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Characterization of an Extracellular Lipase in *Burkholderia* sp. HY-10 Isolated from a Longicorn Beetle

Doo-Sang Park[1], Hyun-Woo Oh[1], Sun-Yeon Heo[1], Won-Jin Jeong[1], Dong Ha Shin[2], Kyung Sook Bae[1], and Ho-Yong Park[1]*

[1]*Biological Resource Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon 305-806, Republic of Korea*
[2]*Insect Biotech Co. Ltd., Daejeon 305-811, Republic of Korea*

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***Burkholderia* sp. HY-10 isolated from the digestive tracts of the longicorn beetle, *Prionus insularis*, produced an extracellular lipase with a molecular weight of 33.5 kDa estimated by SDS-PAGE. The lipase was purified from the culture supernatant to near electrophoretic homogenity by a one-step adsorption-desorption procedure using a polypropylene matrix followed by a concentration step. The purified lipase exhibited highest activities at pH 8.5 and 60°C. A broad range of lipase substrates, from C_4 to C_{18} ρ-nitrophenyl esters, were hydrolyzed efficiently by the lipase. The most efficient substrate was ρ-nitrophenyl caproate (C_6). A 2485 bp DNA fragment was isolated by PCR amplification and chromosomal walking which encoded two polypeptides of 364 and 346 amino acids, identified as a lipase and a lipase foldase, respectively. The N-terminal amino acid sequence of the purified lipase and nucleotide sequence analysis predicted that the precursor lipase was proteolytically modified through the secretion step and produced a catalytically active 33.5 kDa protein. The deduced amino acid sequence for the lipase shared extensive similarity with those of the lipase family I.2 of lipases from other bacteria. The deduced amino acid sequence contained two Cystein residues forming a disulfide bond in the molecule and three, well-conserved amino acid residues, Ser^{131}, His^{330}, and Asp^{308}, which composed the catalytic triad of the enzyme.**

Keywords: *Burkholderia* sp. HY-10, lipase, *Prionus insularis*, insect gut microorganism

Lipases [EC 3.1.1.3] catalyze the hydrolysis of triglyceride at the interface between the insoluble substrate and water. They are ubiquitous in nature and are produced by various animals, plants, fungi, bacteria and archia. Among them, extracellular bacterial lipases are of considerable commercial importance, because of their diverse substrate specificity, stero-specificity, and tolerance against heat and various organic solvents (Jaeger *et al.*, 1999; Gupta *et al.*, 2004). Therefore, they are widely used in food technology, in the detergent and chemical industries, and in biomedical sciences (Pandey *et al.*, 1999; Jaeger and Eggert, 2002; Reetz, 2002).

Bacterial lipases are divided into six groups based on their biochemical and molecular biological characteristics (Jaeger *et al.*, 1999). Group I lipases are divided into six subgroups (Group I.1-I.6) based on the phylogenetical analysis of the amino acid sequence (Jaeger *et al.*, 1999; Kim, 2003). At present, lipases originated from *Pseudomonas* and *Burkholderia* are those most commonly used in household detergents and in the trans-esterification process in the fine chemical industry and are involved in Group I.1 and Group I.2 (Jaeger *et al.*, 1999; Kim, 2003; Gupta *et al.*, 2004). The subfamily I.1 consists of lipases with a molecular weight of approximately 30 kDa and a single disulfide bond in the molecule. Lipases in this group need a molecular chaperone, the Lif (lipase-specific foldase) protein, for their folding and secretion (Frenken *et al.*, 1993; Jaeger *et al.*, 1994). This property is identical in subfamily I.2; however, in this group, lipases have a molecular weight of approximately 33 kDa and have high amino acid sequence homologies, greater than 80%, between group members. Several kinds of lipases originating from *Burkholderia* species have been identified and their enzymatic properties and crystal structures have been elucidated (Jorgensen *et al.*, 1991; Frenken *et al.*, 1992; Sugihara *et al.*, 1992; Noble *et al.*, 1993; Kim *et al.*, 1997; Gupta *et al.*, 2004). Because of their preference for the hydrolysis of triglycerides with a long chain length (greater than C_8), excellent enantioselectivity (Weissfloch and Kazalauskas, 1995), and tolerance to solvents and high temperature (Sugihara *et al.*, 1992; Rathi *et al.*, 2000; Rathi *et al.*, 2001), *Burkholderia* lipases were extensively studied during the past two decades for industrial use.

