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Production of Ethyl (*R*)-2-Hydroxy-4-phenylbutanoate *via* Reduction of Ethyl 2-Oxo-4-phenylbutanoate in an Interface Bioreactor

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Ethyl (*R*)-2-hydroxy-4-phenylbutanoate [(*R*)-EHPB], a useful intermediate for the synthesis of various *anti*-hypertension drugs, was produced *via* microbial reduction of ethyl 2-oxo-4-phenylbutanoate [EOPB] in an interface bioreactor. *Rhodotorula minuta* IFO 0920 and *Candida holmii* KPY 12402 were selected as the best type culture and isolated yeasts, respectively. The highest enantiomeric excess of (*R*)-EHPB produced by *R. minuta* and *C. holmii* were 95 and 94%, respectively. *C. holmii* was used for the reduction of EOPB in a pad-packed interface bioreactor (inner volume, 3 liter). After incubation for 4 days, 4.4 g of (*R*)-EHPB was obtained *via* extraction with methanol followed by column chromatography. The overall yield, chemical purity, and enantiomeric excess of (*R*)-EHPB were 58%, 99.1%, and 90%, respectively.

Key words: interface bioreactor; stereoselective reduction; non-aqueous bioreactor; microbial transformation; bioconversion

Angiotensin-converting enzyme (ACE) inhibitors such as enalapril and lisinopril are useful for the treatment of hypertension because ACE (kininase II, peptidyldipeptide hydrolase, EC 3.4.15.1) catalyzes both the production of vasoconstrictor angiotensin II and inactivation of vasodilator bradykinin.[1,2]

Ethyl (*R*)-2-hydroxy-4-phenylbutanoate [(*R*)-EHPB] is an important intermediate for the synthesis of ACE inhibitors. For example, it was reported that enalaprilat and lisinopril were efficiently prepared from (*R*)-EHPB.[3] A number of methods for the preparation of (*R*)-2-hydroxy-4-phenylbutanoic acid [(*R*)-HPB] *via* optical resolution have been reported, *i.e.*, microbial hydrolysis of racemic 2-hydroxy-4-phenylbutyronitrile,[4] chemical resolution of racemic HPB,[5] enzymatic or microbial hydrolysis of HPB esters,[6] and enzymatic esterification of HPB.[7] However, in principle, maximal yield of (*R*)-HPB cannot exceed 50% for these methods.

Recently, enzymatic or microbial reduction have been used for the preparation of (*R*)-HPB[8] or its esters.[9] The yield of (*R*)-HPB produced *via* reduction of 2-oxo-4-phenylbutanoic acid [OPB] is generally higher than that *via* the optical resolution of racemic HPB. However, the former procedures had some disadvantages, such as toxicity appearance of HPB and/or OPB, use of much biocatalyst, or difficulty of separation of the biocatalyst and HPB.

We have reported that interface bioreactors, which are microbial transformation devices between a hydrophilic carrier and a hydrophobic organic solvent, can be used in several types of microbial transformation, such as oxidation, reduction, hydrolysis, and esterification.[10] Concerning microbial reduction, 2-octanone,[10] citronellal,[11] and 6-methyl-5-hepten-2-one[12] are efficiently converted to 2-octanol, citronellol, and (*R*)-sulcatol, respectively. In the interface bioreactors, the toxicity of hydrophobic substrates and/or products can be efficiently alleviated, and recycling of coenzyme proceeds smoothly. Moreover, the separation of the biocatalyst immobilized on a carrier surface from the product is much easier than that for an aqueous bioconversion system. In this work, we applied interface bioreactors to the production of (*R*)-EHPB *via* microbial reduction of EOPB (Fig. 1).

Materials and Methods

Microorganisms, media, and chemicals. Fifty-five type culture yeasts purchased from IFO and 499 strains of isolated yeasts were used for screening. The basal medium (pH 6.0) containing 5.0 g of peptone, 3.0 g of

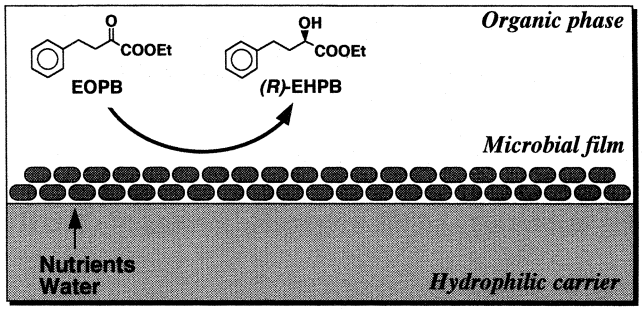

Fig. 1. Microbial Reduction of Ethyl 2-Oxo-4-phenylbutanoate [EOPB] to Ethyl (*R*)-2-Hydroxy-4-phenylbutanoate [(*R*)-EHPB] in an Interface Bioreactor.

