doi: https://doi.org/10.15407/microbiolj83.04.054

LYTIC EXOENZYMES OF SOIL STRAINS OF *BACILLUS* REPRESENTATIVES AND MANIFESTATIONS OF THEIR BIOLOGICAL ACTIVITY

Yu.V. Korzh, I.V. Dragovoz, L.V. Avdeeva

Zabolotny Institute of Microbiology and Virology, NAS of Ukraine,
154 Acad. Zabolotny Str., Kyiv, 03143, Ukraine
e-mail:Jullinka35@meta.ua

Bacteria of the genus Bacillus make up a significant (8–12 %) part of the soil microbiome. Manifestation of their biological activity, in particular, the antagonistic and lytic activity against other microorganisms directly depends on their exometabolites. According to the literature, such properties of soil bacteria of the genus Bacillus thus can be related to their various lytic exoenzymes. ***Aim.*** *To evaluate the role of lytic exoenzymes of the studied soil bacteria strains of the genus Bacillus in the manifestation of their biological (antagonistic, lysing) activity.* ***Methods.*** *The antagonistic activity of bacteria strains of the genus Bacillus against phytopathogenic micromycetes was determined by the method of double culture in Petri dishes on potato-glucose agar. For qualitative analysis of the presence of extracellular enzymes, strains of bacteria of the genus Bacillus were plated on Petri dishes with solid mineral-salt medium and a suitable substrate inducer. The ratio of the diameter of substrate hydrolysis zone to the diameter of the colony was taken as the relative enzymatic activity of the culture. Bacteriolytic activity of the studied strains was determined by the change in optical density of living cells of phytopathogenic bacteria suspension at 540 nm.* ***Results.*** *Six strains of bacteria of the genus Bacillus were selected by the results of preliminary screening, with at least five types of lytic activity, namely proteolytic, chitinase, amylolytic, cellulase, and xylanase of different levels (low, average, high). Analysis of the antagonistic activity of the selected strains of bacteria of the genus Bacillus to the main groups of phytopathogenic bacteria (six test cultures) singled out the strain Bacillus sp. 41 for a careful study of the nature and spectrum of its antagonism. Analysis of the level of antagonistic activity of the selected Bacillus strains against the phytopathogenic micromycetes showed that the minimum decrease of antagonism (the decrease of growth inhibition zones) during the observation period (at the 3rd and 7th days) was in Bacillus sp. 41 strain. Therefore, only this strain showed a stable and relatively wide range of antagonistic activity against phytopathogens of bacterial and fungal etiology. The nature of this antagonism is probably complex and conditioned by the participation of various biochemical mechanisms, in particular, the synthesis of a complex of lytic exoenzymes. To assess the lysing activity of Bacillus strains, three strains with the highest proteolytic and cellulolytic activity of exoenzymes were taken from the six previously chosen. Only Bacillus sp.1913 strain showed high (70 %) lytic activity against gram-negative polyphagous phytopathogen Pseudomonas syringae pv. syringae UCM B-1027^{T}. Such activity of the strain did not manifest against the rest of the phytopathogenic test cultures. The high lytic activity of Bacillus sp. 1913 strain may be associated with high activity of exogenous proteases and cellulases of the lytic complex, which is quite consistent with the literature data on the lytic activity of bacteria of the genus Bacillus.* ***Conclusions****. The spectrum and activity of lytic exoenzymes of strains of the studied soil bacteria of the genus Bacillus indicate the indirect participation of these enzymes in the manifestation of biological activity (antagonistic and lytic).*

Keywords: bacteria of the genus Bacillus, lytic exoenzymes, bacterial and fungal phytopathogens, antagonistic and lytic activity.

The microorganisms of soil, including rhizosphere, that are known to have biocontrol properties, make up less than 10 % of the total population of soil microbiota [1, 2]. In this group of microorganisms, aerobic spore-forming bacteria of the genus *Bacillus* deserve special attention as potential biocontrol agents. The manifestation of their biological activity against other microorganisms directly depends on the spectrum of their synthesized exometabolites. In particular, this applies to the ability of *Bacillus* strains to exhibit antagonistic and lytic activity.

Such properties of soil strains of bacteria of the genus *Bacillus* may be related to their various lytic enzymes that they excrete into the environment. Thus, chitinases-producing strains of *Bacillus* sp. participate in the manifestation of antifungal activity [3, 4]. The ability of bacteria of the genus *Bacillus* to synthesize exoglucanases was also revealed and their participation in biocontrol was proved [5]. It is known that some thermophilic bacteria of the genus *Bacillus* synthesize lytic exoenzymes capable of destroying the cell walls of certain species of yeast and gram-negative bacteria [6]. In particular, a complex preparation of lytic enzymes was obtained from the culture liquid of *B. licheniformis* 234 strain, which showed high proteolytic, glucanase and glycosidase activity. It is concluded that the main role in the lytic process belongs to the proteolytic enzymes [6].

