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Crops biological protection: Phytopathogens growth inhibition by the entomopathogens

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ABSTRACT

Background: The safety of agrarian preparations used for crop protection is a critical issue. In these regards, the minimization of usage of chemical pesticides containing artificial harmful substances is very important. Thus, the elaboration of innovative harmless biopesticides, based on biological enemies of pests, is very desirable. Microbe-based biopesticides can significantly increase the quality of agricultural production.

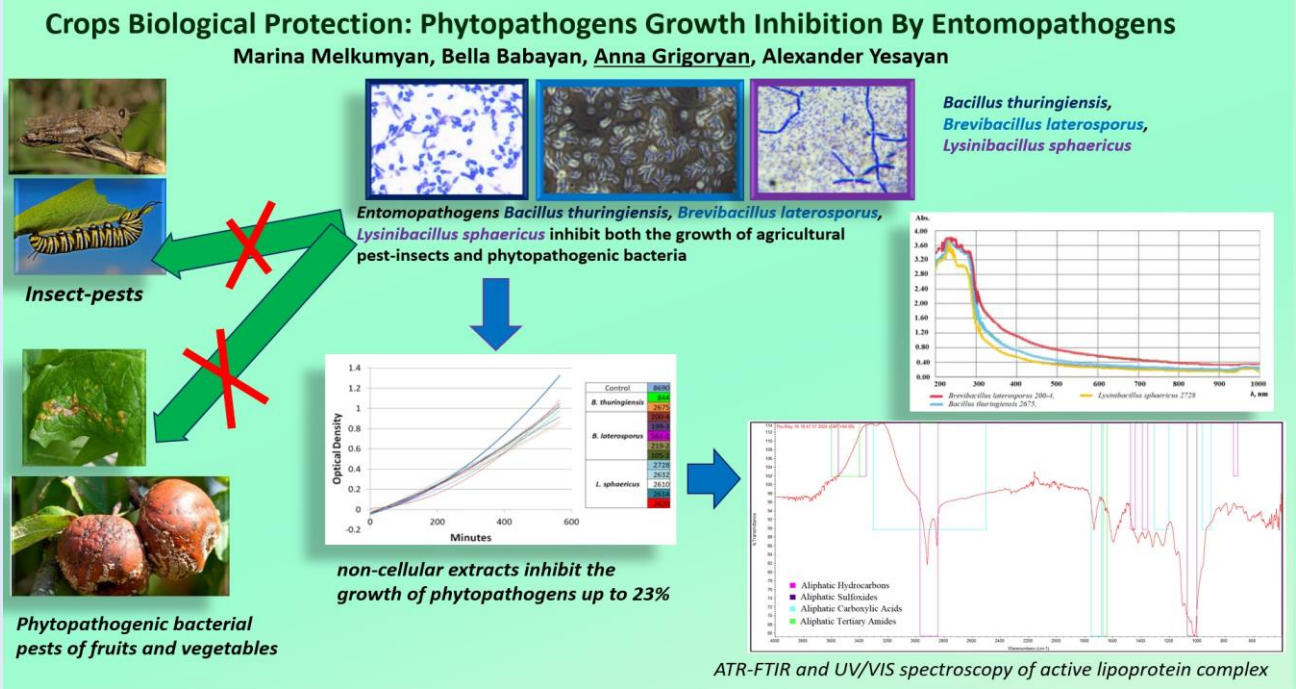
Objectives: This research aims to develop ecologically safe, entomopathogenic microbe based biopesticides for use in green agriculture to improve agricultural production qualityIt includes the quality of vegetables, cereals, and fruits, as well as meat, milk, and eggs from animals that consume plant-based feed cultivated using recommended methodologies.

Context and purpose of this study: This article was devoted to the consideration of bactericide effect of non-cellular extracts of entomopathogens against the various phytopathogens, as the potential prospective innovation for elaboration of plant protection safe biopesticides.

Results: The majority of the studied entomopathogens can synthesize the compounds which inhibit the growth of phytopathogens. Entomopathogenic strains *BT 2675, BL 200-4*, and *LS 2728* were found to significantly inhibit the growth of phytopathogens such as *Pectobacterium, Xanthomonas, Rhodococcus, Curtobacterium*, and *Pseudomonas* genera. According to spectroscopy the antiphytopathogenic effect is caused by an active component which is probably lipoprotein complex. Further detailed research on this is planned.

Conclusions: Non-cellular extracts of entomopathogenic *Bacillus* are effective against the various plant pathogens. The identified antimicrobial properties of non-cellular extracts of entomopathogenic can be potentially used for the elaboration of plant protection safe biopesticides.

