Journal of Biotechnology 168 (2013) 493–498

Contents lists available at ScienceDirect

Journal of Biotechnology

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

Scalable biocatalytic synthesis of optically pure ethyl (*R*)-2-hydroxy-4-phenylbutyrate using a recombinant *E. coli* with high catalyst yield

Ye Ni*, Yuning Su, Haidong Li, Jieyu Zhou, Zhihao Sun

The Key Laboratory of Industrial Biotechnology, Ministry of Education, School of Biotechnology, Jiangnan University, Wuxi 214122, Jiangsu, China

ARTICLE INFO

Article history:
Received 19 July 2013
Received in revised form
26 September 2013
Accepted 30 September 2013
Available online 10 October 2013

Keywords:
Asymmetric reduction
Coexpression
Carbonyl reductase
(*R*)-2-hydroxy-4-phenylbutanoate
Biocatalysis

ABSTRACT

Ethyl (*R*)-2-hydroxy-4-phenylbutanoate [(*R*)-HPBE] is a versatile and important chiral intermediate for the synthesis of angiotensin-converting enzyme (ACE) inhibitors. Recombinant E. coli strain coexpressing a novel NADPH-dependent carbonyl reductase gene iolS and glucose dehydrogenase gene gdh from *Bacillus subtilis* showed excellent catalytic activity in (*R*)-HPBE production by asymmetric reduction. IolS exhibited high stereoselectivity (>98.5% ee) toward α-ketoesters substrates, whereas fluctuant ee values (53.2–99.5%) for β-ketoesters with different halogen substitution groups. Strategies including aqueous/organic biphasic system and substrate fed-batch were adopted to improve the biocatalytic process. In a 1-L aqueous/octanol biphasic reaction system, (*R*)-HPBE was produced in 99.5% ee with an exceptional catalyst yield (gproduct/gcatalyst) of 31.7 via bioreduction of ethyl 2-oxo-4-phenylbutyrate (OPBE) at 330 g/L.

1. Introduction

Chiral alcohols are useful and important starting materials for the synthesis of various pharmaceuticals and other fine chemicals (Kataoka et al., 2003). In comparison with chemical methods, asymmetric reduction of prochiral carbonyl compounds using isolated enzymes or whole-cell systems is a promising method for the production of chiral alcohols, which has several advantages such as low cost, high yield, environmentally benign, and remarkable chemo-, regio-, and stereoselectivity (Xie et al., 2010; Ni et al., 2011; Jakoblinnert et al., 2013). One major challenge to chiral alcohols synthesis through bioreduction is expensive cofactors such as NAD(P)H/NAD(P)$^+$ are usually required. Several convenient and useful methods have been developed to solve this issue, including enzyme-coupled and substrate-coupled systems (Nakamura et al., 2003; Kroutil et al., 2004; Goldberg et al., 2007; Schroer et al., 2007; Hollmann et al., 2010; Savile et al., 2010; Wang et al., 2011).

Ethyl (*R*)-2-hydroxy-4-phenylbutanoate [(*R*)-HPBE] is a versatile and important chiral intermediate for the synthesis of angiotensin-converting enzyme (ACE) inhibitors, which are useful for the treatment of hypertension, such as enalapril, benazepril, and lisinopril etc. (Oda et al., 1998; Iwasaki et al., 1989). Various chemical and biological approaches have been explored for the synthesis of (*R*)-HPBE. Zhu et al. presented an effective strategy for the conversion of inexpensive OPBE directly into optically (*R*)-HPBE by asymmetric hydrogenation. And up to 92.6% enantiomeric excess (*ee*) was achieved with [RuCl(benzene)(S)-SunPhos]Cl as the catalyst and 1 M aq HBr as the additive (Zhu et al., 2011). A lipase from *Pseudomonas cepacia* was employed for the kinetic resolution of (*R*)-HPBE, resulting (*R*)-2-hydroxy ester with 99.5% *ee* and a space-time yield of 275 g/L d^{-1} (Liese et al., 2002). In our previous study, a highly potent carbonyl reductase-producing strain *Candida krusei* SW2026 was isolated for the enantioselective reduction of OPBE, giving (*R*)-HPBE in 97.4% *ee* and 82.0% yield at 20 g/L of OPBE in water/dibutyl phthalate biphasic system. Furthermore, the carbonyl reductase from *C. krusei* was purified to homogeneity through three chromatography columns and its enzymatic properties were investigated (Zhang et al., 2009; Li et al., 2010). In recent years, asymmetric synthesis of (*R*)-HPBE catalyzed by recombinant reductases has shown significant advantages. A recombinant *E. coli* BL21 overexpressing YiaE from *E. coli* and glucose dehydrogenase (GDH) from *Bacillus subtilis* was constructed, resulting (*R*)-HPBE in 98% *ee* (Yun et al., 2005). A recombinant *E. coli* strain harboring both CgKR2 and GDH encoding genes was successfully applied in preparing (*R*)-HPBE with desirable *ee* and yield (99% and 100%, respectively) at 1 M (approximately 206 g/L) OPBE, and the space-time yield of (*R*)-HPBE production reached 700 g/L d^{-1} (Shen et al., 2012).

