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Purification and characterization of a pH and heat stable esterase from *Geobacillus* sp. TF17

[pH ve ısıl kararlı bir esterazın *Geobacillus* sp. TF17 suşundan saflaştırılması ve karakterizasyonu]

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ABSTRACT

Objective: To purify and characterize an esterase from a thermophilic bacterium, *Geobacillus* sp. TF17.

Methods: The crude esterase was purified by using acetone precipitation and ion exchange choromatography methods and characterized.

Results: The optimum temperature and pH of the enzyme were found to be 50°C and 7.5, respectively. The purified enzyme was extremely stable in the range of pH 4.0-9.0 after 72 hour incubation at 4°C. The thermal stability profile shows that this enzyme is stable in the range of 30-50°C after 72 h incubation. The K_m and V_{max} values for this esterase in the presence of *p*-nitrophenyl butyrate (pNPB) as substrate were found as 0.056 mM and 19.38 U/mg protein, respectively. The enzyme activity was inhibited more than about 60% in the presence of some organic solvents such as isopropanol and acetonitrile. Additionally, it was detected that some metal ions affect the enzyme activity at different ratio.

Conclusion: An esterase was purified and characterized from *Geobacillus* sp. TF17. The pH and thermal stability of purified enzyme are quite high. The data obtained from this study show that the purified esterase has advantages for industrial or biotechnological applications in terms of especially its high thermal- and pH-stability.

Key Words: *Geobacillus*, thermophile, esterase, characterization, pH stability, thermal stability

Conflict of Interest: The authors declare that there was no conflict of interest in this work.

ÖZET

Amaç: Termofilik bir bakteri olan *Geobacillus* sp. TF17 suşundan esteraz enziminin saflaştırılması ve karakterize edilmesidir.

Yöntemler: Ham enzim, aseton çöktürme ve iyon değişim kromatografisi metodları kullanılarak saflaştırıldı ve karakterize edildi.

Bulgular: Saf enzimin optimum pH ve sıcaklığı sırasıyla 7,5 ve 50°C olarak bulunmuştur. Saf enzimin, 4°C'de pH 4,0-9,0 aralığında 72 saatlik inkübasyon süresi sonunda oldukça kararlı olduğu gözlenmiştir. Termal kararlılık eğrisi göstermektedir ki saf enzim, 30-50°C arasındaki sıcaklıklarda, 72 saat inkübasyon süresinden sonra kararlıdır. *p*-nitrofenil butirat (pNPB) substratı varlığında esteraz enziminin K_m ve V_{max} değerleri, sırasıyla, 0,056 mM ve 19,38 U/mg proteindir. İzopropanol ve asetonitril gibi bazı organik çözücülerin varlığında enzim aktivitesinin %60'ından fazlası inhibe olmuştur. Ayrıca bazı metal iyonlarının enzim aktivitesini farklı oranlarda etkilediği tespit edilmiştir.

Sonuç: *Geobacillus* sp. TF17 suşundan bir esteraz saflaştırılmış ve karakterize edilmiştir. Saf enzimin pH ve ısıl kararlılığı oldukça yüksektir. Elde edilen veriler, özellikle termal ve pH kararlılığı dikkate alındığında enzimin endüstriyel ya da biyoteknolojik uygulamalar açısından avantajlı olduğunu göstermektedir.

Anahtar Kelimeler: *Geobacillus*, termofil, esteraz, karakterizasyon, pH kararlılık, ısıl kararlılık

Çıkar Çatışması: Yazarlar çıkar çatışması bulunmadığını beyan eder.

Introduction

Two kinds of lipolytic enzymes (esterases and lipases) are known as α/β-hydrolases (EC 3.1.1.X) [1]. Their special catalytic properties make them very attractive for industrial applications such as detergent industry, oleochemical industry, pulp and paper industry and resolution of chiral drugs [2,3]. While they can be used as a catalyst to cleavage ester bounds in the presence of water in reaction media, they catalyze formation of ester bound via esterification, inter-esterification and trans-esterification reactions in organic media [4]. It was also declared that esterases degraded natural materials and industrial pollutants such as cereal wastes, plastics and other toxic chemicals [5].

Most of the lipases used in industry are of microbial (fungal or bacterial origin) enzymes [6]. The researches on esterases from thermophilic microorganisms are interesting in terms of understanding the stabilities of esterases and improving new esterases for application to the industrial processes. Additionally, enzymes from thermophilic microorganisms are widely used in commercial applications because of their inherent stability [7]. Many researchers have focused on searching novel esterases for various industrial production processes. So, different esterases are produced from various sources. Considering biocatalytic applications, it is of great interest to study the specificity and selectivity of novel enzymes.

The strain TF17 used in this study was isolated from a hot spring in Ömerbeyli/Aydın, a province of Turkey and identified as *Geobacillus* sp. by a group of researchers in the Department of Biology in Karadeniz Technical University, based on morphological, physiological, biochemical properties and 16S rRNA analysis (unpublished data).

