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Bacillus thuringiensis ELASTASES WITH INSECTICIDE ACTIVITY

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The purpose of research was a screening of proteases with elastase activity among *Bacillus thuringiensis* strains, their isolation, partially purification, study of physicochemical properties and insecticide activity in relation to the larvae of the Colorado beetle. The objects of the investigation were 18 strains of *B. thuringiensis*, isolated from different sources: sea water, dry biological product "Bitoksibatsillin" and also from natural populations of Colorado beetles of the Crimea, Kherson, Odesa, Mykolaiv and Zaporizhiia regions of Ukraine. Purification of enzymes with elastase activity isolated from above mentioned strains was performed by gel-chromatography and insecticide activity was studied on the 3–4 larvae instar of Colorado beetle. The ability of a number of *B. thuringiensis* strains to synthesize the proteases with elastase activity has been established. The most active enzymes were obtained from strains IMV B-7465, IMV B-7324 isolated from sea water, and strains 9, 902, Bt-H and 0-239 isolated from Colorado beetles. The study of the physicochemical properties of the partially purified proteases of these strains showed that they belong to enzymes of the serine type. Peptidases of a number of *B. thuringiensis* strains (IMV B-7324, IMV B-7465, 902, 0-239, 9) are metal-dependent enzymes. Optimal conditions of action of all tested enzymes are the neutral and alkaline pH values and the temperatures of 30–40 °C. The studies of influence of the complex enzyme preparations and partially purified ones of *B. thuringiensis* strains on the larvae instar of Colorado beetles indicated that enzymes with elastase activity may be responsible for insecticide action of the tested strains.

Key words: *Bacillus thuringiensis*, elastase activity, insecticide activity.

Bacillus thuringiensis is a gram-positive spore-forming bacterium, which synthesizes insecticide toxin and a number of virulent factors. At the beginning of sporulation and during the stationary phase of growth bacterium produces parasporal crystalline inclusions, consisting of wide spectrum of insecticide crystalline (*Cry*) and cytolytic (*Cyt*) proteins, also known as δ-endotoxins. After a penetration in the organism of insects, these crystals are dissolved in a middle intestine, whereupon it is activated by proteases of insect and contact with specific receptors, located on a cellular membrane, that results in death of insect [1, 2]. These proteins are toxic to the insects of orders of *Lepidoptera, Dipteran*, *Coleoptera*, *Hymenoptera*, *Homoptera*, *Orthoptera*, *Mallophage*, and also to nematodes [3]. In addition, *B. thuringiensis* secretes thermo stable, water soluble low-molecular (700 Da) insecticide exotoxins [4], and also it is reported [5–7] that some extracellular chitinases and proteases of these bacteria also can be one of virulent factors of their multicomponent systems [8]. It is possible to assume that proteases possess hydrolyzing activity in relation both to proteins of animal tissues and insects and as a result is death of insect. The targets of action of these enzymes can be proteins both of membranes, cuticle and enzymes of insect hemocoel. Therefore, the research of *Bacillus* enzymes with insecticide activity, in particular *B. thuringiensis*, can be actually.

We have previously demonstrated [8–10] that *B. thuringiensis* IMV B-7324 synthesizes a serine peptidase which has high affinity to number of native proteins of animal origin (elastin, fibrin, fibrinogen, collagen, casein). The purpose of this work was screening of proteases with elastase activity among *B. thuringiensis* strains, their isolation,

partially purification, study of their physicochemical properties and insecticide activity in relation to the larvae of the Colorado beetle.

Materials and Methods

The objects of the investigation were 18 strains of *Bacillus thuringiensis*: IMV B-7324, IMV B-7465, 5, 8, 9, 0-239, O-293, 187, 98, 218, 240, 297, 304, 902, 275, 315, LH-4 and Bt-H. *B. thuringiensis* IMB B-7324 was obtained by chemical mutagenesis from *B. thuringiensis* 27 which was isolated from the Black Sea water [11]. *B. thuringiensis* var. *israelensis* IMV B-7465 was isolated from the Black Sea water near Snake Island. *B. thuringiensis* 98 is the component of the dry biological product "Bitoksibatsillin". Other strains tested were isolated from natural populations of Colorado beetles of the Autonomous Republic of Crimea, Kherson, Odesa, Mykolaiv and Zaporizhiia regions of Ukraine.

