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Characterization of a novel thermophilic phospholipase B from *Thermotoga lettingae* TMO: applicability in enzymatic degumming of vegetable oils

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Abstract A novel phospholipase B (TLPLB) from *Thermotoga lettingae* TMO has been cloned, functionally overexpressed in *Escherichia coli* and purified to homogeneity. Gas chromatography indicated that the enzyme could efficiently hydrolyze both the *sn*-1 and *sn*-2 ester bonds of 1-palmitoyl-2-oleoyl phosphatidylcholine as phospholipase B. TLPLB was optimally active at 70 °C and pH 5.5, respectively. Its thermostability is relatively high with a half-life of 240 min at 90 °C. TLPLB also displayed remarkable organic solvent tolerance and maintained approximately 91–161 % of its initial activity in 20 and 50 % (v/v) hydrophobic organic solvents after incubation for 168 h. Furthermore, TLPLB exhibited high degumming activity towards rapeseed, soybean, peanut and sunflower seed oils, where the phosphorus contents were decreased from 225.2, 189.3, 85.6 and 70.4 mg/kg to 4.9, 4.7, 3.2 and 2.2 mg/kg within 5 h, respectively. TLPLB could therefore be used for the degumming of vegetable oils.

Keywords Phospholipase B · Thermophilic · Organic solvent tolerance · Degumming · Vegetable oil

Introduction

Phospholipases belong to hydrolytic enzymes that can catalyze the hydrolysis of phospholipids into fatty acids and phosphoglycerate [10, 12]. The enzymes are found in various organisms including animals, plants and microorganisms and can be classified as phospholipase A, B, C, or D depending on the site at which they hydrolyze the ester bond of their substrate [9, 16, 28]. Phospholipases play essential roles in a number of different physiological processes, including phospholipid metabolism, signal transduction, cell cycle progression, cytoskeletal organization and inflammatory responses [3–5, 7].

Phospholipase B (PLB, EC 3.1.1.5) showed three different activities: a *sn*-1 and *sn*-2 fatty acid ester hydrolase (PLA_1 and PLA_2), a lysophospholipase, and a transacylase activity [16]. So these enzymes have been used in the food and pharmaceuticals industries such as enzymatic degumming process of vegetable oils and production of valuable phospholipid derivatives, respectively [8, 31]. PLB plays a critical role during the refining process of edible vegetable oils, which could effectively convert non-hydratable phospholipids to the corresponding hydratable forms, which could be separated and removed by centrifugation [6, 11]. Enzymatic degumming of vegetable oil has proved to be much more effective and environmentally friendly than traditional methods because of the reduction in the amounts of acid and base used, wastewater generated and cost in operating, and enhancement in product yields [30]. Several PLBs have been isolated and characterized from *Moraxella bovis* [9], *Pseudomonas fluorescens* BIT-18 [13], *Aspergillus fumigatus* [16], *Cryptococcus neoformans* [18], *Streptomyces* sp. strain NA684 [21], *Candida albicans* [22], rat [20], guinea pig [23] and human epidermis [28].

To date, most reports in the literature pertaining to the use of phospholipases in the enzymatic degumming of oils have focused predominantly on phospholipases A and C. To our knowledge, only one PLB (Pf-PLB from *P. fluorescens* BIT-18) has been reported to be used in the enzymatic

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degumming of vegetable oils [8, 14]. *Thermotoga lettingae* TMO is a thermophilic methanol-oxidizing bacterium that grows optimally at 65 °C and pH 7.0, which makes it a potential source for thermostable enzymes [1]. In this study, we report the cloning, expression and biochemical characterization of a novel phospholipase B (TLPLB) from *Thermotoga lettingae* TMO. Furthermore, TLPLB is identified as the first phospholipase B-type enzyme from a hyperthermophilic bacteria and also displays high degumming activity towards the phospholipids of vegetable oils.

Materials and methods

Strains and plasmids

The genomic DNA of T. *lettingae* TMO was obtained from Professor Jian Xu at the Qingdao Institute of BioEnergy and BioProcess Technology, Chinese Academy of Sciences, China. The plasmid pET15b (Novagen, USA) was modified with *Nde*I changed to *Nco*I in order to facilitate the purification [26]. *Escherichia coli* DH5α and BL21-CodonPlus (DE3)-RIL (Stratagene, CA) were used as the cloning and expression hosts, respectively.