Due to the presence of a sufficient number of various substrate inducers in the soil, lytic enzymes that can destroy the residues of bacterial or fungal cell walls are widely represented in bacteria of the genus *Bacillus* [7]. Their main function is to transform available carbon sources in a variety of organic substrates [8]. Therefore, the role of these enzymes remains unclear, in particular in the lysis of living microorganisms. The involvement of these bacterial exoenzymes in the manifestation of microbial antagonism has not been fully elucidated. The biotic regulation of antagonism of bacteria of the genus *Bacillus*, due to lytic enzymes (glycosidases, peptidases, etc.), is also not entirely clear [9].

Therefore, **the aim** of our work was to evaluate the participation of lytic exoenzymes of the studied soil strains of bacteria of the genus *Bacillus* in the manifestation of their biological (antagonistic and lytic) activity.

Materials and methods. The soil strains of *Bacillus* sp. A1, *Bacillus* sp. A 23/2, *Bacillus* sp. 24, *Bacillus* sp. 41, *Bacillus* sp. 15-(2)-34, *Bacillus* sp. 1913 and *B. coagulans* MC-11 were taken for the study from the Collection of Microorganisms of the Department of Antibiotics of D.K. Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine.

The antagonistic activity of *Bacillus* strains against phytopathogenic bacteria (FPB) was determined by the method of radial strokes on potato agar [10]. Strains from the collection of the Department of Phytopathogenic Bacteria and the Ukrainian Collection of Microorganisms of the IMV NAS of Ukraine were used as test cultures: *Clavibacter michiganensis* 10_2, *Xanthomonas campestris* pv. *campestris* 8003b, *Pseudomonas syringae* pv. *syringae* UCM B-1027^T, *P. fluorescens* 8573, *Agrobacterium tumefaciens* 8628, *Pectobacterium carotovorum* subsp. *carotovorum* UCM B-1095^T. The level of antagonistic activity of *Bacillus* strains was determined by the diameter of the zones of growth inhibition (ZGI) of test cultures of phytopathogenic bacteria. If that parameter was 15 mm or more, the *Bacillus* strain was considered highly active; if it ranged from 10 to 14 mm, the level of activity of the *Bacillus* strain was average; at 1–9 mm of ZGI diameter, the level of activity of the *Bacillus* strain was low [11].

The antagonistic activity of *Bacillus* strains against phytopathogenic micromycetes was determined by the method of double culture in Petri dishes on potato-glucose agar [12]. Strains of pathogenic micromycetes of cereal diseases, *Fusarium graminearum* 9G and *Bipolaris sorokiniana* from the collection of the Department of Antibiotics of the IMV NAS of Ukraine were used as test cultures. For diameter of ZGI in the range of 5–9 mm, the antagonistic activity of studied strains of bacilli was considered low, for 10–19 mm it was average, for 20 mm and more it was high. If ZGI of micromicetes was 0–4 mm in diameter, the *Bacillus* culture was considered inactive.

For qualitative analysis of the extracellular enzymes, *Bacillus* strains were plated on Petri dishes with solid mineral salt medium of the following composition (g/L): $K_2HPO_4 \cdot 3H_2O$ – 6.0; KH_2PO_4 – 2.0; $MgSO_4 \cdot 7H_2O$ – 0.1; $(NH_4)_2SO_4$ – 1.4; agar-agar – 20.0; distilled water – up to 1.0 L; pH 7.0–7.2. The following induction substrates were used as a carbon source: starch (0.5 %) for amylolytic activity; gelatin (0.5 %) and casein (0.5 %) for proteolytic activity; xylan (0.5 %) for xylanolytic activity; Na^+ CMC-cellulose (0.5 %) for cellulolytic one; olive oil (0.5 %) for lipolytic activity; pectin (0.5 %) for pectinolytic activity; colloidal chitin (0.5 %) for chitinolytic activity. The cultures of microorganisms were incubated in a thermostat at a temperature of 37 °C for three days. Zones of substrate hydrolysis around a colony were evaluated visually and measured in millimeters. The ratio of the diameter of the zone of substrate hydrolysis to the diameter of the colony was taken as the relative enzymatic activity of the culture.

Bacteriolytic activity of the studied strains was determined by the change in optical density at 540 nm in suspension of living cells of phytopathogenic bacteria [13]. The activity was expressed as a percentage decrease in the suspension density.

The calculation was performed according to the formula:

$$E, \% = \frac{A - A_1}{A} \times 100 , \qquad [13]$$

where A – is the initial suspension density at 540; A_1 – is the final suspension density at 540 nm.

