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A Novel Low-Temperature Alkaline Lipase from *Acinetobacter johnsonii* LP28 Suitable for Detergent Formulation

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Summary

A strain LP28 that produces alkaline and low-temperature lipase was isolated from the soil collected from the Bay of Bohai, PR China and identified as *Acinetobacter johnsonii* using 16S rDNA sequencing. The lipase was purified to homogeneity by centrifugation, followed by ammonium sulphate precipitation, dialysis, ion exchange chromatography on cellulose DE-52 and gel filtration chromatography on Sephadex G-75. The enzyme was purified about 34-fold with a final yield of 13 % and the relative molecular mass of the enzyme was determined to be 53 kDa by SDS-PAGE. The purified enzyme exhibited maximum activity at 30 °C and pH=9.0, and retained 94.53 % of its maximum activity at 20 °C. The enzyme was stable at 50 °C and retained 80.9 % of its original activity for 30 min. It was also highly stable in a pH range of 8.0–11.0. The enzyme hydrolyzed a wide range of oils and showed a high level of lipase activity in hydrolyzing tributyrin. The enzyme activity was promoted in the presence of Na^+, Ca^{2+}, K^+, Mg^{2+} and sodium citrate. Ba^{2+}, Mn^{2+}, Cr^{3+} and Co^{2+} did not affect the enzyme activity, whereas the presence of Al^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Zn^{2+} and EDTA reduced the enzyme activity. Regarding the stability of detergent process, the enzyme was highly stable in the presence of various oxidizing agents, some commercial detergents and alkaline protease, and its activity was also promoted by most of the surfactants, *viz.* Tween 20, Tween 80, sodium cholate, sodium taurocholate and saponin. For these characteristics, the lipase from *Acinetobacter johnsonii* LP28 showed good potential as an additive in laundry detergent formulation.

Key words: Acinetobacter johnsonii, alkaline lipase, low-temperature lipase, detergent formulation

Introduction

Lipases (triacylglycerol ester hydrolases, EC 3.1.1.3) are enzymes that catalyze the breakdown of fats and oils with subsequent release of free fatty acids, diacylglycerols, monoacylglycerols and glycerol (*1*). They are ubiquitous in nature and are produced by various animals, plants, fungi and bacteria. Furthermore, microbial lipases are currently receiving much attention with the rapid development of enzyme technology (*2*). Many microbial lipases are available for use as commercial products, the majority of which are used in detergents, cosmetics, production processes, food flavourings, and organic syntheses. Major commercial lipase-producing bacteria are *Achromobacter*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Burkholderia*, *Chromobacterium* and *Pseudomonas* (*3*). Nevertheless, the interest in *Acinetobacter* lipase has increased recently (*4–10*). *Acinetobacter* sp. is a strictly aerobic, Gram-negative coccobacillus that is ubiquitous in geographical distribution (*11*). Several lipolytic strains of *Acinetobacter* sp. that produce lipases at low temperature have been isolated

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(*12–15*). However, little attention has been paid to the process stability for detergent formulations. Typically a detergent lipase needs to be active and stable in alkaline environments encountered under harsh washing conditions (pH=8–11, temperature 20–50 °C) and in the presence of salt and surfactants (*7*). Here, a strain LP28 producing low-temperature alkaline lipase in a stable process is described.

