ELSEVIER

Journal of Molecular Catalysis B: Enzymatic 18 (2002) 233–241

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

www.elsevier.com/locate/molcatb

Asymmetric reduction of aryl ketones with a new isolate *Rhodotorula* sp. AS2.2241

Ye Ni, Jian-He Xu*

Laboratory of Applied Biocatalysis, State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai 200237, PR China

Received 4 February 2002; received in revised form 14 March 2002; accepted 14 March 2002

Abstract

A yeast strain, *Rhodotorula* sp. AS2.2241, capable of reducing acetophenone and α-bromoacetophenone with high stereoselectivity, was isolated from soil samples through a novel screening procedure in which acetophenone was supplied in vapor state as the sole carbon and energy source. The biosynthesis of the ketone reductase in the yeast cells reached a maximum of 41.0 U/l at 20 h of cultivation. The reductase isolated from the *Rhodotorula* sp. cells was partially purified by 52.6-fold through a single column chromatography of DEAE–cellulose. The catalytic performance of the partially purified reductase was examined, and the highest activity was observed at pH 6.5 and 50 °C. The short-chain alkyl aldehydes such as acetaldehyde and those aldehydes or ketones with a benzoyl group were found to be good substrates for the reductase. In the preparative bioreductions of 50 mM acetophenone and 2 mM α-bromoacetophenone using resting cells of *Rhodotorula* sp. AS2.2241, (*S*)-(−)-1-phenylethanol (>99.5% enantiomeric excess (e.e.), 34.7% yield) and (*R*)-(−)-2-bromo-1-phenylethanol (>99.9% e.e., 19.9% yield) were obtained, respectively.

Keywords: Acetophenone; α-Bromoacetophenone; Asymmetric reduction; Ketone reductase; Aldehyde reductase; *Rhodotorula* sp.

1. Introduction

Stereochemically pure compounds are widely employed as useful building blocks in many areas, especially in pharmaceutical and agrochemical industries. Asymmetric reduction of acetophenone and α-haloacetophenone is a convenient way to synthesize enantiopure aryl alcohols, of which α-halohydrins are also potentially useful for preparing epoxides and 1,2-diols. All these chiral intermediates are very useful in the synthesis of some optically active natural products [1] and many important chiral pharmaceutical products [2]. Since 1980s, both biological and chemical methods have often been reported [1,3–9]. As a rule, biocatalytic approaches have remarkable predominance due to their high enantioselectivity, mild reaction conditions and environmental compatibility.

Several alcohol dehydrogenases and aldehyde reductases [10–14] have been isolated and applied for the preparation of chiral aryl alcohols. *Saccharomyces cerevisiae* has often been used for the reduction of α-haloaryl ketones and other aromatic ketones to obtain chiral aryl alcohols [15–20]. Various microorganisms such as *Corynebacterium* sp. [21], *Cryptococus macerans* [22], *Hansenula capsulata* [23] and *Geotrichum* sp. [24,25] were also used for the preparation of optically pure aryl alcohols. However, only a few of these previous studies [10,11,21,23,25] could

* Corresponding author. Tel.: +86-21-6425-2498; fax: +86-21-6425-3904.
E-mail address: jianhexu@ecust.edu.cn (J.-H. Xu).

1381-1177/02/$ – see front matter
PII: S1381-1177(02)00101-7

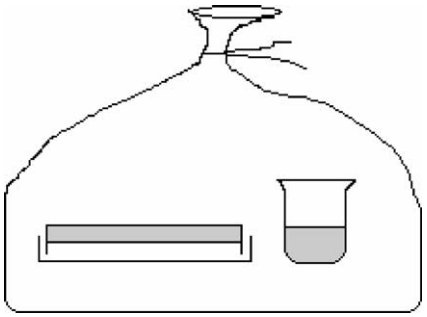

Fig. 1. Schematic illustration of a screening model for isolation of acetophenone-utilizing microorganisms.

afford aryl alcohols with >98% enantiomeric excess (e.e.) which is usually necessary for production of enantiopure chiral drugs. It has been well known that screening among the wide variety of microorganisms living in the soil is an efficient method to obtain desired enzyme towards an unnatural substrate [26]. In our screening mode which was different from previous ones, acetophenone was provided in the vapor state from a separate container to a solid medium for enrichment culture without any other carbon and energy sources (Fig. 1). As a result, a yeast isolate, designated as *Rhodotorula* sp. AS2.2241, showed highly stereospecific reductase activity towards acetophenone and α-bromoacetophenone. This paper describes the isolation of microorganisms for stereospecific reduction of acetophenone and α-bromoacetophenone, and also the preparation of the corresponding aryl alcohols using resting cells of the new isolate, *Rhodotorula* sp. AS2.2241.