The experiments were performed in triplicate; the obtained data were processed statistically using a software package Microsoft Excel. Differences in averages were considered significant at a level of $P < 0.05$.

Results. According to the results of screening for exogenous enzymatic activity of 12 strains of bacteria of the genus *Bacillus*, six strains were found that showed relatively high lytic activity on different substrates (casein, gelatin, chitin, starch, CMC-cellulose, and xylan). These strains were able to utilize five types of substrate producing five classes of relevant lytic enzymes, namely proteases, chitinases, amylase, cellulase, xylanase. Data on the relative enzymatic activity of the strains are shown in Table 1.

Table 1

Exogenous enzymatic activity of bacteria of the genus Bacillus

Strains of *Bacillus* sp.	Relative enzymatic activity, D_z/D_c					
	Proteolytic		chitinase	amylolytic	cellulase	xylanase
	caseinolytic	gelatinase				
A1	3.6±0.16	4.2±0.16	1.0±0.09	3.0±0.15	4.9±0.14	1.3±0.14
A23/2	2.5±0.13	3.2±0.16	1.0±0.12	3.7±0.16	2.8±0.13	1.3±1.13
24	0	1.0±0.12	1.0±0.11	2.1±0.11	2.7±0.17	3.7±0.16
41	3.8±0.16	4.3±0.13	1.2± 0.06	2.7±0.13	4.4±0.12	1.2±0.11
15-(2)-34	2.6±0.13	3.1±0.14	2.6±0.13	2.0±0.11	1.7±0.11	3.5±0.16
1913	2.9±0.14	5.5±0.13	2.3±0.11	2.9±0.15	2.7±0.13	5.2±0.12

Legend: D_z/D_c is the relative activity of culture calculated as the ratio of the diameter of the zone of substrate hydrolysis to the diameter of the colony.

In almost all selected strains with the exception of *Bacillus* sp.24, high and average levels of relative activity of exoenzymes (D_z/D_c in the range of 2 to 5) were observed. At the same time, a fairly low level of chitinase activity in four of the six selected strains (*Bacillus* sp.A1, *Bacillus* sp.23/2, *Bacillus* sp.24, *Bacillus* sp.41), and exoxylanase activity in three of six strains (*Bacillus* sp.A1, *Bacillus* sp.23/2, *Bacillus* sp.41) was noted.

Notably, all studied strains of bacteria of the genus *Bacillus* did not show pectinesterase, polygalacturonase, and lipase activity.

Thus, the spectrum of synthesized exoenzymes and the level of their activity may indicate the wide potential of selected *Bacillus* strains to metabolize different substrates, which is probably related to their saprotrophic life style in soil. All studied strains of bacteria of the genus *Bacillus* are soil microorganisms isolated from different regions of Ukraine. Hence, the spectrum and level of activity of their exoenzymes may indicate their high ecological plasticity.

The next step was to investigate the antagonism of selected strains against test cultures of phytopathogenic bacteria and micromycetes. Antagonistic activity of the studied strains against phytopathogenic microorganisms is shown in Table 2.

The vast majority of strains were weakly antagonistic against test cultures, with some exceptions. In particular, the strain *Bacillus* sp.A1 showed an average level of antagonism against *X. campestris* (a polyphagous causative agent of vascular bacteriosis), and the strain *Bacillus* sp.23/2 was similarly antagonistic against *P. carotovorum* (a causative agent of soft tissue rot). Of particular note is the antagonistic activity of the strain *Bacillus* sp.41 against the test cultures of phytopathogenic bacteria.

This strain was highly antagonistic (ZGI = 22–27 mm) to *P. carotovorum* and *X. campestris* and moderately antagonistic (ZGI = 15 mm) to *A. tumefaciens* (polyphagous pathogen causing bacterial cancer). The test cultures of phytopathogenic bacteria represented the main groups of the most harmful and dangerous microorganisms. Hence, the strain *Bacillus* sp.41 deserves attention for further careful study of the nature and spectrum of its antagonistic activity.

In the study of antagonism of selected *Bacillus* strains against micromycetes *F. graminearum* 9G and *B. sorokiniana*, ZGI were evaluated on the 3rd and 7th days of passage of both cultures. The results are shown in Table 3.