Materials and Methods

Isolation and screening of lipase-producing microorganisms

Alkaline lipase-producing microorganisms were isolated from the soil with an olive oil alkaline plate, which contained olive oil as the sole carbon source and the soil was collected from the Bay of Bohai, PR China. Soil samples were inoculated in 50 mL of enrichment medium composed of (in g/L of distilled water): olive oil (Moreno, Spain) 20, yeast extract (Oxoid, Ltd, Basingstoke, UK) 5, K_2HPO_4 1, $MgSO_4{\cdot}7H_2O$ 0.5, $CaCl_2$ 0.01, pH=9.5. The flasks were incubated at 25 °C and 180 rpm for 72 h. After inoculation to another set of enrichment flask, the culture was spread on the screening plates containing (in g/L of distilled water): Victoria Blue B (Sinopharm Chemical Reagent Co. Ltd, Shanghai, PR China) 2, K_2HPO_4 1, $NaNO_3$ 3, $MgSO_4{\cdot}7H_2O$ 0.5, $FeSO_4{\cdot}7H_2O$ 0.01, olive oil emulsion 20, agar (Bejing Solarbio Science & Technology Co, Ltd, Bejing, PR China) 20, pH=9.5. Plates were incubated at 25 °C for 48 h, growing colonies with blue zones were isolated and transferred to slants which contained (in g/L of distilled water): peptone (Oxoid, Ltd) 10, yeast extract 5, NaCl 10, pH=8.0. The lipase activity was estimated by Victoria Blue plate method, as described below. By rough estimation, the activity of lipase from *Acinetobacter johnsonii* strain LP28 was high at low temperature and in the alkaline range. This lipase has the highest stability at room temperature, so strain LP28 was chosen for the following experiments.

Identification of strain LP28 was conducted using 16S ribosomal DNA (rDNA) analysis (*16*). The genomic DNA was extracted according to the method described by the instructions of the Genome DNA Extraction Kit (Beijing Biofuture Institute of Bioscience & Biotechnology Development, Beijing, PR China). Two primers, F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and R (5′-CTA CGG CTA CCT TGT TAC GA-3′), were used for the PCR (*17*). PCR amplification was carried out as follows: 94 °C for 1 min; 94 ºC for 45 s; 55 °C for 45 s; 72 °C for 90 s, 30 cycles; and 72 °C for 10 min. PCR were analyzed by agarose gel electrophoresis, and DNA was screened by gel documentation system (GeneGenius, Syngene, Frederick, MD, USA). The sequence analysis was performed by Shanghai Sunny Biotech Co, Ltd (Shanghai, PR China). A homology search to reference the strains registered in DDBJ/EMBL/GenBank was performed using NCBI BLAST.

Lipase production

Acinetobacter johnsonii LP28 was grown in a liquid medium containing (in g/L of distilled water): soluble starch 10, bean flour 20, corn syrup 20 (all obtained from Bejing Shuangxuan Microbe Culture Medium Products Factory, Bejing, PR China), K_2HPO_4 1, and olive oil emulsion 20, pH=9.0. Olive oil was emulsified 1:3 (by volume) in a blender (FSH-2, Jintan Ronghua Instrument Manufacture Co, Ltd, Jintan, PR China) in distilled water containing 20 g/L of polyvinyl alcohol (PVA) at the maximum speed for 10 min. Culture conditions were 28 °C and 180 rpm in a rotary shaker (HYG II, Shanghai Xinrui Automation Equipment Company, PR China), in 250-mL flasks containing 30 mL of medium. An aliquot of 0.6 mL of a 10-hour preculture in the above medium without olive oil was used as inoculum.

Crude enzyme was obtained by centrifugation (CR21G, Hitachi Ltd, Tokyo, Japan) at 10 000 rpm and 4 °C for 10 min. The cell-free supernatant was considered as crude enzyme.

Lipase activity

Lipase activity was determined by the following three methods. The first one is Victoria Blue plate method (*18*). The plate containing 10 % (by mass per volume) emulsion of olive oil with 0.2 % (by mass per volume) Victoria Blue B was adjusted to different pH values using 0.1 M phosphate buffer (pH=5.0–9.0) and 0.05 M glycine--NaOH buffer (pH=9.0–10.6). To estimate the lipase activity, 4-mm holes were punched into the agar and filled with 20 μL of cell-free culture supernatant. The plate was incubated for 24 h at 25 °C. Lipase activity was determined by the change in the blue zones.

