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Overexpression of *Serratia marcescens* lipase in *Escherichia coli* for efficient bioresolution of racemic ketoprofen

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Abstract

Lipase from *Serratia marcescens* ECU1010 was cloned and overexpressed in *E. coli*. After optimization, the maximum lipase activities reached 5000–6000 U/l and this recombinant lipase could enantioselectively hydrolyze (*S*)-ketoprofen esters into (*S*)-ketoprofen. Among six alkyl esters of racemic ketoprofen investigated, this lipase showed the best enantioselectivity for the kinetic resolution of ketoprofen ethyl ester, with an ee_p (enantiomeric excess of product) of 91.6% and *E*-value of 63 obtained at 48.2% conversion. Twelve nonionic surfactants were tested for enhancing the enantioselectivity of this lipase in the bioresolution of ketoprofen ethyl ester. A very high *E*-value of 1084 was achieved, with an optical purity of >99% ee_p and a yield of 42.6% in the presence of 3% Brij 92V. Further studies showed that the selectivity of the lipase was improved with the increase of Brij 92V concentration. The substrate (ketoprofen ethyl ester) does not inhibit the lipase activity, while the product (*S*)-ketoprofen inhibits the lipase activity to some extent. These results indicate that the *S. marcescens* lipase is very useful for biocatalytic production of chiral profens such as (*S*)-ketoprofen.

Keywords: *Serratia marcescens* lipase; Chiral resolution; Ketoprofen; Surfactant; Overexpression

1. Introduction

Lipases (EC 3.1.1.3) are ubiquitous enzymes that catalyze the hydrolysis of fats and oils, playing important roles in pharmaceutical, fine chemical industries and other industrial areas such as detergents, oil/fats, cheese making, hard-surface cleaning, leather and paper processing, etc. [1,2]. Among many successful examples of lipases application, the lipase from *Serratia marcescens* (SmL) is well-known in pharmaceutical industry for its excellent enantioselectivity in biocatalytic hydrolysis of *trans*-3-(4′-methoxyphynyl)glycidic acid methyl ester [(±)-MPGM] to produce (2*R*, 3*S*)-3-(4′-methoxyphenyl)glycidic acid methyl ester [(−)-MPGM], a key intermediate for the synthesis of diltiazem hydrochloride [3]. Recently, Bae et al. [4] have reported that a lipase from *S. marcescens* ES-2 was used for kinetic resolution of racemic flurbiprofen, giving an optically pure (*S*)-flurbiprofen (98.5% ee) with a very high enantioselectivtiy ($E = 332$). Jaeger et al. [5] tried to use the lipase from *S. marcescens* SM6 for the kinetic resolution of other racemic esters such as isopropylideneglycerol acetate in organic solvent but the reactions were failed. The lipase gene from *S. marcescens* Sr41 had been cloned by screening a DNA library encoding a protein of 613 amino acids [6]. The lipase gene from *S. marcescens* SM6 and *S. marcescens* ES-2 was also cloned and then overexpressed in *E. coli* [5,7,8].

Ketoprofen or 2-(3′-benzoylphenyl)propionic acid, belonging to the family of 2-arylpropionic acids (profens), is one kind of the nonsteroidal anti-inflammatory drugs. Its (*S*)-enantiomer has the therapeutic activity of reducing inflammation and relieving pains, while the (*R*)-enantiomer shows the gastrointestinal side effect and toxicity [9]. Various efforts have therefore been

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made to obtain optically pure (*S*)-ketoprofen. Lipases from *Candida rugosa* (CrLs) and *Acinetobacter* sp. ES-1, and many other microbial esterases such as esterases from *Trichosporon brassicae* and *Pseudomonas* sp. S34 were used for kinetic resolution of racemic ketoprofen to produce (*S*)-ketoprofen [10–18].

Recently, we have found a bacterial strain *S. marcescens* ECU1010 being able to enantioselectively hydrolyze (±)-MPGM to give a (−)-isomer with a high enantiomeric ratio ($E > 100$) [19]. In the present research, we have cloned and overexpressed the lipase gene (*lip*A) from *S. marcescens* ECU1010 in *E. coli* and firstly report the application of this lipase for the production of optically pure (*S*)-ketoprofen and the positive effect of a nonionic surfactant on the enantioselectivity of this lipase.

2. Experimental

2.1. Chemicals

Ketoprofen was provided by Xi'nan Synthetic Pharmaceutical Factory, Chongqing, China. The racemic ethyl and 2-chloroethyl esters of ketoprofen were prepared using the method described by Moreno and Sinisterra [20]. The surfactant OP-10 was bought from Tianjin Tiantai Reagent Co., China. Triton X-45, Triton X-114 and Tween-80 were from Shanghai Dazhong Pharmaceutical Factory, China. Other surfactants were from Fluka, Switzerland. All other chemicals were also obtained commercially and of analytical grade.