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Abbreviations: EHPB, ethyl 2-hydroxy-4-phenylbutanoate; EOPB, ethyl 2-oxo-4-phenylbutanoate; ACE, angiotensin-converting enzyme; HPB, 2-hydroxy-4-phenylbutanoic acid; OPB, 2-oxo-4-phenylbutanoic acid; e.e., enantiomeric excess.

yeast extract, 3.0 g of malt extract, 1.0 g of $MgSO_4 \cdot 7H_2O$, 10.0 g of glucose, and 1.0 liter of distilled water was used. Agar powder (15 g) was added to 1.0 liter of the basal medium for the preparation of an agar plate interface bioreactor. To examine the effects of the amount of glucose on (*R*)-EHPB production, the glucose contents were changed between 1–5% in the agar plate. EOPB and (*R*)-EHPB were purchased from Aldrich and Fluka Co., Ltd., respectively. All other chemicals were also commercially available.

Screening of EOPB-reducing strains in an agar plate interface bioreactor. Fifty-five type culture yeasts and 499 strains isolated from soil samples were examined. A cell suspension (200 μl, 1 loop/2 ml-medium) was spread on a nutrient agar plate of which the surface area was 38.5 cm^2. After removal of the excess moisture by allowing the plate to stand, 8 ml of a 1% (for type culture yeasts) or 3% (for isolated yeasts) solution of EOPB in decane was added to the agar plate, and incubation was done at 30°C by allowing the plate to stand for 3 (for type culture yeasts) or 5 days (for isolated yeasts). After the incubation, a 100-μl sample of the decane solution was analyzed by HPLC: column, TSK-Gel silica 60 (Tosoh Co., Ltd.; diameter, 4.6 mm; length, 250 mm); eluent, hexane-2-propanol (975:25); flow rate, 0.8 ml/min. The retention times of EOPB and EHPB were 5.5 and 8.5 min, respectively.

Identification of products. The products were identified by GC-MS by comparison with authentic samples. The GC-MS measurement was done by the EI method with GCMS-QP-1000EX (Shimadzu Co., Ltd.) under the following conditions: ionization method, electron impact; ionization voltage, 70 eV; ion source temperature, 250°C; column, CBS-20-M50-025 (Shimadzu Co., Ltd.; diameter, 0.2 mm; length, 50 m); carrier gas, He (20 ml/min).

Conversion of EOPB in an agar plate interface bioreactor. The method of inoculation was identical with that for screening. After removal of excess moisture, a microorganism was cultured at 30°C by allowing the plate to stand for 0-4 days (precultivation). The incubation was started by adding a solution of EOPB in decane (8 or 10 ml). In the case of the examination for the effects of additives on (*R*)-EHPB production, they were added to the decane layer at the concentration of 0.5%.

Determination of absolute configuration and enantiomeric excess of EHPB. After the incubation, 8–10 ml of the organic layer was put on a silica gel column (Wakogel C-200, 5 g). After removal of decane by elution with 30 ml of hexane, EOPB and EHPB were eluted with 20 ml of ethyl acetate. The solvent was evaporated and EHPB was purified by thin layer chromatography (Silica 60 F_{254}) developed with a solvent system of benzene-ethyl acetate (9:1). The R_f values of EOPB and EHPB were 0.44 and 0.26, respectively. The absolute configuration and the enantiomeric excess of EHPB were determined by HPLC: column, Chiralcel OD (Daisel Chemical Industries Co., Ltd.; diameter, 4.6 mm; length, 250 mm); eluent, hexane-2-propanol (95:5); flow rate, 1.0 ml/min. The retention times of (*S*)- and (*R*)-EHPB were 7.2 and 9.7 min, respectively.

