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Microbial synthesis of chiral amines by (*R*)-specific transamination with *Arthrobacter* sp. KNK168

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Abstract *Arthrobacter* sp. KNK168 shows (*R*)-enantioselective transaminase [(*R*)-transaminase] activity, which converts prochiral ketones into the corresponding chiral (*R*)-amines in the presence of an amino donor. The cultural conditions and reaction conditions for asymmetric synthesis of chiral amines with this microorganism were examined. The transaminase was inducible, and its production was enhanced by the addition of *sec*-butylamine and 3-amino-2,2-dimethylbutane to the culture medium. (*R*)-1-Phenylethylamine was a good amino donor for amination of 3,4-dimethoxyphenylacetone with *Arthrobacter* sp. KNK168. Under the optimum conditions, 126 mM (*R*)-3,4-dimethoxyamphetamine (DMA) [>99% enantiomeric excess (ee)] was synthesized from 154 mM 3,4-dimethoxyphenylacetone and 154 mM (*R*)-1-phenylethylamine through the whole cell reaction with an 82% conversion yield. (*R*)-Enantiomers of other amines, such as (*R*)-4-methoxyamphetamine, (*R*)-1-(3-hydroxyphenyl)ethylamine and (*R*)-1-(3-hydroxyphenyl)ethylamine, were also synthesized from the corresponding carbonyl compounds through asymmetric amination with *Arthrobacter* sp. KNK168.

Introduction

Recently, chiral amines have become important as starting materials for the synthesis of some pharmaceuticals and agricultural chemicals. Many attempts at the biological synthesis of chiral amines have been made, being mainly focused on kinetic resolution of racemic amines with lipase (Jaeger et al. 1996; Reeve 1999; Balkenhohl et al. 1997), oxidase (Ayse et al. 2000), and ω-amino acid aminotransaminase (Stirling 1992; Shin and Kim 1997; Shin et al. 2001). For a kinetic resolution process, the maximal yield of one isomer is 50%, and the undesired isomer is wasted or has to be reused through complicated additional processes including racemization. On the other hand, the asymmetric synthesis of chiral amines from prochiral precursors is advantageous from the economical point of view because the theoretical yield is 100%. Recently, some attempts at the asymmetric synthesis of chiral amines with ω-amino acid transferase were reported (Shin and Kim 1999, 2001). Nakamichi et al. (1990) also reported the asymmetric synthesis of the (*S*)-forms of amphetamine derivatives with *Brevibacterium linens*. However, only (*S*)-amines could be produced with ω-amino acid aminotransferase because the reported enzymes were (*S*)-enantioselective and the chiral amine productivities were not enough from the industrial point of view. In the previous study (Iwasaki et al. 2003), we isolated a microorganism, *Arthrobacter* sp. KNK168, exhibiting (*R*)-enantioselective transaminase [(*R*)-transaminase] activity, which forms (*R*)-3,4-dimethoxyamphetamine (DMA) from 3,4-dimethoxyphenylacetone with a high optical yield.

In this paper, we describe the cultural conditions for (*R*)-transaminase production and the reaction conditions for the synthesis of DMA with *Arthrobacter* sp. KNK168. In addition, the application of (*R*)-specific transaminase of this microorganism to the preparation of several chiral amines is reported.

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Materials and methods

Chemicals

3,4-Dimethoxyphenylacetone, 4-methoxyphenylacetone, 3′-hydroxyacetophenone, 3′-methoxyacetophenone, and (*R*)-

1-phenylethylamine were purchased from Wako Pure Chemicals Industries, Ltd. (Osaka, Japan). PRO-EX(A12P), acid hydrolysate of gluten from corn, was purchased from Bansyu Chyoumiryou Co., Ltd. (Hyogo, Japan). All other chemicals used were of analytical grade and commercially available.

