Scientific Bulletin. Series F. Biotechnologies, Vol. XXVII, No. 1, 2023
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

# ANTIMICROBIAL ACTIVITY OF MICROORGANISMS ISOLATED FROM SILT OF THE "LA IZVOR" LAKE SYSTEM (CHISINAU MUNICIPALITY)

**Tamara SÎRBU, Svetlana BURȚEVA, Maxim BÎRSA, Nina BOGDAN-GOLUBI, Valerina SLANINA, Cristina MOLDOVAN, Olga ȚURCAN**

Institute of Microbiology and Biotechnology of the Technical University of Moldova,
1 Academiei Street, Chisinau, Republic of Moldova

Corresponding author email: tamara.sirbu@imb.utm.md

***Abstract***

*Microorganisms are the main source of various bioactive substances used in diverse fields of biotechnology. A current problem in agriculture is the fight against various phytopathogens, which cause crop diseases. Current trends worldwide are green agricultural methods. Actinobacteria, bacteria, and micromycetes are known to naturally associate with plants and have a beneficial effect on their growth. Thus, the groups of microorganisms listed above were isolated from the silt samples of the "La izvor" lake system (Chisinau municipality), and their screening was performed to combat phytopathogenic agents that cause plant diseases. The following strains of microorganism actively inhibit the growth of phytopathogenic fungi: A. alternata - 8 micromycetes, 2 bacteria; A. niger - 1 micromycete, 3 actinobacteria; B. cinerea - 5 micromycetes, 4 bacteria; F. oxysporum - 7 micromycetes, 3 actinobacteria, 2 bacteria. The growth of phytopathogenic bacteria was actively inhibited only by micromycetes: B. subtilis - 15 strains; X. campestris - 8 strains; C. michiganensis - 5 strains; A. tumefaciens - 3 strains; E. carotovora - 5 strains.*

***Key words****: actinobacteria, antibacterial activity, antifungal activity, bacteria, micromycetes.*

## INTRODUCTION

Water ecosystems of lakes in various regions of the Earth provide irreplaceable water resources to humans. The microbiomes of lake ecosystems are suitable bioresources for agriculture, industry and related sectors. An analysis of the microflora presents in the waters of lakes around the world showed that the most common types are *Proteobacteria*, *Cianobacteria*, *Actinobacteria*, *Flavobacterium*, *Firmicutes*, *Acidobacteria*, as well as various representatives of fungi such as micromycetes.

The natural community is an important center of potential microbial resources; only it can serve as a source of microbiological objects for various kinds of research. Diversity of microorganisms is the main source for the creation of new biotechnological processes and products, and screening of microorganisms for potentially useful traits plays an important role. (Borneman & Triplett, 1997; Denisova et al., 1999; Yadav et al., 2018).

Recently, interest has increased in studying the structure and composition of microbial communities, which are one of the key components of aquatic ecosystems. Microorganisms, which are small in size, make up a significant part of the biomass in water bodies. High reproduction rate and wide adaptive abilities enable microorganisms to adapt to various environmental conditions (Bel'kova et al., 2003).

Currently, the world, and especially in developing countries, is experiencing nutritional deficiencies due to a number of causes, including loss of agricultural yields due to diseases of critical crops caused by phytopathogenic fungi and bacteria.

Despite the current trend in the development of ecological farming, the protection of plants from diseases is based mainly on the use of chemical means of protection (pesticides) against pathogens of bacterial and fungal etiology. Intensive and often unregulated use of pesticides leads to environmental pollution due to the accumulation of these substances in soil and natural waters. That is why, much attention is paid to the development of environmentally friendly biological methods for combating plant diseases, which are considered as an alternative to the traditional use of chemical pesticides (Ab Rahman et al., 2018; Burtseva & Sirbu, 2009; Burțeva et al., 2008; Hyakumachi et al., 2014;

Palaniyandi et al., 2013). The biological method of protecting plants from pathogenic microorganisms is based on the use of antagonist microorganisms. Currently, a number of microorganisms have been described that have an antagonistic effect on phytopathogens. Their mechanism of action includes competition for food, effective colonization of the rhizosphere and leaf surfaces, and the synthesis of antibiotic substances (Azizbekyan et al., 2001; Chausaria et al., 2018; Van der Meij et al., 2017).

Microorganisms play a huge role in the control of plant diseases. In recent decades, research into the biological control of plant pathogens has seen a marked acceleration due to the risk of using synthetic pesticides. Therefore, a promising element of modern agroecology is the use of preparations based on microorganisms or their metabolites, which exhibit phytoprotective and growth-stimulating properties, increase plant resistance against pathogens and stress factors (El-Sabbagh et al., 2013; Hata et al., 2015; Hyakumachi et al., 2014; Jeon et al., 2016; Tiwari & Gupta, 2013; Van der Meij et al., 2017).

Biological farming, which is based on the ecological stabilization of agroecosystems, is gaining great popularity in the world. Naturally, there is an increasing interest in microbial preparations to improve plant nutrition, regulate their growth and development, as well as protect against phytopathogens and pests. An important factor in increasing the productivity of agroecosystems is the activation of microbial-plant interaction. Environmentally friendly microbial preparations are being developed and introduced into the system of necessary agrotechnical measures. They contribute to the intensification of physiological and biochemical processes in plants, increase their resistance to diseases and have a positive effect on soil microorganisms. In practice, they are created on the basis of microorganisms isolated from natural biocenoses, including water bodies. They do not pollute the environment and are safe for animals and humans (Andriyuk et al., 2001; Omelyants et al., 2008; Safronova, 2007).

The importance of protecting crops from pests and diseases is becoming a determining factor in increasing the yield and quality of crop products. At the same time, the importance of phytosanitary in the field of environmental safety issues is rapidly increasing. This is convincingly evidenced by the growing volumes of production and use of pesticides in the world. According to Bayer and the Ministry of Agriculture of Japan, in recent years there has been an increase in the resistance of phytopathogenic fungi to fungicides: 20 species of phytopathogens have been found to be resistant to 15 fungicides (Pavlyushin, 2013). In this regard, new strains of actinobacteria, bacteria, and micromycetes were found and included in the lists of agents against plant diseases (Hyakumachi et al., 2014).

The purpose of the research was to screene and finding new strains of microorganisms with antimicrobial properties, which were isolated from the silt sediments of the "La izvor" lake system, Chisinau municipality.

## MATERIALS AND METHODS

The following research was conducted within the National Collection of Non-pathogenic Microorganisms of the Institute of Microbiology and Biotechnology of the Technical University of Moldova.

The geographical coordinates of lake system "La izvor" (Republic of Moldova, Chisinau municipality) where samples were collected are: 1) 47°02'44.2"N, 28°47'18.9"E; 2) 47°02'53.7"N, 28°47'42.5"E; 3) 47°02'59.6"N, 28°47'59.3"E. Altogether 11 points were sampled in August 2020. Random samples of silt sediments were collected in sterile containers. The samples were not pretreated. After that, serial dilutions were carried out using distillate water to dilute the samples to $10^{-1}$-$10^{-6}$ (Hussein et al., 2018; Yu et al., 2015).

