Journal of Molecular Catalysis B: Enzymatic 64 (2010) 48–52

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Deracemization of aryl secondary alcohols *via* enantioselective oxidation and stereoselective reduction with tandem whole-cell biocatalysts

Yun-Long Li, Jian-He Xu*, Yi Xu

Laboratory of Biocatalysis and Bioprocessing, State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, 130 Meilong Road, Shanghai 200237, China

ARTICLE INFO

Article history:
Received 10 December 2009
Received in revised form 26 January 2010
Accepted 26 January 2010
Available online 4 February 2010

Keywords:
Deracemization
Stereoinversion
Aryl secondary alcohols
Microbacterium oxydans
Rhodotorula sp.

ABSTRACT

Deracemization of racemic 1-phenylethanol, *i.e.*, stereoinversion of (*R*)-1-phenylethanol to (*S*)-1-phenylethanol, has been successfully realized *via* concurrent enantioselective oxidation and stereoselective reduction employing whole-cell biocatalysts of an alcohol dehydrogenase and a ketone reductase with opposite stereoselectivity in one-pot. One biocatalyst is *Microbacterium oxydans* ECU2010 which catalyzes stereoselective oxidation of (*R*)-secondary alcohols to corresponding ketones and another is *Rhodotorula* sp. AS2.2241 which reduces the ketones to (*S*)-secondary alcohols. Each of the whole-cell biocatalysts has its own *in vivo* cofactor regeneration system so that there is no need to add the expensive cofactor and/or the oxidoreductase for the cofactor regeneration. To explore the generality of this method, a broad range of racemic aryl secondary alcohols were efficiently deracemized to their (*S*)-enantiomers by combination of the two microorganisms, affording optically pure secondary alcohols in high yields (86.5–99%) and excellent optical purity (>99% *ee*). Our method represents an easy going, cheap and environmentally benign way for the biocatalytic synthesis of chiral aryl secondary alcohols from their racemates.

1. Introduction

Optically pure secondary alcohols are widely used in pharmaceuticals, flavors, agricultural chemicals and specialty materials. Nevertheless, only one enantiomer (A) is usually needed for the synthesis of a final product, and there is little (or no) use for the opposite enantiomer (B), it has to be regarded as "waste" and thus represents a considerable economic burden [1]. Therefore, stereoinversion of undesired enantiomer (B) to the other (A) or deracemization of racemates (A/B) are both two ideal ways to produce the desired optically pure enantiomer (A). Since the first comprehensive review by Carnell [2] on deracemization of chiral secondary alcohols by stereoinversion, an impressive number of reports [3–9] appeared recently and several recent reviews have touched this topic in part [1,10–12].

The aim to obtain a highly valuable enantiopure product in 100% yield and with 100% *ee* (enantiomeric excess) from a cheap racemic substrate or the "waste" enantiomer in a one-pot process with multiple catalysts is currently a hot topic [3,13–15]. Catalytic (asymmetric) tandem reactions are processes in which multiple catalysts operate concurrently in one pot, circumventing the often time-consuming, yield-reducing isolation and purification of intermediates in multiple-step synthesis [16]. In nature, multiple biocatalysts are common within a microorganism for metabolizing natural compounds such as glucose. Nevertheless, a microorganism often does not have the appropriate active and selective enzymes for desired multiple-step chemical synthesis when non-natural compounds are used as substrates [17]. Alternatively, the best enzyme for each reaction step may be selected from different microorganisms, mixed in different forms, and adjusted to the desired ratio for efficient tandem transformation. It is perhaps most important that the different catalytic reactions should not interfere with one other. Hummel and Riebel [18] had reported a stepwise route to enantiomerically pure alcohols from the corresponding racemates by employing two stereocomplementary alcohol dehydrogenases. The stereoinversion and deracemization of *sec*-alcohols by free oxidoreductases were also reported by Kroutil and his coworkers in their recent publication [16]. However, external addition of both the cofactor and the isolated or commercially purified enzymes were necessary in these reports. Comparing with isolated enzymes, the application of the whole-cell biocatalysts for biotransformation brought about more advantages, during which the enzymes are often more stable due to the presence of their natural environment inside the cell and the cost is much lower because of no requirements for tedious separation of enzymes and the regeneration of expensive cofactors (NADH/NADPH). There were only limited reports on the deracemization of secondary alcohol or hydroxyl acid by using growing or resting cells as biocata-

* Corresponding author. Tel.: +86 21 6425 2498; fax: +86 21 6425 2250.
E-mail address: jianhexu@ecust.edu.cn (J.-H. Xu).

