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Original Research

Biodiversity of Microorganisms in the Aquatic Environment as a Source for Combating Phytopathogenic Fungi

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Abstract

The protection of plants from diseases is mainly based on the use of chemical pesticides against pathogenic agents of fungal or bacterial etiology. That is why, in the past two decades, much attention has been paid to the development of ecological biological methods of combating plant diseases, which are considered an alternative to the traditional use of chemical pesticides. The aquatic environment is a good source for the detection of new strains of antagonistic microorganisms in relation to phytopathogens. The aim of the research was to determine the potential of the biodiversity of the microbiota isolated from the aquatic environment to combat the development of phytopathogenic fungi. Among actinobacteria, maximal inhibition of test cultures was achieved by strains of the genera *Streptomyces* (against *A. alternata*), *Micromonospora* (against *A. niger*), *Actinoplanes,* and *Nocardia* (against *F. oxysporum*). Bacteria of the genera *Bacillus* and *Micrococcus* possess the highest activity against *A. alternata*, *B. cinerea*, and *F. oxysporum*. The highest activity against phytopathogenic fungi was achieved by micromycetes of the genus *Trichoderma* (growth inhibition zones reached 40.0 mm). Cyanobacteria *Oscillatoria brevis* and *Oscillatoria acutissima* are active against *A. niger* and *F. oxysporum*, respectively. As a result, strains that were promising for use in phytosanitary control were discovered.

Keywords: antifungal activity, actinobacteria, bacteria, micromycetes, cyanobacteria, microalgae, biofilm, water, silt

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Introduction

Despite the current development trend in ecological agriculture, the protection of plants from diseases is mainly based on the use of chemical means of protection (pesticides) against pathogenic agents of fungal or bacterial etiology. In this sense, in advanced countries, including the Republic of Moldova, much attention is paidto the development of ecological biological methods of combating plant diseases, which are considered an alternative to the traditional use of chemical pesticides. Biological preparations based on microorganisms and their metabolites, which are characterized by high effectiveness, are increasingly used. Bacteria, actinobacteria, and micromycetes naturally associate with plants and have a beneficial effect on plant growth by mitigating biotic and abiotic stress [1-4]. Considering the ecological role of actinobacteria in microbial communities associated with plant roots, the latest achievements in the study of the complex interaction between streptomycetes, plants, and pathogenic and symbiotic organisms, as well as the results of studies of the antagonistic effect of streptomycetes on phytopathogenic organisms that contribute to the formation of symbiotic interactions between plant roots and microorganisms, were described [5-7].

The biological method of protecting plants from pathogens is based on the use of antagonistic microorganisms [8-10]. The importance of protecting crops from pests, diseases, and weeds becomes a determining factor in increasing the yield and quality of plant products. Fungicides are estimated to increase farm income in the U.S. by nearly $13 billion annually. Therefore, researchers have been searching for fungicides with low toxicity, high selectivity, and high activity against fungal strains that are resistant to other fungicides [11].

Oceans, seas, rivers, lakes, and plants are new sources for the detection of new strains of antagonistic microorganisms in relation to phytopathogens. The water space is a wide source for the isolation of new microorganisms with the potential to form biologically active substances. The discovery of new antimicrobial substances produced by aquatic microorganisms has great potential, as it contributes to the further treatmentof infectious diseases [12-14].

Actinobacteria of the genus *Streptomyces*, the most numerous in terms of species, are capable of producing compounds with different chemical structures and physiological activity that can have both positive and negative effects on living organisms [15]. Often, the active antagonism of streptomycetes is due to the synthesis of several metabolites simultaneously, which makes it difficult to form resistance to them among phytopathogens [16].

Among the producers of medical and agricultural antibiotics, one of the leading places is occupied by actinobacteria of the genus *Micromonospora* [17]. Also, it was reported that strains of the genera *Nocardia*, *Actinoplanes*, *Streptosporangium*, and *Actinomadura* were noted among the producers [18].