Digestive enzymes, such as protease, lipase and phospholipase C, are reportedly produced from several pathogenic microbes and are regulated by quorum sensing (Whitehead *et al.*, 2001; Fuqua and Greenberg, 2002; Carvalho *et al.*, 2007). To date, different profiles of exoenzyme productions were monitored from several *Burkholderia cepacia* complex (BCC) strains (Carvalho *et al.*, 2007), which may account for the differences in the pathogenic potentials of each BCC species. We have reported several extracellular, hydrolytic, enzyme-producing bacteria derived from the digestive tracts of insects (Lee *et al.*, 2004; Heo *et al.*, 2006; Kwak *et*

* To whom correspondence should be addressed.
(Tel) 82-42-860-4650; (Fax) 82-42-860-4659
(E-mail) hypark@kribb.re.kr

al., 2007). Among them, a *Burkholderia* strain, highly related to *B. pyrrocinia*, showed high lipolytic activity and cell density-dependent lipase production (Park *et al.*, 2007), which is a common characteristic of quorum sensing circuitry. In this study, an extracellular lipase in the *Burkholderia* strain previously isolated from the digestive tract of a longicorn beetle was purified, characterized and cloned.

Materials and Methods

Bacterial strains and culture conditions

The *Burkholderia* strain, HY-10, previously isolated from the adult longicorn beetle, *Prionus insularis*, was used in this work (Park *et al.*, 2007). This bacterium was routinely grown at 30°C in M9 medium containing 0.5% yeast extract and 0.5% olive oil (M9YO). The *E. coli* strain DH5αMCR was used as a transformation host for cloning DNA fragments and was grown at 37°C in Luria-Bertani (LB) broth supplemented with ampicillin (100 μg/ml) as needed. The plasmid, pGEM-T Easy (Promega, USA), was used for cloning genomic DNA fragments of *Burkholderia* sp. HY-10.

Enzyme assays

Lipase activity was measured according to Ryu *et al.* (2006) with a slight modification. The reaction mixture consisted of 5 μl of 10 mM substrate (C_4-C_{12} ρ-nitrophenyl ester, pNP ester) in acetonitrile, 5 μl of ethanol, and 180 μl of 50 mM phosphate buffer (pH 7.4) containing 10 μl of the enzyme. The enzyme reaction was conducted for 10 min at 25°C. The amount of ρ-nitrophenol liberated during the reaction was measured by its absorbance at 405 nm. For long chain pNP-esters (C_{12}-C_{18}), 10 μl of lipase solution was added to 170 μl of reaction buffer containing 50 mM phosphate buffer (pH 7.4), 0.1% gum arabic and 0.1% deoxycholate. After 5 min incubation at 25°C, the reaction was initiated by adding 20 μl of 8 mM substrate in isopropanol. After 10 min incubation at 25°C, the absorbance at 405 nm was measured.

Characterization of enzyme activities

Substrate specificities for different pNP esters were determined by using the previously described spectrophotometric assay (Ryu *et al.*, 2006). The pNP esters, ranging between C_4 and C_{18}, were determined using pNP-butyrate, pNP-caproate, pNP-caprate, pNP-laurate, pNP-palmitate, and pNP-stearate. The highest activities of pNP-caproate observed during the enzyme assay were defined as 100% level. The enzyme activity was assayed at 37°C at various pH values (6.5-11.0) to determine optimal pH. The buffers used for the pH ranges were 50 mM phosphate buffer for pH 6.5-7.5, 50 mM Tris-HCl for pH 7.5-8.5, 50 mM boric acid-NaOH for pH 8.5-10.0, and 50 mM $NaHCO_3$-NaOH for pH 10.0-11.0. To measure the optimum reaction temperature, lipase activities were measured at 10-90°C under the enzyme reaction conditions. To test the effect of metal ions on enzyme activity, 2 mM of each metal ion was added to the reaction mixture. The effects of chemical reagents were tested at 1 mM or 0.1-0.2% concentration. The data presented were based on comparisons to lipase activity with or without metal ion (100%).

Thin layer chromatography

To determine the lipolytic activity of the enzyme, the hydrolysis products of olive oil were analyzed by thin-layer chromatography (TLC). A reaction mixture composed of 0.5 g of highly refined olive oil (Sigma, USA), 3 ml of 50 mM Tris buffer (pH 8.0), and 50 units of the enzyme was incubated at 50°C for an hour with magnetic stirring at 500

Table 1. Oligonucleotide primers used at PCRs

Sequence	Purpose
5'-CTCGAGTAC(T)TGGTAC(T)GG-3'	Forward (from lipase 71-LEYWYG-76)
5'-GCGCTGCTC(T)TCG(T)TGCATC(T)TG-3'	Reverse (from foldase 282-QMQQDDA-288)
5'-CGAGTTTCAGCAGCCCCTG-3'	5'-end DNA walking (1^{st})
5'-GATCGTCGTCACCGACGCGAC-3'	"
5'-GCGACGTAGCGGGACGTCAG-3'	"
5'-CGTCGCCGCGTATGTATCG-3'	5'-end DNA walking (2^{nd})
5'-CCTGGAACGCATCGATCTGGC-3'	"
5'-ACGGTACGAACGACATCGAATCGAC-3'	"
5'-CTGACCGACGCGCAGAAG-3'	3'-end DNA walking
5'-AGATCGCGCAATTGCGGAAG-3'	"
5'-GCGCAACTGACGCAGTCGCTC-3'	"