Table 2

Antagonistic activity of *Bacillus* sp. strains against test cultures of phytopathogenic bacteria

Test cultures of phytopathogenic bacteria	Studied strains of *Bacillus* sp.					
	A1	A23/2	24	41	15-(2)-34	1913
	Growth inhibition zones of phytopathogenic bacteria, mm (M±m)					
Pseudomonas syringae pv. *syringae* UCM B-1027^{T}	0	8.0±1.7	0	0	3.0±1.1	2.0±0
Pseudomonas fluorescens 8573	2.3±0.7	3.0±1.3	0	6.3±4.0	3.3±0.6	2.0±0
Pectobacterium carotovorum subsp. *carotovorum* UCM B-1095^{T}	4.0±0.2	12.7±1.3	3.0±0	22.0±3.0	3.0±1.3	2.0±0
Xanthomonas campestris pv. *campestris* 8003b	15.0±3.5	11.0±0	4.0±0.7	26.7±4.7	6.3±0.7	3.3±1.3
Clavibacter michiganensis subsp. *michiganensis* 10_2	5.0±1.1	3.7±0.7	7.0±1.1	5.3±0.7	7.0±1.1	7.0±0
Agrobacterium tumefaciens 8628	3.0±0.7	0	0	14.7±5.6	3.3±1.3	2.0±0

Table 3

Antagonistic activity of bacteria of the genus *Bacillus* to phytopathogenic micromycetes

Strains	Growth inhibition zones, mm (M±m)			
	F. graminearum 9G		*B. sorokiniana*	
	3rd day	7th day	3rd day	7th day
Bacillus sp .A_1	9.3 ± 2.5	5.3 ± 1.0	20.0 ± 1.5	12.3 ± 0.9
Bacillus sp. A 23/2	7.2 ± 1.6	4.2 ± 1.4	23.0 ± 4.3	14.8 ± 2.2
Bacillus sp. 24	8.5 ± 1.0	4.0 ± 0.8	16.8 ± 2.2	11.0 ± 1.8
Bacillus sp. 41	11.7 ± 1.7	8.5 ± 0.5	20.0 ± 2.1	15.3 ± 1.2
Bacillus sp. 15-(2)-34	1.3 ± 0.7	0	4.2 ± 1.8	0
Bacillus sp. 1913	1.2 ± 0.9	0	3.1 ± 1.1	0

Only the strain *Bacillus* sp.41 was moderately antagonistic against *F. graminearum* 9G after three days (ZGI of pathogen = 12 mm), the other strains were weakly antagonistic. On the 7th day of observations, the antagonistic activity of all studied strains decreased significantly, and in strains *Bacillus* sp.15-(2)-34, *Bacillus* sp.1913 it disappeared completely. At the same time, the level of antagonistic activity of the strain *Bacillus* sp.41 fluctuated within the average.

Regarding *B. sorokiniana*, the antagonistic activity of the studied strains after three days of observation was as follows: it was high in strains of *Bacillus* sp.A1, *Bacillus* sp.A23/2, *Bacillus* sp.41, average in strain *Bacillus* sp.24, and low in the other strains. On the 7th day of observations, the antagonistic activity slightly decreased. In particular, strains of *Bacillus* sp.23/2 and *Bacillus* sp.41 showed average, and strains of *Bacillus* sp.A1 and *Bacillus* sp.24 low level of antagonism, in other strains the antagonism was absent.

Therefore, only the strain *Bacillus* sp.41 of the studied strains of *Bacillus* sp. deserved attention based on the obtained data on the antagonism to phytopathogenic microorganisms. After all, this strain showed an average level of antagonism against *F. graminearum* 9G on both the 3rd and 7th days, and relatively high antagonism (15.3–20.0 mm) against *B. sorokiniana*. Strains *Bacillus* sp.A1 and *Bacillus* sp.A23/2 also were significantly (12–23 mm) antagonistic against *B. sorokiniana*, which is considered a superficial biotrophic polyphagous phytopathogen. At the same time, the level of antagonism of these strains was quite low (4–9 mm) against *F. graminearum* 9G, which is a more stable and adapted polyphagous biotroph. Therefore, among the selected strains of bacteria of the genus *Bacillus*, the least reduction of antagonism against phytopathogenic micromycetes was found only in the strain *Bacillus* sp.41.

Thus, only the strain *Bacillus* sp.41 of the six studied strains of bacteria of the genus *Bacillus* with a wide range of activity of lytic exoenzymes showed real and relatively wide range of antagonistic activity to phytopathogens of bacterial and fungal etiology. According to literature, the nature of this antagonism can be complex and formed by various biochemical mechanisms: the synthesis of

antibiotic compounds, a complex of lytic enzymes, siderophores, pigments, and so on [13].

It is known that bacteria of the genus *Bacillus* can lyse living cells of microorganisms of different systematic groups, in particular, gram-negative bacteria of the genus *Pseudomonas* [6]. The complex of exoenzymes of these *Bacillus* bacteria includes enzymes with proteolytic and α- and β-glucanase activity, which are able to hydrolyze the rigid layer of peptidoglycans of *Pseudomonas* [14]. The proteolytic activity of exoenzymes is known to play a major role in the lysing ability of the exogenous enzyme complex of *Bacillus* cells. Therefore, the next step was to assess the lysing activity of *Bacillus* strains selected by spectrum and activity of exogenous lytic enzymes. To study the lysing activity, three of the previously selected six strains of soil bacteria of the genus *Bacillus* that showed high proteolytic and cellulosolytic activity were taken, namely *Bacillus* sp.A1, *Bacillus* sp.41, and *Bacillus* sp.1913. Six test cultures of phytopathogenic microorganisms were used as study objects to evaluate the lysing activity of exoenzymes of the bacteria of the genus *Bacillus*. The results are shown in Table 4.

