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## Antagonistic activity of dominant bacteria isolated from the rhizosphere of spring barley against phytopathogenic micromycetes

**Abstract.** The purpose of the study was to examine the manifestation of representatives of the bacterial biome that dominate the spring barley rhizosphere, antagonistic activity against phytopathogenic micromycetes. The standard diffuse method of double culture in Petri dishes was used to examine the antagonistic properties of bacterial strains, dominating the rhizosphere. The level of antagonistic activity of microorganisms was evaluated by indicator (%) of inhibition of growth and development of mycelium of micromycetes *Fusarium sporotrichioides* Sherb. 23.2, *Alternaria alternata* (Fr.) Keissl. 3.45, *Nigrospora oryzae* (Berk. & Broome) Petch. 18.77. As a result of the conducted studies, it was identified that bacteria *Bacillus methylotrophicus* 10 had an inhibitory effect on *Fusarium sporotrichioides* Sherb. 23.2 – 77.4%, *Alternaria alternata* (Fr.) Keissl. 3.45 – 66.6% and *Nigrospora oryzae* (Berk. & Broome) Petch. 18.77 – 86.7%, while bacteria *Phyllobacterium ifriqiyense* 1 showed activity against phytopathogens by 45.1%, 63.1%, and 65.0%, respectively. Thus, both strains of dominating rhizospheric bacteria have high antagonistic activity against phytopathogenic micromycetes, which gives grounds for their further study

**Keywords:** *Bacillus methylotrophicus* 10, *Phyllobacterium ifriqiyense* 1, antagonistic properties, phytopathogenic micromycetes

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## INTRODUCTION

Nature has laid down all the mechanisms for controlling the most important biosphere processes: nitrogen fixation, antagonism of certain microorganisms to pathogens, synthesis by microorganisms of biologically active substances that can substantially affect the physiological state of plants and their immunity, etc. (Andreiuk, 1992). In the root zone of plants, all the positive and negative aspects of inter-microbial and plant-microbial interaction are most active (Kiroiants, 2020).

During the study of the features of the formation of the microbial complex of chernozem typical in the agrophytocenosis of spring barley, a comparative characterisation of the number of the main physiological and taxonomic groups of microorganisms is conducted. The qualitative composition, structure, and diversity of the microbial complex formed in the ontogenesis of spring barley under various farming systems are analysed (Novikova, 2011). Microbiota formation is analysed using the Shannon and Simpson biodiversity indices in various phases of spring barley ontogenesis, and strains of microorganisms dominating the spring barley rhizosphere are identified. Laboratory molecular-biological methods identified the dominating bacteria – *Bacillus methylotrophicus* 10 and *Phyllobacterium ifriqiyense* 1 (registered in GenBank database MK947056, MK947049 (Berg et al, 2016); <https://www.ncbi.nlm.nih.gov/nucleotide/MK947056>; <https://www.ncbi.nlm.nih.gov/nucleotide/MK947049>).

Rhizospheric bacteria can exhibit high antagonistic activity against phytopathogenic microorganisms due to the synthesis of exometabolites of various chemical nature, and act as potential elicitors with which it is possible to control and reduce the level of phytopathogenic damage at the stages of plant ontogenesis (Goudjal, 2016).

The study of the properties of highly active microorganisms as potential bioagents of drugs, in particular, protective action for use in environmentally safe agricultural technologies, is one of the priority tasks of modern agroecology.

The purpose of the study was to research the manifestation of representatives of the bacterial biome that dominate the spring barley rhizosphere, antagonistic activity against phytopathogenic micromycetes.

## MATERIALS AND METHODS

The studies used identified and classified strains of dominant microorganisms – *Bacillus methylotrophicus* 10 and *Phyllobacterium ifriqiyense* 1 (GenBank MK947056, MK947049). Strains of phytopathogenic micromycetes *Fusarium sporotrichioides* Sherb. 23.2, *Alternaria alternata* (Fr.) Keissl. 3.45, *Nigrospora oryzae* (Berk. & Broome) Petch 18.77 (Fig.1) were provided from the collections of the Plant Biochemistry and Bioenergetics V.F. Peresyphkin Department of Phytopathology of the National University of Life and Environmental Sciences Of Ukraine.



**Figure 1.** Phytopathogenic micromycetes

**Note:** a) *Fusarium sporotrichioides* Sherb. 23.2; b) *Alternaria alternata* (Fr.) Keissl. 3.45; c) *Nigrospora oryzae* (Berk. & Broome) Petch. 18.77

Antagonistic properties were determined by the diffuse method of double culture in Petri dishes on a glucose-peptone agarose medium (GPA-Zviagintsev). The studies used the classical block method (Ehorov, 2004) involving dominant rhizospheric bacteria *Bacillus methylotrophicus* 10 and *Phyllobacterium ifriqiyense* 1.