In laboratory experiments, the effect of streptomycetes and cyanobacteria on phytopathogenic fungi of the genus *Fusarium* was studied. The changes revealed in the population structure of the phytopathogenic fungus *F. oxysporum* as a result of seed treatment with antagonistic crops give hope of success in the creation of artificial phototrophic-heterotrophic associations [19], because the ability of representatives of different genera of actinobacteria to influence the development and growth of *F. oxysporum* [20], *Alternaria brassicicola*, *Botrytis* sp., and *Rhizoctonia solani* is widely known [21].

To protect plant seedlings from microdamage, as biofungicides with low toxicity, numerous compounds for agricultural needs have been obtained from newly isolated strains of actinobacteria. There are also reports of antimicrobial compounds synthesized by strains of the genera *Actinoplanes*, *Actinomadura*, *Frankia*, *Nocardiopsis* sp., *Nocardia* sp., *Pseudonocardia*, and *Rhodococcus* [22-24].

Micromycetes of the genera *Penicillium*, *Talaromyces*, and *Trichoderma* are of interest as producers of various metabolites with antibiotic effects against phytopathogenic fungi.

Strains of the genus *Penicillium* have a high antagonistic activity against a wide range of phytopathogenic microorganisms – causative agents of fusariosis, alternariosis, gray mold, white rot, spring black stem, leaf spot, and helmintosporiasis [25].

Another important aspect is the potential of plant pathogenic fungi to rapidly develop resistance mechanisms against licensed and widely used fungicides, which are common on farms worldwide. Fungican adapt to them by *de novo* mutation or selection from standing genetic variation, leading to resistance and loss of fungicide efficacy [26].

Detailed knowledge of *Trichoderma* properties, including metabolic activity and type of interaction with plants and other microorganisms, can ensure its effective use in agriculture. It acts through various complex mechanisms, such as mycoparasitism, degradation of pathogen cell walls, competition for nutrients and space, and induction of plant resistance. With the constant exposure of plants to a variety of pathogens, the main challenge is the development of biological protection alternatives [27].

The plant – *Trichoderma* – pathogen system is a complicated network of numerous processes. *Trichoderma* spp. are avirulent, opportunistic plant symbionts. In addition to being successful plant symbiotic organisms, *Trichoderma* spp. also behaves as a low-cost, effective, and environmentally friendly biocontrol agent. Stimulation of each process involves the biosynthesis of target metabolites such as plant growth regulators, enzymes, siderophores, antibiotics, etc. From an application point of view, the evidence provided here strongly supports the possibility of

using *Trichoderma* as a safe, ecological, and effective biocontrol agent for various crop species [28].

The most commonly used species of bacteria in microbiology is *Bacillus subtilis*, however, there are other antagonistic species, such as *B. methylotrophicus* and *B. amyloliquefaciens*, which have been poorly studiedbut have been considered efficient colonizers that are widespread in different habitats due to their ability to form spores, grow in a wide range of temperatures, and synthesize antibiotics that inhibit the growth of phytopathogens, in addition to being plant growth promoters. Antagonistic bacteria constitute the majority of the microbial population in soil, which is the main natural reservoir where the balance of microbial diversity varies, contributing to disease reduction in crop and non-crop plants. Microbial control agents are commonly used to manage bacterial and fungal diseases in crops and are becoming more widely used, with a possible reduction in the use of synthetic pesticides [29].

Eukaryotic microalgae such as *Nannochloropsis* sp., *Phaeodactylum* sp., *Scenedesmus* sp., and *Chlorella* sp. have antimicrobial properties againstmany microorganisms, including pathogenic fungi [30]. For example, a study examining the inhibition of mycelial growth of fungal pathogens in the *Fusarium* complex by *Nannochloropsis* sp. found that carotenoid (β-carotene and astaxanthin) and various phenolic extracts exhibit antagonistic activities. In addition, cell extracts from marine diatoms, such as *Phaeodactylum tricornutum,* are considered antimicrobial agents [31]. Unlike *P. tricornutum,* Marrez et al. reported considerable antifungal activities of the freshwater microalga *Scenedesmus obliquus* against *A. flavus*, *A. steynii*, *A. westerdijikiae*, and *A. carbonarius* [32]. Moreover, noteworthy antifungal activities of the chlorophyte *Chlorella vulgaris* were highlighted in *in vitro* studies by Vehapi et al. [28]. The authors concluded that *C. vulgaris* could be used as a natural fungicide due to its strong antagonistic effects against multiple pathogens. According to various studies, photosynthetic prokaryotes, such as the cyanobacterium *Spirulina* sp., exhibit fungicidal activities [31]. Biologically active compounds of eukaryotic and prokaryotic microalgae should effectively suppress the growth of phytopathogenic fungi.

