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Evaluation of acetophenone monooxygenase and alcohol dehydrogenase activities in different fungal strains by biotransformation of acetophenone derivatives

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Abstract

Experimental conditions using whole cells to select fungal strains for specific bioreduction of ketones and formation of Baeyer–Villiger oxidation products were studied. *Epicoccum nigrum* SSP 1498 was effective in the bioreduction leading to the chiral alcohols in up to 98% enantiomeric excess. High acetophenone monooxygenase activity was observed by the use of the fungus *Emericella nidulans* CCT 3119 as biocatalyst.

Keywords: Biotransformation; Baeyer–Villiger oxidation; Bioreduction; Fungus; Ketones; Phenols; Monooxygenase; Dehydrogenase

1. Introduction

In recent years, the use of biological systems in the synthesis of several compounds, such as pharmaceutical intermediates and commodity chemicals have attracted much attention [1]. The current interest in applying biocatalysis in organic synthesis is mainly related to the preparation of optically active compounds with high stereoselectivity under environmentally friendly conditions [2]. The biocatalytic transformations can be carried out by means of many classes of enzymes, which are present in bacteria, fungi and yeasts [2,3]. These enzymes can be employed in a purified form, in cell extracts or using a living organism. According to the required chemical reaction and to the scale of the process, the enzymatic systems should be chosen taking into account the cost and efficiency of the whole process. In several cases, the substrates used for biocatalytic reactions are artificial compounds. Therefore, the generalization of a specific enzymatic reaction for a wide range of substrates is still hard to predict. In this way, the discovery of novel enzymes with the desired activity and specificity by screening is constantly needed [4].

In preliminary studies, we succeeded in the preparation of chiral alcohols with high enantioselectivity by bioreduction of ketones or alcohol deracemization [5]. The sources of the enzymatic systems employed in these studies were *Daucus carrota* root [5a], whole fungal [5b–d] and bacterial cells [5e]. In the biotransformation using whole fungal cells it was obtained the chiral alcohols plus phenols as side-products formed through a competitive Baeyer–Villiger reaction [5b,c]. These results suggest that the fungi employed in these biotransformations present promising enzymatic systems for bioreduction and Baeyer–Villiger reaction of aromatic ketones. This last reaction makes use of acetophenone monooxygenases [6], which are responsible for microbial degradation of acetophenones. Recently, some reports were focused on the study of aromatic monooxygenases, including their application in Baeyer–Villiger reactions [7].

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In this work, we selected acetophenone (**1a**), a well-known pro-chiral ketone employed to detect acetophenone monooxygenase and alcohol dehydrogenase activities [6,8], to test fungal strains isolated from environments scarcely explored for chemical purposes, such as bioreduction and Baeyer–Villiger reaction of aromatic ketones (Scheme 1). The most promising strains would be used to study the chemo- and stereoselectivity of the mentioned reactions towards substituted acetophenones with different electronic demands.

2. Experimental

2.1. General methods

Acetophenone, 4′-methoxyacetophenone, 4′-methylacetophenone, 4′-chloroacetophenone, phenol, 4′-methoxyphenol, 4′-methylphenol and 4′-chlorophenol are commercially available and were used without further purification. Thin-layer chromatography (TLC) was performed using precoated plates (Aluminum foil, silica gel 60 F_{254} Merck, 0.25 mm). Merck 60 silica gel (230–400 mesh) was used for flash chromatography. GC analyses were performed in a Shimadzu GC-17A instrument with a FID detector, using hydrogen as a carrier gas (100 kPa). Mass spectra were recorded on a Shimadzu GCMS P5050A (70 eV) spectrometer. The fused silica capillary columns used were a J&W Scientific DB-5 (30 m × 0.25 mm) and a chiral column Chirasil–Dex CB β-cyclodextrin (25 m × 0.25 mm) for determination of the enantiomeric excesses. Optical rotations were determined on a JASCO DIP-378 polarimeter.

