

Full Paper

Identification of a newly isolated *Sphingomonas* sp. LZ1 and its application to biosynthesize chiral alcohols

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A strain LZ1, which showed efficient asymmetric reduction of 3,5-bis(trifluoromethyl) acetophenone to enantiopure (*S*)-[3,5-bis(trifluoromethyl)-phenyl]ethanol, which is the key intermediate for the synthesis of a receptor antagonist and antidepressant, was isolated from a soil sample. Based on its morphological, 16S rDNA sequence, and phylogenetic analysis, the strain LZ1 was identified to be *Sphingomonas* sp. LZ1. To our knowledge, this is the first reported case of the species *Sphingomonas* exhibiting stricter *S*-enantioselectivity and its use for the asymmetric reduction of 3,5-bis(trifluoromethyl) acetophenone. Some key reaction parameters involved in the bioreduction catalyzed by whole cells of *Sphingomonas* sp. LZ1 were subsequently optimized, and the optimized conditions for the synthesis of (*S*)-[3,5-bis(trifluoromethyl)-phenyl]ethanol were determined to be as follows: phosphate buffer pH 7.5, 70 mM of 3,5-bis(trifluoromethyl) acetophenone, 30 g/L of glucose as a co-substrate, 300 g (wet weight)/L of resting cell as the biocatalyst, and a reaction for 24 h at 30°C and 180 rpm. Under the above conditions, a best yield of 94% and an excellent enantiomeric excess of 99.6% were obtained, respectively. *Sphingomonas* sp. LZ1 could also asymmetrically reduce a variety of prochiral ketones to their corresponding optical alcohols with excellent enantioselectivity. These results indicated that *Sphingomonas* sp. LZ1 had a remarkable capacity to reduce 3,5-bis(trifluoromethyl)acetophenone to its corresponding (*S*)-[3,5-bis(trifluoromethyl)-

phenyl]ethanol, and might be a new potential biocatalyst for the production of valuable chiral alcohols in industry.

Key Words: 3,5-bis(trifluoromethyl)acetophenone; asymmetric reduction; biocatalysis; carbonyl reductase; *Sphingomonas* sp.

Introduction

Chiral alcohols are important building blocks, which are widely applied in the introduction of a chiral center into many useful industrial chemicals, in areas such as pharmaceuticals, agrochemicals and natural products (Itoh, 2014; Ni and Xu, 2012; Zheng and Xu, 2011). Various strategies, such as chromatographic separation, enantioselective resolution or dynamic kinetic resolution, and asymmetric reduction, have been developed for the synthesis of chiral alcohols (Zheng et al., 2017). Asymmetric biocatalytic reduction of ketones is an efficient and most powerful way to prepare optically pure alcohols because of its high enantioselectivity, 100% theoretical yield, and environmentally friendly and meeting green chemistry guidelines (Luo et al., 2018; Zhu et al., 2019). Many microorganisms or isolated enzymes have been applied as biocatalysts in the asymmetric biocatalytic reduction process (Shah et al., 2018; Wang et al., 2017; Wu et al., 2015). However, the scale-up of asymmetric biocatalytic reduction reactions has been restricted due to the limited commercially available biocatalysts, narrow substrate specificity, expensive cofactor dependency, and low initial substrate concentration and insolubility. Consequently, the discovery of highly efficient novel biocatalysts is al-

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ways of special interest in the field of biocatalysis, especially in biocatalytic asymmetric synthesis.

