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Synthesis and biocatalytic ene-reduction of Knoevenagel condensation compounds by the marine-derived fungus *Penicillium citrinum* CBMAI 1186

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ABSTRACT

The chemoselective bioreduction of α,β-unsaturated compounds is an important synthetic tool that can have applications in the synthesis of many fine chemicals and pharmaceutical molecules. The synthesis of aromatic malononitrile derivatives through Knoevenagel condensation by microwave radiation under green chemistry conditions using methanol like solvent, free base and free catalyst is here reported. In addiction the biocatalytic reduction of the C–C double bond of aromatic malononitrile derivatives by whole cells of the marine-derived fungal *Penicillium citrinum* CBMAI 1186 was also tested. The products catalyzed by the fungus ene-reductase were obtained in very good yields (up to >98%).

1. Introduction

The chemoselective bioreduction of α,β-unsaturated compounds is an important synthetic tool that can have applications in the synthesis of many fine chemicals and pharmaceutical molecules.[1,2]

Chemoselective reduction of benzylidene malononitrile has attracted much attention in the last decade because the reduction product that is very important intermediates in organic synthesis mainly on the production of medicinal active compounds.[3] However, numerous methods developed for this organic synthesis use $NaBH_4$, $InCl_3$–$NaBH_4$, and other expensive catalysts such as rhodium complexes, and additional harsh experimental conditions also associated with environmental problems.[1,4]

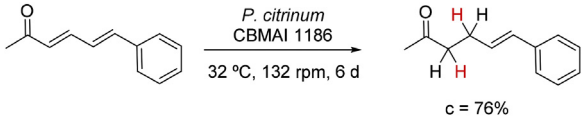
The biotransformation process is only one reaction or a set of simultaneous reactions in cascade performed by enzymes or in the whole cells of microorganisms that can be used complementarily to chemical synthetic methods.[5,4]

Many of those enzymes responsible by chemoselective bioreduction of C–C double bond α,β-unsaturated group are named of ene-reductases (ERs).

Although these catalysts (isolated microorganisms and enzymes) are known since a long time, it has more recently been used as chemical reagents in organic synthesis.

However, the synthetic potential of these enzymes started to be explored in recent decades mainly in the semi-synthesis of natural products, leading to interesting biologically active molecules, drugs or intermediate key compounds.[2,6] The catalytic process by these enzymes occurs on the reduction of C–C double bonds activated by electron-withdrawing group, such as, carbonyl, nitroolefin, cianoolefin, and imines.[7,8]

In addition, whole cells of the fungus filamentous marine-derived *Penicillium citrinum* CBMAI 1186 has been also successfully used on the chemoselective bioreduction of chalcones and enones α,β-, α,β,γ,δ- or di-α,β-unsaturated as it was recently shown in our research group (Scheme 1).[9]



Scheme 1. Chemoselective bioreduction with whole cells of the fungus *P. citrinum* CBMAI 1186.

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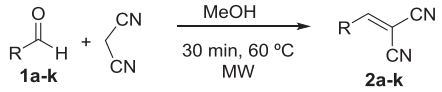
0040-4020/

2. Results and discussion

Due to the versatility on the chemoselective biohydrogenation of enones by fungus *P. citrinum* CBMAI 1186,[9] in this work we extend its application to perform the reduction of different aromatic malononitriles, commonly prepared by Knoevenagel condensation.

Firstly, different Knoevenagel adducts under microwave radiation in methanol were synthesized. All products were obtained in goods yields (Table 1).