Table 2. Purification of liapse from the culture supernatant of *Burkholderia* sp. HY-10

	Total activity (unit)	Total protein (mg)	Specific activity (unit/mg)	Purification fold	Yield (%)
Culture supernatant	2,760	1,008	2.7	1	100
Adsorption/Desorption	830	18.5	44.8	54.5	30

rpm (Sugihara *et al.*, 1992). After incubation, 20 ml of ethyl ether were added to extract the reaction products. Aliquots of the ether layer were applied to a silica gel 60 plate (Merck, Germany), which was then developed with a 96:4:1 (by volume) mixture of chloroform/acetone/acetic acid. Pure oleic acid (Sigma, USA) and olive oil were used as references. The spots were visualized by spraying the plate with 10% (v/v) H_2SO_4 in ethanol and heating on a hot plate until charring occurred.

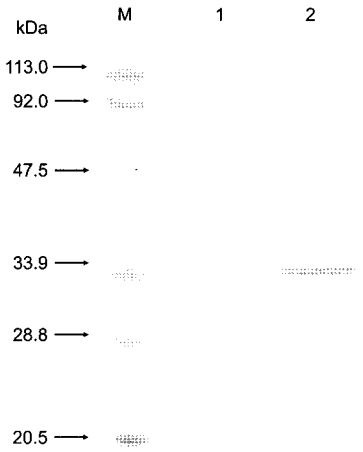

Fig. 1. SDS-PAGE of the purified lipase from *Burkholderia* sp. HY-10. M, molecular weight marker (the molecular weights are marked at the left side of the gel); lane 1, culture supernatant; 2, purified lipase. The gels were stained with Coomassie Brilliant Blue R-250.

Stability of the lipase on temperature and organic solvents

To examine thermostability, the enzyme was incubated at 50°C, 60°C and 70°C for 2 h. An aliquot was collected every 30 min in which residual activity was measured at 25°C and pH 7.4. The stability of the enzyme in the organic solvents, ethanol, methanol, isopropyl alcohol, acetone, and hexane in 50% concentration, was examined by incubation of the enzyme in 50% concentration and residual activity was determined.

Enzyme purification

Purification of the lipase was carried out based on the single-step purification method developed by Gupta *et al.* (2005). The *Burkholderia* sp. HY-10 strain was cultured in M9YO for 48 h at 30°C and 180 rpm. Extracellular fractions were obtained by centrifugation of the culture medium at 5,000×g for 10 min followed by lyophilization. For adsorption, 5 g of polypropylene matrix (Accurel MP-1000, Membrana GmbH, Germany) was wetted with 75 ml of butanol. To this, 425 ml of distilled water and 75 ml of phosphate buffer (50 mM,