Chemicals

Restriction enzymes, Pyrobest DNA polymerase, DNA ligase and a DNA Gel extraction kit were purchased from TaKaRa Biotechnology (Dalian, China). 1-Palmitoyl-2-oleoyl phosphatidylcholine (POPC) was purchased as the chromatographic grade from Larodan Co. (Sweden). Soybean oil and peanut oil were supplied by Jin Taiyang oil Ltd. (Nantong, China), with a phosphorus content of 189.3 and 85.6 mg/kg, respectively. Sunflower seed oil with a phosphorus content of 70.4 mg/kg was kindly provided by Qihua Oil Food Co., Ltd (Hebi, China). Crude rapeseed oil with a phosphorus content of 225.2 mg/kg was mechanically squeezed in the laboratory. All the other chemicals were of the highest reagent grade and obtained from Sangon (Shanghai, China). Nickel columns and Superdex 200 gel filtration columns were from GE Healthcare (USA).

Cloning and expression of the *tlplb* gene

The gene (Tlet_0745) encoding for a putative α/β hydrolase domain-containing protein from *T. lettingae* TMO was amplified using PCR with the forward primer (5′-GCCCATATGCGGTTGAAAAAAATTGACGGG-3′, with an *Nde*I site) and the reverse primer (5′-GGCGTCGACTCAATGCCTTCTCAGCCAATCAAC-3′, with a *Sal*I site). The amplified fragment was digested with *Nde*I and *Sal*I and cloned into pET15b to generate a plasmid named as pET15b/TLPLB. DNA sequencing was performed to confirm that no unintended mutation had occurred. The constructed pET15b/TLPLB was transformed into host *E. coli* BL21-CodonPlus (DE3)-RIL for the gene expression. The transformed cells were grown in LB medium containing 100 mg/l ampicillin and 34 mg/l chloramphenicol at 37 °C and 150 rpm. When the cultures reached an OD_{600} of 0.6–0.8, isopropyl-β-D-thiogalactopyranoside was added to a final concentration of 0.2 mM. After a further 12 h of growth at 30 °C, the cells were harvested by centrifugation and lyophilized by vacuum-freezing.

Purification of the recombinant TLPLB

The harvested cells were resuspended in buffer A (50 mM Tris–HCl, pH 7.4, 100 mM NaCl) and then disrupted by sonication before being incubated at 70 °C for 30 min. The cells were then centrifuged at 12,000×*g* for 30 min, and the soluble fraction was loaded onto a nickel column (GE Healthcare, Buckinghamshire, UK) pre-equilibrated with buffer A. The recombinant enzymes were eluted with a linear imidazole gradient from 20 to 500 mM at a flow rate of 1 ml/min. For further purification, the enzymes were loaded on a Superdex 200 (16/60) gel filtration column (GE Healthcare, Buckinghamshire, UK), which was pre-equilibrated with buffer B (50 mM Tris–HCl, pH 7.4, 200 mM NaCl). The fraction size was 0.5 ml and the flow rate was 0.5 ml/min. The peak fractions were collected, concentrated, and analyzed by SDS-PAGE (12 % polyacrylamide). The protein concentration was determined according to the Bradford method and stored in the buffer A containing 25 % glycerol at −80 °C.

Enzymatic activity assay

The activity of the TLPLB enzyme was measured using a pH–stat assay with a phospholipid emulsion according to the conditions described by Jiang et al. [15]. The phospholipid emulsion was prepared from 25 % POPC and 4 % polyvinyl alcohol solution, which were emulsified at a volume ratio of 1:4. The standard reaction mixture, containing 4 ml of the POPC emulsion, 5 ml of a 100 mM citric acid buffer (pH 5.5), and 1 ml of the purified enzyme solution (20–30 μg/ml) was incubated at 70 °C for 10 min. The reaction was terminated by the addition of 95 % ethanol (15 ml) after incubation, and the released fatty acids were titrated with 50 mM NaOH. Blanks were measured with heat-inactivated TLPLB samples (100 °C, 60 min). One unit of PLB was defined as the amount of enzyme releasing 1 μmol of titratable free fatty acids per minute under the standard conditions. The POPC hydrolysates generated with the TLPLB enzyme were methyl-esterified and detected with a gas chromatograph analysis (GC-6820, Agilent) [24]. The

analysis conditions were as follows: ECD detector with HJ.FFAP column (30 m × 0.32 mm × 0.25 μm), injector temperature of 250 °C, column temperature of 215 °C, ECD detector temperature of 250 °C; flow pressure 30 kPa, makeup gas 75 kPa, hydrogen 50 kPa and air 50 kPa.

