Cloning, expression and characterisation of phospholipase B from *Saccharomyces cerevisiae* and its application in the synthesis of L-alpha-glycerylphosphorylcholine and peanut oil degumming

Yihan Liu<sup>a,c,d</sup>, Mingjie Li<sup>b,#</sup>, Lin Huang<sup>a,b,#</sup>, Shuang Gui<sup>#</sup>, Leibo Jia<sup>b,#</sup>, Dong Zheng<sup>b,#</sup>, Yu Fu<sup>a,#</sup>, Yutong Zhang<sup>#</sup>, Jinqiu Rui<sup>##</sup> and Fuping Lu<sup>a,#</sup>,<sup>c,#</sup>

<sup>a</sup>Key Laboratory of Industrial Fermentation Microbiology, Ministry of Education, Tianjin, PR China; <sup>b</sup>Tianjin Key Laboratory of Industrial Microbiology, Tianjin, PR China; <sup>c</sup>National Engineering Laboratory for Industrial Enzymes, Tianjin, PR China; <sup>d</sup>The College of Biotechnology, Tianjin University of Science and Technology, Tianjin, PR China

**ABSTRACT**

L-alpha-glycerylphosphorylcholine (GPC) has been shown to enhance cognitive performance. Meanwhile, vegetable oils must be refined to remove the impurities for them to be edible. Phospholipase B (PLB), having the ability of hydrolyzing both the sn-1 and sn-2 acyl ester bonds of phospholipids, can produce GPC using PC as substrate and transform the non-hydratable phospholipids into their hydratable forms. The *Saccharomyces cerevisiae* *plb* gene, which encodes PLB, was cloned and expressed in *Pichia pastoris* GS115 to produce recombinant PLB (rPLB). Fermentation optimisation yielded rPLB activity levels as high as 1723 U/mL. rPLB demonstrated maximum enzymatic activity at 40°C and pH 5.5 and was stable at temperatures between 30 and 40°C and pH values between 5.0 and 6.0. rPLB synthesised GPC with a conversion rate of 17% (w/w) and exhibited high degumming activity towards peanut oil, decreasing the phosphorus content from 91.8 to 3.7 mg/kg within 3 h. This study describes a candidate phospholipase for potential applications involving the modification of phospholipids and vegetable oil degumming.

**Introduction**

L-alpha-glycerylphosphorylcholine (GPC; sn-glycero-3-phosphocholine), a common choline compound found in the brain, is a derivative of phosphatidylcholine (PC; 1,2-diacyl-sn-glycero-3-phosphocholine) that is synthesised via the deacylation of sn-1 and sn-2. Clinical trials have shown that the cholinergic precursor improves dementia, memory and cognitive ability in patients and is effective in the treatment of Alzheimer’s disease and dementia [1]. Therefore, GPC has been widely used in medicines and supplements. To date, GPC has been produced via the chemical or enzymatic deacylation of PC. Compared with chemical methods, which involve complicated processes, limited material availability, and high substrate toxicity, the enzymatic preparation of GPC offers several advantages, including high specificity, environmentally friendly processes and easy product recovery.

Vegetable oils, which are derived from oil-bearing seeds such as soybeans, peanuts, sunflowers and rapeseeds, must be refined to remove impurities before they are edible. Degumming, the first step in the refining process for vegetable oils, removes phospholipids and mucilaginous gums from crude vegetable oils, and this process improves the quality of the final product and facilitates subsequent refining steps, including neutralisation, bleaching and deodorisation [2]. Traditional degumming processes, including water degumming, acid degumming and super degumming, do not produce the high-quality oil with low phosphorus content (<10 mg/kg) that is required for industrial applications [3]. Water degumming only removes hydratable phospholipids, and acid and super degumming only remove a portion of the non-hydratable phospholipids (NHPs), resulting in large amounts of NHPs remaining in vegetable oils [4]. Enzymatic degumming, a process through which phospholipase hydrolyses the acyl bonds of phospholipids and then transforms NHPs into their hydratable forms, has been applied to the vegetable-oil refining process to enhance product yields and reduce wastewater generation and operating costs [5].