Table 4

Lysing activity of strains of bacteria of the genus *Bacillus* against the test cultures of phytopathogenic bacteria

Test cultures of phytopathogenic bacteria	Lysing activity (%)		
	Bacillus sp. A1	*Bacillus* sp. 41	*Bacillus* sp. 1913
Pseudomonas syringae pv. *syringae* UCM B-1027^{T}	0	0	70.0
Pseudomonas fluorescens 8573	0	0	0
Pectobacterium carotovorum subsp. *carotovorum* UCM B-1095^{T}	0	2.5	0
Xanthomonas campestris pv. *campestris* 8003b	2.0	2.0	0
Clavibacter michiganensis subsp. *michiganensis* 10_2	2.0	2.0	0
Agrobacterium tumefaciens 8628	0	5.0	0

Only the strain *Bacillus* sp.1913 showed high (70%) lytic activity against the gram-negative bacterium *P. syringae* pv. *syringae* UCM B-1027^{T}. Against the rest of the test cultures, this strain was not active. The lytic activity of the other two strains of bacteria of the genus *Bacillus* (*Bacillus* sp.A1, *Bacillus* sp.41) was, firstly, very low (2–5%), and secondly, manifested only against the bacteria of the genera *Pectobacterium*, *Xanthomonas*, *Clavobacter* and *Agrobacterium*.

Thus, high (70 %) lysing activity is shown in the strain *Bacillus* sp.1913, which has a high level of proteolytic and cellulolytic activity against the phytopathogenic polyphagous strain *P. syringae* pv. *syringae* UCM B-1027^{T}. Our results confirm the published data on such a manifestation of biological (lysing) activity of some bacteria of the genus *Bacillus* [6].

Discussion. Antagonistic soil bacteria of the genus *Bacillus* are able to synthesize a wide range of exometabolites of different physiological direction, such as antibiotics, vitamins, enzymes, phytohormones, etc. [15–17]. In particular, the synthesis of certain exoenzymes by bacteria of the genus *Bacillus* increases the bioavailability of mineral compounds (organo- and mineralophosphates) for own needs and plants [18]. In turn, the ability to synthesize such hydrolytic enzymes as chitinase and glucanase enhances the manifestation of their antagonistic activity to phytopathogenic micromycetes [4]. In addition, according to Melentyev et al., about 19 % of the total population of soil strains of bacteria of the genus *Bacillus* are able to fix atmospheric nitrogen due to the presence of nitrate-reductase complexes [4, 19], which improves nitrogen nutrition of plants.

Using screening, we selected six strains of bacteria of the genus *Bacillus* with high and average levels of exogenous enzymatic activity and capable of hydrolysis of five types of substrate. The spectrum and activity of exoenzymes of these strains of bacteria of the genus *Bacillus* indicate their high adaptive potential for the metabolism of organic residues. This characteristic of the strains is probably associated with their saprotrophic life style in a particular econiche (soil). In the study of antagonistic activity of selected strains of bacteria of the genus *Bacillus*, only one strain, namely *Bacillus* sp.41, showed high and average levels of antagonism to three of the six test cultures of phytopathogenic bacteria, which are considered

the most harmful. Given that the strain *Bacillus* sp.41 is also characterized by a significant activity of certain classes of exoenzymes, in particular, proteolytic and cellulolytic, indirect participation of exoenzymes is not ruled out in the manifestation of its antimicrobial activity. This assumption is supported by studies of other authors. In particular, it has been shown that some strains of *B. subtilis* that synthesize proteases are able to lyse cells of gram-positive and gram-negative microorganisms [20]. A complex of bacteriolytic exoenzymes of bacteria of the genus *Bacillus* with similar properties is also described by Lin Chi-li et al. [21]. The synthesis of lytic enzymes is considered an inducible process [22], which involves different classes of hydrolytic enzymes [23]. The strain *Bacillus* sp.41 studied in present study showed a medium and high level of antagonism to three of the six test cultures of phytopathogenic bacteria and had a high activity of exoenzymes, namely proteases and cellulases. Thus, we can assume the participation of the latter in the antibacterial activity of the strain. At the same time, it should be noted that the role of individual exoenzymes in the manifestation of antimicrobial properties by bacteria of the genus *Bacillus* requires further thorough research.