Here, we report the isolation and purification of an intracellular esterase from the thermophilic bacterium *Geobacillus* sp. TF17. Additionally, the esterase was biochemically characterized in detail. The data obtained from this study are important in terms of pH and heat stability of investigated esterase and providing a new enzyme source for industrial applications.

Materials and Methods

Chemicals

Substrates were purchased from Sigma Chemical Co. (St. Louis) and other reagents were of analytical grade and used as obtained.

Bacterial strain, culture conditions and enzyme extraction

Geobacillus sp TF17 isolated from the Ömerbeyli Jeotermal in Aydın, Turkey was grown aerobically at 55°C in Luria-Bertani (LB) medium. The cells were collected by centrifugation (10000 rpm, 4°C and 10 min.), resuspended in 20 mM Tris-HCl buffer (pH 8.0) containing 10 mg/ml lysozyme, incubated at 37°C for 30 min and finally sonicated (Bandelin-Sonopuls, Germany) in an ice bath (80% amplitude, 1.0 cycle for 5 min). The sonicate was removed by centrifugation at 10000 rpm for 10 min at 4°C and supernatant used as the cell-free extract.

Enzyme purification

The crude extract was precipitated by using an equal volume of cold acetone (chilled -30°C). The crude extract-acetone mixture was stored at +4°C for 2 hours. The acetone precipitate was collected by centrifugation (5000 rpm and 10 min +4°C) and left for 24 h at 4°C to remove acetone. After that, the precipitate was resuspended in 50 mM Tris-HCl buffer (pH 8.0). After being stirred for 5 min, the suspension was centrifuged at 5000 rpm for 5 min. The supernatant was applied to a Q-Sepharose Fast Flow Anion Exchange column (1.5 x 30 cm, Sigma St. Louis, MO, USA) equilibrated with 20 mM Tris-HCl (pH 8.0), at a flow rate of 1 ml min^{-1}. After loading, the column was washed with 200 ml of the equilibration buffer to elute unbound proteins. Bound proteins were eluted by a linear gradient of NaCl (0-0.6 M). The presence of proteins in collected fractions was monitored by measuring absorbance at 280 nm. Fractions were examined for esterase activities using *p*-NPB as a substrate. Active fractions were pooled and concentrated by ultrafiltration (Ultracell Membrane 10.000 MWCO Millipore, Amicon, USA).

The protein concentrations were determined according to Lowry at al. [8] with bovine serum albumin as a standard. The values were obtained from calibration curve prepared at 650 nm.

Native PAGE and activity staining

Enzyme purity was monitored by Native-PAGE with Comassie Brillant Blue staining. Non-denaturing polyacrylamide gel electrophoresis was performed at 4°C by using a 5% stacking gel and a 10% separating gel [9]. Esterase activity staining was performed by incubating the gel in 100 ml of 50 mM Tris-HCl buffer (pH 8.0) containing 2% (v/v) *β*-naphthyl acetate (30 mM, dissolved in acetone) at 50°C for 25 min and then adding 0.04% (w/v) Fast Blue B salt [10].

Enzyme assay

Esterase activity was determined by measuring the amount of *p*-nitrophenol released by esterase catalyzed hydrolysis [11]. The substrate mixture contained 10 mM *p*NPB, ethanol and Tris- HCl buffer (pH 8.0, 20 mM) in the ratio of 1:4:95 (v/v/v), respectively. The reaction mixture was incubated at 50ºC for 20 min and then the change in absorbance at 405 nm was monitored. The non-enzymatic reaction was monitored as a control in the presence of *p*NPB. One unit of esterase activity was defined as the amount of enzyme that produced 1 µmol of *p*-nitrophenol per minute [11].

Substrate specificity and kinetic studies

The substrate specificity of purified esterase was analyzed using *p*-nitrophenyl esters. The solutions of different chain-length fatty acid nitrophenyl esters such as *p*-nitrophenyl acetate (*p*NPA), *p*-nitrophenyl butyrate (*p*NPB) and *p*-nitrophenyl laurate (*p*NPL) were prepared at 10 mM concentrations. The esterase activity was assayed using the standard method described previously [11].

Effect of substrate concentration on esterase activity

A substrate saturation curve was obtained by plotting the final substrate concentration in the range of 0.005-0.5 mM versus esterase activity in the presence of *p*NPB as substrate. The Michaelis-Menten constant (K_m) and the maximum velocity of the reaction (V_{max}) were calculated from Lineweaver-Burk plot [12].

Effects of pH and temperature on enzyme activity

Esterase activity was measured at different pH values by using the following buffers Mcilvaine (pH 4.0-7.0) and Tris-HCl (pH 7.5-9.0). The activity was expressed as percent relative activity with respect to maximum activity which was considered as 100%.

Esterolytic activity, as a function of temperature, was determined in a temperature range of 20-80°C with 10°C increments at the optimum pH value obtained for *p*NPB, using a thermoblock. The mixtures of buffer and the substrate solution were pre-incubated for 5 min at the indicated temperatures. The purified enzyme was added to the mixture and the relative activity was determined spectrophotometrically at 405 nm [11].

pH- and thermal stability of the esterase

To investigate pH stability, the enzyme solutions were incubated at different pH values Mcilvaine (pH 4.0-7.0) and Tris-HCl (pH 7.5-9.0) for 3 days at 4°C. Enzyme activities were examined under standard reaction conditions for the calculation of the residual activities. The percentage residual esterase activity was calculated by comparison with unincubated enzyme.