In preliminary study, a NADPH-dependent carbonyl reductase gene *iolS* was cloned from *B. subtilis* and co-expressed with *gdh* gene for cofactor regeneration, and the recombinant *E. coli* strain was

* Corresponding author. Tel.: +86 0510 85329265; fax: +86 0510 85329265.
E-mail addresses: yni@jiangnan.edu.cn, niyer75@hotmail.com (Y. Ni).

0168-1656/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jbiotec.2013.09.021

applied for (*R*)-HPBE synthesis in aqueous phase (Su et al., 2012). In this study, the kinetics, substrate specificity and stereoselectivity of purified IolS were characterized. To solve the substrate-tolerance obstacle and enhance the process productivity, substrate feeding and aqueous/organic biphasic system were successfully adopted, giving an unprecedented catalyst yield of 31.7 and a space-time yield of 660 g/L d^{-1}.

2. Material and methods

2.1. Protein expression

For the recombinant expression of carbonyl reductase *iolS*, *E. coli*/pET20b-*iolS* (Su et al., 2012) was cultivated in LB medium and induced with 0.4 mM IPTG at 25 °C when the OD_{600} reached around 0.6. To achieve high-level expression of both *iolS* and *gdh*, two-enzyme coexpressed strain *E. coli* BL21(DE3)/pET-G-T7-I (Su et al., 2012) was grown in LB medium containing ampicillin and kanamycin and induced with 0.8 mM IPTG at 25 °C when the OD_{600} reached around 0.6.

2.2. Enzymatic activity and stereoselectivity assays

Carbonyl reductase IolS and GDH activities were determined spectrophotometrically by monitoring the increase or decrease in the absorption of NADPH at 340 nm under 30 °C. The reaction mixture for IolS was composed of 100 mM phosphate buffer (pH 6.0), 1.0 mM OPBE, 5.0 mM NADPH, and an appropriate amount of enzyme in a final volume of 0.25 mL. The reaction mixture for GDH comprised 75 mM Tris-HCl (pH 8.0), 2.0 mM $NADP^+$, 0.1 mM glucose, and an appropriate amount of enzyme in a final volume of 0.25 mL. One unit of activity was defined as the amount of enzyme required for catalyzing the oxidation of 1 μmol of NADPH per minute (IolS) or the reduction of 1 μmol $NADP^+$ per minute (GDH). The protein concentration was measured by Bradford method, using bovine serum albumin as the standard.

The *ee* values of (*R*)-HPBE and other chiral products were determined using Varian CP 3900 gas chromatograph (USA) equipped with Chirasil-Dex CB column (CP 7502, 25 m × 0.25 mm × 0.25 m, VARIAN, USA).

2.3. Protein purification

Briefly, the recombinant cells were harvested after 4 h induction, and resuspended in buffer A (20 mM sodium phosphate, pH 7.4, containing 500 mM NaCl and 5 mM imidazole). The cells were disrupted by ultrasonication (285 W, pulse 1 s, pause 3 s) for 10 min. Cell debris was removed by centrifugation at 8000 × *g* for 15 min at 4 °C, and the supernatant was applied to a HisTrap-FF crude chelating affinity column equilibrated with buffer A. The bound enzyme was eluted by applying a stepwise gradient of imidazole concentration, using buffer A containing 5 mM imidazole to 1 M imidazole. The protein samples were analyzed by SDS–PAGE.

2.4. Substrate specificity of IolS and optimization of OPBE bioreduction

Substrate specificity and stereoselectivity of IolS were determined using various α- and β-ketoesters, as well as aromatic ketones. Reaction mixture containing 1 g wet cells (ca. 0.18 g dry cells), 50 g/L glucose, 0.1 mM $NADP^+$, 1 g/L of each substrate in 20 mL potassium phosphate buffer (0.1 mol/L, pH 6.0) was carried out at 30 °C for 24 h. The supernatant was separated by centrifugation (12,000 × *g*, 15 min) and extracted using ethyl acetate. The organic layer was dried using anhydrous magnesium sulfate, and the supernatant was subjected to chiral GC analysis to determine the product yield and *ee* value as described in Section 2.2.

Glucose concentration was optimized in the asymmetric reduction of OPBE by recombinant *E. coli* cells. Reaction mixture containing 1 g wet cells (ca. 0.18 g dry cells), 20–400 g/L glucose, 0.1 mM $NADP^+$, 20 g/L of OPBE in 20 mL potassium phosphate buffer (0.1 mol/L, pH 6.0) and 20 mL octanol was carried out at 30 °C for 15 h.

