

ACTIVITY OF *STREPTOMYCES* spp. FROM AGRICULTURE SOILS AGAINST PHYTOPATHOGENS

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Summary. Antimicrobial activity of Moldovian selected strains of streptomycetes revealed that the growth of test cultures was actively delayed by 11 *Streptomyces* strains. Strain *Streptomyces* sp. 9 caused the formation of zones of lacking growth in 6 out of the 11 phytopathogens tested. Metabolites of the strains *Streptomyces* spp. 10 and 33 completely suppressed *Alternaria alternata* growth, while *Streptomyces* spp. 10 and 17 – *Botrytis cinerea*, strain *Streptomyces* sp. 10 – *Fusarium graminearum*. Growth of *Clavibacter michiganensis* was completely suppressed by the metabolites of the strain *Streptomyces* sp. 145. Results confirm the prospective to continue the research in bio-pesticide usage.

Keywords: biocidal activity; bio-pesticide; growth inhibition zone; soil borne phytopathogens.

Introduction

Soil is a complex system whose biological activity is strongly influenced by microflora and microfauna. It is an arena of enormous variety of interactions between physical, geochemical and biological processes. Living organisms that inhabit the soil interact each other and with the abiotic environment, based on their trophic or metabolic nature. They synthesize different products (vitamins, amino acids, auxins, antibiotics, enzymes, etc.) adsorbed by plants determining their important role in plant growth. The most active producers of substances or exometabolites with biological properties are strains of the genera *Bacillus*, *Pseudomonas* and *Streptomyces*. These substances have an important role in plant protection especially against phytopathogens (mainly bacteria and fungi) [5] and parasites (soil insects and plant parasitic nematodes) [21]. Moreover, they play a role in biocenoses processes and mineralization of organic residues which are important factors in the ecosystems [6].

Currently, many pests and parasites (phytopathogenic fungi, bacteria, viruses, insects, plant parasitic nematodes and weeds) cause significant damage to numerous crops [4, 13]. According to information, plants are subject to attack by over 50,000 different pathogens [7]. Yield losses caused by these pests cause a nutritional deficiency in the world, especially in developing countries. Many years of experience in active chemical control of harmful species of pests have shown the complete futility of a unilateral approach to ensuring ecological balance in intensive agroecosystems [17].

Despite the current trend towards the development of agriculture production, plant protection against parasites and pathogens is mainly based on the use of pesticides. Intensive and often unregulated use of pesticides leads to environmental pollution due to the accumulation of these substances in soil and natural waters [17]. Therefore, also in the Republic of Moldova, in the last years much more attention has been focused on the development of ecological and biological control methods against plant diseases with the aim to obtain control tools alternative to the use of pesticides.

Biological control methods to protect plants from pathogenic microorganisms is based on the use of antagonistic microorganisms so-called biological control agents. Currently, a number of microorganisms have been described to have an antagonistic effect on phytopathogens [22] and parasites [15].

The mechanism of action of these microorganisms includes competition for nutrition, effective colonization of the rhizosphere and leaf surfaces, synthesis of antibiotic substances and exometabolites with biocidal effects [14].

The aim of this research was to detect and evaluate the biocidal activity of strains of the genus *Streptomyces* spp., isolated from Moldavian agricultural soils, which are in active use for various crops growth.

Materials and methods

Soil samples were collected from the central part of the Republic of Moldova in different localities.

The list of the localities in which soil samples were collected is here reported:

I - Bacioi commune (Chisinau district). Black soil carbonate (humus content 2.5 %) from field with corn monoculture (46°54' N, 28°53' E);

II - Ghidighici village (Chisinau district). Black soil carbonate and medium loamy (humus 3.5 %) from arable land (47°09' N, 28°56' E);

III - Chisinau Municipality. Alkaline black soil and heavy loamy (humus 3.3%) from a vineyard (47°07' N, 28°86' E).

Soil samples were dehydrated by aeration for 7 days and then sieved through a 4 mm sieve. Then soil samples were pretreated with CaCO₃ and incubated for 10 days at 28°C according to procedure suggested by Rizk *et al.* [20] before proceeding to *Streptomyces* spp. isolation.

From the collected soil samples different strains were isolated by the Koch's method [9].

Streptomyces strains isolated from the different soil samples were: 41 (from 1 to 41), from site I; 25 (from 122 to 146), site II; 27 (from 147 to 173), site III.