The most notorious species of pathogenic fungi, including *Sclerotium rolfsii*, *Rhizoctonia solani*, *Botrytis cinerea*, and *Alternaria alternata*, caused a major economic impact on global harvests or production losses [32].

Thus, the aim of the research was to determine the potential of the biodiversity of the microbiota (strains of actinobacteria, bacteria, micromycetes, cyanobacteria, and microalgae) isolated from the aquatic environment (water column, biofilm, slit sediments) of the "La izvor" lake system (Chisinau municipality) to combat the development of phytopathogenic fungi, which pose a danger to the crops of the Republic of Moldova.

Materials and Methods

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

Sampling

Altogether, 11 points were sampled (water column, biofilm, silt sediments) in August 2020 from the "La izvor" lake system (Republic of Moldova, Chisinau municipality). Microorganisms of actinobacteria, bacteria, micromycetes, cyanobacteria, and microalgae were isolated [33].

Isolation of Microorganism Strains

Eight genera of actinobacteria were isolated on special selective nutrient media (which include mineral salts, sources of carbon and nitrogen, and certain antibiotics like streptomycin, nystatin, etc.) in Petri dishes by inoculation of diluted samples.

The bacteria were isolated by serial dilution technique on nutrient agar medium Liofilchem (Italy). The spread plate technique was carried out for isolation, and incubated for 24 hours at 37ºC.

Micromycetes were typically isolated by plating a sample on a Petri dish containing wort agar (5.0°B, pH = 5.8-6.0), and incubated for 7-10 days at 28-30ºC.

Isolation of microalgae and cyanobacteria was made on medium Gromov 6. Cultivation lasted 2 weeks under the light at a temperature of 20ºC.

Antifungal Activity Determination

After purification by several passages, the strains were tested for potential antifungal activity. The selected strains were subcultured in Petri dishes to obtain a bacterial lawn with the diffusion of antimicrobial substances on the agar substrate.

Antifungal activity was tested against: *Alternaria alternata*; *Aspergillus niger*; *Botrytis cinerea*; *Fusarium oxysporum*; and *Fusarium solani*.

Phytopathogenic fungi tests were subcultured on wort agar (5.0°B, pH = 5.8-6.0).

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. 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 28ºC for 72 h, respectively [34]. There were three replications for each test, and the biocidal assessment was performed twice.

Results and Discussion

The tables show the results of the antifungal activity of the studied strains. Growth inhibition zones against test cultures with a diameter greater than 15.0 mm presented interest.

Antifungal Activity of Actinobacteria

Analysis of the results of determining the antifungal activity of actinobacteria isolated from the water column of the lake system, according to the data presented in Table 1, showed that the *Actinomadura* strain A 1.2 more actively inhibited the growth of *A. alternata* (zones up to 22.0 mm); the rest of the tested cultures had extremely small zones (10.0 mm). Three strains of the genus *Actinoplanes* also inhibited the growth of the test cultures, forming zones ranging in size from 9.0 to 21.66 mm.

The growth of *A. niger* with zones up to 21.66 mm was inhibited by the strain *Micromonospora* A 5.3. In comparison with other test cultures, this strain of *Actinobacteria* and other strains of the genus *Micromonospora* were less active; the growth inhibition zones of the test cultures ranged from 13.66 to 19.66mm.

Actinobacteria strains attributed to the genus *Nocardia* were also characterized by low antifungal activity: metabolites of actinobacteria of this genus caused the formation of growth inhibition zones in tested fungi, with zones ranging from 9.66 to 18.33 mm. The strain *Streptomyces* A 8.5 showed high activity only against *A. alternata* (growth inhibition zone of 24.0 mm).

