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Biochemical Properties and Substrate Specificity of Lipase from *Staphylococcus aureus* B56

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Abstract A lipase of *Staphylococcus aureus* B56 was purified from a culture supernatant and its molecular weight was estimated to be 45 kDa by SDS-PAGE. The optimum temperature and pH for the hydrolysis of olive oil was 42°C and pH 8–8.5, respectively. The enzyme was stable up to 55°C in the presence of Ca^{++} at pHs 5–11. The lipase gene was cloned using the PCR amplification method. The sequence analysis showed an open reading frame of 2,076 bp, which encoded a preproenzyme of 691 amino acids. The preproenzyme was composed of a signal sequence (37 aa), propeptide (255 aa), and mature enzyme (399 aa). Based on a sequence comparison, lipase B56 constituted of a separate subgroup among the staphylococcal lipase groups, such as *S. aureus* PS54 and *S. haemolyticus* L62 lipases, and was distinct from other lipases in their optimum pH and substrate specificity.

Key words: Lipase, *Staphylococcus aureus*, substrate specificity

A lipase (triacylglycerol acylhydrolase, EC 3.1.1.3) catalyzes the hydrolysis of the ester bonds of triacylglycerols in an oil-water interface. Many diverse bacterial lipases have been purified, biochemically characterized, and their respective genes cloned and sequenced [2]. So far, they have been classified into four groups (Group I-IV) based on molecular structure and sequence homology [3].

Most *Staphylococcus* strains produce extracellular lipases, which constitute a separate bacterial lipase group (Group IV) [3]. Many lipase genes have been identified from *S. aureus* PS54, *S. aureus* NCTC8530, *S. epidermidis* 9, *S. epidermidis* RP62AM, *S. hyicus*, and S. *haemolyticus* L62 [11]. Genetic analysis has shown that they all produced a 75 kDa-sized preproenzyme and processed extracellulary to 45 kDa-sized mature forms during and after secretion. Group IV lipases have a relatively high sequence homology (99–50%) among themselves, whereas they share little homology with the other lipase groups.

Many *Staphylococcus* lipases have been purified and their biochemical properties studied in isolated systems. Among them, certain staphylococcal lipases are important biotechnologically as well as academically for their extraordinary biochemical properties. That is, *S. hyicus* lipase can hydrolyze neutral lipids almost irrespective of their chain length as well as phospholipids [14, 15], although most staphylococcal lipases can only hydrolyze lipids with a short chain length and exhibit no phospholipase activity.

In addition, both *S. aureus* NCTC8530 and *S. epidermidis* RP62A lipases are active within an acidic pH range with an optimum pH around 6 [11]. This preference for acidic conditions is quite unusual among bacterial lipases, which in most cases exert their highest activities at an alkaline pH.

Staphylococcus lipases are also important physiologically for the nutrition and dissemination of bacteria, plus as an invasive factor [8, 10]. The preincubation of granulocytes with purified *S. aureus* lipase produces a progressive reduction of granulocyte chemotaxis [8]. The phagocytic killing of *S. aureus* by granulocytes preincubated with its lipase is reduced in a dose-dependent manner. *S. aureus* lipase can also cause the monophasic aggregation of human granulocytes and subsequent release of lactoferrin [10]. Thus, *S. aureus* lipases have been demonstrated to be potential virulence factors interacting directly with the human immune system.

S. aureus strain B56 producing an extracellular lipase was isolated from a sewage treatment plant in Korea. The lipase B56 enzyme was purified, and its biochemical properties were studied and compared with other staphylococcal lipases.