Various amount of $NADP^+$ was supplemented in the reaction mixture to evaluate its effect on the asymmetric reduction of OPBE by recombinant *E. coli* cells. The reaction mixture containing 1 g wet cells (ca. 0.18 g dry cells), 200 g/L glucose, 20 g/L of OPBE, $NADP^+$ (0, 0.05, 0.1 mM) in 20 mL potassium phosphate buffer (0.1 mol/L, pH 6.0) and 20 mL octanol, and was carried out at 30 °C and 15 h.

2.5. Bioreduction of OPBE to (R)-HPBE in a 1 L biphasic system

The reaction mixture (1 L) contained 60 g wet cells (ca. 10.4 g dry cells), 200 g/L glucose, and 0.05 mM $NADP^+$ in a biphasic system of 500 mL potassium phosphate buffer (0.1 mol/L, pH 6.0) and 500 mL octanol. Substrate OPBE (30 g) was fed once per hour. The biphasic bioreduction was performed with magnetic agitation at 30 °C for 12 h. After the reaction, the mixture was centrifuged (8000 rpm for 10 min) for the separation of two phases. The organic layer was analyzed directly by GC and evaporated under reduced pressure in an oil bath to afford 263.9 g of optically pure (*R*)-HPBE with >98% chemical purity, 99.5% *ee* and 79.2% overall yield. The structure of (*R*)-HPBE was also confirmed by ^1H and ^{13}C NMR analysis as follows: ^1H NMR ($CDCl_3$ 400 MHz): δ1.29 (3H, t, OCH_2CH_3), 1.90–1.99 (1H, m, $ArCH_2CHH$), 2.07–2.16 (1H, m, $ArCH_2CHH$), 2.73–2.79 (2H, m, $ArCH_2$), 3.14 (1H, s, O*H*), 4.16–4.22 (3H, m, OCH_2CH_3,*CH*OH), 7.16–7.29 (5H, m, Ar-*H*); ^{13}C NMR ($CDCl_3$ 100 MHz): δ14.11, 30.96, 35.90, 61.64, 69.63, 125.94, 128.37, 141.13, 175.14.

3. Results and discussion

3.1. Kinetic parameters of IolS

In preliminary study, a 933-bp *iolS* gene (GenBank accession No. JQ782389) from *B. subtilis* was cloned and expressed in *E. coli*. It encodes 310 amino acids with a calculated molecular mass of 36 kDa and an estimated pI of 5.50 (Su et al., 2012). Based on amino acid sequence analysis, IolS belongs to aldo-keto reductases (AKRs) superfamily. His-tagged recombinant protein IolS was purified to electrophoretic homogeneity by HisTrap-FF crude chelating affinity column as confirmed by SDS-PAGE. The specific activity of purified IolS was 5.1 U mg^{-1}, corresponding to a 3.4-fold increase in activity compared with crude extract.

As the maximum reaction rate of carbonyl reductase IolS was attained at 5.0 mM OPBE and 2.0 mM NADPH, its kinetic parameters were estimated over concentration ranges of OPBE (0.1–5.0 mM) and NADPH (0.04–2.0 mM) at 30 °C in phosphate buffer. The V_{max} and K_m were obtained from Lineweaver–Burk plot, specifically, 2.61 mM and 4.18 μmol/min·mg for OPBE, 0.69 mM and 5.26 μmol/min mg for NADPH. Compared with other carbonyl reductases (Table 1), IolS exhibited higher K_m (2.61 mM) toward substrate OPBE than that from *Candida glabrate* (0.1 mM), while lower K_m than those from *Trichosporon fermentans* (20.1 mM) and *Candida magnoliae* (7.9 mM). The results indicate that IolS has a moderate K_m and reaction rate compared with previous reported reductases. Also, K_{cat} and K_{cat}/K_m for OPBE and NADPH were calculated to be 0.409 s^{-1} and 0.157 mM^{-1} s^{-1}, 0.515 s^{-1} and 0.746 mM^{-1} s^{-1}, respectively.

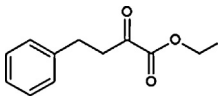
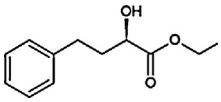
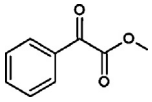
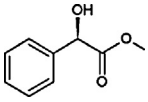
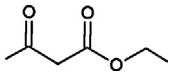
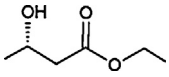
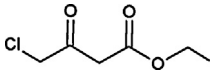
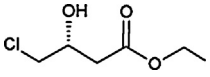
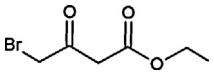
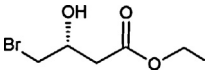
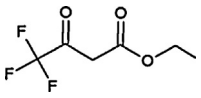
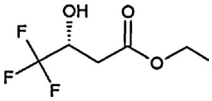
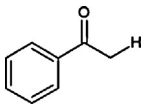
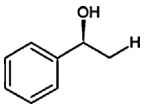
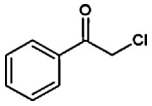
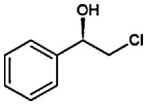
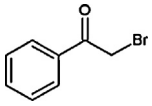
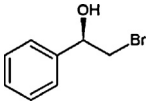
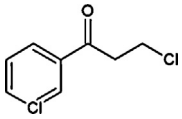
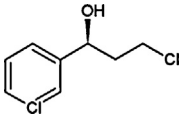
Table 1
Comparison of kinetic parameters of carbonyl reductases originated from different microorganisms.