2.2. Microorganisms library

The microorganisms *Epicoccum nigrum* SSP 1948, *Penicillium decumbens* SSP 1944, *Curvularia lunata* SSP 117, *Geotrichum candidum* SSP 1170, *Pestalotiopsis* sp. SSP 1946, *Aspergillus niger* SSP 1078 and *Aspergillus terreus* SSP 1498 were obtained from the Fungal Culture Collection of the Botanical Institute of São Paulo (São Paulo, Brazil) [9]. *Aspergillus foetidus* CCT 2683, *Emericella nidulans* CCT 3119 and *Rhizopus oryzae* CCT 4964 were obtained from the Culture Collection of the André Tosello Foundation [10].

2.3. Preservation of the microorganisms

The microorganisms were conserved as suspension in distilled water. All the strains were stored at 4 °C in our laboratory.

2.4. Culture media

The following culture media were selected after a growing test for all microorganisms selected. Composition for the culture medium: [A] = potato extract 4 g/L, glucose 20 g/L–BDA (Oxoid); [B] = dextrose 20 g/L (Oxoid), peptone 10 g/L (Oxoid); [C] = glucose 20 g/L, yeast extract 20 g/L (Oxoid), peptone 5 g/L (Oxoid), KH_2PO_4 1 g/L, K_2HPO_4 2 g/L, $NaNO_3$ 2 g/L, KCl 0.5 g/L, $MgSO_4 \cdot 7H_2O$ 0.5 g/L, $FeSO_4 \cdot 7H_2O$ 0.02 g/L; [D] = yeast extract 4 g/L (Oxoid), starch 15 g/L, K_2HPO_4 1 g/L, $MgSO_4 \cdot 7H_2O$ 0.5 g/L.

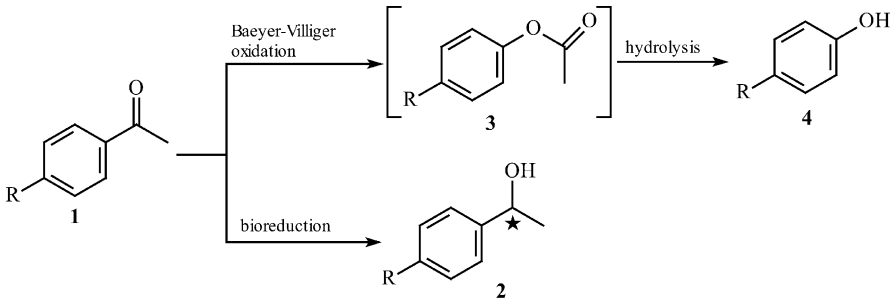
2.5. Synthesis of the chemical standards for analysis correlation by gas chromatography

2.5.1. Synthesis of racemic alcohols **2a–d**

The preparation of the alcohols **2a–d** was carried out by reduction of the corresponding acetophenones with sodium borohydride in ethanol [5c].

2.5.2. Synthesis of acetates **3a–d**

To a solution of the appropriate alcohols (**2a–d**) (0.40 mmol) in CH_2Cl_2 (1 mL), acetic anhydride (0.2 mL) and pyridine (0.2 mL) were added. The reaction mixture was stirred overnight at room temperature. After this period, the reaction mixture was worked-up by addition of saturated aqueous solution of NH_4Cl (4 mL) followed by extraction with ethyl acetate (2 × 15 mL). The organic phases were combined and dried over $MgSO_4$. The solvent was removed in vacuum giving an oil, which was used without further purification as a standard for GC analyses. The acetates (**3a–d**) were identified by GC–MS analyses. The structures were confirmed by comparison with a Mass Spectral Database (CLASS-5000/Wiley).



R = H (**1a-4a**); Cl (**1b-4b**); Me (**1c-4c**); MeO (**1d-4d**)

Scheme 1.

