ECOLOGIA BALKANICA
2019, Vol. 11, Issue 2 December 2019 pp. 279-290

Production of Extracellular Phospholipase C by Species of Genus Bacillus with Potential for Bioremediation

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Abstract. The application of phospholipases produced by species of genus *Bacillus* in different industries reduces the negative impact on the environment by reducing the need of toxic chemicals, consumed energy, and produced carbon emissions. In the current study one hundred sixty-six bacterial strains belonging to the genus *Bacillus* were tested for phospholipase C production. Eighty-seven percent of the studied strains demonstrated phospholipase C activity on egg-yolk agar. Strain *Bacillus thuringiensis* was selected as the most promising for the phospholipase production with initial activity of 19.61 U/ml. The nutrient medium composition and cultivating conditions were optimized for achieving higher enzyme yields. The highest phospholipase production was achieved on the following conditions of the liquid medium: 1% of yeast extract as a source of nitrogen; 0.5% NaCl; 0.4% of glucose as a carbon source; $NaHCO_3$ – 3 g/l; Na_2HPO_4 – 0.4 g/l; 1 mM $ZnCl_2$; pH 7; inoculated with 3% (1.4 x 10^9 cfu/ml) subculture and 8 hours duration of the cultivation. The production of phospholipase C by the selected strain was scaled up in a bioreactor with a volume of 2l.

Key words: phospholipase C, *Bacillus*, bioremediation.

Introduction

The human civilization is facing a global environmental crisis, that could change the lives of everyone. A lot of different measures are being taken to avoid further environmental problems, but the main responsibility lies on the global industry. At the moment some of the older industrial production technologies are being replaced with green alternatives, based on green chemistry and green biotechnology. The main goal is the reduction of used dangerous chemicals and materials, reduction of the used water and energy, eliminating the produced dangerous byproducts and to reduce the production of greenhouse gases.

One of the possible solutions to these problems is the use of biocatalysts – enzymes, in different kinds of chemical processes. They can effectively increase the speed of the occurring chemical reactions, they are active at a lower temperature, which leads to a reduction in the consumed energy for heating, they reduce the quantity of the used toxic chemical compounds and they can be produced from by-products and wastes of other industries. For the production of enzymes, bacterial species are often preferred. Of them *Bacillus* spp. have many favorable properties. Some of the species have GRAS status - they are considered as safe for food and food

additives production. They have short division cycles, they can be cultured in short periods, they can secrete the desired proteins in the medium, which makes the enzyme easier for purification. All these properties are making production cost-effective (SEWALT *et al.*, 2016).

In the food and beverage production industries enzymes also find broad field off application. For example, phospholipases can be used in the refining process of vegetable oils. The biological catalyzers can be used as a replacement to the classical refining technologies, which are relying on a caustic process using strong alkaline and phosphoric acid solutions for the removal of phospholipids, which reduce the quality of the oils. Data form industrial experiments at Bunge's oil refining plants (USA) show, that the used enzymes for degumming have reduced the amount of consumed energy and the produced greenhouse gases. For the refining of 266 000 tons of soy oil the consumed energy is reduced with 112 000 GJ, the carbon emissions are reduced with 12 000 tons, SO_2 emissions are reduced with 140 tons, PO_4 emissions are reduced with 100 tons, and the produced ethylene is with 4 tons less (DE MARIA *et al.*, 2007). On an annual basis, the food industry throws away tons of materials rich in keratin in the form of hair, fur, skin, nails, and other by-products which are considered dangerous waste, but they could be a good source of amino acids. It is possible these waste materials to be used as a substrate for enzymatic hydrolysis process using proteolytic enzymes. The final products will be rich in single amino acids and small peptides, which could be used as a valuable nutrition source for animals (SINGH & BAJAJ, 2017). Another valuable application is in the baking industry. The quality of the produced bread and similar products is declining in time with the crystallization of the starch in the products. In this case, amylase enzymes can be used to increase the shelf life and the quality of the products. Careful calculations show that the financial loses of producing bread with short shelf life are bigger than the cost of the treatment of the drought with amylases (JEGANNATHAN & NIELSEN, 2013).