2.2. Bacterial strains, plasmids, and culture conditions

S. marcescens ECU1010 [19] was used as the lipase gene source for the cloning experiment. *E. coli* DH5α and BL21 (DE3) were employed as host strains in the gene manipulation and protein expression, respectively. The plasmids used for the cloning and protein expression were pMD18-T vector (Takara, Dalian, China) and pET-24a (+) (Novagen), respectively.

S. marcescens ECU1010 was grown at 30 °C in a nutrient broth (0.3% beef extract, 0.5% peptone and 0.5% NaCl). Wild-type and recombinant *E. coli* cells were cultured regularly in a Luria–Bertani (LB) medium at 37 °C, and an appropriate amount of ampicillin (25 μg/ml) or Kanamycin (25 μg/ml) was added for recombinant *E. coli* cells when needed.

Isopropyl-β-D-thiogalactopyranoside (IPTG) was added to the culture media or solid plates when needed. The cell growth was determined by measuring the optical density at 600 nm using a spectrophotometer.

2.3. DNA manipulations, sequence determination and analysis

DNA manipulations and transformations were carried out according to standard procedures [21]. The nucleotide sequence was determined by the dideoxy chain termination method [22]. Nucleotide and amino acid data were compared with annotated sequences from various genomes and protein data banks at the National Center for Biotechnological Information (NCBI).

2.4. Cloning and overexpression of lipA gene in E. coli

A 2.2-kb DNA fragment was amplified by PCR (using Pfu polymerase, Takara, Dalian, China) containing the intact *lip*A with the chromosomal DNA from *S. marcescens* ECU1010 as template. Primers were designed according to the reported *lip*A sequences from *S. marcescens* Sr41 and *S. marcescens* SM6 [6,7]: forward, 5′-CCG CATACCAATAACGTTTCATCA-3′; reverse, 5′-CAGCAGTGGTTCCGCCTTCGCAAG-3′. After adenine (A) were added to the two blunt ends, the PCR fragment was ligated to a plasmid of pMD18-T vector (Takara, Dalian, China) and then transformed into *E. coli* DH5α. Transformants were screened on tributyrin plates [23] and one lipase-producing clone, carrying a plasmid designated as pBCLipC1, which contained a 2.2-kb inserted fragment, was selected by clear-halo formation after 24 h of incubation at 37 °C. Nucleotide sequencing was performed on both strands using an automatic sequencer (ABI PRISM^{TM} 3730 × L, Perkin-Elmer). Double-stranded DNA or PCR-amplified fragments were used as the template with either universal or synthetic primers when needed.

The *lip* gene was reamplified by PCR using Pfu DNA polymerase (Takara, Dalian, China) and a combination of forward (5′-ACT*CATATG*GGCATCTTTAGCTATAAGGATC-TG-3′) and reverse (5′-TGC*AAGCTT*TTAGGCCAACACCA-CCTGATCGGT-3′) primers, where the underlines represent the NdeI and HindIII sites, respectively, and the ATG codon for the initiation of the translation and the sequence complementary to the termination codon TAA are shown in italics. The plasmid (pBCLipC1) was used as a template in this experiment. The resultant 1.8-kb DNA fragment was digested with NdeI and HindIII and ligated to the large NdeI-HindIII fragment of plasmid pET-24a (+) to create an overexpression plasmid for the *lip* gene. The resulting plasmid was designated as pBCLipE1 and an overproducing strain was constructed by transforming *E. coli* BL21 (DE3) with this recombinant plasmid.

2.5. Protein induction and expression

All liquid cultures were grown in a shaker with a shaking speed of 150 rpm. A single bacterial colony was inoculated into 4 ml of LB medium containing 25 mg/l Kanamycin at 37 °C. The overnight culture (0.6 ml) was diluted into 30 ml of a fresh LB medium containing Kanamycin in a 250-ml flask and incubated at 37 °C until turbidity of the culture reached an OD_{600} of between 0.7 and 0.8. The cell culture was induced by the addition of an inducer, either IPTG or lactose.

