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REVIEW ARTICLE

Phospholipases C from the Genus *Bacillus*: Biological Role, Properties and Fields of Application

Yu. A. Merkulyeva[a, b], D. N. Shcherbakov[a, b], E. A. Sharlaeva[a], and V. Yu. Chirkova[a, 1]

[a] *Altai State University, Barnaul, 656049 Russia*

[b] *State Research Center of Virology and Biotechnology Vector, Koltsovo, Novosibirsk oblast, 630559 Russia*

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Abstract—Phospholipases are enzymes of the class of hydrolases that catalyze the cleavage of bonds in phospholipids; they are found in almost all organisms. Enzymes of microbial origin are of the greatest interest. The popularity of bacterial enzymes is due to their huge variety and technological properties: high specific activity, thermal stability, and wide substrate specificity. The production of recombinant bacterial phospholipases and their improvement remain an urgent task, for which it is necessary to deepen and systematize knowledge about the enzymes of this group. This review describes the properties, structure, and mode of action of bacterial phospholipases C, which are widely used in various areas of human practice: scientific research, medicine, food, chemical industry, etc.

Keywords: phospholipids, phospholipase, acyl hydrolase, phosphodiesterase, bacterial enzymes, Bacillus, industrial enzymes

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INTRODUCTION

Phospholipases (PL) (EC 3.1.1, 3.1.4) are enzymes that hydrolyze various bonds in phospholipids (Fig. 1) and which, along with glycolipids and cholesterol, are among the main components of biological membranes. Acting on the lipid–water interface, phospholipases act as biocatalysts for phase transfer catalysis [1, 2]. These enzymes are widespread in nature and perform various functions: they participate in maintaining the lipid composition of membranes, play an essential role in the development of the inflammatory process and triggering the synthesis of inflammatory mediators, take part in the work of the inositol phosphate system, which provides transmembrane transmission of hormonal signals, and act as active components of hemolytic snake venom, etc. [3, 4].

Depending on the site of cleavage of the bond in the phospholipid molecule, the four main families of phospholipases are distinguished: A, B, C and D (Fig. 2) [5]. Phospholipases of types A and B are acyl hydrolases, types C and D are phosphodiesterases. Phospholipases A1 (PLA1) and A2 (PLA2) hydrolyze the SN-1 or SN-2 acyl chain to release free fatty acid and 1- or 2-acyl-lysophospholipid, respectively. In the case of the cleavage of both fatty acids, one speaks of phospholipase type B (PLB).

Phospholipase C (PLC) cleaves the glycerophosphate bond to form diacylglycerol and a phosphate-containing polar group, while phospholipase D (PLD) hydrolyzes the bond between the phosphate and alcohol groups, thereby releasing phosphatidic acid and alcohol.

Several isoforms are isolated for each type of phospholipases, depending on the molecular weight, cell localization, regulation method, and substrate specificity [3, 4, 6]. For example, 10 phospholipase C isoforms have been identified in human cells, and various phospholipase D isoforms have been found in viruses, bacteria, yeast, mucus, plants, and mammals [7].

BACTERIAL PHOSPHOLIPASES

A wide variety of phospholipases are produced by pathogenic and nonpathogenic bacteria. Some of the bacterial phospholipases exhibit the properties of toxins, while others, not being toxins, play an important role in the pathogenesis of diseases. The toxic effect of phospholipases is manifested both directly in lysis and in changes in the metabolism of host cells. The accumulating products of their catalysis—lysophospholipids—have strong surface-active properties, which leads to the destruction of cell lipoprotein structures and the activation of hydrolases responsible for the autolytic degradation of cellular polymers [8, 9].

All types of phospholipases are found in microorganisms, differing in the position of the hydrolyzable

[1] Corresponding author: phone: +7 (913) 218-47-06; e-mail: varvara.chirkova@gmail.com.

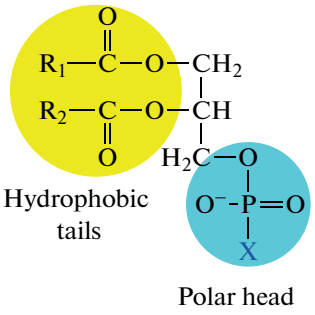

Fig. 1. General structure of phospholipids: substituents R_1 and R_2 are residues of fatty acids, X is the head group: choline, ethanolamine, glycerol, inositol or serine.

bond. Phospholipase A1 (EC 3.1.1.32), cleaving the SN-1 acyl chain, has a wide substrate specificity, Ca^{2+} is a cofactor [10]. PLA1 found in bacteria *Serratia* spp., *Yersinia enterocolitica*, *Streptomyces alboflavus*, *Escherichia coli*, *Bacillus subtilis*, *B. megaterium*, *Mycobacterium phlei* [11, 12]. Phospholipase A1 of gram-negative bacteria is one of the virulence factors, which arouses increased interest in the study of this phospholipase. It enhances the hemolytic properties of bacterial cells and increases their invasiveness [13].