1381-1177/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2010.01.031

lysts, such as *sec*-alcohol [19], mandelic acid [20] and 1,2-octanediol [21]. In the former case, the result was not satisfactory for the deracemization of **1a** and the product (*R*)-**1a** was obtained in only 80% yield and 85% *ee* [19].

In this paper, a tandem biocatalysts system was successfully developed for the stereoinversion of (*R*)-1-phenylethanol and deracemization of various aryl secondary alcohols by using highly selective biocatalyst for each step and mixed them in suitable forms for efficient tandem transformation. A variety of enantiopure (*S*)-alcohols were successfully prepared in a one-pot procedure in good yield and excellent *ee*.

2. Experimental

Gas chromatographic (GC) analyses were performed using a chiral GC-column (Betadex-120, 30 m × 0.25 mm × 0.25 μm, Supelco, USA). HPLC analyses were performed using a chiral column (Chiralcel OD, Ø0.46 cm × 25 cm, Daicel Chemical Industries, Japan).

2.1. Cultivation of microbes

Microbacterium oxydans ECU2010, which was deposited in China General Microbiological Culture Collection Center with an accession number of CGMCC No. 1875 was grown in a medium consisting of glucose (15 g/L), beef extract (30 g/L), peptone (20 g/L), KH_2PO_4 (0.5 g/L), K_2HPO_4 (0.5 g/L), NaCl (1 g/L) and $MgSO_4$ (0.5 g/L) while *Rhodotorula* sp. AS2.2241, which was also deposited in China General Microbiological Culture Collection Center with an accession number of CGMCC No. 1735 was grown in another medium consisting of glucose (15 g/L), yeast extract (5 g/L), peptone (5 g/L), KH_2PO_4 (0.5 g/L), K_2HPO_4 (0.5 g/L), NaCl (1 g/L) and $MgSO_4$ (0.5 g/L). After adjusting to pH 7.0 using 2 M NaOH solution, 100 mL of the medium was placed in a 500-mL Erlenmeyer flask, sterilized (121 °C, 20 min) and inoculated with the preincubated culture, shaken at 30 °C and 180 rpm for 24 h.

2.2. Selective oxidation of aryl secondary alcohols with M. oxydans ECU2010

Fresh cells (0.02 g) of *M. oxydans* ECU2010 and substrate (0.02 mM) were suspended in a screw-capped 10-mL test tube containing Tris–HCl buffer (1 mL, 50 mM, pH 8.5) and shaken at 180 rpm for 24 h at 30 °C. Then the biotransformation was stopped by addition of the same volume of ethyl acetate and extracted for two times. After centrifugation at 12,000 rpm for 10 min, the organic phase was dried over anhydrous Na_2SO_4. Conversion and *ee* of products were determined by GC or HPLC analysis.

2.3. Deracemization of various aryl secondary alcohols by combination of resting cells of M. oxydans ECU2010 and Rhodotorula sp. AS2.2241

To obtain enantiopure aryl secondary alcohols (*S*)-**1**, *rac*-**1** (20–40 mg) and resting cells of *M. oxydans* ECU2010 (wcw: 0.2 g) and *Rhodotorula* sp. AS2.2241 (wcw: 1.8 g) were suspended in Tris–HCl buffer (10 mL, 50 mM, pH 8.5). The reaction mixtures were incubated in a screw-capped 100-mL flask, shaken at 30 °C and 180 rpm for time necessary to obtain an appropriate conversion. The biotransformation was stopped by addition of the same volume of ethyl acetate and extracted for two times. After centrifugation at 12,000 rpm for 10 min, the organic phase was dried over anhydrous Na_2SO_4. Chemical yield and *ee* of the products were determined by GC or HPLC analysis. The product was identified by ^{1}H NMR analysis. The absolute configuration was determined by the specific rotation and comparison with the literature or by chiral GC or HPLC analysis and comparison with known racemates.