2.6. Enzyme assay and SDS-PAGE

The cell cultures (20 ml) were harvested by centrifugation at 4000 × *g* for 10 min, and the cell pellet was washed twice with a 0.85% NaCl solution. The cell pellet was then re-suspended in 2 ml of 50 mM Tris–HCl (pH 8.0) and was lysed by sonication. The cell lysate was centrifuged at 15,000 × *g* for 30 min. The protein expression was determined by gel electrophoresis of cell-free extract and cell debris on a SDS-PAGE as described by Laemmli [24]. Both pellet resuspension and cell-free extract

Table 1
Enhancement of lipase production by recombinant cells at lower induction temperature[a]

Temperature (°C)	IPTG			Lactose		
	DCW (g/l)	Activity (U/l)	Spec. activity (U/g DCW)	DCW (g/l)	Activity (U/l)	Spec. activity (U/g DCW)
20	4.1 ± 0.2	4549 ± 153	1110 ± 17	3.8 ± 0.1	5680 ± 203	1495 ± 13
30	3.5 ± 0.1	1275 ± 65	364 ± 8	3.6 ± 0.1	1618 ± 116	449 ± 20

[a] The final concentration of 1 g/l of glucose and 100 mM of Ca^{2+} were added to the culture accompanied with 0.1 mM of IPTG or 15 g/l of lactose when OD_{600} of the culture reached 0.7–0.8, and then incubated at 30 °C for 4 h, at 20 °C for 12 h. After incubation, 20 ml of cell broth was used to prepare cell lysate by sonication. The lipase activity in the supernatant of cell lysate was determined and calculated to the initial culture medium volume. All results were represented as an average of three independent cultures ± standard deviation.

were used to determine their lipase activities. Lipase activity was routinely determined by a pNPA assay to measure the amount of *p*-nitrophenol formed from *p*-nitrophenylacetate, as described previously [19].

2.7. Enzymatic hydrolysis of ketoprofen esters

To 1 ml of cell-free extract, 10 mM of racemic ketoprofen ester was added. DMSO (5%) or a surfactant (3%, w/v) was added to the system when needed. The mixture was incubated at 30 °C for 24 h in a thermomixer (Eppendorf Co., Germany) at 1000 rpm, and the reaction mixture was extracted with ethyl acetate and analyzed by chiral HPLC as described previously [25].

2.8. Analysis

The (*R*)- or (*S*)-ketoprofen and (*R*)- or (*S*)-ketoprofen ethyl esters were determined by HPLC detected at UV 254 nm, using a chiral column (25 cm, Chiralcel OJ, Daicel, Japan) with hexane/2-propanol/acetic acid (90:10:0.05, by volume) at 1.0 ml/min as a mobile phase [25]. The enantiomeric excess (ee), conversion (*c*) and enantiomeric ratio (*E*) were calculated as follows:

- ee = ([(*S*)-ketoprofen] − [(*R*)-ketoprofen])/([(*S*)-ketoprofen] + [(*R*)-ketoprofen])
- c = ([(*S*)-ketoprofen] + [(*R*)-ketoprofen])/[(*R*,*S*)-ketoprofen ethyl ester]$_0$
- $E = \ln[1 - c(1 + ee)]/\ln[1 + c(1 - ee)]$ [26]

3. Results and discussion

3.1. Cloning and overexpression of S. marcescens lipase gene in E. coli

The lipase gene was first identified by screening transformants on tributyrin agar plate, and three clear-halo forming clones were obtained among 189 colonies screened. A plasmid containing a 2.2-kb inserted fragment from *S. marcescens* ECU1010, designated as pBCLipC1, was achieved after sequencing. The sequencing result showed that the full length of DNA sequence was 2218 base pairs (bp), including an ORF of 1845 bp encoding 614 amino acids, which shares 94–96% identities on DNA level and 96–98% identities on amino acid level with those of *S. marcescens* Sr41, *S. marcescens* SM6 and *S. marcescens* ES-2, respectively [6–8]. The nucleotide sequence has been registered in GenBank, with an accession number of DQ884880.

The *lip* gene was then overexpressed in *E. coli*. The *lip* gene was reamplified by PCR using Pfu DNA polymerase (Takara) and ligated to the plasmid pET-24a (+) to create an overexpression plasmid. The resulting plasmid was designated as pBCLipE1 and an overproducing strain was constructed by transforming *E. coli* BL21 (DE3) with this recombinant plasmid. Many physical and chemical factors such as inducers, glucose [27], Ca^{2+} [6,28] and temperatures may affect the production and correct folding of LipA overexpressed in *E. coli*. For the recombinant strain, we firstly optimized the fermentation and induction conditions to enhance the soluble expression of the *lip*A gene. After optimization, the lipase production was greatly enhanced to 5000–6000 U/l from an initial level of 150 U/l when induced by IPTG or lactose at 20 °C for 12 h (Table 1). From the SDS-PAGE (Fig. 1), it can be seen that the soluble fraction of recombinant LipA expressed at 20 °C has been greatly enhanced as compared to that induced at 30 °C. The lipase expressed in soluble fraction was estimated to be about 30% of the total proteins in the cell-free extract after optimization. Subsequently, the recombinant lipase was used for the bioresolution of racemic ketoprofen esters.