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MATERIALS AND METHODS

Production and Purification of Lipase B56 Enzyme

The *S. aureus* B56 was cultivated in 50 ml of an LB medium (1% tryptone, 0.5% yeast extract, and 0.5% NaCl) at 37°C for 8 h. Five ml of the culture was inoculated into 300 ml of the LB medium and cultivated at 37°C for 20 h Next, ammonium sulfate was added to the culture supernatant to 70% saturation, then the precipitate was collected by centrifugation at 12,000 ×g for 15 min, dissolved in 10% (w/v) $(NH_4)_2SO_4$ in a 10 mM potassium phosphate buffer (pH 7.0), and dialyzed. The dialysate was applied to a Phenyl-Sepharose CL-4B column (2.5×20 cm), and then the column was washed with a 10 mM potassium phosphate buffer (pH 7.0). The bound lipase enzyme was eluted with 1% (v/v) Triton X-100 (TX-100) in a 10 mM potassium phosphate buffer. The active fractions were pooled, dialyzed against 0.02% (v/v) TX-100 in a 10 mM potassium phosphate buffer (pH 5.8), and loaded onto a CM-Sepharose CL-6B column (2.5×20 cm). While most of the proteins bound to the CM-Sepharose, the lipase enzyme flowed through the column. The lipase enzyme thus purified was then concentrated and stored at −20°C.

Lipase Assay

The lipase activity was measured by titrating the free fatty acids released by the hydrolysis of olive oil using the pH-stat method [6]. An olive oil emulsion was prepared by emulsifying 5 ml of olive oil in 495 ml of a 20 mM NaCl, 1 mM $CaCl_2$, and 0.5% (w/v) gum arabic solution for 2 min at maximum speed in a Waring blender. After the pH of the substrate emulsion (20 ml) was adjusted to 8.0 by the addition of 10 mM NaOH solution, an appropriate amount (10–50 µl) of the enzyme solution was added. The rate of the fatty acid released was measured with a pH titrator (718 Stat Titrino, Metrohm) for 5 min at 42°C. One lipase unit was defined as the amount of enzyme liberating 1 µmole of fatty acid per min.

The lipase activity toward various triacylglycerols was measured using the same pH-stat method. Twenty millimolar triacylglycerols in a 20 mM NaCl, 1 mM $CaCl_2$, and 0.5% (w/v) gum arabic solution were emulsified and used as the substrate solution.

The substrate specificity toward various *p*-nitrophenyl esters (*p*-NPE) was analyzed using the spectrophotometric method, as described previously [7].

The lipolytic activity of the protein on SDS-PAGE gel was detected using tributyrin (TBN) agar plates. After gel running, the gel was washed sequentially with a 50 mM Tris buffer (pH 8.0) containing 1% (v/v) TX-100 and then washed twice with a 50 mM Tris buffer. The gel was overlaid on a TBN agar plate prepared with an agar (1.5%) and tributyrin emulsion (1% tributyrin, 20 mM NaCl, 1 mM $CaCl_2$, and 0.5% (w/v) gum arabic).

PCR-Cloning of Lipase B56 Gene

To clone the *S. aureus* lipase B56 gene, two primers were made: primer 1, 5'-GAA CAT ATG TTA AGA GGA CAA GAA-3' and primer 2, 5'-CTT GGA TCC ACT TGC TTT CAA TTG TGT-3'. The nucleotide sequences of Primers 1 and 2 were designed based on the 5'- and 3'-terminal sequences of the *S. aureus* PS54 lipase gene, respectively [4]. These two primer sets and the *S. aureus* B56 chromosomal DNA made a 2.1 kb-sized PCR product. The PCR product was digested with *Nde*I and *Bam*HI and ligated with the plasmid. *E. coli* XL1 Blue was transformed with this ligation mixture and plated on LB plates containing 100 $\mu g\ \mu l^{-1}$ of ampicillin. After overnight incubation at 37°C, the transformed colony was selected and its plasmid purified and sequenced.

RESULTS

Identification of Strain B56

The lipase-producing strain B56 was isolated from a sewage treatment plant in Korea. Strain B56 was found

Table 1. Microbial characteristics of strain B56.

