Mar Biotechnol (2015) 17:736–742
DOI 10.1007/s10126-015-9644-x

ORIGINAL ARTICLE

Stereoselective Bioreduction of α-Azido Ketones by Whole Cells of Marine-Derived Fungi

Lenilson C. Rocha[1,2] · **Mirna H. R. Seleghim**[3] · **João V. Comasseto**[4] · **Lara D. Sette**[5] · **André L. M. Porto**[1]

Received: 17 October 2014 / Accepted: 18 May 2015 / Published online: 14 August 2015

Abstract Seven strains of marine-derived fungi (*Aspergillus sclerotiorum* CBMAI 849, *Cladosporium cladosporioides* CBMAI 857, *Penicillium raistrickii* CBMAI 931, *Penicillium citrinum* CBMA 1186, *Mucor racemosus* CBMAI 847, *Beauveria felina* CBMAI 738, and *Penicillium oxalicum* CBMAI 1185) and terrestrial fungus *Penicillium chrysogenum* CBMA1199 were screened as catalysts for the asymmetric reduction of α-keto azides 5–8 to their corresponding β-azidophenylethanols 9–12. The marine fungi showed Prelog and anti-Prelog selectivities to the reduction α-keto azides 5–8. The fungi *A. sclerotiorum* CBMAI 849, *C. cladosporioides* CBMAI 857, *P. raistrickii* CBMAI 931, and *P. citrinum* CBMA 1186 catalyzed the reduction of azido ketone 6 to the corresponding (*R*)-2-azido-1-(4-methoxyphenyl)ethanol (10) with good conversions (68–100 %) and excellent enantiomeric excesses (>99 % *ee*) according to Prelog rule.

Keywords Azido alcohols · Biotransformation · Biocatalysis · Marine fungi · Mycelia

Electronic supplementary material The online version of this article (doi:10.1007/s10126-015-9644-x) contains supplementary material, which is available to authorized users.

✉ André L. M. Porto
almporto@iqsc.usp.br

[1] Instituto de Química de São Carlos, Universidade de São Paulo, Av. João Dagnone, 1100, J. Santa Angelina, 13563-120 São Carlos, SP, Brazil

[2] Faculdade Ingá - Uningá, Rodovia PR 317, 6114, 87035-510 Maringá, PR, Brazil

[3] Departamento de Ecologia e Biologia Evolutiva, Universidade Federal de São Carlos, Via Washington Luís, Km 235, 13565-905 São Carlos, SP, Brazil

[4] Universidade Federal de São Paulo, Rua Prof. Artur Riedel, 275 J. Eldorado, 09972-270 Diadema, SP, Brazil

[5] Divisão de Recursos Microbianos, Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrícolas, CPQBA, Universidade Estadual de Campinas, Rua Alexandre Casellato, 999, 13140-000 Paulínia, SP, Brazil

Introduction

Biocatalysis, also known as "white biotechnology," has been applied in many spheres of activity aiming of more environmentally friendly processes, such as the production of renewable raw materials and clean energy, and the remediation of the environment contaminated by toxic compounds (Gavrilescua and Chisti 2005). It is estimated that by the year 2020, biocatalysis will be responsible for 20 % of global production of all synthetic chemicals (Hans-Peter and Werbitzky 2011).

Biocatalysts, in the form of enzymes or whole microorganisms, are currently established as useful tools in the production of fine chemical (Liu et al. 2004). Factors such as production costs, biological activity, and regulations issues have led to a growing demand for enantiopure products for chiral drugs (Matsuda et al. 2009; Wang et al. 2009). A novel one-pot tandem biohydrogen transfer process for obtaining enantiopure secondary alcohols was described in the literature (Bisogno et al. 2009). There is a number of works on enzymatic reactions involving biocatalysts of terrestrial origin, but little using marine organisms or their purified enzymes (Trincone 2010; Veberlen et al. 2006).

Marine organisms such as seaweeds, sponges, mollusks, and microorganisms have sets of enzymes that present very useful characteristics for biocatalysis, such as stability at high and low temperatures or at extremes pH, and activity at high

pressure (Antranikian et al. 2005). Marine fungi produce extracellular enzymes and secondary metabolites different from terrestrial microorganisms, because they are adapted to the specific conditions in marine ecosystems, such as high salinity, which can be used in biocatalytic reactions (Bugni and Ireland 2004; Cheng et al. 2009). The use of whole cells of microorganisms is advantageous, particularly to obtain enantiomerically pure compounds.