During the study of rhizospheric bacteria, they were cultured at 28°C for a day for the formation and accumulation of biologically active metabolites in an agarose nutrient medium. Sample preparation of ten-day test cultures of phytopathogenic micromycetes was conducted under incubation conditions by the block method and the temperature range from 24 to 28°C.

The antagonistic activity of rhizospheric prokaryotes was evaluated by the degree of inhibition of growth and development of test micromycetes. The colony diameter was measured on day 14, and the percentage of growth inhibition of fungi colonies was determined by the formula:

$$\text{Growth inhibition (\%)} = \frac{D_k - D_o}{D_k} \times 100\%,$$

where:  $D_k$  – diameter of the fungal colony in the control, mm;  $D_o$  – diameter of the mushroom colony in the experiment, mm.

The colony diameter was measured twice on radially opposite sides, and the arithmetic mean was calculated.

The degree of antagonistic activity of the tested rhizosphere bacteria strains was defined according to the size of the test strain growth inhibition zone around the agar block. Growth retardation zones were considered after 3 and 10 days of cultivation.

Statistical processing was performed using Microsoft Excel.

## RESULTS AND DISCUSSION

The results of studies of the inhibitory activity of bacterial strains against phytopathogenic micromycetes are presented in Table 1.

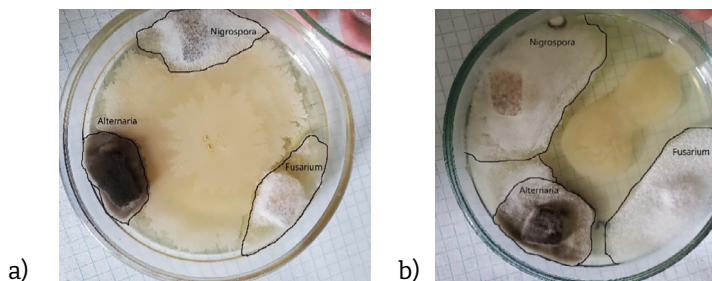
**Table 1.** Inhibitory activity of dominant spring barley rhizosphere bacteria

<i>Bacillus methylotrophicus</i> 10 Do day 10 (mm)	<i>Phyllobacterium ifriqiyense</i> 1 Do day 10 (mm)	Phytopathogenic micromycetes (control), $D_k$ (mm)		Inhibitory activity $P$ (%)	
				<i>Bacillus methylotrophicus</i> 10	<i>Phyllobacterium ifriqiyense</i> 1
34 ± 1.6	14 ± 0.8	<i>Fusarium sporotrichioides</i> Sherb. 23.2	62 ± 3.8	45.1	77.4
21 ± 2.4	19 ± 1.3	<i>Alternaria alternata</i> (Fr.) Keissl. 3.45	57 ± 4.5	63.1	66.6
29 ± 1.4	11 ± 1.9	<i>Nigrospora oryzae</i> (Berk. & Broome) Petch. 18.77	83 ± 3.6	65.2	86.7

**Note:**  $D_k$  – diameter of the fungal colony in the control, mm;  $D_o$  – diameter of the fungus colony in the experiment, mm;  $P$  – indicator of inhibition

As a result of the conducted studies, it was identified that bacteria *Bacillus methylotrophicus* 10 and *Phyllobacterium ifriqiyense* 1 inhibited the growth of phytopathogenic micromycetes *Fusarium sporotrichioides* Sherb. 23.2 – 45.1% and 77.4%, respectively, test cultures *Alternaria alternata* (Fr.) Keissl. 3.45 – 63.1% and 66.6%, *Ni-*

*grosso* *oryzae* (Berk. & Broome) Petch. 18.77 – 65.2% and 86.7%. The conducted studies of antagonistic activity indicate (Fig. 2) that rhizospheric bacterial agents of *Bacillus methylotrophicus* 10 and *Phyllobacterium ifriqiyense* 1 inhibit the growth of micromycetes, which is evidence of their antifungal activity.



**Figure 2.** Antagonistic activity of rhizospheric bacterial strains, (day 10)

**Note:** a) *Bacillus methylotrophicus* 10; b) *Phyllobacterium ifriqiyense* 1

Bacteria *Phyllobacterium ifriqiyense* 1 populate the substrate more quickly and use nutrient resources accordingly, while micromycetes lose the ability to grow and do not have the opportunity to populate the substrate further away, and there is a tendency to die. Thus, evidently, the detected antagonistic activity is associated with competition. In natural conditions, antagonism of this type is most often

observed in the soil environment of the plant rhizosphere, where there is competition between microorganisms for food sources (root exudates) (Hadzalo, 2015).

