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Isolation of an *Acinetobacter junii* SY-01 Strain Producing an Extracellular Lipase Enantioselectively Hydrolyzing Itraconazole Precursor, and Some Properties of the Lipase

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Abstract Water-sludge bacteria were screened to find a lipase enantioselectively hydrolyzing itraconazole precursor, which is well known as the starting material of antifungal drug agents. A bacterial strain was isolated and identified as *Acinetobacter junii* SY-01. After the strain was cultivated, the enzyme was purified 39.4-fold using ultrafiltration and gel filtration through a Sephadex G-100 chromatographic column and the activity yield was 34.9%. The molecular weight of the enzyme was about 40 kDa, as measured by SDS-PAGE, and the optimum pH was 7.0–9.0 and stable at pH 6.0–9.0. The optimum temperature was 45–50°C, and 73% of the enzymes activity remained after incubation at 70°C for 1 h. Enzyme activity was enhanced by gall powder, sodium deoxycholate, a cationic detergent Tween 80, and a non-ionic detergent Triton X-100, but was markedly inhibited by metal ions such as Hg^{2+}, Cu^{2+}, Ni^{2+}, Ca^{2+}, and an anionic-surfactant sodium dodecylsulfate. The K_m values for (R)- and (S)-enantiomers of the itraconazole precursor were 0.385 and 21.83 mM, respectively, and the V_{max} values ($\mu M \cdot min^{-1}$) were 6.73 and 6.49, respectively. The acetyl group among the different acyl moieties of itraconazole precursor showed the highest enantioselectivity for the hydrolysis by the *Acinetobacter junii* SY-01 lipase, and the lipase from *Acinetobacter junii* SY-01 displayed better enantioselectivity than that of commercially available lipases and esterases.

Key words: Lipase, enantioselective, chiral, screening, activity

In many cases, only one of the enantiomers of a chiral compound shows biological activity, and the other can either show a nonbiological activity or an adverse effect [1, 8, 12, 15]. Therefore, interest in the preparation or isolation of chiral starting materials for the use in pharmaceuticals and agrochemicals has rapidly increased in recent years [3, 6, 19]. The use of enzymes to achieve enantioselective synthetic procedures is becoming increasingly important in the production of optically active compounds [13, 20, 22, 25, 28]. In particular, lipases with their high enantioselectivity, which are able to function without cofactors and broad substrates specificity, have been widely used in the production of optically pure compounds [2, 5, 14, 17, 24, 26, 29].

Itraconazole, a racemic mixture having the formula $C_{35}H_{38}C_{12}N_8O_4$, is a synthetic antifungal agent commercially available as SPORANOX®. It has now been discovered that the optically pure isomer of itraconazole is an effective agent for treating local and systemic fungal, yeast, and dermatophyte infections. It is devoid of adverse effects associated with the administration of racemic mixtures, including but not limited to hepatotoxicity, arrhythrogenicity, dizziness, elevations in serum liver enzymes, hypersensitivity reactions, urticaria, headache, nausea, vomiting, and abdominal pain [10, 23]. An optically pure compound of itraconazole precursor is difficult to chemically synthesize. Alternately, a lipase-catalyzed kinetic resolution of the racemic itraconazole precursor can be used for the production of desired optically pure itraconazole [16]. However, most of commercially available lipases do not satisfactorily resolve itraconazole precursor, and good enantiomeric purities can not be obtained. Therefore, isolation of the strain producing a lipase that is capable of improving enantioselectivity in the resolution of itraconazole precursor is needed to develop an enzymatic process of optically pure itraconazole.

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This report deals with the isolation of a lipase-producing bacteria that enantioselectively hydrolyzes an itraconazole precursor, and investigates some properties of the enzyme.

MATERIALS AND METHODS

Chemicals

The racemic itraconazole precursor, *cis*-2-bromomethyl-2-(2,4-dichlorophenyl)-1,3-dioxolane-4-methanol acetate (Fig. 1), was synthesized chemically. The TRIZMA Base for Tris-HCl buffer was purchased from Sigma Chemical Company (St. Louis, MO, U.S.A.), *n*-Hexane and isopropyl alcohol were from J. T. Baker Incorporation (Phillipsburg, NJ, U.S.A.), and yeast extract, tryptone, peptone, beef extract were from Difco Laboratories (Detroit, MI, U.S.A.). Other chemicals were obtained from commercial sources.

