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An organic solvent and thermally stable lipase from *Burkholderia ambifaria* YCJ01: Purification, characteristics and application for chiral resolution of mandelic acid

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ABSTRACT

A solvent-tolerant bacterium *Burkholderia ambifaria* YCJ01 was newly isolated by DMSO enrichment of the medium. The lipase from the strain YCJ01 was purified to homogeneity with apparent molecular mass of 34 kDa determined by SDS-PAGE. The purified lipase exhibited maximal activity at a temperature of 60 °C and a pH of 7.5. The lipase was very stable below 55 °C for 7 days (remaining 80.3% initial activity) or at 30 °C for 60 days. PMSF significantly inhibited the lipase activity, while EDTA had no effect on the activity. Strikingly, the lipase showed distinct super-stability to the most tested hydrophilic and hydrophobic solvents (25%, v/v) for 60 days, and different optimal pH in contrast with the alkaline lipase from *B. cepacia* S31. The lipase demonstrated excellent enantioselective transesterification toward the *S*-isomer of mandelic acid with a theoretical conversion yield of 50%, ee_p of 99.9% and ee_s of 99.9%, which made it an exploitable biocatalyst for organic synthesis and pharmaceutical industries.

1. Introduction

Lipases (triacylglycerol acylhydrolase, EC 3.1.1.3) are an important group of biotechnologically relevant enzymes, and they have immense applications in food, dairy, detergent and pharmaceutical industries [1]. Lipase can perform various reactions, such as esterification, transesterification with high regio- and stereo-selectivity [2]. The use of organic solvents is especially advantageous when the substrates are unstable or poorly soluble in water. High yield and high concentration for organic synthesis could be achieved in a non-aqueous solvent system. Furthermore, many side-reactions that are water dependent can be prevented [3]. However, most enzymes, including lipase, are not stable in solvents [4]. To overcome this limitation, some methods such as chemical modification, lyophilization in the presence of additives, immobilization and protein engineering have been employed to enhance the stability of enzymes in organic solvents [5]. If enzymes are naturally stable in the presence of organic solvents, more stable enzymes could be easily exploited from them.

Some microorganisms from oil or a chemically contaminated area can combat destructive effects and thrive in the presence of various organic solvents through various adaptations [6]. It was hypothesized that extracellular enzymes secreted by organic solvent-tolerant microorganisms were stable in the presence of organic solvents [7]. Besides organic-solvent stability, thermal stability is also an important influencing factor for the lipase to be an ideal biocatalyst in practical applications [5]. Some lipases, such as that from *Bacillus* sp. J33 [8], *B. cepacia* S31 [9], and *Acinetobacter* sp. RAG-1 [10], have been reported as possessing solvent-tolerance as well as thermostability. However, lipases from different sources have large variations in specific activity, substrate specificity, enantioselectivity, optimal temperature and pH [11]. Screening of valuable lipases from nature is still an important approach to accelerate the study and applications of lipases.

In this study, we describe the isolation, purification, characteristics and cloning of a solvent-stable lipase with distinct thermostability from the isolated organic solvent-tolerant *B. ambifaria* YCJ01. The application of the lipase for chiral resolution of racemic mandelic acid was also investigated.

2. Materials and methods

2.1. Chemicals and materials

DEAE Sepharose FF and Phenyl Sepharose FF were purchased from Amersham Biosciences (Uppsala, Sweden). All *p*-nitrophenyl esters and high-performance liquid chromatography (HPLC)-grade solvents were purchased from Sigma (St. Louis, MO, USA). All other chemicals were also commercial sources and of analytical grade.

2.2. Isolation of solvent stable lipase producing strains

Soil samples contaminated by crude oil and chemicals were collected and added to the enrichment medium (Olive oil 1%, peptone

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0.2%, $(NH_4)_2SO_4$ 0.35%, Na_2HPO_4 0.2%, KH_2PO_4 0.2%, $MgSO_4{\cdot}7H_2O$ 0.05%) which contains 15% of toluene or 15% of DMSO. The cultivation vessel was plugged with a chloroprene-rubber stopper to prevent evaporation of the organic solvent. Cultivations were conducted in 10 ml of medium in 50 ml test tubes for 2 days with shaking at 180 rpm at 30 °C. After twice transferring to the fresh enrichment medium containing same organic solvent, the broths were diluted and spread on LB-tributyrin agar plates (yeast extract 1.0%, tryptone 0.5%, NaCl 1%, tributyrin 0.5%, and agar 1.8%), and halo-forming colonies were isolated after incubation overnight. The colonies were then inoculated to the optimized culture medium (Olive oil 1%, yeast extract 0.5%, tryptone 0.5%, urea 0.5%, KH_2PO_4 0.2%, $MgSO_4{\cdot}7H_2O$ 0.05%, Triton X-100 0.05%, pH 7.0) and incubated at 30 °C, 180 rpm. Lipase activity of the cultured supernatant was measured spectrophotometrically. The solvent-stable lipases were subsequently confirmed by determining the residual activity after incubation of the crude enzyme with 25% (v/v) of various solvents. The lipase with broad tolerance to various organic solvents was selected for the following research.

