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POLYFUNCTIONAL PROPERTIES OF THE *Bacillus thuringiensis* var. *thuringiensis* INDUSTRIAL STRAIN 800/15

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Abstract

Crop losses caused by pests can reach 40-50 % and even more. Application of biological methods for regulation of the harmful species is promising and providing ecological safety. Biological preparations based on the living cultures of microorganisms and their metabolites meet these requirements. Currently, the crystal-forming bacterium *Bacillus thuringiensis* is considered to be the most important species for production of biological insecticides, since this bacterium exhibits high specificity in relation to the target pathogens, safety for humans and the environment. At ARRIAM, the biological preparation based on the *Bacillus thuringiensis* var. *thuringiensis* (*Bt*H_1) 800/15 strain was developed. The strain was isolated from larvae of the Colorado potato beetle (*Leptinotarsa decemlineata* Say.) in the Leningrad region, studied for culture-morphological, biochemical and serological properties and identified according to the classification of De Barjac and Bonnefoi. Sequencing of the genes encoding 16S RNA and B-subunit of the DNA-gyrase (GyrB) confirmed that the isolated strain 800/15 belongs to *Bacillus thuringiensis* var. *thuringiensis.* The BtH_1 800/15 strain was deposited in the Russian Collection of Agricultural Microorganisms (RCAM) under the registration number 611 (Patent of the Russian Federation RU 2514211 C1 of 27.04.2014). This paper is the first to report that the BtH1 800/15-based biologicals increases the germination of seeds, the height of seedlings and the root length of various crops, and also revealed the inhibitory activity against phytopathogenic fungi. The goal of this study was to investigate whether the biological preparation based on the BtH_1 800/15 strain has multifunctional properties including entomocidal activity against mass insect pests of crops, growth-stimulating effect on economically significant plant species and antifungal activity against phytopathogenic fungi. The preparation based on the BtH_1 800/15 strain is a liquid that is easily diluted with water to the required concentration and contains the components of the cultural medium, spores and entomocidal exo- and endotoxins. The initial values of the biological activity of the preparation were as follows: titer was 3.5×10^9 CFU/ml, exotoxin content for the *Musca domestica* Linn. larvae in LC_{50} was 3.1 μl/g of feed, entomocidal activity for the larvae of the Colorado beetle *Leptinotarsa decemlineata* Say. in LC_{50} was 0,28 %. The paper presents the data of field trials of the effectiveness of the preparation carried out on different agricultural crops in the period of 2014-2017 in the Leningrad Region, Krasnodar and Primorsky Krai against phytophagous insects, the Colorado beetle (*L. decemlineata* Say.), the 28-spotted potato ladybird (*Henosepilachna vigintioctomaculata* Motsch.), the diamondback moth (*Plutella xylostella* L.), the cabbage white and the small white (*Pieris brassica* L., *P. rapae* L.), cabbage moth (*Barathra brassicae* L.), gooseberry sawfly (*Pteronidea ribesii* Scop.), red spider mite (*Tetranichus urtica* Koch.) and whitefly (*Trialeurodes vaporariorum* West.). Field tests demonstrated the effectiveness of this biological preparation against harmful phytophagous insects (66.7-100 %). The laboratory tests revealed that the preparation did not exhibit phytotoxicity, moreover, it showed a growth-stimulating effect on the seed germination (up to 32 %), as well as the height of seedlings and root length (up to 52 %). The efficacy of the preparation against phytopathogenous fungi did not exceed 54 % and was inferior to the preparation based on the BtH_{10} strain 56. The combined use of the biological preparation based on the BtH_1 800/15 strain with the chemical insecticide Decis Extra, CE (emulsion concentrate) on potato against *H. vigintioctomaculata* Motsch. was very efficient (100 %) even if the application rates were reduced 2 and 3 times, respectively. This combination of biological and chemical insecticides is economically valuable and can be successfully used in potato fields when they are pest-infected, with the predominance of larvae of older ages and imago, which allows a significant reduction of the pesticide load. Overall, data obtained show that the biological preparation based on the *Bacillus thuringiensis* var. *thuringiensis* strain 800/15 has multifunctional properties, including entomocidal, antifungal and growth-stimulating activities, and is also promising for joint use with chemical insecticides.

Keywords: *Bacillus thuringiensis*, phytophagous insects, phytopathogens, polyfunctional properties, entomocidal, antifungal and growth-stimulating activity

The world market for chemical pesticides has recently entered a slowdown, while the market for biologicals is on the rise. The two markets are expected to equate by 2050 [1]. Biologicals are state-of-the-art alternatives to conventional chemical pesticides. They are eco-friendly; safe for humans, warm-blooded animals, and beneficial organisms; highly selective; effective against specific pests [2, 3].