All isolated *Streptomyces* strains are maintained in the National Collection of Non-pathogenic Microorganisms of the Institute of Microbiology and Biotechnology of Technical University of Moldova.

To assess biocidal activity of *Streptomyces*, different soil borne plant pathogens and pathogenic bacteria were selected and considered. The antifungal activity was tested against *Alternaria alternata*, *Aspergillus niger*, *Botrytis cinerea*, *Fusarium graminearum*, *F. oxysporum*, *F. solani* and *Rhizoctonia solani*, whereas the antibacterial efficacy was verified against *Agrobacterium tumefaciens* 8628 (now, *Rhizobium radiobacter* 8628), *Clavibacter michiganensis* 13^a, *Erwinia carotovora* 8982 (now, *Pectobacterium carotovorum* 8982), *Xanthomonas campestris* 8003^b [2, 26]. Antifungal and antibacterial activities were verified by test cultures considering that these pathogens cause severe diseases and yield losses to numerous crops in Republic of Moldova.

Biocidal activities were determined by disk diffusion's method on agar. Test cultures were deep sub-cultured in Petri dishes [20].

There were three replications for each test and the biocidal assessment was performed twice.

Results and discussion

Ninety-three free *Streptomyces* strains were isolated from soil samples collected in the 3 locations of the central part of the Republic of Moldova. They were identified at level genus as *Streptomyces* spp. [11].

Eleven strains, which were characterized by active growth on the Czapek agar medium with glucose, were selected for the assessment of their biocidal activity as reported in Table 1.

In the last two decades, many studies have been carried out to find new *Streptomyces* strains active producers of metabolites with antifungal activity [1].

The antifungal activity of the *Streptomyces* strains isolated from soil samples is reported in Table 1. The *Streptomyces* spp. 10 and 33 completely suppressed growth of *Alternaria alternata* which causes black spot in numerous crops. Exometabolites produced by *Streptomyces* spp. 9 and 12 caused the formation of inhibition zones of 29.0 ± 0 and 25.3 ± 1.3 mm, respectively for the same pathogen. The other strains showed the ability to inhibit *A. alternata* growth by zones from 12.0 ± 0 to 24.67 ± 0.65 mm.

The growth of *Aspergillus niger*, the agent of black mold on various plants, was delayed in different ways by the isolated *Streptomyces* strains. Metabolites from *Streptomyces* spp. 9, 10, 17, 37 and 155 caused the formation of growth inhibition zones from 12.3 ± 0.6 to 24.0 ± 0 mm.

The most widespread phytopathogen in the Republic of Moldova is *Botrytis cinerea*, which has a very wide range of host plants in which various tissues and organs are infected. It causes gray mold of fruits, berries and vegetables both in greenhouses and in open field conditions. A comparative analysis of the antifungal activity of *Streptomyces* strains isolated from different soil samples showed that the most effective antagonist strains of this phytopathogen are *Streptomyces* spp. 10 and 17 which cause a complete suppression of the fungus growth (Table 1). *Streptomyces* spp. 9, 12 and 33 showed inhibition zones with diameters from 22.0 ± 0 to 29.3 ± 0.6 mm. Metabolites of the other *Streptomyces* strains were less effective decreasing fungal growth inhibition zones from 10.7 ± 0.9 to 13.7 ± 0.65 mm (Table 1).

Table 1

Antifungal activity of *Streptomyces* strains isolated from soil samples collected in three sites of the central part of the Republic of Moldova

Diameter of the growth inhibition zones, (mm)	<i>Streptomyces</i> spp.	Test cultures						
		<i>Alternaria alternata</i>	<i>Aspergillus niger</i>	<i>Botrytis cinerea</i>	<i>Fusarium graminearum</i>	<i>F. oxysporum</i>	<i>F. solani</i>	<i>Rhizoctonia solani</i>
	9	29.0 ± 0	24.0 ± 0	29.3 ± 0.6	28.0 ± 0	33.7 ± 1.7	28.7 ± 0.6	29.0 ± 1.1
	10	C. i.	22.0 ± 1	C. i.	C. i.	0	14.0 ± 0	0
	12	25.3 ± 1.3	17.3 ± 0.6	22.0 ± 0	-	17.8 ± 1.4	-	17.0 ± 1.1
	14	16.3 ± 0.6	11.0 ± 0	16.7 ± 0.6	24.0 ± 0	0	14.0 ± 0	15.3 ± 0.6
	17	24.7 ± 0.6	22.8 ± 0.3	C. i.	23.0 ± 0	0	11.0 ± 1.1	0
	23	12.0 ± 0	17.0 ± 0	0	0	0	12.3 ± 0.6	8.8 ± 0.3
	33	C. i.	0	24.3 ± 0.6	30.0 ± 1.1	0	0	0
	37	14.3 ± 0.6	12.3 ± 0.6	17.0 ± 0	24.7 ± 0.6	17.7 ± 2.1	16.2 ± 2.3	-
	122	14.7 ± 0.6	0	10.7 ± 0.9	-	-	10.3 ± 0.6	0
	145	0	0	0	0	0	12.0 ± 1.1	0
	155	0	17.0 ± 0	13.7 ± 0.65	0	28.0 ± 0	0	0