2.4. Assignment of the absolute configuration of the alcohols *1a–1g*

Compound (*S*)-**1a**: $[\alpha]_D^{25} = -58.2$ (*c* 1.03, $CHCl_3$) {lit. [22] $[\alpha]_D^{25} = -55.1$ (*c* 1.63, $CHCl_3$), *S*}.
Compound (*S*)-**1b**: $[\alpha]_D^{25} = -31.9$ (*c* 1.2, EtOH) {lit. [23] $[\alpha]_D^{25} = -29.7$ (*c* 2.59, EtOH), *S*}.
Compound (*S*)-**1c**: $[\alpha]_D^{25} = -29.5$ (*c* 1.11, $CHCl_3$) {lit. [24] $[\alpha]_D^{25} = -39.5$ (*c* 1.21, $CHCl_3$), *S*}.
Compound (*S*)-**1d**: $[\alpha]_D^{25} = -43.1$ (*c* 1.15, $CHCl_3$) {lit. [22] $[\alpha]_D^{25} = -37.9$ (*c* 1.13, $CHCl_3$), *S*}.
Compound (*S*)-**1e**: $[\alpha]_D^{25} = -30.6$ (*c* 0.98, $CHCl_3$) {lit. [25] $[\alpha]_D^{25} = -27.3$ (*c* 0.53, $CHCl_3$), *S*}.
Compound (*S*)-**1f**: $[\alpha]_D^{25} = -25.1$ (*c* 0.41, $CHCl_3$) {lit. [26] $[\alpha]_D^{25} = -30.3$ (*c* 0.996, $CHCl_3$), *S*}.
Compound (*S*)-**1g**: $[\alpha]_D^{25} = -39.9$ (*c* 1.07, MeOH) {lit. [27] $[\alpha]_D^{25} = -37.2$ (*c* 1.01, MeOH), *S*}.

The ^{1}H NMR spectra of these compounds were in agreement with those reported in the literatures [28–30].

3. Results and discussion

In our previous work, a novel microbial strain was isolated from soil and identified as *M. oxydans* ECU2010 [31], which could enantioselectively catalyze the dehydrogenation of (*R*)-enantiomer of *rac*-1-phenylethanol *rac*-**1a** to acetophenone **2a** in an *anti*-Prelog mode *via* a NAD^+-dependent (*R*)-alcohol dehydrogenase (ADH) initiated pathway, retaining (*S*)-1-phenylethanol (*S*)-**1a** in high enantioselectivity. A red yeast strain, *Rhodotorula* sp. AS2.2241, was also successfully isolated from soil samples, which could stereoselectively reduce **2a** to yield (*S*)-**1a** in excellent *ee* *via* a NADPH-dependent (*S*)-stereoselective ketoreductase (KER) [30,32,33].

3.1. Deracemization of rac-1-phenylethanol by whole-cell biotransformation

We attempted to put two kinds of biocatalysts together to realize the stereoinversion of (*R*)-**1a** to (*S*)-**1a** *via* a cascade one-pot process involving the enantioselective oxidation of (*R*)-**1a** to **2a** catalyzed by *M. oxydans* ECU2010 followed with the asymmetric

Table 1
Biocatalytic stereoinversion or deracemization of (*R*)-1-phenylethanol through a tandem stereoselective oxidation–reduction sequence with fresh cells of *Microbacterium oxydans* ECU2010 and *Rhodotorula* sp. AS2.2241.

Entry	Substrate	Concentration (mM)	*M. oxydans* ECU2010 (g L^{-1})	*Rhodotorula* sp. AS2.2241 (g L^{-1})	Time (h)	(*S*)-**1a**[a] (%)	*ee*[b] (%)
1	(*R*)-**1a**	30	20	180	32	97.2	>99
2	(*R*)-**1a**	50	20	180	48	85.1	92.9
3	*rac*-**1a**	30	20	180	24	98.2	>99
4	*rac*-**1a**	70	30	270	32	96.7	>99

[a] The relative amount was determined by GC analysis.
[b] The enantioselectivity was determined by GC analysis, $ee = ([S] - [R]/[S] + [R]) \times 100\%$, where [*S*] and [*R*] denote the concentrations of (*S*)-**1a** and (*R*)-**1a**.