Phospholipase A2 (CF 3.1.1.4), splitting off SN-2 acyl chain, acts on phosphatidylethanolamine, cholineplasmalogen and phosphatides. Ca^{2+} is also a cofactor [14]. PLA2 is found in bacteria *E. coli*, *Streptomyces coelicolor*, *St. violaceoruber*, *Helicobacter pylori* [15], *Yersinia enterocolitica* [16]. In cells of *E. coli* phospholipase A2 increases the level of lysophospholipids and fatty acids in the membrane, increasing its permeability and thus participating in the release of bacteriocin toxin from the cell.

Phospholipase B (L) (EC 3.1.1.5) is also called lysophospholipase. The enzyme acts on lysolecithin (lysophosphatidylcholine), which is formed as a result of the action of phospholipase A1 on lecithin (phosphatidylcholine) [17]. PLB has the activities of phospholipases A1 and A2 as it cleaves both SN-1 and SN-2 acyl chains of the phospholipid. Phospholipase B has no cofactor. Some PLB inhibitors are diisopropyl fluorophosphate and *P*-chloromercurbenzoic acid; all without exception are surfactants. Phospholipase B is found in *Pseudomonas fluorescens*, *Bacillus subtilis*, *Streptomyces* sp., *Mycoplasma laidlawii*, *M. phlei*, *Serratia plymuthica*, *Dictyostelium discoideum* [18–20].

Phospholipase C (EC 3.1.4.3) hydrolyzes the glycerophosphate bond, which leads to the formation of diacylglycerol and a phosphate-containing polar group. PLC is a key enzyme in phosphatidylinositol metabolism and lipid signaling pathways. It hydrolyzes phosphatidylinositol into two secondary mediators, inositol triphosphate and diacylglycerol, which, being involved in signaling pathways, activate the calcium channels of the endoplasmic reticulum and protein kinase C, respectively. The cofactor of this enzyme is Zn^{2+} [21]. Phospholipase C is found in *Listeria monocytogenes*, *Clostridium perfringens*, *Bacillus cereus*, *B. mycoides*, *B. anthracis*, *Pseudomonas aeruginosa*, *P. cepacia*, *P. fluorescens*, *Burkholderia pseudomallei*, *Legionella pneumophila*, *Acinetobacter calcoaceticus*, and *Staphylococcus aureus* [22, 23]. The PLC activity depends not only on the composition of the phospholipids of cell membranes, but also on the components of the medium, for example, a decrease in the activity of phospholipase C of microorganisms *Clostridium perfringens* and *Bacillus cereus* observed under the action of phosphate- and glycerol-containing compounds, possibly due to their competition with the substrate for the corresponding binding sites with the enzyme [2, 24–26].

Phospholipase D (CF 3.1.4.4) hydrolyzes the bond between the phosphatidylcholine and alcohol groups of phosphatidylcholine, thus releasing phosphatidic acid and soluble choline [27]. PLD activators are

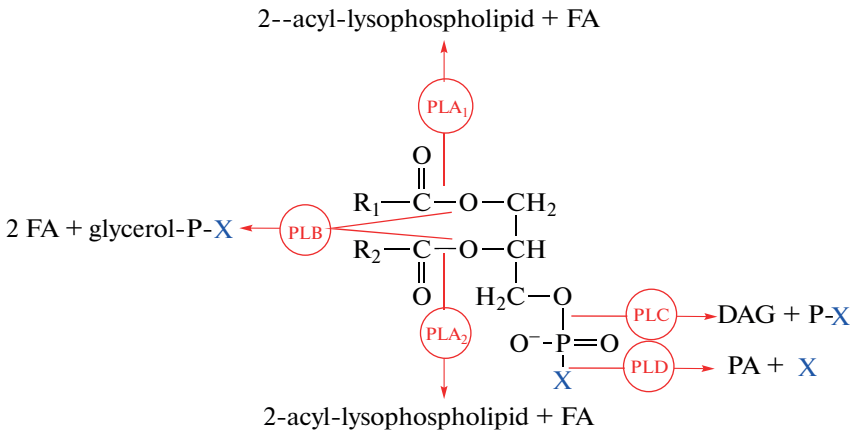

Fig. 2. Reactions catalyzed by phospholipases: FA is fatty acid, PA is phosphatidic acid, DAG is diacylglycerol, X is the head group, and PX, the phosphorylated head group [5].

Table 1. Groups of bacterial phospholipases by structural similarity

Source of phospholipases	Group	Microorganisms
Gram-positive bacteria	Zn-dependent metallophospholipases C (α-toxin, BC-PLC)	*Clostridium perfringens* *Bacillus cereus*
	Sphingomyelinase	*Bacillus cereus* *Staphylococcus aureus*
	Phospholipase C, hydrolyzing phosphatidylinositol (PLC-A)	*Bacillus cereus* *Bacillus thuringiensis* *Listeria monocytogenes*
Gram-negative bacteria	Phospholipase C *Pseudomonas* sp. (PLC-H and PLC-N)	*Pseudomonas aeruginosa*
	Phospholipase C *Legionella* sp.	*Legionella* sp.

anionic surfactants and inhibitors are cationic. Phospholipase D is found in *Acinetobacter baumanii*, *E. coli*, *Neisseria gonorrhoeae*, *Yersinia pestis*, *Chlamydia trachomatis*, *Pseudomonas aeruginosa*, *Streptomyces* sp. PMF, *Rickettsia conorii*, and *R. prowazekii* [28].

