Biosci. Biotechnol. Biochem., **63** (11), 1959–1964, 1999

JSBA

Purification and Characterization of a Novel Extracellular Lipase Catalyzing Hydrolysis of Oleyl Benzoate from *Acinetobacter* nov. sp. Strain KM109

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Received June 14, 1999; Accepted July 23, 1999

A new lipase (OBase) which efficiently hydrolyzes oleyl benzoate (OB) was found in the culture supernatant of *Acinetobacter* nov. sp. strain KM109, a new isolate growing in a minimum medium containing OB as the sole carbon source. OBase was purified to homogeneity with 213-fold purification and 0.8% yield. The molecular weight was estimated to be 62,000±1,000 by SDS-PAGE under denatured-reduced conditions and to be 50,000±1,000 by gel-filtration HPLC under native conditions; these findings indicate that OBase is a monomeric enzyme. The optimum temperature and pH of OBase were about 45°C and pH 8. Temperature and pH stabilities were at or lower than 35°C and in a range of pH 6–8, respectively. Purified OBase preferentially hydrolyzed *p*-nitrophenyl benzoate (pNPB) over *p*-nitrophenyl acetate (pNPA) or *p*-nitrophenyl caproate (pNPC) [pNPB/pNPA=20 and pNPB/pNPC=5.4], indicating that OBase has a high affinity for benzoyl esters. Partial amino-acid sequences of OBase fragments obtained after lysyl endopeptidase treatment showed no similarity with known proteins.

Key words: lipase; *Acinetobacter*; oleyl benzoate

Lipases [EC 3.1.1.3] are widely distributed in various organisms, and catalyze hydrolysis of triacylglycerol to mono- and diacylglycerol and free fatty acids. In addition to hydrolyzing esters in the presence of water, lipases also catalyze transesterification,[1,2] esterification,[3] and aminolysis or oximolysis[4] under nearly anhydrous conditions. Because these nearly anhydrous reactions in non-polar solvents are very useful for organic synthesis, it has become evident that lipases can be used in many aspects of industrial processes.[5,6]

With increasing demand for variety in the biochemical characteristics of lipase, lipases or esterases with specified properties have been reported. Examples would include a lipase with an optimum alkaline pH[7–10] and a lipase that is active at high temperatures.[9,11,12] However, the major disadvantage of commercially available lipases for use in organic synthesis lies in the narrow substrate specificity, namely, such lipases cannot use substrates containing bulky substituents near an ester carbonyl group. To overcome this drawback, several groups have recently identified a lipase or an esterase that can recognize somewhat bulky substrates.[13–16] However, there are only a few reports on lipases that can hydrolyze benzoate esters.[17,18]

In the course of our screening to find general-purpose lipases that can hydrolyze sterically hindered substrates such as esters of *tert*-butyl alcohol,[19] we chose OB as a model ester containing an aromatic ring next to the ester carbonyl group. In the present study, we report the isolation and taxonomic identification of KM109, a strain that produces an extracellular lipase (OBase) capable of efficiently hydrolyzing OB. Furthermore, OBase was purified to homogeneity and characterized biochemically for future application in organic synthesis.

Materials and Methods

Chemicals. Colorine 102, an antifoam emulsifier, was obtained from Sanyo Chemical Industries, Ltd. (Kyoto, Japan). pNPA and pNPC were obtained from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan). OB, pNPB, and tribenzoylglycerol (TBG) were synthesized by acylation of oleyl alcohol, *p*-nitrophenol, and glycerol, respectively, with benzoyl chloride.