Keywords: *Bacillus thuringiensis (BT), Brevibacillus laterosporus (BL), Lysinibacillus sphaericus (LS)*, non-cellular extract, phytopathogen, entomopathogen, safe biopesticide



INTRODUCTION

Plant protection is one of the most urgent problems today. And the pesticide application is a classic method against parasites, pathogenic microbes and other pests, which are harmful for crops. In XX century, they were one of the usable agrarian preparations in the world. And now, in the XXI century they are presented by the various classes of preparations, produced in different countries. Chemically and toxicologically, they mainly can be classified into four general groups: organochlorines, organophosphates, carbamates, and synthetic pyrethroids. The majority of them are harmful to both human health and the environment [1,2]. Their

accumulation in soil and water has very negative effects. It detrimentally impacts the fertility of farmed agricultural soils and decreases overall soil quality. Additionally, it can have potentially severe consequences for agricultural production and human health as well [3]. In these regards, the elaboration of effective methods of plant protection with the minimization of their ecological risks is one of the most urgent problems of modern green agriculture. Thus, the various techniques are being advised, including the elaboration of biopesticide, the derivatization of native antimicrobial compounds (tartaric acid derivatives, etc.), the complex application of semisynthetic compounds and biocontrol agents, etc. [4].

The development of biopesticides is one of the promising directions for advancing green agriculture. Biopesticides are based on natural-sourced components, including active compounds isolated from plant or animal tissues, bacterial cells, such as l minerals [5]. According to data provided by the United States Environmental Protection Agency (EPA), biopesticides can be categorized into microbial and biochemical pesticides, as well as plant-incorporated protectants, which operate based on different principles. Plant-incorporated protectants are synthesized in plants that have been genetically modified [6]. By August 31, 2020, there were 390 registered active ingredients for biopesticides [7, 8]. Biochemical pesticides are natural compounds with minimal toxicity, which can control the pest's population, unlike the conventional synthetic pesticides which directly deactivate or kill pests. One of the examples of biochemical pesticides are insect sex hormones (pheromones and their synthetic analogues) that disrupt mating as well as different scented plant extracts, which can attract pests to traps [9].

Microbial pesticides are based on bacteria, fungi, etc. and their active ingredients. Some of the microbial-based pesticides can target a wide range of pests, while some compounds are specific to the phytopathogens of certain species. One of the used biopesticides are the predatory (carnivorous) nematophagous fungi, which can form traps for the inactivation and the consumption of phytopathogenic nematodes [10]. One of the most common microbial pesticides is strains and subspecies of the Bacillus genus. Representatives of entomopathogenic *Bacillus thuringiensis* (Bt) are very promising. Different strains of *BT* produce unique protein mixtures that target specific insect larvae. Some strains of *Bacillus thuringiensis* (Bt) can serve as biocontrol agents for moth larvae on plants, while others target fly and mosquito larvae. The effectiveness of these microbes is due to the specific proteins they produce. Therefore, the efficiency of Bt-based preparations depends on factors such as pH, humidity, and other conditions that affect the synthesis of these active proteins [11].

To avoid the overall use of pesticides and some disadvantages associated with microbial preparations, various plant protection techniques focus on incorporating genes that encode these proteins directly into the plant genome. But the application of transgene techniques and the cultivation of GMO crops (genetically modified organisms) is not always advisable and carries some potential risks both for native biodiversity alterations and human health [12-14]. Thus, the development of novel biopesticide compositions, such as nano biopesticides, is highly relevant due to their numerous advantages in safety and efficacy compared to traditional synthetic chemical pesticides and the risks associated with transgenic products [15,16]. Some entomopathogenic microorganisms, such as *Brevibacillus laterosporus* (*BL*) and *Lysinibacillus sphaericus* (*LS*) representatives are known for their potential usage as bio pesticide components. They have several effects against phytopathogenic microbes. Also, these bacteria not only have pesticidal properties but also a wide range of other biological activities, which also makes them effective against mixed infections [17-21]. The plant world is constantly affected by different phytopathogenic microorganisms, phytophagic organisms, insects and

other pests, posing a significant threat to crop producers and agricultural products such as fruits and vegetables. The fight against such pests has been carried out for a long time and quite successfully, which resulted in the emergence and spread of pests resistant to modern plant protection means. On the other hand, modern means of crop protection are not ecologically safe, because they increase the concentration of harmful substances in the soil. The fight against resistant phytopathogens implies the search for innovative ecologically safe ways for solution of the mentioned problem [21].

In current research, the possibility of using entomopathogenic bacilli *BT, LS, BL* to suppress the growth of widespread phytopathogens form *Pectobacterium, Pseudomonas, Xanthomonas, Curtobacterium* and *Rhodococcus* genera is considered.

MATERIALS AND METHODS

Bacterial Cultures: For all the experiments the 300 strains of entomopathogenic bacteria of following species: *BT, BL, LS* and the 30 strains of phytopathogenic bacteria of following species: *P. carotovorum, P. syringae, Rh. fascians, X. beticola, X. vesicatoria, C. flaccumfaciens pathovar beticol*a were taken from the National Culture Collection of Microorganisms of the Microbial Depository Center (MDC), Scientific and Production Center (SPC) "Armbiotechnology", National Academy of Sciences, Republic of Armenia (NAS RA). The studied phytopathogens isolation sources are presented on table 1. For the maintenance and cultivation of pure cultures of studied microbes, the solid agar medium (1.8%) and LB (Luria-Bertani) liquid cultural media were used [22].