But enzymes can be used not only in manufacturing processes. They can be used for the treatment of chemical contaminations in the environment. CHRISTOVA *et al.* (2019) have identified a strain of *Bacillus cereus*, which has the ability to degrade up to 93% of the oil hydrocarbons in the examined medium for about 48 hours. The research group has immobilized the isolated strain and their results show that the preparation remains almost fully active for 47 days, at 28°C up to 20 cycles of usage. Other authors also have reported similar results. SAKTHIPRIYA *et al.* (2015) have isolated strain of *Bacillus cereus*, which can degrade up to 80% of the oil hydrocarbons in the contaminated environment. Enzymes can be used also for the removal of toxic agrochemical contaminants in the soil, like pesticides, herbicides, and fungicides. Some strains of *Bacillus amyloliquefaciens* have hy-1 gene with a length of 858 bp, which is coding protein, which can hydrolyze the fungicide carbendazim to 2-aminobenzimidazole. The isolated protein is identified as a type of phospholipase, which successfully can remove the fungicide off the surface of contaminated cucumbers. This experiment proves that enzymes can be used as an effective tool in the bioremediation processes (LI *et al.*, 2019). There are already developed technologies that use *Bacillus subtilis* and other species that produce highly active lipase and phospholipase enzymes that hydrolyze contaminated with fats and oils waste waters. The produced free fatty acids and alcohols can then be easily digest by the active sludge in the water treatment plant (Patent No. PCT/JP2013/055504).

Although lipases and phospholipases are hydrolytic enzymes in nature, they could be used in valuable biosynthesis industrial processes. Their ability to carry on transesterification reactions can be used for the biosynthesis of fatty acids alkyl esters, which commercially are better known as

biodiesels – an important source of renewable fuels (ANOBOM *et al.*, 2014).

The aim of the current work is to examine the extracellular phospholipase C production capabilities of strains of genus *Bacillus* and to optimize the condition for the enzyme production.

Materials and Methods

Bacterial strains

Strains of genus *Bacillus* (166 strains) from the microbial collection of the Department of "Biochemistry and microbiology", Faculty of Biology, University of Plovdiv, Bulgaria were examined for phospholipase C activity. Cultures of the organisms were maintained on nutrient agar medium at 4°C for routine laboratory use. For long-term use, the strains were maintained in nutrient agar under paraffin layer at 4°C.

The initial screening was based on the ability of the strains to hydrolyze phospholipids in egg yolk agar medium.

Hydrolysis of phospholipids

Each strain was inoculated on egg yolk agar (one egg yolk; nutrient broth – 3 g; yeast extract – 0.5 g; glucose – 0.5 g; 5 ml 0.1M $CaCl_2.2H_2O$; 95 ml H_2O; pH 7.2) and incubated for 24 to 48 hours at 37°C. The formed halo around the colony was measured. Strains that have formed halo larger than 5 mm were taken for further analyses.

Fermentation conditions and separation of culture filtrates

A subculture was prepared prior to the fermentation by inoculation of the strain in nutrient broth and incubation for 8h on a rotary shaker at 37°C, 120 rpm. The cell density of the culture was then modified to 6.0 McF units.

Erlenmeyer flasks with volume of 300 ml, containing 30 ml growth medium (casein hydrolysate – 10 g/l; Bacto Peptone – 10 g/l; NaCl – 5 g/l; glucose – 4 g/l; $NaHCO_3$ – 3 g/l; Na_2HPO_4 – 0.4 g/l; $ZnCl_2$ – 0.01 g/l; pH 7; sterilized for 15 minutes at 121°C) were inoculated with 3% (6.0 McF) subculture (GERASIMENE *et al.*, 1980). The inoculated flasks were set on a rotary shaker at 37°C, 120 rpm for 12 - 48 hours. For the separation of the cells, 10 ml culture medium were centrifuged at 4°C, 14 000 rpm for 20 minutes. The supernatant was collected for further analysis.

Optimization of the culture conditions for enzyme production

Optimum phospholipase production was studied at different incubation periods (0 – 36 h), with different volumes of the inoculum between 1 – 10%, carbon sources (mannose, fructose, maltose and ribose), concentrations of the carbon source (0.2 – 0.8%), different sources of nitrogen (bactopeptone, casein hydrolysate, yeast extract, beef extract, KNO_3, $(NH_4)_2SO_4$), and different metal ions (Mg^{2+}, Mn^{2+}, Zn^{2+}, Ca^{2+}).

Fermentation in bioreactor

Fed-batch cultivation was performed in a 2 L bench-top bioreactor (Minifors, Infors HT, Switzerland) equipped with turbine impeller and connected to a digital control unit. The set points for temperature was 37°C, constant pH was not maintained, no compressed air was supplied, the agitation speed was maintained to 100 rpm, no antifoam agent was used. The composition of the medium was identical to the established optimal medium composition in flasks experiments. Every 2 hours, 20 ml sample was taken from the vessel for the determination of the phospholipase C activity, cell density, and pH of the culture.

Enzyme assays

The determination of phospholipase C activity is based on the enzymatic digestion of L-α-Phosphatidylcholine, extraction of the produced phosphate compounds, and determination of their concentration according to the method described by TAKAHASHI *et al.* (1981).

