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Biochemical characterization of a new thermostable lipase from *Bacillus pumilus* strain

[*Bacillus pumilu*s suşundan elde edilen yeni termostabil lipazın biyokimyasal karakterizasyonu]

Faouzi Laachari[1],
Fatimazahra El Bergadi[1],
Adel Sayari[3],
Soumya Elabed[2],
Iraqui Mohammed[1],
El Hassan Harchali[1],
Saad Koraichi Ibnsouda[1]

[1]Laboratoire de Biotechnologie Microbienne. Université Sidi Mohamed Ben Abdellah, B.P. 2202, Route d'Imouzzer, Maroc, Fes
[2]Université Sidi Mohamed Ben Abdellah, Centre Universitaire Régional d'Interface, Fès- Maroc
[3]Laboratoire de Biochimie et de Génie Enzymatique des Lipases, Enis, BPW 3038 Sfax, Tunisia

Correspondence Address
[Yazışma Adresi]

Faouzi Laachari, MD.

Université Sidi Mohamed Ben Abdellah, Laboratoire de Biotechnologie Microbienne, B.P. 2202, Route d'Imouzzer, Maroc, Fes
Phone: +212 666038407
Fax: +212 535608214
E-mail: faouzi.laachari@usmba.ac.ma

Translated by [Çeviri] Dr. Özlem Dalmızrak

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ABSTRACT

Objective: Research and characterization of new thermostable lipases from bacterial strains isolated from tannery waters in the old medina of Fez.

Methods: Gene which encodes the 16S ribosomal RNA for a bacterial species was amplified via PCR and sequenced (*Bacillus pumilus* HF544325). The extracellular lipase from *B. pumilus* is purified by gel filtration (Sephacryl S-200) and cation exchange chromatography (Mono S sepharose cation exchanger). The N-terminal sequences of purified *Bacillus pumilus* lipase were determined by automated Edman's degradation, using an Applied Biosystems 470 A protein sequencer equipped with PTH 120A analyser. The activity of lipase was examined within the pH range of 6.0-10.0 and the effect of pH on lipase stability was determined by incubating the lipase fraction in various buffer solutions ranging from 3.0 to 10.0 for 24 h at room temperature.

Results: The results showed that *Bacillus pumilus* is a strain that produce non-inducible lipase. This enzyme has a molecular weight of 27 kDa and presents a maximal activity at pH 8 and 45°C. The 18 N-terminal amino acid residues showed a high degree of homology with other Bacillus lipase sequences. After treatment in 100°C for 5 min, the thermostable enzyme maintains 60% of its activity, which is greater than that those founded in previous works. The enzyme retained 100% of its activity after 30 min incubation at 70°C.

Conclusion: This newly isolated lipase is thermostable and it has a significant difference which was observed when the biochemical properties of the *Bacillus pumilus* lipase were compared to others microbial lipases. The *Bacillus pumilus* lipase can be considered as a good candidature for industrial and biotechnological applications.

Key Words: *Bacillus pumilus*, lipase, purification, thermostable

Conflict of Interest: The authors have no conflict of interest.

ÖZET

Amaç: Çalışmanın amacı Fez şehrinde bulunan tabakhane sularından izole edilen bakteri suşlarından, sıcaklığa dayanıklı yeni bir lipaz enzimini saflaştırarak karakterize etmektir.

Metod: Bakteri türleri için 16S ribosomal RNA'yı kodlayan gen PCR ile çoğaltılarak sekanslanmıştır (*Bacillus pumilus* HF544325). Ekstraselüler lipaz jel filtrasyonu (Sefakril S-200) ve katyon değiştirici kromatografi kullanılarak (Mono S Sefaroz katyon değiştirici) *B. pumilus*'den saflaştırılmıştır. Saflaştırılan *Bacillus pumilus* lipazının N-terminal dizisi Edman degredasyon yöntemi (Applied Biosystems 470 A protein sekanslama cihazı ile PTH120A analizör) ile saptanmıştır. Lipaz aktivitesi 6.0-10.0 pH aralığında incelenmiş, pH'nın lipaz enziminin dayanıklılığına olan etkisi lipaz fraksiyonunu pH'sı 3.0 ile 10.0 arasında değişen çeşitli tampon çözeltilerinde 24 saat oda sıcaklığında inkübe ederek araştırılmıştır.

Bulgular: Sonuçlar *Bacillus pumilus* suşunun indüklenmeyen lipaz enzimini ürettiğini göstermektedir. Lipaz enzimi 27 kDa molekül ağırlığına sahip olup, en yüksek aktiviteyi pH 8 ve 45°C'de göstermektedir. Amino ucundaki 18 amino asit diğer Bacillus lipaz dizileri ile yüksek oranda homoloji göstermektedir. Sıcaklığa dayanıklı olan bu enzim 100°C'de 5 dakika muamele edildiğinde aktivitesinin %60'ını korumaktadır. Bu değer daha önce yapılan çalışmalardakinden daha yüksek bir değerdir. Enzim 30 dakika 70°C'de inkübe edildiğinde aktivitesinin tamamını korumaktadır.

