

OPTIMIZATION OF ELASTOLYTIC PEPTIDASE BIOSYNTHESIS BY *Bacillus thuringiensis* IMV B-7324

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Received 28.04.2014

The cultivation conditions of *Bacillus thuringiensis* IMV B-7324 for synthesis of the elastolytic peptidase were studied. By mono- and two-factorial experiments it was optimized the nutrient medium and conditions of growth for the synthesis of *B. thuringiensis* IMV B-7324 elastolytic peptidase. It was established that maximal synthesis of the enzyme (5.15 U/mg of the protein) occurs at the exponential phase of growth on 18 h in submerged cultivation. As a result of screening experiments it was shown that all components of the basic medium except gelatin are significant for the enzyme biosynthesis. The elimination of gelatin leads in 9.8-fold increase of the elastolytic activity (50.55 U/mg of the protein). The influence of the nitrogen and carbon sources on the enzyme synthesis was studied. It was established that the optimal sources are the ammonium sulfate and arabinose. Their usage allows us to increase in 17.4 and 4.6 times the elastolytic activity (90 and 24 U/mg of the protein). The optimal concentrations of the ammonium sulfate and arabinose in the medium which allow to increase the elastolytic activity in 24.7 times (127.45 U/mg of the protein) was determined by the bifactorial experiment on three levels. The optimized nutrient medium contains (g/l): arabinose — 13.0; $(\text{NH}_4)_2\text{SO}_4$ — 14.0; KH_2PO_4 — 1.6; $(\text{CH}_3\text{COO})_2\text{Zn}$ — 0.25; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ — 0.75. The elastolytic activity of *B. thuringiensis* IMV B-7324 was 132.5 U/mg of protein during the growth by submerged cultivation for 18 h in 200 ml of the optimized nutrient medium at initial pH 7.0, on a shaker at 220 rpm at 37 °C.

Key words: *Bacillus thuringiensis*, elastolytic peptidase, bifactorial experiment.

Elastases (E.C. 3.4.21.36, E.C. 3.4.21.37) are enzymes which belong to peptidases that are able to split poorly soluble elastin, the protein which together with collagen provides mammalian connective tissue with mechanic properties. Elastin contains approximately 95% of non-polar amino acid residues and a small amount of polar amino acid residues. The following amino acids have the highest content: Gly (30.7%), Ala (20.4%), Pro (11.4%) and Val (10.5%). The pancreatic elastase splits elastin with the predominant formation of Ala and Val. This enzyme has applied aspects of industrial use for hydrolysis of raw materials with elastin fibers [1]. So, in food industry these peptidases are used for maturation of meat and herring and salting herring. Traditionally, the protein hydrolysates are obtained using peptidases. Then these hydrolysates can be used as food supplements [2] or in leather manufacturing for skin processing at skin dehydration and softening. It can help to keep the thickness of the final product. Elastases themselves

or in the complex with other peptidases can be used for wound treatment for burns, for decrease of inflammatory processes, bruising and swelling etc [3]. Enzymes possessing high thermostability have also a great practical importance and can be used in washing to remove protein contamination [4]. Taking into consideration that elastase purification from animal tissue is accompanied by difficulty the search of elastase producers among microorganisms is an actual problem. Most known producers belong to the genus *Pseudomonas*, *Bacillus*, *Vibrio*, *Aeromonas*, *Aspergillus*, *Streptomyces* and some others. The main drawback of these strains is their pathogenicity for human, so their usage is limited. The isolation of strain-producers which are safe for human health and the increase of enzyme production are the actual problems. As a result of induced mutagenesis by N-methyl-N-nitro-N-nitrosoguanidine the stable variant of the mutant with the increased elastase activity (4 U/mg) was obtained earlier from the strain *B. thuringiensis* 27 [5]. The

new strain of *B. thuringiensis* considered as a producer of elastolytic peptidase (number IMV B-7324) was registered in the Depository of Zablotny Institute of Microbiology and Virology NAS of Ukraine [6]. The aim of this work was to carry out the optimization of cultivating parameters of *B. thuringiensis* IMV B-7324 to increase elastase synthesis.