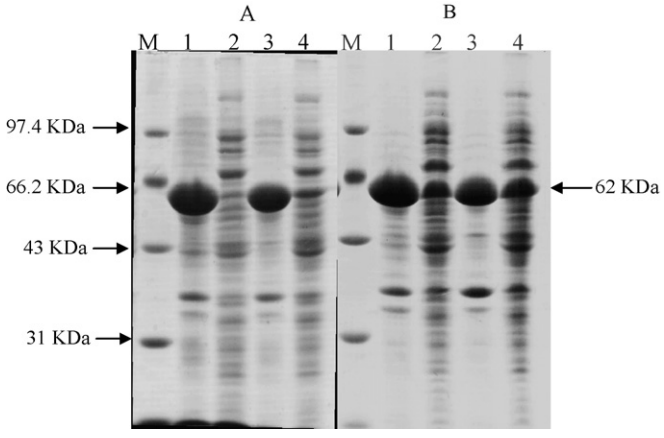

Fig. 1. SDS-PAGE (10% gel) analysis of polypeptides produced in the *E. coli* recombinant cells carrying the *lip*A gene induced at (A) 30 °C for 4 h before optimization and (B) 20 °C for 12 h under optimized conditions. Lanes 1 and 2: cell lysates induced by 0.1 mM IPTG; lanes 3 and 4: cell lysates induced by 1.5% lactose. The gel was stained by Coomassie brilliant blue R-250. Lane M, molecular weight marker; lane 1, precipitate of cell lysate; lane 2, supernatant of cell lysate; lane 3, precipitate of cell lysate; lane 4, supernatant of cell lysate.

Table 2
Enantioselective hydrolysis of *rac*-ketoprofen esters by the *Serratia marcescens* lipase produced in the recombinant *E. coli* cells

Substrate	Conversion (%)	ee_p (%)	*E*
Ketoprofen methyl ester	51.3	69.1	12
Ketoprofen ethyl ester	48.2	91.6	63
2-Chloroethyl ester of ketoprofen	41.0	50.4	4
Ketoprofen isopropyl ester	31.1	59.2	5
Ketoprofen butyl ester	42.4	78.8	15
Ketoprofen octyl ester	47.9	85.2	30

The reactions were carried out at 1000 rpm, 30 °C for 24 h with each reaction system containing 10 mM ketoprofen ethyl ester and 5% DMSO.

3.2. Kinetic resolution of racemic ketoprofen esters with recombinant lipase

As it may be useful for kinetic resolution of other chiral compounds, we tried to use this lipase for enantioselective hydrolysis of racemic ketoprofen esters. The cell-free extract (ca. 5 U/ml, as measured by pNPA assay) was used for kinetic resolution of ketoprofen esters, and the cell-free extract prepared from recombinant cells with no induction was used as a control of the ester hydrolysis. In a standard experiment, 10 mM of each ester and 5% (v/v) of DMSO were added to the reaction systems. As a result, this lipase enantioselectively catalyzed the hydrolysis of racemic ketoprofen esters to (*S*)-ketoprofen, whereas no (*R*)- or (*S*)-ketoprofen was detected in the control experiments (data not shown). As shown in Table 2, six alkyl esters of ketoprofen were investigated, and the highest ee_p (91.6%) and *E*-value (63) were obtained along with a high conversion of 48.2% for the enantioselective hydrolysis of ketoprofen ethyl ester after 24 h of reaction (Fig. 2A). For 2-chloroethyl, isopropyl, *n*-butyl ester and *n*-octyl esters of ketoprofen, an interesting trend was found that the values of ee_p and *E* were enhanced from 50.4 to 85.2% and from 4 to 30***, respectively, with the increase of carbons number of the substituent.