Isolation of OB-using bacteria. Each soil sample, collected from several places in Japan, was suspended in 5 ml of minimum medium [$(NH_4)_2SO_4$ 0.5%, KH_2PO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.05%, NaCl 0.5%, pH 7.2] containing 1.0% OB as the sole carbon source, and the suspension was incubated at 30°C for 2–3 days on a reciprocating shaker at a shaking speed of 120 strokes per min. For cultures that gave a sign of growth, a sample (100 μl) was inoculated into fresh minimum medium (5 ml) containing 1.0% OB and incubated as above to enrich the OB-assimilating bacteria. After three rounds of the enrichment process, the culture was spread on a nutrient broth agar plate (meat extract 1%, polypeptone 1%, NaCl 0.2%, and agar 1.5%) to isolate pure colonies. The assimilation of OB by the isolated colonies

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Abbreviations: OB, oleyl benzoate; OBase, oleyl benzoate hydrolyzing lipase; pNPA, *p*-nitrophenyl acetate; pNPB, *p*-nitrophenyl benzoate; pNPC, *p*-nitrophenyl caproate; TBG, tribenzoyl glycerol; TLC, thin layer chromatography

was confirmed by growth on solid minimum medium containing 1% OB.

To evaluate roughly the OB-hydrolyzing activity of each strain, we used two methods. The first screening was done on solid culture using minimum medium containing 0.1% OB emulsified by 0.2% β-cyclodextrin. After a 7-day incubation at 30°C, OB-hydrolyzing activity was assessed by the diameter of the clearzone around the colonies. The second screening was done by TLC detection of the hydrolyzed products, oleyl alcohol and benzoic acid. Strains that showed an evident clearzone during the first screening were inoculated into 50 ml of LB containing 0.5% OB in 500-ml Sakaguchi flasks, and cultivated for 48 h at 30°C and at a shaking speed of 120 strokes per min. Cells were collected by centrifugation (5,000 × *g*, 20 min, 4°C), suspended in 50 mM potassium phosphate buffer (pH 6.5) containing 1 mM $MgCl_2$, and disrupted by sonication (30 sec, twice, 4°C). OB (0.1 ml) was added to the supernatant obtained by centrifugation; the mixture was then incubated for 15 h at 30°C. The reaction was stopped by the addition of ether, and the ether fraction was analyzed by silica gel TLC using hexane:ethyl acetate (9:1) as the solvent with authentic oleyl alcohol and benzoic acid as standards.

Triglyceride hydrolysis. Positional specificity on the hydrolysis of triglyceride was measured by the method of Uzawa *et al.*,[20,21] using tripalmitin as the substrate. Briefly, dipalmitoyl glycerols produced after the enzyme reaction were recovered by ether extraction, and a free -OH group of the product was silylated with *tert*-butyl dimethylsilyl chloride. The remaining palmitoyl groups in the product were removed by the Grignard reaction, and benzoylated with benzoyl chloride. The dibenzoyl glycerol derivatives were isolated by preparative thin layer chromatography, and analyzed by chiral HPLC on a CHIRALCEL OF column (ϕ4.6 × 250 mm, Daicel Chemical Industries Ltd., Osaka, Japan) using hexane: iso-propyl alcohol (150:1) as a solvent at a flow rate of 0.7 ml/min with detection at 254 nm. Hydrolysis products of TBG were analyzed in a manner similar to that described for analyzing the products of tripalmitin hydrolysis, except that the Grignard reaction and benzoylation were omitted.

Purification of OBase. All the purification procedures were done at 4°C, and lipase activity was monitored with pNPB as a substrate, unless otherwise specified.

Step 1). Preparation of crude powder:

Acinetobacter sp. strain KM109 was cultivated for 40 h in a 100-L jar fermentor with 40 L of lipase production medium at 30°C (agitation rate of 200 rpm, and aeration rate of 40 L/min). The culture broth was cooled by ice, mixed with 400 g of salt mixture (calcium chloride:disodiumhydrogenphosphate = 1:2) to precipitate Colorine 102. Cells as well as precipitated antifoams were removed by continuous centrifugation (S-type high-speed centrifuge, Kansai Ensinbunri Seisakusyo, Osaka, Japan). The supernatant was concentrated to 3.5 L by an ultra filtration module (Asahi Chemical Industry Co., Ltd., Osaka, Japan). Crude OBase in the concentrated supernatant was recovered by 30–60% cold *iso*-propanol precipitation, dried by N_2 flushing and lyophilization, and stored at 4°C until use.