2.6. General procedures for the culture of the microorganisms

(a) The fungi *A. terreus* SSP 1498, *A. niger* SSP 1078, *A. foetidus* CCT 2683, *E. nidulans* CCT 3119 and *R. oryzae* CCT 4964 from slants were cultivated in Erlenmeyer flasks (250 mL) containing 100 mL of the appropriate culture medium at 32 °C in an orbital shaker (160 rpm) for 96 h.
(b) The fungi *E. nigrum* SSP 1948, *P. decumbens* SSP 1944, *C. lunata* SSP 117, *G. candidum* SSP 1170 and *Pestalotiopsis* sp. SSP 1946 from culture plate were cultivated in Erlenmeyer flasks (250 mL) containing 100 mL of the appropriate culture medium at 26 °C in an orbital shaker (120 rpm) for 192 h. Sterile materials were used to perform the experiments and the microorganisms were manipulated in a laminar flow cabinet.

2.7. General procedures for biotransformation

2.7.1. Biotransformation procedure for the evaluation of the enzymatic activities: acetophenone monooxygenase and alcohol dehydrogenase

Erlenmeyer flasks (250 mL) containing 100 mL of the appropriate culture medium (Section 2.4) were inoculated with spores or a disk (5 mm × 5 mm) from a culture plate of the microorganism. Growth was carried out in an orbital shaker [*t* (h), *r* (rpm), *T* (°C), see Section 2.6]. After the incubation time, the appropriate ketones (**1a**–**d**) (liquid compounds: 20 μL or solid compounds: 20 mg in 0.2 mL of ethanol) were added to the flasks and maintained under stirring in an orbital shaker [*r* (rpm), *T* (°C), see Section 2.6] for four days. The progress of the biotransformation was monitored by GC analysis.

2.7.2. Biotransformation procedure for the evaluation of the influence of the substrate concentration on the acetophenone monooxygenase activity of E. nidulans growing cells

The transformation was carried out as mentioned above with few modifications. An additional amount of a phosphate buffer solution (50 mL, pH 7.0, 0.1 M) was added to the culture after the incubation time (96 h). Then an appropriate solution of **1d** (20–400 mg; 20 mg/0.15 mL of ethanol) was added to the biocatalytic system.

2.8. GC analysis for determination of the enzymatic activity: acetophenone monooxygenase and alcohol dehydrogenase

The reaction progress was monitored by collecting 2 mL samples after four days of biotransformation (Tables 1 and 2). These samples were extracted by stirring with ethyl acetate (2 mL) followed by centrifugation (6000 rpm, 5 min). The organic phase (1 μL) was analyzed by GC in a chiral capillary column. The products of the biocatalyzed reactions were compared with racemic mixtures and standard samples previously obtained from chemical reactions (see Section 2.5).

GC conditions (carrier gas—H_2, 100 kPa)—injector 220 °C; detector 220 °C; 110 °C, 3 °C/min up to 180 °C; t_R (min): chiral compounds **2a**–**d**: retention time of (*R,S*)-phenyl-ethanol (**2a**): *R*-enantiomer 4.48 min, *S*-enantiomer 4.75 min; (*R,S*)-1-(4-chloro-phenyl)-ethanol (**2b**): *R*-enantiomer 9.64 min, *S*-enantiomer 10.32 min; (*R,S*)-1-(4-methyl-phenyl)-ethanol (**2c**): *R*-enantiomer 5.61 min, *S*-enantiomer 6.07 min; and (*R,S*)-1-(4-methoxy-phenyl)-ethanol (**2d**): *R*-enantiomer 9.57 min, *S*-enantiomer 9.93 min. Compounds **3a**–**d**: retention time of phenyl acetate (**3a**) 2.12 min, 4-chlorophenyl acetate (**3b**) 4.64 min, 4-methylphenyl acetate (**3c**) 3.04 min and 4-methoxyphenyl acetate (**3d**) 5.95 min. Compounds **4a**–**d**: retention time of phenol (**4a**) 4.38 min, 4-chlorophenol (**4b**) 10.45 min, 4-methylphenol (**4c**) 5.26 min and 4-methoxyphenol (**4d**) 10.45 min.