Crops are increasingly protected by means of biologicals based on the genus *Bacillus*, which is pathogenic to phytophagous insects [4-6], phytopathogens [7-9], and plant pathogenic nematodes [10]. Biologicals based on various subspecies of *B. thuringiensis* Berliner (Bt) take the lead [11-13]. Insecticidal efforts at farms mainly used biologicals based on three pathogenic Bt variants. Pathovar A comprises Bt subspecies (var. *thuringiensis*), whose endotoxin crystals have greatest effects on *Lepidoptera*, Pathovar B comprises Bt subspecies (var. *israelensis*) that affect *Diptera* larvae, and Pathovar C comprises Bt subspecies (var. *tenebrionis*, var. *darmstadiensis*) that affect *Coleoptera* [4]. There has been discovered Pathovar F (for *fungi*) that affects plant pathogenic fungi [14].

The insecticidal traits of Bt arise from the synthesis of various toxins [15-17], the most important family of which is Cry, which comprises crystal delta endotoxins [18, 19]. In an insect's intestines, the protein crystal transforms into a protoxin, which further becomes a true toxin when exposed to serine proteases. The true toxin causes pathological processes and septicemia, a condition that enables microorganisms to colonize the pest's circulatory system, killing it. The thermostable water-soluble nucleotide β-exotoxin is released by bacteria into the environment; this very important substance has a metatoxic effect, as it tampers with the host insect's growth and metamorphosis [4, 20].

Bt-based biologicals have polyenzymatic traits; they have been discovered to produce a variety of hydrolases, which is seemingly what kills pests (insects and fungi) [21, 22]. Bt antifungal effects are also associated with the synthesis of other lytic enzymes, in particular, proteases and chitinases, which destroy the cell walls of phytopathogenic fungi [23, 24]. Recently, *Bacillus* bacteria have been discovered to synthesize lipopeptide antibiotics that have an antagonistic effect [25-27].

One aspect to the multifunctionality of *Bacillus* bacteria is that they can promote plant growth [28-30]. They colonize soil rhizosphere and rhizoplane, where they produce physiological agents that may activate plant resilience genes [31]. R&D of Bt-based biologicals requires finding and selecting active strains from natural sources (soil, affected insects, infected tissue and organs of plants); strains are selectively engineered to be easy to produce, to have strong broad-spectrum effects [32].

The All-Russian Research Institute of Agricultural Microbiology (ARRI-

AM) has developed a liquid broad-spectrum biological against mass plant pests, which is based on *B. thuringiensis* var. *thuringiensis* 800/15 (BtH_1 800/15) (Russian Patent No. 2514211 dd. April 27, 2014) [33]; it can be easily diluted in water to the required concentration and easily disseminated using standard sprayers.

This paper presents the first study of the plant growth promotion effects of the BtH_1 800/15-based biological, as well as its antifungal effects against phytopathogenic fungi. The biological has been found to improve germination rate by 6% to 21%, the seedling height by 4% to 52%, and the root length by 12% to 52% in a variety of crops. Its inhibitory efficacy against phytopathogens is up to 54%.

The goal hereof was to study the multifunctionality of a biological based on the strain *Bacillus thuringiensis* var. *thuringiensis* 800/15.

Techniques. *Bacillus thuringiensis* var. *thuringiensis* 800/15 (BtH_1 800/15) was isolated from dead Colorado beetle larvae collected in the Leningrad Region; the team studied the cultural, morphological, biochemical, and serological traits of the strain to classify it per De Barjac and Bonnefoi [34-36].

Gene sequences encoding 16S rRNA and B-subunits (*gyrB*) were employed to define the isolate by molecular phylogenetics. The strain was cultured in TY medium for 24 hours, after which 10^8 bacterial cells were moved to TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and incubated for 10 minutes at 95 °C. The resulting DNA was used to amplify the sequences encoding 16S rRNA (27f 5′-GTTTGATCMTGGCTCAG-3′ primers), 1492R (5′-TACGGYTACCTTGTTACGACTT-3’) (37), *gyrB* (gyrB_F 5′-CTTGAAGGACTAGARGCAGT-3′, gyrB_Rf 5′-CCTTCACGAACATCYTCACC-3′) [38] by PCR. The reaction mixture had a volume of 20 rl, which comprised 1 rl of bacterial DNA, 0.5 rl of each primer, and 10 rl of Fermentas-DreamTaq green PCR master mix (Thermo Fisher Scientific, USA). PCR was carried out on a T100tm Thermal Cycler (Bio-Rad, USA) using the following procedure: 15 minutes at 95 °C (for initial denaturation); 30 s at 94 °C (denaturation), 30 s at 55 °C (annealing), 1 min at 72 °C (elongation) (31 cycles); 10 minutes at 72 °C (final elongation). Sanger sequencing was performed at the Collective Lab for Genomic Technologies, Proteomics, and Cell Biology, ARRIAM [39]. BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to determine the closest strains in terms of 16S rRNA and *gyrB* sequences.

