Front. Biol. 2016, 11(4): 323–330
DOI 10.1007/s11515-016-1409-z

RESEARCH ARTICLE

Characterization of a newly identified lipase from a lipase-producing bacterium

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BACKGROUND: Lipases differ from one another with respect to certain properties, and such differences can be very important for various industrial applications. Considering the rapidly developing nature of the relevant industries, there is a need for new lipases with characteristics differing from those of existing enzymes.
METHODS: In this study, a bacterium was isolated from both the surface mucus layer and gills of rainbow trout (*Oncorhynchus mykiss*) from Giresun, Turkey. The bacterial species was identified based on its morphological and physiochemical properties, and on its 16S rDNA sequence. The qualitative activity of the bacterial lipase was determined on Rhodamine B and Tween-20 agar plates. The lipase was partially purified from the supernatant of bacterial cultures, and then characterized.
RESULTS: The bacterial strain was identified as *Acinetobacter* sp. strain SU15. The enzyme from *A*sp-SU15 exhibits maximum activity toward *p*-nitrophenyl dodecanoate (C_{12}) at 40°C and pH 8.0. The specific activity of the lipase was calculated to be 10.059 $U \cdot L^{-1}$. The molecular mass of the enzyme was determined to be ~62 kDa *via* SDS-PAGE. However, native-PAGE indicated that the enzyme forms very large active aggregates, with molecular masses exceeding 250 kDa. The catalytic activity of the enzyme is enhanced in the presence of Co^{2+}, Ca^{2+}, and methanol, but is partially inhibited by Ni^{2+}, ethyl acetate, and butanol.
CONCLUSIONS: Further research could examine possible industrial applications for the lipase from *A*sp-SU15.

Keywords *Acinetobacter* sp., lipase, *Oncorhynchus mykiss*, SDS-PAGE

Introduction

Lipases (EC 3.1.1.3) are a highly useful group of enzymes because of their versatility. They find use in many sectors, including the textile, food, biomedical, petrochemical, pharmaceutical, dairy, agrochemical, and cosmetics industries. They are also used in detergent formulations and the synthesis of novel compounds (Haliru and Bukola, 2012; Adan and Arslanoglu, 2013). Lipases are produced by animals, plants, and microorganisms, including Archaea (Wang et al., 2008; Adan and Arslanoglu, 2013). Of these, the lipases produced by microorganisms are the most industrially important and are used the most often (Haliru and Bukola, 2012). Microbial lipases have superior properties; for example, they can be readily obtained in a short time because the nutritional requirements of microorganisms are relatively simple. Moreover, microorganisms generally grow much faster than plants or animals, and they are amenable to convenient lipase-screening procedures. In addition, lipases from microorganisms are generally more stable at an alkaline or neutral pH (Schmidt-Dannert, 1999; Adan and Arslanoglu, 2013).

There are many lipase-producing bacterial strains, yet only a few such strains are commercially important. In particular, these include the genera *Achromobacter, Alcaligenes*, *Arthrobacter, Bacillus*, *Burkholderia, Chromobacterium*, and *Pseudomonas* (Jaeger et al., 1994; Gupta et al., 2004). Furthermore, many studies have been conducted on the lipases obtained from the *Acinetobacter* genus, and several lipolytic strains of *Acinetobacter* spp. have been isolated. These include *A. calcoaceticus* (Pandey et al., 1999;

Received April 15, 2016; accepted June 21, 2016
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Pratuangdejkul and Dharmsthiti, 2000; Wang et al., 2012), *A. radioresistens* (Liu and Tsai, 2003), *A. junii* (Yoon et al., 2004; Anbu et al., 2011), *A. johnsonii* G23 (Wang et al., 2011; Dai et al., 2013), *A. baumannii* (Huda, 2013), and other strains in the genus *Acinetobacter* (Wakelin and Forster, 1997; Kim et al., 2008).

In this study, a psychrotrophic bacterium was isolated from both the surface mucus layer and gills of rainbow trout. The bacterium was identified as *Acinetobacter* sp. strain SU15. It was found to produce a lipase, which was subsequently characterized. Lipase activity was unaffected by the addition of several ions or organic solvents, although its activity was partially inhibited by Ni^{2+}, ethyl acetate, and butanol. The results of this study suggest that future research may investigate possible applications of the lipase secreted from *Acinetobacter* sp. SU15 in various industrial areas.

Further research will be needed to determine the complete biochemical properties of the lipases from the *Acinetobacter* sp. described in this study, and the genes that encode them. This newly described lipase may become commercially important since other lipases from the *Acinetobacter* genus remain very stable in the presence of ions and organic solvents, and at a variety of temperatures and pH ranges (Chen et al., 1998; Wang et al., 2012).

Materials and methods

Bacterial strain and qualitative lipase activity

Fresh rainbow trout (*Oncorhynchus mykiss*) were obtained from a fish farm in Giresun, Turkey. The fish were packed in ice and transferred to the laboratory within 2 h. Samples were obtained from both the surface mucus layer and gills of the fish, and these were then spread on nutrient agar using a sterile swab. Plates were incubated at 30°C and 10°C for 2–3 days, and then bacterial colonies were chosen according to various morphological characteristics such as colony color, shape, and structure. The qualitative lipase activity of the bacteria was determined *via* two methods (Haba et al., 2000; Kukreja and Bera, 2005). The lipase from the bacterium was obtained as described by Rajan et al. (2011).