Production of (R)-EHPB in a pad-packed interface bioreactor. A 3% agar pad holding a stainless-steel frame attached to a stainless-steel net was used as a hydrophilic carrier as mentioned in a former report.[13] Six pieces of agar pad (size, 70 by 130 mm; thickness, 18 mm) were inoculated with a condensed 1-day broth of *Candida holmii* KPY 12402 (200 ml from 1 liter) by dipping. After precultivation at 30°C for 2 days, the agar pads were set in a stainless-steel tank (width, 160 mm; depth, 160 mm; height, 110 mm) with Teflon pads as spacers as mentioned in the former report.[13] Six-hundred ml of a 1.5% solution of EOPB in decane was added, and the preparation was incubated at 30°C with agitation (700 rpm). After the incubation, the decane layer was collected and extracted 5 times with half volumes of methanol. After removal of methanol *in vacuo*, the recovered yellow oil was put on a silica gel column (diameter, 55 mm; length, 350 mm), and (*R*)-EHPB was eluted with benzene-ethyl acetate (9:1). After removal of the eluent, the yield and chemical and optical purities of the (*R*)-EHPB recovered were measured.

Results and Discussion

Screening of (R)-EHPB-producing type culture strains

In this study, decane was used as an organic solvent in the interface bioreactors because decane was a superior hydrophobic organic solvent for EOPB and EHPB. Moreover, the solvent is inexpensive (¥200–500/kg) and has no toxic effect on many microorganisms. First, conversion of EOPB with type culture yeasts was done in an agar plate interface bioreactor. As shown in Table 1, although many yeasts reduced EOPB, the stereoselectivity differs one from another. In some cases, the stereoselectivities were even different among those of the same genus. While *Rhodotorula minuta* IFO 0920 accumulated (*R*)-EHPB (95% e.e.) of the highest e.e., *Debaryomyces hansenii* IFO 0855 afforded (*S*)-isomer (91% e.e.) of the highest one. However, the yield of (*R*)-EHPB produced with *R. minuta* was only 16.0%. It has been reported that aromatic α-hydroxy acids, such as mandelic acid, are oxidized to α-keto acids and decomposed.[14,15] Therefore, it is supposed that the EHPB produced is hydrolyzed to HPB, which in turn is oxidized to OPB, and OPB is decomposed. Indeed, as shown in Fig. 2, although (*R*)-EHPB was accumulated until 12 h, *R. minuta* decomposed it in a prolonged incubation. Next, *R. minuta* was selected as the best strain, and we tried to further improve the e.e. and yield of (*R*)-EHPB.

Production of (R)-EHPB with Rhodotorula minuta IFO 0920 in an agar plate interface bioreactor

In the interface bioreactor, precultivation period is important for the formation of a microbial film, the toxicity alleviation against a substrate, and the microbial

Table 1. Screening for Ethyl 2-Oxo-4-phenylbutanoate-reducing Strains in an Agar Plate Interface Bioreactor

Strain	Concentration (mg/ml)		Yield (%)	e.e. (%)
	EOPB	EHPB		
Rhodotorula minuta IFO 0920	0.0	1.6	16.0	95 (*R*)
Kloeckera corticis IFO 0631	0.1	5.3	53.5	84 (*R*)
Pichia heedii IFO 10019	0.7	3.8	40.9	79 (*R*)
Pichia pastoris IFO 0948	1.3	4.9	56.3	70 (*R*)
Kluyveromyces lactis IFO 1903	0.1	3.8	38.4	61 (*R*)
Hansenula anomala IFO 0146	1.0	4.8	53.3	50 (*R*)
Hansenula anomala IFO 0148	1.3	5.2	59.8	49 (*R*)
Pichia guilliermondii IFO 10107	1.6	3.3	39.3	49 (*R*)
Pichia membranaefaciens IFO 10062	0.7	3.9	41.9	45 (*R*)
Pichia besseyi IFO 1707	3.7	3.9	61.9	34 (*R*)
Pichia carsonii IFO 1989	0.1	6.6	66.7	19 (*R*)
Pichia thermotolerans IFO 10024	5.9	1.4	34.1	13 (*R*)
Pichia quercuum IFO 0949	1.5	2.6	30.6	13 (*R*)
Debaryomyces hansenii IFO 0855	0.1	2.8	28.3	91 (*R*)
Pichia kodamae IFO 10090	1.5	4.9	57.6	59 (*S*)
Pichia scolyti IFO 1280	0.6	2.0	21.3	58 (*S*)
Pichia canadensis IFO 0973	0.1	5.8	58.6	55 (*S*)
Candida utilis IFO 0626	0.7	6.4	68.8	50 (*S*)
Hansenula minuta IFO 0975	0.0	5.3	53.0	46 (*S*)
Candida etchellsii IFO 1229	1.8	3.9	47.6	41 (*S*)
Candida utilis IFO 0619	0.1	6.3	63.6	31 (*S*)
Pichia castillae IFO 1823	0.5	5.7	60.0	26 (*S*)
Pichia pijperi IFO 1290	0.1	6.5	65.7	24 (*S*)
Wickerhamiella domercquii IFO 1857	0.2	6.4	65.3	14 (*S*)