2. Results and discussion

2.1. Isolation of microorganisms for stereospecific reduction of acetophenone and α-bromoacetophenone

No microorganism grew after 3–5 days of cultivation when suspensions of soil samples were sprayed onto agar plates composed of mineral salt medium (MSM) and α-bromoacetophenone (2–5 mM), even if 0.2% of yeast extract was supplemented to the agar medium. This indicated that α-bromoacetophenone produces a strong inhibitory effect on the growth of microorganisms and is thus not a suitable substrate for our screening. Therefore, we turned to acetophenone, a substrate of similar structure. The screening was carried out as described in Section 3. This novel screening procedure was quite efficient. Among 240 soil samples, about 130 strains of microorganisms capable of utilizing gaseous acetophenone as sole carbon source were isolated from the agar plates and analyzed by TLC. Of them, 67 strains with obvious amaranthine spots on TLC plates were further subjected to HPLC analysis with respect to the enantiomeric excesses of the product in the bioreduction. Of the 67 positive strains, 9 strains showed relatively higher values of e.e. (>80%).

Among these active strains, a strain of yeast, originally designated as no. 3–16, was selected for further study due to its high enantioselectivity (>99.9% e.e.) and high activity (>35% conversion) in the subsequent verification experiments.

Morphological, biochemical and physiological tests of the yeast strain no. 3–16 were carried out as described by Barnett et al. in “Yeasts—Characteristics and Identification” [27]. Assimilation and fermentation tests were conducted as described by Kurtzman and Fell in “The Yeasts: A Taxonomic Study” [28]. Cells of the strain are spheroidal or ovoidal, non-motile, non-spore. Colonies on nutrient agar plate were pink, wet and smooth. Lactose was not assimilated and nitrate could be assimilated as the sole nitrogen source. Starch-like substances were not synthesized and fermentative ability was lacking. Presence of urease was positive. All these taxonomic studies of the yeast strain no. 3–16 indicated that it belongs to the genus *Rhodotorula*. Now, the strain is deposited at China General Microbiological Culture Collection Center (CGMCC), with an access number of AS2.2241.

To further characterize the new isolate, time course of the enzyme production was monitored by cultivating *Rhodotorula* sp. AS2.2241 in 250-ml flasks using fermentation medium (FM), as shown in Fig. 2A. The reductase activity was measured with α-bromoacetophenone as substrate, as described in Section 3.4. The ketone reductase activity increased in parallel with cell growth during the first 20 h. The maximum activity of 41.0 U/l was reached at 20 h when the dry mass of cells (DCW) was 5.91 g/l. The

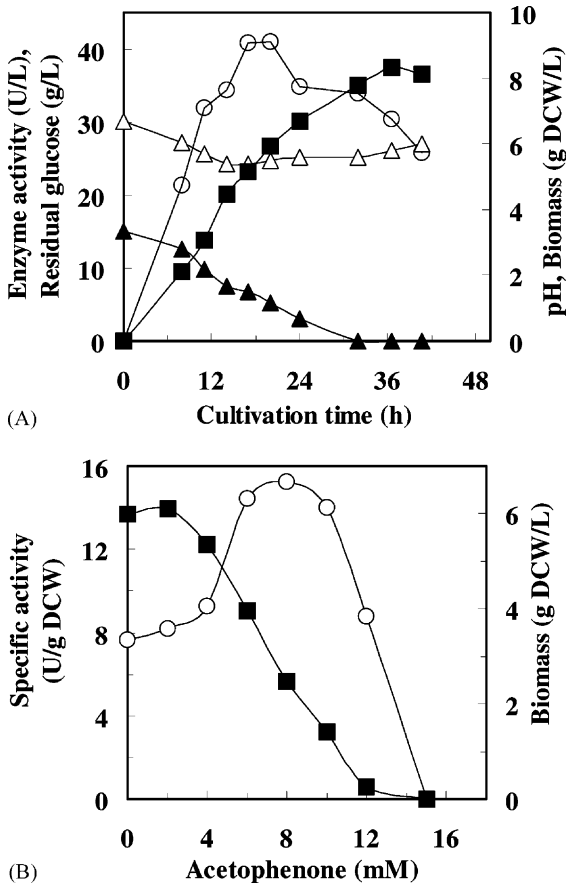

Fig. 2. Production of aryl ketone reductase by *Rhodotorula* sp. AS2.2241. (A) Time courses: (○) reductase activity; (■) biomass; (△) pH; (▲) residual glucose. The reductase activities were determined by HPLC as described in Section 3. (B) Effect of acetophenone as an enzyme inducer: (■) biomass; (○) specific activity.

cell growth continued in proportion to the decrease in the glucose concentration till its depletion. However, the enzyme activity began to decline after 20 h. Reasons are not clear yet, probably because of the cease of reductase synthesis in the aged cells and the degradation of the reductase by the in vivo protease during the later period of growth. The enzyme activity was slightly induced by 0.01 mM of α-bromoacetophenone and the cell could not grow when 0.05 mM was added at the beginning of the cultivation. However, the reductase activity was markedly induced by acetophenone. The specific reductase activity of the cells was doubled when 8 mM of acetophenone was added at the beginning of the cultivation (Fig. 2B). These facts suggest that the reductase is an inducible enzyme and α-bromoacetophenone has a strong inhibitory effect on the cell growth.