Optically pure 1-[3,5-bis(trifluoromethyl)phenyl]ethanol (BTPE) is a very important intermediate for the synthesis of NK-1 receptor antagonist and antidepressant (Pollard et al., 2006; Zhang et al., 2011). Asymmetric biocatalytic reduction of 3,5-bis(trifluoromethyl) acetophenone (BTAP) provides a straightforward approach to produce enantiomerically pure BTPE. In recent years, some microorganisms or isolated enzymes have been reported for their ability to produce (*R*)-[3,5-bis(trifluoromethyl)phenyl] ethanol ((*R*)-BTPE) from BTAP, such as *Leifsonia xyli* (Wang et al., 2014), *Lactobacillus kefir* (Chen et al., 2016), *Penicillium expansum* (Kurbanoglu et al., 2009), *Microbacterium oxydans* (Gai et al., 2013), *Trichoderma asperellum* ZJPH0801 (Li et al., 2013), *Chryseobacterium* sp. CA49 (Liu et al., 2014), *Burkholderia cenocepacia* (Yu et al., 2018), *Leifsonia* sp. S749 (Tang et al., 2019), and immobilized ketoreductase (Li et al., 2015). Some microorganisms or isolated enzymes have also been reported for their ability to produce (*S*)-[3,5-bis(trifluoromethyl)phenyl] ethanol ((*S*)-BTPE) from BTAP, such as *Geotrichum candidum* 90685 (Homann et al., 2004), *Candida tropicalis* 104 (Wang et al., 2011), *Saccharomyces rhodotorula* (Zhang et al., 2011), alcohol dehydrogenase from *Rhodococcus erythropolis* (Pollard et al., 2006), and engineered *Escherichia coli* (Dascier et al., 2014). However, most of these microorganisms mentioned above have some drawbacks, such as a relatively low space-time yield and substrate insolubility, low catalytic functions and a narrow substrate spectrum, etc. These defects greatly limit their industrial application. Therefore, the search for robust biocatalysts with good catalytic efficacies to the asymmetric reduction of BTAP is of great significance.

In the present study, a strain LZ1, which showed an efficient reduction of BTAP to (*S*)-BTPE with an excellent stereoselectivity, was isolated from a soil sample. This strain was identified and named as *Sphingomonas* sp. LZ1. As far as we know, this is the first report case of the species *Sphingomonas* being applied for the asymmetric reduction of BTAP. Some key reaction parameters involved in the reduction process catalyzed by whole cells were consequently systematically investigated, and a substrate-coupled cofactor regeneration system was established, using glucose as a co-substrate, to improve the efficiency of the bioprocess. In addition, a variety of prochiral ketones were also enantioselectively reduced by this new isolate, which yielded corresponding chiral alcohols with an excellent enantiomeric excess (e.e.).

Materials and Methods

Materials and medium. Chemically synthesized BTAP was supplied by Taizhou Jiecheng Chemical Co., Ltd., China. (*R*)-BTPE, (*S*)-BTPE and (*R,S*)-BTPE were purchased from the Aladdin Chemical Co., Ltd., China, as well as other prochiral ketones and the corresponding alcohols. All other biochemical reagents and chemicals used in this study were of analytical grade, and were obtained from commercial sources. The strain *Sphingomonas* sp. LZ1 was isolated from a soil sample collected in

Xiangtan, Hunan Province, China.

The enrichment medium consisted of the following (g/L): (NH₄)₂SO₄ 2.0, KH₂PO₄ 2.0, NaCl 1.0, MgSO₄·7H₂O 0.2, BTAP 12.8 (50 mM). Screening medium plates were prepared with an enrichment medium supplemented with 20 g/L of agar. A seed medium with pH 6.5 contained (g/L): glucose 10, peptone 5, yeast extract 5, (NH₄)₂SO₄ 2, KH₂PO₄ 1, MgSO₄·7H₂O 0.5, and NaCl 0.5, and a slant medium was composed of the seed medium supplemented with 20 g/L of agar. The fermentation medium with pH 6.5 contained (g/L): glucose 30, peptone 20, yeast extract 4, (NH₄)₂SO₄ 2, KH₂PO₄ 1, MgSO₄·7H₂O 0.5, and NaCl 0.5.

Isolation and cultivation. The microbial strains were isolated from soil samples by an enrichment culture technique using an enrichment medium supplemented with 50 mM of BTAP as the sole source of carbon. The isolation process was performed as follows: a soil sample (0.5 g) was suspended into a 250-mL Erlenmeyer flask containing 50 mL of enrichment medium, and the enrichment was cultured for 6 days at 30°C and 180 rpm. The cultured liquid (5.0 mL) was transferred into a fresh enrichment medium for another round of enrichment culture, and then the cultured liquid was diluted appropriately. The diluents (0.2 mL) were plated over the screening medium plates, and incubated at 30°C for 3 days. The microbial strains, which were able to grow on the screening medium plates, were purified by continuous streaking on screening medium plates. The resulting isolated single colonies were inoculated into the slant medium for 3 days at 30°C, and then a full ring of strain cells from the bottom of the slant medium were inoculated into 250-mL flasks containing 100 mL seed medium for 24 h at 30°C and 180 rpm. Then, the cultured seed liquid was inoculated into 500-mL flasks containing 200 mL of fermentation medium with 10% final concentration, and incubated for 48 h at 30°C and 180 rpm. The cells were harvested by centrifugation and washed twice with 0.85% normal saline, and the cells obtained were then used for the bioreduction of BTAP to evaluate their catalytic abilities.