Table 1. The sources of phytopathogenic bacteria isolation.

Phytopathogenic bacterial strain	Source of isolation
Rh. fascians 8628	Strawberries (RF)
P. syringae path. lachrymans 8736	Cucumber (RF)
X. vesicatoria 8647	Tomato (RA)
P. syringae path. tabaci 8656	Tobacco (RA)
P. syringae path. lachrymans 8744	Cucumber (RA)
P. syringae path. lachrymans 8740	Melon (RA)
P. carotovorum 8702	Carrots (RA)
P. carotovorum 8718	Carrots (RA)
P. carotovorum 8705	Carrots (RA)
P. carotovorum 8717	Carrots (RA)
P. carotovorum 8756	Tomato (RA)
P. carotovorum 8764	Carrots (RA)
P. carotovorum 8690	Paprika (RA)
P. carotovorum 8694	Cabbage (RA)
P. carotovorum 8698	Tomato (RA)

The study of Antibiotic-resistance: For qualitative assessment of antibiotic resistance of entomopathogenic and phytopathogenic bacterial strains the sterile solid selective agarised media with 50 mcg/mL and the protocol concentrations of various antibiotics were applied following the generally accepted protocols [22]. For that purpose, the strains were cultivated on the obtained solid selective media (containing the appropriate concentration of each antibiotic) in aerobic conditions at the optimal temperatures of growth of the studied bacteria (at 30-37 °C in DNP-9052 LAB INCUBATOR, CNWTC thermostat), within a period of 72 h. The observation of colony growth was used for the detection of antibiotic-resistance of studied bacteria. For antibiotic-resistance assessment the 13 types of antibiotics from different generations and classes were tested on phytopathogenic and entomopathogenic bacteria. The following antibiotics were considered: Pcn/Penicillin, Amp/Ampicillin, Amx/Amoxicillin, Amc/Augmentin (amoxicillin and clavulanic acid), Cfx/Cefixime, Cro/Ceftriaxone, Stp/Streptomycin, Kan/kanamycin, Gnc/Gentamicin, Tcn/tetracycline, Cam/chloramphenicol, Cip/ciprofloxacin, Azm/ azithromycin (Produced by “Sigma”).

For the quantitative assessment of antibiotic-resistance the generally accepted protocol of disk-diffusion method was applied. The sterile filter-paper disks (d=6.33 mm) were sterilely infiltered by 10 mcL of each tested antibiotic and sterilely placed to the surface of 4.0 mm thickness agarised cultural media in Petri dishes with the appropriate bacterial suspension (a separate Petri dish and suspension prepared in sterile 0.9% NaCl physiological solution were used for each bacterial strain. The incubation and the cultivation of studied bacteria was carried out due to the thermal optimum conditions of appropriate bacteria (aerobic conditions, 30-37 °C) during 72 h[23].

The Study of antimicrobial Effect: The study of antimicrobial effect of entomopathogenic bacteria on phytopathogens was carried out using their non-cellular extracts by the disk-diffusion method [24, 25]. Non-cellular extracts of each studied strain were obtained by the centrifugation of overnight culture (30 °C, aerobic conditions, liquid cultural media) using Spin-X centrifuge tube filters (Produced by "Thermo Fisher") [26, 27]. For chemical characteristics of active compounds of entomopathogens, which are responsible for the target effects on bacterial growth the UV/VIS (ultraviolet and visual) and Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) analyses were carried out. For that purpose, Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer was applied [28, 29].

Statistical Analysis: The statistical assessment of the reliability of the experiments was carried out using generally accepted methods. All our experiments were carried out in five independent series and with three repeats for each sample. MS Excel program package was applied for the data analysis. The Standard Error of the Means (SEM) was ± 0.23–0.37. The significance was tested by the application of Student t-test and the mean of *p*-value was estimated as less than 0.05.

RESULTS

For the entomopathogenic bacteria application potential evaluation in combined and complex plant protection preparations, the resistance of the studied strains to various antibiotics was assessed. Tests were carried out for both entomopathogens and phytopathogenic bacteria. The antibiotic-resistance profiles of the most typical representatives of all the studied phytopathogenic bacteria are presented on table 2-3.

Table 2. Antibiotic resistance of phytopathogenic bacteria.

