Highly enantioselective oxidation of phenyl methyl sulfide and its derivatives into optically pure (S)-sulfoxides with Rhodococcus sp. CCZU10-1 in an n-octane–water biphasic system

ARTICLE *in* APPLIED MICROBIOLOGY AND BIOTECHNOLOGY · OCTOBER 2013

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Highly enantioselective oxidation of phenyl methyl sulfide and its derivatives into optically pure (*S*)-sulfoxides with *Rhodococcus* sp. CCZU10-1 in an *n*-octane–water biphasic system

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Received: 21 July 2013 / Revised: 9 September 2013 / Accepted: 10 September 2013 / Published online: 5 October 2013

Abstract Enantiopure sulfoxides can be prepared via the asymmetric oxidation of sulfides using sulfide monooxygenases. The *n*-octane–water biphasic system was chosen for the bio-oxidation of a water-insoluble phenyl methyl sulfide (PMS) by *Rhodococcus* sp. CCZU10-1. In this *n*-octane–water system, the optimum reaction conditions were obtained. (*S*)-phenyl methyl sulfoxide ((*S*)-PMSO) with >99.9 % enantiomeric excess formed at 55.3 mM in the *n*-octane–water biphasic system. Using fed-batch method, a total of 118 mM (*S*)-PMSO accumulated in 1-L reaction mixture after the 7th feed, and no (*R*)-PMSO and sulfone were detected. Moreover, *Rhodococcus* sp. CCZU10-1 displayed fairly good activity and enantioselectivity toward other sulfides. In conclusion, *Rhodococcus* sp. CCZU10-1 is a promising biocatalyst for synthesizing highly optically active sulfoxides.

Keywords Asymmetric oxidation · Phenyl methyl sulfide · (*S*)-phenyl methyl sulfoxide · Biphasic system · *Rhodococcus* sp. CCZU10-1

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Introduction

Chiral sulfoxides are extensively used as important chiral intermediates and auxiliaries, chiral ligands and catalysts, and chiral drugs (Carreño 1995; Carreño et al. 2009; Fernandez and Khiar 2003; Li et al. 2009; Nakamura et al. 2000; Wojaczyńska and Wojaczyński 2010). The value of chiral sulfoxide functionality is further illustrated by their diverse bioactivities and pharmaceutical uses (Adam et al. 2005; Holland 2001; Li et al. 2009). Usually, only one enantiomer in a chiral compound is responsible for the desired bioactivity (Wojaczyńska and Wojaczyński 2010). Therefore, it is necessary to synthesize enantiomerically pure sulfoxides.

Enantiomerically pure sulfoxides may be obtained by chemical and biological methods. These methods include chiral auxiliary's induction (Bolm and Simic 2001), chiral oxidant's oxidation (Fernandez and Khiar 2003; Lattanzi et al. 2004), chiral resolution (Liao et al. 2003, 2011a), and enantioselective catalysis (Drago et al. 2005; Li et al. 2011b). Among them, the asymmetric oxidation of prochiral sulfides is a more economical method for synthesizing enantiomerically pure sulfoxides than the kinetic resolution of racemic sulfoxides (Huang et al. 2005; Holland et al. 1997, 2003; Li et al. 2009, 2011a). Usually, these asymmetric oxidations of prochiral sulfides into enantiomerically pure sulfoxides with metal catalysts or isolated enzymes (e.g., haloperoxidases, monooxygenases, and peroxidases) are expensive and tedious due to the involvement of chiral metal complexes or the separation of enzymes (Adam et al. 1998; Brunel et al. 1995; Huang et al. 2005; Li et al. 2009). By contrast, asymmetric oxidations of prochiral sulfides catalyzed by whole-cell biocatalysts (e.g., bacteria and fungi) are much cheaper and more convenient, avoiding the involvement of expensive cofactors (NADH/NADPH) and the separation

of enzymes. In the past 20 years, several microorganisms have been successfully employed for asymmetric sulfoxidation (Beecher et al. 1992, 1994; Borges et al. 2009; Bolm and Simic 2001; Colonna et al. 2002; Li et al. 2009; Mascotti et al. 2012; Nakamura et al. 2000; Ohta et al. 1989; Pasta et al. 1995; Pinedo-Rivilla et al. 2007; Ricci et al. 2005). However, low enantioselectivity or poor substrate tolerance was found in aqueous system. *Acinetobacter* sp. NCIMB 9871, *Pseudomonas* sp. NCIMB 9872, *Xanthobacter autotrophicus* DSM 731 (NCIMB 10811), and the Black yeast NV-2 could transform the low concentration (<10 mM) of sulfide (Kelly et al. 1996). In the aqueous systems, phenyl methyl sulfide (PMS) could be oxidized by *Rhodococcus* sp. ECU0066 into (*S*)-phenyl methyl sulfoxide ((*S*)-PMSO) with an enantiomeric excess (*e.e.*) value of about 80 % when the PMS concentration was higher than 10 mM (Li et al. 2011b). *Trichoderma viride* could give the (*R*)-PMSO with 70 % *e.e.*, and *Botrytis cinerea* could afford (*S*)-PMSO with 41 % *e.e.* (Pinedo-Rivilla et al. 2007). *Aspergillus* genus could transform PMS into (*R*)-PMSO with 70 % *e.e.* value (Mascotti et al. 2012). Therefore, it is necessary to develop the bioprocess with higher enzyme activity and better enantioselectivity for asymmetric oxidation of sulfides.