Identification and phylogenetic analysis. The cell morphology of strain LZ1 was observed with a light microscope. The genomic DNA for PCR was extracted by the Guangzhou Aiji Biotechnology Co., Ltd., China. The 16S ribosomal DNA (16S rDNA) genes were amplified by PCR using a universal primer, and a fragment of about 1300 bp was obtained. The amplified fragment was purified and determined by the Guangzhou Aiji Biotechnology Co., Ltd., China. Related sequences were selected from the GenBank database (National Center for Biotechnology Information, NCBI) using the BLAST search program. Multiple alignments of sequences, the construction of a neighbor-joining phylogenetic tree with the nucleotide *p*-distance model, and a bootstrap analysis for the evolution of the phylogenetic topology were performed by CLUSTAL X ver.2.0 and MEGA version 7.0, respectively.

Asymmetric reduction of BTAP using whole cells of strain LZ1 as a biocatalyst. The asymmetric reduction reaction was conducted in a 50-mL flask on an orbital shaker for 24 h at 30°C and 180 rpm. In each flask, the reaction mix-

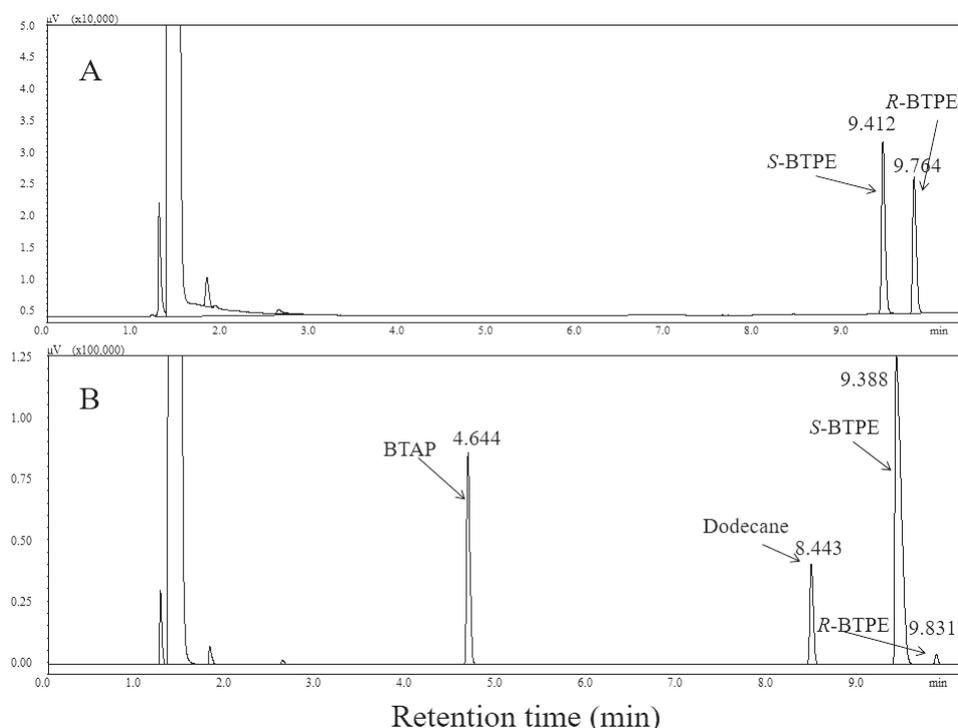


Fig. 1. GC spectrum of BTPE enantiomers.