2.3. 16S rDNA gene of strain YCJ01 and identification of the strain

The nucleotide sequence of 16S rDNA gene of strain YCJ01 has been assigned GenBank Accession No. JQ733582. The strain YCJ01 was identified by combining the analysis of the Biolog Automated Microbiology Identification System (BIOLOG USA) and 16S rDNA sequence.

2.4. Culture conditions for producing lipase

The inoculums were prepared by transferring loopfuls of fresh *B. ambifaria* YCJ01 cells cultured on LB agar plates into an LB medium, followed by incubation at 30 °C with shaking at 180 rpm for 12 h. The 40 ml of the optimized culture medium in a 250 ml Erlenmeyer flask was seeded with 2 ml of the inoculum. The incubation was carried out at 30 °C with shaking at 180 rpm. After 48 h incubation, the supernatant of the culture was obtained by centrifugation and was directly used as crude lipase for purification.

2.5. Assay for lipase activity and substrate specificity

Lipase activity was measured spectrophotometrically using a modified assay based on the hydrolysis of *p*-nitrophenyl palmitate (*p*NPP) [12]. The reaction mixture was composed of 240 μl of *p*NPP solution and 10 μl of lipase solution. The *p*NPP solution was prepared as follows: 1 ml isopropanol (to improve the solubility of substrates) containing 8 μmol *p*NPP was added to 9 ml 0.05 M sodium phosphate buffer (pH 7.5) supplemented with 0.1% gum arabic and 0.6% Triton X-100. The enzyme reaction was incubated at 50 °C for 10 min. The *p*-nitrophenol (*p*NP) liberated was quantified spectrophotometrically at 410 nm. One unit of lipase activity was defined as the amount of enzyme that produced 1 μmol of *p*-nitrophenol (*p*NP) under the conditions mentioned above.

Substrate specificity of the enzyme was determined using *p*-nitrophenyl esters with different chain lengths (C2, C4, C8, C10, C14, C16, C18) as described above. The substrates dissolved in isopropanol were all at the same mole number (8 μmol/ml).

2.6. Purification of lipase YCJ01

The crude lipase was obtained as described in Section 2.4, and then loaded onto a DEAE Sepharose FF column equilibrated with 50 mM Tris–HCl buffer (pH 7.25). The column was washed with two bed volumes of the same buffer and, separately, with the buffer containing 0.2 M NaCl, and then the bound proteins were eluted by 1.0 M NaCl in the same Tris–HCl buffer. Fractions with lipase activity were pooled, concentrated in a dialysis bag (7 kDa molecular weight cut-off) by PEG20000, and dialyzed against the same Tris–HCl buffer overnight. $(NH_4)_2SO_4$ was added to the dialyzed sample to a concentration of 0.7 M, and then loaded onto Phenyl Sepharose FF column which had been equilibrated with the same Tris–HCl buffer containing 0.7 M $(NH_4)_2SO_4$. After washing with 2 bed volumes of 50 mM Tris–HCl buffer and, separately, with the same buffer containing 20% ethanol, the bound proteins were eluted with the same Tris–HCl buffer containing 40% alcohol, dialyzed, and then lyophilized. Purified lipase was analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method described by Laemmli [13].

2.7. Cloning of lipase YCJ01

The Coomassie-stained band of the purified lipase YCJ01 was excised from the gel and submitted to the National Center of Biomedical Analysis (NCBA, Beijing) for analysis of trypsin-digested protein fragments by LC/MS/MS analysis, and the sequences of the fragments were submitted to the Mascot program for possible identity matching. Since some fragments of the lipase were identical to the lipase from *B. cenocepacia* AU1054, the forward primer LU1-5′-CATGCATGGCCAAATCGATGC-3′ and the reverse primer LD1-5′-TCACACGCCCGCGAGTTTCAA-3′ were designed based on the nucleotide sequence of the *B. cenocepacia* AU1054 lipase (NCBI Accession No. YP_624306). PCR amplification was carried out with the primers using the genomic DNA of strain YCJ01 as template. The PCR product was purified and ligated into a PMD18-T simple vector and then sequenced.