Sonuç: Yeniz izole edilen lipaz sıcaklığa dayanıklıdır ve diğer mikrobiyal lipazlar ile karşılaştırıldığında biyokimyasal özellikleri bakımından diğerlerinden oldukça farklıdır. *Bacillus pumilus* lipaz enzimi endüstriyel ve biyoteknolojik uygulamalar için iyi bir aday olarak düşünülebilir.

Anahtar Kelimeler: *Bacillus pumilus*, lipaz, saflaştırma, termostabil

Çıkar Çatışması: Yazarların çıkar çatışması yoktur.

Introduction

Lipases (EC 3.1.1.3) which have been found in many species, plants, bacteria, yeasts and fungi, represent a large group of enzymes in biotechnology [1]. The enzymes of microbial origin are the most interesting, because of their potential applications in different sectors such as food, pharmaceuticals, detergents, biodiesel and cosmetic industries, also the improvement of the biodegradability of effluents from high-fat food industry (dairy, meat and seafood). Lipases used in detergents, amylases and glucose isomerase, used in starch processing and in the bioprocessing of raw materials or in the synthesis of organic chemicals, are very efficient [2,3,4]. Most of lipases are serine enzymes and there is a hydrogen-bond network in their active site, consisting of the triad of Ser, Asp (Glu), and His. Most lipase sequences include the conserved region, Gly-Xxx- Ser-Xxx-Gly or Ala-Xxx-Ser-Xxx-Gly, which is the feature of the lipase sequences from *Bacillus subtilis* [5]. The bacterial lipases receive much attention due the rapid biotechnology development, their substrate specificity and their ability to resist at extremes conditions.

The increase in production of microbial lipases requires not only the techniques of molecular biology which contribute to the overexpression of the corresponding genes, but also the understanding of the molecular and biochemical mechanisms that influence the folding and secretion of these enzymes [6,7,8]. Bacillus lipases have attracted much attention because of their biotechnological potential, which has led to the isolation of several lipolytic enzymes of *B. subtilis* and other species of the genus Bacillus, *Geobacillus* and *Paenibacillus* [9]. The lipases, *B. subtilis* and *B. pumilus* belong to the subfamily I.4 [10], are smaller than other subfamilies, and characterized by the absence of the structure of the cover. Some lipases such as lipase *B. pumilus B26* do not contain the Ca^{2+} binding motif near the catalytic site, its activities and thermostability is independent of Ca^{2+}. In 1990 it was determined the first two 3D structures of lipases by X-ray diffraction: it is a part of the structure of a lipase fungus, *Rhizomucor miehei* lipase [11], also one of the two main digestive enzymes, the HPL [12].

After extensive screening of strains producing lipase, only one bacterial isolated from Fez tannery, give a high lipolytic activity in the solid medium described above. Identification of this strain shows that it is *Bacillus Pumilus*. The thermostable and thermoactive lipases from *Bacillus pumilus* are not previously described. Also, the microbial enzymes may be limited in industrial applications due to relatively lower stabilities and catalytic activities under conditions that characterize industrial practical applications like extremes of pH values or non-aqueous solvents and high degrees of temperatures. In the last years, remarkable works in the engineering of enzymes with appropriate characteristics for industrial processes. Thus, screening of microorganisms with lipolytic activities in extreme habitats could aid the discovery of novel lipases specific characteristics. In this paper for the first time we report the production, the purification and the characterization of a thermoactive and thermstable lipase (BPL) from a newly isolated *Bacillus pumilus* strain. The N-terminal sequence of the BPL was determined and compared to the known bacillus lipases.

Materials and Methods

Chemicals

Tributyrin (99%; puriss) and benzamidine were from Fluka (Buchs, Switzerland); tripropionin (99%, GC) was from Jansen (Pantin, France); phosphatidylcholine, sodium deoxycholic acid (NaDC), Tween 20, yeast extract and ethylene diamine tetraacetic acid (EDTA) were from Sigma Chemical (St. Louis, USA); gum arabic was from Mayaud Baker LTD (Dagenham, United Kingdom); acrylamide and electrophoresis grade were from BDH (Poole, United Kingdom); marker proteins and supports of chromatography used for BPL purification: Sephacryl S-200 and Mono S-Sepharose gels were from Pharmacia (Uppsala, Sweden); PVDF membrane was purchased from Applied Biosystems (Roissy, France); trans-blot cell apparatus was from Bio-Rad (Paris, France); vinyl acetate was from Aldrich (Stenheim, Germany); casein peptone was from Merck (Darmstadt, Germany); and pH-stat was from Metrohm (Switzerland).

Enzymes and proteins

Bacillus Pumilus lipase (BPL) was purified in our laboratory, as described by Sayari [13].

Screening of lipolytic microorganisms

An initial screening of 74 strains from various Moroccain biotopes was carried out. This screening was realized on a solid medium containing 1% olive oil, 1% nutrient broth, 1% NaCl, 1.5% agar and 1% rhodamin B (pH 7). The culture plates were incubated at 37°C, and colonies giving rise to widespread clearing around them were regarded as putative lipase producers. Among the 10 strains retained.