Standard cultural conditions for *Arthrobacter* sp. KNK168

Arthrobacter sp. KNK168 was cultured in R-medium, composed of 15 g glycerol, 2 g yeast extract, 8 g PRO-EX (A12P), 3 g NaCl, 5 g KH_2PO_4, 5 g K_2HPO_4, 1 g $MgSO_4 \cdot 7H_2O$, 0.005 g $FeSO_4 \cdot 7H_2O$, 0.001 g $ZnSO_4 \cdot 7H_2O$, 0.001 g $MnCl_2 \cdot 4H_2O$, and 2 or 4 g *sec*-butylamine in 1 l of medium (pH 7.2). Ten milliliters of the medium in a test tube or 100 ml in a shaking flask (500 ml) was inoculated from a slant culture, followed by incubation for 24–48 h at 30°C with reciprocal shaking.

Culture conditions for *Arthrobacter* sp. KNK in a 5-l fermentor

Thirty milliliters of preculture of *Arthrobacter* sp. KNK168 was transferred to a 5-l fermentor (B.E. Marubishi, Japan) containing 3.5 l of J-medium (40 g glycerol, 3 g yeast extract, 20 g PRO-EX(A12P), 1 g NaCl, 5 g KH_2PO_4, 5 g K_2HPO_4, 1 g $MgSO_4 \cdot 7H_2O$, 0.005 g $FeSO_4 \cdot 7H_2O$, 0.001 g $ZnSO_4 \cdot 7H_2O$ and 0.001 g $MnCl_2 \cdot 4H_2O$ in 1 l of medium, pH 7.2), followed by cultivation at 30°C, with constant stirring at 450 rpm, at an aeration rate of 0.5 vvm (1.75 l/min). The pH of the culture medium was maintained at 7.5 with 5 N sodium hydroxide. At 14 h from the start of the cultivation, (*R*,*S*)-*sec*-butylamine, which had been filtered through a microorganism exclusion filter, was added to give a final concentration of 4 g/l, and then the cultivation was continued for another 30 h.

Standard reaction conditions for (*R*)-3,4-dimethoxyamphetamine synthesis

The cells from 2 ml of cultured broth (R-medium containing 2 g/l of *sec*-butylamine) were added to 1 ml of a reaction mixture composed of 52 mM 3,4-dimethoxyphenylacetone and 62 mM (*R*)-1-phenylethylamine in 0.1 M Tris–HCl (pH 8.5) containing 1 g/l of sodium dodecyl sulfate (SDS), and then incubated in a test tube at 30°C for 24–48 h with shaking. The concentration of DMA formed was determined by high-performance liquid chromatography (HPLC).

Effects of amine compounds on enzyme induction

Cultivation was carried out in a test tube for 29 h as described under "Materials and methods," except for the use of 2 g/l of each amine compound listed in Table 1 in place of *sec*-butylamine. The cells obtained from 2 ml of each cultured broth were added to 1 ml of a reaction mixture composed of 52 mM 3,4-dimethoxyphenylacetone and 62 mM (*R*)-1-phenylethylamine in 0.1 M Tris–HCl buffer (pH 8.5) containing 1 g/L of SDS, and then incubated in a test tube at 30°C for 29 h with shaking. After the reaction, the concentration of DMA formed was determined by HPLC.

Table 1 Effects of amine compounds on enzyme induction

Amine compound	Conversion yield (%)[a]
None	13
iso-Propylamine	35
sec-Butylamine	41
3-Amino-2,2-dimethylbutane	55

Arthrobacter sp. KNK168 was cultivated for 29 h in R-medium containing 2 g/l of each of the amine compounds listed instead of *sec*-butylamine, as described under "Materials and methods." DMA synthesis was performed with the cells from each cultured broth as described under "Materials and methods." Methylamine, ethylamine, *n*-propylamine, *n*-butylamine, *n*-amylamine, putrescine, β-alanine, 4-aminobutyrate, DL-3-aminobutyrate, benzylamine, (*R*,*S*)-3,4-dimethoxyamphetamine and (*R*)-1-phenylethylamine were not effective

[a]Conversion yield was calculated based on the concentration of 3,4-dimethoxyphenylacetone

Amino donor specificity on amination of 3,4-dimethoxyphenylacetone

The cells obtained from 2 ml of cultured broth (R-medium containing 2 g/l of *sec*-butylamine) were added to 1 ml of reaction mixture composed of 52 mM 3,4-dimethoxyphenylacetone and 62 mM of one of the amino compounds listed in Table 2 in 0.1 M Tris–HCl buffer (pH 8.5), and then incubated in a test tube at 30°C for 27 h with shaking. The concentration of DMA formed was determined by HPLC.