2.9. Assignment of the absolute configurations for the alcohols **2a**–**d**

The absolute configurations were attributed by chiral GC correlation with standards (*S*)-phenyl-ethanol (**2a**), (*S*)-1-(4-chloro-phenyl)-ethanol (**2b**), (*S*)-1-(4-methyl-phenyl)-ethanol (**2c**) and (*R*)-1-(4-methoxy-phenyl)-ethanol (**2d**) prepared by us as previously reported [5c].

3. Results and discussion

3.1. Biotransformation of **1a**

In order to evaluate the enzymatic systems of the microorganisms under study on the biotransformation of **1a**, we selected four culture media for their growth. After the growth of each fungal strain in all culture media selected, acetophenone (**1a**) was added to the cells suspensions. After four days of biotransformation, acetophenone monooxygenase and alcohol dehydrogenase activities were evaluate by GC analyses. As can be seen in Table 1, the most enantioselective strain for the bioreduction reaction was *E. nigrum* SSP 1948, which gave (*S*)-1-phenyl-ethanol (**2a**) with high conversion (99%) and enantiomeric excess (98%) (Table 1, Entry 11). The (*S*)-configuration of the alcohol is in accordance with Prelog's rule [11]. Growing *E. nigrum* SSP 1948 in all the culture media selected, the bioreduction of **1a** into **2a** gave the same (*S*)-enantiopreference. The biocatalytic reactions carried out by this last fungal strain using the culture media A and D for its growth showed lower stereoselectivity in the bioreduction of **1a** (Table 1, Entries 1 and 31). Concerning the Baeyer–Villiger reaction, *E. nigrum* SSP 1948 did not show acetophenone monooxygenase activity, except for the culture medium D (Table 1, Entry 31). In this last case, phenol (**4a**) was obtained in low concentration (2%). According to previously reports, we can suppose

Table 1
Effect of the culture media in the biotransformation of the acetophenone (**1a**): bioreduction vs. Baeyer–Villiger oxidation

Entry	Microorganism	Bioreduction, 1-phenyl-ethanol **(2a)**			Baeyer–Villiger oxidation, product **4a**
		Conv. (%)	e.e. (%)	Config.	Conv. (%)
Culture medium A					
1	*Epicoccum nigrum* SSP 1948	99	92	(*S*)	–
2	*Penicillium decumbens* SSP 1944	30	–	–	–
3	*Curvularia lunata* SSP 117	94	83	(*S*)	–
4	*Geotrichum candidum* SSP 1170	–	–	–	–
5	*Pestalotiopsis* sp. SSP 1946	36	37	(*S*)	–
6	*Aspergillus terreus* SSP 1498	7	50	(*S*)	∼3
7	*Aspergillus niger* SSP 1078	16	44	(*R*)	∼1
8	*Aspergillus foetidus* CCT 2683	45	72	(*R*)	∼2
9[a]	*Emericella nidulans* CCT 3119	–	–	–	–
10	*Rhizopus oryzae* CCT 4964	34	73	(*S*)	–
Culture medium B					
11	*Epicoccum nigrum* SSP 1948	99	98	(*S*)	–
12	*Penicillium decumbens* SSP 1944	12	17	(*S*)	–
13	*Curvularia lunata* SSP 117	48	51	(*S*)	–
14	*Geotrichum candidum* SSP 1170	–	–	–	–
15	*Pestalotiopsis* sp. SSP 1946	47	68	(*R*)	40
16	*Aspergillus terreus* SSP 1498	53	51	(*S*)	∼2
17	*Aspergillus niger* SSP 1078	9	76	(*R*)	8
18	*Aspergillus foetidus* CCT 2683	47	89	(*R*)	∼5
19	*Emericella nidulans* CCT 3119	17	58	(*R*)	31
20	*Rhizopus oryzae* CCT 4964	69	77	(*S*)	–
Culture medium C					
21	*Epicoccum nigrum* SSP 1948	99	98	(*S*)	–
22	*Penicillium decumbens* SSP 1944	31	51	(*R*)	–
23	*Curvularia lunata* SSP 117	53	∼6	(*R*)	–
24	*Geotrichum candidum* SSP 1170	–	–	–	–
25	*Pestalotiopsis* sp. SSP 1946	79	28	(*R*)	–
26[a]	*Aspergillus terreus* SSP 1498	–	–	–	–
27	*Aspergillus niger* SSP 1078	∼4	82	(*R*)	9
28	*Aspergillus foetidus* CCT 2683	37	60	(*R*)	8
29	*Emericella nidulans* CCT 3119	12	99	(*R*)	88
30	*Rhizopus oryzae* CCT 4964	62	88	(*S*)	–
Culture medium D					
31	*Epicoccum nigrum* SSP 1948	13	80	(*S*)	∼2
32	*Penicillium decumbens* SSP 1944	11	26	(*R*)	-
33	*Curvularia lunata* SSP 117	46	64	(*R*)	49
34	*Geotrichum candidum* SSP 1170	∼5	28	(*S*)	∼2
35	*Pestalotiopsis* sp. SSP 1946	62	14	(*R*)	∼3
36[a]	*Aspergillus terreus* SSP 1498	–	–	–	–
37	*Aspergillus niger* SSP 1078	∼5	66	(*R*)	11
38	*Aspergillus foetidus* CCT 2683	24	53	(*R*)	13
39[a]	*Emericella nidulans* CCT 3119	–	–	–	–
40	*Rhizopus oryzae* CCT 4964	72	71	(*S*)	–