*- experiment was not carried out; C. i. – complete inhibition.

The antifungal activity of our isolated *Streptomyces* strains was also tested on *Fusarium graminearum* the causal agent of *Fusarium* head blight in wheat, barley and oat. This disease leads to significant yield losses and especially quality through the contamination by different fungal mycotoxins. This pathogen was completely inhibited by *Streptomyces* sp. 10. An interesting control was also showed by *Streptomyces* spp. 9, 14, 17, 33 and 37. They delayed the pathogen growth by inhibition zones from 23.0 ± 0 to 30.0 ± 1.1 mm (Table 1).

Many species of the genus *Fusarium* are plant pathogens causing wilting diseases.

Among them particularly important is *Fusarium oxysporum* which cause *Fusarium* wilt in many horticultural crops [24]. Some *Streptomyces* strains isolated in our study were able to inhibit this soil borne plant pathogen. The best results were obtained by *Streptomyces* sp. 9 (33.7 ± 1.7 mm) and 155 (28.0 ± 0 mm). Less effective were *Streptomyces* spp. 12 and 37 whose metabolites caused the formation of growth inhibition zones of 17.8 ± 1.4 and 17.7 ± 2.1 mm, respectively (Tables 1).

Others *Streptomyces* spp. have also been reported in literature for their antifungal activity against *F. oxysporum* or other species. *S. bikiniensis* HD-087 fluid culture induced the systematic

resistance of cucumber plants against wilting disease caused by *F. oxysporum* f. sp. *cucumerinum* by the increase of peroxidase, phenylalanine, β -1,3-glucanase, chlorophyll and soluble sugars [26].

Moreover, a study on the fungicidal properties of 243 strains of 49 species of actinobacteria by Koch and Loffen revealed that *S. antimycoticus* FZ B 53 strain exhibited fungicidal activity against numerous soilborne plant pathogens, including *F. culmorum* [12].

Streptomyces sp. 9 was the most effective antagonist of *Fusarium solani* (agent of root rot disease). It was able to inhibit the growth of this pathogen culture with inhibition zone of 28.7 ± 0.6 mm. Other isolated strains showed a lower efficacy with inhibition zones ranging between 11.0 ± 1.1 and 16.2 ± 2.3 mm (Table 1).

Rhizoctonia solani causes various plant diseases such as collar rot, root rot, damping off and wire stem. The pathogen attacks its hosts when they are in their early stages of development, such as seeds and seedlings. Only in four selected *Streptomyces* strains it was possible to detect the ability of their metabolites to inhibit *R. solani* growth such as in *Streptomyces* spp. 9 (29.0 ± 1.1 mm), 12 (17.0 ± 1.1 mm), 14 (15.3 ± 0.6 mm) and 23 (8.8 ± 0.3 mm) (Table 1). Some natural fungicides, polyoxins B and D produced by *S. cacaoi* var. *asoensis* were also found to be effective to control *R. solani* [10].

In addition to the antifungal activity, the antibacterial activity of the selected *Streptomyces* strains was also investigated. In literature, there are many findings on the antagonism of actinobacteria towards phytopathogenic bacteria [23].

The tests on the bactericidal activities were carried out on *Agrobacterium tumefaciens* 8628, *Clavibacter michiganensis* 13^a, *Erwinia carotovora* 8982 and *Xanthomonas campestris* 8003^b.

The agent of crown gall disease (formation of tumors), *A. tumefaciens* 8628, was inhibited by *Streptomyces* spp. 9 and 14, zones were 14.3 ± 0.6 and 16.2 ± 1.4 mm, respectively (Table 2).