3.3. Bioresolution of ketoprofen ethyl ester

3.3.1. Effects of surfactants

Surfactants have been widely applied to lipase-catalyzed reactions of insoluble substrates to increase the lipid–water interfacial area, which in turn enhances the enantioselectivity as well as the reaction rate of the kinetic resolution [12]. In addition, surfactants are also involved in hydrogen bonds and hydrophobic action between lipases and surfactants, which can change the enantioselectivity of the lipase [25,29]. As it is a simple and easy-to-use method, we tested several surfactants in the kinetic resolution of ketoprofen ethyl ester and diverse effects were found on the enantioselectivity. As shown in Table 3, compared to DMSO, no evident positive effect was found on its enantioselectivity when OP-10, Triton X-45, Synperonic NP10 or Lgepal CA 630 was utilized as an additive to the reaction. The surfactants of PG 250 de, PG 350 me, PG 400 de and Synperonic PE/F68 markedly lowered the enantioselectivity of this lipase. For the surfactants of Tween-80, Triton X-114 or Synperonic PE/L61, higher ee_p and *E*-value along with lower conversion

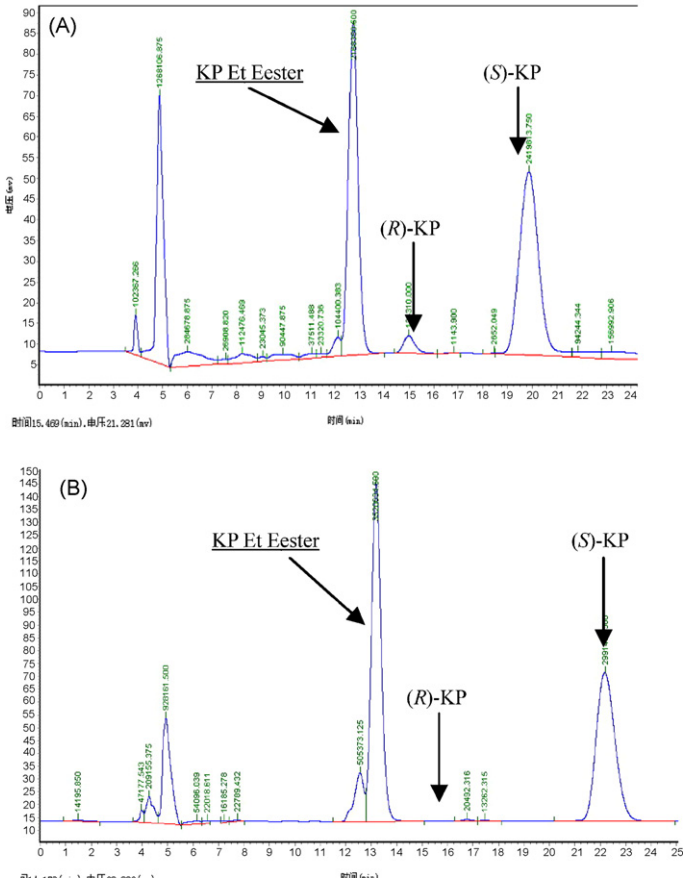

Fig. 2. HPLC figures of ketoprofen (KP) using DMSO as co-solvent or Brij 92V as emulsifier. (A) Resolution of ketoprofen ethyl ester using DMSO as co-solvent; (B) resolution of ketoprofen ethyl ester using Brij 92V as emulsifier.

Table 3
Effects of surfactants on the kinetic resolution of ketoprofen ethyl ester by the *S. marcescens* lipase produced in the recombinant *E. coli* cells[a]

Surfactant [b]	Conversion (%)	ee_p (%)	*E*
Tween-80	36.1	97.2	123
OP-10	36.0	94.6	61
Triton X-114	27.1	97.5	114
Triton X-45	48.2	93.2	80
pg 250 de	49.8	85.6	35
pg 350 me	53.4	82.5	37
pg 400 de	39.9	93.0	52
Synperonic PE/L61	40.4	98.2	221
Synperonic PE/F68	22.6	92.8	35
Synperonic NP10	37.0	94.8	65
Brij 92V	42.6	99.6	1084
Lgepal CA 630	28.0	96.6	84

[a] The reactions were carried out at 1000 rpm and 30 °C for 24 h with each reaction system containing 10 mM ketoprofen ethyl ester and 3% of each surfactant.

[b] OP-10: nonyl phenol polyethyleneoxy ether; pg 250 de, pg 400 de: polyethylene glycol dimethyl ether; pg 350 me: polyethylene glycol monomethyl ether; synperonic PE/L61, synperonic PE/F68, synperonic NP10: block copolymer of polyethylene and polypropylene glycol; Brij 92V: polyethylene glycol oleyl ether; lgepal CA 630: 'Nonidet P 40' nonylphenyl-polyethylenglykol.