Culture conditions

The bacterium was precultured during 12 h at 37°C and 200 rpm in 250 ml shaking flasks with 50 ml of medium A (17 g/l casein peptone, 5 g/l yeast extract (Difco), 2.5 g/l glucose, pH 7.4). Overnight, B. pumillus cultures used as inocula were cultivated in 1-l shaking flasks with 100 ml of medium A. The initial absorbance (OD) measured at 600 nm was adjusted to an approximate 0.2 value. The culture was incubated aerobically for during 72 h on a rotary shaker set at 200 rpm at a temperature of 37°C. Growth was followed by measuring the OD of the cultures at 600 nm.

Lipase activity determination

The lipase activity was measured titrimetrically at pH 8.2 and 45°C with a pH-stat under standard conditions using tributyrin (0.25 ml) in 30 ml of 2.5 mM Tris–HCl pH 8.2, 2 mM $CaCl_2$, 2 mM NaDC or olive oil emulsion (10 ml in

20 ml of 2.5 mM Tris–HCl pH 8.2, 2 mM $CaCl_2$, 4 mM NaDC) as substrate [1]. Lipase activity was also measured at pH 7 and 37°C using TC2 (0.25 ml) or TC3 (0.25 ml) in 30 ml of 2.5 Mm phosphate buffer pH 7, 3 mM $CaCl_2$ as substrate. Some lipase assays were performed in the presence of bile salts. The enzymatic hydrolysis of solutions and emulsions of esters was followed potentiometrically at 25°C and pH 7.0. Assays were carried out in 30 ml of 2.5 mM Tris–HCl buffer pH 7.0 containing 0.1 M NaCl. Standard conditions for measuring enzyme activity at increasing esters concentrations have been described previously [14]. When measuring BPL activity in the absence of $CaCl_2$, we added EDTA or EGTA to the lipolytic system. Lipolytic activity was expressed as units. One unit corresponds to 1 μmol of fatty acid released per minute.

Determination of protein concentration

Protein concentration was determined as described by Bradford [15] using BSA as standard.

Procedure of BPL purification

Culture medium 1 l of, obtained after 48 h of cultivation, was centrifuged for 20 min at 8000 rpm to remove the microbial cells. The supernatant containing extracellular lipase was used as the crude enzyme preparation.

– Ammonium sulfate precipitation: The crude enzyme solution (1–l), containing 15000 Units, was brought to 60% saturation with solid ammonium sulfate (390 g) under stirring conditions at 4°C. After centrifugation (30 min at 10000 rpm), the precipitate was resuspended in 15 ml of buffer A (20 mM sodium acetate pH 5.4, 20 Mm NaCl, and 1 mM benzamidine). Insoluble material was removed by centrifugation at 10000 rpm during 10 min.

– Heat treatment: the supernatant obtained (15 ml) was incubated at 70°C during 30 min. Insoluble material was removed by centrifugation at 10000 rpm during 10 min.

– Filtration on Sephacryl S-200: The supernatant (15 ml) was loaded on a column (3 cm×100 cm) of gel filtration Sephacryl S-200 equilibrated with buffer A. The elution of lipase was performed with the same buffer at a rate of 45 ml/h. The fractions containing the lipase activity (eluted at 1.3 void volume) were pooled.

– Cation exchange chromatography: The pooled fractions of Sephacryl S-200 column were applied to a Mono S sepharose cation exchanger equilibrated in buffer A. The column (2 cm×30 cm) was rinsed with 300 ml of the same buffer. No lipase activity was detected in the washing flow. Adsorbed material was eluted with a linear NaCl gradient (600 ml of 20 to 500 mM in buffer A) at a rate of 45 ml/h. BPL activity was eluted between 150 and 350 mM NaCl.

– The fractions containing the lipase activity were pooled, concentrated and reloaded on a column (2 cm×160 cm) of gel filtration Sephacryl S-200 equilibrated in buffer A. Elution was performed at a rate of 45 ml/h.

Analytical methods

Analytical polyacrylamide gel electrophoresis of proteins in the presence of sodium dodecyl sulfate (0.3 M) and β-mercaptoethanol (0.25 M) or DTT (0.5 M) (SDS/PAGE) was performed as described previously [16]. Sequencing samples were electroblotted according to Bergman and Jornvall [17]. Protein transfer was performed at room temperature during 1 h at 1 mA/cm^2. The molecular mass of BPL was determined by MALDI-TOF (matrix assisted laser desorption ionisation-time of flight).

Amino acid sequencing

The N-terminal sequence of purified BPL was determined by automated Edman_s degradation, using an Applied Biosystems 470 A protein sequencer equipped with PTH 120A analyser [18]. The sequence was kindly determined by Dr. Reinbolt (IBMC, UPR 9002, CNRS-Strasbourg, France).

