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Journal of Molecular Catalysis B: Enzymatic 39 (2006) 24–30
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
www.elsevier.com/locate/molcatb

Biotransformations of *ortho*-, *meta*- and *para*-aromatic nitrocompounds by strains of *Aspergillus terreus*: Reduction of ketones and deracemization of alcohols

João V. Comasseto [a], Leonardo F. Assis [a], Leandro H. Andrade [a], Iracema H. Schoenlein-Crusius [b], André L.M. Porto [a,*]

[a] *Laboratório de Química Fina e Biocatálise-Instituto de Química, Universidade de São Paulo, Av. Prof. Lineu Prestes 748, CEP 05508-900, São Paulo, SP, Brazil*
[b] *Instituto de Botânica, Seção de Micologia e Liquenologia, Av. Miguel Estéfano, CEP 01061-970, São Paulo, SP, Brazil*

Available online 23 March 2006

Abstract

Seven strains of the fungus *Aspergillus terreus* isolated from several provenances in Brazil, catalyzed biotransformations of *ortho*-, *meta*- and *para*-nitrophenyl compounds at different pH values. *ortho*-Nitroacetophenone and *meta*-nitroacetophenone were transformed into (*S*)-(+)-1-(*ortho*-nitrophenyl)ethanol and (*S*)-(−)-1-(*meta*-nitrophenyl)ethanol with high enantiomeric excess (e.e. ≥98%) and conversion (≥98%) by all the strains used. Deracemization of (*RS*)-1-(*meta*-nitrophenyl)ethanol was obtained with high selectivity (e.e. up to ≥98%) and good conversion (*c* 98%). The biotransformations in acidic medium using these fungus strains were more efficient than under basic or neutral conditions.

Keywords: *Aspergillus terreus*; Deracemization; Reduction; Biotransformation; Nitrocompounds

1. Introduction

Nitrocompounds are important building blocks in academic organic synthesis as well as in the pharmaceutical industry [1,2]. In addition, this class of compounds is precursor of the corresponding amines by reduction [3–5]. In view of the importance of the chiral technology in the production of pharmaceuticals [6], it is highly desirable to develop practical and clean methods to access enantiomerically pure molecules containing the nitro group. Chirality has been conveniently introduced into substrates containing this functionality through biocatalytic transformations. As examples, baker's yeast (*Saccharomyces cerevisiae*) was used to reduce acyl groups [7–9], olefins [10] and keto esters [11] in compounds bearing the nitro group, and the enantioselective hydrolysis of nitro substituted phenoxypropylene oxides by *Trichosporon loubierii*—ECU 1040, was the subject of a recent study [12].

On the other hand, asymmetric biocatalytic reduction of ketones [13–16] and deracemization of alcohols [17,18] are important processes in stereoselective synthesis. This fact led us to use the almost unexplored microorganisms native from Brazilian biomes to perform reduction and deracemization of substituted acetophenones [19–21].

The importance of the organic nitrocompounds mentioned before led us to extend this study to the reduction of nitroacetophenones **1**–**3** and the deracemization of (*RS*)-(nitrophenyl)ethanols **1a**–**3a** using seven strains of the fungus *Aspergillus terreus* native from Brazilian biomes and the fungus *A. terreus* SSP 1498 native from USA. As the pH of the reaction medium can influence the course of the biocatalytic transformation, the study was performed at different pH values (Schemes 1 and 2).

2. Experimental

2.1. General methods

Chemical syntheses were monitored by TLC analyses on precoated silica gel foils (aluminum foil, 60 F_{254} Merck). Spots were visualized by *p*-anisaldehyde/sulfuric acid or vanillin followed by heating at about 120 °C. The purification of the compounds was carried out by column chromatography using silica

* Corresponding author. Tel.: +55 11 3091 2287; fax: +55 11 3815 5579.
E-mail address: almporto@iq.usp.br (A.L.M. Porto).

