African Journal of Biotechnology Vol. 11(23), pp. 6327-6334, 20 March, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.4247
ISSN 1684–5315 ©2012 Academic Journals

Full Length Research Paper

Purification and characterization of an extracellular esterase from a moderately halophilic bacterium, *Halobacillus* sp. strain LY5

Xin Li*, Hui-Ying Yu and Yi-Feng Lin

Life Science College, Yuncheng University, Yuncheng, 044000, China.

Accepted 17 February, 2012

A newly moderate halophilic strain LY5 producing extracellular esterase was isolated and identified. Biochemical and physiological characterization, along with 16S rRNA gene sequence analysis placed the isolate in the genus *Halobacillus*. Esterase production started from the mid-exponential phase of bacterial growth and reached a maximum level during the mid-stationary phase. The enzyme was highly active against *p*-nitrophenyl esters with acyl chain lengths from C2 to C10, indicating an esterase activity. Enzyme purification was carried out by combination of 80% ammonium sulphate precipitation, diethylaminoethyl (DEAE)-cellulose ion exchange and Sephacryl S-100 gel filtration chromatography. The molecular weight of the esterase was determined to be 96 kDa by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The purified enzyme was highly active over broad ranges of temperature (30 to 90°C), pH (6.0 to 12.0) and NaCl concentration (0 to 20%), showing optimal activity at 50°C, pH 10.0 and 10% NaCl. Complete inhibition of the esterase by phenylmethylsulfonyl fluoride (PMSF), phenylarsine oxide (PAO) and diethyl pyrocarbonate (DEPC) indicated that serine, cysteine and histidine residues were essential for its catalytic function. Moreover, it exhibited remarkable stability towards SDS and Triton X-100. Results from the present study indicate that the extracellular esterase from *Halobacillus* sp. LY5 may have considerable potential for industrial application from the perspectives of its properties.

Key words: Moderately halophilic bacterium, esterase, purification and characterization, *Halobacillus*.

INTRODUCTION

Carboxyl ester hydrolases, like lipases and esterases, constitute an important group of biocatalysts. These enzymes are essentially distinguished on the basis of their substrate specificity. Lipases preferentially hydrolyze triglycerides made up of long chain fatty acids and esterases hydrolyze only water soluble esters, for example, short-chain fatty acid triglycerides (Fojan et al., 2000). Microbial esterases are currently receiving increase-attention because of their potential applications in synthetic chemistry, food processing and environmental monitoring, such as biodegradation of industrial wastewater and agricultural pesticides (Panda and Gowrishankar, 2005; Li et al., 2008; Wheelock et al., 2008). However, most industrial processes are carried out under harsh physicochemical conditions which may not be definitively adjusted to the optimal points required for the activity of the available enzymes. Therefore, it would be of great importance to develop new enzymes that exhibited optimal activities at various ranges of salt concentration, pH and temperature.

Moderately halophiles are a group of halophilic micro-organisms able to grow optimally in media containing 3 to 15% NaCl (Ventosa et al., 1998). Enzymes from these prokaryotes usually showed high stability under extreme conditions, making them robust biocatalysts with

*Corresponding author. E-mail: lixin-eva@163.com. Tel/Fax: +86 (0)359 2090324.

Abbreviations: DEAE, Diethylaminoethyl cellulose; **SDS-PAGE,** sodium dodecyl sulfate-polyacrylamide gel electrophoresis; **PMSF,** phenylmethylsulfonyl fluoride; **PAO,** phenylarsine oxide; **DEPC,** diethyl pyrocarbonate.

potential applications in harsh industrial processes (Oren, 2002). There are numerous reports about esterase production from halophiles (Amoozegar et al., 2008; Boutaiba et al., 2006; Ozcan et al., 2009; Rohban et al., 2009); however, few studies on their purification and characterization are reported. Enzymatic properties of these esterases were mostly obtained from their crude extracts. Lv et al. (2010) reported a novel carboxylesterase produced by *Thalassobacillus* sp. strain DF-E4, which is the only one paper about esterase purification from halophiles.

In this work, we reported a moderately halophilic strain LY5 with high esterase activity, and purification and biochemical characterization of a novel extracellular esterase was also performed.