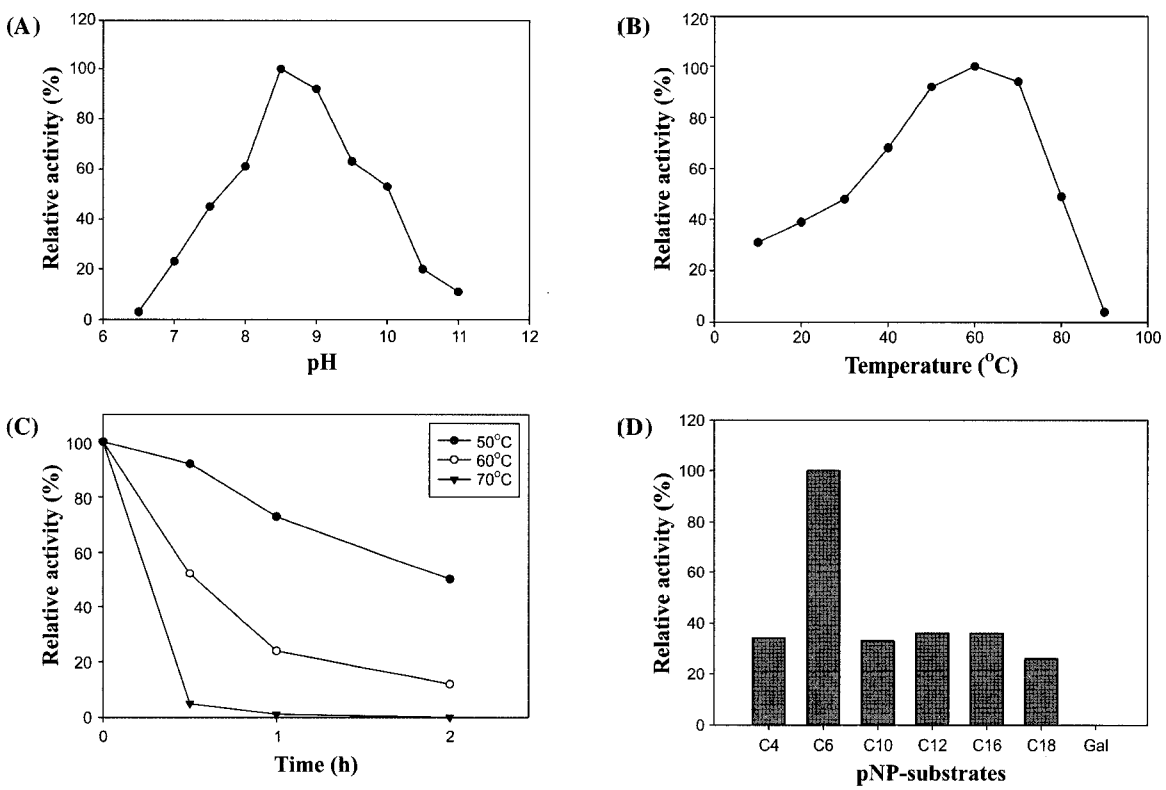

Fig. 2. Properties of lipase from *Burkholderia* sp. HY-10. (A) Effect of pH on the activity of the purified lipase, (B) Effect of temperature on the activity of the purified lipase, (C) The residual activity was measured after incubation of the enzyme at various temperatures for 2 h. (D) Substrate specificity of the enzyme for pNP-substrates; pNP-butyrate (C_4), pNP-caproate (C_6), pNP-caprate (C_{10}), pNP-laurate (C_{12}), pNP-palmitate (C_{16}), and pNP-sterate (C_{18}) were used as pNP esters and pNP-ß-D-galactopyranoside (Gal) was used as a non-lipid negative control.

pH 7.4) were added. The lyophilized culture supernatant (12.5 g) was added to the support. The suspension was shaken at 100 rpm and 25°C for 2 h to allow adsorption. The support was filtered, washed twice with 450 ml of phosphate buffer (50 mM, pH 7.4) to remove unbound protein and air-dried at room temperature. The adsorbed enzyme was eluted twice with the same buffer containing 0.2% triton X-100. The amount of adsorbed enzyme and protein content was calculated from the difference in lipase activity and protein content before and after adsorption. The enzyme was recovered by filtration using a Minimate^{TM} TFF capsule (10K cut off, Pall, USA). The purity of the enzyme was determined by SDS-PAGE (Laemmli, 1970).

Determination of N-terminal amino acid sequence

To obtain the N-terminal sequence of the enzyme, the purified enzyme was fractionated by SDS-PAGE and transferred onto a polyvinylidene difluoride (PVDF) membrane (Bio-Rad, USA) using 10 mM CAPS-NaOH, pH 11.0, and 10% methanol as a transfer buffer. Protein bands were excised and analyzed using Applied Biosystems Procise 491 at the Korea Basic Science Institute (Korea).

Table 3. Stability of lipase from *Burkholderia* sp. HY-10 in organic solvent

Solvent	Residual activity (%)
Ethanol	88
Methanol	95
Isopropyl alcohol	33
Aceton	62
Hexane	103
Control	100

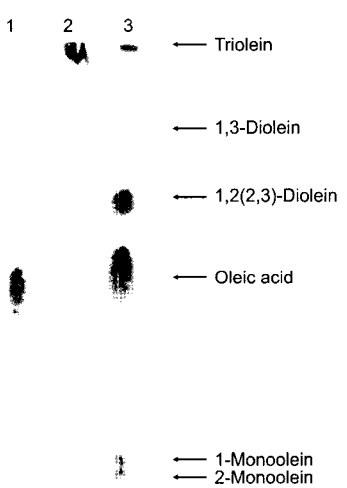

Fig. 3. Thin-layer chromatography of enzyme reaction products obtained with olive oil as the substrate. A reaction mixture containing 50 mM Tris-HCl buffer (pH 8.0), olive oil and purified lipase was incubated for an hour at 50°C. Then ethyl ether was added to extract the reaction products, and aliquots of the ether layer were subjected to TLC. Lane 1, oleic acid; 2, olive oil; 3, hydrolyzed sample.