Microorganism	K_m (mM)	V_{max} (μmol/min mg)	References
Trichosporon fermentans	20.1	3.4	Kira and Onishi (2009)
Candida magnoliae	7.9	20.1	Lee et al. (2003)
Candida viswanathii	0.153	59.2	Soni et al. (2007)
Candida glabrate	0.1	18.5	Shen et al. (2012)
Bacillus subtilis	2.61	4.18	This study

3.2. Substrate specificity and stereoselectivity of IolS

The substrate specificity and stereoselectivity of carbonyl reductase IolS toward various α- and β-ketoesters, as well as aromatic ketones were explored (Table 2). The results indicate that IolS exhibited high stereoselectivity and activity toward α-ketoesters, such as methyl benzoylformate and OPBE. For β-ketoesters, it was noticed that a substitution of halogen group at the 4-position of ethyl 3-oxo-butyrate affects the enantioselectivity significantly, the higher the atomic weight or number of halogen substitution group, the higher the enantioselectivity of product. In comparison with 99.5% and 90.1% *ee* observed for substrates ethyl 4,4,4-trifluoroacetoacetate and ethyl 4-bromoacetoacetate, a significantly lower optical purity of 53.2% was attained for ethyl 4-chloroacetoacetate. Moreover, the enzyme showed no activity toward acetophenone and its various derivatives, while some activity was observed in an ortho-chloro substituent on benzene ring.

Table 2
Asymmetric reduction of various carbonyl compounds by IolS.

	Substrate	Product	Conversion (%)[a]	*ee* (%)[b]
2-Oxo-4-phenylbutyrate			>99	99.5 (*R*)
methyl 2-Oxo-2-phenyl-acetate			>99	98.5 (*R*)
Ethyl acetoacetate			No conversion	
Ethyl 4-Chloro-3-oxobutyrate			>99	53.2 (*R*)[c]
Ethyl 4-Bromoacetoacetate			>99	90.1 (*R*)[c]
Ethyl 4,4,4-Trifluoroacetoacetate			>99	99.5 (*R*)
phenyl methyl ketone			No conversion	
2-Chloroacetophenone			No conversion	
2-Bromoacetophenone			No conversion	
2-Chloro-(3-chlorophenyl)ethanone			>99	32.5 (*R*)

[a] Measured by chiral GC analysis.
[b] The *ee* values were measured by chiral GC or HPLC analysis.
[c] Determined by chiral GC analyses after acetylation of the product.
Reaction conditions: 1 g wet cells (ca. 0.18 g dry cells), 50 g/L glucose, 0.1 mM $NADP^+$, 1 g/L of each substrate in 20 mL potassium phosphate buffer (0.1 mol/L, pH 6.0), 30 °C, 24 h.

Table 3
Screening of organic solvents in the biphasic system for the asymmetric reduction of OPBE.

Organic solvent	Log *P*	Partition coefficient		Conversion (%)
		OPBE	(*R*)-HPBE	
None	–	–	–	60.2
Isobutanol	0.61	19.9	9.2	0.1
Tertiary amyl alcohol	0.89	8.5	10.7	0.1
Ethyl acetate	1.7	11.2	16.3	5.4
octanol	2.9	19.1	29.7	81.6
Cyclohexane	3.2	12.1	30.0	80.9
n-Hexane	3.5	34.3	22.4	24.9
Nonane	5.1	14.8	13.3	9.0
Oleic acid	7.7	10.3	9.2	5.9

Partition coefficient: the molar ratio of the amount of certain compound in the organic phase to its amount in the aqueous phase. Reaction conditions: 1 g wet cells (ca. 0.18 g dry cells), 50 g/L glucose, 0.1 mM $NADP^+$, 10 g/L of OPBE in 20 mL potassium phosphate buffer (0.1 mol/L, pH 6.0) and 20 mL organic solvent, 30 °C, 15 h.