Identification of the bacterial strain

Gram staining was carried out and the bacterial strains were identified based on their morphological, physiological, and biochemical characteristics, as described in *Bergey's Manual of Systematic Bacteriology* (Krieg and Holt, 1984). The colors and shapes of the colonies were determined. The tolerance of the bacteria to NaCl (2%–12%), pH conditions (3.0–11.0), and temperatures (4–37°C) was then established.

For molecular identification of the strain, genomic DNA was extracted according to Sambrook et al. (1989). The 16S rDNA genes were amplified by PCR using genomic DNA and the following oligonucleotide primers: (UNI16SL: 5ATT CTA GAG TTT GAT CAT GGC TTC A3, and UNI16S-R: 5ATG GTA CCG TGT GAC GGG CGG TGT TGT A3).The amplified DNA fragments were sequenced by Macrogen, Europe. The 16S rDNA gene sequences were compared with entries in the updated GenBank database using the *BLAST* server. The sequences were then further characterized by phylogenetic analysis.

Phylogenetic analysis

The nucleotide sequences of the 16S rRNA genes were edited using *EditSeq*. The 16S rRNA gene sequences of one isolate from *Oncorhynchus mykiss*—together with those of 21 closely related species—were used in the phylogenetic analysis. The phylogenetic analysis was performed *via* the neighbor-joining method implemented using *MEGA 5.0* software (Tamura et al., 2004). The reliability of the phylogram was tested by bootstrap analyses of 1000 replicates using *MEGA 5.0*.

Quantitative lipase activity

Lipase activity was quantitatively analyzed using a spectrophotometric assay, with *p*-nitrophenyl dodecanoate (Sigma) as a substrate. The enzyme solution (100 µL) was added to the substrate solution (900 µL), which consisted of 1 part 30 mg *p*-nitrophenyl dodecanoate in 10 mL isopropanol, and 9 parts 0.1 g gum arabic and 2 mL Triton X-100 in 90 mL Tris-HCl buffer (pH 8.0). The reaction mixture was incubated for 15 min at 30°C and then immediately cooled for 10 min to 4°C. Lipase activity was measured at 410 nm (Kumar et al., 2012), and the enzyme activity was calculated in $U \cdot L^{-1}$ (Kumar et al., 2012).

Partial purification of the lipase

The culture of the lipase-producing strain was centrifuged (10000 $\times g$ for 10 min at 4°C) and the supernatant was used for precipitation. Solid ammonium sulfate was added to achieve 30% saturation of the supernatant, which was then stirred for 24 h at 4°C. The suspension was then centrifuged again (10000 $\times g$ for 10 min at 4°C). The precipitate was re-suspended in 50 mM Tris-HCl buffer (pH 8.0) and then stored at 4°C. Following this, ammonium sulfate was added to 50%, then and 80%, saturation of the remaining supernatant. The precipitates were suspended in 50 mM Tris-HCl buffer and stored at 4°C (Ugras et al., 2013). Protein concentration was determined colorimetrically using the Bradford assay (Bradford, 1976).

Effect of temperature and pH on lipase activity

The optimal temperature range for lipase activity was determined to be 10–90°C, at pH 8.0, after which the residual activity was determined. The effect of temperature on lipase

stability was established by analyzing the residual activity after incubation for 5–60 min at the optimal temperature (Lee et al., 1999). The optimal pH of the lipase was determined using various buffer solutions (50 mM), including sodium acetate (pH 4.0 and 5.0), potassium phosphate (pH 6.0 and 7.0), Tris-HCl (pH 8.0), and glycine-NaOH (pH 9.0 and 10.0) (Lee et al., 1999).

Effect of different substrates, metal ions, and organic solvents on lipase activity

The lipase activity was analyzed using various substrates, including *p*-nitrophenyl acetate, *p*-nitrophenyl dodecanoate, and *p*-nitrophenyl butyrate, *via* spectrophotometry (Kumar et al., 2012). The enzyme solution obtained from the bacterial supernatant was incubated at 30°C for 1 h with 5 mM and 10 mM of the metal ions present in $NiCl_2$, $ZnCl_2$, $CuCl_2$, $CoCl_2$, $MgCl_2$, and $CaCl_2$ (Lee et al., 1999), and then the residual activity was determined spectrophotometrically (Kumar et al., 2012). The effect on lipase activity of organic solvents, such as hexane, butanol, isopropanol, methanol, ethyl acetate, and ethanol, was analyzed according to Lee et al. (1999); lipase activity was measured spectrophotometrically (Kumar et al., 2012).

Detection of molecular weight via SDS-PAGE

The SDS-PAGE (12%) procedure was performed according to Laemmli (1970). Samples of the lipase were run on an SDS-PAGE gel and then compared with a marker, which contained 10–225 kDa peptides (Promega, USA). Following electrophoresis, the gel was stained with CBB R250.