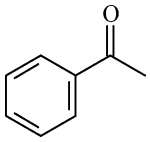
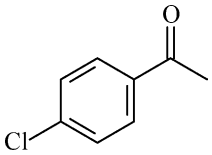
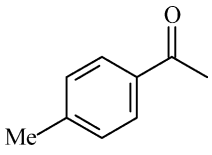
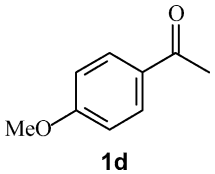
Conv.: conversion determined by GC analysis; e.e.: enantiomeric excess; Config.: absolute configuration. Compounds **3a**–**d** were not detected in any biotransformation.

[a] The acetophenone and the expected products were not detected.

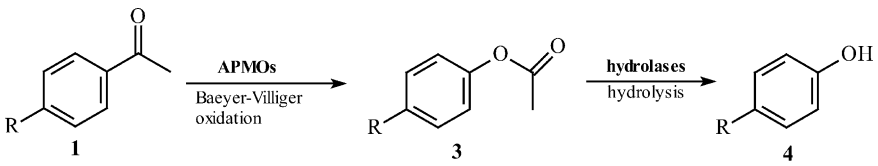
that **4a** was obtained after two sequential reactions [7]. Initially, the action of an acetophenone monooxygenase in the ketone **1a** leads to phenyl acetate (**3a**) followed by reaction with a hydrolase that transforms this compound into phenol (Scheme 2).

Besides *E. nigrum* SSP 1948 other microorganisms performed the reduction of **1a** with good enantioselectivity. Several strains showed enantiopreference for the alcohol with the *R*-configuration. The best *R*-enantioselectivity was observed by the use of *A. foetidus* CCT 2683 (Table 1, Entry 18). In this way depending on the configuration of the chiral alcohol required, we can use different microorganisms in order to obtain both enantiomers. For example, we can use the fungus *E. nigrum* SSP 1948 to prepare (*S*)-1-phenyl-ethanol and *A. foetidus* CCT 2683 to prepare (*R*)-1-phenyl-ethanol (Scheme 3).

Table 2
Biotransformation of substituted acetophenones **1a–d** by *Epicoccum nigrum* SSP 1948

Entry	Ketone	Bioreduction, product **2**		Baeyer–Villiger oxidation, product **4**
		Conv. (%)	e.e. (%)	Conv. (%)
1	 **1a**	98	98 (*S*)	–
2	 **1b**	97	60 (*S*)	–
3	 **1c**	97	92 (*S*)	–
4	 **1d**	63	91 (*S*)	–

Conv.: conversion determined by GC analysis; e.e.: enantiomeric excess; absolute configuration in parenthesis; compounds **3a–d** were not detected in any biotransformation.