3.3. Aqueous/organic biphasic system for the asymmetric reduction of OPBE

In order to enhance the substrate concentration and thus accumulate higher product titer, it is often necessary to develop strategies to alleviate substrate inhibition on the biocatalysts. Herein, we endeavored to conduct the reaction in aqueous/organic biphasic system, a commonly adopted method to overcome the substrate-tolerance obstacle (Li et al., 2007; Carrea and Riva, 2000). With the introduction of organic solvents, the solubility of the water-insoluble substrate could be improved, and the hydrophilic microbial cells (in aqueous phase) could be isolated from the hydrophobic substrate and the newly generated product (in organic phase). As shown in Table 3, eight different organic solvents were chosen, and octanol (log *P* = 2.9) was determined to be the most suitable solvent among various biphasic systems, considering its excellent partition coefficient (19.1 for OPBE and 29.7 for (*R*)-HPBE) and higher product yield (81.6%) compared with aqueous system (60.2%). The asymmetric reaction catalyzed by immobilized cells was however not successful due to the compromised stability and integrity of immobilized particles in the presence of solvents (data not shown).

Therefore, despite of the toxicity of organic solvents, microbial cells demonstrated high activity in aqueous/octanol (1:1) biphasic system owing to the protection of potassium phosphate buffer and alleviated substrate/production inhibition by octanol phase. In this study, concentrations of substrate and product were calculated based on the volume of organic phase.

3.4. Effect of glucose concentration on the asymmetric reduction of OPBE

Glucose, functioning as electron donor, plays an important role in cofactor recycling machinery, and is consequently essential to the smooth proceeding of the biocatalytic reduction (Zhang and Pionnier, 2003). The effect of glucose concentration on the asymmetric reduction of OPBE was investigated (Fig. 1). The product yield increased continuously with increasing in glucose concentration from 20 g/L to 200 g/L, and then stabilized at around 80.5% when glucose concentration was further enhanced up to 400 g/L.

3.5. Effect of $NADP^+$ concentration on the asymmetric reduction of OPBE

Here, the effect of $NADP^+$ concentration on the asymmetric reduction of OPBE was studied, aiming at a lower concentration of the expensive cofactor $NADP^+$. Time courses of bioreduction supplemented with various $NADP^+$ concentrations were shown in Fig. 2. It suggests that a higher amount of $NADP^+$ gave faster bioreduction, while a slightly decreased yield of 67.9% was observed when there is no $NADP^+$ was added. Our result suggests that merely

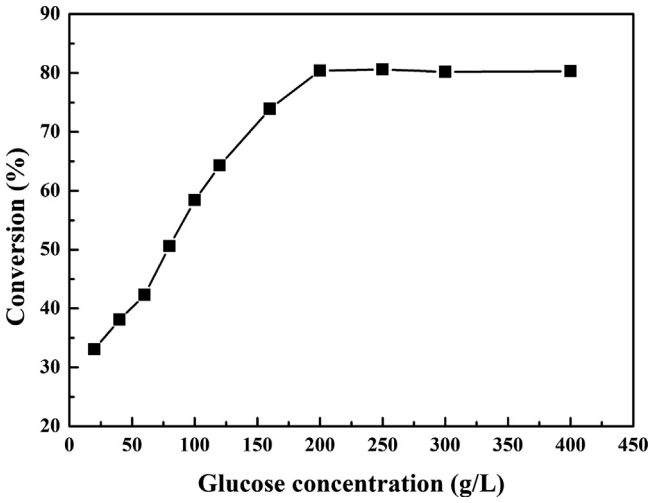


Fig. 1. Effect of glucose concentration on the asymmetric reduction of OPBE. Reaction conditions: 1 g wet cells (ca. 0.18 g dry cells), glucose, 0.1 mM $NADP^+$, 20 g/L of OPBE in 20 mL potassium phosphate buffer (0.1 mol/L, pH 6.0) and 20 mL octanol, 30 °C, 15 h.

0.05 mM $NADP^+$ was sufficient for the bioreduction of OPBE to (*R*)-HPBE.

Similar to Section 3.4, a moderate substrate conversion of 80–85% was achieved when a relative low enzyme loading (1 g wet cells/20 mL buffer) was applied. Using a higher biocatalyst amount (2.4 g wet cells/20 mL buffer), substrate conversion of >99% could be attained at an accumulated substrate concentration of 330 g/L (Fig. 3, results in Section 3.6).

3.6. (R)-HPBE production with substrate feeding in a 1-L biphasic system

In our preliminary studies, 80.5% conversion ratio was attained at 20 g/L OPBE. To enhance the substrate concentration as well as

Table 4
Parameters of asymmetric reduction of OPBE to (*R*)-HPBE in a 1-L biphasic system.

Parameter	
Substrate loading/g/L	330
Reaction time/h	12
Biocatalyst (dry cells) loading/g/L	10.4
Conversion/%	>99%
Chemical purity/%	>98%
ee/%	99.5%
Space-time yield/g/L d^{-1}	660
Catalyst yield ($g_{product}/g_{cat}$)	31.7
Overall yield/%	79.2

Table 5
Comparison of biocatalytic parameters of (*R*)-HPBE and its derivative production by asymmetric reduction in previous reports and this study.