BtH_1 800/15 was deposited in the Russian Collection of Agricultural Microorganisms (RCAM) as a spore microorganism under the registration number 611. BtH_1 800/15 was cultured in dense nutrient media (meat-peptone agar, MPA, and fish agar, FA) at 28-30 °C until the spore generation and crystal endotoxin synthesis were complete. Microscopy was performed on Day 7 [40] using black aniline dye. Strain productivity was measured in yeast-polysaccharide media by submerged cultivation in Erlenmeyer flasks on an aerated rocker at 220 rpm and 28 °C over 72 hours.

ARRIAM’s Ecos branch based in Kolpino, St. Petersburg, used this strain to produce a liquid biological. Cellular population, entomocidal activity (LC_{50} for the larvae of *Leptinotarsa decemlineata* Say from a natural population), and the exotoxin content (LC_{50} for domestic fly *Musca domestica* Linn.) were determined per [32].

The antifungal effects of Bt against various plant pathogenic fungi were determined by the agar block method in Petri dishes [41]. The biological based on BtH_1 800/15 was compared against a reference based on BtH_{10} 56. For tests, the team used the plant pathogenic fungi *Botrytis cinerea* Pers. (strain C-5), *Pythium* spp. (strain C-2), *Bipolaris sorokiniana* (Sacc.) Shoemaker (strain C-20), *Fusarium avenaceum* (Fr.) Sacc. (strain C-8), *F. solani* App. et Wr. (strain C-15), *Verticillium dahlia* Kleb. (strain 27). Liquid Bt biological (5% concentration) was injected in a molten Czapek medium cooled down to 40 °C. Blocks cut out of a 10-day culture of the fungi were placed on the congealed agar. For control, the team used a medium that did not contain the biological. Fungal colony diameters were measured 5 days later. The inhibitory efficacy of Bt was calculated by the Abbot's formula [42]:

$$X = \frac{Dc - Dt}{Dc} \times 100,$$

where X is the degree of inhibiting fungal colony growth, %; D_c and D_t are the diameters of fungal colonies (control and test, respectively), cm.

The phytoregulatory effects of the BtH_1 800/15-based biological were assessed by laboratory germination of seeds in rolls of filter paper at 26 °C and a humidity of 86%. Tomato (*Solanum lycopersicum*), cucumber (*Cucumis sativus*), marrow (*Cucurbita pepo* var. *giromontina*), beet (*Beta vulgaris*), pumpkin (*Cucurbita pepo*), and cabbage (*Brassica oleracea*) seeds were soaked for 3 hours in a liquid culture of BtH_1 800/15, as well as in a nutrient medium for control. Germination was rated in 5 days; seedling and root length was measured in 3 weeks [43].

Field tests were carried out at farms in the Leningrad Region, Krasnodar Territory, and Primorye Territory in 2014-2017. Vegetating *Solanum tuberosum* plants were treated with a liquid biological suspension while the Colorado beetle larvae $L_{1\text{-}2}$ were hatching. In the Krasnodar Territory, soy (*Glycine max*) was treated against red spider mite (*Tetranychus urticae* L.) in the field, while cucumbers were treated against red spider mite and whitefly (*Trialeurodes vaporariorum* West.) indoors. The biological was applied at 15 to 20 l/ha; the solution application rate was 200 to 400 l/ha.

In the Leningrad Region, white cabbage (*Brassica oleracea*) was treated against diamondback moth (*Plutella xylostella* L.), cabbage butterfly, and small white (*Pieris brassicae* L., *P. rapae* L.), cabbage moth (*Barathra brassicae* L.) (10-15 l/ha); gooseberry (*Ribes uva-crispa*) was treated against common gooseberry sawfly (*Pteronidea ribesii* Scop.) (20 l/ha). In the Primorye Territory, potato was treated against 28-spotted potato ladybird (*Henosepilachna vigintioctomaculata* Motsch.) with the tested biological, as well as with Decis Extra, CE (emulsion concentrate, Decis®, Bayer AG, Germany), both applied in recommended dosages (15 and 0.03 l/ha, respectively), also mixed in a tank with reduced dosage (7.5 l/ha and 0.01 l/ha).

The collected data was processed by analysis of variance at 95% CI [44]. For processing, the researchers calculated the means (*M*) and the standard errors of the mean (±SEM). Significance was tested by Student's *t*-test at a confidence interval of 95% ($p < 0.05$).

Results. Following the conventional approach [34-36], the strain 800/15 had earlier been described as *B. thuringiensis* var. *thuringiensis*. We confirmed its taxonomy by molecular systematics. Using BLAST, *Bacillus cereus* VLS-S-1, *Bacillus thuringiensis* HC2, *Bacillus toyonensis* PgBe301 were identified as strains of a nearly identical 16S rRNA sequence. Thus, the 16S rRNA locus is too conservative for accurate identification of the strain's taxonomy, but provides proving the strain to be a *Bacillus*.