The results of determining the antifungal activity of actinobacteria isolated from the biofilm showed that out of 8 genera of actinobacteria, only 3 strains possess the ability to inhibit the growth of test cultures of phytopathogenic fungi. Thus, *Actinoplanes* B 2 .1 showed significant antifungal activity against *A. alternata* and *F. solani* with inhibition zones of 20.0 mm, and against *A. niger,* the inhibition zone was 16.0 mm. Strains of the genera *Geodermatophilus* and *Streptomyces* showed sensitivity only against *A. alternata* with inhibition zones of 19.0 mm and 16.0 mm, respectively.

The growth of *A. alternata* was inhibited by 7 strains (isolated from silt sediments) with zones of 10.0-18.0 mm, and only strain *Actinomadura* N 1.2 was able to inhibit the growth of this phytopathogen with zones up to 23.0 mm.

Against *A. niger*, out of 16 actinobacteria, 11 strains showed antagonism; the growth inhibition zones were 10.0-17.0 mm, and strains from the genus *Micromonospora* (N 5.5), *Nocardia* (N 6.2), and *Streptomyces* (N 8.4) showed inhibition zones of 30.0-35.0 mm.

The representatives of the genus *Fusarium* are also subject to antagonism. It is clear that against *F. oxysporum*, there are more strains with antifungal activity than for *F. solani*. Thus, only 2 strains did not have the ability to inhibit the growth of *F. oxysporum*, while 6 strains had no significant effect on the growth of *F. solani*. The growth inhibition zones of *F. oxysporum* under the influence of actinobacteria metabolites reached sizes of 20.0-26.0 mm, and against *F. solani* 2 strains formed inhibition zones of 21.0-22.0 mm.

None of the Actinobacteria Strains Influenced the Growth of B. cinerea

According to the obtained results, it should be noted that the majority of strains with antifungal activity are among those isolated from silt sediments. Among them, representatives of the genus *Actinomadura* N 1.2, *Actinoplanes* N 2.4, *Micromonospora* N 5.1, N 5.5, *Nocardia* N 6.2, and *Streptomyces* N 8.4 – possess the ability to actively inhibit the growth of phytopathogenic fungi with zones ranging from 21.0 to 35.0 mm.

Antifungal Activity of Bacteria

Table 2 shows the results of determining the antifungal activity of bacteria of the genus *Bacillus* and *Micrococcus* isolated from the biofilm of the "La izvor" lake system. It can be seen that strains of the genus *Bacillus* inhibit the growth of the test cultures in different ways. Four strains of the genus *Bacillus* caused inhibition of the growth of *A. alternata,* with zones from 19.3 to 26.7 mm, and in 2 strains of the genus *Micrococcus*, metabolites inhibited the growth of this pathogen with zones of 20.0-23.3 mm. For *A. niger*, the antifungal activity was lacking.

Against *B. cinerea*, out of 10 bacterial strains, 8 strains inhibited the growth of the test cultures in the range of 16.0 to 36.7 mm. At the same time, both strains of the genus *Micrococcus* showed high activity zones from 31.7 to 35.0 mm, while for the bacteria of the genus *Bacillus,* it was noted either the absence of the ability to inhibit the growth of this phytopathogen, or the growth inhibition zones ranged from 16.0 to 36.7 mm.

A particular relationship between the bacteria isolated from the biofilm and representatives of the genus *Fusarium* was observed: the growth of *F. oxysporum* was delayed only by 4 strains, and the growth of *F. solani* by 3 strains out of 10. Moreover, the diameter of growth inhibition zones of *F. oxysporum* varied from 9.0 to 33.0 mm, and that of *F. solani* was – 15.0-33.0 mm. The best results registered by the genera *Bacillus* B 63 and *Micrococcus* B 57 were that – in *F. solani* and *F. oxysporum*, the growth inhibition zones were 33.0 mm.