For bacteria cultivation it was used a liquid nutrient medium [12] of the following composition (g/l): maltose — 1.0; gelatin — 10.0; yeast autolysate — 0.15; KH_2PO_4 — 1.6; $MgSO_4 \cdot 7H_2O$ — 0.75; $ZnSO_4 \cdot 7H_2O$ — 0.25; $(NH_4)_2SO_4$ — 0.5 (pH 6.8–7.2) [12]. Cultivation was performed by submerged method in the flasks (750 ml) under rotation (220 rpm) at 36 °C for 24 h.

For obtaining complex protein preparations the cells were separated by centrifugation at 5 000 g, 30 min and to the supernatant of culture liquid dry ammonium sulfate with final concentration of 90% was added . The mixture was incubated at 4 °C for 24 h, centrifuged at 5 000 g, 30 min, the formed precipitate was collected and dissolved in 0.01 M of Tris-HCl buffer, pH 7.5, and loaded on the column (1.8×40 cm) with a neutral TSK-gel — Toyopearl HW-55 (Toyosoda, Japan). The elution was carried out with the same buffer and fractions with elastase activity were collected.

Elastase activity was estimated colorimetrically by the color intensity of the solution after enzymatic hydrolysis of elastin stained with Congo red [13]. Reacting mixture consisted of 2.5 ml of 0.01 M Tris-HCl buffer (pH 7.5), 5 mg of elastin stained by 0.002% of Congo red solution and 1 ml of the enzyme solution. The mixture was incubated during 5 h at the temperature of 37 °C. The reaction was stopped by standing the tubes with reacting mixture in an ice bath during 30 min. Unhydrolyzed elastin was separated by centrifugation at 10 000 g, 5 min. The color intensity was measured on a spectrophotometer SF-26 at the wave length 515 nm. The unit of activity was such amount of enzyme that catalyzes the hydrolysis of 1 mg elastin for 1 min.

The protein was measured by the method [14]. The protein content of the fractions obtained from gel-filtration was determined spectrophotometrically at 280 nm.

The study of action of inhibitors, pH and temperature on the activity of the partially purified enzymes was performed on peptidases preparations with protein concentration 1 mg/ml. For inhibitory analysis it was applied the following specific chemical reagents: phenylmethylsulfonyl fluoride (PMSF), 1-ethyl-3-[3-dimethyl-aminopropyl]carbodiimide (EDC), sodium ethylenediaminetetraacetate (EDTA), ethylene glycol tetraacetic acid (EGTA), DL-dithiothreitol (DTT), *p*-chloromercuribenzoic acid (PCMB), N-ethylmaleimide (NEM). Enzyme with reagent (final concentration 10^{-3} M) was incubated at a temperature of 15–20 °C for 60 min. After this the aliquotes were picked out and activity measured as described above. The studies of the effect of pH and temperature of the reaction medium on the peptidase activity were carried out in the range of temperatures from 4 to 90 °C and pH from 3.0 to 11.0 using universal 0.05 M solution of phosphate buffer.

The study of the insecticide activity was carried out on both complex protein preparations and partially purified ones as a result of gel-filtration. The protein content in tested preparations was 0.1 mg/ml. Experiments were performed in triplicate on the 3–4 larvae instar of Colorado beetle. The leaves of potato were placed into a Petri dish and treated by 5 ml enzyme preparations (pH after dialysis against the distilled water was near 6.0). The excess of preparation was not observed. Then 40 larvae were put there and incubated in a dark cool place for 5 days. Distilled water was used as control preparation. Interpretation of results was conducted after 3, 4 and 5 days.

All of experiments conducted no less than in 3–5 replicates. Data are presented as average value ± standard error. Statistical analysis was performed using the Student's *t*-test, estimating authenticity of the results on the the level of significance no less than 95% ($P \leq 0.05$).