Direct detection of lipase activity *via* native-PAGE

The native-PAGE (12%) procedure, which requires non-denaturing conditions, was performed as described by Laemmli (1970). Samples of partially purified lipase were run on a native-PAGE gel. Bovine serum albumin (BSA 1 mg mL^{-1}) was used as a marker. Following electrophoresis, the gel was sliced vertically. The first section, which included the samples of partially purified lipase and BSA, was stained with CBB R250. The other sections of the gel were assayed for direct detection. The direct lipase activity was performed as described in Park et al. (2007).

Results

The bacterial isolate was identified based on morphological, physiological, and biochemical characteristics, in addition to a phylogenetic analysis of 16S rDNA sequences. The 16S rRNA gene sequence consistently revealed a 99% similarity that from *Acinetobacter* sp. P162 (GenBank: KC904087); the phylogenetic analysis of the 16S rRNA genes also supports this identification (Fig. 1). Since the result agreed well with the findings of the biochemical and physiological analysis, the isolated bacterium was identified as *Acinetobacter* sp. P162, strain SU15. The isolate, *A*sp-SU15, is Gram-negative, psychrotolerant, catalase-positive, lipase-positive, and cellulase-positive. It forms smooth, opaque, irregularly shaped, cream-colored colonies on nutrient agar. The strain grows within a temperature range of 4–40°C. Its salt tolerance during growth was determined to be between 2% and 5% (w/v) NaCl, between pH 7.0–9.0, although growth was relatively weak at 45°C and pH 5.0 (Table 1).

In the current study, enzymatic activity was qualitatively indicated by the appearance of a visible precipitate (opaque halos), resulting from the deposition of calcium salt crystals, which was formed by the fatty acids released when either Tween 20 or Tween 80 was enzymatically hydrolyzed (Gopinath et al., 2005). Thus, *Acinetobacter* sp. SU15 secretes a lipase (Fig. 2).

The lipase was partially purified from the supernatant of bacterial cultures *via* ammonium sulfate precipitation (30%, 50%, and 80%). The specific activity of each fraction was calculated to be 5.242 $U \cdot g^{-1}$, 7.293 $U \cdot g^{-1}$, 10.059 $U \cdot g^{-1}$, respectively.

These results demonstrate that the enzyme exhibits optimum activity toward *p*-nitrophenyl dodecanoate (C_{12}) at 40°C, in Tris-HCl buffer at pH 8.0 (Table 2, and Figs. 3 and 4). The activity was calculated to be 9.793 $U \times L^{-1}$.

The activity of lipase obtained from *Asp*. SU15 became enhanced in the presence of Ca^{2+} and Co^{2+}, whereas enzymatic activity decreased in the presence of Ni^{2+}, ethyl acetate, or butanol. Furthermore, there were no significant differences in activity following the addition of ions or organic solvents, with only partial inhibition being observed (Figs. 5 and 6).

As shown in Fig. 7, the molecular mass of the enzyme (~62 kDa) was established by SDS-PAGE (Fig. 7C). However, native-PAGE indicated that the enzyme forms very large, active aggregates with high molecular masses (>250 kDa). Direct enzymatic activity was observed on native-PAGE for the aggregates with masses >250 kDa; however, no such activity was seen in the 62 kDa region of the gel (Fig. 7A and B).

Discussion

In this study, a psychrotolerant bacterium was isolated and identified as *Acinetobacter* sp. strain SU15. It was determined that *Acinetobacter* sp. SU15 produces a lipase. Many studies have been conducted on the lipases obtained from the *Acinetobacter* genus, and several lipolytic strains have been isolated (Liu and Tsai, 2003; Yoon et al., 2004). The present results demonstrated that the enzyme exhibits optimum activity toward *p*-nitrophenyl dodecanoate (C_{12}) at 40°C, in Tris-HCl buffer at pH 8.0. As reported, the optimal conditions