DNA analysis

The chromosomal DNA of *Burkholderia* sp. HY-10 was isolated and used as the PCR template. To amplify the gene fragment encoding lipase (LipA) and lipase foldase (LifA) from the genomic DNA, the degenerate oligonucleotides (Table 1) were used which originated from the highly conserved regions among the *Burkholderia* lipase and lipase foldase gene family. The amplified fragment was cloned into pGEM-T Easy (Promega, USA) and the nulceotide sequence was determined to confirm the identity of the partial gene fragment. The whole DNA fragment containing the lipA and lifA encoding gene was amplified by repeated PCR using the DNA Walking SpeedUp Premix Kit (Seegene, Korea) according to the manufacturer's recommendation (Table 1). The nucleotide sequence was determined from the amplified fragment by Genotech Incorporation (Korea). The nucleotide sequence of *lipA* and *lifA* from *Burkholderia* sp. HY-10 was assigned GenBank accession no. EF562602.

Results

Lipase purification

In a previous study, a bacterial strain retaining high lipolytic activity on agar R2A containing olive oil and Rhodamin B was isolated from the digestive tracts of adult longicorn beetles (Park *et al.*, 2007). The strain demonstrated the highest lipolytic activity among 85 lipase-producing strains screened by agar plate assay and liquid culture from 10 kinds of insects. Phylogenetic analysis and chemotaxonomic characterization demonstrated that the strain belonged to *Burkholderia* sp.. In the present study, the lipase was purified from the culture supernatant of the stationary phase in M9YO using a single step adsorption-desorption method

Table. 4. Effect of various chemicals on the lipase from *Burkholderia* sp. HY-10

Chemical	Relative activity (%)
Control	100
Mn^{2+}	104
Ca^{2+}	112
K^{+}	112
Na^{+}	83
Fe^{2+}	98
Cu^{2+}	28
Co^{2+}	112
Zn^{2+}	162
Mg^{2+}	116
EDTA	90
PMSF	98
2-Mercarptoethanol	122
SDS	0
Triton-X100	125
Tween 80	116
Sodiumdeoxycholate	16