Results and Discussion

Today there is enough information about participation of *B. thuringiensis* proteases in forming of insecticide endotoxins [15]. Moreover, it is shown correlation between the synthesis of protease and endotoxin levels as well as it is established that proteases are one of many factors which are responsible for insecticide activity of the strains [6, 7]. Since there is extremely little information about the possible role of individual proteases as insecticide agents, we have been stated a task to isolate *B. thuringiensis* proteases, investigate their properties and biological activity. We have previously shown [8, 9] that two strains of *B. thuringiensis* IMV B-7324 and *B. thuringiensis* IMV B-7465 [16] synthesize unique proteases hydrolyzing native animal proteins (elastin, collagen, fibrin). This is due to their primary substrate specificity to the hydrophobic amino acid residues, in particular to alanine. It is known also, that the bacillary proteases possessing similar specificity are able to lyze the cells of some microorganisms by cleavage of glycine-alanine bond of peptidoglycan [17]. It is possible that such proteases displaying affinity to the insoluble protein structures also would exert influence on the structural proteins of cuticle and membranes of insects. Thus, 18 strains of *B. thuringiensis* were investigated. It was shown that *B. thuringiensis* IMV B-7324, IMV B-7465, Bt-H, 0-239, 902 and 9 hydrolyzed elastin (Table 1). Therefore these strains were selected for further investigations.

The complex enzyme preparations from the supernatants of culture liquids of IMV B-7324, IMV B-7465, Bt-H, 0-239, 902 and 9 were obtained by ammonium sulfate precipitation (90% of saturation). The further gel-filtration (Fig. 1) permitted to purify enzymes of all *B. thuringiensis* tested strains from significant amounts of undesirable proteins, carbohydrates and nucleic acids. It was achieved the degree of purification up to 10 times and decrease in the carbohydrates and nucleic acids content up to 75–99%.

The studies of dependence of partially purified proteases activity from pH have shown that they have an optimum at the neutral and weakly alkaline values 6.5–8.5 (Fig. 2). Thermo-optimums are at the temperature of 35–45 °C (Fig. 3).

Influence of chemical reagents on elastase activity of partially purified enzymes was investigated. It was demonstrated (Table 2) that all tested elastases are serine peptidases due to reduction of the activity on 44–98% under the action of PMSF. Peptidases

Table 1. **Elastase activity of *B. thuringiensis* strains**

***B. thuringiensis* strain**	**Protein, mg/ml**	**Elastase activity, U/mg of protein**
0-239	2.2 ± 0.11	3.95 ± 0.2
O-293	2.26 ± 0.11	–
Bt-H	2.58 ± 0.13	6.7 ± 0.34
187	2.0 ± 0.10	–
LH-4	2.2 ± 0.11	–
98	2.58 ± 0.13	–
218	1.54 ± 0.08	–
240	1.48 ± 0.07	–
297	2.26 ± 0.11	–
304	1.72 ± 0.08	–
902	2.12 ± 0.11	4.1 ± 0.2
275	2.7 ± 0.13	–
315	1.94 ± 0.09	–
5	2.1 ± 0.11	–
8	1.7 ± 0.08	–
9	2.5 ± 0.12	5.2 ± 0.26
IMV B-7465	2.9 ± 0.14	10.8 ± 0.54
IMV B-7324	2.8 ± 0.14	12.1 ± 0.61

Note: «–» — activity is absence; here and later control — reaction mixture without enzyme.

of *B. thuringiensis* IMV B-7324, IMV B-7465, 902, 0-239 and 9 are metal-dependent enzymes because chelating agent EDTA inhibits their activity on 50–72%. Another chelating agent EGTA inhibits only two enzymes — peptidases of *B. thuringiensis* IMV B-7465 and 902. At the same time EGTA cause an increase in activity on 16–66% for *B. thuringiensis* IMV B-7324, Bt-H and 0-239 peptidases. Thus, it is possible to conclude that the metal ions (probably calcium due to influence of EGTA) play a substantial role in maintenance of molecular active conformation of enzymes and in hydrolysis of the high molecular insoluble protein substrates. However, this assumption requires further investigation.

Investigation of the insecticide activity was made both on the complex protein preparations, obtained by ammonium sulfate precipitation, and on the partially purified enzymes. The larvae of the Colorado beetle (*Leptinotarsa decemlineata*) — insect of family of Leaf beetles were chosen as a test-organism. Beetles and larvae of the Colorado beetle feed on the leaves of plants which are representatives of *Solanaceae* family: potato, tomato, physalis, egg-plant, etc. In favorable years the Colorado beetle is able to destroy up to 40% crop yield and in spite of a progressive development of science it remains dangerous pest of potato.