Results and Discussion

One hundred and sixty-six *Bacillus* strains were tested for production of PLC in a medium with phosphatidylcholine as e substrate. The number of strains by species is presented in Fig. 1a.

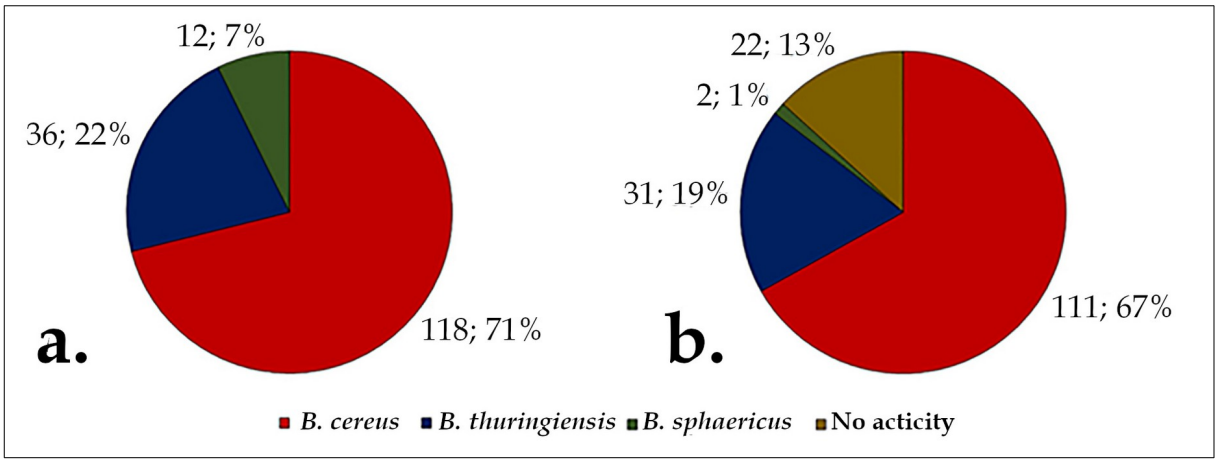

Fig. 1. Enzyme activities of strains of genus *Bacillus* – qualitative analysis. 166 examined strains (a.). Phospholipase C activity of strains of genus *Bacillus* (b.).

After the qualitative assays strains, which formed hydrolysis zones or formed haloes larger than 5 mm, were selected for the quantitative determination of enzyme activity. Of all examined strains thirteen were selected for quantitative determination of their extracellular phospholipase C activity. *Bacillus thuringiensis №17* had highest phospholipase C activity reaching 19.61 U/ml (Fig. 2). In previous research we have shown that these strains can produce also extracellular amylase and protease enzymes with activity within the range of 2.80 – 9.20 U/ml (STEFANOV *et al.*, 2018a; STEFANOV *et al.*, 2018b).

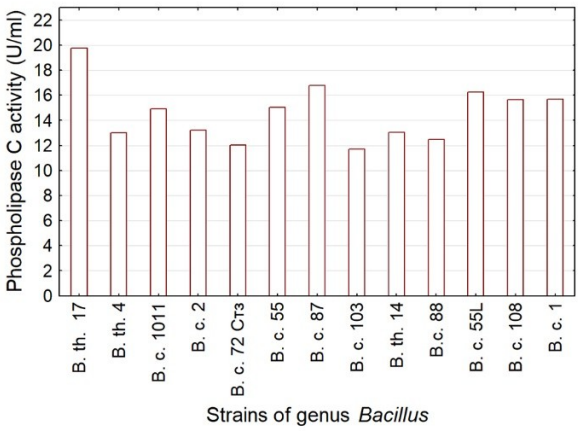

Fig. 2. Quantitative determination of phospholipase C activity of strains of genus *Bacillus*

The present work and in previous studies on protease (STEFANOV *et al.*, 2018a) and amylase (STEFANOV *et al.*, 2018b) activity showed that the examined strains are characterized by a diverse extracellular enzyme production. From all *Bacillus cereus* strains, 94% produced phospholipase C, and 93% produced proteases. Only 29% could produce lipases, 27% amylase enzymes, and 3% had cellulolytic activity. Eighty-seven percent of all examined strains of *Bacillus thuringiensis* had phospholipase C activity, 88% had protease activity, 16% had amylase activity, 15% had lipase activity, and only 2% exhibited cellulolytic activity. All examined strains of *Bacillus sphaericus* exhibited weak extracellular enzyme activity.