2.2. Catalytic performance of a partially purified reductase from Rhodotorula sp. AS2.2241

To further optimize the catalytic conditions for asymmetric reduction, the enzyme responsible for stereospecific reducing activity was partially purified from a cell-free extract of *Rhodotorula* sp. AS2.2241 by fractionation with ammonium sulfate (40–70% saturation) and single column chromatography with DEAE–cellulose. The anion exchange chromatography with DEAE–cellulose was quite effective in purifying the enzyme. In the DEAE–cellulose column chromatography, the only peak of enzyme activity was observed in the first protein peak, which was eluted at 0.10–0.18 M of NaCl. The reductase was purified by about 52.6-fold with an overall yield of 28%. The isolated enzyme also showed high selectivity in the asymmetric reduction of acetophenone and α-bromoacetophenone, giving corresponding products with excellent enantiomeric purity (>99% e.e.).

As shown in Fig. 3, the partially purified enzyme showed the maximal activity between pH 6.0 and 6.5 and the enzyme was stable over a wide pH range from 6.0 to 10.0. The optimum temperature of the enzyme was 50 °C. The enzyme was quite stable at temperatures below 40 °C with nearly 90% of the activity remained, whereas, over 70% of the activity was lost at 60 °C. These results coincided with those of resting cells. The highest activity of resting cells was demonstrated under pH 5.0–8.0 and 50 °C. The activity of intact cells was stable at pH 7.0–10.0 (data not shown). Therefore, the optimal conditions for asymmetric reduction should be pH 6.5 and 50 °C.

Other properties of the partially purified enzyme were also preliminarily examined. The reduction activity of the enzyme was observed in the presence of NADH, while NADPH did not serve as a coenzyme. Using Lineweaver–Bark ($1/v \sim 1/[S]$) plots [29], the K_m values of the partially purified enzyme towards acetaldehyde and NADH were calculated to be 0.095 and 0.23 mM, respectively, which indicated that the enzyme had a high affinity for acetaldehyde. In both cases, typical saturation curves were observed

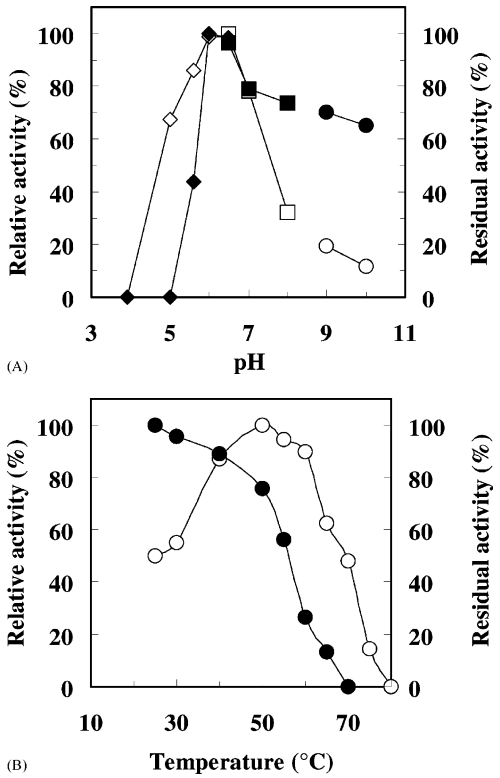

Fig. 3. Catalytic performance of the partially purified enzyme from *Rhodotorula* sp. AS2.2241. (A) Effect of pH: empty symbols, activity measured at various pHs; filled symbols: residual activity after incubation at various pHs for 1 h. Buffers used: (◆, ◇) 50 mM citrate; (■, □) 50 mM potassium phosphate; (●, ○) 50 mM glycine–NaOH. (B) Effect of temperature: (○) activity, measured at various temperatures in 50 mM KPB (pH 7.0); (●) residual activity after incubation at various temperatures for 1 h. All the activities were measured spectrophotometrically using acetaldehyde as the substrate.

(data not shown). Heavy metal ions such as 0.1 mM of Hg^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+} and Ag^{+} strongly inhibited the enzyme, suggesting that the sulfhydryl group is probably essential for the activity. Whereas, chelating reagents such as EDTA did not inhibit the enzyme obviously at a concentration of 0.1 mM. This indicates that metal ions are not essential for the manifestation of activity and the enzyme may not be a metalloenzyme.