Fourteen strains of bacteria, isolated from the water column of the "La izvor" lake system, acted differently on the growth of test cultures: antagonists of *A. niger* were not identified, only 3 strains of bacteria (A 1, A 17, and A 24) inhibited the growth of *F. solani*, and in 3 other tested cultures (*A. alternata*,

Table 1. Antifungal activity of actinobacteria strains isolated from the water column (A), biofilm (B), and silt sediments (N) of the "La izvor" lake system, diameter (mm).

Place of isolation	Genus of actinobacteria	Strain №	*A. alternata*	*A. niger*	*B. cinerea*	*F. oxysporum*	*F. solani*
Water column	*Actinomadura*	A 1.2	22.0±1.13	10.0±0	10.0±0	10.0±0	0
	Actinoplanes	A 2.1	21.66±0.65	12.0±0	0	14.0±1.13	0
		A 2.2	13.0±0	9.0±0	0	14.0±0	0
		A 2.3	17.66±0.65	10.33±0.65	0	10.33±0.65	10.0±0
	Micromonospora	A 5.3	0	21.66±0.65	0	19.33±0.65	13.66±0.65
	Nocardia	A 6.1	16.0±0	10.0±1.13	0	10.66±0.65	0
		A 6.2	18.33±0.65	9.66±0.65	0	0	12.0±1.13
	Streptomyces	A 8.5	24.0±0	0	0	0	0
Biofilm	*Actinoplanes*	B 2.1	20.0±0	16.0±0	0	13.66±1.72	20.0±1.13
	Geodermatophilus	B 4.1	19.0±1.95	12.0±0	10.0±0	12.0±1.3	12.0±0
	Streptomyces	B 8.3	16.0±0	0	0	0	11.66±1.3
Silt sediments	*Actinomadura*	N 1.2	23.0±1.13	10.0±0	0	15.0±1.13	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±1.13	16.0±1.13
		N 2.4	0	14.0±0	0	26.0±0	22.0±1.13
	Frankia	N 3.2	0	14.0±0	0	18.0±1.13	17.0±0
	Geodermatophilus	N 4.1	16.0±0	12.0±1.13	0	17.0±0	18.0±0
		N 4.3	0	11.0±1.13	0	13.0±0	21.0±0
		N 4.5	0	17.0±0	0	16.0±0	0
	Micromonospora	N 5.1	0	12.0±1.13	0	26.0±1.13	0
		N 5.4	0	13.0±1.13	0	20.0±0	0
		N 5.5	0	35.0±1.13	0	21.0±0	0
	Nocardia	N 6.2	0	30.0±1.13	0	26.0±1.13	10.0±0
	Rhodococcus	N 7.1	16.0±1.13	0	0	0	0
		N 7.2	10.0±0	0	0	0	18.0±1.13
	Streptomyces	N 8.4	0	33.0±1.13	0	16.0±0	0

B. cinerea, *F. oxysporum*) the growth inhibition zones varied from 17.0 to 35.3 mm, depending on the tested culture and species membership. For example, strains of bacteria from the genus *Bacillus* showed quite high activity, with zones ranging from 30.0 mm (strain A 1) to 35.3 mm (strain A 13).

In comparison with *B. cinerea*, the range of inhibition zones is quite wide – from 19.3 to 33.0 mm. The best performance was achieved by strains from the genus *Bacillus*: strain A 1 (33.3 mm), strain A 13-30.7 mm, and the strain of the genus *Peribacillus* A 19-29.3 mm.

The growth of *F. oxysporum* was inhibited by 6 out of 14 bacterial strains, and the diameter of the growth inhibition zones ranged from 17.0 mm to 32.7 mm.

Strain A 21 (zones up to 29.0 mm) and strain A 1 (zones 32.7 mm) can be considered the most active.

It can be seen that the bacteria isolated from the silt sediments differ strongly in their relationship with the phytopathogenic fungi selected as test cultures. For example, in relation to the *A.alternata* strain, 3 bacterial strains (N 26, N 28, and N 40) were inactive, strain N 37 inhibited this phytopathogen only by 11.0 mm, other strains possess antagonistic activity with zones from 16.7 to 26.7 mm in diameter.

