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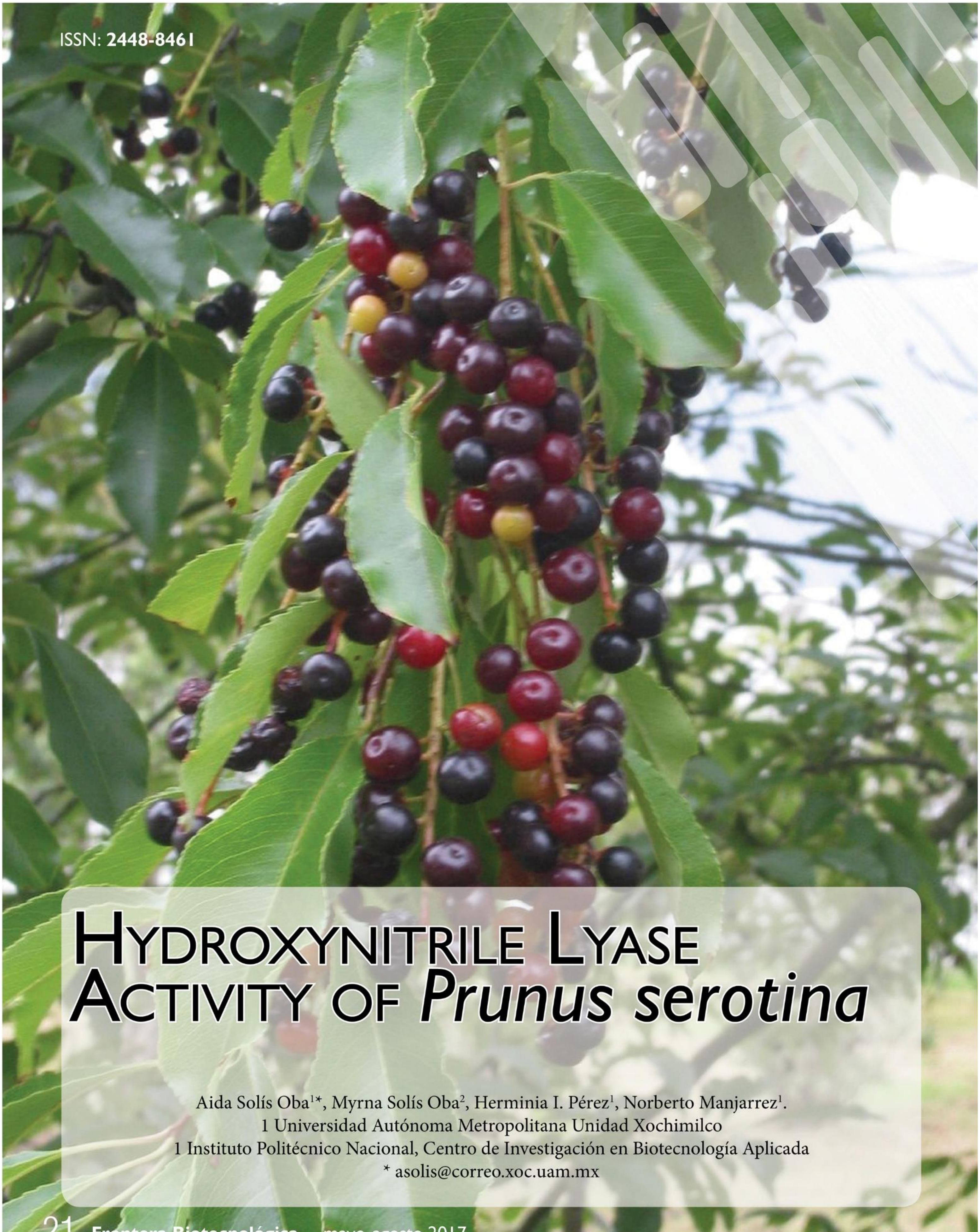

Hydroxynitrile Lyase Activity of *Prunus serotina*

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Abstract

Use of enzymes for chemical catalysis have some advantages, for example they are specific so they only catalyze the reaction you want them to, using lower temperatures and pressures means a lower cost as it saves energy, enzymes work for a long time so after the initial cost of buying them you can continually use them, they are biodegradable and therefore cause less environmental pollution. Other important factors for pharmaceutical industry is that enzymes, besides those, have the ability to accelerate reactions enantio selectively. We study the biocatalytical properties of the HNLs from Prunus serotina var. capuli seeds, like a very cheap enzyme source to catalyze enantiomeric reactions. Towards the addition of HCN to 2-chlorobenzaldehyde, by evaluating the conversion and ee% (enantiomeric excess). We found that this material catalyzes the reaction at 17 to 51% conversion and 43 to 83 ee%. The better conditions are: low concentrations of the aldehyde (0.047 M), 1.23 to 2.43 % aqueous phase and 1 or 2 ratio seed/aldehyde.