For study actinobacteria, were isolated 8 genera on special selective nutrient media in Petri dishes by inoculation of diluted samples:

*Actinomadura* - soluble starch (20.0 g/L), $K_2HPO_4$ (0.5 g/L), $MgSO_4$ (0.5 g/L), $KNO_3$ (1.0 g/L), NaCl (0.5 g/L), $FeSO_4$ (10.0 mg/L), streptomycin (50 mcg/mL), nystatin (50 mcg/mL), agar, pH = 7.2-7.4 (Zakharova et al., 2003);

*Actinoplanes* - oatmeal (2.5 g/L), $K_2HPO_4$ (1.0 g/L), KCl (0.5 g/L), $MgSO_4*H_2O$ (0.5 g/L), $FeSO_4*7H_2O$ (0.01 g/L), streptomycin (50 mcg/mL), nystatin (50 mcg/mL), agar, pH = 7.0 (Zenova & Zvyagintsev, 2002);

*Frankia* - propionic acid (0.5 g/L), $NH_4Cl$ (0.1 g/L), $CaCl_2*2H_2O$ (0.1 g/L), $MgSO_4*H_2O$ (0.2 g/L), $NaH_2PO_4*2H_2O$ (0.67 g/L), agar, pH = 6.8-7.2 (Semenov, 1990);
*Geodermatophilus* - yeast extract (1.0 g/L), glucose (1.0 g/L), soluble starch (1.0 g/L), $CaCO_3$ (1.0 g/L), streptomycin (50 mcg/mL), nystatin (50 mcg/mL), agar, pH=7.0 (Semenov, 1990);
*Micromonospora* - soluble starch (20.0 g/L), $K_2HPO_4$ (0.5 g/L), $MgSO_4$ (0.5 g/L), $KNO_3$ (1.0 g/L), NaCl (0.5 g/L), $FeSO_4$ (0.01 g/L), gentamicin (1 mcg/mL), streptomycin (25 mcg/mL), agar, pH = 7.2-7.4 (Zenova et al., 2004);
*Nocardia* - $NaNO_2$ (2.0 g/L), $Na_2CO_3$ (1.0 g/L), $K_2HPO_4$ (0.5 g/L), gentamicin (1 mcg/mL), agar, pH = 7.0 (Semenov, 1990);
*Rhodococcus* - $KNO_3$ (1.0 g/L), $K_2HPO_4$ (1.0 g/L), $KH_2PO_4$ (1.0 g/L), NaCl (1.0 g/L), $MgSO_4*H_2O$ (0.2 g/L), $CaCl_2*2H_2O$ (0.2 g/L), $FeCl_3$ (0.0001 g/L), yeast extract (1.0 g/L), propionic acid (0.5 g/L), levomycetin (20 mcg/mL), agar, pH = 7.0 (Cheremnykh, 2018);
*Streptomyces* - glucose (20.0 g/L), $KNO_3$ (1.0 g/L), NaCl (0.5 g/L), $MgSO_4$ (0.5 g/L), $K_2HPO_4$ (0.5 g/L), $CaCO_3$ (3.0 g/L), streptomycin (50 mcg/mL), agar, pH = 6.8-7.0 (Semenov, 1990).
The bacteria were isolated by serial dilution technique on nutrient agar medium Liofilchem (Italy): meat extract (1.0 g/L), yeast extract (2.0 g/L); peptone (5.0 g/L), NaCl (5.0 g/L), agar (15.0 g/L), pH = 7.2. Spread plate technique was carried out to isolate the organism form the diluted sample and incubated at 37°C for 24 hours (Kannan et al., 2018).
Fungi are typically isolated by plating a sample on a Petri dish containing wort agar (5.0°B, pH = 5.8-6.0), produced at Rivex SRL Brewery (Republic of Moldova, Chisinau municipality, Gratiesti commune), supporting the growth of a variety of fungi incubated for 7-10 days at 28-30°C (Nevalainen et al., 2014).
The identification of the belongingness of the microorganisms from the samples taken was carried out with optical microscopes (Lomo Mikmed - 2; Optika - B-292) using determinants for bacteria (Birger, 1982; Zarnea, et al., 2004), and fungi (Blagoveshenskaya, 2015; Eremeeva, 2008).
As result were isolated next active microorganisms: 19 strains of actinobacteria group; 12 strains of bacteria group; 15 strains of fungi group. After purification by several passages, the strains were tested for determination of potential antimicrobial activity.
The selected strains were subcultured in Petri dishes to obtain a bacterial lawn with diffusion of antimicrobial substances in agar substrate (Egorov, 2004). Test-cultures are maintained in the National Collection of Non-pathogenic Microorganisms.
Antibacterial efficacy was tested against:
*Agrobacterium tumefaciens* (*Rhizobium radiobacter*) 8628; *Bacillus subtilis* B-117; *Clavibacter michiganensis* (*Corynebacterium michiganense*) 13[a]; *Erwinia carotovora* (*Pectobacterium carotovorum*) 8982; *Xanthomonas campestris* 8003[b].
While antifungal activity was tested against:
*Alternaria alternata*; *Aspergillus niger*; *Botrytis cinerea*; *Fusarium oxysporum*; *Fusarium solani*.
Phytopathogenic bacteria tests were subcultured on nutrient agar (pH = 7.0-7.5), and phytopathogenic fungi tests were subcultured on wort agar (5.0°B, pH = 5.8-6.0) (Krassilnikov & Husein, 1974).
The biocidal activities were determined by the disk diffusion method. The tested cultures were subcultured in Petri dishes. The 8 mm agar blocks were cut with a sterile cork borer from the nutrient substrate where the strains of tested microorganisms grew abundantly. The agar blocks were then transferred to prepared cavities in agar nutrient medium with instantly subcultured tests. Petri dishes were kept in a cool place for 1 hour before incubation to allow the diffusion of biocidal substances. The diameter of the growth inhibition zones was measured after incubation at 37°C for 24 h for bacteria, and at 28°C for 72 h for fungi, respectively (Egorov, 2004; Rizk et al., 2007). There were three replications for each test (was applied to the significance threshold $p = 0.05$) and the biocidal assessment was performed twice.

## RESULTS AND DISCUSSIONS

In order to evaluate the biocidal activity of the studied microorganisms, various plant pathogens were screened. The selected pathogens cause severe disease and yield losses to many agricultural crops in the Republic of Moldova.