Effect of temperature and pH on the activity of TLPLB

The optimum temperature was determined at temperatures ranging from 30 to 100 °C in 50 mM of sodium acetate buffer (pH 5.5). The buffers were adjusted to pH 5.5 for all of the different temperatures being tested. Experiments to determine optimum pH were conducted at 70 °C using pH values in the range of 3.0–9.0. Several buffers (50 mM) were used: sodium citrate (pH 3.0–4.0), sodium acetate (pH 4.5–6.0), sodium phosphate (pH 6.5–8.0), and Tris–HCl (pH 8.5–9.0). In addition, the thermostability of the purified TLPLB was examined in assays with 50 mM of sodium acetate buffer (pH 5.5) at three different temperatures (70, 80, and 90 °C). Each sample (50 μl) was obtained after incubation for 60, 120, 240 and 480 min, respectively. The residual activities were assayed under the standard conditions.

Effect of metal ions and surfactants on the activity of TLPLB

The effect of metal ions and surfactants on the TLPLB activity was investigated by adding each metal salt (5 mM), EDTA (5 mM) and each surfactant (1 and 5 %, w/v) to the assay solution. The reaction mixture was incubated at room temperature for 120 min, and the residual enzyme activity was measured under the standard assay conditions following the addition of substrates.

Effect of organic solvents on the activity of TLPLB

The activity of TLPLB against organic solvents was determined by incubating the enzyme in the presence of organic solvents (20 or 50 %, v/v) in 50 mM sodium acetate buffer (pH 5.5). The mixture was incubated at room temperature with constant shaking at 200 rpm for 1, 24 and 168 h. Blank samples were prepared with the reaction buffer instead of the enzyme.

Enzymatic degumming

Enzymatic degumming process of rapeseed, soybean, peanut or sunflower seed oils by TLPLB was slightly modified according to the method of Yang et al. [29]. One hundred grams of crude oil and 0.1 ml of citric acid buffer (45 %, 45 g/100 ml) were mixed and heated at 75 °C in a well-sealed Erlenmeyer flask with an agitation unit. After being homogenized for 1 min at 10,000 rpm, the mixture was incubated for 20 min at 75 °C with stirring (500 rpm) and then centrifuged at 10,000×*g* for 10 min. The resulting mixture was treated with 0.4 ml of a citric acid buffer (0.1 mM) to give the required pH value. The required quantity of TLPLB (42 U) and 2 ml of water were then added, and the resulting mixture was agitated at a high shear rate (5,000 rpm) for 1 min to provide a large surface area through emulsification. The reaction mixture was conditioned at 70 °C with mechanical stirring at 500 rpm during the process of enzymatic degumming. Samples of the mixtures were taken for phosphorus content and fatty acid analyses. All experiments were carried out at least three times and the data were averaged.

Analysis of phosphorus content and fatty acid of degummed oil

Phosphorus content analysis was carried out as follow: a 10 ml sample of the oil emulsion was heated at 80 °C in a water bath for 10 min, and the resulting mixture was centrifuged at 10,000×*g* for 10 min. A portion of the resulting supernatant oil (5 g) was then collected for phosphorus content analysis using the colorimetric molybdenum blue method (GB/T 5537, National Standard of the People's Republic China, 2008). The free fatty acid (FFA) content of the oil sample was determined according to AOCS Ca 5a-40 method (American Oil Chemist's Society, 1997).

Nucleotide sequence accession number

The nucleotide sequence coding for *tlplb* has been submitted in the National Center of Biotechnology Information (NCBI) GenBank database with the accession number of YP_001470375.