Characteristic	Result	Characteristic	Result
Gram stain	positive	Acid from D-Fructose	+
Cell shape	coccus	D-Mannose	+
Spore	no	Maltose	+
Catalase	positive	Lactose	+
Cell arrangement	irregular	D-Trehalose	+
Colony pigment (carotenoid)	+	D-Mannitol	+
Nitrate reduction to nitrite	+	Xylitol	−
VP reaction	+	D-Melibiose	−
Alkaline phosphatase	+	Raffinose	−
Arginine dihydrolase	+	Xylose	−
Urease	+	Sucrose	+
Coagulase	positive	α-Methyl-D-glucoside	−
Acid from D-Glucose	+	N-Acetyl-glucosamine	+

to be Gram-positive and coccus-shaped. Morphological tests, biochemical tests using an API Staph kit (bioMerieux, France), and a coagulase test identified strain B56 as *Staphylococcus aureus* (Table 1).

Production of Lipase B56

The *S. aureus* B56 strain produced and secreted a lipase enzyme into the culture medium. The extracellular lipase activity started to increase from the early stationary phase and reached up to as much as 4,500 units l^{-1} after 16 h of cultivation (Fig. 1A). A zymogram of the whole cell and culture medium showed that *S. aureus* B56 produced a 75 kDa-sized precursor form of the lipase B56 enzyme, and this precursor enzyme was specifically processed into a 45 kDa-sized mature lipase (Figs. 1B and 1C). A considerable amount of precursor enzyme was detected in the culture medium as well as whole cells, which implies that the removal of the proregion occurred during and after the secretion by a certain extracellular protease [1, 9].

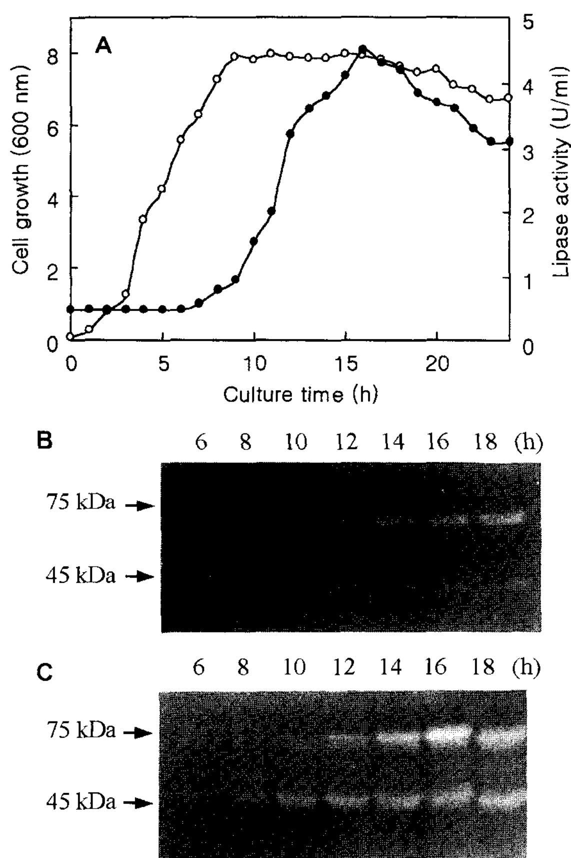

Fig. 1. Lipase B56 production from *S. aureus* B56.

The absorbance at 600 nm (○) and extracellular lipase activity (●) were measured during cell growth (A). The lipolytic activities of the whole cells (B) and extracellular culture supernatants (C) were checked at various cultivation times.

Purification of Lipase B56

The lipase B56 was purified from the culture medium by ammonium sulfate precipitation and hydrophobic and ion exchange column chromatographies. The enzyme bound tightly to the phenyl Sepharose and could be eluted from the column with 1% TX-100. The lipase flowed through the CM column equilibrated with a phosphate buffer (pH 5.8), while most of the other extracellular proteins bound tightly to the CM-Sepharose. A 45 kDa-sized lipase enzyme was purified to homogeneity after these two column chromatographies. The specific hydrolytic activity of the purified lipase was 910 U/mg and the purification yield was calculated to be about 34% of the total activity in the culture medium.