In order to determine the thermal stability of the enzyme, the enzyme was incubated in a thermoblock at various temperatures of 30-80°C with 10°C increments for different periods of time. After the mixture was cooled in an ice bath and brought to room temperature, the enzyme activity was assayed under the standard reaction conditions at optimum pH and temperature values. The percentage residual esterase activity was calculated by comparison with the activity of unheated enzyme.

Effects of different metal ions and organic solvents on enzyme activity

The effect of various metal ions on esterase activity was tested by using the solution of chloride salts of metals such as Na^+, Li^+, Mn^{2+}, Mg^{2+}, Zn^{2+}, Cu^{2+}, Co^{2+} and Ca^{2+} at final concentrations of 1 mM and 10 mM [13]. The residual activities were measured by comparison with standard assay mixture containing no metal ion. Enzyme activity determined in the absence of metal ions was defined as 100%.

In order to determine the effect of the some organic solvents on the esterase activity, organic solvents such as methanol, ethanol, isopropanol and acetonitrile at final concentration of 10% were added to the standard reaction mixture [14]. The residual activities were measured by comparison with standard assay mixture containing no organic solvent. Activity of the enzyme without any additive was taken to be 100%.

Results and Discussion

Esterase purification

In the present study, the purified esterase was obtained by acetone precipitation and ion exchange choromatography. The three-step enzyme purification is summarized in Table 1. The enzyme was purified 57.1 fold and the specific activity of the purified enzyme was found to be 33.15 U/mg protein. The pure enzyme showed a single band on native PAGE with comassie and activity staining (Fig 1). Many studies on esterases with different levels of purification had been reported, 12.5 fold from *Thermoacidophilic archaeon* esterase [15], 42.7 fold from *Bacillus* [16], 52 fold from *Melanocarpus albomyces* [17] and 76.7 fold from *Bacillus licheniformis* S-86 [18]. It is easily seen from the data that choosen purification steps were performed successfully and efficiently.

Substrate specificity and enzyme kinetics

To investigate the substrate specificity of the esterase, the hydrolyzing activity of purified enzyme toward *p*-nitrophenyl (*p*NP) esters of various carbon chain lengths was determined pH 8.0 and 50°C. Esterase had maximal relative activity toward *p*-NP butyrate and also *p*-NP acetate 100 and 61.65%, respectively, whereas *p*-NP esters (*p*-NP laurate and *p*-NP palmitate) with longer chain lengths were converted to *p*-nitrophenol only slightly or not at all. These results show that the purified enzyme is an esterase because of hydrolyzing the short-chain organic acid esters. This result is also consistent with Fu [19] and Chuang [20] for esterolytic enzymes from different sources.

K_m and V_{max} values of the esterase from *Geobacillus sp.* TF17 were determined from the linear regression analysis of $1/V$ versus$1/[S]$. Using *p*-NPB as substrate, K_m and V_{max} values were calculated to be $5.6x10^{-2}$ mM and 19.38 U/mg protein, respectively (Fig 2). K_m value can change as depending on the structure of enzyme, substrate, pH, temperature and ionic strength [21]. The results obtained from this study, especially in terms of K_m value make the study very interesting like

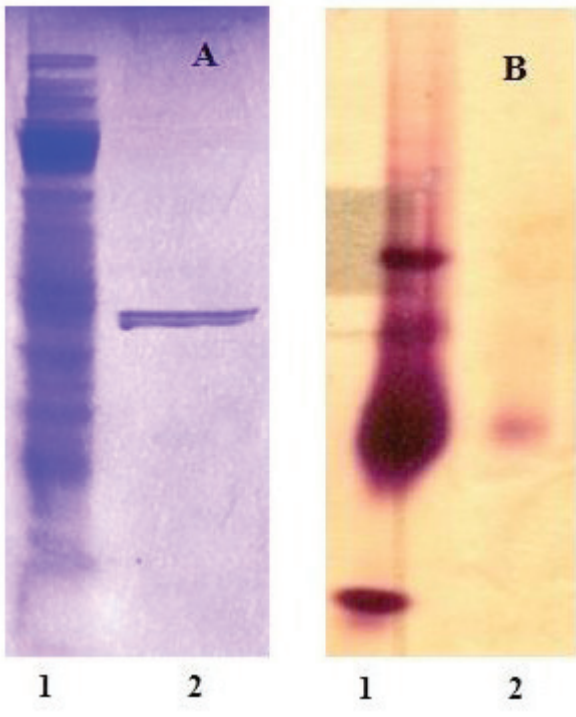

Figure 1. Native PAGE with comassie blue and activity staining. (A) Comassie staining: line 1, crude enzyme extract; line 2, esterase purified by Q-Sepharose column chromatography , (B) activity staining: lane 1, crude enzyme extract; line 2, esterase purified by Q-Sepharose column chromatography.

previously reported esterases [20,22]. It was reported that the K_m values of most industrial enzymes varied within the range of 10^{-1} to 10^{-5} M when acting on biotechnologically important substrates [23]. Therefore, it is obvious that esterase from *Geobacillus sp.* TF17 may be used in these processes.

