

ANTAGONISTIC ACTIVITY OF BACTERIAL ISOLATES AGAINST FUSARIUM PATHOGENS

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Summary. Actinobacteria and bacteria stored in NCNM were tested against *F. oxysporum* CNMN-FF-06 and *F. solani* CNMN-FF-07. In case of antagonistic activity against *F. solani*, results were much higher in comparison with *F. oxysporum*, ranged between 12.3 – 19.3 mm. The highest result of antifungal activity of bacteria against *F. oxysporum* was registered for the strain *Bacillus velezensis* CNMN-BB-16 (25.0 mm). For *F. solani*, the highest result was registered for the strain *Micrococcus yunnanensis* CNMN-BM-19 (33.0 mm). Results of the present research had shown that biocontrol agents can be used as an alternative for chemical pesticides utilized against phytopathogenic fungi.

Keywords: antifungal activity; actinobacteria; bacteria; *Fusarium*; agriculture crops.

Introduction

Plant diseases represent an urgent problem in agriculture. Main pathogens which cause harvest losses are fungi that endanger food security. In addition, many fungal pathogens such as *Fusarium* spp. produce mycotoxins which made agricultural products dangerous for the final consumer [10].

Strains of genus *Fusarium* are very adaptable, regardless of attraction to moderate climates, they contaminate crops all around the world. Such *Fusarium* toxins as trichothecenes, fumonisins, and zearalenone can lead to acute intoxication and even organism death [9].

Majority of *Fusarium* spp. are pathogenic to a vast list of important crops. Among of them are: wheat, barley, maize, bananas, cucumbers, tomatoes, eggplants, dates, cotton, etc. Proliferation of *Fusarium* on vegetal organisms could cause next degradational processes: root or stem rots, cankers, vascular wilts, fruit or seed rots, and leaf diseases. Aftermaths of this fungi on economy, medicine and society because of crop failure increase instability in any region [7].

Below are specific examples of where fusarium possess a threat to various agricultural crops.

Large yield losses are observed in cereal crops, which are a common target for these plant pathogens [7]. There are reports that in the United Arab Emirates, the date production industry is facing with sudden decline syndrome caused by the soil-borne fungi of *Fusarium* spp. In particularly, it is caused by *F. solani* [2, 3]. Passion fruit harvest suffering from *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *passiflorae* [4, 8]. Such demanded crop in markets as banana, has experiencing dramatic losses due to an epidemic of *Fusarium* wilt of banana, caused by *Fusarium oxysporum* f. sp. *cubense* [6]. A very important vegetable which possesses remarkable economic and dietary value is cucumber (*Cucumis sativus* L.) [1]. Each year the cucumber harvest suffer losses because of *Fusarium* wilt, induced by the pathogen *Fusarium oxysporum* f. sp. *cucumerinum* [8, 11]. Another plant not least, as eggplant (*Solanum melongena* L.) is widely cultivated in India, China, Egypt, Iran and Turkey. The common problem for this crop as for mentioned above, is *Fusarium* wilt [16].

Environmentally friendly methods for suppression of diverse *Fusarium* spp. involve the use of microorganisms, especially some groups of bacteria, in the same time, majority of them could promote plant development, many of which have been shown to be efficient. The policy in agriculture should appeal in development of direction for the healthy growth of crops and biological control of phytopathogens using endophytic bacteria [16].

Below are some data on the successful detection of various strains of bacteria capable of suppressing the growth and development of *Fusarium* spp.

Chemical compound β -glucanase, synthesized by streptomyces strain is able to hydrolyze fungal cell wall glucans and suppress *Fusarium* wilt of cucumber [17]. Also, were found five *Streptomyces* spp. which produce chitinases against *Fusarium oxysporum* f. sp. *albedinis* which is responsible for disease of date palm [2, 13].

For protection of banana crop, some *Bacillus* spp. showed moderate but sufficient antagonistic results against *Fusarium* spp. [6].