Isolation and Identification of Enantioselective Lipase-Producing Strain

Water sludge samples taken from industrial complexes in Korea were suspended in sterilized saline water and spread on Tween 80 plates [9] (5 g/l peptone, 3 g/l beef extract, 0.01 g/l $CaCl_2 \cdot 2H_2O$, 20 g/l agar, 10 g/l Tween 80, pH 7.0). The strains which formed opaque halos around the colonies were isolated after incubation at 30°C for 48–96 h. Each of the strains was inoculated on a Rhodamine B plate [11, 30, 31, 32] (5 g/l peptone, 3 g/l beef extract, 0.001 g/l rhodamine B, 20 g/l agar, 10 g/l olive oil, pH 7.0). The strains which formed an orange fluorescent halo in UV irradiation were isolated after incubation at 30°C for 48–96 h. Isolated strains were incubated by shaking cultures at 30°C for 72 h, using a test tube (20 ml) containing 5 ml of the lipase production medium (5 g/l tryptone, 1 g/l dextrose, 5 g/l yeast extract, 1 g/l K_2HPO_4, pH 7.0). The culture broth was tested for lipase activity. Each of the isolated lipase-producing strains was cultivated following the above method and the cells from cultures were removed by centrifugation (12,000 rpm, 10 min). The racemic substrate shown in Fig. 1 was dissolved in 10 g/l of *n*-hexane. One milliliter of the supernatant and 100 µl of the racemic substrate solution were added to 5 ml of 50 mM Tris-HCl buffer (pH 8.0). After the reactions were allowed to proceed for 6 h at 40°C and 250 rpm, the samples were extracted with 1 ml of *n*-hexane, and the organic layer was analyzed for enantioselectivity. The isolated strain was identified by means of an API kit (20NE) Method (bioMieux Inc., France), the MIDI process (Microbial ID, Inc., Newark, Del., U.S.A.), and by way of 16S rDNA sequence determination.

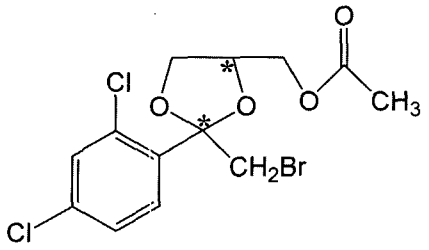

Fig. 1. Molecular structure of (±) *cis*-2-bromomethyl-2-(2,4-dichlorophenyl)-1,3-dioxolane-4-methanol acetate.

Culture Conditions

A 5-l jar fermentor (Korea Fermentor Co. Ltd.) was used for the cultivation of enantioselective lipase-producing strains, with a working volume of 3-l. It was incubated in an appropriate medium (5 g/l tryptone, 1 g/l dextrose, 5 g/l yeast extract, 1 g/l K_2HPO_4, pH 7.0) for enantioselective lipase production with an inoculum size of 3% at 30°C for 24 h. The agitation and aeration rate were 600 rpm and 1.0 vvm, respectively. After cultivation, the culture broth was centrifuged for 10 min at 12,000 rpm, and the supernatant was used for purification of the enzyme.

Purification Procedures

The clear supernatant was concentrated 10-fold using an ultrafiltration unit (Amicon 8200 model, U.S.A.) with a membrane having a nominal molecular mass cutoff of 10 kDa. The concentrate was centrifuged for 10 min at 12,000 rpm to remove any insoluble materials, and the clear concentrate was loaded onto Sephadex G-100 column (150 mm×30 mm) equilibrated with 10 mM Tris-HCl buffer (pH 8.0). Elution was performed with the same buffer at a rate of 20 ml/h.

Assay of Enzyme Activity

A lipase assay was performed with an olive oil emulsion by the procedure of Sigma Bulletin No. 800 based on Tietz and Fiereck [33]. The reaction mixture comprised 3 ml of olive oil emulsion, 1 ml of 200 mM Tris-HCl buffer (pH 8.0), 2.5 ml of distilled water, and 1 ml of enzyme solution, and was incubated at 40°C for 6 h. Immediately after incubation, the emulsion was destroyed by the addition of 3 ml of ethanol, and the liberated free fatty acid was titrated with 0.05 M sodium hydroxide. One unit of lipase was defined as the amount of enzyme which liberated 1 µmol of fatty acid per minute.