To clarify the phylogenetic position of 800/15, wwe sequenced its gene *gyrB* that encodes the B-subunit of DNA gyrase. This locus is less conservative than 16S rDNA, which helps differentiate phylogenetically close groups. The *gyrB* sequence in 800/15 was found to be closer to that of *Bacillus thuringiensis* ATCC 10792 and *Bacillus thuringiensis* Bt 407, earlier classified as serotype BtH_1 [37]. Thus, molecular systematics confirmed that 800/15 was indeed *B. thuringiensis* var. *thuringiensis*.

The next step was to find whether the BtH_1 800/15-based biological was multifunctional. To that end, its samples were first tested for activity. The titer was 3.5-3.6±0.2 billion CFU/ml, with the *Musca domestica* exotoxin concentration at LC_{50} being 3.1-3.2±0.1 rl/g of food and the entomocidal activity against *Leptinotarsa decemlineata* larvae at LC_{50} being in the range 0.26-0.28±0.02%.

In field tests, the biological killed 70% to 100% of insects, see Table 1. Its application improved the yield of potatoes (Leningrad Region, Krasnodar Territory) and cabbage (Leningrad Region) as compared to the controls (no data available).

1. Efficacy of the liquid *Bacillus thuringiensis* var. *thuringiensis* 800/15-based biological as tested on phytophages in different Russian regions over 2014-2017

Pest species	Geographic location	Crop	Biological efficacy, %
Leptinotarsa decemlineata Say.	Krasnodar Territory, Leningrad Region	Potato	95.9-98.8
Henosepilachna vigintioctomaculata Motsch.	Primorye Territory	Potato	66.7-70.0
Tetranichus urticae Koch.)		Cucumber	97.6-98.4
	Krasnodar Territory	Soybean	95.8-96.2
Trialeurodes vaporariorum West.	Krasnodar Territory	Cucumber	99.8-100.0
Plutella maculipennis Curt.	Leningrad Region	Cabbage	80.0-90.1
Pieris brassicae L.	Leningrad Region	Cabbage	94.7-95.6
Pieris rapae L.	Leningrad Region	Cabbage	84.5-96.7
Barathra brassicae L.	Leningrad Region	Cabbage	90.0-92.8
Pteronidear ribesii Scop.	Leningrad Region	Gooseberry	70.8-72.4

N o t e. Efficacies are averaged-based.

Laboratory tests showed the biological was not phytotoxic; on the contrary, it promoted plant growth, e.g. it increased the germination rates of: beet and cabbage by 6%, cucumbers by 10%, marrows by 21%, tomatoes by 11%, and pumpkins by 21%; beet seedlings gained 4% in height, cucumbers gained 8%, marrows gained 52%, cabbage gained 36%, and pumpkins gained 38%; root length rose by 12% in beet, 19% in cucumbers, 52% in marrows, 28% in tomatoes, 48% in cabbage, and 42% in pumpkins, see Table 2. The crops responded differently to BtH_1 800/15. Marrows, tomatoes, cabbage, and pumpkins were stimulated to a far greater extent than beet or cucumbers. Seedlings that sprouted from treated tomato, cabbage, pumpkin, and marrow seeds were 6.4±0.7 to 14±0.75 cm or 36% to 52% higher than controls (4.2±0.65 to 9.9±0.25 cm); the roots of treated cabbage, pumpkin, and marrow were 7.4±0.55; 15.9±0.75, or 22.5±0.9 cm longer than the controls (5.0±0.25; 11.2±0.45, or 14.8±0.75 cm (difference from the controls deemed significant at $p < 0.05$). The biological also proved to significantly boost the pumpkin, cabbage, and marrow root growth (by 42-52%).

The BtH_1 800/15-based biological had antifungal effects, albeit weaker than those of BtH_{10} 56, see Table 3. On average, the fungi *Botrytis cinerea* Pers., *Pythium* spp., *Bipolaris sorokiniana* and *Verticillium dahliae* were inhibited by BtH_1 800/15 by 51.15±1.75; 40.35±1.65; 21.55±1.45, and 18.45±0.45%, respectively. A further selection of BtH_1 800/15 for antifungal effects requires more specific criteria, which will be set forth by further research.

In the Primorye Territory, the BtH_1 800/15-based biological was compared against Decis Extra, CE for protection against 28-spotted potato ladybird. Tests showed mixing both agents in lower doses was as efficient as the chemical reference, as 100% of the pest was dead on the 10th post-treatment day. Thus, combining these agents is appropriate and cost-effective for fields inhabited by this pest, predominantly by older larvae and imago.