PHOSPHOLIPASE WITH BACTERIA OF THE GENUS *Bacillus*: STRUCTURE, PROPERTIES, MODES OF ACTION

Phospholipase C, which catalyzes the stereospecific hydrolysis of phospholipids, has been found in a wide range of gram-positive and some gram-negative bacteria. Bacterial phospholipases C, which are monomeric proteins with typical signal sequences in the structure and secreted into the extracellular space [6], are usually divided into several groups according to the similarity of structures (Table 1).

Genes encoding α-toxin *Clostridium perfringens*, PLC *Bacillus cereus*, PLC from *Clostridium bifermentans* and *Listeria monocytogenes*, were sequenced. A high degree of homology is shown for them, totaling ~250 first amino acids. A-toxin *Clostridium perfringens*, unlike other phospholipases of gram-positive bacteria, has 120 additional amino acid residues per *C*-end. Phosphatidylcholine Specific PLC (PC-PLC) *B. thuringiensis* and *B. cereus* belonging to the same group have a high degree of homology.

The structure and properties of PC-PLC *Bacillus cereus* are the most studied. As a result of extensive research, this protein has received the status of the prototype phospholipase C [29]. The enzyme is a small protein (28 kDa), quite resistant to denaturing agents: urea, sodium dodecyl sulfate and temperature (in the presence of 1 mM Zn^{2+}) [30]. Thanks to stabilizing zinc ions, the protein has the ability to withstand heating up to 100°C for short periods [31]. The optimum action of the enzyme is observed at a temperature of 50°C and pH 8–10. Phospholipase C is highly stable and tolerant to the replacement of Zn^{2+} ions to other divalent cations while maintaining (Co^{2+}, Ni^{2+}, Mg^{2+}, Ca^{2+}, Ba^{2+}) or increasing (Mn^{2+}) enzyme activity [31, 32].

The enzyme is a single polypeptide chain coiled in the form of seven helices forming a coiled structure (Fig. 3a). PLC *Bacillus* spp. synthesized with a signal sequence on *N*-terminal (24 amino acid residues) is secreted into the extracellular space in the form of a propeptide. The active form of the enzyme (amino acid residues 245) is formed by cleavage of 14 *N*-terminal amino acid residues by cellular proteases (Fig. 3b) [33–35].

There are three Zn^{2+} ions in the active center, one of which is loosely coupled. Zn^{2+} ions maintain the conformational stability of the enzyme and participate in the binding of the substrate regardless of its amount [36]. Nine amino acids are involved in the binding of ions: 5 His, 2 Asp, 1 Glu and 1 Trp. It is believed that the first amino acid (Trp) in the mature peptide is essential for enzymatic activity as it facilitates coordination with basic zinc ions. The ions bind to amino acid residues from different helices and therefore stabilize the conformation of the molecule.

PLC bacteria genus *Bacillus* has a wide substrate specificity; it recognizes various phospholipid substrates that differ only in the structure of the head group: phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylglycerol, phosphatidylserine (Fig. 4) [37–39]. Phosphatidylcholine with six carbon atoms in each of the acyl side chains is exposed to the enzyme with a higher catalytic efficiency than the substrate containing C2–C4 fatty acids [40, 41]. The spatial orientation of glycerol side chains on phosphatidylcholine appears to be an important factor promoting binding and catalysis [42].

Eukaryotic and bacterial phospholipases have a common reaction mode: "ping-pong" with an intermediate link, during which the substrate phosphate group covalently binds to the nucleophilic amino acid residue from the active center [23]. The amino acid residues that make up the active site (Glu4, Tyr56, and Phe66) form a scaffold for binding to the substrate base [43]. The carboxyl group Glu4 interacts with the