The second one is a spectrophotometric method (*19*) with *p*-nitrophenyl palmitate (*p*NPP) (Sigma-Aldrich China Inc, Shanghai, PR China) as the substrate. Solution 1 contained *p*NPP (30 mg) dissolved in propane-2-ol (10 mL), solution 2 contained Triton X-100 (Beijing Biofuture Institute of Bioscience & Biotechnology Development, Beijing, PR China) (1 mL) dissolved in 90 mL of buffer (phosphate 0.1 M, pH=8.0). The assay solution was prepared by adding solution 1 to solution 2. The assay mixture contained 900 μL of the emulsion and 100 μL of the appropriately diluted lipase (boiled for 10 min as blank) solution. The reaction was performed at 30 °C for 15 min and terminated at 4 °C for 10 min. The liberated *p*-nitrophenol was measured at 410 nm with a spectrophotometer (TU-1810, Beijing General Instrument Co, Ltd, Beijing, PR China). One unit of lipase was defined as the amount of lipase that releases 1 mmol of *p*-nitrophenol per min at 30 °C (*19*).

Since the *p*NPP was not stable at pH>8.3, all enzyme assays at pH>8.0 were carried out by alkali titration assay as described by Nahas (*20*) with some modifications. The substrate was the emulsion of olive oil, which was adjusted to different pH values using 0.1 M phosphate buffer (pH=5.0–9.0) and 0.05 M glycine–NaOH buffer (pH=9.0–10.6). The reaction mixture contained 4 mL of the substrate, 5 mL of buffer, and 1 mL of crude enzyme solution. After incubation at 30 °C for 1 h, the reaction was terminated by the addition of 10 mL of acetone/ethanol (1:1, by the volume). The resulting mixture was then titrated to pH=10.5 against 0.05 M NaOH. Blanks obtained with enzyme samples boiled for 10 min were subtracted and the activities were expressed as μmol of free fatty acids released. One unit of lipase activity was

defined as the amount of enzyme that releases 1 μmol of free fatty acids per hour and was equal to 0.75 U/mL defined by spectrophotometric assay.

Lipase purification

The cell-free supernatant was obtained by centrifugation at 10 000 rpm at 4 °C for 10 min. To the crude enzyme, ammonium sulphate was slowly added to obtain 25 % saturation at 4 °C. The formed precipitates were separated by centrifugation (8000 rpm for 10 min at 4 °C). These precipitates were then dissolved in 0.1 M phosphate buffer, pH=8.0, dialyzed against distilled water and kept at 4 °C for 18 h. The dialyzed material was centrifuged (8000 rpm for 10 min at 4 °C) and the supernatant was retained for further purification by cellulose DE-52 (Whatman International Ltd, Maidstone, UK) and Sephadex G-75 (Pharmacia, Stockholm, Sweden) column chromatography.

Step 1: Cellulose DE-52 column chromatography

After dialysis, the supernatant containing enzyme protein was applied to a cellulose DE-52 column (1.6×20 cm, Shanghai Huamei Experimental Instrument Factory, Shanghai, PR China), which had been equilibrated with 10 mM Tris-HCl buffer, pH=8.0 (buffer A). After washing with two bed volumes of buffer A, the elution was performed with a negative linear gradient of 0–1.0 M NaCl at a flow rate of 60 mL/h with a constant flow pump (DHL-A, Shanghai Qingpu-Huxi Instruments Factory, Shanghai, PR China). The fractions (4.0 mL) were collected by a fraction collector (DBS-100, Shanghai Qingpu-Huxi Instruments Factory) and analyzed for lipase activity and protein content. The active fractions were pooled and concentrated to 2.0 mL with PEG-20000 (Bio-Sharp, Beijing, PR China).

Step 2: Sephadex G-75 column chromatography

The concentrated sample obtained after cellulose DE-52 column chromatography (1.0 mL) was applied on a Sephadex G-75 column (1.6×80 cm, Shanghai Huamei Experimental Instrument Factory), which was equilibrated with 0.1 M phosphate buffer (pH=8.0) and then the lipase was eluted with the same buffer, pH=8.0, at a flow rate of 42 mL/h, with a constant flow pump. The fractions (5.0 mL) were collected by a fraction collector and assayed for lipase activity and protein content. The active fractions were pooled, concentrated and analyzed for purity by SDS-PAGE (BioDev-Tech, Beijing, PR China).

