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ANTIMICROBIAL ACTIVITY OF SOIL BACILLI AGAINST PHYTOPATHOGENIC MICROORGANISMS ISOLATED FROM AFFECTED CEREAL PLANTS

Aim *of this study was to investigate the antagonistic effect of bacilli isolated from the surface of plants and the rhizosphere zone of the soil against pathogens of barley and wheat diseases.* ***Materials and methods.*** *The material for the study was affected barley and wheat samples used to isolate phytopathogenic microorganisms, as well as samples of healthy plants and the rhizosphere soil zone used to isolate bacteria of the genus Bacillus. Manipulations for the isolation and investigation the biological properties of both phytopathogens and bacilli were carried out by traditional microbiological methods. The antagonistic activity of the isolated strains of bacilli against phytopathogens was studied by the block method.* ***Results.*** *From samples of affected wheat and barley were isolated microorganisms identified on the basis of the studied biological properties as Fusarium oxysporum, Sclerotinia sclerotiorum, Xanthomonas arboricola and Pectobacterium carotovorum. The largest proportion of strains was represented by the micromycete Fusarium oxysporum – 53.9%. From samples of the rhizosphere soil zone and leaf-stem mass of healthy wheat and barley plants, 86 strains of bacteria of the genus Bacillus were isolated by microbiological methods. Antagonistic activity was inherent in 54 strains of bacilli against 25% of phytopathogens. All pathogenic microorganisms were inhibited with different intensity by 14 strains of bacilli.* ***Conclusion.*** *The most active bacillus strains (Bacillus spp. 6, 9, 13, 21, 50) were selected for further research.*

Key words: antimicrobial activity, soil bacilli, phytopathogenic microorganisms, affected cereal plants.

In modern conditions of grain production around the world, there is a tendency to expand the sowing of grain crops as the main source of production of the most important food for people, feed for farm animals and raw materials for industry. Wheat and barley are leading grain crops that are in great demand [1]. Ukraine exports more than 30 million tons of grain every year, ranking 4th in the world, and covers 15% of the world market.

One of the main factors destabilizing grain production is still the affected of plants by pathogens. At present, often due to changes in weather conditions, violation of crop cultivation technology and thoughtless use of chemicals, there is an increase in the development of phytopathogenic organisms, as well as the emer-

gence of new ones that previously had no economic value, often causing the death of agricultural crops [2].

The use of chemical fertilizers, plant protection agents and other pesticides has reached dangerous proportions and disrupts natural biological and physicochemical processes. In this regard, microbiological preparations have an advantage and perspective. They increase the resistance of plants to abiotic and biotic stresses, do not cause the habituation of pests and are environmentally safe [3]. So, the development of biotechnologies for the protection of agricultural crops with the search for alternative ways of reducing the risk of the emergence and spread of pathogens of various etiologies, controlling phytophages and obtaining high-quality plant products is currently a priority and progressive trend [4]. One of the first steps in this direction is the search for and isolation of promising strains of microorganisms-antagonists of phytopathogens [5].

For this reason, the aim of this study was to investigate the antagonistic effect of bacilli isolated from the surface of plants and the rhizosphere zone of the soil against pathogens of barley and wheat diseases.

Materials and methods

The material for the study was plant samples (wheat and barley) affected by phytopathogenic microorganisms, samples of healthy plants, as well as samples of the rhizosphere soil zone.

Samples of affected plants were used to isolate the microorganisms that caused the infections. From healthy plants, as well as soil samples, bacteria of the genus *Bacillus* were isolated.

Taking into account the peculiarities of phytopathogens, using the literature data, the isolation of phytopathogenic microorganisms was carried out on Sabouraud, Czapek-Dox and nutrient agar media [6].

Bacteria of thegenus *Bacillus* were isolated from ther hizosphere zone of the soil and leaf-stem mass of healthy barley and wheat plants on nutrient agar and potato-glucose agar [6].

Manipulations for the isolation of both phytopathogens and bacilli were carried out by traditional microbiological methods [6].