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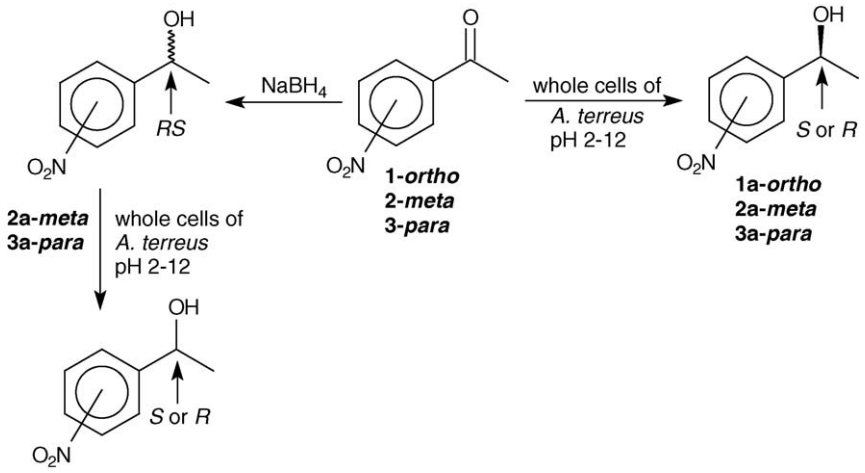
doi:10.1016/j.molcatb.2006.01.022

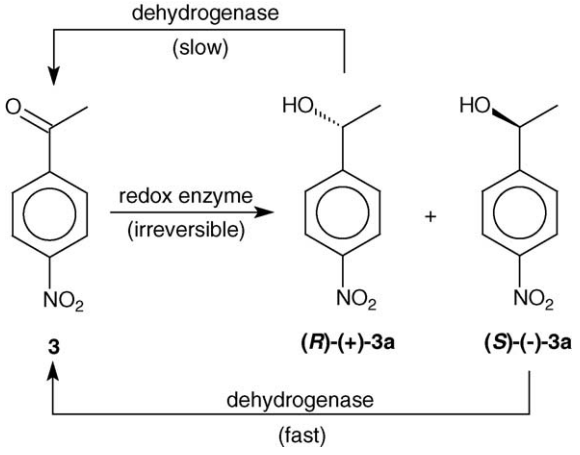


Scheme 1. Reduction of nitroacetophenones and deracemization of (*RS*)-1-(nitrophenyl)ethanols with *A. terreus* strains at different pH values.

gel (230–400 mesh). Conversions and enantiomeric excess of the enzyme catalyzed reactions were determined using a Shimadzu GC-17A gas chromatograph equipped with a chiral capillary column Chirasil-Dex CB β-cyclodextrin (25 m × 0.25 mm). The carrier gas was hydrogen with pressure of 100 kPa. The injection temperature was 220 °C. GC–MS analyses were performed in a Shimadzu equipment (QP 5050A) equipped with a capillary column DB-5 (J & W Scientific 30 m × 0.25 mm × 0.25 μm) and the carrier gas was helium. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker DPX300 (^{1}H: 300.1 MHz; ^{13}C: 75.5 MHz) or AC-200 (^{1}H: 200 MHz; ^{13}C: 50 MHz) spectrometers. Near IR spectra were obtained with a Bomen MB-100 spectrometer. Orbital shakers Tecnal TE-421 or Superohm G-25 were employed for the biocatalyzed transformations.

2.2. Synthesis of the racemic alcohols

The racemic alcohols (**1a**–**3a**) were obtained by reduction of the corresponding ketones (**1**–**3**) (500 mg) with sodium borohydride in methanol (10 mL) [19]. The ^{1}H and ^{13}C NMR spectra of these compounds were in agreement with those reported in literature [7] and the purity of the prepared compounds was determined by GC and GC–MS.



Scheme 2. Deracemization process of (*RS*)-**3a** by *A. terreus* CCT 3320 with enrichment of the mixture in (*R*)-(+)-**3a**.