Key words: Prunus serotine, Hydroxynitrile lyases, cyanohydrins, enantioselectivity

Resumen

El uso de enzimas para catálisis química tiene varias ventajas, por ejemplo son especificas así que solo catalizan la reacción deseada, su uso a bajas temperaturas y presiones significa un ahorro por la reducción de energía, las enzimas actúan por largos periodos por lo que después del desembolso inicial para su adquisición pueden seguirse usando continuamente, son biodegradables por lo que ocasionan menos contaminación. Otro factor importante para la industria farmacéutica es que las enzimas, además de los anteriores, tengan la habilidad de acelerar reacciones de manera enantioselectiva. Se estudió las propiedades biocataliticas de las semillas de Prunus serotina var. Capulí, como una fuente muy económica de enzimas para catalizar reacciones enantiomericas. Mediante la adición de HCN al 2-cloro benzaldehido, mediante la evaluación del porcentaje de conversion y de %ee (exceso enantiomérico). Encontramos que este material cataliza la reacción en un 17 a 51% de conversión y de 43 a 83 de porcentaje de ee. Las condiciones mejores fueron: baja concentración del aldehído (o.047 M), de 1.23 a 2.43 % de fase acuosa y relación semilla a aldehído de 1 o 2.

Palabras clave: Prunus serotine, hidroxinitrilo liasas, cianohidrinas, enantioselectividad.

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Introduction

The application of enzymes as catalysts for chemical synthesis, has become an increasingly valuable tool for the synthetic chemist. Enzymatic transformations, carried out by partially purified enzymes or whole-cell as catalysts, are used for the production of a wide variety of compounds, mainly to assist in synthetic routes to complex molecules of industrial interest. Biocatalytic processes are often cheaper and more direct than their chemical counterparts, and the conversions normally proceed under conditions that are regarded as ecologically acceptable (Wohlgemuth, 2007). The main source of enzymes used in biocatalytic process comes from microorganisms, although enzymes from plants are good alternative have remained less explored (Cordell, 2007).

Hydroxynitrile lyases (HNLs) or oxynitrilases constitute a diverse family of enzymes that have been isolated from a wide variety of plant sources (Asano et. al, 2005; Solís et. al, 2004). Hydroxynitrile lyases are widespread in plants playing a major role in disease suppression; and only recently a bacterial protein with HNL activity in the cyanohydrin cleavage reaction was reported (Hassain et al, 2012), over 3,000 plant species are cyanogenic. Cyanogenesis is defined as the hydroxynitrile lyase catalysed release of a cyanide group in the form of HCN and the corresponding aldehyde or ketone. When a plant is attacked, HCN released is a self defence mechanism. A special characteristic of enzymatic reactions is that all enzymatic reactions are reversible-hydroxynitrile lyases can also be used for the synthesis of enantiomerically pure cyanohydrins which are of great importance in industry (Kassim and Rumbold, 2014). In chemical industries, hydroxynitrile lyase is used as an important industrial biocatalyst for the synthesis of chiral cyanohydrins by exploiting the reversible enzymatic reaction. Cyanohydrins are biologically active compounds used in synthesis of α-amino alcohols, -hydroxy ketones and -hydroxy acids, which have importance as fine chemicals, pharmaceuticals and agrochemicals. Many recombinant hydroxynitrile lyases have been expressed in Escherichia coli, Saccharomyces cerevisiae and Pichia pastoris (Sharma et al, 2005).

Apart from the interesting aspects of their catalytic function, HNLs are of practical importance as biocatalysts for the reverse reaction of cyanogenesis, that is, the stereoselective addition of HCN to carbonyl compounds to produce optically active cyanohydrins (Sherma et. al, 2005). The importance of cyanohydrins is highlighted in several reviews, and special attention is given to the synthetic utility and application of optically active cyanohydrins that can be readily converted into α-hydroxy carboxylic acids, primary and secondary β-hydroxyamines, α-aminonitriles, α-hydroxyketones, α-amino-β-hydroxy carboxylic acids, pantolactones, that have biological activities like insecticides, antibiotics, aziridines, bronchodilators, Cardiac drugs, D- and L-sphingosines, vitamins, thalidomide, hyploglicemics, etc. Also have been studied applications to Industrial scale (Purkarthofer et. al, 2007). (R)-2-chloromandelonitrile is used for the preparation of (R)-2-chloromandelic acid, a key intermediate for the production of clopidogrel, drug widely administered as anticoagulant, that reduces the risk of cardiovascular events in patients with acute coronary syndromes.

The aim of this paper is to study the biocatalytical properties of the HNLs from *Prunus serotina* var. *capuli* (capulin) seeds, like a cheap source of enzymes that catalyzed enantiomeric reactions, towards the addition of HCN to 2-chlorobenzaldehyde.