The resulting pure cultures of microorganisms were studied by investigating a complex of biological properties: morphological, cultural, tinctorial, physiological and biochemical. The biochemical properties of all pure cultures were studied using API (BioMerieux) test systems corresponding to each taxon [7]. In particular, the biochemical properties of bacteria of the genus *Bacillus* were studied using the API 50 test system. The results were interpreted visually and deciphered using the APIWEB database [8]. Isolated strains of microorganisms were identified on the basis of the studied properties.

The antagonistic activity of the isolated strains of bacilli against phytopathogens was studied by the block method [9]. The results were taken into account after 24–48 hours when cultivated at 28 °C, measuring the size of the zones of the absence of growth of phytopathogens.

All studies were carried out in triplicate. Statistical processing of the results of the study was carried out using the MS Excel computer program with the definition of Student's t-criterion. The difference was statistically significant at $p < 0.05$.

Results

When inoculating on Sabouraud, Czapek-Dox, nutrient agar, 100 µl of suspensions prepared from affected samples of wheat and barley, in dilutions of 10^{-2} – 10^{-3}, microorganisms were isolated that differ in the nature of growth on nutrient media. The isolation of pure cultures and the study of their properties: morphological, cultural, tinctorial, physiological and biochemical made it possible to carry out species identification. Most of the isolated cultures formed well-defined aerial and substrate mycelia on dense nutrient media, which made it possible to attribute these isolates to filamentous fungi. It is known that many infectious diseases of plants arise as a result of affected by micromycetes [10]. The results of our research confirm this. However, some of the strains grown on nutrient agar formed colonies whose morphology suggested the bacterial nature of the isolates.

The grown micromycetes differed in their morphological and cultural properties. Most of the solates differed in the rate of colony growth (dimensions varied from 4 to 8 cm), structure, and the presence of aerial mycelium (cotton, flaky, and loose). Color of colonies from white to purple (Fig. 1).

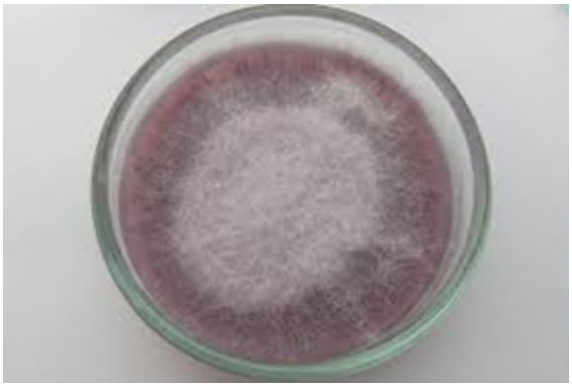


Fig. 1. Colonies morphology of *Fusarium oxysporum* on Czapek-Dox medium

As a rule, the colonies were white, with or without pink inclusions, the aerial mycelium was high, and the color of the reverse was white or pink. All isolates are fast-growing, homogeneous; there were dome-shaped and crater-shaped colonies. On nutrient media, the fungus forms colonies consisting of vegetative mycelium, macro- and microconidia, and chlamydospores. Branching conidiophores, with bottle-shaped phialides, pale yellow or light pink, formed on the mycelium, less often as a mucous layer in sporodochia or pionnots. Chlamydospores are abundant, intermediate or apical on hyphae, usually solitary, sometimes in chains, thick-walled, with a smooth or rough shell, colorless, one- or two-celled, 3.6–7.0 microns in diameter. Macroconidia are not numerous, usually with 3, very rarely with 4 or 5 septa, colorless, narrowly fusiform or ellipsoidal in shape, almost straight or slight-

ly sickle-shaped. The apical cells are short and slightly curved in some isolates. The basal cell had the appearance of a cut or in the form of a leg. Sizes of conidia with 3 septa are 20–50 × 2.5–5.5 μm, more often 25–40 × 3.5–5 μm, with 5 septa 20–60 × 3–5 μm, more often 30–50 × 3–4 μm. Microconidia are always abundant, formed on aerial mycelium on short monophialides, often in false heads, glued together with a mucous substance or in groups directly on hyphae, unicellular, sometimes 2- or 3-celled, ellipsoidal or kidney-shaped, colorless, sizes 5–12 × 2–4 μm. The studied morphological and cultural properties made it possible to attribute these isolates to the genus *Fusarium*.