2.3. Culture collection [22,23]

The following strains were isolated in Brazil:

A. terreus CCT 3320 (isolated by Attili, D.S., 09/93, from the soil of the Atlantic rain forest in Peruíbe, SP).
A. terreus CCT 4083 (isolated by Pfenning, L., 08/94, from pasture soil in Belém do Pará).
A. terreus CCT 5179 (isolated by Pfenning, L., 07/96, from commercial filter paper in Campinas, SP).
A. terreus URM 3371 (isolated in 1993 from rhizosphere soil of *Vernonia herbaceae* in São Paulo, SP).
A. terreus URM 3422 (isolated in 1994 from commercial oatmeal in the Brazilian northeast region).
A. terreus URM 3571 (isolated in 1996 from reservoir waters in the Brazilian northeast region).
A. terreus URM 4602 (isolated in 2003 from mangrove soil of the River Paripe, Ilha de Itamaracá in Pernambuco).

The strain *A. terreus* SSP 1498 was isolated in USA (historic culture, the strain was probably obtained from the soil, by Dr. Enrique Duprat and freeze-dried in 1943, in the Northern Regional Research Laboratories in Illinois, USA; it is part of the collection of the São Paulo Botanical Institute).

2.4. Growth conditions for the microorganisms cultures

The fungi were grown in culture shaker-flasks (170 rpm, 1000 mL, 2000 mL Erlenmeyer) in 500 or 1000 mL of Oxoid malt extract medium (20 g/L, 72–96 h) at 32 °C. The cells were harvested by filtration under vacuum. Sterile material was used to perform the experiments and the microorganisms were manipulated in a laminar flow cabinet (Veco).

Table 1
Bioreduction of *ortho*-, *meta*- and *para*-nitroacetophenones (**1–3**) with whole cells of *Aspergillus terreus*[a,b]