A number of antagonistic microbes, such as *Pseudomonas* spp., *Bacillus* spp., *Streptomyces* spp., *Trichoderma* spp., and *Paenibacillus* spp., were investigated and studied to control various plant pathogens, they were demonstrated as efficient biocontrol agents against cucumber *Fusarium* wilt [8].

Bacterial strains of *Bacillus* sp. SV101 and *Bacillus tequilensis* SV104 isolated from the *Solanum elaeagnifolium* proved efficiency against *Fusarium* wilt [5, 16].

Considering positive results reported by researchers, biological control of fungal diseases is one such approach that offers much toward increasing world crop production. Therefore, the present study aimed at screening of bacteria with antifungal properties on their potential to control the *Fusarium oxysporum* and *Fusarium solani*, pathogens of agriculture crops in the Republic of Moldova.

Materials and methods

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

The study included 20 bacterial strains of genera *Actinomadura*, *Actinoplanes*, *Micromonospora*, *Streptomyces*, *Bacillus*, *Micrococcus*, *Paenibacillus*, *Peribacillus*, *Planococcus* stored and deposited in NCNM.

As a nutritional medium for the cultivation and growth of strains, nutrient agar, Gause and Czapek medium were used. Cultivation was carried out at a temperature of $35\pm 1^\circ\text{C}$, 48 h for bacteria and 28°C , 14 days for actinobacteria.

To assess biocidal activity of selected microorganisms, the antifungal activity was tested against *Fusarium oxysporum* CNMN-FF-06 and *Fusarium solani* CNMN-FF-07; considering that these pathogens have cause severe diseases and yield losses to numerous crops in Republic of Moldova.

Biocidal activities were determined by disk diffusion method on agar. Test cultures were subcultured by pour plate method. The diameter of the growth inhibition zones was measured after 3-4 days of test cultures incubation at 25°C [15].

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

Results and discussion

Below are presented the significant results of antifungal activity of 10 strains of actinobacteria and 10 strains of bacteria against the causative agents of fusarium diseases in agricultural plants, namely *Fusarium oxysporum* CNMN-FF-06 and *Fusarium solani* CNMN-FF-07.

According to results (Table 1), antifungal activity of actinobacteria against *F. oxysporum* CNMN-FF-06 ranged between 9.7 – 13.3 mm. The highest activity had shown by strain of *Actinomadura* sp. 43; low activity, less than 10.0 mm was registered for strain *Streptomyces* sp. 72.

In case of antagonistic activity against *F. solani* CNMN-FF-07, results were much higher, ranged between 12.3 – 19.3 mm. The highest result was registered for strain *Actinoplanes* sp. 26 (diameter of 19.3 mm). Also, significant results were obtained under the influence of strains: *Actinomadura* sp. 36; *Micromonospora* sp. 56; *Streptomyces* spp. 71, 73. Other strains had shown moderate results (less than 15.0 mm).

It should be noted that actinobacteria strains are more active against *F. solani* CNMN-FF-07 than against *F. oxysporum* CNMN-FF-06.

Table 1

Antifungal activity of actinobacteria strains from NCNM

Diameter of the growth inhibition zones, (mm)	Actinobacteria	Test cultures	
		<i>F. oxysporum</i> CNMN-FF-06	<i>F. solani</i> CNMN-FF-07
	<i>Actinomadura</i> sp. 2	10.3±0.7	12.3±1.7
	<i>Streptomyces</i> sp. 21	12.0±1.1	11.7±0.7
	<i>Actinoplanes</i> sp. 26	12.7±1.3	19.3±1.3
	<i>Actinomadura</i> sp. 36	12.0±1.1	16.0±2.0
	<i>Actinomadura</i> sp. 37	12.7±1.3	13.3±1.3
	<i>Actinomadura</i> sp. 43	13.3±1.3	14.3±0.7
	<i>Micromonospora</i> sp. 56	10.3±0.7	15.7±1.3
	<i>Streptomyces</i> sp. 71	10.3±0.7	15.7±2.4
	<i>Streptomyces</i> sp. 72	9.7±0.7	13.7±0.7
	<i>Streptomyces</i> sp. 73	11.3±0.7	17.0±3.0

Bacterial strains (Table 2), on the other hand, showed more significant results. Antifungal activity against *F. oxysporum* CNMN-FF-06 ranged between 11.7 – 25.0 mm. The highest result was registered for the strain *Bacillus velezensis* CNMN-BB-16 (25.0 mm). Also, significant results were obtained under the influence of strains: *Bacillus velezensis* CNMN-BB-17 and *Planococcus chinensis* CNMN-BP-24 (growth inhibition zones more than 20.0 mm).