Phospholipase B (PLB; EC 3.1.1.5) exhibits three distinct activities; specifically, this enzyme acts as an sn-1 and sn-2 fatty acid ester hydrolase, a lysophospholipase...
and a transacylase. PLB hydrolyses both the sn-1 and sn-2 acyl ester bonds of phospholipids to yield free fatty acids, lysophospholipids or glycerophospholipids and transfers free fatty acids to a lysophospholipid to produce a diacylphospholipid. Therefore, PLB catalysed the production of GPC through the decacylation of PC as the substrate, whereas phosphoglycerate, which is more hydrophilic than lysophospholipids, is obtained from NHPs in vegetable oils and is then efficiently removed via centrifugation. Over the past few decades, PLBs have been identified in humans and other mammals, plants, bacteria and fungi, including Cryptococcus neoformans [6], Candida albicans [7], Candida utilis [8], Penicillium chrysogenum [9], Kluveromyces lactis [10], Schizosaccharomyces pombe [11] and Saccharomyces cerevisiae [12]. S. cerevisiae, a nonpathogenic yeast, contains three similar genes (plb₁, plb₂ and plb₃, showing more than 60% identity at the DNA level) that encode PLB [13], and the substrate specificities of these three PLB/lysophospholipase enzymes have been characterised both in vitro and in vivo [13,14]. In addition, PLB encoded by plb₂ demonstrates broad substrate specificity, similar to the PLB encoded by plb₁ [13].

Several recent studies reported the production of GPC through the hydrolysis of PC using phospholipase A₁ (PLA₁) or a combination of lipase and phospholipase A₂ (PLA₂) [15–18]. Therefore, a novel enzymatic procedure utilising PLBs as biocatalysts has been developed for the production of GPC. In addition, although PLBs from Pseudomonas fluorescens BIT-18 and Thermotoga lettingae have been used to degum soybean, peanut, rapeseed and sunflower seed oils [19,20], few studies have investigated the use of PLB for enzymatic degumming processes. In the present study, the plb₂ gene (hereafter referred to as plb) from S. cerevisiae was cloned and expressed in Pichia pastoris GS115, and recombinant PLB (rPLB) was purified, characterised and tested for the production of GPC and peanut oil degumming. Our work will be beneficial for the large-scale production of rPLB and its further application in phospholipid modification and vegetable oil degumming.

Materials and methods

Construction of recombinant P. pastoris

The plb gene was cloned via PCR amplification using the genomic DNA of S. cerevisiae TCC331028 as template with the primers P1 (5'-CCGGAATTCCGACATTGCCTCGTC-CACTACT-3') and P2 (5'-ATAAGAATGCGCCGCTCAATG- GGTGTTGTGGTGGTAATTAGTCACAAATGCTGT-3'); the bold and underlined letters indicate EcoRI and NotI sites, respectively, and the italics indicate the 6-His tag for purification of rPLB. The PCR product was digested with EcoRI and NotI and inserted into pPIC9K digested with the same enzymes to produce the P. pastoris expression vector pPIC9K-plb. The resulting vector was verified by both restriction endonuclease analysis and nucleotide sequencing. The constructed plasmid pPIC9K-plb was then linearised by digestion with SalI and transformed into P. pastoris GS115 via electroporation. The pPIC9K vector was also transformed into P. pastoris GS115 (GS115/pPIC9K) for use as a control. P. pastoris transformants were screened on minimal dextrose (MD) medium plates [1.34% (w/v) yeast nitrogen base (YNB), 4 × 10⁻⁵% (w/v) biotin, 2% (w/v) dextrose, and 2% (w/v) agar] following incubation at 30 °C for 96 h. To identify transformants with multiple inserts, promising transformants harbouring pPIC9K-plb (GS115/pPIC9K-plb) were further screened using a conventional G418 gradient (0.5, 1.0, 1.5 and 2.0 mg/mL) and then identified by colony polymerase chain reaction (PCR).