Entry	pH	*A. terreus* SSP 1498						*A. terreus* CCT 3320					
		o-NO_2-**1**		*m*-NO_2-**2**		*p*-NO_2-**3**		*o*-NO_2-**1**		*m*-NO_2-**2**		*p*-NO_2-**3**	
		c **1a**	e.e. **1a**	*c* **2a**	e.e. **2a**	*c* **3a**	e.e. **3a**	*c* **1a**	e.e. **1a**	*c* **2a**	e.e. **2a**	*c* **3a**	e.e. **3a**
1	2	75[c]	≥98 (*S*)	88	49 (*S*)	98	38 (*R*)	87[d]	≥98 (*S*)	96[d]	34 (*S*)	98	31 (*R*)
2	4	85	≥98 (*S*)	98	86 (*S*)	98	54 (*R*)	95	≥98 (*S*)	98	35 (*S*)	98	46 (*R*)
3	6	92	≥98 (*S*)	98	51 (*S*)	98	59 (*R*)	98	≥98 (*S*)	87	47 (*S*)	98	41 (*R*)
4	7	81	≥98 (*S*)	98	65 (*S*)	98	57 (*R*)	98	≥98 (*S*)	96	34 (*S*)	98	49 (*R*)
5	8	90	≥98 (*S*)	98	46 (*S*)	98	52 (*R*)	98	≥98 (*S*)	92	34 (*S*)	98	41 (*R*)
6	10	88	≥98 (*S*)	38	6 (*S*)	55	50 (*R*)	98	≥98 (*S*)	90	32(*S*)	98	51 (*R*)
7	12	87	≥98 (*S*)	10	10 (*S*)	13	51 (*R*)	98	≥98 (*S*)	57	49 (*S*)	98	35 (*R*)
		A. terreus URM 3371						*A. terreus* URM 3422					
8	2	98[d]	≥98 (*S*)	98	82 (*S*)	98	15 (*S*)	81[d]	≥98 (*S*)	95[d]	97 (*S*)	94[d]	25 (*R*)
9	4	98	≥98 (*S*)	98	69 (*S*)	98	6 (*S*)	98	≥98 (*S*)	95	97 (*S*)	97	27 (*R*)
10	6	97	≥98 (*S*)	98	76 (*S*)	98	10 (*S*)	91	≥98 (*S*)	95	93 (*S*)	98	59 (*R*)
11	7	96	≥98 (*S*)	98	59 (*S*)	98	10 (*S*)	88	≥98 (*S*)	95	97 (*S*)	98	40 (*R*)
12	8	96	≥98 (*S*)	98	57 (*S*)	98	10(*S*)	72	≥98 (*S*)	95	97 (*S*)	98	21 (*R*)
13	10	98	≥98 (*S*)	96	57 (*S*)	86	4 (*R*)	40	≥98 (*S*)	74	90 (*S*)	98	7 (*R*)
14	12	88	≥98 (*S*)	90	41 (*S*)	40	40 (*R*)	23	≥98 (*S*)	85	40 (*S*)	98	7 (*R*)
		A. terreus URM 3571						*A. terreus* CCT 4083					
15	2	87[d]	≥98 (*S*)	92	98 (*S*)	98	4 (*S*)	98[d]	≥98 (*S*)	98[d]	92 (*S*)	72	24 (*R*)
16	4	97	≥98 (*S*)	93	98 (*S*)	98	11 (*S*)	98	≥98 (*S*)	98	≥98(*S*)	90	49 (*R*)
17	6	87	≥98 (*S*)	95	98 (*S*)	98	12 (*R*)	98	≥98 (*S*)	98	≥98(*S*)	72	53 (*R*)
18	7	84	≥98 (*S*)	95	98 (*S*)	98	8 (*R*)	98	≥98 (*S*)	98	≥98(*S*)	93	52 (*R*)
19	8	86	≥98 (*S*)	95	98 (*S*)	98	2 (*R*)	98	≥98 (*S*)	98	≥98(*S*)	91	48 (*R*)
20	10	50	≥98 (*S*)	39	98 (*S*)	23	8 (*R*)	98	≥98 (*S*)	80	83 (*S*)	94	51 (*R*)
21	12	50	≥98 (*S*)	14	98 (*S*)	6	23 (*R*)	98	≥98 (*S*)	80	71 (*S*)	32	11 (*R*)
		A. terreus URM 4602						*A. terreus* CCT 5179					
22	2	98	≥98 (*S*)	98[d]	97 (*S*)	95	7 (*S*)	98[c]	≥98 (*S*)	98[d]	62 (*S*)	98	19 (*R*)
23	4	98	≥98 (*S*)	98	≥98 (*S*)	98	24 (*R*)	98	≥98 (*S*)	98	58 (*S*)	98	34 (*R*)
24	6	98	≥98 (*S*)	97	≥98 (*S*)	98	30 (*R*)	98	≥98 (*S*)	98	59 (*S*)	98	20 (*R*)
25	7	98	≥98 (*S*)	96	≥98 (*S*)	98	33 (*R*)	98	≥98 (*S*)	98	56 (*S*)	98	28 (*R*)
26	8	98	≥98 (*S*)	96	≥98 (*S*)	98	45 (*R*)	98	≥98 (*S*)	98	62 (*S*)	98	25 (*R*)
27	10	79	≥98 (*S*)	97	95 (*S*)	76	19 (*R*)	98	≥98 (*S*)	93	62 (*S*)	98	18 (*R*)
28	12	56	≥98 (*S*)	87	93 (*S*)	70	4 (*R*)	98	≥98 (*S*)	61	67 (*S*)	79	9 (*R*)

c (%): conversion determined by GC analysis; e.e. (%): enantiomeric excess.
[a] The *ortho*-, *meta*- and *para*-aminoacetophenones [7,26] were detected in 2–10% by GC–MS.
[b] Analysis at 24 h.
[c] Analysis at 48 h.
[d] Analysis at 72 h.

2.5. Standard procedure for small scale deracemization and bioreduction reactions

The appropriate substrate (acetophenones **1–3** or alcohols **1a–3a**, 5 μL or 5 mg) was added to a phosphate buffer solution (50 mL) containing a suspension of washed wet cells of *A. terreus* (1.5–2.0 g) in Erlenmeyer flasks (125 mL). The mixtures were stirred on a rotary shaker (32 °C, 170 rpm) for the time indicated in Tables 1–3. The buffer solutions were prepared by the method described in literature [24].