MATERIALS AND METHODS

Bacterial strain and culture conditions

The strain LY5 (CICC 10447) was isolated from the saline soil in Yuncheng, China, and cultivated aerobically at 37°C in the complex medium (CM) of the following composition (g/L): casein peptone 7.5; yeast extract 10.0; sodium citrate 3.0; $MgSO_4 \cdot 7H_2O$ 20.0; KCl 2.0; $FeSO_4 \cdot 7H_2O$ 0.01; NaCl 120.0 and pH 8.0 (Li and Yu, 2011). For esterase production, the isolate LY5 was incubated in CM medium containing 1% (v/v) Tween-20.

Bacterial growth, along with enzyme activity, was measured by spectrometric method (Shimadzu model UV-160A).

Identification of the isolate LY5

Morphological, physiological and biochemical characteristics of LY5 were studied either on CM agar plate (2% agar, w/v) or in CM broth plus 12% NaCl, according to Spring et al. (1996). 16S rRNA gene was amplified using the general bacterial primers 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-TACCTTGTTACGACTT-3′) (Li and Yu, 2011). The PCR product was purified and sequenced in both directions using an automated sequencer by Seq Lab laboratory (Germany). The obtained 16S rRNA gene sequence was aligned with its closely related neighbor sequences retrieved from GenBank and a phylogenetic tree was constructed as previously described (Yoon et al., 2007).

Esterase activity assay

The esterase activity was determined by using *p*-nitrophenyl butyrate (*p*-NPB) as substrate. Briefly, 0.4 ml of substrate solution (10 mM dissolved in 2-propanol) was mixed with 3.6 ml of a solution containing 50 mM Tris-HCl buffer (pH 10.0) and 11.7% NaCl. After pre-incubation at 30°C for 10 min, the enzymatic assay was initialed by adding 0.2 ml of the purified enzyme solution to the reaction mixture and incubated at 50°C for 10 min. Following the addition of 2.0 ml of Na_2CO_3 solution (0.25 M) to stop the reaction, the amount of *p*-nitrophenol (*p*-NP) released was measured at 410 nm against a blank. One unit (U) was defined as the amount of enzyme liberating 1 µmol of *p*-NP per minute. The specific activity was expressed in the units of enzyme activity per milligram of protein.

Esterase purification

Cells were harvested by centrifugation at 7,700 g for 12 min at 4°C. One liter culture supernatant was treated with solid ammonium sulphate to 80% saturation and stirred overnight at 4°C. The precipitate collected by centrifugation was dissolved in 20 mM Tris-HCl buffer containing 10% NaCl (pH 9.0). After dialysis against the same buffer overnight, the sample was applied to a diethylaminoethyl (DEAE)-cellulose column (2.5 × 30 cm). The column was pre-washed with Tris-HCl buffer (20 mM) and bound proteins were eluted with a linear gradient of 0 to 0.8 M NaCl in the same buffer at a flow rate of 0.5 ml/min. Active fractions were pooled and concentrated by freeze-drying. The resulting concentrate was dissolved in Tris-HCl buffer, and then loaded on a Sephacryl S-100 gel filtration column (1.6 × 60 cm) and eluted with the same buffer at a flow rate of 0.2 ml/min. The active fractions (4 ml) were concentrated by ultrafiltration, and used as the purified esterase for further characterization. Protein content was determined by the method of Lowry et al. (1951), using bovine serum albumin as standard.

Molecular weight estimation

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE, 12% acrylamide) was performed to determine the molecular weight of the purified esterase, according to the method of Laemmli (1970). After running the gel, the proteins were stained with Coomassie Brilliant Blue R-250.

Influence of temperature, pH and salt on esterase activity

Effect of the temperature on the purified esterase was determined at various temperatures from 30 to 90°C. Effect of pH on esterase activity was studied in the range of 6.0 to 12.0, using appropriate buffers at a concentration of 0.05 M (6.0 to 7.0, sodium phosphate; 8.0 to 9.0, Tris-HCl; 10.0 to 12.0, glycine-NaOH). Moreover, esterase activity was measured with different NaCl concentrations (0 to 20%) in the reaction mixtures.

Effect of metal ions and chemical reagents on esterase stability

The effect of metal ions and chemical reagents [ethylenediaminetetraacetic acid (EDTA), phenylmethylsulfonyl fluoride (PMSF), phenylarsine oxide (PAO), diethyl pyrocarbonate (DEPC), β-mercaptoethanol, SDS and Triton X-100] on the esterase activity were examined after the enzyme had been pre-incubated with them for 30 min at 50°C, respectively, and then the residual activity was determined using the standard assay method as previously described. Activity in the absence of any additives was taken as 100%.