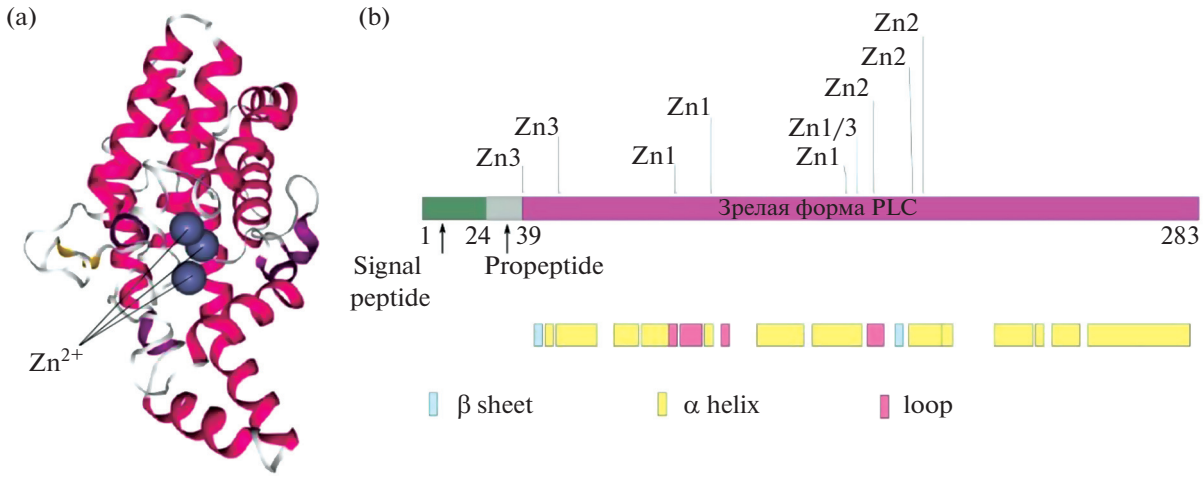

Fig. 3. Tertiary model (a) and secondary (b) structure of phosphatidyl-specific phospholipase C *B. cereus* (PDB: 1AH7) [33–35].

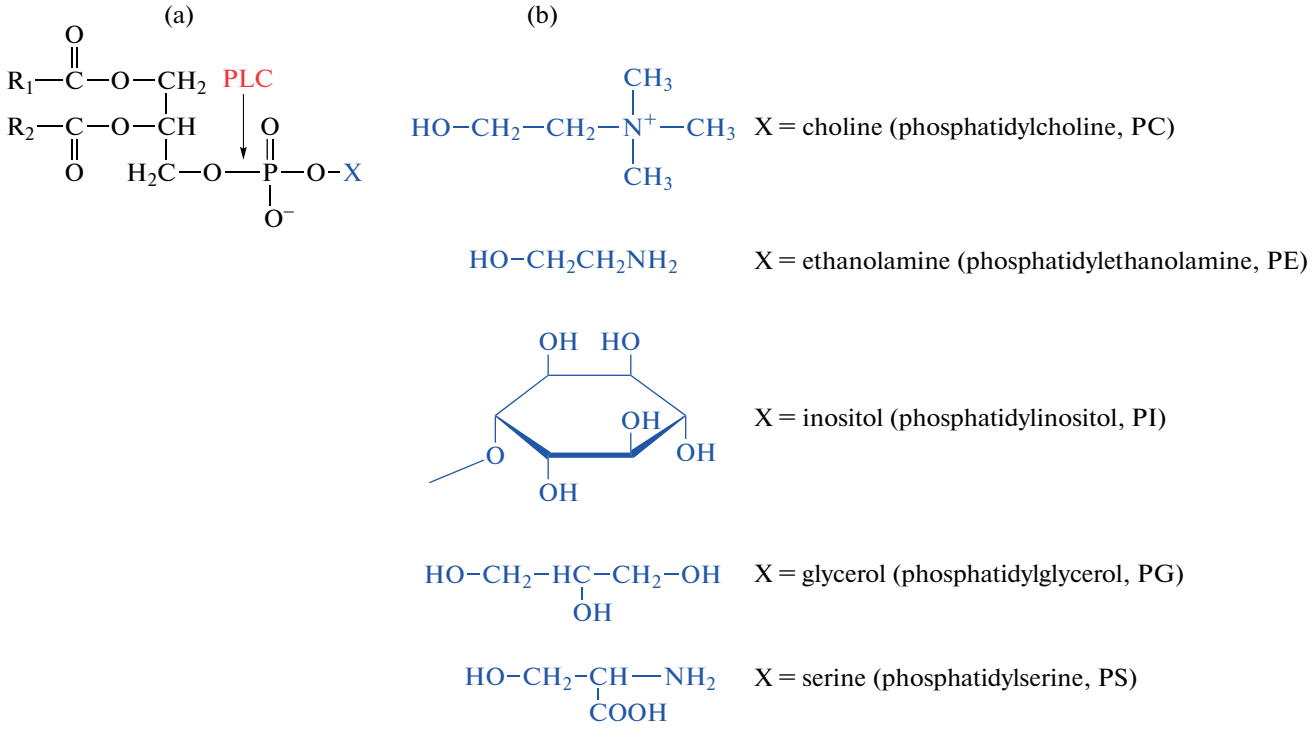

Fig. 4. Spectrum of substrates for phospholipase C *B. cereus*: (a) the general structure of the phospholipid; (b) head group [37].

nitrogen atom in the head group of choline via a polar or ionic bond, while Phe66 interacts via the cation–π interaction [44, 45]. It is assumed that Tyr56 can stabilize a positive charge on an inhibitor or substrate and, apparently, determine the specificity of the enzyme [46].