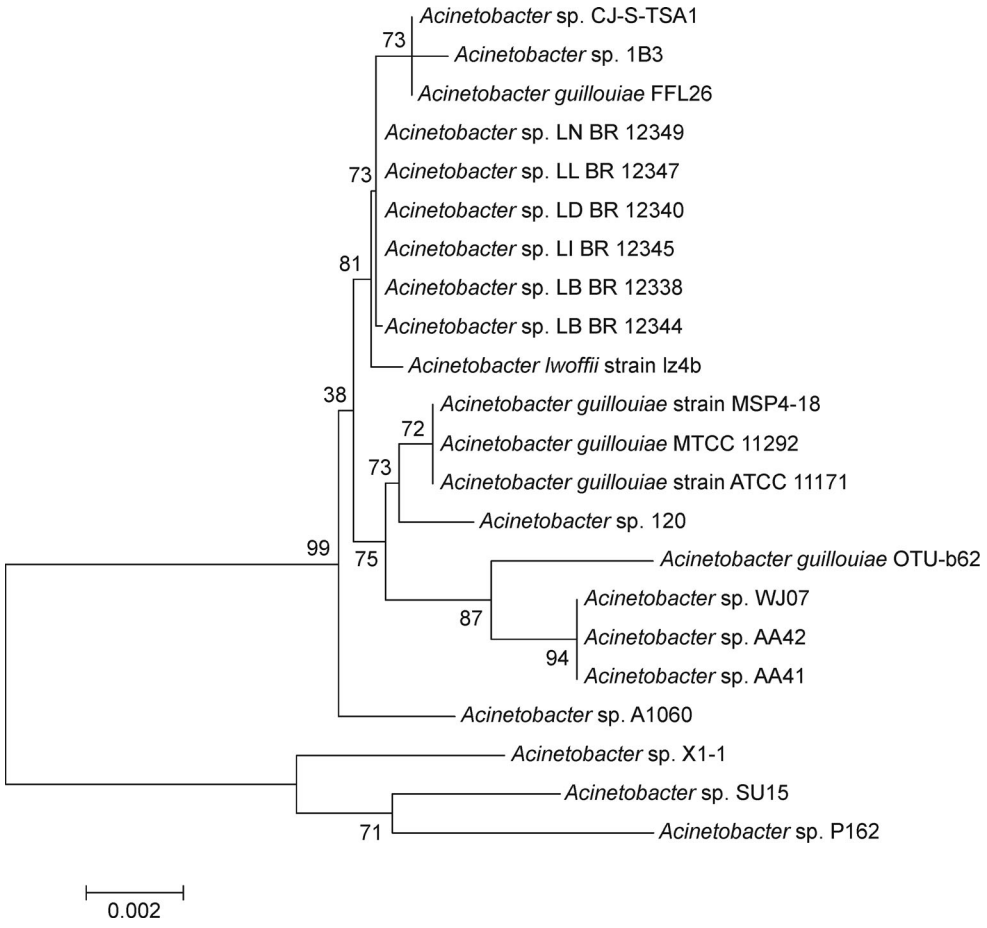

Figure 1 Neighbor-Joining tree of isolate Asp-SU15.

Table 1 Identification of *Acinetobacter* sp. SU15

Tests	Characteristics
Colony morphology	Cream, irregular, smooth, opaque colonies
Gram staining	Gram (−)
Temperature range	4°C–40°C; (45°C)*
NaCl Tolerance	2%–5%
pH range	7.0–9.0; (5.0)*
Enzyme production	
Lipase	+++
Amylase	−
Catalase	+++
Tryptophanase	−
Cellulose	+
Urease	−

(*), Weak growth; (+++), high activity; (+), low activity; (−), no activity.

Table 2 Lipase activity toward different substrates

Time (h)	Substrates	Activity ($U \cdot L^{-1}$)
24	PNPA	3.580
	PNPD	0
	PNPB	3.494
48	PNPA	4.462
	PNPD	8.411
	PNPB	5.154
72	PNPA	4.833
	PNPD	**9.793***
	PNPB	5.649

(*), the highest activity; PNPA, p-Nitrophenyl acetate; PNPD, p-Nitrophenyl dodecanoate; PNPB, p-Nitrophenyl butyrate.

for enzymatic activity differ among the lipases of the *Acinetobacter* genus. For example, the optimal activity of the lipase from *Acinetobacter* sp. O16 occurs at 35°C and pH 7.5 (Breuil and Kushner, 1975a, 1975b), that from *A. calcoaceticus* BD413 achieves its optimum at pH 7.8–8.8 (Kok et al., 1995), that from *A. radioresistens* CMC-1 achieves its optimum at pH 10.5 (Hong and Chang, 1988; Chen et al., 1998), that from *Acinetobacter* nov. sp. KM109 achieves its optimum at pH 8.0 (Mitsuhashi et al., 1999), while that from *Acinetobacter* sp. SY-01 achieves its optimum at 50°C and pH 10.0 (Han et al., 2003). The properties of the lipase described in the current study are similar to those of the others. Although other enzymes from bacteria in the *Acinetobacter* genus are generally stable at alkaline pH (such as 9.0–10.0), and thus are especially suited

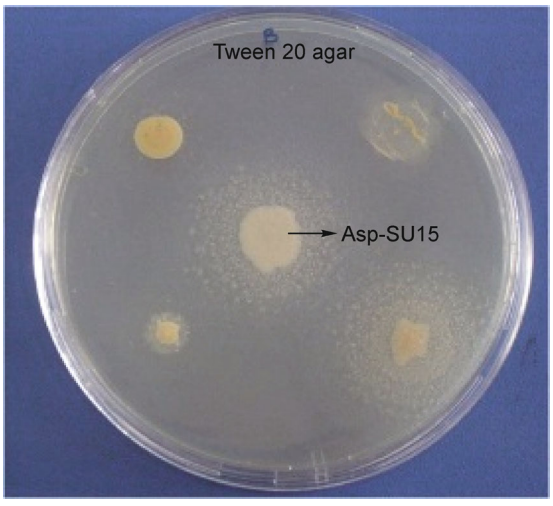

Figure 2 The lipase activity of isolate Asp-SU15.

to applications in the detergent industry (Hong and Chang, 1988; Wang et al., 2012), this enzyme lost 50% of its activity at pH 9.0. Nonetheless, the stability of this enzyme remained unaffected following 1 h at 40°C in Tris-HCl buffer (pH 8.0), permitting its application in a variety of areas.