2.8. Lipase YCJ01 gene sequence

The nucleotide sequence of lipase YCJ01 has been assigned GenBank Accession No. JQ733583.

2.9. Effect of pH and temperature on the activity and stability of lipase YCJ01

To study the effect of pH on lipase activity, the lipase was assayed at various pH values (6.5–9.5). pH stability was tested by preserving the enzyme in buffers of various pH values varying from pH 5.4 to 10.6 at 30 °C for 1 h, and the residual activity was measured according to the *p*NPP method.

To study the effect of temperature on lipase activity, the enzyme activity assay was conducted at various temperatures from 30 to 75 °C at pH 7.5. The thermostability of the enzyme was checked by incubating the enzyme at different temperatures (30–80 °C) at pH 7.0 for 3 h. The residual lipase activity was assayed under standard conditions.

2.10. Effect of metal ions, inhibitors and detergents on enzyme activity

The effect of metal ions and inhibitors on lipase activity was studied by preincubating the lipase with 1 and 10 mM of the ions or inhibitors, or 0.1% and 0.5% surfactants for 1 h at 30 °C. The residual lipase activity was measured by the *p*NPP method.

2.11. Organic solvent stability of the lipase

The effects of organic solvents with different log *P* values at 25% (v/v) and 40% (v/v) concentration on the activity and stability of the purified lipase were investigated following Ogino et al. [14]. Aliquots (1 ml) of organic solvents were added to the purified lipase solution (3 ml), or 1.6 ml of organic solvent to 2.4 ml of the purified lipase with 0.05–0.1 mg/ml protein concentration in a sealed glass

vial. The mixture was incubated at 30 °C with shaking at 150 rpm, and samples were withdrawn periodically to examine the residual lipase activity by the *p*-NPP method.

2.12. Chiral resolution of racemic mandelic acid

Lipase YCJ01 powder preparation was undertaken as follows: Chilled acetone was added to the crude lipase until the ratio was 1:1 (v/v) under magnetic stirring at 0 °C for 4 h. The precipitate was obtained by centrifugation at 10,000 × *g* and 4 °C for 30 min. Finally, the precipitate was air-dried and lipase powder was obtained.

The resolution of racemic mandelic acid by transesterification with vinyl acetate was conducted as referenced in the report by Miyazawa et al. [15]. The reaction mixture consisted of 30 mM racemic mandelic acid and 210 mM vinyl acetate dissolved in 2 ml diisopropyl ether. The reaction was catalyzed by 20 mg of lipase powder (1%, w/v) at 50 °C with shaking at 180 rpm. A total of 100 μl of the samples were withdrawn periodically to analyze the transesterified product and its optical purity by HPLC according to the method established by Dabkowska and Szewczyk [16].

3. Results

3.1. Isolation of a solvent-tolerant-lipase-producing strain

Twelve strains with relatively high lipase activity were obtained. Of them, seven was isolated using 15% DMSO as screening stress and five using 15% toluene. Only three of the twelve strains showed both high lipase activity and good solvent tolerance. The crude lipase secreted by strain YCJ01 (isolated by 15% DMSO as screening stress) showed the best stability in the presence of tested hydrophobic and hydrophilic organic solvents and was selected for further research. By combining the analysis of the Biolog Automated Microbiology Identification System and 16S rDNA sequence, the strain YCJ01 was identified as *B. ambifaria* and deposited in CCTCC (Wuhan, China) with an accession number of CCTCC M 2011058.

3.2. Purification and identification of lipase YCJ01

The extracellular lipase was purified from the cultured supernatant of strain YCJ01 by DEAE Sepharose FF and Phenyl Sepharose FF chromatography. Table 1 shows that 12.5% of the total activity could be recovered with 24.2-fold purification. The purified lipase was homogeneous with an apparent molecular mass of 34 kDa by SDS-PAGE (Fig. 1).

The internal fragments of lipase YCJ01 protein were identified by trypsin digestion and sequencing. These sequences, such as YPIILVHGLTGTDK (Fragment1), VNLVGHSQGGLTSRYVAAVAPDLVASVTTIGTPHR (Fragment2), ASGQNDGLVSKCSALYGKVLSTSYKWNHIDEINQLLGVR (Fragment3), showed significant homology to that of the lipase from *B. cenocepacia* AU 1054. Based on the sequence of the lipase from *B. cenocepacia* AU 1054, the gene encoding the organic-solvent-tolerant lipase of *B. ambifaria* YCJ01 was cloned. A homology search revealed that lipase YCJ01 gene had the highest identity of 92.4% with the lipase from *B. cepacia* S31 (NCBI Accession No. FJ638612). The full-length open reading frame (ORF) of the lipase consisted of 1095 bp encoding 364-amino acid residues and the mature lipase contained 324-amino acid residues.