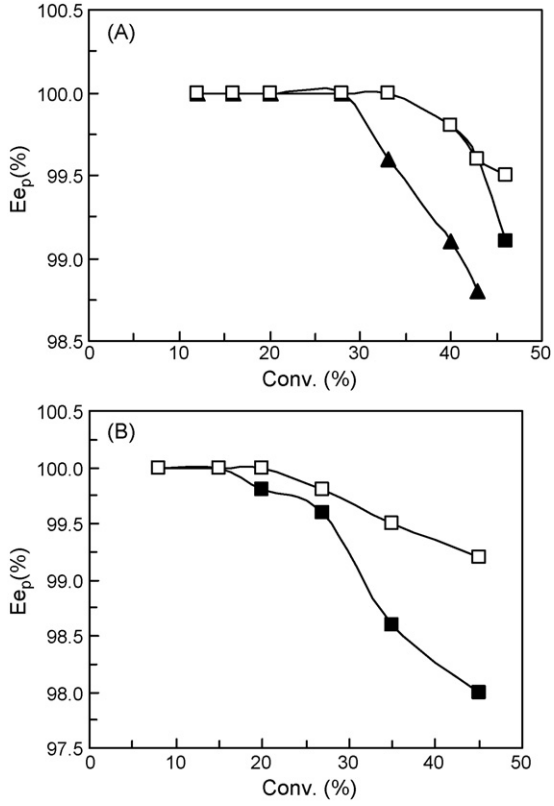

Fig. 3. Effect of Brij 92V concentration on the kinetic resolution of ketoprofen ethyl ester by recombinant *Serratia marcescens* lipase produced in the *E. coli* strain BL21 (DE3). (A) 10 mM ketoprofen ethyl ester; (B) 100 mM ketoprofen ethyl ester: (▲) 1% Brij 92V; (■) 3% Brij 92V; (□) 5% Brij 92V.

were obtained as compared to that of 5% DMSO utilized. The highest *E*-value ($E = 1084$), $ee_p > 99\%$ along with a conversion of 42.6% were achieved when 3% of a nonionic surfactant Brij 92V was utilized (Fig. 2B). These results indicate that Brij 92V is an effective additive for this lipase-catalyzed reaction, making this lipase work more enantioselectively in the asymmetric hydrolysis *rac*-ketoprofen ethyl ester towards optically pure (*S*)-ketoprofen. The concentration effects of Brij 92V, substrate and products on the activity and selectivity of this lipase in the resolution of *rac*-ketoprofen ethyl ester were subsequently carried out using Brij 92V as an emulsifier.

3.3.2. Effect of Brij 92V concentration

To investigate its exact effect, different concentrations of Brij 92V were used as an emulsifier in the recombinant lipase-catalyzed reactions with 10 and 100 mM ketoprofen ethyl ester as substrate, respectively. As shown in Fig. 3, the ee_p was decreased with the increase of substrate conversion while the selectivity of the lipase was improved with the increase of Brij 92V concentration. Fig. 3A shows the relationship of the conversion with ee_p under different concentrations of Brij 92V at 10 mM ketoprofen ethyl ester. The results indicated that the ee_p obtained were higher than 99.9% when the conversion was not higher than 30% and different ee_p were obtained when the conversion was near to 45% under different Brij 92V concentrations, that was, $ee_p < 99\%$ (1% Brij 92V), $ee_p = 99.1\%$ (3% Brij 92V) and $ee_p = 99.5\%$ (5% Brij 92V), respectively. Similar results were obtained when the substrate concentration was increased to 100 mM (Fig. 3B). To obtain a high ee_p ($ee_p > 99\%$) with a high conversion (conv. > 45%), the concentration of Brij 92V has to be increased to 5%.

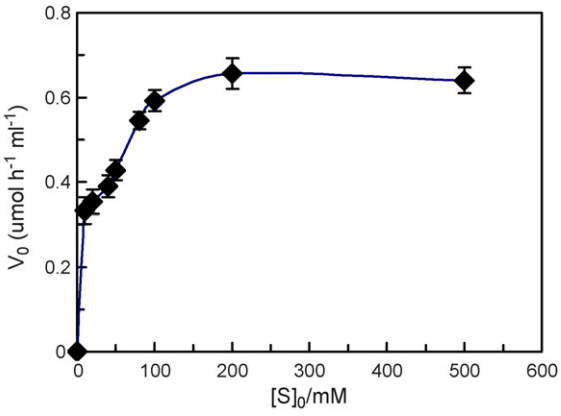

Fig. 4. Effect of ester concentration on the activity of recombinant *S. marcescens* ECU1010 lipase produced in the *E. coli* strain BL21 (DE3). The reactions were carried out at 1000 rpm and 30 °C, with 3% of Brij 92V for 3 h. Data are the means of two experiments ± standard deviation.