The oxidation activity of the partially purified enzyme was also measured spectrophotometrically using various alkyl and aryl alcohols, including methanol, ethanol, *n*-butanol, *n*-octanol and benzyl alcohol. The substrates (5 mM) were added directly to the reaction mixtures. The highest activity was observed with ethanol and *n*-butanol, about 87% of the reduction activity towards acetaldehyde (data not shown). On the other hand, the reduction activity of the isolated enzyme was determined towards a broad variety of carbonyl compounds, including ketones, keto esters and aldehydes. The insoluble substrate, α-bromoacetophenone, was used in 5 mM of emulsion prepared as follows. α-Bromoacetophenone (50 mg) was homogenized in 50 ml of 50 mM potassium phosphate buffer (KPB, pH 7.0, containing 0.2% Tween-80) by heating in boiling water for 2 min and subsequently by sonication at 100 W for 1 min. Other substrates (5 mM) were added directly to the reaction mixtures. As shown in Table 1, the enzyme catalyzed the reduction of not only ketones but also aldehydes. However, keto esters, such as ethyl acetoacetate and ethyl 4-chloroacetoacetate were not reduced at all. The enzyme showed high activity toward aldehydes, including alkyl aldehydes and aryl aldehydes. The highest activity of 49.4 U/mg was observed with acetaldehyde (1667% in comparison with the activity on α-bromoacetophenone), followed in sequence by propionaldehyde and *n*-butyraldehyde. This was why we chose acetaldehyde as the indicative substrate in the *enzyme assay*. Also, benzaldehyde was a suitable substrate for the enzyme. Based on these results, the

Table 1
Relative activities of the partially purified enzyme from *Rhodotorula* sp. AS2.2241 in the reduction of various carbonyl compounds (5 mM)

Substrate	Relative activity (%)[a]
Formaldehyde	367
Acetaldehyde	1667
Propionaldehyde	483
n-Butyraldehyde	400
n-Heptaldehyde	167
Benzaldehyde	383
Phenylacetaldehyde	0
Acetone	133
Acetophenone	33.2
α-Bromoacetophenone	100[b]
Ethyl acetoacetate	0
Ethyl 4-chloroacetoacetate	0

[a] The reductase activity was measured spectrophotometrically.
[b] The activity with α-bromoacetophenone (specific activity, 2.98 U/mg protein) was defined as 100%.

short-chain alkyl aldehydes and those aldehydes or ketones with a benzoyl group seemed to be suitable substrates for this enzyme.

The acetophenone and α-bromoacetophenone were reduced respectively into (*S*)-(−)-1-phenylethanol (>99.5% e.e.) and (*R*)-(−)-2-bromo-1-phenylethanol (>99.9% e.e.). These results showed that the enzyme might possess high stereospecifity towards a series of ketones with a benzoyl group. The bioreductions obeyed the Prelog rule [30], which was the same as baker's yeast [15–20], *Cryptococus macerans* [22] and *Corynebacterium* sp. [21]. It is worthy to note that the absolute configuration of (*R*)-(−)-2-bromo-1-phenylethanol is actually identical with that of (*S*)-(−)-1-phenylethanol, although their stereochemical naming is apparently opposite due to the difference between the sizes of bromomethyl and methyl groups in the sequence rule.

2.3. Asymmetric reduction of acetophenone and α-bromoacetophenone at the optimal substrate concentrations by resting cells of Rhodotorula sp. AS2.2241

As shown in (Fig. 4A and B), the highest initial velocities for the reduction of acetophenone and α-bromoacetophenone were observed at 50 and 2 mM, respectively. Therefore, 50 mM of acetophenone and 2 mM of α-bromoacetophenone were the optimal concentrations in resting-cell reactions. It was obvious that α-bromoacetophenone had much stronger inhibitory effect than acetophenone. The inhibition was extraordinarily remarkable when the α-bromoacetophenone concentration was above 20 mM. And the reaction was almost stopped by 50 mM of α-bromoacetophenone (data not shown). However, the inhibition of acetophenone was quite slight. About 75% of the highest initial velocity was displayed at 100 mM.

According to the results earlier, the asymmetric reduction of acetophenone and α-bromoacetophenone by resting cells of *Rhodotorula* sp. AS2.2241 were performed at their optimal concentrations. Though the optimal conditions were proved to be pH 6.5 and 50 °C, we still chose pH 7.0 and 30 °C in actual preparation allowing for the thermal stability of the enzyme and products. As shown in Fig. 5A, the concentration of 1-phenylethanol increased linearly