Strain		Resistance to Antibiotics (50 mcg/mL)													C
		Tcn	Cro	Cip	Cam	Gnc	Amx	Stp	Cfx	Amp	Amc	Kan	Azm	Pcn	
8736	1	R	R	R	-	R	R	-	R	R	R	-	-	R	+
8740		R	R	R	R	R	R	R	R	R	R	R	R	R	+
8744		R	R	R	-	-	R	-	R	R	R	-	R	R	+
8730		-	R	R	-	-	R	-	R	R	R	-	R	R	+
8732		-	R	-	-	-	R	-	R	-	-	-	-	-	+
8734		-	R	-	-	-	R	-	R	-	-	-	-	-	+
8742		R	R	R	-	-	R	R	R	-	-	-	-	R	+
8738		R	R	R	R	R	R	R	R	-	R	R	R	R	+
8733		-	R	-	R	-	R	-	R	R	R	-	-	R	+
8731		-	-	-	-	-	R	-	-	-	-	-	-	-	+
8656	2	R	R	R	R	-	R	R	R	-	-	R	R	R	+
8663		R	R	-	-	R	R	R	R	-	-	R	-	R	+
8657		-	R	-	-	-	R	-	R	R	R	-	-	R	+
8651	3	-	-	-	-	-	-	-	-	-	-	-	-	-	+
8653		-	-	-	-	-	-	-	-	R	R	-	-	-	+
8647		-	R	-	-	-	R	-	R	R	R	-	-	R	+
8680	4	-	-	-	-	-	-	-	-	-	-	-	-	-	+
8681		-	-	-	-	-	-	-	-	-	-	-	-	-	+
8628	5	-	R	-	-	-	R	-	R	R	R	-	-	R	+

The used antibiotics: Pcn/Penicillin, Amp/Ampicillin, Amx/Amoxicillin, Amc/Augmentin (amoxicillin and clavulanic acid), Cfx/Cefixime, Cro/Ceftriaxone, Stp/Streptomycin, Kan/kanamycin, Gnc/Gentamicin, Tcn/tetracycline, Cam/chloramphenicol, Cip/ciprofloxacin, Azm/azithromycin; C – the positive control; "+" – the growth of bacterial cultures on nutrient agar cultural media without with antibiotics; "R" – resistance to antibiotic; "-" – sensitivity to antibiotic; bacterial cultures: 1 – *P. syringae pathovar lachrymans*; 2 – *P. syringae pathovar tabaci*; 3 – *X. vesicatoria*; 4 – *C. flaccumfaciens pv. beticola*; 5 – *Rh. fascians*.

Table 3. Antibiotic-resistance of phytopathogenic *Pectobacterium carotovorum***.**

Strain	Antibiotics (50 mcg/mL)													C
	Tcn	Cro	Cip	Cam	Gnc	Amx	Stp	Cfx	Amp	Amc	Kan	Azm	Pcn	
8702	-	R	-	-	-	R	-	R	R	R	-	R	R	+
8705	-	R	R	R	-	R	R	R	R	R	R	R	R	+
8717	-	R	R	-	-	R	-	R	R	R	R	R	R	+
8718	-	-	-	R	-	R	-	R	R	R	-	R	R	+
8756	R	R	R	-	-	R	R	R	R	R	R	R	R	+
8764	R	R	-	R	-	R	-	R	R	R	-	R	R	+
8765	-	-	-	-	-	-	-	-	R	R	-	-	R	+
8690	R	R	-	-	R	R	-	R	R	R	-	R	R	+
8694	R	R	-	-	-	R	-	R	R	R	R	R	R	+
8698	-	-	-	-	-	R	-	-	R	-	-	-	R	+

The used antibiotics: Pcn/Penicillin, Amp/Ampicillin, Amx/Amoxicillin, Amc/Augmentin (amoxicillin and clavulanic acid), Cfx/Cefixime, Cro/Ceftriaxone, Stp/Streptomycin, Kan/kanamycin, Gnc/Gentamicin, Tcn/tetracycline, Cam/chloramphenicol, Cip/ciprofloxacin, Azm/azithromycin; C – the positive control; "+" – the growth of bacterial cultures on nutrient agar media without antibiotics; "R" – resistance to antibiotic; "-" – sensitivity to antibiotic.

According to the obtained data, the majority of the studied strains of *Pseudomonas, Xanthomonas, Rhodococcus, Pectobacterium* and *Curtobacterium* genera representatives demonstrate polyresistance or at least the resistance to more than one antibiotic. No strains were found to be absolutely resistant to all of the considered antibiotics, although some strains were sensitive to all of them. The obtained results are demonstrating the main cause of lack of efficiency of classical antimicrobials which can be applied in agriculture for the plant protection, against the infections caused by these microbes, due-to their high level of antimicrobial resistance even to broad spectrum antibiotics. For the next step of research, the resistance of entomopathogens, which are planned to be used against the phytopathogens was assessed (table 4).

Table 4. Antibiotic resistance of entomopathogenic bacteria (*BT, BL, LS*).