Materials and Methods

The object of investigation was the strain *B. thuringiensis* IMV B-7324. To study the dynamic of peptidase synthesis the cultivation of *B. thuringiensis* IMV B-7324 was carried out on the following nutrient medium (g/l): KH_2PO_4 — 1.6; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ — 0.75; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ — 0.25; $(\text{NH}_4)_2\text{SO}_4$ — 0.5; maltose — 1.0; gelatin — 10.0; yeast autolysate — 0.15; pH — 6.5–6.7 (the base medium) under rotating (220 rpm) at 42 °C for 72 h [7]. Erlenmeyer flasks (750 ml) containing 150 ml of nutrient medium were seeded by the inoculum, which was obtained on the same medium in the middle of exponential phase of growth. Concentration of seeded material was 10^4 – 10^5 CFU/ml. The culture growth was determined by measurement of absorbance of bacterial suspension on spectrophotometer SF-26, $\lambda = 590$ nm. Influence of different carbon sources on synthesis intensity was studied by removing from the base medium maltose, gelatin and yeast autolysate and replacing them by carbohydrate compounds in the quantity equivalent to carbon content in the base medium. As the sources of carbon we used monosaccharides (arabinose, galactose, glucose, xylose, mannose, rhamnose and sorbose); disaccharides (maltose, lactose and sucrose); trisaccharides (raffinose), polyhydric alcohols (mannitol, sorbitol and dulcitol) and soybean and corn flour. Influence of nitrogen sources on synthesis intensity was studied by removing from the base cultivating medium all nitrogen-containing compounds [i.e. gelatin, yeast autolysate and $(\text{NH}_4)_2\text{SO}_4$] replacing them by the test substance in the quantity equivalent to nitrogen content in the base medium. The sources of nitrogen were the following: Met, Val, Ser, Thr, Ala, Leu, Asn, Ile, Gly, Asp, Glu, Arg, His, NaNO_3 , $(\text{NH}_4)_2\text{SO}_4$, gelatin and yeast autolysate.

Significant elements of mineral nutrition of the base medium were determined using screening, which revealed significant factors (or components) making essential influence on the analysis accuracy [8]. The lower level of the studied concentrations was taken as

zero and the value of the upper level complied with the concentration of each component in the base cultivation medium. To carry out the medium optimization for elastolytic peptidase biosynthesis we used the following reagents: KH_2PO_4 , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, ZnCl_2 , $(\text{CH}_3\text{COO})_2\text{Zn}$, MgCO_3 , NaH_2PO_4 , CaH_2PO_4 and KCl. To determine the optimal concentration of nitrogen and carbon we carried out a full two-factor investigation (FTI). Factors of nutrient medium were designated as X_n . The influence of each factor was studied at three levels. To increase the elastolytic activity an arabinose and $(\text{NH}_4)_2\text{SO}_4$ taken in correspondent concentrations were used as a source of carbon and nitrogen respectively. To investigate the influence of cultivation parameters the strain was grown on the optimized media for 18 and 48 h under the following changing conditions: pH from 5.0 to 8.0, temperature from 28 °C to 42 °C and volume from 50 to 250 ml. The initial pH value in the medium was established with 1M solutions of NaOH and HCl. The protein content was determined by the method of Lowry et al. [9]. The staining intensity was determined using the spectrophotometer SF-26, $\lambda = 750$ nm. Bovine serum albumin was used as the standard. Elastolytic activity was determined by the colorimetric method measuring the intensity of solution staining by Congo red [10]. The incubation mixture had the following content: 2.5 ml of 0.01 M Tris-HCl buffer (pH 7.5), 5 mg of elastin stained by 0.002 % solution of Congo red and 1 ml of enzyme solution. The mixture was incubated for 5 h at 37 °C. The reaction was stopped by the incubation of the mixture on the ice bath for 30 min. Unhydrolyzed elastin was separated by centrifugation for 5 min at 10 000 g. The staining intensity was determined using the spectrophotometer SF-26, $\lambda = 515$ nm. The unit of enzyme activity was determined as the amount of enzyme which converted 1 mg of elastin per min. The data were statistically tested by Student's test [11]. All experiments were performed in 5–8 replicates. $P < 0.05$ were considered to be statistically significant. We calculated the mean values and standard errors ($M \pm m$). The results which are presented graphically were obtained using Microsoft Excel 2010.