Laboratory tests showed that neither at sublethal dosages nor at higher-than-recommended concentrations in combination did Decis Extra hinder the viability of BtH_1.

2. *Bacillus thuringiensis* var. *thuringiensis* 800/15-based biological and its phytoregulatory effects on different crops

Crop	Seed germination, %			Seedling height, cm			Root length, cm		
	controls	post-treatment		controls	post-treatment		controls	post-treatment	
		total	percent of the control		total	percent of the control		total	percent of the control
Beet	92-96	99	106	4.2-5.0	4.8-5.1	104	3.5-4.0	3.8-4.7	112
Cucumbers	87-90	99	110	4.6-5.3	5.2-5.7	108	4.9-5.2	4.9-5.2	119
Marrows	77-80	91-96	120	9.7-10.2	11.4-15.7	142	14.0-15.9	14.0-15.9	152
Tomatoes	88-91	99	111	4.3-4.8	5.6-7.0	152	12.9-14.0	12.9-14.0	128
Cabbage	92-94	99	106	4.9-5.9	7.1-7.9	136	4.8-5.3	4.8-5.3	148
Pumpkin	77-80	91-98	120	7.4-8.3	10.4-11.3	138	10.7-11.8	10.7-11.8	142

3. Antifungal effects of the strains *Bacillus thuringiensis* var. *thuringiensis* (BtH_1) and *Bacillus thuringiensis* var. *darmstadiensis* (BtH_{10})

Вид фитопатогенного гриба	Ингибирование роста колоний гриба через 5 сут, %	
	BtH_1 800/15	BtH_{10} 56
Botrytis cinerea Pers (штамм С-5)	48,3-54,0	100
Pythium spp. (штамм С-2)	38,7-42,0	83,2-86,5
Bipolaris sorokiniana (Sacc) Shoemaker (штамм С-20)	20,1-23,0	74,1-75,3
Fusarium avenacrum (Fr.) (Sacc) (штамм С-8)	0	50,0-51,2
Fusarium solani App. et Wr. (штамм С-3)	0	25,2-26,0
Verticillum dahlia Kleb. (штамм 27)	18,0-18,9	50,7-52,0

П р и м е ч а н и е. Ингибирующую активность препарата рассчитывали по формуле W.S. Abbot (см. раздел «Методика»).

Combining chemicals and biologicals subjects plants to less stress and is a cost-effective solution, as both agents can thus be applied in lesser quantities. The synergistic effects of *B. thuringiensis*-based biologicals in combination with chemicals have been noted in research literature. Thus, using *B. thuringiensis* var. *israelensis*-based bactoculicide in combination with dimilin, a chitin synthesis inhibitor, at a quarter to an eighth of the recommended dosage did kill up to 91.2% *Lycoriella solani* Winn. while boosting the yield by up to 58.6% [45]. Ivantsova evaluated *B. thuringiensis* var. *darmstadiensis*-based bacicol in combination with Fastac®, BASF SE, Germany, at low-toxic dosage; the agents amplified each other, and the combination was extremely efficient against mustard pests [46]. Bactoculicide has been proven effective against *Cricotopus sylvestris* Fabr. in combinations with Proponit®, Arysta LifeScience S.A.S., France, Basagran®, BASF SE, Germany, 2M-4X MCPA, Bayer AG, Germany, and Ordram®, Syngenta AG, Switzerland. The herbicides had no identifiable negative effects on the viability of *B. thuringiensis* var. *israelensis* [47].

Thus, the *Bacillus thuringiensis* var. *thuringiensis* 800/15-biological has entomocidal, plant growth-promoting, and antifungal effects. It is a promising solution for combating mass pests affecting a wide range of crops, from potatoes and cruciferous crops to berries. Use in combination with a chemical insecticide seems appropriate and cost-effective for fields populated with older larvae and imago of *Leptinotarsa decemlineata* Say. and *Henosepilachna vigintioctomaculata* Motsch.