Table 2
Deracemization of (*RS*)-1-(*para*-nitrophenyl)ethanol (**3a**) with whole cells of *Aspergillus terreus* CCT 3320

Entry	pH	*t*	*c** **3**	*c* **3a**	e.e. **3a**	*t*	*c** **3**	*c* **3a**	e.e. **3a**
1	2	72	6	94	91 (*R*)	120	5	90	88 (*R*)
2	4	72	5	95	92 (*R*)	120	–	100	99 (*R*)
3	6	72	5	95	83 (*R*)	120	62	38	72 (*R*)
4	7	72	56	44	51 (*R*)	120	100	–	–
5	8	72	23	77	49 (*R*)	120	79	–	–
6	10	72	61	29	16 (*R*)	120	79	21	28 (*R*)
7	12	72	1	100	5 (*R*)	120	4	96	7 (*R*)

t: time (h); *c* (%): conversion in alcohol; *c** (%): conversion in ketone; e.e. (%): enantiomeric excess.

Table 3
Deracemization of (*RS*)-1-(*meta*-nitrophenyl)ethanol **2a** and (*R*S)-1-(*para*-nitrophenyl)ethanols **3a** with whole cells of *Aspergillus terreus* at pH 4

Entry	*t*	*A. terreus* SSP 1498						*A. terreus* CCT 3320					
		(*RS*)-1-(*meta*-nitrophenyl)ethanol **2a**			(*RS*)-1-(*para*-nitrophenyl)ethanol **3a**			(*RS*)-1-(*meta*-nitrophenyl)ethanol **2a**			(*RS*)-1-(*para*-nitrophenyl)ethanol **3a**		
		c^* **2**	*c* **2a**	e.e. **2a**	c^* **3**	*c* **3a**	e.e. **3a**	c^* **2**	*c* **2a**	e.e. **2a**	c^* **3**	*c* **3a**	e.e. **3a**
1	24	12	62	77 (*S*)	22	78	13 (*R*)	2	98	26 (*S*)	–	100	5 (*R*)
2	72	10	77	98 (*S*)	–	98	21 (*R*)	2	98	47 (*S*)	1	96	94 (*R*)
3	168	2	90	98 (*S*)	38	60	46 (*R*)	2	98	56 (*S*)	1	97	>99 (*R*)
4	240	–	90	98 (*S*)	10	70	12 (*S*)	2	98	61 (*S*)	15[a]	51	>99 (*R*)
5	320	–	96	≥98 (*S*)	15	74	41 (*S*)	–	90	98 (*S*)	–	23	>99 (*R*)
		A. terreus URM 3371						*A. terreus* URM 3422					
6	24	–	98	2 (*S*)	–	98	12 (*S*)	4	96	64 (*S*)	–	98	11 (*R*)
7	72	–	98	21 (*S*)	–	98	37 (*S*)	–	96	90 (*S*)	2	97	12 (*R*)
8	168	4	96	87 (*S*)	–	98	62 (*S*)	–	95	92 (*S*)	10	88	5 (*R*)
9	240	4	96	89 (*S*)	–	84	68 (*S*)	–	92	91 (*S*)	6	88	7 (*R*)
10	320	4	90	98 (*S*)	–	85	98 (*S*)	–	90	98 (*S*)	5	91	6 (*R*)
		A. terreus URM 3571						*A. terreus* CCT 4083					
11	24	7	93	14 (*S*)	–	98	–	–	100	31 (*S*)	5	95	23 (*R*)
12	72	2	95	36 (*S*)	–	98	5 (*S*)	2	98	78 (*S*)	7	93	44 (*R*)
13	168	2	96	81 (*S*)	–	98	11 (*S*)	3	97	91 (*S*)	15	80	44 (*R*)
14	240	4	90	63 (*S*)	–	98	25 (*S*)	4	96	91 (*S*)	–	–	–
15	320	1	95	98 (*S*)	–	98	41 (*S*)	4	92	97 (*S*)	–	–	–
		A. terreus URM 4602						*A. terreus* CCT 5179					
16	24	10	88	86 (*S*)	3	95	24 (*R*)	2	96	52 (*S*)	2	98	1 (*R*)
17	72	–	90	≥98 (*S*)	2	95	51 (*R*)	2	96	86 (*S*)	–	98	17 (*R*)
18	168	10	90	≥98 (*S*)	7	89	79 (*R*)	2	96	96 (*S*)	–	98	16 (*R*)
19	240	10	90	≥98 (*S*)	5	90	88 (*R*)	2	96	98 (*S*)	–	95	20 (*R*)
20	320	–	90	≥98 (*S*)	–	95	90 (*R*)	2	96	98 (*S*)	–	85	31 (*R*)