Regarding the molecular mode of action of PLC *B. cereus* there are two points of view. According to the first, Asp55 plays the role of a base that attacks the nucleophilic water molecule by deprotonation; water, in turn, launches an attack on phosphorus of phospholipid (Fig. 5a) [38, 41].

According to the second point of view, Zn1 and Zn3 initiate a nucleophilic attack on phosphorus, while Zn2 activates a water molecule to protonate the leaving group (Fig. 5b) [47, 48].

OBTAINING BACTERIAL PHOSPHOLIPASES C

Plants, animals and microorganisms can act as sources of enzymes. However, it is economically and

technologically less profitable to isolate enzymes from plant or animal sources than to obtain them using microorganisms. Using *E. coli* allows the production of a large amount of the inactive form of this enzyme in inclusion bodies. To obtain an active enzyme, the stages of cell destruction, purification, refolding and activation of the protein are additionally carried out, which increases the cost of the final product [49].

The first attempt at extracellular PLC synthesis was carried out using the system *Pichia pastoris*. The *N*-end of the enzyme was combined with the α-factor signal peptide from *Saccharomyces cerevisiae* for secretion and His_6-teg, with further purification after cleavage of the signal peptide [50]. However, the cultivation of this producer requires the presence of methanol as an inducer and expensive media, which makes its use unprofitable. The enzymatic activity of PLC depends on zinc, but the growth activity of *P. pastoris* is inhibited by excess Zn^{2+} (5 mM). Considering this, as well as the duration of cultivation of *P. pastoris* (several days), to obtain PLC on an industrial scale, attempts are being made to use the expression system of *B. subtilis*. Typically, the signal sequence of *B. subtilis* amyE α-amylase is used for secretion [51].

PRACTICAL APPLICATION OF PHOSPHOLIPASE C

Bacterial phospholipases C are used to study the modes of activation of arachidonic acid and protein kinase C in mammalian cells. In addition, they can act as reagents for studying the structure of cell membranes, for example, erythrocyte membranes [52, 53]. Unlike many bacterial toxins, PLC does not require protein internalization, which has attracted the attention of scientists to investigate the possibility of using the enzyme for drug delivery. PLC linked to a suitable antibody can form the backbone of an active cytotoxic agent. Today, phospholipase C is considered one of the key enzymes of the drug delivery system along the endocytosis pathway, since it can catalyze membrane fusion between cell membranes and phospholipids of delivery vehicles – liposomes [54].

PLC has therapeutic potential in thrombohemorrhagic syndrome because it is capable of inactivating thromboplastin, which triggers the blood coagulation process [31]. Hemolytic phospholipase C (α-toxin) *Clostridium perfringens* plays a major role in the pathogenesis of gas gangrene, and therefore is a promising component of a vaccine against this disease [23].

In the food industry, phospholipase C is used to refine oils: soybean, palm, sunflower, rapeseed, etc. Crude vegetable oil consists mainly of triglycerides, but can contain up to 3% phospholipids, which cause darkening of the oil and change in taste during storage [55]. Phospholipases are used to remove phospholipids during refining. The participation of PLC in the refining process does not lead to the formation of free

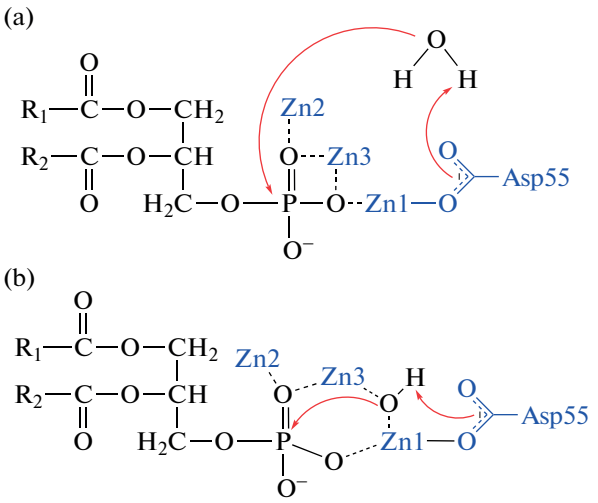

Fig. 5. Supposed modes of phospholipid hydrolysis by phospholipase C *B. cereus*: (a) deprotonation of water, triggering an attack on phosphorus of the phospholipid [38, 41]; (b) nucleophilic attack on phosphorus of a phospholipid and activation of a water molecule [47, 48].

fatty acids, which must be removed. This reduces the loss of raw materials, so the use of PLC is preferable to PLA. Currently, a PLC preparation from *B. anthracis* has been proposed for the production of refined vegetable oil (Purifine™, Verenium) [56]. PLC from *B. cereus* is an optimal enzyme for use in the industrial refining of vegetable oils. It has a high specific activity, the most suitable spectrum of substrate specificity and thermal stability [5].