Substrate specificity

The substrate specificity of the purified esterase was studied by a spectrophotometric assay with various *p*-NP esters (C2 to C18, Sigma) as previously described (Lv et al. 2010). Data were expressed as the percentage of the observed maximal activity obtained with *p*-NPB.

RESULTS

Identification of the isolate LY5

Based on morphological, physiological and biochemical characterization, the isolate LY5 is a Gram-positive, motile,

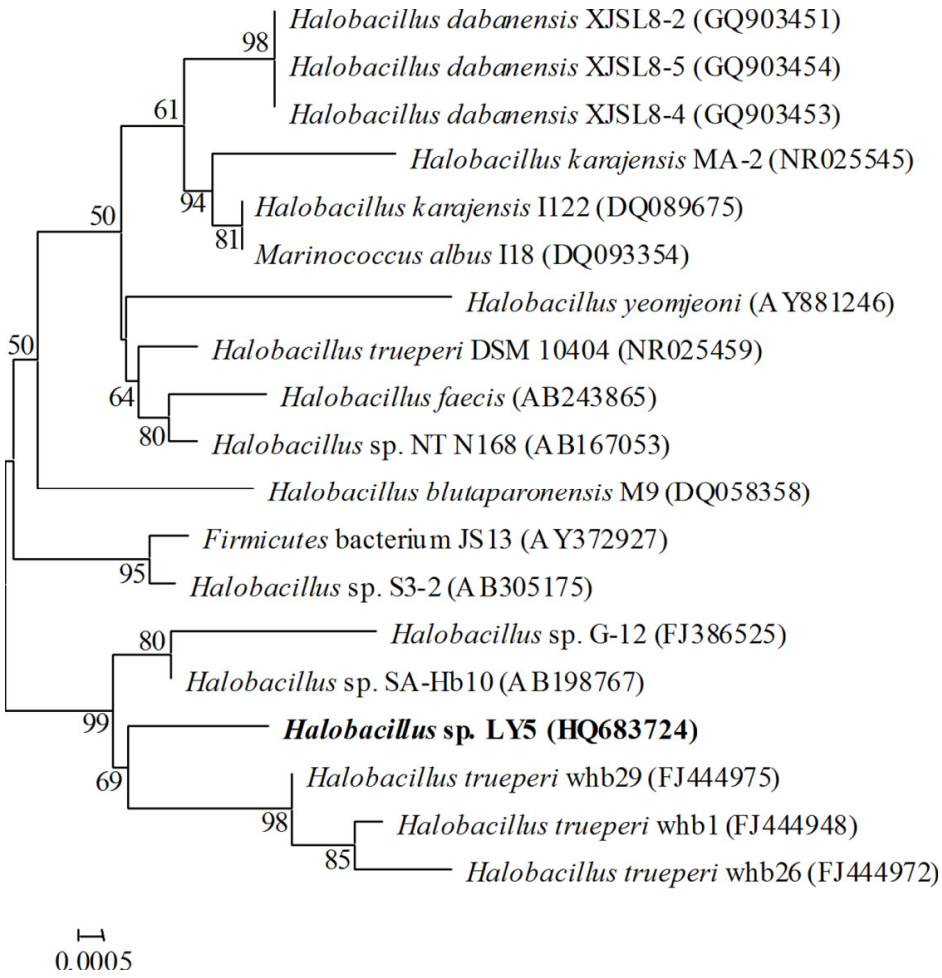

Figure 1. Phylogenetic tree based on 16S rRNA gene sequences, showing the relationship of the isolate LY5 to other members of the genus *Halobacillus*. Type strain was *Halobacillus trueperi*. Accession numbers of the sequences used in this study are shown in parentheses after the strain designation. Numbers at nodes are percentage bootstrap values based on 1,000 replications; only values greater than 50% are shown. Bar 0.0005 substitutions per nucleotide position.

rod shaped and aerobic spore-forming bacterium. Colonies are light yellow, uniformly round, circular and convex on CM agar plate. Growth occurred over a wide range of salt concentrations (0.5 to 15%). Optimal bacterial growth was observed at pH 10.0, 37 to 42℃ and 12% NaCl. H_2S production, methyl red and Voges-Proskauer tests were negative, while nitrate reduction, oxidase and catalase, hydrolysis of Tween 80 and gelatin were positive. Acid was produced from maltose, D-fructose, sucrose and glucose. Phylogenetic analysis based on 16S rRNA gene sequence comparisons revealed that the isolate LY5 (accession number in GenBank: HQ683724) fell within the branch encompassing members of the genus *Halobacillus* and was most closely related to *H. trueperi* (99% 16S rRNA gene sequence similarity) (Figure 1). Therefore, it belonged to moderate halophile and was tentatively named as *Halobacillus* sp. LY5.