REFERENCES

1. Olson S. An analysis of the biopesticide market now and where it is going. *Outlooks on Pest Management*, 2015, 26(5): 203-206 (doi: 10.1564/v26_oct_04).
2. Siegel J.P. The Mammalian Safety of *Bacillus thuringiensis*- Based Insecticides. *Journal of Invertebrate Pathology*, 2001, 77(1): 13-21 (doi: 10.1006/jipa.2000.5000).
3. Raymond B., Federici B.A. In deference of *Bacillus thuringiensis*, the safest and most success the microbial insecticide available to humanity — a response to EFSA. *FEMS Microbiology Ecology*, 2017, 93(7): fix084 (doi: 10.1093/femsec/fix084).
4. Kandybin N.V., Patyka T.I, Ermolova V.P., Patyka I.F. *Mikrobiokontrol' chislennosti nasekomykh i ego dominanta Bacillus thuringiensis.* St. Petersburg—Pushkin, 2009 (in Russ.).
5. Polanczyk R.A., Pires da Silva R.F., Fiuza L.M. Effectiveness of *Bacillus thuringiensis* against *Spodoptera frugipera* (*Lepidoptera*: *Noctuidae*). *Brazilian Journal of Microbiology*, 2000, 31(3): 165-167 (doi: 10.1590/S1517-83822000000300003).
6. Eswarapriya B., Gopalsamy B., Kameswari B., Meera R., Devi P. Insecticidal activity of *Bacillus thuringiensis* IBt-15 strain against *Plutella xylostella*. *International Journal of PharmTech Research*, 2010, 2(3): 2048-2053.
7. Seo D.J. Nguyen D.M.S., Song Y.S., Jung W.J. Induction of defense response against *Rhizoctonia solani* in cucumber plants by endophytic bacterium *Bacillus thuringiensis* GS1. *J. Microbiol. Biotechnol.*, 2012, 22(3): 407-415 (doi: 10.4014/jmb.1107.07027).
8. Martinez-Absalón S., Rojas-Solís D., Hernández-Leyn R., Prieto-Barajas C., Orozco-Mosqueda M., Peña-Cabriales J.J., Sakuda S., Valencia-Cantero E., Santoyo G. Potential use and mode of action of the new strain *Bacillus thuringiensis* UM96 for the biological control of the grey mould phytopathogen *Botrytis cinerea*. *Biocontrol Science and Technology*, 2014, 24(12):