Calculation and graphical presentation of the results of full two-factor experiment were carried out according to Box-Wilson response surface methodology using STATISTICA 8.0.

Results and Discussion

According to [12, 13] the role of peptidases from *B. thuringiensis* has not clarified yet, however, it is known that they take part in sporulation and entomotoxicity. Previously, we showed that strain *B. thuringiensis* IMV B-7324 which was isolated as the result of chemical mutagenesis possessed elastolytic activity [5, 6]. This strain is avirulent and belongs to third class according to classification of microorganism severity: “microorganisms which are moderately safely and have weak general toxic or allergenic effects”. So, the selected strain could be placed among other commercially promising producers which possess the unique property to hydrolyze elastin. So, we had to determine the optimal conditions for biosynthesis of elastolytic peptidase from *B. thuringiensis* IMV B-7324 basing on the dynamics of its accumulation. The study of dynamics of enzyme accumulation from *B. thuringiensis* var. *kurstaki* showed that this strain was able to synthesize extracellular metallopeptidase during the early phase of sporulation [14]. The maximal activity of elastolytic peptidase from *B. cereus* and *B. polymyxa* was observed at the stages of withering away and sporulation correspondingly [15]. Studying the dynamics of growth of *B. thuringiensis* IMV B-7324 and its ability to accumulate the peptidase which has specificity towards elastin we noted that maximal synthesis of the enzyme (5.15 U/mg) was observed during the phase of exponential growth at 18 h of cultivation (Fig. 1). Taking into consideration the individual needs of strain-producer in sources of carbon, nitrogen and microelements we carried out the optimization of liquid nutrient medium for biosynthesis of *B. thuringiensis* IMV B-7324 elastolytic peptidase using the methods of mathematical planning. Our first screening test showed that all components of nutrient medium apart from gelatin are essential for the enzyme biosynthesis (Table 1). When the strain was grown on the medium which does not contain gelatin the level of elastolytic activity was 50.55 U/mg of the protein. That was in 9.8 times higher than in the presence of gelatin.

According to published data concerning the influence of different nutrient media on peptidase synthesis, the certain organic compounds are needed to accumulate these enzymes. Thus, glucose and starch stimulate the growth of alkaline proteinases from *B. licheniformis* CUMC 305 and *B. coagulans* CUMC 512 [16]. At the same time, glucose,

Table 1. Determination of significant factors of the cultivation medium for biosynthesis of elastolytic peptidase from *B. thuringiensis* IMV B-7324

№ experiment	ZnSO ₄ ·7H ₂ O	MgSO ₄ ·7H ₂ O	KH ₂ PO ₄	(NH ₄) ₂ SO ₄	Gelatin	Yeast autolysate	Maltose	Elastolytic activity U/mg of the protein
1	-	+	+	+	+	+	+	5.16±0.26
2	+	-	+	+	+	+	+	6.80±0.34
3	+	+	-	+	+	+	+	0
4	+	+	+	-	+	+	+	9.22±0.46
5	+	+	+	+	-	+	+	50.55±2.53
6	+	+	+	+	+	-	+	10.98±0.55
7	+	+	+	+	+	+	-	7.75±0.39
8	-	-	+	+	+	+	+	9.38±0.47
9	+	-	-	+	+	+	+	0.2±0.01
10	+	+	-	-	+	+	+	0.5±0.03
11	-	-	-	+	+	+	+	3.8±0.19
12	+	-	-	-	+	+	+	0.7±0.04
13	-	-	-	-	+	+	+	6.23±0.31
14	-	+	-	+	+	+	+	1.89±0.09
15	-	+	+	-	+	+	+	2.85±0.14
16	+	-	+	-	+	+	+	9.34±0.47
Regression coefficient, b	-1.1	-2.46	5.25	-0.97	-45.7	-6.12	-2.89	58.85

Note. Here and after: «+» — the presence of the component in the medium; «-» — the absence of the component in the medium.

