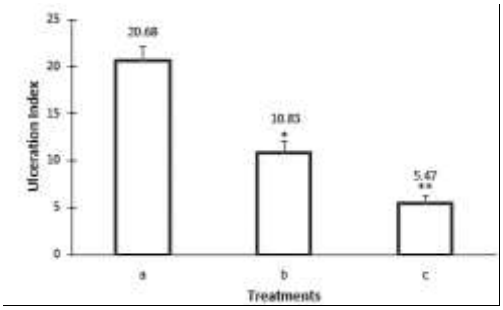


Fig.2. Ulceration index in stomach mice: a) vehicle 10 mL/Kg, b) misoprostol 20 µg/Kg, and c) ethanol-extract *Kalanchoe gastonis-bonnieri* 10 mg/Kg. Values are mean ± SEM. ANOVA – Bonferrioni* post test p<0.05 (n=5).

The hidroalcoholic extract from leaves of *Kalanchoe gastonis-bonnieri* seems to be related with an anti-ulcerogenic activity, which has not been reported. The compounds related to anti-ulcerogenic effect could be terpenes and phenolics, both groups were identified in extracts of *K. gastonis-bonnieri* (2).

Conclusions. The extract from leaves of *K. gastonis-bonnieri* was the most effective treatment with anti-ulcerogenic activity.

Acknowledgements. SIP-IPN by the financial support (SIP 20180804). AAGP thank to Conacyt for the scholarship, and BEIFI-IPN.

References.

1. Yukes J, and Balick M. (2010) Dominican Medicinal Plants: A Guide for Health Care Providers. Second edition Web version (New York Botanical Garden). New York, EUA.44-45.
2. Dasgupta S, Parmar A, Patel, H. (2013). *Int J of Pharm Biol Sci*, 4, 550-557.
3. Silva DM, Martins JLR, Florentino IF, Oliveira DR, Fajemiroye JO, Tresnvenzol LMF, Paula JR, Costa EA. (2016). *Anais da Academia Brasileira de Ciências*, 88(3), 1819-1828.
4. Shuai, W., Yong-rui, B., Yun-Peng, D., Xian-Sheng, M., Ting-Guo, K., 2011. *Afr. J. Microbiol. Res*. 5, 1285–1290.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
VE17

INDUCTION OF HAIRY ROOTS IN *kalanchoe daigremontiana*

Yessica Casales-Tlatilpa, Araceli Urquiza-López, Crescencio Bazaldua, María Asunción Bravo Díaz, Alma Angélica Del Villar-Martínez. Instituto Politécnico Nacional, Centro de Desarrollo de Productos Bióticos. Yautepec, Morelos, México. C.P. 62731. cbazaldua@ipn.mx

Key words: Kalanchoe daigremontiana, Agrobacterium rhizogenes, hairy roots, secondary metabolites.

Introduction. *Kalanchoe daigremontiana* (Crassulaceae) is a medicinal plant that produces glycosides, flavonoids, triterpenoids, and bufadienolides, which pharmacological activity has been reported [(1)]. However, their accumulation in *de*differentiated cell cultures is often low and unstable. By other hand, the production of secondary metabolites through hairy roots culture is an excellent alternative to obtain their stable and higher production [(2)].
The objective of the present study is to establish hairy root cultures from *Kalanchoe daigremontiana* for the production of compounds with biological activity.

Methods. Hairy roots induction by infecting leafs and nodes with three strains of *Agrobacterium rhizogenes* (15834, A4, and K599) was done and transformation efficiency was calculated. After 15 days, detached putative hairy roots were placed in Petri dishes containing hormone-free MS medium at 70 % amended with sucrose (30 g/L) and phytagel (2.6 mg/L), at 25 ± 1 °C. Putative transformed roots were phenotypically selected.

Results. Sprouting of putative hairy roots was observed after seven days of the infection in the node explant; ten days after leaf explants infection roots were evident, except with the K599 strain infection, which did not induce roots in leaves (Figure 1).

Fig.2. Putative hairy roots sprouting in *K. daigremontiana.* Nodes a) 15834, b) A4, c) K599 and, leaves d) 15834, e) A4. The arrows indicate site of infection.

The first selection criteria was the length of putative hairy roots when detached from explant, and their growth capability. The 15834 strain induced the highest number of roots in nodes (41), but the A4 strain induced the largest number of roots in leaves (48). Transformation efficiency depends on strain and explant source. The highest efficiency transformation (100 %) was observed utilizing the 15834 strain (leaves and nodes), and the lowest (50 %) was observed with the K599 strain infecting nodes (Table 1).

Table 1. Transformation efficiency of three strains of *A. rhizogenes*, and number of putative hairy root in nodes and leaves of *K. daigremontiana*.

Agrobacterium rhizogenes strain	Type of explant		Number of putative hairy roots	
	Leaves	Nodes	Leaves	Nodes
15834	100%	100%	11	41
A4	73.9%	100%	48	29
K599	0%	50%	-	18

Putative hairy roots have an enlarged and yellow cap root, which was different from the not induced-*Agrobacterium* roots which caps are smaller and white. (Figure 2).

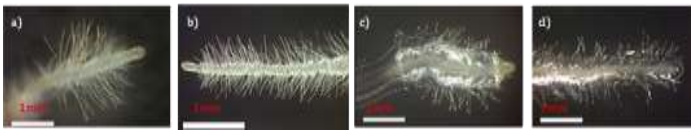


Fig.2. Putative hairy roots sprouted from nodes infected whit three *A. rhizogenes* strains, a) 15834, b) A4, c) K599, d) not transformed root.

Conclusions. *Agrobacteriunm rhizogenes* strain 15843 was the most efficient for the production of hairy roots. Infected explants showed hairy roots whit yellow cap.

Acknowledgements. SIP-IPN by the financial support (SIP 20180804). AUL and YCT thank to Conacyt for the scholarship.

References.

1. Bogucka-Kocka, A., Zidorn, C., Kasprzycka, et al., (2016). Phenolic acid content, antioxidant and cytotoxic activities of four *Kalanchoë* species. *Saudi. J Biol. Sci.*
2. Thwe, A., Valan Arasu, M., Li, X.,et al., (2016). Effect of different Agrobacterium rhizogenes strains on hairy root induction and phenylpropanoid biosynthesis in tartary buckwheat (Fagopyrum tataricum Gaertn). *Front Microbiol.,* 7: 318.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
VE18

EFFECT OF PHOSPHATE CONCENTRATION ON ARBUSCULAR MYCORRHIZAL SYMBIOSIS AND ON PHOTOCHEMICAL ACTIVITY IN *Stevia rebaudiana*

Luis Gerardo Sarmiento López [1], Melina López Meyer [2], Gabriela Sepúlveda Jiménez [1], Luis Cárdenas Torres [3] and Mario Rodríguez Monroy [1]; [1] CeProBi, Instituto Politécnico Nacional, Carretera Yautepec-Jojutla, Km. 6, calle CEPROBI No. 8, Col. San Isidro, Yautepec, Morelos, México. CP 62731. [2] CIIDIR-SINALOA, Instituto Politécnico Nacional, Blvd. Juan de Dios Bátiz 250, Guasave, Sinaloa, México CP 81101. [3] Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, Morelos, México CP 62271. lsarmientol1100@alumno.ipn.mx

Key words: Mycorrhizal symbiosis, Phosphate, Chlorophyll fluorescence.

Introduction. Arbuscular mycorrhizal (AM) symbiosis may uptake water, increase phosphate translocation and induce changes at the photochemical level. It is known that phosphate concentration plays an important role in symbiotic and nutritional development. Additionally, AM symbiosis can generate biotic stress, modifying its photochemical activity [1]. *Stevia rebaudiana* is a specie outstanding by their accumulation of sweaters compounds (steviol glycosides, SG´s). However, studies on these characteristics in *S. rebaudiana* under AM interaction are limited.

The objective of this study was to evaluate the effect of phosphate on the AM symbiosis establishment and determine the response on *S. rebaudiana* photochemical activity.

Methods. Stem cutting of *S. rebaudiana*, were procured from CeProBi-IPN, Yautepec, Mexico. *Rhizophagus irregularis* was procured from Dra. Melina López Meyer at CIIDIR-Sinaloa (Guasave, Mexico). The substrate used was a mixture of 1/1 sand-vermiculite, plants were fertilized twice weekly with ½ strength Hoagland's solution containing 20, 200, 500 and 1000 µM PO_4^{3-} Roots of *S. rebaudiana* were cleared [2] to determine mycorrhiza colonization percentage which was estimated microscopically [3]. Chlorophyll fluorescence parameters were measured using a portable fluorometer OS-30P (Opti-Sciences Inc., USA).

Results. *R. irregularis* successfully established mutualistic symbiosis in *S. rebaudiana* roots (Figure 1), and high percentage of AM was observed in phosphate concentration of 20 and 200 µM. However, it was found a significant decrease at phosphate concentration of 500 and 1000 µM. The maximum quantum efficiency of PSII photochemistry (Fv/Fm) of *S. rebaudiana* plants at two phosphate concentration (200 and 1000 µM), did not show differences between mycorrhizal and non-mycorrhizal treatment (Figure 2).

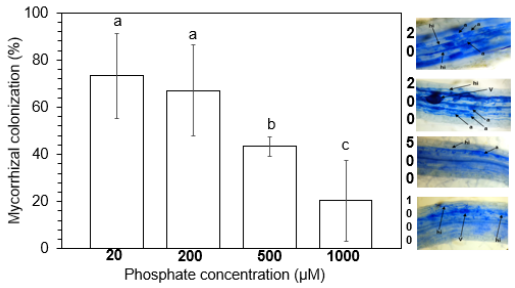


Fig.1 Phosphate concentration effect on the colonization of *R. irregularis* in *S. rebaudiana* roots. Mean ± SD, different letters indicate significant difference (*P*> 0.05) according to the Tukey test.

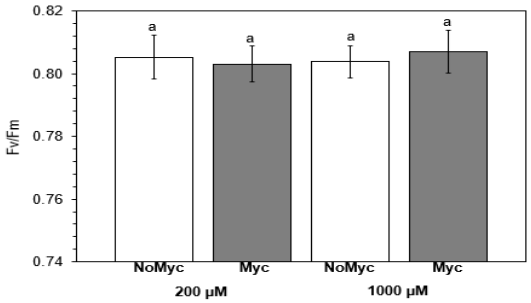


Fig.2 Fv/Fm *in S. rebaudiana* leaves inoculated (Myc) or not (NoMyc) with *R. irregularis* at two phosphate concentration. Mean ± SD, different letters indicate significant difference (*P*> 0.05) according to the Tukey test.

Conclusions. The present work shows that percentage of AM colonization decreases as phosphate concentration increases in the nutrient solution, whereas no changes in Fv/Fm activity was detected in *Stevia* plants regardless of AM symbiosis and phosphate concentrations treatment

Acknowledgements. LGSL to CONACYT (480787) and SIP-IPN (BEIFI) for the scholarship. The work was conducted with support of SIP-IPN (20180427

References.

1.- Xian Z. Feng S. Sheng L. Tie L. (2011). *Plant Soil*. (346): 89-199.
2.- Phillips J. Hayman D. (1970). *Trans. Br. Mycol. Soc*. Vol. (55):18-29.
3.- Trouvelot A, Kough JL, Gianinazzi-Pearson V (1986) *Mesure du taux de mycorhizationVA d'un système radiculaire*. Gianinazzi-Pearson V. INRA edition, Paris, 217-221.

ISSN: 2448-8461

VE19

ENDOPHYTIC BACTERIA OF *Stevia rebaudiana* BERTONI WITH GROWTH PROMOTING ACTIVITY

Alejandra María Montes-Salazar [1]; Ignacio Eduardo Maldonado-Mendoza[2]; Mario Rodríguez-Monroy[1]. 1. CEPROBI del Instituto Politécnico Nacional. Yautepec Morelos 62731. 2. CIIDIR Sinaloa-IPN. Guasave Sinaloa 81101. mariorodriguezmonroy@gmail.com

Key words: Stevia rebaudiana, endophytic bacteria

Introduction. *Stevia rebaudiana* Bertoni is a plant that accumulates in its leaves steviol glycosides, compounds with sweetening power 100 to 400 times greater than sucrose (1). A biotechnological strategy to improve plant growth, may be the use of endophytic bacteria employed as inoculants which benefit yield (2).
The objective of this work was to isolate and identify endophytic bacteria of *S. rebaudiana* cultivated in Mexico and to determine its plant growth promotion activity.

Methods. Leaf, stem and root of a commercial crop of *S. rebaudiana* grown in Yucatan were used. Potential endophytic isolates were tested for hemolysis and were characterized morphologically and identified molecularly by sequencing the 16S-rDNA gene. The strains were characterized in terms of their ability to promote growth: the production of IAA was measured with the Salkowsky reagent; the phosphate solubilization capacity was measured in Pikovskaya medium by the blue molybdenum method and the siderophore production using the Azurol chrome medium.

Results. Table 1 summarizes the results obtained. Strains of all tissues were identified: 3 from leaf, 3 from stem and 6 from root. The isolated strains belong to species of the genera *Enterobacter, Pseudomonas, Pantoea, Bacillus, Acetinobacter* and the Enterobacteriacea family. The strains produced between 31.3 to 72.4 µg of AIA mL^{-1}; *E. cloacae* is the strain with the highest production. The ability of the strains to solubilize phosphate (SI) in solid medium was between 1.4 and 3.7; the best strains also solubilized phosphate in liquid medium (539-795 µg mL^{-1}). All strains isolated from *S. rebaudiana* produced siderophores, with halos from 3 to 6 mm in diameter.

Conclusions. Strains isolated as endophytes of *S. rebaudiana* belong to the genera *Enterobacter, Pseudomonas, Pantoea, Bacillus, Acetinobacter* and the family Enterobacteriacea and have outstanding characteristics to be considered as promoters of plant growth.

Acknowledgements. AMMS to CONACYT and SIP-IPN (BEIFI) for the scholarship. The work was conducted with support of SIP-IPN 20170110, 20170939, 20181778 and 20180427.

References

1. Pande S, Gupta P. (2013). *Journal of Pharmacognosy and Phytotherapy*. 5: 26–33.

2. Santoyo G, Moreno-Hagelsieb G, Orozco-Mosqueda M, Glick R. (2016). *Microbiological Research*. 183: 92–99.

Table 1. Characteristics of growth promotion of endophytic bacteria of *S. rebaudiana*

Strain	Tissue	IAA (µg ml^{-1})	Phosphate Solubilization Agar (SI)*	Líquid (µg mL^{-1})	Siderophores**
Pseudomonas sp.	Leaf	63.4 ± 1.1 ^{d}	3.5	539 ± 7.8 ^{e}	++
Acinetobacter sp.	Leaf	31.3 ± 0.4 ^{g}	2.6	N/E***	++
Enterobacteriaceae bacterium	Leaf	46.8 ± 0.5 ^{f}	2.8	N/E	++
Pantoea sp.	Stem	63.2 ± 1.2 ^{d}	3.0	562 ± 2.5 ^{d}	+++
E. kobei	Stem	64.2 ± 0.8 ^{cd}	3.7	622 ± 3.5 ^{c}	+++
Bacillus sp.	Stem	61.9 ± 0.1 ^{de}	2.6	N/E	+
E. cloacae	Root	60.4 ± 0.5 ^{e}	2.6	N/E	+
E. cloacae	Root	72.4 ± 1.0 ^{a}	0	N/E	++
E. hormaechei	Root	63.4 ± 0.8 ^{d}	3.5	558 ± 3.8 ^{d}	++
Enterobacter sp.	Root	66.9 ± 1.0 ^{b}	3.5	795 ± 2.6 ^{a}	+++
B. safensis	Root	63.4 ± 0.4 ^{d}	1.4	N/E	++
E. xianfangensis	Root	66.2 ± 0.9 ^{bc}	3.5	680 ± 1.5 ^{b}	++

* SI: Solubility index ** the results correspond to the diameter of the halos around the colony: + <3mm; ++ > 3mm < 4mm; +++ > 4mm. *** N/E: not measured. Values in columns followed by different letters are significantly different at $p < 0.05$

ISSN: 2448-8461

ANTI-INFLAMMATORY COMPOUNDS FROM CELL SUSPENSION OF *SPHAERALCEA ANGUSTIFOLIA*

Pilar Nicasio Torres[1*], Juanita Pérez-Hernández[1], Manasés González-Cortazar[1], Jade Serrano Román[1], Mariana Miranda Arámbula[2], Sandra Cabrera Hilerio[3], Maribel Herrera-Ruiz[1]. [1]Centro de Investigación Biomédica del Sur, Instituto Mexicano del Seguro Social, 62790 Xochitepec, Morelos, México. [*]E-mail: pisaliva@yahoo.com.mx [2]Instituto Politécnico Nacional, Centro de Investigación en Biotecnología Aplicada. [3]Facultad de Ciencias Químicas, Benemérita Universidad Autónoma de Puebla.

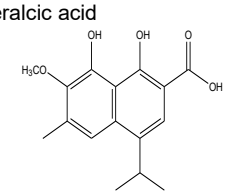
Key words: nutritional stress, phenolic compounds, inflammation models

Introduction. *Sphaeralcea angustifolia* is a plant traditionally used in Mexico for diseases that involve an inflammatory process. Ant-inflammatory activity was demonstrated in acute and chronic inflammation mouse models, as well as in a clinic study (1-2). This plant grows in isolated populations and its collection is controlled by SEMARNAT (NOM-007-RECNAT-1997). Cell suspension cultures were established to produce the active compound scopoletin (3).
The purpose of this study was isolate, and identify the anti-inflammatory compounds produced in *S. angustifolia* cell suspension grown in MS medium with nutritional restriction.

Methods. Cell suspensions were grown in MS medium with total nitrates content reduced at 2.74 mM shaking at 110 rpm and incubated at 26 ± 2 ° C, 16 h light for 8 h of darkness. The cultures were sacrificed at day 16; the cells were filtered and dried. Cells were extracted with CH_2Cl_2:CH_3OH (9: 1); the extract fractionated in silica gel with a gradient system of hexane: ethyl acetate: methanol, and RP-18 silica gel with acetonitrile: water. The purified compounds were analyzed by HPLC, ^{1}H and ^{13}C NMR; and the anti-inflammatory effect was evaluated in acute and chronic mouse models.

Results. The nitrate reduction favored the production of 3 compounds: 2 of them identified by spectroscopy thecnics as 5-hydroxy-6,7-dimethoxycoumarin (tomentin) and 2-1,8-dihydroxy-4-isopropyl-6-methyl-7-methoxy naphthoic acid (sphaeralcic acid), and another compound no yet identified (Figure 1).

Sphaeralcic acid



Tomentin

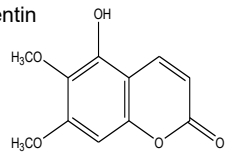


Fig.1 Structure of compounds isolated from *S. angustifolia* cell suspension

The 3 compounds inhibited the development of subplantar edema induced with λ-carrageenan and ear edema induced with TPA (Table 1). Sphaleralcic acid had an Effective Dose 50 (ED_{50}) of 0.54 mg/ear and the compound not yet identified DE_{50} = 0.25 mg/ear. Tomentin and sphalercic acid were active as anti-inflammatories in the kaolin/λ-carrageenan induced monoarthritis model with an ED_{50} = 7.8 mg/kg for sphalercic acid and an ED_{50} = 10.32 mg/kg for tomentin. Likewise, tomentin and sphalercic acid modulate the production of anti-inflammatory (IL-4 and L-10) and pro-inflammatory cytosines (TNF-α and IL-1β).

Table 1. Anti-inflamatory activity of compounds isolated from *S. angustifolia* cell suspension

Compound	Edema inhibition (%)		
	TPA (1 mg/ear)	Subplantar (45 mg/kg, 5 h)	Monoarthritis (15 mg/kg, 10 días)
Control			
Tomentin	47.5 ± 6.7 *	68 ± 5.7 *	63.96*
Sphaeralcic acid	86.6 ± 3.2 **	66 ± 3.7 *	82.42**
No identified	83.0 ± 0.6 **	45 ± 4.9 *	–
Indomethacin	59.3 ± 7.6 *	60 ± 3.3	–
Methotrexate (5 mg/kg)	–	–	54.92

– Not evaluated
** Significant differences according to ANOVA and Tukey's test

Conclusions. *S. angustifolia* cell suspension produces a mixture of compounds with anti-inflammatory activity.

Acknowledgements. Fondo de Investigación en Salud (FIS), Instituto Mexicano del Seguro Social, registration number FIS/IMSS/PROT/G17/1683.

References.

1. García-Rodríguez V, Chamorro G, Siordia G, Jiménez-Arellanes A, Chávez MA, Meckes M (2012). *Bol Latinoam Caribe Plant Med Aromat* 11(5): 454-465.
2. Romero-Cerecero O, Meckes-Fischer M, Zamilpa A, Jiménez-Ferrer JE, Nicasio-Torres P, Pérez-García D, Tortoriello J (2013). J Ethnopharmacol 147: 467-473.
3. Pérez-Hernández J, González-Cortazar M, Marquina S, Herrera-Ruiz M, Meckes-Fischer M, Tortoriello J, Cruz-Sosa F, Nicasio-Torres MP (2014). *Planta Med* 80: 1-6.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
VE21

EVALUATION OF THE ANTIFUNGAL ACTIVITY OF PLANT EXTRACTS ON MONILIOPHTHORA RORERI, COCOA PLAGUE

Wendy A. Mayo-Arellano, Laura J. García Barrera, Pilar Nicasio Torres[3], Mariana Miranda-Arámbula[2] y Sandra Luz Cabrera-Hilerio[1]. [1]Facultad de Ciencias Químicas, Benemérita Universidad Autónoma de Puebla. Ciudad Universitaria, San Manuel, CP. 72570 Puebla, Pue., México, E-mail: cabrerahilerio_sl@hotmail.com
[2]Instituto Politécnico Nacional, Centro de Investigación en Biotecnología Aplicada. [3] Centro de Investigación Biomédica del Sur, Instituto Mexicano del Seguro Social. Argentina 1, Centro, 62790 Xochitepec, Morelos México.
Key words: Moniliophthora roreri, plant extracts, cinnamon

Introduction. Mexico is one of the main producers of cocoa in the world, with 1.6% of total production; however, this production has decreased on 50% (1). Moniliasis is the plague produced by the fungus *Moniliophthora roreri*, which is responsible of the main losses causing the decomposition in cocoa pods. Currently, there are agrochemicals that help control this pest; nevertheless, the use of these harmful environmental products represents high costs and eliminates the value of organic product. To implement alternatives to avoid environmental contamination and cost reduction, natural origin systems had been development to combat pests and insect damage.
Therefore, the objective of this work was to evaluate different botanical extracts for *Moniliophthora roreri* growth inhibition.

Methods. The fungus *M. roreri* was isolated from infected cobs of Tabasco, Mexico, by the method of Barros and Sanchez (1979). Extracts were obtained using different solvents of the following plants: laurel (leaves), thyme, pepper (seeds), garlic and cinnamon. The evaluation of the antifungal activity was conducted by disc method (1mg/mL), in which a standard solution of $1x10^6$ spores /ml was used (3). The zones of inhibition were evaluated every 24 hours for 5 days and the extracts with highest inhibition were selected.

Results. The results show that ether extract of garlic and ethyl acetate extract of cinnamon inhibited the fungus during the 5 days, even with better results than the chemical control (Table 1). Cinnamon showed larger zones of inhibition during the 5 days (Figure 1). The evaluated extracts of thyme and pepper species did not observe any inhibition.

Table 1. Inhibition percentage of the evaluated extracts.

Plant	Vegetable extracts			
	Ethanolic	Athyl acetate	Petroleum ether	Aqueous
Laurel	--	--	70	--
Thyme	--	--	--	--
Cinnamon	--	90	--	--
Garlic	--	--	20	--
Pepper	--	--	--	--
Control	100	100	100	100

-- Not inhibition

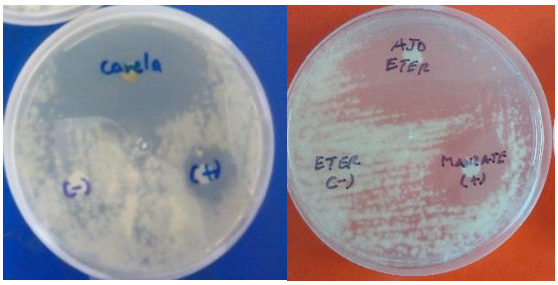


Fig 1. Inhibition zones shown by the garlic and cinnamon extracts, using manzate agrochemical as the positive control, and the solvent as negative control.

Conclusions. From all the extracts evaluated, those of garlic and cinnamon showed high antifungal activity on *Moniliophthora roreri.*

References.

1. Córdova Ávalos, V. (2014). Factores que afectan la producción de cacao (*Theobroma cacao* L.) en el ejido Francisco I. Madero del Plan Chontalpa, Tabasco, México. Ecosistemas y Recursos Agropecuarios, 17(34), 92-100.
2. Barros, O.; Sánchez, J. 1979. Un método de aislamiento del hongo *Moniliophthora roreri,* Cif y Par. Información técnica. El cacaotero Colombiano. No. 11, diciembre.
3. Cañedo V. y Ames T. 2004. Manual de Laboratorio para el Manejo de Hongos Entomopatógenos. Lima, Perú: Centro Internacional de la Papa (CIP) pp. 62.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/2018
San José del Cabo, B.C.S., México
VE22

CALLI INDUCTION IN *Randia echinocarpa* AND EVALUATION OF ITS ANTIOXIDANT ACTIVITY

Valenzuela-Atondo DA[1], Delgado-Vargas F[1], López-Angulo G[1], Calderón-Vázquez CL[2], Cruz-Mendívil A[2].
[1]Universidad Autónoma de Sinaloa, Facultad de Ciencias Químico Biológicas, Culiacán 80030. [2]Instituto Politécnico Nacional, CIIDIR Unidad Sinaloa, Departamento de Biotecnología Agrícola, Guasave 81101. Email: acruzm@conacyt.mx

Key words: Randia echinocarpa, callus, antioxidant

Introduction. *Randia echinocarpa*, commonly known as "papache" in Sinaloa, is an endemic plant from northwest Mexico. Extracts of its fruit have antioxidant, antidiabetic and antimutagenic activities [1, 2]; thus, it shows great potential to be used under sustainable conditions. However, the wild populations of "papache" have decreased by deforestation and are in risk. In this context, the *in vitro* propagation of plant species is a successful strategy employed to recover/preserve plant genetic resources at risk, and also to produce plant secondary metabolites under controlled conditions.
The goal of the present work was to establish a tissue culture protocol for the *in vitro* germination and calli induction in *R. echinocarpa*, as well as to evaluate their antioxidant activity.

Methods. *R. echinocarpa* fruits (Fig. 1A) where obtained from the sierra region of Ocoroni, Sinaloa, Mexico. The seeds were subjected to surface sterilization and *in vitro* germination in half-strength MS medium [3]. Leaf and internodal explants from eight weeks-old plantlets (Fig. 1B) were cultured in six callus induction media (MIC) with different concentrations of AIA (1 and 2 mg/L) and BAP (0.2, 0.6 y 1 mg/L). Finally, methanol extracts (Fig. 1D) were obtained from callus tissue, and their antioxidant activity was assayed by the ABTS and DPPH methods.

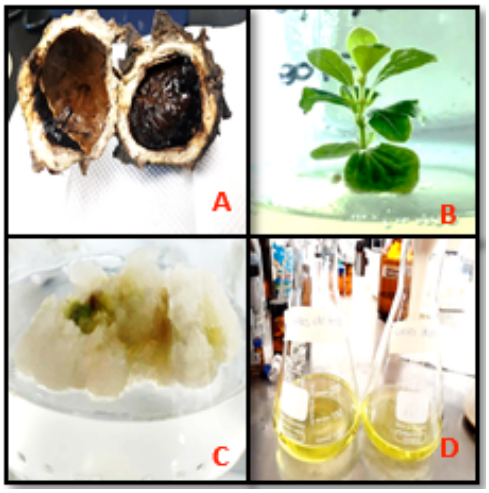


Fig.1 *In vitro* tissue culture of *Randia echinocarpa*. A) "papache" ripe fruit, B) well-developed plantlet after eight weeks of *in vitro* culture, C) Callus from leaf explant after 12 weeks of culture in MIC6, D) methanol extracts from callus tissue.

Results. The percentage of explants with callus was 100% in all treatments, and some morphological differences were observed. The explants cultured in MIC with 2 mg/L AIA formed calli with brown color, compact texture, and small-medium size; whereas, the explants cultured in MIC with 1 mg/L AIA formed calli with green-white color, compact-friable texture and medium-large size. The calli with higher biomass (Fig. 1C) were achieved with MIC6 (1 mg/L AIA + 1 mg/L BAP) after 12 weeks of culture, therefore this treatment was selected for further experiments. The antioxidant activity of calli derived from internodes (CI) was significantly higher ($p \leq 0.05$) than that of calli derived from leaves (CL) in both assays ABTS and DPPH (Table 1). The antioxidant activity values of *R. echinocarpa* in the ABTS assay were higher than those previously reported for 131 medicinal plants from India using the same method [4].

Table 1. Antioxidant activity of extracts from calli of *R. echinocarpa*

Extract	Antioxidant activity (µmol TE/ 100 g dw)	
	DPPH	ABTS
CL	49.3 ± 10.0b	343.0 ± 10.1b
CI	56.0 ± 9.0a	533.2 ± 7.0a

CL: calli from leaves, CI: calli from internodes. TE: Trolox equivalents, dw: dry weight. Different letters within a column indicate significant differences ($p \leq 0.05$).

Conclusions.
A protocol for *in vitro* germination and calli induction from leaves and internodes of *R. echinocarpa* was successfully established. Calli from internodes showed higher antioxidant activities than calli from leaves in both assays (ABTS and DPPH). This is the first report of tissue culture in *R. echinocarpa*, and may be useful for propagation and conservation purposes, as well as for the optimization of culture conditions for the production of specific bioactive compounds.

References.
1. Santos-Cervantes ME, Ibarra-Zazueta M, Loarca-Piña G, Paredes-López O, Delgado-Vargas F. 2007. *Plant Foods for Human Nutrition* 62(2):71-77
2. Cuevas-Juárez E, Yuriar- Arredondo KY, Pío-León JF, Montes-Avila J, López-Angulo G, Díaz-Camacho SP, Delgado-Vargas F. 2014. *J. of Functional Foods* 9:78–88.
3. Cruz-Mendívil A, Rivera-López J, Germán-Báez LJ, López-Meyer M, Hernández-Verdugo S, López-Valenzuela JA, Reyes-Moreno C, Valdez-Ortiz A. 2011. *HortScience* 46:1660-1665.
4. Surveswaran S, Cai Y, Corke H, Sun M. 2007. *Food Chem*. 102: 938–953.

ISSN: 2448-8461

VE23

MOLECULAR CHARACTERIZATION OF *CANDIDATUS* PHYTOPLASMA SPECIES ASSOCIATED TO BUNCHY TOP SYMPTOM OF PAPAYA IN COLIMA, MÉXICO

María José Cinco-Brown, María Elena Santos-Cervantes, Jesús Méndez-Lozano, Edgar Antonio Rodríguez-Negrete, Norma Elena Leyva-López. Instituto Politécnico Nacional, Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional (CIIDIR-IPN, Unidad Sinaloa) Guasave, Sinaloa. CP: 81101. majo_5b@hotmail.com

Key words: Bunchy Top Symptom disease, Candidatus phytoplasma, nested PCR.

Introduction. Papaya (*Carica papaya* L.*)* is the third most cultivated fruit in the world, being Mexico the first exporter. Colima state is the second in production, but the first exporting estate of México [1]. One of the main concerns of papaya producers in Colima is the bunchy top symptom (BTS) disease of papaya associated to phytoplasmas. Typical symptoms of BTS disease are shortening of internodes of the inner crown leaves, giving a bunchy appearance of the crown. Other symptoms include leaf yellowing and crinkling, mosaic, stunting, a marked reduction in latex flow, small fruits, and no flowering or fruit production in the advanced stages [2].

Due to the producers concerns about BTS disease and the high incidence of the disease, the objective of our work is to identify phytoplasmas associated to BTS disease in papaya and to know the distribution of the disease in the main producer areas of Colima, México.

Methods. DNA was extracted (CTAB 3% and PVP 3%) from papaya plants showing BTS disease. To detect and identify the phytoplasma, the 16S rRNA nested PCR amplified products (primers R16mF2/R16mR1 and R16F2N/R16R2) were purified, cloned (pGEM-T Easy Vector System) and sequenced. The phytoplasma sequences were compared with other species using the Genbank (NCBI) by BLASTn. Also, MEGA6 software using the neighbor-joining method and the iPhyClassifier [3] were used to identify the associated phytoplasma.

Results. Phytoplasmas were detected in collected samples from Colima showing the characteristic symptomatology of BTS disease (Fig. 1 and Fig. 2).

Fig.1 Papaya trees with the symptomatology associated to Bunchy Top disease.

On the basis of molecular analysis, we found that the phytoplasma associated to BTS disease of papaya in Colima correspond to 16SrI group (Fig. 3).

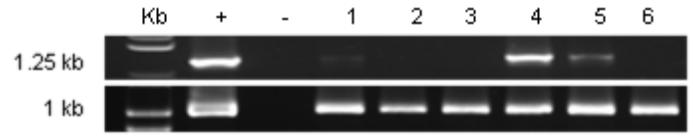


Fig. 2 Nested PCR detection of phytoplasmas. A) Nested PCR using the primers R16mF2/R16mR1 and R16F2N/R16R2. B) Internal PCR control (papaya autosomal gene).

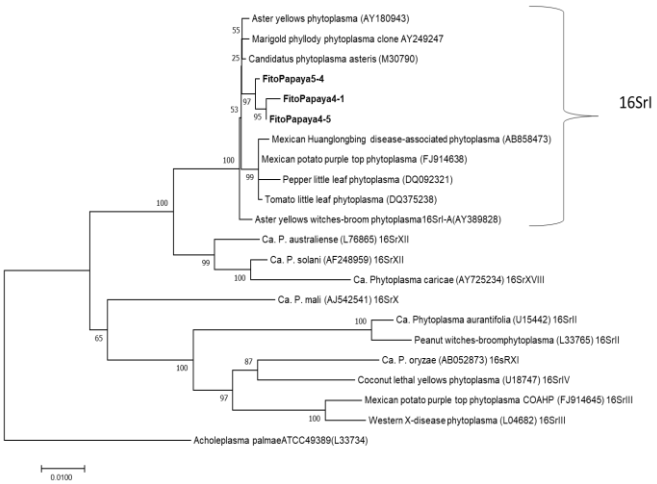


Fig 3. Phylogenetic tree based on the 16S rDNA sequences of phytoplasmas associated to BTS disease and other phytoplasma strains.

Conclusions. We identified the presence of phytoplasmas of the 16SrI group (*Candidatus* Phytoplasma asteris) subgroup AF, in the varieties Maradol y Maradol Cera cultivated in Colima.

Acknowledgements. We thank Instituto Politécnico Nacional, Especialistas en papaya S.A. de C.V. and Conacyt for the financial support.

References.

1. Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (SAGARPA). 2017
2. Acosta, K., Zamora, L., Piñol, B., Fernández, A., Chávez, A., Flores, G., ... & Arocha, Y. (2013). *Crop Protection*, *45*, 49-56.
3. Zhao, Y., Wei, W., Lee, M., Shao, J., Suo, X., & Davis, R. E. (2009). *International Journal of Systematic and Evolutionary Microbiology*, *59*(10), 2582-2593

ISSN: 2448-8461

VE24

EFFECT OF THE USE OF BACTERIA ON THE PRODUCTION OF OVILLO GRASS *(Dactylis glomerata L.)*

Stefani Meza[1], Elibeth Gonzáez[1], Rigoberto Castro[1], Karim Montalvo[1], Francisco Quiroz[2]; Instituto Politécnico Nacional - [1]Centro de Investigación en Biotecnología Aplicada, Tláxcala, México C.P. 90700; [2]Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Sinaloa – IPN. aletsemeza@gmail.com.

Key words: fodder, production, biofertilizer

Introduction.
Fodder-based animal production is an effective, economical and efficient way to produce quality food compared to the exploitation of confined animals, which are feed with concentrates and high-grain diets. Fodders are an important part of the diet of ruminants and represent a high cost in animal production (1). Grazing is considered the "natural" way to produce food of animal origin, which is why it is a well-studied option as an alternative for the economic exploitation of the land. To improve the profitability of pastoral systems, it has been pointed out that animal production per unit area should be increased by increasing the productivity of the forage component (2) (3). We evaluate the application of isolated bacteria on development of Ovillo grass.

Methods. Microorganisms of soil (S), compost (C) and digestate (D) were isolated in different selective media; it was determined if they produce phytoregulators and the germination index with respect to the distilled water control. The cultivation of Ovillo grass *(Dactylis glomerata L.)* in pots of 1.2 kg of soil was established, one series was placed in sterile soil (S) and another in non-sterile soil (N). Five isolated strains were selected and inoculated in the grass cultivation. Treatment with uninoculated seeds, negative control (C (-)) and chemical fertilizer, positive control (C (+)) were used. Digestate was applied at 60% every month, and the grass was cut every five weeks Dry matter weight was determined and the height of the plants was measured for each season.

Results.

The grass performance was higher with the strains applied with respect to the negative control.
Table 1 shows the indole acetic (IAA) and siderophore production and germination index (GI) of the selected stains. All of them produce phyto regulators this could be reason why grass grow better than using controls.
Figure 1 shows the height and dry biomass of grass after 5 weeks. Using the stains improve the development compared with both controls, except with S1, which development was similar than using fertilizer.

Stain	Strain Identification	IAA (µg/ml)	Siderophore Production (%)	GI (%)
C6	*Microbacterium oxydans*	0.0815	28.48	399.66
C31	*Bacillus toyonensis*	0.0812	-	182.35
S1	*Pseudomonas chlororaphis*	0.0813	-	107.22
S17	*Ewingella americana*	0.0813	18.49	229.22
D33	*Ewingella americana*	0.0813	21.47	87.29

Table 1. Strain characterization, IAA and siderophore production and IG index

There was no a patron using sterile or no sterile soil, in some cases using sterile one was higher than using no sterile and in other cases were upside down.

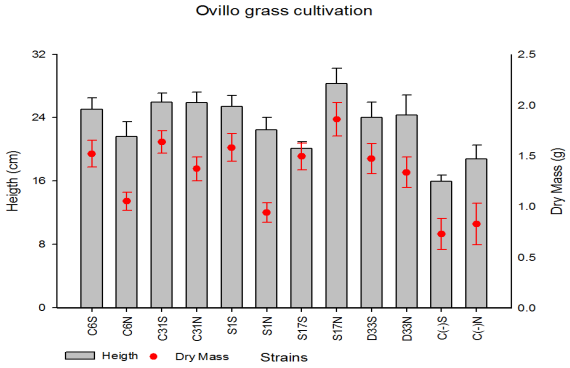


Fig.1. Heigth and dry mass of ovillo grass using stains and contols

Conclusions. Isolated stains could be used as biofertilizer and improve the grass development compared to the use of non-treated soil and chemical fertilizer.

Acknowledgements. The authors would like to thanks CONACYT for the scholarship CVU 856729 and SIP-IPN for the financial support received for this work, project 20170241.

References.
1. Pérez M, Hernández-Garay A, Pérez J, Herrera J. (2002). *Téc Pecu Méx*. México, 2002. pp.251–263.
2. Hernández-Guzmán F, Hernández-Garay A, Ortega-Jiménez E, Enríquez-Quiroz J, Velázquez-Martínez M. (2015). *Agron. Mesoam.* México, 2015. pp.33-42.
3. Villarreal A, Hernández A, Martínez P. (2014). *Revista Mexicana de Ciencias Pecuarias*. México, 2014. pp. 231-245.

ISSN: 2448-8461

VE25

EXPRESSION PROFILE OF STRESS-RESPONSIVE MEXICAN LIME miRNAs IN RESPONSE TO INOCULATION WITH *Candidatus* LIBERIBACTER ASIATICUS

Ángela P. Arce-Leal, Edgar A. Rodríguez-Negrete, Eduardo Rodríguez-Bejarano, María E. Santos-Cervantes, Jesús Méndez-Lozano, Norma E. Leyva-López. Instituto Politécnico Nacional CIIDIR-IPN, Unidad Sinaloa, Departamento de Biotecnología Agrícola. Blvd. Juan de Dios Bátiz Paredes No. 250, San Joachín, C.P 81101, Guasave, Sinaloa, México. E-mail: angela_paulina22@hotmail.com

Key words: miRNAs Huanglongbing Candidatus Liberibacter asiaticus

Introduction. Huanglongbing (HLB), the most devastating disease of citrus, is associated with infection by *Candidatus* Liberibacter asiaticus (CLas) (1). In plants, gene silencing pathways guided by microRNAs (miRNAs) constitute an important system of gene expression regulation and anti-pathogenic mechanism (2). The manipulation of miRNAs and their target genes expression levels in plants is emerging as an effective strategy for improving the responses of plant crops to biotic and abiotic stresses. In this regard, the aim of this study was to analyze, the expression profiling of some stress-responsive miRNA in Mexican lime response to CLas.

Methods. Mexican lime trees infected with CLas were bud inoculated and kept in a greenhouse. Five CLas-infected and five healthy plants were selected and bacterial titer was determined by quantitative PCR (qPCR) (3). The expression profiling of the stress-responsive miRNAs was detected using an RT-qPCR stem-loop RT-PCR method (4).

Results. A significant increase in the bacterial titer in HLB-infected trees and foliar symptoms were induced by CLas during the evaluation period (Fig.1).

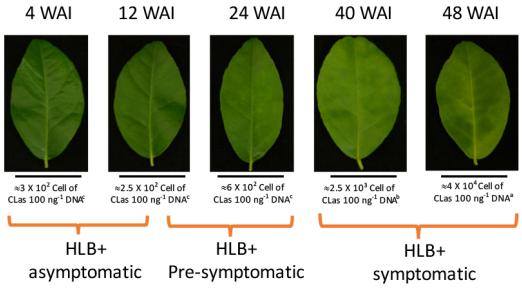


Fig. 1. Foliar symptoms and quantification of CLas in Mexican lime 4, 12 24, 40 and 48 weeks after inoculation (WAI).

The miR160 and miR393 were up-regulated at 12 WAI, nevertheless, a down regulation was observed for miR160 at 40 WAI (symptomatic stage); whereas miRNA399 showed significantly up-regulated in CLas-positive samples in the symptomatic stage (Fig. 2).

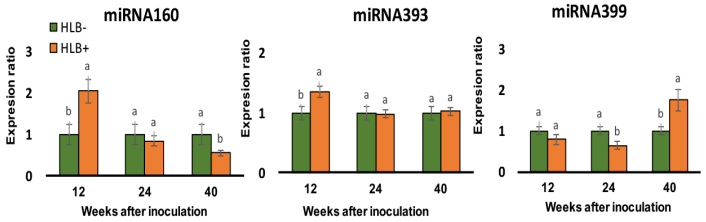


Fig. 2. Expression of miRNAs in Mexican lime trees inoculated CLas

Conclusions. This is the first study were the expression profiling of some stress-responsive conserved miRNAs involved in the response of Mexican lime trees to inoculation with *Candidatus* Liberibacter asiaticus was determined. The differential expression of these miRNAs may reflect synergistic activities at biochemical, physiological and molecular levels such as auxin signaling and sugar response to attenuate growth and development the HLB-infected Mexican lime trees.

Acknowledgements. We thank CONACYT and the Instituto Politécnico Nacional for their financial support of this research project.