Part of the fungal cultures at the beginning of their development formed a visually poorly expressed mycelium, cobwebbed. Subsequently, abundant dichotomous branching of the primary hyphae began and a well-defined layer of dense, white, long-lasting mycelium appeared. On Czapek-Dox it is white (Fig. 2), on nutrient agar it becomes yellowish-dark over time.

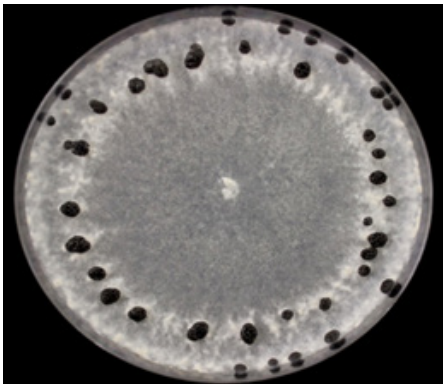


Fig. 2. Colonies morphology of *Sclerotinia sclerotiorum* on Czapek-Dox medium

When sclerotia matured, a putrefactive odor appeared, on Czapek-Dox it was weak, on nutrient agar it was strong, intensifying with the formation of sclerotia. After their full ripening, the smell weakened and was absent in the old culture. On solid media, the formation of sclerotia was registered by us when the mycelium reached the edge of the Petri dish, approximately for 4–5 days. At this time, an annular zone of white mycelium was formed along the edge of the colony 1.0–1.5 cm from the edge of the dish. Here it became thick, then flaky, and on it (at the very edge of the cup or not reaching it) mycelial clots began to appear, and then loose white tubercles – the beginnings of sclerotia. These tubercles gradually increased in size, taking on a more defined shape. The shell of the sclerotium was compacted and on the 10–14-th day from sowing the sclerotium mycelium was formed. Such morphological and cultural properties are typical for representatives of micromycetes of the genus *Sclerotinia*.

Bacteria isolates also differed in their properties. Some of them were straight sticks, 0.5–0.8 × 1.0–2.0 μm in size. They were motile by means of a polar flagellum. Gram-negative, spore-forming aerobic bacteria. On nutrient agar formed round, shiny, smooth colonies with a smooth edge. The studied colonies of these

isolates produced a yellow pigment – xanthomonas, which is a unique characteristic of the genus *Xanthomonas* (Fig. 3).

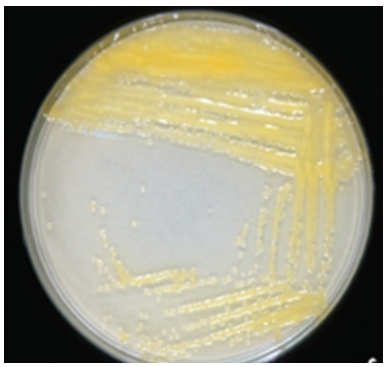


Fig. 3. Colonies morphology of *Xanthomonas arboricola* on nutrient agar

In addition, the colonies were slimy and oily, which is an essential feature that distinguishes pathogenic bacteria of the genus *Xanthomonas* from saprophytes.

One strain of bacteria was short sticks, straight, with rounded edges, their size is approximately 0.6–1.8 × 1.7–5.1 μm. Single, connected in pairs or in short chains, movable. Flagellation is peritrichous. Bacteria did not form capsules or spores. Gram negative, facultative anaerobes. They were grown at a temperature of 30 °C. On nutrient agar, the strain formed grayish-white, shiny, smooth colonies with smooth edges (Fig. 4).

Fig. 4. Colonies morphology of *Pectobacterium carotovorum* on nutrient agar

The studied biochemical properties made it possible to carry out species identification of the isolated strains and establish that 14 of them are representatives of the *Fusarium oxysporum*, 7 are *Sclerotinia sclerotiorum*, 4 are *Xanthomonas arboricola* and one is *Pectobacterium carotovorum* (Fig. 5).

When inoculating on nutrient and potato-glucose agar suspensions prepared from samples of healthy plants and the rhizosphere zone of the soil, morphologically similar colonies were visually determined after 24–48 hours: dense, wrinkled (less often uneven), shiny or matte, convex or flat (Fig. 6).