Determination of molecular mass

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using the method of Laemmli (*21*) on a 4 % (by mass per volume) polyacrylamide-stacking gel and a 12 % (by mass per volume) polyacrylamide-resolving gel in an electrophoresis cell (DYCE-24DN, Beijing Wode-Life Sciences Instrument Co, Beijing, PR China) with the electrophoresis apparatus (DYY-6B, Beijing Wode-Life Sciences Instrument Co). Protein bands were visualized by staining with Coomassie Brilliant Blue R250 (Amresco Inc, Solon, OH, USA) and M_r was estimated by comparison with broad range molecular mass standards from 14.3 to 116 kDa.

Lipase characterization

The effect of temperature on lipase activity was determined by the spectrophotometric assay at different temperatures (10–50 °C) and pH=8.0. For studying thermal stability, 1 mL of lipase was mixed with 9 mL of 0.1 M phosphate buffer (pH=8.0) and incubated in a temperature range of 30–80 °C for 30 min. Residual lipase activity was measured by the spectrophotometric assay described above.

Optimal pH was determined by the alkali titration assay at 30 °C in a pH range of 5.0–11.0 using different buffers. For pH stability studies, 1 mL of lipase was mixed with 9 mL of the buffers in a pH range of 5.0–11.0 (0.1 M phosphate buffer for pH=5.0–9.0, 0.05 M glycine-NaOH buffer for pH=9.0–11.0) and incubated at 30 °C for 1 h. Subsequently, the residual enzymatic activity was determined by the spectrophotometric assay.

Lipase activity on different types of oil and triglycerides was studied using alkali titration assay as described above. The olive oil emulsion was replaced by different types of oil and triglycerides. The effect of 0.1, 1, and 10 mM of various metal ions was determined by incubating the lipase for 1 h at 30 °C in 0.1 M phosphate buffer (pH=8.0). The lipase activity was determined by the spectrophotometric assay.

Lipase from *A. johnsonii* strain LP28 was characterized for its potential application in the detergent industry. The lipase sample was incubated in the presence of surfactants, oxidants, alkaline protease (Noao, Tianjin, PR China) and different kinds of commercial detergents at 1 % for 1 h at 30 °C and lipase activity was determined by the spectrophotometric assay at pH=8.0 and 30 °C. All the experiments of characterization study were performed three times and all the relevant chemicals were procured from the Tianjin Huaxue Company (Tianjin, PR China) unless specified differently.

Results and Discussion

Strain selection and identification

A total of 354 bacterial isolates from the soil were screened for lipase production, among which 49 isolates were isolated for the low-temperature and alkaline lipase activity.

The purpose of searching for new sources of lipases is their possible future applications requiring not only enzyme-substrate specificity but also process stability (*4,22*). Thus, *A. johnsonii* strain LP28 was isolated for its high lipase activity and the highest stability at room temperature, which retained 93.78 % activity after remaining for 48 h at room temperature (Fig. 1).

The full 16S rDNA of *A. johnsonii* strain LP28 was cloned (Fig. 2) and the sequence was analysed using GenBank database, which showed 100 % homology with the sequence of *Acinetobacter johnsonii* (GenBank accession number: DQ911549.1). *Acinetobacter* sp. with the lipase activity had previously been reported. The lipase from *Acinetobacter lwoffii* CR9 exhibited maximum activity at 40 °C and pH optimum at 8.0 (*8*). A novel enantioselective lipase from *Acinetobacter* sp. ES-1 showed a high enantioselectivity, and the optimum temperature and pH were