Table 2

Antifungal activity of bacteria strains isolated from isolated from NCNM

Diameter of the growth inhibition zones, (mm)	Bacteria	Test cultures	
		<i>F. oxysporum</i> CNMN-FF-06	<i>F. solani</i> CNMN-FF-07
	<i>Bacillus subtilis</i> CNMN-BB-01	15.7±0.7	19.7±2.8
	<i>Bacillus velezensis</i> CNMN-BB-16	25.0±3.4	29.3±1.3
	<i>Bacillus velezensis</i> CNMN-BB-17	22.7±1.3	31.3±1.7
	<i>Micrococcus yunnanensis</i> CNMN-BM-19	24.7±0.7	33.0±2.0
	<i>Paenibacillus pabuli</i> CNMN-BP-21	0	0
	<i>Peribacillus simplex</i> CNMN-BP-23	11.7±2.8	24.0±2.0
	<i>Planococcus chinensis</i> CNMN-BP-24	21.0±2.0	27.7±2.8
	<i>Bacillus safensis</i> CNMN-BB-25	0	0
	<i>Bacillus rugosus</i> CNMN-BB-29	0	20.0±7.9
	<i>Micrococcus aloeverae</i> CNMN-BM-30	0	0

By analysis of data obtained for *F. solani* CNMN-FF-07, antifungal activity ranged between 19.7 – 33.0 mm. The highest result was registered for the strain *Micrococcus yunnanensis* CNMN-

BM-19 (33.0 mm). Also, significant results were obtained under the influence of strains: *Bacillus velezensis* CNMN-BB-16; *Bacillus velezensis* CNMN-BB-17; and *Planococcus chinensis* CNMN-BP-24 (growth inhibition zones more than 25.0 mm).

As in case of actinobacteria, the bacteria also showed greater activity against *F. solani* CNMN-FF-07 than against *F. oxysporum* CNMN-FF-06.

According to the literature data, *Actinomadura* sp. COL08 is capable to suppress the growth and development of *F. oxysporum* f. sp. *albedinis* [12], which is in accordance with data obtained for *Actinomadura* sp. 43. Analysis of the results obtained for *Actinoplanes* sp. 26 against *F. solani* CNMN-FF-07, demonstrated that *Actinoplanes* spp. possessed capacity to produce antifungal metabolites active against *Fusarium solani* [2].

Best results obtained for the *Bacillus velezensis* CNMN-BB-16 against *F. oxysporum* CNMN-FF-06 is justified by comparison with research on *B. velezensis* YW17, which could inhibit this pathogen by secreting antifungal lipopeptides, proteins and volatile substances, thereby protecting crops from pathogenic fungal infections [18].

Micrococcus yunnanensis CNMN-BM-19 showed huge activity against *F. solani* CNMN-FF-07, which was confirmed in similar studies provided by Mexican researchers [14].

Conclusions

The screening of autochthonous bacteria with pronounced antifungal effect on phytopathogenic strains *F. oxysporum* CNMN-FF-06 and *F. solani* CNMN-FF-07 was performed. Results of the present research had shown that biocontrol agents can be used as an alternative for chemical pesticides utilized against phytopathogenic fungi.

Strains of bacteria, in addition to antifungal properties also synthesize substances with plant growth-promoting properties, which can lead to an increase in the organic harvest obtained and the quality of the products.

Therefore, the further investigations also will be focused on potential of selected biotechnologically relevant bacteria for plant phytostimulation what contribute to the development of sustainable agricultural through the use of environmentally harmless natural substances.

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