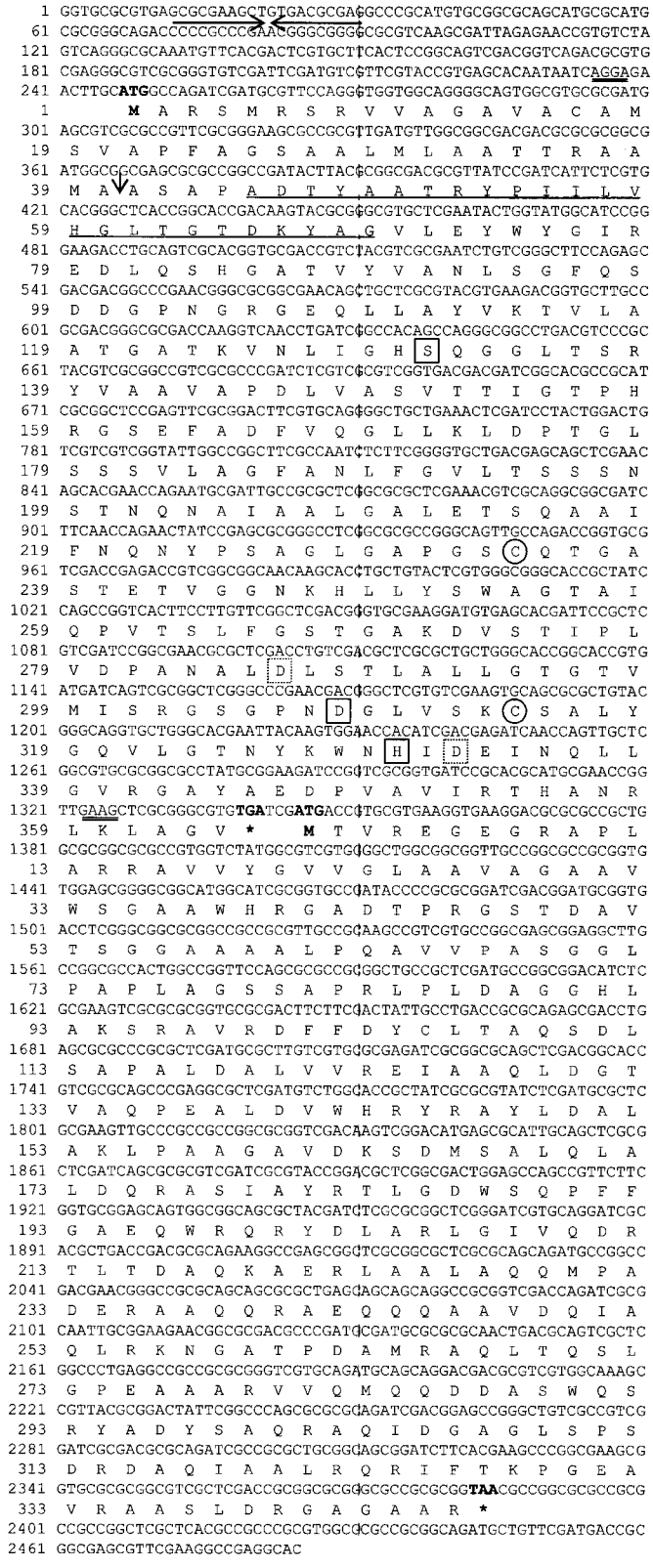

Fig. 4. Nucleotide sequence and deduced amino acid sequences of the PCR cloned DNA fragment containing *lipA* and *lifA* genes from *Burkholderia* sp. HY-10. Two putative ribosome binding sequences are double underlined. The vertical arrow indicates the putative signal sequence cleavage site predicted by SignalP software. The underlined amino acid sequence was the determined N-terminal amino acid sequence of the purified lipase. The conserved Ser, Asp, and His residues that comprise a putative catalytic triad are boxed. Putative Ca^{2+}-binding Asp residues are boxed with dotted line. The two Cys residues comprise a potential disulfide bond are circled. The inverted repeat sequence positioned 5' upstream region was arrowed.

(Gupta *et al.*, 2005). The culture supernatant containing 2.7 units/mg of lipase activity was enriched to 44.8 units/mg after purification and concentration with a recovery yield of 30% (Table 2). The enzyme was purified as a single protein band with a molecular mass of 33.5 kDa on an SDS-PAGE gel (Fig. 1). The enzyme was named LipA.

Enzymatic properties of LipA

The purified lipase exhibited maximum activity at pH 8.5 (Fig. 2A). The highest activity was shown at 60°C (Fig. 2B) and more than 90% of relative activity was retained at 70°C. In thermostability testing, the enzyme retained more than 50% of its relative activity at 50°C for 2 h. After preincubation at 60°C for 30 min, the enzyme exhibited about 50% of relative activity, indicating that the half-life of enzyme activity was 30 min at 60°C (Fig. 2C). The purified lipase exhibited broad range chain-length specificity toward synthetic substrates. The lipase efficiently hydrolyzed pNP-esters with various chain lengths (C_4-C_{18}), showing maximum activity toward pNP caproate (C_6) but did not hydrolyze pNP-β-D-galactopyranoside, a non-ester glucoside substrate (Fig. 2D). After treatment in 50% organic solvent, the lipolytic activity of the enzyme demonstrated relatively stable activities in hexane, ethanol, methanol, and acetone, but its activity decreased when treated in isopropanol (Table 3). Partial hydrolysis of olive oil by the enzyme resulted in six kinds of hydrolyzed products, including 1,3-diolein, 1,2(2,3)-diolein, oleic acid, 1-monoolein, and 2-monoolein (Sugihara *et al.*, 1992) (Fig. 3).

Effect of metal ions and chemicals on enzymatic activity

To investigate the effects of metal ions and chemicals on LipA activity, 2 mM of various metal ions (Ca^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, K^+, Mg^{2+}, Mn^{2+}, Na^+, and Zn^{2+}) and 1 mM EDTA, 1 mM PMSF, 1 mM 2-mercarptoethanol, 0.1% SDS, 0.1% Triton X100, 0.2% Tween 80, and 0.2% sodium deoxycholate were added to the reaction mixtures. Zn^{2+} increased the lipolytic activity by approximately 62% and considerable decreases in activity were observed in the presence of Cu^{2+}, SDS, and sodium deoxycholate (Table 4).

Cloning and sequencing of the lipase and lipase foldase gene

The N-terminal amino acid sequence of the purified LipA was identified as ADTYAATRYPIILVHGLTGTDKYAG. The partial gene fragment of *lipA* and *lifA* was isolated by PCR amplification with degenerate oligonucleotide primers and cloned a 2,485-bp DNA fragment by chromosomal walking as described in Materials and Methods. Determination of the nucleotide sequence revealed that two open reading frames were present in the DNA fragment (Fig. 4). The open reading frames encoded two polypeptides of 364 and 346 amino acids, which were identified as lipase and lipase foldase, respectively. Two putative ribosome binding sites were present at 8 bp upstream of the LipA initiation codon (AGGA) and 18 bp upstream of the LifA initiation codon (GAAG). The N-terminal amino acid sequence of the purified LipA matched the 45^{th} to 69^{th} amino acid sequence deduced from the nucleotide sequence. The enzyme is composed of 320 amino acid residues with a deduced molecular weight of 33,003 Da and a pI value of 5.95. The lipase, LipA, contains a putative catalytic triad composed by Ser^{131}, His^{330}, and Asp^{308}, two Asp residues (Asp^{286} and Asp^{332}) involved in Ca^{2+}-binding, and two Cys residues (Cys^{234} and Cys^{314}) that may form a disulfide bond. The promoter region of the *lipA* gene could not be predicted; however, a characteristic 20 bp inverted repeated sequence was located at approximately 180 bp upstream of the translation initiation site (Fig. 3).