Listed below are diseases caused by test bacteria and fungi:

- *Agrobacterium tumefaciens* (*Rhizobium radiobacter*), which cause neoplastic diseases in plants like crown gall disease of woody plants such as pome (apple, pear, etc.) and stone (cherry, apricot, etc.) fruit, and nut (almond, walnut, etc.) trees (Escobar & Dandekar, 2003; Gohlke & Deeken, 2014; Kado, 2002);
- *Bacillus subtilis*, causative agent of potato disease of baked goods (Zavorohina, 2018);
- *Clavibacter michiganensis* (*Corynebacterium michiganense*), is the main causative agent of bacterial canker on solanaceous crops like eggplant, pepper and tomato (Ansari et al., 2019);
- *Erwinia carotovora* (*Pectobacterium carotovorum*), infects a much broader host of plants and cause soft rot of tomato and potato (Akbar et al., 2014);
- *Xanthomonas campestris*, cause black rot and vascular or leaf spot diseases of brassica species (Vicente & Holub, 2013);
- *Alternaria alternata*, cause *Alternaria* blotch of apple, *Alternaria* black spot of strawberry, and stem canker of tomato, respectively (Gat et al., 2012);
- *Aspergillus niger*, produce mycotoxins during developing on seeds, leaves and other plant organs (Soares et al., 2013; Alkhalifah et al., 2022);
- *Botrytis cinerea*, is a major plant pathogen, causing gray mold rot in a variety of cultures like pome fruits, stone fruits, grapes and berries (Rupp et al., 2017; Kahramanoğlu et al., 2022);
- *Fusarium oxysporum*, caused the *Fusarium* wilt in different fruits, berries and vegetables like tomato, watermelon, strawberry, cabbage, etc. (Gordon, 2017);
- *Fusarium solani*, is a pathogen reported on different crops both in nurseries and in fruit production fields, causing wilt and root rot (Villarino et al., 2019).

Activity of actinobacteria, bacteria, and micromycetes to inhibit the growth of the studied phytopathogenic fungi, is different and mainly depends on the characteristics of the synthesized substances with antimicrobial properties. For example, out of total number of 36 strains of actinobacteria belonging to 8 main genera isolated, 17 strains did not show the ability to inhibit the growth of 5 test cultures of phytopathogenic fungi. Next strains of actinobacteria showed activity against *A. alternata*: 2 of genus *Actinomadura*, 4 of genus *Actinoplanes*, 1 of genus *Frankia*, 3 of genus *Geodermatophilus*, 3 of genus *Micromonospora*, 2 of genus *Nocardia*, 2 of genus *Rhodococcus*, and 1 of the genus *Streptomyces*. While the size of growth inhibition zones varied between 10.0-23.0 mm. The strain of the genus *Actinomadura* N 1.2 showed the best result - the diameter of the growth inhibition zone of the test culture was 23.0 mm. The growth of *A. niger* was inhibited by 14 strains of actinobacteria with a zone diameter of 10.0-35.0 mm. The best result - the growth inhibition zones of this phytopathogen reached 30.0-35.0 mm in size and were noted under the influence of metabolites of *Nocardia* N 6.2 strain (30.0 mm), *Streptomyces* N 8.4 strain (33.0 mm), and *Micromonospora* N 5.5 strain (35.0 mm). The ability to inhibit the growth of *B. cinerea* was not seen among the new strains of actinobacteria. For *F. oxysporum*, 15 strains of actinobacteria were noted that have the ability to inhibit the growth of this test phytopathogen. Actively inhibit the growth by zones of 26.0 m next strains: *Actinoplanes* N 2.4, *Micromonospora* N 5.1, *Nocardia* N 6.2, while in other strains the zones varied between 13.0-21.0 mm. The growth of another representative of the phytopathogens of the genus *Fusarium*, *F. solani*, was inhibited by 10 strains of actinobacteria, and the growth inhibition zones varied in the range of 10.0-22.0 mm (Table 1).

Table 1. Antifungal activity of actinobacteria strains isolated from the silt sediments of the "La izvor" lake system, diameter of growth inhibition zones (mm)

| Genus of actinobacteria | Strain No. | *A. alternata* | *A. niger* | *B. cinerea* | *F. oxysporum* | *F. solani* |
|---|---|---|---|---|---|---|
| *Actinomadura* | N 1.2 | 23.0±1.1 | 10.0±0 | 0 | 15.0±1.1 | 14.0±0 |
| | N 1.3 | 18.0±0 | 14.0±0 | 0 | 20.0±0 | 10.0±0 |
| *Actinoplanes* | N 2.2 | 16.0±0 | 10.0±0 | 0 | 18.0±0 | 12.0±0 |
| | N 2.3 | 16.0±0 | 10.0±0 | 0 | 17.0±1.1 | 16.0±1.1 |
| | N 2.4 | 0 | 14.0±0 | 0 | 26.0±0 | 22.0±1.1 |
| | N 2.5 | 12.0±0 | 0 | 0 | 14.0±1.1 | 0 |
| *Frankia* | N 3.2 | 0 | 14.0±0 | 0 | 18.0±1.1 | 17.0±0 |
| *Geodermatophilus* | N 4.1 | 16.0±0 | 12.0±1.1 | 0 | 17.0±0 | 18.0±0 |
| | N 4.3 | 0 | 11.0±1.1 | 0 | 13.0±0 | 21.0±0 |
| | N 4.5 | 0 | 17.0±0 | 0 | 16.0±0 | 0 |
| | N 4.6 | 10.0±1.1 | 0 | 0 | 0 | 0 |
| *Micromonospora* | N 5.1 | 0 | 12.0±1.1 | 0 | 26.0±1.1 | 0 |
| | N 5.4 | 0 | 13.0±1.1 | 0 | 20.0±0 | 0 |
| | N 5.5 | 0 | 35.0±1.1 | 0 | 21.0±0 | 0 |
| *Nocardia* | N 6.1 | 10.0±0 | 0 | 0 | 0 | 0 |
| | N 6.2 | 0 | 30.0±1.1 | 0 | 26.0±1.1 | 10.0±0 |
| *Rhodococcus* | N 7.1 | 16.0±1.1 | 0 | 0 | 0 | 0 |
| | N 7.2 | 10.0±0 | 0 | 0 | 0 | 18.0±1.1 |
| *Streptomyces* | N 8.4 | 0 | 33.0±1.1 | 0 | 16.0±0 | 0 |

Note: p = 0.05

The conducted studies showed that out of total number of 22 bacterial strains assigned to 3 genera *Arthrobacter*, *Bacillus*, *Paenibacillus*, 9 bacteria did not show antagonism in relation to 5 strains of phytopathogenic fungi selected as test cultures, in the remaining strains of antifungal activity was ambiguous and was manifested by the formation of growth inhibition zones of a wide range – diameter between 6.0-30.0 mm (Table 2). Thus, the bacteria were able to inhibit the growth of *A. alternata* with zones between 11.0-26.7 mm. As can be seen from the data in Table 2, the metabolites of *Bacillus* 39 and *Paenibacillus* 43 formed inhibition zones with a size of 26.7 and 25.7 mm, while under the influence of metabolites of other strains of bacteria, the zones were between 11.0-21.7 mm in size. The growth of *A. niger* was inhibited only by 5 strains in small zones: 6.0-7.0 mm for bacteria of the genus *Bacillus* and 16.0-19.3 mm for bacteria of the genus *Paenibacillus*.

The studied bacteria inhibited the growth of *B. cinerea* more actively: the size of the zones was noted between 19.3-30.0 mm. Of the 11 bacterial strains marked by the ability to actively inhibit the growth of this phytopathogen, it should be noted *Bacillus* 32 (zones 25.0 mm), *Arthrobacter* 35 (zones 28.3 mm), *Bacillus* 31 (zones 29.9 mm), and especially *Paenibacillus* 47, causing the formation of inhibition zones with a size of 30.0 mm.