Recently, we have isolated a bacterial strain from soil, *Rhodococcus* sp. CCZU10-1 (He et al. 2012), which harbors high sulfide-monooxygenase activity and could enantioselectively transform PMS into (*S*)-PMSO with >99.9 % *e.e.* (He et al. 2013a). However, low yield of (*S*)-PMSO was obtained when PMS concentration was >10 mM in the aqueous system (Fig. 1). To effectively oxidize high concentration of PMS, various factors were systematically examined and optimized to improve the yield and keep the excellent *e.e.* values of (*S*)-PMSO in the organic–aqueous biphasic system. Furthermore, asymmetric oxidation of PMS and its derivatives by *Rhodococcus* sp. CCZU10-1 in the organic–aqueous biphasic system was successfully demonstrated.

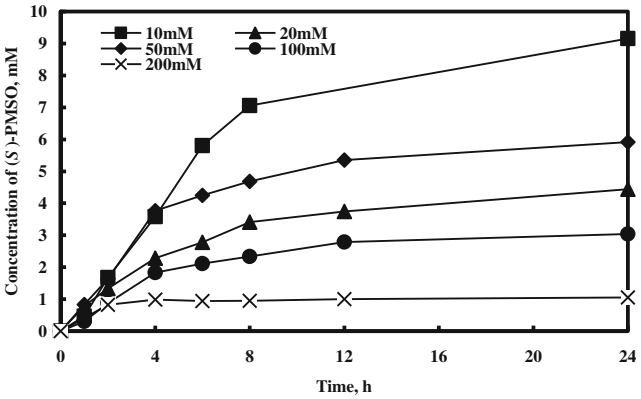


Fig. 1 Time course for the oxidation of PMS in aqueous system

Materials and methods

Chemicals

PMS was purchased from Aladdin reagent Co., Ltd. (Shanghai, China). Peptone and yeast extract were purchased from Sinopharm Group Chemical Reagent Co. Ltd. (Shanghai, China). Other chemicals were obtained from commercial sources and were of analytical grade.

Microorganism and its culture

Rhodococcus sp. CCZU10-1 with the sulfide monooxygenase activity was preserved in our laboratory (He et al. 2013a), and its sequence data of 16S rDNA was deposited in Genbank database (accession no., JF272470). It has been deposited at China General Microbiological Culture Collection Center with an accession numbers of CGMCC no. 4911. *Rhodococcus* sp. CCZU10-1 was cultured in 500-mL Erlenmeyer flasks containing 100 mL growth medium (glucose, 10.0 g/L; peptone, 10.0 g/L; yeast extract, 5.0 g/L; KH_2PO_4, 2.0 g/L; NaCl, 1.0 g/L; $MgSO_4$, 0.20 g/L; and PMS, 1.0 mM; pH 7.5) at 30 °C in a rotary shaker (180 rpm). After 48 h, the cells were harvested by centrifugation (10,000×*g*, 5 min), and then suspended in the KH_2PO_4–K_2HPO_4 buffer (100 mM, pH 8.0) for further use.

Oxidation of PMS in the aqueous single-phase system

Asymmetric oxidations of PMS by *Rhodococcus* sp. CCZU10-1 in the aqueous single-phase system were performed in 500-mL shake flasks with screw caps. The harvested resting cells of *Rhodococcus* sp. CCZU10-1 (8.0 g, wet weight; ca. 1.58 g dry cell weight) was suspended in 100 mL KH_2PO_4–K_2HPO_4 buffer (100 mM, pH 8.0). Various concentrations of PMS were oxidized by the resting cells of *Rhodococcus* sp. CCZU10-1. The substrate concentrations were varied from 10 mM to 200 mM. Reactions were carried out at 30 °C and 180 rpm. A 1.0-mL aliquot was withdrawn from the reaction media, saturated with NaCl, and mixed with 0.6 mL of the internal standard solution (5 mM 4-nitroacetophenone in ethyl acetate (EtOAc)). Following centrifugation (8,000×*g*, 5 min), the EtOAc phase was dried over Na_2SO_4 and then subjected to GC for assaying (*S*)-PMSO and sulfone concentrations and HPLC for assaying *e.e.* of (*S*)-PMSO.

Oxidation of PMS in different aqueous–organic solvent biphasic systems

Asymmetric oxidations of PMS by *Rhodococcus* sp. CCZU10-1 in biphasic systems were performed in 500-mL shake flasks with screw caps. The harvested resting cells of