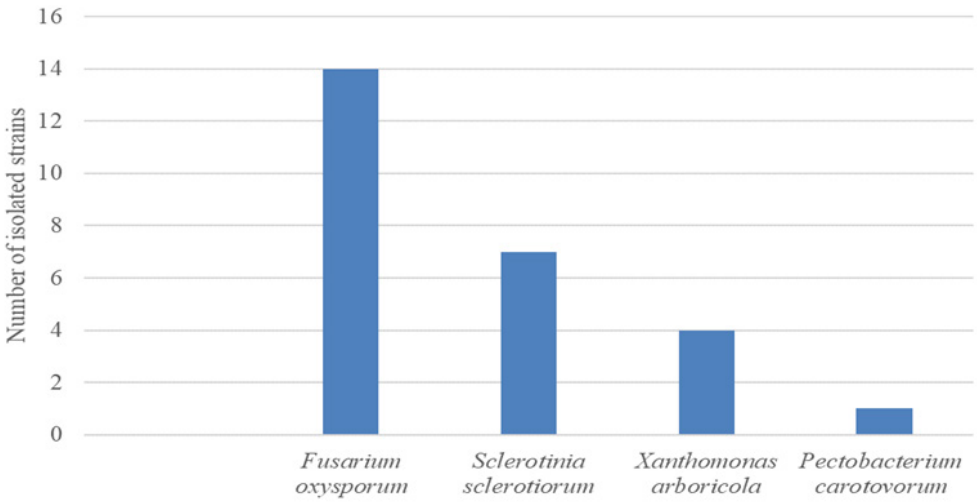
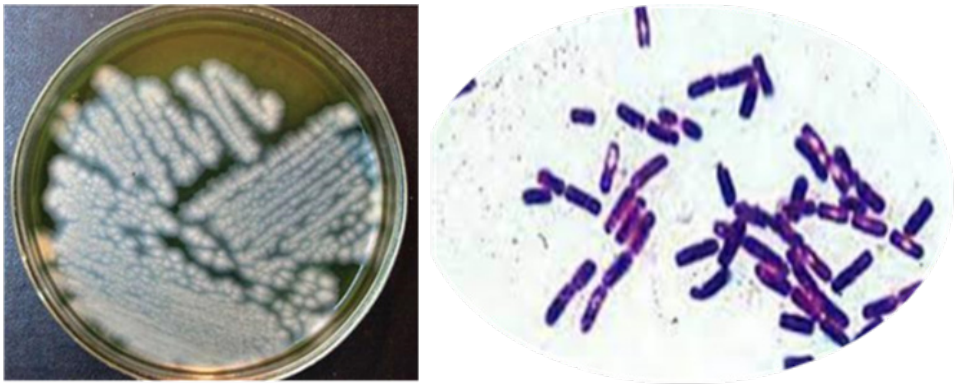


Fig. 5. Species diversity of strains isolated from affected plants



a) **b)**

Fig. 6. Morphology of colonies (a) and cells (b) of the strain *Basilus sp.* 13

Microscopy of Gram-stained preparations revealed gram-positive rods, the sizes of which ranged from 5 to 10 microns for different strains, were arranged in pairs, chains, on the second day of cultivation they formed spores, which were located in the cells mainly in the center, sometimes at the ends.

The study of biochemical properties showed that the isolated bacteria were somewhat variable, especially in the catabolism of such substrates as galactose, xylose and mannitol. The complex of results obtained during the study of biological properties made it possible to identify the isolated bacteria of the genus *Bacillus*. To carry out species identification, unfortunately, the identified properties are not enough and the use of molecular genetic methods is necessary.

Thus, 54 strains (which accounted for 62.8% of all isolates) and 32 strains (37.2%) of bacteria of the genus *Basillus* were isolated from the rhizosphere soil zone and leaf-stem mass of healthy plants (barley and wheat), respectively (Fig. 7).