3.3.3. Effect of substrate concentration

Both substrate and product are significant factors, which may affect enzyme activity in the kinetic resolution processes. Effect of ketoprofen ethyl ester concentration (0–500 mM) on the recombinant lipase activity was investigated in Fig. 4. The results indicated that the initial rate (for racemic substrate) of this lipase-catalyzed reaction was enhanced with the increase of the substrate concentration when the ketoprofen ethyl ester concentration was lower than 200 mM. No evident decrease in the initial rate was observed even when the ketoprofen ethyl ester concentration was increased up to 500 mM, suggesting that there was no substrate inhibition in this lipase-catalyzed reaction.

3.3.4. Effect of products concentration

The main product (*S*)-ketoprofen and co-product ethanol were produced in the lipase-catalyzed resolution of *rac*-ketoprofen ethyl ester. The effect of (*S*)-ketoprofen concentration on the activity of this lipase was shown in Fig. 5. Different concentrations of (*S*)-ketoprofen were initially added to the reaction system to examine its effect on the lipase activity with 10 mM of substrate. As shown in Fig. 5, the lipase activity (for racemic substrate) decreased with the increase of (*S*)-ketoprofen concentration, and about 80% of the initial lipase activity were preserved when the (*S*)-ketoprofen concentration was increased to 50–80 mM, suggesting that (*S*)-ketoprofen inhibits the activity of this lipase to some extent. As shown in Fig. 6, the effect of ethanol as a co-product on lipase activity (for racemic substrate) was investigated between 0 and 1000 mM of ethanol. The results showed that no inhibition of the lipase activity was observed, on the contrary, lipase activity was slightly activated by ethanol.

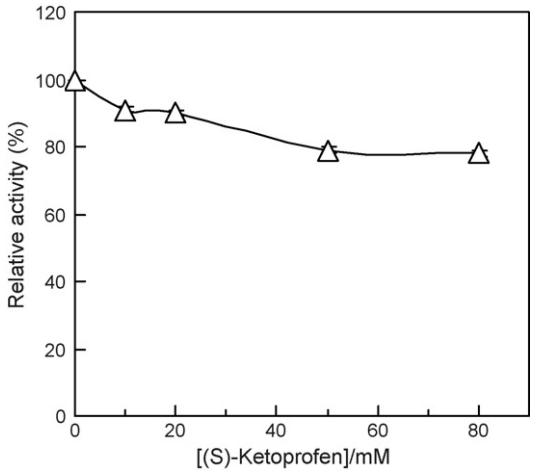

Fig. 5. Effect of (*S*)-ketoprofen concentration on the activity of recombinant *S. marcescens* ECU1010 lipase produced in the *E. coli* strain BL21 (DE3). The reactions were carried out at 1000 rpm and 30 °C for 3 h with each reaction system containing 10 mM ketoprofen ethyl ester and 3% of Brij 92V. Data are the means of three experiments ± standard deviation.

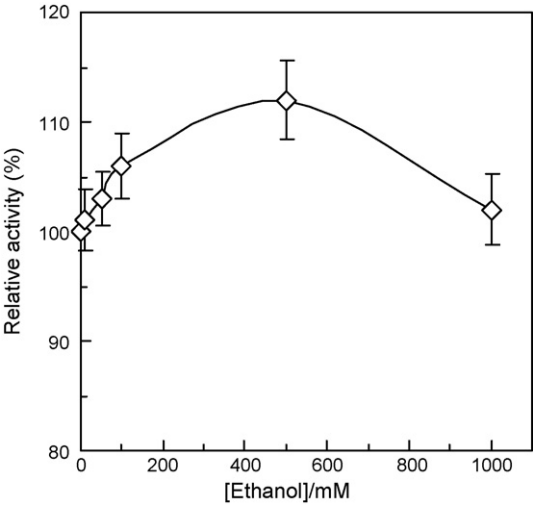

Fig. 6. Effect of ethanol concentration on the activity of recombinant *S. marcescens* ECU1010 lipase produced in the *E. coli* strain BL21 (DE3). The reactions were carried out at 1000 rpm and 30 °C for 3 h with each reaction system containing 10 mM ketoprofen ethyl ester and 3% of Brij 92V. Data are the means of three experiments ± standard deviation.

4. Conclusions

The lipase gene (*Lip*A) from *S. marcescens* ECU1010 was cloned and expressed in *E. coli*. This lipase enantioselectively hydrolyzed *rac*-ketoprofen esters into (*S*)-ketoprofen. A high ee_p (>99%) and *E*-value (1084) were obtained under 10 mM of *rac*-ketoprofen ethyl ester in the presence of 3% Brij 92V. Further studies showed that the lipase selectivity was enhanced with the increase of Brij 92V concentration. No substrate inhibition was observed while the product (*S*)-ketoprofen inhibits the lipase activity to some extent. These results indicate the usefulness of this lipase for the chiral resolution of racemic profens including (*R*,*S*)-ketoprofen, and on the other hand it also suggests the possibility of utilizing various additives including surfactants for the improvement of enzyme enantioselectivity in biocatalytic resolutions.