Each strain was inoculated on a nutrient agar plate of which the surface area was 38.5 cm^2 and cultured at 30°C by allowing the plate to stand for 1 day. After precultivation, 8 ml of a 1% solution of EOPB in decane was added, and incubation was done at 30°C by allowing the plate to stand for 3 days.

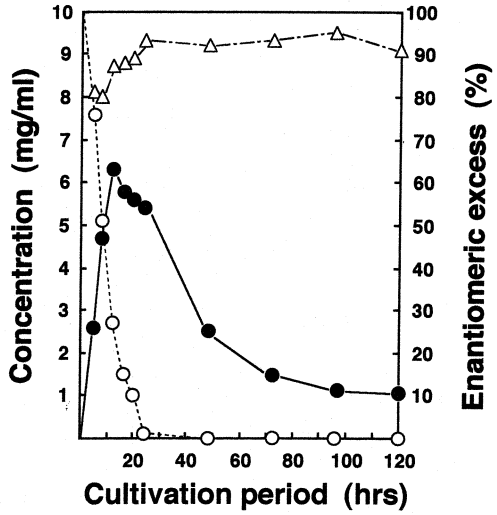

Fig. 2. Time Course of Reduction of Ethyl 2-Oxo-4-phenylbutanoate to Ethyl (*R*)-2-Hydroxy-4-phenylbutanoate with *Rhodotorula minuta* IFO 0920 in an Agar Plate Interface Bioreactor.

Symbols: ○, concentration of EOPB; ●, concentration of (*R*)-EHPB; △, % e.e. of (*R*)-EHPB. *R. minuta* was inoculated on a nutrient agar plate of which the surface area was 38.5 cm^2. After precultivation for 1 day, 8 ml of a 1% solution of EOPB in decane was added, and incubation was done at 30°C by allowing the plate to stand.

transformation.[16] First, the effects of precultivation period on the production of (*R*)-EHPB was examined in the agar plate interface bioreactor. As shown in Table 2, when EOPB was added immediately after the inoculation (no precultivation period), the conversion of EOPB to (*R*)-EHPB was inhibited by the toxic substrate. On the other hand, when precultivation was done for 1–4 days, while e.e. of (*R*)-EHPB was little affected, the accumulation of (*R*)-EHPB decreased according to the precultivation period. Thus, the optimum precultivation period found to be 1 day.

Table 2. Effects of Precultivation Period on Ethyl (*R*)-2-Hydroxy-4-phenylbutanoate Production with *Rhodotorula minuta* IFO 0920

Precultivation period (day)	Concentration (mg/ml)		Yield (%)	e.e. (%)
	EOPB	EHPB		
0	6.8	1.0	31.3	35 (*R*)
1	0.1	5.0	50.5	90 (*R*)
2	0.1	3.4	34.3	94 (*R*)
3	0.1	4.3	43.4	90 (*R*)
4	0.1	4.6	46.5	90 (*R*)

R. minuta was inoculated on a nutrient agar plate of which the surface was 38.5 cm^2. After precultivation for 0–4 days, 10 ml of a 1% solution of EOPB in decane was added, and incubation was done at 30°C by allowing the plate to stand for 2 days.