Table 1
Knoevenagel synthesis of aromatic and heteroaromatic malononitriles under MW[a]



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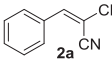
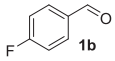
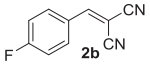
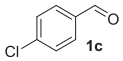
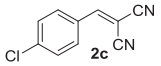
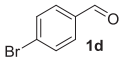
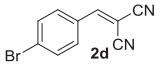
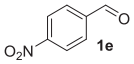
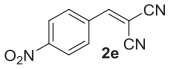
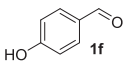
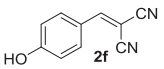
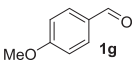
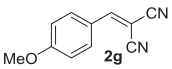
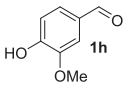
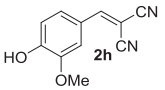
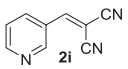
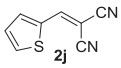
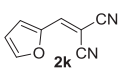
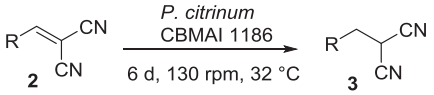
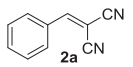
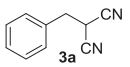
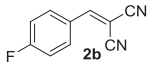
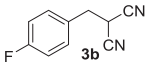
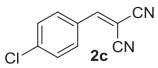
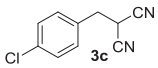
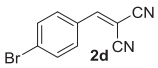
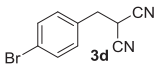
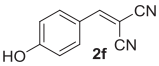
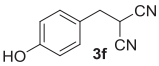
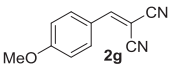
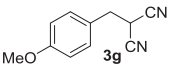
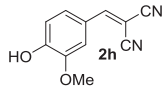
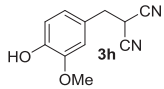
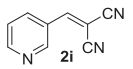
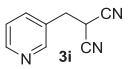
1			98
2			98
3			95
4			93
5			95
6			93
7			99
8			98
9			90
10			85
11			95

[a] General reaction conditions: benzaldehyde (1 mmol), malononitrile (1.1 mmol), MW power: 20 W, 60 °C, 30 min. The reactions were monitored by TLC. The crystals were filter out, wash them with water (3×5 mL) and recrystallized in a mixture of hexane–dichloromethane (1:1).
[b] Isolated yields obtained after purification.

The results from all biotransformations of aromatic and heteroaromatic malononitriles with *P. citrinum* CBMAI 1186 are summarized in Table 2. The reaction of biotransformation of the compound **2a** by fungus marine-derived *P. citrinum* CBMAI 1186 gave exclusively to the formation of the compound **3a** with 97% yield (Entry 1, Table 2), resulting from the action of the ERs on the C–C double bond in the **2a**. The mechanism of reduction of the C–C double bond by ERs is known by the nucleophilic attack of a hydride transfer from the flavin cofactor onto a β-carbon atom of the C–C double bond in the presence of an electron-withdrawing substituent. Then, a tyrosine residue delivers a proton to the α-carbon atom, typically on the alkene opposite face, affording *anti*-hydrogen addition.[8,10]

Table 2
Bioreduction of Knoevenagel adducts by mycelium of the marine-derived fungus *P. citrinum* CBMAI 1186



Entry	Knoevenagel adducts (**2**)	Products (**3**)	Isolated yields (%)
1			97
2			98
3			95
4			93
5			12
6			84
7			66
8			93
9			99

Based on this observation we tried to investigate and confront this enzyme catalytic mechanism by examining the action of whole cells of the fungus *P. citrinum* CBMAI 1186 in the reduction the C–C double bond of different aromatic malononitriles with substituent electron withdrawing and electron donating.

The bioreduction of benzylidene malononitriles bearing electron withdrawing substituents such as 2-(4-fluorobenzylidene) malononitrile (**2b**), 2-(4-chlorobenzy-lidene)malononitrile (**2c**), and 2-(4-bromobenzylide-ne)malononitrile (**2d**) promoted the corresponding reduced products in the C–C double bond with excellent yields (98, 95 and 93%, respectively). The reactions were carried out for 6 days of the inoculation with whole cells of fungus marine-derived *P. citrinum* CBMAI 1186 (Entries 2–4, Table 2). However, the compound **2e**, which also has an electron withdrawing substituent ($-NO_2$) followed a completely different catalytic pathway as its biotransformation led to yielding a mixture of products (Scheme 2). At the end of the biotransformation it was