The activity of the lipase was enhanced in the presence of Ca^{2+} and Co^{2+}. No significant difference in activity occurred upon the application of organic solvents or other ions, with only partial inhibition from butanol, ethyl acetate, and Ni^{2+}. Many studies have shown that the activity of lipases from *Acinetobacter* sp. O16, *A. calcoaceticus* BD413, and *Acinetobacter* sp. SY-01 can be increased Ca^{2+} ions (Breuil and Kushner, 1975a; Kok et al., 1995; Han et al., 2003). Similarly, in the current findings, the activity of the lipase increased upon addition of Ca^{2+} ions, which may arise from a dependence on Ca^{2+} for fatty acids release, or from stabilization of the structure of the enzyme upon binding of Ca^{2+} (Kouker and Jaeger, 1987; Wang et al., 2012).

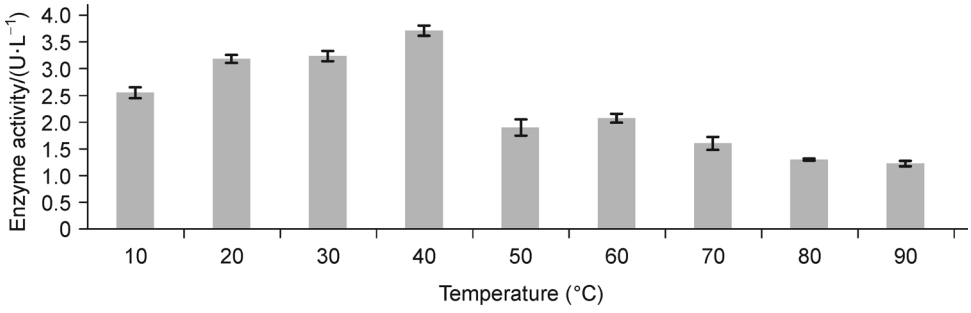

Figure 3 Effect of temperature on enzyme activity.

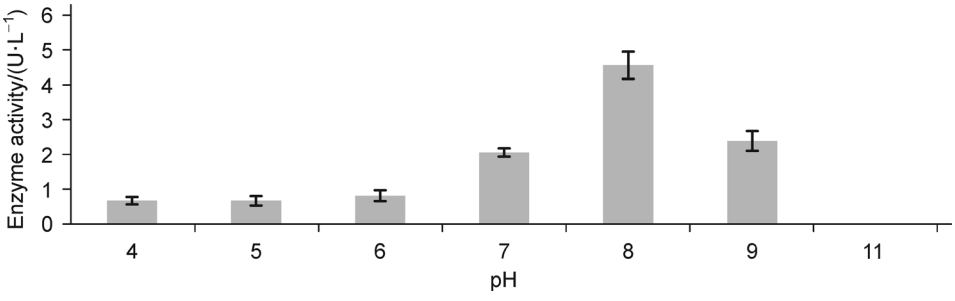

Figure 4 Effect of pH on enzyme activity.

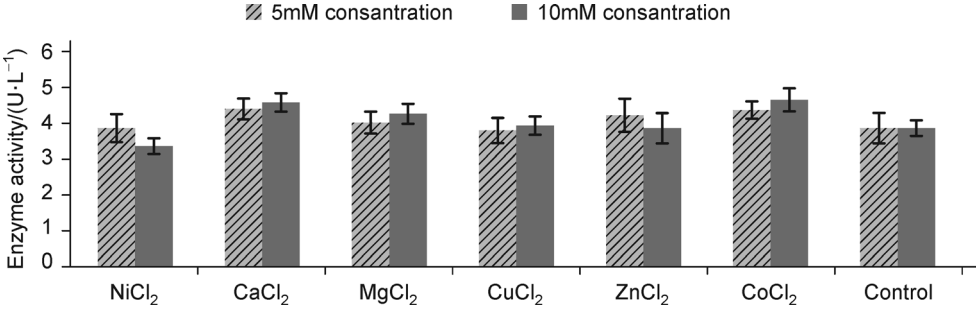

Figure 5 Effect of metal ions on enzyme activity.

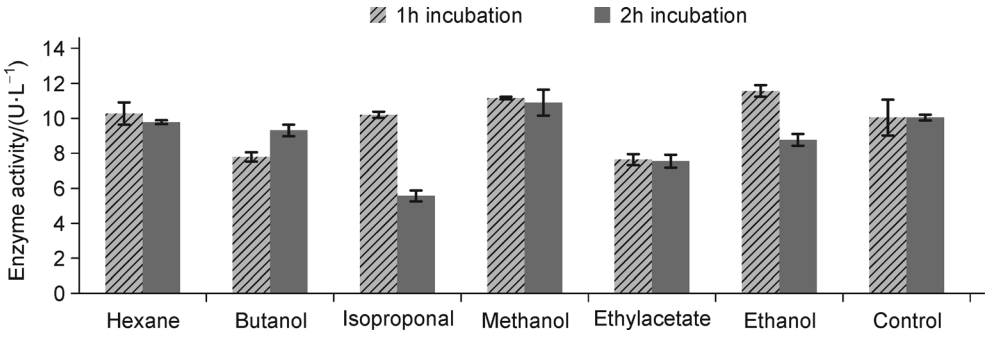

Figure 6 Effect of organic solvents on enzyme activity.