Protein Assay and Electrophoresis

Protein concentration was measured by the Micro protein determination method (Sigma Procedure No. 690) which is the modified Lowry's method [21]. Bovine serum albumin was used as a standard. The protein content in the fractions collected during each chromatographic procedure was determined by measuring absorbance at 280 nm. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-

Table 1. Isolation of the enantioselective lipase-producing strains.

Number of isolated strains	Halo size[1] in Tween 80 medium	Halo size in rhodamine B medium	Lipase activity (U/ml)	c (%)[2]	ee_p (%)[3]	E[4]
YLL[a]	+	++	4.59	42.3	31.2	2.4
PCL[b]			2.56	67.1	1.1	1.0
CRL[c]			4.63	61.6	5.2	0.5
9	+	+	0.31	22.7	63.1	5.4
11	+	++	2.41	26.4	43.0	2.9
12	++	++	4.56	25.8	41.1	2.7
13	+	-	0.36	13.0	40.5	2.5
26	+	+	0.11	25.4	66.8	6.3
36	+	+	0.48	80.9	16.4	2.5
46	+	+	0.76	43.4	31.3	2.4
47	+	+	0.25	27.8	48.6	3.5
54	+	+	0.48	28.2	55.6	4.3
59	+	+	0.50	55.3	22.2	2.0

-: no detectable; +: low; ++: medium; +++: large.
[1,3,4]See Materials and Methods.
[a]YLL: lipase from *Yarrowia lipolytica* (KCCM 50038).
[b]PCL: lipase from *Pseudomonas cepacia* (Sigma Cat. # L-9156).
CRL: lipase from *Candida rugosa* (Sigma Cat. # L-1754).

PAGE) was carried out as described by Laemmli [18], and the gel was stained with Coomassie brilliant blue R-250.

Chiral Assay

The conversion of the reactant and estimation of the enantiomeric purity were determined by means of the HPLC system (Younglin Instrument Co., Korea) under the following conditions: column, Chiralcel OD (Chiral Technologies, Exton, PA, U.S.A.); column size, 4.6 mmx250 mm; mobile phase, *n*-hexane containing 5% isopropyl alcohol; flow rate, 1 ml/min; detection, UV 230 nm. Conversion(c), enantiomeric excess(ee) and enantioselectivity(E) are defined as follows [4]:

$$\text{Conversion, } c(\%)=\frac{[(R,S)-\text{alcohol}]}{[(R,S)-\text{ester}]+[(R,S)-\text{alcohol}]}$$

$$\times 100=\frac{ee_s}{ee_s+ee_p}100$$

$$\text{Enantiomeric excess for substrate, } ee_s(\%)$$

$$=\frac{([(S)-\text{ester}]-[(R)-\text{ester}])}{([(S)-\text{ester}]+[(R)-\text{ester}])}\times 100$$

Table 2. Biochemical characterizations of isolated strain according to API kit (20NE).

Characterizations	Isolated strain No. 26	*Acinetobacter junii*
Reduction of nitrates to nitrites	-	-
Indole production	-	-
Glucose acidification	-	-
Arginine dihydrolase	-	-
Urease	-	-
Esculin hydrolysis (β-glucosidase)	-	-
Gelatin hydrolysis (protease)	-	-
β-Galactosidase	-	-
Glucose assimilation	-	-
Arabinose assimilation	-	-
Mannose assimilation	-	-
Mannitol assimilation	-	-
N-Acetyl-glucosamine assimilation	-	-
Maltose assimilation	-	-
Gluconate assimilation	-	-
Caprate assimilation	+	+
Adipate assimilation	-	-
Malate assimilation	+	+
Citrate assimilation	-	+
Phenyl-acetate assimilation	-	-
Cytochrome oxidase	-	-

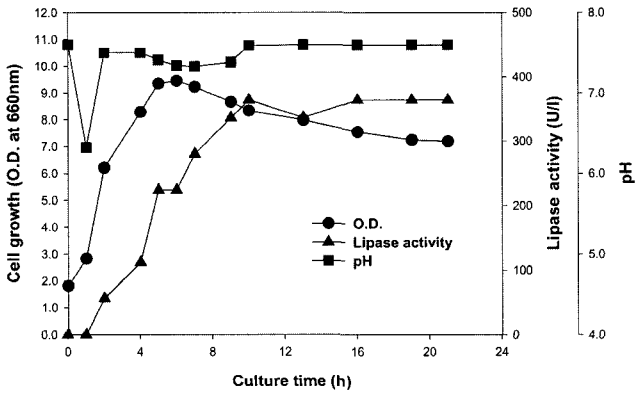

Fig. 2. The profile of the enantioselective lipase production during cultivation of *Acinetobacter junii* SY-01 in the jar fermentor.