Bacillus species are known for their ability to secrete a vast variety of proteins in the culture medium. They can produce more than 40 different extracellular enzymes. Transcriptomic analyses of the species during different stages of cultivation show that they produce more than 3800 coding transcripts but in varying quantity. It has been shown that *Bacillus pumilus* transcribes genes that are coding two lysophospholipases and three phospholipases. Their expression is initiated during the exponential phase of growth, but its maximum is reached during the early stationary phase of growth. Some species of

Bacillus have several genes that code different types of extracellular proteases, which are form bio-technical interest. It is shown that the expression of epr and subE begins at the exponential phase of growth, while aprE, aprX, and wprA are highly expressed during the stationary phase of growth. The major proteolytic activity of the isolated supernatant is contributed to the products of these 3 genes. All this shows that the expression of the different proteases is controlled by different regulatory mechanisms and may even have different secretion paths (HAN *et al.*, 2017).

The production of a vast variety of extracellular enzymes makes some strains of genus *Bacillus* valuable tools for saving the environment and avoiding ecological disasters. It has been shown that strains that produce lipolytic, proteolytic, and/or amylolytic enzymes could efficiently reduce the biological oxidation demand (BOD), chemical oxidation demand (BOD), nitrates and, phosphates by up to 56.25% after 72 hours (SONUNE & GARODE, 2018). This could prevent the depletion of oxygen in the water and the suffocation of its main consumers.

Chromatographic analysis of the products from the hydrolysis reactions

The identification of the products of the hydrolysis reaction of L-α- phosphatidylcholine (Sigma-Aldrich, USA), mediated by phospholipase C, isolated from *Bacillus thuringiensis №17*, was done using thin-layer chromatography (Fig. 3). The reduced amount of the substrate (Fig. 3b) correlates with the appearance of the reaction products (Fig. 3a). It can be seen from the chromatogram that phospholipase C from *B. thuringiensis №17* hydrolyzed the highest amount of the substrate.

The used substrate for the hydrolysis reaction was sonicated as a pretreatment to form lipid vesicles. Phospholipase enzymes prefer phospholipid substrates in the form of small vesicles with a diameter of 20-100 nm instead of single molecules. It was established by other researchers that the produced phospholipases from species of genus *Bacillus* bind strongly to vesicles with a high content of phosphatidylcholine. Although the strong binding between the substrate and the enzymes, which lasts for an average of 378 ± 49 ms, the high phosphatidylcholine concentration reduces the enzyme activity. Phospholipases also prefer smaller vesicles with higher surface tension (YANG *et al.*, 2015).

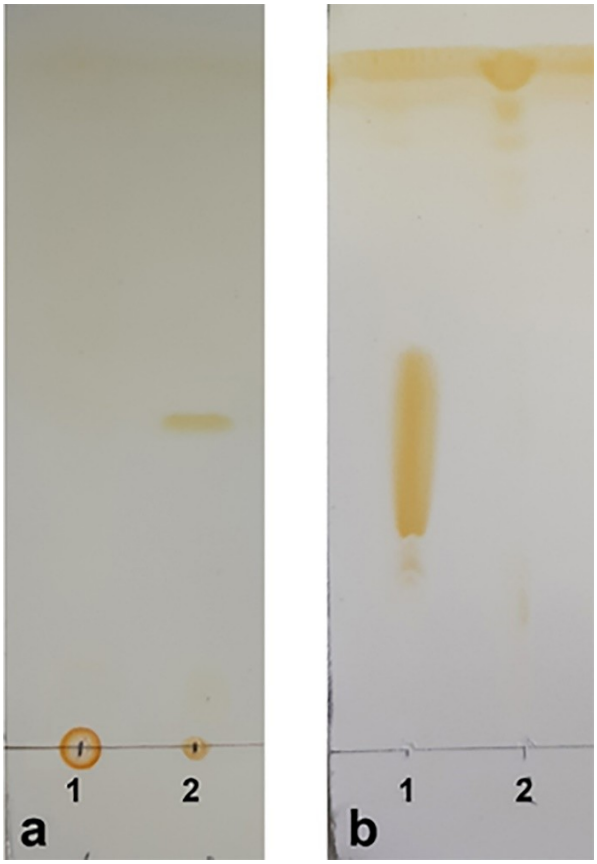

Fig. 3. Thin-layer chromatography of the substrate and the products of the enzyme hydrolysis with phospholipase C from *B. thuringiensis №17*. (1) L-α-phosphatidylcholine (99%, Sigma-Aldrich, USA); (2) the substrate after hydrolysis with PLC from *B. thuringiensis №17*. System (a) - petroleum ether:diethyl ether:acetic acid (6:4:0.2); System (b) - chloroform:methanol:water (65:25:4).