Rhodococcus sp. CCZU10-1 (8.0 g, wet weight; ca. 1.58 g dry cell weight) was suspended in 85 mL phosphate buffer (100 mM, pH 8.0) and mixed with 15 mL of organic solvent (e.g., cyclohexane, dichloromethane, ethyl acetate, *n*-heptane, *n*-hexane, *iso*-octane, *n*-octane, petroleum ether, or toluene) to form the aqueous–organic solvent biphasic system (Table 1). Reactions were carried out at 30 °C and 180 rpm. Unless otherwise specified, the reported substrate and product concentration refers only to the concentration in the reaction media based on total volume of reaction media. Given its high hydrophobicity, the substrate PMS is primarily partitioned in the organic phase, while the product (*S*)-PMSO and the possible by-product sulfone are mainly dissolved in the aqueous phase (Li et al. 2011b). Individual aliquots (200 μL) of sample were withdrawn from the organic phase at different time intervals and mixed with 200 μL of an EtOAc solution of 4-nitroacetophenone (25 mM) as an internal standard. The mixture was then subjected to GC analysis for determining the substrate concentration remaining within the organic phase. A 1.0-mL aliquot was withdrawn from the aqueous phase, saturated with NaCl, and mixed with 0.6 mL of the internal standard solution (5 mM 4-nitroacetophenone in EtOAc). Following centrifugation (8,000×*g*, 5 min), the EtOAc phase was dried over Na_2SO_4 and then subjected to GC for assaying (*S*)-PMSO and sulfone concentrations and HPLC for assaying *e*.*e*. of (*S*)-PMSO. The amounts of starting material (substrate and cells) and the phase volume ratio were adjusted according to the requirements of each specific experiment.

Phase volume ratio was calculated as follows:

$$\text{Phase volume ratio} = (\text{Volume of organic solvent})/(\text{Volume of water})$$

Yield was calculated as follows:

$$\text{Yield} = (\text{Concentration of } (S)\text{−PMSO in reaction media})/(\text{Initial concentration of substrate in reaction media}) \times 100\%$$

Analytical methods

The *e*.*e*. of (S)-PMSO and concentrations of PMS, (*S*)-PMSO and phenylmethyl sulfone were assayed by HPLC or GC analysis (Li et al. 2009). Assays of PMS-oxidizing activity were assayed by GC. One unit of cellular activity (U) was assayed as the amount of cells (g dry cell weight, g DCW) required for 1 μmol/min of PMS decrease at 30 °C and pH 8.0. All experiments were performed in triplicate.

Table 1 Effects of organic solvent on the PMS-oxidizing activity

Organic solvent	Log *P*	Relative activity (%)[a]
None	–	100±2.7
Toluene	2.5	23.6±1.0
Ethyl acetate	0.68	33.5±1.1
Dichloromethane	1.0	90.5±2.1
Petroleum ether	3.1	136±2.7
Iso-octane	4.5	161±2.8
n-hexane	3.5	166±2.9
n-heptane	4.0	187±3.1
Cyclohexane	3.2	245±3.2
n-octane	4.5	892±19.2

[a] Using 20 mM PMS as substrate, specific activity of *Rhodococcus* sp. CCZU10-1 was 0.71±0.02 μmol min^{-1} g^{-1} DCW) in monophasic system (pH 8.0, 100 mM) at pH 8.0 and 30 °C, and the enzyme activity was taken as 100 %. After 10 min of reaction, the PMS-oxidizing activities were determined by GC. All experiments were performed in triplicate.

Optimization of reaction conditions in *n*-octane–aqueous system.

The effect of reaction pH on the oxidation of PMS was determined using reaction mixtures adjusted to specified pH values as follows: pH 4.0–6.0, 100 mM citric acid–Na_3 citrate buffer; pH 6.0–8.0, 100 mM KH_2PO_4–K_2HPO_4 buffer; pH 8.0–10.0, glycine–NaOH; under otherwise similar conditions. The effect of reaction temperature on the oxidation of PMS was examined at different temperatures from 25 to 50 °C by assaying their activities at the respective temperatures, under otherwise similar conditions. Furthermore, the oxidation reaction conditions by *Rhodococcus* sp. CCZU10-1 were optimized in the *n*-octane–water system. The effects of cell concentration on the PMS-oxidizing activity were examined at 0.01–0.20 g (wet weight)/mL, under otherwise similar conditions. The effects of PMS concentration on the PMS-oxidizing activity were examined at 20–150 mmol/L, under otherwise similar conditions.

Fed batch reaction

Eighty grams of wet resting cells were suspended in 850 mL KH_2PO_4–K_2HPO_4 buffer (100 mM, pH 8.0). This cell suspension was then mixed with 150 mL *n*-octane containing 20 mmol PMS in reaction media. The reaction was carried out at 30 °C in a 5-L reaction jar fermenter with stainless steel three-bladed turbine impellers (Shanghai Guoqiang Bioengineering Equipment Co., Ltd., China). The agitation rate (400 rpm) and aeration rate (2.4 L/min) were controlled

by a peed-varible motor and a submerged sparger (Li et al. 2011b), respectively. Fed-batch reaction was monitored by HPLC and GC. PMS (20 mmol) was fed in the subsequent six feeds, and the certain volume of reaction mixture was periodically withdrawn and assayed by HPLC and GC for probing the product (*S*)-PMSO formation.

Results

Oxidation of PMS in the aqueous single-phase system

Various concentrations of PMS were oxidized by the resting cells of *Rhodococcus* sp. CCZU10-1. The substrate concentrations were varied from 10 to 200 mM. Figure 1 shows the time course for the (*S*)-PMSO production with resting cells. All *e.e*. values remained above 99.9 % (data not shown). These finding were better than those observed in aqueous system (PMS=10 mM) (Li et al. 2009), the use of aqueous systems for PMS oxidation by *Rhodococcus* sp. ECU0066 resulted in *e.e*. values of about 80 % when the substrate concentrations were higher than 10 mM. As shown in Fig. 1, the asymmetric oxidation of PMS could be successfully conducted with substrate of up to 10 mM in aqueous system. Ten micromolars of PMS could be oxidized into (*S*)-PMSO in a yield of 91.6 % after 24 h. When the PMS concentration was incresed to 20 mM, the (*S*)-PMSO was obtained in the yield of 29.6 % after 24 h. Further increase of PMS concentration, low yields of (*S*)-PMSO were obtained (Fig. 1). To effectively synthesize (*S*)-PMSO, it is necessary to build an appropriate reaction system and optimize the reaction conditions.