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References

[1] M.T. Reetz, Curr. Opin. Biotechnol. 6 (2002) 145.
[2] K.-E. Jaeger, T. Eggert, Curr. Opin. Biotechnol. 13 (2002) 390.
[3] H. Matsumae, M. Furui, T. Shibatani, J. Ferment. Bioeng. 75 (1993) 93.
[4] H.A. Bae, K.W. Lee, Y.H. Lee, J. Mol. Catal. B: Enzyme 40 (2006) 24.
[5] K.E. Jaeger, B. Schneidinger, F. Rosenau, M. Werner, D. Lang, B.W. Dijkstra, K. Schimossek, A. Zonta, M.T. Reetz, J. Mol. Catal. B: Enzyme 3 (1997) 3.
[6] H. Akatsuka, E. Kawai, K. Omori, S. Komatsubara, T. Shibatani, T. Tosa, J. Bacteriol. 176 (1994) 1949.
[7] X.Y. Li, S. Tetling, U.K. Winkler, K.-E. Jaeger, M.J. Benedik, Appl. Environ. Microbiol. 61 (1995) 2674.
[8] K.W. Lee, H.A. Bae, Y.H. Lee, J. Microbiol. Biotechnol. 17 (2007) 74.
[9] A.J. Hutt, J. Caldwell, Clin. Pharmacokinet. 9 (1984) 371.
[10] C.S. Cobbs, M.J. Barton, L. Peng, A. Goswami, A.P. Malick, J.P. Hamman, G.J. Calton, US Patent 5,108,916 (1992).
[11] D. Shen, J.H. Xu, H.Y. Wu, Y.Y. Liu, J. Mol. Catal. B: Enzyme 18 (2002) 219.
[12] G.J. Kim, G.S. Choi, J.Y. Kim, J.B. Lee, D.H. Jo, Y.W. Ryu, J. Mol. Catal. B: Enzyme 17 (2002) 29.
[13] K.W. Lee, H.A. Bae, G.S. Shin, Y.H. Lee, Enzyme Microbiol. Technol. 38 (2006) 443.
[14] N.D. Antona, P. Lombardi, G. Nicolosi, G. Salvo, Process Biochem. 38 (2002) 373.
[15] M.G. Kim, E.G. Lee, B.H. Chung, Process Biochem. 35 (2000) 977.
[16] G.J. Kim, E.G. Lee, B. Gokul, M.S. Hahm, D. Prerna, G.S. Choi, Y.W. Ryu, H.S. Ro, B.H. Chung, J. Mol. Catal. B: Enzyme 22 (2003) 29.
[17] J.N. Jin, S.H. Lee, S.B. Lee, J. Mol. Catal. B: Enzyme 26 (2003) 209.
[18] Y.Y. Liu, J.H. Xu, H.Y. Wu, D. Shen, J. Biotechnol. 110 (2004) 209.
[19] L. Gao, J.H. Xu, X.J. Li, Z.Z. Liu, J. Ind. Microbiol. Biotechnol. 31 (2004) 525.
[20] J.M. Moreno, J.V. Sinisterra, J. Mol. Catal. A: Chem. 98 (1995) 171.
[21] J. Sambrook, E.F. Fritsch, T. Maniatis, Molecular cloning: A laboratory manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989.
[22] F. Sanger, S. Nicklen, A.R. Coulson, Proc. Natl. Acad. Sci. U.S.A. 74 (1977) 5463.
[23] W. Kugimiya, Y. Otani, Y. Hashimoto, Y. Takagi, Biochem. Biophys. Res. Commun. 141 (1986) 185.
[24] U.K. Laemmli, Nature (London) 228 (1970) 680.
[25] Y.Y. Liu, J.H. Xu, Y. Hu, J. Mol. Catal. B: Enzyme 10 (2000) 523.
[26] C.S. Chen, Y. Fujmito, G. Girdaukas, C.J. Sih, J. Am. Chem. Soc. 104 (1982) 7294.
[27] D. Kazan, A. Camurdan, A. Hortacsu, Process Biochem. 30 (1995) 269.
[28] K. Amada, H.J. Kwon, M. Haruki, M. Morikawa, S. Kanaya, FEBS Lett. 509 (2001) 17.
[29] J.H. Liu, Y.Y. Zhang, L.H. Qiu, F.K. Yang, L. Ye, Y.M. Xia, J. Ind. Microbiol. Biotechnol. 31 (2004) 495.