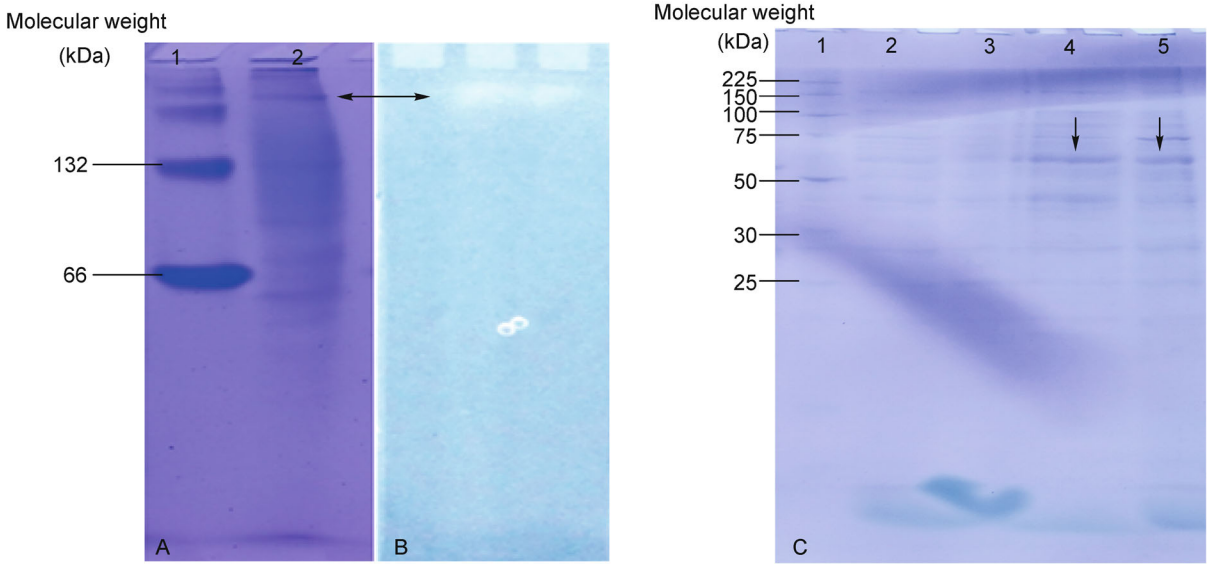

Figure 7 (A and B) Native-PAGE analysis of partially purified lipase enzyme. (A) 1, Marker; 2, partially purified lipase enzyme with 80% ammonium sulfate precipitation. (B) direct detection of lipase activity. The arrows correspond to the activity of lipase enzyme. (C) SDS-PAGE analysis of partially purified lipase enzyme. 1, Marker; 2, 0% ammonium sulfate precipitation (control); 3, 30% ammonium sulfate precipitation; 4, 50% ammonium sulfate precipitation; 5, 80% ammonium sulfate precipitation. The arrows correspond to the lipase enzyme.

The molecular mass of the enzyme was judged to be ~62 kDa by SDS-PAGE. The molecular masses of lipases from the *Acinetobacter* genus have been reported in previous studies, for example, 32 kDa from both *Acinetobacter* sp. ES-1 (Kasana et al., 2008) and *A. calcoaceticus* BD413 (Kok et al., 1995), 23 kDa from *A. calcoaceticus* LP009 (Pratuangdejkul and Dharmsthiti, 2000), 38 kDa from *A. radioresistens* CMC2 (Ng et al. 1999), 43.8 kDa from *Acinetobacter* sp. SY-01 (Han et al., 2003), 45 kDa from *A. radioresistens* CMC-1 (Hong and Chang, 1988; Chen et al., 1998), 53 kDa from *A. johnsonii* LP28 (Wang et al., 2011), and 62 kDa from *Acinetobacter* sp. KM109 (Mitsuhashi et al., 1999). The molecular mass of the newly described enzyme matches that of the lipase from *Acinetobacter* sp. KM109; however, as was discovered during native-PAGE, the new lipase forms very large, active aggregates (>250 kDa). An earlier study reported that *Acinetobacter* sp. O16 produces a lipase that forms aggregates greater than 200 kDa (Breuil and Kushner, 1975a, 1975b). Furthermore, Salameh and Wiegel found that the thermophilic alkaline lipases from *Thermosyntropha lipolytica* also form high molecular mass aggregates ($\geqslant 280$ kDa) on a gradient native-PAGE (Salameh and Wiegel, 2010). In addition, that study found that SDS prevented aggregation, and a monomeric species was observed following SDS-PAGE. Moreover, several other lipases have been found to form aggregates, including those from *Burkholderia cepacia* (Dunhaupt et al., 1992), *Geobacillus thermocatenulatus* (Rúa et al., 1997), *Bacillus subtilis* (Lesuisse et al., 1993) and a *Moraxella* sp. (Feller et al., 1990).

As a result of this study, a psychrotolerant bacterium was identified, and shown to be *Acinetobacter* sp., strain *A*sp-SU15. The strain *A*sp-SU15 produces industrially important enzymes such as lipases, cellulases, and catalases. The lipase was characterized, and found to exhibit optimal activity

toward *p*-nitrophenyl dodecanoate (C_{12}) at 40°C, in Tris-HCl buffer at pH 8.0. Its properties are both similar to, and different from, other lipases obtained from *Acinetobacter* strains. The results of this study suggest that future research should investigate possible applications of the lipase secreted from *Acinetobacter* sp. SU15 in different industrial processes.