Oxidation of PMS in the aqueous–organic solvent biphasic system

Organic solvent could influence the enzyme activity (He et al. 2012; Kansal and Banerjee 2009). Therefore, a potential biocompatible organic solvent could improve the oxidation of PMS. In this study, nine organic solvents were tested (Table 1), including cyclohexane, dichloromethane, EtOAc, *n*-heptane, *n*-hexane, *iso*-octane, *n*-octane, petroleum ether, and toluene. The influence of various organic solvents on catalytic activity and enantioselectivity of *Rhodococcus* sp. CCZU10-1 was investigated using different aqueous–organic solvent (15:85, v/v) biphasic systems. The *e.e*. values of (*S*)-PMSO produced in all the aqueous–organic solvent biphasic systems were in excess of 99.9 % (data not shown). As listed in Table 1, oxidation activities were well correlated with solvent log *P* values. The oxidation activity of PMS increased with increases in the log *P* value of the solvent. As shown in Table 1, dichloromethane (log *P*=1.0), ethyl acetate (log *P*=0.68) and toluene (log *P*=2.5) gave lower PMS-oxidation activity, whereas petroleum ether (log *P*=3.1), cyclohexane (log *P*=3.2), *n*-hexane (log *P*=4.0), *iso*-octane (log *P*=4.5), and *n*-octane (log *P*=4.5) gave a higher PMS-oxidizing activity. However, using the resting cells of *Rhodococcus* sp. ECU0066 as biocatalyst, no product (*S*)-PMSO was detected in the EtOAc–aqueous biphasic system (Li et al. 2011b), and the *iso*-octane–water biphasic system was employed for the oxidation of PMS. In the case of *n*-octane, the substrate partitioned mainly in the organic phase while the product (*S*)-PMSO was dissolved mainly (>98 %) in the aqueous phase. In this study, therefore, *n*-octane was chosen to be a more suitable organic phase for achieving a better separation between the substrate PMS and the product (*S*)-PMSO.

In the aqueous–organic biphasic system, the volumetric phase ratio influences not only the interfacial areas but also the organic phase effect on microbial cells (Gong and Xu 2005; He et al. 2012; Li et al. 2011b). In the *n*-octane–water biphasic system, the volumetric phase ratio had significantly effects on the oxidation activity of PMS (Fig. 2). However, *e.e*. values of (*S*)-PMSO produced in the different *n*-octane–water biphasic systems were in excess of 99.9 % (data not shown). As shown in Fig. 2, the oxidation activity reached the maximum when the content of *n*-octane was 15 % (*v*/*v*), which was comparable with the results obtained in single aqueous phase of phosphate buffer (100 mM, pH 8.0). As a result, the optimal ratio of water phase to *n*-octane phase was considered to be 85:15 (*v*/*v*).

Reaction pH, reaction temperature, cell and substrate concentrations have significant effects on the biocatalysis activity (He et al. 2010, 2013b; Kansal and Banerjee 2009; Li et al. 2011a, b). In this study, effects of these factors on PMS-oxidizing activity were also investigated in *n*-octane–water (15:85, *v*/*v*) biphasic system. The oxidation reaction was also

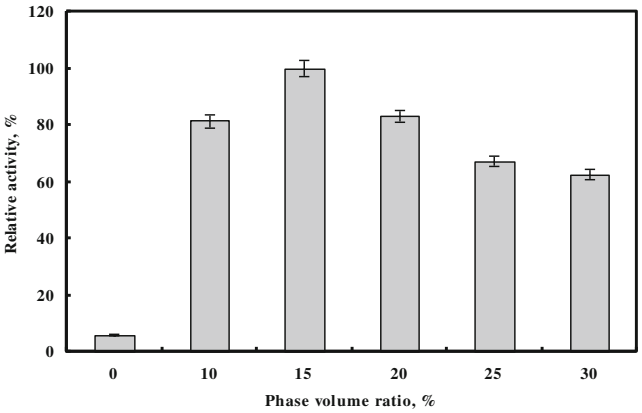


Fig. 2 Effects of *n*-octane phase volume ratio on the PMS-oxidizing activity in biphasic system. Using 20 mM PMS as substrate, specific activity of *Rhodococcus* sp. CCZU10-1 was 6.4±0.18 μmol min^{-1} g^{-1} DCW in *n*-octane–water (15:85, *v*/*v*) biphasic system at pH 8.0 and 30 °C, and the enzyme activity was taken as 100 %. After 10 min of reaction, the PMS-oxidizing activities were determined by GC. All experiments were performed in triplicate