Step 2). Sepharose CL-6B gel-filtration chromatography: The crude powder from step 1 was suspended in 100 mM potassium phosphate buffer (pH 6.5) containing 0.1% Noigen HC (buffer B) in a ratio of 10 mg-powder/ml-buffer B. The suspension was left overnight at 4°C with mild stirring, and insoluble materials were removed by centrifugation (8,000 × *g*, 10 min, 4°C). The supernatant was put on a Sepharose CL-6B gel-filtration column (ϕ46 × 500 mm, Amersham Pharmacia Biotech) equilibrated with buffer B, and eluted with 1800 ml of the same buffer. The solution was collected in 18-ml increments. Fractions containing OBase activity (fractions 36–45, 180 ml) were pooled and concentrated to 20 ml by ultrafiltration (UP-20, M_r cut off 20,000, Toyo Roshi Kaisha Ltd., Japan). The concentrated sample was dialyzed against a 100-fold volume of 10 mM Tris-HCl buffer (pH 8.0) containing 0.1% Noigen HC (buffer C).

Step 3). DEAE-Sephacel ion-exchange column chromatography: The sample from step 2 was put on a DEAE-Sephacel column (ϕ25 × 90 mm, Amersham Pharmacia Biotech) equilibrated with buffer C, and eluted with a linear gradient of NaCl concentrations from 0 to 0.5 M. Fractions containing the OBase activity (fractions 27–41, 75 ml) were pooled and concentrated to 2.5 ml by ultrafiltration.

Step 4). Gel-filtration HPLC: The sample from step 3 was separated in portions of 80 μl each on a TSK gel G2000SW$_{XL}$ column (ϕ0.78 × 30 cm, Tosoh Corporation, Tokyo, Japan) equilibrated with buffer B containing 0.2 M NaCl at a flow rate of 0.5 ml/min. Protein was detected by UV at 280 nm, and fractions were collected for each 30 sec. Activity appeared at 17–22 min, and fractions from 17.5 min to 21 min were pooled, concentrated, and dialyzed against 100-fold volume of buffer B.

Step 5). MonoQ ion-exchange chromatography: The sample from step 4 was separated by a Mono Q PC1.6/5 column (ϕ1.6 × 50 mm) on a SMART system (Amersham Pharmacia Biotech). OBase was eluted with a linear gradient of NaCl concentrations from 0 to 0.1 M.

SDS-PAGE. SDS-PAGE was done with a ready-made 10–20% linear gradient gel (Daiichi Pure Chemicals Co. Ltd., Osaka, Japan) with a minigel apparatus (Daiichi Pure Chemicals Co. Ltd.). Proteins in the gel were detected by a Silver Stain Kit II Wako (Wako Pure Chemical Industries, Ltd., Osaka, Japan), according to the manufacturer's protocol.

Enzyme and protein assay. OBase activity was routinely assayed by monitoring the release of *p*-nitrophenol from *p*-nitrophenyl benzoate as described previously.[22] One unit of enzyme activity was defined as the amount of enzyme liberating 1 μmol of *p*-nitrophenol/min. Protein concentration was assayed by dye-binding (Protein Assay Kit, Bio-Rad) with bovine serum albumin as the standard.

Peptide sequence. Purified OBase (200 pmol) was hydrolyzed with lysyl endopeptidase (lipase/enzyme=50 mol/mol, Wako Pure Chemical Industries Ltd.) in 0.2 ml of 20 mM Tris-HCl, pH 9.0 for 16 h at 30°C. Fragment peptides were purified by a Superdex 75 column on a SMART system with 200 mM NaCl in 10 mM Tris-HCl (pH 8.0). Four fragments were isolated, lyophilized, and analyzed by an automated protein sequencer (Applied Biosystems 476A).