A. Racemic BTPE standard; B. Whole cell biocatalysis produced (*S*)-BTPE. The sample was analyzed under the conditions described in Analytical methods.

ture consisted of 5.0 mL of phosphate buffer (200 mM, pH 8.0 or 7.5), 50 mM of BTAP, 200 g (wet weight)/L of resting cells, and 20 g/L of glucose as the co-substrate. The reaction was quenched by removing biomass from the reaction mixture through centrifugation. Ethyl acetate was used to extract the product from the reaction mixture, and the ethyl acetate layer obtained was dried with anhydrous MgSO_4 and then analyzed by GC.

Analytical methods. The yield and e.e. of product were determined by a GC instrument with an FID detector and a varian CP-Chirasil-Dex CB column (25 m \times 0.25 mm \times 0.25 μm). Detection conditions: carrier gas was nitrogen (2 mL/min), injector temperature and FID detector temperature were 250°C, the split ratio was 1:15 and the injection volume was 1 μL . The column temperature was held at 80°C for 2 min and was increased to 180°C at a rate of 8°C/min, and then kept constant for 10 min. The absolute configuration of the products were identified by comparing the retention times with that of the standard samples. The retention times of the products were as follows: (*S*)-BTPE (9.388 min), (*R*)-BTPE (9.831 min); (*R*)-1-phenylethanol (9.848 min), (*S*)-1-phenylethanol (10.121 min); (*R*)-1-[*p*-(trifluoromethyl)phenyl]ethanol (11.268 min), (*S*)-1-[*p*-(trifluoromethyl)phenyl]ethanol (11.683 min); (*R*)-1-(*p*-fluorophenyl)ethanol (10.384 min), (*S*)-1-(*p*-fluorophenyl)ethanol (10.687 min); (*R*)-1-(*o*-methylphenyl)ethanol (12.112 min), (*S*)-1-(*o*-methylphenyl)ethanol (12.656 min); (*R*)-1-(*p*-methoxyphenyl)ethanol (14.887 min), (*S*)-1-(*p*-methoxyphenyl)ethanol (15.082 min). Split ratio was 1:50: (*R*)-1-phenyl-1-propanol (11.544 min), (*S*)-1-phenyl-1-propanol (11.672 min); (*S*)-2,2,2-trifluoro-1-phenylethanol (11.806 min), (*R*)-2,2,2-trifluoro-1-phenylethanol (12.052

min); ethyl (*R*)-3-hydroxybutyrate (6.084 min), ethyl (*S*)-3-hydroxybutyrate (6.343 min).

Results and Discussion

Isolation and identification of strain LZ1

After several cycles of continuous isolation, hundreds of colonies exhibiting different morphologies appeared on the screening medium plates. The biocatalytic activity and enantioselectivity of these isolates were evaluated for their ability to reduce BTAP to enantiomerically pure BTPE. The yield and e.e. of the bioreduction product were determined by GC analysis as described above, and the GC spectra of BTPE enantiomers are shown in Fig. 1 (dodecane was the internal standard). One isolate, namely LZ1, was isolated from a soil sample, and exhibited stricter *S*-enantioselectivity and a higher yield for the preparation of (*S*)-BTPE. Therefore, strain LZ1 was chosen for further experiments.

Colonies of the strain LZ1 on screening medium plates were round, smooth and wet, slightly uplifted, yellow and transparent. The cell morphology was rod-shaped under a light microscope. The partial 16S rDNA sequence of strain LZ1 (1358 bp) was determined. To elucidate the phylogenetic position of strain LZ1, sequence analysis of the 16S rDNA genes of strain LZ1 and related species were used to construct a phylogenetic tree using the neighbor-joining method (Fig. 2). Strain LZ1 was closely clustered with *Sphingomonas* sp. *S*-2 (GenBank accession No. AY081166.1), and sharing a high similarity of 99.85%. Hence, the strain LZ1 was designated as *Sphingomonas* sp. LZ1. To our knowledge, this is the first report case of the species *Sphingomonas* being capable of reducing