References

1. Bové J. (2006). *Plant Pathol. J.* 88: 7–37.
2. Peláez P., Sanchez F. (2013). *Front. Plant Sci.* 4:1-16.
3. Li W., Hartung J., Levy L. (2006). *J. Microbiol. Methods*. 66:104-115.
4. Varconyi-Gasic E. Wu R., Wood M., Walton E., Hellens R. (2007). *Plant Methods.* 12:1-12.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México

VE26

MORPHOLOGY AND PHYTOCHEMICAL ANALISYS OF *Stevia rebaudiana* PLANTS PROPAGATED IN BIOREACTORS AND BY CUTTINGS

Dumas Gabriel Oviedo Pereira, Silvia Evangelista Lozano[1], Gabriela Sepúlveda Jiménez[1], Jorge Molina Torres[2] y Mario Rodríguez Monroy[1]; Instituto Politécnico Nacional, Centro de Desarrollo de Productos Bióticos[1], Yautepec, Morelos, 62731; Centro de Investigación y Estudios Avanzados[2] , Unidad Irapuato, Irapuato, Guanajuato,3682; Oviedo.pereria1991@gmail.com

Key words: Stevia rebaudiana Bertoni, Steviol glycoside, Temporary Immersion Biorreactor (TIB), Propagations

Introduction. *Stevia rebaudiana* Bertoni is a plant with commercial interest, due to their natural sweetener properties and antioxidant properties of the compounds present in the leaves. The propagation method traditional of *S. rebaudiana* plants is via plant cuttings; but, the plants generated show morphological and phytochemical profiles very heterogeneous. Alternatively, the plants micropropagation in bioreactors is a technique recognized that generated clonal plants. The objective of this work was to compare the morphological development, the contents of steviol glycosides (SG), phenolic compounds (PC) and flavonoids (F) from *S. rebaudiana* plants propagated using a temporal immersion bioreactor (TIB) and the method of plant cuttings.

Methods. Due the high SG content (72.3 y 58.6 mg de SG/ g DW, respectively), two *S. rebaudiana* plants were selected from a sample of ten mother plants. The plants were propagated in TIB (Figure 1) and by cuttings.

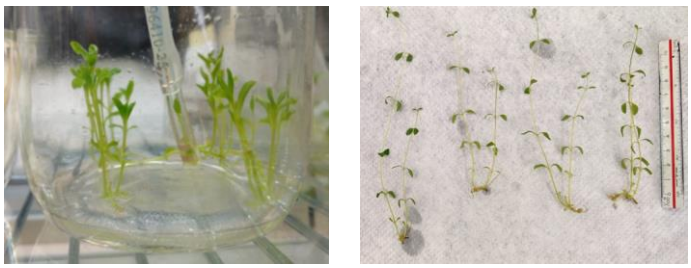


Fig.1 *S. rebaudiana* plants obtained in TIB and cuttings

The plants were grown in 10 L pots using a substrate based on peat, perlite and vermiculite (6: 2: 2) and under greenhouse conditions for 2 months. The morphological development was evaluated measuring the plant length and leaves number. SG content was determined by HPTLC according to the methodology reported by Villamarin-Gallegos et al. (1). PC and F contents was evaluated according to the methodology reported by Bobo-García et al. (2), Chang et al. (3) and modified by Villamarin-Gallegos et al. (1).

Results. Plants propagated in TIB shown a size between 10 and 15 cm, while plants propagated via cuttings reached 5 to 25 cm. The leaves number was less variable in the plants obtained in TIB (4-12 leaves per plant), than those plants propagated by cuttings (4-20 leaves per plant). Plants propagated in TIB were more homogeneous than those plants obtained by cuttings. In the same way, plants generated in TIB shown a minor variations in the contents of secondary metabolites (SG content was 15 – 60 mg SG/g DW, FC content was 10 – 20 mg EGA/g DW and F content was 20 – 50 mg EQ/g DW) than those plants obtained by cuttings (SG content was 15 – 175 mg SG/g DW, FC content was 10 – 80 mg EQ/g DW and F content was 20 – 80 mg EAG/g DW).

Conclusions. The propagation of *S. rebaudiana* plants by TIB culture generates populations with lower variability of morphological and phytochemical profile.

Acknowledgements. DGOP to CONACYT (702975/589456) and SIP-IPN (BEIFI) for the scholarship. The work was conducted with support of SIP-IPN (20180427).

References.

1. Villamarín-Gallegos, D. J. (2016). Tesis para obtener el título de Maestría en Ciencias en Desarrollo de Productos Bióticos. Instituto Politécnico Nacional. Yautepec, México. pp.1-89.
2. Bobo-García, G, Davidov-Pardo, G, Arroqui, C, Vírseda, P, Marín-Arroyo, M. R, and Navarro, M. (2014). *J. Sci. Food Agric*, *95* (1), 204–209.4.
3. Chang, C.-C., Yang, M.-H., Wen, H.-M., y Chern, J. (2002). *J Food Drug Anal*, *10*(3), 178–182.

ISSN: 2448-8461

VE27

A method for *in vitro* production of *Sclerotinia sclerotiorum* ascospores, and estimation of their viability

Valentina del Rosario Leal-Cervantes, Melina López-Meyer, Claudia María Ramírez-Douriet. Instituto Politécnico Nacional CIIDIR-SINALOA, Departamento Biotecnología Agrícola, Guasave, Sinaloa, 81101; cmramirez@ipn.mx

Key words: apotecium, ascospores, viability

Introduction. White mold is the most important fungal disease in common bean crops in the Northwest of Mexico, and it is caused by *Sclerotinia sclerotiorum*. This fungus can form resistance structures called sclerotia, which can remain alive and infective in the soil for several years, even in the absence of a host; when temperature and humidity are suitable, they can germinate either as mycelium, or form apotecia, which produce ascospores. Synthetic fungicides can control white mold disease; however, this strategy can cause contamination and human health problems. Then, other alternatives to control this disease must be investigated, and in order to do this, it is needed a way to induce the disease in control manner, either at greenhouse or field conditions. The induction of the desease by ascospore germination on senescent tissues would be an optimal strategy; however, ascospore production and collection under laboratory conditions is not a trivial process. Then, the objective of this work was to establish a methodology to obtain apotecia and ascospores in the laboratory from *S. sclerotiorum* sclerotia, as well as to determine their viability when they are storage in water at 4°C.

Methods. Sclerotia were disinfected with 0.5% commercial sodium hypochlorite, then submerged in water at 4°C with pumping air for eight weeks, according to Cobb and Dillar (2004), as well as for four weeks. Then, sclerotia were put in sterile sand saturated with water at 19°C (photoperiod 12h/12h) for two weeks. When apotecia formed, a scoscope collection was done by the method described by Steadman (1974), as well as by agitation of excised apotecia in water. Finally, viability of ascospores was tested by germinating them on PDA-neon plates according to Peres *et al*. (2002). Viability of ascospores was monitored at the time of collecting the ascospores (time 0), and after one, two and three weeks of storage at 4°C in water.

Results. The formation of apotecia was improved when sclerotia was conditioned for four weeks compared to eight weeks, because at this time the structures formed did not turned into mature apotecia, whereas at four weeks, proper apotecia formation was observed (Fig 1). For ascospore collection, the agitation of apotecia in water rendered a higher number of ascospores compared to the method described by Steadman (1974). Finally, we found that viability of ascospores storage at 4°C decrease to about 5% after three weeks.

Fig.1 Apotecia produced under laboratory conditions from esclerotia of *S. sclerotiorum*

Conclusions. Conditioning time of *S. sclerotiorum* sclerotia for the production of apotecia was reduced from eight to four weeks. Agitation of apotecia in water was a more efficient method to obtain ascospores compared to that reported by Steadman (1974). Storage of ascospores in water at 4°C reduced their viability to 5% in three weeks.

Acknowledgements. Project SIP 20161795.

References.

1. Cobb, CA, y Dillard H.R., 2004. Producción de apotecios y ascosporas de Sclerotinia sclerotiorum. El instructor de Sanidad Vegetal. DOI: 10.1094/PHI-T-2004-0604-01.
2. Peres, A.P., Nasser, L.C.B. y Machado, J. C. 2002.Use of semi-seletive media for detection of Sclerotinia sclerotiorum on bean and soybean seeds. Fitopatologia Brasileira 27:123-127.
3. Steadman, J. R., and Cook, G. E. 1974. A simple method for collecting ascospores of Whetzelinia sclerotiorum. Plant Disease Reporter. Vol. 58. N°2. pp 190.

ISSN: 2448-8461

VE28

A NEW EXPERIMENTAL SYSTEM FOR FUNCTIONAL ANALYSIS OF microRNAs ASSOCIATED TO INFECTION OF NON-CULTIVABLE BACTERIA OF GENERA *Candidatus* LIBERIBACTER

María Cristina Hernández-Peraza, Edgar Antonio Rodríguez-Negrete, María Elena Santos-Cervantes, Jesús Méndez-Lozano, Norma Elena Leyva-López. Instituto Politécnico Nacional, Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional (CIIDIR-IPN, Unidad Sinaloa) Guasave, Sinaloa. CP: 81101. *hpcristina.1407@gmail.com*

Key words: Candidatus Liberibacter solanaceraum, miRNAs, grafting.

Introduction. The growing impact of phloem-limited bacteria on high-value crops has led to a renewed interest in understanding how they cause disease. The genera "*Candidatus* Liberibacter" is associated with economically devastating diseases in many crops around the world (1). *Canditatus* Liberibacter asiaticus affects citrus and cause HLB (Huanglongbing) and *Candidatus* Liberibacter solanaceraum (Lso) is associated to Zebra Chip (ZC) of potatoes and other vegetable crops (2). Phloem-limited pathogens have small genomes and lack many genes required for core metabolic processes, which is, in part, an adaptation to the unique phloem environment (3). Recently, several miRNAs have been implicated in plant stress-response and play a vital role in regulating host responses to pathogen infection. Understanding different process of defenses in plants against non-cultivable bacteria is crucial for proposing new control strategy models.
The objective of this work was to implement an experimental system to study the infective process of non-cultivable bacteria of the genera *Candidatus* Liberibacter.

Methods. Potato (Var. Citlali, INIFAP 5-10) Lso-infected tubers treated with Gibberellic Acid to break tuber dormancy were cultivated in greenhouse. ZC disease was verified in emerging plantlets by the presence of foliar symptoms and molecular pathogen detection by PCR with primers OI2c /OA2. PCR products were purified, cloned and sequenced. The Lso obtained sequences were compared with another species using the Genbank (NCBI) by BLASTn Potato plants Lso-PCR positive were used as inoculum source to inoculate the bacteria (by graft) to potato and tomato plants. Real time PCR using the primers CLsoF/ CLsR was used to determinate the pathogen title in the inoculated plants.

Results. The sequence analysis of the amplified PCR Lso fragment from potato (Var. Citlali) revealed a similarity of 99% with Haplotype B of Lso associated to ZC. Tomato and potato Lso inoculated plants showed typical symptoms associated to the disease. Lso was detected in all the inoculated potato plants at 24 dpi. In inoculated tomato plants Lso was detected at 24 dpi in 1 out of 4, and in 2 out of 4 tomato plants at 32 dpi (Fig. 1 and Fig. 2).

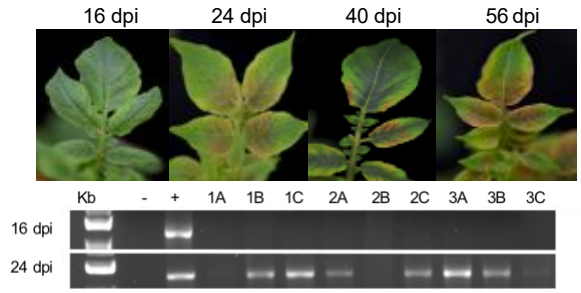


Fig. 1. Symptomatology associated to Lso in potato plants (Zebra Chip) throughout 56 dpi and molecular detection of Lso by PCR.

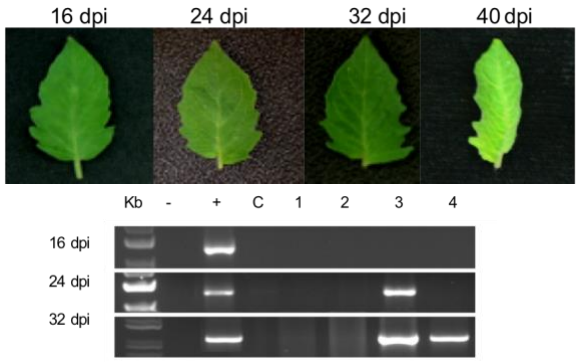


Fig. 2. Symptomatology associated to Lso in tomato plants throughout 40 dpi and molecular detection of Lso by PCR.

Conclusions. Functional studies of miRNAs involved in the response against non-cultivable bacteria associated with economically devastating diseases of citrus and potato are very limited. This new system, coupled with Mir-VIGS technology, would be used to determine the functional roll of miRNAs involved in the response against the infection of this pathogen.

Acknowledgements. We thank Instituto Politécnico Nacional (BEIFI) and CONACYT for the financial support.

References.

1. Wang N, Pierson E, Setubal J, Xu J, Levy J, Zhang Y, Li J, Rangel L, Martins J. (2017). A*nnu Rev Phytopathol*. 55:451–82
2. Haapalainen N. (2014). *Ann Appl Biol*. 165:172–198
3. Bendix C, Lewis J. (2017). *Mol Plant Pathol*. 19:38-254

ISSN: 2448-8461

VE29

COMPATIBILITY AND BIOCONTROL EFFECT OF YEAST MIXTURES FOR CONTROL OF *Colletotrichum gloeosporioides, Fusarium* sp. AND *Penicillium digitatum.*

Rose Meena A. Edward, Erika A. De la Cruz-Arguijo, José A. Narváez-Zapata, Claudia Patricia Larralde-Corona. Instituto Politécnico Nacional, Centro de Biotecnología Genómica, Laboratorio de Biotecnología Industrial, Reynosa (Tam) México, C.P. 88710. E-mail: redwardr1400@alumno.ipn.mx

Key words: Biocontrol; Yeasts; Filamentous fungi

Introduction. In the world, the major producers of citrus are Brazil, China, United States of America, Mexico, India, Spain, Iran, Italy, Nigeria and Turkey. Mexico ranked 5th place in world production even though oranges are not native plant. Most common postharvest diseases are Clear rot or Green/Blue mold (*Penicillium* spp) and Anthracnose (*Colletotrichum gloeosporioides*) [1]. Biological control using microbial antagonists (BCA) individually has received a great deal of attention as a promising alternative to chemicals.
In this work we explored the possibility of achieving a good biocontrol activity by using yeast mixtures with a high number of viable cells to be used to control *Citrus* spp phytopathogens.

Methods. Microorganisms used belong to LBI-CBG yeast and filamentous fungi collection. Yeast used are LCBG-03 (*Pichia guilliermondii*), LCBG-27 (*Macalpinomyces bursus*), LCBG-30 (*Pseudozyma sp.*), L10B2 (*Rhodotorula mucilaginosa)* and 49 *(Saccharomyces cerevisiae* 3D6*)*. Phytopathogenic fungi were *C. gloeosporioides* (AL-13), *Fusarium* sp (AL-21), *Penicillium digitatum* (AL-38) and Phoma sp (H3A). The best performing yeasts were selected for testing their compatibility and biocontrol performance. Compatibility amongst yeasts: Aliquots of 100 µL of each yeast combinations tested at initial inoculum concentration of $1x10^8$ cells/mL, and were evenly distributed on plates of 50% potato-dextrose agar (PDA) using a glass rod, incubated at 29°C. Each treatment was replicated two times. Combined growth was reported as CFU/ml. Biocontrol effect of mixed yeasts**:** Biocontrol effect of mixed yeasts was assessed. An agar plug of 0.5 cm of diameter of the most infectious citrus fungus, obtained from the edge of a 7 d colony of each of the fungi tested growth on PDA were placed facing the center of the plates and incubated at 29°C. The colonies radius were recorded periodically for 5 d. Each treatment was replicated three times.

Results. The best performing yeast combinations were LCBG-03, LCBG-03+LCBG-27, L10B2, LCBG-03+L10B2, LCBG-27, LCBG-27+L10B2 as shown in Table 1.

Table 1. Percentage of biocontrol and initial yeast concentration

Fungus / Yeast Combo	LCBG03	LCBG03 + LCBG27	L10B2	LCBG03 + L10B2	LCBG27	LCBG27 + L10B2
***C.g* AL13**	69	68	83	49	72	81
***F. sp* AL21**	53	56	48	56	51	64
***Pd.* AL38**	29	67	28	44	4	36
***P.* H3A**	33	25	64	54	27	39
Combined inhibition effect	184	216	223	203	154	220
CFU/ml (alone or in mixture)	1.13E+08	8.05E+07	1.53E+07	1.35E+08	1.07E+08	1.69E+07

Based on the percentage of inhibition of radial growth the best combinations were selected, as well as their initial cell counts, as it was observed that some yeast combinations were antagonistic to each other (data not shown). Our results demonstrate that not all combination of yeasts are compatible, and also that biocontrol efficacy depended on the fungus being controlled, being the mixture of LCBG-03 with LCBG-27 one of the best in terms of number of fungi controlled in more than 50%.

Conclusions. There are some reports in the literature about the performance of yeast combinations as biocontrol agents in horticulture crops but almost none in *Citrus* sp. This work contributes in the search of those compatible yeast combinations aimed to diminish the fungal losses of citrus fruits.

Acknowledgements. We thank the finantial support of projects CONACYT Ciencia Básica 2013-221289 and SIP2018-1748 and SIP2018-0983 (Instituto Politécnico Nacional), as well as support BEIFI-IPN given to RMAE.

References.

1. Timmer L.W., Garnsey, S.M., Graham, J.H., (Eds) 2000. Compendium of Citrus Diseases. American Phytopathological Society Press, St. Paul, MN, 92 pp.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
VE30

APPLICATION OF BACTERIA ON THE PRODUCTION OF LETTUCE CULTIVATION *(Lactuca sativa L.)*

Jeisel Delgado[1], Laura García[1], Javier Ruiz[1], Ismael Eulogio[1] , Gisela Aguilar[2]; [1]Instituto Politécnico Nacional Centro de Investigación en Biotecnología Aplicada, Tlaxcala, México; [2]Instituto de Investigación de Zonas Desérticas Universidad Autónoma de San Luis Potosí infinitium_solem@hotmail.com

Key words: PGP, digestate, biofertilizer

Introduction. The increase in the world population, has caused an increasing demand of food, especially of those of vegetable origin, and as a consequence also, the uncontrolled use of agrochemicals to produce yields that satisfy that demand. The objective of this work was to evaluate the yield of lettuce cultivation (*Lactuca sativa L.)* based on the behavior of production variables in response to the controlled application of a biofertilizer which will be made up of bacterial plant growth promoters isolated from different sources.

Methods. Microorganisms of soil (S), compost (C) and digestate (D) were isolated in different selective media; it was determined if they produce phytoregulators and the germination index with respect to the distilled water control. A crop cycle will be evaluated in each season of the year in pots with 20% compost and 80% soil, fertilized every 15 days with digestate at a concentration of 80%. Three bacterial strains will be applied alone and in every posible combination. There will be treatments with sterile (S) substrate and non-sterile substrate (NS). Among other crop variables, plant height and leaf area index will be evaluated compared with a positive control (treated with chemical fertilizer NPK 17-17-17 every 15 days) and a negative control (soil without treatment). Data obtained from the evaluation of 2018 sping season are shown.

Results. Table 1 shows the indol acetic (IAA) and siderophore production and germination index (GI) of the selected stains. All of them showed characteristics of plant growth promoters.

Figure 1 shows the height lettuce inoculated with strains A and B. Treatments with bacteria inoculated in lettuce exceed the values of the negative and positive controls.

Figure 2 shows leaf area index of the best treatments compare with the controls. Selected bacteria inoculated in lettuce recorded higer leaf area index with respect to the controls.

Stain	Strain Identification	IAA (µg/ml)	Siderophore Production (%)	GI (%)
D37 (A)	Bacillus simplex	0.0812	-	178.16
C32 (B)	Microbacterium sp	0.0811	47	151.0
S21 (C)	Bacillus megaterium	0.0812	-	175.50

Table 1. Strain characterization, IAA and siderophore production and IG index

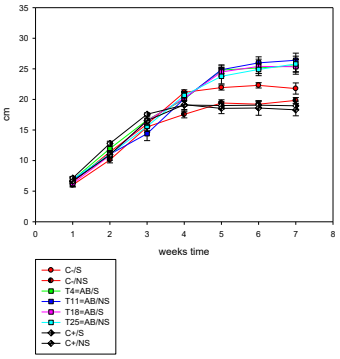


Fig.1. Height of lettuce inoculated with strains A and B

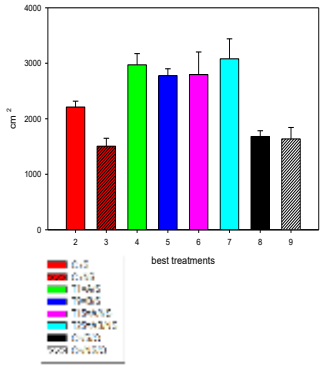


Fig. 2. Leaf area index of best treatments

Conclusions. Sterilized soil presented higher values than in non-sterilized soil in height and leaf area index. *Bacillus simplex* and *Microbacterium sp*. showed the highest values ($p < 0.05$) in the variables evaluated when they were inoculated as pure bacteria and in combination.

Acknowledgements. The authors would like to thanks CONACYT for the scholarship CVU 854823 and SIP-IPN for the financial support received for this work, project 20170313.

References.

1. Breugelmans, P. Leroy, B., Bers, K., Dejonghe, W., Wattiez, R., De Mot, R., & Springael, D. (2010). *Research in Microbiology*, *161*(3), 208–218.
2. Coronado, M., Vega y León, S., Gutiérrez, R., Vázquez, M., & Radilla, C. (2015). *Revista Chilena de Nutrición*, *42*(2), 206–212.
3. Gerardo-montoya, L., & Armenta-bojorquez, A. D. (2010). Ra Ximhai, *6*, 69–72.

ISSN: 2448-8461

VE31

SELECTION OF RHIZOSPHERIC BACTERIA FOR BIOLOGICAL CONTROL OF AGRICULTURAL IMPORTANCE FUNGI IN COMMON BEAN CROPS (*Phaseolus vulgaris* L.)

Bojórquez-Armenta, Yolani, Verdugo-Navarrete, Candelario, Martínez-Álvarez, Juan C., Mora-Romero, Guadalupe A.
Instituto Politécnico Nacional CIIDIR IPN Unidad Sinaloa. Dpto. Biotecnología Agrícola. Guasave Sinaloa, CP: 81101; yolaniba09@gmail.com

Key words: biocontrol, antagonism, bacteria

Introduction. Bean production is reduced by the presence of different pathogens such as fungi. Genus such as *Fusarium*, *Rhizoctonia*, *Botrytis* and *Sclerotium* are some examples of phytopathogens that cause diseases in bean cultivars (1). Most of the strategies to combat these pathogens are based on the use of chemical pesticides. There is a need to find pest control alternatives that guarantee the care of ecosystems and low economical investment. Use of plant growth promoting rhizobacteria (PGPR) could improve the health of plants reducing the use of chemicals in crops (2).

The aim of this study was the creation and *in vitro* assessment of a scientific collection from bean plants rhizobacteria with potential antagonism against important agricultural phytopathogen fungi.

Methods. Soil samples from bean plants roots were serial diluted in saline solution (0.85%, NaCl, p/v), plated on LB agar medium, incubated at 30 °C for 24-48 h. After that, pure bacterial colonies were selected and sub-cultivated and preserved. *In vitro* assays were performed to measure antagonistic activity against *F. verticillioides, F. oxysporum radices lycopersici, S. rolfsii, Rhizoctonia sp.* and *Botrytis sp*. according to (3) with some modifications. Percentage of inhibition was calculated by the equation previously used by (4).

Results. A collection of 63 bacterial isolates from bean plants rhizosphere was obtained. Eighteen isolates showed antagonistic ability *in vitro* against *Rhizoctonia sp, Sclerotium rolfsii and Botrytis sp*. (Table 1). The isolates BA 17 and BA 18 were antagonists against these three fungi, with inhibition up to 78% (Table 1, fig 1).

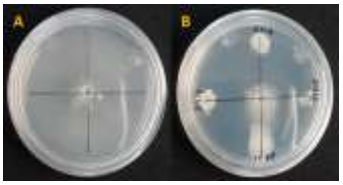


Fig 1. *In vitro* antagonistic test. A) Control (*Botrytis sp*); B) Isolated BA17, BA18 and BA19 against pathogen.

Table 1. Percentage of inhibition in antagonistic test.

Isolated	*Rh (%)*	*Bs (%)*	*Sr (%)*	*Isolated*	*Rh (%)*	*Bt (%)*	*Sr (%)*
BA 11	-	26	-	**BA 35**	70	-	-
BA 12	-	47	35	**BA 39**	-	43	-
BA 14	-	56	30	**BA 40**	63	-	-
BA 15	-	65	-	**BA 41**	45	-	-
BA 16	-	52	25	**BA 42**	54	-	-
BA 17	36	78	30	**BA 50**	-	39	25
BA 18	27	78	45	**BA 53**	-	47	-
BA 19	-	69	-	**BA 64**	-	47	35
BA 34	-	-	35	**BA 76**	-	47	-

Conclusions. Bacteria from the collection created can inhibit the growth of fungi of agricultural importance in bean crops.

Acknowledgements. Thanks to CONACyT, Instituto Politécnico Nacional and Universidad Autónoma de Occidente

References.

1. Apodaca-Sánchez, M. (2013). *Memory of Technology Transfer Conference of Bean Crop*. 7-26.
2. Bhattacharyya, P., & Jha, D. (2012). *World J Microbiol Biotechnol.* Vol. *28*(4): 1327-1350.
3. Martínez-Álvarez, J., Castro-Martínez, C., Sanchez-Peña, P., Gutiérrez-Dorado, R., & Maldonado-Mendoza, I. (2016). *World J Microbiol Biotechnol*. vol. 32(5): 1-10.
4. Paneerselvam, A., Kumar D., Thenmozhi, R., Anupama P., Nagasathya A., & Thajuddin N. (2012). *J Microbiol Biotechnol Res*. vol. (2):78–89.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
VE32

ISOLATION AND SELECTION OF FLUORESCENT PSEUDOMONADS WITH POTENTIAL BIOLOGICAL CONTROL OF WEED *AMARANTHUS* SP AND PHYTOPATHOGENIC FUNGI

Verdugo Navarrete Candelario, Bojórquez Armenta Yolani de Jesús, Martínez Álvarez Juan Carlos;
Instituto Politécnico Nacional CIIDIR-IPN Unidad Sinaloa. Dpto. Biotecnología Agrícola, Blvd. Juan de Dios Bátiz Paredes C.P.81101.Guasave, Sinaloa México.
Cande_v13@hotmail.com, jcmartinezal@ipn.mx

Key words: Pseudomonas, biocontrol, Amaranthus sp

Introduction. In agriculture there are problems due to the presence of weeds, which involves the excessive use of pesticides generating environmental damage (1). Biological control of weeds with bacteria is an ecological alternative to chemical products (2); the most promising agents are from the *Pseudomonas* group (3).
The main objective of this study is to isolate and select rhizobacteria with potential for biological control of *Amaranthus* sp. weed and phytopathogenic fungi.

Methods. A scientific collection of rhizobacteria was created from samples of soil and roots of *Amaranthus* weed, carrying out isolation and purification of fluorescent pseudomonads.
Germination and antagonism tests were performed on *Amaranthus* sp seeds and 5 phytopathogenic fungi respectively.

Results. The collection was generated with a total of 60 fluorescent isolates which were tested and 4 of these were selected based on the low percentage of germination as shown in figure 1. The isolate TR18 produced antibiosis against 2 fungi in the antagonism test (Fig. 2). In addition, in figure 3, isolate TR18 (B) showed a reduction of 75.8 % in the elongation *of Amaranthus* seedlings (A).

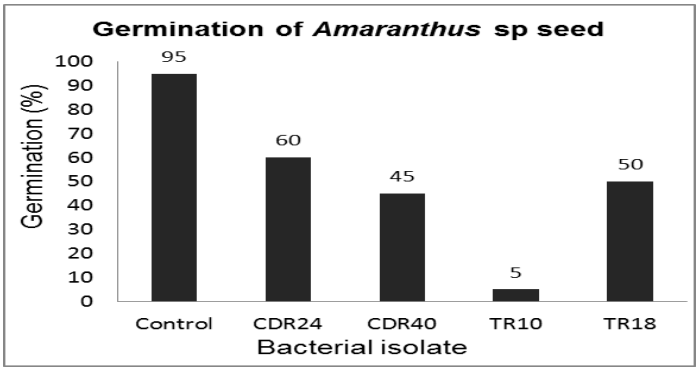


Fig.1 Shows the percentages of germination presented by the isolates tested.

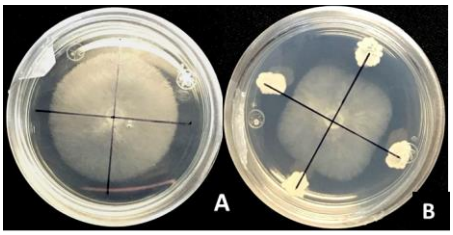


Fig. 2 Shows that isolate CDR24 against fungus *Botrytis* sp (B) and a control experiment with *Botrytis* sp fungus (A).

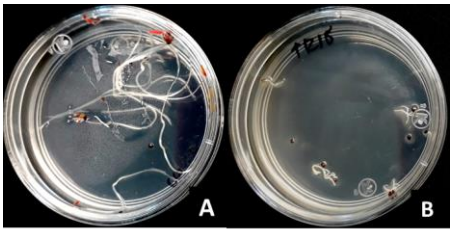


Fig. 3 Shows the elongation in the seedlings of *Amaranthus sp* in a control experiment (A) and seedlings of *Amaranthus* sp with exposure to isolate TR18 (B).

Conclusions. It was possible to obtain fluorescent pseudomonads isolates with potential to use in biological control of *Amaranthus* sp weed and phytopathogenic fungi.

Acknowledgements. The authors acknowledge the financial support from IPN (SIP 20180963) and CONACyT.

References.

1. Paredes, E. (2011). Manejo agronómico de malezas. Manual para la adopción del manejo agroecológico de plagas en fincas de la agricultura suburbana, Inisav-Inifat, 1: 173-201, 279.

2. Boyetchko, S. M. (1997). Efficacy of rhizobacteria as biological control agents of grassy weeds. Paper presented at the Proceedings of the Soils and Crop Workshop

3. Patil, V. S. (2014). Isolation, characterization and identification of rhizospheric bacteria with the potential for biological control of *Sida acuta*. *Journal of Environmental Research and Development, 8*(3), 411.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México

VE33

PROMOTER EFFECT OF SEED GERMINATION GROWTH WITH SOIL ACTINOMYCETES OF PROTECTED NATURAL AREAS THE HILL CULIACÁN, GTO. MEX.

Blanca Estela Gómez Luna; Rafael Alejandro Veloz García; Juan Carlos Ramírez Granados; César Díaz Pérez, Adan Topiltzin Morales Vargas, Laura Mejia Teniente. University of Guanajuato, Department of Agroindustrial Engineering, Celaya, C.P. 38060; be.gomez@ugto.mx

Key words: actinomycetes, soil, germination

Introduction.

The hill Culiacan was declared as a Protected Natural Area in the category of sustainable use area and in soil exist benefic bacterias named plant growth promoting bacteria and are a group of bacteria that inhabit the root of the plants and soil attached to it, this space is known as rhizosphere (Cassán et al, 2009).

This group of bacteria provides benefits to plants through several mechanisms: N_2 fixation, phytohormone production, solubilization of phosphates, synthesis of enzymes such as ACC deaminase that reduces ethylene levels, biological control, production of siderophores, antibiotics, activation of the induced systemic response and production of lytic enzymes (Glick 1995). The products generated by the different mechanisms, have direct and indirect effects on the plant in the development and growth, such as: improvement in germination, greater development of the root, stems, leaves and fruits or defense against phytopathogenic organisms (Glick, 1995, Dobbelaere et al., 2003; Esquivel-Cote et al., 2013).

In this work, we seek to isolate soil bacteria from protected natural areas and use their biotechnological potential in plant growth.

Methods.

Twenty strains were selected and inoculated in 15 ml of potato broth and incubated for 24 h at 28 ° C, the seeds of lentil (*Lens culinaris*), radish (*Raphanus sativus*) and cucumber (*Cucumis sativus*), (20) seeds were placed in 15 mL of the inoculum each for each strain and left in agitation for 30 min, the seeds were removed from the broth with inoculum and placed in sterile petri dishes with moistened paper, incubated for 48 h at 28 °C and evaluated the percentage of germination, development of the seedling and the length of the root.

Results.

For lentil seeds the control had a 60% germination and with the strains 100% was reached with the strains 254, 256 and 265; with radish the control presented 73% of germination and with the strains it was reached 100% with the strains 121, 125, 210, 225, 255; with cucumber the control presented 80% of germination and with the strains 100%, Figure 1. In addition to improving the percentage of germination, the seeds treated with the strains formed radicle in less time, more long and with abundant presence of root hairs.

Fig.1 Seeds germination with effect promoter of actinomycetes whit different plants lentil, cucumber and radish.

Conclusions.

We were able to appreciate the beneficial effect of bacterial isolates from soils of Protected Natural Areas in diverse plants. These results indicate the biotechnological potential of natural resources and from these sites bacteria can be isolated that help preserve these ecosystems, but it is also feasible to apply to plants of agronomic interest.

References.

Cassán, F., Perrig, D., Sgroy, V., Masciarelli, O., Penna, C. y Luna, V. (2009). European Journal of Soil Biology, 45(1), 28 – 35.
Dobbelaere, S. (2003). Critical Reviews in Plant Science. 107-149.
Esquivel-Cote, Rosalba; Gavilanes-Ruiz, Mariana; Cruz-Ortega, Rocío; Huante, Pilar. (2013). Revista Fitotecnía Mexicana, 36(3):251-258.
Glick, B. R. (1995). Can. J. Microbiol. 41:109-117.
Instituto de Ecología del Estado. (2015). 28-38.

ISSN: 2448-8461

VE34

KLEBSIELLA VARIICOLA IS CAPABLE TO ESTABLISH AN ENDOSYMBIOTIC INTERACTION WITH *ANTHURIUM ANDREANUM* AND *ARABIDOPSIS THALIANA* PLANTS

Patricia Samaniego, Angélica Martínez-Navarro, Ulises Garza and Nelson Avonce; Centro de Investigación en Dinámica Celular de la Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos C.P 62209; Patricia Samaniego patysc10@gmail.com

Key words: Arabidopsis thaliana, Anthurium andreanum, Klebsiella variicola

Introduction. *Anthurium andreanum* is a highly economically important ornamental plant, being the second tropical plant most traded in the world (1). The production of this ornamental requires good practices on its care and good nutrition which it one of the major factors on its price on the market. Here we show the results of the infection of *A. andreanum* and *A. thaliana* plants with *Klebsiella variicola*, an endosymbiotic bacteria originally isolated from Banana (2, 3). *K. variicola* has been described to promote plant grow by synthesizing and secreting of auxins and gibberellins, as well as its nitrogen-fixing properties (4, 5). It has been found both in soil but also as an endosymbiotic agent in corn, rice and sugarcane. We show results on the bacterial infection and localization in the plant tissue, as well as the observed phenotypes.

The main aim of the present work is to determine the effects of the infection of *Klebsiella variicola* on *Anthurium andreanum* and *Arabidopsis thaliana*.

Methods. *K. variicola* strains F2R9 and VI were transformed with plasmid pJOQmCherry, which allows us to localize the bacteria in plant tissues by fluorescent analysis. Once the identity of the bacteria was confirmed by PCR-multiplex (6), we proceded to infect a set of 5-day-old plantlets and 9-month-old plants of *Anthurium andreanum* L., as well as 10-day-old plantlets of *A. thaliana*. We evaluated the growth development of infected plants and compared againts non-transformed plants during several months by evaluating leaf number and plant height. Bacterial localization was analyzed by fluorescent microscopy and DNA isolation (*Reyna*,2016).

Results. We found that *Klebsiella variicola* is capable to infect and establish an endosymbiotic interaction with *Anthurium andreanum*. After two months of the infection event, we found the bacteria is located mostly in stem, which indicates that the bacteria has migrated from root to stem tissues. At this stage of the proyect, we have not detect any difference on growth and development between the infected and non-infected plants.

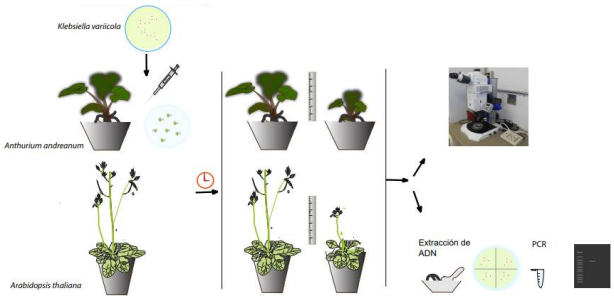


Fig.1 Schematic representation of the scientific approach

Conclusions. Our data clearly shows that *K. variicola* is capable to infect and establish a symbiotic relationship with *Anthurium*, although we have not seem any effects on growth and development so far.

Acknowledgements. We thanks to Ing. Konrad Muller Olmedo by providing tha *Anthurium* plants and Mariano Oropeza Soza president of CEPOMAC by its support and facilities to perform this project.

References.

1. Hernández, L. (2004). *EL CULTIVO DEL ANTHURIUM. CultivosTropicales*, 25 (4), 41-51.
2. Rosenblueth, M., Martínez, L., Silva, J., & Martínez-Romero, E. (2004). *Klebsiella variicola, a novel species with clinical and plant-associated isolates. Systematic and applied microbiology,* 27(1), 27-35.
3.Reyna, Fernando (2016). *IDENTIFICACIÓN Y ANÁLISIS COMPARATIVO ENTRE Klebsiella variicola y Klebsiella pneumoniae EN AISLAMIENTOS CLÍNICOS Y AMBIENTALES*
4. Martínez, E., Rodríguez, N., Beltrán, M., Toribio, J., & Garza, U. (2017). *Klebsiellavariicola and Klebsiella quasipneumoniae genomic analysis provides insights into their capacity to adapt to clinical and plant settings.* saludpública de méxico, 9-10.
5. Defez, Roberto, Anna Andreozzi, and Carmen Bianco. (2017) *The overproduction of indole-3-acetic acid (IAA) in endophytes upregulates nitrogen fixation in both bacterial cultures and inoculated rice plants.* Microbial ecology 74.2 441-452.
6. Garza-Ramos, U. et al. (2015*) Development of a multiplex-PCR probe system for the proper identification of Klebsiella variicola.* BMC Microbiol.15, 64.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
VE35

IN VITRO SELECTION OF MUTANT PLANTS OF BLACKBERRY (*Rubus fruticosus* Cv. Tupi) TOLERANT TO *Botrytis cinerea*

Huerta-Olalde, Ana Maria.[1]*, Hernández-García, Alejandra. [1], López-Gómez, Rodolfo.[1], Zavala-Páramo, Maria Guadalupe.[2], Salgado-Garciglia, Rafael.[1]

[1]Instituto de Investigaciones Químico Biológicas, Universidad Michoacana de San Nicolás de Hidalgo (UMSNH), Avenida Francisco J. Múgica S/N Ciudad Universitaria, C.P. 58030, Morelia, Michoacán, México. Phone (+52) (443) 322 3500 Ext. 4218; [2]Centro Multidisciplinario de Estudios en Biotecnología, Facultad de Medicina Veterinaria y Zootecnia, UMSNH, C.P. 58194, Morelia, Michoacán, México.

*Email: amho10@hotmail.com

Key words: *Blackberry, gamma rays, tolerance*

Introduction. The blackberry (*Rubus fruticosus*) is a crop of economic importance in México, mainly in the State of Michoacán. Its production is affected by various factors but the gray mold, disease caused by *Botrytis cinerea*, causes serious losses (1). As an alternative to generate plants tolerant to *B. cinerea*, gamma irradiated mutant plants of *R. fruticosus* Cv. Tupi were *in vitro* selected using fungus filtrate (2).

The aim of this work was to perform *in vitro* tolerance assays with *B. cinerea* in mutant plants of *R. fruticosus* Cv. Tupi.

Methods. The mutant plants of *R. fruticosus* Cv. Tupi were obtained from *in vitro* shoots gamma irradiated with 30.82 Gy (LD_{50}) and selected in 4 g/L (LC_{50}) of sterile filtrate of *B. cinerea*. Plantlets were regenerated on MS medium with 1.0 mg/L of benzyladenine (BA) and 0.06 mg/L of indole butyric acid (IBA) under room culture conditions (25°C, 16-h light/8-h photoperiod, 132 µmol/m^2s white fluorescent light) (2).

Subsequently, selected mutant plantlets were subjected to *in vitro* tolerance bioassays in detached-leaf and plantlets, inoculating 5 µL of spores ($1x10^3$ spores/mL) of *B. cinerea*, to evaluate responses of plant mutant lines against *B. cinerea* infection.

Results. Ninety-two mutant plantlet lines of *R. fruticosus* Cv. Tupi were *in vitro* propagated from shoots irradiated with 30.82 Gy and cultured on the LC_{50} of sterile fungal filtrate. After 30 days of culture, thirty-two lines were selected considering tolerant plant mutants. Detached-leaves (Fig. 1) and plantlets (Fig. 2) of these plant mutant lines were inoculated with *B. cinerea*.

After inoculation using detached-leaf and plantlet dual culture test, a total of five plant mutant lines were considered as tolerant to *B. cinerea* by present the absence or minimal symptoms of the disease. The perspective of this research is to test the tolerance to *B. cinerea* in at least three mutant lines in greenhouse assays and realize agronomical and molecular characterization

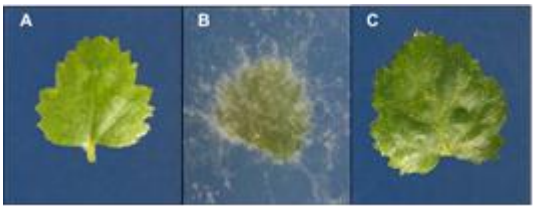


Fig.1 Leaves of *R. fruticosus* Cv. Tupi inoculated with *B. cinerea* at five days of the culture. A) Leaf without inoculum (negative control); B) Leaf with inoculum (positive control); C) Leaf of mutant plantlet with inoculum (RFUM5).

Fig. 2 Plantlets of *R. fruticosus* Cv. Tupi inoculated with *B. cinerea* at ten days of the culture. A) Plantlet without inoculum (negative control); B) Plantlet with inoculum (positive control). C) Mutant plantlet with inoculum (RFUM5).

Conclusions. The use of the sterile filtrate of *B. cinerea* as a selection agent of mutant plants of blackberry (*Rubus fruticosus* Cv. Tupi) gamma irradiated, resulted in the obtaining of mutant plantlets tolerant to *B. cinerea*, finding a direct relationship between the tolerance to the filtrate and the tolerance to fungus in *in vitro* bioassays. The mutant lines RFUM5, RFUM6, RFUM16, RFUM17 and RFUM18 were tolerant to *B. cinerea*.

Acknowledgements. This research was supported by the Universidad Michoacana de San Nicolás de Hidalgo and the Consejo Nacional de Ciencia y Tecnología (CONACyT).

References.

1. Rebollar-Alviter, A. 2011. *Manejo del Mildiú y del Moho gris de la Zarzamora en Michoacán*. Rebollar-Alviter, A. Universidad Autónoma Chapingo. México. May 2011. 34p.

2. Huerta-Olalde, A.M., *et al*. 2016. IV Simposio de Herramientas en Biotecnología para una Agricultura Sustentable (30 de noviembre-2 de diciembre), Auditorio del Instituto de Investigaciones en Ecosistemas y Sustentabilidad (IIES) de la UNAM, Morelia, Michoacán, México.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
VE36

MICROPROPAGATION OF *Hedeoma piperita* (LAMIACEAE) AN AROMATIC MEDICINAL HERB OF MICHOACAN, MEXICO

Torrez-Sosa, S., Huerta-Olalde A.M.*, Hernández-García, A., Salgado-Garciglia, R.
[1]Laboratotio de Biotecnología Vegetal, Instituto de Investigaciones Químico Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Avenida Francisco J. Múgica S/N Ciudad Universitaria, C.P. 58030, Morelia, Michoacán, México. Phone (+52) (443) 322 3500 Ext. 4218
*Email: amho10@hotmail.com

Key words: *In vitro, tabaquillo, shoots, plantlets*

Introduction. Plant tissue culture is being used widely for study the medicinal properties and for the commercial propagation of a large number of plant species, including many medicinal plants (1). *Hedeoma piperita* Benth. (tabaquillo) is herbaceous plant with aromatic stems and leaves, which habits in template forests of Central and Northern Mexico. This species is used in the treatment of digestive stomach inflammation, poor digestion, but mainly to relieve stomach pain. The populations of this plant have declined by over-exploitation in the Michoacán State, for this it is necessary to develop alternatives to its propagation. In the present study we develop a suitable protocol using stem axillary buds *in vitro* for its rapid multiplication.