Effect of Temperature and pH on Lipase B56 Activity and Stability

The optimum temperature for lipase B56 was 42°C (Fig. 2A). The lipase exhibited a Ca^{2+}-dependent stability at

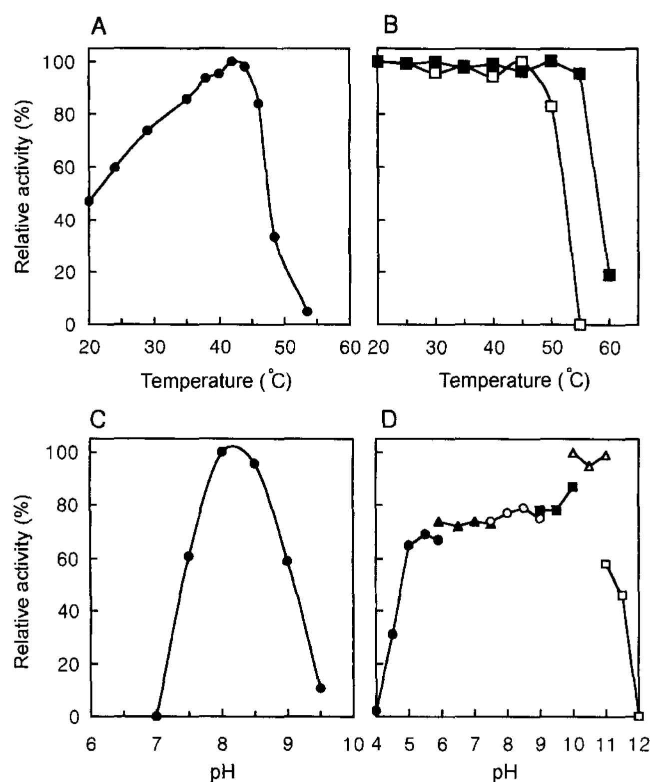

Fig. 2. Effects of temperature and pH on enzyme activity and stability.

The lipase activity was measured at different temperatures (A). The enzyme was preincubated at various temperatures for 30 min in the presence of 10 mM Ca^{2+} (■) and 5 mM EDTA (□) and the residual activities were measured (B). The lipase activity was measured at different pHs (C). The enzyme was preincubated with various pH buffers for 24 h at 4°C and the residual activity was measured. ●, 0.1 M sodium acetate (pH 4–6); ▲, 0.1 M potassium phosphate (pH 6–7.5); ○, 0.1 M Tris-HCl (pH 7.5–9); ■, 0.1 M Glycine-KOH (pH 9–10); △, 0.1 M Na_2HPO_4-NaOH (pH 10–11); □, 0.1 M $NaHCO_3$-NaOH (pH 11–12).

elevated temperatures (50–55°C) (Fig. 2B). That is, in the presence of Ca^{2+}, it was stable up to 55°C, then a drastic decrease in stability occurred above 55°C, plus its thermal stability decreased by 5 degrees in the absence of Ca^{2+}.

Lipase B56 showed a high activity at pH 8–8.5 when assayed at 42°C (Fig. 2C). Although the lipase activity differed somewhat depending on the incubation buffer used, it remained fairly stable for 24 h from pH 5 to pH 11 (Fig. 2D).

Substrate Specificity of Lipase B56

As shown in Fig. 3A, the hydrolysis of various triacylglycerols by lipase B56 depended significantly on the acyl chain length in the substrate molecules. Tripropionin (C_3) and tributyrin (C_4) were the most preferred substrates, while trilaurin (C_{12}), trimyristin (C_{14}), and tripalmitin (C_{16}) were moderately well-hydrolyzed substrates.

When linear synthetic esters, such as *p*-nitrophenyl esters, were used as substrates, lipase B56 showed a preference activity toward short and medium chain lengths (C_2-C_8) (Fig. 3B). No activity was observed against phosphatidylcholine (data not shown), implying that it had no phopholipase activity.