Expression and purification of rPLB

A single transformant colony was cultured overnight in 5 mL of yeast extract peptone dextrose (YPD) medium [1% (w/v) yeast extract, 2% (w/v) peptone, 2% (w/v) dextrose] at 30 °C with shaking at 220 rpm. The culture was then inoculated into 50 mL of buffered minimal glycerol-complex (BMGY) medium [1.34% (w/v) YNB, 1% (w/v) yeast extract, 2% (w/v) peptone, 1% (v/v) glycerol, 4 × 10⁻⁵% (w/v) biotin] and shaken at 30 °C until an OD₆₀₀ of approximately 2.0 was reached. The cells were collected via centrifugation and resuspended in a 250 mL shake flask containing 50 mL of buffered minimal methanol-complex (BMMY) [1.34% (w/v) YNB, 1% (w/v) yeast extract, 2% (w/v) peptone, 0.5% (v/v) methanol, 4 × 10⁻⁵% (w/v) biotin]. Every 12 h, 0.5% (v/v) methanol was added to induce rPLB expression with a total induction time of 120 h. To maximise rPLB yield, the following conditions were optimised: the concentration of methanol in BMMY medium (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0%, v/v), pH value (5.0, 5.5, 6.0, 6.5, 7.0 and 7.5), culture volume (10, 20, 30, 40 and 50 mL), temperature (24, 26, 28, 30, 32 and 34 °C), and shaking speed (220, 240, 260, 280 and 300 rpm). Fermentation supernatant was collected for enzyme assays.

To purify the rPLB with the 6-His tag, culture supernatant was collected by centrifugation (8000 × g at 4 °C for 20 min) and loaded onto a Ni-NTA agarose gel column equilibrated with lysis buffer (20 mmol/L Tris-HCl, 500 mmol/L NaCl, and 20 mmol/L imidazole, pH 7.4). The resin was washed twice with wash buffer (20 mmol/L Tris-HCl, 500 mmol/L NaCl, and...
50 mmol/L imidazole, pH 7.4) and the target protein was eluted by adding elution buffer (20 mmol/L Tris-HCl, 500 mmol/L NaCl and 500 mmol/L imidazole, pH 7.4). The purity and apparent molecular mass of the rPLB were monitored by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE was performed following the method described by Laemmli [21] using 12% (w/v) separating gel and 5% (w/v) stacking gel. The marker proteins were protein molecular weight standards in the range of 14.4–116 kDa. Protein bands were visualized by Coomassie brilliant blue R-250 staining.

**PLB activity assay**

The hydrolytic activity of PLB was measured via sulfhydryl group release using 1,2-bis(Heptanoylthio)-1,2-dideoxy-sn-glycero-3-phosphocholine [1,2-bis(heptanoylthio)glycerophosphocholine] as the substrate. The reaction was initiated by the addition of 40 µL of enzyme extract to 160 µL of 200 mmol/L Tris-HCl (pH 5.5) containing 20 mmol/L CaCl2 and 0.5 mmol/L 5.5-dithiobiis-(2-nitrobenzoic acid) (DTNB) in a final volume of 200 µL at 40 °C for 10 min. The absorbance at 410 nm of the resulting mixture was measured using a microplate reader. One unit (U) of PLB activity was defined as the amount of enzyme that hydrolysed 1 µmol of 1,2-bis (heptanoylthio) glycerophosphocholine per minute under the assay conditions.

**Synthesis and optimisation of GPC**

For determination of the optimal rPLB-catalysed hydrolysis conditions, reactions were initiated by adding rPLB (1000, 1500, 2000, 2500, 3000 and 3500 U) in 10 mL of buffer (200 mmol/L bis Tris-HCl buffer (pH 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0) containing 20 mmol/L CaCl2) to PC (100, 120, 140, 160, 180 and 200 mg). The mixture was stirred at 500 rpm with a magnetic stirring apparatus at different temperatures (30, 35, 40, 45 and 50 °C) for 12, 16, 20, 24, 28, 32 or 36 h. The biotransformation reaction products were quantified by liquid chromatography-mass spectrometry (LC-MS). The yield (%) of PC to GPC was defined as follows:

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\text{Yield} \text{ (%)} = \frac{\text{GPC (g)}}{\text{Initial PC (g)}} \times 100 \%
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**rPLB application for degumming**