carried out in 100 mM buffers of various pH values. In addition, the oxidation rate exhibited a maximum at pH 8.0 (Fig. 3a). Moreover, the *e.e.* value of the product was not affected by temperature and pH changes (data not shown). All *e.e.* values remained above 99.9 %. The oxidation reaction was also carried out in different temperatures. The oxidation rate increased with a rise in temperature, reaching a maximum at 30 °C (Fig. 3b). At temperatures above 30 °C, the oxidation rate decreased considerably, possibly due to thermal deactivation of enzymes in the cells during the reaction. The optimal reaction conditions of pH 8.0 and 30 °C were employed for the following experiments. The concentration of biocatalyst is an important factor in enzymatic reactions because it affects both enantioselectivity and reaction rate (Li et al. 2011b). To investigate the effect of cell concentration on PMS oxidation in *n*-octane–water system, the *e.e.* values and PMS-oxidizing activities were determined at different cell concentrations. As shown in Fig. 3c, the concentration of cells had a marked effect on the PMS-oxidizing reaction, with 0.08 g (wet weight)/mL being the optimum value. However, all *e.e.* values remained above 99.9 % (data not shown). When the cell concentration was above 0.08 g (wet weight)/mL, the viscosity of the aqueous phase increased so that the low dissolved oxygen (DO) in the aqueous phase became prominent, and the PMS-oxidizing reaction rate decreased significantly. In *n*-octane–water biphasic systems, the experiments with substrate concentrations ranging from 20 to 150 mM were employed for the asymmetric oxidation of PMS to (*S*)-PMSO under the optimal conditions obtained above. The highest reaction rate (31.8 μmol min^{-1} g^{-1} DCW)) was recorded at 100 mM PMS (Fig. 4a). Substrate inhibition resulted when the PMS-oxidizing rate was significantly decreased at 150 mM PMS. Moreover, the *e.e.* value of the product was not affected by substrate concentration changes. All *e.e.* values remained above 99.9 % (data not shown). As shown in Figs. 1 and 4a, therefore, it could be concluded that this aqueous–organic solvent biphasic system was advantageous over the monophasic aqueous system (Li et al. 2011b) for the enantioselective oxidation of PMS catalyzed by the resting cells of *Rhodococcus* sp. CCZU10-1 as it kept both high oxidation activity and high enantioselectivity of the biocatalyst used, which is essential for the efficient production of (*S*)-PMSO with high concentration and high *e.e.* value.

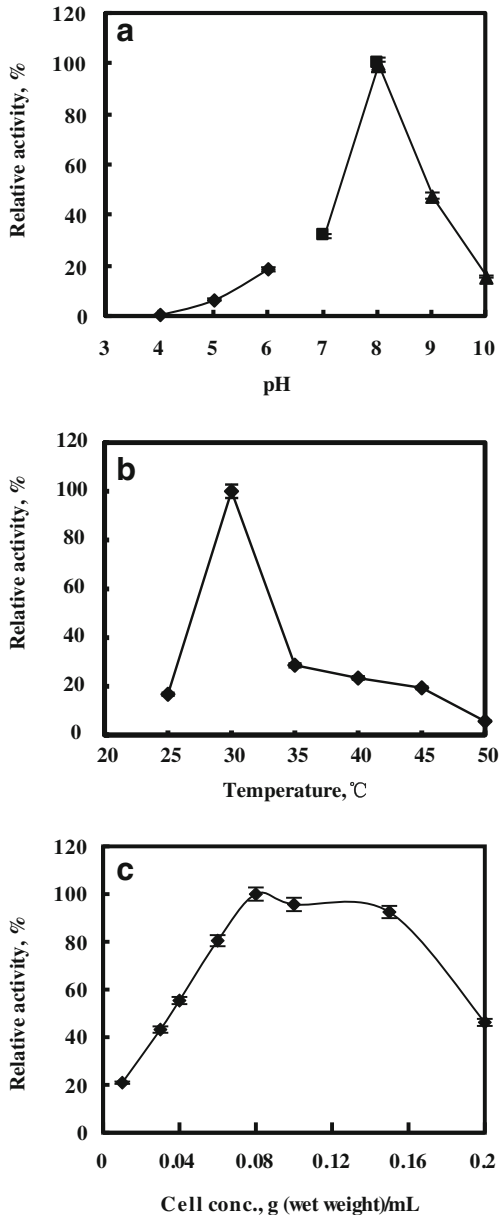


Fig. 3 Effects of reaction pH (**a**), reaction temperature (**b**), and cell concentration (**c**) on the PMS-oxidizing activity. Using 20 mM PMS as substrate, specific activity of *Rhodococcus* sp. CCZU10-1 was 6.4± 0.18 μmol min^{-1} g^{-1} DCW in *n*-octane–water (15:85, *v*/*v*) biphasic system at pH 8.0 and 30 °C, and the enzyme activity was taken as 100 %. After 10 min of reaction, the PMS-oxidizing activities were determined by GC. All experiments were performed in triplicate

Based on the above experiments, the optimum reaction temperature, reaction pH, cells, and substrate concentration were 30 °C, 8.0, 0.08 g (wet weight)/mL, and 100 mM, respectively. Under these reaction conditions, different concentrations of PMS were oxidized by *Rhodococcus* sp. CCZU10-1 in *n*-octane–water (15:85, *v*/*v*) biphasic system. As shown in Fig. 4b, the sample of PMS (20 mM) could be oxidized into (*S*)-PMSO in the yield of 73.7 % after 24 h. When the PMS was 100 mM, (*S*)-PMSO yielded 55.3 % in 24 h. When the PMS was 150 mM, the (*S*)-PMSO presented with a yield of 23.7 % in 24 h. However, using the resting cells of *Rhodococcus* sp. ECU0066 as catalysts, the final product (*S*)-PMSO concentration could reach 16.7 and 18.3 mM from the oxidation of 100 and 150 mM PMS, respectively (Li et al. 2011b). Significantly, using the resting cells of *Rhodococcus* sp. CCZU10-1 as catalysts, (*S*)-PMSO formed at a higher concentration and a greater enantiomeric excess (55.3 mM and >99.9 % *e.e.*) in this *n*-octane–water biphasic system than