The intensity of growth and development of bacteria dominating the rhizosphere of spring barley plants around agar blocks of phytopathogenic micromycetes on days 3 and 10 of the experiment is presented in Table 2.

**Table 2.** Determination of the growth retardation zone of phytopathogenic micromycetes under the influence of dominant bacteria on days 3 and 10 of the experiment

Phytopathogenic micromycetes (control, mm)		<i>Bacillus methylotrophicus</i> 10		<i>Phyllobacterium ifriqiyense</i> 1	
		Day 3 (mm)	Day 3 (mm)	Day 3 (mm)	Day 10 (mm)
<i>Fusarium sporotrichioides</i> Sherb. 23.2	62 ± 1.4	3 ± 0.2	1 ± 0.1	9 ± 0.6	2 ± 0.1
<i>Alternaria alternata</i> (Fr.) Keissl. 3.45	57 ± 0.9	11 ± 0.6	3 ± 0.2	13 ± 0.8	5 ± 0.4
<i>Nigrospora oryzae</i> (Berk. & Broome) Petch. 18.77	83 ± 2.2	5 ± 0.4	2 ± 0.1	11 ± 0.7	7 ± 0.5

Based on the results obtained, bacteria *Bacillus methylotrophicus* 10 and *Phyllobacterium ifriqiyense* 1 on day 3 of the experiment suppressed the growth and development of phytopathogenic micromycetes, in particular, the zones of growth retardation of micromycetes were as follows: *Fusarium sporotrichioides* Sherb. 23.2 – 3 and 9 mm, respectively, *Alternaria alternata* (Fr.) Keissl. 3.45 – 11 and 13 mm, and *Nigrospora oryzae* (Berk. & Broome) Petch. 18.77 – 5 and 11 mm, respectively. On the 10<sup>th</sup>

day of the experiment bacteria *Bacillus methylotrophicus* 10 and *Phyllobacterium ifriqiyense* 1 inhibited the growth of fungi *Fusarium sporotrichioides* Sherb. 23.2 – 1 and 2 mm, *Alternaria alternata* (Fr.) Keissl. 3.45 – 3 and 5 mm, and *Nigrospora oryzae* (Berk. & Broome) Petch. 18.77 – 2 and 7 mm, respectively.

Consequently, the antagonistic activity of the bacterial strains dominating the rhizosphere of spring barley was manifested already on the third day of the experiment and

increased its influence on the growth and development of phytopathogenic micromycetes during the experiment.

### CONCLUSIONS

Rhizospheric bacteria *Bacillus methylotrophicus* 10 and *Phyllobacterium ifriqiyense* 1, have antagonistic activity against phytopathogenic micromycetes, and also they have an inhibitory effect on the growth and development of phytopath-

ogenic micromycetes, and therefore the ability to compete with specific plant phytopathogens under study.

Therefore, further research of these bacteria and the search for new effective ones with polyfunctional action are promising for the development of biotechnologies for their use as preparative forms for crop production (alternative ecological technological means for chemical plant protection and mineral fertilisers).

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03041, вул. Героїв Оборони, 15, м. Київ, Україна**Антагоністична активність домінантних бактерій,  
виділених з ризосфери ячменю ярого,  
щодо фітопатогенних мікроміцетів**

**Анотація.** Метою дослідження було вивчення прояву представниками бактеріального біома, що домінують у ризосфері ячменю ярого, антагоністичної активності щодо фітопатогенних мікроміцетів. Для вивчення антагоністичних властивостей штамів бактерій, що домінують у ризосфері, використовували стандартний дифузний метод подвійного культивування в чашках Петрі. Рівень антагоністичної активності мікроорганізмів оцінювали за показником (%) пригнічення росту і розвитку міцелію мікроміцетів *Fusarium sporotrichioides* Sherb. 23,2, *Alternaria alternata* (Fr.) Keissl. 3.45, *Nigrospora oryzae* (Berk. & Broome) Petch. 18.77. В результаті проведених досліджень встановлено, що бактерії *Bacillus methylotrophicus* 10 мали інгібуючий вплив на *Fusarium sporotrichioides* Sherb. 23,2 - 77,4%, *Alternaria alternata* (Fr.) Keissl. 3,45 - 66,6% та *Nigrospora oryzae* (Berk. & Broome) Petch. 18,77 - 86,7%, тоді як бактерії *Phyllobacterium ifriqiyense* 1 проявляли активність проти фітопатогенів на 45,1%, 63,1% та 65,0% відповідно. Таким чином, обидва штами домінуючих ризосферних бактерій мають високу антагоністичну активність проти фітопатогенних мікроміцетів, що дає підстави для їх подальшого вивчення

**Ключові слова:** *Bacillus methylotrophicus* 10, *Phyllobacterium ifriqiyense* 1, антагоністичні властивості, фітопатогенні мікроміцети