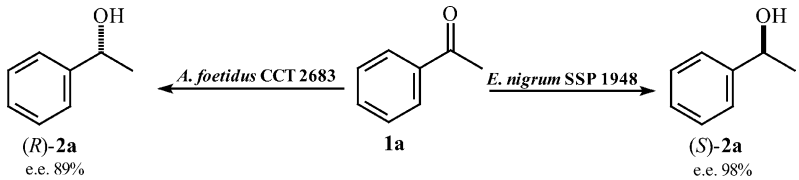


R = H (**1a-4a**); Cl (**1b-4b**); Me (**1c-4c**); MeO (**1d-4d**)
APMOs = acetophenone monooxygenases

Scheme 2.

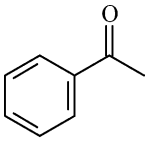
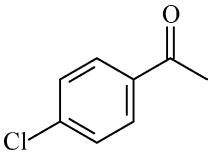
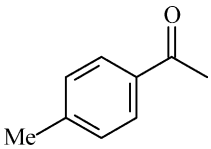
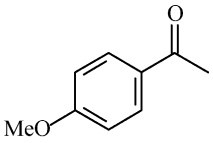
An interesting feature observed for the biotransformation performed by *Pestalotiopsis* sp. SSP 1946 was that by changing the culture medium both enantiomers of the chiral alcohol **2a** with low enantiomeric excess were obtained (Table 1, Entries 5 and 15). Besides the alcohol **2a** obtained by using the culture medium B for growth of this strain, we also observed a large amount of **4a** (40%) (Table 1, Entry 15).

Concerning the Baeyer–Villiger reaction, the strain with higher acetophenone monooxygenase activity was found to be *E. nidulans* CCT 3119. Growing this strain in culture medium C, the biotransformation of **1a** led to the alcohol **2a** (12%) and phenol (**4a**) (88%) (Table 1, Entry 29). The use of both culture media A and D for growing *E. nidulans* CCT 3119 led to complete biodegradation of **1a** (Table 1, Entries



Scheme 3.

Table 3
Biotransformation of substituted acetophenones **1a–d** by *Emericella nidulans* CCT 3119

Entry	Ketone	Bioreduction, product **2**		Baeyer–Villiger oxidation, product **4**
		Conv. (%)	e.e. (%)	Conv. (%)
1	 **1a**	16	99 (*R*)	70
2	 **1b**	90	95 (*R*)	4
3	 **1c**	25	98 (*R*)	75
4	 **1d**	–	–	97

Conv.: conversion determined by GC analysis; e.e.: enantiomeric excess; absolute configuration in parenthesis; compounds **3a–d** were not detected in any biotransformation.

29 and 39). Besides *E. nidulans* CCT 3119, *A. terreus* SSP 1498 showed the same biodegradation behavior by the use of media C and D (Table 1, Entries 26 and 36).

3.2. Biotransformation of acetophenones (1a–d)

In view of the results described above using **1a** as the substrate, we selected *E. nigrum* SSP 1948 (culture medium B) to study the bioreduction of substituted acetophenones (**1a–d**) (Table 2) and *E. nidulans* CCT 3119 (culture medium C) to study the Baeyer–Villiger reaction with the same substrates (Table 3).

As can be seen in Table 2, the products of a Baeyer–Villiger and hydrolysis reaction (**4a–d**) were not observed for all the biotransformations performed by growing cells of *E. nigrum* SSP 1948 and the ketones (**1a–d**). Concerning the enantiopreference all the ketones studied were reduced by this fungal strain to their corresponding (*S*)-alcohol with high conversion and stereoselectivity. Reduction of **1a** by this fungus showed the best enantioselectivity observed in this study (Table 2, Entry 1). Reduction of 4′-chloroacetophenone (**1b**) into its corresponding alcohol **2b** occurred in high conversion, but in lower selectivity (e.e. 60%) than the others acetophenone derivatives (Table 2, Entries 1–4).

