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Screening and Immobilization *Burkholderia* sp. GXU56 Lipase for Enantioselective Resolution of (*R*,*S*)-Methyl Mandelate

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Abstract Microorganisms producing lipase were isolated from soil and sewage samples and screened for enantioselective resolution of (*R*,*S*)-methyl mandelate to (*R*)-mandelic acid. A strain designated as GXU56 was obtained and identified as *Burkholderia* sp. Preparing immobilized GXU56 lipase by simple adsorption on octyl sepharose CL-4B, the optimum temperature was shifted from 40 °C (free lipase) to 50 °C (immobilized lipase), and the optimum pH was shifted from 8.0 (free lipase) to 7.2 (immobilized lipase). The immobilized enzyme displayed excellent stability in the pH range of 5.0–8.0, at the temperatures below 50 °C and in organic solvents compared with free enzyme. Enantioselectivity ratio for (*R*)-mandelic acid (*E*) was dramatically improved from 29.2 to more than 300 by applying immobilized lipase in the resolution of (*R*,*S*)-methyl mandelate. After five cycles of use of immobilized lipase, conversion and enantiomeric excess of (*R*)-mandelic acid were 34.5% and 98.5%, respectively, with enantioselectivity ratio for (*R*)-mandelic acid (*E*) of 230. Thus, octyl-sepharose-immobilized GXU56 lipase can be used as a bio-resolution reagent for producing (*R*)-mandelic acid.

Keywords *Burkholderia* sp. GXU56 · Immobilization · Lipase · (*R*)-Mandelic acid

Introduction

Mandelic acid and its enantiomers are valuable reagents that are widely employed in synthetic industry and for various pharmaceuticals. (*R*)-mandelic acid is an important resolving reagent [1], and can be used as a precursor for the manufacture of semi-synthetic penicillin, cephalosporin [2], and antiobesity medicine [3]. (*R*)-mandelic acid can be obtained by chemical resolution of the racemic form [4–5] and enzymatic resolution

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involving dehydrogenase [6], lipase [7], and nitrilase [8]. Chemical resolution is a difficult and expensive undertaking and can be environmentally detrimental. In contrast, enzymatic resolution is a natural process that is relatively benign to the environment. In particular, lipase (EC 3.1.1.3) does not require cofactors for activity, retains its activity in organic solvents, and is capable of catalyzing hydrolysis, esterification, transesterification, acidolysis, and aminolysis [7, 9–11]. The application of free lipase in resolution reactions is often tempered by some drawbacks, which include low enzymatic stability, low conversion, or low enantioselectivity. However, these shortcomings can be overcome by the use of immobilized enzyme [9, 12, 13]. Lipase can be immobilized on supports via adsorption, covalent attachment, and entrapment, which alters some of its properties, improves stability, and renders the enzyme reusable.

In this paper, we report on the isolation and identification of *Burkholderia* sp. GXU56, which produces an extracellular lipase with highly enantioselective resolution of (*R*,*S*)-methyl mandelate to (*R*)-mandelic acid but low stability. To improve its stability and enantioselectivity, we prepared immobilized lipase. Stability and enantioselectivity of enzyme were dramatically improved after simple adsorption on octyl sepharose. Thus, octyl-sepharose-immobilized GXU56 lipase may represent a useful bio-resolution reagent for producing (*R*)-mandelic acid.

Materials and Methods

Materials

(*R*,*S*)-methyl mandelate, (*R*)-mandelic acid, (*S*)-mandelic acid, 4-nitrophenyl butyrate (PNPB), TritonX-100, hexadecyl trimethyl ammonium bromide (CTAB), and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Octyl sepharose CL-4B was purchased from Amersham Biosciences (Uppsala, Sweden). Restriction enzymes, Taq DNA polymerase, T_4DNA ligase, and DNA marker were obtained from Promega (Madison, WI, USA). Olive oil (chemical grade) was purchased from Shanghai Reagents Company (Shanghai, People's Republic of China). All other chemicals were of analytical grade.