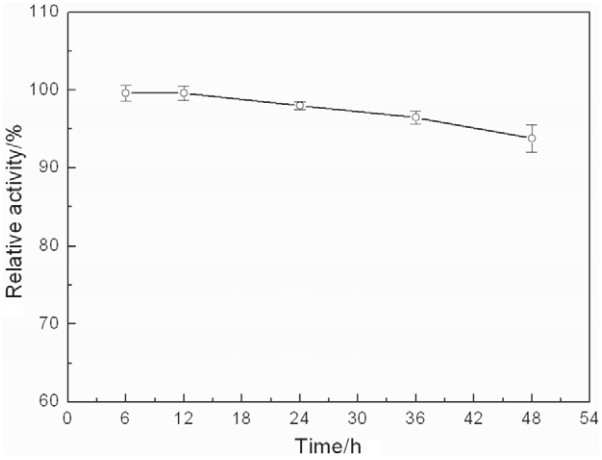

Fig. 1. The stability of lipase from *Acinetobacter johnsonii* LP28 at room temperature

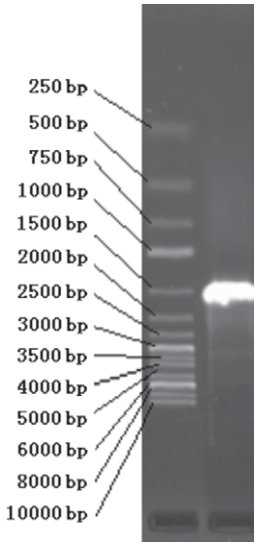

Fig. 2. Agarose gel electrophoresis analysis of 16S rDNA from *Acinetobacter johnsonii* LP28

40 ºC and 7.0, respectively (*7*,*23*). A lipase from *Acinetobacter* sp. RAG-1 was studied and showed good potential for use as a biocatalyst (*24*). *Acinetobacter calcoaceticus* LP009 isolated from raw milk produced a lipase that had the ability to improve fat hydrolysis in soybean meal and in premixed animal feed (*25*). In another investigation, the lipase from *Acinetobacter junii* SY-01 displayed a good enantioselectivity and the optimum pH and temperature were 7.0–9.0 and 45–50 °C, respectively (*5*).

However, limited scientific data is available on the lipase from *Acinetobacter johnsonii*. This has been the first report about *Acinetobacter johnsonii* producing the low--temperature alkaline lipase to our knowledge, and the lipase has shown a potential application in the detergent industry.

Enzyme purification

In previous investigations, a three- or four-step purification protocol had been followed by most of the workers, and the lipase from *Acinetobacter johnsonii* LP28 was purified employing a three-step procedure. As summarized in Table 1, the enzyme was purified about 34-fold with a final yield of 13 % after gel filtration on Sephadex G-75 column.

As shown in Fig. 3, the purified lipase was homogenous and had a molecular mass of 53 kDa determined by analytical SDS-PAGE. As reported, the molecular mass of lipase from *Acinetobacter* sp. was variable to some extent, from 23 to 62 kDa. The most frequently reported molecular mass for lipase from *Acinetobacter* sp. was 32 kDa, *viz*. *Acinetobacter* sp. ES-1 (*7*), *Acinetobacter venetianus* RAG-1 (*9*), *Acinetobacter* sp. RAG-1 (*24*), and *A. calcoaceticus* BD413 (*26*). Other investigated strains were *A. junii* SY-01 40 kDa (*5*), *Acinetobacter* sp. B2 60 kDa (*6*), *A. calcoaceticus* LP009 23 kDa (*25*), *A. radioresistens* CMC-1 45 kDa (*27*), *Acinetobacter* sp. KM109 62 kDa (*28*) and *Acinetobacter* CMC-2 38 kDa (*29*). In the present study a purified lipase from *Acinetobacter johnsonii* LP28 with a molecular mass of 53 kDa has been found.