Effects of additives on the reaction

The cells obtained from 2 ml of cultured broth (R-medium containing 2 g/l of *sec*-butylamine) were added to 2 ml of a reaction mixture composed of 154 mM 3,4-dimethoxyphenylacetone, 207 mM (*R*)-1-phenylethylamine, and 30 g/l of one of the compounds listed in Table 4 in 0.1 M Tris–HCl buffer (pH 8.5), and then incubated in a test tube at 30°C for 46 h with shaking. The concentration of DMA formed was determined by HPLC.

Preparation of (R)-3,4-dimethoxyamphetamine with *Arthrobacter* sp. KNK168

One and a half liters of a reaction mixture, composed of the cells of *Arthrobacter* sp. KNK168 obtained from 1.5 l of cultured broth in a 5-l fermentor, 45 g 3,4-dimethoxyphenylacetone, 28 g (*R*)-1-phenylethylamine, 65 g oleic acid,

Table 2 Amino donor specificity on the synthesis of DMA from 3,4-dimethoxyphenylacetone

Amino donor	Relative activity (%)[a]
Ethylamine	0
Propylamine	0
Butylamine	0
Benzylamine	10
iso-Propylamine	0
sec-Butylamine	4
2-Pentylamine	7
2-Heptylamine	25
(*R*)-1-Phenylethylamine	100
(*S*)-1-Phenylethylamine	0
(*RS*)-1-Phenylethylamine	63
β-Alanine	0
4-Aminobutyric acid	0
Putrescine	0
DL-Alanine	0
D-Alanine	0
L-Alanine	0
DL-Lysine	0
DL-Ornithine	0
NH_3Cl	0

Reaction conditions are given under "Materials and methods"
[a]Relative activity (%) was calculated based on that of (*R*)-1-phenylethylamine, which was taken as 100%. Relative activity of less than 1% is expressed as zero

and 0.1 M Tris–HCl (pH 8.5), was stirred for 39 h at 30°C. The reaction mixture was adjusted at pH 2.0 with 4 N hydrochloric acid, and then extracted with 3 l of toluene to remove 3,4-dimethoxyphenylacetone and acetophenone formed from (*R*)-1-phenylethylamine. The aqueous layer was adjusted to pH 12 with 5 N sodium hydroxide and then extracted with 3 l of toluene again. The organic layer was concentrated in vacuo and 31 g of DMA (total yield 69%), as a colorless liquid, was obtained on vacuum distillation (boiling point, 127°C/5 mmHg). ^{1}H NMR ($CDCl_3$, 400 MHz): 6.81 (1H, d), 6.71–6.74 (2H, s, d), 3.87 (3H, s), 3.86 (3H, s), 3.10–3,18 (1H, m), 2.67 (1H, dd), 2.44 (1H, dd), 1.41 (2H, broad), 1.12 (3H, d).

The specific rotation of DMA is −32.1 (20°C, *c*=2.27, chloroform). The reported value for the (*R*)-isomer is $[\alpha]_D$: −30.9 (*c*=4.13, chloroform) (Anthony 1957).

Syntheses of various chiral amines by asymmetric synthesis with *Arthrobacter* sp. KNK168

The cells obtained from 400 ml of cultured broth (R-medium containing 2 g/l of *sec*-butylamine) were added to 200 ml of a reaction mixture composed of 2 g of one of the ketone compounds listed in Table 5 and 1.2-fold equivalent moles of (*R*)-1-phenylethylamine in 0.1 M Tris–HCl buffer (pH 8.5) in a shaking flask (500 ml) and then incubated at 30°C for 40 h with shaking (135 rpm). The concentrations and optical purities of the amines formed were determined by HPLC, as described under "Materials and methods."