Screening Microorganisms Producing Lipases with Enantioselective Resolution of (*R*,*S*)-Methyl Mandelate to (*R*)-Mandelic Acid and Identification

Soil and sewage samples (n=210) were collected from various locations including a restaurant, market, slaughterhouse, automobile repair shop, and a food-industry factory. One gram of each sample was suspended in 10 ml of 0.85% (w/v) saline solution. A volume of 5 ml of the suspension was inoculated into 250 ml flasks containing 50 ml enriched medium with the following ingredients (all in grams per liter): olive oil (10), peptone (2), $Na_2HPO_4{\cdot}12H_2O$ (3.5), KH_2PO_4 (1.0), $MgSO_4{\cdot}7H_2O$ (0.2; final pH of 7.0). The inoculated medium was cultivated at 32 °C and 200 rpm for 48 h. Then, 0.5 ml of the culture was inoculated into 50 ml of fresh enriched medium and cultivated as before. The process was repeated. Aliquots (0.1 ml) of the final culture were serially diluted, and 0.1 ml portions of the appropriate dilution were spread on enriched agar plates, which were composed of 1.1% (w/v) agar and the same ingredients as enriched medium, and were emulsified thoroughly by an agitator before autoclave. The plates were cultivated at 32 °C for 72 h. Each colony

with a clear halo around was picked up and streaked on a fresh plate to obtain pure colonies.

Single colonies were obtained and inoculated into 25 ml flasks containing 8 ml of enriched medium, cultivated at 32 °C and 200 rpm for 48 h. Cells were collected by centrifugation (10,000×*g*, 5 min) and washed twice with 25 mM sodium phosphate buffer (pH 7.5). A screening reaction mixture (2.5 ml) contained 100 mg resting cells, 25 mM (*R*,*S*)-methyl mandelate, 5% (*v*/*v*) DMSO, 25 mM sodium phosphate buffer (pH 7.5), and 20 μl of 0.1% (*w*/*v*) bromothymol blue indicator. The reaction mixture was incubated at 32 °C and 200 rpm. The screening reactions were terminated by adjusting the pH to 2.0 using 1 M HCl.

After removing cells by centrifugation (10,000×*g*, 5 min), each reaction mixture was extracted with two times volume of ether. The extracted material was evaporated to dryness and the residue dissolved in an appropriate volume of cold toluene. The precipitate was collected, dried, and dissolved in 1 ml of distilled water for high-pressure liquid chromatography (HPLC). Strains exhibiting the greatest enantioselectivity for the production and conversion of (*R*)-mandelic acid were stored at −80 °C for further study.

The isolated strain was grown on enriched agar plates at 32 °C, 72 h for observation of colonial characteristics and subjected to Gram test and electron microscope (Hitachi, model H-500, Tokyo) after straining with 1% phosphotungstic acid sodium. The physiological, biochemical tests including aerobic, oxidase, arginine by-hydrolyase, the hydrolysis of starch, the liquefaction of gelatin and antibiotic susceptibility, and cultural characteristics including carbon source, nitrogen source, and cultivation temperature of the isolated strain were identified according to the general procedure of *Bergey's Manual of Systematic Bacteriology*.

The 16S rDNA sequence of the isolated strain was amplified by polymerase chain reaction (PCR) with the following pair of primers (5′-AGAGTTTGATCATGGCTCAG-3′) and (5′-TAGGGTTACCTTGTTACGACTT-3′). The PCR product was 1.5 Kb nucleotide and was isolated, ligated to pGEM-T Easy vector (Madison, WI, USA), transformed into *Escherichia coli* DH5α. *Escherichia coli* DH5α was grown at 37 °C in Luria broth consisting of 1% (*w*/*v*) peptone, 0.5% (*w*/*v*) yeast extract, 0.5% (*w*/*v*) NaCl. When necessary, the medium was solidified with 1.2% (*w*/*v*) agar and supplied with ampicillin (50 μg/ml), isopropylthiogalactoside (40 μg/ml) and X-gal (40 μg/ml). The positive transformant with 16S rDNA sequence was kept at −20 °C, and the nucleotide sequence of 16S rDNA was determined by the dideoxy chain-termination method. The 16S rDNA sequence finally obtained was analyzed on the website of National Center for Biotechnology Information using BLASTN.

Lipase Assay

Lipase activity was investigated by measuring the increase in the absorbance of *p*-nitrophenol produced by hydrolysis of PNPB according to the method of Dong-Woo et al. [14], slightly modified as described subsequently. The assay mixture contained 3 μl of 0.1 M PNPB/DMSO, 20 μl free lipase solution or 20 mg immobilized lipase, and 0.1 M sodium phosphate buffer (pH 7.0) in a final volume of 3 ml. *p*-Nitrophenol was monitored spectrophotometrically (Utrospect 3300, Amersham) at 405 nm and 30 °C. The molecular extinction coefficient of *p*-nitrophenol under the assay conditions above was 9,835 l mol^{-1} cm^{-1}. One unit lipase activity (U) was defined as the amount of enzyme that released 1 μmol of *p*-nitrophenol from PNPB per minute of above conditions.