In Table 3 (Entry 4), we can observe that 4′-methoxyacetophenone (**1d**) was the best substrate for the Baeyer–Villiger reaction with growing cells of *E. nidulans* CCT 3119. The experimental results shown in Table 3 suggest the following reactivity sequence for the substrates **1a–d** towards Baeyer–Villiger reaction with *E. nidulans* CCT 3119: *para*-methoxy > *para*-methyl > *para*-hydrogen > *para*-chloro. This reactivity sequence can be attributed to the electron donating groups at the *para* position of the aromatic ring [7b]. In addition to the Baeyer–Villiger reaction, we also observed the bioreduction of all the ketones employed except **1d**, which suffered preferentially the Baeyer–Villiger reaction (Table 3, Entry 4). The (*R*)-alcohols **2a–c** were formed in this study (Table 3, Entries 1–3).

In view of the good results of the Baeyer–Villiger reaction by *E. nidulans* CCT 3119 with **1d**, we investigated this biotransformation at different substrate concentration (Fig. 1). The tolerance of the fungus for substrate **1d** was observed to occur up to 100 mg for 100 mL suspension of growing cells. The best concentration of **1d** for this reaction was found to be 20 mg/100 mL. Phenol **4d** was produced at a good rate using up to 80 mg/100 mL of **1d** concentration. Besides the Baeyer–Villiger reaction, we also observed the formation of 1-(4-methoxy-phenyl)-ethanol (**2d**) using the ketone precursor **1d** in concentration of 60–100 mg/100 mL. This competitive reaction can be occurring due to a possible enzymatic inhibition of acetophenone monooxygenase by increasing **1d** concentration. The above results show that the best **1d**

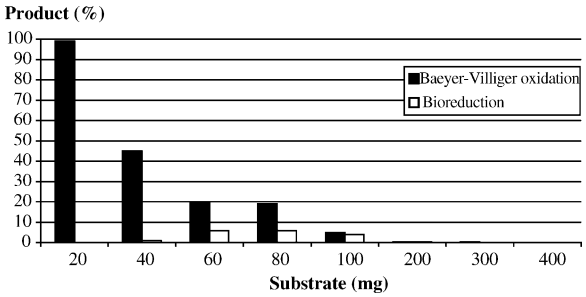


Fig. 1. Influence of the substrate concentration in the biotransformation of the 4′-methoxyacetophenone (**1d**) using *E. nidulans* CCT 3119 growing cells. Baeyer–Villiger oxidation product, 4-methoxyphenol (**4d**); bioreduction product, (*R*)-1-(4-methoxy-phenyl)-ethanol (**2d**).

concentration for the acetophenone monooxygenase would be between 20 and 40 mg/100 mL suspension of *E. nidulans* CCT 3119 growing cells.

4. Conclusion

In summary, experimental conditions using whole cells to select fungal strains for specific bioreduction reaction of acetophenones and formation of Baeyer–Villiger oxidation products were studied. *E. nigrum* SSP 1948 showed good results in the bioreduction reaction, leading to the chiral alcohols in up to 98% enantiomeric excess. (*S*)- and (*R*)-alcohols were prepared by reduction of the corresponding ketones using different fungal strains. High acetophenone monooxygenase activity was observed with the fungus *E. nidulans* CCT 3119. These results provide useful information for further investigations aiming to obtain purified enzyme systems from these fungi.

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References

[1] (a) A. Schmid, J.S. Dordick, B. Hauer, A. Kiener, M. Wubbolts, B. Witholt, Nature 409 (2001) 258–268;
(b) S.G. Burton, D.A. Cowan, J.M. Woodley, Nat. Biotechnol. 20 (2002) 37–45;
(c) J.D. Rozzell, Biorg. Med. Chem. 7 (1999) 2253–2261;
(d) H.E. Schoemaker, D. Mink, M.G. Wubbolts, Science 299 (2003) 1694–1697.

[2] (a) K. Faber, Biotransformations in Organic Chemistry, fourth ed., Springer, Berlin, 2000;
(b) A. Liese, K. Seelbach, C. Wandrey, Industrial Biotransformations, Wiley-VCH, Verlag GmbH, Weinheim, 2000.