Amino acceptor specificity on deamination of (R)-1-phenylethylamine

The cells obtained from 1 ml of cultured broth (R-medium containing 2 g/l of *sec*-butylamine) were added to 2 ml of a reaction mixture composed of 41 mM (*R*)-1-phenylethylamine and 41 mM of one of the compounds listed in Table 6 in 0.1 M Tris–HCl buffer (pH 8.5) and then incubated in a test tube at 30°C for 2 h with shaking. The concentration of acetophenone formed was determined by HPLC.

Kinetic resolution of 1-phenylethylamine

The cells obtained from 100 ml of cultured broth (R-medium containing 2 g/l of *sec*-butylamine) were added to 100 ml of a reaction mixture composed of 1.0 g racemic 1-phenylethylamine and 0.44 g pyruvate in 0.1 M Tris–HCl buffer (pH 8.5) in a shaking flask (500 ml) and then incubated at 30°C for 6 h with shaking (135 rpm). The concentration of 1-phenylethylamine was determined by HPLC. The reaction mixture was adjusted to pH 2.0 with 4 N hydrochloric acid and then extracted with 100 ml of ethyl acetate to remove acetophenone formed. The aqueous layer was adjusted to pH 12 with 5 N sodium hydroxide and then extracted with 100 ml of ethyl acetate again. The organic layer was washed with a small amount of saturated aqueous sodium chloride, dried with anhydrous Na_2SO_4, and then concentrated in vacuo to give (*R*)-1-phenylethylamine as a slightly yellow oil [0.43 g, 86% yield, 98% enantiomeric excess (ee)].

Analytical methods

Identification and determination of amines, such as 3,4-dimethoxyamphetamine, 4-methoxyamphetamine, 1-(3-hydroxyphenyl)ethylamine, 1-(3-methoxyphenyl)ethylamine, and 1-phenylethylamine, and ketones, such as 3,4-dimethoxyphenylacetone, 4-methoxyphenylacetone, 3′-hydroxyacetophenone, 3′-methoxyacetophenone, and acetophenone, were performed by HPLC. HPLC was carried out on a Cosmosil AR C_{18} (4.6×250 mm) column (Nakalai Tesque, Japan) at room temperature and a flow rate of 1 ml/min (eluent, H_2O/acetonitrile/KH_2PO_4/sodium hexane sulfonate/H_3PO_4=1500 ml/300 ml/4.55 g/1.69 g/3.0 g). The absorbance of the eluate was monitored at 254 nm. 3,4-Dimethoxyamphetamine, 4-methoxyamphetamine, 1-(3-methoxyphenyl)ethylamine, 1-(3-hydroxyphenyl)ethylamine, and 1-phenylethylamine were eluted at 11.2, 13.6, 10.2, 13.8, and 8.0 min, respectively.

The optical purities of 3,4-dimethoxyamphetamine, 4-methoxyamphetamine, 1-(3-hydroxyphenyl)ethylamine, and 1-(3-methoxyphenyl)ethylamine were determined by HPLC

analysis of the corresponding diastereomers with L-leucine *N*-carboxy anhydride (L-Leu-NCA), as described in the previous paper (Iwasaki et al. 2003). Chiral analysis of 1-phenylethylamine was performed by HPLC on a Crownpak CR column (Daicel Co., Japan) at room temperature and a flow rate of 0.5 ml/min [eluent, perchloric acid (60% w/w)/methanol=90/10].

A ^{1}H NMR spectrum was recorded with a FT-NMR, JNM-400 spectrometer (400 MHz, JEOL, Japan). Chemical shifts are expressed in parts per million (ppm), with tetramethylsilane as an internal standard. Optical rotations were determined with a digital polarimeter (SEPA-200, Horiba, Japan).