Lipase Immobilization

For the preparation of immobilized lipase, 5 ml of the lipase solution from the ammonium sulfate precipitate [30–50% (*w*/*v*) saturation] was added to 20 ml of octyl sepharose CL-4B equilibrated with 25 mM sodium phosphate buffer containing 0.8 M ammonium sulfate (pH 7.5), mixed, and kept at 4 °C with intermittent stirring. After 4 h, the immobilization mixture was thoroughly washed with 25 mM sodium phosphate buffer (pH 7.5) until lipase activity could not be detected in the supernatant. The immobilized lipase (octyl-lipase) was stored at 4 °C until required.

Characterization of Free Lipase and Octyl-Lipase

After 48 h cultivation, a cell-free supernatant obtained by centrifugation (10,000×*g*, 15 min) was fractionated with ammonium sulfate [30–50% (*w*/*v*) saturation]. The precipitates were harvested and dissolved in distilled deionized water and then applied to a Hiprep 26/10 desalting column equilibrated with 25 mM sodium phosphate buffer (pH 7.5) and eluted with the same buffer in ÄKTA explorer P-100 system (Amersham Biosciences). The protein fractions with lipase activity were collected and used for enantioselective resolution and partial properties of free lipase. The optimum temperature of the lipase activity was studied by controlling the temperature in the range of 20–80 °C. The thermal stability of enzyme was determined by preserving free lipase and octyl-lipase in the various temperatures for 2 h, and the residual activity was measured in the standard conditions. The optimum pH of enzyme activity was determined in different pH buffers. The pH stability of free lipase was determined by preserving the enzyme in various pH buffers for 2 h, and the residual activity was measured using standard conditions. The pH stability of octyl-lipase was determined by preserving the enzyme in various pH buffers for 24 h, and the residual activity was measured by standard conditions. The effect of organic solvents on enzyme activity was determined at 10% (*v*/*v*) concentrations of solvent in the standard buffer at 30 °C. The effect of organic solvents on enzyme stability was determined by the residual activity after preserving it for 2 h at 10% (*v*/*v*) concentrations of solvent in the standard buffer at 30 °C.

Enantioselective Resolution of (*R*,*S*)-Methyl Mandelate by Free and Immobilized Lipase

The enantioselective resolutions were performed at 32 °C and 100 rpm in a total volume of 4 ml containing 25 mM (*R*,*S*)-methyl mandelate, 10% (*v*/*v*) DMSO, 25 mM sodium phosphate buffer (pH 7.5), and different lipases. During the reaction, the pH value was maintained at 6.5±0.5 with 1.0 M NaOH. The immobilized lipase was recovered by filtration at the end of the resolution. All resolution reactions were handled as described in the section of screening and subsequently.

Analysis of Mandelic Acid

(*R*) and (*S*)-mandelic acids were analyzed by HPLC according to the method of Arai et al. [4]. HPLC (Waters 2695, Milford, MA, USA) was equipped with Shim-pack VP-ODS column (Shimadzu, Kyoto, Japan, 150×4.6 mm, 5 μm). The solvent systems were 6 mM phenylalanine and 3 mM CuSO4/methanol (85:15, *v*/*v*) at the flow rate of 0.8 ml/min. The products were detected at 254 nm by a Waters 2996 photodiode array detector. The

retention times of (*R*)-mandelic acid and (*S*)-mandelic acid were 10–11 and 11–12 min, respectively.