Database searching and phylogenetic analysis revealed that the LipA could be grouped with lipases belonging to the Subgroup I.2 family (Jaeger *et al.*, 1994; Sullivan *et al.* 1999) (Fig. 5). The amino acid sequence similarity levels were determined to be 87% with *B. cepacia*, 86% with *B.*

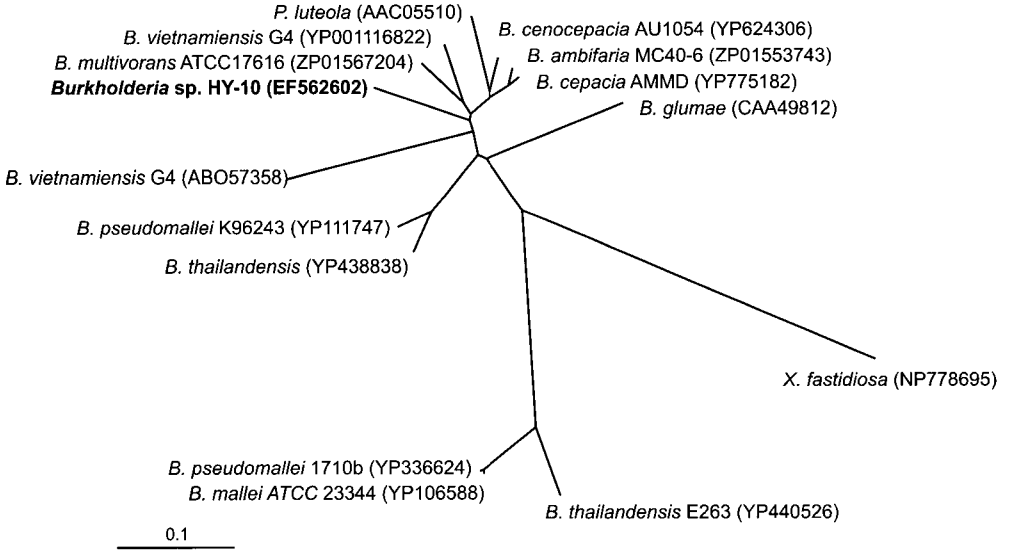

Fig. 5. Phylogenetic tree of family I.2 lipases. The amino acid sequences of lipases were aligned by CLUSTAL algorithm (CLUSTAL X ver. 1.83), and the phylogenetic tree was constructed based on the alignment by PHYLIP program. The GenBank accession numbers for the sequences used to calculate the tree are shown in parentheses.

cenocepacia, *B. ambifaria* and *B. vietnamiensis* G4, 85% with *B. multibvorans*, and 70-80% with other known *Burkholderia* species lipases.

Discussion

A new lipase was identified from the culture supernatant of *Burkholderia* sp. HY-10 isolated from the digestive tract of a longicorn beetle. Because the lipase occurred in the culture supernatant, could be isolated using simple purification tools, exhibited moderate thermal stability, was stable in various organic solvent and demonstrated strong lipolytic activities against various artificial substrates and triolein, the enzyme is an attractive candidate for various industrial applications, such as for removal of fatty residues in laundry, or use in dishwashers and for cleaning clogged drains.

The lipase seems to fall into the group I.2 family on the basis of several pieces of evidence. The molecular weights estimated from the purified enzyme and the amino acid sequence deduced were approximately 33.0 kDa like those of other lipases belonging to the same family. The amino acid sequence homologies deduced from the nucleotide were similar to more than 80% of those in several I.2 family lipases from other bacteria. The catalytic triad residues, composed the active site, and two Cys residues, comprising a potential disulfide bond, were also present in the open reading frame of LipA. The conserved pentapeptide sequence around the Ser in the active site was GHSQG which was GHSHG in the Group I.1 lipases (Ollis *et al.*, 1992). LifA, a molecular chaperone essentially required for LipA folding (Jorgensen *et al.*, 1991), was succeeded by LipA with 3 bp gapped together in an operon.