Chiral β-azido alcohols are precursors in the synthesis of chiral aziridines and amino alcohols (Besse et al. 1994). There has been growing interest in chiral aziridines and amino alcohols in organic synthesis due to the increasing importance of bioactive molecules containing these functionalities, e.g., enzyme inhibitors, such as salmeterol and albuterol (Pàmies and Bäckvall 2001; Brenelli and Fernandes 2003).

In addition, marine organisms are rich sources of compounds that exhibit significant antitumor, anti-inflammatory, analgesic, antiviral, and antiallergic activities (Newman and Cragg 2004; San-Martín et al. 2008). Recently, we employed marine fungi to catalyze the transformation of several xenobiotic compounds (Rocha et al. 2009, 2010; Martins et al. 2011). In the present study, we report the first screening of marine-derived fungi for asymmetric reduction of α-keto azides 5–8.

Experimental

General Methods

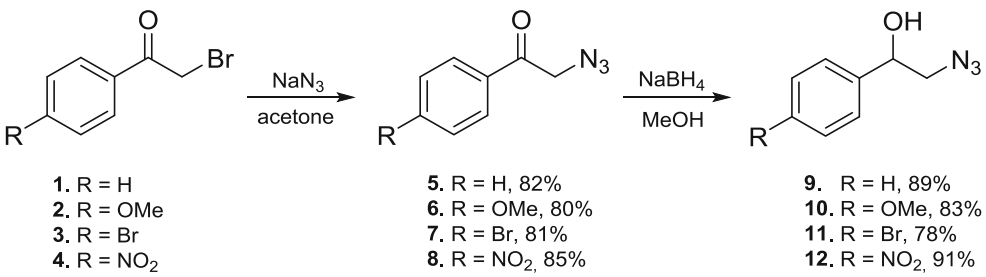
The reagents 2-bromo-1-phenylethanone (1), 2-bromo-1-(4-methoxyphenyl)ethanone (2), 2-bromo-1-(4-bromophenyl)ethanone (3), and 2-bromo-1-(4-nitrophenyl)ethanone (4), and solvents were purchased from Sigma-Aldrich (São Paulo, SP, Brazil). All manipulations involving the fungi (*Aspergillus sclerotiorum* CBMAI 849, *Cladosporium cladosporioides* CBMAI 857, *Penicillium raistrickii* CBMAI 931, *Penicillium citrinum* CBMA 1186, *Mucor racemosus* CBMAI 847, *Beauveria felina* CBMAI 738, *Penicillium chrysogenum* CBMAI 1199, and *Penicillium oxalicum* CBMAI 1185) were carried out under sterile conditions in a Veco laminar flow cabinet. Technal TE-421 or Superohm G-25 orbital shakers were employed in the biocatalyzed experiments. The products were purified by column chromatography (CC) on silica gel (230–400 mesh) eluting with mixtures of *n*-hexane and EtOAc (9:1, 8:2, 7:3). The fractions were monitored by TLC, on pre coated plates with silica gel 60F254 layers (aluminum-backed: Sorbent).

The products were analyzed using Shimadzu LC-10AD® or Shimadzu 20AT® chromatographs with UV detector (190–254 nm) equipped with a Chiralcel® OD-H chiral column (0.46 cm×25 m; 5 μm); the mobile phase was hexane and 2-propanol (95:5), flowing at 0.5 mL min^{-1}. The Shimadzu LC-10AD model chromatograph was equipped with a photodiode array SPD-M10A detector, a DGU-14A degasser, a SCL-10A control center, and a manual Rheodyne® injector. The Shimadzu LC-20AT model was equipped with a diode array SPD-M20A detector, a DGU-20A5 degasser, a CBM-20A control center, a Sil-20A automatic injector, and a CTO-20A oven. Acquisition and data analysis were performed using the LCSolution/CLASS-VP software. A Shimadzu GC 2010 plus gas chromatography system coupled to a mass selective detector (Shimadzu MS 2010 plus) in electron ionization (EI, 70 eV) mode and equipped with a DB5 fused silica column (J&W Scientific 30 m×0.25 mm×0.25) was employed for obtaining the mass spectra. ^{1}H and ^{13}C NMR spectra were registered on a Bruker AC-200 spectrometer (^{1}H at 200 MHz and ^{13}C at 50 MHz), using chloroform-*d* ($CDCl_3$) as solvent and tetramethylsilane (TMS) as internal standard; the chemical shifts are given in parts per minute. Near-IR spectra were recorded on a Bomem MB-102 spectrometer.

Separations of the enantiomers of β-azidophenylethanols were archived by HPLC analysis on a chiral column. The retention times of the compounds were the following: (*R*-9, 35.0 min; *S*-9, 33.0 min), (*R*-10; 27.0 min; *S*-10, 23.0 min), (*R*-11, 24.0 min; *S*-11, 28.0 min), and (*R*-12, 21.2 min; *S*-12, 23.1 min).