Dynamics of the phospholipase production during growth

The production of phospholipase enzymes started after the second hour of

cultivation and it slowly increased, reaching its maximum at the 8-th hour with an activity of 20.69 U/ml. After twelve hours of cultivation, the enzyme production slowly dropped reaching 2.42 U/ml at the 24-th hour. The pH of the culture medium doesn't change dramatically during the cultivation. In the beginning, the medium was weakly acidified to pH 6.75 at the fourth hour and then the medium gradually was alkalized to pH 7.90 at the 24-th hour. The analysis of the cell density showed that the exponential phase of growth begins nearly at the begging of the cultivation and it is over at the 8-th hour (Fig. 4).

The maximum enzyme production was reached between the late exponential phase of growth and the beginning of the stationary phase. The rate of phospholipase production correlates with cell density. This type of fermentation, in which phospholipase C is produced from species of genus *Bacillus* is classified as growth associates (SHILOACH *et al.*, 1973). This phenomenon may be explained by the fact that many (primary) enzymes, produced from *Bacillus*, are needed for the survival and development of the bacterial culture especially in the early stages of the fermentation (BLANCO *et al.*, 2016). It has been discovered that the synthesis of phospholipases is under the control of the quorum-sensing system of the bacterial community (ELLEBOUDY *et al.*, 2011; DONG *et al.*, 2002), but the presence of these enzymes is not due to cell lysis or sporulation. Thus, higher phospholipase production is achieved at certain cell density. The enzymes are secreted in the medium (ELLEBOUDY *et al.*, 2011).

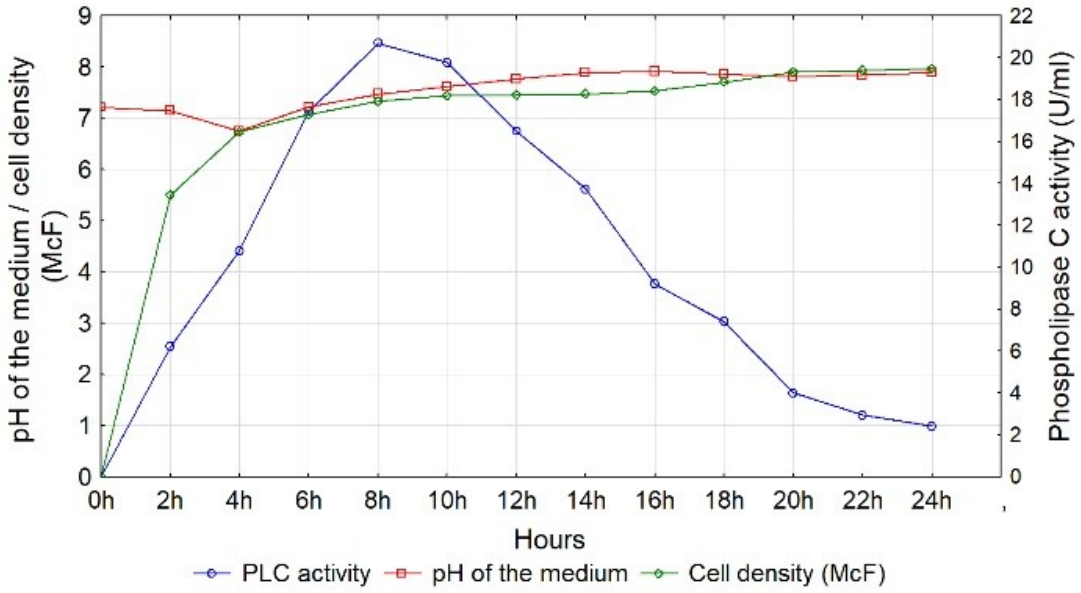

Fig. 4. Growth and phospholipase C activity of *Bacillus thuringiensis* strain *17* in a liquid medium of GERASIMENE *et al.* (1980).

Effects of different carbon sources

To determine the effects of different carbon sources on the phospholipase C production, glucose, fructose, ribose, inulin, or maltose, respectively, at a concentration of 0.4%. were added to the base medium. The control medium contained glucose as a source of carbon (Fig. 5). Our results showed that maximum enzyme production was achieved when the medium was supplemented with glucose. High enzyme activity was also achieved when the strain was incubated in medium, containing maltose. The production was reduced when

the medium contained ribose or inulin (Fig. 5). The results of THAYUMANAVAN & BOOPATHY (2005) also show that glucose is a good choice as a source of carbon for the production of phospholipase C from *B. thuringiensis*. *Bacillus thuringiensis* can utilize other carbohydrates like fructose, sucrose, and lactose, but more complex carbon sources (like starch) are less preferable. This can reduce the cell density and the production of extracellular enzymes respectively (BULLA *et al.*, 1980). It has been discovered that the use of molasses as a cheaper source of carbon results in lower phospholipase production (THAYUMANAVAN & BOOPATHY, 2005).