Bacterial growth and enzyme production

As shown in Figure 2, the lag phase of bacterial growth was short (2 h), and after 26 h the bacterial growth reached to the stationary phase. No esterase activity was detected during the early-exponential growth phase. Esterase production started from the mid-exponential phase (14 h) and reached a maximum level during the mid-stationary phase (40 h).

Enzyme purification and molecular weight determination

The esterase was purified by combination of 80% ammonium sulphate precipitation, DEAE- cellulose ion exchange and Sephacryl S-100 gel filtration chromatography. The results of each purification procedure are

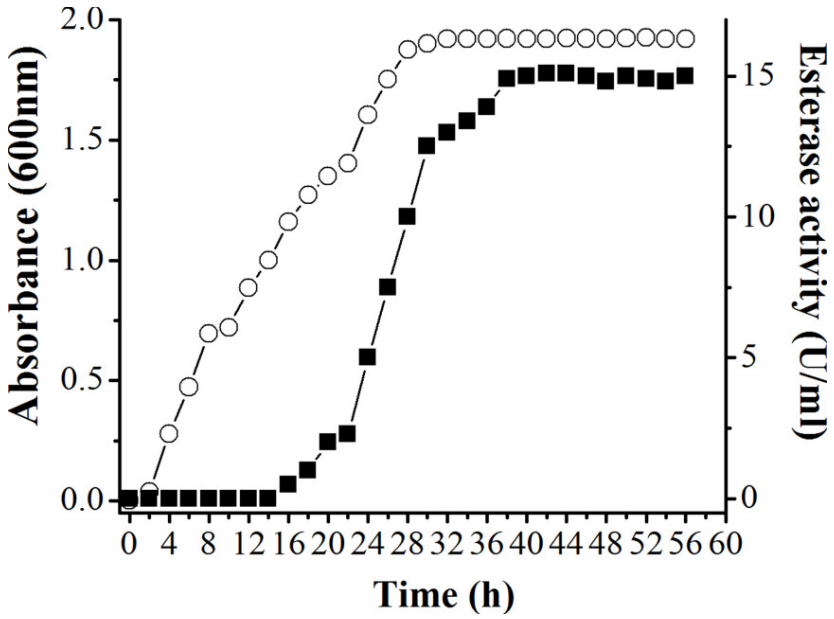

Figure 2. Bacterial growth (○) and extracellular esterase production (■) of *Halobacillus* sp. LY5 in CM broth containing 12% NaCl at 37°C. Results represent the means of three independent experiments.

Table 1. Summary of the purification of the esterase from *Halobacillus* sp. LY5.

Purification step	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Purification (fold)	Yield (%)
Crude enzyme	1520	67.5	22.5	1.0	100
80% $(NH_4)_2SO_4$	1135	19.3	58.8	2.6	74.7
DEAE-cellulose	452	5.4	83.7	3.7	29.7
Sephacryl S-100	157	0.8	196.3	8.7	10.3

shown in Table 1. The enzyme was purified 8.7-fold with a recovery rate of 10.3% and a specific activity of 196.3 U/mg (protein). The purified esterase showed a single band corresponding to an apparent molecular weight of 96 kDa on SDS-PAGE (Figure 3).

Effect of temperature, pH and salt on esterase activity

The esterase showed activity over a broad temperature range with an optimum at 50°C and 60% of the activity remained at 90°C (Figure 4a). The esterase was high active over a wide pH range (6.0 to 12.0). The optimal pH for the enzyme was found to be 10.0. It retained 40% of activity at pH 12.0 (Figure 4b).

Enzyme activity was also determined with different NaCl concentrations at 50°C and pH 10.0 (Figure 4c). The highest activity was shown at 10% NaCl. Under higher (20%) salinity, 60% of the activity was shown. Meanwhile, in the absence of NaCl, the enzyme still retained more than 70% of its activity.

Effect of metal ions and chemical reagents on esterase stability

The presence of Cu^{2+} and Hg^{2+} strongly inhibited the esterase with activity lost by 40 and 32%, respectively, while other metal ions tested did not affect the enzyme. The esterase was highly stable towards surfactants, such as SDS and Triton X-100. The effect of some known enzyme inhibitors revealed that EDTA and β-mercaptoethanol had no significant influence on the enzyme, whereas PMSF, PAO and DEPC inactivated the esterase completely (Table 2).