Phospholipase C is also used for pretreatment of oil in the production of biodiesel. With the simultaneous use of PLC and lysophospholipases, a synergistic effect is observed, which makes the enzymatic process of biodiesel production more economical and environmentally friendly. The combination of refining and transesterification of released fatty acids in this process can be carried out with the simultaneous use of phospholipases and liquid lipase Callera Trans L. [57].

In the formation of the structural and mechanical properties of milk, as well as in the ripening of cheese, phospholipids play an important role, and therefore phospholipases are widely used in cheese making. The commercial product PLA1 is currently used from *A. oryzae* (YieldMAX® PL, Novozymes), PLC *B. cereus* and PLD *St. chromofuscus*. In cheese production, most of the phospholipids are removed along with the whey. When phospholipases are used, only the polar group is removed as a result of phospholipid hydrolysis, which allows an increase in the product yield [58].

Another promising area of using phospholipase C is the production of diacylglycerides with a definite enantiomeric structure, which are used in the synthesis of stereospecific compounds and also act as cell

mediators [59]. The sequential action of two classes of phospholipases C and D can be used, for example, for the synthesis of dihydroxyacetone phosphate (DHAP), an intermediate of fine chemical synthesis, or such an important bioactive compound for medicine and pharmaceuticals as sphingosine-1-phosphate (S1P), which is the main regulator of the vascular and the immune system [60, 61].

Improving the efficiency of the PLC can be achieved by immobilizing the enzyme, because catalysts obtained by covalent addition have a higher specific activity and are suitable for repeated use [62].

CONCLUSIONS

Bacterial phospholipases not only play an important role in biological processes, but also find wide application in scientific research, medicine, food, chemical and other industries. The currently obtained recombinant enzymes are distinguished by improved technological properties (high specific activity, thermal stability, wide substrate specificity), which open up additional opportunities for their use; therefore, the creation of effective phospholipase producers based on nonpathogenic bacteria remains an important biotechnological task.

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COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain a description of any research carried out by the authors of this work, with the participation of humans and animals as objects.

CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

REFERENCES

1. Aloulou, A., Ali, Y.B., Bezzine, S., Gargouri, Y., and Gelb, M.H., *Methods Mol. Biol.*, 2012, vol. 861, pp. 63–85. https://doi.org/10.1007/978-1-61779-600-5_4
2. Litvinko, N.M., *Izv. Nats. Akad. Nauk Belarusi, Ser. Khim. Nauk*, 2015, no. 4, pp. 109–121.
3. Ulbrich-Hofmann, R., *ChemBioChem*, 2012, vol. 13, pp. 2148–2149. https://doi.org/10.1002/cbic.201200542
4. Fil'kin, C.Yu., Lipkin, A.V., and Fedorov, A.N., *Usp. Biol. Khim.*, 2020, no. 60, pp. 369–410.
5. Borrelli, G.M. and Trono, D., *Int. J. Mol. Sci.*, 2015, vol. 16, pp. 20774–20840. https://doi.org/10.3390/ijms160920774
6. Titball, R.W., *Symp. Ser. Soc. Appl. Microbiol.*, 1998, vol. 27, p. 127.
7. Ramenskaia, G.V., Melnik, E.V., and Petukhov, A.E., *Biomed. Khim.*, 2018, vol. 64, pp. 84–93. https://doi.org/10.18097/PBMC20186401084
8. Titball, R.W., *Microbiol. Rev.*, 1993, vol. 57, pp. 347–366.
9. Songer, J.G., *Trends Microbiol.*, 1997, vol. 5, pp. 156–161.
10. Scandella, C.J. and Kornberg, A., *Biochemistry*, 1971, vol. 10, pp. 4447–4456.
11. Richmond, G.S. and Smith, T.K., *Int. J. Mol. Sci.*, 2011, vol. 12, pp. 588–612. https://doi.org/10.3390/ijms12010588
12. Murayama, K., Kano, K., Matsumoto, Y., and Sugimori, D., *J. Struct. Biol.*, 2013, vol. 182, pp. 192–196. https://doi.org/10.1016/j.jsb.2013.02.003
13. Bakholdina, S.I., Tischenko, N.M., Sidorin, E.V., Isaeva, M.P., Likhatskaya, G.N., Dmitrenok, P.S., Kim, N.Yu., Chernikov, O.V., and Solov'eva, T.F., *Biochemistry*, 2016, vol. 81, pp. 47–57. https://doi.org/10.1134/s0006297916010053
14. Hanahan, D.J., Brockerhoff, H., and Barron, E.J., *J. Biol. Chem.*, 1960, vol. 235, pp. 1917–1923.
15. Ishiwata, S., *Dainihon Sanshi Kaiho*, 1901, vol. 114, pp. 1–5.
16. Kohler, G.A., Brenot, A., Haas-Stapleton, E., Agabian, N., Deva, R., and Nigam, S., *Biochim. Biophys. Acta*, 2006, vol. 1761, pp. 1391–1399. https://doi.org/10.1016/j.bbalip.2006.09.011
17. Saito, K., Sugatani, J., and Okumura, T., *Methods Enzymol.*, 1991, vol. 197, pp. 446–456. https://doi.org/10.1016/0076-6879(91)97170-4
18. Jiang, F., Huang, S., Imadad, K., and Li, C., *Bioresour. Technol.*, 2012, vol. 104, pp. 518–522. https://doi.org/10.1016/j.biortech.2011.09.112
19. Matsumoto, Y., Mineta, S., Murayama, K., and Sugimori, D., *FEBS J.*, 2013, vol. 280, pp. 3780–3796. https://doi.org/10.1111/febs.12366
20. Masayama, A., Kato, S., Terashima, T., Molgaard, A., Hhemmi, H., Yoshimura, T., and Moriyama, R., *Biosci. Biotechnol. Biochem.*, 2010, vol. 74, pp. 24–30. https://doi.org/10.1271/bbb.90391
21. Taguchi, R. and Ikezawa, H., *Arch. Biochem. Biophys.*, 1978, vol. 186, pp. 196–201.
22. Djordjevic, J.T., *Front. Microbiol.*, 2010, vol. 1, pp. 1–13. https://doi.org/10.3389/fmicb.2010.00125
23. Pokotylo, I., Pejchar, P., Potocký, M., Kocourková, D., Krčková, Z., Ruelland, E., Kravets, V., and Martinec, J., *Prog. Lipid Res.*, 2013, vol. 52, pp. 62–79. https://doi.org/10.1016/j.plipres.2012.09.001
24. Ivinskene, V.L., *Entomopatogennye bakterii i ikh rol' v zashchite rastenii: sbornik nauchnykh trudov* (Entomopathogenic Bacteria and Their Role in Plant Protection: Collection of Scientific Articles), Novosibirsk: VASKhNIL, Sib. Otd., 1987, pp. 57–75.
25. Ikezawa, H., Nakabayashi, T., Suzuki, K., Nakajima, M., Taguchi, T., and Taguchi, R., *J. Biochem.*, 1983, vol. 93, pp. 1717–1719. https://doi.org/10.1093/oxfordjournals.jbchem.a134315