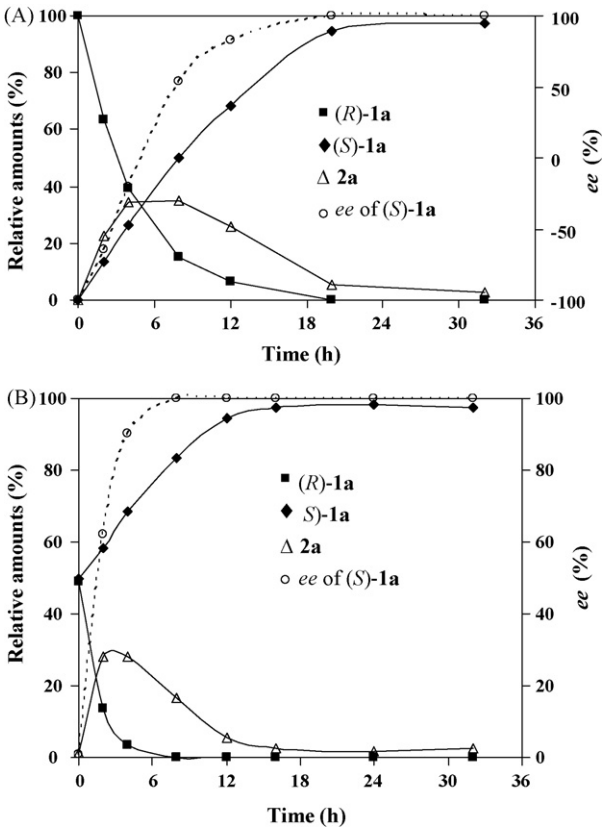


Fig. 1. Relative amounts of enantiomers of **1a** in (A) stereoinversion of (*R*)-**1a** (30 mM) and (B) deracemization of *rac*-**1a** (30 mM) with tandem whole-cell biocatalysts of ECU2010 and AS2.2241.

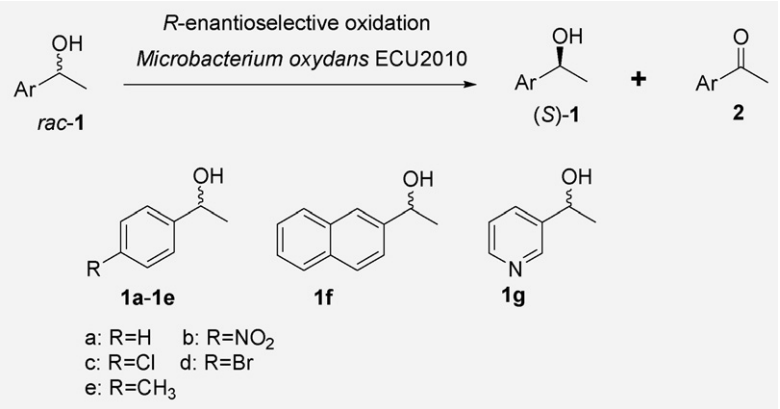
reduction of **2a** to (*S*)-**1a** catalyzed by *Rhodotorula* sp. AS2.2241 in tandem.

In initial explorative experiments, we attempted to combine the lyophilized cells of *M. oxydans* ECU2010 and *Rhodotorula* sp. AS2.2241 for stereoinversion of (*R*)-**1a** to (*S*)-**1a**. But the result was not satisfactory: only moderate yield and *ee* value were obtained. Similar results were given using lyophilized cell free extract (data not shown). As the lyophilization process will partially permeabilize the cell membrane, the *in vivo* cofactor regeneration system inside the cells may be affected. Further study revealed that the cell free extract (CFE) of *M. oxydans* ECU2010 can catalyze not only the oxidation of (*R*)-**1a** to **2a** using NAD^+ as cofactor, but also the reduction of **2a** to (*R*)-**1a** with NADPH as cofactor. The CFE (cell free extract) of *Rhodotorula* sp. AS2.2241 can catalyze the reduction of **2a** to (*S*)-**1a** and also the oxidation of (*S*)-**1a** to **2a** at the same time. The reaction rate of reduction was faster than that of oxidation. When using the freshly harvested cells as biocatalyst, only one direction of reaction happened, that is, *M. oxydans* ECU2010 only catalyze the selective oxidation of (*R*)-**1a** to **2a** and *Rhodotorula* sp. AS2.2241 only catalyze the asymmetric reduction of **2a** to (*S*)-**1a**. Indeed, the combination of freshly harvested cells of *M. oxydans* ECU2010 and *Rhodotorula* sp. AS2.2241 finally led to a successful system for concurrent stereoinversion.