Substrate specificity of the purified esterase

The esterase was highly active against a wide range of *p*-NP esters with acyl chain lengths from C2 to C10 (Figure 5). Maximal activity was obtained against *p*-NPB (C4). Esterase activity declines along with longer chain-length, reaching 90% activity with *p*-NPH (C6), 80% with *p*-NPO (C8) and 60% with p-NPD (C10), respectively. Little

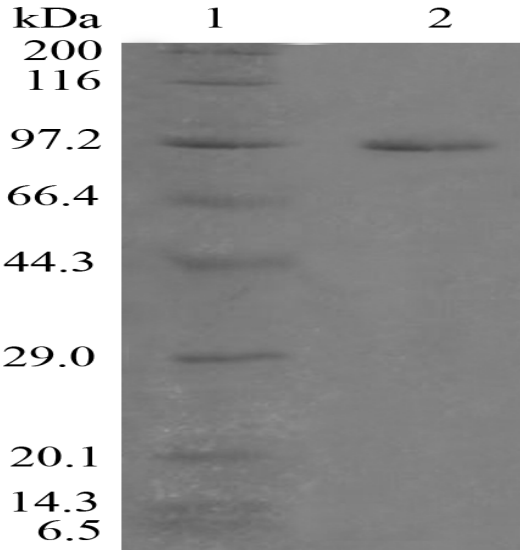

Figure 3. SDS-PAGE analysis of the purified esterase. Lane 1, Molecular mass markers; lane 2, purified esterase. SDS-PAGE, Sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Table 2. Effect of different metal ions and chemical reagents on esterase stability.

Substance	Final concentration (mM)	Residual activity (%)
Control	-	100
Ca^{2+}	5	88 ± 1.1
Zn^{2+}	5	85 ± 0.5
Fe^{2+}	5	99 ± 0.7
Fe^{3+}	5	98 ± 0.5
Cu^{2+}	5	60 ± 1.5
Mn^{2+}	5	98 ± 1.5
Hg^{2+}	5	68 ± 1.2
Mg^{2+}	5	99 ± 1.5
EDTA	2	98 ± 0.1
SDS	2	88.1 ± 1.3
β-Mercaptoethanol	2	99.3 ± 1.1
Triton X-100	2	90.1 ± 1.3
PMSF	2	0
DEPC	2	0
PAO	2	0

Residual activity was determined as described in “Materials and Methods” and expressed as the percentage of the control value (without any additives). Values are expressed as the averages of three independent experiments ± standard deviations.

activity was detected with *p*-NP esters with acyl chain lengths longer than C12.

DISCUSSION

In recent years, although halophilic microorganisms have had increasing interest, most studies have been performed on extreme halophiles. However, the ability of the moderate halophiles to grow and produce enzymes over a very wide range of salinities make them very attractive for research and for screening of novel enzymes with unusual properties. In this investigation, several strains of moderate halophilic bacteria were isolated from salt lake