Kinetic study

Lipase activities were measured as a function of various substrate (TC_4, TC_8 or TC_{18}) concentrations (0–40 mM). The Michaelis–Menten constant (KMapp.) and the maximum velocity (Vmax) for the reaction with TC_4, TC_8 or TC_{18} as substrate were calculated by Lineweaver-Burk plot.

Effect of pH and Temperature on the Activity and Stability of BPL

The activity of lipase was examined within the pH range of 6.0-10.0 .The lipase activity was measured titrimetrically at pH 8.0 and 37°C with a pH-stat under standard conditions using tributyrin (0.25 mL) in 30 mL of 2.5 mM Tris-HCl, pH 8.5, 3 mM $CaCl_2$ or olive oil (10%) emulsion (10 mL in 20 mL of 2.5 mM Tris-HCl, pH 8.5, 3 mM $CaCl_2$) [19] as substrate. The effect of pH on lipase stability was determined by incubating the lipase fraction in various buffer solutions ranging from 3.0 to 10.0 for 24 h at room temperature. After the incubation period, the residual activity was determined, after centrifugation, under standard assay method [19]. The optimum temperature

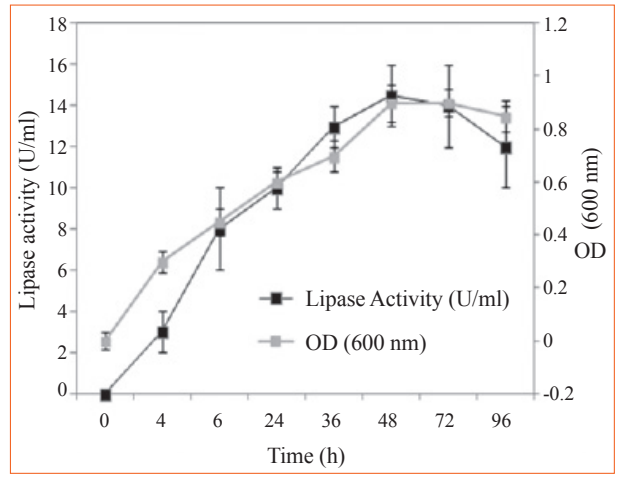

Figure 1. The time courses of lipase production. The culture was carried out at 37°C in shaking at 200 rpm in the presence or in the absence of triacylglycerols or esters.

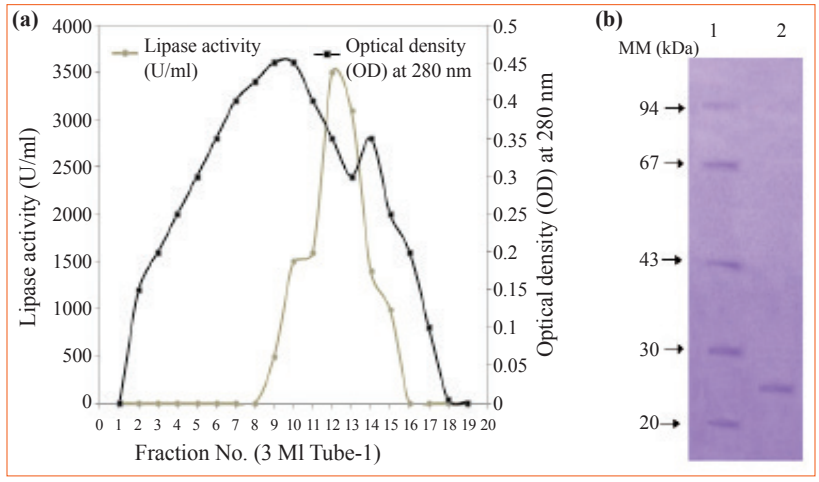

Figure 2. (a) Chromatography of BPL on Sephacryl S-200. The column (3×100 cm) was equilibrated with buffer A (20 mM sodium acetate pH 5.4, 20 Mm NaCl, 1 mM benzamidine). The elution of lipase was performed with the same buffer at a rate of 45 ml/h. Lipolytic activity was measured under standard conditions at pH 8.0 and 45°C using a pH-stat. **(b)** SDS/PAGE (15%). Lane 1, molecular mass markers (Pharmacia); lane 2, characterisation of the PBL obtained after Mono-S chromatography chromatography.

for the *Bacillus pumilus* lipase activity was determined by carrying out the enzyme assay at different temperatures (20–90°C) at pH 8.0. The effect of temperature on lipase stability was determined by incubating the enzyme solution at different temperatures (20–90°C) for 30 min. The residual activity was determined, after centrifugation, under standard assay method [19].

Results

Production of lipase

Culture of *B. pumilus* in medium A was realised at 37°C. The maximal production of lipase was obtained after 48 h of incubation. This production atteind 15 U/ml, with an inoculum size of 4.10^8 cells/ml (Figure 1). BPL production was not induced by the presence of triacylglycerols (TC4 or olive oil) or esters (Tween-20) (data not shown).