Results

Effects of amine compounds on enzyme induction

We examined the cultural conditions for *Arthrobacter* sp. KNK168 for the production of (*R*)-transaminase at the beginning of the study. *Arthrobacter* sp. KNK168 was cultured in R-medium supplemented with 2 g/l of the amine compounds listed in Table 1, and the washed cells obtained from each cultured broth were assayed for DMA synthesis activity. As shown in Table 1, the activity increased when some kinds of *sec*-amines, such as *iso*-propylamine, *sec*-butylamine and 3-amino-2,2-dimethylbutane, were added to the medium. On the other hand, 3,4-dimethoxyamphetamine (DMA), which was used as the sole nitrogen source for the screening for this microorganism, was not a good inducer for (*R*)-transaminase production.

Amino donor specificity on the amination of 3,4-dimethoxyphenylacetone

The amino donor specificity on the amination of 3,4-dimethoxyphenylacetone by strain KNK168 is shown in Table 2. Among the amino compounds tested, (*R*)-1-phenylethylamine was the best amino donor. No detectable amount of DMA was formed with (*S*)-1-phenylethylamine, showing (*R*)-enantioselectivity for the amino donor. Other chiral amines, *iso*-butylamine, 2-pentylamine, and 2-heptylamine, showed reactivities, but these reactivities were lower than that of (*R*)-1-phenylethylamine. None of the simple amines whose nitrogen atoms are attached to the terminal carbon atoms, except for benzylamine, showed any reactivity. Inorganic ammonium salts and DL-α-amino acids, including α,ω-diaminomonocarboxylic acids such as DL-lysine and DL-ornithine, did not serve as amino donor. Typical substrates of ω-amino acid transaminase such as β-alanine, 4-aminobutyric acid, and putrescine (Yonaha et al. 1977; Yonaha and Toyama 1980) were inactive. The ee values of the DMA formed were above 99% (*R*) in all cases with racemic 1-phenylethylamine, 2-pentylamine, and 2-heptylamine as well as (*R*)-1-methylbenzulamine as the amino donor (data not shown). These results indicate that racemic amine could be used as an amino donor for asymmetric amination. However, twofold amount of racemic amine is required because only (*R*)-enantiomer is active.

Enzymatic synthesis of (*R*)-3,4-dimethoxyamphetamine with cell-free extract of *Arthrobacter* sp. KNK168

The cells from 100 ml of cultured broth (R-medium containing 2 g/l of *sec*-butylamine) were suspended in 25 ml of 0.1 M Tris–HCl (pH 8.0) and then disrupted by ultrasonication. After centrifugation, the resulting supernatant was used as the cell-free extract. A reaction mixture composed of 2 ml of the cell-free extract, 30 mM 3,4-dimethoxyphenylacetone, 0.1 M Tris–HCl (pH 8.0), and 36 mM (*R*)-1-phenylethylamine or ammonium chloride was incubated in a test tube at 30°C for 22 h with shaking. After the reaction, no DMA was formed with ammonium chloride. However, 23 mM (*R*)-DMA and 22 mM acetophenone were accumulated in a reaction mixture with (*R*)-1-phenylethylamine, indicating that the (*R*)-DMA-forming reaction proceeds in a manner of enzymatic transamination (Fig. 1).

Effects of pH and temperature on the amination of 3,4-dimethoxyphenylacetone with cells of *Arthrobacter* sp. KNK168

The effects of pH and temperature on the synthesis of DMA from 3,4-dimethoxyphenylacetone and (*R*)-1-phenylethylamine with cells of *Arthrobacter* sp. KNK168 are shown in Fig. 2. The activity was higher under weak alkaline conditions, and the optimum pH range for the reaction was about 8.5–9.0. The optimal temperature for the reaction