26. Volwerk, J.J., Koke, J.A., Wetherwax, P.B., and Griffith, O.H., *FEMS Microbiol. Lett.*, 1989, vol. 61, pp. 237–241. https://doi.org/10.1111/j.1574-6968.1989.tb03629.x
27. Jenkins, M.G. and Frohman, M.A., *Cell. Mol. Life Sci.*, 2005, vol. 62, pp. 2305–2316. https://doi.org/10.1007/s00018-005-5195-z
28. Selvy, P.E., Lavieri, R.R., Lindsley, C.W., and Brown, H.A., *Chem. Rev.*, 2011, vol. 111, pp. 6064–6119. https://doi.org/10.1021/cr200296t
29. Sakurai, J., Nagahama, M., and Oda, M., *J. Biochem.*, 2004, vol. 136, pp. 569–574. https://doi.org/10.1093/jb/mvh161
30. González-Bulnes, P., González-Roura, A., Canals, D., Delgado, A., Casas, J., and Llebaria, A., Bioorg. Med. Chem., 2010, vol. 18, pp. 8549–8555. https://doi.org/10.1016/j.bmc.2010.10.031
31. Otnaess, A.-B., Little, C., Sletten, K., Wallin, R., Johnsen, S., Flengsrud, R., and Prydz, H., *Eur. J. Biochem.*, 1977, vol. 79, pp. 459–468. https://doi.org/10.1111/j.1432-1033.1977.tb11828.x
32. Elleboudy, N.S., Aboulwafa, M.M., and Hassouna, N.A., *Asian Pacific J. Trop. Med.*, 2014, vol. 7, pp. 860–866. https://doi.org/10.1016/s1995-7645(14)60150-4
33. Hough, E., Hansen, L.K., Birknes, B., Jynge, K., Hansen, S., Hordvik, A., Little, C., Dodson, E., and Derewenda, Z., *Nature*, 1989, vol. 338, pp. 357–360. https://doi.org/10.1038/338357a0
34. Rose, A.S., Bradley, A.R., Valasatava, Y., Duarte, J.M., Prlić, A., and Rose, P.W., in *Proceedings of the 21St International Conference on Web3D Technology – Web3D'16*, 2016, pp. 185–186. https://doi.org/10.1145/2945292.2945324
35. Rose, A.S. and Hildebrand, P.W., *Nucleic Acids Res.*, 2015, vol. 43, pp. W576–W579. https://doi.org/10.1093/nar/gkv402
36. Beecher, D.J. and Wong, A.C.L., *Microbiology*, 2000, vol. 146, pp. 3033–3039. https://doi.org/10.1099/00221287-146-12-3033
37. Lyu, Y., Ye, L., Xu, J., Yang, X., Chen, W., and Yu, H., *Biotechnol. Lett.*, 2016, vol. 38, pp. 23–31. https://doi.org/10.1007/s10529-015-1962-6
38. Antikainen, N.M., Hergenrother, P.J., Harris, M.M., Corbett, W., and Martin, S.F., *Biochemistry*, 2003, vol. 42, pp. 1603–1610. https://doi.org/10.1021/bi0267285
39. Shinitzky, M., Friedman, P., and Haimovitz, R., *J. Biol. Chem.*, 1993, vol. 268, pp. 14109–14115.
40. El-Sayed, M.Y., DeBose, C.D., Coury, L.A., and Roberts, M.F., *Biochim. Biophys. Acta*, 1985, vol. 837, pp. 325–335. https://doi.org/10.1016/0005-2760(85)90056-6
41. Martin, S.F., Follows, B.C., Hergenrother, P.J., and Trotter, B.K., *Biochemistry*, 2000, vol. 39, pp. 3410–3415. https://doi.org/10.1021/bi9919798
42. Snyder, W.R., *Biochim. Biophys. Acta*, 1987, vol. 920, pp. 155–160.
43. Benfield, A.P., Goodey, N.M., Phillips, L.T., and Martin, S.F., *Arch. Biochem. Biophys.*, 2007, vol. 460, pp. 41–47.