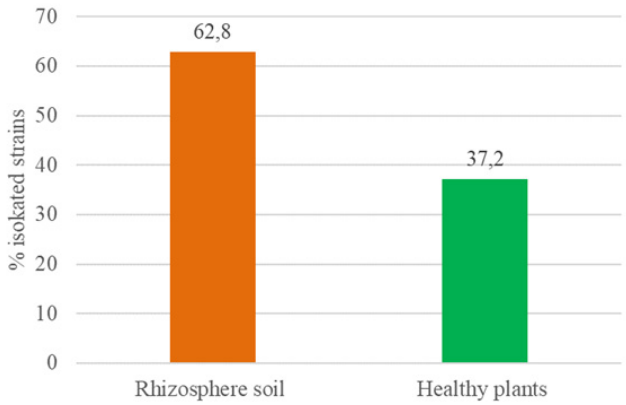


Fig. 7. Proportion of isolated strains of bacteria of the genus *Bacillus* from different natural sources

Microbial preparations based on bacilli are widely popular in various fields of medicine, veterinary medicine and agricultural production. Thanks to the synthesis of various metabolites, bacilli are able to suppress the development of many pathogenic microorganisms [11].

One of the first stages on the way to creating bacillary preparations is testing the ability of strains to show antagonistic activity against pathogens. Therefore, the next stage of the study was the investigation of the ability of isolated strains of bacteria of the genus *Bacillus* to prevent the growth of phytopathogenic microorganisms.

The results of the research showed that not all strains of bacilli inhibited the growth of phytopathogens isolated from the affected organs of wheat and barley. Of the 86 strains of bacilli isolated, the growth of a 25% of phytopathogens was inhibited by 54 strains (62.8%), the growth of half of phytopathogens was inhibited by 35 strains (40.7%), 75% of pathogens – 27 (31.4%) strains of bacilli (Fig. 8).

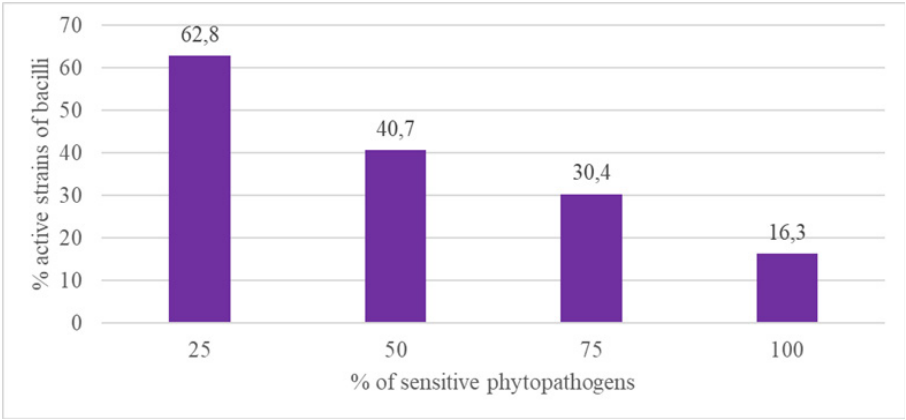


Fig. 8. Proportion of isolated strains of bacilli that showed antagonism to various phytopathogens

Note that these were phytopathogenic microorganisms of different structure. Among those studied, 14 strains of bacilli were found, which inhibited the growth of all phytopathogens with varying intensity, which was manifested in the size of the zones of absence growth.

The most active, that is, the metabolites of which significantly inhibited the growth of all isolated phytopathogenic microorganisms were strains of *Bacillus spp.* 6, 9, 13, 21, 50. Note that all these strains were isolated from samples of the rhizosphere soil zone. At the same time, the sizes of the absence zones of growth of phytopathogens were larger than 15 mm. In Fig. 9 is showen as an example, the antagonistic activity of *Bacillus sp.* 13 against certain strains of phytopathogenic microorganisms.