Results and Discussion

Screening and Identification of Lipase-Producing Microorganisms with Enantioselective Resolution of (*R*,*S*)-Methyl Mandelate to (*R*)-Mandelic Acid

Screening lipase-producing microorganisms was carried out through enrichment by using olive oil as the sole carbon source for growth. The lipases produced by microorganisms convert the insoluble olive oil to soluble triglyceride and/or fatty acid, developing a clear zone around the colonies in the emulsified enriched plates [15]. In total, 151 lipase-producing strains were obtained. To rapidly detect lipase-catalyzed hydrolysis of (*R*,*S*)-methyl mandelate, we developed a visual screening method using the pH-sensitive indicator of bromothymol blue. With the production of mandelic acid, the pH of reaction mixtures became more acidic, which was evident as a color change of the indicator from blue to yellow (Fig. 1). HPLC analysis identified a strain designated GXU56 as possessing the highest conversion of (*R*,*S*)-methyl mandelate and enantioselectivity to produce (*R*)-mandelic acid. The fermentation of the carbon source (olive oil) released fatty acids, which produced a decrease in pH to below 7.0, which was in turn evident by the indicator-related color change of the culture supernatant. However, since a similar color change was evident in some of the supernatants, it proved impossible to use bromothymol blue as an indicator when supernatants of broths were applied in screening reaction. The screening approach of applying supernatant, which is taxing in terms of effort and time, was thus abandoned. Instead, we utilized resting cells but not the supernatant for screening of the 151 strains for

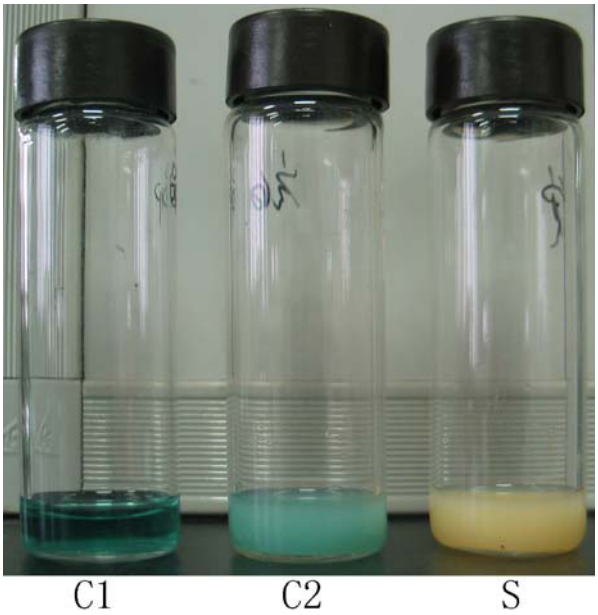

Fig. 1 Visual method to screen strains which hydrolyze (*R*,*S*)-methyl mandelate using bromothymol blue. The reaction liquid (RL) used in the 2.5-ml screening volume consisted of 5% (*v*/*v*) DMSO, 25 mM sodium phosphate buffer (pH 7.5), and 20 μl 0.1% (*w*/*v*) bromothymol blue. *C1* 25 mM (*R*,*S*)-methyl mandelate + RL; *C2* resting cells 100 mg + RL; *S* resting cells 100 mg + 25 mM (*R*,*S*)-methyl mandelate + RL

the production of extracellular lipase. Lipase activity could not be detected before reaction, while the enzymatic activity could be detected at the end of screening reaction in the reaction mixtures, which indicated that the resting cells still secreted extracellular lipase during the screening reaction.

When grown on agar plate containing emulsified olive oil, the circular, clear edge and smooth-surfaced colonies of strain GXU56 were yellow in color, and a halo was formed around the colony as well. Biochemical tests showed that the cells were nonmotile, rod-shaped, Gram-negative, aerobic, oxidase-positive, arginine-by-hydrolyase-negative, and capable of the hydrolysis of starch and the liquefaction of gelatin. GXU56 was sensitive to chloromycetin, gentamycin, spectinomycin, and kanamycin, was inhibited by ampicillin, and was resistant to tetracyclin. GXU56 utilized glucose, sucrose, starch, mannito, and some lipids including olive oil, tween80, peanut oil, bean oil, and lard as sole carbon source for cell growth and lipase production, but much more lipase was produced when lipid served as sole carbon source. It also utilized various organic and inorganic nitrogen sources including ammonium sulfate, urea, peptone, yeast extract, and beef extract as sole nitrogen source for growth and lipase production. It grew well in the temperature from 25 to 37 °C. 16S rDNA sequence analyses revealed a fairly high identity (99%) with *Burkholderia anthina* (accession no. AJ544072.1), *Burkholderia cepacia* (accession no. AB252073.1), and *Burkholderia* sp. (accession no. DQ847125.1). Therefore, strain GXU56 was identified as *Burkholderia* sp. GXU56.