Substrate loading (g/L)	NADPH (mmol L^{-1})	Catalyst yield ($g_{product}/g_{cat}$)	Product *ee* (%)	Space-time yield (g/L d^{-1})	Scale (L)	References
1.4	0	0.368	81	1.4	0.1	Lacerda et al. (2006)
20	1.0	2.5	98	1.7	0.05	Yun et al. (2005)
1.03	0	0.02	92	1.03	0.05	Shi et al. (2009)
206	0	4.12	>99	700	0.01	Shen et al. (2012)
330	0.05	31.7	>99	660	1	This study

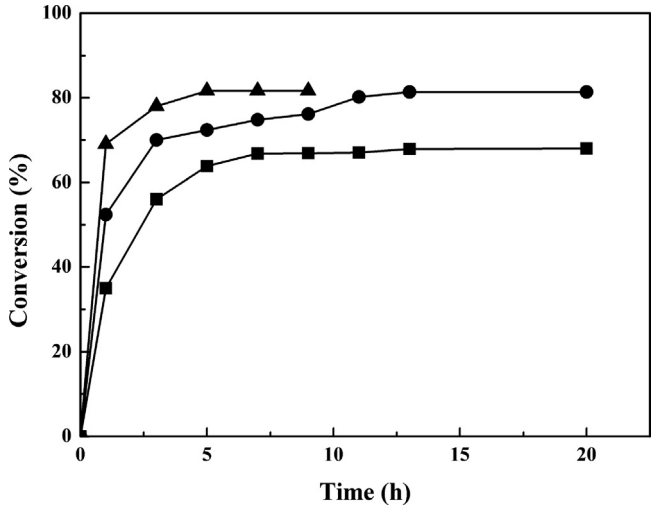


Fig. 2. Reduction of OPBE by recombinant *E. coli* cells supplemented with 0 – 0.1 mM $NADP^+$. (▲) 0.1 mM $NADP^+$; (●) 0.05 mM $NADP^+$; (■) no $NADP^+$. Reaction conditions: 1 g wet cells (ca. 0.18 g dry cells), 200 g/L glucose, $NADP^+$, 20 g/L of OPBE in 20 mL potassium phosphate buffer (0.1 mol/L, pH 6.0) and 20 mL octanol, 30 °C, 15 h.

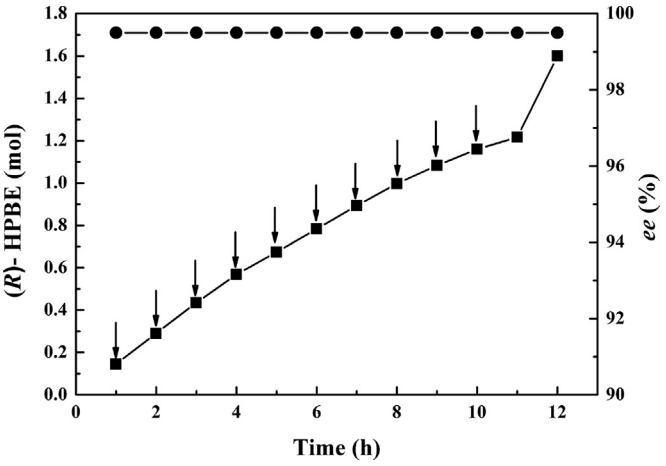


Fig. 3. Time course of (*R*)-HPBE production in a water-octanol biphasic system with substrate feeding. (●) *ee*; (■) concentration of (*R*)-HPBE. Reaction conditions: 60 g wet cells (ca. 10.4 g dry cells), 200 g/L glucose, 0.05 mM $NADP^+$, 500 mL potassium phosphate buffer (0.1 mol/L, pH 6.0) and 500 mL octanol, OPBE (30 g) was fed once per hour. The two phase bioreaction was performed by magnetic agitation at 30 °C for 12 h.

productivity, a substrate feeding strategy was attempted (Fig. 3). In a 1-L aqueous/octanol biphasic reaction system, substrate OPBE (total 330 g) was fed once (30 g) per hour. At the same time, the reaction system was titrated with 1 M Na_2CO_3 solution to neutralize the gluconic acid generated in glucose oxidation. As no substrate was added after 10th h, a slightly faster increase in product was noted. It is also likely due to the enhanced membrane permeability (or even enzyme leakage) in the end of reaction. After 12 h of reaction, a surprisingly high substrate conversion of >99% and space-time yield of 660 g/L d^{-1} were obtained, which was attributed to the biphasic system and substrate-feeding strategies adopted in this study. The catalytic parameters are summarized in Table 4. Compared with previous reports (as shown in Table 5), substrate loading of 330 g/L was reached in this study, which is 1.6 times higher than the highest value reported so far (Shen et al., 2012). Additionally, a stunning catalyst yield of 31.7 was attained in this study, representing the highest catalyst yield in the asymmetric reduction of OPBE. In previous reports, considerably lower catalyst yields of 0.02–4.12 were reached (Lacerda et al., 2006; Yun et al., 2005; Shi et al., 2009; Shen et al., 2012). Moreover, this highly efficient reaction system was conducted in liter scale, which is more practical in contrast with previous studies carried out in volumes of 10–100 mL. As far as we know, this is the first report on the application of IolS from *Bacillus sp.* as an excellent biocatalyst for the asymmetric reduction of OPBE, and the product *ee* of 99.5% is one of the highest among recombinant strains reported so far. Therefore, IolS is a robust biocatalyst with great potential in enantioselective reduction of OPBE to produce (*R*)-HPBE.