In the study of antagonism of the selected strains of bacteria of the genus *Bacillus* against two phytopathogenic micromycetes (polyphagous biotrophs), only the strain *Bacillus* sp.41 on the 3rd and 7th day of observations was moderately antagonistic against *F. graminearum* 9G and relatively highly antagonistic against *B. sorokiniana*. Therefore, among the studied strains of bacteria of the genus *Bacillus*, a minimum decrease in antagonistic activity against phytopathogenic micromycetes is shown only in the strain *Bacillus* sp.41. The literature presents quite contradictory data on the participation of certain exoenzymes in the manifestation of antagonism by bacteria of the genus *Bacillus*. In particular, the important role of chitinases is pointed out in the manifestation of antifungal activity of the strain *Bacillus* sp.739 [24]. On the other hand, the analysis of mycolytic and antifungal activity of exometabolites of 26 soil strains of the antagonistic bacteria of the genus *Bacillus* did not reveal any correlation between these two indicators [7]. At the same time, the bacterial strains with high antifungal activity against *F. graminearum* showed the ability to synthesize several classes of hydrolytic enzymes [25]. Due to the fact that the enzymes of the mycolytic complex of bacteria of the genus *Bacillus* do not have a direct negative effect on the growth of phytopathogenic fungi *in vitro*, some researchers evaluate their indirect participation in enhancing the antifungal activity of certain metabolites, including antibiotics, in 10–70 % [26]. Therefore, the antifungal activity of the strain *Bacillus* sp.41 is probably due to the synthesis of low molecular weight antibiotic compounds. Also, different classes of exoenzymes (chitinase, protease, etc.) in one way or another may enhance the action of antibiotic compounds of the antagonist strain.

In further work, the lysing activity was studied on three strains of bacteria of the genus *Bacillus* that showed relatively high exogenous enzymatic activity (in particular, proteolytic, amylolytic, cellulolytic). Only the strain *Bacillus* sp.1913 showed high (70 %) lysing activity against gram-negative phytopathogenic strain-polyphage of *P. syringae* pv. *syringae* UCM B-1027^{T}. It showed low antibacterial and very low antifungal activity against the studied test cultures of phytopathogens. There are reports of the participation of bacterial exoenzymes in the lysis of cell membranes of phytopathogenic micromycetes [7], yeast and gram-negative bacteria, in particular, of the genus *Pseudomonas* [6]. It has been found that the main role in the lysis process is played by proteolytic enzymes of exogenous hydrolase complex [13]. Such complex may include different classes of hydrolases: glucanases, lyases, proteases, amylases, acetylhexosaminidases and/ or other enzymes with different substrate specificity [27]. Information on the participation of exoenzymes in the manifestation of lysing activity of bacteria of the genus *Bacillus* is also quite contradictory. Some authors believe that the ability to synthesize exoenzymes by bacteria of the genus *Bacillus* is related to their competition for econiche, which is realized by expanding the variability of available food sources [28]. Our results of high lysing activity of *Bacillus* sp.1913 strain are noteworthy because, firstly, it is expressed against the classical polyphagous phytopathogen, which is common in Ukraine on different cultures. Secondly, given the peculiarities of plant infection with *P. syringae* strains (airborne, superficial and wound infection), the selected strain of bacteria of the genus *Bacillus* can be considered as a potential agent for plant biocontrol. It may be considered as the basis of a biological preparation; however, this requires further careful studies of the lysing activity of the strain against various strains and/or pathogens of *P. syringae*.

Conclusions. The obtained results indicate the indirect participation of exoenzymes of the studied strains of bacteria of the genus *Bacillus* in the manifestation of their biological (antagonistic and lysing) activity. The particularities of this participation of exoenzymes remain the subject of further research.

ЛІТИЧНІ ЕКЗОФЕРМЕНТИ ҐРУНТОВИХ ШТАМІВ БАКТЕРІЙ РОДУ *BACILLUS* І ПРОЯВ ЇХ БІОЛОГІЧНОЇ АКТИВНОСТІ

Ю.В. Корж, І.В. Драговоз, Л.В. Авдєєва

Інститут мікробіології і вірусології ім. Д.К. Заболотного НАН України, вул. Академіка Заболотного, 154, Київ, 03143, Україна