Table 3. Comparison of the fatty acid profiles of isolated strain and *Acinetobacter calcoaceticus*.

(Unit: %)

Fatty acid	Isolated strain No. 26	*Acinetobacter calcoaceticus*
n-Decanoic acid (10:0)	2.13	ND
n-Dodecanoic acid (12:0)	1.48	4.70
Unknown 12.484	0.33	0.33
2-Hydroxy dodecanoic acid (12:0 2OH)	3.80	1.57
3-Hydroxy dodecanoic acid (12:0 3OH)	4.94	3.48
n-Tetradecanoic acid (14:0)	0.66	0.68
15:0	ND	0.39
16:1 w7c alcohol	ND	1.43
n-Hexadecenoic acid (16:1 ω9c)	0.98	ND
Sum In Feature 3 2-hydroxy-15-methyl-hexadecenoic acid (16:1 ω7c/15 iso 2OH)	22.04	26.32
n-Hexadecanoic acid (16:0)	18.32	16.23
15-Methylhexadecanoic acid (17:0 iso)	0.47	1.13
n-Heptadecenoic acid (17:1 ω8c)	0.53	0.53
n-Heptadecanoic acid (17:0)	0.49	0.49
n-Octadecatrienoic acid (18:3 ω6c (6,9,12))	2.02	0.68
n-Octadecenoic acid (18:1 ω9c)	40.11	29.89
n-Octadecenoic acid (18:1 ω7c)	0.74	2.80
n-Octadecanoic acid (18:0)	0.94	3.08

ND: No detectable.

Enantiomeric excess for substrate, $ee_p(\%)$

$$= \frac{([(S)-\text{alcohol}]-[(R)-\text{alcohol}])}{([(S)-\text{alcohol}]+[(R)-\text{alcohol}])} \times 100$$

Enantioselectivity, E

$$= \frac{\ln[(1-c)(1-ee_s)]}{\ln[(1-c)(1+ee_s)]} = \frac{\ln[(1-c)(1-ee_p)]}{\ln[(1-c)(1+ee_p)]}$$

Hydrolysis of Itraconazole Precursor

A reaction mixture containing 1 ml of itraconazole precursor (10 g/l solution in *n*-hexane), 99 ml of 100 mM TRIZMA buffer solution (pH 8.0), and enzyme solution was incubated at 40°C with shaking at 150 rpm. After the reaction, the samples were extracted with 1 ml of *n*-hexane and the organic layer was analyzed to determine the conversion rate and the enantiomeric purity.

Results and Discussion

Isolation and Identification of Enantioselective Lipase-Producing Strain

Two-hundred isolates from 100 soil or water sludge samples were screened for lipolytic activity, and 60 strains were examined for enantiomeric purity at the hydrolysis of itraconazole precursor. Table 1 shows the results for the most selective strains, although only strain no. 26 was highly selective. PCL (Lipase from *Pseudomonas cepacia*, Sigma Cat. # L-9156), CRL (Lipase from *Candida rugosa*, Sigma Cat. # L-1754), and YLL (Lipase from *Yarrowia lipolytica*, KCCM 50038) were used as controls. Biochemical characteristics of the isolate no. 26 according to an API kit (20NE) are shown in Table 2. The isolate was identified as *Acinetobacter junii*. Fatty acid analysis by the MIDI System (Microbial ID, Inc.) gave the profiles shown in Table 3. When these profiles were compared with the AEROBE Bacterial Library (Microbial ID, Inc.), a match with the *Acinetobacter calcoaceticus* GC subgroup was determined at 0.343. The 16S rDNA sequence of the isolate had a 98.9% homology with that of *Acinetobacter junii* ATCC 17908T strain (results not shown). According to the above results, it was concluded that the isolate was most likely *Acinetobacter junii*, therefore, named as *Acinetobacter junii* SY-01. This strain was deposited in the Korean Culture Center of Microorganisms (KCCM) with the collection number KFCC-11111.