Characterization of the lipase for detergent formulation

As shown in Fig. 4, the optimum temperature for the lipase from *Acinetobacter johnsonii* LP28 was 30 °C, lower than all the other lipases reported from *Acinetobacter* (35–55 ºC) (*6–9*,*12*). It retained 87.89 and 94.53 % of its maximum activity at 15 and 20 °C, respectively. On the other hand, compared to lipase from *Acinetobacter* sp. RAG-1 (*24*) and *A. calcoaceticus* LP009 (*25*), the enzyme showed higher stability at high temperature as it retained 80.9 % of the activity at 50 °C and 30.6 % at 60 °C (Fig. 4). Thus, both characteristics, activity at low temperature and thermostability suggest its usefulness in industrial applications.

Acinetobacter johnsonii LP28 was found to be the most active at pH=9.0 (Fig. 5) and showed activity in a wide pH range (8.0–11.0), similar to that reported for lipase from *Acinetobacter* sp. RAG-1 (pH=9.0) (*24*) and *A. junii*

Table 1. Purification of the lipase from *Acinetobacter johnsonii* LP28

Purification step	Volume	Total lipase activity	*m*(total protein)	Specific lipase activity	Yield	Purification
	mL	U	mg	U/mg	%	fold
crude	300.00	927	480.00	1.93	100	1.00
precipitation	69.00	673	70.38	9.57	73	4.96
ion exchange	9.30	273	6.60	41.28	29	21.39
gel filtration	2.90	125	1.89	66.09	13	34.24

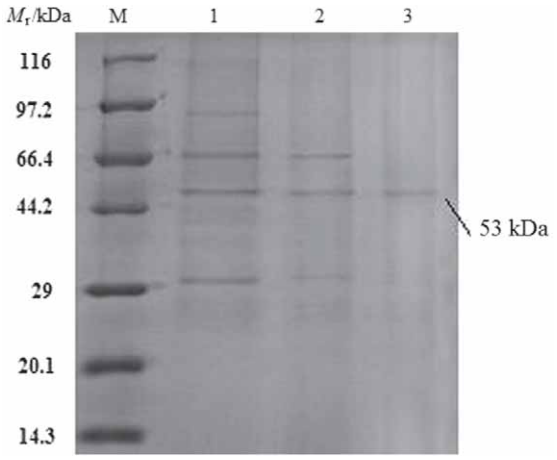

Fig. 3. SDS-PAGE analysis of lipase from *Acinetobacter johnsonii* LP28 at various stages of purification: lane M: molecular mass markers, lane 1: crude enzyme, lane 2: ion exchange, lane 3: gel filtration

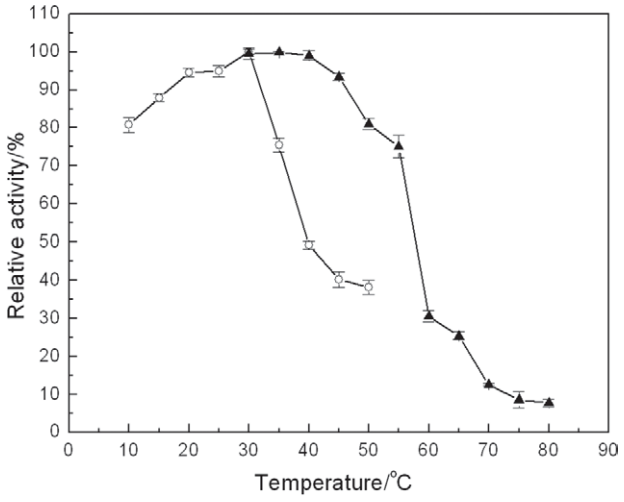

Fig. 4. Effect of temperature on the activity (–O–) and the stability (–▲–) of lipase from *Acinetobacter johnsonii* LP28