Growth inhibition of *F. oxysporum* was noted under the influence of 5 strains of bacteria, and 2 strains of them were active (*Bacillus* 32 and 33), because the zones were 27.7-29.7 mm in size, while 3 strains formed growth inhibition zones of a smaller size - 15.3-19.0 mm. In relation to another representative of the phytopathogens of the genus *Fusarium* - *F. solani*, bacteria isolated from silt sediments showed a different level of antifungal activity: a high activity in two strains (*Paenibacillus* 43 and *Bacillus* 39, respectively), while in other strains the level of activity was lower (zones 15.3-22.0 mm in size). The studied strains of bacteria showed the least antifungal activity against *A. niger* - 5 strains of bacteria formed growth inhibition zones with a diameter of 6.0-19.3 mm.

Table 2. Antifungal activity of bacteria strains isolated from the silt sediments of the "La izvor" lake system, diameter of growth inhibition zones (mm)

| Genus of bacteria | Strain No. | *A. alternata* | *A. niger* | *B. cinerea* | *F. oxysporum* | *F. solani* |
|---|---|---|---|---|---|---|
| *Arthrobacter* | 35 | 21.7±1.73 | 0 | 28.3±1.73 | 0 | 0 |
| *Bacillus* | 26 | 0 | 0 | 20.7±1.31 | 0 | 0 |
| | 28 | 0 | 0 | 23.3±3.27 | 0 | 0 |
| | 31 | 16.7±1.73 | 0 | 29.0±1.13 | 17.3±0.65 | 15.3±0.65 |
| | 32 | 19.0±1.13 | 6.0±1.13 | 25.0±1.13 | 27.7±0.65 | 20.0±1.13 |
| | 33 | 21.3±1.73 | 7.0±1.13 | 23.0±1.13 | 29.7±1.73 | 17.7±1.31 |
| | 39 | 26.7±1.73 | 0 | 21.0±4.08 | 0 | 29.3±1.31 |
| | 40 | 0 | 0 | 19.3±1.31 | 0 | 0 |
| *Paenibacillus* | 37 | 11.0±1.13 | 0 | 24.0±1.96 | 0 | 22.0±1.13 |
| | 43 | 25.7±0.65 | 16.0±1.13 | 0 | 15.3±0.65 | 26.7±1.31 |
| | 46 | 20.7±1.31 | 19.3±1.31 | 0 | 19.0±1.13 | 20.0±2.26 |
| | 47 | 20.7±1.31 | 19.3±1.31 | 30.0±2.26 | 0 | 0 |

Note: p = 0.05

Results of determining the antifungal activity of micromycetes isolated from the silt sediments of the "La izvor" lake system are present in Table 3. The data obtained showed that only one strain out of total number of 16 strains isolated did not show antifungal activity against 5 strains of test cultures of phytopathogens selected for the experiment. For other strains, the results are rather ambiguous: there is a high activity of micromycete strains (growth inhibition zones reach 40.0 mm in diameter) or insignificant (zones with a diameter of 10.7-11.3 mm). The fact of differences in the antagonism of the studied micromycetes in relation to one or another test phytopathogen also draws attention.

Table 3. Antifungal activity of micromycetes strains isolated from the silt sediments of the "La izvor" lake system, diameter of growth inhibition zones (mm)

| Genus of micromycetes | Strain No. | *A. alternata* | *A. niger* | *B. cinerea* | *F. oxysporum* | *F. solani* |
|---|---|---|---|---|---|---|
| *Penicillium* | N 1 | 18.0±2.26 | 0 | 0 | 14.0±1.13 | 15.0±1.13 |
| | N 2 | 18.3±2.36 | 0 | 14.7±0.65 | 10.7±1.31 | 12.7±0.65 |
| | N 5 | 17.3±1.31 | 0 | 16.7±1.31 | 0 | 14.7±0.65 |
| | N 6 | 17.7±2.85 | 0 | 0 | 0 | 16.3±1.73 |
| | N 8 | 16.0±1.13 | 0 | 16.3±0.65 | 0 | 15.7±0.65 |
| *Talaromyces* | N 3 | 39.0±1.13 | 0 | 14.3±1.73 | 18.0±2.26 | 15.0±1.13 |
| | N 4 | 39.0±1.13 | 0 | 13.3±1.31 | 11.3±1.31 | 15.3±0.65 |
| | N 7 | 17.3±1.31 | 28.3±1.73 | 13.3±1.31 | 26.3±1.31 | 18.7±1.31 |
| | N 28 | 27.7±2.85 | 0 | 14.3±1.73 | 27.7±2.85 | 22.0±2.26 |
| | N 29 | 22.0±2.26 | 23.0±2.99 | 16.3±0.65 | 18.0±2.26 | 25.3±0.65 |
| *Trichoderma* | N 9 | 28.7±1.31 | 24.7±0.65 | 40.0±1.13 | 29.0±4.08 | 40.0±2.26 |
| | N 10 | 40.0±2.26 | 16.0±1.13 | 40.7±1.73 | 40.0±1.13 | 40.0±1.96 |
| | N 12 | 40.0±2.26 | 18.0±2.26 | 40.7±1.73 | 40.0±1.13 | 40.0±1.96 |
| | N 13 | 31.7±3.27 | 18.0±2.26 | 40.0±2.26 | 40.0±1.96 | 40.0±2.26 |
| | N 14 | 40.0±2.26 | 18.7±1.31 | 41.3±1.31 | 40.3±1.73 | 40.3±1.73 |

Note: p = 0.05

For example, 8 strains of fungi showed no activity against *A. niger*, while only 2 strains against *B. cinerea*. Those there is a clear selectivity. In addition, we should also note the level of antifungal activity of fungi in relation to each of the test phytopathogens: if micromycetes inhibited the growth of *A. niger* by zones from 16.0 to 28.3 mm, then, for example, the growth of *B. cinerea*, *F. solani*, and *F. oxysporum* fungi inhibited by zones from 13.3 to 40.7 mm, or from 10.7 to 40.3 mm.

Comparing the degree of antifungal activity of the studied micromycetes, it should be noted that it manifested itself least of all in relation to *A. niger*: only one strain *Talaromyces* N 7 had the ability to inhibit the growth of this phytopathogen with zones of 28.3 mm, in other strains, the zone sizes varied between 16.0-24.7 mm.

The growth of *A. alternata* was more actively inhibited by all 15 strains of micromycetes. The activity was higher and the zones varied from 16.0 to 22.0 mm and even were from 27.7 to 40.0 mm in size, and the zones of 39.0-40.0 mm in size formed by 5 strains of micromycetes (Table 3).

An interesting pattern was noted in micromycetes in relation to *B. cinerea*: some strains showed low activity - zones of 13.3-16.6 mm under the influence of metabolites of 8 strains or rather high - zones reached 40.0-41.3 mm under the influence of 5 strains of micromycetes.