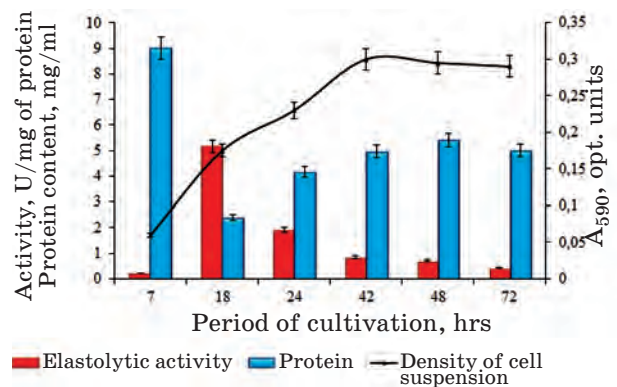


Fig. 1. Dynamics of accumulation of peptidases with elastolytic and fibrinolytic activity from *B. thuringiensis* IMV B-7324

starch and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ are optimal sources of nutrition for intensification of biosynthesis of elastolytic peptidase from *Bacillus* sp. EL31410 [17]. Maximal activity of alkaline proteinase from *B. licheniformis* NCIM-2042 was observed in case of the introduction to the culture medium the starch as a source of carbon, soy flour as a source of nitrogen and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ as a source of sulphate [18]. Investigation of the influence of different sources of carbon and nitrogen on synthesis of peptidases from *B. licheniformis* (TD4) showed that xylose and urea are able to increase the enzyme activity [19]. Under these conditions the optimal source of mineral nutrition was 1 M NaCl. That gave us possibility to increase peptidase activity almost in 2 times.

Comparative study of the influence of some compounds on the specific elastolytic activity of the investigated enzyme from *B. thuringiensis* IMVB-7324 showed that amino acids Met, Val, Thr, Ala and Arg promoted the increased enzyme synthesis as compared with the control (base medium, Fig. 2). Besides, it was revealed that simple sources of nitrogen such as NaNO_3 and $(\text{NH}_4)_2\text{SO}_4$ also increased the specific elastolytic activity in 2.4 and 4.6 times correspondingly. The usage amino acids is not economically profit, so we used $(\text{NH}_4)_2\text{SO}_4$ for further optimization of the nutrient medium.

Investigation of the influence of different carbon sources (arabinose, galactose, glucose, xylose, mannose, rhamnose, sorbose, maltose, lactose, sucrose, raffinose, mannitol, sorbitol, dulcitol, soybean and corn flour) was carried out using one-factor experiment. In these experiments $(\text{NH}_4)_2\text{SO}_4$ was used as a source

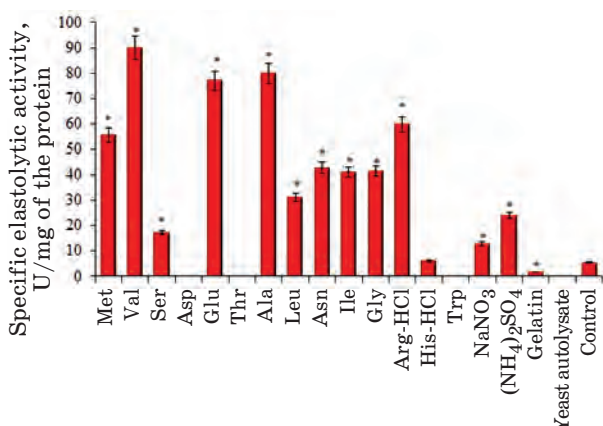


Fig. 2. Specific elastolytic activity from *B. thuringiensis* IMV B-7324 at cultivation on the media with different nitrogen sources: Here and after: * — $P < 0.05$ as compared with the control (base medium)

of nitrogen. The maximal specific elastolytic activity was observed in case of growing of *B. thuringiensis* IMV B-7324 on the medium containing rhamnose and arabinose: 72 and 90 U/mg of the protein correspondingly (Fig. 3).