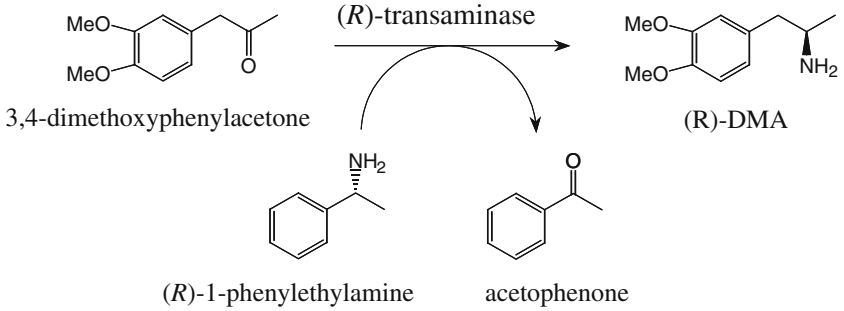

Fig. 1 Reaction scheme of the synthesis of (*R*)-DMA

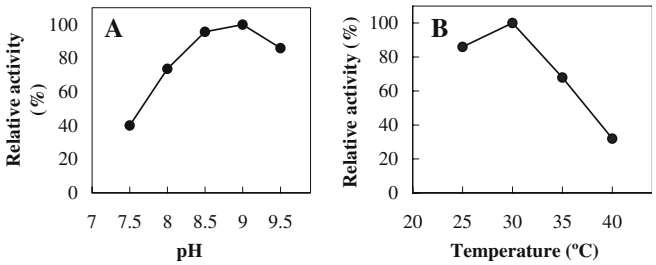

Fig. 2 Effects of pH (**a**) and temperature (**b**) on the synthesis of DMA. The reaction was performed according to the standard conditions for DMA synthesis, as described under "Materials and methods," except that the initial pH of the reaction mixture at 30°C (**a**) and the reaction temperature (**b**) were changed as indicated in the figure

was 30°C, and the activity decreased rapidly at temperatures higher than 30°C.

Effects of additives

Inhibition and inactivation of (*R*)-transaminase by substrates and products amines were observed (data not shown). In particularly, inactivation of the enzyme by ketones such as 3,4-dimethoxyphenylacetone and acetophenone was a severe problem. No transaminase activity remained after preincubation with 20 g/l of 3,4-dimethoxyphenylacetone or 20 g/l of acetophenone for 20 h at 30°C (Table 3).

Therefore, probably due to this inactivation, the reaction stopped within a short period, which resulted in a low yield, especially with high concentrations of substrates. To enhance the reaction yield, we tried to find suitable additives and found some types of surfactants and long-chain fatty acids that effectively increased the reaction yield (Table 4). The conversion yield was enhanced twofold on the addition of SDS, linoleic acid, and oleic acid. The addition of other organic solvents, such as ethylacetate, butylacetate, hexane, toluene, and benzene, was not so effective (data not shown).

Table 3 Inactivation of the transaminase activity by substrates and products

Compound	Remaining activity (%)[a]
None	49
(*R*)-1-Phenylehylamine	52
DMA	31
3,4-Dimethoxyphenylacetone	0
Acetophenone	0

Cells obtained from 2 ml of cultured broth were added to 1 ml of 0.1 M Tris–HCl buffer (pH 8) containing 2% (w/v) of each of the compounds listed, followed by incubation at 30°C for 20 h with shaking. Cells were centrifuged off and washed with the buffer, and then the DMA synthesis activity of the cells was measured according to the standard conditions for DMA synthesis, as described under "Materials and methods"

[a]Remaining activity (%) was calculated based on the activity of the cells before incubation, which was taken as 100%

Table 4 Effects of additives on the synthesis of (*R*)-DMA

Additive	Conversion (%)[a]
None	34
iso-Valeric acid	33
Decanoic acid	45
Oleinic acid	61
Linoleic acid	62
SDS	65
Triton X-100	49

DMA syntheses were performed with the addition of one of the compounds listed in Table 4 to the reaction mixture, as described under "Materials and methods"

[a]Conversion was calculated based on the concentration of 3,4-dimethoxyphenylacetone

Figure 3 shows the time courses of the reactions with and without SDS. Without SDS, the reaction stopped within 20 h. On the other hand, when SDS was added, the reaction proceeded after 20 h, a greater than 70% conversion yield being reached in 64 h.