Table 4. Summary of purification procedure.

Purification steps	Total protein (mg)	Total activity (U)	Specific activiy (U/mg)	Yield (%)	Purification fold
Curture supernatant	1,575	98.0	0.062	100	1
Ultrafiltration (MW: 10,000)	283	53.8	0.190	54.9	3.1
Gel filtration (Sephadex G-100)	14	34.2	2.443	34.7	39.4

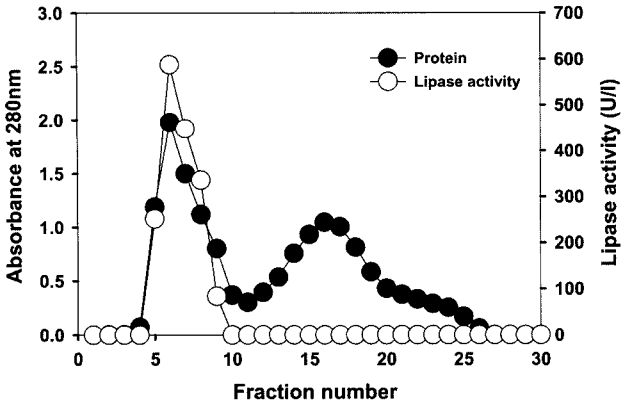

Fig. 3. Gel filtration chromatogram of lipase on Sephadex G-100 column.
Elution was carried out with 10 mM Tris-HCl buffer (pH 8.0). Flow rate: 20 ml/h, Fraction: 10 ml/tube.

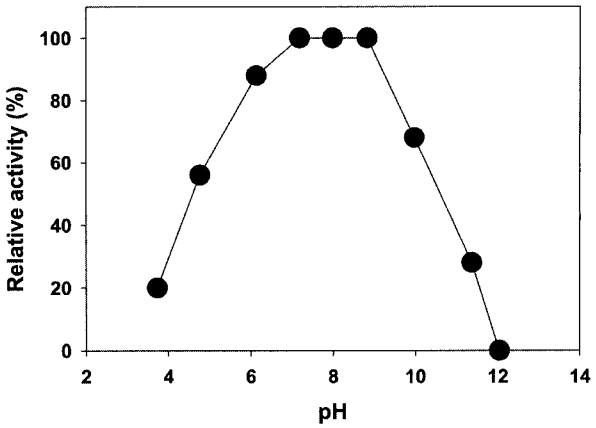

Fig. 5. Effect of pH on the lipase activity.
The lipase activity was measured by standard assay method at each pH. Britton-Robinson buffer was used. The enzyme unit at 100% relative activity was 2.8 unit.

Enzyme Purification

The enantioselective lipase-producing strain, *Acinetobacter junii* SY-01, was cultivated at an initial pH of 7.0 and at 30°C for 22 h in a medium containing 0.5% olive oil, 5 g/l tryptone, 5 g/l yeast extract, and 1 g/l K_2HPO_4. As shown in Fig. 2, cell growth reached its maximum after 6 h, and the maximum enzyme activity occurred after 10 h. Secretion of extracellular lipase occurred during the logarithmic growth phase and was greatest when cultures reached the stationary growth phase. The purification processes and yields are summarized in Table 4. The enzyme was purified 39.4-fold with 34.9% recovery, and its specific activity was 2.44 U/mg protein. Figure 3 represents a chromatogram of gel filtration on Sephadex G-100. The purified enzyme appeared homogeneous on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

Molecular Weight

The enzyme showed a single protein band with a molecular mass of about 40 kDa on SDS-PAGE (Fig. 4A), comparing the relative mobility of the size marker and the purified enzyme as shown in Fig. 4B.

Effects of pH on Enzyme Activity and Stability

The effect of pH on the enzyme activity was examined, and the result is shown in Fig. 5. The lipase was most active at pH 7.0–9.0. In order to determine the effect of pH on the stability of the enzyme, the lipase solution was incubated in Britton-Robinson buffer of various pHs at 30°C for 24 h. After adjusting the pH of the solution to 8.0, the remaining activity was determined. As shown in Fig. 6, the lipase was stable at pH between 6.0 and 9.0.