Preparation of the α-Azido Ketones 5–8

A mixture of the α-bromoacetophenones 1–5 (14.00 mmol) and sodium azide (1.82 g, 28.00 mmol) in acetone (250 mL) was stirred at room temperature, and the reaction was monitored by TLC (Scheme 1). The mixture was poured into water and extracted with EtOAc (3×30 mL); the organic layer was dried with $MgSO_4$, filtered, concentrated under reduced pressure, and purified by crystallization or by column



Scheme 1 Synthesis of (±)-β-azidophenylethanols 9–12 from α-bromoacetophenones 1–4

chromatography (Patonay et al. 2002). The spectroscopic data (^{1}H and ^{13}C NMR, MS and IR) of the α-azido ketones 5–6 and 8 were in agreement with those reported in the literature (Patonay et al. 2002; Patonay and Hoffman 1994).

2-Azido-1-(4-bromophenyl)ethanone (7): ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.53 (s, 1H); 7.66 (d, 2H, J=8.0 Hz); 7.79 (d, 2H, J=8.0 Hz). IR (KBr) 2102 (N_3), 1690 (C=O), 1554, 1228, 1215, cm^{-1}.

Preparation of the (±)-β-Azidophenylethanols 9–12

(±)-β-Azidophenylethanols 9–12 were obtained by reduction of the α-azido ketones 5–8 with sodium borohydride in ethanol (Scheme 1) (Pavia et al. 1999). The spectroscopic data (^{1}H and ^{13}C NMR, MS and IR) of the (±)-β-azidophenylethanols 9–12 were in agreement with those reported in the literature (Kamal et al. 2004a, b; Brenelli and Fernandes 2003; Mesas-Sánchez et al. 2013; Ankati et al. 2008).

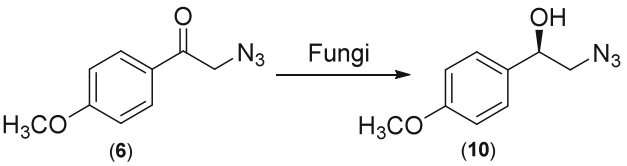
Marine Fungal Strains

The marine fungal strain *P. raistrickii* CBMAI 931 was isolated from the sponge *Chelonaplysylla erecta*. The strains *P. citrinum* CBMAI 1186, *P. oxalicum* CBMAI 1185, and *B. felina* CBMAI 738 were isolated from a marine alga *Caulerpa* sp. (De Vita-Marques et al. 2008). The fungal strains *A. sclerotiorum* CBMAI 849, *C. cladosporioides* CBMAI 857, and *M. racemosus* CBMAI 847 were isolated from the Brazilian cnidarian zoanthids, i.e., *Palythoa variabilis*, *Palythoa caribaeorum*, and *Mussismilia hispida*, respectively, collected in São Sebastião City, northern coast of the State of São Paulo, Brazil (Santos et al. 2010). The terrestrial fungal strain *P. chrysogenum* CBMAI 1199 was donated by Prof. Dra. Mirna Helena Regali Seleghim from the Department of Ecology and Evolutionary Biology (DEBE) at UFSCar, São Carlos, Brazil. The fungi used in this work were identified by both conventional and molecular methods at the Chemical, Biological and Agricultural Multidisciplinary Research Center (CPQBA) at UNICAMP, São Paulo, Brazil (http://www.cpqba.unicamp.br/).

Bioreduction of the α-Azido Ketones 5–8 by Whole Cells of Marine Fungi

The marine-derived fungi were grown in culture media and artificial seawater (Rocha et al. 2009). Small slices of solid medium (0.5×0.5 cm) bearing mycelia of the marine-derived fungi (*A. sclerotiorum* CBMAI 849, *C. cladosporioides* CBMAI 857, *P. raistrickii* CBMAI 931, *P. citrinum* CBMA1186, *M. racemosus* CBMAI 847, *B. felina* CBMAI 738, *P. chrysogenum* CBMAI 1199, and *P. oxalicum* CBMAI 1185) were cut from the stock solid culture. They were incubated at 32 °C for 9 days in a rotary shaker (130 rpm), filtered using Buchner funnels, and suspended in 250-mL Erlenmeyer flasks containing 100 mL of phosphate buffer solution. The each biocatalytic reactions was carried out with 5.0 g mycelium (wet weight), 0.50 mmol of the α-azido ketones 5–8, and inoculated into 2-L Erlenmeyer flasks containing 1000 mL of the liquid culture medium dissolved in 300 μL