4. Conclusion

In this study, recombinant *E. coli* strain coexpressing *iolS* and *gdh* was successfully applied in the asymmetric reduction of OPBE to (*R*)-HPBE. IolS demonstrated over 98.5% enantioselectivity toward α-ketoesters, whereas moderate (53.2% *ee*) to excellent (99.5% *ee*) enantioselectivity toward β-ketoesters substrates. In a 1-L biphasic system, the recombinant *E. coli* cells gave (*R*)-isomer of 99.5% *ee* with an unprecedented catalyst yield of 31.7 and high conversion (>99%) at 330 g/L OPBE.

Acknowledgements

We are grateful to the National Basic Research and Development Program of China (973 Program, 2011CB710800), Natural Science Foundation of China (21276112), New Century Excellent Talents in University (NCET-11-0658), Natural Science Foundation of Jiangsu Province (BK2011150), the Program of Introducing Talents of Discipline to Universities (111-2-06), and a Project Funded by the Priority Academic Program, for the financial support of this research.

References

Carrea, G., Riva, S., 2000. Properties and synthetic applications of enzymes in organic solvents. Angew. Chem. Int. Ed. Engl. 39, 2226–2254.
Goldberg, K., Schroer, K., Lütz, S., Liese, A., 2007. Biocatalytic ketone reduction – a powerful tool for the production of chiral alcohols – part I: processes with isolated enzymes. Appl. Microbiol. Biotechnol. 76, 237–248.
Hollmann, F., Arends, I.W.C.E., Buehler, K., 2010. Biocatalytic redox reactions for organic synthesis: nonconventional regeneration methods. Chem. Cat. Chem. 2, 762–782.
Iwasaki, G., Kimura, R., Numao, N., Kondo, K., 1989. A practical and diastereoselective synthesis of angiotensin converting enzyme-inhibitors. Chem. Pharm. Bull. 37, 280–283.
Jakoblinnert, A., van den Wittenboer, A., Shivange, A.V., Bocola, M., Heffele, L., Ansorge-Schumacher, M., Schwaneberg, U., 2013. Design of an activity and stability improved carbonyl reductase from *Candida parapsilosis*. J. Biotechnol. 165, 52–62.
Kataoka, M., Kita, K., Wada, M., Yasohara, Y., Hasegawa, J., Shimizu, S., 2003. Novel bioreduction system for the production of chiral alcohols. Appl. Microbiol. Biotechnol. 62, 437–445.

Kira, I., Onishi, N., 2009. Purification and characterization of a (*R*)-1-phenyl-1,3-propanediol-producing enzyme from *Trichosporon fermentans* AJ-5152 and enzymatic (*R*)-1-phenyl-1,3-propanediol production. Biosci. Biotechnol. Biochem. 73, 1640–1646.

Kroutil, W., Mang, H., Edegger, K., Faber, K., 2004. Recent advances in the biocatalytic reduction of ketones and oxidation of sec-alcohols. Curr. Opin. Chem. 8, 120–126.

Lacerda, P.S.B., Ribeiro, J.B., Leite, S.G.F., Ferrara, M.A., Coelho, R.B., Bon, E.P.S., Silvalima, E.L., Antunes, O.A.C., 2006. Microbial reduction of ethyl 2-oxo-4-phenylbutyrate. Searching for (*R*)-enantioselectivity. New access to the enalapril like ACE inhibitors. Tetrahedron Asymmetr. 17, 1186–1188.

Lee, J.K., Kim, S.Y., Ryu, Y.W., 2003. Purification and characterization of a novel erythrose reductase from *Candida magnolia*. Appl. Environ. Microbiol. 69, 3710–3718.

Li, N., Ni, Y., Sun, Z.H., 2010. Purification and characterization of carbonyl reductase from *Candida krusei* SW2026 involved in enantioselective reduction of ethyl 2-oxo-4-phenylbutyrate. J. Mol. Catal. B-Enzym. 66, 190–197.

Li, Y.N., Shi, X.A., Zong, M.H., Meng, C., Dong, Y.Q., Guo, Y.H., 2007. Asymmetric reduction of 2-octanone in water/organic solvent biphasic system with Baker's yeast FD-12. Enzyme Microb. Technol. 40, 1305–1311.