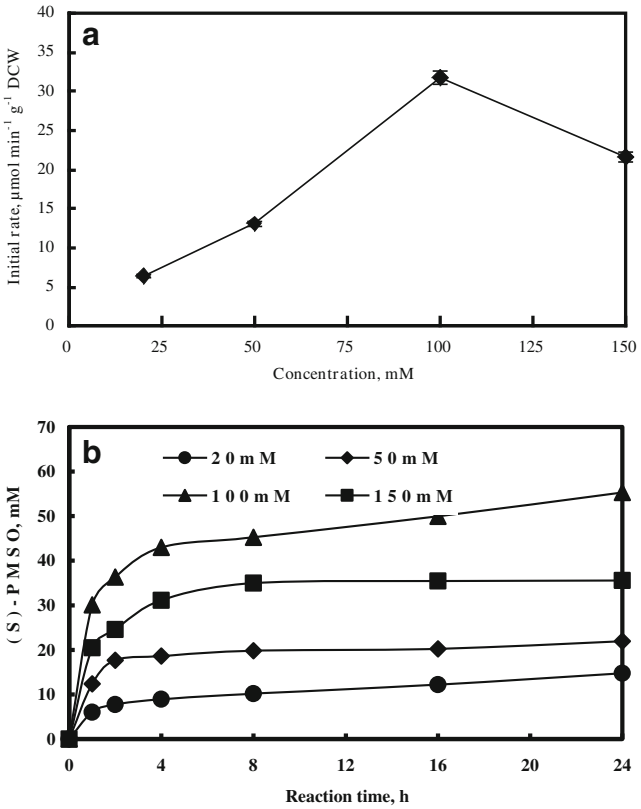


Fig. 4 Effects of PMS concentration on the PMS-oxidizing activity (**a**). Time course for the oxidation of PMS in *n*-octane–water system (**b**)

in an aqueous single-phase system (9.2 mM and >99.9 % *e.e.*) (Figs. 1 and 4b).

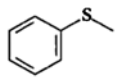
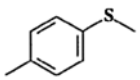
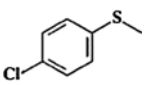
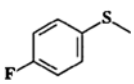
Oxidation of sulfides with resting cells of the *Rhodococcus* sp. CCZU10-1

The resting cells of *Rhodococcus* sp. CCZU10-1 were used to transform PMS and its derivatives with the groups (–CH_3), (–Cl), and (–F) at the *para*-position of the aromatic ring in *n*-octane–water (15:85, *v*/*v*) biphasic system. As listed in Table 2, these substrates generated reasonably high yields (>50 %). All *e.e.* values remained above 99.9 %. The electron-withdrawing groups (–Cl or –F) and an electron-donating one (methyl-) had different effects on the oxidation. *Rhodococcus* sp. CCZU10-1 showed a high oxidation activity on the derivative with an electron-donating group (methyl-) at the *para*-position of the aromatic ring. However, when a substrate with the substitution (–Cl) or (–F) at the *para*-position of the aromatic ring, the oxidation activity was reduced significantly, and *Rhodococcus* sp. CCZU10-1 showed a relatively low oxidation activity on the derivative with an electron-withdrawing group (−F) at the para-position of the aromatic ring.

Fed batch reaction in 1-L *n*-octane–water biphasic system and identification of product

For efficient production of (*S*)-PMSO in 1-L *n*-octane–water biphasic system, a fed-batch reaction was designed to relieve

Table 2 Enantioselective oxidation of sulfides to sulfoxides with the resting cells of *Rhodococcus* sp. CCZU10-1

Substrate	Conc., mM	Relative activity, %[a]	Yield, %	e.e., % (Config.)[b]	Ref.
	100	100 ± 2.8	55.3 ± 2.6	> 99.9 (*S*)	This study
	5	-	44.2	99.0 (*S*)	Li et al. 2009
	100	112 ± 3.1	78.7 ± 2.1	> 99.9 (*S*)	This study
	5	-	85.7	97.3 (*S*)	Li et al. 2009
	100	88.5 ± 2.2	53.6 ± 1.4	> 99.9 (*S*)	This study
	5	-	60.0	98.8 (*S*)	Li et al. 2009
	100	83.7 ± 2.4	50.3 ± 1.3	> 99.9 (*S*)	This study
	5	-	69.6	91.7 (*S*)	Li et al. 2009

The reaction, consisting of 100 mM of substrate and 8.0 g wet weight (ca. 1.58 g DCW) of resting cells in 100 mL *n*-octane–aqueous biphasic system (pH 8.0, 100 mM), was conducted at 30 °C and 180 rpm for 24 h on the shaker. Using 100 mM PMS as substrate, specific activity of in *Rhodococcus* sp. CCZU10-1 was 31.7±0.9 μmol min^{-1} g^{-1} DCW at pH 8.0 and 30 °C, and the enzyme activity was taken as 100 %. All experiments were performed in triplicate