For *F. solani*, 5 strains can be considered active antagonists, which formed zones up to 40.0 mm, the remaining 10 strains differed in a variety of low activity values: zones 12.7-18.7 mm or 22.0-25.3 mm in two strains (*Talaromyces* 28 and *Talaromyces* N 29, respectively). In relation to *F. oxysporum*, 4 strains showed active antagonism (zones 40.0-40.3 mm), low activity - 3 strains (zones 10.7-18.0 mm) and medium activity - 3 strains (zones size - 26.3, 27.7, and 29.0 - strains *Talaromyces* N 7, *Talaromyces* N 28, and *Trichoderma* N 9, respectively).

The antibacterial activity of the studied representatives of actinobacteria was, as can be seen when comparing the data presented in Tables 1 and 4, noticeably less than the antifungal activity: the growth inhibition zones of 5 strains of phytopathogenic bacteria selected as test cultures varied between 9.0-16.0 mm. Growth inhibition of *A. tumefaciens* by zones of 9.0-13.0 mm was caused by 6 strains belonging to genera *Geodermatophilus*, *Micromonospora*, *Nocardia*, and *Streptomyces*. Seven strains of actinobacteria retarded the growth of *B. subtilis* by zones of 11.0-16.0 mm, they belonged to the genera *Geodermatophilus*, *Micromonospora*, *Nocardia*, and *Streptomyces*.

Table 4. Antibacterial activity of actinobacteria strains isolated from the silt sediments of the "La izvor" lake system, diameter of growth inhibition zones (mm)

| Genus of actinobacteria | Strain No. | *A. tumefaciens* 8628 | *B. subtilis* B-117 | *C. michiganensis* 13[a] | *E. carotovora* 8982 | *X. campestris* 8003[b] |
|---|---|---|---|---|---|---|
| *Geodermatophilus* | N 4.3 | 11.0±0 | 13.0±0 | 14.0±0 | 11.0±0 | 13.0±0 |
| *Micromonospora* | N 5.1 | 0 | 12.0±0 | 0 | 0 | 0 |
| | N 5.4 | 12.0±1.1 | 14.0±0 | 13.0±0 | 9.0±0 | 10.0±0 |
| *Nocardia* | N 6.1 | 12.0±1.1 | 14.0±1.1 | 15.0±0 | 13.5±0.6 | 14.0±0 |
| *Streptomyces* | N 8.1 | 13.0±1.1 | 16.0±1.1 | 16.0±0 | 12.0±0 | 15.0±1.1 |
| | N 8.3 | 11.0±0 | 12.0±0 | 14.0±0 | 14.0±0 | 14.0±1.1 |
| | N 8.6 | 9.0±0 | 11.0±1.1 | 9.0±0 | 9.0±0 | 0 |

Note: p = 0.05

The growth of *C. michiganensis* was inhibited by 6 strains of actinobacteria by zones of 9.0-16.0 mm. For *E. carotovora* also 6 strains showed weak antagonism - zones 9.0-14.0 mm. The growth inhibition of *X. campestris* was formed by 5 strains of actinobacteria, the zones were also small in size - 10.0-15.0 mm (Table 4).

The results of determining the antibacterial activity of the studied micromycetes are presented in Table 5. It can be seen that 15 strains differently caused the formation of growth inhibition zones against test bacteria. So, in relation to *A. tumefaciens*, 5 strains showed the ability to inhibit the growth of the test culture with zones of 25.3-28.0 mm, other strains against this test bacterium caused the formation of zones by 16.3-24.7 mm in size. In relation to *B. subtilis*, micromycetes quite actively showed the ability to inhibit growth – zones of 30.3-38.0 mm in size were noted. At the same time, zones with a size of 30.3-34.0 mm caused by metabolites of 9 strains, and zones with a size of 35.7-38.0 mm - 6 strains of the studied fungi. The growth of *C. michiganensis* was inhibited by 10 strains of micromycetes (zones between 10.7-23.7 mm), and in 5 strains metabolites formed growth inhibition zones between 25.0-26.0 mm. In relation to *E. carotovora*, 5 strains of micromycetes did not show antibacterial activity, turned out to be higher, which was reflected in the size of growth inhibition zones - their diameter was 28.0-30.7 mm. In relation to *X. campestris*, in 7 strains of micromycetes antibacterial activity was manifested by the formation of zones with a size of 12.7- 23.3 mm, and other 8 strains were distinguished by the ability to cause the appearance of zones of the absence of growth of this phytopathogen with a diameter of 25.3-30.7 mm (Table 5).

Table 5. Antibacterial activity of micromycetes strains isolated from the silt sediments of the "La izvor" lake system, diameter of growth inhibition zones (mm)

| Genus of micromycetes | Strain No. | *A. tumefaciens* 8628 | *B. subtilis* B-117 | *C. michiganensis* 13[a] | *E. carotovora* 8982 | *X. campestris* 8003[b] |
|---|---|---|---|---|---|---|
| *Penicillium* | N 2 | 22.7±3.46 | 38.0±1.13 | 20.7±1.31 | 20.7±1.31 | 25.7±0.65 |
| | N 5 | 25.3±0.65 | 33.0±4.0 | 25.0±1.13 | 24.3±2.36 | 22.3±2.85 |
| | N 6 | 25.7±0.65 | 34.0±1.96 | 23.3±1.31 | 0 | 21.7±3.27 |
| | N 8 | 21.3±1.31 | 30.3±0.65 | 19.3±1.31 | 0 | 23.3±1.73 |
| | N 11 | 28.0±1.96 | 35.7±1.31 | 26.0±2.26 | 30.0±2.26 | 30.7±1.31 |
| *Talaromyces* | N 3 | 16.3±0.65 | 31.3±1.31 | 10.7±1.31 | 0 | 20.0±2.26 |
| | N 4 | 18.3±1.73 | 31.3±1.31 | 15.0±1.13 | 28.0±2.26 | 19.3±1.31 |
| | N 7 | 22.0±2.26 | 36.3±1.31 | 17.3±1.31 | 17.3±0.65 | 26.0±1.13 |
| | N 28 | 25.3±0.65 | 37.3±1.31 | 25.7±1.31 | 0 | 25.3±0.65 |
| | N 29 | 23.3±1.31 | 35.7±0.65 | 26.0±1.96 | 0 | 28.3±1.73 |
| *Trichoderma* | N 9 | 21.0±1.13 | 33.7±1.73 | 17.3±1.31 | 20.7±1.31 | 12.7±1.31 |
| | N 10 | 24.0±2.26 | 33.3±3.27 | 25.7±1.31 | 30.7±1.31 | 29.3±1.31 |
| | N 12 | 24.3±1.31 | 36.0±1.96 | 20.7±1.31 | 28.7±2.61 | 25.7±3.64 |
| | N 13 | 24.7±1.73 | 34.0±1.96 | 20.7±1.31 | 23.0±1.96 | 22.3±2.85 |
| | N 14 | 26.7±1.73 | 30.7±1.31 | 23.7±1.73 | 28.7±1.73 | 26.7±1.73 |