Резюме

Бацили складають значну (8–12%) частину мікробіому ґрунту. Прояв їх біологічної активності по відношенню до інших мікроорганізмів безпосередньо залежить від їх екзометаболітів. Зокрема, йдеться про здатність бацил до антагонізму та їх лізуючу активність. За даними літератури, такі властивості ґрунтових бацил можуть бути пов'язані зі спектром і активністю літичних екзоферментів. **Мета.** Оцінити роль літичних екзоферментів досліджених ґрунтових штамів бацил у прояві їх біологічної (антагоністичної, лізуючої) активності. **Методи.** Антагоністичну активність штамів бацил до фітопатогенних бактерій визначали методом радіальних штрихів на картопляному агарі. Антагоністичну активність штамів бацил до фітопатогенних мікроміцетів визначали методом подвійної культури в чашках Петрі на картопляно-глюкозному агарі. Для якісного аналізу наявності позаклітинних ферментів штами бацил висівали на чашки з твердим мінерально-сольовим середовищем і відповідним субстратом-індуктором. За відносну ферментативну активність культури брали відношення діаметра ореолу гідролізу субстрату до діаметру колонії. Бактеріолітичну активність досліджуваних штамів визначали за зміною оптичної густини при 540 нм суспензії живих клітин фітопатогенних бактерій. **Результати.** За результатами попереднього скринінгу відібрано шість штамів бацил, що проявляють, принаймні, п'ять типів літичної активності різного рівня (низький, середній, високий), а саме: протеолітичну, хітиназну, амілолітичну, целюлозолітичну, ксиланазну. Аналіз антагоністичної активності відібраних штамів бацил до основних груп фітопатогенних бактерій (6 тест-культур) показав, що штам *Bacillus* sp.41 заслуговує на увагу щодо ретельного дослідження природи і спектру його антагонізму. Аналіз рівня антагоністичної активності відібраних штамів бацил до фітопатогенних мікроміцетів показав, що найменший рівень втрати антагонізму (зменшення зон затримки росту) впродовж періоду спостереження (3-тя і 7-ма доба) був у штаму *Bacillus* sp.41. Отже, тільки цей штам проявляв стабільний і відносно широкий спектр антагоністичної активності до фітопатогенів бактеріальної і грибної етіології. Природа цього антагонізму, ймовірно, є комплексною і може формуватися за участю різних біохімічних механізмів, зокрема, синтезу комплексу літичних екзоферментів. Для оцінки лізуючої активності штамів бацил з шести раніше відібраних було взято три штами з максимально високою протеолітичною і целюлолітичною активністю екзоферментів. Показано, що тільки штам *Bacillus* sp.1913 проявляв високу лізуючу (70%) активність до грамнегативного фітопатогена-поліфага *Pseudomonas syringae* pv. *syringae* УКМ B-1027^{T}. До решти тест-культур фітопатогенів така активність штаму не проявлялась. Висока лізуюча активність штаму *Bacillus* sp.1913 може бути пов'язана з високою активністю екзогенних протеаз і целюлаз літичного комплексу, що цілком збігається з даними літератури щодо лізуючої активності бацил. **Висновки**. Спектр і активність літичних екзоферментів штамів досліджених ґрунтових бацил свідчать про їх опосередковану участь у прояві біологічної активності (антагоністичної та лізуючої).

Ключові слова: бактерії роду *Bacillus,* літичні екзоферменти, бактеріальні і грибні фітопатогени, антагоністична та літична активність.

1. Rosenbluth M, Martinez-Romero E. Bacterial endophytes and their interactions with hosts. Mol Plant-Microbe Interact. 2006; 19:827–837.
2. Sturz AV, Nowak J. Endophytic communities of rhizobacteria and the strategies required to create yield enhancing associations with crops. Applied soil ecology. 2000; 15(2):183–190.
3. Maksimov IV, Khairullin RM. The role of *Bacillus* bacterium in formation of plant defence: mechanism and reaction. In: Gupta VK, Shar-