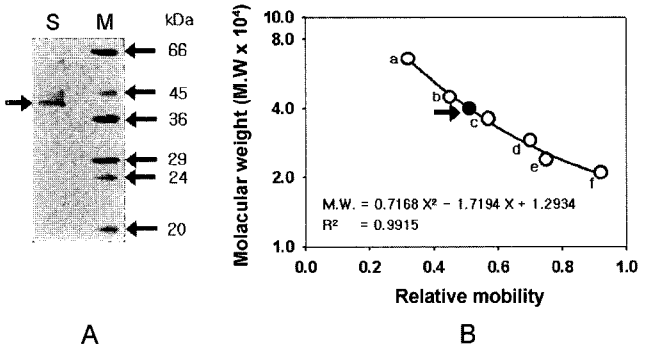

Fig. 4. SDS-PAGE pattern of the purified enantioselective lipase.
Determination of molecular weight of enzyme subunits by SDS-polyacrylamide gel electrophoresis. The standard markers are as follows: Bovine serum albumin (a: 66,000), Egg albumin (b: 45,000), Glyceraldehyde-3-phosphate dehydrogenase (c: 36,000), Carbonic anhydrase (d: 29,000), Trypsinogen (e: 24,000), Trypsine inhibitor (f: 20,000).

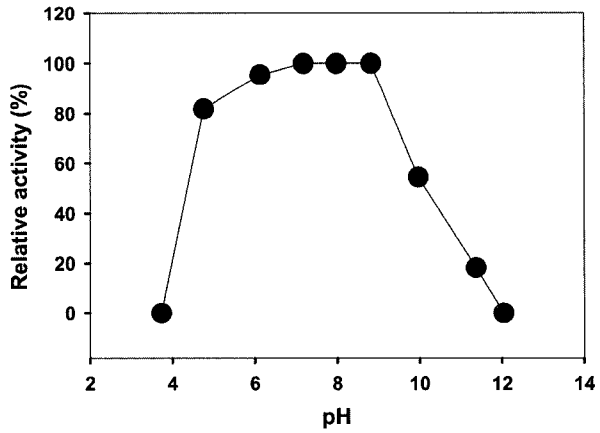

Fig. 6. Effect of pH on the lipase stability. The enzyme solution was treated at each pH for 24 h at 30°C.
The residual activity of lipase was measured by standard assay method. The enzyme unit at 100% relative activity was 2.8 unit.

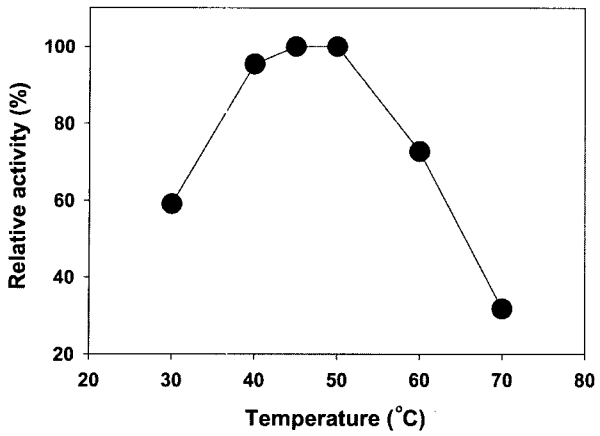

Fig. 7. Effect of temperature on the lipase activity.
The lipase activity was measured by standard assay method at each temperature. The enzyme unit at 100% relative activity was 2.8 unit.

Effects of Temperature on Enzyme Activity and Stability

The optimum temperature for the lipase activity was examined. The enzyme was most active at 45-50°C, as shown in Fig. 7. To determine the heat stability of the enzyme, the enzyme solution in 20 mM Tris-HCl buffer at pH 8.0 was incubated at various temperatures for 1 h. Subsequently, the enzyme assay was performed as described in Materials and Methods, as shown in Fig. 8. The remaining activity after treatment of the enzyme at 70°C for 1 h was 73%, and the enzyme was completely stable at 60°C for 1 h: The enzyme activity was relatively stable up to 70°C.