Note: p = 0.05

Analysis of the obtained results showed the dependence of antimicrobial activity in the studied strains of actinobacteria, not only on species characteristics, but also on belonging to a particular genus. Thus, according to the data, it is clear that the strains of these 8 genera are not the same in their ability to inhibit the growth of one or another phytopathogen: out of 5 strains of the genus *Actinomadura*, only 2 strains had antifungal activity (zones between 10.0-23.0 mm), 4 from 5 strains of the genus *Actinoplanes* inhibit the growth of test fungi by zones of 10.0-26.0 mm. Low antifungal activity were in 1 from 2 strain of genus *Frankia* (zones 14.0-18.0 mm). Out of 6 strains from the genus *Micromonospora*, 3 strains showed high antifungal activity (especially, 26.0 and 35.0 mm zones under the influence of metabolites of strains N 5.1 and N 5.5, respectively). The strains of the genus *Nocardia* differed sharply in their antifungal activity: strain N 6.1 had a weak antifungal activity, and strain N 6.2 showed good results - zones against *A. niger* (30.0 mm), and *F. oxysporum* (26.0 mm). Weak antifungal activity was registered for strains of *Rhodococcus*. Only 1 strain out of 6 *Streptomyces* strains showed the ability to actively inhibit the growth of *A. niger* - zones up to 33.0 mm. The obtained results make it possible to choose for further studies a number of active strains of actinobacteria, which were characterized by high antifungal activity against phytopathogenic fungi. The results obtained are consistent with the literature data: out of 8 actinobacteria genera, the highest antifungal activity showed strains of the genus *Micromonospora*, *Nocardia*, and *Streptomyces* (Hata et al., 2015; El-Sabbagh et al., 2013). These strains include some strains of "rare" genera, in particular, *Actinomadura* and *Actinoplanes*, since they caused the formation of growth inhibition zones by 23.0-26.0 mm against *A. alternata* and *F. oxysporum* (N 1.2 and N 2.4). That is, these strains of actinobacteria, due to their antagonistic activity against phytopathogenic fungi, as a biocontrol, can replace chemical fertilizers and pesticides in the future. Moreover, according to the literature, out of 23,000 registered biologically active metabolites, more than 10,000 compounds are produced by actinobacteria, which is 45% of all biologically active metabolites of microbial origin (Aktuganova et al., 2007).

Determination of the dependence of the level of antimicrobial activity on the belonging of new strains of micromycetes to one or another genus showed that for 5 strains belonging to the genus *Penicillium*, a low antifungal activity is generally characteristic of the selected test cultures of phytopathogenic fungi: the strains either did not affect the growth of the test culture at all, or caused the formation of zones of growth inhibition with a diameter of 10.7-18.3 mm (Table 3). Four strains of micromycetes were assigned to the genus *Talaromyces*, of which 2 strains proved to be active antagonists against *A. alternata* (strain N 3 and N 4 - zones 39.0 mm), they did not retard the growth of *A. niger*, and in relation to representatives of the *Fusarium* genus, their activity was either low (zones 11.3-

18.0 mm) or significantly higher (zones 26.3-27.7 mm under the influence of metabolites of strains N 7 and N 28). One of the strains of this genus (N 28) also had the ability to inhibit the growth of *A. alternata* (zones up to 27.7 mm).

The strains of the genus *Trichoderma* were characterized by the ability to actively inhibit the growth of test cultures (except for *A. niger*) by forming zones up to 40.0-41.3 mm or inhibit growth zones by 18.0-24.7 mm (in the variant of experiments with test fungus *A. niger*). That is, micromycetes isolated from silt sediments, assigned to the genus *Penicillium*, *Talaromyces*, and *Trichoderma*, given their active antagonism against the selected test phytopathogenic fungi, can also be considered effective biocontrol.

The growing interest in the application of *Trichoderma* is due to their potential for direct and indirect biocontrol against a wide range of soil phytopathogens. They act through various complex mechanisms such as mycoparasitism, degradation of pathogen cell walls, competition for substrate and space, and induction of plant resistance (Sood et al., 2022; Tyśkiewicz et al., 2022; Guo et al., 2019).

Bacteria isolated from silt sediments and assigned to 3 genera *Arthrobacter*, *Bacillus*, and *Paenibacillus* differed markedly by antifungal activity from actinobacteria and micromycetes: their activity was, in general, higher than that of actinobacteria, but noticeably less than that of micromycetes. As can be seen in Table 3, a strain of the genus *Arthrobacter* No. 35 had the ability to inhibit growth of *A. alternata* and *B. cinerea* (21.7 and 28.3 mm, respectively), a representative of bacteria of the genus *Paenibacillus* No. 37 also had low activity (11.0-22.0 mm and 24.0 mm), while in representatives of the genus *Bacillus* it varied within a fairly wide range - from 6.0, 15.3 to 30.0 mm, and strains that actively inhibit growth of *B. cinerea* (No. 35, 31, and 47 - 28.3-30.0 mm) or the growth of representatives of the genus *Fusarium* (zones 26.7-29.7 mm under the influence of metabolites of strains No. 32, 33, 39, 43). That is, out of 12 strains of bacteria, 7 strains of the genus *Bacillus*, capable of showing antagonism against such phytopathogens that are often found in the Republic of Moldova, such as *B. cinerea* and phytopathogenic fungi that cause various kinds of fusariosis, may be of particular interest. The data obtained are consistent with the literature, which emphasizes the possibility of considering individual bacterial strains as clear candidates for bacterial control agents against fungal pathogens that cause significant damage to agriculture (Grabova et al., 2017; Lemanova, 2019; Pliego et al., 2011).

## CONCLUSIONS

The conducted studies have shown that microorganisms (actinobacteria, bacteria, and micromycetes) isolated from the silt sediments of the "La izvor" lake system have a different effect on the growth of phytopathogenic fungi and bacteria, and it should be noted that most of the studied isolates are characterized by a greater degree of antifungal activity than antibacterial.

These new strains of microorganisms, due to their antifungal and antibacterial activity, will replenish the National Collection of Non-pathogenic Microorganisms as a real source of metabolites with antimicrobial properties, as example the substances that contribute to better preservation of agricultural products, or as biopesticides.

The next stage of our research will be determination of the synthesized antibiotic substances by these microorganisms and compare them with well-known antibiotics.

## ACKNOWLEDGEMENTS

This research work was carried out with the support of State Program Project 20.80009.7007.09 “Conservation and use of microbial biodiversity as a support for the development of sustainable technologies and agriculture, the integration of science and education” (2020-2023), financed by NARD of the Republic of Moldova.

## REFERENCES

Ab Rahman, S.F.S., Singh, E., Pieterse, C.M.J., & Schenk, P.M. (2018). Emerging microbial biocontrol strategies for plant pathogens. *Plant Science, 267*, 102–111. doi: 10.1016/j.plantsci.2017.11.012.

Akbar, A., Din, S.U., Ahmad, M., Khan, G., & Alam, S. (2014). Effect of phytobiocides in controlling soft rot of tomato. *Journal of Natural Sciences Research, 4*(11), 99–102.

Aktuganova, G.E., Melentiev, A.I., & Galimzyanova N.F. (2007). Features of antagonistic interaction of various

taxonomic groups of bacilli with soil fungi. In Abstracts of the international scientific conference "Microorganisms and Biosphere" (Ed.), November 19-20, Moscow (pp. 3–4) (in Russian).