Results and Discussion

Isolation of OB-hydrolyzing bacteria

To isolate OB-hydrolyzing bacteria, over 700 soil samples were cultivated in liquid minimum medium containing 0.1% OB as described in Materials and Methods, and 182 bacteria were isolated after 3 rounds of enrichment procedure. For further selection, they were cultivated on solid minimum medium containing 0.1% OB emulsified with 0.2% β-cyclodextrin, and 52 strains were selected for their ability to form a clearzone around the colony arising from OB hydrolysis. To measure intracellular and extracellular OBase activity, strains were cultivated in 50 ml of LB medium containing 1.0% OB in 500-ml shaking flasks at 30°C for 48 days. Seventeen strains showed pNPB-hydrolyzing activity (Table 1). When Colorine 102 was added to the medium as an antifoam emulsifier, six strains (KM016, KM069, KM083, KM109, KM252, and KM264) showed increased extracellular activities toward pNPB or pNPA (Table 1). Considering that extracellular enzymes can be purified more easily than intracellular enzymes, 6 strains showing extracellular lipase activity were selected for further analysis, such as the pNPB/pNPA hydrolysis ratio, positional specificity on triglyceride hydrolysis, and ability to hydrolyze TBG (Table 2). Only strain KM109 hydrolyzed TBG, thus strain KM109 was finally selected. The lipase of strain KM109 specifically hydrolyzed TBG at the *sn*-3 position (data not shown).

Morphological, chemical, and nutritional characteristics of strain KM109 are summarized in Table 3, which indicates that strain KM109 belongs to the genus *Acinetobacter*, and was designated as *Acinetobacter* nov. sp. strain KM109.

Culture conditions for producing OBase

To maximize the OBase production, culture conditions of *Acinetobacter* nov. sp. strain KM109 were investigated (Table 4). Basal medium composition, such as LB or NB, did not much affect the amount of OBase, but no activity was detected in the MB medium. pNPA-hydrolyzing activity (*i.e.*, esterase activity) in LB and NB were 0.09 and 0.22 units/ml, respectively, suggesting that cultivation in NB resulted in the production of both OBase and esterase, while LB afforded mainly OBase. It is well known that addition of long-chain fatty acids is required for the effective induction of a lipase such as that from *Geotrichum candidum*.[23,24] Hence, several oils were added to the culture media to test the effects of such oils on OBase production by *Acinetobacter* nov. sp. strain KM109. Only the addition of OB was effective, with an optimum effect at 1.0% (Table 4). However, other oils (olive oil, palm oil, and soybean oil) did not affect the level of enzyme activity. Detergents have sometimes been reported to be important for the production of extracellular lipases.[25] To see the effects of a detergent on OBase production, Colorine 102,

Table 1. Screening of OB-hydrolyzing Bacteria

Strain	Clear zone Formation[a]	Lipase activity (units/ml)							
		LB+OB[b]				LB+OB+Colorine 102[b]			
		Intracellular[c]		Extracellular[c]		Intracellular[c]		Extracellular[c]	
		pNPB	pNPA	pNPB	pNPA	pNPB	pNPA	pNPB	pNPA
KM016	+	0.42	4.94	0.06	0.41	0.12	1.37	0.03	0.81
KM034	+	0.05	0.69	0.04	0.14	0.06	0.82	0.01	0.08
KM039	++	0.46	1.66	0.33	0.10	0.27	0.78	0.09	0.11
KM069	++	0.42	1.32	0.05	0.08	0.28	0.37	0.11	0.08
KM083	++	0.36	1.33	0.03	0.41	0.04	0.24	0.11	0.01
KM100	++	0.03	1.89	0.14	0.09	ND	0.24	0.03	0.01
KM109	++	0.88	1.69	0.06	0.15	0.46	0.80	0.26	0.07
KM237	+++	0.15	0.33	0.03	0.69	ND	0.34	0.02	0.09
KM238	+	0.13	5.17	0.02	0.58	ND	0.18	0.06	ND
KM252	+	0.64	7.97	0.01	0.93	0.34	2.54	0.06	1.69
KM253	+	0.30	6.39	ND	0.28	0.19	3.31	0.02	0.15
KM257	+	0.05	2.76	0.01	4.29	0.01	0.54	ND	0.06
KM260	+	0.22	2.15	0.02	0.17	0.11	0.75	0.11	0.07
KM264	+	0.05	3.45	ND	1.99	0.01	3.17	0.31	13.76
KM267	+	0.58	3.46	0.02	0.28	0.43	0.84	0.01	0.12
KM268	+	0.54	3.34	0.07	0.32	0.52	0.95	0.01	0.09
KM322	+	0.13	1.71	0.01	0.26	0.59	0.82	0.01	0.13

