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ESTIMATION OF PRODUCTIVITY BACILLUS THURINGIENSIS ON DIFFERENT MEDIA

***Aim**: Studying the nutrient medium influence on productivity and insecticidal activity of entomopathogenic bacteria Bacillus thuringiensis (Bt).* ***Materials and methods.** The reference exotoxin strain B. thuringiensis var. thuringiensis (Bt H1) 800 and selective strain (Bt H1) 87 were used. The common nutrient medium, such as: meat infusion agar, Luria Bertrani (LB), and optimized laboratory–industrial medium: yeast-polysaccharidic composition, technological medium of molasses (4.0%) was used for cultivation. During the development optimal cultivation conditions there were determined the following parameters: producing capacity pure culture through boundary dilutions, the rate of formation entomocidal metabolites by percentage of biotest deaths – larvae of Leptinotarsa decemlineata Say.* ***Results.** It was shown the different technological effect and expression of entomopathogenic activity in different media – from 1.6 to 3.3 billion spores / ml, and from 85.0 to 96.8%, in accordance. Additionally, a high crystal endotoxin productivity was observed in the yeast polysaccharidic medium in comparison with common media. Expressive maximum death of larvae (L_{1-2}) is detected on the 10-th day of the experiment on infection load of 1: 1.* ***Conclusions.** The most conduced interrelation was protein-vitamin complex to corn-meal 2: 1 (3.0 and 1.5% in accordance). This ensures the highest titer of entomocidal components in Bt cultures – 2.8 and 3.3 billion spores/ml.*

Key words: Bacillus thuringiensis, nutrient medium, entomopathogenic, properties, cultivation conditions, spores titer.

Bacillus thuringiensis (Bt) – gram positive spore-forming bacterium is the most spreading biopesticide in the biological control market, accounting for 90% of all biopesticides sold all over the world. It is known that this bacterium characteristic feature is its ability to produce crystalline inclusions proteins called endotoxin during sporulation and/ or stationary phase. The *Bt* preparations are based on endotoxin proteins along with the spores and have a great potential to control a great number of pest insects belonging to the order *Lepidoptera, Diptera* and *Coleoptera.*

Optimization of biotechnological production of entomopathogenic preparations based on bacteria *Bacillus thuringiensis (Bt)* is connected with the solution of some

problems of microbiological synthesis in combination with selective reseach of pure growth and conditions of cultivation. [6] A unique feature of bacteria consists in its ability to multiply quickly where there is a number of resources needed for energy, constructive substrate and electron donor. This physiological properties of bacteria and fractional composition of cells are closely related to the growth rate, which depends on external conditions, including the composition of the nutrient medium [1].

Industrial cultivation of *Bt* strains-producers aimed at maximizing output of entomocidal components (exotoxins, endotoxins, related biologically active metabolites) that determines the insecticidal activity of biological products to wide range susceptible insects-"pests" [1, 8]. It is known that the process of crystal and spores formation of entomopathogenic culture *Bt* depends on the sources of nitrogen, carbon and their correspondence, existence in the environment necessary concentration of mineral compounds, sugars [2, 7]. Using high levels of substrate components without corresponding adjustments concentrations on sources of nitrogen can cause changes of pH parameters (from 6.0 to 8.0), which practically leads to slower processes of toxin-, sporogenesis. The main parameters that are monitored during the biotechnological production are also related to the ranges of temperature (30˚C ± 2 °C), aeration modes etc. As technological substrates today currently actively use various agricultural and industrial by-products, such as maize glucose, peptone, water after pressing fruits,soybean flour, cereals, grains, beans,oilseeds, peanuts, fish and meat meals, etc [5].

The aim of this work was studying the nutrient medium influence on productivity and insecticidal activity of entomopathogenic bacteria Bacillus thuringiensis (Bt).

Materials and methods

The reference exotoxin strain *B. thuringiensis var. thuringiensis (Bt H1) 800*, which is a producer bio-agent of preparation Bitoksibatsillin and stored in the collections of cultures of non-pathogenic microorganisms agricultural purposes: Institute of Agricultural Microbiology and Agricultural Production, NAAS of Ukraine (Chernigiv), the Federal State Budget Institution All-Russian Research Institute of Agricultural Microbiology (St. Petersburg, Pushkin). Selective protection type strain *B. thuringiensis var. thuringiensis (Bt H1) 87*, isolated from larvae of Colorado potato beetle IV generation in Chernigiv region (collection of useful soil microorganisms) is used in the paper.

Obtaining of pure cultures, preparation serial dilutions of bacterial suspensions, cultivation on liquid and agar nutrient media were conducted by the classical scientific and methodological works in microbiology [1, 9].

The common nutritional medium, such as: meat infusion agar, Luria Bertrani (LB), and optimized laboratory and industrial medium yeast-polysaccharidic composition (3.0% of protein-vitamin complex + 1.5% cornmeal), technological medium of molasses (4.0%) [1], creating the appropriate selective conditions for the development of specifically adapted bacteria *Bt* were used for cultivation.