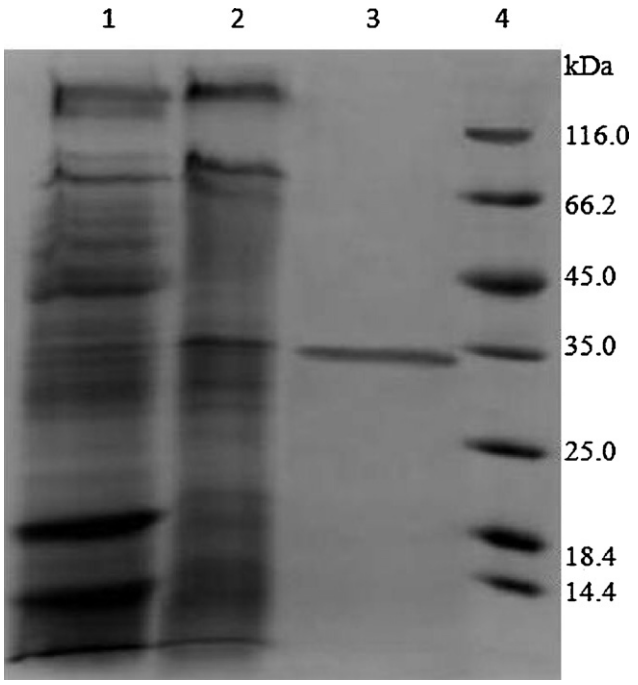

Fig. 1. SDS-PAGE analysis of lipase from *B. ambifaria* YCJ01 at each stages of purification. Lane 1: culture supernatant, lane 2: crude enzyme after DEAE Sepharose FF chromatograph, lane 3: lipase purified by Phenyl Sepharose, lane 4: molecular weight marker.

BLAST-based analysis of the Swiss-port database also revealed that the amino acid sequence of the lipase (between position 41 and 364) from strain YCJ01 showed the highest similarity (96.6%) to the lipase from *B. cepacia* S31. Eleven amino acid residues (Val^{187}, His^{198}, Ser^{207}, Ala^{214}, Leu^{262}, Val^{264}, Val^{279}, Gly^{303}, Gln^{320}, Ile^{331}, Tyr^{344}) in the mature peptide from the lipase from *B. cepacia* S31 were substituted with Ala, Asn, Ala, Ser, Ile, Ala, Ile, Ala, Lys, Leu, Asn in the lipase from *B. ambifaria* YCJ01, respectively.

3.3. Effect of temperature and pH on the activity and stability of the lipase

The optimal reaction temperature of the lipase YCJ01 was 60 °C (Fig. 2). The lipase retained 97.4% activity after incubation at and below 60 °C for 3 h. The lipase was very stable below 55 °C for 7 days (remaining 80.3% initial activity) or at 30 °C for 60 days. The lipase exhibited maximum lipase activity at pH 7.5. It was stable between pH values from 6.0 to 8.0 with pH 7.0 for the best stability (Fig. 3).

3.4. Effects of metal ions, inhibitors and detergents on the lipase and its substrate specificity

The effects of metal ions, inhibitors and detergents on lipase activity are depicted in Table 2. The metal chelator, EDTA, had no effect on the lipase activity. Lipase activity was strongly inhibited by the PMSF, while DTT and β-mercaptoethanol caused nearly no inhibition of lipase activity. This suggests that residual serine was involved in the reaction center of the lipase. The nonionic surfactant, Triton X-100, Tween-80 had significant activation on the

Table 1
Purification of the lipase from *B. ambifaria* YCJ01.