Experimental

1. Chemicals

Enantiomeric excesses were determined by HPLC, using a Chiracel OD column and hexanes–isopropanol as eluent in a Hewlett–Packard 1050 series, equipped with a diode array detector. Conversion percentages were determined by NMR. 1H NMR spectra were recorded on a Varian instrument at 400 MHz, using CDCl3 as a solvent and TMS as internal reference.

The capulin seeds were obtained from fresh fruits purchased in local grocery stores. The upper layer of the seeds was cracked with a hammer to give the soft kernels inside, and the fleshy cover was removed to obtain the seeds, then they were blended three times with acetone, after filtration by suction the powder was air dried and stored at 4°C,7 this defatted meal was used as biocatalyst source.

2. Biocatalytic addition of HCN to 2-chlorobenzaldehyde

To a solution of HCN in diisopropyl ether and KCN/citric acid or NaOH/citric acid buffer solution, was added the defatted meal, then the aldehyde, the mixture was stirred 38 h at 4°C, after that time it was filtered and the solvent evaporated to dryness. The product was analyzed by HPLC using a Chiralcel OD column to determine enantiomeric excess (ee) % and by 1H NMR the conversion.

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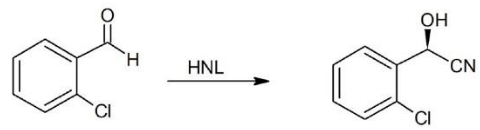
Results and Discussion

The reaction evaluated was the follow:



1 2-chlorobenzaldehyde — **2** 2-chlorobenzaldehyde cyanohidryn

Table 1 shows results obtained considering the different reaction conditions tested.

Table 1. Enantiomeric excess of 2 under different reaction conditions.

Entry	2 chlorobenzaldehyde [M]	Aqueous phase (%)	Ratio seed: aldehyde	%conv	ee %
1	0.2	2	1.8	43	48
2	0.12	90	2.5	nd	64
3	0.12	50	2.5	nd	58
4	0.12	25	2.5	nd	62
5	0.12	12.5	2.5	nd	72
6	0.047	5.2	2	51	80
7	0.047	5.2	1	38	76
8	0.047	2.43	2	30	83
9	0.047	2.43	1	31	81
10	0.047	1.23	1	17	81
11	0.047	1.23	1	17	81
12	0.047	1.23	4.28	38	78
13	0.047	0.5	4.28	23	63
14	0.047	1.25	2.14	41	75

From results in **Table 1** we can observe the following facts that have an important influence on the enantioselectivity of the reaction:

- Prunus serotina var. capuli is a good source of enzyme to catalysis the addition of HCN to 2-chlorobenzaldehyde with good ee%, from 48 to 83%.

- The lower ee% was obtained with the higher concentration of 2 chlorobenzaldehyde, as it can be observed, more concentrated solutions yield lower ee (Entry **1**, ee 48%).

- Considering the aqueous phase, it is better to try with medium percentages, because high (12.5 to 90 %) or very low (0.5 %) aqueous phase content, also decrease the enantioselectivity (entries **2, 3, 4** and **5**, 90 to 12.5% aqueous phase, ee 58-72%; entry **13**, 0.5% aqueous phase, ee 63 %).The content of aqueous phase that improve the enantioselectivity is between 1.23 and 5.2%.

- The ratio seed:aldehyde also has an important influence on ee%, is recommended to use ratio 1 or 2; from entries **10, 11, 12, 13 and 14** it can be observed that more enzyme source do not enhance the enantioselectivity, using a ratio of 4.28 (entry **12 or 13**) the ee was 78 or 63% respectively, whereas using a ratio of 1 or 2 (entries **10** and **11**) the ee was 81%.

Besides using seeds and vegetal material as enzyme sources to catalyze enantioselective reactions, it has been used other methods like using chemical catalysts, they are almost invariably rendered chiral (Bauer, 2012). Most enantioselective catalysts are effective at low substrate/catalyst ratios. Given their high efficiencies, they are often suitable for industrial scale synthesis, even with expensive catalysts. The design of new catalysts is very much dominated by the development of new classes of ligands. Certain ligands, often referred to as 'privileged ligands', have been found to be effective in a wide range of reactions. Organocatalysis refers to a form of catalysis, where the rate of a chemical reaction is increased by an organic compound consisting of carbon, hydrogen, sulfur and other non-metal elements. When the organocatalyst is chiral enantioselective synthesis can be achieved (Dalko, 2001)

Conclusions

Seeds like Prunus serotine var. capuli are an interesting and cheap sources of enzymes Hydroxynitrile lyases, that catalyzes enantioselectvity some reactions like the stereoselective addition of HCN to carbonyl compounds to produce optically active cyanohydrins. The better conditions to the addition of HCN to 2-chlorobenzaldehyde are: low concentrations of the aldehyde (0.047 M), 1.23 to 2.43 aqueous phase and 1 or 2 ratio seed/aldehyde.

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