It is known that divalent metal ions are necessary for formation of active conformation and for stabilization of many peptidase molecules. These ions can promote enzyme activity [20]. So, we have found that not only carbon and nitrogen sources are necessary but also mineral elements. As it was not established what was the influencing element (metal cation or anion) we carried out a series of experiments where the strain *B. thuringiensis* IMV B-7324 was cultivated on the media containing the sources of carbon and nitrogen which had previously been selected and $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ was changed for $(\text{CH}_3\text{COO})_2\text{Zn}$ and ZnCl_2 ; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ — for MgCO_3 ; KH_2PO_4 — for KCl , NaH_2PO_4 and CaHPO_4 (experiments 1–7, Table. 2). It was established that for maximal synthesis of elastolytic peptidase (104 U/mg of the protein) $(\text{CH}_3\text{COO})_2\text{Zn}$ had to be added into the medium (experiment 5).

The optimal ratio of the selected sources of carbon and nitrogen (at the stable level of other medium factors) was found with the use of two-factor experiment on three levels. Arabinose and $(\text{NH}_4)_2\text{SO}_4$ were the sources of carbon and nitrogen respectively. Taking into consideration the previous results $(\text{CH}_3\text{COO})_2\text{Zn}$ at the constant concentration was used in our experiments instead of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$. The factors of nutrient medium were designated as following: X1 — $(\text{NH}_4)_2\text{SO}_4$ and X2 — arabinose (Table 3).

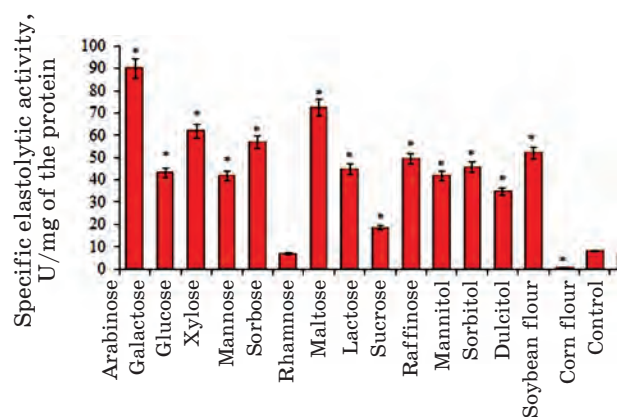


Fig. 3. Specific elastolytic activity from *B. thuringiensis* IMV B-7324 at cultivation on the media with different carbon sources

Table 2. The selection of the mineral nutrition elements in the cultivation medium of *B. thuringiensis* IMV B-7324 for biosynthesis of elastolytic peptidase

№ experiments	Arabinose+ (NH ₄) ₂ SO ₄	KH ₂ PO ₄	MgSO ₄ ·7H ₂ O	ZnSO ₄ ·7H ₂ O	KCl	(CH ₃ COO) ₂ Zn	ZnCl ₂	NaH ₂ PO ₄	CaHPO ₄	MgCO ₃	Specific activity, U/mg of the protein
1 (control)	+	+	+	+	-	-	-	-	-	-	52.2±2.6
2	+	-	+	+	-	-	-	+	-	-	29±1.4*
3	+	-	+	+	-	-	-	-	+	-	52±4.0
4	+	-	+	+	+	-	-	-	-	-	28.7±1.4*
5	+	+	+	-	-	+	-	-	-	-	104±6.7*
6	+	+	+	-	-	-	+	-	-	-	32±1.6*
7	+	+	-	+	-	-	-	-	-	+	24±1.2*

Screening experiments for optimal ratio arabinose and (NH₄)₂SO₄ showed that to obtain maximal accumulation of elastolytic peptidase from *B. thuringiensis* IMV B-7324 we had to increase the concentration of the carbon and nitrogen sources (see Table 4). The supernatant of culture fluid had maximal elastolytic activity when concentration of (NH₄)₂SO₄, and arabinose was 12 g/l.