The LipA from *Burkholderia* sp. seems to be secreted to the external medium through Type II secretion machinery. A two-step secretion processes was required for passage through the inner and outer membranes of Gram-negative bacteria. Passage through the inner membrane was performed by the Sec system which requires a signal peptide at the N-terminal region of the target protein (Von Heijne 1986; Cristobal *et al.*, 1999), and passage through the outer membrane required a Type II secretion complex composed of 14 kinds of gene product (Sandkvist, 2001). A 40 amino acid residue signal sequence was found at the N-terminal from the amino acid sequence deduced for LipA with a cleavage position between Ala^{40}-Ala^{41} predicted by the SignalP 3.0 Server (http://www.cbs.dtu.dk/services/SignalP/). However, the N-terminal amino acid sequencing result showed that the carboxyl terminal of Pro^{44} was the last cleaved site of the active enzyme secreted (Fig 4). The possibility that the polypeptide secreted was modified by a second proteolysis while it folded into its active conformation assisted by Lif, and formed a disulfide bond by the action of Dsb in the periplasm (Rosenau and Jaeger, 2000; Rosenau *et al.*, 2004), could not be excluded.

Our previous data (Park *et al.*, 2007) showed that the lipase production of *Burkholderia* sp. HY-10 seemed to be under the control of catabolic repression and cell density dependent gene expression, or, so called quorum sensing, reported at various Group I family lipase expressions (Dunphy *et al.*, 1997; Reimmann *et al.*, 1997; Lewenza *et al.*, 1999; Rosenau and Jaeger, 2000). The promoter element and dyad symmetric lux-box-like sequences positioned 40 to 60 bp upstream of each transcript start have been studied against *P. aeruginosa* quorum sensing genes (Whiteley and Greenberg, 2001). We speculate on the cis-acting element of *lipA* gene expression; however, no consensus sequence for the promoter region as well as a lux-like sequence could be predicted. Instead, a GC rich 20 bp inverted repeated sequence was found at the upstream region (Fig. 4). The sequence was located approximately -180 to -150 bp upstream from the translation initiation codon and was also found with high homogeneity in the lipase genes of two *B. cepacia* strains and in a *B. vietnamiensis* strain (Fig. 6). More study to identify the promoter and the cis-acting element will be needed.

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HY-10 lipase    CCCCGCCCGAACGGGCGGGG
Bc lipase       CCCCGCCCGAACGGGCGGGG
Bv lipase       CCCCGCCCGAAGCGGCGGGG
Bc DSM3959      CCCCGCCTGAACGGGCGGGG
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Fig. 6. An alignment of the inverted repeat elements of *Burkholderia* sp. HY-10, *B. cepacia* (Bc, YP775182), *B. vietnamiensis* (Bv, YP001116822), and *B. cepacia* DSM3959 strain (Jorgensen *et al.*, 1991) lipase.

Burkholderia cepacia complex (Bcc) species were isolated from variety of natural habitats, such as plant rhizosphere, soil and river water and several urban environments (Fiore *et al.*, 2001; Miller *et al.*, 2002; Vermis *et al.*, 2003). They have useful properties as plant pest antagonists (Bevivino *et al.*, 2000), plant-growth-promoting rhizobacteria (Tran Van *et al.*, 2000), ligin degradative bacteria in the termite gut (Kinya *et al.*, 1998) and degradative agents of toxic substances (Fries *et al.*, 1997; Lee, 2003). In spite of their beneficial characteristics, Bcc species can also colonize and/or infect cystic fibrosis (CF) patients and, therefore, are regarded as opportunistic pathogens in humans (Speert, 2001) and are restricted in their biotechnological application (Federal-Register, 2003). Phylogenetic analysis of the amino acid sequence deduced for LipA showed that it branched from the group composed by *B. cepacia*, *B. ambifaria* and *B. cenocepacia* lipase although they demonstrated the highest homologies in the GenBank data base search (Fig. 5). Similar results were obtained from the phylogenetic analysis of 16S rDNA in that *Burkholderia* sp. HY-10 showed relatedness with those strains which are rarely found in patients with CF, that is, *B. pyrrocinia* and *B. vietnamiensis*, rather than the strains *B. cenocepacia*, *B. cepacia* and *B. multivorans*, prevalent in the lungs of CF patients (Park *et al.*, 2007). The use of *Burkholderia* species could be exempted from existing rigid restrictions and they could be applied as a biopesticide or plant growth promoting reagent if robust evidence on the Bcc strains as harmless microorganisms were generated (Reik *et al.*, 2005; Chiarini *et al.*, 2006).

In the course of screening a symbiotic microorganism from the intestine of an insect that is evolutionally adapted *in vivo* and investigation of its use as a source of an industrial enzyme, a lipase-producing *Burkholderia* strain was isolated which belonged to the potent harmless Bcc species rather than the more abundantly studied *B. cepacia* lipase.

To guarantee the safety of this strain, further study will be required.

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