It was established (Fig. 4) that after 3 days of action of complex enzyme preparations of the strains Bt-H, 902 and IMV B-7324 the amount of the dead larvae is 50–60% (for comparison in control only 2.5%). This index for the purified enzymes is from 7.5 to 35%. After 4 days of treatment (Fig. 5) the effect of purified preparations was more significant than for unpurified. So, for partially purified protease of *B. thuringiensis* IMV B-7324 this index is 85% of dead larvae and for complex enzyme preparation — 65%. It was shown (Fig. 6) that after 5 days almost all of the

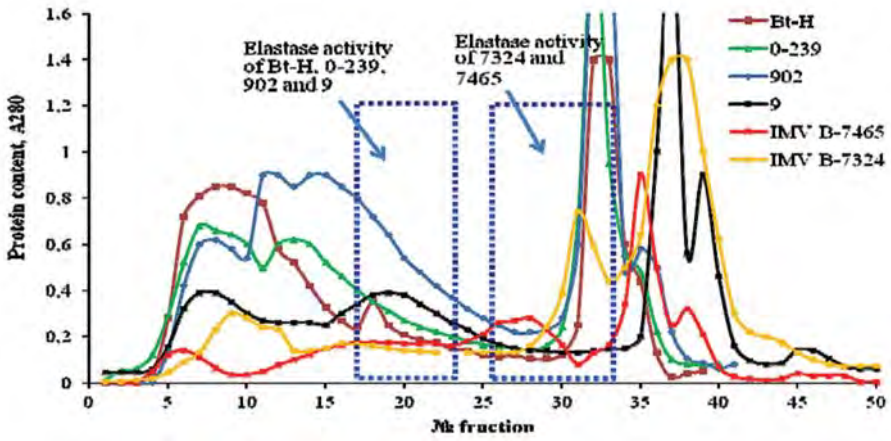

Fig. 1. **Elution profiles of the complex preparations which were obtained by ammonium sulfate precipitation (90% of saturation) from the culture supernatants of different *B. thuringiensis* strains**

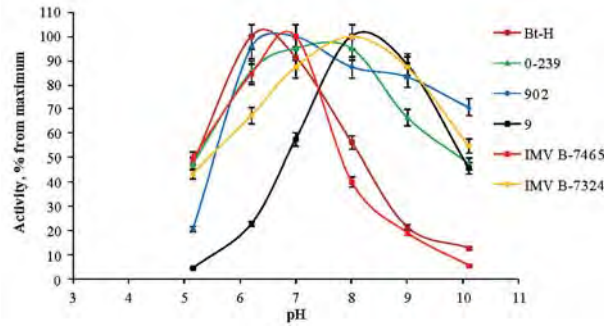

Fig. 2. **Influence of pH on elastase activity of partially purified preparations of *B. thuringiensis* strains**

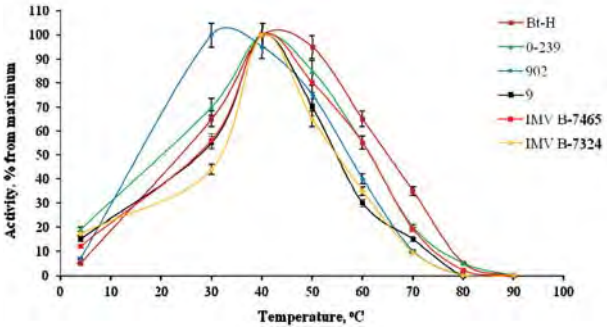

Fig. 3. **Influence of temperature on elastase activity of partially purified preparations of *B. thuringiensis* strains**

Table 2. **Influence of the chemical reagents on elastase activity of the partially purified enzymes of *B. thuringiensis* strains**

Chemical reagent	Elastase activity (%) of *B. thuringiensis* strains:					
	Bt-H	9	902	0-239	IMV B-7465	IMV B-7324
PMSF	66 ± 3.3*	2 ± 0.1*	50 ± 2.5*	33 ± 1.6*	62 ± 3.1*	5 ± 0.2*
EDC	44 ± 2.2*	79 ± 3.9*	50 ± 2.5*	66 ± 3.3*	43 ± 2.2*	15 ± 0.7*
EDTA	100 ± 5.0	28 ± 1.4*	50 ± 2.5*	66 ± 3.3*	29 ± 1.4*	35 ± 1.7*
EGTA	166 ± 8.3*	100 ± 5.0	75 ± 3.8*	116 ± 5.8*	40 ± 2.0*	121 ± 6.1*
NEM	144 ± 7.2*	89 ± 4.4*	10 ± 0.5*	83 ± 4.1*	100 ± 5.0	98 ± 4.9
PCMB	133 ± 6.6*	7 ± 0.35*	100 ± 5.0	83 ± 4.1*	156 ± 7.8*	64 ± 1.3*
DTT	66 ± 3.3*	102 ± 5.1	100 ± 5.0	100 ± 5.0	28 ± 1.4*	86 ± 4.3*
Control	100 ± 5.0	100 ± 5.0	100 ± 5.0	100 ± 5.0	100 ± 5.0	100 ± 5.0