ma GD, Tuohy MG, Gaur, R. The handbook of Microbial Bioresources, Ch. 4. Wallingford: CAB International; 2016. p. 56–80.
4. Melentev AI. [Aerobic spore-forming bacteria *Bacillus Cohn* in agroecosystems]. Moskva: Nauka; 2007. Russian.
5. Chen F, Wang M, Zheng Y, Luo J, Yang X, Wang X. Quantitative changes of plant defense enzymes and phytohormone in biocontrol of cucumber *Fusarium* wilt by *Bacillus subtilis* B579. World Journal of Microbiology and Biotechnology. 2010; 26:675–684.
6. Zholner LG. [Lytic enzymes of thermophillic bacilli] Avtoref. dis. for the degree of Cand. biol. sciences., Kyiv; 1989. p. 22. Russian.
7. Shyrokov AV. [Mycolytic enzymes of *Bacillus Cohn* bacteria and their role in antagonism to soil micromycetes]. Avtoref. dis. for the degree of Cand. biol. sciences., Ufa; 2004. p. 20. Russian.
8. Hong TY, Meng M. Biochemical characterization and antifungal activity of an endo-1, 3-β-glucanase of *Paenibacillus* sp. isolated from garden soil. Applied microbiology and biotechnology. 2003; 61:472–478.
9. Semyonov AV. [Characteristics of the antagonistic activity of bacteria during intermicrobial interactions]. Avtoref. dis. for the degree of Cand. biol. sciences: Special. 03.00.07 "Microbiology", Orenburg; 2009. p. 20. Russian.
10. Egorov NS. [Fundamentals of the doctrine of antibiotics]. Moscow: Higher school; 1986. Russian.
11. Dankevich LA, Lapa SV, Zakharova AN, Avdeyeva LV, Patyka VP. Antagonisticheskiye svoystva nekotorykh shtammov bakteriy roda *Bacillus* k fitopatogennym bakterii roda *Erwinia* na yablone. In: Mezhdunarodnyy simpozium «Zashchita rasteniy – problemy i perspektivy»; 2012 Okt 30–31; Kishinev, Moldova. Kishinev, 2012. p. 196–199. Russian.
12. Kryuchkova LO. [Diseases of winter wheat caused by necrotrophic fungal pathogens and methods for their diagnosis]. Avtoref. dis. for the degree of Dr. biol. sciences. Kyiv; 2007. Ukrainian.
13. Zholner LG, Pavlova IN, Tinyanova NZ. [Proteases and their role in the lytic activity of thermophilic strain *Bacillus* sp.86]. Mikrobiol Z. 1988; 50(1):20–25. Russian.
14. Pirog TP. [General microbiology]. 2th ed. Kyiv: NUXT; 2010. Ukrainian
15. Idriss EE, Makarewicz O, Farouk A, Rosner K, Greiner R, Bochow H, Richter T, Borriss R. Extracellular phytase activity of *Bacillus amyloliquefaciens* FZB45 contributes to its plant-growth-promoting effect. Microbiology. 2002; 148:2097–2109.
16. Ding Y, Wang J, Liu Y, Chen S. Isolation and identification of nitrogen-fixing bacilli from plant rhizospheres in Beijing region. J Appl Microbiol. 2005; 99(5):1271–81.
17. Bais HP, Fall R, Vivanco JM. Biocontrol of *Bacillus subtilis* against infection of *Arabidopsis* roots by *Pseudomonas syringae* is facilitated by biofilm formation and surfactin production. Plant Physiology. 2004; 134:307–319.
18. Chung S, Kong H, Buyer JS, Lakshman DK, Lydon J, Kim S-D, P Roberts D. Isolation and partial characterization of *Bacillus subtilis* ME488 for suppression of soilborne pathogens of cucumber and pepper. Appl Microbiol Biotechnol. 2008; 80:115–123.
19. Emmert EA, Handelsman J. Biocontrol of plant disease: a (gram-) positive perspective. FEMS Microbiology Letters. 1999; 171:1–9.
20. Smirnov VV, Sorokulova IB, Pinchuk IV. [Bacteria of *Bacillus* species – prospective source for biologically active substances]. Mikrobiol Z. 2001; 63(1):72–9. Russian.
21. Pat. 5364623 USA. Antibiotic produced by *Bacillus subtilis* ATCC 55422 capable of inhibiting bacteria. Patel PS, Mayerl F, Mayers E. Publ. 15.11.94.
22. Lam TBT, Iiyama K, Stone BA. The relationship between *in vitro* enzymatic digestibility of cell walls of wheat internodes and compositional changes during maturation. Acta Bot Neerl. 1993; 42(2):175–185.
23. Handelsman J, Stabb EV. Biocontrol of soilborne plant pathogens. The Plant Cell. 1996; 8:1855–1869.
24. Aktuganov GE, Melent'ev AI, Kuz'mina LIu, Galimzianova NF, Shirokov AV. [Chitinolytic activity of *Bacillus Cohn*. – phytopathogen-

ic fungus antagonist]. Mikrobiologiia. 2003; 72(3):356–60. Russian.

25. Asaturova AM, Dubyaga VM, Tomashevich NS, Zharnikova MD. [Selection of perspective biological control agents for fall wheat protection from fusarium diseases]. Scientific journal KubSAU. 2012; 75(1):1–12. Russian.
26. Aktuganov GE, Melent'ev AI, Shirokov AV. Antifungal substances of antagonistic bacteria *Bacillus* sp. 739. In: Abstract Book of lth FEMS Congress of European Microbiologists; 2003 29.06–03.07; Ljubljana, Slovenia. p. 231–232.
27. Avdeeva LV, Kharkhota MA, Kharkhota AV. [The Decomposition of Various Types of Crop Residues by Strains *Bacillus subtilis* IMB B-7516 and *B. licheniformis* IMB B-7515]. Mikrobiol Z. 2016; 78(2):52–60. Ukrainian.
28. Rabinovich ML, Mel'nik MS, Bolobova AV. [Cellulases from microorganisms]. Prikl Biokhim Mikrobiol. 2002; 38(4):355–73. Russian.

Received 10.03.2021