Purification step	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Purification (fold)	Yield (%)
Culture supernatant	2400	12.6	191	1.00	100
DEAE Sepharose FF	1421	0.908	1564	8.20	59.2
Phenyl Sepharose FF	300	0.0650	4616	24.2	12.5

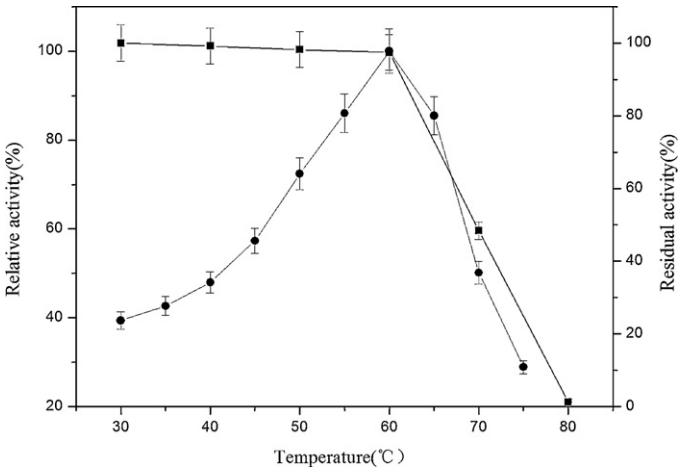

Fig. 2. Effect of temperature on the activity and stability of the lipase. Lipase activities (●) are shown as values relative to that measured at 60 °C (taken as 100%). Thermostability (■) was measured by incubating the lipase in 50 mM sodium phosphate buffer (pH 7.0) at various temperatures for 3 h. Residual lipase activity (%) was calculated relative to the initial activity.

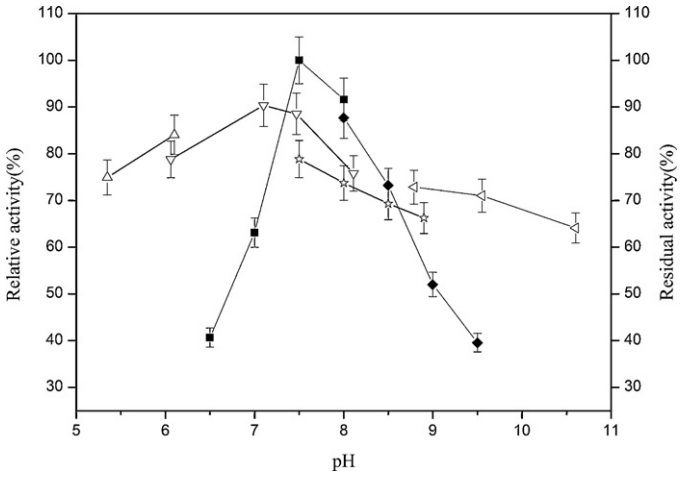

Fig. 3. Effect of pH on activity (solid) and stability (hollow) of the lipase. Lipase activities are shown as values relative to that measured in 50 mM sodium phosphate buffer at pH 7.5 (taken as 100%). Stability of the lipase was determined by incubating at 30 °C for 1 h in the following buffer systems: 50 mM citric acid–citrate sodium, pH 5.4–6.1 (△); 50 mM Na_2HPO_4–$NaHPO_4$, pH 6.1–8.1 (■▽); 50 mM Tris–HCl, pH 7.5–9.5 (☆◆); 50 mM Glycine–NaOH, pH 8.8–10.6 (◁). The remaining activities of the lipase are shown as values relative to the activity of the lipase incubated at pH 7.5 (taken as 100%).

Table 2
Effect of various metal ions, inhibitors and detergents on lipase YCJ01.[a]

Reagent	Residual activity		Metal ions	Residual activity	
	1 mM	10 mM		1 mM	10 mM
Control[b]	100	100	Li^+	95.6	84.7
EDTA	101	98.3	K^+	90.6	91.4
PMSF	35.5	7.60	Ba^{2+}	109	107
DTT	103	98.5	Ca^{2+}	108	138
β-Mercaptoethanol	102	97.6	Mg^{2+}	104	90.5
Triton X-100*	110	123	Mn^{2+}	138	113
Tween-80*	115	125	Co^{2+}	103	77.8
Tween-20*	104	107	Fe^{2+}	84.5	71.6
SDS*	6.00	4.40	Cu^{2+}	110	107
			Zn^{2+}	84.8	70.0

[a] Lipase YCJ01 was incubated with various metal ions, inhibitors (1 mM and 10 mM) and detergents (0.1% and 0.5%) with the superscript * in 50 mM Tris–HCl (pH 7.0) at 30 °C for 1 h.

[b] Lipase activities are shown as values relative to the initial activity without addition of effectors (Control).