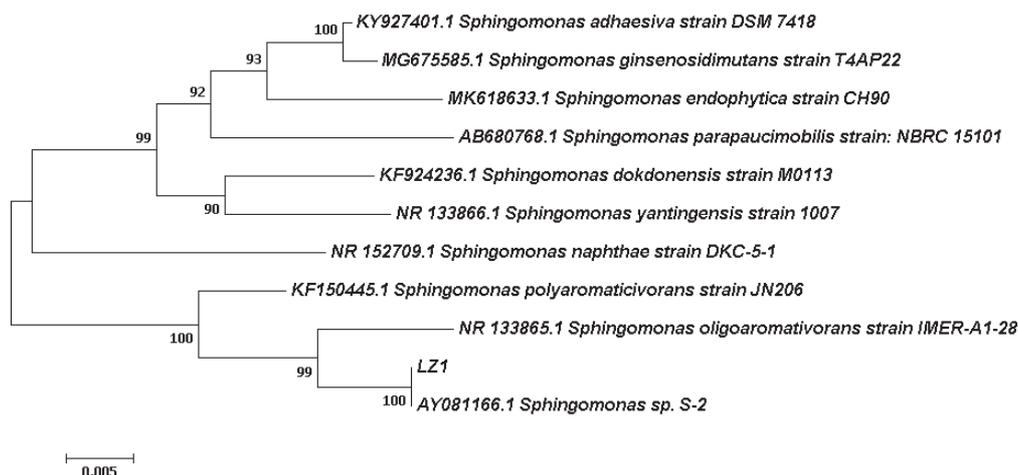


Fig. 2. Phylogenetic tree based on 16S rDNA sequences, constructed by the neighbor-joining method. The bar represents 0.005 substitutions per base position. Bootstrap values expressed as percentages of 1,000 replications are shown at branch points.

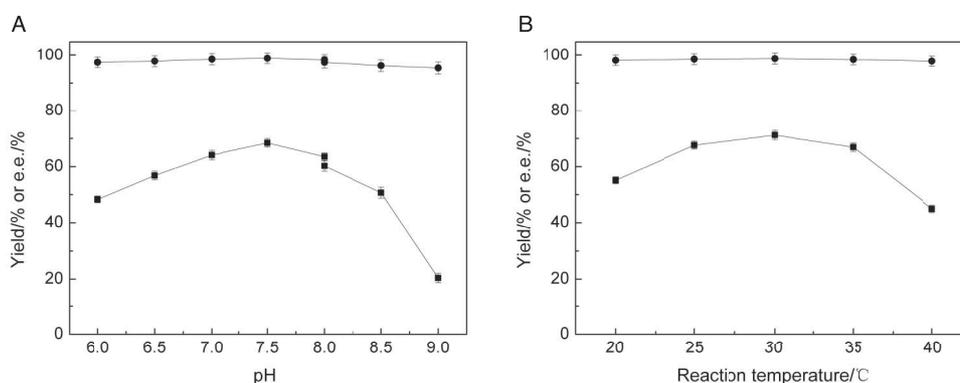


Fig. 3. Effect of pH (A) and reaction temperature (B) on the bioreduction of BTAP.

Symbols: e.e. (solid circles) and yield (solid squares). Reaction conditions (A): 5.0 mL buffer system [200 mM, different pHs ($\text{NaHPO}_4\text{-NaH}_2\text{PO}_4$, 6.0, 6.5, 7.0, 7.5, 8.0; boric acid-borax: 8.0, 8.5, 9.0)], 200 g/L of resting cells, 20 g/L of glucose, 50 mM of BTAP, and reaction for 24 h at 30°C, 180 rpm. Reaction conditions (B): 5.0 mL phosphate buffer (200 mM, pH 7.5), 200 g/L of resting cells, 20 g/L of glucose, 50 mM of BTAP, and reaction for 24 h at different reaction temperatures, 180 rpm.

BTAP. Accordingly strain LZ1 is proposed to be an important contribution to the microbial reductase library, and to provide a new enzyme source for the preparation of (S)-BTPE.

Effects of pH and temperature on the bioreduction of BTAP

The reaction pH and temperature were two crucial variables in biocatalytic processes, as these conditions affect enzyme activity and selectivity (Cui et al., 2017; Wang et al., 2016; Wei et al., 2016). Therefore, the reduction of BTAP using the whole cells of strain LZ1 as the biocatalyst were investigated in the pH range of 6.0–9.0. The results in Fig. 3A indicate that pH has a remarkable effect on product yield, but only a slight influence on enantioselectivity. With pH varying from 6.0–7.5, the yield of (S)-BTPE increased to the highest level of 71%, and then the yield decreased rapidly when the pH was above 8.0, with a yield of 20% at pH 9.0. These results indicate

that strain LZ1 has a better activity under slightly alkaline conditions, and the optimum reaction pH was 7.5.