Strain		Resistance to antibiotics (50 mcg/mL)													C
		Tcn	Cro	Cip	Cam	Gnc	Amx	Stp	Cfx	Amp	Amc	Kan	Azm	Pcn	
2675	1	-	R	-	-	-	-	-	R	-	-	-	-	-	+
2670		-	R	-	-	-	R	-	R	R	R	-	-	R	+
2677		R	R	-	-	-	R	R	R	R	R	-	-	R	+
200-4	2	-	-	-	-	-	R	-	R	R	R	-	-	-	+
561-1		-	-	-	-	-	R	R	R	R	R	-	-	-	+
219-2		R	R	-	-	-	R	-	R	R	R	-	-	R	+
199-3		-	-	-	-	-	-	-	R	R	-	-	-	R	+
105-2		-	R	-	-	-	R	-	R	R	R	-	-	R	+
69-3-5		-	-	-	-	-	-	-	R	R	-	-	-	-	+
754		-	-	-	-	-	-	-	-	R	-	-	-	-	+
2728	3	R	-	-	R	-	R	R	R	R	R	-	-	-	+
2606		-	-	-	-	-	-	-	R	R	-	-	R	-	+
2631		R	-	-	R	-	R	R	-	R	R	-	-	-	+
2632		R	-	-	R	-	R	R	-	R	R	-	-	-	+
728		-	R	-	-	-	R	R	R	R	R	-	-	R	+
2614		R	-	-	-	-	-	R	R	R	-	-	-	-	+
2750		-	R	-	-	-	-	R	R	R	-	-	-	-	+
2851		-	-	-	-	-	-	R	R	-	-	-	-	-	+

Cultures of entomopathogenic bacteria: 1 – *BT*; 2 – *BL*; 3 – *LS*; C – the positive control; "+" – the growth of bacterial strains on nutrient cultural media; The used antibiotics: Pcn/Penicillin, Amp/Ampicillin, Amx/Amoxicillin, Amc/Augmentin (amoxicillin and clavulanic acid), Cfx/Cefixime, Cro/Ceftriaxone, Stp/Streptomycin, Kan/kanamycin, Gnc/Gentamicin, Tcn/tetracycline, Cam/chloramphenicol, Cip/ciprofloxacin, Azm/ azithromycin; "-" – sensitivity to antibiotic; "R" – the resistance to antibiotics.

According to the obtained data, representatives of three species of entomopathogenic bacteria also differed in their resistance profiles, even among different strains within the same species. The same was shown in tests of antibiotic-resistance of phytopathogens. Also, it should be noted that *BL* and *LS* representatives have demonstrated higher resistance than *BT* representatives, what makes them potentially more prospective in combined preparations containing antimicrobials.

Then non-cellular extracts of all the entomopathogens were obtained. Their effects against the considered phytopathogens were compared on different cultural media (table 5-7).

Table 5. Th effect of non-cellular extracts of *BL 200-4* against the phytopathogenic bacteria

Bacterial Phytopathogen	Phytopathogen growth inhibition zone (in mm) by non-cellular extract of BL 200-4			
	Extract	Dilutions of extract		
		1:1	1:10	1:100
8702	8	6	4	-
8705	9	6	5	-
8718	-	-	-	-
8764	15	10	8	-
8690	8	7	4	-
8694	8	8	5	-
8698	15	10	8	-
8628	10	8	5	-
8736	-	-	-	-
8740	13	8	8	6
8744	10	8	5	-
8656	10	7	5	-
8647	-	-	-	-

According to the obtained results the different strains have different characteristics of effect on both liquid and solid cultural media. Some strains of entomopathogens are more active against specific species of phytopathogens, while others exhibit a broad range of bactericidal effects against various species of phytopathogenic bacteria. The maximal bactericide effect against the majority of the considered phytopathogens was detected for three following strains: *BT 2675, BL 200*-4, *LS 2728*. The effect was detected for both pure extracts and their dilutions (tables 6-7).

Table 6. The Effect of *BL 200-4* non-cellular extracts against the phytopathogenic bacteria.

Bacterial Phytopathogen	Phytopathogen growth inhibition zone (in mm) by non-cellular extract of BL 200-4			
	Extract	The dilutions of Extract		
		1:1	1:10	1:100
8702	10	8	6	-
8705	10	8	7	-
8718	16	13	8	5
8764	15	13	9	4
8690	10	8	6	-
8694	10	8	6	3
8698	11	11	7	-
8628	10	8	7	-
8736	5	4	-	-
8740	10	8	4	-
8744	10	8	6	-
8656	9	7	5	-
8647	-	-	-	-

Table 7. The Effect of *BL 200-4* non-cellular extracts on various bacterial phytopathogens.

Bacterial Phytopathogen	Phytopathogen growth inhibition zone (in mm) by non-cellular extract of BL 2675			
	Extract	Dilutions of extract		
		1:1	1:10	1:100
8702	6	4	3	-
8705	9	6	5	-
8718	-	-	-	-
8764	18	12	8	-
8690	9	7	4	-
8694	10	8	3	-
8698	14	10	8	-
8628	10	8	5	-
8736	-	-	-	-
8740	13	7	7	-
8744		6	5	-
8656	10	7	4	-
8647	-	-	-	-

Then the effect of non-cellular extracts of entomopathogens *BT, BL, LS* against the phytopathogens of different species, was studied in liquid media (fig. 1).