Effect of pH and temperature on enzyme activity

As shown in Fig. 3A, the esterase had maximum activity at pH 7.5. Similar results were reported earlier for esterases from *Brevibacterium linens* ATCC 9174 [24] and *Thermus thermophilus* HB27 [25].

Effect of temperature on the enzyme activity was determined at different temperatures ranging from 20 to 80°C. It was observed that the enzyme retained 70% of its maximum activity at 40 and 60°C and showed maximum activity at 50°C. (Fig. 3B). This is similar to earlier findings shown for *Penicillium chrysogenum* [26], *Aspergillus niger* [27,28] and *Aspergillus oryzae* [29] esterases. However, the optimum temperature of *Geobacillus* sp TF17 esterase is higher than the esterases from *Cucurbita pepo* Elc and Ell with an optimum temperature between 30 and 40°C [30] and some *Bacillus* sp. with an optimum temperature at 45°C [17], and is lower than the esterase from *Bacillus licheniformis* S-86 [31] at 60°C and *Fervidobacterium nodosum* Rt17-B1 [32] at 75°C.

pH- and thermal- stability

The pH stability of the purified esterase was determined by monitoring the residual activities after 72 h incubation of enzyme solutions at different pH values at 4°C. The results showed that the enzyme was quite stable at all investigated pH values at 4°C (Fig. 4A), with no loss of activity after 48 h of incubation. At the end of 72 h incubation period, all examined pH values except 7.5, the enzyme retained nearly 60% of its original activity but 100% at pH 7.5. The pH stability profile of *Bacillus sp.* esterase showed that enzyme retained almost its original activity when it was incubated up to 10 h at pH 4.0-8.0 [22]. In the other study for an esterase, it was observed

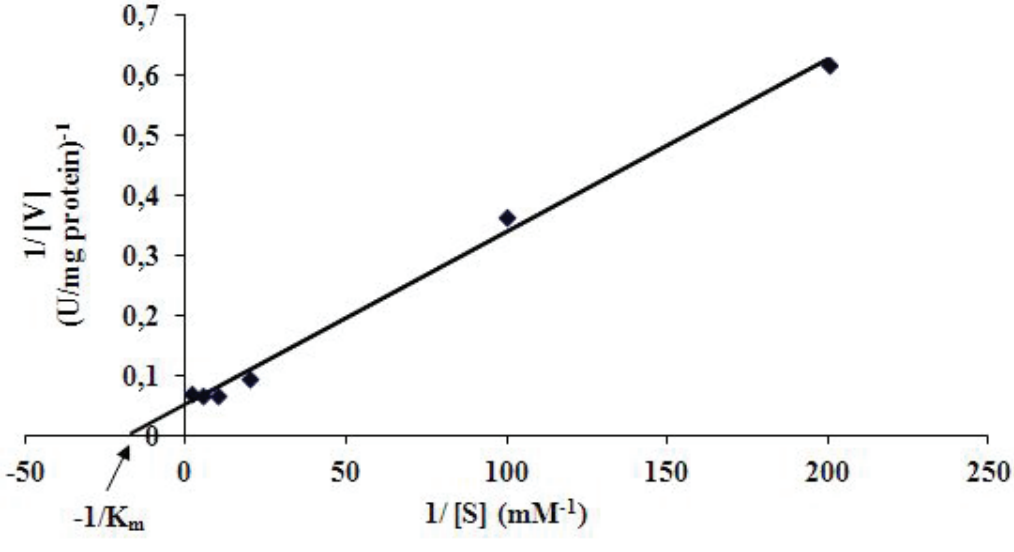

Figure 2. Lineweaver-Burk plot of purified *Geobacillus* sp TF17 esterase.