Partial Characterization of Free and Immobilized Lipase

The effect of temperature on lipase activity and stability are shown in Fig. 2. The optimum temperatures for the activity of free lipase and octyl-lipase were 40 and 50 °C, respectively. The thermal stability of free lipase was very low, with the residual activity being reduced by 88% from the initial activity after preservation for 2 h at 30 °C and pH 7.0. In contrast, octyl-lipase did not display any loss of activity during a 2 h exposure to 50 °C and pH 7.0, or 5 days at 30 °C and pH 7.0.

The effect of pH on lipase activity and stability is shown in Fig. 3. The optimum pH of free lipase was 8.0. pH stability of the free enzyme was low; only 30.7% of the initial

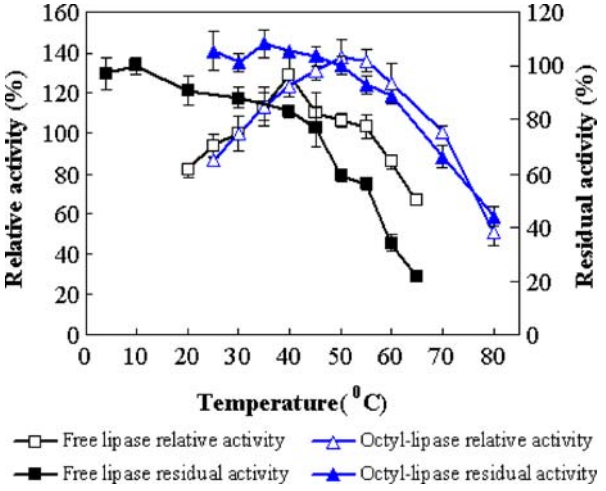

Fig. 2 Effect of temperature on the activity and stability of free and octyl-sepharose-immobilized GXU56 lipase. Lipase activity was assayed as described in "Materials and Methods." The initial activity of free lipase (1,690 U ml^{-1}) and octyl-lipase (2,072 U g^{-1}) assayed in standard conditions were considered as 100% relative activity and residual activity. Values represent the mean±SD of three independent experiments

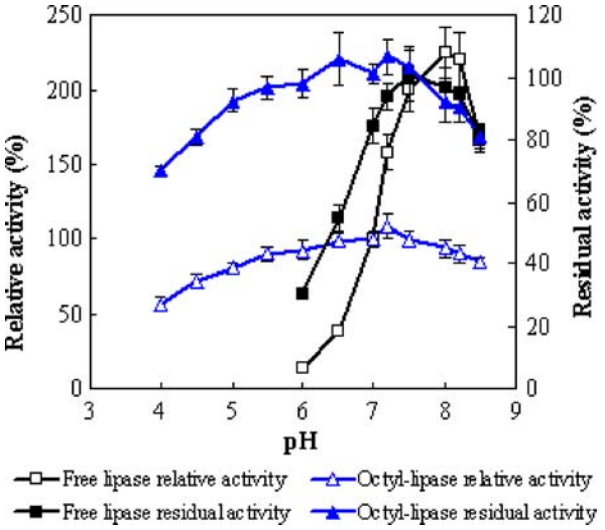

Fig. 3 Effect of pH on the activity and stability of free and octyl-sepharose-immobilized GXU56 lipase. The buffers used were 0.05 M citric acid–sodium citrate, pH 3–6; 0.1 M sodium phosphate, pH 6–7.5; and 0.05 M Tris–HCl, pH 7.5–8.5. Lipase activity was assayed as described in "Materials and Methods." The initial activity of free lipase (1,690 U ml^{-1}) and octyl-lipase (2,072 U g^{-1}) assayed in standard conditions were considered as 100% relative activity and residual activity. Values represent the mean±SD of three independent experiments

activity remained following preservation for 2 h at pH 6.0 and 30 °C. The optimum pH of octyl-lipase was 7.2. In contrast to the free form, octyl-lipase was very stable in an acidic environment; residual activity following preservation for 24 h at pH 5.0 and 30 °C was 92.6% of the initial activity. Free lipase had very low activity in organic solvents except DMSO, but octyl-lipase had relatively high activity and was very stable in organic solvents (Table 1).

Burkholderia lipases are found to have a wide range of optimum temperature and pH for activity and are widely utilized in biocatalysis. *B. cepacia* G63 lipase was reported to have maximum activity at pH 7.0 at 70 °C and to be stable at a range of temperature from 40 to 70 °C [16]. A trehalose-oleate hydrolyzing lipase from *B. cepacia* AKU-883 shows the maximum activity at pH 9.0 and 65 °C and is stable in the range of pH 5–10 and below 60 °C [17].