[a] After 10 min of reaction, the sulfide-oxidizing activities were determined by GC

[b] The absolute configuration of the products were determined according to Li's report (2009). Absolute configurations were assigned by comparison of the specific rotations with the literature values. Enantiomeric excess (*e.e.*) values of products were determined by chiral HPLC

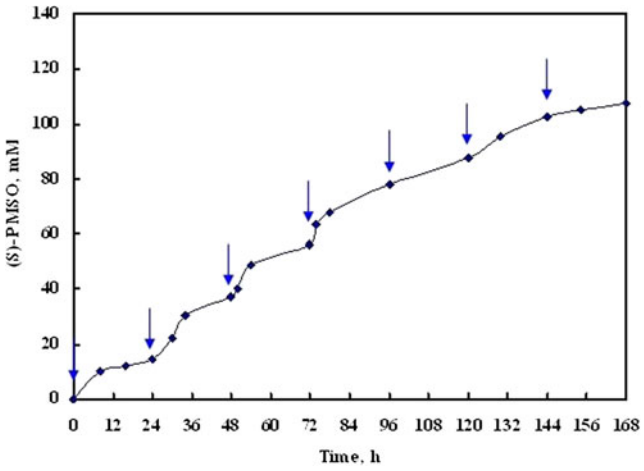


Fig. 5 Accumulation of (*S*)-PMSO in 1-L reaction mixture containing resting cells. The reaction was carried out at 30 °C, and 20 mM PMS was fed after certain time. *Downward-facing arrows* indicate addition of 20 mM PMS at each time

the biocatalyst from potential inhibition. As shown in Fig. 5, substrate feeding (20 mmol) at an interval of 24 h showed no significant inhibitory effect on the rate of formation of (*S*)-PMSO until seven feeds. A total of 118.0 mM (*S*)-PMSO (*e.e.*>99.9 %) was produced in the reaction mixture (Fig. 5). During the bioxidation, no (*R*)-PMSO and sulfone were detected (data not shown). In addition, the volumetric productivity of 1.50 g (*S*)-PMSO L^{-1} h^{-1} was achieved. Significantly, fed-batch bioconversion of PMS with resting cells of *Rhodococcus* sp. CCZU10-1 was a potential process for the production (*S*)-PMSO.

Furthermore, the reaction mixture was centrifuged, and the cells were soaked with EtOAc (80 mL; three times) and the supernatant was extracted with EtOAc (500 mL; twice). The two parts of EtOAc were combined, washed three times with saturated NaCl (100 mL), dried over anhydrous Na_2SO_4, and finally evaporated under reduced pressure. The residue was purified by silica gel column chromatography with petroleum ether-EtOAc (2:1, *v*/*v*) as the elution solvent, yielding pure products of (*S*)-PMSO. Its charateristics were listed as followings: (*S*)-PMSO, isolated yield 67.3 %, *e.e.*>99.9 %, $[\alpha]_D^{25}$ −134.8° (*c* =0.7, acetone) (Li et al. 2009) ($[\alpha]_D^{25}$ −134.6° (*c* = 0.7, acetone)), ^{1}H NMR (300 MHz, $CDCl_3$ δ/ppm): 2.73 (s, 3H, CH_3^-), 7.52–7.54 (m, 3H, ArH), 7.64–7.67 (m, 2H, ArH).

Discussion

Enantiomerically pure sulfoxides are important building blocks in the organic synthesis of natural products and chiral drugs because of their powerful stereodirecting ability in the carbon carbon bond formation (Holland 2001; Wojaczyńska and Wojaczyński 2010; Zhang et al. 2010). The activity of the sulfinyl group in the synthesis of drugs is exemplified by omeprazole, an antiulcer compound, a nonsteroidal anti-inflammatory drug, and an inhibitor of tumoral cell growth (Zhang et al. 2010). Many methods have been used for synthesizing enantiomerically pure sulfoxides (Bolm and Simic 2001; Drago et al. 2005; Fernandez and Khiar 2003; Lattanzi et al. 2004; Liao et al. 2003, 2011a). Among them, asymmetric oxidations of prochiral sulfides catalyzed by whole-cell biocatalysts attract great interest owing to the desirability of conducting such conversions under mild conditions. Moreover, the application of whole cell systems for the asymmetric oxidation of sufildes has many advantages, including the fact that the tedious separation of enzymes, the involvement of chiral metal complexes, and the regeneration of expensive cofactors (NADH/NADPH) are not required. Recently, asymmetric oxidations of prochiral sulfides catalyzed by whole-cell biocatalysts (e.g., bacteria and fungi) have been successfully employed for asymmetric sulfoxidation. However, low enantioselectivity or poor substrate tolerance was found in aqueous system (Kelly et al. 1996; Li et al. 2011b; Mascotti et al. 2012; Pinedo-Rivilla et al. 2007). As shown in Table 3, *Aspergillus japonicus*, *B. cinerea* and *Rhodococcus* sp. ECU0066 could transform PMS into low *e.e.* value of PMSO when the concentration of PMS reached 10 mM. In this study, using *Rhodococcus* sp. CCZU10-1 as a whole cell biocatalyst, highly enantiopure (*S*)-PMSO (>99.9 % *e.e.*) was obtained from ≥10 mM of PMS in the aqueous single-phase system. However, the substrate inhibition of enzyme activity appeared when the initial PMS concentration was higher than 10 mM (Fig. 1). Probably, given