Next, the effects of the glucose content in the carrier on the production of (*R*)-EHPB were examined in the agar plate interface bioreactor. Interestingly, the glucose content in the agar plate did not affect the stereoselective reduction of EOPB (Table 3). It is well known that high concentrations of glucose in a medium repress the activity of alcohol dehydrogenase.[17] Con-

Table 3. Effects of Glucose Content on Ethyl (*R*)-2-Hydroxy-4-phenylbutanoate Production with *Rhodotorula minuta* IFO 0920

Glucose content (%)	Concentration (mg/ml)		Yield (%)	e.e. (%)
	EOPB	EHPB		
1	0.1	4.5	45.5	90 (*R*)
2	0.1	4.6	46.5	87 (*R*)
3	0.1	4.7	47.5	87 (*R*)
4	0.2	5.2	53.1	84 (*R*)
5	0.3	4.9	50.5	89 (*R*)

R. minuta was inoculated on a nutrient agar plate of which the surface area was 38.5 cm². After precultivation for 1 day, 10 ml of a 1% solution of EOPB in decane was added, and incubation was done at 30°C by allowing the plate to stand for 2 days.

Table 4. Effects of Incubation Temperature on Ethyl (*R*)-2-Hydroxy-4-phenylbutanoate Production with *Rhodotorula minuta* IFO 0920

Temp. (°C)	Concentration (mg/ml)		Yield (%)	e.e. (%)
	EOPB	EHPB		
20	0.3	4.5	46.4	78 (*R*)
25	0.1	5.2	52.5	82 (*R*)
30	0.2	5.3	54.1	89 (*R*)
35	2.6	4.3	58.1	86 (*R*)
40	6.0	2.2	55.0	85 (*R*)

R. minuta was inoculated on a nutrient agar plate of which the surface area was 38.5 cm². After precultivation for 1 day, 10 ml of a 1% solution of EOPB in decane was added, and incubation was done at 20–40°C by allowing the plate to stand for 2 days.

cerning the interface bioreactor, NAD-dependent citronellol dehydrogenase in *Hansenula saturnus* IFO 0809 were repressed by glucose in an agar plate when its content was over 4%.[18] However, the activity of EOPB-reducing enzyme in *R. minuta* was not repressed by glucose even when its content is high, as shown in Table 3.

Third, the effects of incubation temperature on the production of (*R*)-EHPB were examined in the agar plate interface bioreactor. As shown in Table 4, the optimum temperature for the activity of EOPB-reducing enzyme was 30°C, while the activity reduced at over 35°C. Enantiomeric excess of (*R*)-EHPB was not affected by the incubation temperature.

Fourth, the effects of substrate concentration on (*R*)-EHPB production were examined. As shown in Table 5, EOPB had a very strong biotoxicity, and the strain could not act on EOPB of over 2% concentration even with the interface bioreactor. In the interface bioreactor, although the toxicity of hydrophobic compounds in an organic phase is drastically alleviated, the toxicity of hydrophilic poisons cannot be alleviated.[10-12,16,19] Indeed, the solubility of EOPB in decane was only 6% because of the relatively high polarity. Therefore, the toxicity of EOPB appeared even in the interface bioreactor.

Fifth, the effects of additives such as alcohols and ketones on the production of (*R*)-EHPB were examined in the agar plate interface bioreactor. It has been reported that the addition of allyl alcohol,[20] methyl vinyl ketone,[21] metal chloride,[22] α,β-unsaturated carbonyl compounds,[23] and various organic solvents[24] affect the stereoselectivity of the microbial reduction. Furthermore, it has been reported that ethanol and 1-propanol inhibit the oxidation of mandelic acid to benzoylformic acid in *Bordetella parapertusis*.[15] As shown in Table 6, while the addition of ethanol, 2-propanol, dioxane, and acetone increased the yield and e.e. of (*R*)-EHPB as compared with that of original reaction, the addition of allyl alcohol, 2-mercaptoethanol, 1-butanol, and cyclohexanol strongly inhibited the reduction of EOPB.

Table 5. Effects of Substrate Concentration on Ethyl (*R*)-2-Hydroxy-4-phenylbutanoate Production with *Rhodotorula minuta* IFO 0920

EOPB concentration (%)	Concentration (mg/ml)		Yield (%)	e.e. (%)
	EOPB	EHPB		
1.2	0.1	4.4	37.0	83 (*R*)
1.4	1.8	6.2	50.8	71 (*R*)
1.6	7.3	4.1	47.1	66 (*R*)
1.8	11.0	2.1	30.0	55 (*R*)
2.0	13.2	0.4	5.9	—

R. minuta was inoculated on a nutrient agar plate of which the surface area was 38.5 cm². After precultivation for 1 day, 8 ml of 1.2–2.0% solutions of EOPB in decane were added, and incubation was done at 30°C by allowing the plate to stand for 3 days.