Purification of BPL

The BPL was purified according to the procedure described in the Materials and methods section 2.8. The protein elution profile obtained at the final step of the purification is shown in (Figure 2a). This figure shows that the lipase was eluted at 1.3 V_0. The results of SDS/PAGE analysis of the pooled fraction of this last step of chromatography are given in (Figure 2a). This figure shows that the enzyme exhibited one band corresponding to a molecular mass of about 27 kDa. The purification flow sheet is given in (Table 1), it shows that BPL is able to hydrolyse triacylglycerols without significant chain length specificity; the specific activity of 2100 U/mg was measured at pH 8 and 45°C with olive oil as substrate in the presence of (2 mM $CaCl_2$, and 2 mM NaDC). BPL was incubated at a temperature of 100°C for 1 to 5 min. The residual activity was determined, after centrifugation, under standard assay method using tributyrin or olive oil (10%) emulsion (Figure 3). BPL was found to be stable at 100°C after 5-min of incubation, maintains about 60% of its activity. Proteins were precipitated by acetone (v/2v) and visualized by SDS-PAGE 15% (Figure 2b), Which is a special case in the world, until now no lipase supports a temperature of 70°C and 100°C [20,21,22].

N-terminal sequence of BPL

The BPL NH_2-terminal sequencing allowed the identifica-

Table 1. Purification table of BP lipase

Purification step	Total activity (U/ml)[a]	Proteins (mg)[b]	Specific activity (U/mg)	Activity recovery (%)	Purification factor
Culture supernatant	15000	1500	10	100	1
$(NH_4)_2SO_4$ precipitation	11200	775	14	74.5	1.4
Heat treatment (30 min at 70 °C)	10100	655	15.5	67	1.55
Sephacryl S-200	8050	90	89	53.5	8.9
Mono-S chromatography	5530	2.63	2100	36	210

[a]1 Unit corresponds to 1 µmol of fatty acid released per minute; [b]Proteins were estimated by Bradford.

tion of 18 residues, A-E-K-F-V-G-K-Q-A-A-E-H-N-P-V-V-M-V. This N-terminal sequence exhibits a high degree of homology with lipases of the same genus previously characterised *Bacillus vallismortis* DV1-F-3, *Bacillus pumilus* ATCC 7061 and *Bacillus subtilis* BSn5 (Table 2).

Activation of BPL by interface

As it has been shown by Ferrato [17], among the short chain triacylglycerols tested as substrates (TC_2, TC_3, TC_4), TC_3 is the best system to check the interfacial activation of lipases. In this study, we have selected TC_3 to evaluate the interfacial activation phenomenon of BPL. The hydrolysis rate of TC_3 emulsified in 0.33% GA and 0.15M NaCl by BPL as a function of substrate concentration shows a normal Michaelis–Menten dependence of the activity on the substrate concentration (Figure 4). The interfacial activation cannot be taken as the unique criterion required to distinguish lipases from esterases "16" as described by Sarda [1]. Lipases are defined as a family of enzymes able to hydrolyse long chain triacylglycerols independently of the presence, or the absence, of an interfacial activation phenomenon. Here, we can say that BPL, which hydrolyses olive oil, is a true lipase.

Effect of pH and Temperature on the Activity and stability of B.pumilus lipase

The maximal activity of BPL was obtained at pH 8.0 (Figure 5a), and a temperature between 40 and 50°C (Figure 5c). The pH-optimum for BPL activity is the same results as others BL [23,24,25]. BPL was found to be stable at pH

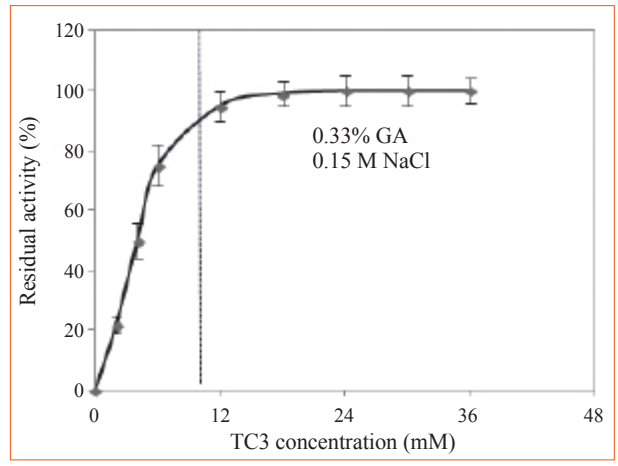

Figure 3. Hydrolysis rate of TC_3 by BPL as function of substrate concentration. The TC_3 solutions were systematically prepared by mixing (3×30 s in a warring blender) a given amount of TC3 in 30 ml of 0.33% GA and 0.15 M NaCl. The release of propionic acid was recorded continuously at pH 8 and 45°C using a pH-stat. The CMC of TC_3 (12 mM) is indicated by vertical dotted lines.

5 and 8 (Figure 5b). In contrast to all Bacillus lipases, this enzyme maintains about 100% of its activity after 30-min incubation at 70°C (Figure 5d).