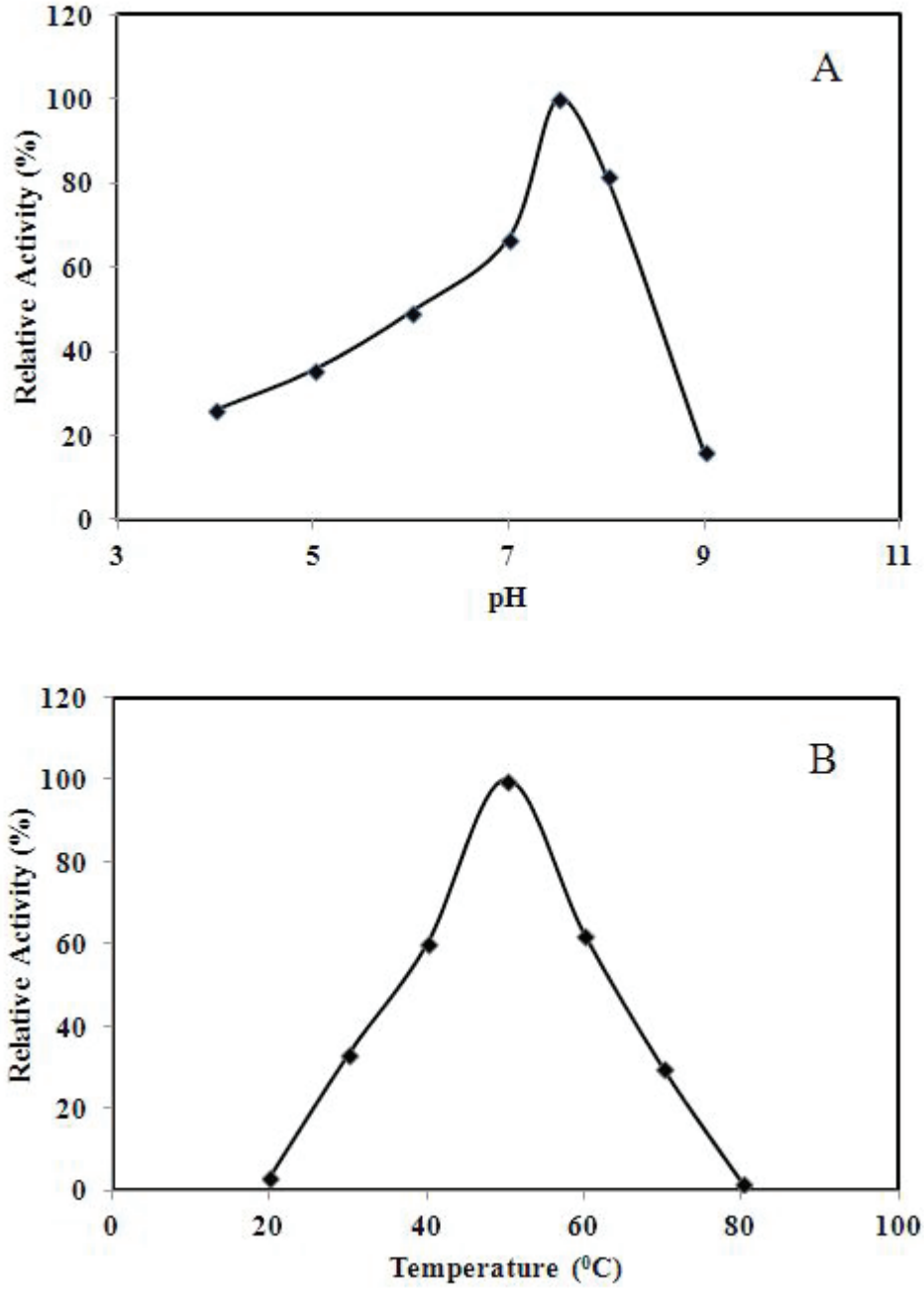

Figure 3. (A) pH -Relative activity (%) and (B) Temperature -Relative activity (%) profiles of the purified esterase. (A) The activity was determined by performing *p*NPB assay in the pH range of 4.0-9.0. The activity at pH 7.5 corresponds to 100%. (B) The reaction mixture containing the purified enzyme and *p*NPB was incubated at various temperatures for 20 min. The esterase activity was measured by standard assay conditions.

that the enzyme retained 50% of its original activity at pH 8.0 after 430 and 286 min incubation period at 50 and 55°C [33]. Consequently, it can be said that *Geobacillus* sp TF17 esterase is fairly stable for pH changes. The stability of the enzyme in acidic and basic pHs suggests its usefulness in industrial applications.

The purified esterase was almost completely stable at 30, 40 and 50°C at the end of 72 h incubation period. Catalytic efficiency of the enzyme did not change at 40 and 50°C while 90% and 30% of the original enzyme activity was retained after pre-incubation at 30 and 60°C for 72 h, respectively. After 4 h incubation period at 70 and 80°C, It was seen that *Geobacillus* sp TF17 was not stable at high temperatures (Fig. 4B). The thermal stability of esterase from *Geobacillus sp.* TF17 was found higher than that observed in esterases from *B. licheniformis* S-86 (50% at 50°C for 1 h) [31] and *Bacillus sp.* (50% at 65°C for 10 h) [22], nearly similar with *P. furiosus* (100% 75°C for 2 h) [34] but lower than *Thermus thermophilus* HB27 esterase (50% at 85°C for 135 min) [35].