1349-1362 (doi: 10.1080/09583157.2014.940846).
9. Heydari A., Pessaraki M. A review on biological control of fungal plant pathogens using microbial antagonists. *Journal of Biological Sciences*, 2010, 1(4): 273-290 (doi: 10.3923/jbs.2010.273.290).
10. Mohammed S.H., El Saedy M.A., Enan M.R., Nasser E.I., Ghareeb A., Salah A.M. Biocontrol efficiency of *Bacillus thuringiensis* toxins against root-knot nematode, *Meloidogyne incognita. J. Cell Mol. Biol.*, 2008, 7(1): 57-66.
11. Pane C., Villecco D., Campanile F., Zaccardelli M. Novel strains of *Bacillus* isolated from compost and compost-amended soils as biological control agents against soil-borne phytopathogenic fungi. *Biocontrol Science and Technology*, 2012, 22(12): 1373-1388 (doi: 10.1080/09583157.2012.729143).
12. Akram W., Mahboob A., Jave A.A. *Bacillus thuringiensis* strain 199 can induce systemic resistance in tomato against *Fusarium* wilt. *European Journal of Microbiology and Immunology*, 2013, 3(4): 275-280 (doi: 10.1556/EuJMI.3.2013.4.7).
13. Tao A., Pang F., Huang S., Yu G., Li B., Wang T. Characterization of endophytic *Bacillus thuringiensis* strains isolated from wheat plants as biocontrol agents against wheat flag smut. *Biocontrol Science and Technology*, 2014, 24(8): 901-924 (doi: 10.1080/09583157.2014.904502).
14. Smirnov O.V. *Patotipy Bacillus thuringiensis i ekologicheskie osnovy ikh ispol'zovaniya v zashchite rastenii. Avtoreferat doktorskoi dissertatsii* [Pathotypes of *Bacillus thuringiensis* and the ecological basis of their use in plant protection. DSc Thesis]. Petersburg—Pushkin, 2000 (in Russ.).
15. Shrestha A., Sultana R., Chae J.-C., Kim K., Lee K.-J. *Bacillus thuringiensis* C25 which is rich in cell wall degrading enzymes efficiently controls lettuce drop caused by *Sclerotinia minor*. *Eur. J. Plant Pathol.*, 2015, 142(3): 577-589 (doi: 10.1007/s10658-015-0636-5).
16. Loseva O., Ibrahim M., Candas M., Koller C.N., Bauer L.S., Bulla Jr L.A. Changes in protease activity and Cry3Aa toxin binding in the Solorado potato beetle: implications for insect resistance to *Bacillus thuringiensis* toxins. *Insect Biochemistry and Molecular Biology*, 2002, 32(5): 567-577 (doi: 10.1016/S0965-1748(01)00137-0).
17. Zhong C.H., Ellar D.J., Bishop A., Johnson C., Lin S.S., Hart E.R. Characterization of *Bacillus thuringiensis* δ-endotoxin which is toxic to insects in three orders. *Journal of Invertebrate Pathology*, 2000, 76(2): 131-139 (doi: 10.1006/jipa.2000.4962).
18. Aronson A.I., Shai Y. Why *Bacillus thuringiensis* insecticidal toxins are so effective: unique features of their mode of action. *FEMS Microbiology Letters*, 2001, 195(1): 1-8 (doi: 10.1111/j.1574-6968.2001.tb10489.x).
19. Knaak N., Rohr A., Fiuza L. In vitro effect of *Bacillus thuringiensis* strains and Cry proteins in phytopathogenic fungi of paddy rice-field. *Brazil. J. Microbiol.*, 2007, 38(3): 526-530 (doi: 10.1590/S1517-83822007000300027).
20. Shternshis M.V., Belyaev A.A., Tsvetkova V.P. Shpatova T.V., Lelyak A.A., Bakhvalov S.A. *Biopreparaty na osnove bakterii roda Bacillus dlya upravleniya zdorov'em rastenii* [*Bacillus*-based biological products for plant health management]. Novosibirsk, 2016 (in Russ.).
21. Wagner W., Möhrlen F., Schnetter W. Characterization of the proteolytic enzymes in the midgut of the European Cockchafer, *Melolontha melolontha* (*Coleoptera*: *Scarabaidae*). *Insect Biochemistry and Molecular Biology*, 2002, 32(7): 803-814 (doi: 10.1016/S0965-1748(01)00167-9).
22. Smirnov O.V., Grishechkina S.D. Polyfunctional activity of *Bacillus thuringiensis* Berliner. *Sel'skokhozyaistvennaya Biologiya* [*Agricultural Biology*], 2011, 3: 123-126 (in Russ.).
23. Reyes-Ramírez A., Escudero-Abarca B.I., Aguilar-Uscanga G., Hayward-Jones P.M., Barboza-Corona J.E. Antifungal activity of *Bacillus thuringiensis* chitinase and its potential for the biocontrol of phytopathogenic fungi in soybean seeds. *Journal of Food Science*, 2004, 69(5): M131-M134 (doi: 10.1111/j.1365-2621.2004.tb10721.x).
24. Xiao L., Xie C.-C., Cai J., Lin Z.-J., Chen Y.-H. Identification and characterization of chitinase producing *Bacillus* slowing significant antifungal activity. *Curr. Microbiol.*, 2009, 58(5): 528-533 (doi: 10.1007/s00284-009-9363-5).
25. Kim P.I., Bai H., Bai D., Chae H., Chung S., Kim Y., Park R., Chi Y.-T. Purification and characterization of a lipopeptide produced by *Bacillus thuringiensis* CMB26. *Journal of Applied Microbiology*, 2004, 97(5): 942-949 (doi: 10.1111/j.1365-2672.2004.02356.x).
26. Yánez-Mendizábal V., Zeriouh H., Viñas I., Torres R., Usall J., de Vicente A., Pérez-García A., Teixidó N. Biological control of peach brown rot (*Monilinia* spp.) by *Bacillus subtilis* CPA-8 is based on production of fengycin-like lipopeptides. *Eur. J. Plant Pathol.*, 2011, 132(4): 609-619 (doi: 10.1007/s10658-011-9905-0).
27. Elkahoui S., Djébalin N., Karkouch I., Hadj Ibrahim A., Kalai L., Bachkouel S., Tabbene O., Limam F. Mass spectrometry identification of antifungal lipopeptides from Bacillus sp. BCLRB2 against *Rhizoctonia solani* and *Sclerotinia sclerotiorum*. *Appl. Biochem. Microbiol.*, 2014, 50(2): 161-165 (doi: 10.1134/S0003683814020082).
28. Choudhary D.K., Johri B.N. Interactions of *Bacillus* spp. and plants — With special reference to induced systemic resistance (ISP). *Microbiological Research*, 2009, 164(5): 493-513 (doi: 10.1016/j.micres.2008.08.007).
29. Kumar P., Dubey R.C., Maheshwari D.K. *Bacillus* strain isolated from rhizosphere showed

plant growth promoting and antagonistic activity against phytopathogens. *Microbiological Research*, 2012, 167(8): 493-499 (doi: 10.1016/j.micres.2012.05.002).