t: time (h); *c* (%): conversion in alcohol; c^* (%): conversion in ketone; e.e. (%): enantiomeric excess.
[a] *c* 5–15% *para*-aminoacetophenone [7,26].

2.6. Standard procedure for preparative scale bioreduction and deracemization reactions

The previously filtered wet cell cultures of *A. terreus* CCT 4083 (15 g) were suspended in a phosphate buffer solution (200 mL) in 500 mL Erlenmeyer flasks. The nitroacetophenones **1** (500 μL), **2** (100 mg) and **3** (100 mg) were added to the flasks. The mixtures were stirred on a rotary shaker (32 °C, 170 rpm) until the starting material was completely consumed.

After the appropriate conversion the mixture was filtered and the biomass was washed with ethyl acetate (4 × 100 mL). The organic phases were combined and dried over $MgSO_4$. The solvent was removed in vacuum and the residue was purified by silica gel column chromatography using a mixture of hexane and ethyl acetate (9:1) as eluent to afford compounds **1a**–**3a**:

Bioreduction: *A. terreus* CCT 4083: (*S*)-(+)-**1a** (yield: 95%, pH 7, 5 days, e.e. > 98%), (*S*)-(−)-**2a** (yield: 80%, pH 4, 7 days, e.e. > 98%) and (*R*)-(+)-**3a** (yield: 70%, pH 7, 7 days, e.e. 73%).
Deracemization: The same procedure used for the bioreduction of **1**–**3** was used in the deracemization of alcohols **2a** and **3a** using 100 μL of **2a** and **3a** and 15 g of wet cells in 500 mL of the buffer solution. The racemic alcohol **2a** was added to the Erlenmeyer flasks (100 μL, 32 °C, 170 rpm) and stirred for the periods indicated below.
A. terreus CCT 4083: (*S*)-(−)-**2a** (yield: 50%, pH 4, 14 days, e.e. 92%).
A. terreus CCT 3320: (*R*)-(+)-**3a** (yield: 52%, pH 4, 14 days, e.e. > 98%).

2.7. General procedure for the alcohols extraction

The reactions were monitored by GC and after the appropriate conversion, the mixture was filtered and the aqueous phase was extracted with ethyl acetate. The yellow organic phase was dried over $MgSO_4$ and evaporated. The residue was purified by silica gel column chromatography using hexane and ethyl acetate (90:10, 80:20 and 50:50) as eluents to yield the desired alcohols.

2.8. Analysis of the course of the biocatalytic reactions

The reaction progress was monitored every 24 h by collecting 1–2 mL samples. These samples were extracted by stirring with ethyl acetate (0.5 mL). The organic phase was analyzed by GC/FID (2–4 μL) in a fused silica chiral capillary column. The products of the biocatalyzed reactions were compared with a racemic mixture previously obtained by chemical reduction.

2.9. GC analysis conditions (chiral column Chirasil-Dex CB-cyclodextrin)

Injector 220 °C; detector 220 °C; column pressure (kPa) 100; split ratio (1:20). (*RS*)-1-(*ortho*-Nitrophenyl)ethanol (**1a**): rate 1 °C/min; oven 150 °C (t = 0 min)–180 °C; retention time [(*R*)-isomer: 8.2 min; (*S*)-isomer: 9.3 min]. (*RS*)-1-(*meta*-Nitrophenyl)ethanol (**2a**): rate 1 °C/min; oven 140 °C (t = 0 min)–180 °C; retention time [(*R*)-isomer: 16.2 min; (*S*)-isomer: 16.5 min]. (*RS*)-1-(*para*-Nitrophenyl)ethanol (**3a**): rate 1 °C/min; oven 150 °C (t = 0 min)–180 °C; retention time [(*R*)-isomer: 13.5 min; (*S*)-isomer: 14.8 min].