Alkhalifah, D.H.M., Damra, E., Khalaf, S.M.H., & Hozzein, W.N. (2022). Biogeography of black mold *Aspergillus niger*: Global situation and future perspective under several climate change scenarios using MaxEnt Modeling. *Diversity, 14*, 845. https://doi.org/10.3390/d14100845

Andriyuk, K.I., Iutynska, G.O., & Antipchuk, A.F. (2001). *Functioning of microbial coenoses under conditions of anthropogenic load.* Kiev: Oberegy (in Ukrainian).

Ansari, M., Taghavi, S.M., Hamzehzarghani, H., Valenzuela, M., Siri, M.I., & Osdaghi E. (2019). Multiple introductions of tomato pathogen *Clavibacter michiganensis* subsp. *michiganensis* into Iran as revealed by a global-scale phylogeographic analysis. *Applied and Environmental Microbiology – ASM, 85,* e02098-19. https://doi.org/10.1128/AEM.02098-19.

Azizbekyan, R.R., Kuzin, A.I., Nikolaenko, M.A., Smirnova, T.A., & Shamshina T.M. (2001). Biological control of plant fungal diseases. Biocontrol agents: Mode of action and interaction with other means of control. *IOBC WPRS Bull., 24*(3), 93–95.

Bel'kova, N.L., Parfenova, V.V., Kostopnova, T.I., Denisova, L.I., & Zaĭchikov, E.F., (2003) Microbial biodiversity in the Lake Baikal water. *Mikrobiologiia, 72*(2), 239–249 (in Russian).

Birger, M.O., (1982). *Handbook of microbiological and virological research methods.* Moscow - Medicine (in Russian).

Blagoveshenskaya E.I., (2015). *Phytopathogenic micromycetes. Educational determinant.* URSS (in Russian).

Borneman, J., & Triplett, E.W. (1997). Molecular microbial diversity in soils from eastern Amazonia: evidence for unusual microorganisms and microbial population shifts associated with deforestation. *Applied and Environmental Microbiology - ASM, 63*(7), 2647–2653. doi: 10.1128/aem.63.7.2647-2653.1997.

Burţeva, S., Sirbu, T., Postolachi, O., & Subina V. (2008). Antimicrobial properties of micromycetes and streptomycetes from Moldovan soils. *Bulletin of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 65*(1), 389–390.

Burtseva, S., & Sirbu T. (2009). Search for antagonists promising in the fight against fungi – pathogens of agricultural crops. *Ştiinţa agricolă, 1,* 44–49 (in Russian).

Chaurasia, A., Meena, B.R., Tripathi, A.N., Pandey, K.K., Rai, A.B., & Singh, B. (2018). Actinomycetes: An unexplored microorganisms for plant growth promotion and biocontrol in vegetable crops. *World Journal of Microbiology and Biotechnology, 34*(9), 132. doi: 10.1007/s11274-018-2517-5.

Cheremnykh, K.M. (2018). *Biodegradation of dehydroabietic acid by actinobacteria of the genus Rhodococcus.* 03.02.03 Microbiology. Abstract of the dissertation for the degree of candidate of biological sciences. Perm. (in Russian).

Denisova, L.Y., Bel'kova, N.L., Tulokhonov, I.I., & Zaichikov, E.F. (1999). Biodiversity of bacteria at different depths of the southern basin of Lake Baikal, identified by 16S rRNA sequences. *Mikrobiologiia, 68*(4), 547–556 (in Russian).

Egorov, N.S., (2004). *Basic teachings about antibiotics. Determination of antibiotic activity of the microorganisms.* Moscow: Science (in Russian).

El-Sabbagh, S.M., Emara, H.A., Metwally, A.M., & Saba H.A. (2013). A new antifungal compound from *Streptomyces exfoliatus*. *Life Science Journal, 10*(4), 2654–2665.

Eremeeva, S.V. (2008). *Mold mushrooms. Methods of isolation, identification, storage.* Astrakhan (in Russian).

Escobar, M.A., & Dandekar, A.M. (2003). *Agrobacterium tumefaciens* as an agent of disease. *Trends in Plant Science, 8*(8), 380–386. doi: 10.1016/S1360-1385(03)00162-6.

Gat, T., Liarzi, O., Skovorodnikova, Y., & Ezra, D. (2012). Characterization of *Alternaria alternata* causing black spot disease of pomegranate in Israel using a molecular marker. *Plant Disease - APS, 96*(10), 1513–1518. doi: 10.1094/PDIS-12-11-1041-RE.

Gohlke, J., & Deeken, R. (2014). Plant responses to *Agrobacterium tumefaciens* and crown gall development. *Frontiers in Plant Science, 5,* 155. doi:10.3389/fpls.2014.00155

Gordon, T.R. (2017). *Fusarium oxysporum* and the *Fusarium* wilt syndrome. *Annual Review of Phytopathology, 55,* 23–39.

Grabova, G.Y., Dankevich, L.A., Dragovoz, I.V. (2017). Antagonism of strains of *Bacillus* sp. C6 and *Bacillus* sp. LG 37S against certain phytopathogenic bacteria of the genera *Pectobacterium* and *Erwinia*. Abstracts of reports of the XV Congress of the Society of Microbiologists of Ukraine named after S.M. Vinohradskyi, September 11-15, (p. 48). Lviv: SPOLOM (in Ukrainian).

Guo, Y., Ghirardo, A., Weber, B., Schnitzler, J.-P., Benz, J.P., & Rosenkranz, M. (2019). *Trichoderma* species differ in their volatile profiles and in antagonism toward ectomycorrhiza *Laccaria bicolor*. *Frontiers in Microbiolology, 10,* https://doi.org/10.3389/fmicb.2019.00891

Hata, E.M., Sijam, K., Ahmad, Z., Yusof, M.T., & Azman N.A. (2015). In vitro antimicrobial assay of actinomycetes in rice against *Xanthomonas oryzae* pv. *oryzicola* and as potential plant growth promoter. *Brazilian Archives of Biology and Technology, 58*(6), 821–832.

Hussein, E.I., Jacob, J.H., Shakhatreh, M.A.K., AlRazaq, M.A.A., Juhmani, A.F., & Cornelison, C.T., (2018). Detection of antibiotic-producing Actinobacteria in the sediment and water of Ma'in thermal springs (Jordan). *Germs, 8*(4), 191–198.

Hyakumachi M., Takahashi, H., Matsubara, Y., Someya, N., Shimizu M., Kobayashi K., & Nishiguchi M. (2014). Recent studies on biological control of plant

diseases in Japan. *Japanese Journal of Phytopathology, 80,* 287–302.

Jeon, B.J., Kim, J.D., Han, J.W., & Kim, B.S., (2016). Antifungal activity of rimocidin and a new rimocidin derivative BU16 produced by *Streptomyces mauvecolo*r BU16 and their effects on pepper anthracnose. *Journal of Applied Microbiology, 120*(5), 1219–1228.

Kado, C.I. (2002). Crown gall. *The Plant Health Instructor*. doi: 10.1094/PHI-I-2002-1118-01.