Results and discussion

Sequence analysis of TLPLB

An ORF (Tlet_0745) consisted of 753 bp encoding a α/β hydrolase domain-containing protein (named TLPLB) of 250 amino acids and was identified from the genomic DNA of *T. lettingae* TMO. Sequence alignment demonstrated that TLPLB shares 30, 27, 20, 20, 18, 17, 16, 16 and 13 % sequence identity with the characterized PLB enzymes from *Mycobacterium parascrofulaceum* (ZP_06850887), *A. fumigatus* (AAQ85122), *M. bovis* (AAK53448), *Dictyostelium discoideum* (AAN03644), *E. coli* (1T16), human granulocytes (EAW96323), *Bifidobacterium animalis* (YP_002967882), *P. fluorescens* BIT-18 (AEB15975) and *Bacillus subtilis* QB928 (YP_006628600), respectively.

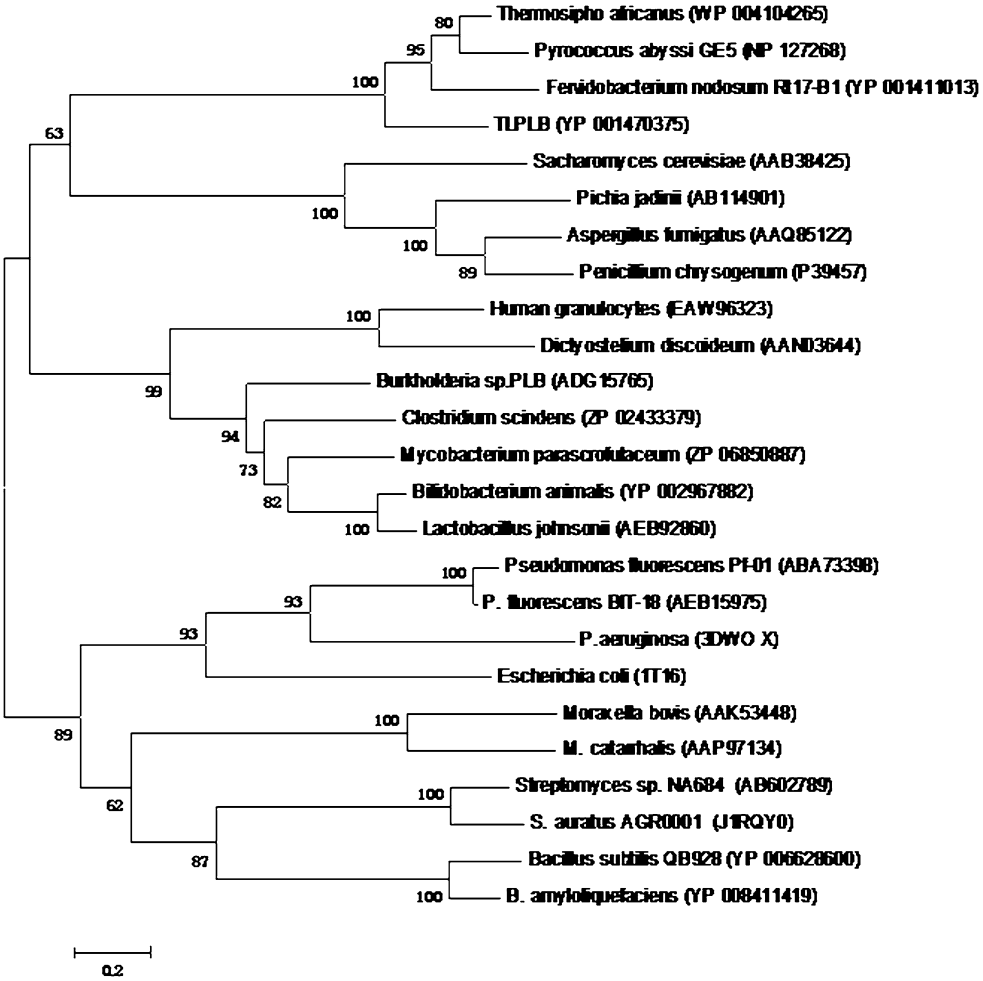

Fig. 1 Phylogenetic tree of TLPLB and related phospholipase B-type enzymes. The tree was constructed using the MEGA 4.1 program with the neighbor-joining method. *Bar* 0.2 substitutions per amino acid site. Sequences were obtained from Genbank and sequenced genomes