Table 1 Effect of organic solvents on the activity and stability of free and octyl-sepharose-immobilized GXU56 lipase.

Solvent (10%)	Relative activity (%)		Residual activity (%)	
	Free lipase	Octyl-lipase	Free lipase	Octyl-lipase
Control	100	100	–	–
DMSO	105.1	123.7	91.6	117.1
Methanol	54.3	117.3	10.7	86.3
Ethanol	64.8	136.0	6.8	85.6
Isopropanol	40.2	98.4	0	77.3
Acetone	54.0	94.9	0	97.8
Ether	60.5	92.6	9.6	90.5
Formaldehyde	0	105.1	–	98.4
Dimethylformamide	43.1	118.5	0	110.7

Lipase activity was assayed as described in "Materials and Methods." The initial activity of free lipase (1,690 U ml^{-1}) and octyl-lipase (2,072 U g^{-1}) assayed in standard conditions were considered as 100% relative activity and residual activity. Values are the means of triplicate determinations.

Table 2 Resolution of (*R*,*S*)-methyl mandelate to (*R*)-mandelic acid using free lipase and octyl-sepharose-immobilized GXU56 lipase.

Enzyme	Total activity (U)	Time (h)	*C* (%)	ee_R (%)	*E*
Free lipase	3,000	4	25.0	91.2	29.2
Free lipase	3,000	8	35.8	86.7	22.4
Free lipase	3,000	12	39.8	79.0	14.2
Free lipase	3,000	16	41.2	79.3	17.2
Free lipase	3,000	24	41.7	78.6	14.6
Octyl-lipase	622	24	29.8	99.9	>300
Octyl-lipase	622	48	41.9	99.9	>300

The resolutions were performed in a total volume of 4 ml containing 25 mM (*R*,*S*)-methyl mandelate, 10% DMSO, and 25 mM sodium phosphate buffer (pH 7.5), and different lipases, at 32 °C and 100 rpm.
C Conversion, $C(\%) = [P]/[S_0] \times 100$, where *P* is total concentration of (*R*)-mandelic acid and (*S*)-mandelic acid, S_0 is the initial concentration of (*R*,*S*)-methyl mandelate; ee_R enantiomeric excess of (*R*)-mandelic acid, $ee_R(\%)=[(R)\text{-mandelic acid}]-[(S)\text{-mandelic acid}]/[(R)\text{-mandelic acid}]+[(S)\text{-mandelic acid}]\times 100$; *E* enantioselectivity ratio for (*R*)-mandelic acid, $E = \mathrm{In}[1 - C(1 + ee_R)]/\mathrm{In}[1-C(1 - ee_R)]$.

The immobilized enzyme technique is a useful method to change characterization and improve stability. Some properties of GXU56 were dramatically changed and were more stable after immobilization on octyl sepharose CL-4B. This is consistent with previous observations that the optimum pH of *Mucor miehei* lipase immobilized on polysiloxane-polyvinyl alcohol magnetic particles is improved to 8.0 from 7.0 of free enzyme [18], the improved thermal stability of *Candida rugosa* lipase following immobilization on octyl-agarose [19], and the enhanced lipase hydrolysis of 4-nitrophenyl palmitate by immobilized lipases from a variety of different bacterial species [20]. The enhanced activity may well be due to a conformational change in the immobilized enzyme and (or) re-orientation of the site of catalysis[18–20].