Compliance with ethics guidelines

Serpil Ugras and Sebnem Uzmez declare that they have no conflict of interest. All institutional and national guidelines for the care and use of laboratory animals were followed.

Acknowledgements

The authors wish to thank Dr. Zihni DEMIRBAG (Black Sea Technical University, Turkey) for helping with the bacterial identification analysis. The authors also wish to thank Dr. Hatice KATI (Giresun University, Turkey) for helping with the enzyme analysis.

References

Adan G A, Arslanoglu A (2013). Purification and biochemical characterization of an extracellular lipase from psychrotolerant *Pseudomonas fluorescens* KE38. Turk J Biol, 37: 538–546

Anbu P, Noh M J, Kim D H, Seo J S, Hur B K, Min K H (2011). Screening and optimization of extracellular lipases by *Acinetobacter* species isolated from oil contaminated soil in South Korea. Afr J Biotechnol, 10: 4147–4156

Bradford M M (1976). A Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem, 72: 248–254

Breuil C, Kushner D J (1975a). Partial purification and characterization of the lipase of a facultatively psychrophilic bacterium (Acinetobacter O16). Can J Microbiol, 21(4): 434–441

Breuil C, Kushner D J (1975b). Lipase and esterase formation by psychrophilic and mesophilic *Acinetobacter* species. Can J Microbiol, 21(4): 423–433

Chen S, Cheng C, Chen T (1998). Production of an alkaline lipase by *Acinetobacter radioresistens.* J Ferment Bioeng, 86(3): 308–312

Dai Y, Zhang Y, Sun G, Li X, Wang H, Zhang L (2013). Optimization of lipase production by *Acinetobacter johnsonii* G23. Res J Biotech, 8 (12): 16–20

Dunhaupt A, Lang S, Wagner F (1992). *Pseudomonas cepacia* lipase: studies on aggregation, purification and on the cleavage of olive oil. Biotechnol Lett, 14: 953–958

Feller G, Thiry M, Arpigny J L, Mergeay M, Gerday C (1990). Lipases from psychrotrophic antarctic bacteria. FEMS Microbiol Lett, 66(1-3): 239–244

Gopinath S C B, Anbu P, Hilda A (2005). Extracellular enzymatic activity profiles in fungi isolated from oil-rich environments. Mycoscience, 46(2): 119–126

Gupta R, Gupta N, Rathi P (2004). Bacterial lipases: an overview of production, purification and biochemical properties. Appl Microbiol Biotechnol, 64(6): 763–781

Haba E, Bresco O, Ferrer C, Marqués A, Busquets M, Manresa A (2000). Isolation of lipase-secreting bacteria by deploying used frying oil as selective substrate. Enzyme Microb Technol, 26(1): 40–44

Haliru M, Bukola C A T (2012). Screening of microorganisms ısolated from different environmental samples for extracellular lipase production. AU J Technol, 15: 179–186

Han S J, Back J H, Yoon M Y, Shin P K, Cheong C S, Sung M H, Hong S P, Chung I Y, Han Y S (2003). Expression and characterization of a novel enantioselective lipase from *Acinetobacter* species SY-01. Biochimie, 85(5): 501–510

Hong M, Chang M (1988). Purification and characterization of an alkaline lipase from a newly isolated *Acinetobacter radioresistens* CMC-1. Biotechnol Lett, 20(11): 1027–1029

Huda J M (2013). Physicochemical Factors Affected the Partial Purified Lipase Activity of *Acinetobacter baumannii* local isolates. Iraqi J Pharm Sci, 22: 82–89

Jaeger K E, Dijkstra B W, Reetz M T (1999). Bacterial biocatalysts: molecular biology, three-dimensional structures, and biotechnological applications of lipases. Annu Rev Microbiol, 53(1): 315–351

Jaeger K E, Ransac S, Dijkstra B W, Colson C, van Heuvel M, Misset O (1994). Bacterial lipases. FEMS Microbiol Rev, 15(1): 29–63

Kasana R C, Kaur B, Yadav S K (2008). Isolation and identification of a psychrotrophic *Acinetobacter* sp. CR9 and characterization of its alkaline lipase. J Basic Microbiol, 48(3): 207–212

Kim S H, Park I H, Lee S C, Lee Y S, Zhou-Yi , Kim C M, Ahn S C, ChoiY L (2008). Discovery of three novel lipase (lipA1, lipA2, and lipA3) and lipase-specific chaperone (lipB) genes present in *Acinetobacter* sp. DYL129. Appl Microbiol Biotechnol, 77(5): 1041–1051

Kok R G, van Thor J J, Nugteren-Roodzant I M, Brouwer M B, Egmond M R, Nudel C B, Vosman B, Hellingwerf K J (1995). Characterization of the extracellular lipase, LipA, of *Acinetobacter calcoaceticus* BD413 and sequence analysis of the cloned structural gene. Mol Microbiol, 15(5): 803–818

Kouker G, Jaeger K E (1987). Specific and sensitive plate assay for bacterial lipases. Appl Environ Microbiol, 53(1): 211–213

Krieg N R, Holt J G (1984) Bergy's Manual of Determinative Bacteriology. volume 1, Baltimore, The Williams and Wilkins Co.