To determine the optimal concentration of arabinose and (NH₄)₂SO₄ when we observed the maximal specific activity of the supernatant of culture fluid from *B. thuringiensis* IMV B-7324 we made three-dimensional image of the surface response (Fig. 4, a) on the base of two-factor experiment data. It was shown that the change of concentrations of nitrogen and carbon sources made influence on the curvature of the 3D surface, the highest level of which was observed at arabinose concentration 13 g/l and (NH₄)₂SO₄ concentration 14 g/l. That was corresponded the maximal value of elastolytic activity (Fig. 4, b).

These concentrations of arabinose and (NH₄)₂SO₄ in the cultivation medium reach the level of specific elastolytic activity in the cultural fluid (127.45 U/mg of the protein) on the first day of producer cultivation. Under these conditions the total amount of carbon was 0.53% and nitrogen — 0.3%. So, during the experiment we could increase the elastolytic activity of the investigated peptidase almost in 24.7 times as compared with the activity which was observed during

Table 3. The values of factors in the natural variables and concentrations the main components of the nutrient medium

Investigated factors	Levels of the investigated factors*		
	1	2	3
(NH ₄) ₂ SO ₄ (g/l), X1	6	12	24
Arabinose (g/l), X2	6	12	24

Note: * — the concentrations of KH₂PO₄, MgSO₄·7H₂O and (CH₃COO)₂Zn in the medium were kept at the constant level (1.6, 0.75 and 0.25 g/l respectively).

Table 4. Results of full two-factor experiments to find optimal ratio of both: arabinose (X1) and (NH₄)₂SO₄ (X2)

№ variant	X1	X2	Elastolytic activity, U/mg of the protein
1	1	1	60.71±3.0
2	1	2	69.18±3.5
3	1	3	56.24±2.8
4	2	1	89.09±4.5
5	2	2	127.45±6.4
6	2	3	55.38±2.8
7	3	1	47.61±2.4
8	3	2	56.53±2.8
9	3	3	42.78±2.1

the growing of *B. thuringiensis* IMV B-7324 on the base medium. These results showed that for each producer the individual selection of the nutrient source was needed.

According to our results of nutrient medium optimization to increase *B. thuringiensis* IMV B-7324 elastase synthesis the optimal sources of carbon and nitrogen were arabinose (1.3%) and (NH₄)₂SO₄ (1,4%) correspondingly, while for *Bacillus* sp. EL31410 the main nutrient sources were casein (1.15%) and corn flour (0.61%) [17]. The level of biosynthesis of elastolytic peptidase depends on the following parameters of producer cultivation: pH, temperature and a volume of the nutrient medium. Maximal synthesis of *B. licheniformis* ZJU31410 elastase was observed when cultivation was carried out for 25 h under rotating (220 rpm) in Erlenmeyer flasks (250 ml) containing 25 ml of nutrient medium and 5 % inoculum was contributed [21]. Synthesis of elastase