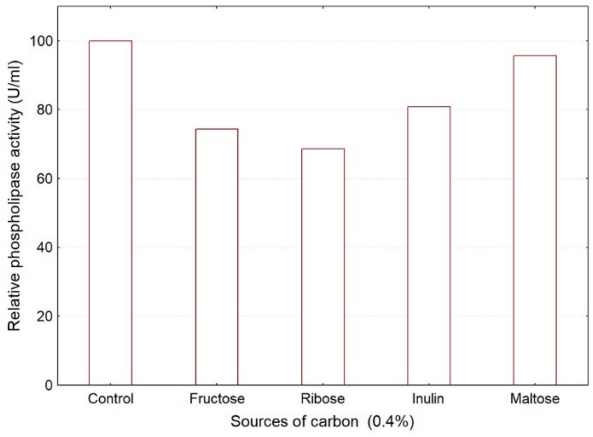

Fig. 5. Effect of the carbon source on the phospholipase C production.
*Control – glucose.

According to ZHAO *et al.* (2018), research high concentration of the glucose, fructose or maltose in the liquid medium may reduce or even inhibit the synthesis of phospholipase enzymes. Our results showed that a gradual increase in the concentration of glucose to a maximum amount of 0.4% leads to a rise in phospholipase C production. Further increase in the concentration results in a decrease and inhibition of the enzyme synthesis. The results of THAYUMANAVAN & BOOPATHY (2005), show that maximum phospholipase production is achieved when the medium contained 0.65% of glucose. For many microorganisms, glucose is the preferred carbon source. When it is present in the medium in excess, it may repress the catabolism of other substrates and the synthesis of the needed enzymes. This phenomenon is called catabolic repression and it is an evolutionary mechanism, which allows the organisms to utilize first the substrate, which guarantees them the fastest growth in the competitive environment (SINGH & BAJAJ, 2004).

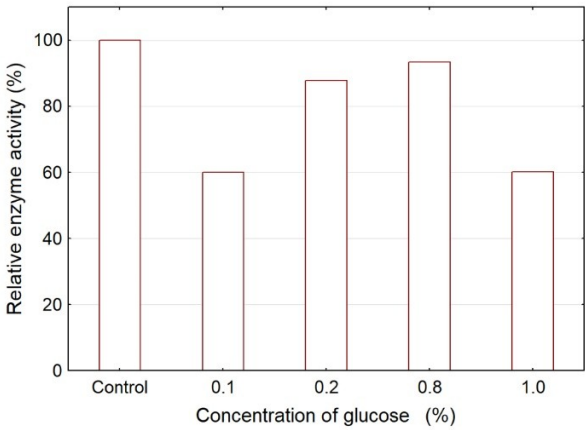

Fig. 6. Effect of glucose concentrations on the phospholipase C production.
*Control - 0.4% glucose.

Effects of different nitrogen sources

The source of nitrogen is another important factor for the growth of the bacterial culture and for the production of phospholipase C. In the current study the influence of six nitrogen sources was studied – peptone, bactopeptone, casein hydrolysate, yeast extract, potassium nitrate, and ammonium sulfate. The results showed that the production of phospholipase C is slightly higher when the growth medium was supplemented with yeast extract. It has been proven that *Bacillus thuringiensis* №17 cannot utilize inorganic sources of nitrogen (Fig. 7). Although some strain can utilize inorganic nitrogen compounds, the majority of *Bacillus thuringiensis* strains prefer organic nitrogen (THAYUMANAVAN & BOOPATHY, 2005; EL-BENDARY, 2006). To ensure the growth of the bacterial culture the medium must be supplemented with source of at least one of the following amino acids – glutamate,

aspartate, valine, leucine, serine or threonine, but the addition of cysteine or cystine will lead to full growth inhibition and the formation of toxic compounds (EL–BENDARY, 2006), but complex organic sources of nitrogen are preferred – peptone, casein hydrolysate, yeast extract and others (ICGEN *et al.*, 2002). When the medium is rich in free proteins and protein hydrolyzates the added free amino acids are used as a source of carbon (EL–BENDARY, 2006).

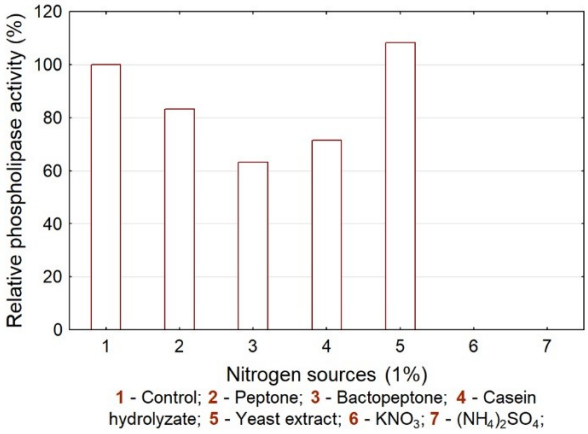

Fig. 7. Effect of nitrogen sources on the phospholipase C.
*Control - the original medium as described by GERASIMENE *et al.* (1980).