[a] Number of + corresponded to the incubation days necessary for clear-zone formation on the plate assay. +++: 2 days, ++: 4 days, +: 7 days.
[b] LB was used for the production of the lipase, and 1.0% OB and 0.2% Colorine 102 were added.
[c] Schemes for preparing extracellular and intracellular sample solutions were described in Materials and Methods. For measurement of activities, pNPA and pNPB were used as substrates.

Table 2. Characteristics of Lipases from Newly Isolated Bacteria

Strain	pNPB/pNPA hydrolysis ratio[a]	Positional specificity[b]	Hydrolysis of tribenzoyl glycerol
KM016	0.04	3>1>2	ND
KM069	1.38	3>1>2	ND
KM083	11.0	3>1>2	ND
KM109	3.71	3>1>2	+
KM252	0.04	3>1>2	ND
KM262	0.002	3>1>2	ND

[a] The values of pNPB and pNPA hydrolyzing activities in Table I (LB+OB+Colorin 102, extracellular) were used.

[b] Positional specificity of crude lipase on the hydrolysis of tripalmitin was analyzed as described in Materials and Methods.

ND, not detected.

Table 3. Identification of the OB Hydrolyzing Bacteria Strain KM109

Morphological characteristics		Nutritional characteristics	
Shape	short rod	D-Mannitol	−
Spore formation	−	D-Glucose	+
Gram staining	−	L-Arabinose	+
Mobility	−	Capric acid	+
Observation of colony		Sodium citrate	+
Color	yellow white	Adipinic acid	+
Shape	circular	*N*-Acetyl glucosamine	−
Surface	smooth	Maltose	−
		DL-Malonate	+
Chemical characteristics		D-Mannose	−
Oxydase	−	Phenyl acetate	+
Urease	−	Potassium gluconoate	−
Esculin hydrolysis	+	Sodium acetate	+
Ubiquinone	Q9	n-Decane	+
Catalase	+		
Gelatin liquefaction	+		
Reduction of nitrate	−		
GC content	37.4%		
OF test	Oxidation		

Tween 80, Triton X-100, and Noigen HC were tested for OBase production. Only Colorine 102 was effective for the production of OBase (data not shown). The highest amount of activity was observed when 0.2% Colorine 102 was used (Table 4). Therefore, the production medium for extracellular OBase was decided to be LB containing 1.0% OB and 0.2% Colorine 102.

The course of OBase production was investigated in a 100-L jar fermentor using 40 L of the OBase production medium at 30°C with an agitation speed of 200 rpm and an aeration rate of 40 L/min. pNPB-hydrolyzing activity appeared after 15 hr of cultivation, and reached a maximum after 40 hr cultivation (data not shown).

Purification of OBase

OBase was purified to homogeneity on SDS-PAGE from the culture supernatant of *Acinetobacter* nov. sp. strain KM109. The purification procedure consisted of four steps (Sepharose CL-6B gel-filtration chromatography, DEAE-Sephacel ion-exchange chromatography, TSK gel G2000SW$_{XL}$ gel-filtration HPLC, and MonoQ PC1.6/5 ion-exchange chromatography on a SMART system), and yielded pure OBase with 213-fold purification and a 0.8% yield (Table 5). SDS-PAGE analysis indicated that denatured OBase has a molecular weight of approximately 62,000±1,000 (Fig. 1). Gel-filtration HPLC indicated a molecular weight of 50,000, presumably due to the weak interaction of OBase with column resin, suggesting that under native conditions, OBase should be present as a monomer.