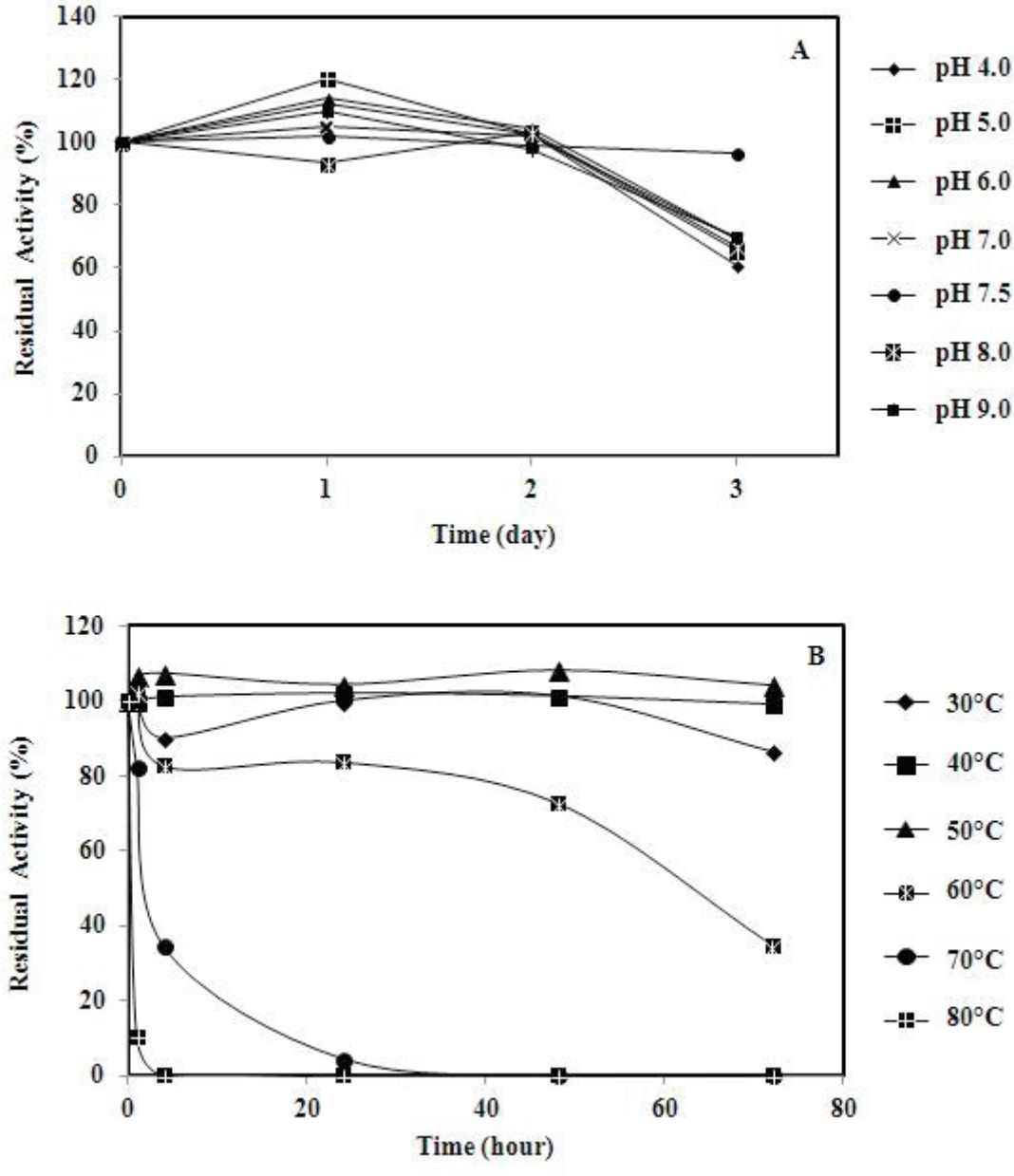

Figure 4. pH and temperature stability profiles of the purified esterase. (A) The pH stability profile of the purified esterase. The enzyme was incubated in various buffers with a pH range from 4.0 to 9.0 for 3 days at 4°C. The reaction mixture contained the enzyme and *p*NPB substrate were assayed by standard assay conditions, and the residual activity was calculated. (B) Thermostability of purified esterase. After 1, 4, 24, 48 and 72 h of incubation at different temperatures, the residual activities were measured by standard assay conditions. Control with non-incubated enzyme was used to determine the 100% activity value.

Effect of organic solvents on esterase activity

The effect of organic solvents (10%, v/v, in final reaction mixture) on esterase activity was examined by determining residual activity of purified enzyme solution. The esterase retained 65% of its original activity in the presence of methanol or ethanol, whereas a stronger inhibition (64% and 82%) was observed in the presence of isopropanol and acetonitrile, respectively. These results are consistent with the literature. *Thermoacidophilic archaeon* esterase activities in the presence of some organic solvents were reported by Kim and Lee [16]. According to this study, esterase had a very high resistance against ethanol. While this enzyme has 40% relative activity against acetonitrile, enzyme activity was almost completely inhibited by methanol and isopropanol. *Kluyveromyces marxionus* CBS 15532 esterase had 82% and 76% residual activities in the presence of methanol and ethanol, respectively [36].

Table 1. Purification steps of the esterase from *Geobacilius sp.*TF17

Purification Step	Total protein (mg)	Total activity (U)	Specific activity (U/mg protein)	Yield (%)	Purification fold
Crude extract	42	24.4	0.58	100	1
Acetone precipitation	38	23.7	0.62	97.1	1.1
Q Sepharose Fast Flow	0.6	19.9	33.15	81.6	57.1

Table 2. Effect of metal ions on esterase activity. The reaction mixture was incubated at 50 °C for 20 min in the presence of various metal ions at concentrations of 1 and 10 mM.