Streptomyces sp. 145, from II site, completely inhibit the growth of *C. michiganensis* 13^a, agent of soft rot disease on tomato. Metabolites of *Streptomyces* sp. 9 delayed the growth of this pathogen by inhibition zones up to 29.7 ± 1.7 mm. For *Streptomyces* spp. 23, 33, 37 and 122 the inhibition zones ranged between 22.7 ± 0.6 and 25.0 ± 1.1 mm. A lower antibacterial efficacy was showed by the other tested *Streptomyces* sp. (12, 14 and 17) with inhibition zones from 14.3 ± 0.6 to 18.0 ± 0 mm (Tables 2). The same bacterium was also inhibited by metabolites of *S. antimycoticus* FZ BS 3 strain as was found in a previous study by Koch and Loffen [12].

Table 2

Antibacterial activity of *Streptomyces* strains isolated from soil samples collected in three sites of the central part of the Republic of Moldova

Diameter of the growth inhibition zones, (mm)	<i>Streptomyces</i> spp.	Test cultures			
		<i>Agrobacterium tumefaciens</i> 8628	<i>Clavibacter michiganensis</i> 13 ^a	<i>Erwinia carotovora</i> 8982	<i>Xanthomonas campestris</i> 8003 ^b
	9	14.3 ± 0.6	29.7 ± 1.7	16.0 ± 1.1	30.0 ± 0
	10	-	15.0 ± 0	-	14.0 ± 0
	12	0	14.3 ± 0.6	0	0
	14	16.2 ± 1.4	15.0 ± 1.1	0	30.0 ± 1.1
	17	0	18.0 ± 0	0	15.0 ± 1.1
	23	0	25.0 ± 1.1	0	9.7 ± 0.6
	33	0	22.7 ± 0.6	-	16.0 ± 0
	37	0	23.0 ± 0	0	10.3 ± 0.6
	122	-	24.3 ± 0.6	-	9.3 ± 0.6
	145	-	C. i.	-	12.0 ± 0
	155	0	15.3 ± 0.6	-	12.7 ± 0.6

*- experiment was not carried out; C. i. – complete inhibition.

Streptomyces strains effective against *Erwinia carotovora*, the agent of soft rot disease, were not found. Only *Streptomyces* sp. 9 showed a partial control of the bacterium growth with inhibition zones of 16.0 ± 1.1 (Tables 2). Delay in the development of infections caused by *E. carotovora* was also found by Reiter *et al.* due to endophytic bacterial communities [19]. Findings by Oskay *et al.* reports that some *Streptomyces* strains isolated from a soil in Turkey inhibited *E. amylovora* and *A. tumefaciens* growth [18].

Since 2000 Yan-Min *et al.* reported the efficacy of some *Streptomyces* strains on the development of phytopathogenic bacteria such as *Xanthomonas campestris* and *E. carotovora* [25]. According to the literature, studies of Hastuti *et al.* and Mingma *et al.* are of some interest because describing results of the influence of rhizosphere and endophytic *Streptomyces* on *X. campestris* and *X. oryzae* [8, 16].

The antibacterial activities of *Streptomyces* spp. 9 and 14 (I soil samples location) were effective on *X. campestris* 8003, agent of black rot disease. They produced metabolites with inhibition zones of 30.0 ± 1.1 mm. For others studied *Streptomyces* spp. (10, 17, 23, 33, 37, 122, 145, 155) antibacterial activity was also detected by the diameter of the inhibition growth zones which ranged between 9.3 ± 0.6 and 16.0 ± 0 mm (Table 2).

A promising element of a modern and sustainable agriculture is the use of bio-pesticides based on microorganisms or their metabolites that exhibit growth-promoting properties that increase plant resistance to pests, pathogens and abiotic stress factors [3].

An analysis of the results on the antimicrobial activity of Moldovian selected strains of streptomycetes revealed that the growth of test cultures was actively delayed by strains isolated from soil samples collected in Bacioi Municipality (I) until complete growth inhibition of pathogens.

Conclusions

Results of our study on the antibacterial and antifungal activity on *Streptomyces* genus allow us to confirm the prospective to continue the research on the identification of *Streptomyces* spp. strains useful for bio-pesticide formulations to use in a sustainable plant protection against harmful phytopathogens and parasites.

Acknowledgments. This research was funded by Government of Republic of Moldova, Ministry of Education and Research, Research Subprogram 020101 “InBioS – Innovative biotechnological solutions for agriculture, medicine and environment”. Institutional financing contract no. 4/FI of 22 February 2024.

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