Discussion

In this study, our findings show that *B. pumilus* is able to produce a thermostable lipolytic enzyme. Also, as lipases are generally produced using carbon source such

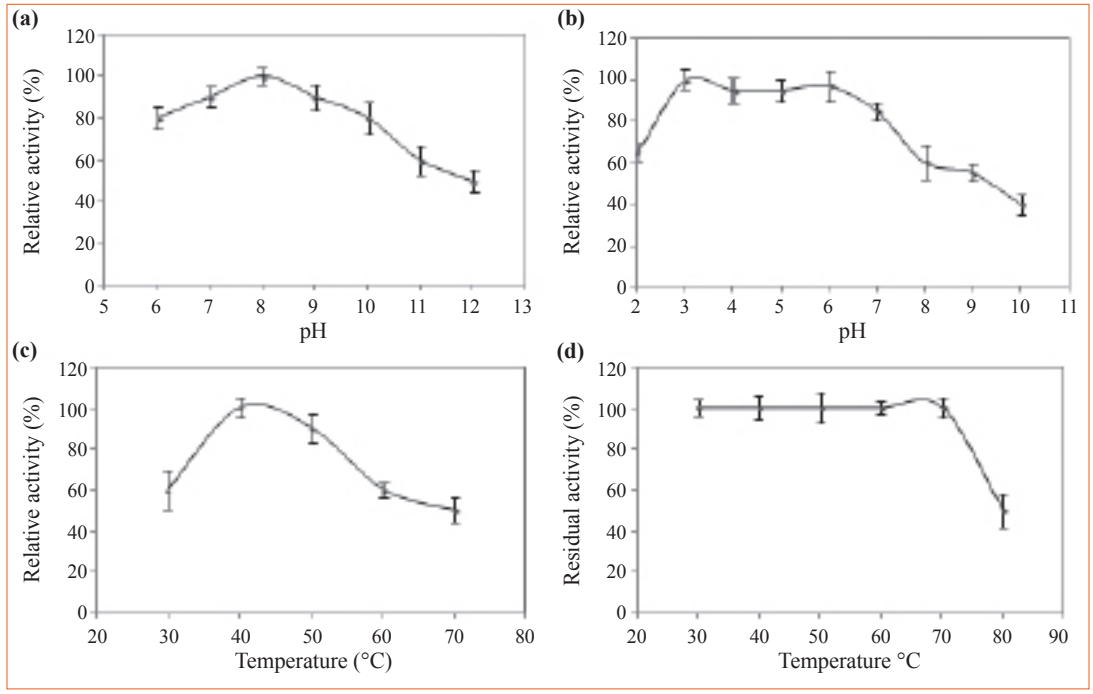

Figure 4. pH effect on enzyme activity **(a)** and stability **(b)** of BPL. Optimal pH was determined with tributyrin at 45°C under the standard conditions. Stability was analysed after preincubating the pure enzyme for 24 h in different buffer solutions at various pH ranging from 3 to 12. Temperature effect of on BPL activity **(c)** and stability **(d)**. For temperature stability the pure enzyme was preincubated at different temperatures for 30 min and the remaining activity was measured under the standard conditions.

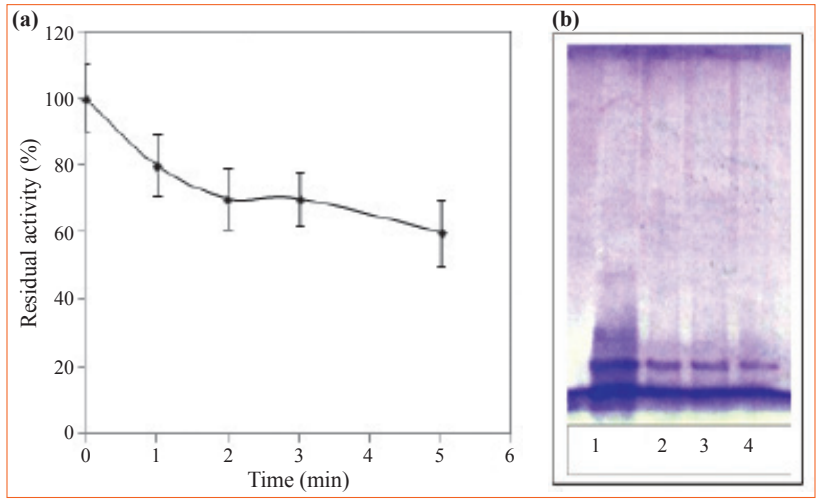

Figure 5. Temperature (100°C) effect on crude enzyme **(a)**. Residual activity was determined with tributyrin at pH 8.00 and 45°C using a pH-stat. SDS/PAGE (15%) **(b)**, characterization of the PBL obtained after heat treatment in 100°C, Lane 1, heat treatment for 1 min; Lane 2 (2 min); Lane 3 (3 min); Lane 4 (5 min).