Because the *N*-terminus of the purified OBase was resistant to Edman degradation, it was concluded that the *N*-terminus was blocked. Therefore, to obtain sequence information, purified OBase was hydrolyzed by lysyl endopeptidase, and 4 fragment peptides were put on an automated protein sequencer, revealing partial amino-acid sequences of G-V(or T)-M-L-E-N-V-V-N-L-F, Y-A-P-L-A-I-A(or G)-Q-V-N-N-Q-X-Y-G-Y-V-L-D-P, Q-I-N-I-X-D-V-V-N-X-F, and L-I-F-V-N-I-F-T-D-L-V-X-L (X represents an ambiguous residue). From *Acinetobacter calcoaceticus*, genes encoding a lipase

Table 4. Effects of Medium Conditions on Lipase Production

Changed factor		Extracellular pNPB hydrolyzing activity (units/ml)
Nutrition	LB	0.29
	NB	0.23
	MB	ND
Inducer oil	Oleyl benzoate	0.20
	Olive oil	0.02
	Palm oil	0.02
	Soybean oil	0.03
Concentration of OB	0%	0.06
	0.1%	0.07
	0.2%	0.13
	0.5%	0.13
	1.0%	0.23
	2.0%	0.19
Concentration of Colorine 102	0%	0.06
	0.1%	0.26
	0.2%	0.34
	0.5%	0.26
	1.0%	0.23

Tested media were LB (Yeast extract 0.5%, Trypton 1.0%, NaCl 1.0%, pH 7.2), NB (Beef extract 1.0%, Polypeptone 1.0%, NaCl 0.2%, pH 7.2) and MB (Malt extract 0.5%, Trypton 1.0%, NaCl 1.0%, pH 7.2). All media had Colorine 102 (0.1%) and OB (1.0%).

For inducer oil and optimum concentration of OB and Colorine 102, LB medium was used as the basal medium.

ND, not detected.

Table 5. Purification of OBase Produced from *Acinetobacter* nov. sp. Strain KM 109

Purification step	Protein (mg)	Lipase activity (units)	Specific activity (units/mg)	Purification (-fold)	Yield (%)
Crude	807	115	0.14	1.00	100
Sepharose CL-6B	203	24.9	0.12	0.87	21.8
DEAE Sephacel	23.4	25.7	1.92	13.5	22.4
G2000SWXL	4.19	16.6	3.96	27.9	14.5
MonoQ PC 1.6/5	0.03	0.91	30.0	213	0.79

and an esterase have been cloned and sequenced.[26,27] These amino-acid sequences showed no similarity to the deduced amino acid sequences of *A. calcoaceticus* lipase and esterase. Furthermore, database searches using FASTA[28,29] found no significant similarity to known proteins.

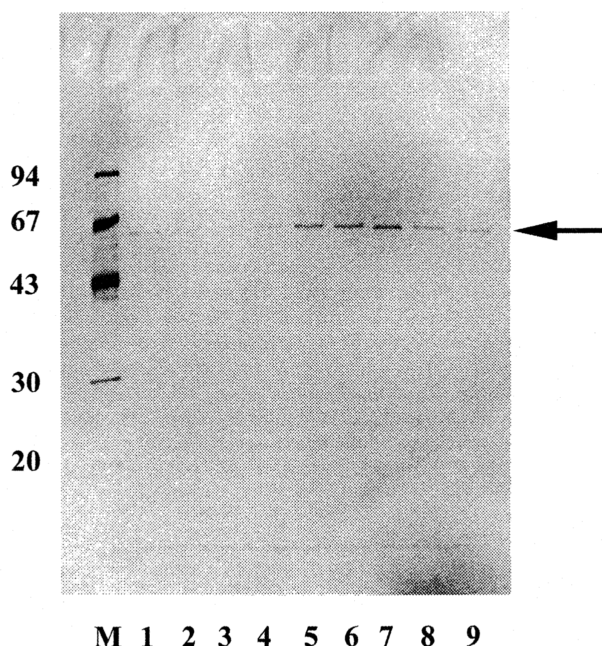

Fig. 1. SDS-PAGE of Purified OBase.

Samples eluted from a Mono Q PC1.6/5 column on a SMART system were collected every 30 sec from 16.5 min to 21 min and analyzed by SDS-PAGE (lane 1–9). Lane M. Molecular weight markers: phosphorylase b (94,000), bovine serum albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), and soybean trypsin inhibitor (20,000). The arrow shows the position of the purified OBase.