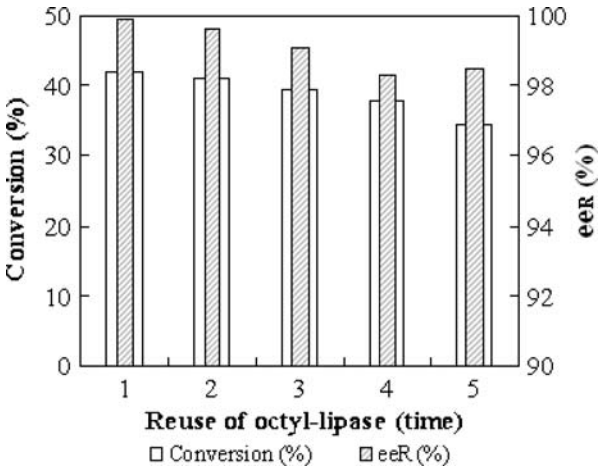

Fig. 4 Repeated use of octyl-sepharose-immobilized GXU56 lipase for resolution of (*R*,*S*)-methyl mandelate to (*R*)-mandelic acid. Experiments were performed in a total volume of 4 ml containing 25 mM (*R*,*S*)-methyl mandelate, 10% DMSO, 25 mM sodium phosphate buffer (pH 7.5), and 622 U octyl-lipases, at 32 °C and 100 rpm. $\text{Conversion}(\%) = [P]/[S_0] \times 100$, where *P* is total concentration of (*R*)-mandelic acid and (*S*)-mandelic acid, S_0 is the initial concentration of (*R*,*S*)-methyl mandelate; ee_R enantiomeric excess of (*R*)-mandelic acid, $ee_R(\%)=[(R)\text{-mandelic acid}]-[(S)\text{-mandelic acid}]/[(R)\text{-mandelic acid}]+[(S)\text{-mandelic acid}]\times 100$

Resolution of (*R*,*S*)-Methyl Mandelate by Free Lipase and Octyl-Lipase

The results of resolution by free GXU56 lipase and octyl-lipase are shown in Table 2. With conversion increasing, ee_R decreased, which demonstrated that free lipase did not have to be strictly enantioselective to (*R*)-methyl mandelate. The highest enantioselectivity ratio for (*R*)-mandelic acid (*E*) was merely 29.2. Moreover, a higher enantiopurity is a requirement for pharmaceutical application, and the low stability of free GXU56 lipase also limited its application. This prompted us to improve the enzyme's stability and enantioselectivity. Some successful cases have been reported. Chaubey et al. [13] immobilized *Arthrobacter* sp. lipase (ASL) by covalent bonding on sepharose and entrapping in silica. Both of two immobilized enzymes displayed excellent stability compared to free ASL, also improved enantioselectivity value of (*R*)-products from 45–47 to 285–473, with enantimeric excess of (*R*)-products from 93% to 99% compared with free ASL in the same conditions in resolution of an intermediate of antidepressant fluoxetine, ethyl 3-hydroxy-3-phenylpropanoate alkyl acylates. *Pseudomonas* sp. lipase immobilized in poly (ethylene oxide) obtained (*R*)-methyl mandelate with enantioselectivity ratio of 1,057 at conversion of 50% and enantiomeric excess of 99% compared with free enzyme under same conditions obtaining enantioselectivity ratio of 230 at conversion of 13% and enantiomeric excess of 15% in the transesterification of (*R*,*S*)-methyl mandelate and vinyl acetate [9]. By immobilizing GXU56 lipase, which could be accomplished simply by adsorption on octyl sepharose CL-4B, the enantioselectivity ratio for (*R*)-mandelic acid (*E*) significantly improved to more than 300 at 29.8% and 41.1% conversion with ee_R of 99.9%. Moreover, repeated use (five times) of the immobilized GXU 56 lipase showed that the enzymatic activity persisted, with 84% of initial activity remaining, and with conversion and ee_R still 34.5% and 98.5% (E=230), respectively (Fig. 4). These observations mirror previous results obtained using a commercial lipase of Novozym 435 (component B of the lipase from *Candida antarctica* immobilized on macroporous polyacrylate resin), in which the catalyzed enantioselective resolution of (*R*,*S*)-methyl mandelate in tert-butanol displayed an enantiomeric excess of (*R*)-mandelic acid of 78% at 19% conversion at the end of 24 h, and enantioselectivity ratio for (*R*)-mandelic acid was 9.6. [7]. Presently, GXU56 lipase displayed a strong hydrophobic interaction with octyl sepharose CL-4B; the adsorbed GXU56 lipase was eluted in presence of 0.3 M TritonX-100 (data not shown). Octyl-lipase has minor conformational changes or re-orientation of enzymatic active structure [12, 19, 20], similar to lipase from *C antarctica* immobilized on octyl agarose [12].

In conclusion, the present study demonstrates that octyl-sepharose-immobilized *Burkholderia* GXU56 lipase can be used as a bio-resolution reagent for producing (*R*)-mandelic acid. Further studies to refine the efficiency of this reaction and to scale up its use to industrially relevant proportions are warranted.

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