Liese, A., Kragl, U., Kierkels, H., Schulze, B., 2002. Membrane reactor development for the kinetic resolution of ethyl 2-hydroxy-4-phenylbutyrate. Enzyme Microb. Technol. 30, 673–681.

Nakamura, K., Yamanaka, R., Matsuda, T., Harada, T., 2003. Recent developments in asymmetric reduction of ketones with biocatalysts. Tetrahedron Asymmetr. 14, 2659–2681.

Ni, Y., Li, C.X., Ma, H.M., Zhang, J., Xu, J.H., 2011. Biocatalytic properties of a recombinant aldo-keto reductase with broad substrate spectrum and excellent stereoselectivity. Appl. Microbiol. Biotechnol. 89, 1111–1118.

Oda, S., Inada, Y., Kobayashi, A., Ohta, H., 1998. Production of ethyl (*R*)-2-hydroxy-4-phenylbutanoate via reduction of ethyl 2-oxo-4-phenylbutanoate in an interface bioreactor. Biosci. Biotechnol. Biochem. 62, 1762–1767.

Savile, C.K., Janey, J.M., Mundorff, E.C., Moore, J.C., Tam, S., Jarvis, W.R., Colbeck, J.C., Krebber, A., Fleitz, F.J., Brands, J., Devine, P.N., Huisman, G.W., Hughes, G.J., 2010. Biocatalytic asymmetric synthesis of chiral amines from ketones applied to sitagliptin manufacture. Science 329, 305–309.

Schroer, K., Mackfeld, U., Tan, I.A., Wandrey, C., Heuser, F., Bringer-Meyer, S., Weckbecker, A., Hummel, W., Daussmann, T., Pfaller, R., Liese, A., Lütz, S., 2007. Continuous asymmetric ketone reduction processes with recombinant *Escherichia coli*. J. Biotechnol. 132, 438–444.

Shen, N.D., Ni, Y., Ma, H.M., Wang, L.J., Li, C.X., Zheng, G.W., Zhang, J., Xu, J.H., 2012. Efficient synthesis of a chiral precursor for angiotensin-converting enzyme (ACE) inhibitors in high space-time yield by a new reductase without external cofactors. Org. Lett. 14, 1982–1985.

Shi, Y.G., Fang, Y., Ren, Y.P., Guan, H.L., Zhang, J.Y., 2009. Applying alpha-phenacyl chloride to the enantioselective reduction of ethyl 2-oxo-4-phenylbutyrate with baker's yeast. J. Chem. Technol. Biotechnol. 84, 681–688.

Soni, P., Kansal, H., Banerjee, U.C., 2007. Purification and characterization of an enantioselective carbonyl reductase from *Candida viswanathii* MTCC 5158. Process Biochem. 42, 1632–1640.

Su, Y., Ni, Y., Wang, J., Xu, Z., Sun, Z., 2012. Construction of two-enzyme coexpression recombinant strain for the asymmetric synthesis of optically pure ethyl (*R*)-2-hydroxy-4-phenylbutyrate. Chin. J. Catal. 33, 1650–1660.

Wang, L.J., Li, C.X., Ni, Y., Zhang, J., Liu, X., Xu, J.H., 2011. Highly efficient synthesis of chiral alcohols with a novel NADH-dependent reductase from *Streptomyces coelicolor*. Bioresour. Technol. 102, 7023–7028.

Xie, Y., Xu, J.H., Xu, Y., 2010. Isolation of a *Bacillus* strain producing ketone reductase with high substrate tolerance. Bioresour. Technol. 101, 1054–1059.

Yun, H., Choi, H.L., Fadnavis, N.W., Kim, B.G., 2005. Stereospecific synthesis of (*R*)-2-hydroxy carboxylic acids using recombinant *E. coli* BL21 overexpressing YiaE from *Escherichia coli* K12 and glucose dehydrogenase from *Bacillus subtilis*. Biotechnol. Prog. 21, 366–371.

Zhang, B.L., Pionnier, S., 2003. Cofactor recycling mechanism in asymmetric biocatalytic reduction of carbonyl compounds mediated by yeast: which is the efficient electron donor? Chem. Eur. J. 9, 3604–3610.

Zhang, W., Ni, Y., Sun, Z.H., Zheng, P., Lin, W.Q., Zhu, P., Ju, N.F., 2009. Biocatalytic synthesis of ethyl (*R*)-2-hydroxy-4-phenylbutyrate with *Candida krusei* SW2026: a practical process for high enantiopurity and product titer. Process Biochem. 44, 1270–1275.

Zhu, L.F., Chen, H.H., Meng, Q.H., Fan, W.Z., Xie, X.M., Zhang, Z.G., 2011. Highly enantioselective hydrogenation of 2-oxo-4-arybutanoic acids to 2-hydroxy-4-arylbutanoic acids. Tetrahedron 67, 6186–6190.