as oils, fatty acids, glycerol or tweens in the presence of organic nitrogen source, in this study, the production of B.pumilus is not induced by the presence of triacylglycerols (like TC_4 or olive oil) or esters (Tween 80). According to SDS-PAGE, we have a protein of 27 KD molecular size which will be the first protein for this type of bacterium with this size (Figure 2b). The importances of alkaline and thermostable lipases for different applications have been growing rapidly. A great deal of research is currently going to develop lipases which will work under alkaline conditions as fat stain removers. Our results show that *B. pumilus* lipase remains active at a pH range of 6.0 to 10. This result can be very attractive and can have a great potential application in many areas. Our protein was found to be stable at 70°C with considerable activity (100%), and at 100°C with half-lives of 5 min (Figure 5). Many enzymes were produced by bacteria and yeast showed maximum activities at high temperatures, such as *Pseudomonas aeruginosa* (70°C), a thermophilic *Bacillus* sp. (60 to 70°C) [26], and the yeast *Kurtzmanomyces* sp. (75°C) [27]. Just few fungal lipases reported other studies presented as a thermophilic behavior. Among fungi of the genus *Penicillium*, which are mesophilic organisms, most lipases showed maximum activities at temperatures of 25 to 45°C [28,6]. Lipase of *B. pumilus* maintains its activity despite the denaturing conditions and treatment at high temperature (70°C and 100°C) (Figure 4d; Figure 5), similar as the lipase from *Burkholderia cepacia* ATCC 25416 and *Bacillus pumilus* RK31 appears to be tolerant to temperature, as were reported to be stable in the range temperature between 30°C to 60°C [29,30]. The same findings were described by fairolniza, the lipase from bacillus sp was thermostable in the temperature range of 55 to 75°C; and considerable activity (75%) was retained. Enzyme activity sequentially decreased as the incubation time is increased [31]; other lipase from *bacillus subtilis* was stable during 273.38, 51.04 and 41.58 min, at 60, 70 and 80°C and has an optimum activity in temperature of 60°C and stable in the pH of 7.0–9.0 and 40–70°C of temperature [32]. Due to the physicochemical properties of *B. pumilus* lipase, it can be the most efficient lipases and the best candidates in the industrial field [33].

Conclusion

BPL was isolated from the culture medium, the newly lipase from *B. pimulis* has been reported for the first time in this paper. BPL demonstrates high activity towards olive oil emulsion and TC_4 and exhibits optimal activity under the condition of 45°C and pH 8.0 and very stable at 70°C with a mass molecular of 27 KD. BPL hydrolyses the long chains more efficiently than the short chain triacyl-

Table 2. N-terminal sequence comparison of BPL with *Bacillus vallismortis* DV1-F-3, *Bacillus pumilus* ATCC 7061 and *Bacillus subtilis* BSn5

***Bacillus pumilus* (27Kda)**	**AEKFVGKQAAEHNPVVMV**	**Present study**
Bacillus vallismortis DV1-F-3	^{30}SLFAVQPSAKAAEHNPVVMV	accession ZP_10511292
Bacillus pumilus ATCC 7061	^{30}SMAFIQPKEVKAAEHNPVVMV	accession ZP_03056417
Bacillus subtilis BSn5	^{30}SLFALQPSAKAAEHNPVVMV	accession YP_004206227

glycérols and presents the interfacial activation phenomenon also there is a significant difference observed when the biochemical properties of the BPL were compared to other lipases.

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Conflict of Interest

There are no conflicts of interest among the authors.

References

[1] Sarda L, Desnuelle P. Actions of pancreatic lipase on esters in emulsions. [Article in French] Biochim Biophys Acta 1958; 30(3):513-21. [Abstract]

[2] Cheetham PSJ. The applications of enzymes in industry, in: Handbook of Enzyme Biotechnology (Wiseman, A. ed.) 1995; pp 420, Ellis Harwood, London.

[3] Rubin B, Dennis EA. Lipases: Part A. Biotechnology Methods in Enzymology 1997; pp. 1-408, Academic Press, vol. 284. New York.

[4] Kazlauskas RJ, Bornscheuer UT. Biotransformations with lipases, in: Rehm HJ, Pihler G, Stadler A, Kelly PJW. (Eds.), Biotechnology 1998; pp. 37-192, vol. 8, VCH, New York.

[5] Nthangeni MB, Patterton H, van Tonder A, Vergeer WP, Litthauer D. Over-expression and properties of a purified recombinant Bacillus licheniformis lipase: a comparative report on Bacillus lipases. Enzyme Microb Technol 2001; 28(7-8):705-12.

[6] Tan Y, Miller KJ. Cloning, expression, and nucleotide sequence of a lipase gene from Pseudomonas fluorescens B52. Appl Environ Microbiol 1992; 58(4):1402-7.

[7] Priest FG. Isolation and identification of aerobic endospore-forming bacteria 1989; p. 27-56. In: Harwood CR (ed.), Bacillus. Plenum Press, New York, NY.

[8] Harwood CR, Coxon RD, Hancock IC. Molecular Biological Methods for Bacillus 1990; pp. 327-89. In: Harwood CR, Cutting SM (Eds.), Wiley UK.

[9] Gupta R, Gupta N, Rathi P. Bacterial lipases: an overview of production, purification and biochemical properties. Appl Microbiol Biotechnol 2004; 64(6):763-81.