[3] B.G. Davis, V. Boyer, Nat. Prod. Rep. 18 (2001) 618–640.

[4] For recent examples, see (a) M.J. Homann, R.B. Vail, E. Previte, M. Tamarez, B. Morgan, D.R. Dodds, A. Zaks, Tetrahedron 60 (2004) 789–797;
(b) B.-Y. Hwang, B.-G. Kim, Enzyme Microb. Technol. 34 (2004) 429–436;
(c) D. Wahler, J.-L. Reymond, Curr. Opin. Biotechnol. 12 (2001) 535–544;
(d) F. Cardenas, E. Alvarez, M.-S. de Castro-Alvarez, J.-M. Sanchez-Montero, M. Valmaseda, S.W. Elson, J.-V. Sinisterra, J. Mol. Catal. B: Enzym. 14 (2001) 111–123;
(e) M.T. Reetz, K.M. Kühling, S. Wilensek, H. Husmann, U.W. Häusig, M. Hermes, Catal. Today 67 (2001) 389–396.

[5] (a) J.V. Comasseto, A.T. Omori, A.L.M. Porto, L.H. Andrade, Tetrahedron Lett. 45 (2004) 473–476;
(b) J.V. Comasseto, A.T. Omori, L.H. Andrade, A.L.M. Porto, Tetrahedron: Asymmetry 14 (2003) 711–715;
(c) J.V. Comasseto, L.H. Andrade, A.T. Omori, L.F. Assis, A.L.M. Porto, J. Mol. Catal. B: Enzym. 29 (2004) 55–61;
(d) L.H. Andrade, A.T. Omori, A.L.M. Porto, J.V. Comasseto, J. Mol. Catal. B: Enzym. 29 (2004) 47–54;
(e) L.H. Andrade, J.V. Comasseto, D.F. Rodrigues, V.H. Pellizari, A.L.M. Porto, unpublished results.

[6] For recent reviews, see (a) N.M. Kamerbeek, D.B. Janssen, W.J.H. Van Berkel, M.W. Fraaije, Adv. Synth. Catal. 345 (2003) 667–678;
(b) M.D. Mihovilovic, B. Müller, P. Stanetty, Eur. J. Org. Chem. (2002) 3711–3730.

[7] (a) A. Tanner, D. Hopper, J. Bacteriol. 128 (2000) 6565–6569;
(b) N.M. Kamerbeek, M.J.H. Moonen, J.G.M. van der Vem, W.J.H. van Berkel, M.W. Fraaije, D.B. Janssen, Eur. J. Biochem. 268 (2001) 2547–2557;
(c) M.J.H. Moonen, I.M.C.M. Rietjens, W.J.H. van Berkel, J. Ind. Microbiol. Biot. 26 (2001) 35–42;
(d) N.M. Kamerbeek, A.J.J. Olsthoorn, M.W. Fraaije, D.B. Janssen, Appl. Environ. Microbiol. 69 (2003) 419–426;
(e) N.M. Kamerbeek, M.W. Fraaije, D.B. Janssen, Eur. J. Biochem. 271 (2004) 2107–2116;
(f) M.W. Fraaije, N.M. Kamerbeek, A.J. Heidekamp, R. Fortin, D.B. Janssen, J. Biol. Chem. 279 (2004) 3354–3360.

[8] K. Nakamura, R. Yamanaka, T. Matsuda, T. Harada, Tetrahedron: Asymmetry 14 (2003) 2659–2681.

[9] Fungal Culture Collection of the Botanical Institute of São Paulo—SSP—Seção de Micologia e Liquenologia, Av. Miguel Estéfano no. 3687, CE 01061-970, São Paulo, SP, Brazil.

[10] André Tosello Fundation, Rua Latino Coelho 1301, CE 13087-010, http://www.fat.org.br/, Campinas, SP, Brazil. CCT—Coleção de Cultura Tropical.

[11] V. Prelog, Pure Appl. Chem. 9 (1964) 119–130.