Table 6. Effects of Additives on Ethyl (*R*)-2-Hydroxy-4-phenylbutanoate Production with *Rhodotorula minuta* IFO 0920

Additive	Concentration (mg/ml)		Yield (%)	e.e. (%)
	EOPB	EHPB		
None	0.2	6.4	65.3	79 (*R*)
Allyl alcohol	7.2	0.3	10.7	—
2-Mercaptoethanol	2.8	1.5	20.8	76 (*R*)
Ethanol	0.2	7.3	74.5	82 (*R*)
1-Propanol	4.9	3.2	62.7	68 (*R*)
2-Propanol	0.2	7.4	75.5	84 (*R*)
1-Butanol	6.7	1.4	42.4	54 (*R*)
Cyclohexanol	5.7	2.0	46.5	60 (*R*)
Dioxane	0.2	6.7	68.4	83 (*R*)
Acetone	0.1	7.0	70.7	87 (*R*)
Cyclohexanone	1.8	6.2	75.6	75 (*R*)
2-Octanone	1.8	5.4	65.9	74 (*R*)
Ethyl acetate	1.0	6.7	74.4	77 (*R*)

R. minuta was inoculated on a nutrient agar plate of which the surface area was 38.5 cm² and precultured at 30°C by allowing the plate to stand for 1 day. After precultivation, 10 ml of a 1% solution of EOPB in decane containing 500 μl of an additive. Incubation was done at 30°C by allowing the plate to stand for 2 days.

Production of (R)-EHPB with Candida holmii KPY 12402 in an agar plate or a pad-packed interface bioreactor

Next, we screened for superior strains among the 499 yeasts we kept from those isolated from 219 soil samples. Through the screening, 6 strains were found to be able to convert EOPB of 3% solution and accumulate 6.7–17.6 mg/ml of (*R*)-EHPB with 60–88% e.e. Among the 6 strains, KPY 12402 was selected as the best

Table 7. Effects of Precultivation Period on Ethyl (*R*)-2-Hydroxy-4-phenylbutanoate Production with *Candida holmii* KPY 12402

Pecultivailon period (day)	Concentration (mg/ml)		Yield (%)	e.e. (%)
	EOPB	EHPB		
0	13.9	10.7	66.5	94 (*R*)
1	3.3	16.9	63.3	88 (*R*)
2	5.2	14.3	57.7	89 (*R*)
3	19.2	5.1	47.2	81 (*R*)
4	23.3	3.5	52.2	83 (*R*)
5	23.7	3.5	55.6	80 (*R*)

C. holmii was inoculated on a nutrient agar plate of which surface area was 38.5 cm². After precultivation for 0–5 days, 8 ml of a 3% solution of EOPB in decane was added, and incubation was done at 30°C by allowing the plate to stand for 5 days.

Table 8. Effects of Glucose Content on Ethyl (*R*)-2-Hydroxy-4-phenylbutanoate Production with *Candida holmii* KPY 12402

Glucose content (%)	Concentration (mg/ml)		Yield (%)	e.e. (%)
	EOPB	EHPB		
1	4.8	15.5	61.5	89 (*R*)
2	3.1	16.2	60.2	90 (*R*)
3	4.0	15.0	57.7	87 (*R*)
4	4.5	14.3	56.1	89 (*R*)
5	4.5	13.7	53.7	88 (*R*)

C. holmii was inoculated on a nutrient agar plate of which the surface area was 38.5 cm². After precultivation for 1 day, 8 ml of a 3% solution of EOPB in decane was added, and incubation was done at 30°C by allowing the plate to stand for 5 days.