[10] Arpigny JL, Jaeger KE. Bacterial lipolytic enzymes: classification and properties. Biochem J 1999; 343:177-83.

[11] Brady L, Brzozowski AM, Derewenda ZS, Dodson E, Dodson G, et al. A serine protease triad forms the catalytic centre of a triacylglycerol lipase. Nature 1990; 343(6260):767-70.

[12] Winkler FK, D'Arcy A, Hunziker W. Structure of human pancreatic lipase. Nature 1990; 343(6260):771-4.

[13] Sayari A, Agrebi N, Jaoua S, Gargouri Y. Biochemical and molecular characterization of Staphylococcus simulans lipase. Biochimie 2001; 83(9):863-71.

[14] Stöcklein W, Sztajer H, Menge U, Schmid RD. Purification and properties of a lipase from Penicillium expansum. Biochim Biophys Acta 1993; 1168(2):181-9.

[15] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976; 72:248-54.

[16] Shastry BS, Rao MR. Studies on rice bran lipase. Indian J Biochem 1971; 8(4):327-32.

[17] Ferrato F, Carriere F, Sarda L, Verger R. A critical reevaluation of the phenomenon of interfacial activation. Methods Enzymol 1997; 286:327-47.

[18] Simons JW, Boots JW, Kats MP, Slotboom AJ, Egmond MR, et al. Dissecting the catalytic mechanism of staphylococcal lipases using carbamate substrates: chain length selectivity, interfacial activation, and cofactor dependence. Biochemistry 1997; 36(47):14539-50.

[19] Rathelot J, Julien R, Canioni P, Coeroli C, Sarda L. Studies on the effect of bile salt and colipase on enzymatic lipolysis. Improved method for the determination of pancreatic lipase and colipase. Biochimie 1975; 57(10):1117-22.

[20] Ruizhi L, Xiaolu J, Haijin M, Huashi G, HueyMin H, et al. A novel low-temperature resistant alkaline lipase from a soda lake fungus strain Fusarium solani N4-2 for detergent formulation. Biochem Eng J 2009; 46:265-70.

[21] Supakdamrongkul P, Bhumiratana A, Wiwat C. Characterization of an extracellular lipase from the biocontrol fungus, Nomuraea rileyi MJ, and its toxicity toward Spodoptera litura. J Invertebr Pathol 2010; 105(3):228-35.

[22] Rivera-Pérez C, del Toro Mde L, García-Carreño F. Purification and characterization of an intracellular lipase from pleopods of whiteleg shrimp (Litopenaeus vannamei). Comp Biochem Physiol B Biochem Mol Biol 2011; 158(1):99-105.

[23] Ma J, Zhang Z, Wang B, Kong X, Wang Y, et al. Overexpression and characterization of a lipase from *Bacillus subtilis*. Protein Expr Purif 2006; 45(1):22-9.

[24] Manoj S, Kumar S, Neha S, Krishnan K. Lipase Production by *Bacillus subtilis* OCR-4 in Solid State Fermentation Using Ground Nut Oil Cakes as Substrate. Current Research Journal of Biological Sciences 2010; 2(4):241-5.

[25] Shah KR, Bhatt SA. Purification and characterization of lipase from *Bacillus subtilis* Pa2. J Biochem Tech 2011; 3(3):292-5.

[26] Nawani N, Kaur J. Studies on lipolyticisoenzymes from a thermophilicBacillus sp: production, purification and biochemical characterization. J Enzyme Microb Technol 2006; 40:881-7.

[27] Kakugawa K, Shobayashi M, Suzuki O, Miyakawa T. Purification and characterization of a lipase from the glycolipid-producing yeast Kurtzmanomyces sp. I-11. Biosci Biotechnol Biochem 2002; 66(5):978-85.

[28] Jesus MFCO, Branco RN, Santanna GLJ, Freire DMG, Silva JGJ. Penicilliumrestrictumlipases: A comparative study and characterization of enzymes with different degrees of purity. Braz J Chem Eng 1999; 16:113-8.

[29] Wang X, Yu X, Xu Y. Homologous expression, purification and characterization of a novel high alkaline and thermal stable lipase from Burkholderia cepacia ATCC 25416. Enzyme and Microbial Technol 2009; 45:94-102.

[30] Rakesh K, Arpit S, Arun K, Deepak S. Lipase from *Bacillus pumilus* RK31: Production, Purification and Some Properties. World Appl Sci J 2012; 16(7):940-8.

[31] Shariff FM, Rahman RN, Basri M, Salleh AB. A newly isolated thermostable lipase from Bacillus sp. Int J Mol Sci 2011; 12(5):2917-34.

[32] Olusesan AT, Azura LK, Forghani B, Bakar FA, Mohamed AK, et al. Purification, characterization and thermal inactivation kinetics of a non-regioselective thermostable lipase from a genotypically identified extremophilic *Bacillus subtilis* NS 8. N Biotechnol 2011; 28(6):738-45.

[33] Vakhlu J, Kour A. Yeast lipases. Enzyme purification, biochemical properties and gene cloning. Electron J Biotechnol 2006.