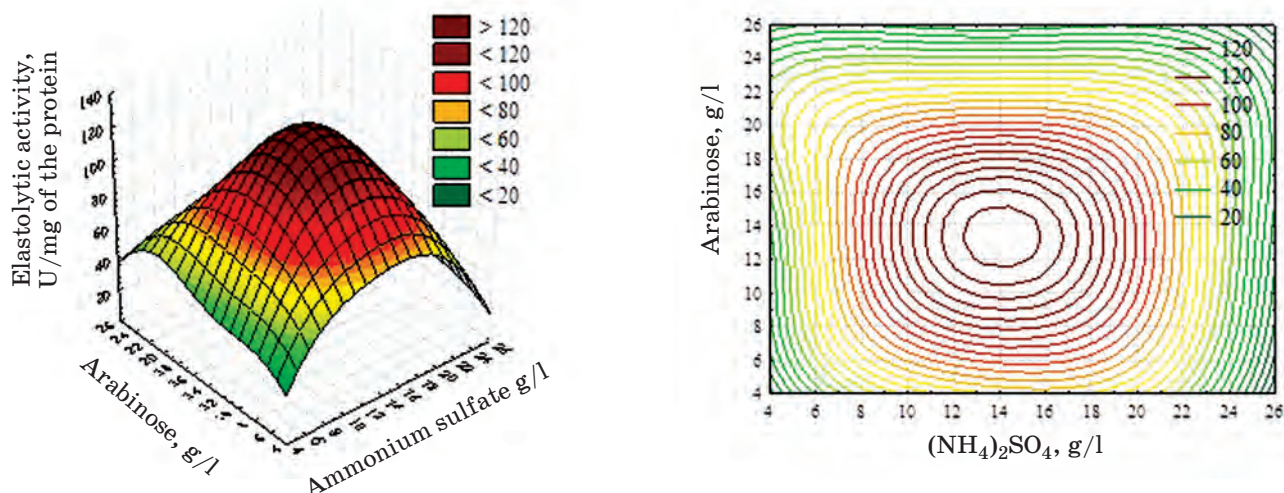


Fig. 4. Three-dimensional image of the surface response (a) and its projection on the plane (b), that shows the influence effect of different concentrations of arabinose and $(\text{NH}_4)_2\text{SO}_4$ on the specific elastolytic activity of *B. thuringiensis* IMV B-7324

Table 5. Influence of cultivation conditions on the elastolytic activity of the supernatant cultural liquid from *B. thuringiensis* IMV B-7324

Cultivation conditions		Elastolytic activity, U/mg of the protein
Initial pH of the medium	5.0	57.1±2.86
	6.0	98.7±4.94*
	7.0	131.4±6.57*
	8.0	119.8±6.00*
Temperature, °C	28	93.8±4.69
	32	114.5±5.73*
	37	129.5±6.48*
Medium volume, ml	50	63.8±3.19
	100	100.4±5.02*
	150	122.7±6.14*
	200	132.5±6.63*
	250	97.9±4.9*

from *B. thuringiensis* IMV B-7324 depended also on the cultivation conditions: the growing at the initial pH value 7.0 in optimized medium led to the increase of enzyme activity 131.14 U/mg of the protein (Table 5). The study of the influence of temperature of producer cultivation showed that the highest value of elastolytic activity (129.5 U/mg of the protein) in the supernatant of producer cultural liquid was at 37 °C. The level of aeration of the nutrient medium made also the

essential influence on the biosynthesis of the investigated peptidase. When *B. thuringiensis* IMV B-7324 was grown in flasks with 200 ml of the medium the elastolytic activity was increased till 132.5 U/mg of the protein.

So, for accumulation of elastolytic peptidase in the supernatant cultural liquid from *B. thuringiensis* IMV B-7324 all components of basic nutrient medium were significant except gelatin. Removing this component from the

cultivation medium promoted the increase of elastolytic activity of the supernatant cultural liquid till 50.55 U/mg of the protein. The optimal carbon and nitrogen sources were arabinose and $(\text{NH}_4)_2\text{SO}_4$, correspondingly. The maximal specific elastolytic activity was noted with

the following content of the nutrient medium (200 ml), g/l: arabinose — 13.0; $(\text{NH}_4)_2\text{SO}_4$ — 14.0; KH_2PO_4 — 1.6; $(\text{CH}_3\text{COO})_2\text{Zn}$ — 0.25; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ — 0.75; pH 7.0, 37 °C.