Table 1 Bioreduction of 2-azido-1-(4-methoxyphenyl)ethanone (6) by marine-derived fungi (32 °C, 130 rpm)



(6) (10)

Time (days)	*c* (%) 6	c (%) / yield (%) 10	*ee* 10 (ac)
A. sclerotiorum CBMAI 849			
1	75	25	>99 (*R*)
2	63	37	>99 (*R*)
3	42	58	>99 (*R*)
5	29	71	>99 (*R*)
7	12	88	>99 (*R*)
9	0	100/94	>99 (*R*)
C. cladosporioides CBMAI 857			
1	81	19	>99 (*R*)
2	67	33	>99 (*R*)
3	42	58	>99 (*R*)
5	27	73	>99 (*R*)
7	29	80	>99 (*R*)
9	1	99/91	>99 (*R*)
P. raistrickii CBMAI 931			
1	89	11	>99 (*R*)
2	78	22	>99 (*R*)
3	63	37	>99 (*R*)
5	47	53	>99 (*R*)
7	40	60	>99 (*R*)
9	32	68/62	>99 (*R*)
P. citrinum CBMAI 1186			
1	76	24	>99 (*R*)
2	62	38	>99 (*R*)
3	41	59	>99 (*R*)
5	29	71	>99 (*R*)
7	8	86	>99 (*R*)
9	1	99/93	>99 (*R*)

The biocatalytic reactions were carried out with 5.0 g (wet weight) of mycelium and 0.50 mmol of α-azido ketones 5–8 dissolved in 300 μL of DMSO, added to a 250-mL Erlenmeyer flask containing 100 mL of buffer solution (Na_2HPO_4/KH_2PO_4, pH 7, 0.1 M). Conversion determined by HPLC analysis with a Chiracel® OD-H chiral column

c concentration, c conversion, % isolated yield, *ee* (%) enantiomeric excess, *ac* absolute configuration

DMSO in the buffer solution (0.1 M, Na_2HPO_4/KH_2PO_4, pH 7). The mixtures were incubated for 9 days in an orbital shaker at 32 °C and 120 rpm. The progress of the reactions was checked by TLC analysis for 2.0 mL of samples. For compound 6, the reactions were periodically analyzed (1–9-day intervals) by HPLC (Table 1). The samples were extracted with ethyl acetate (1.0 mL), and then centrifuged (6000 rpm, 6 min) and analyzed by GC-MS and HPLC. The products were purified by column chromatography over silica gel to yield the (*R*)- or (*S*)-β-azidophenylethanols 9–12 (Table 2).

Assignment of the Absolute Configuration

The optical rotations of the β-azidophenylethanols 9–12 were measured in a PerkinElmer (Waltham, MA, USA) 241 polarimeter with a 1-dm cuvette and referenced to the Na-D line. The absolute configurations of the β-azidophenylethanols 9–12 were determined by comparing the sign of the measured specific rotation with those reported in the literature (Kamal et al. 2004a, b; Brenelli and Fernandes 2003; Mesas-Sánchez et al. 2013; Ankati et al. 2008).

Table 2 Bioreduction of α-azido ketones 5–8 by marine-derived fungi (32 °C, 130 rpm, 9 d)

2-Azido-1-phenylethanone (5)			2-Azido-1-(4-methoxyphenyl)ethanone (6)		
c (%) 5	c (%)/yield (%) 9	*ee* 9 (ac)	*c* (%) 6	c (%)/yield (%) 10	*ee* 10 (ac)
A. sclerotiorum CBMAI 849					
2	98/90	85 (*S*)	0	100/94	>99 (*R*)
C. cladosporioides CBMAI 857					
–	–	–	1	99/91	>99 (*R*)
P. raistrickii CBMAI 931					
10	90/82	90 (*R*)	32	68/62	>99 (*R*)
P. citrinum CBMAI 1186					
–	–	–	1	99/93	>99 (*R*)
M. racemosus CBMAI 847					
10	90/86	72 (*S*)	100	0	–
B. felina CBMAI738					
0	100/91	80 (*R*)	100	0	–
P. chrysogenum					
100	0	–	100	0	–
2-Azido-1-(4-bromophenyl)ethanone (7)			2-Azido-1-(4-nitrophenyl)ethanone (8)		
c (%) 7	c (%)/yield (%) 11	*ee* (ac) 11	*c* (%) 8	c (%)/yield (%) 12	*ee* (ac) 12
A. sclerotiorum CBMAI 849					
25	75/66	39 (*S*)	–	–	–
C. cladosporioides CBMAI 857					
2	98/90	44 (*S*)	–	–	–
P. raistrickii CBMAI 931					
68	32/30	70 (*S*)	–	–	–
P. citrinum CBMAI 1186					
52	48/25	39 (*S*)	5	95/17	28 (*S*)
M. racemosus CBMAI 847					
21	79/65	29 (*S*)	–	–	–
B. felina CBMAI 738					
100	0	–	–	–	–
P. chrysogenum					
100	0	–	2	98/36	41 (*R*)
P. oxalicum CBMAI 1185					
–	–	–	4	96/42	51 (*S*)