Due to different catalytic activities, the resting cells of *M. oxydans* ECU2010 (8.9 U g^{-1} wet cell weight (wcw)) and *Rhodotorula* sp. AS2.2241 (0.15 U g^{-1} wcw) were combined at different ratios of wcw for stereoinversion of (*R*)-**1a** (Table 1). (*R*)-**1a** (30 mM) could be transformed into (*S*)-**1a** (>99% *ee*) in 97.2% yield with only a very little amount of **2a** (Table 1, entry 1) within 32 h. A typical time course of the stereoinversion process is shown in Fig. 1A, indicating that the oxidation step is the faster step of the overall reaction. When increasing the substrate concentration to 50 mM, (*S*)-**1a** was obtained in 85.1% yield with 92.9% *ee* (Table 1, entry 2).

As the stereoinversion process was quite efficient, we applied this method to the deracemization of *rac*-**1a**. It can be seen from Fig. 1B that the amount of (*R*)-**1a** decreased rapidly to zero at 8 h due to the oxidation; **2a** was formed and further transformed to

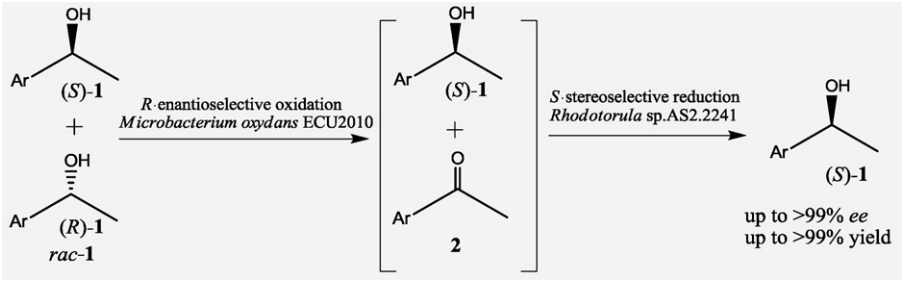
Table 2
Selective oxidation of various aryl secondary alcohols catalyzed by *Microbacterium oxydans* ECU2010.



Entry	Substrate	Time (h)	Conversion[a] (%)	*ee*[a] (%)	Configuration[c]
1	*rac*-**1a**	24	>49.9	>99	*S*
2	*rac*-**1b**	24	>49.9	>99	*S*
3	*rac*-**1c**	24	>49.9	>99	*S*
4	*rac*-**1d**	24	>49.9	>99	*S*
5	*rac*-**1e**	24	>49.9	>99	*S*
6	*rac*-**1f**	24	>49.9[b]	>99[b]	*S*
7	*rac*-**1g**	40	>49.9	>99	*S*

[a] Determined by GC analysis.
[b] Determined by HPLC using Chiralcel OD-H column.
[c] Absolute configurations were assigned by comparison of the specific rotation with the literature values.

Table 3
Deracemization of various aryl secondary alcohols through a tandem stereoselective oxidation–reduction sequence with fresh cells of *Microbacterium oxydans* ECU2010 and *Rhodotorula* sp. AS2.2241.



Entry	Substrate	*S*-**1**[a,b] (%)	*ee*[a] (%)	Configuration[d]
1	*rac*-**1a**	95.1 (56.3)	>99	*S*
2	*rac*-**1b**	98.2 (73.6)	>99	*S*
3	*rac*-**1c**	>99 (68.1)	>99	*S*
4	*rac*-**1d**	98.1 (84.3)	>99	*S*
5	*rac*-**1e**	98.3 (42.6)	>99	*S*
6	*rac*-**1f**	86.5[c] (53.6)	>99[c]	*S*
7	*rac*-**1g**	>99 (77.2)	>99	*S*

[a] Determined by GC analysis.
[b] Isolated yield in parentheses.
[c] Determined by HPLC using Chiralcel OD-H column.
[d] Absolute configurations were assigned by comparison of the specific rotation with the literature values.