44. Burley, S.K. and Petsko, G.A., *Adv. Protein Chem.*, 1988, vol. 39, pp. 125–189. https://doi.org/10.1016/s0065-3233(08)60376-9
45. Dougherty, D.A., *Science*, 1996, vol. 271, pp. 163–168. https://doi.org/10.1126/science.271.5246.163
46. Celandroni, F., Salvetti, S., Senesi, S., and Ghelardi, E., *FEMS Microbiol. Lett.*, 2014, vol. 361, pp. 95–103. https://doi.org/10.1111/1574-6968.12615
47. Sundell, S., Hansen, S., and Hough, E., *Protein Eng.*, 1994, vol. 7, pp. 571–577. https://doi.org/10.1093/protein/7.4.571
48. Liao, R.Z., Yu, J.G., and Himo, F., *Phys. Chem.*, vol. 114, pp. 2533–2540. https://doi.org/10.1021/jp910992f
49. Martin, S.F., Spaller, M.R., and Hergenrother, P.J., *Biochemistry*, 1996, vol. 35, pp. 12970–12977. https://doi.org/10.1021/bi961316
50. Seo, K.H. and Rhee, J.I., *Biotechnol. Lett.*, 2004, vol. 26, pp. 1475–1479. https://doi.org/10.1023/b:bile.0000044447.15205.90
51. Durban, M.A., Silbersack, J., Schweder, T., Schauer, F., and Bornscheuer, U.T., *Appl. Microbiol. Biotechnol.*, 2007, vol. 74, pp. 634–639. https://doi.org/10.1007/s00253-006-0712-z
52. Kent, C., Evers, A., and Haun, S.S.L., *Arch. Biochem. Biophys.*, 1986, vol. 250, pp. 519–525.
53. Parkinson, E.K., *Carcinogenes*, 1987, vol. 8, pp. 857–860. https://doi.org/10.1093/carcin/8.6.857
54. Shimanouchi, T., Kawasaki, H., Fuse, M., Umakoshi, H., and Kuboi, R., *Colloids Surf. B: Biointerfaces*, 2013, vol. 103, pp. 75–83.
55. Mounts, T.L. and Nash, A.M., *J. Am. Oil Chem. Soc.*, 1990, vol. 67, pp. 757–760. https://doi.org/10.1007/bf02540486
56. De Maria, L., Vind, J., Oxenboll, K.M., Svendsen, A., and Patkar, S., *Appl. Microbiol. Biotechnol.*, 2007, vol. 74, pp. 290–300. https://doi.org/10.1007/s00253-006-0775-x
57. Cesarini, S., Haller, R.F., Diaz, P., and Nielsen, P.M., *Biotechnol. Biofuels*, 2014, vol. 7, pp. 1–12. https://doi.org/10.1186/1754-6834-7-29
58. Casado, V., Martin, D., Torres, C., and Reglero, G., *Lipases and Phospholipases: Methods and Protocols*, New York: Springer, 2012, vol. 861, pp. 495–523.
59. Arrigo, P.D. and Servi, S., *Trends Biotechnol.*, 1997, vol. 15, pp. 90–96. https://doi.org/10.1016/s0167-7799(97)01012-3
60. Schümperli, M., Pellaux, R., and Panke, S., *Appl. Microbiol. Biotechnol.*, 2007, vol. 75, pp. 33–45. https://doi.org/10.1007/s00253-007-0882-3
61. Morigaki, E., Miura, Y., Takahata, K., Tada, M., Nakajima, S., and Baba, N., *J. Chem. Res.*, 1998, vol. 12, pp. 774–775. https://doi.org/10.1039/a804770g
62. Anthonsen, T., D'Arrigo, P., Pedrocchi-Fantoni, G., Secundo, F., Servi, S., and Sundby, E., *J. Mol. Catal.*, 1999, vol. 6, pp. 125–132. https://doi.org/10.1016/s1381-1177(98)00141-6

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