The different reaction temperatures were also investigated and the results are shown in Fig. 3B. Within the tested temperature range from 20–40°C, (S)-BTPE was prepared with high optical purity (>98%). The yield of the product was improved with an increase of reaction temperature from 20–30°C, and then a higher temperature led to a sharp drop in the yield, with a yield of 45% at 40°C, which could be attributed to the partial inactivation of the enzyme at the relatively higher reaction temperature. Therefore, the optimum reaction temperature was 30°C.

Effect of co-substrate on the bioreduction of BTAP

Cofactor regeneration is essential in efficient bioreduction processes to produce chiral alcohols from ketones, and efficient and cost-effective cofactor regeneration systems, such as enzyme- and substrate-coupled systems, have been developed (Kawano et al., 2011). In

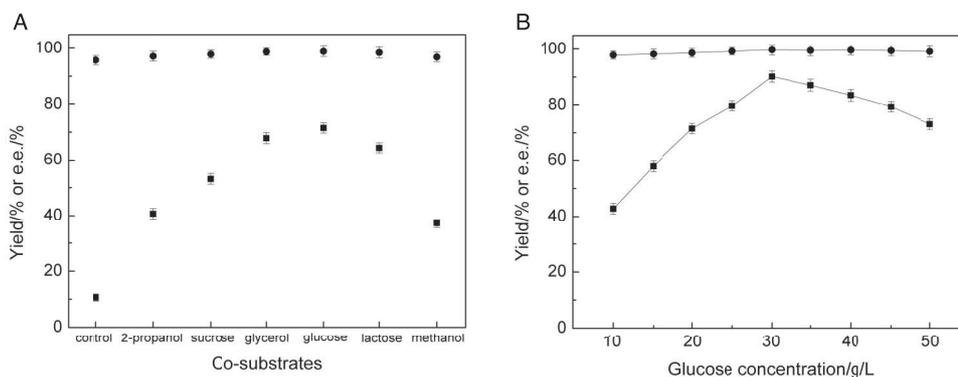


Fig. 4. Effect of different co-substrates (A) and glucose concentration (B) on the bioreduction of BTAP.

Symbols: e.e. (solid circles) and yield (solid squares). Reaction conditions (A): 5.0 mL phosphate buffer (200 mM, pH 7.5), 200 g/L of resting cells, 50 mM of BTAP, 20 g/L of co-substrate, and reaction for 24 h at 30°C, 180 rpm. Reaction conditions (B): 5.0 mL phosphate buffer (200 mM, pH 7.5), 200 g/L of resting cells, 50 mM of BTAP, different concentrations of glucose, and reaction for 24 h at 30°C, 180 rpm.

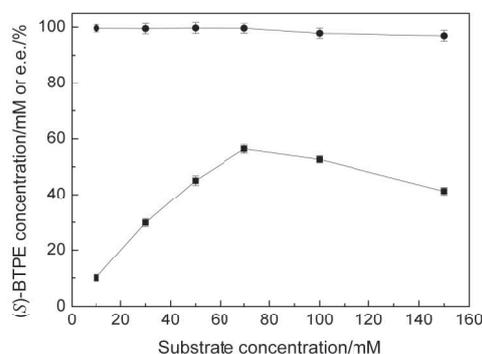


Fig. 5. Effect of substrate concentration on the bioreduction of BTAP.

Symbols: e.e. (solid circles) and (S)-BTPE concentration (solid squares). Reaction conditions: 5.0 mL phosphate buffer (200 mM, pH 7.5), 200 g/L of resting cells, 30 g/L of glucose, different concentrations of BTAP and reaction for 24 h at 30°C, 180 rpm.

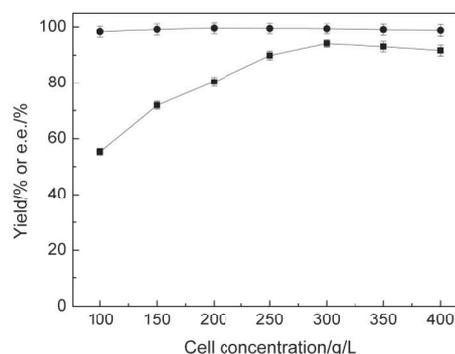


Fig. 6. Effect of cell concentration on the bioreduction of BTAP.