Large-scale preparation of (*R*)-DMA with *Arthrobacter* sp. KNK168

Arthrobacter sp. KNK168 was cultured in a 5-l fermentor as described under "Materials and methods." One and a half liters of a reaction mixture composed of cells of *Arthrobacter* sp. KNK168 obtained from 1.5 l cultured broth, 45 g 3,4-dimethoxyphenylacetone (154 mM), 28 g (*R*)-1-phenylethylamine (154 mM), 65 g oleic acid, and 0.1 M Tris–HCl (pH 8.5) was prepared, and the reaction was performed with stirring at 30°C. After 39 h, 37 g DMA (126 mM) was formed with 82% conversion yield. DMA was extracted from the reaction mixture, and 31 g of DMA (total yield

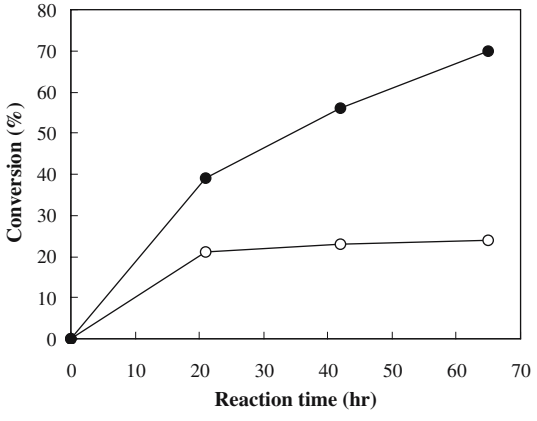

Fig. 3 Effect of SDS on the synthesis of (*R*)-DMA from 3,4-dimethoxyphenylacetone. The reaction conditions were as follows. The cells obtained from 50 ml of cultured broth were added to 25 ml of a reaction mixture composed of 155 mM 3,4-dimethoxyphenylacetone, 207 mM (*R*)-1-phenylethylamine, and SDS in 0.1 M Tris–HCl buffer (pH 8.5) in a shaking flask, followed by incubation at 30°C with shaking (135 rpm). ○, none; •, 3% (w/v) SDS. Conversion yield was calculated based on the concentration of 3,4-dimethoxyphenylacetone

Table 5 Amination of various prochiral ketones with *Arthrobacter* sp. KNK168

Substrate	Product	% ee
DMPA	DMA	99.6 (*R*)
4-Methoxyphenylacetone	4-Methoxyamphetamine	99.4 (*R*)
3′-Hydroxyacetophenone	1-(3-Hydroxyphenyl) ethylamine	99.1 (*R*)
3′-Methoxyacetophenone	1-(3-Methoxyphenyl) ethylamine	99.1 (*R*)

Reaction conditions are given under "Materials and methods"

69%) was obtained by vacuum distillation (5 mmHg, 127°C) as described under "Materials and methods." The chemical purity and optical purity of DMA obtained were 97.4% and 99.6% ee for (*R*).

Syntheses of various chiral amines by asymmetric synthesis with *Arthrobacter* sp. KNK168

Arthrobacter sp. KNK168 catalyzed the transamination of not only 3,4-dimethoxyphenylacetone but also several kinds of prochiral ketones. 4-Methoxyphenylacetone, 3′-hydroxyacetophenone, and 3′-methoxyacetophenone were converted to the corresponding amines. As shown in Table 5, all products were optically active and their optical purities were very high for (*R*).

Kinetic resolution of 1-phenylethylamine

Kinetic resolution is useful for the preparation of a chiral amine as well as asymmetric synthesis. As mentioned above, *Arthrobacter* sp. KNK168 showed (*R*)-enantioselectivity toward 1-phenylethylamine. We attempted kinetic resolution of racemic 1-phenylethylamine with this microorganism. Pyruvate was a good amino acceptor on the deamination of (*R*)-1-phenylethylamine (Table 6). The cells obtained from 100 ml of culture broth were incubated with 100 ml of a reaction mixture composed of 1.0 g racemic 1-phenylethylamine (83 mM) and 0.44 g pyruvic acid (50 mM) in 0.2 M Tris–HCl (pH 8.5) in a shaking flask (500 ml) at 30°C for 6 h with shaking (135 rpm). Under these conditions, (*S*)-1-phenylethylamine of 98% ee was obtained in a 51% conversion yield. 1-Phenylethylamine was extracted from the reaction mixture, and 0.43 g (*S*)-1-phenylethylamine (98% ee) was obtained as described under "Materials and methods."