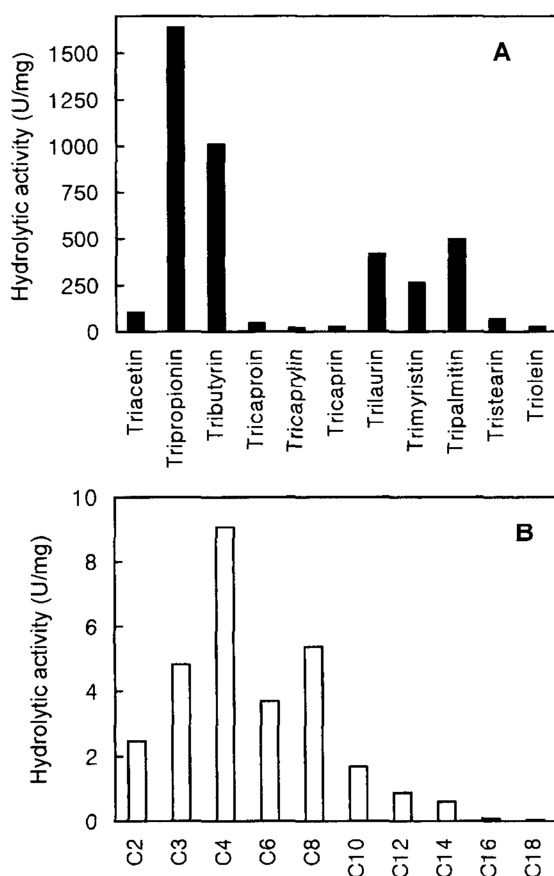

Fig. 3. Lipase activity of lipase B56 on various triacylglycerols (A) and *p*-nitrophenyl esters (B).

Nucleotide Sequence of Lipase B56 Gene

The N-terminal amino acid sequence of lipase B56 was determined from the 45 kDa-protein band on SDS-PAGE gel. Based on a sequence comparison, the sequence of lipase B56 was found to be similar to that of *S. aureus* PS54 lipase [4]. The lipase B56 gene was cloned by PCR amplification using *S. aureus* B56 chromosomal DNA and two primers designed based on the coding sequence of the *S. aureus* PS54 lipase gene. The nucleotide sequence of the PCR product showed one open reading frame of 2,076 bp, which encoded a polypeptide of 691 amino acid residues (Fig. 4). A 37 amino acid signal sequence was suggested from a sequence comparison with the *S. aureus* PS54 lipase sequence [4]. In addition, a 255 amino acid pro-region and cleavage site between Val 292 and Arg 293 were also assigned by the N-terminal amino acid sequence of the 45 kDa mature lipase. Therefore, the deduced sequence of the mature lipase contained 399 amino acids and corresponded to a calculated molecular weight of 44,631 Da. A sequence alignment showed that Ser121 in the mature enzyme was located in the conserved G-X-S-X-G sequence around the active site serine residue. In addition, Asp312 and His354, which appeared to be members of a catalytic triad, were found together with Ser121 in the enzyme.

DISCUSSION

The amino acid sequence of *S. aureus* B56 lipase was compared with those of most staphylococcal lipases using the Clustal method (Fig. 5A). The mature part of lipase B56 exhibited 99%, 54%, 67%, 54%, 55%, and 50% homologies with *S. aureus* PS54, *S. aureus* NCTC8530, *S. haemolyticus* L62, *S. epidermidis* RP62A, *S. epidermidis* 9, and *S. hyicus* lipases, respectively.

Lipase B56 exhibited high homology (99%) with *S. aureus* PS54 lipase, yet relatively low homology (54%) with *S. aureus* NCTC8530 lipase, even though they all originate from the same staphylococcal species. In fact, *S. aureus* NCTC8530 lipase had a rather high homology (85%) with *S. epidermidis* lipases (Fig. 5A).

Since there has been no previous report on the biochemical properties of *S. aureus* PS54 lipase, it was impossible to compare lipase B56 with *S. aureus* PS54 lipase. However, the reaction properties of lipase B56 were quite different from those of *S. aureus* NCTC8530 lipase, at least in respect to the optimum pH; the former was pH 8–8.5, whereas that of the latter is pH 6 [5, 12].