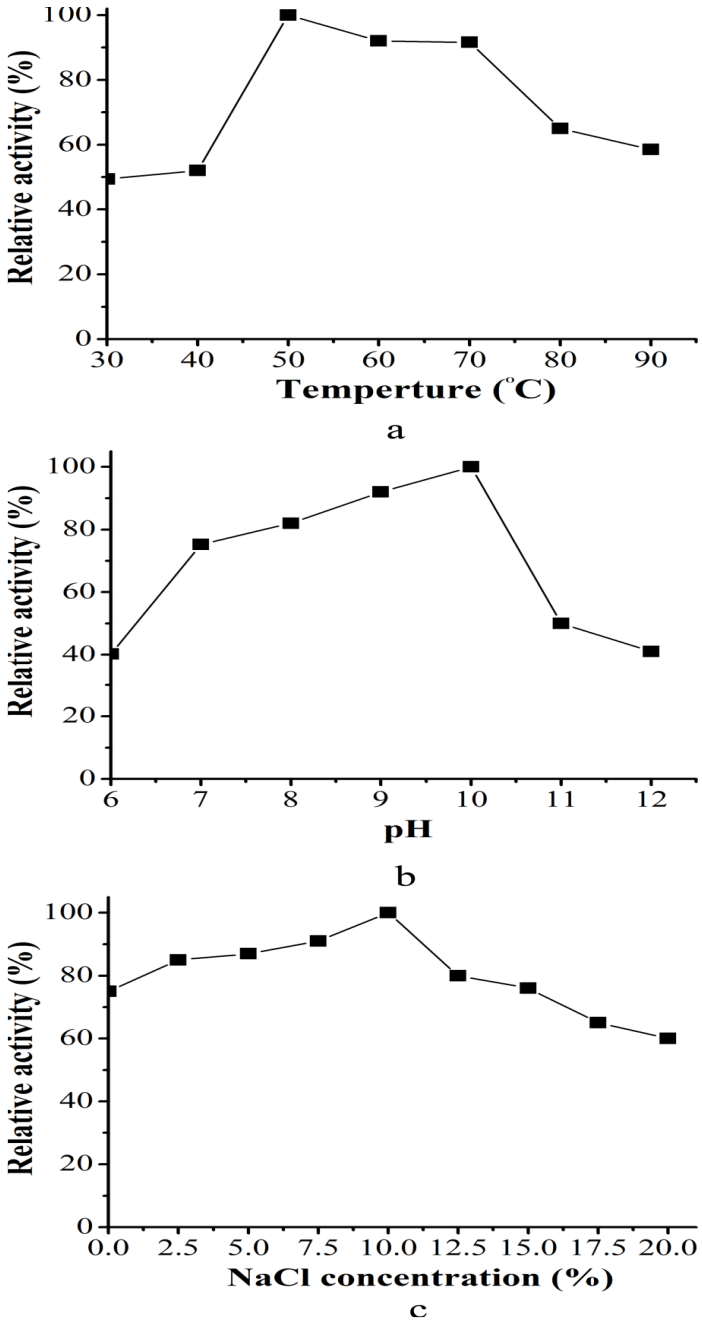

Figure 4 Effect of temperature (a), pH (b) and NaCl concentration (c) on the activity of the purified esterase. The relative activity was defined as the percentage of activity detected with respect to the maximum enzyme activity. Values are averages of three independent experiments.

of Yuncheng, China. Among these, the isolate LY5 was selected for further studies because it appeared to be the best producer of extracellular esterase. As determined by 16S rRNA gene sequence analysis, the isolate was closely related to the species of the genus *Halobacillus* (Figure 1). Thus, it was tentatively named as *Halobacillus* sp. LY5.

Esterase production by strain LY5 started from the mid-exponential phase of bacterial growth and reached its maximum level during the mid-stationary phase, while in other moderate halophiles, such as *Marinobacter lipolyticus*, *Salinivibrio* sp. strain SA-2 and *Thalassobacillus* sp. strain DF-E4, lipolytic activity was detected at the end of the exponential growth phase

Table 2. Effect of different metal ions and chemical reagents on esterase stability.

Substance	Final concentration (mM)	Residual activity (%)
Control	-	100
Ca^{2+}	5	88 ± 1.1
Zn^{2+}	5	85 ± 0.5
Fe^{2+}	5	99 ± 0.7
Fe^{3+}	5	98 ± 0.5
Cu^{2+}	5	60 ± 1.5
Mn^{2+}	5	98 ± 1.5
Hg^{2+}	5	68 ± 1.2
Mg^{2+}	5	99 ± 1.5
EDTA	2	98 ± 0.1
SDS	2	88.1 ± 1.3
β-mercaptoethanol	2	99.3 ± 1.1
Triton X-100	2	90.1 ± 1.3
PMSF	2	0
DEPC	2	0
PAO	2	0

Residual activity was determined as described in "Materials and Methods" and expressed as the percentage of the control value (without any additives). Values are expressed as the averages of three independent experiments ± standard deviations.

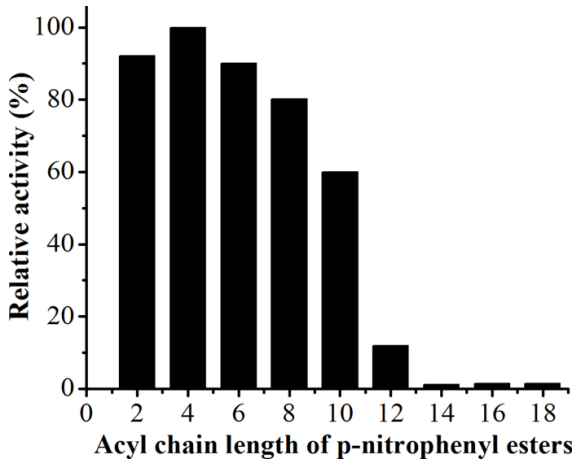

Figure 5. Substrate specificity test of the purified esterase towards the *p*-NP esters with different acyl chain lengths. Assays were done with 10% of NaCl at 50°C and pH 10.0. Data represent the means of three independent experiments.