Kahramanoğlu, I., Panfilova, O., Kesimci, T.G., Bozhüyük, A.U., Gürbüz, R., & Alptekin, H. (2022). Control of postharvest gray mold at strawberry fruits caused by *Botrytis cinerea* and Improving fruit storability through *Origanum onites* L. and *Ziziphora clinopodioides* L. volatile essential oils. *Agronomy, 12,* 389. https://doi.org/10.3390/agronomy12020389

Kannan, M.N., Sethi, S., Badoni, A., Chamoli, V. & Bahuguna N.C. (2018). Isolation and characterization of bacterial isolates from agriculture field soil of Roorkee region. *Journal of Pharmacognosy and Phytochemistry, 7*(5S), 108–110.

Krassilnikov, N.A., & Husein A., (1974). *Biology of selected groups of Actinomycetes*. Published for the U.S. Department of Agriculture, Agricultural Research Service and the National Science Foundation Washington, D.C. by the Indian National Scientific Documentation Centre, New Delhi.

Lemanova, N.B. (2019). Use of PGPR bacteria for protection sugar beet from root rot. *Agricultural science, 2,* 72–74. https://doi.org/10.32634/0869-8155-2019-326-2-72-74 (in Russian).

Nevalainen, H., Kautto, L., & Te'o, J. (2014). Methods for isolation and cultivation of filamentous fungi. *Environmental microbiology: Methods and protocols. Methods in Molecular Biology, 1096*, 3–16. Springer Science+Business Media, LLC. doi: 10.1007/978-1-62703-712-9_1

Omelyants, T.G., Kovalenko, N.K., & Golovach T.M. (2008). Safety assessment of microbial biotechnology products and hygienic regulation. *Mikrobiolohichnyi Zhurnal, 79*(2-3), 124–128 (in Ukrainian).

Palaniyandi, S.A., Yang, S.H., Zhang, L., & Suh, J.W. (2013). Effects of actinobacteria on plant disease suppression and growth promotion. *Applied Microbiology and Biotechnology, 97*(22), 9621–9636. doi: 10.1007/s00253-013-5206-1.

Pavlyushin, V.A. (2013). Problems of modern plant protection. In "Problems of mycology and phytopathology in the XXI century" (Ed.), *Mater. int. scientific conf.* (pp. 202–205). St. Petersburg, Russia (in Russian).

Pliego, C., Ramos, C., de Vicente, A., Cazorla, F.M. (2011). Screening for candidate bacterial biocontrol agents against soilborne fungal plant pathogens. *Plant and Soil, 340,* 505–520. https://doi.org/10.1007/s11104-010-0615-8

Rizk, M., Abdel-Rahman, T., & Metwally, H. (2007). Screening of antagonistic activity in different *Streptomyces* spp. against pathogenic microorganisms. *Journal of Biological Sciences, 7*(8), 1418–1423.

Rupp, S., Weber, R.W.S., Rieger, D., Detzel, P., & Hahn, M. (2017). Spread of *Botrytis cinerea* strains with multiple fungicide resistance in German horticulture. *Frontiers in Microbiology, 7,* 2075. doi: 10.3389/fmicb.2016.02075

Safronova, G.V., Sukhovitskaya, L.A., & Korolenok, N.V. (2007). Influence of inoculants and pesticides on the development of leguminous rhizobial symbiosis and productivity of leguminous plants. In Agricultural microbiology (Ed.), *Compilation of interdisciplinary subjects of science* (pp. 62–73). Chernihiv, Ukraine. (in Russian).

Semenov, S.M. (1990). *Laboratory media for actinomycetes and fungi*. Handbook - Moscow, RU: Agropromizdat. (in Russian).

Soares, C., Calado, T., & Venâncio, A. (2013). Mycotoxin production by *Aspergillus niger* aggregate strains isolated from harvested maize in three Portuguese regions. *Revista Iberoamericana de Micología, 30*(1), 9–13. doi: 10.1016/j.riam.2012.05.002.

Sood, M., Kapoor, D., Kumar, V., Sheteiwy, M.S., Ramakrishnan, M., Landi, M., … & Sharma, A. (2020). *Trichoderma*: The "Secrets" of a multitalented biocontrol agent. *Plants (Basel), 9*(6), 762. doi: 10.3390/plants9060762.

Tiwari, K., & Gupta, R.K. (2013). Diversity and isolation of rare actinomycetes: an overview. *Critical Reviews in Microbiology, 39*(3), 256–294. doi: 10.3109/1040841X.2012.709819.

Tyśkiewicz, R., Nowak, A., Ozimek, E., & Jaroszuk-Ściseł J. (2022). *Trichoderma*: The current status of its application in agriculture for the biocontrol of fungal phytopathogens and stimulation of plant growth. *International Journal of Molecular Sciences, 23*(4), 2329. https://doi.org/10.3390/ijms23042329

Van der Meij, A., Worsley, S.F., Hutchings, M.I., & Van Wezel, G.P., (2017). Chemical ecology of antibiotic production by actinomycetes. *FEMS Microbiology Reviews, 41*(3), 392–416.

Vicente, J.G., & Holub, E.B. (2013). *Xanthomonas campestris* pv. *campestris* (cause of black rot of crucifers) in the genomic era is still a worldwide threat to brassica crops. *Molecular Plant Pathology, 14*(1), 2–18. doi: 10.1111/j.1364-3703.2012.00833.x.

Villarino, M., De la Lastra, E., Basallote-Ureba, M.J., Capote, N., Larena, I., Melgarejo, P., & De Cal, A. (2019). Characterization of *Fusarium solani* populations associated with Spanish strawberry crops. *Plant Disease – APS, 103*(8), 1974–1982. doi: 10.1094/PDIS-02-19-0342-RE.

Yadav, N., Kour, D., & Yadav, A.N. (2018). Microbiomes of freshwater lake ecosystems. *Journal of Microbiology & Experimentation, 6*(6), 245–248.

Yu, J., Zhang, L., Liu, Q., Qi, X., Ji, Y., & Kim B.S. (2015). Isolation and characterization of actinobacteria from Yalujiang coastal wetland, North China. *Asian Pacific Journal of Tropical Biomedicine, 5*(7), 555–560.

Zakharova, O.S., Zenova, G.M., & Zvyagintsev, D.G. (2003). Selective techniques for isolating actinomycetes of the genus *Actinomadura* from the soil. *Mikrobiologiia, 72*(1), 126–130 (in Russian).

Zarnea, G., Mihăescu, G.R., & Velehorschi, V., (1992). *Principii şi tehnici de microbiologie generală. Volumul I.* Bucureşti, RO.

Zavorohina, N.V. (2019). New technologies for the production of wheat bread long-term storage under the conditions of new industrialization. *Advances in Social Science, Education and Humanities Research, 240*. 61–65.

Zenova, G.M., & Zvyagintsev, D.G. (2002). *Diversity of actinomycetes in terrestrial ecosystems*. Moscow, RU: MGU (in Russian).

Zenova, G.M., Zakalyukina, Y.V., Semin, V.V., & Zvyagintsev, D.G. (2004). Isolation and growth of soil acidophilic actinomycetes of the genus *Micromonospora*. *Soil Science*, 7, 847–852 (in Russian).