Metal ion	Residual activity (%)	
	(1 mM final concentration)	(10 mM final concentration)
None	100±3	100±3
Mn^{2+}	98±3	60±2
Co^{2+}	95±3	46±2
Na^{+}	91±3	79±2
Li^{+}	87±3	81±3
Mg^{2+}	87±2	78±2
Ca^{2+}	83±3	55±2
Cu^{2+}	78±2	9±1
Zn^{2+}	72±2	1±1

Effect of some metal ions on the enzyme activity

In the presence of all assayed metal ions at 1mM concentration, it was shown that the purified enzyme retained >70% of its original activity. However, Mn^{2+}, Co^{2+} and Ca^{2+} at 10 mM concentration reduced original esterase activity more than 40%. *T. terreum* [37], *C. pepo* [30] and *B. subtilis* RRL 1789 [38] esterases were inhibited by Mn^{2+}. Similar inhibitory effects of Co^{2+} on the activity of different esterases have been confirmed by several authors [19,37]. In the presence of Ca^{2+}, esterases from rice bran [20] and *S. thermophilus* [39] was slightly inhibited, whereas an esterase from salt-tolerant *Bacillus* [17] was strongly inhibited. The addition of 10 mM Zn^{2+} and Cu^{2+} to the reaction mixture almost completely inhibited the enzyme activity (Table 2). Similar results have been reported for the esterases *Cucurbita pepo* [30] whereas the esterases from deep sea metagenomic library [19] and *Acinetobacter baumannii* BD5 [40] lost their activity more than 55% in the presence of Zn^{2+} and Cu^{2+} at 10 mM concentration.

In conclusion, this study describes the successful purificaion and characterization of an esterase from thermophilic *Geobacillus sp.* TF17. This enzyme is characterized in terms of pH and temperature optima, thermal- and pH-stability and kinetic parameters. Biochemical characterization revealed several properties of *Geobacillus sp.* TF17 esterase and suggested that the enzyme may be used in the suitable biotechnological applications.

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Conflict of Interest: The authors declare that there was no conflict of interest in this work.

References

[1] Ollis DL, Shea E, Cygler MB, Dijkstra B, Frolow F. The α/β hydrolase fold. Protein Eng 1992; 5:197-211.

[2] Jeager KE, Schneidinger B, Rosenau F, Werner M, Lang D, et al. Bacterial lipases for biotechnological applications. J Mol Catal-B 1997; 3:3-12.

[3] Jeager KE, Reetz MT, Microbial lipases form versatile tools for biotechnology. Trends Biotechnol 1998; 16:396-403.

[4] Kawamoto T, Sonomoto K, Tanaka A. Esterification in organic solvents: Selection of hydrolases and effects of reaction conditions. Biocatalysis 1987; 1:137-145.

[5] Panda T, Gowrishankar BS. Production and applications of esterases. Appl Microbiol Biot 2005; 67:160-169.

[6] Gandhi NN. Applications of lipase. J Am Oil Chem Soc 1997; 74:621-634.

[7] Haki GD, Rakshit SK. Developments in industrially important thermostable enzymes: a review. Bioresour Technol 2003; 89:17-34.

[8] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951; 193:265-275.

[9] Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual 1989; 2nd ed., Spring Harbor Laboratory Press, New York.

[10] Zhou X, Scharf ME, Sarath G, Meinke LJ, Chandler LD, et al. Partial purification and characterization of a methyl-parathion resistance-associated general esterase in *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). Pestic Biochem Phys 2004; 78:114-125.

[11] Lee D, Koh Y, Kim K, Kim B, Choi H, et al. Isolation and characterization of a thermophilic lipase from *Bacillus thermoleovorans* ID-1. FEMS Microbiol Lett 1999; 179:393-400.

[12] Lineweaver H, Burk D. The determination of enzyme dissociation constants. J Am Chem Soc 1934; 56:658-666.

[13] Hess M, Katzer M, Antranikian G. Extremely thermostable esterases from the thermoacidophilic euryarchaeon *Picrophilus torridus*. Extremophiles 2008; 12:351-364.

[14] Nawani N, Singh R, Kaur J, Immobilization and stability studies of a lipase from thermophilic *Bacillus sp*: The effect of process parameters on immobilization of enzyme. Electron J Biotechn 2006; 9:559-565.

[15] Kim S, Lee SB. Thermostable esterase from a thermoacidophilic archaeon: Purification and characterization for enzymatic resolution of a chiral compound. Biosci Biotechnol Biochem 2004; 68:2289-2298.

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

[17] Kontkanen H, Tenkanen M, Reinikainen T. Purification and characterization of a novel steryl esterase from *Melanocarpus* albomyces. Enzyme Microb Tech 2006; 39:265-273.

[18] Torres S, Baigori MD, Pandey A, Castro GR. Production and purification of a solvent-resistant esterase from *Bacillus licheniformis* S-86. Appl Biochem Biotech 2008; 151:221-232.