Cultivation was carried out in Erlenmeyer's flasks on biotechnology shaker with termoplatform (200 rpm, the temperature 30 °C for 48–72 hours). Medium, of volume 50, 100 ml, the amount of inoculum – at least 4.0% by volume of the medium (the titer of colony forming units, CFU, 2.5–3.0 x10^9 spores/ml of the culture liquid, which was determined by inoculation on agar and counted in Goryaev chamber). The study of the morphology of bacterial cells was performed by microscopy of fixed preparations stained by carbolic fuchsin , and differentiated color technique V. Smirnoff [9]. Microsamples were performed using immersion in light microscope Axio Scope of photographic images (x 100), microscope without immersion on Polivar microscope (x 40).

During the development of optimal process the conditions of cultivation *Bt* strains determined the following parameters: producing capacity of pure culture through boundary dilutions, the rate of formation entomocidal metabolites (spore-crystal complex) the percentage of biotest deaths – larvae of *Leptinotarsa decemlineata* Say. $L_{1\text{-}2}$ when infected culture liquid at dilution of 1:1; 1:10; without dilution. The biological activity of liquid formulations *Bt* strains evaluated in model experiments on intact and contact populations of *Leptinotarsa decemlineata* Say. $L_{1\text{-}4}$ three replications (25 larvae in each).The number of dead beetles account for 5, 7, 10 day experiment according to Abbot formula:

$$A = \frac{M_0 - M_c}{100 - M_c} \times 100,$$

where A – entomocidal activity, (%); M_0 – the percentage of dead larvae in experiment; M_c – the percentage of dead larvae in the control. Death in control will not exceed 15.0%.

Processing of the results was carried out using the descriptive methods (variational) statistics and the analysis of variance on PC using software MS Excel 10.0 and STATISTICA.

Results and discussion

It is known that periodic culture is initiated when seeding in fresh sterile culture medium and runs four main phases: lag-phase (positive growth acceleration), exponential, stationary and bacterial die-away. On the exponential phase the culture medium is constantly changing. At the same time due to the inability to provide equal conditions for the total population relevant part of the cells pass into a state of stress (with the possibility of dying out). Thus, balanced growth is seen as a conditional concept. The main influence on the properties of the cells in the growth process and therefore the properties of exponentially growing population provides a composition of specially selected medium [3, 4].

During the cultivation of *Bt* bacteria there are significant changes for the cultural-morphological, physiological, biochemical, technological characteristics. Thus, a series of experiments, we found that the vegetative stage of growth strains of *B. thuringiensis var. thuringiensis (Bt H1) 800* and *87/3* characterized by uniform cells that are connected in pairs or chains. Going to sporogonic is usually charac-

terized by increased growth and development, and the presence of small, isolated cells. Choosing the right nutrient medium ingredients is essential to the success of biotechnological production for quality seed (inoculum) with the efficiency of toxigenic activity of the end products of metabolism. There were investigated nutrient medium balance for the main sources of carbon and nitrogen on development cultures of strains *B. thuringiensis var. thuringiensis (Bt H1) 800* and *87*. Research results have shown a positive effect of trophic resources selected for the cultivation of *Bt*, at efficiency and entomotoxic components activity, in particular spore-crystal complex (table).

Table

Influence of trophic resources on productivity of entomopathogenic strains *B. thuringiensis var. thuringiensis* (model research)

Nutrient media/strain-producer	Productivity,1x10^9 spores/ml		Death *Leptinotarsa decemlineata* Say. L_{1-2} of spore-crystal complex (interrelation 1: 1) % on 10 day	
	strain № 87	strain № 800	strain № 87	strain № 800
Laboratory – industrial (Yeast polysaccharide resource)	3,0 *	2,4 *	96,8 ± 0,6	95,6 ± 1,2
Industrial (with molasses)	2,7 *	2,6 *	92,0 ± 0,8	94,3 ± 0,5
Common I (meat infusion agar)	1,6 *	1,8 *	85,0 ± 1,1	88,3 ± 1,2
Common II (LB)	2,2 *	1,9 *	87,7 ± 1,8	89,0 ± 1,3

Note: * The results of three experiments presented where titre of viable spores is given maximum limits of variation in productivity *Bt* culture.

Thus, the study of the effect of four growth media (yeast-polysaccharidic, molasses, meat infusion agar, LB) on the productivity of *Bt* strains first serotype has shown different technological effect and expression of entomopathogenic activity – from 1.6 to 3.3 billion spores/ml, and from 85.0 to 96.8%, in accordance. Additionally, in the yeast polysaccharidic medium-synchronic sporogenesis observed which was conducted by a high crystal endotoxin yield in comparison with common media.

Most conduced interrelation was protein-vitamin complex to cornmeal 2: 1 (3.0% and 1.5% in accordance). This ensures the highest titer of entomocidal components in Bt cultures – 2.8 and 3.3 billion spores/ml. Expressive maximum death larvae *Leptinotarsa decemlineata Say* younger generation (L_{1-2}) on the 10-th day of the experiment is detected on infection load of 1: 1. Thus, on the 10-th day of the experiment there were about 96.0–97.0% death larvae. When infected of bacterial suspensions Bt with lower spores titer (1.5–1.7 billion spores/ml) on meat infusion agar and LB media options entomocidal variables that does not exceed 89.0% are obtained.