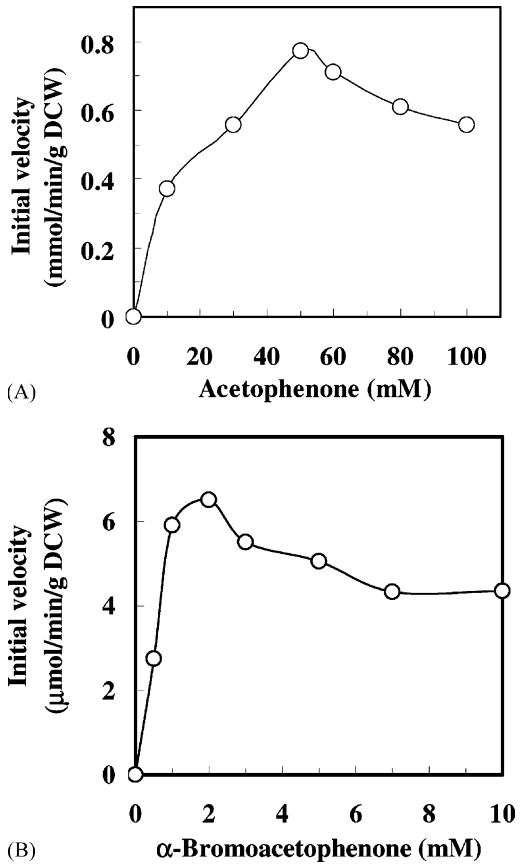

Fig. 4. Effect of substrate concentration on asymmetric reduction of acetophenone and α-bromoacetophenone by resting cells of *Rhodotorula* sp. AS2.2241: (A) acetophenone; (B) α-bromoacetophenone. The cell concentrations used in the reaction mixtures for acetophenone and α-bromoacetophenone were 28.8 and 4.39 g DCW/l, respectively. The concentrations of the products were determined by HPLC.

during the first 6 h. After 30 h of conversion, 27 mM of product was obtained. The e.e. of the product maintained >99.5% throughout the course. The time course of α-bromoacetophenone reduction is shown in Fig. 5B. The initial reaction rate calculated over first 15 min was 6.14 μmol/min g DCW. After 3 h of conversion, 2 mM of substrate was completely transformed into 2-bromo-1-phenylethanol. The e.e. maintained >99.9% throughout the course.

Finally, in the preparative bioreductions of 50 mM acetophenone and 2 mM α-bromoacetophenone using resting cells of *Rhodotorula* sp. AS2.2241, 780 mg of (*S*)-(−)-1-phenylethanol (>99.5% e.e.) and 240 mg of

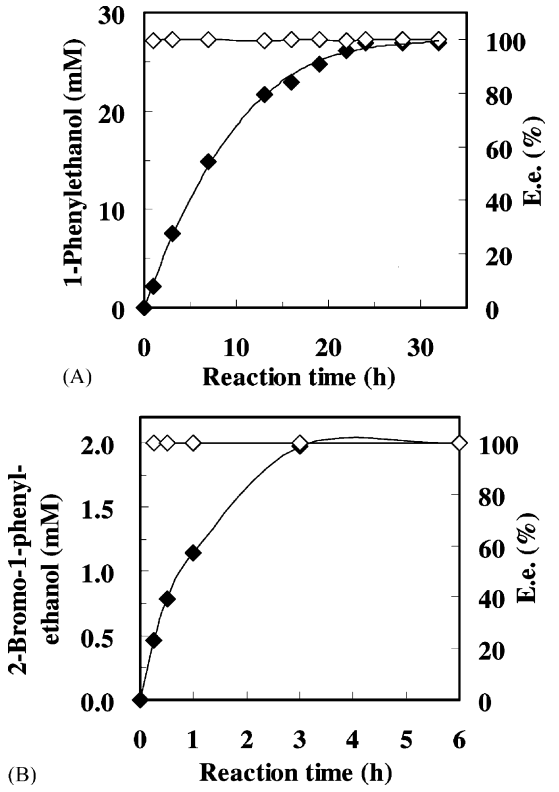

Fig. 5. Time-courses of asymmetric reductions of acetophenone and α-bromoacetophenone by resting cells of *Rhodotorula* sp. AS2.2241: (A) acetophenone; (B) α-bromoacetophenone. (◇) Enantiomeric excess of product; (◆) concentration of product. The cell concentrations used in the reaction mixtures for acetophenone and α-bromoacetophenone were 23.7 and 3.76 g DCW/l, respectively.

(*R*)-(−)-2-bromo-1-phenylethanol (>99.9% e.e.) was obtained with an overall yield of 34.7 and 19.9%, respectively. Their chemical structures were confirmed by NMR and IR, as indicated in the following section.

3. Experimental

3.1. Chemicals and media

α-Bromoacetophenone and ethyl 4-chloroacetoacetate were purchased from Acros Organics, USA. All other chemicals were from local suppliers and of analytical grade. Soil samples were collected from different locations in several regions of China.

Mineral salt medium (MSM): 0.2% $(NH_4)_2SO_4$, 0.2% KH_2PO_4, 0.1% NaCl, 0.02% $MgSO_4{\cdot}7H_2O$, pH 7.0.