The biocatalytic reactions were carried out with 5.0 g (wet weight) of mycelium and 0.50 mmol of α-azido ketones 5–8 dissolved in 300 μL of DMSO, added to a 250-mL Erlenmeyer flask containing 100 mL of buffer solution (Na_2HPO_4/KH_2PO_4, pH 7, 0.1 M). Conversion determined by HPLC analysis equipped with a Chiracel® OD-H chiral column

c concentration, c conversion, (%) isolated yield, *ee* (%) enantiomeric excess, *ac* absolute configuration

(*R*)-(−)-2-azido-1-phenylethanol (**9**): $[\alpha]_D^{25}$=−20.2 (*c* 0.82, CH_3OH; 90 % *ee*); Lit. (Ankati et al. 2008) $[\alpha]_D^{22}$=+104.9 (*c* 0.67, CH_3OH, 99 % *ee*, for *S*-enantiomer). The *R*-9 was obtained by reduction of ketone 5 with the fungus *P. raistrickii* CBMAI 931.

(*R*)-(−)-2-azido-1-(4-methoxyphenyl)ethanol (10): $[\alpha]_D^{25}$=−76.0 (*c* 0.85, CH_3OH, >99 % *ee*); Lit. (Ankati et al. 2008) $[\alpha]_D^{22}$=+100.9 (*c* 1.17, CH_3OH, 99 % *ee*), for *S*-enantiomer. The *R*-10 was obtained by reduction of ketone 6 with the fungus *C. cladosporioides* CBMAI 857.

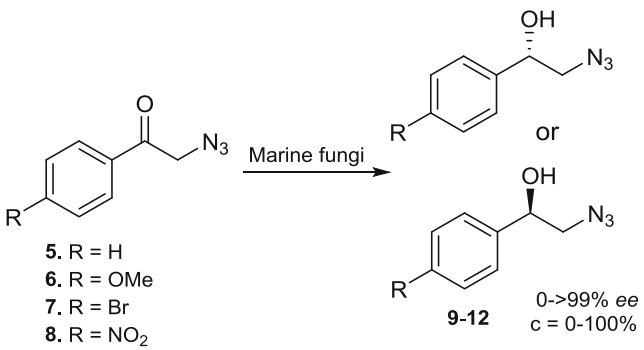
(*S*)-(+)-2-azido-1-(4-bromophenyl)ethanol (11): $[\alpha]_D^{25}$=+40.3 (*c* 1.12, CH_3OH, 39 % *ee*); lit. (Ankati et al. 2008) $[\alpha]_D^{25}$=+61.3 (*c* 0.67, CH_3OH, >99 % *ee*). The *S*-11 was obtained by reduction of ketone 7 with the fungus *A. sclerotiorum* CBMAI 849.

(*S*)-(+)-2-azido-1-(4-nitrophenyl)ethanol (12): $[\alpha]_D^{25}$=+30.5 (*c* 0.64, MeOH, 28 % *ee*); Lit. (Ankati et al. 2008) $[\alpha]_D^{25}$=+87.9 (*c* 0.17, CH_3OH, 99 % *ee*). The *S*-12 compound was obtained by reduction of ketone 8 with the fungus *P. citrinum* CBMAI 1186.

Results and Discussion

Bioreduction of α-azido ketones 5–8 was performed for whole mycelia of seven marine fungi (*A. sclerotiorum* CBMAI 849, *C. cladosporioides* CBMAI 857, *P. raistrickii* CBMAI 931, *P. citrinum* CBMA1186, *M. racemosus* CBMAI 847, *B. felina* CBMAI 738, *P. oxalicum* CBMAI 1185), and terrestrial fungus *P. chrysogenum* CBMAI 1199. The fungi were screened for the stereoselective reduction of ketones 5–8. The results are shown in Scheme 2 and in Tables 1 and 2.

For compound 6, the reactions were periodically analyzed (1–9-day intervals) by HPLC (Table 1). After establishing the best reaction time (9 days) for all reactions, the products were analyzed by HPLC on a chiral column, to determine the conversions and enantiomeric excesses (Table 2). The progress of the reactions for compounds 5, 7–8 was followed by TLC analysis of aliquots collected from 3 to 9-day reaction time.