BLAST-P analysis revealed a high level of similarity to other α/β hydrolase fold proteins, including putative phospholipases. Phylogenetic analysis showed that TLPLB and its homologs, WP_004104265 from *Thermosipho africanus* (47 %), NP_127268 from *Pyrococcus abyssi* GE5 (47 %) and YP_001411013 from *Fervidobacterium nodosum* Rt17-B1 (46 %), could be grouped together into a distinct group (Fig. 1). Multiple sequence alignment for TLPLB and its homologs revealed that TLPLB has a conserved motif Gly-His-Ser-Leu-Gly–Gly-Leu (amino acid residues 83–89) containing an active site serine 85, similar to a classic catalytic motif GXSXG found in the esterase and lipase family (Supplementary Fig. 1) [19, 26].

Expression, purification and characterization of TLPLB

The recombinant plasmid pET15b/TLPLB was constructed to determine the catalytic properties of TLPLB. TLPLB was expressed in *E. coli* BL21-CodenPlus (DE3) and purified to homogeneity after heat treatment, Ni–NTA affinity and Superdex 200 gel filtration chromatography. The purified recombinant TLPLB with a polyhistidine (6× His) on the N terminus showed a major band with a calculated mass of 30 kDa by SDS-PAGE (Fig. 2). The specific activity of the purified TLPLB reached 158 U/mg using POPC as a substrate in citric acid buffer at pH 5.5 and 70 °C. The enzyme was finally purified to 5.6-fold with a yield of 33.7 % (Table 1). Gas chromatography demonstrated that TLPLB could release the free palmitic acid and oleic acid from the substrate POPC and is thus capable of catalyzing the hydrolytic cleavage of both the *sn*-1 and *sn*-2 acyl ester bonds of phospholipids (Supplementary Fig. 2). These results demonstrate that the TLPLB enzyme exhibits type B phospholipase activity and is a novel PLB [16].

Effects of temperature and pH on enzyme activity

TLPLB exhibited its maximum activity at 70 °C and pH 5.5, and showed half of its maximum activity at temperature and pH values in the 30–100 °C and 4.5–7.0, respectively, (Fig. 3a, b). The temperature optimum was higher than that of the reported PLBs including those from *M. Bovis* [9], *P. fluorescens* BIT-18 [13], *A. fumigatus* [16], *C. neoformans* [18], *Streptomyces* sp. strain NA684 [21],

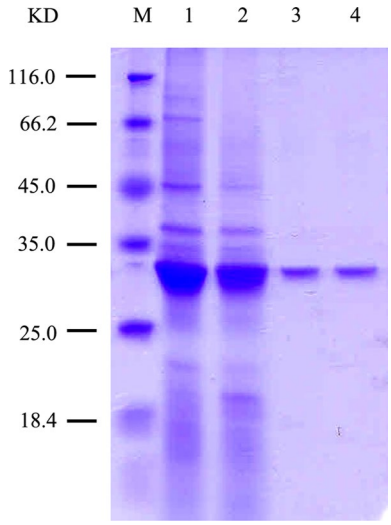

Fig. 2 SDS-PAGE of the recombinant enzyme TLPLB. *Lanes 1* crude cell extract, *2* supernatant after heat treatment, *3* the TLPLB after nickel affinity chromatography and *4* the purified TLPLB after Superdex 200 gel filtration chromatography

and *C. albicans* [22]. The thermostability of TLPLB was examined at 70, 80, and 90 °C with increasing incubation times up to 480 min. Most of the enzymatic activity was maintained after incubation at 70 °C for at least 480 min, whereas incubation at 90 °C for 240 min reduced the maximum activity by approximately 50 % (Fig. 3c). These results indicated that TLPLB possesses high stability at high temperatures and is a thermophilic PLB. Several thermophilic enzymes from archaea and hyperthermophilic bacteria have recently attracted considerable attention due to their high thermal and chemical stability [2, 25]. With this in mind, it is therefore necessary to explore the novel thermophilic PLB at elevated reaction temperature of the hydrolysis of phospholipids compounds. To our knowledge, the TLPLB enzyme is the most thermophilic and first reported thermostable PLB to date.