(*S*)-**1a**; the amount of (*S*)-**1a** increased with time until 16 h; and nearly nothing happened between 16 h and 32 h. Finally, (*S*)-**1a** was obtained in 98.2% yield with >99% *ee* (Table 1, entry 3).

To achieve higher product concentration, deracemization of 70 mM *rac*-**1a** with higher concentrations of *M. oxydans* ECU2010 cell (30 g L^{-1}) and *Rhodotorula* sp. AS2.2241 cell (270 g L^{-1}) was performed, giving (*S*)-**1a** in 96.7% yield and >99% *ee* (Table 1, entry 4). The specific activity for the reduction with *Rhodotorula* sp. AS.2.4421 cells was rather low (0.15 U g^{-1} wcw), as compared to the oxidation with *M. oxydans* ECU2010 cells (8.9 U g^{-1} wcw). Therefore, engineering and use of a recombinant strain expressing highly active KER (ketoreductase) may be considered in the future to further improve the productivity of such a tandem biocatalysts system.

3.2. Selective oxidation of aryl secondary alcohols by M. oxydans ECU2010

To examine the generality of this method, several aryl secondary alcohols **1a**–**1g** were subjected to the enantioselective oxidation reactions catalyzed by *M. oxydans* ECU2010 in an *anti*-Prelog mode. The results are summarized in Table 2. *M. oxydans* ECU2010 displayed high yield (>49.9%) and excellent enantioselectivity (>99% *ee*) for all the substrates tested (**1a**–**1g**).

3.3. Deracemization of various aryl secondary alcohols

With the optimal conditions and the substrate range in hand, we explored the deracemization of aryl secondary alcohols (**1a**–**1g**) by resting cells of *M. oxydans* ECU2010 and *Rhodotorula* sp. AS2.2241 in one pot. A variety of aromatic alcohols bearing various *para*-substituents underwent the kinetic reaction with high enantioselectivity (>99%) and 95–99% yield in the process (Table 3, entries 1–5). Although (*S*)-1-(2-naphthyl)-ethanol (*S*)-**1f** was obtained in merely 86.5% yield, (*S*)-1-pyridin-3-ylethanol (*S*)-**1g** was achieved with excellent *ee* (>99%) and high yield (>99%) in 24 h.

4. Conclusions

In summary, a tandem biocatalysts system was successfully developed for the stereoinversion of (*R*)-1-phenylethanol and deracemization of various aryl secondary alcohols with resting cells of *M. oxydans* ECU2010 and *Rhodotorula* sp. AS2.2241. A variety of enantiopure (*S*)-aryl alcohols were successfully prepared in a one-pot procedure with good yield and excellent *ee*. Our method represents an easy going, cheap and environmentally benign way for the biocatalytic synthesis of chiral aryl secondary alcohols from their racemates.

Acknowledgements

The financial supports from National Natural Science Foundation of China (Grant Nos. 20402005 and 20773038), Ministry of Science and Technology (Grant Nos. 2006AA02Z205 and 2007AA02Z225) and China National Special Fund for State Key Laboratory of Bioreactor Engineering (Grant No. 2060204) are gratefully acknowledged.