Staphylococcus lipases have been classified as a different group of enzyme among bacterial lipases [3]. Figure 5B shows the further classification of *Staphylococcus* lipases into three subgroups based on their amino acid sequences. Subgroup I consists of *S. aureus* B56, *S. aureus* PS54, and

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ATGTTAAGAGGACAAGAAGAAAGAAAGTATAGTATTAGAAAGTATTCAATAGGCGTGGTGTCAGTGTTAGCGGCTACAATGTTTGTTGTG   90
 M  L  R  G  Q  E  E  R  K  Y  S  I  R  K  Y  S  I  G  V  V  S  V  L  A  A  T  M  F  V  V   30

TCATCACATGAAGCACAAGCCTCGGAAAAAACACCAACGTCCAATGCAGCGGCACAAAAAGAAACACTAAATCAACCGGGAGAACAAGGG  180
 S  S  H  E  A  Q  A  S  E  K  T  P  T  S  N  A  A  A  Q  K  E  T  L  N  Q  P  G  E  Q  G   60

AACGCGATAACGTCACATCAAATGCAGTCAGGAAAGCAATTAGACGATATGCATAAAGAGAATGGTAAAAGTGGAACAGTGACAGAAGGT  270
 N  A  I  T  S  H  Q  M  Q  S  G  K  Q  L  D  D  M  H  K  E  N  G  K  S  G  T  V  T  E  G   90

AAAGACACGCTTCAATCATCGAAGCATCAATCAACACAAAATAGTAAAACAATCAGAACGCAAAATGATAATCAAGTAAAGCAAGATTCT  360
 K  D  T  L  Q  S  S  K  H  Q  S  T  Q  N  S  K  T  I  R  T  Q  N  D  N  Q  V  K  Q  D  S  120

GAACGACAAGGTTCTAAACAGTCACACCAAAATAATGCGACTAATAATACTGAACGTCAAAATGATCAGGTTCAAAATACCCATCATGCT  450
 E  R  Q  G  S  K  Q  S  H  Q  N  N  A  T  N  N  T  E  R  Q  N  D  Q  V  Q  N  T  H  H  A  150

GAACGTAATGGATCACAATCGACAACGTCACAATCGAATGATGTTGATAAATCACAACCATCCATTCCGGCACAAAAGGTATTACCCAAT  540
 E  R  N  G  S  Q  S  T  T  S  Q  S  N  D  V  D  K  S  Q  P  S  I  P  A  Q  K  V  L  P  N  180

CATGATAAAGCAGCACCAACTTCAACTACACCCCCGTCTAATGATAAAACTGCACCTAAATCAACAAAAGCACAAGATGCAACCACGGAC  630
 H  D  K  A  A  P  T  S  T  T  P  P  S  N  D  K  T  A  P  K  S  T  K  A  Q  D  A  T  T  D  210

AAACATCCAAATCAACAAGATACACATCAACCCGCGCATCAAATCATAGATGCAAAGCAAGATGATACTGTTCGCCAAAGTGAACAGAAA  720
 K  H  P  N  Q  Q  D  T  H  Q  P  A  H  Q  I  I  D  A  K  Q  D  D  T  V  R  Q  S  E  Q  K  240

CCACAAGTTGGCGATTTAAGTAAACATATCGATGGTCAAAATTCCCCAGAGAAACCGACAGATAAAAATACTGATAATAAACAACTAATC  810
 P  Q  V  G  D  L  S  K  H  I  D  G  Q  N  S  P  E  K  P  T  D  K  N  T  D  N  K  Q  L  I  270

AAAGATGCGCTTCAAGCGCCTAAAACACGTTCGACTACAAATGCAGCAGCAGATGCTAAAAAGGTTCGACCACTTAAAGCGAATCAAGTA  900
 K  D  A  L  Q  A  P  K  T  R  S  T  T  N  A  A  A  D  A  K  K  V  R  P  L  K  A  N  Q  V  300

CAACCACTTAACAAATATCCAGTTGTTTTTGTACATGGATTTTTAGGATTAGTAGGCGATAATGCACCTGCTTTATATCCAAATTATTGG  990
 Q  P  L  N  K  Y  P  V  V  F  V  H  G  F  L  G  L  V  G  D  N  A  P  A  L  Y  P  N  Y  W  330