Effects of metal ions and surfactants on enzyme activity

Addition of 5 mM of various metal ions (Mg^{2+}, Ca^{2+}, Zn^{2+}, Mn^{2+}, Fe^{2+}, Ni^{2+} and EDTA) did not significantly affect the enzymatic activity of TLPLB after incubation for 120 min at room temperature, indicating the enzyme was not a metal ion-independent PLB (Table 2). However, Pb^{2+} and Cu^{2+} reduced the enzyme activity to 44 and 23 %, respectively. In the presence of Al^{3+}, PLB activity was completely inhibited. Enzyme activity was also inhibited by SDS (1 and 5 %), but not by Tween 20 or Triton-X100 (1 and 5 %). The enzyme activity was activated by 5 mM DTT (130 %).

Stability of TLPLB in organic solvents

The purified recombinant TLPLB exhibited high activity and stability in the presence of organic solvents tested (Table 3). A good measure of polarity of an organic solvent is the logarithm of the partition coefficient (log*P*) of the standard *n*-octane/water biphasic system [17]. Some typical solvents with various polarities such as acetone (log$P$ = −0.23), ethanol (log$P$ = −0.24), methanol (log$P$ = −0.5), DMF (log$P$ = −1.0), DMSO (log$P$ = −1.3), benzene (log$P$ = 2.0), toluene (log$P$ = 2.5), xylene (log$P$ = 3.1), *n*-hexane (log$P$ = 3.5) and heptane (log$P$ = 4.0), were investigated. Incubation with 20 and 50 % (v/v) organic solvents for 1 h did not cause a drastic loss of enzyme activity, which was not apparently related to their log *P*. After 168 h incubation, the enzyme retained only 11–38 % of its residual activity in hydrophilic organic solvents (log$P$ < 0), while it was quite stable in hydrophobic organic solvents (log$P$ > 2) at both 20 and 50 % (v/v) concentrations, retaining approximately 91–161 % of its original activity. These results demonstrated that TLPLB was apparently more stable in non-polar organic solvents (those with a high log*P*) than in polar organic solvents (those with a low log*P*). Although some work on PLBs have been published [11, 13, 16, 21, 28], there are no other reported phospholipases that exhibit high stability in the presence of organic solvents, which make it potentially useful for biocatalyzing non-aqueous solvents.

Degumming of vegetable oils by TLPLB

The reduction of phosphorus content (<10 mg/kg) in the degummed oil meets the demands of industrial refining. During the degumming process, the addition of citric acid could cause coagulation and precipitation of phospholipids,

Table 1 Purification of TLPLB expressed in *E. coli*

Step	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Purification fold	Yield (%)
Crude cell extract	38.5	1,080	28	1	100
Heat treatment	14.9	810	54	1.9	75.0
Ni–NTA affinity	5.6	658	118	4.2	60.9
Superdex-200 gel filtration	2.3	364	158	5.6	33.7