TGY medium (TGYM): 1% glucose, 0.5% yeast extract, 0.5% peptone and 0.2% KH_2PO_4, pH 7.0.

Fermentation medium (FM): 1.5% glucose, 0.5% yeast extract, 0.5% peptone, 0.1% KH_2PO_4, 0.1% K_2HPO_4, 0.1% NaCl, 0.05% $MgSO_4{\cdot}7H_2O$, pH 7.0.

3.2. Isolation of acetophenone-utilizing microorganisms with highly stereospecific reductase activity towards aryl ketones

By supplying acetophenone in its vapor state, acetophenone-utilizing microorganisms were isolated from soil samples. Appropriate amount of soil samples was mixed with 20 ml of tap water, and then 0.2 ml of the supernatant was sprayed onto the MSM agar plates (containing 1.5% agar). The plates and a 25-ml beaker filled with acetophenone were placed in a sealed plastic bag at 30 °C for 3–5 days. Monocolonies appeared on the plates were inoculated individually into 10 ml of TGYM in 100-ml (25 mm × 200 mm) test tubes which were constantly shaken on a slope for 1 day (120 rpm, 30 °C). The cells were collected by centrifugation (4000 rpm, 10 min) and re-suspended in 2 ml of 50 mM KPB (pH 7.0) to which a final concentration of 10 mM α-bromoacetophenone was added. After 1 day of conversion, the reactants were extracted with 2 ml of ethyl acetate and analyzed by thin layer chromatography (TLC) with hexane/ethyl acetate (6/1 v/v) as eluent. Then, the TLC plate was sprayed with vanilla solution (5 g/l, in concentrated sulfuric acid) and baked on a hot plate. The samples showing an apparent amaranthine spot on TLC plate at the same position ($R_f = 0.54$) with the standard 2-bromo-1-phenylethanol indicate the aryl ketone reductase activity. Finally, samples with an obvious product spot were further analyzed by HPLC for determination of conversion ratio and enantiomeric excess (e.e.).

3.3. Culture conditions

The isolated strain, *Rhodotorula* sp. AS2.2241, was shaken aerobically at 120 rpm and 30 °C for 40 h in 250-ml Erlenmeyer flasks with 50 ml of FM. The FM was inoculated with a 12-h pre-culture. At each time

interval, two flasks were withdrawn for determination of enzyme activity, dry mass of cells (DCW), residual glucose concentration and pH. The enzyme activity was measured by HPLC analysis as described in Section 3.4. The dry mass of cells was measured after drying the wet cells at 50 °C till a constant weight. The concentration of residual glucose was measured using a commercial kit of glucose oxidase.

3.4. Enzyme assay

The activity of aryl ketone reductase in the culture broth was determined by HPLC as follows. The cells harvested from 1.5 ml of broth were washed with saline solution (0.85% NaCl) and re-suspended in 1.98 ml of 50 mM KPB (pH 7.0). The mixtures were vortexed and pre-incubated on a shaker (120 rpm, 30 °C) for 10 min. Then, 20 μl of 0.20 M α-bromoacetophenone in ethanol were added, giving a final concentration of 2 mM. The reaction was carried out on the same shaker and the first 15 min was calculated as the initial velocity. The reaction mixture was saturated with NaCl and extracted by 2 ml of ethyl acetate. The extract was dried over anhydrous Na_2SO_4 and subjected to HPLC analysis to determine the quantity of the alcohol produced. One unit of reductase activity was defined as the amount of enzyme catalyzing the formation of 1.0 μmol 2-bromo-1-phenylethanol/min under above conditions.

The reductase activity of the partially purified enzyme was determined spectrophotometrically at 30 °C by measuring the initial velocity of the decrease in the absorbance of NADH at 340 nm. Instead of α-bromoacetophenone, acetaldehyde was used as an indicative substrate because of its high sensitivity. The standard reaction mixture consisted of 15 μmol of acetaldehyde, 0.4 μmol of NADH, 150 μmol of KPB (pH 7.0) and an appropriate quantity of enzyme solution, in a total volume of 3 ml. The reverse reaction of the enzyme was also measured at 340 nm in 3 ml of reaction mixture containing 15 μmol of an alcohol (e.g. ethanol), 4.5 μmol of NAD^+, 150 μmol of glycine–NaOH (pH 10.0) and an appropriate quantity of enzyme solution. After 5 min of pre-incubation without substrate, the reaction was initiated by the addition of the substrate. The blank contained buffer instead of the substrate. One unit of the enzyme activity was defined as the amount of enzyme that catalyzes the oxidation of 1 μmol NADH or the reduction of 1 μmol NAD^+/min under the conditions specified.