(Martin et al., 2003; Amoozegar et al., 2008; Lv et al., 2010).

Substrate specificity test revealed that the extracellular enzyme was high active against *p*-NP esters with acyl chain lengths from C2 to C10 (Figure 5). Meanwhile, lipolytic activity on Rhodamine B agar plates (Kouker and Jaeger, 1987) indicated it could not hydrolyze olive oil (data not shown). These results confirm the esterase nature of this enzyme, as being more active on short-chain fatty acid esters.

The extracellular esterase can be classified as moderately thermoactive enzyme because of its optimal activity at 50°C. However, it was worth noting that the enzyme showed relatively high activity (>50%) at 90°C. This characteristic made it obviously different from other esterases described previously, which were usually inactive under temperatures higher than 80°C (Ozcan et al., 2009; Lv et al., 2010). Optimal pH for this enzyme was found to be 10.0, indicating its alkali-stable nature. Moreover, Boutaiba et al. (2006) reported that lipase from *Natronococcus* sp. exhibited an optimum activity at pH 7.0, while Ozcan et al. (2009) reported a range of pH from 8.0 to 8.5 for optimal esterase activities of five halophilic archaeal strains. The esterase showed activity under high salinity (20%).

This finding makes the enzyme an interesting candidate for future investigation as biocatalysis in non-aqueous solvents, since it could be considered as active and robust biocatalyst at low water activity. Similar extreme halotolerance has been reported in other esterases from *Haloarcula marismortui* (Camacho et al., 2009) and *Halobacterium* sp. NRC-1 (Camacho et al., 2010). As we all know, the catalytic activity of most enzymes from halophilic microorganisms that normally live in a saline environment falls off dramatically and irreversibly when the enzyme is exposed to lower salt concentration. However, the esterase from *Halobacillus* sp. LY5 was found to be highly active without NaCl. Similarly, some halophilic enzymes were also reported to retain their activity at low salt concentrations or even in its absence (Johnsen and Schonheit, 2004; Lv et al., 2010).

Complete inhibition by PMSF indicated that the purified esterase was a serine enzyme. Meanwhile, it was also strongly inhibited by DEPC, a histidine modifier (Wragg et al., 1997) and PAO, a cysteine enzyme inhibitor (Lv et al., 2010), suggesting that cysteine and histidine residues were essential for its catalytic function. However, no significant effect was detected with EDTA and β-mercaptoethanol, indicating it was not a metalloenzyme and disulfide bonds did not have important role for its catalysis.

These structural characteristics were similar to the carboxylesterase produced by *Thalassobacillus* sp. strain DF-E4 (Lv et al., 2010). Nevertheless, in comparison with other halophilic esterases (Sana et al., 2007; Rao et al., 2009; Lv et al., 2010), the enzyme from LY5 had higher molecular weight, which was determined to be 96 kDa by SDS-PAGE (Figure 3). In addition, the esterase exhibited remarkable stability towards surfactants, such as SDS and Triton X-100, and may be useful in food industries.

In the present investigation, we described a novel moderately thermoactive, surfactant stable, alkali-stable and halotolerant esterase from *Halobacillus* sp. LY5, with serine, cysteine and histidine residues locating in its active site. All of these results led us to conclude that the enzyme may have considerable potential for industrial application from the perspectives of its properties.

ACKNOWLEDGMENTS

This work was financially supported by Shanxi Provincial Science and Technology Foundation (grants no. 20110021) and Natural Science Fund of Shanxi Province (grants no. 2011021031-4).

REFERENCES

Amoozegar MA, Salehghamari E, Khajeh K, Kabiri M, Naddaf S (2008). Production of an extracellular thermohalophilic lipase from a moderately halophilic bacterium, *Salinivibrio* sp. strain SA-2. J. Basic Microbiol. 48(3): 160-167.

Boutaiba S, Bhatnagar T, Hacene H, Mitchell DA, Baratti JC (2006). Preliminary characterization of a lipolytic activity from an extremely halophilic archaeon, *Natronococcus* sp. J. Mol. Catal. B. Enzyme, 41: 21-26.

Camacho RM, Mateos JC, González-Reynoso O, Prado LA, Córdova J (2009). Production and characterization of esterase and lipase from *Haloarcula marismortui*. J. Ind. Microbiol. Biotechnol. 36(7): 901-909.