Methods. *H. piperita* plant was collected from a community of Zacapu, Michoacan (19°49′00″N 101°47′27″O) and maintained in the greenhouse. Sections of the stems with buds (1-2 cm in length) were dissected and immersed in 70 % ethanol (v/v), rinsed in sterile distilled water, then soaked in 10 % detergent for 5 min with 0.5 % fungicide (Tecto 60) (w/v) for 5 min, followed in 1.2 % sodium hypochlorite with 0.5 % Tecto 60 (w/v) and finally rinsed by three washings with sterile distilled water. Damaged tissue surrounding the buds was cut off and the stems buds were cultured aseptically on MS medium with 0.05 mg/L of benzyladenine (BA) (3). To establish the optimum treatment for multiple shoot induction, the *in vitro* shoots were used as explants and cultivated on MS medium (2) supplemented with different combination of auxin (naphthalene acetic acid, NAA) and cytokinin (BA) (Table. 1) (ten flasks per treatment). The cultures were maintained at 25 ± 1 ° C, at a light intensity of 132 µmol/m^{2}s white fluorescent light (16-h light/8-h photoperiod). The obtained data regarding number and lenght of shoots, the ANOVA (variation analysis) test was used, and the means were compared using Tukey test.

Results. Shooting from the basal part of the stems buds was observed within 15 days, at 30 days of culture, the largest number of shoots (24.8 shoots/explante) was obtained in the medium containing 0.5 mg/L BA and 0.1 mg/L NAA (Fig. 1A), confirming that the use of nodal explants is the best for the propagation of herbaceous species (3). Rooting was achieved in half-strength MS medium supplemented with 1 mg/L of indol butyric acid (IBA) (Fig. 1B).

Table 1. Number and length of shoots/explant of *H. piperita* (30 days of culture.

Treatment	BA (mg/L)	NAA (mg/L)	Number	Lenght
T1	0	0	1.1±0.12^{d}	0.7±0.02^{d}
T2	0.5	0.1	1.4±0.16^{d}	0.88±0.04^{c}
T3	0.5	0.5	2.1±0.31^{c}	1.1±0.09^{c}
T4	0.5	1.0	24.8±2.2^{a}	1.96±0.09^{a}
T5	1.0	0.5	21.2±1.8^{b}	2.68±0.17^{b}
T6	1.0	1.0	2.3±0.31^{c}	0.68±0.04^{d}

Values are means ± standard error; different letters are significantly different (P ≤ 0.05) according to Tukey test.

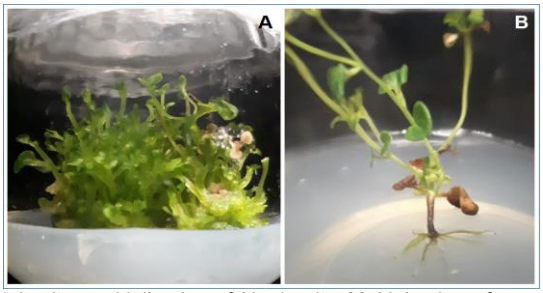


Figure. 1 *In vitro* multiplication of *H. piperita*. Multiple shoot formation (A); plantlets rooted on half-strength MS medium (B).

Conclusions. This study reports for the first time, a successful rapid method for micropropagation of *Hedeoma piperita*. In a short period of time is possible its propagation, an alternative for medicinal exploitation and conservation.

Acknowledgements. This research was supported by the Universidad Michoacana de San Nicolás de Hidalgo.

References.

1. Khan, S and Bibi, T. 2012. *Pak. J. Bot.*, 44:1965-1971.
2. Murashige, T. and Skoog F.A. 1962. *Physiol Plant*., 15:473-497.
3. Chishti, N., et al. 2006. *Pak. J. Biol. Sci*., 9:1416-1419.

ISSN: 2448-8461

VE37

ESTABLECIMIENTO DE CULTIVO SEMIHIDROPÓNICO BAJO CONDICIONES DE INVERNADERO DE *PHYSALIS AFF. RYDBERGII*

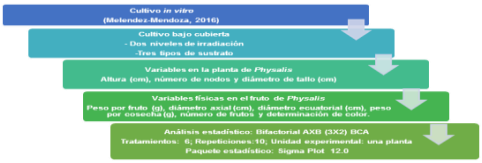
Jeanette Melendez-Mendoza; Elsa Ventura Zapata; Raúl Nava Juárez, Waldir Felipe Núñez Guzmán. CEPROB-IPN, Yautepec, Morelos, México. C.P. 62731. jmelendezm@ipn.mx

Palabras clave: Physalis rydbergii, sustratos, irradiación

Introducción. El género *Physalis* pertenece a la familia de las *Solanaceas* y comprende de 75 a 120 especies a nivel mundial. México ocupa el segundo lugar en su familia en cuanto al número de éstas, la mayoría silvestres, por ello es considerado centro de diversificación.[1,2,3] Muchas de sus especies son motivo de estudio debido a que sus frutos son comestibles, y que muchas de ellas contienen alto valor alimenticio y son fuente de importantes sustancias con usos medicinales[4,5],sin embargo, la gran mayoría aún no han sido estudiadas, tal es el caso de *P. aff. rydbergii*, la cual es una especie nativa de México. Ésta se encuentra de manera silvestre en la parte noreste del estado de Puebla, en donde los pobladores consumen sus frutos.

En el 2016 se propagó por cultivo *in vitro*, por consiguiente, fue necesario establecer las condiciones de cultivo bajo cubierta para explorar posteriormente el potencial medicinal y nutricional de sus frutos

Métodos.



Resultados.

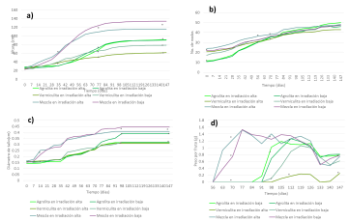


Fig.1 Interacción entre el tipo de sustrato y nivel de irradiancia en: a) altura; b) Número de nodos; c) Diámetro de tallo y e) Peso por fruto de *Physalis rydbergii*, durante los 147 días de experimentación

En la mezcla de sustratos se obtuvo mayor altura en irradiancia alta y baja (115.6 y 133.9 cm, respectivamente) presentando diferencia significativa durante la experimentación (Fig. 1a).

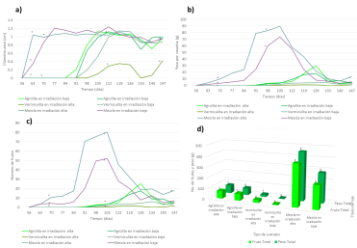


Fig. 2 Interacciones entre el tipo de sustrato y nivel de irradiancia en: a) Diámetro axial del fruto; b) Peso por cosecha; c) Número de frutos y d) Número y peso total del fruto de *Physalis rydbergii* durante los 147 días [a), b) y c)] y al finalizar la experimentación [d)].

El tipo de sustrato y nivel de irradiancia no presentaron efecto en el número de nodos (Fig. 1b), sin embargo, para el diámetro de tallo fue en la mezcla de sustratos donde se registraron los valores más altos: 0.4 cm en irradiancia alta y 0.44 en irradiancia baja (Fig.1c). La fructificación se presentó a los 63 días en la mezcla de sustratos, a los 98 en agrolita y a los 105 en vermiculita, a pesar de ello, el peso por fruto, diámetro ecuatorial y axial fue similar en mezcla y agrolita. El peso por cosecha y en el número de frutos durante y al finalizar la experimentación, fue mayor en mezcla en irradiancia alta con un número total de frutos de 390 que equivale en peso a 455.03 gramos (Fig. 2).

Conclusiones. La mezcla de sustratos en alta irradiación fueron las mejores condiciones para el cultivo de *P. aff. rydbergii*.

Agradecimientos. Los autores agradecen a la Secretaría de Investigación y Posgrado del IPN el financiamiento otorgado a ésta investigación, a través del proyecto: SIP20181241.

Referencias.

1. Whitson M., Manos P.S. (2005**)** *Syst bot***,** 30, 216-230.
2. Cuevas C., Vargas O., Rodríguez A. (2008) *RMB***.** 79, 90-99.
3. Martinez M., Vargas P., Rodríguez A., Chiang F. (2017) *Bot Sci***,** 95,131-145.
4. Valdivia L., Rodríguez Z., Sánchez J. (2016) *Sci Hortic*, 200, 83-94.
5. Puente L., Pinto C, Castro E., Cortés M. (2011) *Food Res Int* 44, pp. 1733-1740.

ISSN: 2448-8461

DETECTION OF CATECHINS IN STEMS OF *Pouteria campechiana*

José Fernando Pérez Barcena[1], Antonia De Jesús Sánchez[1], Silvia Evangelista Lozano[1]; Instituto Politécnico Nacional, Centro de Desarrollo de Productos Bióticos[1], Yautepec, Morelos, 62731

Keywords: vegetative propagation, sapotaceas, histochemistry

Introduction. Among the fruit trees with commercial potential is *Pouteria campechiana*, tree, produces fruits, which for its exquisite taste and nutritional properties has acceptance in the market (1). In vegetative propagation, the time of collection of the propagation material is an important factor to ensure success (2). It has been reported that the presence of catechin is responsible for the impediment of propagation by plant tissues (3); the catechins can be detected both biochemically and histologically; therefore, the objective of this study was to quantify biochemically and histologically the presence of catechin in propagation material (cuttings, semi-hardwood stakes, hardwood stakes) of *P. campechiana* during different times of the year to determine the appropriate period of time to collect the propagation material.

Methods: The presence of catechin in propagation tissues of *P. campechiana* was quantified biochemically and histologically during different times of the year for the determination of the adequate period of propagation, based on the type of propagation material (cutting, semi-hard and / or hard wood stake).) and at the lowest level of (+) - catechin. The quantification of catechin in the propagation material was carried out by HPLC and histological sections stained with 4-dimethylamino-cinnamaldehyde (DMACA) (4), the sections were observed in a NIKON® microscope attached to a SONY® camera.

Results: Catechin was found in all the samples collected during the seasonal transition months, observing that in May, August and September the concentrations of this molecule are higher, August was the month with the highest concentration 4.1 µg of catechin / mg of dry extract in the semi-hard wood stakes and having statistical differences with the other propagation materials (Figure 1). Different results were obtained in the months of March and January, where hardwood stakes with a concentration of 0.87 and 0.92 µg of catechin / mg of dry extract respectively being the best materials to collect.

In all the months of collection the catechins are found in cells close to the xylem and the phloem and the catechin deposits are not uniformly distributed in the cellular tissue. At the level of the tissues, the outer layers of the plant contain higher levels of phenolic that those located in their internal parts So with this can explain the presence of catechin in cells near the xylem and phloem, is special in cribosas cells since these are directly involved in transport (5).

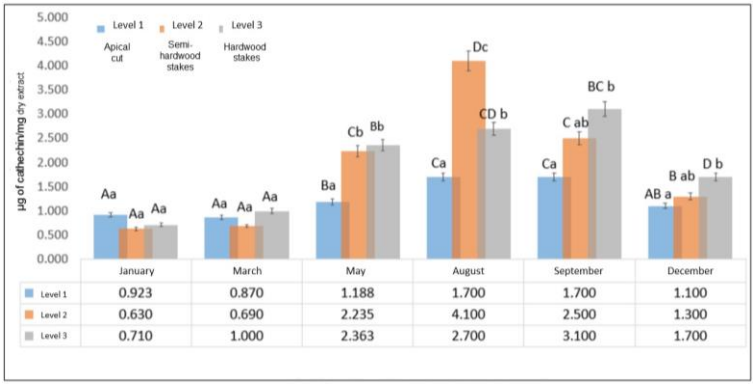


Figure.1 Catechin concentration in different months of collection of *P. campechiana* propagation material

Conclusions: The results of the biochemical detection of the catechin and the histological identification are related, and allow to better understand the evolution of the catechin content, providing an approach that allowed the association of quantitative and spatial data. In this study it was shown that both techniques are complementary and equivalent, being able to confirm the catechin dynamics in *P. campechiana* propagation material, finding in both that, the best collection period with respect to the lowest concentration of catechin was in March and occupying hardwood propagation stems, followed by January with the same characteristics, and in August and September, the period with the least desirable characteristics to carry out the propagation by stems of *P. campechiana.*

Acknowledgements: IPN, BEIFI, CONACYT

References.

1. Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (SAGARPA) y Sistema de Información Agroalimentaria de Consulta (SIACON). 2013. Sistema de información agroalimentaria de consulta de producción frutícola. México, CDMX Tomado de http://www.siap.gob.mx el día 30 de junio del 2018
2. González, G. Ruíz, B. (1999). Respuesta del chicozapote Achras sapota L. a cinco tipos de injerto y tres épocas de injertado. Horticultura Méxicana. VIII Congreso de Horticultura. Manzanillo, Col. México. 7(1): 25
3. Errea, P. (2001). Early detection of graft incompatibility in apricot Prunus armeniaca using in vitro techniques. Physiologia Plantarum 112: 153-141.
4. Gutmann, M., Feucht, W. 1991. A new method for selective localization of catechins and procyanidins. Acta Horticulturae. 381: 797-801.
5. Azcón, J. (2008). Fundamentos de Fisiología Vegetal. 2da ed. McGraw-Hill. Interamericana de España. 66-67 pp.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
VE39

RHIZOSPHERE SOIL ENZYMES OF *Bromelia hemisphaerica* AND *Jacaratia mexicana*

Silvia Evangelista-Lozano[1], Sarahi Gamarra-Morales[1], Amado Rodríguez-López[1] María Isabel Cortes-Vázquez[1]; Instituto Politécnico Nacional, CEPROBI-IPN[1], Departamento de Biotecnología. Yautepec, Morelos, 62731

Keywords: associated crop, soil enzymatic activity, soil recovery.

Introduction. Among the ecological forest units in Morelos stands out the low deciduous forest (1), this one suffers negative effects as the change of land use, a situation that banks to look for alternatives of vegetation studies to improve the landscape and soil conditions. Knowledge of soil enzymes involved in the transformation of complex organic compounds into assimilable substances by plants. The objective was to analyze the enzymatic activity in a stony hillside soil of low deciduous forest in associated crop, established 30 years ago of *Bromelia hemisphaerica* (timbirichi) and *Jacaratia mexicana* (bonete) as a strategy for recovering degraded soils.

Methods. Quadrants of 400 m² were delimited, on stony hillside (1063 masl upper part and 1060 masl lower part); one with intercropping plants of Timbirichi-bonete Quadrant 1 (C1-1: 4), and another with dominant vegetation of Zacatón Quadrant 2 (C2-1: 4). The soil collection was in different periods: dry season (February and December) and rainy season (June and August). The quantified enzymes were: cellulase (2), protease (3) and polyphenol oxidase (4). In addition, an exploration of the microbial density, the percentage of organic matter and available nitrogen was carried out.

Results. The enzymatic indicators: cellulase, protease and polyphenol oxidase have higher activity in soil with the intercropping of timbirichi-bonete, but not in Quadrant 2. In timbirichi-bonete intercropping soil in August (C13) the protease showed higher activity; a similar situation happened with protease and the polyphenol oxidase (Figure 1). Microbial density (bacteria, fungi and actinomycetes); in rain season, the largest number of bacterial colonies was at C13 of 19.66 x10 4 compared to C23 (2 x10 4); in fungi was similar C13 of 18.66 x10 2; compared to C23 1.33 x10 2 colonies /g of soil; and in actinomycetes in C13 with 12.66 x10 3 in C23 of 1.33 x10 3 colonies /g of soil. The organic matter in intercropping timbirichi-bonete in rainy season was 7.87%, which explains the higher enzymatic activity in this quadrant since the organic matter is related to the activity of cellulase and polyphenol oxidase in soil; nitrogen in rainfall was 14 ppm; the organic matter and nitrogen in the quadrant with zacatón were minor in all seasons. Nitrogen is related to the present protease enzyme activity. The intercropping of *B. hemisphaerica* and *J. mexicana* had a positive effect on soil quality; therefore, both species can be an alternative to be planted in stony hillside soil, areas not suitable for agriculture

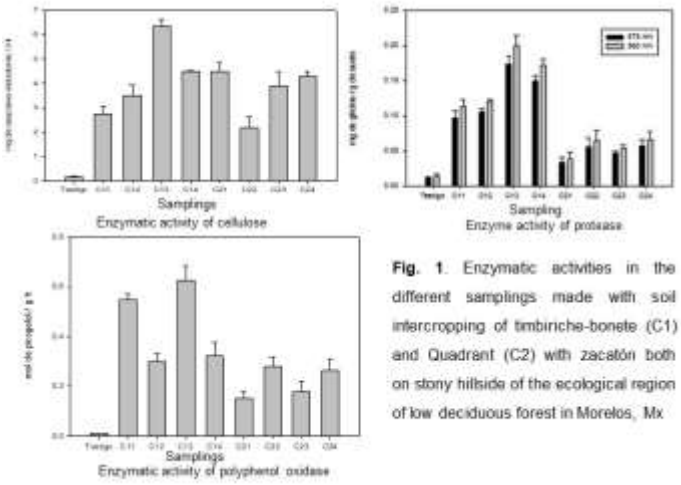


Fig. 1. Enzymatic activities in the different samplings made with soil intercropping of timbiriche-bonete (C1) and Quadrant (C2) with zacatón both on stony hillside of the ecological region of low deciduous forest in Morelos, Mx

Conclusion. The enzymatic activity of protease and polyphenol oxidase was higher in timbirichi-bonete intercropping (C1), cellulase with little difference with C2; Rainy season marked the difference, both the microbial density, the percentage of organic matter and available nitrogen; the results indicate that the intercropping timbirichi-bonete promotes a better enzymatic activity in the presence of microorganisms and improvement in nutrimental quality of the soil.

Acknowledgments to: al Instituto Politécnico Nacional (SIP, BEIFI) CONACYT.

References.

1. Contreras- Macbeath T., Monroy J. & Boyas D. C. 2004. La diversidad biológica en Morelos, estudio del estado. *Edit. UAEM*. pp: 8-70.
2. Deng S. & Tabatabai M. 1994. *Soil Biol Biochem* 26:1347–1354.
3. Wei-xian W., Qing-fu M. & Hang. 2004. J. *Soil Biol.* 40. 15–22.
4. Sinsabaugh, R., Antibus, R., Linkins, A., McClaugherty, C., Rayburn, L., Repert, D. & Weiland, T. 1992. *Soil Biol Biochem*. 24, 743–749.

ISSN: 2448-8461

VE40

EFECTO EN EL DESARROLLO Y MICROBIOTA DE PLANTULAS DE CHILE *CAPISCUM ANNUUM*, CON RESIDUOS DE LA PRODUCCIÓN DE SETAS (*PLEUROTUS SPP*) APLICADOS COMO BIOFERTILIZANTE

Jaime Herrera Gamboa[1,2], Néstor Naranjo Jiménez[2], René Torres Ricario[2], Natividad Uribe Soto[2] Laura Anabel Páez Olivan[2], Silvia Soto Ibarra[2], Nalleli Trancoso Reyes[2] y Imelda Rosas Medina[3]

[1]Instituto Tecnológico del Valle del Guadiana, Tecnológico Nacional de México. Carretera Durango-México km 22.5, Durango, Dgo. Tel: 618-5961822, [2]Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional Unidad Durango del Instituto Politécnico Nacional, Sigma 119, Fraccionamiento 20 de noviembre II, Durango, Dgo., 34200. Tel/Fax: 618-8142091, [3]Secretaria de investigación y posgrado- Instituto Politécnico Nacional, Av. Luis Enrique Erro S/N, Unidad Profesional Adolfo López Mateos, Zacatenco, Delegación Gustavo A. Madero, Ciudad de México, México.
Correo electrónico: gherrera-j@hotmail.com

Key words: *Fungi, waste, bio-fertilizers, seedlings, agriculture*

Introducción. La agricultura orgánica consiste en la comprensión del funcionamiento y conservación del suelo mediante la dinámica de las propiedades físicas, químicas y biológicas del suelo[1], en relación a la productividad de alimentos de forma sustentable, con mayor cantidad de compuestos bioactivos que aportan beneficios a la salud del consumidor[2]. En el cultivo y producción de hongos Pleurotus se emplean subproductos agrícolas como pajas de cereales (avena, trigo, cebada, entro otros); y se generan residuos al término de la producción conocidos como Spent Mushroom Substrate[3] (SMS), estos materiales presentan características benéficas para su aplicación en suelo como biofertilizante, ya que presentan avances de mineralización por la acción enzimática del hongo, contienen nutrientes disponibles para plantas, su alta composición de materia orgánica, influye en la microbiota del suelo, responsable de transformar componentes orgánicos e inorgánicos hasta elementos que puedan ser asimilados por las plantas, contribuyendo en la fertilidad del suelo, por lo anterior el objetivo de este trabajo fue evaluar el efecto microbiológico de residuos de la producción de hongos *Pleurotus* spp., elaborados con paja de avena como biofertilizante en suelo, en producción de plántulas de chile *Capsicum annuum* bajo condiciones de invernadero como sustrato.

Metodología. En un diseño experimental de bloques al azar, de acuerdo a los siguientes tratamientos: un testigo blanco con suelo al 100%, posteriormente en proporciones (1:1) entre residuo y suelo: 5, 10, 15, 20, 40, 60, 80 y 100% de SMS, las variables a evaluar fueron; altura de plántula, diámetro del tallo, numero de hojas, longitud radicular, bacterias, hongos y actinomicetos.

Resultados. Se observaron diferencias significativas ($p< 0.05$) en las poblaciones de bacterias, hongos y actinomicetos adicionados con SMS, en comparación al suelo sin aditivos, siendo T8 (12.75) UFC/g suelo para hongos, T6 (9.09) para bacterias y T2 (6.65) para actinomicetos. En cuanto al efecto en plantas, cada tratamiento difiere estadísticamente, el mejor tratamiento corresponde al T2 dónde se registró una altura promedio de 4.98 cm en comparación a los demás tratamientos, la menor altura se registró en T6 de 2.22 cm.

Conclusiones. La adición de SMS proporcionó condiciones necesarias para el desarrollo de plántulas de chile *Capsicum annuum*, favoreció en la longitud radicular en algunos tratamientos, esto puede permitir un mejor éxito en el proceso de trasplante con mayor capacidad de absorción de agua y nutrientes. Además, favorece la actividad de los microorganismos y por consecuencia contribuye a la fertilidad del suelo conforme aumento la cantidad de residuo, por lo cual sería interesante para estudios posteriores su aplicación en campo y agricultura orgánica.

Agradecimientos. Al CIIDIR-IPN Unidad Durango, CONACYT Y COCYTED

Referencias.

1. Velten S, Leventon J, Jager N, Newig J. What Is Sustainable Agriculture? A Systematic Review. *Sustainability*. 2015;7(6):7833-7865. doi:10.3390/su7067833
2. Reganold JP, Wachter JM. Organic agriculture in the twenty-first century. *Nat plants*. 2016;2(February):15221. doi:10.1038/nplants.2015.221
3. Rinker DL. Spent Mushroom Substrate Uses. *Edible Med Mushrooms*. 2017;(October):427-454. doi:10.1002/9781119149446.ch20

ISSN: 2448-8461

VE41

CHARACTERIZATION OF Arabidopsis PLANTS OVER-EXPRESSING THE *AtPAO3*-uORF

Juan Antonio Zapata-Martínez[1], Margarita Rodríguez-Kessler[2], Pablo Delgado-Sánchez[1], Maria de la Luz Guerrero-González[1]*;
[1] Biotechnology Lab, Faculty of Agronomy and Veterinary, Universidad Autónoma de San Luis Potosí, Soledad de Graciano Sánchez, SLP., Mexico. CP. 78321. [2] Facultad de Ciencias, Universidad Autónoma de San Luis Potosí. CP.
*Corresponding author: luz.guerrero@uaslp.mx

Key words: AtPAO3, uORF, regulation

Introduction. Putrescine (Put), spermidine (Spd), and spermine (Spm) are the most abundant polyamines in nature. In plants, these amines and their catabolic products participate in several processes including embryogenesis, growth and development, and in biotic and abiotic stress tolerance. *Arabidopsis thaliana* Polyamine oxidase 3 (AtPAO3) catalyzes the back-conversion reaction of Spm to Spd, then to Put (1). Through the generation of H_2O_2, AtPAO3 play a critical role during elongation of pollen tube by modulating a plasma membrane channel (2). *AtPAO3* mRNA contains an upstream open reading frame (uORF) in the 5´-UTR region (3). Usually, uORFs repress the translation of the main ORF, however, *AtPAO3* uORF function it has not been experimentally demonstrated. Here we show evidence that suggest that AtPAO3 uORF is involved in translational regulation of *AtPAO3* mRNA.

Methods. As a first approach to obtain functional information about *AtPAO3* uORF, Arabidopsis plants over-expressing the *AtPAO3* uORF were generated. Polyamino oxidase (PAO) activity was determined spectrophotometrically from leaf extracts of 15-day-old *A. thaliana* Col-0 (WT) and AtPAO3-uORF over-expression lines (ovuO-L2 and ovuO-L4) according to the protocol described by Jasso-Robles et al. (4). Germination assay under ABA treatments: thirty seeds of WT Arabidopsis, *Atpao3*$^{-/-}$ insertion mutant, and AtPAO3-uORF over-expression lines (ovuO-L2 and ovuO-L4) were germinated on 0.5x MS medium in presence of different ABA concentrations (0, 3, 5, and 8 µM). The seeds were observed daily and regarded when the radicle emerged from the seed coat.

Results. It was detected a decrease in Spd-oxidation activity in the AtPAO3-uORF over-expression lines, as compared to WT plants. In the case of Spm-oxidation activity, a significative reduction was observed only in the ovuO-L2 line (Fig. 1). It is tempting to think that the decrease in activity is due to the fact that the uORF is affecting the *AtPAO3* translation.

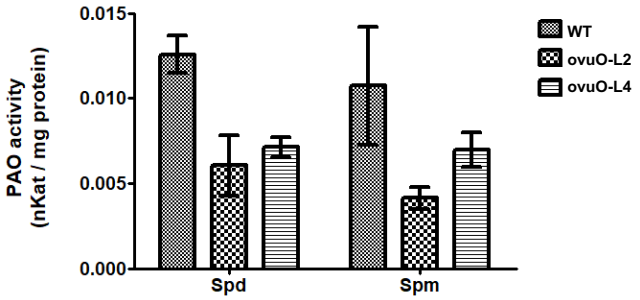


Fig.1 Polyamine oxidase activity in WT Arabidopsis, *Atpao3*$^{-/-}$ insertion mutant, and AtPAO3-uORF over-expression lines.

To gain further evidence into the control of *AtPAO3* uORF, over-expression lines were analyzed under ABA concentrations. For all of the ABA concentrations assessed, the AtPAO3-uORF over-expression lines showed a higher speed of germination than WT seeds. The behavior of over-expression lines was similar to the phenotype observed in the *Atpao3*$^{-/-}$ mutant line. Thus, these results reinforce the notion that AtPAO3 uORF is able to repress the translation of *AtPAO3*.

Conclusions. We show evidence that indicates that AtPAO3 uORF is able to act in a specific manner in translational regulation of *AtPAO3*. These findings will contribute to our understanding on the mechanisms through which uORFs regulate gene expression.

Acknowledgements. This work was financially supported by CONACYT: Investigación Ciencia Básica CB-2015-256574.

References.

1. Takahashi Y, Cong R, Sagor GHM, Niitsu M, Berberich T, & Kusano T. (2010). *Plant Cell Rep*, 29(9): 955-965.
2. Wu J, Shang Z, Jiang X, Moschou PN, Sun W, Roubelakis-Angelakis KA, et al. (2010). *Plant J.* 63:1042-1053.
3. Guerrero-González ML, Rodríguez-Kessler M, & Jiménez-Bremont J F. (2014). *Mol Biol Rep*, *41*(4): 2427-2443.
4. Jasso-Robles F, Jiménez-Bremont, J, Becerra-Flora, A, Juárez-Montiel M, Gonzalez, M, Pieckenstain, F, ... & Rodríguez-Kessler, M. (2016). *Plant Physiol Biochem*. *102*: 115-124.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
VE42

EFFICIENT in vitro MICROPROPAGATION PROTOCOLS FOR CACTUS AND SUCCULENTS

Estrella Karina Hernández-Vázquez[1], Hye Hyeong Kim[2], Gee Young Lee[2], Youn Hee Kim[2], María de la Luz Guerrero-González[1], Jae Hong Lee[2], Jae Hong Jung[2], Sang Dug Lee[2], Pablo Delgado-Sánchez[1]*.

[1] Biotechnology Lab, Faculty of Agronomy and Veterinary, Universidad Autónoma de San Luis Potosí, Soledad de Graciano Sánchez, SLP., México. CP. 78321.

[2] Cactus and Succulents Research Institute, Goyang-si, Gyeonggi-do, Republic of Korea. CP. 10224.

*Corresponding author e-mail addresses: pablo.delgado@uaslp.mx

Key words: Astrophytum myriostigma, Haworthia truncata, Organogenesis.

Introduction. Despite Mexico is the country with the greatest diversity of cacti species and some succulents, the micropropagation of this kind of plants has not come to fully enter competitive schemes of production for national and international markets (1). Trade in these species is attractive and not very demanding in quality standards (2). The objective of this work was to establish the optimal conditions for *in vitro* propagation of *Haworthia truncata* and *Astrophytum myriostigma*.

Methods. Both species plants were disinfected following a protocol generated in the Biotechnology Lab-FAV-UASLP (3). Leaf explants were dissected and placed in 0.5X and 1X MS culture media added with glucose (30 g/L) and phytagel (2.2 g/L). These media were supplemented with plant growth regulators Naphthaleneacetic acid (NAA), Thidiazuron (TDZ), and N6-benzyladenine (BA) for callus and shoot induction, and plant regeneration.

Results. We found that in both species the apex was the best explant for callus induction. However, the plants showed a differential response to the different combinations of plant growth regulators.

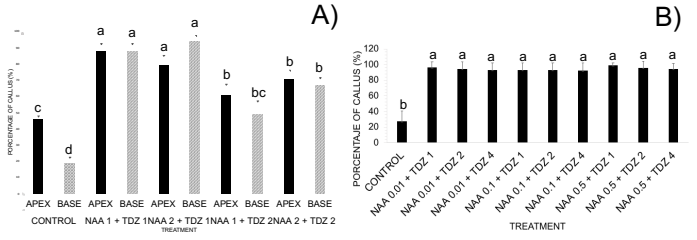


Fig.1 Percentage of callus obtained with different mediums and explants from A) *Haworthia truncata* and B) *Astrophytum myriostigma.*

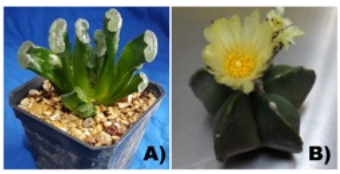


Fig 2. Plants of the species used for *in vitro* culture. A) *Haworthia truncata* and B) *Astrophytum myriostigma.*

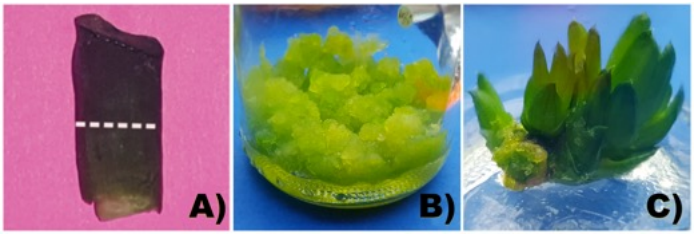


Fig 3. Phases of *in vitro* culture of the *Haworthia truncata*. A) Cuts made in the explants (apex and base); B) Lime green callus, and C) Plant regenerated *in vitro*.

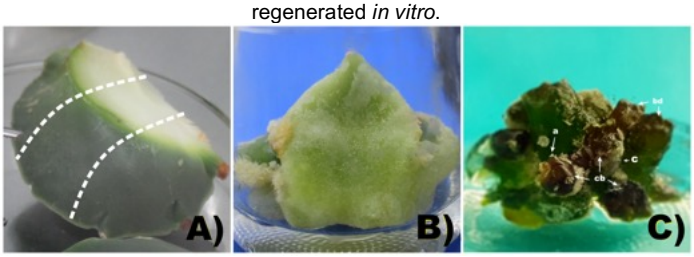


Fig 4. Phases of *in vitro* culture of the species *Astrophytum myriostigma*. A) Cuts made in the explants (apex, central, and base); B) Callus generated *in vitro;* and C) Shoots regenerated *in vitro*; a- areolas; bd- differentiated shoot; c- callus; cb- shoot with callus.

Conclusions. The protocol used for the decontamination of plant material for the species *H. truncata* and *A. myriostigma* were the indicated. The use of growth regulators such as NAA, TDZ, and BA improved the percentage of callus induction and plant regeneration. Shoots growth for these species is possible even without the presence of plant growth regulators.

Acknowledgements. This research was supported by the international cooperative research of Rural Development Administration (RDA) from Republic of Korea, Project No. PJ012429012016.

References.

1. Arredondo A. 2009. *Estandares de calidad requeridos en la produccion de cactaceas ornamentales.* INIFAP. 1-3 pp.
2. INIFAP. S/F. *Tecnologia para la micropropagación y producción in vitro de cactaceas ornamentales amenanzadas de extinción.* INIFAP. 19-20 pp.
3. Hernández E. 2018. *Desarrollo de protocolos de cultivo in vitro para crasuláceas con fines de conservación y producción.* Facultad de Agronomía y Veterinaria, UASLP. 101 p.

ISSN: 2448-8461

VE43

ANALYSIS OF THE ANTIOXIDANT ACTIVITY OF THE SEAGRASS *PHYLLOSPADIX TORREYI*

Clemente Robles-Geraldo, Mauricio Muñoz-Ochoa, Dora Luz Arvizu-Higuera, Yoloxochitl Elizabeth Rodríguez-Montesinos.
Centro Interdisciplinario de Ciencias Marinas, Laboratorio Química de Algas Marinas, La Paz, Baja California Sur, México CP. 23090; clementewv@gmail.com
Key words: seagrass, antioxidant, phenols

Introduction. Currently, one of the most researched biological activities is the antioxidant, due to the benefits they can provide to human health, in a world where oxidative stress is the causative agent of many degenerative diseases such as premature aging, various cancers, heart diseases, etc[1]. Seagrasses have been studied as a source of compounds with biological activity, which has led to knowledge of their uses. The antioxidant activity has been evaluated in different species, however, in the case *of Phyllospadix torreyi*, there are no references of their study, so this work aims to expand the knowledge about *P. to rreyi*, as a source of compounds with antioxidant activity.

Methods. The ethanolic extract (FA) of the seagrass *P. torreyi* was fractionated by means of a chromatographic column using an elution system composed of dichloromethane: methanol in polarity gradient, after which the fractions obtained were determined their antioxidant potential using the free radical 2,2- diphenyl-1-picrylhydrazyl (DPPH). The most active fraction was subjected to a second fractionation under the same conditions and the resulting fractions were re-evaluated in the DPPH assay. All the fractions were determined their phytochemical profile by means of thin layer chromatography in order to identify the type of active compounds, additionally the average efficient concentration (EC_{50}) was calculated. Finally, a quantification of polyphenols was carried out using the most active fractions; gallic acid was used as reference standard.

Results. Both the ethanolic extract and all its fractions showed scavenging activity of the DPPH free radical, the fractions with the highest polarity were the most active (Fig1). The EC_{50} of the active fractions FA and bF4 was 0.209 and 0.122 mg mL^{-1} respectively.
The phytochemical analysis revealed the presence of triterpenes, sterols, phenols, tannins, flavonoids, coumarins and saponins. Most fractions show the presence of phenolic compounds, which may be responsible for the antioxidant activity observed in the active fractions. The compounds that corresponded to the active zones were mainly flavonoids and tannins they have already been reported for their antioxidant activity in other seagrass species[2,3].

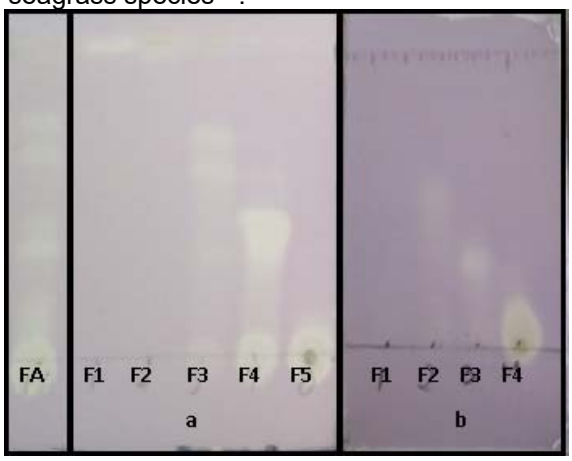


Fig.1 Thin layer chromatography (CH_2Cl_2:MeOH; 9:1, for FA and a, CH_2Cl_2:MeOH; 9:2, for b) revealed with DPPH at 0.04% in MeOH. FA= active fraction; the plate a and b are first and second fractionation respectively. The cleared areas indicate active zones.

Conclusions. The presence of sequestering activity of the DPPH stable free radical of the seagrass extract *P. torreyi* offers the option of continuing a deeper research on antioxidant activity with the possibility of isolating and characterizing active compounds useful for various industrial branches such as food and medical.

Acknowledgments. We thank the IPN for the financial support through the SIP project and COFAA exclusivity scholarship.

References.

1. Kannan, R., Arumugam, R, Meenkashi, S., & Anantharaman, P. (2010). *Int.J. ChemTech Res*, 2(3): 1526-1530,
2. Kannan, R., Arumugam, R., Thangaradjou, T., & Anantharaman, P. (2013). *Food Res Int*, 54(1): 1229-1236.
3.# Kashina, E., Kolenchenko, E., Sgrebneva, M., Kovalev, V. & Khotimchenko, Y. (2003). *Russian Journal of Marine Biology*, 29(4): 259-261.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
VE44

INDUCTION OF HAIRY ROOTS IN *CASTILLEJA TENUIFLORA*

Crescencio Bazaldúa Muñoz, Gabriela Trejo Tapia, Guadalupe Salcedo Morales, José Luis Trejo Espino.
Instituto Politécnico Nacional, Centro de Desarrollo de Productos Bióticos, Departamento de Biotecnología. Yautepec, Morelos, México. C.P. 62731. cbazaldua@ipn.mx

Key words: Hairy roots, Phenylethanoids glycosides, Castilleja tenuiflora

Introduction. *Castilleja tenuiflora* Benth (Orobanchaceae) is a wild plant used in traditional Mexican medicine to treat various ailments [(1)]. Several studies and clinical trials have shown that extracts of this species possess pharmacological properties, which are related with different groups of secondary metabolites such as iridoid glycosides, phenylethanoid glycosides (PhGs), flavonoids and lignans [(2,3,4)]. Biotechnological systems are an alternative source of the bioactive compounds and among these, PhGs are produced by *in vitro* root cultures of *C. tenuiflora* [(4)]. Higher quantities of secondary metabolites have been obtained through hairy roots cultures [(5)] but in previous attempts, *C. tenuiflora* was recalcitrant to transformation with *Agrobacterium rhizogenes.*
The objective of this work was to establish hairy roots cultures of *Castilleja tenuiflora* as a potential source of the secondary metabolites with pharmacological activity.

Methods. Dra. Guadalupe Salcedo facilitated *C. tenuiflora in vitro* plantlets. Putative hairy roots were obtained by infecting nodes and roots with *A. rhizogenes*. Two types of explant infection were evaluated: 1) sonicating the apex of the plantlet during 5, 10 or 15 seconds; and, 2) by direct infection of nodes and roots, with three *A. rhizogenes* strains (15834, A4 and K599). Putative hairy roots were detached and placed in Petri dishes containing hormone-free MS at 70% culture medium, amended with 3% sucrose and 0.26% phytagel; then were kept at 25 ± °C, and photoperiod 16/8 LD.

Results. Putative hairy roots were induced by direct infection of nodes and roots of *C. tenuiflora* and only by two *A. rhizogenes* strains (15834 and A4), because K599 did not induced putative hairy roots. The infection by sonication was not effective. Figure 1 shows the sprout of putative hairy roots from nodes, and roots. The sprout of putative hairy roots was strain-dependent, but not explant-dependent.

Fig 1. Sprouting of putative hairy roots after 15 days node and root infection (a, and b respectively), 30 days after nodes infection (c) 22 days after root infection (d).

The highest efficiency of transformation was achieved with 15834 strain (Table 1).

Table 1. Effect of different bacterial strains on percentage of putative hairy root induction in nodes and leaves of *C. tenuiflora.*

A. rhizogenes strain	Nodes direct infection	Roots direct infection	Number of putative hairy roots	
			Nodes	Roots
15834	22.5%	30%	36	9
A4	3.0%	5%	6	3

Putative transformed roots induced by the 15834 strain infection were “hairier” than those induced with the A4 strain (Figure 2).

Fig.2. Putative hairy roots sprouted from nodes infected with two *A. rhizogenes* strains: a) 15834, b) A4, and not transformed root (c).

Conclusions. The highest transformation efficiency was obtained by infecting directly roots of *Castilleja tenuiflora* with strain 15834 of *A. rhizogenes*. *C. tenuiflora* was recalcitrant to the infection with the strain K599.

Acknowledgements. This work was partially financed by CONACyT (grant CB-2013-220007) and by SIP-IPN (grant SIP-20181383.).

References.

1. Graham JG, Quinn ML, Fabricant DS, Farnsworth NR (2000). *J. Ethnopharmacol*. 73:347–377.
2. Jiménez ME, Padilla ME, Reyes ChR, Espinosa LM, Melendez E, Lira–Rocha A (1995) *Biochem System Ecol.* 23:455–456.
3. Herrera-Ruiz M, López-Rodríguez R, Trejo-Tapia GB, Domínguez-Mendoza BE, González-Cortazar M, Tortoriello J, Zamilpa A (2015) *Molecules* 20:13127-13143.
4. Gómez-Aguirre YA, Zamilpa A, González-Cortazar M, Trejo-Tapia G (2012) *Ind Crops Prod*. 36:188-195.
5. Rekha K, Thiruvengadam M. (2017) Secondary Metabolite Production in Transgenic Hairy Root Cultures of Cucurbits In: Transgenesis and secondary metabolism. Springer International Publishing Switzerland S. Jha (ed.) 267-293

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México

VE45

ANALYSIS OF THE MATING TYPE AND ITS RELATION TO VIRULENCE IN USTILAGO MAYDIS

González Prieto Juan Manuel [1], Melgarejo Hernández Keyla Andrea [1,2], Hernández Delgado Sanjuana [1], Villota Salazar Nubia Andrea [1]

[1]*Centro de Biotecnología Genómica-Instituto Politécnico Nacional. Blvd. del Maestro esq. Elías Piña s/n. Col. Narciso Mendoza 88710. Reynosa, México. Correo electrónico: jmgonzalezp@ipn.mx*

[2]*Universidad Francisco de Paula Santander, Cúcuta, Colombia.*

Key words: Huitlacoche, virulence, locus b

Introduction

Ustilago maydis (De Candolle) Corda is a pathogenic fungus that causes the disease of "common charcoal", or "huitlacoche" in corn. The formation of tumors in the aerial tissues of the plant is the main characteristic of the disease (1). During its life cycle, *U. maydis* has two forms of growth: outside the plant it is yeast-like, haploid, saprophytic and non-pathogenic; while inside the plant it is mycelial form, diploid and pathogenic. Mating and pathogenesis are controlled by two loci called *a* and *b*: The biallelic *a* locus is required for the fusion of sexually compatible sporidia, and the multiallelic *b* locus controls filamentous growth and pathogenicity (2). The objective was to evaluate the relationship of the mating type of the fungus with its virulence in maize plants.

Methods

Different fungal isolates from Mexico, Colombia and the United States were confronted with corn seedlings. The sexual type of the fungus was determined by the fuz reaction, PCR amplification and sequencing. The virulence tests were carried out in a greenhouse using the Cacahuazintle and Jalisco maize varieties. The statistical analysis of the results obtained in pathogenicity tests was carried out by the Kruskal-Wallis method in the IBM SPSS 23.0 software.