Effects of different metal ions

More than half of the discovered enzymes are metalloenzymes, including the phospholipases, but according to other researchers, only 30% of the enzymes require metal ions to carry out their normal functions (WALDRON & ROBINSON, 2009). Phospholipases, produced by species of genus *Bacillus* contain two zinc atoms in their protein structure. The removal of one of the zinc atoms results in partial reduction of the enzyme activity. The removal of the second atom results in the full inhibition of the activity. This effect is reversible if zinc ions are added to the enzyme solution (LITTLE & OTNASS, 1975). Some metal ions can enhance the enzyme activity and others can inhibit it. In the current study, the influence of 4 sources of metal ions in 1 mM concentration ($CaCl_2$, $ZnCl_2$, $MgCl_2$, $MnCl_2$) on the phospholipase C production was studied. As a control was used a medium that didn't contain any metal ions. The results showed that the enzyme production is reduced when the medium contained manganese ions. The production of phospholipase C is greatly enhanced when the medium contained zinc ions. Other researchers also report that the presence of metal ions in the medium is of great importance for the growth of the bacterial producer and for the enzyme production (THAYUMANAVAN & BOOPATHY, 2005). Zinc ions can directly participate in the enzyme reaction or they may maintain the protein structure stable. They also function in all catalytic sites as Lewis acid – chemical compound, containing free orbital, which is capable of accepting electron pairs.

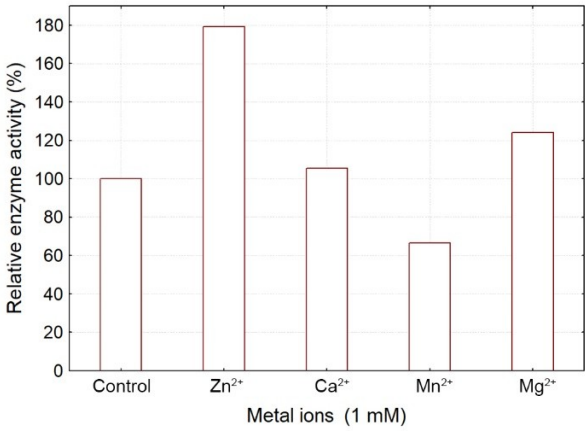

Fig. 8. Effect of metal ions on the phospholipase C activity of *B. thuringiensis*.
*Control - medium with no metal ion supplementation.

Effects of the volume of inoculum

To determine the effects of the volume of the inoculum over the production of phospholipase C, Erlenmeyer flasks with a sterile medium where inoculated with varying volumes (between 1 – 10%) of 8-th hour bacterial culture with a cell density of 1.4×10^9 cfu/ml. It was found that maximum enzyme production occurs when the medium is inoculated with 7.5% of

inoculum. The quantity of the produced enzyme between the different examined volumes of inoculum is not significant, but the tendency is clearly visible. The rate of enzyme production rises with the larger volumes of inoculum. The reduction of the enzyme production when using volumes larger than 7.5% may be explained with the more intensive consumption of nutrients in the medium. The bacterial culture also produces compounds that are inhibiting the growth and development of the culture. (HAYES & LOW, 2009).

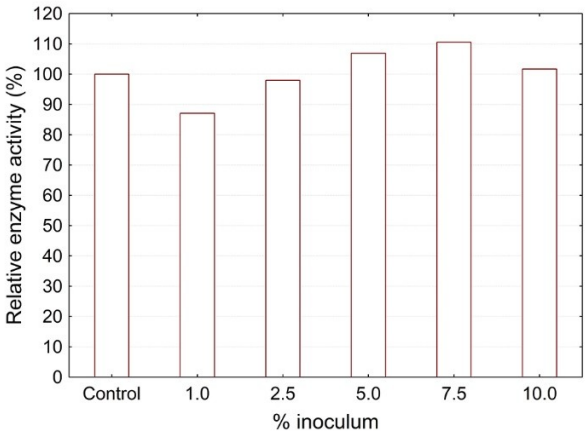

Fig. 9. Effect of inoculum concentration on the phospholipase C production.
*Control - 3% inoculum.