3.5. Enzyme purification

All purification operations were conducted at 4 °C, and 20 mM of Tris–HCl buffer (pH 7.5) containing 1 mM EDTA and 2 mM cysteine was used throughout the purification. Centrifugation was usually conducted at 18,000 × *g* for 20 min. The protein concentration was estimated by measuring the absorbance at 280 nm or using the method of Bradford [31] with bovine serum albumin as a standard protein. The enzyme activity was spectrophotometrically analyzed as described in Section 3.4.

Wet cells (4 g) harvested from 140 ml of culture broth were suspended in 20 ml of buffer and then sonicated at 400 W for 25 min (each pulse of 3 s was followed by an interval of 7 s). After centrifugation, the supernatant was fractionated with ammonium sulfate (40–70% saturation). The precipitated protein was dissolved in 3 ml of Tris–HCl buffer and dialyzed against 300 ml of the same buffer for 24 h. The dialyzed enzyme solution (4.1 ml) was applied onto a DEAE–cellulose column (1.5 cm × 14 cm, bed volume: 25 ml) pre-equilibrated with the 20 mM buffer. The enzyme was washed with 60 ml of 20 mM buffer and then eluted with a linear gradient of NaCl solution (0–1.0 M in the buffer, 300 ml). The active fractions were combined and used for further characterization.

3.6. Bioreduction with isolated enzyme

The bioreduction of acetophenone and α-bromoacetophenone with isolated enzyme was performed in the test tubes containing 3 ml of mixture composed of 15 μmol of each substrate, 0.4 μmol of NADH, 30 μmol of ethanol for coenzyme recycling, 150 μmol of KPB (pH 7.0) and an appropriate quantity of enzyme solution. The reaction proceeded by shaking for 24 h at 25 °C and 120 rpm. The concentrations and enantiomeric excesses of the products were determined by HPLC.

The optimum pH of the partially purified enzyme was measured directly in buffers of various pHs. The pH stability (residual activity) of the enzyme was determined after incubation at 4 °C for 1 h in various buffers. The optimum temperature of the partially

purified enzyme was measured at temperatures ranging from 25 to 70 °C after pre-incubation for 10 min. The thermal stability of the enzyme (residual activity) was determined at 30 °C after incubation at different temperatures for 1 h in 50 mM KPB (pH 7.0).

3.7. Asymmetric reduction of acetophenone and α-bromoacetophenone by resting cells of Rhodotorula sp. AS2.2241

The small-scale resting-cell reduction of acetophenone and α-bromoacetophenone were performed in test tubes as described in Section 3.4. For acetophenone, it was prepared into an emulsion of 0.50 M using Tween-80 as an emulsifier (200 mg Tween-80/100 ml). The first 30 min was calculated as the initial velocity. For α-bromoacetophenone, 0.20 M α-bromoacetophenone in ethanol was used. The first 15 min was calculated as the initial velocity. The time-course of the bioconversion was monitored by withdrawing two test tubes at each time interval for HPLC analysis of the concentration and e.e. of the products.

Gram-scale preparation of acetophenone and α-bromoacetophenone were performed as follows. The cells (wet weight, 50 g) harvested from 1500 ml culture broth were washed and suspended in 337.5 ml of 50 mM KPB (pH 7.0), then 37.5 ml of 0.50 M acetophenone emulsion was added to give a final concentration of 50 mM. The 375-ml mixture was equally divided into three and added into three 500-ml Erlenmeyer flasks. The flasks were shaken at 120 rpm and 30 °C. After incubation for 30 h, the mixture was centrifuged. The supernatant was saturated with NaCl and then extracted three times with ethyl acetate. The organic solution was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The alcohol was purified by silica gel chromatography with ethyl acetate/hexane (1:9). The (*S*)-(−)-1-phenylethanol (780 mg, 34.7% yield, >99.5% e.e.) was obtained as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$), δ_{ppm}: 1.49 (3H, d, C**H**$_3$), 1.83 (1H, s, O**H**), 4.9 (1H, d, C**H**), 7.3 (5H, m, Ar**H**); $[\alpha]_D^{15}$ −44.8° (c = 0.12, $CHCl_3$) (Lit. $[\alpha]_D^{23}$ −52.5°, c = 2.27, CH_2Cl_2) [16].

Preparation of (*R*)-(−)-2-bromo-1-phenylethanol was the same as above. From 2 mM α-bromoacetophenone, (*R*)-(−)-2-bromo-1-phenylethanol (240 mg, 19.9% yield, >99.9% e.e.) was obtained as a colorless oil. IR (film) 3400 (OH), 1490 (C=C) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$), δ_{ppm}: 2.82 (1H, br s, O**H**), 3.52 (2H, m, C**H**$_2$), 4.82 (1H, dd, C**H**), 7.3 (5H, m, Ar**H**); $[\alpha]_D^{20}$ −38.6° (c = 0.29, $CHCl_3$) (Lit. $[\alpha]_D^{25}$ −39.7°, c = 8.4, $CHCl_3$) [15].