Effects of Metal Ions and Reagents

To determine the effect of metal ions, various metal ions were added to the enzyme solution (Table 5). The enzyme activity was assayed after preincubation of the enzyme solution containing each ion at room temperature for 30 min. Hg^{2+}, Cu^{2+}, Ni^{2+}, and Ca^{2+} inhibited the enzyme activity, but Na^{+}, Mg^{2+}, Co^{2+}, and Fe^{3+} had no effect on the activity. Table 6 shows the effects of various reagents on the enzyme activity. The enzyme was markedly stimulated by gall powder, sodium deoxycholate, a cationic detergent like Tween 80, and a non-ionic detergent like Triton X-100, but inhibited by an anionic detergent like sodium dodecyl sulfate.

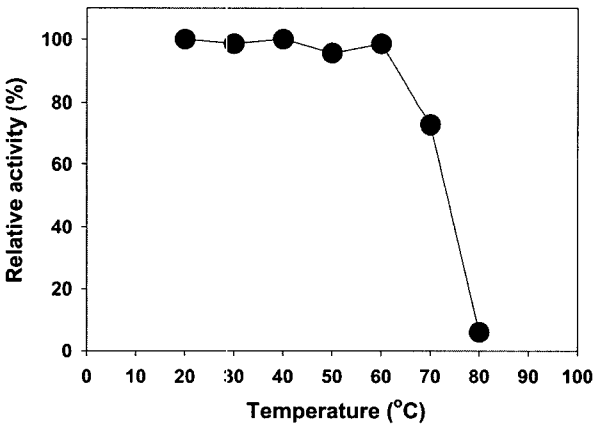

Fig. 8. Effect of temperature on the lipase stability.
Enzyme solution was treated at each temperature for 1 h. After heat treatment, the residual activity of lipase was measured by standard assay method. The enzyme unit at 100% relative activity was 2.8 unit.

Table 5. Effects of metal ions on the lipase activity.

Metal ions (5 mM)	Relative activity (%)
None	100.0
NaCl	100.0
$MgCl_2$	110.0
$HgCl_2$	25.0
$MnCl_2$	75.0
$CuCl_2$	35.0
$CoCl_2$	115.0
$BaCl_2$	75.0
$NiCl_2$	50.0
KCl	95.0
$CaCl_2$	55.0
$FeCl_3$	105.0

The enzyme unit at 100% relative activity was 2.8 unit.

Kinetic Properties

Kinetic constants of the enantioselective lipase on the (R)- and (S)-enantiomer of the itraconazole precursor were examined. The influence of each enantiomer of the racemic substrate on the initial hydrolysis rate was investigated at various substrate concentrations. A Lineweaver-Burk plot was performed for the kinetics (Fig. 9). The K_m (mM) for the (R)- and (S)-enantiomers of the itraconazole precursor were 0.385 and 21.83, and the V_{max} (mM·min^{-1}) were 6.73 and 6.49, respectively. The K_m value of the (R)-enantiomer increased approximately 57 times compared to the (S)-enantiomer, whereas the V_{max} value remained unchanged. The above results indicate that the enantioselectivity was

Table 6. Effects of various reagents on the lipase activity.

Reagents	Concentration	Relative activity (%)
None	-	100.0
EDTA	1 mM	110.0
Phenylmethylsulfonyl fluoride	1 mM	74.4
2-Mercaptoethanol	1 mM	100.0
Sodium deoxycholate	0.2%	162.8
Gall powder	0.2%	139.5
Tween 80	0.1%	144.2
Triton X-100	0.1%	120.9
Sodium dodecyl sulfate	0.1%	4.7

The enzyme unit at 100% relative activity was 2.8 unit.

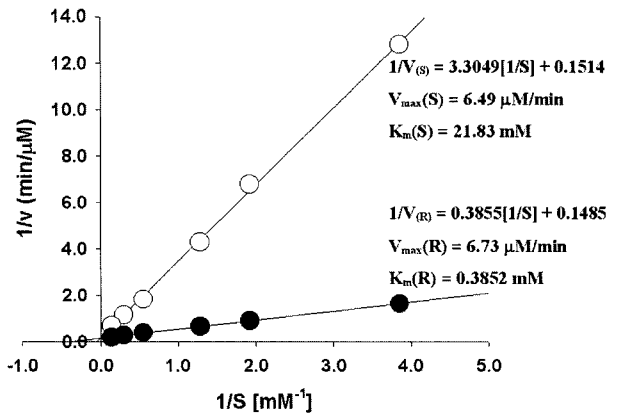

Fig. 9. Lineweaver-Burke plot showing the chiral activity at the hydrolysis of itraconazole precursor using the lipase from *Acinetobacter junii* SY-01.
(○: (S)-enantiomer, ●: (R)-enantiomer).

mainly due to substrate binding ability rather than catalytic ability for each of the (R)- and (S)-enantiomers.