Effects of temperature and pH on activity and stability

With the purified OBase, the optimum reaction temperature was around 45°C (data not shown) using pNPB as the substrate. OBase was stable for the duration of a 30-min treatment of up to 35°C, but lost 20% and 58% activity after 30-min incubations at 40 and 50°C, respectively. Similarly, OBase showed an optimum reaction pH of around 8.0, and was stable between pH 6–8 for 24 h at 4°C in 50 mM potassium phosphate buffer (data not shown).

Hydrolysis of p-nitrophenyl esters and OB

To compare the substrate specificity of purified OBase with that of commercial lipases, hydrolysis of *p*-nitrophenyl esters and OB was investigated. Among the 10 commercial lipases tested, 4 hydrolyzed pNPB (Table 6). The highest activity was observed with lipase OF from *Candida cylindracea*, but this level was only 24% of that observed with OBase. The pNPB/pNPA and pNPB/pNPC ratios shown in Table 6 show that OBase hydrolyzes pNPB at least 12-fold more preferentially than the other lipases tested.

Hydrolysis of OB was detected for 3 commercial lipases (Lipase OF and two *Pseudomonas* lipases, Table 6), although their activity was quite low compared to that of OBase. Furthermore, three lipases showing pNPB-hydrolyzing activity did not show any OB hydrolysis, even in the presence of ten times the amount of enzyme (10 mg/ml, data not shown).

Table 6. Comparison of Activities with OBase and Commercial Lipases

Origin	pNPB hydrolysis (units/mg)	pNPA hydrolysis (units/mg)	pNPB/pNPA ratio	pNPC hydrolysis (units/mg)	pNPB/pNPC ratio	OB hydrolysis[k]
OBase	30.0	1.49	20.1	5.57	5.39	+
Candida cylindracea[a]	7.07	4.40	1.61	8.13	0.87	+
Candida antarctica[b]	ND	0.01	—	0.04	—	−
Pseudomonas sp.[c]	ND	7.40	—	54.7	—	±
Pseudomonas sp.[d]	ND	47.9	—	346	—	+
Pseudomonas fluorescens[e]	ND	2.68	—	3.89	—	−
Rhizopus japonicus[f]	ND	0.04	—	0.01	—	−
Rhizopus delemer[g]	0.21	1.86	0.11	0.03	7.00	−
Mucor javanicus[h]	0.03	0.17	0.19	0.08	0.38	−
Geotrichum candidum[i]	0.04	0.33	0.13	0.51	0.08	−
Porcine pancreas[j]	ND	0.02	—	0.04	—	−

Enzyme solutions of commercial lipases were prepared by resolving into buffer A at a concentration of 1 mg/ml.
[a] Lipase OF (Meito Sangyo, Nagoya, Japan).
[b] Novozyme 435 (Novo Nordisk Industry, Denmark).
[c] Lipase LP-051-S (Toyojozo Co., Tokyo, Japan).
[d] Lipoprotein lipase A (Toyobo Co. Ltd.).
[e] Lipase P (Amano Pharmaceutical Ltd., Nagoya, Japan).
[f] Olipase 4SD (Nagase Biochemicals Ltd., Osaka, Japan).
[g] Lyophilized lipase (Seikagaku Kogyo Co. Ltd., Tokyo, Japan).
[h] Lipase M (Amano Pharmaceutical).
[i] Lipase GEM (Funakoshi, Tokyo, Japan).
[j] Lipase Type II (Sigma, USA).
[k] +, oleyl alcohol and benzoic acid were detected; ±, very weak signals were detected; −, not detected.
ND: not detected.

Therefore, it can be concluded that OBase from *Acinetobacter* nov. sp. KM109 is a novel lipase that catalyzes the hydrolysis of benzoate esters, including oleyl benzoate and *p*-nitrophenyl benzoate. Chemical synthesis using OBase as a catalyst is underway in our laboratory, and will show the usefulness of this enzyme in organic synthesis involving bulky substituents near ester groups.

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