Crude peanut oil samples (50 g) were heated to 70 °C in a water bath, and 0.13 mL of 45% (w/v) citric acid solution was added. After homogenisation for 1 min at 10,000 rpm, the mixture was incubated for 25 min at 70 °C with stirring at 500 rpm and then centrifuged at 3000 × g for 10 min. The resulting mixture was cooled to 40 °C, and a 45% (w/v) citric acid solution was then added until the mixture reached a pH of 6.0. Two millilitres of water and 5500 U of rPLB were then added, and the resulting mixture was cooled to 40 °C with stirring at 500 rpm to initiate enzymatic degumming reactions. After 3 h of enzymatic treatment, the reaction mixture was heated to 90 °C for 10 min to inactivate rPLB and was then centrifuged at 3000 × g for 10 min to separate the oil and water phases. Samples of the mixtures were collected for residual phosphorous content analysis. The phosphorus content was determined using the molybdenum blue method in accordance with GB/T 5537–2008 [22].

**Data analysis**

Data are presented as mean values with standard deviation (±SD) from triplicate experiments. Data analysis was performed using Statgraphics Plus version 5.1 statistical package.

**Results and discussion**

**Construction of yeast strains for rPLB expression**

The plb gene was amplified by PCR using S. cerevisiae TCCC31028 genomic DNA as a template. The amplified plb gene was digested with EcoRI and NotI and ligated into the expression vector pPIC9K, which was linearised with the same enzymes. The resulting vector was transformed into E. coli JM109 and screened on an LB/agar plate containing 100 µg/mL ampicillin, yielding isolates containing the recombinant expression plasmid pPIC9K-plb. The pPIC9K-plb plasmid was then digested with SalI and electroporated into the expression host P. pastoris GS115 to generate the recombinant strain P. pastoris GS115/pPIC9K-plb.

**rPLB expression and purification**

After 120 h of methanol induction, the supernatant of P. pastoris GS115/pPIC9K-plb was collected via centrifugation and used for protein purification. The examination of rPLB activity under optimal conditions [1.0% (v/v) methanol in BMMY medium, pH 6.0, 32 °C, culture volume of 20 mL, and shaking at 280 rpm] indicated that rPLB reached a maximum activity of 1723 U/mL. P. pastoris GS115/pPIC9K-plb produced a distinct SDS-PAGE band at a position equivalent to a molecular mass of approximately 75 kDa, which is consistent with the
calculated molecular weight (Figure 1a, lane 2). No corresponding band was present in the control strain GS115/pPIC9K (Figure 1a, lane 1). After Ni-affinity chromatography, the purified rPLB appeared as a single band (Figure 1b), which is convenient for functional analysis.

**Characterisation of rPLB**

The optimal temperature for the rPLB enzyme was 40 °C (Figure 2a), which is similar to those observed for the PLB enzymes of *C. neoformans* (37 °C) [6], *C. utilis* (40 °C) [8], *K. lactis* (35 °C) [10] and *S. pombe* (35 °C) [11]. The optimal pH for the rPLB enzyme was 5.5 (Figure 2b), which is more acidic than the optimum pH for the PLB from *Streptomyces* sp. (pH 8.4) [23]. In addition, rPLB retained more than 35% of its maximum activity over a broad pH range of 4.5–8.5 (Figure 2), whereas other PLBs display no or low activity at pH values ranging from 5.0–9.0 [6,8,10,11].

As shown in Figure 3a, rPLB retained 47% and 31% of its initial activity after incubation at 30 and 40 °C for 120 min, respectively, and 22% of its original activity, after incubation at 60 °C for 40 min. Thus, rPLB was more thermally stable than other PLBs from *Streptomyces* sp. [23], *P. fluorescens* [24], *C. neoformans* [6] and *S. pombe* [11]. rPLB exhibited 44% and 30% of its maximal activity after incubation for 10 h at 4 °C and pH 5.0 and 6.0, respectively (Figure 3b). Compared with the PLB from *S. pombe*, which is inactive after incubation at 30 °C and pH 9.0 for 30 min [11], rPLB was still active at an alkaline pH (8.0–9.0) after incubation for 10 h. Based on these results, rPLB exhibits broad pH stability, and due to its observed properties, rPLB has potential applications in various industries, including the production of GPC and vegetable oil degumming.