Effects of Alkyl Chain Length on Enantioselectivity

Effects of alkyl chain length on enantioselectivity for the hydrolysis of itraconazole precursor using the lipase from *Acinetobacter junii* SY-01 was examined, and the results are shown in Table 7. The alkyl chain length markedly affected enantioselectivity, but did not affect hydrolytic activity. Acetyl group showed the highest enantioselectivity and the others displayed poor selectivity.

Table 7. Effect of alkyl chain length on enantioselectivity at the hydrolysis of itraconazole precursor using the lipase from *Acinetobacter junii* SY-01.

Alkyl group of substrate[1]	Time (h)	c (%)[2]	ee_s (%)[3]	E[4]
R=methanol acetate	3.5	74.8	95.6	6.4
R=methanol propanoyl	3.5	71.0	11.7	1.2
R=methanol butanoyl	3.5	88.0	4.9	1.0
R=methanol pentanoyl	3.0	68.2	14.7	1.3
R=methanol hexanoyl	3.0	85.4	0.9	1.0
R=methanol heptanoyl	3.0	59.2	4.1	1.1

[1]Substrate: *cis*-2-bromomethyl-2-(2,4-dichlorophenyl)-1,3-dioxolane-4-R.
[2,3] See Materials and Methods.
Enzyme addition: 4.0 unit.
Initial substrate concentration: 100 mg/l.

Comparison with Commercial Enzymes

Several commercially available enzymes were examined for their enantioselectivity for the hydrolysis of itraconazole precursor, and compared with the lipase from *Acinetobacter junii* SY-01 (Table 8). The result showed that the enantioselectivity of the lipase from *Acinetobacter junii* SY-01 was better than that of commercially available lipases and esterases. In particular, the lipase from *Acinetobacter junii* SY-01 showed better enantioselectivity than CAL-B (Lipase from *Candida antarctica*, Fraction B), which is the most successfully used lipase in the chiral resolution process [7, 27]. We are in a process to mass produce the enantioselective lipase and its application to other racemates.

Table 8. Comparison of enantioselectivity at the hydrolysis of itraconazole precursor using the lipase from *Acinetobacter junii* SY-01 and other commercial enzymes.

Enzymes	c (%)[1]	ee_s (%)[2]	ee_p(%)[3]	E[4]	Major enantiomer of products
Lipase from *Acinetobacter junii* SY-01	74.8	95.6	32.2	6.40	(R)
CAL-B[a]	64.0	72.1	40.5	4.80	(R)
CRL[b]	78.4	14.3	3.9	1.21	(S)
CRL, purified[c]	30.3	17.3	39.9	2.75	(S)
CAL-A[d]	43.0	15.2	20.2	1.73	(S)
PSL[e]	27.4	1.7	4.5	1.11	(S)
PPL[f]	18.9	13.9	59.6	4.51	(R)
TLL[g]	15.4	2.9	16.2	1.43	(R)
MML[h]	38.4	28.9	46.5	3.59	(R)
ASL[i]	44.8	15.5	19.1	1.69	(S)
PLE[j]	34.8	16.3	30.6	2.19	(R)

[1,2,3,4]See Materials and Methods.
[a]CAL-B: lipase from *Candida antarctica*, Fraction B.
[b]CRL: lipase from *Candida rugosa.*
[c]CRL, purified: purified lipase from *Candida rugosa.*
[d]CAL-A: lipase from *Candida antarctica*, Fraction A.
[e]PSL: lipase from *Pseudomonas* sp.
[f]PPL: lipase from porcine pancreas.
[g]TTL: lipase from *Thermomyces lanuginose.*
[h]MML: lipase from *Mucor miehei.*
[i]ASL: lipase from *Alcaligenes* sp.
[j]PLE : esterase from porcine liver. The above enzymes were obtained from Lipase & Esterase Screening Set 2 (Roche Diagnostics GmBH, Germany).

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