Symbols: e.e. (solid circles), yield (solid squares). Reaction conditions: 5.0 mL phosphate buffer (200 mM, pH 7.5), 70 mM of BTAP, 30 g/L of glucose, different concentrations of resting cells, and reaction for 24 h at 30°C, 180 rpm.

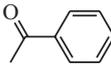
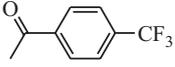
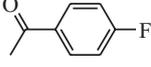
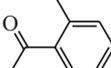
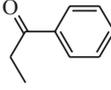
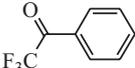
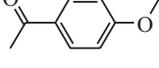
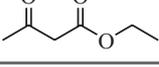
this study, a substrate-coupled cofactor regeneration system was established to improve the efficiency of the bioprocess. As shown in Fig. 4A, glucose, sucrose, lactose, glycerol, 2-propanol and methanol, were employed as co-substrates for cofactor recycling, and improved significantly the yield of (S)-BTPE but had only a slight influence on enantioselectivity. As is indicated, using glucose as a co-substrate achieved the highest yield, and this yield was increased 6.74-fold than without a co-substrate (control). Therefore, a substrate-coupled biotransformation was demonstrated with glucose as a co-substrate for driving the cofactor regeneration.

In order to achieve a higher product yield, the effects of glucose concentration on yield and enantioselectivity were measured. As shown in Fig. 4b, the highest yield of 90% was obtained at a concentration of 30 g/L of glucose, which is almost an 8.5-fold increase in contrast to no addition of co-substrate. Meanwhile, the e.e. value also improved slightly from 95.7% (in the absence of a co-substrate) to 99.7% (using glucose as the co-substrate). Therefore, the optimal glucose concentration was considered to be 30 g/L, and was used for the following experiments.

Effect of substrate concentration on the bioreduction of BTAP

The amount of substrate loading is a key issue for the potential of biocatalytic application. An excessive substrate concentration either results in substrate inhibition or affects the utilization of alcohol dehydrogenase because of a short of cofactors (Zhang et al., 2014). Thus, the effect of substrate concentration on the bioreduction of BTAP was investigated and the results are shown in Fig. 5. The concentration of (S)-BTPE increased almost linearly with the increase of substrate concentration within the range of 10–70 mM, while the e.e. of the product was constantly above 99.0%. With a further increase of the substrate concentration to 150 mM, the concentration of (S)-BTPE notably decreased, and the e.e. value also decreased slightly, for which a likely reason may be some substrate and/or product inhibition, and/or a toxicity to microbial cells. Taking these results into account, a substrate concentration of 70 mM was adopted for the production of (S)-BTPE.

Table 1. Bioreduction of different substrates by *Sphingomonas* sp. LZ1^a.

Prochiral ketone	Structure	Conversion ^b /%	e.e. ^c /%
Acetophenone		65	96.7 (<i>S</i>)
<i>p</i> -(Trifluoromethyl)acetophenone		81	99.1 (<i>S</i>)
<i>p</i> -Fluoroacetophenone		58	97.3 (<i>S</i>)
<i>o</i> -Methylacetophenone		34	81.5 (<i>S</i>)
Propiophenone		44	89.3 (<i>S</i>)
2,2,2-Trifluoroacetophenone		77	98.4 (<i>R</i>)
<i>p</i> -Methoxyacetophenone		84	95.3 (<i>S</i>)
Ethyl acetoacetate		99	88.5 (<i>S</i>)

^a The values are averages of three replicas.

^b The conversions and ^c e.e. values were determined by chiral GC analysis. The absolute configuration of the products were identified by comparing the retention times with the standard samples. Reaction conditions: 5.0 mL phosphate buffer (200 mM, pH 7.5), 30 g/L of glucose, 300 g (wet weight)/L of resting cells, 50 mM of substrates, and reaction for 24 h at 180 rpm and 30°C.