2.10. Assignment of the alcohols *1a–3a* absolute configuration

Optical rotation values were measured in a Jasco DIP-378 polarimeter. The reported data refer to the Na-line value using a 1 dm cuvette. The absolute configurations were determined by comparison of the measured optical rotation signs with the values from literature [7].

(*S*)-(+)-1-(*ortho*-Nitrophenyl)ethanol (**1a**): $[\alpha]_D^{20} + 30.6°$ (*c* 3.06, $CHCl_3$), e.e. > 98%.
(*S*)-(−)-1-(*meta*-Nitrophenyl)ethanol (**2a**): $[\alpha]_D^{20} - 30.0°$ (*c* 2.99, $CHCl_3$), e.e. 92%.
(*S*)-(−)-1-(*meta*-Nitrophenyl)ethanol (**2a**): $[\alpha]_D^{20} - 14.67°$ (*c* 3.83, $CHCl_3$), e.e. 73%.
(*R*)-(+)-1-(*para*-Nitrophenyl)ethanol (**3a**): $[\alpha]_D^{20} + 30.7°$ (*c* 3.45, $CHCl_3$), e.e. > 98%.

3. Results and discussion

3.1. Bioreduction of ortho-, meta- and para-nitroacetophenones *1–3*

The microbial reductions of nitroacetophenones **1**–**3** were carried out with whole cells of *A. terreus* in buffer solutions at different pH values (pH 2, 4, 6, 7, 8, 10 and 12) [24]. The best results for the bioreduction were obtained when whole cells of *A. terreus* were used with *ortho*-nitroacetophenone **1** (Table 1). The reaction produced (*S*)-(+)-1-(*ortho*-nitrophenyl)ethanol (**1a**) with good conversion (*c* 98%) and excellent enantiomeric excess (e.e. ≥ 98%) in all the cases. The enantioselectivity observed is in accordance with Prelog's rule [25]. In this case the pH had no influence in the stereoselectivity. However, a decrease in the conversion was observed when the reaction was performed in basic medium (Entries 13–14, 20–21 and 27–28, Table 1). The reaction of *ortho*-nitroacetophenone **1** with the fungus *A. terreus* CCT 4083 was carried out in a preparative scale (15 g of wet cells of *A. terreus* CCT 4083 and 500 μL of compound **1**) affording (*S*)-(+)-1-(*ortho*-nitrophenyl)ethanol (**1a**) in 95% isolated yield with >98% e.e. after 120 h (see Section 2.6).

The bioreduction of *meta*-nitroacetophenone **2** was also promoted by whole cells of all strains of *A. terreus* under study, with high conversion, but with different stereoselectivity, as can be observed in Table 1. When the reaction was performed in small scale (5 mg/2 g of wet cells) with *A. terreus* CCT 4083, *meta*-nitroacetophenone **2** was reduced to the corresponding (*S*)-(−)-1-(*meta*-nitrophenyl)ethanol (**2a**) with good conversion and enantiomeric excess (*c* 98%, e.e. 92%). After 3 days the reactions

showed high enantioselectivity (e.e. > 98%). The preparative scale reduction (100 mg of **2**) produced the alcohol (*S*)-(−)-**2a** in 80% yield and 99% e.e. In this case, *meta*-aminoacetophenone was isolated in 5% yield [7,26].

p-Nitroacetophenone (**3**) was reduced by all the fungal strains studied and under different pH values, leading in most cases predominantly to the anti-Prelog (*R*)-(+)-**3a** product in low stereoselectivity (Table 1). A semi-preparative scale reduction of **3** was performed with whole cells of *A. terreus* CCT 4083 producing (*S*)-(−)-**3a** in 70% yield and 73% e.e. in 168 h. In this case it could be observed that the reduction was followed by a deracemization, since the initial product was the (*R*)-(+)-**3a** isomer (Entry 18, pH 7, Table 1, e.e. 52%). This process was recently investigated in detail by us using the alcohol **3a** and the fungus strain *A. terreus* CCT 4083 at pH 7 [19,21].