[19] Fu C, Hu Y, Xie F, Guo H, Ashforth EJ, et al. Molecular cloning and characterization of a new cold-active esterase from a deep-sea metagenomic library. Appl Microbiol Biotechnol 2011; 90:961-970.

[20] Chuang HH, Chen PT, Wang WN, Chen YT, Shaw JF. Functional proteomic analysis of rice bran esterases/lipases and characterization of a novel recombinant esterase. J Agr Food Chem., 2011; 59:2019-2025.

[21] Doğan S, Salman U. Partial characterization of lettuce (*Lactuca sativa* L.) polyphenol oxidase. Eur Food Res Technol 2007; 226: 93-103.

[22] Ateslier ZBB, Metin K. Production and partial characterization of a novel thermostable esterase from a thermophilic *Bacillus sp.* Enzyme Microb Tech 2006; 38:628-635.

[23] Fullbrook PD. Practical Applied Kinetics, Industrial Enzymology 1996; 2nd. Edition, Stockholm Pres, New York.

[24] Rattray FP, Fox PF. Purification and characterization of an intracellular esterase from *Brevibacterium linens* ATCC 9174. Int Dairy J 1997; 7:273-278.

[25] Fucinos P, Atanes E, Lopez OL, Cerdan ME, Siso MIG, et al. Production and characterization of two N-terminal truncated esterases from *Thermus thermophilus* HB27 in a mesophilic yeast: effect of N terminus in thermal activity and stability. Protein Expres Purif 2011; 78:120-130.

[26] Sakamoto T, Nishimura S, Kato T, Sunagawa Y, Tsuchiyama M, et al. Efficient extraction of ferulic acid from sugar beet pulp using the culture supernatant of Penicillium chrysogenum. J Appl Glycosci 2005; 52:115-120.

[27] Kroon PA, Faulds CB, Williamson G. Purification and characterization of a novel esterase induced by growth of Aspergillus niger on sugar-beet pulp. Biotechnol Appl Biochem 1996; 23:255-262.

[28] Levasseur A, Benoit I, Asther M, Asther M, Record E. Homologous expression of the feruloyl esterase B gene from Aspergillus niger and characterization of the recombinant enzyme. Protein Expres Purif 2004a; 37:126-133.

[29] Tenkanen M, Schuseil J, Puls J, Poutanen K. Production, purification and characterization of an esterase liberating phenolic acids from lignocellulosics. J Biotechnol 1991; 18:69-84.

[30] Fahmy AS, Abo-Zeid AZ, Mohamed TM, Ghanem HM, Borai IH, et al. Characterization of esterases from *Cucurbita pepo* cv. "Eskandrani". Bioresource Technol 2008; 99:437-443.

[31]Torres S, Baigori MD, Swathy SL, Pandey A, Castro GR. Enzymatic synthesis of banana flavour (isoamyl acetate) by *Bacillus licheniformis* S-86 esterase. Food Res Int 2009; 42:454-460.

[32] Yu S, Zheng B, Zhao X, Feng Y. Gene cloning and characterization of a novel thermophilic esterase from *Fervidobacterium nodosum* Rt17-B1. Acta Biochimica et Biophysica Sinica 2010; 42:288-295.

[33] Vafiadi C, Topakas E, Biely P, Christakopoulos P. Purification, characterization and mass spectrometric sequencing of a thermophilic glucuronoyl esterase from *Sporotrichum* thermophile. FEMS Microbiol Lett 2009; 296:178-184.

[34] Almeida RV, Alqueres SMC, Larentis AL, Rössle SC, Cardoso AM, et al. Cloning, expression, partial characterization and structural modeling of a novel esterase from *Pyrococcus furiosus*. Enzyme Microb Tech 2006; 39:1128-1136.

[35] Fucinos P, Pastrana L, Sanroman A, Longo MA, Hermoso JA, et al. An esterase from *Thermus thermophilus* HB27 with hyperthermoalkalophilic properties: purification, characterization and structural modeling. J Mol Catal B- Enzym 2011; 70:127-137.

[36] Monti D, Ferrandi EE, Righi M, Romano D, Molinari F. Purification and characterization of the enantioselective esterase from *Kluyveromyces marxianus* CBS 1553. J Biotechnol 2008; 133:65-72.

[37] Ertunga NS, Cakmak U, Colak A, Faiz O, Sesli E. Characterization of esterolytic activity from two wild mushroom species, *Amanita vaginata var. Vaginata* and T*richoloma terreum*. Food Chemistry 2009; 115:1486-1490.

[38] Kaiser P, Raina C, Parshad R, Johri S, Verma V, et al. A novel esterase from *Bacillus subtilis* (RRL 1789): Purification and characterization of the enzyme. Protein Expes Purif 2006; 45:262-268.

[39]Liu SQ, Holland R, Crow VL. Purification and properties of intracellular esterases from *Streptococcus* thermophilus. Int Dairy J 2001; 11:27-35.

[40] Park IH, Kim SH, Lee YS, Lee SC, Zhou Y, et al. Gene cloning, purification, and characterization of a cold-adapted lipase produced by *Acinetobacter baumannii* BD5. J Microbiol Biotechnol 2009; 19:128-135.