For *A. niger*, only 3 strains of bacteria showed the ability to inhibit the growth of this phytopathogen, and only strains N 46 and N 47 showed as more visible in comparison with others – 19.3 mm diameter zones.

Table 2. Antifungal activity of bacteria strains isolated from the water column (A), biofilm (B), and silt sediments (N) of the "La izvor" lake system, diameter (mm).

Place of isolation	Genus of bacteria	Strain №	*A. alternata*	*A. niger*	*B. cinerea*	*F. oxysporum*	*F. solani*
Water column	*Bacillus*	A 1	30.0±2.26	0	33.3±1.31	32.7±1.31	23.0±1.13
		A 13	35.3±1.31	0	30.7±1.31	20.3±1.15	0
		A 14	30.0±2.26	0	23.3±1.31	0	0
		A 15	33.0±1.13	0	22.0±1.26	0	0
		A 22	30.7±1.31	0	25.7±1.31	27.3±1.31	0
		A 24	24.7±0.65	0	22.0±2.26	0	21.3±1.73
	Planococcus	A 8	0	0	23.3±1.73	17.0±1.13	0
		A 17	0	0	20.7±1.31	17.0±1.13	24.7±0.65
	Kocuria	A 18	0	0	19.3±1.31	0	0
	Peribacillus	A 5	0	0	26.3±7.36	28.0±2.26	0
		A 19	0	0	29.3±1.31	0	0
		A 21	29.3±1.31	0	24.7±1.31	29.0±1.96	0
		A 30	17.7±0.65	0	22.3±2.85	0	0
	Paenibacillus	A 37	11.0±1.13	0	24.0±1.96	0	22.0±1.13
Biofilm	*Bacillus*	B 48	19.3±1.31	0	0	0	0
		B 50	0	0	30.7±1.31	0	0
		B 51	20.7±1.31	11.3±1.31	27.7±2.36	0	0
		B 53	0	0	0	23.7±1.73	0
		B 54	24.3±1.31	0	29.0±1.13	0	0
		B 56	0	0	23.3±1.31	9.0±1.13	0
		B 60	0	0	16.0±1.13	0	0
		B 63	26.7±1.73	0	36.7±1.73	19.0±1.13	33.0±1.13
	Micrococcus	B 57	23.3±1.73	7.0±1.96	35.0±1.96	33.0±1.13	18.3±0.65
		B 61	20.0±2.26	0	31.7±1.73	20.7±1.73	15.0±1.13
Silt sediments	*Arthrobacter*	N 35	21.7±1.73	0	28.3±1.73	0	0
	Bacillus	N 26	0	0	20.7±1.31	0	0
		N 28	0	0	23.3±1.27	0	0
		N 31	16.7±1.73	0	29.0±1.13	17.3±0.65	15.3±0.65
		N 32	19.0±1.13	6.0±1.13	25.0±1.13	27.7±0.65	20.0±1.13
		N 33	21.3±1.73	7.0±1.13	23.0±1.13	29.7±1.73	17.7±1.31
		N 40	0	0	19.3±1.31	0	0
		N 43	25.7±0.65	16.0±1.13	0	15.3±0.65	26.7±1.31
		N 46	20.7±1.31	19.3±1.31	0	19.0±1.13	20.0±2.26
		N 47	20.7±1.31	19.3±1.31	30.0±2.26	0	0
	Paenibacillus	N 37	11.0±1.13	0	24.0±1.96	0	22.0±1.13
		N 39	26.7±1.73	0	21.0±4.08	0	29.3±1.31

Table 3. Antifungal activity of micromycetes strains isolated from the water column (A), biofilm (B), and silt sediments (N) of the "La izvor" lake system, diameter (mm).