3.8. Analytical methods

The concentrations and enantiomeric excesses of the 2-bromo-1-phenylethanol and 1-phenylethanol were determined by HPLC using a chiral column (Chiralcel OD, Daicel, Japan; 250 mm in length, Ø 4.6 mm). The mobile phase was hexane/2-propanol (98/2, v/v) and its flow rate was 1.0 ml/min. Detection was made at 212 nm. The retention times for (*S*)-, (*R*)-2-bromo-1-phenylethanol and (*S*)-, (*R*)-1-phenylethanol were 20.8, 26.0, 19.6 and 17.7 min, respectively.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (Grant no. 29776018 and 20176011). We are grateful to Ms. H.-Y. Wu for her helpful discussion and Mr. Q.-S. Tan for his technical assistance.

References

[1] H.C. Brown, G.G. Pai, J. Org. Chem. 48 (1983) 1784.
[2] E.J. Corey, J.O. Link, Tetrahedron Lett. 31 (1990) 601.
[3] M.M. Mildland, Chem. Rev. 89 (1989) 1553.
[4] E.J. Corey, S. Shibata, R.K. Bakshi, J. Org. Chem. 53 (1988) 2861.
[5] R.V. Malyala, C.V. Rode, M. Arai, S.G. Hegde, R.V. Chaudhari, Appl. Catal. A 193 (2000) 71.
[6] P. Cognet, A.G. Lakhal, J. Berlan, A.M. Wilhelm, H. Delmas, P.L. Fabre, Chem. Eng. Sci. 55 (2000) 2571.
[7] M.F.P. Ma, K. Li, Z. Zhou, C. Tang, A.S.C. Chan, Tetrahedron: Asymm. 11 (2000) 2435.
[8] K. Molvinger, M. Lopez, J. Court, J. Mol. Catal. A: Chem. 150 (1999) 267.
[9] E. Dorrestijn, S. Hemmink, G. Hulstman, L. Monnier, W.V. Scheppingen, P. Mulder, Eur. J. Org. Chem. 3 (1999) 607.
[10] W. Hummel, Biotechnol. Lett. 12 (1990) 403.
[11] C.W. Bradshaw, W. Hummel, C.-H. Wong, J. Org. Chem. 57 (1992) 1532.
[12] A. Tani, Y. Sakai, T. Ishige, N. Kato, Appl. Environ. Microbiol. 66 (2000) 5231.

[13] N. Itoh, K. Yoshida, K. Okada, Biosci. Biotech. Biochem. 60 (1996) 1826.
[14] N. Itoh, R. Morihama, J.-C. Wang, K. Okada, N. Mizuguchi, Appl. Environ. Microbiol. 63 (1997) 3783.
[15] M. Carvalho, M.T. Okamoto, Tetrahedron 47 (1991) 2073.
[16] H.-X. Liu, J.-Y. Su, L.-M. Zeng, Huaxue Tongbao (1) (1996) 36.
[17] X. Liu, T.-S. Zhu, P.-D. Sun, J.-H. Xu, Synth. Commun. 31 (2001) 1521.
[18] P. Nikoiova, O.P. Ward, Biotechnol. Bioeng. 39 (1992) 870.
[19] D.R. Griffin, F.-X. Yang, G. Carta, J.L. Gainer, Biotechnol. Prog. 14 (1998) 588.
[20] D.R. Griffin, J.L. Gainer, G. Carta, Biotechnol. Prog. 17 (2001) 304.
[21] N. Itoh, N. Mizuguchi, M. Mabuchi, J. Mol. Catal. B: Enzym. 6 (1999) 41.
[22] M. Imuta, K. Kawai, H. Ziffer, J. Org. Chem. 45 (1980) 3352.
[23] Y. Hasegawa, S. Adachi, R. Matsuno, J. Ferment. Bioeng. 85 (1998) 322.
[24] Z.-L. Wei, Z.-Y. Li, G.-Q. Lin, Tetrahedron 54 (1996) 1826.
[25] K. Nakamura, T. Matsuda, J. Org. Chem. 63 (1998) 8957.
[26] Y. Asano, J. Biotechnol. 94 (2002) 65.
[27] J.A. Barnett, R.W. Payne, D. Yarrow, Yeasts—Characteristics and Identification: Cambridge University Press, Cambridge, 1983.
[28] C.P. Kurtzman, J.W. Fell, The Yeasts: A Taxonomic Study, 4th ed., Elsevier, Amsterdam, 1998.
[29] H. Lineweaver, D.J. Bark, J. Am. Chem. Soc. 56 (1934) 658.
[30] V.H. Prelog, Pure Appl. Chem. 9 (1964) 119.
[31] M. Bradford, Anal. Biochem. 72 (1976) 248.