References

[1] C.C. Gruber, I. Lavandera, K. Faber, W. Kroutil, Adv. Synth. Catal. 348 (2006) 1798–1805.
[2] A.J. Carnell, Adv. Biochem. Eng. Biotechnol. 63 (1999) 57–72.
[3] C.V. Voss, C.C. Gruber, W. Kroutil, Angew. Chem., Int. Ed. 47 (2008) 741–745.
[4] G.J. Cardus, A.J. Carnell, H. Trauthwein, T. Riermeir, Tetrahedron: Asymmetry 14 (2003) 711–715.
[5] T.M. Beard, N.J. Turner, Chem. Commun. 3 (2002) 246–247.
[6] L.F. Assis, E. Kagohara, A.T. Omori, J.V. Comasseto, L.H. Andrade, A.L.M. Porto, Food Technol. Biotechnol. 45 (2007) 415–419.
[7] J.V. Comasseto, L.F. Assis, L.H. Andrade, I.H.S. Crusius, A.L.M. Porto, J. Mol. Catal. B: Enzym. 39 (2006) 24–30.
[8] 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.
[9] J.V. Comasseto, A.T. Omori, L.H. Andrade, A.L.M. Porto, Tetrahedron: Asymmetry 14 (2003) 711–715.
[10] K. Nakamura, T. Matsuda, T. Harada, Chirality 14 (2002) 703–708.
[11] R.N. Patel, Curr. Opin. Biotechnol. 12 (2001) 587–604.
[12] U.T. Strauss, U. Felfer, K. Faber, Tetrahedron: Asymmetry 10 (1999) 107–117.
[13] K.B. Borges, W.S. Borges, R. D-Patron, M.T. Pupo, P.S. Bonato, I.G. Collado, Tetrahedron: Asymmetry 20 (2009) 385–397.
[14] S. Shekhar, B.D. Faulk, J. Am. Chem. Soc. 128 (2006) 6745–6754.
[15] A. Ajamian, J.L. Gleason, Angew. Chem., Int. Ed. 43 (2004) 3754–3760.
[16] C.V. Voss, C.C. Gruber, K. Faber, T. Knaus, P. Macherous, W. Kroutil, J. Am. Chem. Soc. 130 (2008) 13969–13972.
[17] Y. Xu, X. Jia, S. Panke, Z. Li, Chem. Commun. 12 (2009) 1481–1483.
[18] W. Hummel, B. Riebel, Ann. N. Y. Acad. Sci. 799 (1996) 713–716.
[19] G. Fantin, M. Fogagnolo, P.P. Giovannini, A. Medici, P. Pedrini, Tetrahedron: Asymmetry 6 (1995) 3047–3053.

[20] E. Takahashi, K. Nakamura, M. Furui, J. Ferment. Bioeng. 80 (1995) 247–250.
[21] L.S. Chen, S.M. Mantovani, L.G. Oliveira, M.C.T. Durate, A.J. Marsaioli, J. Mol. Catal. B: Enzym 54 (2008) 50–54.
[22] H. Nagaoka, Biotechnol. Prog. 20 (2004) 128–133.
[23] M.J. Homann, R.B. Vail, E. Previte, M. Tamarez, B. Morgan, D.R. Dodds, A. Zaks, Tetrahedron 60 (2004) 789–797.
[24] S.D. Yang, Y. Shi, Z.H. Sun, Y.B. Zhao, Y.M. Liang, Tetrahedron: Asymmetry 17 (2006) 1895–1900.
[25] H. Shimizu, D. Igarashi, W. Kuriyama, Y. Yusa, N. Sayo, T. Saito, Org. Lett. 9 (2007) 1655–1657.
[26] J.I. Ito, S. Ujiie, H. Nishiyama, Chem. Commun. 16 (2008) 1923–1925.
[27] K. Okano, K. Murata, T. Ikariya, Tetrahedron Lett. 41 (2000) 9277–9280.
[28] I.N. Lykakis, C. Tanielian, M. Orfanopoulos, Org. Lett. 5 (2003) 2875–2878.
[29] T. Itoh, Y. Matsushita, Y. Abe, S.H. Han, S. Wada, S. Hayase, M. Kawatsura, S. Takai, M. Morimoto, Y. Hirose, Chem. Eur. J. 12 (2006) 9228–9237.
[30] W. Yang, J.H. Xu, Y. Xie, Y. Xu, G. Zhao, G.Q. Lin, Tetrahedron: Asymmetry 17 (2006) 1769–1774.
[31] Y. Xu, L.N. Zhang, J.H. Xu, X.X. Chen, L. Ou, Method for manufacturing optically pure aryl sencondary alcohol with *Microbacterium oxydans*. Chinese Patent, CN 101016526, 2007-08-15.
[32] Y. Ni, J.H. Xu, J. Mol. Catal. B: Enzym. 18 (2002) 233–241.
[33] W. Yang, J.H. Xu, J. Pan, Y. Xu, Z.L. Wang, Biochem. Eng. J. 42 (2008) 1–5.