Table 6 Amino acceptor specificity on the deamination of (*R*)-1-phenylethyiamine

Amino acceptor	Relative activity (%)[a]
Pyruvate	100
Glyoxylate	3
α-Ketoglutarate	2
3,4-Dimethoxyphenylacetone	7

Reaction conditions are given under "Materials and methods"
[a]Relative activity (%) was calculated based on that of pyruvate, which was taken as 100%

Discussion

The (*R*)-transaminase of *Arthrobacter* sp. KNK168 is induced by *sec*-amines such as *sec*-butylamine during the cultivation, and catalyzes a transamination between *sec*-amine and ketone compound. Taking these results into consideration, it is likely that the (*R*)-transaminase is associated with the biosynthesis or the metabolism of *sec*-amines. However, further investigation of this enzyme is necessary to elucidate its role in this microorganism.

The (*R*)-transamase catalyzes the transfer of an amino group from an amine substrate to a prochiral ketone (*R*)-enantioselectively and forms a chiral (*R*)-amine from the ketone. This makes the (*R*)-transaminase an attractive biocatalyst for production of chiral amines. The (*R*)-enantiomers (>99% ee) of secondary amines, such as DMA, 4-methoxyamphetamine, 1-(3-hydroxyphenyl)ethylamine, and 1-(3-methoxyphenyl)ethylamine, can be produced from the corresponding ketone compounds in the presence of an amino donor through asymmetric amination. (*R*)-1-Phenylethylamine is a good amino donor for DMA formation. Alanine does not act as an amino donor substantially, although pyruvate, a deaminated form of alanine, serves as an amino acceptor well. During the production of (*R*)-DMA through asymmetric amination of 3,4-dimethoxyphenylacetone using (*R*)-1-phenylethylamine as the amino donor with cells of *Arthrobacter* sp. KNK168, inactivation of the enzyme by ketone compounds was a severe problem, preventing the use of a high substrate concentration of 2% (w/v) or more. The addition of some kinds of organic solvents is expected to minimize the toxic effect of a substrate or product through its extraction into the organic layer. Organic solvents were not so effective for increasing the productivity of DMA. However, when SDS and some long-chain fatty acids were added to the reaction mixture, the reaction yield increased. The underlying reasons for the effectiveness of these additives are not yet clear. Probable reasons are as follows. Not only are these additives supposed to minimize the toxic effects of ketone compounds by extracting toxic ketones from the aqueous layer, but it is also supposed that their acidic natures are favorable for minimizing the inhibition by a product amine through the formation of some kinds of salts.

Under optimum conditions, 126 mM (*R*)-DMA was formed from 154 mM 3,4-dimethoxyphenylacetone and 154 mM (*R*)-1-phenylethylamine with 82% conversion yield by means of a whole cell reaction with *Arthrobacter* sp. KNK168. It has been reported that (*S*)-amines, such as (*S*)-1-methyl-3-phenylprophylamine and (*S*)-1-phenylethylamine, were synthesized by asymmetric amination with ω-transaminase from *Vibrio fluvialis* JS17 (Shin and Kim 1999). However, the concentration of the chiral amine formed in the whole cell reaction was very low (about

20 mM), and a ten times excess of the amino donor as to the substrate ketone was needed to obtain a high conversion yield. The asymmetric synthesis of (*R*)-amine with *Arthrobacter* sp. KNK168, demonstrated in this study, is much more advantageous from the viewpoints of productivity and its enantioselectivity for (*R*).

To our knowledge, the synthesis of a (*R*)-amine by means of asymmetric amination with an enzyme and a microorganism has not been previously reported. According to the properties described here, the (*R*)-transaminase of *Arthrobacter* sp. KNK168 seems to be a novel enzyme. Further characterization of this enzyme and its application to the manufacture of chiral amines are now in progress.

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