Scheme 2 Bioreduction of α-azido ketones 5–8 to (*S*)- or (*R*)- β-azidophenylethanols 9–12 by marine-derived fungi

The fungi *A. sclerotiorum* CBMAI 849 and *M. racemosus* CBMAI 847 were able to catalyze the reduction of 2-azido-1-phenylethanone (5) to the corresponding (*S*)-2-azido-1-phenylethanol (9). *A. sclerotiorum* CBMAI 849 gave *S*-9 in 98 % conversion and 85 % *ee*, and *M. racemosus* CBMAI 847 gave *S*-9 in 90 % conversion and 72 % *ee*, in accordance with the anti-Prelog rule. The (*S*)-azido-alcohol 9 was obtained with 90 and 86 % isolated yields, respectively, after purification by column chromatography. *P. raistrickii* CBMAI 931 and *B. felina* CBMAI 738 catalyzed the reduction of the keto azide 5 to the corresponding (*R*)-2-azido-1-phenylethanol (9) in high conversion and enantiomeric excess following the Prelog rule (Table 2). The (*R*)-azido-alcohol (9) was isolated with 82 % (*P. raistrickii* CBMAI 931) and 91 % (*B. felina* CBMAI 738) yields. *C. cladosporioides* CBMAI 857, *P. citrinum* CBMAI 1186, and *P. chrysogenum* CBMAI 1199 were unable to catalyze the reduction of the keto azide 5.

The fungi *A. sclerotiorum* CBMAI 849, *C. cladosporioides* CBMAI 857, *P. raistrickii* CBMAI 931, and *P. citrinum* CBMA 1186 catalyzed the bioreduction of 2-azido-1-(4-methoxyphenyl)ethanone (6) to the corresponding (*R*)-2-azido-1-(4-methoxyphenyl)ethanol (10) with high conversions (*c*=68–100 %) and enantiomeric excesses (>99 % *ee*) in 9-day reaction time, following the Prelog rule (Table 2). The (*R*)-azido-alcohol 10 was obtained with 62–94 % yield after purification by column chromatography. The fungi *M. racemosus* CBMAI 847, *B. felina* CBMAI 738, and *P. chrysogenum* CBMAI 1199 were unable to catalyze the bioreduction of the keto azide 6.

The fungi *A. sclerotiorum* CBMAI 849, *C. cladosporioides* CBMAI 857, *P. raistrickii* CBMAI 931, *P. citrinum* CBMAI 1186, and *M. racemosus* 847 CBMAI catalyzed the bioreduction of 2-azido-1-(4-bromophenyl)ethanone (7) to the corresponding (*S*)-2-azido-1-(4-bromophenyl)ethanol (11) with different conversions (*c*=32–98 %) and enantiomeric excesses (29–70 % *ee*) in 9-day reaction time, following the anti-Prelog rule. In this case, only the (*S*)-azido-alcohol 11 was obtained from the fungus *P. raistrickii* CBMAI 931 with 30 % isolated yield. The fungi *B. felina* CBMAI 738 and *P. chrysogenum* CBMAI 1199 did not catalyze the reduction of the keto azide 7 after 9-day reaction time (Table 2). Bioreduction of the α-azido ketones 5–7 by *Saccharomyces cerevisiae* was reported with 91–94 % isolated yields and 97–100 % enantiomeric excesses (Yadav et al. 2001). The same compounds were reduced by *Geotrichum candidum* (isolated yield 93–99 and 40–99 % *ee*), *Rhodotorula glutinis* (isolated yield 88–97 and >99 % *ee*) (Fardelone et al. 2006), and *Daucus carota* root (isolated yield 58–77 and 97–100 % *ee*) (Yadav et al. 2002).

The 2-azido-1-(4-nitrophenyl)ethanone (8) is unstable under light even at room temperature. Possibly the low solubility in aqueous media made the bioreduction of keto azide 8 harder to perform. However, the fungi *P. citrinum* CBMAI 1186,

P. chrysogenum CBMAI 1199, and *P. oxalicum* CBMAI 1185 were able to catalyze the bioreduction of the keto azide 8. *P. oxalicum* CBMAI 1185 and *P. citrinum* CBMAI 1186 catalyzed the bioreduction of keto azide 8 to the corresponding (*S*)-2-azido-1-(4-nitrophenyl)ethanol (12) in high conversion and modest enantiomeric excess (Table 2). The (*S*) and (*R*)-azido-alcohols 12 were produced with 17–42 % yields after purification by column chromatography (Table 2).