**GPC synthesis and optimisation**

To date, GPC has only been prepared via chemical synthesis or enzymatic catalysis. Compared with the chemical method, the enzymatic production of GPC has several advantages, including high efficiency, excellent specificity, and environmental friendliness. In studies conducted by Bang et al. [15], GPC was successfully prepared through the hydrolysis of soy PC with PLA₁ in a hexane-water biphasic medium. However, the organic

![Figure 1. Analysis of the expression and purification of rPLB by SDS-PAGE. (a) SDS-PAGE analysis of rPLB expressed by *Pichia pastoris* GS115. Lane M: protein molecular weight marker; lane 1: supernatant of GS115/pPIC9K; lane 2: supernatant of GS115/pPIC9K-plb. (b) SDS-PAGE analysis of purified rPLB. Lane M: protein molecular weight marker (Thermo Scientific); lane 1: purified rPLB.

![Figure 2. Effects of temperature (a) and pH (b) on the activity of rPLB. Note: The enzyme activity at the optimum temperature and pH was defined as 100%. All measurements were performed in triplicate.](image-url)
solvent is not suitable for the synthesis of GPC because GPC is intended for medicinal applications and use as a food additive.

Based on the above observations, the present study investigated the hydrolysis of soy PC for GPC production in a water-based medium. An LC-MS spectral analysis of the reaction products showed a major peak in the positive-ion electrospray mode \([M + H]^+\) at a \(m/z\) of 258.2, which corresponds to GPC (Figure 4), indicating that GPC was successfully synthesised from PC by rPLB in the water-based medium. To maximise the GPC yield, we systematically assessed several variables, including the PC and rPLB concentrations, the buffer pH, the reaction temperature and the reaction time. The optimised formulation for the synthesis of GPC from PC by rPLB is summarised as follows: 160 mg of PC, 3000 U of rPLB, pH 5.5, temperature of 40 °C and a reaction time of 28 h. Under these conditions, the maximum yield of GPC reached 17% (w/w).

**Application of rPLB in peanut oil degumming**

The low phosphorus content (<10 mg/kg) in degummed oil meets the demands of industrial refining. During the degumming process, the addition of citric acid causes the coagulation and precipitation of phospholipids, resulting in a lower phosphorus content in acid-treated oil than in crude oil. After citric acid treatment, the oil was then degummed by rPLB. Enzymatic hydrolysis reduced the phosphorus content of peanut oil from 91.8 to 3.7 mg/kg within 3 h. The PLBs from *P. fluorescens* BIT-18 and *T. lettingae* reduced the phosphorus content to levels below 10 mg/kg, yielding levels of 4.96 mg/kg (soybean oil), 3.54 mg/kg (peanut oil) and 4.9 mg/kg (rapeseed oil) or 4.7 mg/kg (soybean oil), 3.2 mg/kg (peanut oil), and 2.2 mg/kg (sunflower seed oil), respectively [19,20]. However, limited studies have investigated the use of PLB in enzymatic degumming. Therefore, the rPLB produced in our study, which demonstrated high peanut
oil degumming activity, might be a potential candidate for the enzymatic degumming of other vegetable oils.

Conclusions

In the present study, a novel strategy for the production of GPC and peanut oil degumming was successfully implemented using rPLB expressed in P. pastoris GS115. Based on our findings, rPLB is a potential candidate for the enzymatic modification of phospholipids and vegetable oil degumming in commercial applications.

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Yihan Liu and Fuping Lu are designated as co-corresponding authors.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

[1] De Jesus Moreno Moreno M. Cognitive improvement in mild to moderate Alzheimer’s dementia after treatment with the acetylcholine precursor choline alfoscerate: a multicenter, double-blind, randomized, placebo-controlled trial. Clin Ther. 2003;25:178–193.

[2] Xie MZ, Dunford NT. Lipid composition and emulsifying properties of canola lecithin from enzymatic degumming. Food Chem. 2017;218:59–164.

[3] Jiang FY, Wang JM, Kaleem I, et al. Degumming of vegetable oils by a novel phospholipase B from Pseudomonas fluorescens BIT-18. Biocatal Bioserv. 2011;102:8052–8056.

[4] Liu KT, Gao S, Chung TW, et al. Effect of process conditions on the removal of phospholipids from Jatropha curcas oil during the degumming process. Chem Eng Res Des. 2012;90:1381–1386.