Note: Control — reaction mixture with enzyme and without chemical reagent. Here and later * — $P < 0.05$ as compared with control.

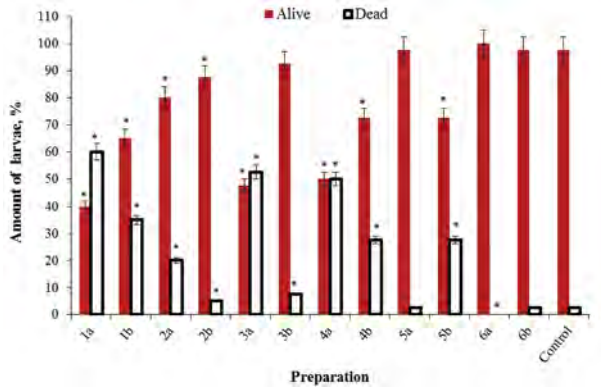

Fig. 4. **Insecticide activity of the tested protease preparations of *B. thuringiensis* after 3 days of treatment. Strains of *B. thuringiensis*: *1* — Bt-H; *2* – IMV B-7465; *3* — IMV B-7324; *4* — 902; *5* — 0–239; *6* — 9. Preparations:** here and later: *a* — complex enzyme preparation; *b* — partially purified protease; control — the amount of the larvae placed into a Petri dish with the potato leaves treated by distilled water

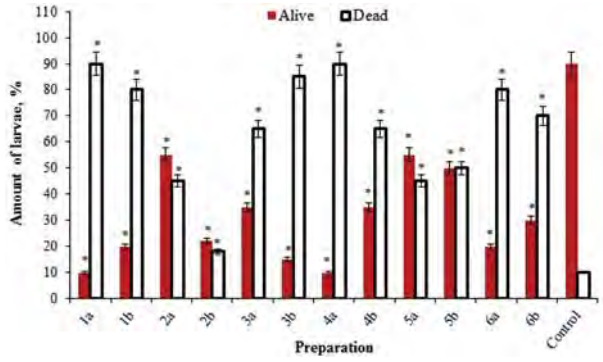

Fig. 5. **Insecticide activity of the tested protease preparations of *B. thuringiensis* after 4 days of treatment. Strains of *B. thuringiensis*:** *1* — Bt-H; *2* — IMV B-7465; *3* — IMV B-7324; *4* — 902; *5* — 0–239; *6* — 9

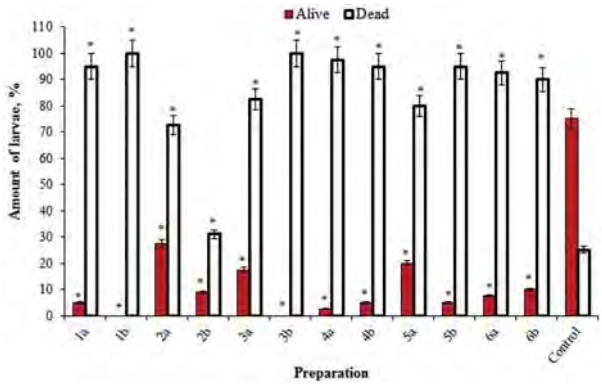

Fig. 6. **Insecticide activity of the tested protease preparations of *B. thuringiensis* after 5 days of treatment. Strains of *B. thuringiensis*:** *1* — Bt-H; *2* — IMV B-7465; *3* — IMV B-7324; *4* — 902; *5* — 0–239; *6* — 9

tested partially purified enzymes led to death of 90–100% larvae while complex preparations only of a number of *B. thuringiensis* Bt-H, 902, 9 caused similar action (from 90 to 100%).