In summary, enantiomerically pure azido alcohols 9–12 were obtained by bioreduction of the corresponding ketones using whole cells of marine-derived fungi. These compounds are precursors of chiral aziridines and amino alcohols used in drugs synthesis. For example (*R*)-(−)-octopamine a potent chiral drug possessing β-adrenergic activity was prepared from (*R*)-2-azido-1-phenylethanol (9). The (*R*)-2-azido-1-(4-methoxyphenyl)ethanol (10) was employed in the synthesis of (*R*)-(−)-tembamide and (*R*)-(−)-aegeline used in traditional Indian medicine. It has been shown that these compounds have good hypoglycemic activity (Sadyandy et al. 2005).

Finally, the strains of marine-derived fungi used in this study showed potential for reduction of azido ketones in good conversions and selectivities.

Conclusion

In conclusion, marine-derived fungi demonstrated to be excellent biocatalysts for the reduction of α-keto azides, affording the corresponding azido alcohols 9–12 in good yields and enantiomeric excesses. The reductases of the marine-derived fungi studied showed Prelog and anti-Prelog selectivity in the bioreduction α-keto azides. The fungi *A. sclerotiorum* CBMAI 849, *C. cladosporioides* CBMAI 857, *P. raistrickii* CBMAI 931and, and *P. citrinum* CBMAI 1186 catalyzed the reduction of the azido ketone 6 to the corresponding (*R*)-2-azido-1-(4-methoxyphenyl)ethanol (10) with good conversions (68–100 %) and >99 % enantiomeric excess in 9 days following the Prelog rule.

Acknowledgments The authors thank Prof. Dr. Timothy Brockson (UFSCar) for the optical rotation analysis and Prof. Dr. Antonio Gilberto Ferreira (UFSCar) for NMR analyses. LCR thanks CAPES for a fellowship. ALM Porto acknowledges CNPq and FAPESP for financial support. The text was reviewed by Timothy Roberts (MSc) native English.

References

Ankati H, Yang Y, Zhu D, Biehl ER, Hua L (2008) Synthesis of optically pure 2-azido-1-arylethanols with isolated enzymes and conversion to triazole-containing β-blocker analogues employing click chemistry. J Org Chem 73:6433–6436

Antranikian G, Vorgias CE, Bertoldo C (2005) Extreme environments as a resource for microorganisms and novel biocatalysts. Adv Biochem Eng Biotech 96:219–262

Besse P, Veschambre H, Chênevert R, Dickman M (1994) Chemoenzymatic synthesisof chiral β-azidoalcohols. Application to the preparation of chiral aziridines and aminoalcohols. Tetrahedron-Asymmetry 5:1727–1744

Bisogno FR, Lavandera I, Kroutil W, Gotor V (2009) Tandem concurrent processes: one-pot single-catalyst biohydrogen transfer for the simultaneous preparation of enantiopure secondary alcohols. J Org Chem 74:1730–1732

Brenelli ECS, Fernandes JLN (2003) Stereoselective acylations of 1,2-azidoalcohols with vinyl acetate, catalyzed by lipase Amano OS. Tetrahedron-Asymmetry 14:1255–1259

Bugni TS, Ireland CM (2004) Marine-derived fungi: a chemically and biologically diverse group of microorganisms. Nat Prod Rep 21: 143–163

Cheng Z, Pan J, Tang W, Chen Q, Lin Y (2009) Biodiversity and biotechnological potential of mangrove-associated fungi. J For Res 20:63–72

De Vita-Marques AM, Lira SP, Berlinck RGS, Seleghim MHR, Sponchiado SRP, Tauk-Tornisielo SM, Barata M, Pessoa C, De Moraes MO, Coelho B, Nascimento GGF, De Souza AO, Galetti FCS, Silva CL, Silva M, Pimenta EF, Thiemann O, Passarini MRZ, Sette LD (2008) A multi-screening approach for marine-derived fungal metabolites and the isolation of cyclodepsipeptides from *Beauveria felina*. Quim Nova 31:1099–1103

Fardelone LC, Rodrigues JAR, Moran PJS (2006) Bioreduction of 2-azido-1-arylethanones mediated by *Geotrichum candidum* and *Rhodotorula glutinis*. J Mol Catal B Enzym 39:9–12

Gavrilescua M, Chisti Y (2005) Biotechnology - a sustainable alternative for chemical industry. Biotechnol Adv 23:471–499

Hans-Peter M, Werbitzky O (2011) How green can the industry become with biotechnology. In: Junhua AT, Kazlauskas R (eds) Biocatalysis for green chemistry and chemical process development. Wiley, Hoboken