Results

The differences in virulence showed that the fungal isolate that had the b19 allele was the most virulent in the Cacahuazintle variety (fig. 1). Similarly, the sporidia with the b13 allele was the most virulent in the Jalisco variety (fig. 2). In both trials, the isolates that presented the b18 allele was found to be the less virulents (figs 1 and 2).

Conclusions

The sporidias that have alleles b19 and b13 showed greater virulence in corn plants. We suggest that the variety of corn has a close relationship with the susceptibility to the fungus.

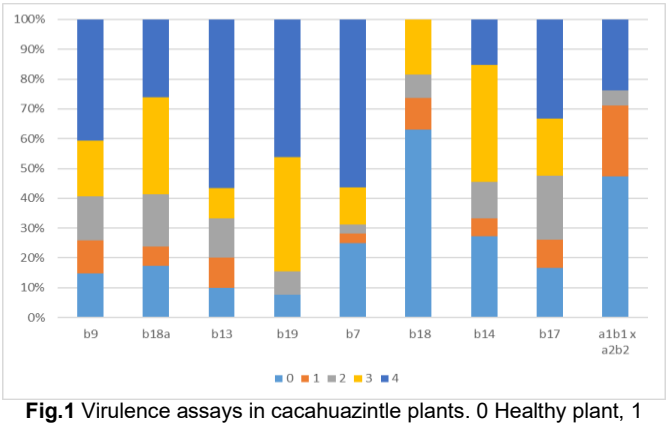


Fig.1 Virulence assays in cacahuazintle plants. 0 Healthy plant, 1 chlorosis and anthocyanins, 2 Small tumor, 3 Large tumor, 4 Dead plant.

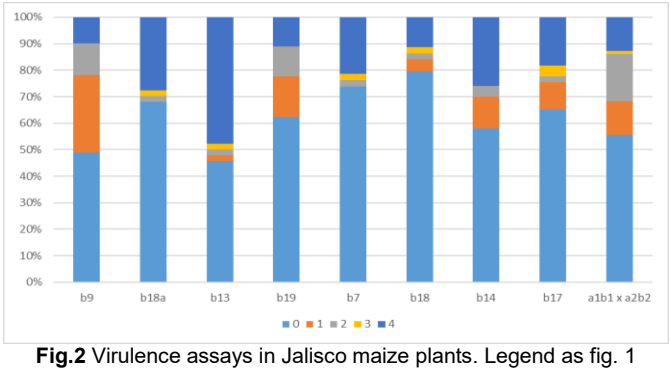


Fig.2 Virulence assays in Jalisco maize plants. Legend as fig. 1

Acknowledgements.
Proyecto SIP-IPN. Glez-Prieto foundation.

References

1. Ruiz Herrera, J. (2008). Ustilago maydis: ascenso de un hongo mexicano de la gastronomía local al mundo científico. Nova Scientia, 1(1).
2. Banuett, F. (1995). Genetics of Ustilago maydis, a fungal pathogen that induces tumors in maize. Annual review of genetics, 29(1), 179-208.

ISSN: 2448-8461

VE46

EFFECT OF DROUGHT STRESS ON PHYSIOLOGICAL AND TRANSCRIPTOMIC TRAITS OF THREE *Phaseolus vulgaris* CULTIVARS

Analilia Arroyo-Becerra[1], Josefat Gregorio-Jorge[2], Karen Lizeth Chavarría-Alvarado[1], Selma Ríos-Melendez[1], Melina López-Meyer[3], Flor de Fátima Rosas Cárdenas[1], Silvia Luna Suárez[1], Miguel Ángel Villalobos-López[1].

[1]Instituto Politécnico Nacional, Centro de Investigación en Biotecnología Aplicada. Laboratorio de Biología Molecular y Biotecnología de Plantas. Tlax. México. 90700. [2]Consejo Nacional de Ciencia y Tecnología. [3]Instituto Politécnico Nacional, Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional. Departamento de Interacción Microorganismos-Plantas. Guasave, Sin. México. 81049.

e-mail alarroyo@ipn.mx; analilia_arroyo@hotmail.com

Key words: Phaseolus vulgaris, drought stress, transcriptomics

Introduction. Common bean (*Phaseolus vulgaris*) is one of the most important vegetable protein sources in developing countries. In our country there are bean varieties capable of resist water deficit stress conditions that have developed a series of physiological, morphological and metabolic adaptations, governed by changes in the regulation of gene expression. Despite the economic importance of common beans and their genetic diversity, with approximately 2900 records of cultivated varieties (1), genomic information sources of *P. vulgaris* cultivars are still scarce (2, 3).

The aim of this study was to evaluate drought resistance of three *P. vulgaris* varieties that are among the most sown in our country. The variety with the highest resistance level was selected to obtain genomic information and differential gene expression in response to drought.

Methods. Three varieties of *P. vulgaris* were evaluated: Negro Plus (NP), Azufrado Higuera (AH), and Pinto Saltillo (PS). Drought resistance tests were carried out under greenhouse conditions with phenotypic and physiological parameters (photosynthetic efficiency, aerial height, root length, fresh /dry weight ratio). The variety with the highest resistance level was selected to make a transcriptomic analysis by RNAseq with Illumina technology.

Results. Common bean plants were submitted to a period of progressive water deficit for two weeks by suppression of irrigation. Visual inspection showed that PS suffered less damage in leaves that AH and NP, even though differences at glance in height between control and drought-treated plants suggested that NP was the variety with less growth reduction during drought (Fig. 1a). To determine whether these bean varieties can recover after the drought treatment, normal irrigation of all drought-treated plants was re-established. Two weeks later, post-drought recovery plants were assessed by visual inspection, finding that PS was greener and robust than the other bean varieties (Fig. 1b)

Quantitative assessment of physiological and morphological features of three common bean varieties subjected to drought and then recovery, indicate that PS resist drought stress more than AH and NP varieties (data not shown.

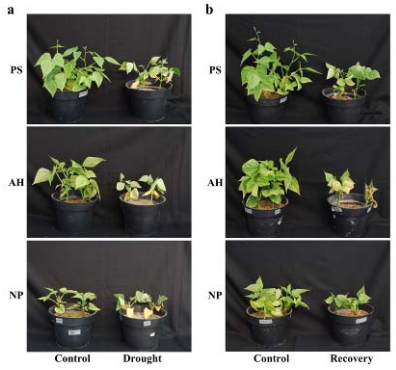


Fig.1 Effect of drought in three common bean varieties. a) Drought stressed plants. b) Two-weeks recovery.

RNA-seq analysis shows that several genes of the PS variety are dynamically modulated by drought (Fig.2)

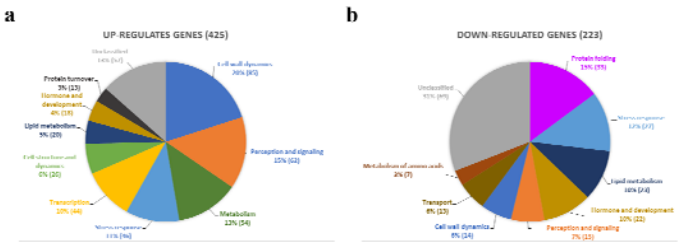


Fig.2 Classification of PS DEGs according to cellular processes in response to drought stress. a) Up-regulated genes. b) Down-regulated genes.

Conclusions. Functional and enrichment analysis of DEGs suggest that most of the up-regulated genes in PS in response to drought belong to processes related to carbohydrate metabolism within the cell periphery, whereas down-regulated genes are associated to abiotic stress response.

Acknowledgements. We thank Instituto Politécnico Nacional-SIP and CONACYT1452 for financial support.

References.

1. Singh SP. (2001). *Crop Science* 41:1659-1675.
2. Meloto M, Monteiro-Vitorello CB, Bruschi A, Carmago LEA. (2005). *Genome* 48:562-570.
3. Ramírez M, Graham MA, Blanco-López L, Silvente S, Medrano-Soto A, Blair MW, Hernández G, Vance CP, Lara M. (2005). *Plant Physiol.*, 137(4): 1211-1227.

ISSN: 2448-8461

VE47

MINING HALOPHYTES AND ANALYSIS OF THEIR POTENTIAL USE AS BIOFERTILIZERS IN COMMON BEAN AND MAIZE

Miguel Angel Villalobos López[a], Andrea Salvador Muñoz[a], Selma Ríos Meléndez[a], Cecilio Mauricio Ramos[a], Lourdes Girard Cuesy[b], Martha D. Bibbins Martínez[a], and Analilia Arroyo Becerra[a]. [a] Centro de Investigación en Biotecnología Aplicada-Instituto Politécnico Nacional, Tepetitla, Tlaxcala, México. [b]Centro de Ciencias Genómicas-UNAM, Cuernavaca, Morelos, México. Email: alarroyo@ipn.mx, Phone: 52 55 57296000 ext. 87805.

Key words: Drought, Salinity, Biofertilizer

Introduction. The application of Rhizobiaceae bacteria as biofertilizers can improve the performance of *P. vulgaris* in regards to grain production, biomass, N content, and a moderate better response to abiotic (including drought and salinity) and biotic factors, among other benefits. Several strategies have been successfully used to improve rhizobium-induced plant tolerance to drought: rhizobium overexpressing the trehalose-6-phosphate synthase gene in order to increase the trehalose content (1), enhanced expression of cbb3 oxidase to increase respiratory capacity (2), and use of validamycin as a trehalase inhibitor (3). Unfortunately, because of regulatory and economic facts, the use of these strategies on field is not viable at the moment.
Thus, it is necessary the hunting for novel autochthonous rhizobium strains adapted to extreme climate conditions for its potential use as biofertlizers.

Methods. Soils from arid and salty areas were used to isolate *P. vulgaris* associated bacteria. Our isolates were characterized using physiological and molecular approaches. The ability of selected isolates to enhance the salinity and drought tolerance of *P. vulgaris* and maize was analyzed under greenhouse conditions.

Results. Using *P. v ulgaris* Negro Plus as tramp-plant under greenhouse conditions, a total of 101 Gram-bacterial isolates were obtained from soils collected in different regions of Hidalgo, Tlaxcala, and Puebla States. 57 of these isolates were isolated from fertile soils with low levels of salinity (Low Salt Soils), whereas the rest (44) were obtained from soils with very high levels of salinity (High Salt Soils). The bacterial collection was classified according to morphological and molecular characteristics (Fig. 1). Also, the ability to growth under high osmotic and saline conditions was evaluated, and also their ability to solubilize phosphate and to fix atmospheric nitrogen. Interestingly, several isolates are able to enhance the tolerance of *P. v ulgaris* and maize under drought and saline conditions.

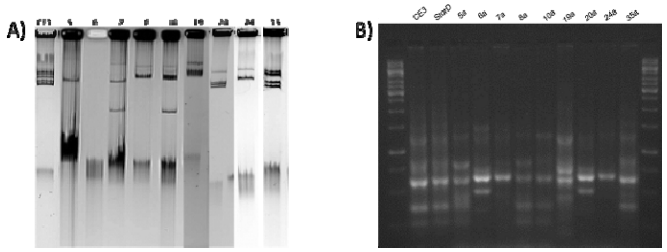


Fig.1 Molecular classification of isolates obtained from fertile Low Salt Soils. A) Eckhardt gel electrophoresis showing the nine megaplasmid band patterns found between the 57 isolates. B) The band profile obtained by TP-RAPD confirm the genetic differences for the nine groups previously determined by Eckhardt gel electrophoresis

Conclusions. -Both, saline or no saline soils harbors bacteria able to induce *P. vulgaris* nodulation. -For the non saline soils isolates we found at least nine different patterns of megaplasmids. -Regardless of the soil of origin, we found osmotolerant and halotolerant isolates, although the High Salt Soils seems to be enriched on those abiotic stress tolerant bacteria. –Some isolates are able to induce a stronger nitrogen fixation to P. vulgaris under drought conditions. –Some isolates clearly enhances drought and salinity tolerance to maize and common bean plants.

Acknowledgements. We thank Instituto Politécnico Nacional-SIP 20181190 for financial support.

References.

1. Suárez et al., (2008) *Mol Plant Microbe Interact*, 21:958–966
2. Talbi et al., (2012) *J Exp Bot*, 63:5035-5043
3) López et al., (2009) *J Plant Phys*, 166:1218-122.

.

ISSN: 2448-8461

VE48

EFFECT ON PERFORMANCE OF *Pennisetum purpureum*, AT DIFFERENT CONCENTRATIONS OF MYCORRHIZAL INOCULANTS

Primavera Pérez Ramos[1], Yuri Villegas Aparicio, Rigoberto Castro Rivera[2;.]
[1] Posgrado en Ciencias en Productividad en Agroecosistemas-Instituto Tecnológico del Valle de Oaxaca. Ex hacienda de Nazareno, Xoxocotlán, Oaxaca. CP. 71230. Tel. (01) 951 5170444. Correo electronico: primaveraperez39@gmail.com.
[2]Instituto Politécnico Nacional, CIBA Tlaxcala. Ex-Hacienda San Juan Molino Carretera Estatal Tecuexcomac-Tepetitla Km 1.5, Tlaxcala C.P. 90700, Tel.: 01-248-48707-65

Key words: Pennisetum Purpureum, Inoculant, Performance.

Introduction.

The elephant grass *Pennisetum purpureum*, has extensive use as feed for livestock, and is used for its high efficiency and dry matter production (Ramos-Trejo et al., 2015). Nevertheless; increase their yields with the use of bio-fertilizers, favors the increase of phenological variables and forage quality. Given the above the aim of this experiment was to evaluate the effect of inoculation with mycorrhizal fungi at different concentrations in the phenological variables of elephant grass *Pennisetum purpureum*, in the region of the Central Valleys of the State Oaxaca, México.

Methods.

9 cotypes of *Pennisetum purpureum* were planted, which were given a period of 6 months establishment, and on this date the uniformity cut was made to reduce the effect of covariate; likewise, a mycorrhizal consortium was inoculated. It was established in a completely randomized design with 9x4 factorial arrangement, with the “A” factor corresponding to the 9 ecotypes of *P. Purpureum*. The "B" corresponds to the inclusion levels of the consortium. The variables evaluated were: Dry matter, plant height and shoot length. The data obtained were analyzed using SAS statistical software, using the GLM procedure and comparing means by Tukey (P <0.05).

Results.

Ecotype Mott was the one recorded the highest values of dry matter, number of shoots, leaf and stem production, plant height and regrowth length, being higher (P <0.05) than CT-115 and Roxo ecotype.

Although ecotypes were evaluated under the same conditions of temperature and soil, the responses of these were differential, so should continue to evaluate the effects of inclusion of mycorrhizae in soil.

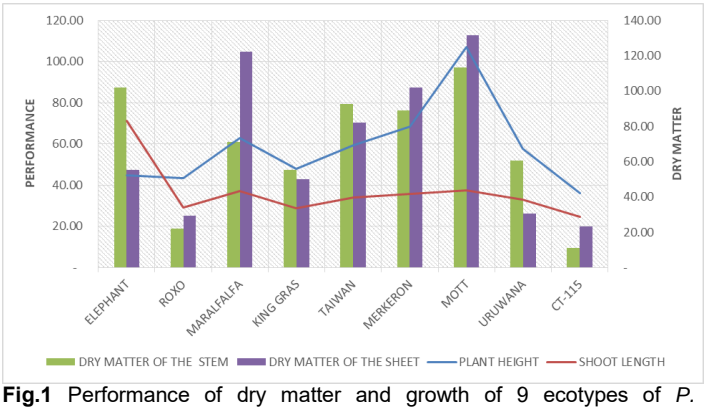


Fig.1 Performance of dry matter and growth of 9 ecotypes of *P. purpureum* in the rainy period.

Fig 2. Data logging of variables evaluated

Conclusions.

Ecotypes have different responses in the dry matter yield even though were in similar conditions of production, so the response can be explained by the inter-species genetic differenciation of the plant or by the interaction between microorganisms and varieties.

References.

1. Ramos-Trejo, O. Santiago, Victoria-Graniel, C. Arturo, y Sandoval-Gío, J. José. .2015. Season, fertilization, and yield of varieties of *Pennisetum purpureum*. Rev. Agrociencia, *49*(8), 837-844.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México

VE49

DEVELOPMENT OF A PLATFORM FOR RECOMBINANT PROTEIN EXPRESSION IN TOBACCO CELL SUSPENSIONS USING BIOLISTIC

Lourdes J. Germán-Báez, Claudia D. Norzagaray-Valenzuela, Angel Valdez-Ortiz*; Facultad de Ciencias Químico-Biológicas, Universidad Autónoma de Sinaloa, Culiacán, Sinaloa, México, Cp. 80030. *avaldez@uas.edu.mx

Key words: Microparticule bombardment, tobacco cell suspensions, Recombinant protein

Introduction. Nowadays, there is an increase in global demand for recombinant proteins with therapeutics, nutraceutics and food applications. Transgenic plant cell suspensions represents an attractive model of expression since can reach high production rates in a short period of time, representing less cost of production [1,2]. Biolistic has turned out to be an easy, efficient and robust biotechnological tool which has been used for genetic transformation of diverse variety of living cells and organisms [3]. This study aimed to develop a system for the expression of recombinant proteins in tobacco cell suspensions by using genetic transformation by biolistic.

Methods. The cell culture methodology was based on Santos-Ballardo *et al* [4]. The genetic transformation method was achieved by evaluating the effect of three factors: the osmotic medium, the rupture-disc pressure and the bombardment distance throughout the expression of the reporter gene *gusA*. The % of transformation of the different treatments were analyzed using multifactorial analysis of variance and means were compared by the Fisher's test ($p<0.5$) using Statgraphics Centurion XV software. Treatment with the highest % of transformation was used for the transformation of cell suspensions with the amarantin cDNA [4]. Molecular PCR analyses were carried out in hygromycin-resistant cells, and recombinant protein expression was confirmed by Western blot.

Results. Through the *GUS* biochemical test the different treatments were compared (Fig. 1). According with the multivariate analysis of variance, the best transformation efficiency (60%) was obtained through the combination of 900 psi, shooting distance of 7 cm and osmotic pretreatment with 0.5 M sorbitol. Using these conditions, transgenic cells (Fig.2, a-b) expressing recombinant amarantin were obtained (Fig. 2c).

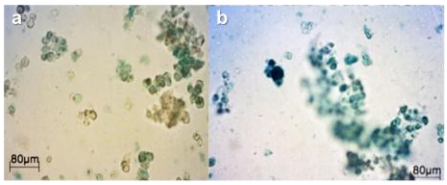


Fig.1 Histochemical *GUS* assay of tobacco transformed cells expressing the *gusA* gene transiently (a), and stably (b).

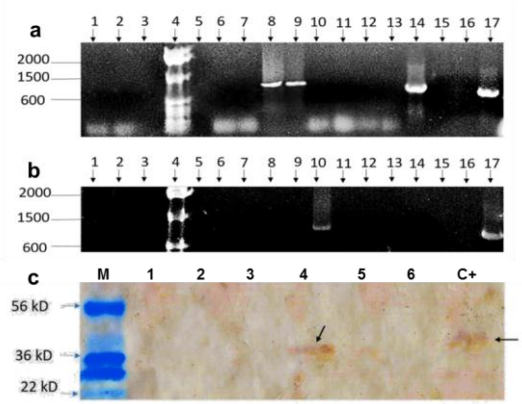


Fig.2 Molecular and biochemical analyses of transgenic tobacco cell suspensions. a and b, PCR of hygromycin-resistant cells, some of which showed the presence of the amarantin cDNA fragment. c, Western blot analysis of total protein extract from transgenic tobacco cell suspensions. M, molecular weight marker; 1, negative control (protein extract from untransformed tobacco cells); 2-5, protein extracts of transformed cells with pBioamarKDEL; 6, empty line; C+, positive control of amarantin expressed in *E. coli*.

Conclusions. In the present work, the development of a platform for protein expression in transgenic tobacco cell suspensions by biolistic was achieved. The best transformation condition, based on the *gusA* assay, reached around 60% of transformation efficiency. It was possible to validate the transformation system through the incorporation of the amarantin cDNA, in where the molecular analysis showed the expression of this recombinant protein with nutraceutical applications. The protocol of genetic transformation established here could be used for expression of different recombinant proteins with biotechnological applications.

References.

1. Huang T, McDonald K (2009) *Biochem Eng J* 4(3):168-184.
2. Castillo-Reyna J (2010) *Tesis doctoral*. ITESM, Monterrey, México.
3. Sanford John C (1988) *Trends in Biotechnology* 6 (12): 299-302.
4. Santos-Ballardo D, Germán-Báez L, Cruz-Mendívil A, Milán-Carrillo J, Reyes-Moreno C, Valdez-Ortiz A (2013) *PCTOC* 113(2):315-322

ISSN: 2448-8461

VE50

DEVELOPMENT OF A SELECTABLE MARKER BASED ON PHOSPHITE FOR CHLOROPLAST TRANSFORMATION IN *Chlamydomonas reinhardtii*

José Sandoval-Vargas[1], Noé V. Durán-Figueroa[1], Jesús A. Badillo-Corona[1]
[1]Instituto Politécnico Nacional, Unidad Profesional Interdisciplinaria de Biotecnología, 07340, Mexico City, Mexico.
*Corresponding author: jbadilloc@ipn.mx

Key words: phosphite, ptxD, Chlamydomonas reinhardtii

Introduction. Genetic engineering of algae offers relevant opportunities to develop species with traits that can help broaden the repertoire of naturally occurring products. Foreign genes can be expressed from the chloroplast genome for molecular farming and for metabolic engineering. Chloroplast transformation requires the use of selectable markers based on the use of antibiotics or herbicides. However, given the concerns with the potential risk of horizontal transfer of genes that confer resistance to antibiotics, recently the interest in developing new antibiotic-free selectable markers has increased. Phosphite (PO_3^{-3}, Phi), a reduced form of phosphorous (PO_4^{-3}, Pi), has been shown to be a strong candidate to replace the use of antibiotics. In most organism Pi is the only form of assimilable phosphorous. However, in some bacteria Phi can be assimilated by the action of the NAD-dependent phosphite dehydrogenase (encoded in the *ptxD* gene from *Pseudomonas stutzeri* WM 88), which catalyzes the conversion of Phi to Pi. Selection of transgenic lines based on their ability to grow on phosphite has been demonstrated for *E. coli* (Hirota *et al.* 2017), and for nuclear transformation of the plants *Nicotiana tabacum* and *Arabidopsis thaliana* (Lopez and Herrera, 2013), *Zea mays* (Nahampun et al. 2016), *Oriza sativa* (Mohan et al., 2017), the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* (Kanda, 2015), and the green alga *C. reinhardtii* (Loera-Quezada, 2016). We have recently demonstrated that phosphite assimilation can be enabled when the *ptxD* gene is expressed from the chloroplast genome of *C. reinhardtii* (Sandoval-Vargas et al., 2018). In the present work we demonstrate that the *ptxD* gene can also be efficiently used as a selectable marker for chloroplast transformation in *C. reinhardtii*.

Methods. Wild-type cells of *C. reinhardtii* (Chlamydomonas Resource Center, University of Minnesota, USA), grown in phosphate-free TAP media (TA media), were used for bombardment (Guzmán-Zapata et al., 2016) with the previously reported p320-*ptxD* vector (Sandoval-Vargas et al., 2018). In this vector, expression of the codon optimized *ptxD* gene (ATUM, USA) is under the control of the *psbD* promoter and the *rbcL* terminator. Transformed lines were identified as single colonies growing in phosphite-containing phosphate-free TAP media (TAPhi media).

Results. Phosphorous-starved cultures of *C. reinhardtii*, showed the appearance of phosphite-assimilating colonies three weeks after bombarded with tungsten particles carrying the p320-*ptxD* vector (Fig. 1). Transplastomic lines were taken through three rounds of cultivation to establish homoplastomic lines. The presence of the *ptxD* gene and of the PTXD enzyme were determined by PCR and western blotting, respectively. The efficiency of our selectable marker is similar to other selectable markers with the added benefit of not requiring the use of antibiotics.

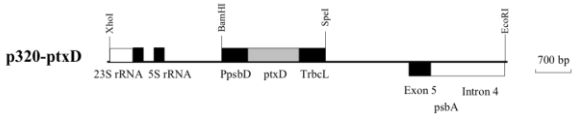


Fig.1 Schematic representation of chloroplast transformation vector p320-*ptxD*.

Conclusions. We have shown that the NAD-dependent phosphite dehydrogenase, encoded by the *ptxD* gene from *Pseudomonas stutzeri* WV 88, can be used as a positive selectable marker based on phosphite for chloroplast transformation.

Acknowledgements. Funding in the laboratory of JABC comes from Instituto Politécnico Nacional (SIP-IPN). JMSV is the recipient of a CONACYT PhD studentship.

References.

1. Hirota, R., et al. (2012). *J Biosci Bioeng.* 113 (4): 445–450
2. Lopez, D., and Herrera, L. (2013). *Plant Biotech J* .11:516–525
3. Nahampun, H., et al. (2016). *Plant cell rep.* 35(5):1121–113
4. Mohan, V., et al. (2017). *Plant Biotech J.* (2017).15: 1493–1508
5. Kanda, K., et al. (2014). *J Biotech.* 182–183(20):68-73
6. Loera, M. et al. (2016). *Plant Biotech J.* 14 (10):1–11
7. Guzmán, D., et al. (2016). Production of recombinant proteins in the chloroplast of the green alga Chlamydomonas reinhardtii. *Recombinant Proteins from Plants: Methods and Protocols*, MacDonald, J. Humana Press, USA. 69-85.
8. Sandoval-Vargas, JM., et al. (2018). *Algal res. 33:291-297.*

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
VE51

SEAWEED AS POTENTIAL PLANT GROWTH STIMULANTS FOR AGRICULTURE IN MEXICO

Rosalba Mireya Hernández-Herrera[1], Fernando Santacruz-Ruvalcaba[1], Diego Ramón Briceño-Domínguez[2], Dania Andrea Di Filippo-Herrera[3] and Gustavo Hernández-Carmona[3]

[1]Laboratorio de Biotecnología, Departamento de Botánica y Zoología, CUCBA, Universidad de Guadalajara. Col. Nextipac, Zapopan, Jalisco, 45110. México. [2]Laboratorio de Ciencias Básicas, ITS de Felipe Carrillo Puerto. Felipe Carrillo Puerto, Quintana Roo, 77200. México, [3]Laboratorio de Química de Algas Marinas. Departamento de Desarrollo de Tecnologías, CICIMAR-IPN. Av. Instituto Politécnico Nacional S/N Col. Playa Palo de Santa Rita, La Paz, Baja California Sur, 23096. México. e-mail: gcamona@ipn.mx

Key words: agriculture, biostimulants, seaweeds extracts

Introduction.

One of the promising directions in agriculture is the rational use of biologically active substances or plant growth stimulators obtained from local raw materials. In Mexico, seaweed can be considered a cheap, abundant, and accessible local resource along the Mexican coast. It represents great potential for eventual commercial exploitation as a source of plant growth promoters. Previous reports have emphasized the importance of seaweed extracts and their utilization with significant results to improve seed germination, seedling development, growth, and yield of plants, increasing crop productivity (1).

Methods.

A general description of the state and context of the application of marine algae extracts in Mexican agriculture was made. Through a search of bibliographic information which includes aspects of algae diversity with potential as a biofertilizer, the Mexican industry of seaweed extracts, the effect of seaweed and its derivatives on germination and the current research of extracts from algae and its effect on some crops.

Results.

We provided information about the regulation for harvesting of some of the more abundant marine seaweed in México (*Macrocystis pyrifera, Gelidium robustum, C. canaliculatus, Gracilariopsis lemaneiformis*), and the research carried out in Mexico. One of the uses included the production of seaweed liquid extract. We describe the analysis of the chemical composition of 12 species to test biological efficacy on promoting plant growth, as well as elicitors of disease defense caused by pathogens.

Fig.1 Activity of the extracts of *M. pyrifera* on the adventitious root formation from bean mung plants and IBA as reference (control).

Table 1. Seaweed used as bioestimulants in crops

Geographic regions	Seaweed used to produce the extracts	Type of extracts	Crops	Beneficial effects under crops	References
I Baja California	*Macrocystis pyrifera*	Alkaline	Mungo bean Tomato	Growth promoter	Briceño-Domínguez *et al.*, 2014
		Alcoholic	Reed radish	Root promoter	Hernández-Alarcón, 2014
	Eisenia arborea (= *Ecklonia arborea*)	Alkaline	Mungo bean Lettuce Reed radish	Growth promoter	Martinez-Morales, 2015
II Gulf of California	*Acanthophora spicifera* *Ulva lactuca*	Alkaline	Mungo bean Lettuce Reed radish	Root promoter	Martinez-Morales, 2015
	Ulva lactuca	Acid	Mungo bean	Root promoter	Castellanos *et al.*, 2017
III Tropical Pacific	*Caulerpa sertularioides* *Padina gymnospora* *Sargassum liebmannii* *Ulva lactuca*	Neutral Alkaline	Mungo bean Tomato	Enhance germination Root promoter	Hernández-Herrera *et al.*, 2014, 2016
	Sargassum liebmannii	Neutral	Jicama	Germination promoter	Nicolás-Álvarez *et al.*, 2015
IV Gulf of México	*Ulva lactuca*	Un	Un	Un	Garduño-Solórzano *et al.*, 2005
V Mexican Caribbean	*Hydroclothrus clathratus* *Sargassum filipendula* *Sargassum fluitans* *Turbinaria tricostata* *Turbinaria turbinaria*	Un	Un	Growth promoter	Robledo-Ramírez & Freile-Pelegrín, 1998

Un = **Unspecific**

Conclusions.

Seaweeds from Mexico have enough potential for the extraction of biologically active compounds that could increase agriculture productivity. The development of effective strategies to use seaweed extracts is essential for the future of Mexican agriculture.

Acknowledgements. G. Hernández thanks the Instituto Politécnico Nacional for financially supporting this research.

References.

(1) Khan, W., U. P. Rayirath., S. Subramanian., M. N. Jithesh., P. Rayorath., D. M. Hodges., A. T. Critchley., D. Laporte., J. Vera., N. P. Chandía., E. A. Zúñiga., B. Matsuhiro & A. Moenne. 2007. Structurally unrelated algal oligosaccharides differentially stimulate growth and defense against tobacco mosaic virus in tobacco plants. Journal of Applied Phycology 19: 79-88. DOI:10.1007/s10811-006-9114-y

ISSN: 2448-8461

AL1

ANTINOCICEPTIVE AND ANTI-INFLAMMATORY EFFECTS OF *BRASSICA OLERACEAE* VAR. *ITALICA* SPROUTS IN PAIN MODELS

María Eva González-Trujano[1], Omar Guadarrama-Enríquez[1], Agustina Cano[2], Diego A. Moreno[3]. [1]Laboratory of Neuropharmacology of Natural Products, Neuroscience Department, National Institute of Psychiatry "Ramón de la Fuente Muñiz" 14370 Mexico, City; [2]Physiology Department of Cardiology National Institute Ignacio Chávez, 14080 Mexico, City. [3]Phytochemistry Laboratory, Department of Food Science and Technology, CEBAS-CSIC, 30100 Murcia, Spain. evag@imp.edu.mx.

Key words: Brassica oleraceae var. *italica; pain; experimental models*

Introduction. Pain is associated with a wide range of injuries and diseases often caused by inflammation. The significant adverse effects of current analgesic medication have promoted a greater interest in natural alternatives -such as dietary supplements and herbal remedies-, which have been used for centuries to reduce pain and inflammation, not only for mild to moderate aches but also for chronic pain. *Brassica oleracea* var. *italica* (broccoli) is considered a rich source of health-promoting bioactive compounds related to the reduction of oxidative stress and inflammation such as glucosinolates, isothiocyanates phenolic compounds, among others(1).

The aim of the study was to obtain evidence of the potential of broccoli chemically enriched for therapy of pain and inflammation using experimental models.

Methods. Pharmacological evaluation of the analgesic and anti-inflammatory effects of broccoli sprouts aqueous extract -after enhanced phytochemical quality (1)- was done using intraperitoneal (i.p.) doses of 30, 100, and 300 mg/kg in comparison to the isothiocyanate sulforaphane (0.1 mg/kg, i.p.) and the reference analgesic drug ketorolac (20 mg/kg, i.p.) in the plantar and carrageenan tests as experimental thermal nociceptive and inflammatory pain models in rats, respectively. Histological analysis of the inflamed paws was included using the Masson´s trichrome staining.

Results. Significant antinociceptive (plantar test, table 1) and anti-inflammatory effects (100-300 mg/kg during the first 6 h, and also at 30 mg/kg after 24 h on the carragenan-induced edema) (Fig. 1A and 1B) were found after treatment with broccoli and sulforaphane. Tissue injury was also reduced in rats treated with broccoli (300 mg/kg) in comparison to ketorolac improving preservation of muscular fibers and cell infiltration (red arrows in Fig. 1B), where a dosage of 30 mg/kg produced similar response than the vehicle group (data not showed). Our results reinforce health properties of broccoli (2) and sulforaphane (3) against nociceptive pain and inflammation.

Table 1. Analgesic effect in the thermal stimulus-induced nociception.

TREATMENT	DOSE (mg/kg, i.p.)	Maximum posible effect (%)
Vehicle	-	0.0 ±8
Broccoli sprouts	30	41±8**
	100	29±5*
	300	40±11**
Sulforaphane	0.1	27±4*
Tramadol	50	82±10**

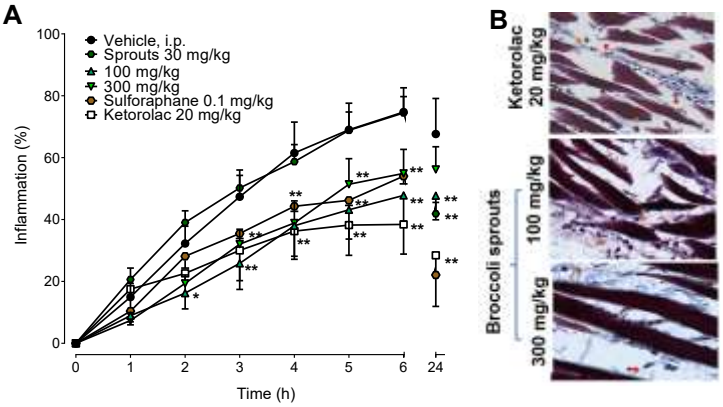


Fig.1 Temporal course curves of the anti-inflammatory response (A) and histological evaluation of inflamed paw treated with broccoli sprouts (100 and 300 mg/kg) and ketorolac (20 mg/kg) (B). Data represent the mean ± standard error. ANOVA followed by Tukey´s test.*p<0.05, **P<0.01.

Conclusions. Broccoli sprouts possesses chemical constituents that are source of bioactive compounds (for example: sulforaphane) as natural products with potential usefulness for therapy of pain.

Acknowledgements. CONACYT-226454/256448 and fellowship 781549 Biological Sciences Post-degree UNAM for Omar Guadarrama. MINECO AGL2013-466247-P/project 19900/GERM/15 in Spain. CORNUCOPIA Thematic Network, CYTED Program action 112RT0460.

References. 1. Baenas N, García-Viguera C, Moreno DA. 2014. *J. Agric. Food Chem*. 62:1881-1892. 2. Baenas N, González-Trujano ME, Guadarrama O, Pellicer F, García-Viguera C, Moreno DA. 2017. *Food Funct.* 8:167-176.3. Redondo A, Ferreira-Chamorro FA, Riego G, Leánez S, Pol O. 2017. *J. Pharmacol. Exp. Ther.* 363:293-302.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México

AL2

EFFECT OF ELECTRICAL VOLTAGE ON THE THERMAL INACTIVATION OF AGARICUS BISPORUS TYROSINASE USING OHMIC HEATING

[1]Eduardo Morales Sánchez; [2]Marcela Gaytán Martínez, [1]Oscar Yael Barrón García.
[1]Instituto Politécnico Nacional. CICATA-IPN Unidad Querétaro. Cerro Blanco 141.Qro,Qro. [2]Universidad Autónoma de Querétaro. Facultad de química. Cerro de las campanas, Qro. Qro. Corresponding autor: emoraless@jpn.mx

Key words: Ohmic heating, tyrosinase enzyme; thermic inactivation

Introduction. Tyrosinase (Tir) is a metallic binuclear enzyme that catalyzes two different reactions in the presence of molecular oxygen using phenolic compounds as a substrate. Studies on fungal tyrosinases have taken on importance due to that tyrosinase is the responsible of the darkening, in the presence of oxygen of fungi, fruits or vegetables when they are cut or damaged (Ismaya et al., 2011; Kampmann et al., 2015). By other side, ohmic heating (OH) is an emergent technology based on the Joule effect, it occurs when an electric current circulates internally through the food, causing an increase in the internal temperature of the food (Ramaswamy et al., 2014) The objective of the present work was to evaluate the effect of the electrical voltage on the inactivation of Tyrosinase.

Materials and Methods.

Conventional heating (CH): Aliquots of 2 ml of Tyrosinase from Agaricus bisporus (Sigma-Aldrich, T3824, CAS 9002-10-2) were placed in a glass cell with a lid and heated in a water bath at different temperatures (50-60 ° C) for several times: 0, 1, 3, 5, 10 and 15 min after reaching the desired temperature.

Ohmic heating (OH): Aliquots of 2 ml of Tyrosinase were set in a glass cell with a lid and heated at different voltages (25, 30, 35V), temperatures (50-60°C) and times (0, 1, 3, 5, 10 and 15 minutes). The samples were cooled in an ice bath to subsequently measure the residual activity (Brochier et al., 2016).

Determination of the polyphenol oxidase activity: To calculate the polyphenol oxidase (PFO) activity, tyrosinase was used at a concentration of 25 ppm (0.025 mg / mL) and catechol (Sigma-Aldrich, 135011, CAS 120-80-9). (Zhou et al., 2017). The initial velocity of each reaction was calculated from the linear region of the absorbance-time curve and was used as the enzymatic activity (Makroo et al., 2017).

Results. Fig. 1 shows PFO residual activity for CT (Conventional Treatment) and OH at 25,30 y 35 Volts. It is clear that OH treatments reach very low residual activity than CT. This means that the electric field applied increase the velocity of reaction and reach the same residual activity but in less time.

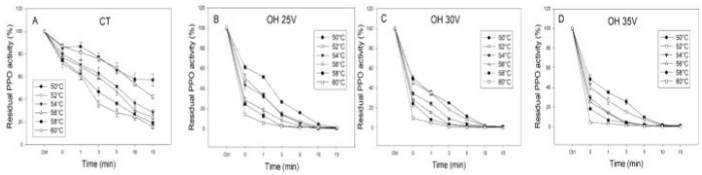


Fig. 1 PFO residual activity for CH and OH (25, 30 y 35 V).

It has been reported that the presence of electric field (electrical voltage) could eliminate the metallic prosthetic groups present in the active site of the polyphenol oxidases enzymes, therefore, resulting in a greater loss of enzymatic activity compared to the purely thermal treatment (Makroo et al., 2017).

Conclusions. In the present work it is shown that the voltage significantly decreases the enzymatic activity of PFO in synergy with temperature. This additional effect attributed to the electrical voltage will allow to design processes with moderate operating temperatures that help maintain the original characteristics and obtain minimally processed products.

Acknowledgements. This work was partially funded through IPN-SIP grant: SIP20161290 and 20171461.

References.

Brochier, B., G. D. Mercali, y L. D. F. Marczak. Influence of moderate electric field on inactivation kinetics of peroxidase and polyphenol oxidase and on phenolic compounds of sugarcane juice treated by ohmic
heating. LWT - Food Science and Technology. Vol. 74, Págs. 396–403, 2016.
Ismaya, W. T., H. J. Rozeboom, A. Weijn, J. J. Mes, F. Fusetti, H. J. Wichers, y B. W. Dijkstra.. Crystal structure of agaricus bisporus mushroom tyrosinase: Identity of the tetramer subunits and interaction with tropolone. Biochemistry. Vol. 50, Págs. 5477–5486, 2011.
Kampmann, M., A.-C. Hoffrichter, D. Stalinski, y R. Wichmann. Kinetic characterization of tyrosinase containing mushroom (Agaricus bisporus) cells immobilized in silica alginate. Journal of Molecular Catalysis B: Enzymatic. Vol. 116, Págs. 124–133, 2015.
Makroo, H. A., J. Saxena, N. K. Rastogi, y B. Srivastava. Ohmic heating assisted polyphenol oxidase inactivation of watermelon juice: Effects of the treatment on pH, lycopene, total phenolic content, and color of the juice. Journal of Food Processing Preservation. Págs. 1–9, 2017.
Ramaswamy, H. S., M. Marcotte, S. Sastry, y K. Abdelrahim. Ohmic Heating in Food Processing. CRC Press. 2014.

ISSN: 2448-8461

AL3

ISOLATION OF LACTIC ACID BACTERIA PRODUCERS OF BIOSURFACTANTS

Guillermo Cruz-Nicolás, Marisol Sánchez-Esgua and Edhit Olvera-Uribe

Centro de Investigación en Biotecnología Aplicada, IPN. Ex-hacienda San Juan Molinos, Carretera Estatal Santa Inés Tecuexcomac – Tepetitla de Lardizabal, Tlaxcala. CP 90700. email: cruzng7@hotmail.com

Keywords: Biosurfactant, LAB, Bioconservation.

Introduction. Biosurfactants (BS) are biomolecules with high surface activity, they are produced by bacteria, yeasts and fungi. The BS have applications in the industries: food, pharmaceutical, cosmetics, paper, textile, plastic, petrochemical, have also been attributed biological activities such as immunomodulators, antitumor and antimicrobial. The BS have acquired great importance as an alternative to the use of chemically synthesized surfactants. The current approach is to obtain more microorganisms that produce BS, as well as optimizing their production.

Lactic acid bacteria (LAB) are Gram-positive, tolerant acid (pH between 3.5-9.6). The industrial importance of LAB lies in being considered GRAS. Recently, the search for BS produced by BAL has been used because of its food safety and the potential of applications in food for humans and animals.

The objective of this work is isolate strains of LAB capable of producing BS that can be applied in the processing and conservation of food.

Methods. Fermented food samples were collected for LAB isolation. The LAB were propagated on MRS, M17 and GYC agar. The formation of emulsions of cell-free cultures with mineral and vegetable oils was measured, the surface tension was measured by the ring method, finally the hemolysis produced by extracts of induced LAB cultures was evaluated

Results. Thirty LAB strains were isolated in MRS medium, the strains were identified by the APICHL50 system, which belong to the genera *Lactococcus*, *Lactobacillus* and *Pediacoccus.* 25% of the isolates had phase separation in the emulsions produced. 10% of them showed a decrease in water TS from 72.4 mN/m to 36.9 mN/m. 30% of the strains tested showed hemolysis on blood agar plates.

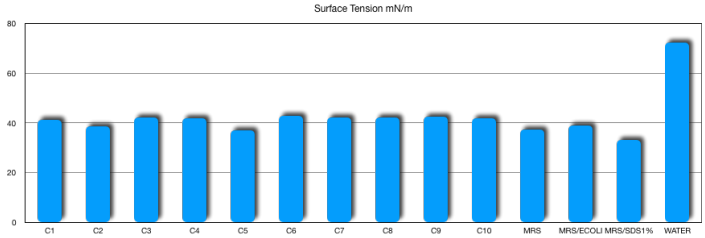


Fig.1 Surface tension of cultures of LAB strains induced with mineral oil.

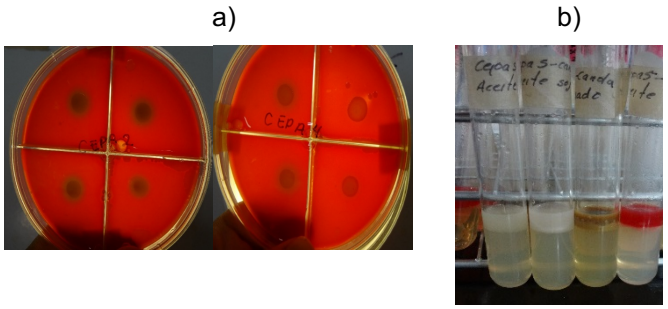


Fig.2 a) Hemolysis produced by LAB strains C2 and C4. b) Formation of emulsions with LAB cultures with two mineral oils and two vegetable oils.

Conclusions. The tests showed that 5 strains are able to form stable emulsions, decrease ST and produce hemolysis of red blood cells. The values of the decrease of the ST are similar to those produced by biosurfactants of *Candida antarctica* (lipids of manosyleritritol) and sophorolipids of *Torulopsis bombicola.*

Acknowledgements. This work was carried out thanks to the financing granted by CONACyT PEI 2018.