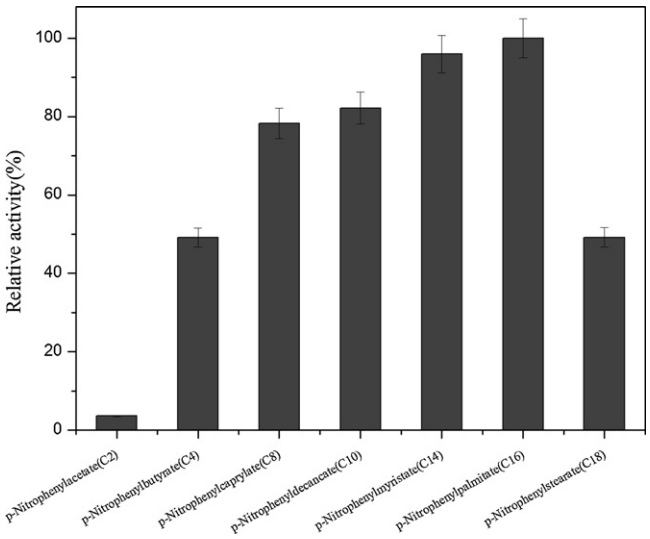

Fig. 4. Substrate specificity of the lipase. The activities of lipase toward different *p*-nitrophenyl esters were determined and calculated relative to the maximum activity measured toward *p*-nitrophenyl palmitate (taken as 100%).

lipase. In contrast, the anionic surfactant, SDS inhibited the most activity of the lipase. Among the tested metal ions, Ba^{2+}, Ca^{2+}, Mn^{2+} and Cu^{2+} activated the lipase at certain degrees.

The hydrolytic activity of the lipase YCJ01 toward a series of *p*-nitrophenyl esters was studied (Fig. 4). The lipase preferred to hydrolyze *p*-nitrophenyl esters with medium-long chain fatty acid and exhibited high activity toward *p*-nitrophenyl palmitate (C16) and *p*-nitrophenyl myristate (C14).

3.5. Effect of organic solvent on the activity and stability of the lipase

No significant effects of 25% various organic solvents treating for 1 h on the lipase were observed (data not shown). Interestingly, lipase YCJ01 was very stable for 60 days in the presence of organic solvents (25%, v/v) of various hydrophilic and hydrophobic solvents with different log*P* values (−1.35 to 8.7) (Fig. 5). Some solvents, such as hexane and heptane, even activated the lipase with 135% and 119% of the initial activity, respectively. A few solvents, including diisopropyl ether and acetonitrile, showed weak deactivation

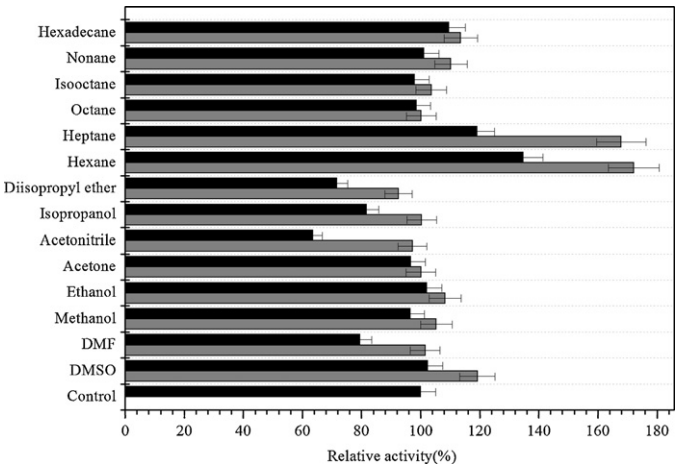

Fig. 5. Organic solvent stability of the lipase: residual activities of the lipase were measured after incubation at 30 °C for 1 day (gray box) and 60 days (black box) with 25% (v/v) of various organic solvents, and were calculated relative to the initial activity without addition.

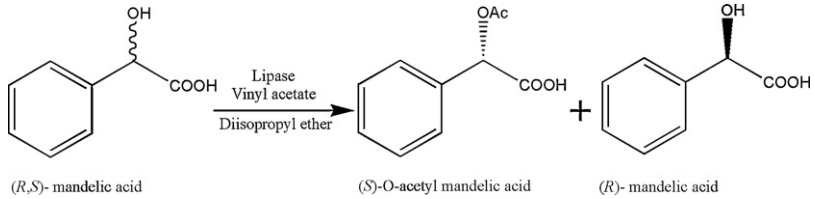

Fig. 6. Transesterification of (*R*,*S*)-mandelic acid catalyzed by the lipase.

on the lipase, 72% and 64% of the initial activity remained after 60 days. The lipase was also very stable for 16 days in the presence of organic solvents (40%, v/v) of various hydrophobic character and several hydrophilic solvents. Strikingly, methanol and DMSO at a final concentration of 40% also activated the lipase with 118% and 113% of the initial activity, respectively, after incubation for 16 days (data not shown).