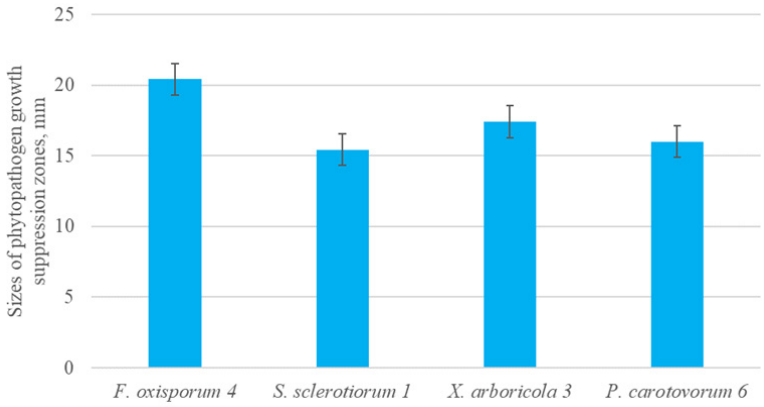


Fig. 9. Antagonistic activity of *Bacillus sp.* 13 against phytopathogens

As can be seen from the data shown in Fig. 9, the most sensitive to the action of *Bacillus sp.* 13 were *Fusarium oxisporum* 4 and *Xanthomonas arboricola* 3 (the sizes of the absence zones of growthwere 20.42 ± 0.03 mm and 17.42 ± 0.03 mm, respectively).

When evaluating the data obtained, it should be noted that the manifestation of antagonistic activity depends on the specific strains taken in the experiment and on the conditions of the experiment.

Studies have shown that wheat and barley plants can be affected by phytopathogenic microorganisms of different structures. Microorganisms of fungal and bacterial nature were isolated from samples of affected plants. The frequency of their isolation was not the same with the dominance of representatives of filamentous fungi isolated in 80.8% of cases.

From the rhizosphere zone of the soil and leaf-stem mass of healthy barley and wheat were isolated, respectively, 62.8% and 37.2% of bacteria of the genus *Basillus*, which is evidence of the widespread distribution of bacteria of this genus in nature, due to the peculiarities of the structure, metabolic activity and high adaptive capabilities.

Evaluating the antagonistic potential of bacilli against phytopathogens, we note that more than half of the isolated strains of bacilli inhibited the growth of

25% of strains of phytopathogenic microorganisms. The growth of all pathogens with different intensity was suppressed by 14 strains isolated exclusively from the soil. The most active strains (*Bacillus spp.* 6, 9, 13, 21, 50) were selected for further research on the creation of microbial preparations for agriculture.

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АНТИМІКРОБНА АКТИВНІСТЬ ҐРУНТОВИХ БАЦИЛ ЩОДО ФІТОПАТОГЕННИХ МІКРООРГАНІЗМІВ, ВИДІЛЕНИХ ІЗ УРАЖЕНИХ ЗЛАКОВИХ РОСЛИН

Реферат

Метою *роботи було дослідження антагоністичної дії бацил, виділених з поверхні рослин та ризосферної зони ґрунту, проти збудників хвороб ячменю та пшениці.* ***Матеріали та методи.*** *Матеріалом для дослідження були зразки уражених ячменю та пшениці, з яких виділяли фітопатогенні мікроорганізми, а також зразки здорових рослин і ризосферної зони ґрунту, з яких виділяли бактерії роду Bacillus. Маніпуляції з виділення та дослідження біологічних властивостей як фітопатогенів, так і бацил проводили з використанням традиційних мікробіологічних методів. Антагоністичну активність виділених штамів бацил щодо фітопатогенів досліджували блоковим методом.* ***Результати.*** *Із зразків ураженої пшениці та ячменю виділено мікроорганізми, ідентифіковані на основі вивчених біологічних властивостей як Fusarium oxysporum, Sclerotinia sclerotiorum, Xanthomonas arboricola та Pectobacterium carotovorum. Найбільшою часткою штамів був представлений мікроміцет Fusarium oxysporum – 53,9%. Із проб ризосферної зони ґрунту та листо-стебелової маси здорових рослин пшениці та ячменю мікробіологічними методами виділено 86 штамів бактерій роду Bacillus. Антагоністична активність притаманна 54 штамам бацил, які пригнічували ріст 25% фітопатогенів. Ріст усіх патогенних мікроорганізмів з різною інтенсивністю пригнічували 14 штамів бацил, виділених із ґрунту.* ***Висновок.*** *Для подальших досліджень відібрано найбільш активні штами бацил (Bacillus spp. 6, 9, 13, 21, 50).*

Ключові слова: антимікробна дія, ґрунтові бацили, фітопатогенні мікроорганізми, уражені злакові рослини.

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