References.

1. Vecino X., Devesa Rey R., Cruz J.M., Moldes A. B. (2013). *J. of Environ Manage*. 128 655-660
2. Rodrigues L. R., Teixeira J. A., Oliveira R. (2006). *Biochem Eng Journal* 32 (2006) 135-142.
3. Gudiña J. E., Texeira A. J., Rodrigues R. L. (2009). *Colloids Surf B Biointerfaces* 76 (2010) 298-304.

ISSN: 2448-8461

AL4

MEXICAN PAPAYA MATERIALS WITH OUTSTANDING PHENOLIC CONTENT

María Elena Santos-Cervantes[1], Fernando Rubio-Gutiérrez[2], Laura Gabriela Espinosa-Alonso[1], Norma Elena Leyva-López[1], Cristian Iván Delgado-Salas[3], Jesús Mendez-Lozano[1], Maribel Valdez-Morales[4*]

[1]Instituto Politécnico Nacional, CIIDIR Unidad Sinaloa, Dpto de Biotecnología Agrícola, Guasave, Sinaloa 81010, [2]Instituto Tecnológico Superior de Guasave / Ingeniería en Industrias Alimentarias, Ej. El Burrioncito, Guasave, Sinaloa, [3] Especialistas en Papayas SA de CV, [4]Cátedras CONACyT-Instituto Politécnico Nacional / CIIDIR Unidad Sinaloa, Dpto de Biotecnología Agrícola Guasave, Sinaloa 81010.
*mvaldezmo@conacyt.mx

Key words: Carica papaya, phenolic acids, flavonoids

Introduction. Papaya (*Carica papaya* L.) is the third tropical fruit most produced around the world. All cultivars of papaya have several attractive characteristics to the consumers such as: their flavor, texture, color, high nutritional composition and antioxidant compounds content (Septembre-Malaterre et al., 2016). The beneficial effects of papaya on human health include chemopreventive, antiviral, blood glucose regulation, anticancerigen, neuroprotective, and others; these benefits are conferred by diverse compounds such as: fiber, vitamins, carotenoids, several polyphenols, and others (Kelebek et al., 2015; Vij and Prashar, 2015). Nowadays, scientist and farmers are interested in generating high value-added papaya cultivars containing high concentrations of nutraceutical compounds. Thus, the objective of this work was to determine the profile of phenolic compounds in new materials of papaya generated in Mexico, in order to know their nutraceutical potential, and to give more add value to the productive chain of papaya.

Methods. The content of phenolic compounds in 16 materials of papaya generated in Mexico was analyzed. The pulp was lyophilized, and then a fine flour was obtained, using a mill. An ethanolic (80%) extraction was done, and then total phenolic content was measured by Folin-Ciocalteu methodology (Swain and Hills, 1959); and, the profile of phenolic compounds was determined by high-resolution liquid chromatography (reverse-phase, C18 column, DAD detector). All determinations were done by triplicate in at least two independent experiments.

Results. Attractive concentrations of total phenolics were found in the different materials of papaya (Figure 1). Table 1 presents a summary of the materials of papaya that showed the major results of phenolic acids (gallic, ferulic, chlorogenic, caffeic, *p*-coumaric, *o*-coumaric, and 2,5-dihydroxybenzoic acids) and flavonoids (catechin, epicatechin, quercetin, and rutin). The content of gallic an ferulic acids, quercetin, and rutin, of some materials are better than the reported previously, highlighting the material CI-9.

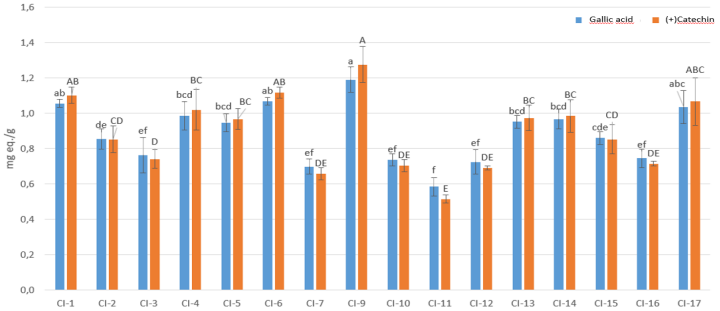


Fig.1 Total phenolic content of ethanolic extracts from papaya pulp of different Mexican materials. Different letters in bars of the same color, means values with statistically significant difference.

Table 1. Summary of papaya materials with the major contents of phenolc acids and flavonoids.

Phenolic compound		Material with the highest content	Concentration ug/g
Phenolic acids	Gallic	CI-9	43.0 ± 0.5
	Ferulic	CI-9	48.4 ± 0.4
	Chlorogenic	CI-2	3.7 ± 0.1
	Caffeic	CI-13	14.7 ± 0.3
	p-coumaric	CI-9	2.35 ± 0.1
	o-coumaric	CI-9	8.64 ± 0.6
	2,5-dihidroxy benozic	CI-9	16.5 ± 0.2
Flavonoids	(+)-catechin	CI-16	44.0 ± 0.2
	Epicatechin	CI-9	22.8 ± 0.1
	Quercetin	CI-9	27.0 ± 0.3
	Rutin	CI-14	54.7 ± 0.4

Conclusions. Outstanding contents of total and individual phenolics were found in new Mexican materials of papaya, such as: gallic and ferulic acids, besides rutin; this fact can give add value to this important fruit.

Acknowledgements. The authors wish to express their gratitude to Especialistas en Papaya S. A de C.V, and to Instituto Politécnico Nacional for their financial SIP 2017-2018 No. 1891, SIP20170351 and POD17-0002-0127 projects support and technical facilities.

• **References.** 1. Vij T, Prashar Y. (2015) Asian P J Trop Dis 5(1):1-6.
2. Septembre-Malaterre A, Stanislas G, Douraguia E, Gonthier M-P. (2016) Food Chem. 212:225-233.
3. Kelebek H, Selli S, Gubbuk H, Gunes E. (2015) Food Chem. 173:912-919.

ISSN: 2448-8461

AL5

Biocontrol of *Botrytis cinerea* using a seed extract of *Moringa oleifera LAM*

Francisco Javier García-García[1], M. Valentina Angoa-Pérez[1], José Roberto Medina-Medrano[1], Ernesto Oregel-Zamudio[1], Carlos Mendez-Inocencio[1], José Luis Montañez-Soto[1,] [1] Instituto Politécnico Nacional CIIDIR-Michoacán, Justo Sierra 28, Colonia Centro. Jiquilpan, Michoacán., C.P. 59510, México. e-mail: **montasoto@yahoo.com.mx**

Key words: Seeds, Phytochemicals, Moringa, phytopathogen.

Introduction. Many crops are affected by phytopanogenic fungi such as *Botritys c inerea* which causes necrosis during harvest and post-harvest time [1]. For its control are used chemical fungicides which causes to severe environmental damages; to avoid this, the researchers are resorting to the use of plant extracts [2]. The *Moringa oleifera* Lam is commonly called the tree of life or miracle tree [3]. The extracts of almost all its parts have potential as controllers of phytopathogens, this due to the presence of phytochemicals with antimicrobial capacity such as tannins, alkaloids, flavonoids, saponins among others [4].

The objective of this work was to control *Botrytis cinerea* with seed extract of *Moringa olei fera* Lam, using chloroform as a solvent.

Methods. 50 g of ground moringa seed and 200 mL chloroform were placed in an Erlenmeyer flask, mixed and rested by 5 days. The mixture was sonicated for 20 min, stirred for 24h, filtered through filter paper and later using a membrane of 0.45 µm. The extract was concentrated in rotaevaporator, drying in an oven at 40°C for 72h and storage in amber colored vials at 15 ± 2°C. A standard solution of dry extract in chloroform at 2mg/mL was prepared, from which four solutions with (0.01, 0.05, 0.25, 0.5) mg/mL were prepare. 1 mL of each one of these solutions was deposited on Petri dishes with PDA medium and spread uniformly. Then Petri dishes were left open for 40 min and in its center were placed 8 mm diameter discs, which contain *B. cinerea*, and they were incubated at 25 ± 2°C by 7 days. As controls were used only fungus, chloroform-fungus (white) and a control with fungus and commercial chemical fungicide at 0.2 mg/mL. The inhibition percentage was determined according Rodríguez et al. (2017) [5]. The results were evaluated by means of an analysis of variance and the comparison test of means of Tukey ($p \leq 0.05$).

Results. All solutions inhibited the *B. cinerea* growth after incubation for 48 h. After incubation by 7 days to 25 ± 2°C, the reached inhibition percentages on the growing of *B. cinerea* by the extracts of *M. oleifera* seeds with 0.01, 0.05, 0.25, 0.5 mg / mL were 14.36, 20.99, 22.65 and 24.31% respectively. There were significant stadistic difference between the lowest and highest concentration according to the grouping made with the statistical program SAS (Tukey $\rho \leq 0.5$). The extract concentrations evaluated showed a trend of higher concentration of the extract higher percentage of inhibition. It should be mentioned that the chemical treatment generated an inhibition of 100% from 48 hours of incubation.

Conclusions. The seed extracts of *Moringa oleifera* LAM showed ability for the control of phytopathogens such as *B. cinerea* . It is convenient to increase the dose or the extract concentration to try to reach 100% inhibition of the growth of the phytopathogen and analyze the phytochemical compounds involved in said inhibition.

Acknowledgements. The researchers greatly appreciates the financial support and provision of materials, equipment and facilities to the CIIDIR-IPN-Unidad Michoacán. We also thank to Productores de Moringa de La Cienega S.A de C.V for providing the seeds used in this research.

References.

[1] Daniel, C. K., Lennox, C. L., & Vries, F. A. (2015). *In-vitro* effects of garlic extracts on pathogenic fungi *Botrytis cinerea*, *Penicillium expansum* and *Neofabraea alba* . *South African Journal of Science, 111*(7-8), 1-8.

[2] Pârvu, M., & Pârvu, A. E. (2011). Antifungal plant extracts. *Science against m icrobial pat hogens: comm unicating current research and t echnological adv ances. For matex Research Center, Badajoz, Spain, 2*, 1055-1062.

[3] Idris, M. A., Jami, M. S., Hammed, A. M., & Jamal, P. (2016). *Moringa oleifera* seed extract: A review on its environmental applications. *Int. J. Appl. Environ. Sci, 11*, 1469-1486.

[4] Shukla, P., Tripathi, M., & Dwivedi, N. (2016). Qualitative Phytochemical Screening and Development HPTLC Fingerprint Profile of *Moringa ole ifera* lamk. Leaf. *Int. J. Rec. Biotech, 4*(1), 18-24.

[5] Rodríguez, D. J., Salas-Méndez, E. d. J., Rodríguez-García, R., Hernández-Castillo, F., Díaz-Jiménez, M., Sáenz-Galindo, A., Peña-Ramos, F. (2017). Antifungal activity in vitro of ethanol and aqueous extracts of leaves and branches of *Flourensia* spp. against postharvest fungi. *Industrial Crops and Products*.

ISSN: 2448-8461

AL6

Analysis of pozol fermentation through metaproteomic approach

Jocelin Rizo[1], Carmen Wacher[2], Sergio Encarnación[3], Romina Rodríguez-Sanoja[1]. [1]Insituto de Investigaciones Biomédicas, UNAM. Departamento de Biología Molecular y Biotecnología. México, CDMX 04510. [2]Facultad de Química, UNAM. Departamento de Alimentos y Biotecnología. México, CDMX 04510. [3]Centro de Ciencias Genómicas, UNAM. Genómica Funcional de Procariotes. Cuernavaca, Morelos 62210.
marari_qa@hotmail.com, romina@biomedicas.unam.mx

Key words: Pozol, metaproteomic, traditional fermented food

Introduction. Pozol is a Mexican refreshing, non-alcoholic acid beverage, made from fermented nixtamalized maize dough. It is consumed during the working day, as food or as a refreshment at any hour of the day. Ethnological studies have reported health benefits related to pozol consumption, such as the control of diarrhea, the reduction of fever and healing of superficial infections and wounds (1). Besides, in the fermented dough there is a nutritional improvement, to which both the nixtamalization process and the fermentation process contribute. Indeed, pozol has higher content of essential amino acids, vitamins and soluble protein than maize (2). Benefits possibly due to the diverse microbiota, rich in potentially probiotic bacteria and yeasts that develop in the nixtamalized dough (3).
In order to understand the process and eventually be able to establish control parameters to ensure the benefits of pozol in the diet, a metaproteomic approach is proposed. Thus, the first objective of this work was to obtain the metaproteome at different fermentation times to validate the origin of the prevailing proteins with the previous microbiological studies.

Methods. The pozol dough was purchased at the Villa Hermosa market from two independent producers. Doughs were mixed and 300 g balls were formed and wrapped in banana leaves. A triplicate was considered for each sampled time (0, 9, 24 and 48 hours). Metaproteome was extracted according to the protocol previously standardized in the laboratory. Extracted proteins were separated in SDS-PAGE for sequencing in the IRCM-Montreal, Canadá. MS/MS samples were analyzed using Mascot (Matrix Science, London, UK) set up to search in UniProt database assuming the digestion enzyme trypsin. Scaffold_3.6.1 (Proteome Software Inc., Portland, OR) was used to validate MS/MS based peptide and protein identifications.

Results. A total of 2109 proteins were identified. The most abundant proteins were those of vegetable origin (60.5%), followed by bacteria (28.5%) and fungi (9.4%). In addition, archaea (1.6%) proteins were identified (Figure 1).

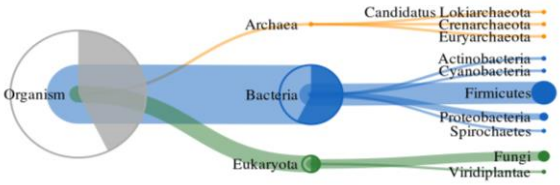


Fig.1. Treemap. Only bacteria, fungi and archaea diversity in pozol metaproteome is showed. Multipeptide analysis performed with deduplicated peptides and equating isoleucine and leucine (4).

From the total of identified bacteria proteins, 48% belong to the genus *Streptococcus*, widely described in this fermentation, 7% of the proteins fit in the *Lactobacillus* genus and also proteins of *Leuconostoc* and *Enterococcus* were identified. Of the total prokaryotic proteins found, 53.59% corresponds to lactic bacteria.
In the case of fungi, the major proteins belong to the genera *Neurospora*, *Schizosaccharomyces*, *Saccharomyces* and *Aspergillus*.

Conclusions. The profile of prokaryotic proteins found perfectly agree with the microbiological reports, while for the fungi the information is so scarce that it cannot be established if the proteomic results approach to microbiology, although it is clear that the identified fungi proteins correspond to fungi previously reported in other fermented foods.

Acknowledgements. The project was supported by UNAM-DGAPA grant IN223917 and CONACYT 131615. Rizo J. belongs to Posgrado en Ciencias Biológicas, UNAM.

References.

1. Ulloa M, Herrera T, Lappe P. (1987). Fermentaciones tradicionales indígenas de México. *Serie de Investigaciones Sociales*. Instituto Nacional Indigenista. México. 13-20.
2. Cravioto O, Cravioto G, Massieu H, Guzman J. (1955). *Ciencia*. 15:27-30.
3. Wacher C, Cañas A, Bárzana E, Lappe P, Ulloa M, Owens D. (2000). *Food Microbiol*. 17:251-256.
4. Mesuere B, Van der Jeugt F, Willems T, Naessens T, Devreese B, Martens L, Dawyndt P. (2018). *J Proteomics*. 171:11-22.

ISSN: 2448-8461

AL7

STUDY BY INFRARED SPECTROSCOPY OF ULTRASONIC TREATMENT EFFECT ON ISOLATED WHEY PROTEIN

Juanita Barajas; Raúl Delgado; Genaro Amador; Valentín López; Orlando Zaca.
Centro de Investigación en Biotecnología Aplicada (CIBA), Instituto Politécnico Nacional (IPN), Ex-Hacienda San Juan Molino Carretera Estatal Tecuexcomac-Tepetitla Km 1.5, Tlaxcala, Tlax, C.P. 90700., México

Key words: proteins, ultrasound, spectroscopy.

Introduction. The potential of milk proteins as food ingredients has been a popular topic of research for the past 40 years (Nicolai et al., 2011). In this work, the effect of high intensity ultrasound on isolates of whey proteins was determined, modifying the medium in which it is found to observe a greater effect on the structure of the proteins (denaturation) and their subsequent aggregation. Therefore, the objective of this work was to determine the effect of sonication treatment on Whey Protein Isolate (WPI), associated with changes in the infrared band of the amide I region.

Methods. The purpose with which the technique Infrared spectroscopy was used is to identify changes in the secondary structure of the protein, using a Bruker® brand Vertex 70 spectrometer in the Attenuated Total Reflectance mode (ATR). For the analysis, an aliquot of 10 µL of each sample were placed and the spectrum was collected within the medium infrared region between 4000-650 cm^{-1}, making 3 measurements per sample, the sampling times were 0, 6 and 24 hours (H).

Results. Figure 1 shows the spectra of the samples obtained after 4 minutes of the ultrasonic treatment, in the spectra are shown times 0, 6 and 24 H. They allow us to determine that, if there was irreversible denaturation due to the effect of the ultrasound treatment.

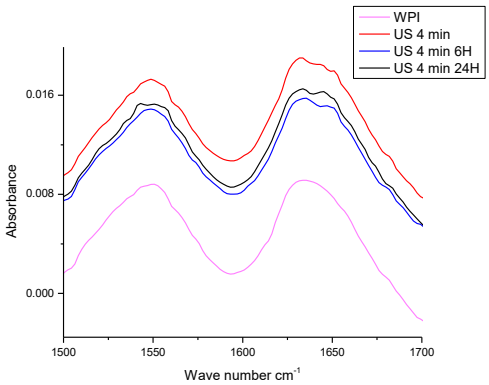


Fig.1 Spectra of whey protein isolated in ultrasonic water model solution for 4 min at 3% and control.

Figure 2 shows the spectra of samples obtained after 6 minutes of the ultrasonic treatment, in the spectra are show times 0, 6 and 24 H, in both figures is possible observe peaks that are defined after 6 hours located at 1635 cm^{-1}, 1651 cm^{-1} and 1683 cm^{-1} corresponding to leaves β, α-helices and spin structures respectively, all this in the region of Amida I (1600-1700 cm^{-1}). These peaks were previously identified by several authors as Antony et al., (2005) and more current Barth, (2007).

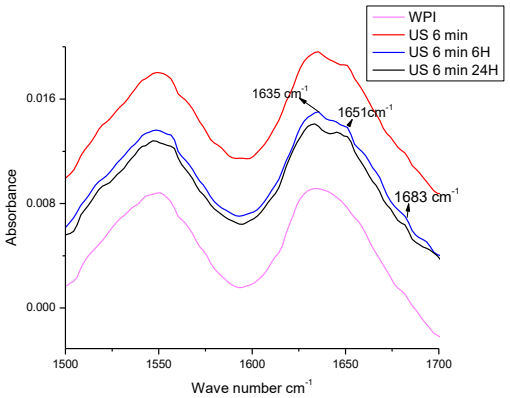


Fig.2 Spectra of whey protein isolated in ultrasonic water model solution for 6 min at 3% and control.

Conclusions. The peaks behavior at 6 and 24 H were very similar to the spectra behavior at 0 H which tell us an irreversible proteins denaturation.

Acknowledgements. To the scholarship CONACyT and BEIFI-SIP and to the grant SIP 2018423.

1. Nicolai, T., Britten, M., & Schmitt, C. (2011). β-Lactoglobulin and WPI aggregates: Formation, structure and applications. *Food Hydrocolloids*, *25*(8), 1945–1962. http://doi.org/10.1016/j.foodhyd.2011.02.006.
2. Antony, B. Schmidt, C. Schütte, (2005), Nonadiabatic effects on peptide vibrational dynamics induced by conformational changes, J. Chem. Phys. 122 014309.
3. Barth, A. (2007). Infrared spectroscopy of proteins. *Biochimica et Biophysica Acta - Bioenergetics.* https://doi.org/10.1016/j.bbabio.2007.06.004.

ISSN: 2448-8461

AL8

ACIDIC SUBUNIT OF AN 11S GLOBULIN MODIFIED WITH ANTIHYPERTENSIVE PEPTIDES: EXPRESSION AND THERMAL STABILITY

Jocksan Ismael Morales-Camacho[1], Edgar Espinosa-Hernández[2], Silvia Luna-Suárez[2]
[1] Universidad de las Américas Puebla, Depto. de Ingeniería Química y Alimentos, San Andrés Cholula, Puebla C.P. 72810
[2] Centro de Investigación en Biotecnología Aplicada, Instituto Politécnico Nacional, Tepetitla, Tlaxcala C.P. 90700
silvials2004@yahoo.com.mx
Key words: 11S globulin, biopeptides, variable regions

Introduction. Protein engineering is a tool to transform biomolecules in order to study the structure and function of proteins (enzymes), develop functional proteins, or create therapeutic proteins through deletion or insertion of an aminoacid or peptide(s) into a target protein [1].
In this sense, our study was focused to modify acidic subunit of an 11S globulin from amaranth through insert an antihypertensive peptide (VYVYVYVY) into IV variable region in order to create a new therapeutic protein (AACM.4). We compared the expression level and thermal stability of the new protein with other proteins previously reported for our group (unmodified protein AAC, protein modified into III variable region AACM.3 and doubly modified protein, AACM.3.4) [2-3].

Methods. The construction of plasmid to express AACM.4 was carried out using as template pET-AC-6His [2]. The expression of recombinant proteins was made by *E. coli* BL21-CodonPlus(DE3)-RIL harboring the specific plasmid to express AAC, AACM.3, AACM.3.4, and AACM.4. In order to achieve high levels of proteins, the fermenter cultures were performed in a 5 L bioreactor Biostat A (Sartorius). Protein concentration was determined using BCA (bichinconinic acid) assay and the presence of proteins were confirmed by western blot assays. The purification of proteins was made by IMAC chromatography with Protino Ni-TED resin. The thermal stability of proteins was measured by thermofluor assay using Sypro Orange as a fluorescent dye [4]. All experiments were done in triplicate and ANOVA analysis was carried out to observe significant difference ($P < 0.05$).

Results. The proteins showed an accumulative yield (g/L) as a function of expression time. AAC and AACM.4 showed high yields to difference with proteins modified in third variable regions, AACM.3 and AACM.3.4 (Table 1).
At fermenter level the higher productivity (g/L*h) was achieved at 6 h of expression. Therefore, at this time, the cells were recovered and all proteins were purified. The thermofluor assays showed that AACM.4 (*Tm* 37.2 °C) is more thermal resistant than AAC (*Tm* 34 °C). AACM.3 and AACM.3.4 not showed thermal transition curves (Fig. 1).

Table 1. Protein yields (g/L) obtained by *E. coli* BL21-CodonPlus(DE3)-RIL

Protein	Expression time (h)		
	3	6	24
AAC	0.30 [a]	0.56 [c]	0.95 [i]
AACM.3	0.22 [b]	0.44 [d]	0.69 [g]
AACM.4	0.33 [a]	0.56 [c]	0.81 [f]
AACM.3.4	0.20 [b]	0.33 [e]	0.56 [j]

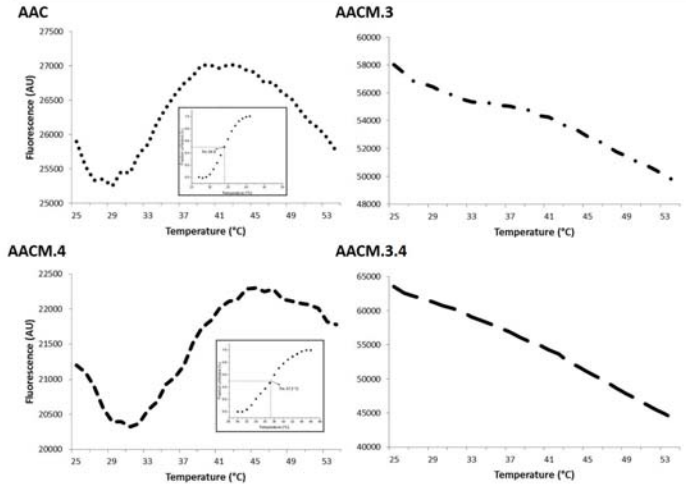


Fig.1 Thermal transition curves obtained by Thermofluor assay. Adapted from Morales-Camacho et al. [5].

Conclusions. There are differences in the protein yields and thermal stability of proteins as result of insertions done. Maybe the third variable regions is important to maintain a correct folding of protein.

References.

1. Tobin P., Richards D, Callender R, Wilson C (2014) *Curr Drug Metab* vol. 15(7):743-756.
2. Luna-Suárez S, Medina-Godoy S, Cruz-Hernández A, Paredes-López O. (2008) *Biotechnol J* 3:209-219.
3. Castro-Martínez C, Luna-Suárez S, Paredes-López O. (2012) *J Biotechnol* 158:59-67.
4. Pantoliano M, Petrella E, Kwasnoski J, Lobanov V, Myslik J, Graf E. (2001) *J Biomol Screen* 6:429-440.
5. Morales-Camacho J, Paredes-López O, Espinosa-Hernández E, Fernández Velasco D, Luna-Suárez S. (2016) *Electron J Biotechnol* 22: 44-51.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/2018
San José del Cabo, B.C.S., México

AL9

EXPRESSION OF THE FRAGMENTS THAT CONSTITUTE THE SOYBEAN 2S ALBUMIN IN *Escherichia coli*.

Jara-Romero Guadalupe Janet[1], Nava-Cabrera Ana María[1], Luna-Suárez Silvia[1],
[1]Centro de investigación en Biotecnología Aplicada, Instituto Politécnico Nacional, Tepetitla, Tlaxcala C.P. 90700.
silvials2004@yahoo.com.mx

Key words: 2S albumin, lunasin, E coli morphology

Introduction. The *gm2s* gene coding for a 2S albumin from soybean (*glycine max*). The protein is composed of its signal peptide, and two subunits and one signal peptide. the small subunit have encrypted the peptide called lunasin which expressed in *Escherichia coli* cause elongated non-septate cells. This effect is, caused by cell cycle interruption [1], for which it was proposed as anticancer peptide. However the effect caused by the whole protein or its separate subunits has not been studied.
The objective of this work was to analyze the effect of the expression of the different fragments of the gm2s in the *E. coli* growth and morphology

Methods. Oligonucleotides were designed to isolate and clone in *E coli* from soybean seed genomic DNA the following *gm2S* fragments: Small subunit (F2), Large subunit (F3), small subunit + large subunit (F2.3), peptide signal + small subunit (F1.2) and peptide signal +small subunit + large subunit (F1.2.3). This fragments were cloned into pet32b(+) and *E. coli* BL21-CodonPlus(DE3)-RIL were transformed with the expression vectors [2]. Growth kinetics were performed measuring the bacterial growth by optical density (OD) at 600 nm and the morphological changes were monitored by confocal microscopy.

Results. The clones expressing F3 and F2.3 had a lower absorbance regarding control, it is expected that the clones containing the subunit small had a lower absorbance since it belongs to the lunasin peptide. It was taken as elongated cells those with a greater length than 7µm, the expression of the F2 fragment (containing the lunasin peptide), causing the reduction of normal cells by up to 50% respect to the control and the expression of a reserve protein that does not cause morphological changes in cells (R1); followed by the expression of F1.2 with a reduction of 24.3%, a Tukey test with a confidence level of 95% shows that the expression of this fragments have a significant difference, being the expression of these fragments those that generate a greater percentage of elongated non-septated cells (Fig. 1).

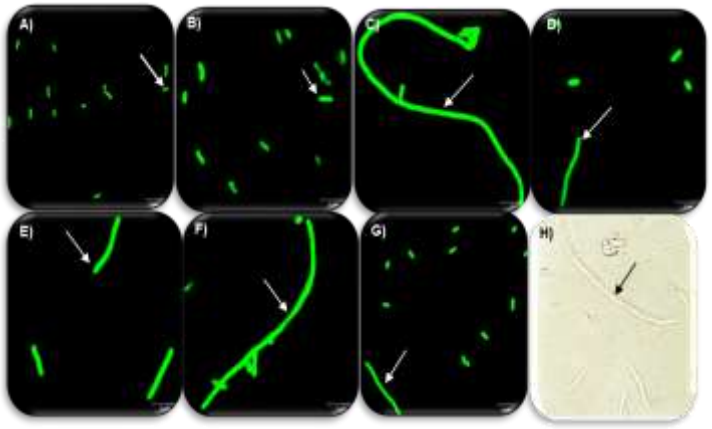


Fig.1 Morphology observed by confocal microscopy in *E.coli* cells expressing the different fragments of soybean 2S albumin, scale at 5µm A) pET32b+, with arrow a cell with normal morphology is indicated, B) R1 protein of reserve, with arrow a cell with normal morphology is indicated, C) F2, D) F3, E) F2.3, F) F1.2, G) F1.2.3 and H) Lunasin, taken from Galvez & De Lumen (1). In the rest of the images, those cells with a length greater than 7µm without the presence of septation are indicated.

Conclusions.
The presence of lunasin bound to other fragments (F1.3) can generate anomalies in the morphology of *E. coli*. For this case high absorbance values could not be related to a greater number of cells, but to a few very elongated cells.

References.
1. Galvez, F. A. & De Lumen, O. B., 1999. A soybean cDNA encoding a chromaton-binding peptide inhibits mitosis of mammalian cells. Nature America, Volumen 17, pp. 495-500.
2. Sambrook, J. & Russell W., D., 2001. Molecular cloning: A laboratory manual. Tercera ed. New York, USA: Cold Spring Harbor.

ISSN: 2448-8461

AL10

PRESENCE OF *Salmonella* AND ENUMERATION OF MICROBIAL GROUPS ON AVOCADOS (*Persea americana* var. Hass) COMMERCIALIZED IN MARKETS

Ramón García-Frutos[1], Liliana Martínez-Chávez[2], Elisa Cabrera-Díaz[3], Marco Antonio Cardona-López[4], Porfirio Gutiérrez-González[2], **José Luis Montañez-Soto**[1], and N. E. Martínez-Gonzáles[2,*]. [1]Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional-Unidad Michoacán, Instituto Politécnico Nacional, Jiquilpan, Michoacán, 59510, México. [2]Dptos. de Farmacobiología y Matemáticas, CUCEI, Universidad de Guadalajara, Guadalajara, Jalisco 44430, México. [3]Dpto. de Salud Pública, CUCBA, Universidad de Guadalajara, Zapopan, Jalisco 45110, México. [4]Dpto. de Ciencias Médicas y de la Vida, CUCIENEGA, Universidad de Guadalajara, Ocotlán, Jalisco 47820, México. *nanci.martinez@cucei.udg.mx

Key words: Avocados, microbial groups, Salmonella

Introduction. The avocado is a fruit consumed raw, as a base or component of some dishes. The consumption of contaminated avocados has not been implicated in outbreaks attributed to *Salmonella*. However, this fruit can be contaminated with the enteric pathogen as result of handling practices and hygiene in the crop and harvest (1). In Mexico, local markets are important points of sale for avocados, where they are stored at room temperature and exposed to environmental and human contamination sources.
The aim of this study was determine the presence of *Salmonella* and enumeration of microbial groups on avocados commercialized in local markets.

Methods. Avocados (200±50 g) with dark green or black epicarp (n=225) were obtained from 45 establishments located in 16 local markets in Guadalajara, Mexico. Each avocado was placed into 200 mL of buffer peptone water (BPW) and homogenized in an ultrasonic bath at 300 W and 40 kHz for 1 min. Decimal dilutions were prepared from the rinse liquid to determine the counts of coliforms and *Escherichia coli* with the Petrifilm™ method. The remaining rinse liquid was stored at room temperature for 1 h. Afterward filtered and the membrane enriched in 50 mL BPW for 24 h for the isolation of *Salmonella* by culture method (2). Isolates were checked by biochemical tests, and confirmed by PCR (3). The mean counts for coliforms and *E. coli* were expressed by log CFU/piece. Statistically significant differences (P<0.05) in the mean counts of microbial groups on avocado were calculated by analysis of variance and LSD test, using Statgraphics Centurion XV ver.15.2.06.

Results. *Salmonella* was isolated in 3.2 % (8/225) of analyzed samples. The positive samples corresponded to 12.5 % (2/16) of markets. The isolation frequency for coliforms and *E. coli* on the avocado surface was 74.7 % (168/225) and 11.5 % (26/225), respectively. Mean coliform count was higher (P<0.05) than those for *E. coli*. The count for coliforms range was from 3.3 to 8.4 log CFU/piece, from those, 87 % avocados ranged from 3.3 to 7 log CFU/piece, whereas that 13 % samples contained from >7 to 8.4 log CFU/piece. The samples with enumerable levels of *E. coli* showed counts ranging from 3.3 to 6.2 log CFU/piece, from those, 88 % avocados ranged from 3.3 to 5.7 log CFU/piece, and 12 % samples contained 6.2 log CFU/piece.
The eight avocado samples positives to *Salmonella* showed coliforms counts ranging from 3.3 to 7.2 log CFU/piece, while *E. coli* was only detected in an avocado sample with 4.7 log CFU/piece.

Conclusions. Hass avocado commercialized in local markets may contain *Salmonella*. The frequency and variable levels of coliforms and *E. coli* on avocados indicates the need for improved handling practices, and public education for the importance of avocado decontamination for reducing the risk associated to its consumption.

Acknowledgements. This project was funded by Universidad de Guadalajara and CIIDIR-Michoacán, IPN. We thank the National Council for Science and Technology, México, for providing a scholarship to Ramón García-Frutos.

References.

1. Rodríguez-García, M.O. (2011). Bases experimentales para el diseño de prácticas agrícolas sanitarias durante el cultivo y la cosecha del aguacate (*Persea americana*, var. Hass). [Tesis, doctorado]. Universidad de Guadalajara. Puerto Vallarta, Jalisco, México.
2. Wallace H., Jacobson A. and Hammack T. (2016) Bacteriological Analytical Manual. Chapter 5. *Salmonella*. [Internet] [Consultado el 15 de junio 2017]. Disponible en: https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070149.htm
3. Kawasaki, S., Horikoshi, N., Okada, Y., Takeshita, K., Sameshima, T. and Kawamoto S. (2005). Multiplex PCR for Simultaneus Detection of *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* O157:H7 in Meat Samples. *Journal of Food Protection,* 68 (3):551-556.

ISSN: 2448-8461

AL11

ANTIOXIDANT ACTIVITY OF EXTRACTS OF THE FRUITING BODIES OF THE WILD MUSHROOM *Lactarius indigo*.

Yolanda Cornejo-Hernández[4], Maura Téllez-Téllez[2], Luis F. Candia Sánchez[3], Gerardo Díaz-Godínez[1]*
[1]Centro de Investigación en Ciencias Biológicas, Universidad Autónoma de Tlaxcala, Tlax. México
[2]Centro de Investigaciones Biológicas, Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos, México. C.P. 62209.
[3]Maestría en Biotecnología y Manejo de Recursos Naturales, Universidad Autónoma de Tlaxcala.
[4]Licenciatura en Naturopatía, Universidad Autónoma de Tlaxcala.
*gerardo.diaz@uatx.mx

Key words: Enzymes, Antioxidant activity, wild mushrooms.

Introduction. The consumption of antioxidants in the diet can protect the human body from the effects of free radicals, reactive oxygen species, lipid peroxidation and slow the progress of chronic diseases (1). A large variety of antioxidant compounds from different natural sources are being widely used in foods and medicines (2). It has been reported that fungi have antioxidant activity (3). *Lactarius indigo*, is a species of edible mushroom of the family Russulaceae, is associated with pines or oaks with those who form mycorrhizas. Its fruiting body presents colors ranging from dark blue in fresh specimens to pale blue gray in the more mature. A feature of this species is that when it is cut or wounded, an indigo blue latex emanates, which upon contact with the air turns green. In this study, the antioxidant activity of aqueous extracts and eatnol/acetone of powder from fruiting bodies of *Lactarius indigo* recollected in the oak forest of the Municipality of Temetzontla Tlaxcala was evaluated.

Methods. Freshly harvested fruiting bodies were dehydrated by air flow, pulverized and sieved. To determine the antioxidant activity and the content of total phenols, extracts were obtained with water and with ethanol/acetone (1: 1); in both cases a concentration of 50 mg/mL was used, they were kept under stirring for 10 min at room temperature, centrifuged for 10 min and filtered. The antioxidant activity was determined in the extracts by the methods of the DPPH and ABTS radicals and the total phenol content using the Folin-Ciocalteu reagent (4).

Results. An approximate yield of 10% mushroom powder was obtained with respect to its fresh weight. Table 1 shows the results of antioxidant activity and content of total phenols present in the aqueous extract and ethanol/acetone extract of the fruiting bodies of *Lactarius indigo*, it can be seen that the highest activity with both methods was present in the aqueous extract, being approximately 10% lower the activity obtained in the ethanol/acetone extract. The content of total phenols was also higher in the aqueous extract, so it can be suggested that these compounds participate in an important way in the antioxidant activity of this fungus.

Table 1. Antioxidant activity and total phenols content infruiting bodies of *Lactarius indigo*.

Extracts	Radical scavenging activity (% inhibition)		Total phenols content (mgGAE/g)
	ABTS	DPPH	
Aqueous	84.71	76.8	0.445
Ethanol/acetone	75.90	62.0	0.127

Conclusions. The fruiting body of *Lactarius indigo* showed antioxidant activity, so its consumption is recommended, however since it is an ectomycorrhizal fungus, at this moment it can only be obtained in a wild way. It is suggested to make submerged cultures with this fungus looking for the production of bioactive compounds in a short time.

Acknowledgements.
To the biotechnology laboratory of the Autonomous University of Tlaxcala.

References.

1.Jayakumar T, Sakthivel M, Thomas, PA, Geraldine P. (2008). *Pleurotus ostreatus* an oyster mushroom, decreases the oxidative stress induced by carbon tetrachloride in rat kidneys, heart and brain. Chemico-Biol. Inter., 176, 2-3, 108–120,.
2.Yaltirak T, Aslim B, Ozturk S, Alli H. (2009). Antimicrobial and antioxidant activities of *Russula delica* Fr. Food Chem Toxicol: International Journal Published for the British Ind. Biol. Res. Association, 47, 8, 2052–2056,
3.Preeti A, Pushpa S, Sakshi S, Jyoti A. (2012). Antioxidant mushrooms: a review. IRJP, 3, 6, 65–70.
4. González-Palma I, Escalona-Buendía HB, Ponce-Alquicira E, Téllez-Téllez M, Gupta VK, Díaz-Godínez G, Soriano-Santos J. (2016). Evaluation of the antioxidant activity of aqueous and methanol extracts of *Pleurotus ostreatus* in different growth stages. Frontiers in Microbiology. 7, 1099.

ISSN: 2448-8461

AL12

LACCASES PRODUCED BY *Humphreya coffeatum* GROWN IN SUBMERGED CULTURE

Alma Agapito-Ocampo[1, 2], Lourdes Acosta-Urdapilleta[1], Gerardo Díaz-Godínez[3], Edgar Martínez-Fernández[1], Elba Villegas[4], Maura Téllez-Téllez[1]*

[1]Centro de Investigaciones Biológicas, Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos, México. C.P. 62209.
[2]Maestría en Manejo de Recursos Naturales, Universidad Autónoma del Estado de Morelos.
[3]Centro de Investigación en Ciencias Biológicas, Universidad Autónoma de Tlaxcala, Tlax. México
[4]Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos.
*maura.tellez@uaem.mx

Key words: Enzymes, pH, white-rot fungus, laccases.

Introduction. Laccases (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) are multicopper oxidase proteins, which can be constitutive or inducible, catalyze the oxidation of phenolic compounds and therefore, there is great interest for their application in many biotechnological processes, including the food industry [1]. Its presence has been reported in different organisms, saprophytic fungi are a source of importance. *Humphreya coffeatum* is a basidiomycete fungus, saprophyte of white rot which is characterized by the zonations of the pileus surface with shades ranging from light brown to dark brown and sometimes orange-brown [2]. In this work the laccases activity of *H. coffeatum* grown in a culture medium without the presence of inductors was determined.

Methods. *H. coffeatum* was grown in potato dextrose broth, incubated at 30°C with orbital shaking at 130 rpm. The biomass was quantified every 24 h by dry weight difference and the activity of laccases using 2,6-dimethoxyphenol as substrate at different pH (6.5, 5.5, 4.5 and 3.5).

Results. A maximum biomass of 8.7 g/L was obtained (Fig. 1) and a specific growth rate of 0.09. h^{-1}. The activity of laccases was higher in acid pH, since as the pH increased, the activity decreased. The maximum activity was 210 U/L at pH 3.5 (432 h), at pH 4.5 it was 143 U/L (432 h) and 174 U/L (480 h), at pH 5.5 it was 61 U/L (480 h) and pH 6.5 was 45 U/L (Fig. 2). Contreras et al. (2016) reported that *Ganoderma lucidum* grown in dextrose potato broth presented higher activities at acidic pH, almost three times more than those determined in this research work. When presenting laccase activity in a medium without inductors, it is suggested that in *H. coffeatum* these enzymes are constitutive, however culture conditions must be optimized to increase the activity.

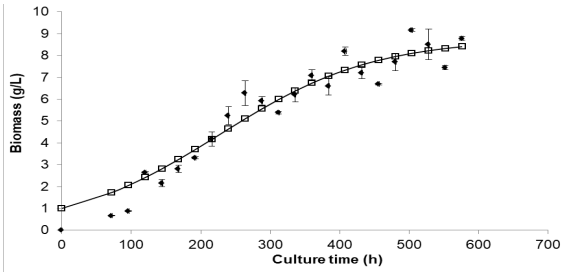


Fig.1 Biomass of *H. coffeatum* grown in submerged culture.

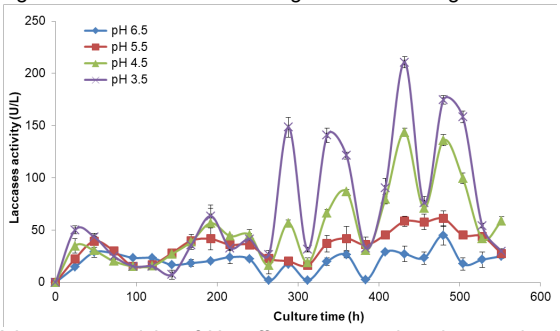


Fig.2 Laccases activity of *H. coffeatum* grown in submerged culture.

Conclusions. The strain of *H. coffeatum* showed laccase activity in the absence of inductors and with greater activity at acid pH.

Acknowledgements.
To CONACYT for the postgraduate scholarship awarded to Alma Agapito (No. 628052) and the UAEM.

References.

1. Imran M, Asad M.J, Hadri S.H, Mehmood S. (2012). *Journal of Cell & Molecular Biology*, 10(1).
2. Mata M, Halling R, M. Muller G. (2003). Macrohongos de Costa Rica. Costa Rica: editorial INBio. 2: 146-147.
3. Contreras D.L.R, Zuluaga-Jimenez F, Díaz-Godínez G, & Téllez-Téllez, M. (2016). *3rd Biotechnology Summit 2016, Ciudad Obregón, Sonora, Mexico, 24-28 October 2016*, 209-212.

ISSN: 2448-8461

AL13

CHEMICAL CARACTERIZATION OF ORANGE SWEET POTATO AND THERMAL ANALYSIS OF ITS STARCH

Sandra Luz Cabrera-Hilerio, E. Adriana Camarillo-Jiménez, Henoc Flores-Segura, Lucía Vargas Sosa, Albino Moreno-Rodríguez, Genaro Carmona-Gutiérrez; Facultad de Ciencias Químicas, Benemérita Universidad Autónoma de Puebla, 14 sur y Av. San Claudio, C.P. 72570 Puebla, Puebla. México. E-mail: sandra.cabrera@correo.buap.mx

Key words: DSC, gelatinization, sweet potato starch.

Introduction. Native starch is characterized because its functional and physicochemical properties are influenced by its molecular weight, content and granular organization. The physicochemical properties are very important for its use in food processing and other applications. Differential scanning calorimetry (DSC) is a technique that allows to determine the temperatures fusion and crystallization temperatures, and glass transition among others, as well as the energy required during a transition such as the gelatinization of starch (1)

The aim of this work was to determine the thermochemical properties like temperature of gelatinization and enthalpy of gelatinization of the starch of orange sweet potato (*Ipomea batata*) and proximal chemical characterization for orange sweet potato.