Thus, as a result of the investigations it was shown the ability of *B. thuringiensis* strains to synthesize the proteases with elastase activity. The study of the physicochemical properties of the partially purified proteases showed that they belong to proteases of the serine type. Peptidases of a number of *B. thuringiensis* strains (IMV B-7324, IMV B-7465, 902, 0-239, 9) are metal-dependent enzymes. Optimal conditions of action of all tested enzymes are the neutral and alkaline pH values and the temperatures of 30–40 °C. The studies of influence of the complex enzyme preparations and partially purified ones of *B. thuringiensis* strains tested on the larvae of Colorado beetle indicated that enzymes with elastase activity may be responsible for insecticide action of the tested strains.

REFFERENCES

1. *Kandibin N. V., Patyka T. I., Ermolova V. P., Patyka V. F.* Micro biocontrol of population of insects and dominant *Bacillus thuringiensis*. Ed. Kandibin N.V. *PublisherVIZR, St. Petersburg, Pushkin.* 2009, 252 p. (In Russian).
2. *Patyka V. F., Patyka T. I.* Ecology of *Bacillus thuringiensis*. *PGAA, Kyiv.* 2007, P. 120–128. (In Ukrainian).
3. *Palma L., Munoz D., Berry C., Murillo J., Caballero P. Bacillus thuringiensis* toxins: an overview of their biocidal activity. *Toxins.* 2014, V. 6, P. 3296–3325.
4. *Liu X. Y., Ruan L. F., Hu Z. F., Peng D. H., Sao of S. Y., Yu Z. N., Liu Y., Zheng J. S., Sun M.* Genome-wide screening reveals the genetic determinants of antibiotic insecticide in *Bacillus thuringiensis*. *J. Biol. Chem.* 2010, V. 285, P. 39191–39200.
5. *Barboza-Corona J. E., Delgadillo-Angeles J. L., Castaneda-Ramirez J. C., Barboza-Perez U. E., Casados-Vazquez L. E., Bideshi D. K., del Rincon-Castro M. C. Bacillus thuringiensis* subsp. *kurstaki* HD1 as and factory to synthesize alkali-labile ChiA74Δsp chitinase inclusions, Cry crystals and spores for applied use. *Microb. Cell Fact.* 2014, 13 (15). doi: 10.1186/1475-2859-13-15.
6. *Luo X., Chen L., Huang Q., Zheng J., Zhou W., Peng D., Ruan L., Sun M. Bacillus thuringiensis* metalloproteinase Bmp1 functions as and nematicidal virulence factor. *Appl. Environ. Microbiol.* 2013, 79 (2), 460–468.
7. *Harrison R. L., Bonning B. C.* Proteases as insecticidal agents. *Toxins.* 2010, V. 2, P. 935–953.
8. *Matseliukh O. V., Nidialkova N. A., Varbanets L. D.* Purification and physico-chemical properties of *Bacillus thuringiensis* IMV B-7324 peptidase with elastolytic and fibrinolytic activity. *Ukr. Biokhim. Zh.* 2012, 84 (6), 25–36. (In Ukrainian).
9. *Matseliukh O. V., Varbanets L. D., Ivanitsa V. O.* 2012. Strain *Bacillus thuringiensis* IMV B-7324 — producer of extracellular elastase. *UA Patent* 97906 C2, March 26 2012. (In Ukrainian).
10. *Matseliukh O. V.* Substrate specificity of *Bacillus thuringiensis* IMV B-7324 serine alkaline peptidase. *Microbiology and Biotechnology.* 2014, 2 (26), 24–33. (In Ukrainian).
11. *Matseliukh O. V.* Obtaining of mutants of *Bacillus* sp. with enhanced elastase production. *Biotekhnolohiia.* 2010, 3 (2), 42–47. (In Ukrainian).
12. *Koltukova N. V., Vaskivniuk V. T.* Selection of the methods for isolating the proteolytic enzymatic complex from *Bacillus mesentericus* 316m in stab culture. *Mikrobiol. Zh.* 1980, 42 (2), 245–249. (In Russian).
13. *Trombridg G. O., Moon H. D.* Purification of human elastase. *Proc. Soc. Exp. Biol. Med.* 1972, 141 (13), 928–931.
14. *Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J.* 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193 (1), 265–275.
15. *Brar S. K., Verma M., Tyagi R. D., Surampalli R. Y., Barnabe S., Valero J. R. Bacillus thuringiensis* proteases: Production and role in growth, sporulation and synergism. *Proc. Biochem.* 2007, 42 (5), 773–790.
16. *Nidialkova N. A., Varbanets L. D., Ivanitsa V. O.* A bacterial strain *Bacillus thuringiensis* var. *israelensis* — producer of extracellular collagenase. *UA Patent* 97906. January 26 2015. (In Ukrainian).
15. *Salazar O., Asenjo J. A.* Enzymatic lysis of microbial cells. *Biotechnol. Lett.* 2007, 29 (7), 985–994.