Kukreja V, Bera M B (2005). Lipase from *Pseudomanas aeriginosa* MTCC 2488: Partial purification, characterization and calcium dependent thermostability. Int J Biotechnol, 4: 222–226

Kumar R, Sharma A, Kumar A, Singh D (2012). Lipase from *Bacillus pumilus* RK31: Production, purification and some properties. W Appl Sci J, 16: 940–948

Laemmli U K (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227(5259): 680–685

Lee D, Koh Y, Kim K, Kim B, Choi H, Kim D, Suhartono M T, Pyun Y (1999). Isolation and characterization of a thermophilic lipase from *bacillus thermoleovorans* ID-1. FEMS Microbiol Lett, 179(2): 393–400

Lee K W, Bae H A, Shin G S, Lee Y H (2006). Purification and catalytic properties of novel enantioselective lipase from *Acinetobacter* sp. ES-1 for hydrolysis of (*S*)-ketoprofen ethyl ester. Enzyme Microb Technol, 38(3-4): 443–448

Lesuisse E, Schanck K, Colson C (1993). Purification and preliminary characterization of the extracellular lipase of *Bacillus subtilis* 168, an

extremely basic pH-tolerant enzyme. Eur J Biochem, 216(1): 155–160

Liu I L, Tsai S W (2003). Improvements in lipase production and recovery from Acinetobacter radioresistens in presence of polypropylene powders filled with carbon sources. Appl Biochem Biotechnol, 104(2): 129–140

Mitsuhashi K, Yamashita M, Hwan Y S, Ihara F, Nihira T, Yamada Y (1999). Purification and characterization of a novel extracellular lipase catalyzing hydrolysis of oleyl benzoate from *Acinetobacter* nov. sp. strain KM109. Biosci Biotechnol Biochem, 63(11): 1959–1964

Ng I S, Tsai S W, Chen S J (1999). Purification and characterization of extracellular lipase from Acinetobacter radioresistens CMC-2. J Chin Inst Chem Eng, 30: 355–362

Pandey A, Benjamin S, Soccol C R, Nigam P, Krieger N, Soccol V T (1999). The realm of microbial lipases in biotechnology. Biotechnol Appl Biochem, 29(Pt 2): 119–131

Park H J, Jeon J H, Kang S G, Lee J H, Lee S A, Kim H K (2007). Functional expression and refolding of new alkaline esterase, EM2L8 from deep-sea sediment metagenome. Protein Expr Purif, 52(2): 340–347

Pratuangdejkul J, Dharmsthiti S (2000). Purification and characterization of lipase from psychrophilic Acinetobacter calcoaceticus LP009. Microbiol Res, 155(2): 95–100

Rajan A, Kumar D R S, Nair J (2011). Isolation of a novel alkaline lipase producing fungus *Aspergillus fumigatus* MTCC 9657 from aged and crude rice bran oil and quantification by HPTLC. Inter J Biol Chem, 5 (2): 116–126

Rúa M L, Schmidt-Dannert C, Wahl S, Sprauer A, Schmid R D (1997). Thermoalkalophilic lipase of *Bacillus thermocatenulatus* large-scale production, purification and properties: aggregation behaviour and its effect on activity. J Biotechnol, 56(2): 89–102

Salameh M A, Wiegel J (2010). Effects of detergents on activity, thermostability and aggregation of two alkali thermophilic lipases from *Thermosyntropha lipolytica.* Open Biochem J, 4: 22–28

Sambrook J, Fritsch E F, Maniatis T (1989). Molecular Cloning: a laboratory manual. (Cold Spring Harbor Laboratory Press, 1659

Schmidt-Dannert C (1999). Recombinant microbial lipases for biotechnological applications. Bioorgan Med Chem, 7: 2123–2130

Tamura K, Nei M, Kumar S (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. Proc Natl Acad Sci USA, 101(30): 11030–11035

Ugras S, Sezen K, Katı H, Demirbag Z (2013). Purification and characterization of the bacteriocin Thuricin Bn1 produced by *Bacillus thuringiensis* subsp. *kurstaki* Bn1 isolated from a hazelnut pest. J Microbiol Biotechnol, 23(2): 167–176

Wakelin N G, Forster C F (1997). An investigation into microbial removal of fats, oils and greases. Bioresour Technol, 59(1): 37–43

Wang C H, Guo R F, Yu H W, Jia Y M (2008). Cloning and sequence analysis of a novel cold-adapted lipase gene from strain lip35 (*Pseudomonas* sp.). Agric Sci China, 7(10): 1216–1221

Wang H, Zhong S, Ma H, Zhang J, Qi W (2012). Screening and characterization of a novel alkaline lipase from *Acinetobacter calcoaceticus* 1-7 Isolated from bohai bay in china for detergent formulation. Braz J Microbiol, 43(1): 148–156

Wang H K, Shao J, Wei Y J, Zhang J, Qi W (2011). A novel low-temperature alkaline lipase from *Acinetobacter johnsonii* LP28 suitable for detergent formulation. Food Technol Biotechnol, 49: 96–102

Yoon M Y, Shin P K, Han Y S, Lee S H, Park J K, Cheong C S (2004). Isolation of an *Acinetobacter junii* SY-01 strain producing an extracellular lipase enantioselectively hydrolyzingitraconazole precursor and some properties of the lipase. J Microbiol Biotechnol, 14: 97–104