Place of isolation	Genus of micromycetes	Strain №	*A. alternata*	*A. niger*	*B. cinerea*	*F. oxysporum*	*F. solani*
Water column	*Penicillium*	A 1	21.7±1.27	0	14.3±1.36	0	15.7±0.65
		A 2	20.7±1.31	15.0±1.13	19.0±1.13	22.0±2.26	22.0±2.26
		A 4	22.3±0.65	20.0±2.26	19.3±1.31	23.3±1.31	22.7±1.31
		A 5	21.3±1.31	21.0±1.13	18.7±1.73	28.0±2.26	24.0±2.26
		A 6	26.0±1.13	22.3±0.65	16.0±1.13	24.7±1.31	25.3±1.31
		A 8	0	0	17.7±1.31	13.3±1.31	12.7±1.31
		A 10	23.0±1.13	21.0±1.13	11.3±1.31	30.3±0.65	27.3±1.31
	Talaromyces	A 3	23.3±1.27	25.3±0.65	0	26.0±1.13	15.0±1.13
	Trichoderma	A 11	0	19.3±1.31	31.3±1.31	31.7±3.27	26.7±3.27
		A 13	14.0±2.26	16.0±1.13	28.0±2.26	19.0±1.13	28.7±1.31
		A 14	0	0	15.7±0.65	0	0
		A 15	21.7±3.27	0	16.0±1.13	24.7±2.85	21.3±1.31
		A 16	21.7±1.27	0	33.7±3.64	26.3±1.73	37.0±1.13
Biofilm	*Talaromyces*	B 1	0	0	14.3±0.65	12.0±2.26	13.7±1.73
	Trichoderma	B 3	0	0	14.7±0.65	16.3±1.73	15.7±0.65
		B 5	0	23.0±2.99	17.0±1.13	35.0±1.13	22.3±2.85
Silt sediments	*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

The bacterial strains from the silt sediments inhibited the growth of *B. cinerea* much more actively. Of particular note are strains N 35, N 31, and N 47 (zones 28.3-30.0 mm in diameter).

Antifungal Activity of Micromycetes

Table 3 shows the results of determining the antifungal activity of micromycetes isolated from the water column of the "La izvor" lake system, which were assigned to the genera *Penicillium*, *Talaromyces*, and *Trichoderma*. It can be seen that the growth of the test culture *A. alternata* was inhibited by 10 out of 13 micromycete strains, and the range of the growth inhibition zones was 14.0-26.0 mm. In strains of the genus *Penicillium*, the zones were from 20.7 mm to 26.0 mm, in strains of the genus *Talaromyces* (A 3) – zones up to 25.3 mm, and in strains of the genus *Trichoderma* – 21.7 mm.

Table 4. Antifungal activity of cyanobacteria and microalgae strains isolated from the water column of the „La izvor" lake system, diameter (mm).

Genus of microalgae and cyanobacteria	*A. alternata*	*A. niger*	*B. cinerea*	*F. oxysporum*	*F. solani*
Anabaena variabilis	20.0±1.13	0	21.0±0	0	22.33±2.84
Aphanizomenon flos-aquae	22.0±0	0	0	0	0
Chlorella vulgaris	21.0±0	20.0±1.13	23.0±0	0	24.0±0
*Nostoc verrucosu*m	22.0±0	0	0	0	21.0±1.13
Oscillatoria brevis	20.0±2.26	40.0±0	0	0	0
Oscillatoria acutissima	24.33±1.72	22.33±1.72	24.0±1.13	30.0±1.13	20.0±1.13
Oscillatoria planctonica	20.0±0	21.0±0	22.0±2.99	0	20.0±0
Spirulina major	25.0±0	0	22.33±0.65	0	21.0±1.13

Of particular interest were the results of measuring the diameter of the growth inhibition zones of *B. cinerea* under the influence of metabolites of micromycetes isolated from water. For example, strains of the genus *Penicillium* were observed to induce zone formation with a diameter of 11.3-19.3 mm, while strains of the genus *Trichoderma* were noted to inhibit the growth of this phytopathogen from 15.7 to 33.7 mm. The best results were presented by strains A 11, A 13, and A 16, in which the inhibition zones of the test culture had a diameter of 31.3, 28.0, and 33.7 mm, respectively.

A sufficiently wide range of sizes of the growth inhibition zones of 2 representatives of the genus *Fusarium* was presented by strains of micromycetes isolated from water. Small (12.7, 13.3, or 15.0 mm) and rather large (28.0 to 37.0 mm) zones were observed.