ЕЛАСТАЗИ *Bacillus thuringiensis* З ІНСЕКТИЦИДНОЮ АКТИВНІСТЮ

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Метою роботи було провести скринінг протеаз із еластазною активністю серед штамів *Bacillus thuringiensis*, їх ізолювання, часткове очищення, вивчення фізико-хімічних властивостей та інсектицидної активності стосовно личинок колорадського жука. Об'єктами досліджень були 18 штамів *B. thuringiensis*, виділених із різних джерел: морської води, сухого біологічного продукту «Бітоксибацилін», а також із природних популяцій личинок колорадського жука з регіонів Криму, Херсона, Одеси, Миколаєва і Запоріжжя. Очищення ензимів з еластазною активністю, ізольованих із вищезазначених штамів, здійснювали гель-фільтрацією, а інсектицидну активність досліджували на личинках колорадського жука.

Вивчено здатність низки штамів *B. thuringiensis* синтезувати протеази з еластазною активністю. Найактивніші ензими було одержано зі штамів IMB B-7465, IMB B-7324, ізольованих з морської води, і штамів 9, 902, Bt-H та 0-239, виділених із колорадських жуків. Вивчення фізико-хімічних властивостей частково очищених протеаз цих штамів свідчить про їх належність до ензимів серинового типу. Пептидази штамів *B. thuringiensis* (IMB B-7324, IMB B-7465, 902, 0-239, 9) є металозалежними ензимами. Оптимальними умовами дії цих ензимів є нейтральні й лужні значення pH і температура 30–40 °C. Дослідження впливу комплексних і частково очищених ензимних препаратів штамів *B. thuringiensis* на личинки колорадського жука свідчать, що ензими з еластазною активністю можуть впливати на інсектицидну активність досліджуваних штамів.

Ключові слова: *Bacillus thuringiensis*, еластазна активність, інсектицидна активність.

ЭЛАСТАЗЫ *Bacillus thuringiensis* С ИНСЕКТИЦИДНОЙ АКТИВНОСТЬЮ

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Целью работы было проведение скрининга протеаз с эластазной активностью среди штаммов *Bacillus thuringiensis*, их изолирования, частичной очистки, изучение физико-химических свойств и инсектицидной активности по отношению к личинкам колорадского жука. Объектами исследований были 18 штаммов *B. thuringiensis*, изолированных из различных источников: морской воды, сухого биологического продукта «Битоксибациллин», а также естественных популяций личинок колорадского жука из регионов Крыма, Херсона, Одессы, Николаева и Запорожья. Очистку энзимов с эластазной активностью, изолированных из вышеупомянутых штаммов, осуществляли гель-фильтрацией, а инсектицидную активность исследовали на личинках колорадского жука. Изучена способность ряда штаммов *B. thuringiensis* синтезировать протеазы с эластазной активностью. Наиболее активные энзимы получены из штаммов ИМВ B-7465, ИМВ B-7324, изолированных из морской воды, и штаммов 9, 902, Bt-H и 0-239, выделенных из колорадских жуков. Изучение физико-химических свойств частично очищенных протеаз этих штаммов свидетельствует об их принадлежности к энзимам серинового типа. Пептидазы штаммов *B. thuringiensis* (ИМВ B-7324, ИМВ B-7465, 902, 0-239, 9) являются металлозависимыми энзимами. Оптимальные условия действия этих энзимов — нейтральные и щелочные значения pH и температура 30–40 °C. Исследования влияния комплексных и частично очищенных энзимных препаратов штаммов *B. thuringiensis* на личинки колорадского жука свидетельствуют, что энзимы с эластазной ативностью могут влиять на инсектицидную активность изученных штаммов.

Ключевые слова: *Bacillus thuringiensis*, эластазная активность, инсектицидная активность.