Methods. The sweet potato starch was extracted following the methodology indicated in the literature (2). The thermochemical properties of the starch were determined by DSC. For each sample a heating ramp was programed in a range of 25 °C to 100 °C. The proximal composition was made according to the standardized methodology described in AOAC (1990) (3).

Results. The table 1 shows the thermochemical properties obtained of sweet potato starch and the figure 1 shows the obtained thermogram from the thermal analysis of starch.

Table 1. Thermochemical properties of sweet potato starch

Humidity/%	Gelatinization temperature (*T*g)/°C	Gelatinization entalpy (Δ*H*g)/kJmol^{-1}
60	61.16 ± 0.41	1.00 ± 0.04
80	62.35 ± 0.17	0.68 ± 0.05

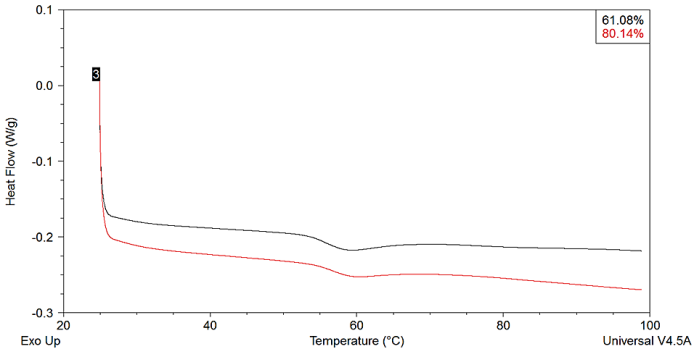


Fig.1 Thermogram of the starch gelatinization process.

The edothermic peak, indicates the gelatinization temperatura (*T*g), and the under curve area represents the gelatinization enthalpy (Δ*H*g). The proximal chemical analysis results shows that the sweet potato is a carbohydrate rich food source (table 2).

Table. 2. Proximal chemical analysis results

Analysis	Dry base/(%
Humidity	81.42 ± 0.13
Ashes	0.67 ± 0.04
Lipids	1.80 ± 0.1
Proteins	2.04± 0.4
Carbohydrates	14.07± 0.23

Conclusions. This kind of starch is feasible for inclusion in products that do not require handling at high temperatures due to its low gelatinization temperature.

References.

1. S. Suriñach, M.D. Baro, S. Bordas, N. Clavaguera, M. T. Clavaguera-Mora. (1992) Bol. Soc. Esp. Ceram. Vidr. 31, (1): 11-17.
2. Z. N. Ganga, H. Corke. Physical properties of starch of Asian-adapted potato varieties. (1999) J. Sci. Food Agric. 79, 1642-1646.
3. AOAC (Association of Official Analytical Chemist). 1990. Official methods of analysis. 15th ed. AOAC, WA, USA.

ISSN: 2448-8461

AL14

FLOURESCENT NANOIMMUNOSENSOR FOR THE DETECTION AND CUANTIFICATION OF *Staphylococcus aureus*.

Leslie Susana Arcila-Lozano, Marlon Rojas-López , Minerva Romero-Sánchez; Instituto Politécnico Nacional, CIBA-Tlaxcala, Tepetitla, Tlaxcala 90700, México; email: marlonrl@yahoo.com.mx

Key words: biosensor, gold nanoparticles, Staphylococcus aureus.

Introduction. Foodborne illnesses are usually infectious or toxic and caused by bacteria, viruses, parasites, or chemical substances entering the body through contaminated food or water. *Staphylococcus aureus* has been indicated as the fifth causative agent of all reported outbreaks. *S. aureus* is an ubiquitous bacterium that is both a human and zoonotic commensal and is a common cause of foodborne poisoning worldwide from the ingestion of heat-stable toxins produced in food. *S. aureus* may be transmitted to food by hands or drippage from the nose and mouth (1).Traditional methods for the detection of bacteria are laborious, time consuming and material intensive (2). Biosensors offer several advantages over existing techniques that include reduced analysis time, high throughput screening, improved sensitivity and real-time analysis. Among the nanomaterials used in biosensors are gold nanoparticles, which possess unique optoelectronic properties and provide high surface-to-volume ratios with biocompatibility using appropriate ligands like proteins that make them scaffolds for the fabrication of biosensing systems (3).
The objective of the work was the design of a fluorescent-nanoimmunosensor colloidal solution for the cuantification of *Staphylococcus aureus*.

Methods. The methodology is described by L.S. Arcila-Lozano et. al 2017 (4).

Results. The fluorescent nanoimmunosensor was mixed 1:1 with *S. aureus* from 1.2 x10^8 to 1.2 x10^0 cell/ml and incubated at 37ºC for 1 h to promote the labeling of the cell membrane of *S. aureus* with the biosensor. Then, each bioconjugates (biosensor-bacteria) were passed through cellulose acetate membranes (0.22 µm). The permeated (biosensor without links to *S. aureus*) were characterized by their photoluminescence. Figure 1 shows the photoluminescence spectra of the filtered biosensor that serve as emission centers during laser excitation. The filtrated samples were analyzed in a quartz cuvette. No intrinsic photoluminescence of 1.2 x10^8 cell/ml *S. aureus* was observed. An intense band at 574 nm from the tetramethylrhodamine of the streptavidin in the biosensor was present. After performing spectral normalization of the band at 521 nm from the quartz cuvette, a decrease of the photoluminescence intensity was observed from the band at 574 nm with the bacteria concentration. Thus, for high bacteria concentrations, few biosensor were obtained after filtration. On the other hand, for low-bacteria concentrations, the number of biosensor and photoluminescence increased after filtration. The sensitivity limit was 1 x10^5 cell/ml.

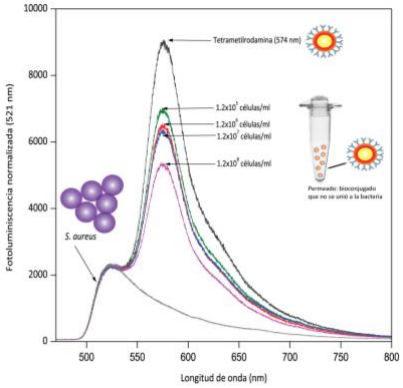


Fig.1 Photoluminescence spectra of the filtered biosensor. The emission band at 574 nm is from the tetramethylrhodamine in the bioconjugate and is proportional to the bacteria concentration**.**

Conclusions. A fluorescent nanoimmunosensor, which is based in the use of gold nanoparticles stabilized with tetramethylrhodamine labeled streptavidin and biofunctionalized with biotinylated anti-*S. aureus* antibody was constructed. Photoluminescence emission of filtered biosensor that were previously mixed with the bacteria provided a direct measurement of the bacteria concentration with a detection limit of 1 x10^5 cell/ml.

Acknowledgements. The authors thank support from SNI-CONACYT, COFAA-IPN, SIP-IPN, CNMN-IPN, and Instituto Nacional de Rehabilitación, México.

References.

1.Podkowik M., Park J.Y., Seo K.S., Bystro J., Bania J. (2013). Int. J. Food Microbiol. 163:34–40.
2.Arora P., Sindhu A., Dilbaghi N., Chaudhury A. (2011). Biosens. Bioelectron. 28 (1):1–12.
3.Tokel O., Inci F., Demirci U. (2014). Chem. Rev. 114 (11):5728–52.
4.Arcila-Lozano L.S., Ríos-Corripio M.A., García-Pérez B.E., Jaramillo-Flores M.E., González C. A., Rocha-Gracia R. C., Gracia-Jiménez J. M., Rojas-López M. (2017). Anal. Lett. 50:7, 1150-11.

ISSN: 2448-8461

AL15

DETERMINING AND QUANTIFYING HEAVY METALS IN MILK MATRIX USING SPECTROSCOPIC AND CHEMOMETRIC TECHNIQUES

Alfredo C. Benítez-Rojas, Raúl J. Delgado-Macuil
Centro de Investigación en Biotecnología Aplicada, Instituto Politécnico Nacional. Ex Hacienda San Juan Molino Carretera Estatal Tecuexcomac-Tepetitla Km. 1.5, Tlaxcala, C. P. 90700, MEXICO, alfcesar@gmail.com

Key words: Heavy Metals, FTIR, Chemometrics

Introduction. Heavy metals are among the oldest toxic substances known to man even at very low levels. In the present, due to the innumerable sources of exposure such as water, food or polluted air, they are an important target in public health (1). Milk as a raw material in cheese can be contaminated with heavy metals in an exogenous way, the most important elements that can appear in milk are Cu, Pb, Zn, Sn, Fe, Hg, Al, As and Mo (2). At this moment, the most widely used method to identify and quantify heavy metals are atomic absorption spectrometry (graphite and flame), mass spectrometry coupled to inductance and hydride generation (3); However, these techniques are expensive, laborious, complex and require high volume of sample. By the other hand, many works have been reported using FTIR and chemometrics methods applied to analytical chemistry (4) and although, them were made in organic matrices looking for organic analytes there are a few that uses both or any of those techniques for studying ions interactions (5) or quantifying inorganic species (6).

The present work aims to develop a methodology based on the technique of infrared spectroscopy by Fourier transform and chemometric analysis for identification of heavy metals in a milk matrix and validated by Atomic Absorption Spectrophotometry.

Methods. Obtaining spectra by means of Fourier Transform Infrared Spectroscopy (FTIR, Brooker, Vertex 70) using certified primary standards (Perkinelmer) and pure compounds (Merck) starting on 1000 ppm of As, Hg, Pb, Cd and Cr and making serial dilutions until 0.1 ppb in different matrices such as water, ultrafiltrate of milk, milk proteins and milk lipids. Use of Principal Component Analysis (PCA) in each of the spectra obtained to identify those components that can present a pattern in each matrix and concentration to finally use these results to stablish a correlation curve between FTIR-PCA-concentration and AAS-concentration (AAS, Aanalyst 200, PerkinElmer).

Results. Figure 1 shows the effect of Pb(II) on water spectrum, it can see that addition of this ion affects the scissoring and OH stretching regions, around 2360 cm^{-1} appeared some bands which absorbance is according to concentration. Besides, there is a couple of inverse bands located in 2917 and 2850 cm^{-1} that didn't appear in others heavy metals spectra.

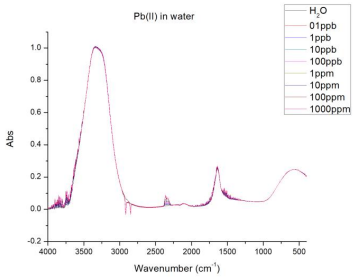


Fig.1 Effect of Pb(II) ion on water spectrum

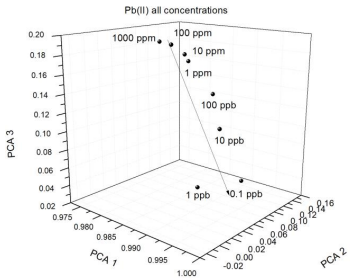


Fig.2 PCA plot of Pb(II) according with its concentration

With this spectra, one for each concentration, was applied a PCA analysis on 2965-2820 cm-1 and 2400-2280 cm-1 regions and were obtained the first three principal components for each one and plotted (figure 2). It can be see that there is a clear trend between all concentrations and PCA.

Conclusions. FTIR coupled with chemometric methods can be used to identify and quantify heavy metal ions in aqueous matrix and probably un milk matrix too.

References

1. Lucho-Constantino C.A., y col. (2005). Chemical fractionation of boron and heavy metals in soils irrigated with wastewater in central Mexico, Agriculture, Ecosystems & Environment. 108(1): 57-71.
2. Castro-González N.P., Calderón-Sánchez F., Castro de Jesús J.,Moreno-Rojas R., Tamariz-Flores J.V., Pérez-Sato M. & Soní-Guillermo E. (2018). Heavy metals in cow's milk and cheese produced in areas irrigated with waste water in Puebla, Mexico, Food Additives & Contaminants: Part B. 11(1), 33-36.
3. Dean J.R.& Ma R. (2009). Chapter 14 Atomic Absorption, Atomic Emission, and Inductively Coupled Plasma Spectroscopies in Food Analysis In: Handbook of Food Analysis Instruments. CRC Press, N.Y., U.S.A. 319-346
4. Kumar N., Bansal A., Sarma G.S., Rawal R.K. (2014). Chemometrics tools used in analytical chemistry: An overview. Talanta Vol (123) t186-199.
5.Fischer W. B., Fedorowics A., Koll A. (2001). Structured water around ion FTIR difference spectroscopy and quantum-mechanical calculations. Phys. Chem. Chem. Phys, Vol (3) 4228-4234.
6. Bosch-Reig F., Gimeno-Adelantado J.V. & Moya-Moreno M.C.M. 2002). FTIR quantitative analysis of calcium carbonate (calcite) and silica (quartz) mixtures using the constant ratio method. Application to geological samples. Talanta 58, 811-821.

ISSN: 2448-8461

AL16

EXPRESSION OF BUTYRATE RECEPTOR GPR43 IN RATS COLON AND THE DIETARY FIBER FROM *Prunus serotina var. capuli*.

Rafael Ortiz-Alvarado, Berenice Yahuaca-Juárez, Victor Meza-Carmen. Faculty Químico Farmacobiología, Universidad Michoacana de San Nicolás de Hidalgo, Morelia, Michoacán, street Tzintzuntzan No.173, Col. Matamoros, C.P.5800, Tel. +52 443 3142152 ext. 208, rortizalvarado@gmail.com

Key words: Short chain fatty acids, GPR-43, FFAR-2, Prunus serotine var. capuli.

Introduction. Short chain fatty acids (SCFA) like, acid acetic, propionate and butyrate are the major molecules produced by the bacterial fermentation of dietary fiber (DF) in colon. Recently, the butyrate has been recently studied because is important to maintain colonic functions phisological and because it has been related with a protective effect in colorectal cancer, which is mainly, explained by its potential to regulate gene expression by inhibiting enzyme histonedeacetylase (HDAC). Several investigations shown that SCFA receptor GPR43 (FFAR-2) is involved insignal transduction mechanisms once they bind to ligands such as propionate and butyrate to generate different physiological effects in the colonocytes the mammals like the rats. Objective: Determine if dietary fiber consumption from *Prunus serotina var. capuli* containing a ratio of soluble-insoluble fiber 40/60, has a direct influence on the quantitative expression of butyrate-specific receptor GPR43.

Methods. Wistar rats were fed with four different diets formulated at different concentrations of dietary fiber of 0%, 5%, 10% and 15% of dietary fiber from Prunus serótina var. capuli, with base of AIN-93 (dietary rodent American Institute of Nutrition), for 14 days, *ad libitum*, respectively and the expression was determined with rt-pcr quantitative, with this sequences, fwd 5-ACC ATC GTC ATC ATC GTT-3 and rvd 5-CAC CGA GAA CAA ATT CAC-3 (sequence NM 001005877.1 for FFAR-2 in rat) an for B-actine from rat, NM 031144.2 like gene constitutive.

Results. The results shown an increase in the expression of GPR43 (529%) when rats was fed with a 5% fiber diet, using β-actin as a reference gene. The results of this research will contribute to determinate the relation of diet (dietary fiber) with intestinal health for the purpose of expanding the knowledge of butyric acid on colonic functions physiological.

Table 1. Relative expression for GPR43 gene, from day 14th of the induction of dietary fiber in rats fed with *Prunus serotine var. capuli* *n=4.*

% Fiber dietary	β-actine	GPR-43
0	0.8010609	0.62416527
5	0.8030700	3.34035168
10	0.8010539	4.22807216
15	0.8010853	7.67411295

Conclusions. The results of this research will contribute to determinate the relation of diet (dietary fiber) with intestinal health for the purpose of expanding the knowledge of butyric acid on colonic functions physiological, from others sources like *Prunus serotine var capuli,* this fruit is part from Taxonomic Family *Rosaceae* like genus Rubus spp. (blackberry and raspberry).

Acknowledgements. We are grateful financial support from Consejo de Investigación Científica, Universidad Michoacana de San Nicolás de Hidalgo, Grant 2018–2019.

References.

1.- Louis P[1], Flint HJ. (2009). Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. FEMS Microbiol Lett. 294(1):1-8.

2.- Pryde SE[1], Duncan SH, Hold GL, Stewart CS, Flint HJ. (2002). The microbiology of butyrate formation in the human colon. FEMS Microbiol Lett. 217(2):133-9.

3.- Rivero-Cruz B.(2014). Simultaneous quantification by HPLC of the phenolic compounds for the crude drug of Prunus serotina subsp. Capulí. Pharm Biol.52(8):1015-20.

4.- Scott KP[1], Martin JC, Duncan SH, Flint HJ. (2014). Prebiotic stimulation of human colonic butyrate-producing bacteria and bifidobacteria, in vitro. FEMS Microbiol Ecol. 87(1):30-40.

ISSN: 2448-8461

NUTRITIONAL AND FUNCTIONAL PROPERTIES OF GLUTEN-FREE BEAN FLOURS, OBTAINED FROM CO-PRODUCTS OF AZUFRADO HIGUERA PROTEIN CONCENTRATES EXTRACTION

Laura Gabriela Espinosa-Alonso[1*], Jocelyn de Jesús Galvez-Morales[1], Maribel Valdez-Morales[2], Sergio Medina-Godoy[1]

[1]Instituto Politécnico Nacional, CIIDIR Unidad Sinaloa, Dpto de Biotecnología Agrícola, Guasave, Sinaloa 81010, [2]Cátedras CONACyT-Instituto Politécnico Nacional / CIIDIR Unidad Sinaloa, Dpto de Biotecnología Agrícola Guasave, Sinaloa 81010. *lespinosaa@ipn.mx

Key words: protein concentrates, gluten free, techno-functional, been co-products, valorization

Introduction. Common beans had high nutrimental and nutraceutical value food, also considered gluten-free[1]. The overproduction and marketing problems of Azufrado Higuera in the Northwest of Mexico is generating economic losses[2]. Protein concentrates and flours for the food industry represents an opportunity to give added value. Protein concentrates obtained by alkaline extraction and isoelectric precipitation, generate 80% of three co-products during the process that should be exploited[3]. The objective is the chemical and techno-functional characterization of co-products dry flours from Azufrado Higuera bean.

Methods. Co-products were recovered from milling (GC), alkaline extraction (AEC) and isoelectric precipitation (IPC) stages. After freeze-drying to get flours, it was measured granulometry, color, pH, proximal composition, total and damaged starch and the techno-functional properties: water absorption index (WAI), oil absorption capacity (OAI), foam capacity and stability (FC, FS), and emulsion activity and stability (EA, ES). All determinations were done by triplicate in at least two independent experiments.

Results. Co-products GC, AEC, IPC had 35.59, 31.93, 12.50 g/100 g bean, and 6.55, 8.72 and 3.9 pH, respectively. All flours have a light color with yellow tendencies. GC had high protein, carbohydrates of which 90% are insoluble. AEC contain a high carbohydrates, Figure 2 and 3 show the techno-functional properties mainly starch and insoluble fiber. IPC has the highest protein content and almost the middle of carbohydrate low in fiber and high Ca, Fe Mg and Zn (data not shown) (Table 1). The particle size profile show that AEC had the allowed size for flours (≤ 40 µm, 53.3%) (Figure 1).

Composition (g/100 g)	GC	AEC	IPC
Humidity	9.15 ± 0.13	3.01 ± 0.4	5.65 ± 1.78
Total lipids	0.75 ± 0.01	0.50 ± 0.01	0.10 ± 0.07
Protein	28.19 ± 0.06	6.08 ± 0.13	36.10 ± 0.06
Ashes	5.63 ± 0.04	2.11 ± 0.01	16.68 ± 0.2
NFE	57.78	89.01	46.38
Total dietary fiber	39.22	19.65	6.45
Insoluble	35.56 ± 0.53	16.32 ± 0.25	5.6 ± 0.38
Soluble	3.66 ± 0.30	3.33 ± 0.08	0.85 ± 0.01
Total starch	21.25 ± 3.4	61.61 ± 4.3	1.99 ± 0.1
Damaged starch	0.16 ± 0.05	0.66 ± 0.14	2.04 ± 0.56
Amilose	20.15 ± 0.73	19.79 ± 0.66	-

Table 1. Nutrimental composition of bean co-product flours

Acknowledgements. To Instituto Politécnico Nacional for their financial SIP20143993 and 20164879 Innovation projects support and technical facilities.

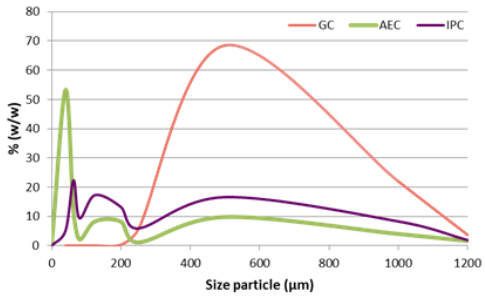


Fig.1 Particle size distribution profile of bean co-products flours

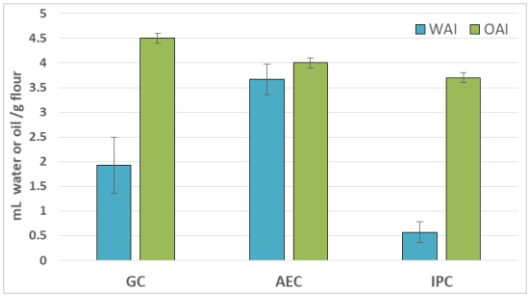


Fig.2. Water and oil absorption indices from bean co-products flours

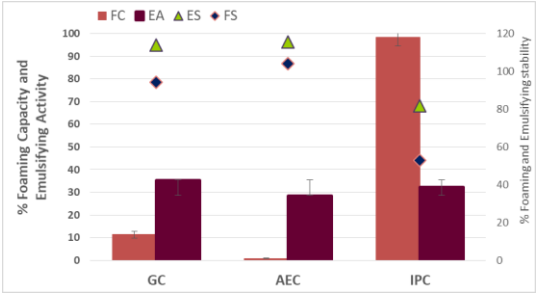


Fig. 3 Foaming capacity and emulsifying activity and stability from bean co-products flours.

Conclusions. By the nutrimental quality and functionality, bean co-products flours present versatility for use in the food industry, as ingredient in sauces and dressings by its thickeners capacity. AEC flour can be used as a functional ingredient, because of its high fiber and mineral content in the baking gluten-free industry, or for starch extraction. Due to the binding properties, IPC can be used in the manufacture of chewing gums and GC for animal feed, due to its high protein content. Bean co-products can provide value-added products and improve the bean production chain.

References. 1. FAO (2016). http:www.fao.org/documents/card/es/c/2255c9fc-a643-42e4-b116-3b190ebb3f92/. 2. Financiera Agrícola Rural (2016). https://www.gob.mx/cms/uploads/attachment/file/200638/Panorama_Agroalimentario_Frijol_2016.pdf. 3. Gálvez-Morales J.J (2014). Caracterización de los subproductos obtenidos en el proceso de extracción de concentrados proteicos de frijol (Phaseoulus vulgaris, L.) Azufrado Higuera. Tesis maestría. Instituto Politécnico Nacional. CIIDIR Sinaloa.

ISSN: 2448-8461

AL18

DESCRIPTION OF THE MICROBIOTA ASSOCIATED TO ARTISANAL ADOBERA CHEESE, A GENUINE MEXICAN CHEESE

José Martín Ruvalcaba-Gómez[1,2], Ramón Ignacio Arteaga-Garibay[3], Raúl Jacobo Delgado-Macuil[1], Juan Lara-Aguilera[3].
[1]Instituto Politécnico Nacional, Centro de Investigación en Biotecnología Aplicada, Tepetitla de Lardizábal, Tlaxcala, 90700.
Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias: [2]Campo Experimental Centro Altos de Jalisco;
[3]Centro Nacional de Recursos Genéticos, Tepatitlán de Morelos, Jalisco, México. E-mail: arteaga.ramon@inifap.gob.mx

Key words: Adobera cheese, massive sequencing, raw milk

Introduction. Adobera is a fresh cheese from Jalisco, Mexico, with a creamy and crumbly texture that melt upon heating and a distinctive smooth, salty flavor. It exhibits a mild acidity produced by native microflora[1]. However, just a few reports have been published about adobera cheese, mainly related with its microbiological safety. In order to generate more information related with characteristics of adobera cheese, it results necessary to carry out descriptive studies. 16S rRNA massive sequencing, allow the generation of thousands of sequences useful to discriminate among the species present in a sample[2]. The objective of this study is describe the structure and composition of adobera cheese microbiota made from raw milk.

Methods. Two samples of artisanal adobera cheese were taken directly from cheese-factory located in Los Altos Region in Jalisco, in two seasons of the year (dry and rainy season). Metagenomic DNA was extracted from samples and used for the amplification of 7 of 9 hypervariable regions of 16S rRNA gene using 16S metagenomics kit and Ion PGM system for next generation sequencing. Data were analyzed on Ion reporter software and Microbiome Analyst software. Identity assignation was performed using Greengenes taxonomy database.

Results. An average of 36,000 reads per sample were obtained and were assigned to a total of 1033 operational taxonomic units (OTU´s). 589 OTU´s with more than two counts were identified and assigned to 4 phyla, at least 6 classes, 11 orders, 12 families and 16 genera. Composition of adobera cheese samples shown a similar composition among seasons of the year, mainly represented by genus *Lactococcus, Streptococcus, Leuconostoc* and *Lactobacillus*. Changes in structure of this microbiota were observed in relation with the season of the year in which samples were taken. In this context, higher abundance of *Streptococcus* was registered in the dry season sample in comparison with rainy season (25 % vs 1 % respectively); conversely, *Lactococcus* was the predominant genus in rainy season cheese in relation with dry season cheese (28 % and 15 % respectively).

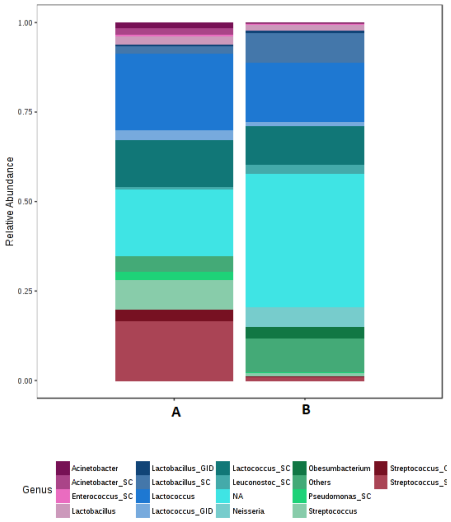


Fig. 1. Relative abundance at genus level in adobera cheese samples from Los Altos de Jalisco region. (A = Dry season, B = Rainy season)

However, at genus level, a significant amount of sequences was not assigned to a specific genus, mainly in rainy season sample; for these reason is necessary to confirm the identity assignation by using specific regions of the gen, in order to improve the taxonomic identification.

Conclusions. Bacterial community of Artisanal adobera cheese consists in a diverse microbiota, strongly represented for a few bacterial genera. Its structure seems to be conditioned for the season of the year of their elaboration.

References.

1. Villegas de Gante A., Cervantes E. F.,Cesín V. A., Espinoza O. A., Hernández M. A, Santos M. A, Martínez C. 2014. Atlas de los Quesos Mexicanos Genuinos. Montecillo, Texcoco, México. Colegio de Postgraduados.
2. Yarza P., Yilmaz P., Pruesse E., Glöckner F.O., Ludwig W., Schleifer K.H., Whitman W.B., Euzéby J., Amann R., Rosselló-Móra R. 2014. Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. NATURE REVIEWS | MICROBIOLOGY.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
AL19

DEVELOPMENT OF AN OPTICAL BIOSENSOR BASED ON POROUS SILICON FOR PUTRESCINA DETECTION

José Contreras Rozo, Raúl Delgado Macuil, Francisco severiano, Orlando zaca moran, Valentin Lopez Gayou
Instituto Politécnico Nacional, Centro de Investigación en Biotecnología Aplicada Unidad Tlaxcala, carretera a Santa Inés Tecuexcomac, a 1.5 Km, Ex-Hacienda San Juan Molino, 90700, Tlaxcala, Tlax., México; E-mail address: jacr1291@gmail.com

Key words: Biogenic amines, porous silicon, biosensor.

Introduction.

The high content of biogenic amines (BA) in foods has been studied extensively due to their toxicity in human health. A technological alternative for the detection of AB are the optical biosensors based on porous silicon (PS) manufactured by self-assembled monolayers (SAM's). The PS offers an increase in sensitivity, reduced energy demand, low cost and has a very large surface area.

The objective of this project is to obtain PS substrates with gold nanoparticles (NPAu's) in their structure and immobilize the DAO by means of SAM's, perform the characterization of the assembly and subsequent detection of putrescine using infrared Fourier transform spectroscopy (FTIR).

Methods.

Chemical etching assisted metal salts [1] obtained the substrates of PS with NPAu's. SAM's for the immobilization of the DAO and the determination of putrescine was carried out as reported by [2] with some modifications in the sensing.

Results

PS samples were obtained with NPAu's by the chemical etching method assisted with metallic salts. The parameters used for anodizing were previously standardized, highlighting this technique for being reproducible, easy to control the process and low cost.

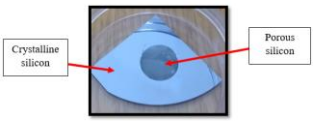


Fig.1 Sample of PS with NPAu's in its structure.

Figure 2 shows the characterization in each of the stages followed by FTIR in the transmission mode. The bands at 625, 675 and 914 cm^{-1} are characteristic of the hydride flexure in SP [3]. The bands between 950-1250 cm^{-1} are associated with the vibrational modes of siloxane bonds (Si-O-Si) [4]. The band of 460 cm^{-1} is due to the OH vibrations of silanol [5]. The appearance of the band at 550 cm^{-1} associated with the hydride flexion is observed when the crosslinker is incorporated and finally, when the DAO is immobilized, an attenuation of the aforementioned bands is observed.

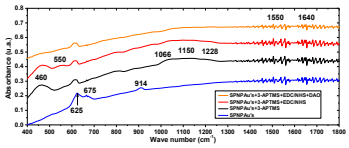


Fig 2. FTIR characterization in the step-by-step transmission mode of the built biosensor.

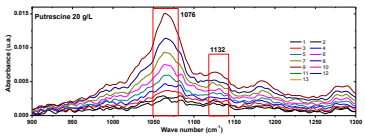


Fig 3. Detection of 20 g/L of putrescine with the biosensor built.

Finally, as shown in Figure 3, the detection with the biosensor constructed of 20 g / L putrescine diluted in Tris buffer. For practical purposes, every 5 spectra was graphed using the Origin 8 software. The signal generated in 1132 cm^{-1} is associated with the formation of ammonium, which is one of the reaction products of the DAO when it comes into contact with the AB.

Conclusions.

It was possible to detect the analyte of interest using SP with NPAu's and as a biological recognition element DAO through the formation of ammonium.

Acknowledgements.

To CONACyT scholarship, and to SIP-IPN project.

References.

[1] Severiano F *et al*. (2017). In situ inclusion of Au nanoparticles in porous silicon structure. *Applied Physics A: Materials Science and Processing*, *123*(1-7).

[2] Severiano F *et al.* (2017). POROUS SILICON BASED BIOSENSOR FOR DETECTION OF BIOGENIC AMINE (SPERMIDINE). *Frontera biotecnológica.*

[3] Young T & Chen C. (2000). Study on the Si Si Vibrational States of the Near Surface Region of Porous Silicon. *Journal of Porous Materials*, *343*, 339–343.

[4] Queeney K *et al.* (2004). Infrared spectroscopic analysis of an ordered Si/SiO2 interface. *Applied Physics Letters*, *84*(4), 493–495.

[5] Queeney K *et al.* (2000). *Journal of Applied Physics*, *87*(3), 1322–1330.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México

AL20

EVALUATION OF THE ACTIVITY OF AN ENZYMATIC OPTICAL BIOSENSOR FOR THE DETECTION OF BIOGENIC AMINES

Stephane Pastrana-Cruz[1,3], José Antonio Gallardo-Lozada[1,3], Aaron González-Espinosa[1,3], Raúl Jacobo Delgado-Macuil[1], Leslie Susana Arcila-Lozano[1,2], Cynthia Cristina Arcila-Lozano[3]; [1]Instituto Politécnico Nacional, Centro de Investigación en Biotecnología Aplicada, Tepetitla, Tlaxcala 90700; [2]Consejo Nacional de Ciencia y Tecnología, CONACYT, Ciudad de México 03940;[3]Tecnológico Nacional de México. Instituto Tecnológico Superior de Perote, Perote, Veracuz 91270; email: rdmacuil@yahoo.com.mx

Key words: biosensor, biogenic amines, diamine oxidase.

Introduction. The biogenic amines (BA) are organic bases of low molecular weight, produced by the decarboxylation of amino acids that can accumulate in food they have an important role in diverse physiological functions, however, their consumption in high quantities can produce intoxications. The best known technique to determine BA is HPLC, which has shown to have a high cost and its analysis time is prolonged. Currently an alternative is the use of biosensors, which for their advantages are easy to use, low cost and short time of analysis what makes them efficient in the control of the processes to determine the quality of the food (1).
The objective of the work was to evaluate the activity of an enzymatic optical biosensor for the detection of several biogenic amines using diamine oxidase (DAO) as the biological recognition element which was immobilized onto crystalline silicon substrates used as an optical transducer.

Methods The optical biosensor was self-assembled according to standardized protocols (2). The enzymatic assay of diamine oxidase was performed using the protocol described by SIGMA-ALDRICH.

Results. The stability of the biosensor was determined measuring the enzymatic activity of the immobilized DAO with different biogenic amines standards as substrates for approximately thirteen days of trials. Absorbance values are the result of oxidation of biogenic amines in the presence of DAO. Those values are the following: histamine initial absorbance of 0.1165 and final absorbance 0.0940; spermine initial absorbance 0.1404 and final absorbance 0.1414; spermidine initial absorbance 0.5072 and final absorbance 0.1832; putrescine initial absorbance 0.7251, final absorbance 0.1075; cadaverine initial absorbance 0.1402, final absorbance 0.0969, figure 1. It should be noted that different biosensors were used for each of the enzymatic assays in order to analyze the DAO`s affinity for their substrates.

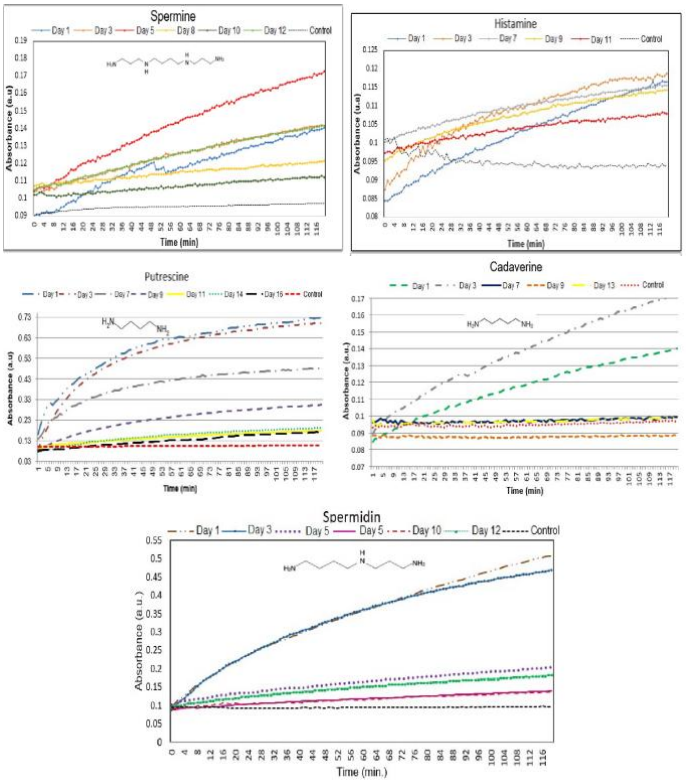


Fig.1 Enzymatic activity assays of the optical biosensor using biogenic amines standards as substrates.

Conclusions. The optical biosensor showed enzymatic activity during the evaluation period and it was demonstrated that it could be reused for different assays to detect biogenic amines for at least one week.

Acknowledgements. The authors thank support from CONACYT.

References

1.Jiménez C., León, D. (2009). Vitae,16 (1), pp. 144-154.
2. Caso-Vargas L.R. Tesis Doctoral. (2016). IPN-CIBA.

ISSN: 2448-8461

AL21

PREPARATION OF A COVER WITH ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY FOR THE CARE AND IMPROVEMENT OF THE SHELF LIFE OF THE STRAWBERRY

Marlene Caballero Reyes, Esther Bautista Ramírez, Luis Torres Bustillos, Yolanda Gómez y Gómez; Instituto Politécnico Nacional, UPIBI, Depto. Bioprocesos, CDMX México. ygomezipn@hotmail.com

Key words: Edible coating, antioxidant, antimicrobial, Fragaria ananassa

Introduction. One of the problems presented by horticultural products is the post-harvest life, it has been estimated that during this period 40% of the total harvested losses, depending on the product, and the time of year. This is mainly caused by infection during transportation and handling of horticultural products caused by fungi of various genera and bacteria.
The effect of edible coatings (RC) of sodium alginate added with cinnamon extract on the changes in ripening in the strawberry was evaluated.

Methods. Strawberries (*Fragaria ananassa*) and cinnamon (*Cinnamomum verum*) were obtained from a local market of CDMX. The phytochemical profile was made with the acetone extract of cinnamon. Antioxidant activity with DPPH radical. A solution was prepared with the 2,2-diphenyl-1-picrylhydrazyl (DPPH) 6 * 10-5M radical in methanol (1). The antimicrobial activity was evaluated by the Kirby-Bauer method (2). Film formulation.- Strawberries were covered with 2% sodium alginate, 1% calcium chloride and 0.3% cinnamon (3).
The fruits were stored for 7 days at 4 °C, evaluating the changes in quality (weight, firmness and Brix degrees) every third day.

Results. The phytochemical study of cinnamon gave positive to phenols, flavonoids, alkaloids, coumarins, tannins, cardiac glycosides, saponins and steroids.
Cinnamon extract inhibited the growth of the following bacteria; *Bacillus cereus, Listeria monocytogenes, Escherichia coli, Pantoea agglomerans, Staphylococcus aureus, Salmonella typhi, Yersinia enterocolitica, Pectobacterium, Klebsiella rhinoscleromatis* from 8 to 10.5 eq of cephalosporin C/g sample.

The thickness of the alginate film was 0.057mm and that of alginate-cinnamon 0.058mm.

Table 1 represents the % inhibition of the antioxidant capacity of the strawberries covered with alginate and alginate-cinnamon, as well as the control group.

Table 1. Antioxidant capacity of *Fragaria ananassa* at 4 ºC

Storage days	1	5
	% inhibition DPPH	% inhibition DPPH
Strawberry without coating	53.731 ± 0.373	42.341 ± 1.653
Strawberry with alginate coating	61.132 ± 3.068	44.067 ± 1.538
Strawberry with alginate and cinnamon coating	64.303 ± 1.028	46.602 ± 5.480

In the 7 days of storage of the strawberry covered with alginate and strawberry covered with alginate-cinnamon, it lost 19% of weight, while the strawberry without coating lost 28% of the weight.

The firmness of the strawberry remained constant during the first 7 days with the treatment of alginate-cinnamon (3.97 N), while the group of alginate (6.14 N) and the control (5.3 N) increased the hardness.

Conclusions. The cover of alginate-cinnamon presented good antimicrobial and antioxidant activity in the protection of the strawberry, added to this I present the least weight loss and I retain the firmness of the fruit during the 7 days of storage.

Acknowledgements. Secretaria de Ciencia, Tecnología e Innovación de la Ciudad de México, for Grant SECITI / 085 / 2017 / Folio 154.

References.

1. *Kuskoski ME, Agustín G. Asuero AG, Troncoso A, Mancini-Filhoj J, Fett R. (2005). Aplicación de diversos métodos químicos para determinar actividad antioxidante en pulpa de frutos. Ciênc. Tecnol. Aliment., Campinas, 25(4): 726-732.*
2. Bernal M, Guzmán M. (1984). El antibiograma de discos técnica de Kirby-Bauer. *Biomédica*. Vol. 4(3): 112-121.
3. Guzmán L, Acevedo D, Romero, Estrada J. (2015). Elaboración de una Película Comestible a Base de Colágeno Incorporado con Nisina como Agente Antimicrobiano. *Información Tecnológica*. Vol. 26 (3), 17-24.

ISSN: 2448-8461

AL22

COMPARATIVE STUDY OF THE CONTENT OF BIOACTIVE COMPOUNDS OF EDIBLE MUSHROOMS: LENTINULA EDODES, PLEUROTUS OSTREATUS AND HERICIUM ERINACEUS WITH MODIFIED SUBSTRATE WITH THE ADDITION OF AAS10^{-2}

Lizelena Guevara[a], María Eugenia Meneses[b, c], Helios Escudero Uribe[c], Porfirio Morales Almora[c], Mercedes Sobal[c], Daniel Claudio Martínez Carrera[c]

[a] Universidad Popular Autónoma del Estado de Puebla (UPAEP), [b] Conacyt-Colegio de Postgraduados, *Campus* Puebla, [c] Biotecnología de Hongos Comestibles, Funcionales y Medicinales, Colegio de Postgraduados *Campus* Puebla, Heroica Puebla de Zaragoza; lizzie_guelo@hotmail.com

Key words: Edible Fungi, Bioactive compounds, Functional Properties

Introduction. There is evidence of the beneficial properties of edible mushrooms derived from the action of bioactive compounds, however there is little scientific information that assesses the change of these bioactive compounds in the modification of substrate, which is why fungi represent an enormous potential as a raw material for biotechnology activity.
Compare in dehydrated mushrooms and in extracts derived of the species *L. edodes, P. ostreatus* and *H. erinaceus* the amount of bioactive compounds by means of the addition of AAS in the substrate.

Methods. Three techniques were used: ORAC for the measurement of antioxidant capacity concentrations (1). Megazyme Kit for the measurement of total glucan concentrations (2) and the Folin Ciocalteu for the measurement of polyphenol concentrations (3). Statistical significance was determined by single-factor ANOVA (Tukey's multiple comparison). The differences were considered significant at $p < 0.05$.

Results. The addition of AAS increased the antioxidant capacity of the extracts of the three species and the amount of total glucans in both dehydrated mushrooms and extracts.

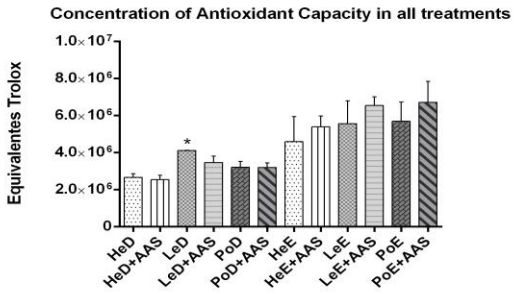


Fig.1 Averages from the concentration of antioxidant capacity in all treatments

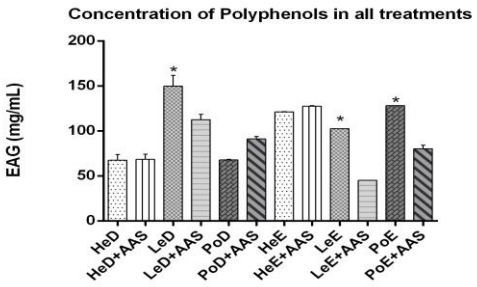


Fig.2 Averages from the concentration of polyphenols in all treatments for all fungal species

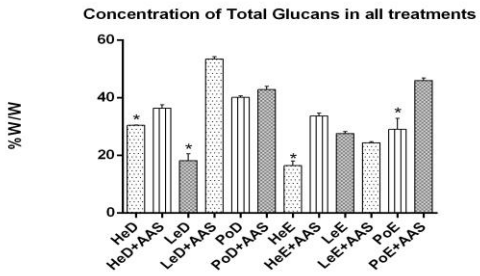


Fig. 3 Average of total glucans in all fungal species

Conclusions. Acetylsalicylic acid (AAS) added to the substrate where the different fungus species were grown had an effect on the concentration and potentiation of the bioactive compounds.

