Annals of Advanced Agricultural Sciences, Vol. 1, No. 2, November 2017
https://dx.doi.org/10.22606/as.2017.12003 

# Estimation of the Genus Bacillus Bacterial Strains Antagonist Properties Against Pathogenic Micromycetes

[1]Maria Boiko, Post Graduate Student; [1]Mykola Patyka, Doc Agr Sci, Corresponding Member of NAAS, Head of the Chair; [2] Yuliya Vintskovs'ka, Junior Research Worker

[1]National University of Life and Environmental Sciences of Ukraine, 03041, Kyiv, 13, Heroi Oborony St.,
[2]Institute of Horticulture, NAAS of Ukraine, 03027, Kyiv-27, 23, Sadova
Email: maryaulina@gmail.com; yuliya_vinz@i.ua

**Abstract:** The authors have investigated the antagonistic properties of the strains of the g. Bacillus (*B. thuringiensis 87/3* – selected in vitro and *B. thuringiensis 800*– reference strain) concerning the test organism *Venturia spp* (the apple scab causative agent). The activity of bacteria-antagonists to the test cultures were determined by agar blocks. There are significant changes of the micromycetes morphogenesis under the influence of the strain *Bt 87/3*, in particular, expression of the lysis specific zones, changes of the density, thickness, the direction of the mycelium growth, and of the inhibition of the conidia germination within 86±0,8– 93±0,9%. The inhibitory effect difference in the degree of the *B. thuringiensis* strains cultural liquid was shown on the test cultures *Fusarium spp.* — from 54.0±0,4 to 60.0±0,7%. The metabolites of spore-forming bacteria, besides the entomocidal activity against phytophages have appeared to be of considerable interest as for the microbial interaction and as the basis of the preparations of the multifunctional actions. Those metabolites present selective activity and do not brake the natural biocoenosis.

**Keywords:** Bacillus thuringiensis, antagonistic activity, phytopathogenic micromycetes, bacteria-antagonist, preparations.

## 1 Introduction

Microbiological method of protecting plants using biotic origin components and metabolic preparations based on living cultures of microorganisms are important scientific and practical dimension of biotechnology in agricultural science. The effectiveness of modern approaches, are primarily determined by the selection and breeding of antagonistic microorganisms — producers of biologically active substances that can provide protection of crops from pests during the growing season. The comprehensive studies and selection of strains-producers as the basic components of biological preparations are extremely urgent issue today [8].

Predominantly, the basis of the bacterial preparations for biocontrol are rhizosphere microorganisms, including bacteria of the genus *Bacillus*, showing high antagonistic activity against phytopathogenic microorganisms by synthesis exometabolites of different chemical nature [2]. Some species of bacteria *Bacillus* are known as potential elicitors, through which it is possible to control and significantly reduce the level of destruction pathogens of plants at different stages of ontogeny. Having a selective action microorganisms of this group provides active participation of other natural regulators to control the number of agents of diseases and weeds. Therefore there is a necessity of scientifically grounded, systematic approaches in studying the role of bacteria of the genus *Bacillus* in the formation and functioning of microbial communities [1].

The most widespread and investigated representatives of the rhizosphere bacteria from g. *Bacillus* (*B. subtilis, B. mycoides B. sphaericus*) show high antagonistic activity against microorganisms g. *Fusarium* sp p ., *Erwinia* sp p ., *Pseudomonas* sp p ., *Aspergillus* s p p. and others due to exometabolites different chemical nature [10, 11]. Some bacillus species are known as potential elicitors, through which it is possible to control and significantly reduce the level of destruction of plant pathogens at different stages of ontogenesis. So, the bacteria g. *Bacillus* takes the special place in agrobiocenosis and characterized by different contagious levels and showing high protective effect against complex diseases during the growing season (bacterioses of oats, cucumber, tomato and potato blight, etc.) and rots

 

during roots storage. Having a selective action entomopathogens *B. thuringiensis* ensures the active participation of other natural regulators to control the number of phytophages and causative agents of diseases and weeds, including *S. marcescens, P. fluorescens* and others [6]. Pathogenic forms of soil microbiota use living plant material as a carbon source, lyse plant cells, and lead to crops damage and a serious threat to agricultural production. The defeat of plant pathogens of bacterial or fungal etiology causes decreases of yield, worsening marketable product quality, suppression overall condition and plant resistance [3].

Application of the traditional methods of protection such as crop rotation, selection resistant to disease breeds, spraying pesticides is not sufficient to control contamination of plants. It is possible to reduce using of chemicals, which can serve as an alternative biotechnological application of antagonistic microorganisms. Thus, the importance of biological control of pathogens increases. This also conduces the necessity of pesticide load reduction on agrocenosis and searching ecological alternative to chemicals. Therefore, studying the g. *Bacillus* new strains multifunctional properties is relevant to expanding the effective using of bio-agents in agricultural technologies.

**Objectives-** determine the *B. thuringiensis* strains antagonistic properties against the test cultures *Venturia spp., Fusarium spp.* with the prospect of their practical application.

## 2 Materials and Methods.

Research was conducted on the base of the Institute of Horticulture NAAS of Ukraine, laboratory of plant physiology and microbiology and National University of Life and Environmental Sciences of Ukraine. In this work we have used new entomopathogenic bacteria strain *B. thuringiensis var. thuringiensis (Bt H1) №87 / 3* selected in vitro, isolated from the larvae of leaf-eating insects natural populations of *Leptinotarsa decemlineata* Say. For the cultivation and storage of bacteria standart nutrient media of different composition were used: peptone-glucose agar, GPA (g / l distilled water): peptone - 20.0 g glucose - 10.0 g, NaCl - 5.0 g, agar-agar - 15.0 g, pH = 7.2 and medium Luria Bertani (LB), of the following composition (g / l distilled water), peptone (trypton) - 10.0 g , yeast extract - 5.0 g, NaCl - 10.0 g , agar-agar - 15.0 g, pH = 7.0 [2,4].