Production of phospholipase C in bioreactor

The dynamics of phospholipase production was studied in a scale-up experiment using a bioreactor with a volume of 2 liters. *Bacillus thuringiensis* №17 was inoculated in a medium with the determined optimum composition. The production of phospholipase C enzymes began at the second hour of cultivation, slowly increased and reached its maximum at the 8th hour of cultivation with activity of 14.87 U/ml after which the production dropped down. During the cultivation, the medium was slowly acidifying reaching pH 6.21 at the twelve hours of cultivation. The bacterial culture entered almost immediately in the exponential phase of growth, which ended after the 6-th hour of cultivation. In our scale-up experiment, maximum phospholipase production was achieved in the early stationary phase of cultivation with pH 6.17 of the medium.

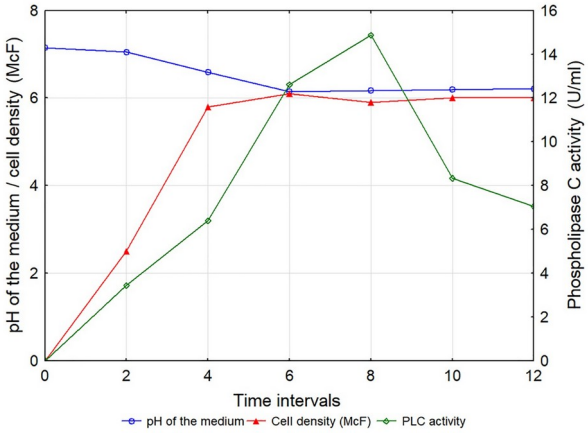

Fig. 10. Dynamics of the phospholipase production using bioreactor with volume of 2 liters.

Although during the cultivation in bioreactor and Erlenmeyer flasks the production of phospholipase C was in identical time intervals, the cell density, pH of the medium and enzyme activity varied. During the cultivation in flasks, the medium first slowly acidified to pH 6.75, but later the medium was alkalized to pH 8. During the cultivation in a bioreactor, the medium was steadily acidified to pH 6. The difference in the cell density of the two cultures was quite significant. In flask maximum enzyme production was achieved when the culture had a cell density of 7.4 McF, but in bioreactor only 5.9 McF. The difference equals 0.5 x 10^9 cfu/ml. Even the enzyme activity was lower when the strain was cultivated in a bioreactor. This can be explained with many factors that are important during scale-up fermentation – the form of the vessel, the speed of agitation, the supplementation with oxygen, production of foam, the influence of antifoaming agents and others. Many researchers also describe similar effects – although using similar medium composition and fermentation conditions the results

between flask and bioreactor fermentation differ from one another (BEG *et al.*, 2003). The lower cell density may correlate with the lower enzyme production. It is discovered that cell communication in the form of quorum sensing is regulating the gene expression in the bacterial community. In gram-positive bacteria, cell communication is using cytoplasmic sensors, regulated by secreted and reimported signal peptides. Quorum sensors in genus *Bacillus* include Rap, NprR, and PlcR, which are part of the RNPP protein family. Besides Rap these RNPP proteins are transcriptional factors, which directly regulate the gene expression. In this way, quorum-sensing regulates many important functions in the *Bacillus cereus* group. In this situation, quorum-sensing regulates the expression of phospholipase enzymes (which expression is regulated by PlcR) (SLAMTI *et al.*, 2014). If the cell density is not high enough, the cell to cell communication is hampered and the expression of some key enzymes is reduced.

Conclusion

It was determined that *B. thuringiensis №17* was the best producer of phospholipases between the studied species of genus *Bacillus*, with an activity of 19.61 U/ml. The strain showed consistent extracellular phospholipase C activity. The production of phospholipase C was detected at the second hour of cultivation and it reached its maximum at the 8-th hour of cultivation during the end of the exponential and the beginning of the stationary phase of growth. Such early production of extracellular lipolytic enzymes is a feature of economic value. The highest phospholipase C production was achieved when the medium was supplemented with 0.4% of glucose, yeast extract as a source of nitrogen and 1 mM $ZnCl_2$ and inoculated with 7.5% of inoculum. After the optimization of the medium, the duration of the cultivation, and the volume of used inoculum, the activity reached 31 U/ml. Scale-up study with a benchtop bioreactor with a volume of 2 liters achieved the highest exoenzyme production at the 8 hours of cultivation, which correlated with the flask experiments. The selected strain and studied phospholipase enzyme have a significant potential for application in the field of bioremediation – cleaning contaminated soils, digesting of spilled oils and fats in wastewaters and even reducing the energy consumption and the generated greenhouse gas emissions in some industries. Replacing the old chemical technology of refining oils with enzyme degumming using phospholipases results in reduced production of carbon emissions, reduced consumption of dangerous chemical compounds and product with higher quality. Phospholipases can be a sustainable and effective alternative to some old and inefficient technologies.

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Received: 02.12.2019
Accepted: 23.12.2019