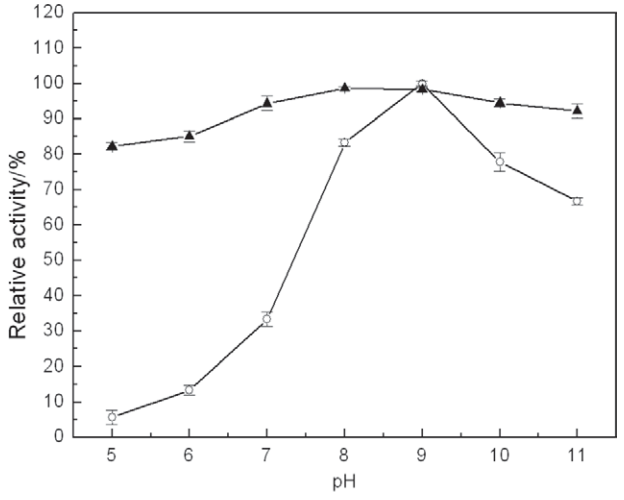

Fig. 5. Effect of pH on the activity (–O–) and stability (–▲–) of lipase from *Acinetobacter johnsonii* LP28

SY-01 (pH=10.0) (*5*). However, the optimal pH of the lipase from *Acinetobacter johnsonii* LP28 was higher than of other lipases reported from *Acinetobacter* (pH=7.0–8.5) (*7–9,12*). On the other hand, the results from Fig. 5 showed the stability from pH=5.0–11.0, especially in the alkaline range. It can be seen that the lipase from *Acinetobacter johnsonii* LP28 had better stability and activity at alkaline pH, both useful characteristics in detergent applications.

Acinetobacter johnsonii LP28 lipase hydrolysed a wide range of different oils (Table 2) and showed a high level of enzyme activity in hydrolyzing tributyrin (186 % of relative activity). In other studies of *Acinetobacter* sp. lipase, *Acinetobacter* species SY-01 (*30*) lipase was reported to hydrolyse a wide range of fatty acid esters of *p*-nitrophenyl, but preferentially to hydrolyse short-length acyl chains (C2 and C4). Its mutant (*31*) increased its selectivity for the short-chain C2 and C4 and the long-chain esters, C10, C12, C14 and C16, but decreased its selectivity for the middle-chain esters, C6 and C8. Kasana *et al.* (*7*) reported that lipase from *Acinetobacter lwoffii* CR9 showed an ability to hydrolyse tributyrin, Tween 80, soybean oil, mustard oil and olive oil.

Table 2. Substrate specificity of lipase from *Acinetobacter johnsonii* LP28

Natural oils/triglycerides	Relative lipase activity/%
olive oil	100.00
mustard oil	106±1
soybean oil	107±1
peanut oil	103±1
palm oil	106±1
sunflower oil	99±1
coconut oil	114±1
tributyrin	186±2

Results are the relative lipase activity expressed as the percentage of olive oil; data are means of triplicate determinations± standard deviation

The effect of different metal ions on the activity of lipase is shown in Table 3. The enzyme activity was promoted in the presence of Na^+, Ca^{2+}, K^+, Mg^{2+} and sodium citrate. The addition of 0.1, 1 and 10 mM $CaCl_2$ increased the lipase activity by 40–60 %, there is most probably a Ca^{2+}–binding pocket in the lipase of *Acinetobacter johnsonii* LP28 that leads to the active-site configuration. As summarized by Snellman and Colwell (*11*), a nearly universal property of *Acinetobacter* lipase is the positive effect of Ca^{2+} on enzyme stabilization and activity. Ions of Ba^{2+}, Mn^{2+}, Cr^{3+} and Co^{2+} showed no significant effect on the enzyme activity, whereas the presence of Al^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Zn^{2+} and EDTA reduced the enzyme activity. In contrast to *A. johnsonii* LP28, the lipase from *Acinetobacter* sp. RAG-1 (*24*) showed a decrease in the activity when incubated with Cu^{2+} and an increase with Fe^{3+} and Zn^{2+}.