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**ОПТИМІЗАЦІЯ БІОСИНТЕЗУ
ЕЛАСТОЛІТИЧНОЇ ПЕПТИДАЗИ
Bacillus thuringiensis ІМВ В-7324**

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Метою роботи було визначення умов культивування *Bacillus thuringiensis* ІМВ В-7324 для біосинтезу еластолітичної пептидази. Оптимізацію живильного середовища та умов культивування для синтезу еластолітичної пептидази *B. thuringiensis* ІМВ В-7324 здійснювали за допомогою одно- та двофакторних експериментів. Встановлено, що максимальний синтез ензиму (5,15 од/мг протеїну) відбувається в експоненційній фазі росту на 18-у год за умов глибинного культивування. З використанням відсіювального експерименту показано, що для біосинтезу ензиму значущими є всі компоненти базового середовища, окрім желатину, вилучення якого сприяє підвищенню еластолітичної активності в 9,8 раза (50,55 од/мг протеїну). Вивчено вплив різних джерел азоту та вуглецю на синтез ензиму. Виявлено, що оптимальними джерелами є сульфат амонію та арабіноза, використання яких дає змогу збільшити еластолітичну активність в 17,4 і 4,6 рази (90 і 24 од/мг протеїну) відповідно. За допомогою двофакторного експерименту на трьох рівнях було визначено оптимальні концентрації сульфату амонію та арабінози в середовищі, за яких можливе підвищення еластолітичної активності в 24,7 рази (127,45 од/мг протеїну). Оптимізоване середовище містило (г/л): арабінозу — 13,0; $(\text{NH}_4)_2\text{SO}_4$ — 14,0; KH_2PO_4 — 1,6; $(\text{CH}_3\text{COO})_2\text{Zn}$ — 0,25; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ — 0,75. Під час вирощування *B. thuringiensis* ІМВ В-7324 у глибинних умовах культивування протягом 18 год у 200 мл оптимізованого живильного середовища за початкового значення рН 7,0, температури 37 °С, швидкості обертання качалки 220 об/хв еластолітична активність становила 132,5 од/мг протеїну.

Ключові слова: *Bacillus thuringiensis*, еластолітична пептидаза, двофакторний експеримент.

**ОПТИМИЗАЦИЯ БИОСИНТЕЗА
ЕЛАСТОЛИТИЧЕСКОЙ ПЕПТИДАЗЫ
Bacillus thuringiensis ИМВ В-7324**

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Целью работы было определение условий культивирования *Bacillus thuringiensis* ИМВ В-7324 для биосинтеза эластолитической пептидазы. Оптимизацию питательной среды и условий культивирования для синтеза эластолитической пептидазы *B. thuringiensis* ИМВ В-7324 проводили с помощью одно- и двухфакторных экспериментов. Установлено, что максимальный синтез энзима (5,15 ед/мг протеина) происходит в экспоненциальной фазе роста на 18-м ч в условиях глубинного культивирования. С использованием отсеивающего эксперимента было показано, что для биосинтеза энзима значимыми являются все компоненты базовой среды, кроме желатина, удаление которого приводит к повышению эластолитической активности в 9,8 раза (50,55 ед/мг протеина). Изучено влияние разных источников азота и углевода на синтез энзима. Выведено, что оптимальными источниками являются арабиноза и сульфат аммония, использование которых позволяет увеличить эластолитическую активность в 17,4 и 4,6 раза (90 и 24 ед/мг протеина), соответственно. С помощью двухфакторного эксперимента на трех уровнях были определены оптимальные концентрации сульфата аммония и арабинозы в среде, при которых возможно повышение эластолитической активности в 24,7 раза (127,45 ед/мг протеина). Оптимизированная среда содержала (г/л): арабинозу — 13,0; $(\text{NH}_4)_2\text{SO}_4$ — 14,0; KH_2PO_4 — 1,6; $(\text{CH}_3\text{COO})_2\text{Zn}$ — 0,25; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ — 0,75. При выращивании *B. thuringiensis* ИМВ В-7324 в условиях глубинного культивирования в течение 18 ч в 200 мл оптимизированной питательной среды при исходном значении рН 7,0, температуре 37 °С, скорости оборотов качалки 220 об/мин эластолитическая активность составила 132,5 ед/мг протеина.

Ключевые слова: *Bacillus thuringiensis*, эластолитическая пептидаза, двухфакторный эксперимент.