Kamal A, Shaik AA, Sandbhor M, Malik MS (2004a) Synthesis of enantiopure β-azidoalcohols from their ketoazides by reduction with $NaBH_4$ in the presence of alumina and in situ lipase resolution. Tetrahedron-Asymmetry 15:935–939

Kamal A, Shaik AA, Sandbhor M, Malik MS (2004b) Chemoenzymatic synthesis of (*R*)- and (*S*)-tembamide, aegeline and denopamine by a one-pot lipase resolution protocol. Tetrahedron-Asymmetry 15: 3939–3944

Liu Z, Weis R, Gliede A (2004) Enzymes from higher eukaryotes for industrial biocatalysis. Food Technol Biotechnol 42:237–249

Martins MP, Mouad AM, Boschini L, Seleghim MHR, Sette LD, Porto ALM (2011) Marine fungi *Aspergillus sydowii* and *Trichoderma* sp. catalyze the hydrolysis of benzyl glycidyl ether. Mar Biotechnol 13: 14–20

Matsuda T, Yamanaka R, Nakamura K (2009) Recent progress in biocatalysis for asymmetric oxidation and reduction. Tetrahedron-Asymmetry 20:513–557

Mesas-Sánchez L, Díaz-Álvarez AE, Dinér P (2013) Non-enzymatic kinetic resolution of 1,2-azidoalcohols using a planar-chiral DMAP derivative catalyst. Tetrahedron 69:753–757

Newman DJ, Cragg GM (2004) Marine natural products and related compounds in clinical and advanced preclinical trials. J Nat Prod 67:1216–1238

Pàmies O, Bäckvall J-E (2001) Syntheses and transformations of α-azido ketones and related derivatives. J Org Chem 66:4022–4025

Patonay T, Hoffman RV (1994) A general and efficient synthesis of α-azido ketones. J Org Chem 59:2902–2905

Patonay T, Juhász-Tóth E, Bényei A (2002) Base-induced coupling of α-azido ketones with aldehydes - an easy and efficient route to trifunctionalized synthons 2-azido-3-hydroxy ketones. Eur J Org Chem 2002:285–295

Pavia DL, Lampman GM, Kriz GS, Engel RG (1999) Introduction to organic laboratory techniques, 3rd edn. Sunders College Publishing, Orlando

Rocha LC, Ferreira HV, Pimenta EF, Berlinck RGS, Seleghim MHR, Javaroti DCD, Sette LD, Bonugli RC, Porto ALM (2009) Bioreduction of α-chloroacetophenone by whole cells of marine fungi. Biotechnol Lett 31:1559–1563

Rocha LC, Ferreira HV, Pimenta EF, Berlinck RGS, Rezende MOO, Landgraf MD, Seleghim MHR, Sette LD, Porto ALM (2010) Biotransformation of α-bromoacetophenones by the marine fungus *Aspergillus sydowii*. Mar Biotechnol 12:552–557

Sadyandy R, Fernandes RA, Kumar P (2005) An asymmetric dihydroxylation route to (*R*)-(−)-octopamine, (*R*)-(−)-tembamide and (*R*)-(−)-aegeline. ARKIVOC iii:36–43

San-Martín A, Rovirosa J, Astudillo L, Sepúlveda B, Ruiz D, San-Martín C (2008) Biotransformation of the marine sesquiterpene pacifenol by a facultative marine fungus. Nat Prod Res 22:1627–1632

Santos RCB, Durrant LR, Silva M, Sette LD (2010) Production of laccase, manganese peroxidase and lignin peroxidase by Brazilian marine-derived fungi. Enzym Microb Technol 46:32–37

Trincone A (2010) Potential biocatalysts originating from sea environments. J Mol Catal B Enzym 66:241–256

Veberlen PJ, De Schutter DP, Delvaux F, Verstrepen KJ, Delvau FR (2006) Immobilized yeast cell systems for continuous fermentation applications. Biotechnol Lett 28:1515–1525

Wang Y, Liu D, Meng Q, Zhang W (2009) Asymmetric hydrogenation of simple ketones with planar chiral ruthenocenyl phosphinooxazoline ligands. Tetrahedron-Asymmetry 20:2510–2512

Yadav JS, Reddy BVS, Nanda S, Rao AB (2001) A facile synthesis of (*R*)-(−)-2-azido1-aryethanols from 2-azido-1-arylketones using baker's yeast. Tetrahedron-Asymmetry 12:63–67

Yadav JS, Nanda S, Reddy BVS, Rao AB (2002) Efficient enantioselective reduction ketones with *Daucus carota* root. J Org Chem 67:3900–3903