Table 3 Enantioselective oxidation of PMS with microorganisms in aqueous system

Microorganism	Concentration (mM)	Yield (%)	*e.e.* (%; configuration)	References
Aspergillus japonicas	10 25	99 27	70 (*R*) 30 (*R*)	Mascotti et al. (2012)
Botrytis cinerea	10	58	41 (*S*)	Pinedo-Rivilla et al. (2007)
Rhodococcus sp. ECU0066	5 10	44.2 >95 %	99.0 (*S*) 80.0 (*S*)	Li et al. (2009)
Rhodococcus sp. CCZU10-1	10 20	91.6 29.6	>99.9 (*S*) >99.9 (*S*)	This study

the toxicity and hydrophobicity of PMS (Kelly et al. 1996), the substrate inhibition of enzyme activity appeared when the initial PMS concentration was higher than 10 mM, resulting in incomplete transformation of the substrate even if the reaction time was extended. In our previous report, the aqueous–organic biphasic systems could low the substrate inhibition (He et al. 2012). To effectively synthesize (*S*)-PMSO, several aqueous–organic biphasic systems were employed for the asymmetric oxidations of PMS (Table 1). Among these oranic solvents, *n*-octane (log *P*=4.5) was chosen to be a more suitable organic phase for achieving a better separation between the substrate PMS and the product (*S*)-PMSO. Moreover, the optimal ratio of water phase to *n*-octane phase was found to be 85:15 (*v*/*v*). This result might be explained by this fact that an increase in the aqueous phase volume would result in less chance for the cells to contact with substrate molecules dissolved in *n*-octane phase, thus reducing the inactivation effect of substrate and/or *n*-octane therein on biocatalyst. Conversely, as the ratio of *n*-octane phase increased, it became more and more difficult for the substrate PMS therein to diffuse in to the aqueous phase, thus decreasing the reaction rate and the final yield of product (*S*)-PMSO. Furthermore, the reaction conditions were optimized in *n*-octane–water (15:85, v/v) biphasic system, and the optimum reaction temperature, reaction pH, cell and substrate concentration were 30 °C, 8.0, 0.08 g (wet weight)/mL, and 100 mM, respectively. The biosynthesis of (*S*)-PMSO from the oxidaiton of PMS by *Rhodococcus* sp. CCZU10-1 was successfully demonstrated in *n*-octane–water biphasic system. (*S*)-PMSO (>99.9 % *e*.*e*.) formed at a higher concentration (55.3 mM) in *n*-octane–water biphasic system than in an aqueous single-phase system (Figs. 1 and 4b). In this *n*-octane–water (15:85, *v*/*v*) system, such a PMS concentration (100 mM) with a good conversion and *e*.*e*. value still represents the highest level that has been reported so far for whole cell-mediated sulfoxidation (Kelly et al. 1996; Mascotti et al. 2012; Li et al. 2011b; Pinedo-Rivilla et al. 2007).

Moreover, this bacterial strain also displayed fairly good activity and enantioselectivity toward other sulfides (Table 2). All *e*.*e*. values remained above 99.9 %. Significantly, *Rhodococcus* sp. CCZU10-1 showed higher oxidation activity on the PMS derivative with an electron-donating group (methyl-) at the *para*-position of the aromatic ring than the derivatives with the electron-withdrawing groups (–F or –Cl) at the para-position of the aromatic ring. Similar biotransformations were reported by Li et al. (2009). However, all *e*.*e*. values of these products remained ≤99.0 %. These data presented suggest that the use of this strain *Rhodococcus* sp. CCZU10-1 for (*S*)-PMSO and its derivatives production may have potential scientific and commercial applications, although in-depth exploration of this subject is needed.

For a preparative synthesis of (*S*)-PMSO, a fed-batch reaction was attempted in 1-L *n*-octane–water biphasic system (Fig. 5). Using fed-batch method, a total of 118 mM (*S*)-PMSO accumulated in 1-L reaction mixture after the 7th feed, and the volumetric productivity of 1.50 g (*S*)-PMSO L^{-1} h^{-1} was achieved. The product (*S*)-PMSO was further prepared and assayed by ^{1}H NMR and chiral HPLC. After being assayed by HPLC and GC (data not shown), no (*R*)-PMSO and sulfone were detected during oxidation of PMS. Significantly, fed-batch bioconversion of PMS with resting cells was a potential process for producing high *e*.*e*. value (>99.9 %) of (*S*)-PMSO.

In conclusion, asymmetric oxidation of PMS and its derivatives by *Rhodococcus* sp. CCZU10-1 in *n*-octane–water biphasic system was successfully demonstrated. Significantly, the sulfide monooxygenase from *Rhodococcus* sp. CCZU10-1 is a promising biocatalyst for synthesizing optically active (*S*)-sulfoxides (*e*.*e*.>99.9 %), although in-depth exploration of this subject is needed.

Acknowledgments This work was financially supported by the National Natural Science Foundation of China (no. 21102011), the Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions, the University Student's Science and Technology Creation Foundation of Changzhou University (no. 102006), the Open Project Program of the State Key Laboratory of Bioreactor Engineering, Jiangsu Key Laboratory of Advanced Catalytic Materials and Technology (no. BM2012110), and the Key Laboratory of Guangxi Biorefinery (no. GXBF11-03).

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