Acknowledgements. Research work supported by the National Council of Science and Technology (CONACYT; www.conacyt.mx) in Mexico, through the Research Project FORDECYT-273647

References. 1. Huang, D., Ou, B., Hampsch-Woodill, M., Flanagan, J. A., & Prior, R. L. (2002). *Journal of Agricultural and Food Chemistry*, vol. (50.): 4437–4444. 2. Megazyme KIT (2011).*Mushroom and Yeast Beta-Glucan Assay Procedure.* Ireland: Megazyme International Ireland. 3. Koren, E., Kohen, R., & Ginsburg, I. (2010). *Experimental Biology and Medicine*, vol. (235.): 689–699.

ISSN: 2448-8461

AL24

SYNTHESIS OF SUGAR FATTY ACID ESTERS BY SEQUENTIAL ENZYMATIC REACTIONS OF TRANSFRUCTOSYLATION AND ACYLATION

Amador Campos[a], Leticia Casas[a], Georgina Sandoval[a], Francisco Plou[c], Lázaro Hernández[b], Javier Arrizon[a]*

[a]Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco A.C., Biotecnología Industrial, C.P. 44270 Guadalajara, Jal., México.
[b]Centro de Ingeniería Genética y Biotecnología, Grupo Tecnología de Enzimas, C.P. 6162, La Habana 10600, Cuba.
[c]Instituto de Catálisis y Petroleoquímica, CSIC, 28049 Madrid, Spain.

Key words: Carbohydrate fatty acid esters, Enzymatic acylation, Fructooligosaccharides

Introduction. Carbohydrate fatty acid esters (CFAE) are broadly employed as surfactants in food, cosmetic and pharmaceutical industries[1]. Their surfactant properties depend on the fatty acid and sugar moieties that compose them. The CFAE with fructooligosaccharides (FOS) have been mainly obtained by acylation of inulin endohydrolysis products, due to their commercial availability. This inulin fatty acid esters and other acylated FOS have shown interesting tensoactive properties in comparison with their analog CFAE[2]. As FOS can be produced enzymatically by the fructosylation of sucrose, which is a cheaper and more abundant substrate than commercial FOS, it is possible to develop a low-cost enzymatic process of CFAE synthesis by sequential transfructosylation and acylation reactions using sucrose as the initial substrate. Depending on their origin, fructosyltransferases can produce different type of fructans. For instance, 1-sucrose:sucrose fructosyltransferase from the monocot plant *Schedonorus arundinaceus* (Sa1-SST) exclusively yields 1-kestose and nystose[3]*, while levansucrase from the bacterium *Gluconacetobacter diazotrophicus* (LsdA) produces a mixture of FOS with β-2,1 and β-2,6 linkages in addition to the polymer levan[4*].
The objective of this study was to produce and characterize the CFAEs obtained by sequential enzymatic reactions of transfructosylation followed by acylation with vinyl laurate, to perform a more specific and controlled CFAE synthesis from FOS mixtures of low degree of polymerization (DP).

Methods. The fructosylation of sucrose (600 g/L) was performed independently with LsdA and Sa1-SST. The two FOS mixtures were then acylated with different concentrations of vinyl laurate (15-60 mg/mL) in 2-methyl-2-butanol by the commercial enzyme Lipozyme 435. The acylation performance was determined from the final concentrations of vinyl laurate and lauric acid in the medium; and the degree of acylation of CFAEs was determined by mass spectrometry using MALDI-TOF.

Results. The reactions of LsdA and Sa1-SST on sucrose produced FOS with a DP between 3-15, and 3-4 respectively. The highest acylation yield was achieved for the reaction with 60 mg/mL of vinyl laurate, reaching 40% for the FOS produced by LsdA and 29% for the FOS produced by Sa1-SST. For both acylated FOS mixtures, the MALDI-TOF analysis showed the presence of a similar CFAE where only mono- and diesters where found, and DP 3 CFAE were predominant.

Conclusions. We present the first report of a successful enzymatic acylation of fructooligosaccharides obtained by sequential enzymatic reactions of transfructosylation and acylation. The 40% and 29% of the initial acyl donor was employed respectively for the acylation of the FOS mixtures produced by LsdA and Sa1-SST, respectively. The mass spectrometry showed the presence of mainly DP 3 mono- and diesters.

Acknowledgements. This research is supported by the Energy Sustainability Fund (ESF) of SENER and CONACYT.

1. Ballesteros, A., Plou, F., & Alcalde, M. (2006). Biocatalysis in the Pharmaceutical and Biotechnology Industries, 463–488.
2. Casas-Godoy, L., Arrizon, J., Arrieta-Baez, D., Plou, F. J., & Sandoval, G. (2016). Food Chemistry, 204, 437–443.
3. Hernández et al. (1995) The Biochemical Journal 309:113-118.
4.Hernández et al. (2018) Journal of Biotechnology 266:59-71

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México

AL25

BIOFUNCTIONALIZATION OF HYDROGEN AMORPHOUS SILICON CARBIDE FILMS FOR THE DEVELOPMENT OF AN OPTICAL BIOSENSOR

Hiram CancinoJavalois*[a], Abdú Orduña Díaz[a], Claudia Reyes-Betanzo[b]
[a] Instituto Politécnico Nacional. CIBA-IPN. San Juan Molino Km 1.5 de la Carretera Estatal Sta. Inés Tecuexcomac-Tepetitla, Tlaxcala, México C.P. 90700
[b]Instituto Nacional de Astrofísica Óptica y Electrónica, INAOE, Luis Enrique Erro no. 1, Santa María Tonatzintla, 72840 Puebla, Puebla.
javaloa_2012@hotmail.com
Key words: Biofuntionalization, a.SiC:H, biosensor.

Introduction.

Self-assembled monolayers (SAMs) is a very useful technique to cover a surface with a layer of certain chemical functionality. The terminal functional groups in the SAMs constitute the reactive centers to carry out subsequent chemical modifications. In this context, SAMs with terminal functional groups OH, NH_2 and C=O are used to promote surface synthesis processes for the immobilization of antibodies capable of detecting *E. coli* in food. In this paper we present the results of infrared spectroscopy by Fourier transform (FTIR) of the biofunctionalization of hydrogenated amorphous silicon carbide (a-SiC:H) with 3-aminopropyltrimethoxysilane (APTMS) and glutaraldehyde for the immobilization of IgG antibodies in the development of an optical biosensor.

Methods.

Hydrogenated amorphous silicon carbide films were obtained by Plasma-enhanced chemical vapor deposition (PECVD) [1]. For the SAMs process, the methodology was followed [2] with some modifications.

Results

Figure 1 (a) shows the spectrum of a-SiC:H with the characteristic bands at 640 cm^{-1} Si-H_n, 750 at 780 cm^{-1} Si-C, 1000 and 1245 cm^{-1} CH_n and Si-CH_3 respectively and in 2080 cm^{-1} of SiH_2 [3]; at the same time, the spectrum of silanization with APTMS of a-SiC:H with the characteristic band at 1020-1140 cm^{-1} corresponding to Si-O-Si. On the other hand, in the region of 1400 to 1700 cm^{-1}, there are the amino-terminal groups of the APTMS molecule and in 2800 to 2980 cm^{-1}, the CH_2 groups belonging to said molecule. Antibody immobilization (IgG) shows the characteristic bands of amide I (C=O) and amide II (N-H, C-N) in 1650 and 1550 cm^{-1}, characteristics of the binding of the biological recognition element with the terminal end C=O of glutaraldehyde and an increase in these bands due to the polypeptide chains of the antibodies [4]. Finally, in figure 1 (b) a decrease in the amide bands of the antigen-antibody interaction was observed due to a probable conformational change of the antibody.

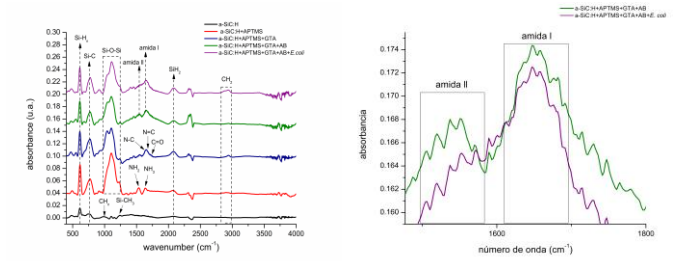


Figure 1. (a) FTIR spectrum of the SAMs process, (b) antigen-antibody interaction

Conclusions.

Examined him the process of biofuncionalization and interaction antigen-antibody on a-SiC:H. The functional groups observed correlated bands themselves for FTIR amino, aldehyde and amida I and II at 1500-1700 cm^{-1} characteristic of the processes. The interaction antigen-antibody showed a decrease in the bands of the amida groups.

Acknowledgements.

To CONACyT scholarship

References.

[1] Herrera J., Reyes C., Orduña A. (2017). Interdigitated Microelectrode Arrays Based on Non-Cytotoxic a-Si_XC_{1-X}:H for *E. coli* Detection. *Journal of Electrochemical Society,* 164 (13), 641-650.
[2] Herrera J., Reyes C., Orduña A., Itzmoyotl A., Pérez A. (2015). A-Si_xC_{1-x}:H thin films with subnanometer surface roughness for biological applications. *Journal of Vacuum Science & Technology A,* 33 (5), 05E108.
[3] Herrera J., Reyes C., Orduña A., Itzmoyotl A. (2016). Effects of hydrogen dilution and B-doping on the density of Si–C bonds and properties of a-Si_xC_{1-x}:H films. *Superficies y Vacíos*, 29 (2), 38-42.
[4] Gunda N.S.K., Singh M., Norman L., Kaur K. & Mitra K. (2014). Optimization and Characterization of Biomolecule Immobilization on Silicon Substrates Using (3-aminopropyl) Triethoxisilane (APTES) and Glutaraldehyde Linker. *Applied Surface Science,* 305, 522-530.

ISSN: 2448-8461

AL26

COMPARATIVE STUDY OF TWO TECHNIQUES (ULTRASOUND AND MECHANICAL AGITATION) FOR THE EXTRACTION OF MUCILAGE FROM THE SEED OF *HYPTIS SUAVEOLENS*

Rafael A. Veloz García, Brenda E. García Mier, Juan Carlos Ramírez Granados, César Díaz Pérez, Guadalupe Piña Sánchez, **Alejandrina Martínez Flores**; Universidad de Guanajuato, División de Ciencias de la Salud e Ingenierías, Departamento de Ingeniería Agroindustrial. Priv. de Arteaga s/n, Col. Centro, C.P. 38000 Salvatierra, Guanajuato, México.
janimf6@gmail.com

Key words: Mucilage, Ultrasound, Mechanical Agitation

Introduction. Mucilages are considered a group of hydrocolloids, composed mainly of polysaccharides and proteins that interact strongly with water. The use of mucilage depends on its unique functional properties, such as viscosity and foaming, gelling, as well as, its bioactive role in the prevention and / or treatment of certain diseases. There is literature concentrated towards the investigation of hydration and extraction of mucilage considering modifications of temperature solvents, and additions of salts to the extraction medium, among others. *Hyptis suaveolens* commonly referred as Chan, produces a seed, which, when soaked in water, is covered with a mucilaginous polysaccharide. Explaining the above, with the present project we sought to establish an efficient system to extract mucilage from the seed of *H. suaveolens*.

Methods. The present project focused on comparing mechanical agitation and ultrasound in the extraction of mucilage from the seed of *Hyptis suaveolens*. For this purpose, the effect of mechanical agitation at 600 RPM and ultrasound at 40 KHz was evaluated. For both extraction processes, the evaluated variables were: temperature (80 and 100 ^{o}C), pH (6, 7, 8 and 9) and seed-water ratio (1:20, 1:30 and 1:40). For all the evaluations, 10 g of seeds were used.

Results. The lowest yield of mucilage was 0.24 ± 0.06 g with a water-seed ratio of 1:40 (Figure 1) and mechanical agitation. The best yields of mucilage were 0.48 ± 0.07 g for the extraction at pH of 6 with a seed-water ratio of 1:20 and mechanical agitation, and of 0.47 ± 0.04 g and 0.47 ± 0.06 g when the temperature was 100º C with sonication and seed-water ratios of 1:20 and 1:30, respectively (Figure 1.

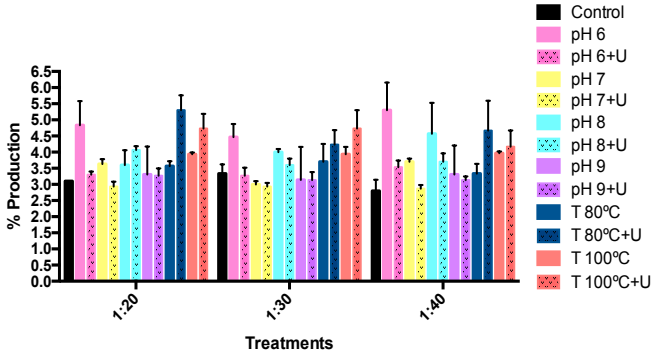


Fig.1. Yield of the mucilage extraction process.

Conclusions. The application of ultrasound in the extraction of mucilage from the seeds of *Hyptis suaveolens* has generated advantageous alternatives since the results in the three hydration ratios stand out the treatments at temperature of 80 and 100ºC with ultrasound and although the treatment at pH 6 also stands out, with the statistical method was found that the treatment with greater significance was that with a seed-water ratio of 1:20 at 80ºC with ultrasound. That method is considered the best since less water is used, less time is required, and greater yield of mucilage is obtained**.**

Acknowledgements. This work was carried out thanks to the funding of DAIP of the Universidad de Guanajuato by means of the projects 9792016 and 1692016.

References.

1. Ziolkovska A. (2012). *Food Hydrocoll*. Vol. (26): 197-204,
2. Gowda C (1984) *Phytochemistry*. Vol. (23):337-338.
3. Muñoz L., Cobos A., Díaz O., Aguilera J. (2012). *J. Food Eng*. Vol. (108): 216-224

ISSN: 2448-8461

AL27

SEQUENCE OF THE MARKER *psb*A-*trn*H and *mat*K of SWEET XOCONOSTLE (*Stenocereus stellatus*) FOR THE CONTRIBUTION OF ITS MOLECULAR ANALYSIS

Clara Cervantes[1]., José Mendoza[2]., Fernando Díaz de León[1]., Clara Pelayo [1]., Edgar Sierra[2]., Angélica Román [1].

Laboratory of Horticultural Products, Biochemical Group of Macromolecules, Department of Biotechnology[1] Universidad Autónoma Metropolitana Unidad Iztapalapa, CDMX, México. Laboratory of Natural Products[2], Casa Libertad Campus. Universidad Autónoma de la Ciudad de México. Calzada Ermita Iztapalapa Num. 4163, Col. Lomas de Zaragoza, C.P. 09620. México.

cjpba_100@hotmail.com

Molecular characterization, sweet xoconostle, Stenocereus stellatus, psbA-trnH, matK

Introduction. Molecular tools today are an important advance for the identification and classification of different organisms (1)(2). Some of them are based on the use of molecular markers. In the analysis of plants among the most used, the *psb*A-*trn*H and *mat*K (3)(4). The sweet xoconostle (*Stenocereus stellatus*) has four variants currently identified by their different colors (white, red, orange and purple). However, the factor that influences the expression of this characteristic is not known.
The objective in this investigation was to evaluate if the color of the variants studied can be attributed to a genetic difference or is a consequence of environmental factors.

Methods. It started from the four variants; white, red, orange and purple from orchards located in the San Juan Joluxtla region, municipality of Chazumba, State of Oaxaca, Mexico. For the comparison of the sequences, DNA extraction of the four variants was performed. The PCR technique (polymerase chain reaction) was carried out to obtain the amplification products for the markers *psb*A-*trn*H and *mat*K. The PCR products were cloned in the PJET plasmid, *Escherichia coli* DH5α from which the plasmid was isolated was used in the transformation process. Finally, the PCR products cloned from the four variants were sent to the Institute of Biotechnology of the UNAM for sequencing.

Results. The sequences of the four variants were obtained for the markers *psb*A-*trn*H and *mat*K, sequences that were then compared between them. This analysis allowed to observe differences in the number of nucleotides and in the order for the white variant with respect to the red, orange and purple variants, however among the latter there was no difference between the number and the order for the marker *psb*A-*trn*H.

Conclusions. The molecular analysis allowed to identify the four variants through the sequences of the markers *psb*A-*trn*H and *mat*K. In addition to differentiate these variants through these sequences. Thus giving the possibility of identifying through these markers species with great similarity.

Acknowledgements. In this work we thank the support of the National Council of Science and Technology (CONACYT) for its support in the development of this research that is part of my PhD in Biotechnology.

References.

1. Nock C., Waters D., Edwards M., Bowen S., Rice N., Cordeiro G., Henry R. (2011). *Plant Biotechnol J*. (9): 328-333.
2. Marcial J., Mendoza J., Sierra E. (2015). *J Plant Sci*. (10): 116-127.
3. Song J., Yao H., Li Y., Li X., Lin Y., Liu C., Chen, S. (2009). *J Ethnopharmacol*. (124): 434-439.
4. Hao D., Chen S., Xiao P. (2009). *Pharm Biotechnol*. (16): 490-494.

ISSN: 2448-8461

AL28

MINERAL CONTENT, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF FRUITS HARVESTED IN THE STATE OF ZACATECAS

Jesús Aguayo Rojas* (Universidad Autónoma de Zacatecas, Químico en Alimentos, Zacatecas, Zacatecas, 98160), Jesús Jaime Rochín Medina (Instituto Tecnológico de Culiacán, Ingeniería Bioquímica, Culiacán, Sinaloa, 80220), José Manuel Jáuregui López (Instituto Tecnológico Superior de Jerez, Ingeniería en Industrias Alimentarias, Jerez, Zacatecas, 99863), Ricardo Omar Navarro Cortez (Universidad Autónoma del Estado de Hidalgo, Instituto de Ciencias Agropecuarias, Tulancingo, Hidalgo, 43600), Manuel de Jesús Macías Patiño (Universidad Autónoma de Zacatecas, Unidad Académica de Ciencias Químicas, Zacatecas, Zacatecas, 98000), Saraíd Mora Rochín (Universidad Autónoma de Sinaloa, Facultad de Ciencias Químico Biológicas, Culiacán, Sinaloa, 80000). *chuy_aguayo@hotmail.com

Key words: Mineral content, antioxidant activity, antimicrobial activity

Introduction. Fruits contain high levels of biologically active components that impart health benefits beyond basic nutritional value. Within the biologically active components, natural antioxidants have attracted interest because of their safety and potential therapeutic effect. Epidemiological studies have revealed a positive association between fruit and vegetable consumption and a reduced risk of certain degenerative diseases. The best way to give antioxidant nutrients to the human body is to eat generous servings of fruits and vegetables rich in antioxidants, such as polyphenols (1) Many current human health problems relate to diets. Micronutrients are involved in numerous biochemical processes and an adequate intake of certain micronutrients relates to the prevention of deficiency diseases (2).
The state of Zacatecas has the first places in fruit production in Mexico, as guava, peach and Pointleaf Manzanita, for this reason is important to evaluate their nutraceutical potential of these fruits.

Methods. Samples of guava, peach and Pointleaf Manzanita were collected in the state of Zacatecas, The plant material was dried in a forced-air convection oven at 35°C for 2 days. The dried fruits were ground in a domestic to obtain grits that passed through a 10 US mesh (2 mm) screen. A dry ground sample of 1 g was mixed with 10 mL of chilled mehanol–water (80:20, v/v) for 30 min in a shaker at 50 rpm. The blends were centrifugated (3000g, 10 min) in order to recover the supernatant. The extracts were concentrated to 2 mL at 45ºC using a vacuum evaporator. Mineral content as Ca, Mg, Fe, Zn and P, were determined by atomic absorption spectroscopy. Antioxidant activity was evaluated by the methods ABTS and DPPH. Methanolic extracts were realized to evaluate the antimicrobial potential with strains of *Escherichia coli, Salmonella thyphimurium, Staphylococcus aureus* and *Listeria monocytogenes,* the antimicrobial potential was reported as millimeters of inhibition in the diameter of agar ring (3, 4).

Results..

Table 1. Mineral content (mg/kg)

Fruit	Ca	Mg	Fe	Zn	P
Guava	1931	590	259	0	10
Peach	849	454	285	0	1
Manzanita	13,450	107	1,695	42	137

Table 2. Antioxidant activity[1] and total phenolics[2]

Fruit	DPPH	ABTS	ORAC	Phenolics
Guava	7421	10,956	10,616	862
Peach	3858	6215	5519	452
Manzanita	6214	8465	8890	209

1 mg equivalents of gallic acid/ 100 g
2 mg equivalents of Trolox/ 100 g

Table 3. Antimicrobial activity[1]

Fruit	*E.coli*	*Salmonella*	*S. aureus*	*L. monocytogenes*
Guava	8.9	14	8.6	11.6
Peach	9.9	8.8	7.8	7.2
Manzanita	7.5	9.1	6.7	6.4

1 mm of inhibition in the diameter of agar ring

Conclusions. Guava presented the highest values of antioxidant activity, the fruits evaluated satisfy the daily riquirements for Ca, Mg and Fe. The antimicrobial effect, over the strains Gram + y -, could be due to the different types of phytochemicals contained in the fruits.

References.

1. Giampieri F, Alvarez-Suarez JM, Battino M. (2014). Strawberry and human health: Effects beyond antioxidant activity. *J Agric Food Chem*. 62: 3867–3876.
2. Kumari M, Gupta S, Lakshmi A, Prakash J. (2004). Iron bioavailability in green leafy vegetables cooked in different utensils. *Food Chem*. 86: 217–222.
3. Ou B, Hampsch-Woodill M, Prior RL. (2001). Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *J Agric Food Chem*. 49: 4619–4626.
4. Singleton VL, Orthofer R, Lamuele-Raventós RM. (1999). Analysis of total phenols and other oxidation substrates and antioxidant by means of Folin-Ciocalteu reagent. *Method enzymol*. 299:152-165.

ISSN: 2448-8461

AL29

PHYTOCHEMICALS AND ANGIOTENSIN-CONVERTING ENZYME INHIBITION BY PRICKLY PEARS VARIETIES FROM ZACATECAS

Jesús Aguayo Rojas* (Universidad Autónoma de Zacatecas, Químico en Alimentos, Zacatecas, Zacatecas, 98160), Guillermo Quiñones Reyes (Universidad Autónoma de Zacatecas, Químico en Alimentos, Zacatecas, Zacatecas, 98160), Jesús Jaime Rochín Medina (Instituto Tecnológico de Culiacán, Ingeniería Bioquímica, Culiacán, Sinaloa, 80220), Ricardo Omar Navarro Cortez (Universidad Autónoma del Estado de Hidalgo, Instituto de Ciencias Agropecuarias, Tulancingo, Hidalgo, 43600), Saraíd Mora Rochín (Universidad Autónoma de Sinaloa, Facultad de Ciencias Químico Biológicas, Culiacán, Sinaloa, 80000) Xochitl Tovar Jiménez (Universidad Politécnica de Pachuca, Ingeniería en Biotecnología, Zempoala, Hidalgo, 43830); *chuy_aguayo@hotmail.com

Key words: Phytochemicals, antioxidant activity, antihypertensive

Introduction. Angiotensin I-Converting Enzyme (ACE) is a key component in regulation of blood pressure by virtue of the rennin-angiotensin system. ACE converts the inactive decapeptide, angiotensin I, into the potent vasopressor octapeptide, angiotensin II, and inactivates bradykinin, which has a vasodilating action. So, inhibition of ACE has become a major target in control of hypertension (1). It is well known that the consumption of fruits could provide health benefits by lowering the risk of chronic diseases such as metabolic syndrome diseases including type 2 diabetes and cardiovascular disease. Plants belonging to the genus (2). *Opuntia* spp. are the most abundant of the Cactaceae family, grown throughout America. Its fruit, known as cactus pear or prickly pear, is an oval berry grouped in different colors. Some studies have shown its antioxidant activities which may help in preventing chronic pathologies such as diabetes. The prickly pear is a cactaceae that have chemical compounds that act as natural antioxidants (3).

The objective of the study was to evaluate the phytochemical content and antihypertensive effect of four varieties of prickly pear, in order to determinate their nutraceutical potential

Methods. Mature fruits were collected in Zacatecas. The plant material was dried in a forced-air convection oven at 35°C for 2 days. The dried fruits were ground in a domestic to obtain grits that passed through a 10 US mesh (2 mm) screen, and packed in plastic bags (1 kg). A dry ground sample of 1 g was mixed with 10 mL of chilled mehanol–water (80:20, v/v) for 30 min in a shaker at 50 rpm. The blends were centrifugated (3000g, 10 min) in order to recover the supernatant. The extracts were concentrated to 2 mL at 45ºC using a vacuum evaporator

The potential antihypertensive activity of prickly pear varieties, was evaluated by their ability to inhibit Angiotensin-Converting Enzyme (ACE). The ACE inhibitory property was assayed using ACE from rabbit lung and hippuryl-histidyl-leucine as the substrate (4, 5).

Results.

Table 1. Antihypertensive effect.

Variety	Antihypertensive activity (% ACE Inhibition)
Chaveña	55±1.4
Blanca Cristalina	61±0.5
Duraznillo	61±1
Xoconostle	65±1

Table 2. Phytochemical content.

Variety	Phenolics[1]	Flavonoids[2]	Tannins[3]	Coumarins[4]
Chaveña	124±1.4	735±1.4	61±0	49±3
Blanca Cristalina	61±0.5	246±0.5	28±1	68±18
Duraznillo	61±1	780±1	44±1	50±1
Xoconostle	65±1	293±1	32±3	68±17

1 mg equivalents of gallic acid /100 g, **2**. mg equivalents of catechin / 100 g, **3** mg equivalents of tannic acid /100 g, **4** mg equivalents of coumarin /100 g

Conclusions. This study shows a high percentage of ACE inhibition by prickly pears varieties which lead to consider this species as a potential source of ACE inhibitor secondary metabolites. The methanolic extracts contained high values of phenolic compounds, possible secondary metabolites responsible for the inhibition of the enzyme.

References.

1. Johnston CI. (1992). Renin-angiotensin system: a dual tissue and hormonal system for cardiovascular control. J Hypertens. 10 (7):13–26.
2. Alves RE, Brito EA, Rufino MSM, Sampaio CG. (2008). Antioxidant activity measurement in tropical fruits: A case study with acerola. *Acta Hortic*. 773: 299–305.
3. Astello-García MG, Cervantes I, Nair V, Santos-Díaz M, Reyes-Agüero A, Guéraud F, Negre-Salvayre A, Rossignol M, Cisneros-Zevallos L, Barba de la Rosa AP. (2015). Chemical composition and phenolic compounds profile of cladodes from *Opuntia spp*. cultivars with different domestication gradiente. *J Food Compost Anal*. 43 (1): 119-130.
4. Astello-García MG, Cervantes I, Nair V, Santos-Díaz M, Reyes-Agüero A, Guéraud F, Negre-Salvayre A, Rossignol M, Cisneros-Zevallos L, Barba de la Rosa AP. (2015). Chemical composition and phenolic compounds profile of cladodes from *Opuntia spp*. cultivars with different domestication gradiente. *J Food Compost Ana*l. 43 (1): 119-130.

ISSN: 2448-8461

AL30

CANDIDATE GENE POLYMORPHISMS EFFECT ON MEXICAN BEEF FATTY ACID COMPOSITION

Diana Vela[1], Ana Sifuentes[1], Williams Arellano[1], Pascuala Ambriz[1], Víctor Moreno-Medina[1], Gaspar Parra-Bracamonte[1], María Rubio[2] and Felipe Rodríguez Almeida[3]; Centro de Biotecnología Genómica IPN[1], Facultad de Medicina Veterinaria y Zootecnia UNAM[2], Facultad de Zootecnia y Ecología UACH[3], Reynosa, Tamaulipas 87710; dvelav1600@alumno.ipn.mx.

Key words: bovine meat, SNPs, fatty acid synthesis genes

Introduction. Beef is one of the main components of a human balanced diet; however, different studies have linked its consumption with an increased risk of some diseases because of their fatty acids (FA) composition. Currently, different studies have been aimed to find molecular markers with an effect on meat FA composition (1), in order to use this molecular information as a tool to assist in the selection of animals with a favorable genetic background for FA-synthesis and composition (2).
Objective: Evaluate the effect of a selected panel of SNPs on the composition of fatty acids in Mexican beef.

Methods. Ninety three *Longissimus dorsi* muscle samples were collected from different producers all of them representing two beef commercial systems (Specialized and Non-specialized). Samples were used to FA extraction using the Lipid Extraction Kit MAK174, SIGMA-ALDRICH® for the specialized system and the method proposed by the AOAC (3) for the non-specialized system and they were also used to obtain the DNA used to genotyping with a 35 SNPs located in candidate genes for organoleptic quality and FA synthesis. A general linear model procedure (GLM) that included the fixed effects of FA extraction method, commercial system, fattening days as a linear covariable and the random effect of genetic background, was used to obtain the values of adjusted phenotypes and isolate genetic component. In order to select the molecular markers with a putative effect on FA composition, a multiple stepwise regression method was computed, markers with a $P<0.05$ were then analyzed by another GLM to determine the effect of each genotype on FA composition. A means comparison was performed with a PDIFF statement. All procedures were performed using SAS 9.0 software.

Results. From the tested panel seven markers were monomorphic and were excluded from the association analysis. Six molecular markers showed important effects on the concentration and composition of fatty acids (Table 1). These markers are located in six different genes. Interestingly, the marker located in the SLC2A4 gene showed effect on five fatty acids (C15:0, C16:0, C17:0, C14:1 y C16:1), as well as the markers located in the MEF2C and SCD genes since they were associated with the composition of two fatty acids. MEF2C associated with C14:0 and total fatty acid concentration, and SCD702 associated with the composition of the fatty acids C16:1 and C17:1.

Table 1. SNPs with significant effect on the deposition of fatty acids of mexican beef.

Gene	SNP_ID	Alleles[1]	Fatty acid	P- Value
LPL	ss65478732	C/**T**	C12:0 (Lauric acid)	0.0169
MEF2C	ss65449641	**G**/T	C14:0 (Myristic acid)	0.0590
		C/T	C15:0 (Pentadecanoic acid)	0.0453
		C/T	C16:0 (Palmitic acid)	0.0352
SLC2A4	ss62538460	**C**/T	C17:0 (Heptadecanoic acid)	0.0395
		C/T	C14:1 (Myristoleic acid)	0.0300
		C/T	C16:1 (Palmitoleic acid)	0.0328
INSIG2	rs134478878	**G**/C	C16:1 (Palmitoleic acid)	0.0360
SDC762	SCD762T>C	**T**/C	C16:1 (Palmitoleic acid)	0.0084
		T/C	C17:1 (cis-10-Heptadecanoic acid)	0.0342
SRPR	rs110036978	G/**C**	TOTAL	0.0209
MEF2C	ss65449641	T/**G**	TOTAL	0.0514

[1]favorable allele in bold.

Conclusions. Six molecular markers were identified with effect on the concentration and composition of fatty acids in Mexican beef. The association of the molecular markers: LPL ss65478732, MEF2C ss38329156 and SLC2A4 ss62538460 with the composition of fatty acids in beef have not been previously reported. The validation of the effect of SLC2A4 is of particular interest since it showed an effect on five fatty acids.

Acknowledgements. The authors would like to thank CONACYT and IPN for financing this research through the project number 294826, SIP 20171674, and also Ing. Paola Mares and the staff of Agroindustry Cohuayana for the meat samples donation.

References. 1. De Lemos, M, Pereira, A, Regatieri, I, Feitosa, F, and Baldi, F. (2017). Genetic Factors that Determine the Meat Fatty Acids Composition. In: *Fatty Acids*. Catala A. IntechOpen, United Kingdom. 221-231.2. Sevane, N, Armstrong, E, Cortés, O, Wiener, P, Wong, R, Dunner, S, and GemQual Consortium. (2013). Meat Sci. 94(3): 328-335. 3. AOAC Official Method 996.06, Fat (total, saturated, and unsaturated in foods), hydrolytic extraction gas chromatographic method, first action 1996, revised 2001. Urbana, IL: AOCS Press.

ISSN: 2448-8461

AL31

Modified atmosphere for the conservation of phenols and capacity antioxidant in cooked chickpea (*Cicer arietinum* L.)

Perez-Perez LM[1]., Wong-Corral FJ[1]., Rosas-Burgos EC[1]., Huerta-Ocampo JA[2]., Ruiz-Cruz S[3]., González Vega RI[1]., Borboa-Flores J. [1], Rodríguez-Figueroa J.C. [1], Del -Toro-Sánchez CL[1]

[1] Universidad de Sonora, Rosales y Niños Heroes S/N, 83000, Hermosillo, Son, México

[2]Centro de Investigación en Alimentación y Desarrollo. Carretera a La Victoria km 0.6 C.P. 83304, Hermosillo, Sonora, México.

[3]Instituto Tecnológico de Sonora-ITSON, Cd. Obregón, Sonora, México

liliana.pz.1.9@gmail.com

LILIANA MARIBEL PEREZ PEREZ

Key words: chickpea atmosphere antioxidant

Introduction.

Mexico is inside on the firsts producing country of chickpea in the world. The consumption of chickpea present benefits to the human healthy as antioxidants source which the phenolic compounds are responsible. The phenolic compound in the chickpea can be present in three different ways, free, conjugated and linked, however these compounds can be degraded during storage. An new alternative for the preservation of these compound would be use the modified atmospheres technology.

Methods. The chickpea was packed in bags of polypropylene under different gases (CO_2, N_2) and air, monitoring at 0, 25 and 50 days, under different temperatures (-20, 4, 25 and 50 °C)[1]. Free, conjugated and linked phenolic compounds were extracted. Total phenols was measured by Folin-Cioalteu and the antioxidant capacity was obtained using the radical DPPH• and ABTS•+.

Results. In general the phenolic compounds and the antioxidant capacity were maintained in the N_2 atmosphere at -20° C and 4° C.

Table 1. Antioxidant capacity of extract using the radical DPPH and ABTS in chickpea flour raw and cooked

	DPPH		ABTS		Folin-Ciocalteu	
	µM Trolox Equivalent				mg GAE/g	
	FC Raw	FC Cooked	FC Raw	FC Cooked	FC Raw	FC Cooked
Free phenols	18.43 $^{a\,A}$ ± 1.9	9.46 $^{b\,A}$ ± 1.4	25.01^{aA} ± 2.7	0.11 $^{b\,A}$ ± 4.3	0.99^{aA} ±0.2	0.3 ^{bA} ± 0.0
Conjugate phenols	18.98 $^{a\,A}$ ± 0.9	15.19 $^{b\,B}$ ±1.9	31.63^{aB} ± 4.6	4.53 ^{bB} ± 2.8	0.49 $^{a\,A}$ ±0.0	0.36 ^{bA} ± 0.0
Linked phenols	41.27 $^{a\,B}$ ± 4.9	30.88 $^{b\,C}$ ± 1.5	34.46^{aC} ± 1.7	34.73^{aC} ±0.6	2.56 $^{a\,B}$ ±0.3	2.61 ^{aB} ± 0.2

The data are shown as the mean ± standard deviation of at least three repetitions. Capital letter represent significant differences per column. Minuscule letter represents significant differences per column per line.
CF: Chikpea flour GAE: gallic acid equivalent

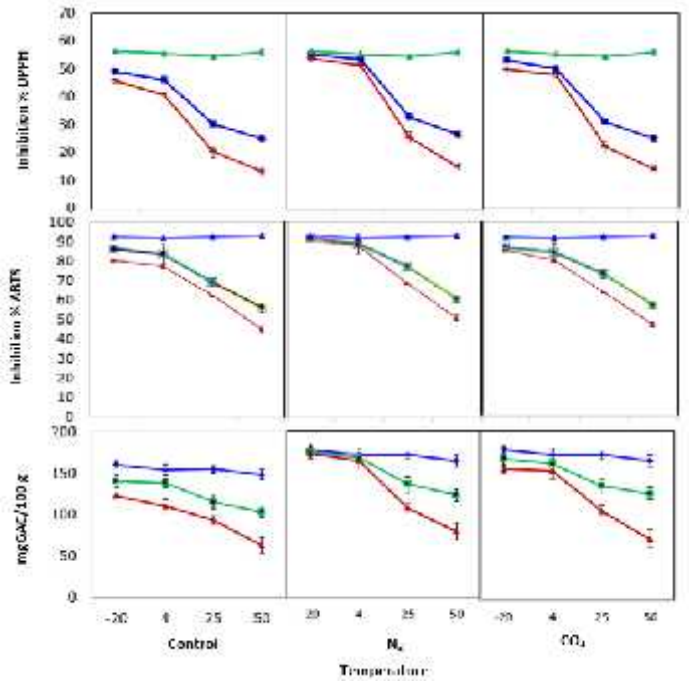


Figure 1.Inhibition percentage to radicals (DPPH, ABTS) and phenols quantification to linked extract in chickpea cooked.

Conclusions. The atmosphere of N_2 was the best for the conservation of phenolic compounds and antioxidant capacity in general, and under the temperatures of -20° C and 4° C. This method helps to that the phenolic compounds remain for long time

Acknowledgements. This study was supported by "Programa para el Desarrolo Profesional Docente, para el Tipo Superior (PRODEP)" de la Secretaría de Educación Pública (SEP).

References.

1. La-Zazzera. M., Cornacchia ML., Colelli A.G. 2010. Effect of atmosphere composition on quality of a ready to cook complex soup including fresh-cut vegetables and seeds.Proc 10 Intern Controlled and modified atmosphere.325-332..

ISSN: 2448-8461

AL32

ANTIHYPERTENSIVE ACTIVITY OF PLUM AND QUINCE HARVESTED IN ZACATECAS

Jesús Aguayo Rojas* (Universidad Autónoma de Zacatecas, Químico en Alimentos, Zacatecas, Zacatecas, 98160), Guillermo Quiñones Reyes (Universidad Autónoma de Zacatecas, Químico en Alimentos, Zacatecas, Zacatecas, 98160), Manuel de Jesús Macías Patiño (Universidad Autónoma de Zacatecas, Unidad Académica de Ciencias Químicas, Zacatecas, Zacatecas, 98000), Ricardo Omar Navarro Cortez (Universidad Autónoma del Estado de Hidalgo, Instituto de Ciencias Agropecuarias, Tulancingo, Hidalgo, 43600), Saraíd Mora Rochín (Universidad Autónoma de Sinaloa, Facultad de Ciencias Químico Biológicas, Culiacán, Sinaloa, 80000) Xochitl Tovar Jiménez (Universidad Politécnica de Pachuca, Ingeniería en Biotecnología, Zempoala, Hidalgo, 43830); *chuy_aguayo@hotmail.com

Key words: Phytochemicals, phenolics, antihypertensive

Introduction. Angiotensin I-Converting Enzyme (ACE) is a key component in regulation of blood pressure by virtue of the rennin-angiotensin system. ACE converts the inactive decapeptide, angiotensin I, into the potent vasopressor octapeptide, angiotensin II, and inactivates bradykinin, which has a vasodilating action. So, inhibition of ACE has become a major target in control of hypertension (1). It is well known that the consumption of fruits could provide health benefits by lowering the risk of chronic diseases such as metabolic syndrome diseases including type 2 diabetes and cardiovascular disease. (2). A number of compounds from plants has been identified to possess *in vitro* ACE inhibitory activity, including hydrolysable tannins, phenylpropanes, proanthocyanidins, flavonoids, xanthones, fatty acids, terpenoids, alkaloids oligosaccharides and peptide amino acids, among others. Nevertheless, only a few works on bioprospecting plant species for this molecular target has been reported. (3).
The objective of the study was to evaluate the phytochemical content and antihypertensive effect of plum and quince, in order to determinate their nutraceutical potential

Methods. Mature fruits were collected in Zacatecas. The plant material was dried in a forced-air convection oven at 35°C for 2 days. The dried fruits were ground in a domestic to obtain grits that passed through a 10 US mesh (2 mm) screen, and packed in plastic bags (1 kg). A dry ground sample of 1 g was mixed with 10 mL of chilled mehanol–water (80:20, v/v) for 30 min in a shaker at 50 rpm. The blends were centrifugated (3000g, 10 min) in order to recover the supernatant. The extracts were concentrated to 2 mL at 45ºC using a vacuum evaporator
The potential antihypertensive activity was evaluated by their ability to inhibit Angiotensin-Converting Enzyme (ACE). The ACE inhibitory property was assayed using ACE from rabbit lung and hippuryl-histidyl-leucine as the substrate (4, 5).

Results.

Table 1. Antihypertensive effect.

Variety	Antihypertensive activity (% ACE Inhibition)
Plum	51±0.1
Quince	45±0.5

Table 2. Phytochemical content.

Variety	Phenolics[1]	Flavonoids[2]	Tannins[3]	Coumarins[4]
Plum	240±7	558±29	66±7	68±18
Quince	122±11	422±4	58±8	43±1

1 mg equivalents of gallic acid /100 g, **2**. mg equivalents of catechin / 100 g, **3** mg equivalents of tannic acid /100 g, **4** mg equivalents of coumarin /100 g

Conclusions. This study shows a considerable percentage of ACE inhibition by plum and quince which lead to consider this species as a potential source of ACE inhibitor secondary metabolites. The methanolic extracts contained high values of phenolic compounds, possible secondary metabolites responsible for the inhibition of the enzyme and a potential high antioxidant source.

References.

1. Johnston CI. (1992). Renin-angiotensin system: a dual tissue and hormonal system for cardiovascular control. J Hypertens. 10 (7):13–26.
2. Alves RE, Brito EA, Rufino MSM, Sampaio CG. (2008). Antioxidant activity measurement in tropical fruits: A case study with acerola. *Acta Hortic*. 773: 299–305.
3. Braga FC, Wagner H, Lombardi JA, Oliveira AB. (2000). Screening the Brazilian flora for antihypertensive plant species for in vitro angiotensin-I-converting enzyme inhibiting activity. *Phytomedicine*. 7: 245.
4. Astello-García MG, Cervantes I, Nair V, Santos-Díaz M, Reyes-Agüero A, Guéraud F, Negre-Salvayre A, Rossignol M, Cisneros-Zevallos L, Barba de la Rosa AP. (2015). Chemical composition and phenolic compounds profile of cladodes from *Opuntia spp*. cultivars with different domestication gradiente. *J Food Compost Anal*. 43 (1): 119-130.
5. Serra CP, Côrtes SF, Lombardi JA, Oliveira AB, Braga FC. (2005). Validation of a colorimetric assay for the in vitro screening of inhibitors of angiotensin-converting enzyme (ACE) from plant extracts. *Phytomedicine*. 12: 424.

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México
AL33

EFFECT OF A MICROFILTRATION PROCESS TO OBTAINED ANTIOXIDANTS COMPOUNDS OF CAPULIN JUICE

Jesus Antonio Valencia-Arredondo [a], Claudia Stephania Rivera-Gaytan [b], Jorge Yáñez-Fernandez [a]*
[1] Instituto Politécnico Nacional, Laboratorio de Alimentos de posgrado de la Unidad Profesional Interdisciplinaria de Biotecnología. Av. Acueducto de Guadalupe s/n, Barrio la Laguna Ticomán, C.P. 07340, México, D.F.
[2] Universidad Tecnológica de Tecámac, Área Químico-Biológicas. Carretera Fed. Mex-Pac. K.m 37.5, Predio Sierra Hermosa, Tecámac, Edo de México, C.P. 55740.
e-mail: jyanezfe@ipn.mx
Key words: Microfiltration (MF), anthocyanins, antioxidant activity.

Introduction. Phenolic compounds are one of the most frequent sources of phytochemical nutrients, associated with health benefits found in many vegetables and fruits like the capulin. The beneficial effects derived from phenolic compounds have been attributed to their antioxidant activity [1], but the deterioration factors of these compounds, the low yields of obtaining processes have limited their qualities. Membrane processes are today consolited system in the food industry and in several productive sectors, besides being a friendly process, athermal and involves no phase change or chemical agents, advantages that can be exploited in obtaining bioactive components.
The aim of this study is to analyze the microfiltration process in order to clarify capulin juice, analyzing the viability the process and the effect in obtaining the antioxidants.

Methods. Capulin fruit was obtained from local market (Michoacan, Mexico). The extract was obtained by solid-liquid extraction and microfiltration (MF) process (polysulfone hollow fiber capsule filter 5575 cm^2, 0.05 μm, analyzing the permeate flux (*JP*) on function of operating time at different TMP; it was analyzed the concentration of total phenols (TP) and antioxidant activity (Aa).