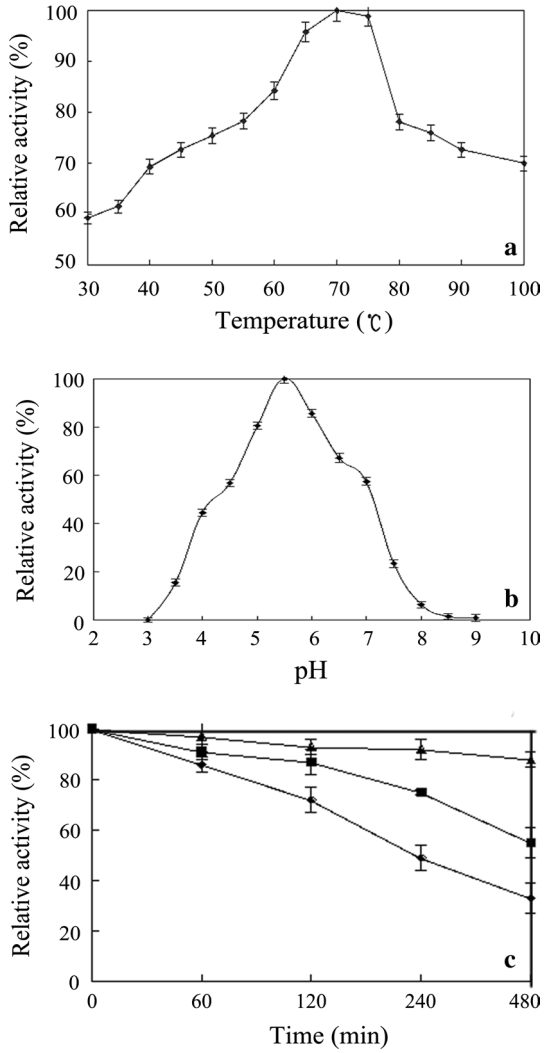

Fig. 3 **a** Effects of temperature on the activity of TLPLB. Optimal temperature of TLPLB was determined with POPC as substrates in 50 mM of sodium acetate buffer (pH 5.5) at different temperatures ranging from 30 to 100 °C. **b** Effects of pH on the activity of TLPLB. Optimal pH of the enzyme at pHs ranging from 3.0 to 9.0 was measured for 60 min at 70 °C. **c** Thermostability of TLPLB. The residual enzyme activity was measured after incubation of the purified enzyme at 70 °C (*triangles*), 80 °C (*boxes*), and 90 °C (*diamonds*), respectively. The values are means of three independent experiments

which make the phosphorus content to be lower than that of the crude oils [12, 14]. In the present study, the phosphorus content of the rapeseed oil, soybean oil, peanut oil and sunflower seed oil were reduced from 225.2, 189.3, 85.6, 70.4 mg/kg to 45.3, 32.1, 29.4, and 26.8 mg/kg, respectively, following citric acid treatment. The addition of the TLPLB enzyme to the oils prior to the degumming process led to a reduction in the residual phosphorus content to less than 10 mg/kg after only 3 h (Fig. 4). The final phosphorus contents were further reduced to 4.9 (rapeseed oil), 4.7 (soybean oil), 3.2 (peanut oil) and 2.2 mg/kg (sunflower

Table 2 Effect of metal ions and surfactants on the activity of TLPLB[a]

Metals or inhibitors	Concentration	Relative activity (%)
None	–	100
Mg^{2+}	5 mM	109 ± 2
Ca^{2+}	5 mM	87 ± 3
Zn^{2+}	5 mM	93 ± 5
Mn^{2+}	5 mM	79 ± 5
Ni^{2+}	5 mM	83 ± 4
Fe^{2+}	5 mM	72 ± 3
Pb^{2+}	5 mM	44 ± 1
Cu^{2+}	5 mM	23 ± 3
Al^{3+}	5 mM	0
EDTA	5 mM	105 ± 3
DTT	5 mM	130 ± 3
Tween 20	1 % (w/v)	86 ± 3
	5 % (w/v)	106 ± 2
Triton X-100	1 % (w/v)	91 ± 2
	5 % (w/v)	99 ± 1
SDS	1 % (w/v)	33 ± 3
	5 % (w/v)	10 ± 2

[a] The values are means of three independent experiments

Table 3 Effect of organic solvents on the activity of TLPLB[a]

Organic solvents	Concentration (%)	Relative activity (%)		
		1 h	24 h	168 h
Control	0 (v/v)	100	100	100
Ethanol	20	108 ± 4	48 ± 2	25 ± 2
	50	99 ± 3	52 ± 1	18 ± 3
Acetone	20	113 ± 3	63 ± 1	41 ± 6
	50	95 ± 2	55 ± 5	37 ± 3
Methanol	20	96 ± 1	58 ± 5	36 ± 3
	50	91 ± 2	51 ± 3	15 ± 4
DMF	20	108 ± 5	48 ± 4	38 ± 6
	50	94 ± 3	44 ± 2	14 ± 5
DMSO	20	108 ± 3	40 ± 1	19 ± 4
	50	94 ± 2	24 ± 3	11 ± 3
Benzene	20	106 ± 4	124 ± 3	129 ± 2
	50	97 ± 1	117 ± 4	121 ± 5
Toluene	20	102 ± 4	119 ± 6	127 ± 3
	50	99 ± 6	107 ± 2	135 ± 6
Xylene	20	103 ± 2	123 ± 4	145 ± 3
	50	105 ± 4	131 ± 6	161 ± 4
n-hexane	20	115 ± 3	101 ± 1	95 ± 2
	50	123 ± 4	93 ± 3	91 ± 3
Heptane	20	113 ± 4	124 ± 2	106 ± 6
	50	99 ± 1	116 ± 2	109 ± 2