Effect of resting cell concentrations on the bioreduction of BTAP

The amount of incubated whole-cell (cell loading) is also a key parameter which should be optimized for biocatalytic processes. As shown in Fig. 6, the product yield showed an obvious enhancement as the cell loading increased. With an increase of cell loading from 100 to 300 g/L, the yield increased significantly and the best yield of 94% was attained at 300 g/L. However, the yield decreased slightly as the cell loading was further increased. A likely reason for this might be that excessive cells could not be uniformly dispersed and thus not be sufficiently in contact with the substrate in the reaction system. Additionally, all the tested cell concentrations hardly have any influence on enantioselectivity. Therefore, the most suitable cell concentration was taken to be 300 g (wet weight)/L.

Preparative scale bioreduction of BTAP to (*S*)-BTPE

The preparative scale asymmetric bioreduction of BTAP to (*S*)-BTPE by whole cells of *Sphingomonas* sp. LZ1 was carried out in a 5.0-L bioreactor. The reaction mixture contained 1.0 L phosphate buffer (200 mM, pH 7.5), 5.0 mM of BTAP, 300 g (wet weight)/L of resting cell, and 30 g/L of glucose. After reaction for 24 h at 30°C and 500 rpm, the substrate was transformed to (*S*)-BTPE with >99% yield and >99% e.e. The reaction mixture was extracted with ethyl acetate and the ethyl acetate layer obtained was collected, and concentrated by vacuum distillation in a

rotary evaporator. The concentrate solution was purified by silica gel column chromatography (eluent: petroleum ether/ethyl acetate, 8:1, v/v), followed by evaporation under vacuum, and an isolated yield of 78% was achieved. The purified product was characterized with the standard samples on GC, the optical rotation and ¹H NMR. The GC spectrum and retention time for the product were consistent with the standard sample of (*S*)-BTPE, and ¹H NMR data [¹H NMR (600 MHz, CDCl₃): 7.83 (s, 2H), 7.78 (s, 1H), 5.04 (q, 1H, *J* = 6.48 Hz), 2.08 (d, 1H), 1.55 (d, 3H, *J* = 6.48 Hz)] and [α]_D²⁵ = -21.4 (*c* = 1.0, CHCl₃).

Bioreduction of different substrates

To extend the application of *Sphingomonas* sp. LZ1, another eight prochiral ketones were subjected to asymmetric bioreduction under the above-mentioned conditions for BTAP. The results are presented in Table 1, which shows that strain LZ1 exhibited a stricter *S*-enantioselectivity of all the tested substrates. Ethyl acetoacetate was nearly completely reduced but the optical purity of the product was only 88.5%, and the reduction of propiophenone and *o*-methylacetophenone also did not achieve a high stereoselectivity. The reduction of acetophenone, *p*-(trifluoromethyl)acetophenone, *p*-fluoroacetophenone, *p*-methoxyacetophenone, and 2,2,2-trifluoroacetophenone were satisfactory (e.e. of above 95%), while the relatively low conversions might be improved by optimizing the cultivation and/or reaction con-

ditions. In view of the above-mentioned results, *Sphingomonas* sp. LZ1 had a relatively broad substrate spectrum, and showed a high potential for the synthesis of chiral alcohols.

Conclusions

In conclusion, a newly isolated *Sphingomonas* sp. LZ1 was isolated from a soil sample using BTAP as the sole carbon source, and the optimum reaction conditions for the preparation of (*S*)-BTPE were found to be as follows: 5.0 mL of phosphate buffer (200 mM, pH 7.5), 70 mM of BTAP, 300 g (wet weight)/L of resting cell, 30 g/L of glucose, and reaction for 24 h at 30°C, 180 rpm. Under the above optimal conditions, the strain LZ1 exhibited a good yield (94%) and an excellent e.e. (99.6%), which was superior to the reported *Candida tropicalis* 104 (Wang et al., 2011) (92% yield and 99.9% e.e. of (*S*)-BTPE, at 50 mM of BTAP) and *Saccharomyces rhodotorula* (Zhang et al., 2011) (78% yield and 97.8% e.e. of (*S*)-BTPE, at 5.5 mM of BTAP). Strain LZ1 could also reduce a variety of prochiral ketones with a high optical purity of products. All these features indicated that the strain LZ1 had a remarkable capacity to reduce BTAP to its corresponding (*S*)-BTPE, and might be a new potential biocatalyst for the production of valuable chiral alcohols in industry.

Acknowledgments

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