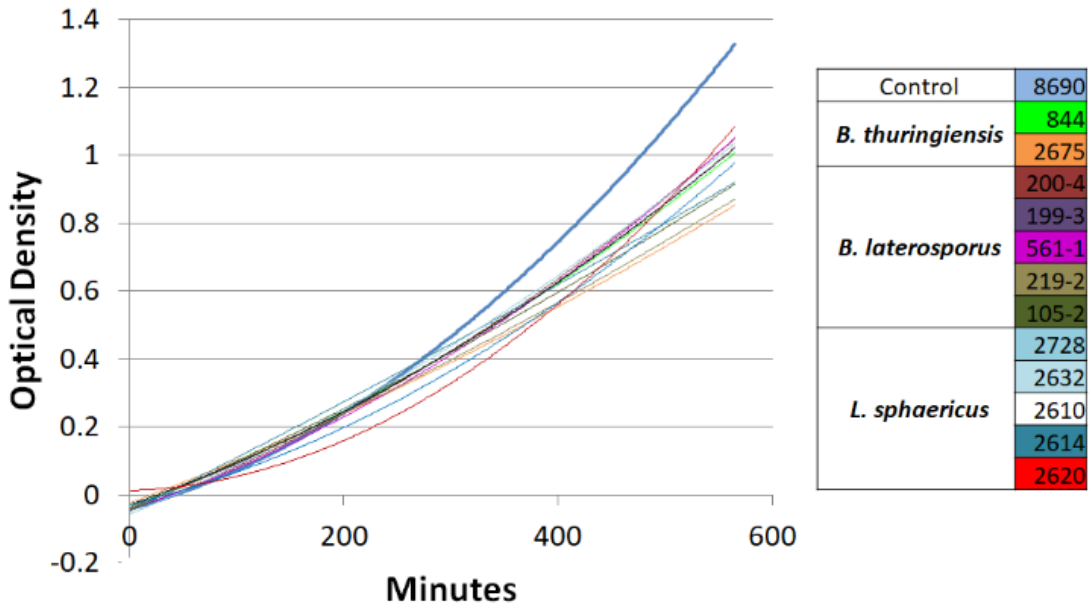


Fig. 1. Phytopathogen growth inhibition by non-cellular extracts of entomopathogens in liquid cultural media.

Also, the effect of dilutions of the considered non-cellular extracts was studied. According to the results, the growth of phytopathogenic bacteria was inhibited by up to 23%. In case of dilution effect, the growth inhibition was decreased. For better understanding the chemical nature of active components of non-cellular extracts of entomopathogenic microbes, their UV/VIS and IR analyses were carried out (Fig. 2 – 3).

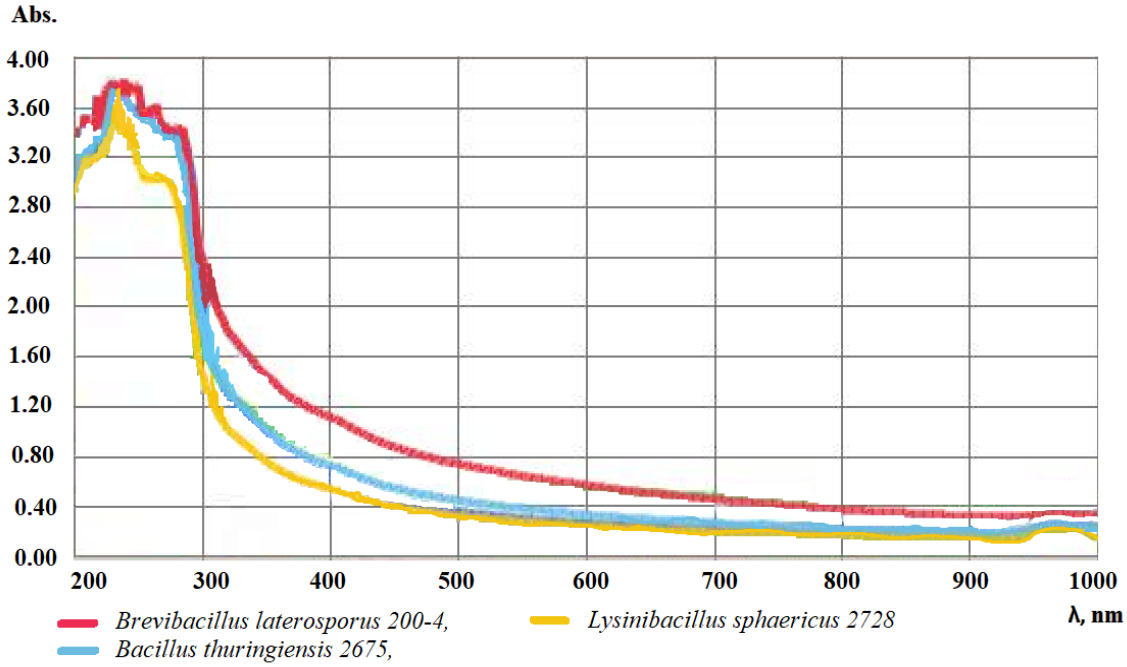


Fig. 2. Spectrophotometric analyses of non-cellular extracts of entomopathogenic bacteria: *BL 200-4, LS 2728, BT 2675*