Results. The table 1 show the effects to the different pressures on the bioactive components, the best pressure was 34 kPa, who maintains the best conditions in the permeate with 5.56 mg·mL^{-1} EGallic acid and 82.80% Aa

Table 1. Properties evaluated in the microfiltration process on the effect of bioactive components.

BEFORE CLARIFICATION	AFTER CLARIFICATION			
Total phenols mg · mL^{-1} EGalic acid	Pressure		Total phenols mg · mL^{-1} EGalic acid	Activity antioxidant DPPH %
2.72 ± 0.20	0 kPa	Permeate	0.34 ± 0.14	80.06 ± 3.7
		Retentate	2.79 ± 0.19	67.86 ± 2.0
Activity antioxidant DPPH %	34 kPa	Permeate	5.56 ± 0.21	82.80 ± 0.9
		Retentate	3.17 ± 1.79	42.08 ± 2.3
81.69 ± 1.89	69 kPa	Permeate	2.21 ± 0.35	56.77 ± 1.8
		Retentate	6.70 ± 1.92	60.43 ± 0.6
	103 kPa	Permeate	2.57 ± 0.17	60.19 ± 1.2
		Retentate	4.16 ± 0.29	61.10 ± 0.8

compared to the raw extract before being processed that is of 2.72 mg·mL^{-1} EGallic acid of TP and 81.69% Aa, while for the other conditions these values are low and in some cases the highest are retained in the system.

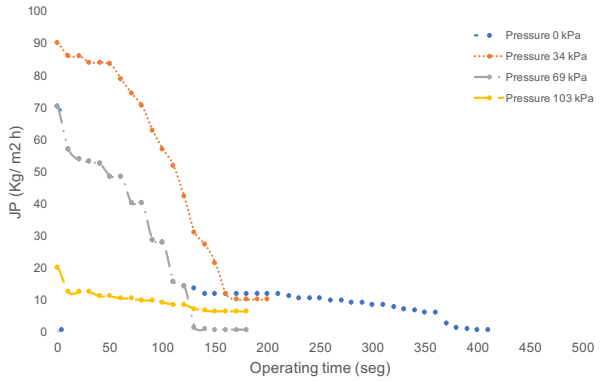


Figure 1. Behavior of *JP* on function of operating time at different transmembrane pressures

Figure 1 show *JP* versus operation time, it can be noted that the best operating condition is linked to the increase in JP so that so the polarization phenomenon limits the flow in the other pressures that can be and equally limits the concentration of the antioxidant [2].

Conclusions. The highest recovery of phenolic components and antioxidant capacity in the capulin extract was obtained at a TPM of 34 kPa**.** Membrane separation technology is a promising approach to concentrate and separate bioactive compounds.

Acknowledgements. To CONACYT and SIP (20180365) for funding.

References.

1. Heim K., Tagliaferro A., Bobilya D. (2002). Flavonoid antioxidants: cemestry metabolism and structure-activity relationship. The Journal of Nutritional Biochemestry. 13, 572-584.
2. Cassano A., Jiao B. and Drioli. (2004). Production of concentrated kiwifruit juice by integrated membrane process. Food Research International. 37, 139-148.

ISSN: 2448-8461

AL34

ISOLATION AND SELECTION OF ETHANOLIC FERMENTING YEASTS FOR THE PRODUCTION OF MAIZE BEVERAGES

Nadia Morales, Gonzálo Velázquez de la Cruz, Regina Hernández Gama; Instituto Politécnico Nacional; Centro de Investigación en Ciencia Aplicada y Tecnología Avanzada, unidad Querétaro; Biotecnología, Querétaro, CP76090 4422302379; reginagama99@gmail.com

Key words: ethanolic fermentation, maize, yeast.

Introduction. Ethanolic fermentation of alternative grains presents a good opportunity to generate distinctive products for breweries and distilleries, using national raw material. Corn is a grain with a high starch content, it has multiple varieties and it allows the production of beverages for people suffering from celiac disease.
However, the standard fermentation has been developed based on barley as raw material, so it is necessary to carry out the optimization of malting and fermentation processes based on corn. The aim of this work was to cultivate and select yeasts for their capacity to produce ethanol from maize.

Methods. Yeasts were grown from ingredients used in the manufacture of traditional fermented corn beverages: germinated maize of the varieties VC-42, VC-152 and FAC-M-40 [1]; panela, piloncillo, cinnamon, cloves, beans, chamomile flower, sugar cane, peach blossom, arbutus leaf, rice, papaya, plum, strawberry, guava, peach, pineapple, tepache, tejuino, pozol, tascalate and atole [2]. Samples were inoculated in corn meal extract with amoxicillin 50 mg/mL. From each yeast culture, DNA was purified and PCR products of the ITS1-ITS4 region were sequenced and analyzed for the taxonomic positioning of the cultures.
Fermentation kinetics were used to compare the production of ethanol of the yeasts against the *Saccharomyces cerevisiae* strain "Whiskey" from the Stillspirits distillery [1]. Maize VC-42, was steeped during 24 h, it was germinated during 4 days, and it was finely ground [3]. The green malt obtained was boiled 3 h with a malt:water ratio of 1:10. The extract was cooled, then, enzymes alpha-amylase and beta-glucosidase (GRANOTEC) were added, and each of the previously selected yeasts was tested in fermentation kinetics for 120 h, taking samples every 24 h and following the consumption of sugars (glucose, fructose and maltose) and ethanol production, by HPLC measurements.

Results. Ten yeast cultures were obtained, two of which were consortia and the rest axenic cultures. The table 1 shows the taxonomic position and origin of the yeasts.

Table 1. Yeast cultures and taxonomic position.

Isolate	Origen	Taxonomic position
1	Maize 1 VC-152	*Candida intermedia*
2	Maize 2 VC-152	*Meyerozima carbbica* 1
3	Maize VC-42	*Kluyveromyces marxianus*
4	Maize FAC-M-40	Consorcio 1
5	Tejuino 1	Zygotorulaspora florentina culture
6	Tejuino 2	Hanseniaspora uvarum
7	Tepache	Candida bordini
8	Masa	Consorcio 2
9	Guayaba	Meyerozima carbbica 2
10	Piña	Hanseniaspora sp

Three of the cultures obtained *Meyerozima carbbica* 2, *Candida intermedia* and Consorcio 2, generated a higher production of ethanol than Whisky reference strain (Fig 1).

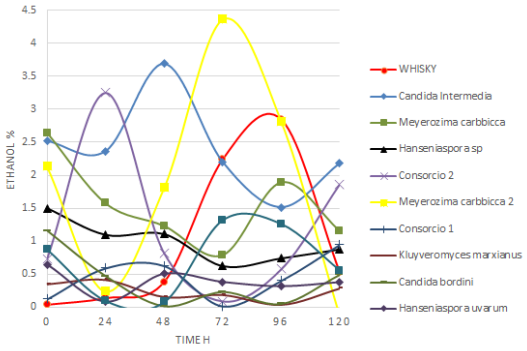


Fig.1 Ethanol accumulation during the 120 h kinetics.

Conclusions. It was possible to obtain competitive yeasts for the fermentation of maize and the production of alcoholic beverages

Acknowledgements. This work was part of the SIP 20170189 project.

References.

1. Rodriguez E, (2018), IPN, Optimización del proceso de malteado y fermentación para la producción de whisky artesanal de maíz, tesis. IPN.
2. Vallejo A, Miranda P, Flores-Félix D, Sánchez-Juanes F, Ageitos M, González-Buitrago M, Velázquez E, Villa G. (2013), *Syst Appl Microbiol.*36(8):560-4.
3. Iwouno, O., Ojukwu M. (2012), *Afr. J. Food Sci. Technol.*, 3(10) pp. 252-259.

ISSN: 2448-8461

AL35

PHYSICOCHEMICAL CHARACTERIZATION OF THE FRUIT OF *Byrsonima crassifolia* (NANCHE)

Alejandra Lozada-Zarate, Yolanda Gómez y Gómez, Heidi.Gorostiola-Espinosa, María Fernández Martínez y Jorge Yáñez-Fernández.
Food Biotechnology Laboratory, Unidad Profesional Interdisciplinaria de Biotecnología, Instituto Politécnico Nacional. Av.Acueducto de Guadalupe S/N, Mexico City. e-mail: jyanezfe.ipn@gmail.com

Key words: Nanche, bioactive, phytochemical

Introduction. Fruits and vegetables, being rich in phenolic compounds, carotenoids, glucosinolates, vitamin C and tocopherols, are plentiful in bioactive compounds[1,2]. These bioactive compounds have high antioxidant capacity and are important for preventing oxidative stress and chronic disease. *Byrsonima crassifolia* is known as Changunga or Nanche,it is a fruit native from tropical America and the southeast of Mexico, it is a yellow fruit with a bittersweet flavor whose size varies between 1.7 and 2 cm in diameter; Empirically, health benefits have been conferred to him, but there is little evidence of this[4].Mexico has a wide variety of native, wild and non-commercially cultivated fruits; although few information about their constituents are available. Thus, the aim of this investigation was doing an exploratory evaluation of some bioactive chemical constituents in *Byrsonima crassifolia* fruits.

Methods.

Samples. Nanches were obtained from a local market in Patzcuaro Michoacan, Mexico. The fruits were obtained in an appropriate stage of maturity and the juice was extracted from fruit maceration with water at 40°C during 40 min. **Physicochemical characterization of the fruit**. The determinations were made on dry basis following the standard methodology of A.O.A.C; percentage of humidity (925.30 AOAC), total ash (923.03 AOAC), total fat (920.39 AOAC), protein (955.04 AOAC), fiber (985.29 AOAC), total soluble solids (932.12 AOAC), vitamin C (967.12 AOAC), pH (Method 945.27, AOAC 1990). Ascorbic Acid, Tritable Acidity, and pH. Ascorbic acid was measured by the titration method based on the reduction of the sodium salt of the dye 2,6-dichlorophenolindophenol by ascorbic acid. The titratable acidity expressed as citric acid was assessed by standard procedures. pH was measured with a OAKTON 510 pHmeter. **Total phenolic compound analysis**. Total soluble phenols in ethanol extracts were determined with Folin−Ciocalteau reagent. The results were expressed as micrograms of galic acid equivalents per mL of juice. All analyses were made in triplicate. DPPH assay was performed as reported Kimm et. al.,(2002), with some modifications[4].

Results. Table 1 shows the physicochemical composition of the Nanche.

Table 1. Composition of the Nanche

Humidity	83.267% ±2.35	pH	3.83 ± 0.1
Total solids	16.73% ±2.34	Ash	16.39% ±0.13
soluble solids	8.3 °Brix ±0.12	Protein	4.39% ±0.34
Citric acid	0.14% ±0.1	Fibre	4.12% ±0.39
Vitamin C	21.975 mg ±0.4		

The aqueous extract showed 185.06 µg gallic acid / mL and 18.38 µg quercetin/mL for total phenols and flavonoids respectively. The antioxidant capacity in aqueous extract presented 30.11% of inhibition. The phytochemical preliminary assay showed the presence of flavonoids and other phenolic compounds.

Conclusions. Nanche showed the presence of bioactive compounds, such as flavonoids, tannins, reducing sugars, cardiac glycosides and quinones, which have gained great importance in the food industry as potentially bioactive compounds.

Acknowledgements. Financial support received for COFFA-IPN and Conacyt.

References. .1. Aguilera, Y., Martin-Cabrejas, M. A., & de Mejia, E. (2016). Phytochemistry Reviews, 15(3), 405e423.
2. Barba, F. J., Esteve, M. J., & Frigola, A. (2014). Bioactive components from leaf vegetable products. Studies in Natural Products Chemistry, 41, 321e346.
3. Carbonell-Capella, J. M., Barba, F. J., Esteve, M. J., & Frigola, A. (2014a). Quality pa- rameters, bioactive compounds and their correlation with antioxidant capacity of commercial fruit-based baby foods. Food Science and Technology International, 20(7), 479e487.
4. Vázquez, C., Batis, A., Alcocer, M., Gual, M.,y Sánchez, C. (1999). . Reporte técnico del proyecto J084. CONABIO - Instituto de Ecología, UNAM.
5. Kim, D. O.; Lee, K. W.; Lee, H. J.; Lee, C. Y. Vitamin C equivalent antioxidant capacity (VCEAC) of phenolic phytochemicals. J. Agric. Food Chem. 2002, 50, 3713–3717

ISSN: 2448-8461

AL36

DIVERSIDAD GENÉTICA DE LEVADURAS INVOLUCRADAS EN LA FERMENTACIÓN DEL MEZCAL DEL ESTADO DE DURANGO

Sandra Martínez-Estrada, Julio Grijalva-Avila, Israel Martínez-Rivera, J. Natividad Gurrola-Reyes, Isaías Chairez-Hernández; Instituto Politécnico Nacional, CIIDIR IPN Durango Calle Sigma #119, Fracc. 20 de Noviembre, Durango (Durango). con_sandr@hotmail.com

Palabras clave: levadura, fermentación, mezcal

Introducción. El mezcal es una bebida artesanal Mexicana obtenida de la destilación de los jugos fermentados de las piñas cocidas de agave (1). Las bebidas destiladas implican una población compleja de microorganismos durante la etapa de fermentación, donde las levaduras son responsables de la producción de varios compuestos químicos (2).
El objetivo de este trabajo fue identificar y evaluar la diversidad de las levaduras que están presentes en diferentes etapas de fermentación artesanal (inicio, medio y final) del mosto obtenido a partir de *Agave durangensis.*

Metodología. Las muestras fueron tomadas a diferentes tiempos (inicio, medio y final) durante la fermentación de una destilería artesanal, ubicada en Durango, Dgo., México. Las levaduras se aislaron en medio YPDA, La extracción de ADN se realizó con el Kit de purificación de ADN genómico Wizard© (PROMEGA). Se amplifico la región 26S ADNr utilizando los oligonucleótidos NL1 y NL4. La reacción se llevó a cabo en un secuenciador ABI 3130. Para obtener la identidad de cada uno de los aislados se tomaron secuencias de referencia de la página NCBI (GenBank) mediante el algoritmo Blast, se elaboraron árboles de distancias génicas para la región analizada.

Resultados. Los 25 aislados obtenidos fueron clasificados de acuerdo a su origen y etapa de fermentación. La etapa inicial de la fermentación fue donde se observó mayor diversidad, siendo *Saccharomyces cerevisiae* y *Kluyveromyces marxianus* las que aparecen en mayor número de aislamientos, sin embargo ya que S. cerevisiae es una levadura tolerante a altas concentraciones de etanol, desplaza a las demás especies al final de la fermentación, disminuyendo la diversidad conforme transcurre la fermentación.

Se encontraron 5 especies diferentes de levaduras, quedando de la siguiente manera: 13 cepas de *S. cerevisiae*, ocho cepas de *K. marxianus,* dos *Pichia kluyveri,* una cepa de *Torulaspora delbruekii* y un aislado de *Hanseniaspora lachancei.*

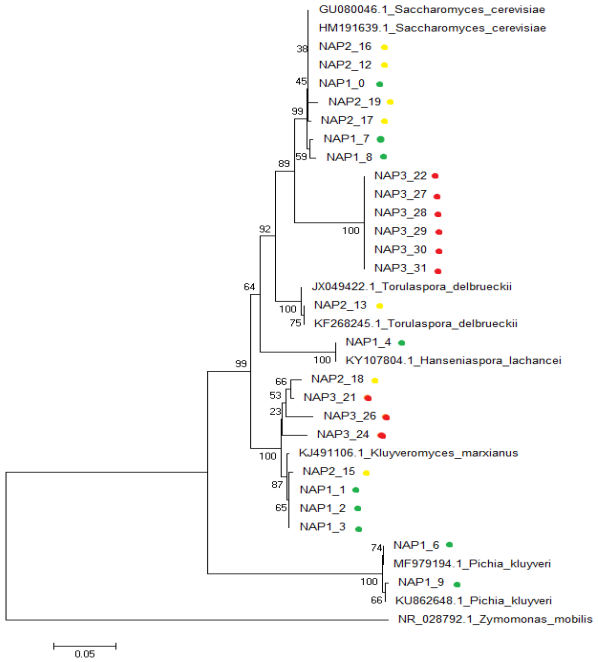


Fig.1. Análisis filogenético de la región 26S rDNA. (Verde) Inicio, (Amarillo) medio y (Rojo) final de la fermentación..

Conclusiones. La destilería mostró alta diversidad debido a que conserva un proceso de fermentación tradicional.

Agradecimientos. Agradecemos el apoyo financiero del Instituto Politécnico Nacional y CONACYT.

Referencias.

1. Flores Berrios, E.P., Alba González, J.F., Arrizon Gaviño, J.P., Romano, P., Capece, A. (2005). The uses of AFLP for detecting DNA polymorphism, genotype identification and genetic diversity between yeasts isolated from Mexican agave-distilled beverages and from grape musts. *Lett Appl Microbiol.* 41 (2) 147-152.
2. Escalante-Minakata, P., Blashek, H.P., Barba de la Rosa, A.P., Santos, L., De León-Rodríguez, A. (2008). Identification of yeast and bacteria involved in the mezcal fermentation of Agave *salmiana*. *Lett Appl Microbiol.* 46 (6) 626-630.

ISSN: 2448-8461

DETERMINATION OF THE STRESS RESISTANCE OF *Listeria spp*. ISOLATED MATURE CHEESE

Flores Magallon Rebeca[1] y Vazquez Navarrete Jesus[2]
[1]Instituto Politécnico Nacional. Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Unidad Michoacán. Jiquilpan, Michoacán, México CP 59510, Tel. y fax 353 53 30218.
[2] INIFAP Cenid Microbiología.

E-mail: rbk_fm@hotmail.com Phone: 3535330083

Key words: Cheese, Resistance, *Listeria spp*.

Introduction

The microorganisms become resistant to their adaptation capacity, developing mechanisms of resistance that inhibit the action of medicines, which constitutes a serious problem for public health and forces the development and use of new antimicrobial agents, more expensive and sometimes more toxic substances commonly used (Otto *et al*., 2009). However, little attention has been paid to the presence of resistant microorganisms in foods intended for human consumption, especially those of animal origin and that presumably can be carriers of resistance. Hence the need to evaluate patterns of sensitivity and resistance to certain antimicrobials commonly used in the treatment of listeriosis in strains of *Listeria spp*.

Materials and methods

To determine the presence or absence of *Listeria spp.* in matured cheeses was made according to the methodology proposed by NOM-147-SSA1-1995.

The susceptibility and antimicrobial resistance tests of the isolated microorganisms are determined by the agar disc diffusion method, in which they are used in the brand's multi-disc BIORAD, gram positive (CAT.71080180) and gram negative (CAT.71080280).

Results

From 100 pieces of cheese analyzed, 5 strains of *Listeria spp* to which the susceptibility and resistance tests were determined.

Of the 5 strains evaluated, they showed intermediate sensitivity to 4 of the 12 antimicrobials (PSU, TLO, FE and FFC). On the other hand, the percentage of strains showed resistance to 8 antimicrobials (VAN, FLAN, PSU, FE, TLO, FFC, AM, LIC). None of the 5 strains were sensitive to the 12 antimicrobials, although 4 of them were sensitive to AMX, OXT, TMP and EXT.

Arslan and Ozdemir in 2007 presented results very similar to those obtained in the present investigation using the disc diffusion method on Miuller-Hinton agar.

Ebrahim *et al.,* 2010, studied the susceptibility of 54 strains of Listeria isolated from milk and dairy products by means of the disc diffusion method, with which they found that 98.2% of the strains were resistant to one or more antimicrobials, obtaining results very similar to those obtained in the present investigation, since all were sensitive to vancomycin, 12.7%, resistant to Ampicillin, 20% to ciprofloxacin, 1.8% to gentamicin, 34%, 5% to penicillin and 27% to tetracycline. However, they differ in resistance to erythromycin (12.8%) since all strains showed intermediate sensitivity to these antimicrobials. Other studies, which use the same disk diffusion method, with strains of Listeria spp. isolated chicken products, ready-to-cook (RTC) and ready-to-eat (RTE), obtained the following patterns of microbial susceptibility: resistance to ampicillin (35.5%), ciprofloxacin (30.0 %), erythromycin (6.4%), penicillin (35.5%), rifampicin (7%), tetracycline (32%) and vancomycin (0%). Results similar to those of the present investigation.

Conclusions

The strains of *Listeria spp* of food origin that have been tested in this study, have shown generalized resistance to several antimicrobials commonly used in the treatment of listeriosis.

References

Harakeh, S., Saleh, I., Zouhairi, O., Baydoun, E., Barbour, E., Alwan, E. 2009. Antimicrobial resistance of *Listeria monocytogenes* isolated from dairy-based food products. *Science of The Total Environment* (407):4022-4027.

Gallegos, G.J., Arrieta, S.G., Salim, M.RP., Trespalacios, C.A. 2007. Frequency of *Listeria spp* in colombian cheeses Costenos. Revista MVZ. Cordoba. 12(1):996 -1012.

ISSN: 2448-8461

IMPACT OF STEVIA ON THE VIABILITY OF A LACTOBACILLUS.

Yadira Rivera Espinoza, Zaira Hernández Casiano, Humberto Hernández Sánchez, Yoja Gallardo Navarrol; Depto. de Ingeniería Bioquímica, ENCB-Instituto Politécnico Nacional, Ciudad de México, 07738; yadirariveraespinoza1@gmail.com

Key words: Stevia, lactobacillus, growth.

Introduction. In recent years there has been an excessive consumption of hypercaloric foods (1). Given this problem, guidelines have been proposed to develop alternatives that can prevent the onset and growth of diseases related to the consumption of high caloric intake (2). In this sense, microorganisms of the genus Lactobacillus and low-calorie natural sweeteners such as *Stevia rebaudiana* Bertoni (stevia), are of great importance for the development of products that provide health benefits.

The objective of this work was to evaluate the capacity of *Lactobacillus brevis* Lb9H (3) to remain viable or grow in the presence of different estevia products, in order to stablish the bases for the subsequent development of new products.

Methods. To evaluate the viability and growth of *L. brevis* an inoculum concentration of $1x10^7$ CFU / ml was used in both mineral medium (MM) and MRS broth (10% inoculum volume) with different commercial samples of stevia (1, 2 and 3) (1 g / 100 ml) and without it. For reference, glucose was used in the media used. The growth and viability of L. brevis was also evaluated in an infusion of stevia leaves using the same inoculum concentration and leaf weight / volume of water. The growth experiments were carried out at 37 ° C for 5 days under constant agitation and the viability was carried out at rest at 1 ° C for 24 days. The growth of lactobacillus was evaluated by turbidimetric methods and by plate count. The pH was measured during the entire process. For the data analysis, the MINITAB EXPRESS program was used.

Results. An increase in colony forming units (CFU) of *L. brevis* was observed in MRS with or without stevia. These samples did not show significant differences ($p < 0.05$). In MM with stevia there was no cell death or growth since it was maintained at 10^7 CFU/mL for 5 days. The pH in MRS medium added with stevia fell from 6.5 to 4.5. The pH of the MM added with stevia did not present variations during the time of the experiment. On the other hand, no growth was observed when the microorganism was evaluated directly in an infusion of stevia leaves, however, it managed to remain viable for 15 days in refrigeration. L. brevis grew in MRS media added with stevia sweeteners. The total cell concentration increased from 10^7 to 10^9 CFU/mL until 24 hours of incubation. On the other hand, it was observed that L. brevis reached a concentration in the order of 1010 CFU/mL until 5 days of incubation in all MRS media without significant differences with respect to control ($P = 0.9990$) and in mineral media no growth was recorded during the 5 days of incubation, however, the microorganism managed to remain viable in the order of 10^7 CFU / mL. No modification was observed in the CFU when the microorganism was evaluated in the infusion during 9, 12 and 24 h of growth. *L. brevis* showed a decrease (from 10^6 to 10^5 CFU/mL) when kept in incubation under refrigeration for 15 days.

Conclusions. With the above, it can be concluded that stevia sweeteners at a concentration of 10 g / L did not exert an inhibitory effect, nor a prebiotic effect in L. brevis Lb9H.

Acknowledgements. This research was funded by Instituto Politécnico Nacional (20181206).

References.

(1) Moran, C. P., Fellow, G., & Shanahan, F. (2014). Best Practice & Research Clinical Gastroenterology Gut microbiota and obesity : Role in aetiology and potential therapeutic target. *Best Practice & Research Clinical Gastroenterology*, *28*(4), 585–597. https://doi.org/10.1016/j.bpg.2014.07.005.

(2) Martin, A. A., (2016). Why can´t we control our food intake? The downside of dietary variety on learned satiety responses. Physiology & Behaviour 162, 120-129.

(3) Reyes-Nava L. A, Garduño-Siciliano L, Estrada-de los Santos P., Hernández-Sánchez H., Arauz J., and Rivera-Espinoza Y. Use of bile acids as a selection strategy for lactobacillus strains with probiotic potential.. J Food Nutr Disor 2015, 4 (6) 1-10. http://dx.doi.org/10.4172/2324-9323.1000185

ISSN: 2448-8461

AL39

Fermentation of chia flour by *Lactobacillus sp. antioxidant effect*

Salazar-Bautista Edgar; Jara-Romero G. Janet, Luna-Suárez Silvia; Centro de investigación en Biotecnología Aplicada, Instituto Politécnico Nacional, Tepetitla de Lardizabal C. P. 90700; silvials2004@yahoo.com.mx

Key words: chía, antioxidant peptides, Lactobacillus

Introduction. Bioactive peptides are inactive within the sequence of the parent protein and can be released by enzymatic proteolysis, once they are liberated in the body, bioactive peptides may act as regulatory compounds with hormone-like activity. Thus, these peptides represent potential health enhancing components for food and pharmaceutical applications (1). A way to produce peptides are hydrolysis with microorganism as Lactic acid bacteria (LAB) that have been used for centuries as starters or adjunct cultures in dairy fermentations because have a potent enzymatic system (2). By other hand, chia (*Salvia hispánica L.*) is a native specie from Mexico and Guatemala (3). Its seeds are rich in proteins (15–25%) (4) These seeds can be a source to produce bioactive peptides.
The aim of this work was to know de effect of *Lactobacillus sp.* over chia flour and the relation on the antioxidant activity by fermentation

Methods. As a medium growth, defatted chia flour (10%) was added to a solution of glucose in water (8%). It was sterilized and 1x10^7 cells of *Lactobacillus sp*. were inoculated. Samples were taken every determined time, these were centrifuged and the supernatant was taken to evaluate its antioxidant capacity with the DPPH method

IC50 was calculated using the following equation:

IC50= 100-(100(sample abs/blank abs))

Results. The growth kinetic of Lactobacillus on chia flour is shown in Fig. 1. The 50% inhibition concentration (IC50) is shown there, where an increase in the antioxidant effect over the time is observed. A lower IC50 value indicate a better antioxidant power.
As can be seen, the number of *Lactobacillus* cells grow in the chia flour as time passes.

The IC50 obtained at 52 h was better than the informed by Orona-Tamayo et al., 2015 (5), who reported an IC50 value of 250 µg/ml from chia enzymatic hydrolysates.

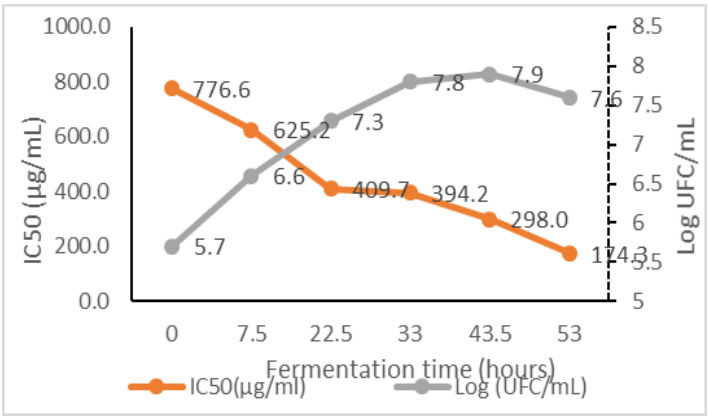


Fig.1 Antioxidant activity of hydrolysates from chia seeds when *Lactobacillus sp.* fermented the flour.

Conclusions.
The chia flour can be fermented by *Lactobacillus sp*. when this microorganism broke the macromolecules contained in, can produce molecules exhibiting antioxidant capacity, the amount of these molecules produced during fermentation increases as time increases.
The longer the fermentation, the greater the antioxidant activity.

Acknowledgements. The source of any financial support received for your work can be indicated in this section.

References.

1.Meisel H. (1998). Overview on milk protein-derived peptides. *Int Dairy J*. vol. (8):63-73.
2. Mengjin L., Jumamurat R., Renckens B., Nauta A., Siezen R. (2010). The proteolytic system of lactic acid bacteria revisited: a genomic comparison. *BMC Genomics*. 11:36.
3. Mohd A., N., Yeap S. K., Ho W. Y., Beh B. K., Tan S. W., Tan S. G. (2012). The promising future of chia, Salvia hispanica L. *Journal of Biomedicine and Biotechnology*, 1-9.
4. Segura M, Ciau N., Rosado G., Chel L., Betancur D. (2014). Chemical and Functional Properties of Chia Seed (Salvia hispanica L.) Gum. *Int. J. of Food Science.* 1-5
5. Orona-Tamayo, D., Valverde, M.E, Nieto-Rendón, B; Paredes-López, O. (2015). Inhibitory activity of chia (*Salvia hispanica L.*) protein fractions against angiotensin I converting enzyme and antioxidant capacity. *LWT - Food Science and Technology.* 64, 236-242

ISSN: 2448-8461
11° Encuentro Nacional de Biotecnología
2nd Biotechnology World Symposium
16-20 Octubre/October 2018
San José del Cabo, B.C.S., México

AL40

ENZYMATIC ACTIVITY DURING SOLID STATE FERMENTATION OF *Opuntia ficus indica* WITH *Trametes polizona*

Sánchez-Pardo, María Elena, Pérez-Díaz, Diana Larissa, Escutia-López, Karina Nathalie, García Rojas, Vanessa, Alcántara Capitán, Yelitza. Instituto Politécnico Nacional. Escuela Nacional de Ciencias Biológicas. Depto. Ingeniería Bioquímica. Unidad Profesional Adolfo López Mateos, Av. Wilfrido Massieu Esq. Cda. Miguel Stampa s/n, C.P.07738 Delegación Gustavo A. Madero Ciudad de México. alimentoselena@hotmail.com

Key words: Solid state fermentation, lignocellulolytic enzymes, Trametes polyzona

Introduction. Solid state fermentation (SSF) is a process widely used in Eastern countries. Through this process some microorganisms catalyze the nutrients, synthesize the secondary metabolites and complete other physiological activities under aerobic or anaerobic conditions. During the process, the desired microorganisms or microbial metabolites accumulate so they have three elements of the research: the product, the producing strain and the formation environment (nutrients, pH, temperature and oxygen) (Hongzhang, 2013). Currently, agricultural production generates a large amount of waste that doesn't receive any kind of treatment: excessive storage and open burning are still some of the cheapest and easiest ways to get rid of plant materials.

The objective is extract and evaluates the lignocellulolytic enzymes produced by *Trametes polyzona* during solid fermentation on vegetable cactus leaves using *Opuntia ficus-indica* as a substrate.

Methods. The microorganism used was donated by the Molecular Microbiology Laboratory, Department of Biotechnology of the Polytechnic University of Pachuca P(UP), isolated in previous works (Cruz et al., 2012) where the capacity of the microorganism for the production of enzymes is demonstrated lignocellulolytics. Said microorganism is Trametes polyzona isolated in Huejutla Hidalgo, conserved in inclined tubes of 15 mL of potato dextrose agar (PDA) at 4 ° C. 4.

Results. Trametes polyzona can grow on *Opuntia ficus-indica* due to its high content of cellulose and hemicellulose, which induce the production of enzymes. The established conditions (temperature, humidity, oxygen, inoculum concentration) are suitable for growth. The fungus *Trametes polyzona* is able to grow on *Opuntia ficus-indica* roots due to its high content of cellulose and hemicellulose, which induce the production of enzymes.

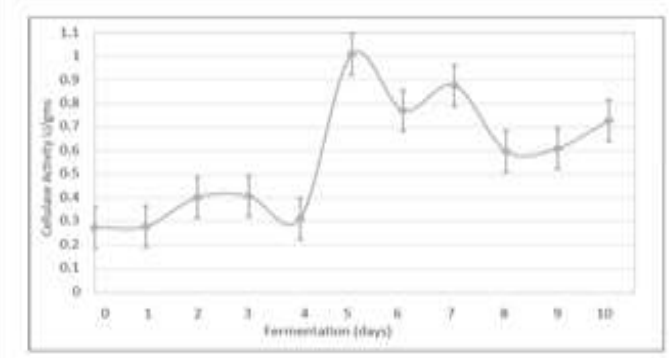


Figure 1. Cellulase activity of *Trametes polyzona* on *Opuntia ficus-indica*

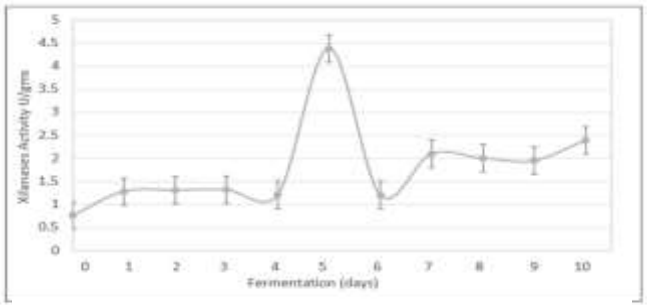


Figure 2. Xilanase activity of *Trametes polyzona* on *Opuntia ficus indica*

Conclusions. The maximum activity of xylanases and cellulases was obtained after 5 days of solid fermentation

Acknowledgements. Financial support received for our work: SIP-IPN

References.

1. Cruz M, G., Rivera J, M., Tellez A, Maqueda A, P., Mercado Y, & Arana C, A. (2012). Screening for thermotolerant ligninolytic fungi with laccase, lipase, and protease activity isolated in Mexico. *J Environ Manage*, 256-259.
2. Hongzhang, C. (2013). Solid-State Fermentation Connotation. *Modern solid state fermentation,* 1-21.
3. Peláez Acero, A., Meneses Mayo, M., Miranda Romero, A., Ayala Martínez, M., Crosby Galván, M., Loera Corral, O., & Megías Rivas, D. (2011). Enzimas fibrolíticas producidas por fermentación en estado sólido para mejorar los ensilajes de caña de azúcar. *Agrociencia*, 675-685.

ISSN: 2448-8461

AL41

FUNCTIONAL CHARACTERIZATION OF PEANUT (*Arachis hypogaea*) WASTE

Sánchez-Pardo, María Elena, Enríquez Guerra, Vianey E., Ortiz-Moreno, Escutia-López, Karina Nathalie, García Rojas, Vanessa, Alcántara Capitán, Yelitza. A. Instituto Politécnico Nacional, Escuela Nacional de Ciencias Biológicas, Departamento de Ingeniería Bioquímica. Unidad Profesional Adolfo López Mateos, Av. Wilfrido Massieu Esq. Cda. Manuel Stampa s/n, C.P.07738, Delegación Gustavo A. Madero, México. alimentoselena@hotmail.com

Key words: peanut, cuticle, functionally

Introduction. An important characteristic of the residues is the content of compounds; the capacity of retention ions is the ability of the fiber to bind to cations and is related to the amount of minerals that are absorbed at the intestinal tract by the fiber, so a high value is bad for because it would cause decalcification. Noting that some of these are found in the pericarp or cuticle. Currently, only the pericarp is used in other areas, such as food for cattle, vehicle for pesticides or fertilizers, as well as a medium for growing fungi, among others.

The objective of the present work was the functional characterization of the Virginia variety peanut residues from the state of Morelos, Mexico.

Methods. Swelling capacity. In a graduated tube 0.5 grams of the sample was weighed, measuring the volume occupied (Vo); then 5 mL of water was added to stand for 24 hours. Subsequently the volume was measured and the calculations were made (gram of water / gram of dry sample). Water absorption capacity 0.5 g of the sample was placed, which will be p1; in a falcon tube, then 5 milliliters of water were added and stirred for 30 minutes; then centrifuged for 10 minutes at 3000 rpm, the supernatant removed and concluded by weighing the supernatant, weight was p2; finally we will proceed to make its calculation and report in mL of water / gram of dry sample. Oil absorption capacity. In a tube Falcon were placed 0.5 g of the sample (p1), which was added 5 mL of extra virgin olive oil and stirred for 30 minutes; taking the sample centrifuged for 10 minutes at 3000 rpm, remove the supernatant after even though the sediment (p2), and finally it was reported in gram of oil/gram of sample.

Results. As for the functional properties, the water retention capacity has to do with the size of the bolus of food that it will take in the organism and with it, the effect of satiety is presented; it was obtained from 0.39 mL of water/g dry sample, a value not far from that obtained in the work of Guerrero-Colin et al 2016. With 0.95 and 0.98 mL of water /g regarding the cuticle, a value of 8.75 mL of water /g dry sample was obtained. The result obtained from the peanut cuticle for this determination was very low (1.91x10-5 meq/g sample), which is considered suitable for human consumption since it would avoid decalcification problems as mentioned; although for the case of the shell, it was obtained that it was 0.0207 meq/g sample equally low, which is favorable; in this case, a reference margin was not found either

Table 1. Functional properties of pericarp and cuticle of peanut

Functional properties	Pericarp	Cuticle
Water absorption capacity	3.83 ± 0.22 $mL\ water\ /\ dry\ sample$	7.32 ± 0.64 $mL\ water\ /\ dry\ mass$
Oil absorption capacity	2.45 ± 0.11 $(g\ oil)/(g\ dry\ sample)$	6.32 ± 0.87 $(g\ oil)/(g\ dry\ sample)$
Capacity of retention ions	$0.02 \pm 0.41x10^{-4}$ $\frac{meq[H]}{gdry\ sample}$	$1.91x10^{-5} \pm 3.5x10^{-7}$ $\frac{meq\,[H]}{gdry\ sample}$
Water retention capacity	0.39 ± 0.28 mL H 2 O / g dry sample	8.75 ± 0.43 mL H 2 O / g dry sample

Conclusions. Only the peanut cuticle is viable to be treated due to its functional properties.

Acknowledgements. Financial support received for our work: SIP-IPN

References.

1. Casas L. y Coral G. 2014. Enzimas en la valorización de residuos agroindustriales, Rev. Digital Universitaria, Vol. 15, p. 4.
2. Guerrero-Colín, J. I., Trejo-Márquez M., Moreno-Lara J., Lira-Vargas A. A., Pascual- Bustamante, S. (2016). Memoria de Congreso. Investigación y Desarrollo en Ciencia y Tecnología de Alimentos. Extracción de fibra de los desechos agroindustriales de cacahuate, para su aplicación en el desarrollo de alimentos, 1(2), 806–812.

ISSN: **2448-8461**

Índice de Autores / Author Index

ÍNDICE DE AUTORES / AUTHORS INDEX

A

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

U

V

W

X

Y

Z

ISSN: **2448-8461**

Instituciones Internacionales Participantes / International Institutions

INSTITUCIONES INTERNACIONALES PARTICIPANTES
INTERNATIONAL INSTITUTIONS

Bangladesh Agricultural Research Institute

Biomedical Research Center, Slovak Academy of Sciences

Centro de Edafología y Biología Aplicada, España

Centro de Ingeniería Genética y Biotecnológica, Cuba

Charles University, Czech Republic

Czech Academy of Sciences

Escuela Politécnica Nacional, Ecuador

Imperial College London, United Kingdom

Instituto Tecnológico de Costa Rica

Slovak Academy of Sciences

Slovak University of Technology

Universidad de Sucre, Colombia

Universidad Agraria de La Habana, Cuba

Universidad de Córdoba

Universidad de Santander UDES

Universidad de Santiago de Compostela, España

Universidad Distrital Francisco José de Caldas, Colombia

Universidad Industrial de Santander, Colombia

Universidad Nacional Mayor de San Marcos, Perú

Université des Antilles

Université Paris-Saclay

University of California

University of French West Indies

University of Science and Technology, Kwame Nkrumah, Ghana

ISSN: **2448-8461**

Instituciones Nacionales Participantes / National Institutions

INSTITUCIONES NACIONALES PARTICIPANTES
NATIONAL INSTITUTIONS

Agrobiotecnología del Bajío SA de CV

Álvarez & Arraola Radiólogos

Benemérita Universidad Autónoma de Puebla

Biodesarrollos Valmex SA de CV

Centro de Biotecnología Genómica

Centro de Ciencias Genómicas, UNAM

Centro de Desarrollo de Productos Bióticos

Centro de Investigación Biomédica del Sur, IMSS

Centro de Investigación en Biotecnología Aplicada

Centro de Investigación en Ciencia Aplicada y Tecnología Avanzada Querétaro

Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, AC

Centro de Investigaciones Biológicas del Noroeste

Centro de Investigaciones y de Estudios Avanzados del IPN

Centro Interdisciplinario de Ciencias Marinas

Centro Nacional de Recursos Genéticos, INIFAP

Centro de Investigación e Innovación Biotecnológica, Agropecuaria y Ambiental

Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Durango

Centro Interdisciplinario de Investigación para El Desarrollo Integral Regional, Oaxaca

Centro Interdisciplinario de Investigación para El Desarrollo Integral Regional, Michoacán

Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Sinaloa

Centro Universitario de Ciencias Biológicas Y Agropecuarias, U de G

Centro Universitario de Ciencias Exactas e Ingenierías, U de G

Centro Universitario de La Ciénega, U de G

Comisión Nacional de Áreas Naturales Protegidas

Escuela Nacional de Ciencias Biológicas

Escuela Nacional de Medicina y Homeopatía

Escuela Superior de Ingeniería Mecánica y Eléctrica, Zacatenco

Escuela Superior de Medicina

Especialistas en Papayas SA de CV

Grupo Multidisciplinario Integral GMI SC

Hospital Civil de Culicán

Hospital General de Culiacán

Instituto de Biotecnología, UNAM

Instituto de Ecología AC

Instituto de Investigaciones Biomédicas

Instituto Nacional de Astrofísica, Óptica Y Electrónica

Instituto Nacional de Neurología y Neurocirugía, Manuel Velasco Suárez

Instituto Nacional de Psiquiatría Ramón de la Fuente

Instituto Tecnológico de Durango

Instituto Tecnológico de Oaxaca

Instituto Tecnológico de Orizaba

Instituto Tecnológico de Sonora

Instituto Tecnológico y de Estudios Superiores de Monterrey

Instituto Tecnológico Superior de Felipe Carrillo Puerto, Quintana Roo

Instituto Tecnológico Superior de Guasave, Sinaloa

Secretaría de Salud, México

Tecnológico Superior de Perote, Veracruz

Unidad Profesional Interdisciplinaria en Ingeniería y Tecnologías Avanzadas

Unidad Profesional Interdisciplinaria de Ingeniería

Universidad Autónoma de Baja California

Universidad Autónoma de Baja California Sur

Universidad Autónoma de Campeche

Universidad Autónoma de Chihuahua

Universidad Autónoma de Nuevo León

Universidad Autónoma de San Luis Potosí

Universidad Autónoma de Sinaloa

Universidad Autónoma de Tlaxcala

Universidad Autónoma de Zacatecas

Universidad Autónoma del Estado de Hidalgo

Universidad Autónoma del Estado de Morelos

Universidad Autónoma Indígena de México

Universidad Autónoma Intercultural de Sinaloa

Universidad Autónoma Metropolitana, Iztapalapa

Universidad de Colima

Universidad de Guadalajara

Universidad de Guanajuato

Universidad de Occidente, Sinaloa

Universidad de Sonora

Universidad Interserrana del Estado de Puebla-Ahuacatlán

Universidad Michoacana de San Nicolás de Hidalgo

Universidad Nacional Autónoma de México

Universidad Politécnica de Cuautitlán Izcalli, México

ISSN: **2448-8461**

CIBA

INVESTIGACIÓN +

POSGRADOS

- Maestría en Biotecnología Aplicada
- Maestría en Biotecnología Productiva
- Doctorado en Biotecnología Aplicada
- Doctorado en Biotecnología Productiva

IPN

Centro de Investigación en Biotecnología Aplicada
Ex-Hacienda San Juan Molino Carretera Estatal
Tecuexcomac - Tepetitla K. 1.5, Tlaxcala, C.P. 90700, México