[5] Galhardo F, Hitchman T. Has degumming with enzymes come of age? Oil Mill Gazetteer. 2012;117:1–5.

[6] Chen SC, Wright LC, Golding JC, et al. Purification and characterization of secretory phospholipase B, lysophospholipase and lysophospholipase/transacylase from a virulent strain of the pathogenic fungus Cryptococcus neoformans. Biochem J. 2000;347:431–439.

[7] Mirbod F, Banno Y, Ghannoum MA, et al. Purification and characterization of lysophospholipase-transacylase (h-LPTA) from a highly virulent strain of Candida albicans. BBA-Lipids Lipid Metabol. 1995;1257:181–188.

[8] Fujino S, Akiyama D, Akaboshi S, et al. Purification and characterization of phospholipase B from Candida utilis. Biosci Biotechnol Biochem. 2006;70:377–386.

[9] Saito K, Sugatani J, Okumura T. Phospholipase B from Penicillium notatum. Methods Enzymol. 1991;197:446–456.

[10] Oishi H, Morimoto T, Watanabe Y, et al. Purification and characterization of phospholipase B from Kluyveromyces lactis, and cloning of phospholipase B gene. Biosci Biotechnol Biochem. 1999;63:83–90.

[11] Oishi H, Tsuda S, Watanabe Y, et al. Purification and some properties of phospholipase B from Schizosaccharomyces pachyamo phiae. Biosci Biotechnol Biochem. 1996;60:1087–1092.

[12] Lee KS, Patton JL, Fido M, et al. The Saccharomyces cerevisiae PLB1 gene encodes a protein required for lysophospholipase and phospholipase B activity. J Biol Chem. 1994;269:19725–19730.

[13] Merkel O, Fido M, Mayr JA, et al. Characterization and function in vivo of two novel phospholipases B/lysophospholipases from Saccharomyces cerevisiae. J Biol Chem. 1999;274:28121–28127.

[14] Merkel O, Oskolkova OV, Raab F, et al. Regulation of activity in vitro and in vivo of three phospholipases B from Saccharomyces cerevisiae. Biochem J. 2005;387:489–496.

[15] Bang HI, Kim IH, Kim BH. Phospholipase A1-catalyzed hydrolysis of soy phosphatidylcholine to prepare l-a-glycercylophosphorylcholine in organic-aqueous media. Food Chem. 2016;190:201–206.

[16] Blasi F, Cossignani L, Simonetti MS, et al. Enzymatic decylation of l2-diacyl-sn-glycerol-3-phosphocholines to sn-glycerol-3-phosphocholine. Enzyme Microb Tech. 2006;39:1405–1408.

[17] Zhang KY, Liu YF, Wang XG. Enzymatic preparation of l-alpha-glycerylphosphorylcholine in an aqueous medium. Eur J Lipid Sci Technol. 2012;114:1254–1260.

[18] Zhang KY, Wang XG, Liu YF. Aqueous medium enzymatic preparation of l-alpha-glycerylphosphorylcholine optimized by response surface methodology. Eur Food Res Technol. 2012;234:485–491.

[19] Huang S, Liang ML, Xu YH, et al. Characteristics and vegetable oils degumming of recombinant phospholipase B. Chem Eng J. 2014;237:23–28.

[20] Wei T, Xu CP, Yu X, et al. Characterization of a novel thermophilic phospholipase B from Thermotoga lettingae TMO: applicability in enzymatic degumming of vegetable oils. J Ind Microbiol Biotechnol. 2015;42:515–522.

[21] Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 1970;227:680–685.

[22] National Standard of the People’s Republic of China. Phosphorus content analysis of edible oils.GB/T 5537–2008. Beijing: General Administration of Quality Supervision, Inspection and Quarantine of the People’s Republic of China and Standardization Administration of the People’s Republic of China. 2008.

[23] Matsumoto Y, Mineta S, Murayama K, et al. A novel phospholipase B from Streptomyces sp. NA684-purification, characterization, gene cloning, extracellular production and prediction of the catalytic residues. FEBS J. 2013;280:3780–3796.

[24] Jiang FY, Huang S, Imadad K, et al. Cloning and expression of a gene with phospholipase B activity from Pseudomonas fluorescens in Escherichia coli. Biocatal Bioserv. 2012;104:518–522.