Table 3. Effects of different compounds on the activity of lipase from *Acinetobacter johnsonii* LP28

Compound	Remaining lipase activity/%		
	c/mM		
	0.1	1	10
NaCl	111±2	108±1	97±2
KCl	117±1	110±2	102±2
$MgCl_2$	119±2	140±2	122±3
$CaCl_2$	151±1	160±2	144±2
$BaCl_2$	102±1	96±2	92.3±0.5
$CoCl_2$	103±1	101±1	97.3±0.3
$CrCl_3$	94±2	98±2	94±1
$MnCl_2$	101±0.3	96±2	90±1
$AlCl_3$	64±2	63±2	58±1
$CuCl_2$	83±2	90±1	73±2
$FeSO_4$	50.1±0.8	64±1	53.1±0.6
$Fe_2(SO_4)_3$	50±2	52±1	55±2
$ZnSO_4$	52±2	46±2	50±2
EDTA	56±1	70±1	62.2±0.4
sodium citrate	105±1	110±2	90±1

Results are the relative lipase activity expressed as the percentage of the maximum activity recorded without the addition of a compound; data are means of triplicate determinations±standard deviation

Stability in the presence of surfactants and oxidizing agents is a prerequisite of enzymes used in detergent formulations. According to the results in Table 4, the lipase from *Acinetobacter johnsonii* LP28 was stable in some surfactants and retained 75.71, 101.14, 101.26, 129.91, 103.33 and 100.11 % of its control activity in the presence of Triton X-100, Tween 20, Tween 80, saponin, sodium cholate and sodium taurocholate, respectively. The above finding was similar to that of Dharmsthiti *et al*. (*14*) and Lee *et al*. (*23*), who found that lipase from *A. calcoaceticus* LP009 and *Acinetobacter* sp. ES-1 showed appreciably good stability in the presence of Triton X-100, Tween 20 and Tween 80. However, when another lipase from *Acinetobacter* sp. was incubated in the presence of surfactants, a significant reduction in its activity was observed, especially with SDS and Triton X-100, which caused a 70 % reduction of lipase activity (*9*). In our study, *Acinetobacter johnsonii* LP28 lipase also retained 60.87, 78.25 and 83.88 % of its activity after exposure to hydrogen peroxide, sodium hypochlorite and sodium perborate, respectively (Table 4), which confirms that this lipase is stable against oxidizing agents. Furthermore, the lipase had very little loss in the activity after exposure to various commercial detergents (Table 4), indicating that it could be used as an additive to these indigenous detergents. Thus, the lipase from *Acinetobacter johnsonii* LP28 suggested a good potential application in the detergent industry.

Conclusion

In this study, *Acinetobacter johnsonii* LP28, a strain that produces alkaline lipase at low-temperature, was isolated from the soil collected from the Bay of Bohai, PR China. To our knowledge this has been the first report about *Acinetobacter johnsonii* producing a low-temperature and alkaline lipase. The lipase was purified and the relative molecular mass of the enzyme was determined to be 53 kDa by SDS-PAGE. The results obtained in this study show that *Acinetobacter johnsonii* LP28 lipase exhibited maximum activity at 30 °C and pH=9.0. The lipase was also highly stable in the presence of various oxidizing agents, some commercial detergents and alkaline protease, and its activity was further promoted by most of the surfactants. All these results indicate that *Acinetobacter johnsonii* LP28 lipase has a good potential for the application in the detergent industry.

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Table 4. Lipase stability in the presence of surfactants, protease, oxidizing agents and detergents

Surfactants/detergents/oxidizing agents (1 %)		Relative lipase activity %	Surfactants/detergents/oxidizing agents (1 %)		Relative lipase activity %
surfactants	Triton X-100	76±1	oxidizing agents	hydrogen peroxide	61±2
	Tween 20	101±1		sodium hypochlorite	78±1
	Tween 80	101±1		sodium perborate	83.9±0.5
	saponin	129.9±0.4	commercial detergent	Liby	89±1
	sodium cholate	103±2		Bilang	80.3±0.3
	sodium taurocholate	100.1±0.5		OMO	80±1
alkaline protease	10 000 U/mL	76±2		Tida	90±1
				Diao	84±1

Results are the relative lipase activity expressed as the percentage of the maximum activity recorded without the addition of compound; data are means of triplicate determinations±standard deviation

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