3.2. Deracemization of (RS)-1-(nitrophenyl)ethanols 1a–3a

The preliminary result obtained in the above mentioned deracemization reaction, led us to investigate the behavior of (*RS*)-(nitrophenyl)ethanols **1a**–**3a** towards all the fungus strains studied (Scheme 1). The deracemization reaction of (*RS*)-**3a** was initially investigated with the fungus *A. terreus* CCT 3320, with different incubation periods at several pH values (Table 2). The deracemization process gave best results in acidic medium (pH 4), producing the (*R*)-alcohol **3a** with high conversion and selectivity (Entry 2, Table 2).

In view of this result the deracemization reaction of **1a**–**3a** was performed with all the fungus strains under study at pH 4. Compound (*RS*)-**2a** was deracemized by the eight strains of *A. terreus* leading to (*S*)-(−)-**3a** in ≥98% e.e. in several cases (Table 3). The low isolated yields of the transformation (50%) can be attributed to the long reaction time. The deracemization of (*RS*)-**3a** by the *A. terreus* strains under study in most cases occurred in modest enantiomeric excesses, both enantiomers being obtained as the main product, depending on the fungus strain used (Table 3). In a preceding work we observed that the fungus strain *A. terreus* CCT 3320 did not deracemize (*RS*)-**3a** at pH 7 [21]. Now we found that this compound is deracemized by *A. terreus* CCT 3320 at pH 4 with high enantiomeric excess, high conversion and in a short reaction time (Entry 5, Table 3). The semi-preparative scale was performed with whole fungal cells of *A. terreus* CCT 3320 leading to (*R*)-(+)-**3a** in 52% yield and 99% e.e. (100 μL, 14 days, pH 4).

(*RS*)-1-(*ortho*-Nitrophenyl)ethanol (**1a**) was not deracemized by the eight strains of *A. terreus* investigated under the employed conditions.

The deracemization of **2a** and **3a** by the fungus strains under study, probably occurred by pathways similar to the one exemplified in Scheme 2 for the deracemization of (*RS*)-**3a** by *A. terreus* CCT 3320 (Table 3). Initially **3a** was oxidized to **3** by a dehydrogenase present in *A. terreus* CCT 3320. In a second step a redox enzyme also present in the fungus, reduced **3** to **3a**. As the oxidation of (*R*)-(+)-**3a** is slower than the oxidation of (*S*)-(−)-**3a**, with the reaction progress the mixture becomes enriched in (*R*)-(+)-**3a** (Scheme 2).

4. Conclusion

This study showed that several strains of the fungus *A. terreus* isolated from different provenances in Brazil present high potential for biotransformations of nitrocompounds. In some cases the reduction of the nitroacetophenones **1**–**3** and the deracemization of the (*RS*)-(nitrophenyl)ethanols **2a**–**3a** occurred in high yield and enantiomeric excess. The biotransformations are sensitive to the pH of the reaction medium.

Acknowledgements

The authors express their gratitude to the donors of the fungal strains from the Brazilian Culture Collections of the Botanical Institute of São Paulo (São Paulo, SP), of the Federal University of Pernambuco (Recife, PE) and of the "Fundação André Tosello" (Campinas, SP). A.L.M. Porto and L.H. Andrade thank FAPESP for fellowships. J.V. Comasseto thanks FAPESP and CNPq for support. We are grateful to Edna Kagohara for the technical support.

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[22] Instituto de Botânica e Jardim Botânico Av. Miguel Stéfano, 3687-CEP 04301-902, Água Funda, Tel.: +11 5073 6300, fax: +11 5073 3678, São Paulo, SP, Brazil, http://www.ibot.sp.gov.br.

[23] André Tosello Foundation, Rua Latino Coelho 1301, CEP 13087-010, fax: +19 3242 7827, E-mail: atosello@fat.org.br; http://fat.org.br, Campinas, São Paulo, Brazil. CCT—Coleção de Cultura Tropical.
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