3.6. Chiral resolution of racemic mandelic acid

Chiral mandelic acid and their derivatives are valuable medical intermediates. The lipase YCJ01 was found to be highly selective toward (*S*)-mandelic acid. After primary optimization, (*S*)-mandelic acid in diisopropyl ether was specifically acylation by the lipase powder using vinyl-acetate as an acyl donor (Fig. 6). The time course of resolution of mandelic acid showed that only the product of the (*S*)-mandelic acid was detected with an almost theoretical conversion yield of 50% after 26 h reaction. The enantiomeric excess ee_p of (*S*)-mandelic acid and ee_s of the residual (*R*)-mandelic acid reached excellent values of 99.9% (Fig. 7).

4. Discussion

Recently, solvent-tolerant bacteria as a relatively novel group of extremophilic microorganisms with unique ability to live in the presence of organic solvents have attracted the great attention of many researchers [17]. This property of tolerating organic solvents makes these bacteria better candidates for exploiting naturally solvent-stable enzymes [6]. Hydrophobic organic solvents, such as benzene, toluene, and cyclohexane, are generally used in screening for solvent-tolerant bacteria [18–21]. There are few reports of the isolation of organic-solvent-tolerant microorganisms using hydrophilic organic solvent ($\log P < 1$) enrichment, which is known to be extremely toxic to cells and enzymes [6]. In this study, we modified the screening strategy to obtain distinct microorganisms secreting an excellent solvent stable lipase. Using DMSO ($\log P$ −1.0) enrichment, only a few strains of solvent-tolerant bacteria, such as *B. ambifaria, Bacillus licheniformis*, *Acinetobacter johnsonii*, were isolated from 200 soil samples. The lipase from *B. ambifaria* showed greater stability to various hydrophobic and hydrophilic solvents and suggested that it could be used as a biocatalyst in non-aqueous media.

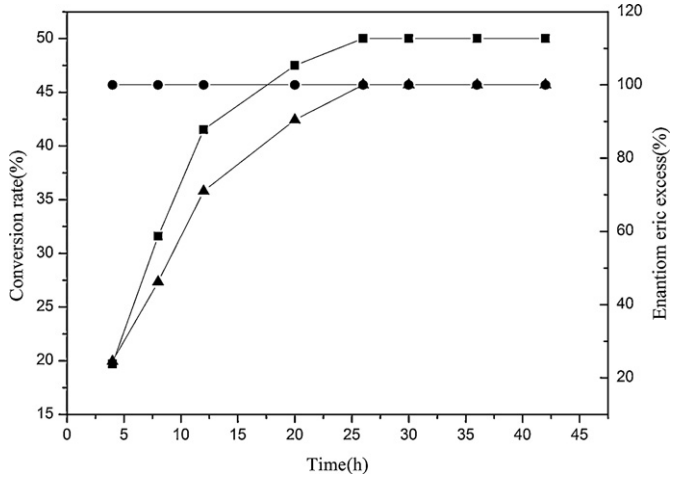

Fig. 7. Time course of bioresolution of (R,S)-mandelic acid catalyzed by Lipase YCJ01. Symbols: (■) conversion yield; (▲) ee_s, enantiomeric excess of residual substrate; (●) ee_p, enantiomeric excess of product. The reaction mixture consisted of 30 mM mandelic acid and 215 mM vinyl acetate and was catalyzed by 1% (w/v) enzyme powder at 50 °C, 180 rpm.

Many solvent-tolerant lipases have been obtained from *Pseudomonas* and *Bacillus* genus. However, lipases from different genus, species and even strains have large variations in substrate specificity, enantioselectivity, as well as solvent stability. Many isozymes, such as lipases with different characteristics from the same species were studied and developed. In this study, the amino acid sequence of the lipase from *B. ambifaria* YCJ01 showed the highest similarity (96.6%) to the lipase from *B. cepacia* S31. Eleven amino acid residues (Val^{187}, His^{198}, Ser^{207}, Ala^{214}, Leu^{262}, Val^{264}, Val^{279}, Gly^{303}, Gln^{320}, Ile^{331}, Tyr^{344}) of the lipase from *B. cepacia* S31 were substituted with Ala, Asn, Ala, Ser, Ile, Ala, Ile, Ala, Lys, Leu, Asn in the lipase YCJ01, respectively. From the structure of YCJ01 lipase modeled using lipase from *Pseudomonas cepacia* (PDB ID: 4LIP) as the template (95.6% identity to lipase YCJ01), ten of these eleven residuals were located at the surface of the lipase protein. Interestingly, the two lipases with the highest homology showed different optimal reaction pH values, 7.5 for lipase YCJ01 and 9.0 for the alkaline lipase from *B. cepacia* S31.