Camacho RM, Mateos-Díaz JC, Diaz-Montaño DM, González-Reynoso O, Córdova J (2010). Carboxyl ester hydrolases production and growth of a halophilic archaeon, *Halobacterium* sp. NRC-1. Extremophiles, 14(1): 99-106.

Fojan P, Jonson PH, Petersen MT, Petersen SB (2000). What distinguishes an esterase from a lipase: A novel structural approach. Biochimie, 82: 1033-1041.

Johnsen U, Schonheit P (2004). Novel xylose dehydrogenase in the halophilic archaeon, *Haloarcula marismortui*. J. Bacteriol. 186: 6198-6207.

Kouker G, Jaeger KE (1987). Specific and sensitive plate assay for bacterial lipase. Appl. Environ. Microbiol. 53: 211-213.

Laemmli UK (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680-685.

Li G, Wang K, Liu YH (2008). Molecular cloning and characterization of a novel pyrethroid-hydrolyzing esterase originating from the Metagenome. Microb. Cell. Factories, 7: 38-48.

Li X, Yu HY (2011). Extracellular production of beta-amylase by a halophilic isolate, *Halobacillus* sp. LY9. J. Ind. Microbiol. Biotechnol. 38(11): 1837-1843.

Lowry OH, Rosebrough NJ, Farr AL, Randall JR (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-275.

Lv XY, Guo LZ, Song L, Fu Q, Zhao K, Li AX, Luo XL, Lu WD (2010). Purification and characterization of a novel extracellular carboxyl-esterase from the moderately halophilic bacterium, *Thalassobacillus* sp. strain DF-E4. Ann. Microbiol. 61: 281-290.

Martin S, Marquez M, Sánchez-Porro C, Mellado E, Arahal D, Ventosa A (2003). *Marinobacter lipolyticus* sp. nov, novel moderate halophile with lipolytic activity. Int. J. Syst. Evol. Microbiol. 53: 1383-1387.

Oren A (2002). Molecular ecology of extremely halophilic archaea and bacteria. FEMS Microbiol. Ecol. 39: 1-7.

Ozcan B, Ozyilmaz G, Cokmus C, Caliskan M (2009). Characterization of extracellular esterase and lipase activities from five halophilic archaeal strains. J. Ind. Microbiol. Biotech. 36: 105-110.

Panda T, Gowrishankar BS (2005). Production and applications of esterases. Appl. Microbiol. Biotechnol. 67: 160-169

Rao L, Zhao X, Pan F, Xue Y, Ma Y, Lu JR (2009). Solution behavior and activity of a halophilic esterase under high salt concentration. PLoS. One, 4(9): e6980.

Rohban R, Amoozegar MA, Ventosa A (2009). Screening and isolation of halophilic bacteria producing extracellular hydrolyses from Howz Soltan Lake, Iran. J. Ind. Microbiol. Biotech. 36(3): 333-340.

Sana B, Ghosh D, Saha M, Mukherjee J (2007). Purification and characterization of an extremely dimethylsulfoxide tolerant esterase from a salt-tolerant *Bacillus* species isolated from the marine environment of the Sundarbans. Process Biochem. 42: 1571-1578.

Spring S, Ludwig W, Marquez MC, Ventosa A, Schleifer KH (1996). *Halobacillus* gen. nov., with descriptions of *Halobacillus litoralis* sp. nov. and *Halobacillus trueperi* sp. nov., and transfer of *Sporosarcina halophila* to *Halobacillus halophilus* comb. nov. Int. J. Syst. Bacteriol. 46: 492-496.

Ventosa A, Nieto JJ, Oren A (1998). Biology of moderately halophilic aerobic bacteria. Microbiol. Mol. Biol. Rev. 62: 504-544.

Wheelock CE, Phillips BM, Anderson BS, Miller JL (2008). Applications of carboxylesterase activity in environmental monitoring and toxicity identification evaluations (TIEs). Rev. Environ. Contamin. Toxicol. 195: 117-178.

Wragg S, Hagen FK, Tabak LA (1997). Identification of essential histidine residues in UDP-N-acetyl-D-galactosamine: Polypeptide N-acetylgalactosaminyltransferase-T1. Biochem. J. 328: 193-197.

Yoon JH, Kang SJ, Jung YT, Oh TK (2007). *Halobacillus campisalis* sp. nov., containing meso-diaminopimelic acid in the cell-wall peptidoglycan, and emended description of the genus *Halobacillus*. Int. J. Syst. Evol. Microbiol. 57: 2021-2025.