[a] The values are means of three independent experiments

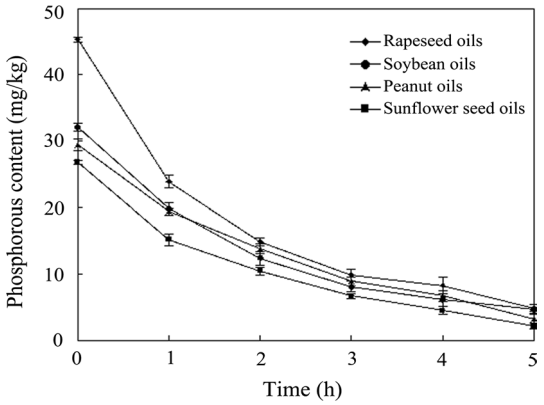

Fig. 4 Residual phosphorous content in rapeseed oil, soybean oil, peanut oil and sunflower seed oil as assayed at different reaction time from 0 to 5 h

Table 4 Changes in FFA content during the enzymatic degumming of vegetable oil by TLPLB[a]

Reaction time (h)	FFA content[b] (%)			
	Rapeseed oils	Soybean oils	Peanut oils	Sunflower seed oils
0	2.87 ± 0.04	1.52 ± 0.03	2.31 ± 0.02	1.85 ± 0.05
1	2.91 ± 0.05	1.55 ± 0.02	2.33 ± 0.03	1.88 ± 0.04
3	2.94 ± 0.03	1.58 ± 0.01	2.36 ± 0.05	1.91 ± 0.02
5	2.96 ± 0.02	1.59 ± 0.04	2.37 ± 0.04	1.92 ± 0.03

[a] The reaction conditions of degumming the different vegetable oils: TLPLB dosage, 420 U/kg oil; pH 4.7; temperature, 70 °C; water content, 20 ml/kg

[b] The values are means of three independent experiments

seed oil) by enzymatic hydrolysis for 5 h. The only PLB enzyme to have been found with a high activity towards the degumming of vegetable oils was the Pf-PLB from *P. fluorescens* BIT-18 [13, 14].

Enzymatic degumming of crude oil with PLB enzymes could cause a slight increase in the concentration of FEAs. As shown in Table 4, it was observed that the concentration of FFAs in the rapeseed, soybean, peanut and sunflower seed oils increased about 0.06–0.09 % following the enzymatic degumming of the crude oils with TLPLB for 5 h. It has been reported that the concentration of FFAs increased by approximately 0.2 % when the phosphorus content was reduced to 100 mg/kg during the enzymatic degumming process [13]. So the increase in FFAs observed in the current study therefore fitted well with the decrease in the phosphorus content (about 26–41 mg/kg), which indicated that the increase in the concentration of FFAs was the consequence of the hydrolysis of phospholipids and that TLPLB was a true phospholipase B rather than a lipase. Several microbial enzymes with both phospholipases and lipase activities have been applied in the degumming of different vegetable oils [6, 27, 29], but these enzymes are not well-suited for degumming as the hydrolysis of triglycerides in vegetable oil causes a decrease of oil yields. Taken together, these results demonstrated that TLPLB has high activity performance of the vegetable oil degumming, and that this enzyme could be a potential candidate for enzymatic degumming of vegetable oils in commercial applications.

Conclusions

In this study, a novel thermophilic phospholipase B TLPLB from *T. lettingae* TMO was cloned, purified and characterized. TLPLB displayed high activity and extreme stability in the presence of hydrophobic organic solvents for 168 h. TLPLB exhibited high activity for degumming of vegetable oils since phosphorus contents were reduced to less than 10 mg/kg within 5 h at 70 °C. To our knowledge, TLPLB is the first characterized thermophilic phospholipase B-type enzyme with high degumming activity from hyperthermophilic bacteria. These results indicate that TLPLB could be a potential candidate for enzymatic degumming of vegetable oils in commercial applications.

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