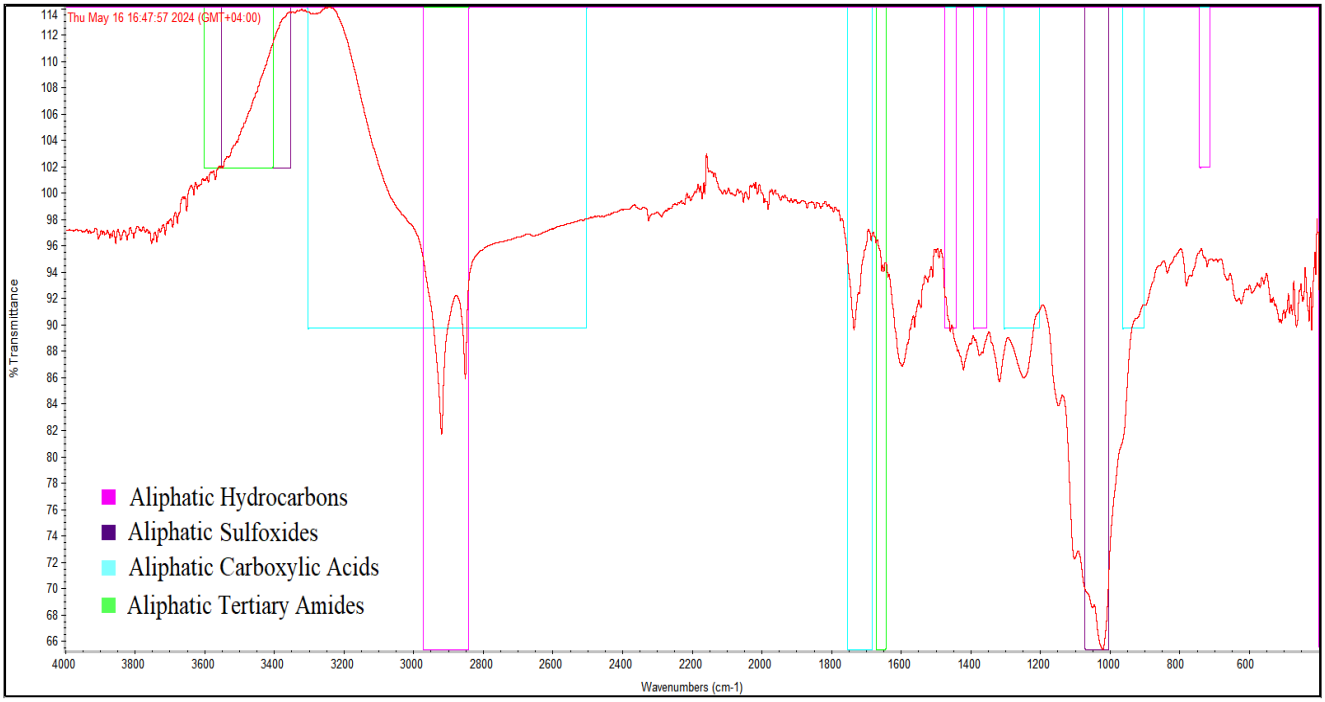


Fig. 3. ATR-FTIR spectrometry of non-cellular extract of *Brevibacillus laterosporus 200-4*

According to the obtained data, the active component of all the studied non-cellular extract might be the lipoprotein complex.

DISCUSSION

The investigation of microbiomes that promote plant protection against phytopathogens and the design of new microbiomes is highly relevant. In the present work, the screening of entomopathogens, which are active against the phytopathogens was carried out with the aim to use them in creating special communities to combat various bacterial pests of crops [30, 31]. As a result of experiments in solid and liquid media, the ability of 42 strains of spore-forming entomopathogens to suppress

the growth of 15 phytopathogenic bacteria was investigated. According to the presented data, some strains of *BT*, *BL* and *LS* display anti-phytopathogenic activity. The strain of *BT 2675* suppresses the growth of 5 strains of *P. carotovorum* phytopathogens. *BL 105-2* and *BL* 200-4 suppress the growth of 9 strains of *P. carotovorum*. According to the obtained data, *BL*105-2 and *LS* 2728 strains suppress the growth of all phytopathogens of this group of microorganisms, while *BL 219-2, BL 200-4* and several *LS* strains have a wide range of effects on phytopathogens of this group. Among the investigated strains of *BT*, growth inhibitors of *P. syringae pv. lachrymans 8744*, *8740* phytopathogens were not identified. The strains of *BT* 2675, *BL 105-2, BL 200-4, LS 2728* that can suppress the growth of 15 strains of various phytopathogens have been identified, and it was discovered that the strains differ in their spectrum of action. *BT 2675, BL 105-2, BL 200-4, LS 2728* have the broadest spectrum of phytopathogen suppression. *P. carotovorum 8702* proved to be the most resistant to entomopathogens, its growth was suppressed only by *BT 2675*. In some cases, a certain specificity of the interaction of the entomopathogen-phytopathogen can be observed. For example, *P. syringae path. lachrymans 8744*, *8740* phytopathogens showed resistance to all tested *BT* strains, although these strains are sensitive to almost all the entomopathogens of *BL* and *LS*. The representatives of *X. vesicatoria 8647* and *Rh. fascians 8628* exhibited greater resistance to entomopathogens in comparison with other phytopathogens. Their growth is suppressed by a relatively smaller number of entomopathogens [32]. When the extracts were appropriately diluted, the results showed an inhibitory effect. Also, the effect was present in the case of both freezing of extract (4 °C, 30 min), and heating (90 °C, 30 min). The dilution degree is directly related to inhibitory effect decrease. Thus, it might be concluded about the several thermal stability diapasons of active components. Due to the literature data, similar compounds were identified in various bacteria [33, 34].