GGTGGAAATAAATTTAAAGTTATCGAGGAATTGAGAAAGCAAGGCTATAATGTACATCAAGCAAGTGTAAGTGCATTTGGTAGTAACTAT 1080
 G  G  N  K  F  K  V  I  E  E  L  R  K  Q  G  Y  N  V  H  Q  A  S  V  S  A  F  G  S  N  Y  360

GATCGCGCTGTAGAACTTTATTATTACATTAAAGGTGGTCGCGTAGATTATGGCGCAGCACATGCAGCTAAATACGGACATGAGCGCTAT 1170
 D  R  A  V  E  L  Y  Y  Y  I  K  G  G  R  V  D  Y  G  A  A  H  A  A  K  Y  G  H  E  R  Y  390

GGTAAGACTTATAAAGGAATCATGCCTAATTGGGAACCTGGTAAAAAGGTACATCTTGTAGGGCATAGTATGGGTGGTCAAACAATTCGT 1260
 G  K  T  Y  K  G  I  M  P  N  W  E  P  G  K  K  V  H  L  V  G  H  S  M  G  G  Q  T  I  R  420

TTAATGGAAGAGTTTTTAAGAAATGGTAACAAAGAAGAAATTGCCTATCATAAAGCGCATGGTGGAGAAATATCACCATTATTCACTGGT 1350
 L  M  E  E  F  L  R  N  G  N  K  E  E  I  A  Y  H  K  A  H  G  G  E  I  S  P  L  F  T  G  450

GGTCATAACAATATGGTTGCATCAATCACAACATTAGCAACACCACATAATGGTTCACAAGCAGCTGATAAGTTTGGAAATACAGAAGCT 1440
 G  H  N  N  M  V  A  S  I  T  T  L  A  T  P  H  N  G  S  Q  A  A  D  K  F  G  N  T  E  A  480

GTTAGAAAAATCATGTTCGCTTTAAATCGATTTATGGGTAACAAGTATTCGAATATCGATTTAGGATTAACGCAATGGGGCTTTAAACAA 1530
 V  R  K  I  M  F  A  L  N  R  F  M  G  N  K  Y  S  N  I  D  L  G  L  T  Q  W  G  F  K  Q  510

TTACCAAATGAGAGTTACATTGACTATATAAAACGCGTTAGTAAAAGCAAAATTTGGACATCAGACGATAATGCTGCCTATGATTTAACG 1620
 L  P  N  E  S  Y  I  D  Y  I  K  R  V  S  K  S  K  I  W  T  S  D  D  N  A  A  Y  D  L  T  540

TTAGATGGCTCTGCAAAATTGAACAACATGACAAGTATGAATCCTAATATTACGTATACGACTTATACAGGTGTGTCTTCACATACTGGT 1710
 L  D  G  S  A  K  L  N  N  M  T  S  M  N  P  N  I  T  Y  T  T  Y  T  G  V  S  S  H  T  G  570

CCATTAGGTTATGAAAATCCTGATTTAGGTACATTTTTCTTAATGGATACAACGAGTAGAATTATTGGTCATGATGCAAGAGAAGAATGG 1800
 P  L  G  Y  E  N  P  D  L  G  T  F  F  L  M  D  T  T  S  R  I  I  G  H  D  A  R  E  E  W  600

CGTAAAAATGATGGTGTCGTACCAGTGATTTCGTCGTTACATCCATCCAATCAACCATTTATTAATGTTACGAATGATGAACCTGCCACA 1890
 R  K  N  D  G  V  V  P  V  I  S  S  L  H  P  S  N  Q  P  F  I  N  V  T  N  D  E  P  A  T  630

CGCAGAGGTATCTGGCAAGTTAAACCAATCATACAAGGATGGGATCATGTCGATTTTATCGGTGTGGACTTCCTGGATTTCAAACGTAAA 1980
 R  R  G  I  W  Q  V  K  P  I  I  Q  G  W  D  H  V  D  F  I  G  V  D  F  L  D  F  K  R  K  660