Table 9. Effects of Substrate Concentration on Ethyl (*R*)-2-Hydroxy-4-phenylbutanoate Production with *Candida holmii* KPY 12402

EOPB concentration (%)	Concentration (mg/ml)		Yield (%)	e.e. (%)
	EOPB	EHPB		
1	1.8	3.9	47.6	79 (*R*)
2	0.3	13.3	67.5	89 (*R*)
3	1.7	19.6	69.3	89 (*R*)
4	22.9	12.6	73.7	84 (*R*)
5	28.3	17.3	79.7	82 (*R*)
6	28.7	16.3	52.1	81 (*R*)

C. holmii was inoculated on a nutrient agar plate of which the surface area was 38.5 cm². After precultivation for 1 day, 8 ml of 1–6% solutions of EOPB in decane were added, and incubation was done at 30°C by allowing the plate to stand for 5 days.

strain and identified as *Candida holmii* with an Api identification kit (Vio Mérieux, France). This strain could accumulate (*R*)-EHPB of 88% e.e. up to 17.6 mg/ml. Next, we tried to raise the e.e. and yield of (*R*)-EHPB with *C. holmii* KPY 12402.

A prolonged precultivation period, over 3 days, decreased (*R*)-EHPB production and the optimum precultivation period was found to be 1 day (Table 7). Glucose content in the nutrient agar plate had little effect on the yield and stereochemistry of (*R*)-EHPB, similar to *R. minuta* IFO 0920 (Table 8). Thus, EOPB-reducing enzyme in *C. holmii* was also not repressed by high concentrations of glucose. As shown in Table 9, the strain had a high tolerance to EOPB compared with *R. minuta* and efficiently reduced 1–3% EOPB to (*R*)-EHPB. However, EOPB inhibited the reduction at over 4% concentration because of its biotoxicity.

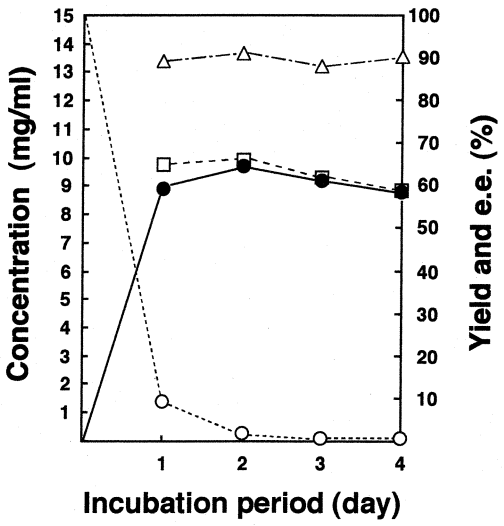

Fig. 3. Production of Ethyl (*R*)-2-Hydroxy-4-phenylbutanoate *via* Reduction of Ethyl 2-Oxo-4-phenylbutanoate with *Candida holmii* KPY 12402 in a Pad-packed Interface Bioreactor.

Symbols: ○, concentration of EOPB; ●, concentration of (*R*)-EHPB; △, % e.e. of (*R*)-EHPB; □, yield of (*R*)-EHPB. Six pieces of nutrient agar pad (size, 70 by 130 mm; thickness, 18 mm) were inoculated with a condensed 1-day broth culture of *C. holmii* (200 ml from 1 liter) by dipping. After precultivation for 2 days, the agar pads were set in a stainless-steel tank (width, 160 mm; depth, 160 mm; height, 110 mm) with Teflon pads as spacers. Six hundred ml of a 1.5% solution of EOPB in decane was added, and the preparation was incubated at 30°C with agitation (700 rpm).

Finally, the reduction of EOPB to (*R*)-EHPB with *C. holmii* was done in a pad-packed interface bioreactor. Six pieces of 3% agar pad were used as the carrier, and 600 ml of a 1.5% solution of EOPB in decane was used as the organic phase. As shown in Fig. 3, the reduction of EOPB to (*R*)-EHPB was almost finished in 2 days, and the resulting (*R*)-EHPB decreased after 3 days to some extent. Then, (*R*)-EHPB was purified from the decane layer *via* extraction with methanol followed by column chromatography. The yield of (*R*)-EHPB was 4.4 g (58%), and the chemical purity and e.e. of (*R*)-EHPB were 99.1% and 90%, respectively. Thus, (*R*)-EHPB could be preparatively synthesized *via* microbial reduction of EOPB with *C. holmii* in the pad-packed interface bioreactor. It is assumed that the pad-packed interface bioreactor can be used repeatedly because cells attached on the carrier surface live after the reduction.

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