Strikingly, the lipase YCJ01 showed distinct super-stability to the most tested hydrophilic and hydrophobic solvents (25%, v/v) for 60 days. Lipase YCJ01 remained 100% and 80% in 25% ethanol and acetonitrile for 30 days (data not shown), while only 43% and 71% residual activity of the lipase from *B. cepacia* S31 remained for 6 h at the same concentration of 25% ethanol and acetonitrile [9]. The lipase *B. cepacia* ATCC 25416 retained 89.9%, 61.6% and 52.7% residual activity in 30% methanol, ethanol and isopropanol after incubation for 2 h [22]. However, lipase YCJ01 showed higher stability in the high concentration of hydrophilic solvents and retained 100%, 82.5% and 80.2% residual activity in the presence of 40% methanol, ethanol and isopropanol for 2 days. Furthermore, the lipase was also activated by the tested hydrophobic solvents. After 60 days incubation, hexane and heptane significantly facilitated the lipase with 135% and 119% of the initial activity, respectively. These properties make this excellent solvent-stable lipase a promising biocatalyst for enzymatic synthesis in the presence of organic solvents, a particular interest that relies on the pharmaceutical industry.

It was found that the amino acid residues located at the surface of an enzyme played an important role in its organic solvent-stability by research into the PST-01 protease [23] and a lipase from *Pseudomonas* sp. KWI-56 [24]. The difference in 11-amino acid residues might contribute to the organic solvent stability of the lipase YCJ01. Further studies on this lipase could provide an insight into the structure–function relationship of its solvent tolerance.

The practical industrial processes could be characterized: high temperature and a nonaqueous reaction system. The advantages of employing high temperature are as follows: higher process rates

(reaction temperature that increases from 25 °C to 75 °C will result in about a 100-fold increase in the process rate) [25], fewer diffusional limitations and lower viscosity of the medium, decreased bacterial contamination and a shift in thermodynamic equilibrium in case of endothermic reactions [5]. Lipase YCJ01 showed thermostability with an optimal reaction temperature at 55–60 °C. After incubation at 60 °C for 3 h, below 55 °C for 7 days or at 30 °C for 60 days (data not shown), there was hardly any lose of lipase activity. The superiority of the thermostability of lipase YCJ01 provided another excellent property for its use in biocatalysis.

Enantiomers of mandelic acid and their derivatives are valuable chemicals that have been utilized extensively for important pharmaceutical intermediates as well as stereo-chemical investigations. Lipases-catalyzed resolution is an important means of preparing chiral mandelic acid. However, these approaches were frustrated by poor organic solvent-tolerance, relative low stereoselectivity of lipase and the low solubility of mandelic acid. Yadav reported that among several commercial lipases, Novozym 435 was found to be the most effective in the hydrolysis of (*R*)-methyl mandelate with an optical purity of 78% [26]. Kinetic resolution of (*R,S*)-methyl mandelate on the concentration of 1.5 mM can be better achieved by using the lipase from *Pseudomonas* sp. immobilized in poly(ethylene oxide) with e.e. >99% at 50% conversion, while only 13% conversion was obtained with the free form [27]. Fortunately, lipase YCJ01 in free form was found to catalyze specifically the transesterification of (*S*)-mandelic acid at a concentration of 30 mM using vinyl-acetate as an acyl donor in diisopropyl ether. It reached the theoretical boundary with a conversion yield of 50% and an ee_p of 99.9%. The bioresolution process of racemic mandelic acid by lipase YCJ01 showed significant industrial potency. The mechanism of the lipase in resolution of mandelic acid with high stereoselectivity will be further analyzed according to the crystal structure of the lipase and the model of enzyme-substrate complex.

In summary, an extracellular lipase from a newly isolated solvent-tolerant bacterium *B. ambifaria* YCJ01 was purified and identified. The lipase exhibited thermostability and super-stability to the various hydrophilic and hydrophobic solvents. Furthermore, the lipase showed high activity especially toward the transesterification of (*S*)-mandelic acid in diisopropyl ether. These results indicate that lipase YCJ01 possesses attractive potency in biocatalysis and the resolution of pharmaceutical intermediates.

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