It can be observed that only 3 of the micromycetes isolated from the biofilm of the "La izvor" lake system possess antifungal activity against some phytopathogens tested. These strains did not act on *A. alternata*, and 2 strains on the pathogen *A. niger*.

In comparison with *B. cinerea*, the activity of these micromycete strains was low; the diameter of the growth inhibition zones of this test varied from 14.3 to 17.0 mm. The antifungal activity was also low in relation to *F. solani* – the zone with a diameter of 13.7-22.3 mm, while in relation to *F. oxysporum,* a sensitivity was observed in strain B 5 of the genus *Trichoderma* – the growth inhibition zone reached 35.0 mm in diameter.

Next are presented the results of determining the antifungal activity of 15 strains of micromycetes from the genera *Penicillium*, *Talaromyces*, and *Trichoderma* isolated from the silt sediments. It should be noted that 5 strains of micromycetes belonging to the *Penicillium* genus did not show high activity in relation to these test cultures; the diameter of the growth inhibition zones varied from 10.7 to 18.3 mm. Among 5 representatives of the genus *Talaromyces*, strains with low activity were noted, in relation to *B. cinerea* (13.3-14.3 mm), or in relation to *F. solani* (11.3 mm), or with a high value in relation to *A. alternata* (39.0 mm).

Of the 15 strains of micromycetes, only 7 strains showed the ability to inhibit the growth of *A. niger* with zones of 16.0-28.3 mm. Particular attention is drawn to the results of determining the antifungal activity of 5 strains of micromycetes of the genus *Trichoderma*, which differ in activity by strain in relation to *A. alternata* (28.7-40.0 mm), in 2 strains of the genus *Fusarium* (29.0-40.0 mm), and in *B. cinerea* (40.0-41.3 mm).

Thus, among the micromycetes isolated from the "La izvor" lake system, it should be noted that 5 strains of the genus *Trichoderma* have the highest activity in relation to the test cultures, especially those strains isolated from the silt sediments. For example, the size of growth inhibition zones of *A. alternata*, *B. cinerea*, *F. oxysporum*, and *F. solani* reached 40.0-41.3 mm.

Antifungal Activity of Microalgae and Cyanobacteria

The results of determining the antifungal activity of cyanobacteria and microalgae isolated from water are shown in Table 4, which clearly shows that the activity is not the same and not all strains of microalgae possess it. The growth of *F. oxysporum* could only be stopped by the *Oscillatoria acutissima* strain with a sufficiently high level of activity – the growth inhibition zone of this phytopathogen reached 30.0 mm. Of note is the *Oscillatoria brevis* strain, which has the ability to cause inhibition of the growth of such phytopathogens as *A. niger* with zones up to 40.0 mm, while other strains caused the formation of growth inhibition zones in this test culture with a size of 20.0-22.0 mm. In this sense, certain strains can be considered sources of substances with antifungal activity.

Discussion of Results

Soil fungi represent a major problem for greenhouse and ornamental crops, as they infect the plant throughout its development and cause diseases, the distribution

of which is focal. Death of an ornamental plant species is rarely caused by a single soil phytopathogen, but is usually caused by a complex infection with several pathogens. Therefore, the search for new strains - antagonists of a number of phytopathogens of bacterial or fungal nature - is so important [35].

Currently, there is an increase in the number of diseases caused by bacterial, fungal, and viral infections. Infections affect plants at different stages of agricultural production. Depending on climatic conditions and the phytosanitary state of crops, disease incidence can reach 70-80% of the total plant population [36].

The most active antagonism against phytopathogens showed strains isolated from silt sediments, which is in accordance with other research [37-41].

Conclusions

As a result of this study, strains of actinobacteria, bacteria, micromycetes, cyanobacteria, and microalgae, characterized by significant antagonism towards phytopathogenic fungi (*A. niger*, *A. alternata*, *B. cinerea*, *F. solani*, and *F. oxysporum*), were identified and selected. The selected strains, with significant antifungal potential, will be further studied to identify the most promising ones for the purpose of obtaining preparations for phytosanitary use.

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Conflict of Interest

The authors declare no conflict of interest.

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