GGTGCAGAACTTGCCAACTTCTATACAGGTATTATAAATGACTTGTTGCGTGTGGAAGCGACTGAAAGTAAAGGAACACAATTGAAAGCA 2070
 G  A  E  L  A  N  F  Y  T  G  I  I  N  D  L  L  R  V  E  A  T  E  S  K  G  T  Q  L  K  A  690

AGTTAA                                                                                     2076
 S ***                                                                                      691
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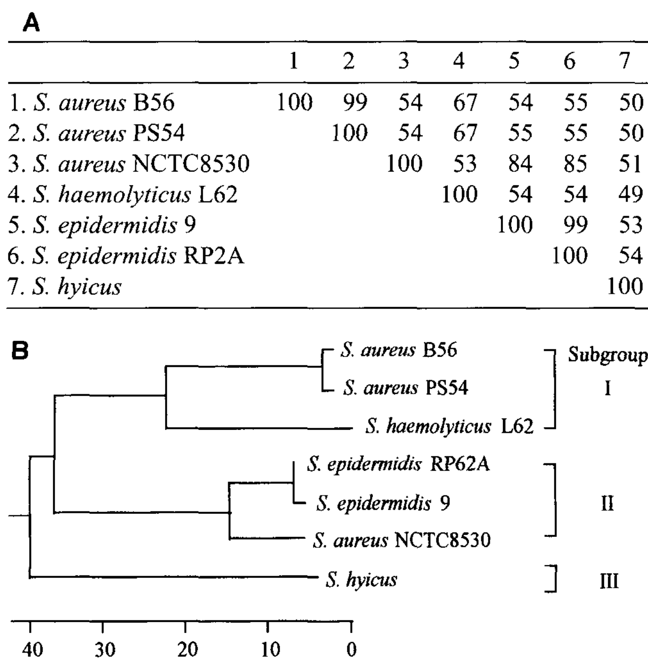
Fig. 4. Nucleotide and amino acid sequence of lipase B56.
The N-terminal five amino acid sequence of the proenzyme and mature enzyme are underlined. The sequence has been submitted to the GenBank under accession number AY028918.

S. haemolyticus L62 lipases, which have a higher than 67% homology. Subgroup II consists of *S. aureus* NCTC8530, *S. epidermidis* 9, and *S. epidermidis* RP62AM, which have the highest homology (≥84%). Finally, subgroup III contains *S. hyicus* lipase. Each subgroup of lipases also has certain enzymatic properties in common that are distinct from the



A

	1	2	3	4	5	6	7
1. *S. aureus* B56	100	99	54	67	54	55	50
2. *S. aureus* PS54		100	54	67	55	55	50
3. *S. aureus* NCTC8530			100	53	84	85	51
4. *S. haemolyticus* L62				100	54	54	49
5. *S. epidermidis* 9					100	99	53
6. *S. epidermidis* RP2A						100	54
7. *S. hyicus*							100

Fig. 5. Sequence homology and phylogenetic tree of staphylococcal lipases.

The amino acid sequence homologies of seven staphylococcal lipases were calculated (A). Their phylogenetic tree was built based on the amino acid sequences (B). The bottom line indicates the percent divergence.

other subgroups. First of all, the optimum pHs of subgroups I and III are pH 8–8.5 [7, 16], while those of subgroup II are pH 6.0–6.5 [12, 13]. Secondly, subgroups I and II exhibit a typical preference for short chain lipids, whereas subgroup III can hydrolyze any neutral lipid almost irrespective of their chain length as well as phospholipids [14, 15].

In conclusion, it is suggested that *Staphylococcus* lipases can be classified into at least three subgroups and that lipase B56 belongs to lipase subgroup I together with *S. aureus* PS54 and *S. haemolyticus* L62 lipases. It was also demonstrated that lipase B56 can be distinguished from the subgroup II lipases based on its optimum reaction pH (alkaline pH) and from the subgroup III lipases based on its substrate specificity (short chain preference).

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