Creating a variety of microorganism culture conditions is possible to find out which ones are the most favorable for the identifying the potential production of

biologically active components. Research of accumulation *Bt* culture biomass in different medium (maximum titer of viable spores and crystals to 3.0 billion spores/ml) and liquid preparations functional capacity (biotesting on insecticidal, death *Leptinotarsa decemlineata Say*. L_{1-2} more than 80.0%) provides an opportunity to deepen the scientific theoretical knowledge and practical approaches in the field of biotechnology and analysis of cultures in gradient media, which compositionally is close to natural.

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ОЦІНКА ПРОДУКТИВНОСТІ *BACILLUS THURINGIENSIS* НА РІЗНИХ ПОЖИВНИХ СЕРЕДОВИЩАХ

Реферат

***Мета роботи**: Вивчення впливу живильного середовища на продуктивність та інсектицидну активність ентомопатогенних бактерій Bacillus thuringiensis (Bt). **Матеріали та методи.** В роботі використані референтний екзотоксиногенний штам B. thuringiensis var. thuringiensis (Bt H1) 800 та селекційний штамм (Bt H1) 87. Для культивування використовували універсальні поживні середовища: м'ясо-пептонний бульон (МПБ), Луріа Бертрані (LB),а також оптимізовані лабораторно-промислові середовища дріжджо-поліцукридного складу (3,0% білково-вітамінний комплекс +1,5% кукурудзяне борошно), технологічні середовища з мелясою (4,0%). В процесі розробки оптимальних технологічних параметрів культивування штамів Bt визначали такі показники: продуктивність аксенічної культури шляхом граничних розведень, інтенсивність утворення ентомоцидних метаболітів в біотесті за відсотком загибелі личинок Leptinotarsa decemlineata Say. **Результати.** Показано різний технологічний ефект та прояв ентомоцидної активності на різних середовищах – від 1,6 до 3,3 млрд спор/мл та від 85,0 до 96,8%, відповідно. Крім цього, на дріжджо-поліцукридному середовищі спостерігали більш високий вихід кристалічного ендотоксину, ніж на звичайних універсальних середовищах. Виразний максимум загибелі личинок (L_{1-2}) зафіксовано на десяту добу досліду при інфекційному навантаженні 1:1. **Висновки.** Найбільш сприятливим виявилося співвідношення білково-вітамінного комплексу до кукурудзяного борошна 2:1 (3,0% і 1,5% відповідно). При цьому досягається найбільший титр ентомоцидних компонентів в культурах Bt – 2,8 і 3,3 млрд спор/мл.*

Ключові слова: Bacillus thuringiensis, живильні середовища, ентомопатогенні властивості, умови культивування, титр спор.

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ОЦЕНКА ПРОДУКТИВНОСТИ *BACILLUS THURINGIENSIS* НА РАЗНЫХ ПИТАТЕЛЬНЫХ СРЕДАХ

Реферат

***Цель работы**: Изучение влияния питательной среды на продуктивность и инсектицидную активность энтомопатогенных бактерий Bacillus thuringiensis (Bt).* ***Материалы и методы.** В работе использованы референтный экзотоксиногенный штамм B. thuringiensis var. thuringiensis (Bt H1) 800 и селекционный штамм (Bt H1) 87. Для культивирования использовали универсальные питательные среды: мясо-пептонный агар (МПА), Луриа Бертрани (LB), а также оптимизированные лабораторно-промышленные среды дрожже-полисахаридного состава (3,0% белково-витаминный комплекс +1,5% кукурузная мука), технологическая среда с мелясой (4,0%). В процессе разработки оптимальных технологических параметров культивирования определяли такие показатели: продуктивность аксеничной культуры путём граничных разведений, интенсивность образования энтомоцидных метаболитов в биотесте по проценту гибели личинок Leptinotarsa decemlineata Say.* ***Результаты.** Показан разный технологический эффект и энтомопатогенная активность на различных средах от 1,6 до 3,3 млрд. спор/мл и от 85,0 до 96,8% соответственно. Кроме того, высокая производительность кристаллов эндотоксина наблюдалась в дрожжевой полисахаридной среде, по сравнению с универсальными питательными средами. Выраженный максимум гибели личинок (L_{1-2}) обнаружен на 10-й день эксперимента при инфекционной нагрузке 1: 1.* ***Выводы.** Наиболее благоприятным оказалось соотношение белково-витаминного комплекса и кукурузной муки 2:1 (3,0% и 1,5% соответственно). При этом достигается наибольший титр энтомоцидных компонентов в культурах Bt – 2,8 и 3,3 млрд спор/мл.*

Ключевые слова: Bacillus thuringiensis, питательные среды, энтомопатогенные свойства, условия культивирования, титр спор.

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