30. Chebotar' V.K., Naumkina T.S., Borisov A.Yu. *Kompleksnoe mikrobnoe udobrenie «Bisolbimiks»* /Pod redaktsiei A.Yu. Borisova [Complex microbial fertilizer Bisolbimix. A.Yu. Borisov (ed.)]. St. Petersburg, 2015 (in Russ.).
31. Partida-Martínez L.P., Heil M. The microbe-free plant root: fact or artifact? *Frontiers in Plant Science*, 2011, 2(100): 1-16 (doi: 10.3389/fpls.2011.00100).
32. Grishechkina S.D., Ermolova V.P., Minina G.N., Safronova V.I., Bologova E.V. *Metodika. Kollektsiya shtammov bakterii-simbiontov vrednykh nasekomykh i gryzunov, prigodnykh dlya biokontrolya chislennosti vreditelei sel'skokhozyaistvennykh rastenii* [Collection of bacterial symbionts in harmful insects and rodents, suitable for biocontrol of agricultural plant pests]. St. Petersburg, 2014 (in Russ.).
33. Tikhonovich I.A., Ermolova V.P., Grishechkina S.D., Romanova T.A. *Shtamm bakterii Bacillus thuringiensis var. thuringiensis №800/15 v kachestve sredstva dlya polucheniya entomotsidnogo biopreparata.* Patent RU 2514211 S1. Zayavl. 10.10.2012. Opubl. 27.04.2014. Byul. № 12 [The strain of *Bacillus thuringiensis* var. *thuringiensis* №800/15 for entomocidal biopreparation. Patent RU 2514211 S1. Claims 10.10.2012. Publ. 27.04.2014. Bul. № 12] (in Russ.).
34. De Barjac H., Bonnefoi A. Essai de classification biochmique et seroloque de 24 coucher de *Bacillus* du type *B. thuringiensis. Entomophaga*, 1962, 7(1): 5-31 (doi: 10.1007/BF02375988).
35. De Barjac H., Bonnefoi A. A classification strains of *Bacillus thuringiensis Berliner* with a key to their differentiation. *Journal of Invertebrate Pathology*, 1968, 11(3): 335-347 (doi: 10.1016/0022-2011(68)90182-1).
36. De Barjac H., Bonnefoi A. Misse au point sur la classification des *Bacillus thuringiensis. Entomophaga*, 1973, 18(1): 5-17 (doi: 10.1007/BF02373009).
37. Lane D.J. 16S/23S rRNA sequencing. In: *Nucleic acid technics in bacterial systematics.* E. Stackebrandt, M. Goodfellow (eds.). John Wiley & Sons, Chichester, NY, 1991: 115-175.
38. Punina N.V., Zotov V.S., Parkhomenko A.L., Parkhomenko T.U., Topunov A.F. Genetic diversity of *Bacillus thuringiensis* from different geo-ecological regions of Ukraine by analyzing the 16S rRNA and *gyrB* genes and by AP-PCR and saAFLP. *Acta Naturae*, 2013, 5(1): 90-100.
39. Sanger F., Nicklen S., Coulson A.R. DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences*, 1977, 74(12): 5463-5467 (doi: 10.1073/pnas.74.12.5463).
40. Kol'chevskii A.G., Rybina L.M., Kolomiets V.Ya. *Vydelenie i otbor vysokovirulentnykh kul'tur Bacillus thuringiensis var. galleriae. Metodicheskie rekomendatsii* /Pod redaktsiei V.A. Pavlyushina [Isolation and selection of highly virulent cultures of *Bacillus thuringiensis* var. *galleriae*. Guidelines. V.A. Pavlyushin (ed.)]. Leningrad, 1987 (in Russ.).
41. *Metody eksperimental'noi mikologii* /Pod redaktsiei V.I. Bilai [Methods of experimental mycology. V.I. Bilai (ed.)]. Kiev, 1982 (in Russ.).
42. Abbot W.S. A method for computing the effectiveness of insecticide. *Journal of Economic Entomology*, 1925, 18(2): 265-267 (doi: 10.1093/jee/18.2.265a).
43. Benken A.A., Khatskevich L.K., Grishechkina S.D. *Uskorennyi metod otsenki bolezneustoichivosti sortoobraztsov yachmenya i paraziticheskikh svoistv vozbuditelya fuzarioznoi kornevoi gnili. Informatsionnyi listok* [A rapid assessment of disease resistance in barley varieties and parasitic activity of the Fusarium root rot causative agent]. St. Petersburg, 1983 (in Russ.).
44. Dospekhov B.A. *Metodika polevogo opyta* [Methods of field trials]. Moscow, 1985 (in Russ.).
45. Mokrousova E.P., Glazunova I.N., Kandybin N.V., Ermolova V.P. *Sovmestnoe primenenie ingibitorov sinteza khitina s mikrobiologicheskimi preparatami protiv vreditelei shampin'onov. Problemy entomologii v Rossii* [Combined use of chitin synthesis inhibitors with microbiological preparations against champignon pests. Problems of entomology in Russia. Vol. 2]. St. Petersburg, 1998, tom 2: 40-41 (in Russ.).
46. Ivantsova E.A. *Optimizatsiya fitosanitarnogo sostoyaniya agrobiotsenozov Nizhnego Povolzh'ya. Avtoreferat doktorskoi dissertatsii* [Optimization of the phytosanitary state of agrobiocenoses in the condition Lower Volga region. DSc Thesis]. Saratov, 2009 (in Russ.).
47. Stus' A.A., Ermolova V.P. Kandybin N.V. *Byull. VNIISKHM*, 1989, 52: 25-27 (in Russ.).