According to ATR-FTIR spectroscopy data, the studied non-cellular extract exhibits peaks characteristic of lipid components and radicals of sulfur-containing amino acids within peptides or proteins [35]. Thus, the studied components in all the considered bacteria could potentially be discussed as a complex compound, possibly a lipopeptide or lipoprotein derivative. Due to the obtained data the observed compounds are like lipid and protein complexes. According to the literature data, the proteins have a peak in zone of 280 nm, such as the aromatic amino acids and nucleic acids in 260 nm. Also, the absorption in 220 –230 nm region is present due to the amide bonds in the protein's peptide backbone. In comparison to proteins, the lipids have a comparably weak absorption in UV peaks in zone 200 – 300 nm. The main chromophore groups of lipids are unsaturated fatty acids residues, which ensure the absorption peaks forming on spectrograms. For example, for conjugated diens containing fatty acids the peaks are in zone around 230 – 235 nm, while for conjugated triens containing ones it's around 260 – 270 nm. Non-conjugated ones have no strong absorption peaks in UV light. Also, the sterols can have a peak, such as cholesterol which has a peak of absorption in zone around 205 nm. Thus, due to the obtained spectral data it might be concluded that the active component of non-cellular extract of the studied entomopathogenic bacteria might be from the lipoprotein-like or lipopeptide-like complex compounds class. The literature data evidences that the compounds with the analogous effect are lipopeptides [36].

Taking in consideration all the data, which was obtained within a period of research, it might be concluded, that the considered entomopathogens and their active components of non-cellular extracts are recommended for further research as the prospective agents of bacterial phytopathogens biocontrol.

In summary, the obtained data reveal that different strains of entomopathogens can exhibit either a broad spectrum of antimicrobial effects or a narrow spectrum with high bactericidal activity against several species. Thus, it might be used in case of mixed infections, which are very common in worm humid agroclimatic zones. This is very important because these agroclimatic zones provide optimal conditions for the cultivation of most vegetables, crops, fruit trees, and other plants. The maximal effect was indicated for the strains *BL 200-4, LS 2728, BT 2675.* The investigated antimicrobial influence of the considered entomopathogenic representatives of *BL, LS* and *BT* can potentially be used for crop protection against antibiotic-resistant bacterial pathogens. Besides, due to the high level of antimicrobial resistance of entomopathogenic representatives of *BL, LS* and *BT*, they can be used in combining preparations simultaneously with some fungicidal antimicrobials. The same approach can also be used for protecting medicinal plants, which is important due to their potential application in the development of innovative functional foods [37,38]. Further detailed research is planned to explore the antiphytopathogenic components of non-cellular extracts from the studied entomopathogens, including their potential fungicidal and nematocidal properties against phytopathogenic fungi (e.g., *Monilinia fructigena*, *Taphrina deformans*) and phytopathogenic nematodes (e.g., *Globodera rostochiensis*). This study may also be particularly promising in investigating their effects on pest insect larvae such as *Pieris brassicae* and *Manduca quinquemaculata*.

Abbreviations: Amc, Augmentin (amoxicillin and clavulanic acid); Amp, Ampicillin; Amx, Amoxicillin; ATR-FTIR, Attenuated Total Reflectance Fourier Transform Infrared spectroscopy; Azm, azithromycin; BL, *Brevibacillus laterosporus*, BT, *Bacillus thuringiensis*; Cam, chloramphenicol; Cfx, Cefixime; Cro, Ceftriaxone; Cip, ciprofloxacin; EPA, Environmental Protection Agency; Gnc, Gentamicin; GMO, genetically modified organisms; Kan, kanamycin; LB, Luria-Bertani; LS, *Lysinibacillus spaericus*; Microbial Depository Center, MDC; MPA, Meat-Peptone Agar; NAS, National Academy of Sciences; Pcn, Penicillin; PIPs, Plant-Incorporated-Protectants; RA, Republic of Armenia; RF, Russian Federation; SPC, Scientific and Production Center; Stp, Streptomycin; Tcn, tetracycline; UV/VIS, Ultraviolet and Visual spectroscopy.

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