The antifungal activity of strains g. *Bacillus* is in conjunction with test cultures of pathogenic micromycetes g. *Venturia ssp , Fusarium ssp*, which were isolated from infected plants of Ruddy climber apple and Redhaven peach ( plantings IH NAAS), in accordingly. The activity of bacteria-antagonist to the test cultures were determined by agar block [2]. The culture of bacteria were plated solid "lawn" on the surface of the nutrient agar (medium LB) and cultivated at 28 ° C for three days. Incubation period ranged from three to six days at a temperature of 28-30 ° C. Antagonistic activity was estimated by zones of absence or growth inhibition around test-micromycetes pathogen cells.

**Figure 1.** Petri dishes with the test culture of phytopathogen

Note: The diameter of the growth inhibition zones formed around the block was taken into account. Inhibitory activity was calculated based on the diameter of the colonies in the control and experiment variants.

 

Inhibitory effect of bacterial culture metabolites was calculated using the formula (1): $\frac{D_c - D_{exp}}{D_c} x\ 100\%$ (1) where $D_c$ and $D_{exp}$ — diameter of test culture accordingly in the control and experimental variant, cm.

## 3 Results and Discussion.

Laboratory model experiments showed that under the influence of *B. thuringiensis 87/3* strain spore-crystal complex were significant morphogenesis test culture changes in all variants, especially the lysis specific zones expression, changing the density, thickness and direction of mycelium growth, and the inhibition degree of conidia germination within 86±0,8 – 93±0,9%.. In the control (without introducing *B. thuringiensis* culture bacterial metabolites) average micromycetes diameter for 10 days was 2.1 cm compared to research variants, which observed mycelium growth less than 0.4 - 0.7 cm (Fig. 2.0, 3.0).

The *B. thuringiensis 87/3* strain showed different technological effect and accumulation of biomass cultures in different nutrient media and titer of viable spores and crystals — from 2.0 to 3.6 billion spores/ ml. Besides, synchronous sporogenesis was observed in the yeast-polysaccharide medium. It was accompanied by the high output of crystal endotoxin than in conventional common media (2,0-2,4 billion / ml). The most favorable correlation is appeared of protein-vitamin complex and cornmeal 2: 1 (3.0 and 1.5% accordingly). This ensures the *Bt* cultures entomocide components highest titer —2,8 -3,6 billion / ml.

The inhibitory effect difference in the degree of the *B. thuringiensis* strains cultural liquid was shown on the test cultures *Fusarium spp.* — 54.0±0,4 to 60.0±0,7%., with appropriate darkening of the mycelium (Table 1). In control the average diameter of the test *Fusarium spp.* at 10 day was equal to 4.5 cm compared with experimental variants of the strains, which experienced growth of mycelium less than 1.8 cm. The combination of these experimental data shows a selective effect of bacteria *B. thuringiensis* metabolic complex as a multifunctional agent with the potential activity of enzymatic components (for background entomocidal and antifungal effects in relation to insect pests and plant pathogens).

**Table 1.** Determination of antagonistic *B. thuringiensis 800* strain properties against the test culture *Fusarium* spp. in double replication (8 day of mycelium growth)

| Strain | D micromycetes (aver.), cm | D loosening of mycelium (aver.), cm | D lysis zone (aver.), cm |
|---|---|---|---|
| Bacillus thuringiensis 800 (1) | 1.3±0.006 | 0.3±0.001 | 0.6±0.003 |
| Bacillus thuringiensis 800 (2) | 1.4±0.007 | 0.52±0.002 | 0.67±0.003 |

A) Peptone-glucose agar B) LB

**Figure 2.** Inhibition of micromycetes ( g. Venturia spp.) growth on the 6th day of cultivation under the influence of bacterial strain B. thuringiensis 87/3 metabolites on different nutrient media (compared to control)

 

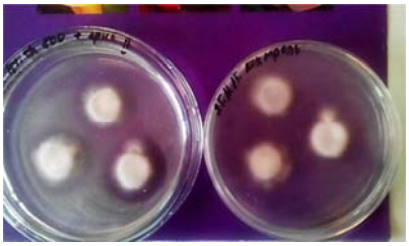

**Figure 3.** Stasis (inhibition of growth) micromycetes (g.Fusarium spp.) due to the influence of bacteria B. thuringiensis

It is known that some *Bacillus* species produce lytic enzymes of protease, chitinase types in medium / substrate, which are able to lysis the micromycetes-pathogens cell walls. In addition, micromycetes hyphae components become a nutritional source for bacteria. Besides, bacillus feature has been found by the production of cyclic lipopeptide antibiotic substances which are responsible for the antagonistic effect. Scientists have been linked the antibiotic activity of *B. thuringiensis* with the crystal δ-endotoxin, produced by bacteria and conceive that this effect is associated with separation of target objects oxidative phosphorylation and respiration [9]. It is probable that the strain *B. thuringiensis 87/3* high entomotoxic and antifungal activity existence are caused by common metabolic complex. However, the selective ability concerning to different target objects or separate test cultures may indicate the ability of bacteria to the formation of a wide spectrum of biologically active substances.

## 4 Conclusion and Recommendations.

Evaluation of antifungal properties of *B. thuringiensis 87/3* strain in model experiments on pathogenic micromycetes showed that it can be successfully used in plant pathogens system control, in particular against pathogens of fruit crops scab and mixed infections. Special attention should be focused on research on breeding selections of strains that have polyfunctional features and high antagonistic activity in the field conditions.

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