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ANTIBIOTIC AND PHYTOTOTOXIC ACTIVITIES OF CULTURAL FILTRATES OF SOME MICROMYCETE STRAINS

I.G. Rubezhniak

National University of Life and Environmental sciences of Ukraine,
15 Heroyiv Oborony st., Kyiv, 03041, Ukraine
e-mail: rubezhnyak60@gmail.com

*The **aim** of work was to test the antibiotic and phytotoxic activity of cultural filtrates of 125 micromycete strains isolated from different ecological niches. **Methods.** The activity was studied using different test-organisms – Gram-negative phytopathogenic bacteria, yeasts and green algae. **Results.** It is shown that 57 cultures were developed biological activities only. The wide spectrum of antibiotic action and middle antifungal activity was characterized for the cultures of genus Aspergillus and Penicillium. A fungistatic activity was shown only by strains as A. parvulus 31, A. versicolor 60, P. brevicompactum 144, P. rubrum 91and 93. A significant group consisted of cultures with phytotoxic activity: A. alliaceus 16, A. parvulus 30, A. versicolor 56, Aureobasidium pullulans 41 and 44, Chaetomium globosum 38, Curvularia inaequalis 84, Fusarium merismoides 151, Mortirella vinacea 74 and 82, Paecilomyces lilacinus 127, P. chrysogenum 163. Most strains with antibiotic activity did not show phytotoxic activity against test culture. **Conclusions.** As it is no findings about phytotoxic effect of strains of A. pullulans and M. vinacea and from the data obtained it might be inferred that the phytotoxic potential of these genus to be the most promising for further researches.*

Keywords: micromycetes, antibiotic activity, phytotoxic activity, test-organisms.

During the second century the identification of micromycetes that produce bioactive compounds is of great interest in the finding of new molecules to fight against many plant diseases. Fungi produce a wide range of metabolites as enzymes, antibiotics, vitamins, cytotoxic substances, insecticides, compounds that promote or inhibit growth, attractor, repellent, etc. [1, 2].

System researches concerning ability to produce the biologically active substances were conducted by different authors on the separate strains of molds [2, 3]. Wide potential of micromycetes had not been fully studied up to now.

Intensive use of chemicals such as fertilizers and pesticides in agriculture forwards to increase of anthropogenic load of the environment. Chemicals accumulate in plants and pass to human beings cause intoxication, chronic diseases and cancer. The elimination of these consequences is possible only through a search new high- performance and ecologically safe chemicals as biopesticides.

The aim of our work was to test the antibiotic and phytotoxic activity of cultural filtrates of 125 micromycete strains as a starting point for further investigations of the most perspective strains for feasible obtaining of biopesticides.

Materials and methods. In this work it was used 125 micromycete strains of the genus *Penicillium, Cladosporium, Botrytis, Beauveria, Chaetomium, Curvularia, Endomyces, Fusarium, Gliocladium, Aspergillus, Alternaria, Aureobasidium, Paecilomyces, Mortirella, Nigrospora, Pseudallescheria, Scopulariopsis, Trichoderma, Ulocladium* and separate strains *Nectria* sp., *Phialophora* sp. and *Verticillium dahliae,* which were isolated from different ecological niches such as air, dwelling walls, soil of Chernigovska area and rhizosphere of plants.

The micromycetes were grown on a basic nutritive Czapek medium [5]. The culture media were distributed in Erlenmeyer flasks (250 ml) with 50 ml medium. For inoculation ten-day suspension (1 x 10^8 cells per ml) of micromycetes grown on Czapek medium by 26°C were used. After 14 days the mycelium was removed through filtration. The culture liquid filtrates were tested against Gram-negative phytopathogenic bacteria such as *Agrobacterium tumefaciens* 8464 and *Pectobacterium carotovorum* 8636. *A. tumefaciens* causes crown gall disease on various plant species and *P. carotovorum* is a ubiquitous plant pathogen with a wide host range (carrot, potato, tomato, leafy greens, etc.). Antifungal activity was tested using test culture of yeast *Trichosporon cutaneum* 1502. Phytotoxic properties of culture liquid filtrates were studied using green algae *Chlorella vulgaris* 190 [5].

Bacterial test organisms have been incubated on the medium of agarose gel for 24 hours. The yeast strain have been grown on wort agar at 26 ° C for 48 hours, the algal culture was grown in test tubes on a medium [5] at 26° C and illumination of 4000 lux for 5 days.

The screening study of culture liquid filtrate of micromycete strains using standard agar well diffusion method was followed. Eight-millimetre diameter wells were cut from the agar using a sterile cork-borer, and 100 µl of filtrate were delivered into the wells [5]. A diameter of inhibition zones of Petri dish culture was measured. The inhibition zones of bacteria were measured after 18-24 hours. In a similar way, the antifungal activity of culture liquid filtrates using yeast after 48 hours of incubation was studied. Phytotoxic properties of culture liquid filtrates were determined after incubation of Petri dishes with *C. vulgaris* 190 for 48 -72 hours.

Bacterial and yeast strains were collected from the Department of Physiology and Taxonomy of Micromycetes, Danylo Zabolotny Institute Microbiology and Virology NAS of Ukraine.

Results. It can be seen in Table 1 that only 57 from 125 strains shown antibiotic activity and inhibit the growth of test organisms. Among them, strains such as *A. alternata* 116, *A. pullulans* 41*, C. cladosporiodes* 8 and 9*, F. merismoides f. merismoides* 151*, P. variotii* 67-69*, P. aculeatum* 123 and 124, *P. funiculosum* 1-6, *P. brevicompactum* 144*, P chrysogenum* 163*, P. ochrochloron* 145 and 156, *P. purpurogenum* 143, *T. harzianum* 157 and *V. dahlia* 168 have a broad spectrum of antibiotic activity against phytopathogenic bacteria.

The separate strains such as *A. alleaceum* 118*, A. parvulus* 31, *A. terreus* 119*, A. versicolor* 58 and 61*, A. pullulans* 43-46, *M. vinacea* 62 - 63, *P. funiculosum* 7*, P. rubrum* 91 and *T. viride* 120 displayed antibiotic activity against *P. carotovorum* only. These strains didn't show the antifungal and phytotoxic activities except *A. parvulus* 31. The strains as *A. parvulus* 30, *A. versicolor* 60 and *G. virens* 39 shown antibiotic activity against *A. tumefaciens* (inhibition zone is 30, 13 and 18, respectively). Most strains inhibited the growth of bacteria for 24-48 hours (bacteriostatic activity).

The strains of the genus *Aspergillus* such as *A. parvulus* 31, *A. versicolor* 60, *Penicillium* genus such as *P. brevicompactum* 144 and *P. rubrum* 91, 93 showed fungistatic activity against *T. cutaneum* 1502 (inhibition zone from 15 to 10 mm). Strains of *C. cladosporiodes* 10 and 11 also visualized very weak fungistatic effect.

The study of phytotoxic effect of micromycete strains indicated that *A. alliaceus* 16*, A. parvulus* 30*, A. versicolor* 56*, A. pullulans* 41 and 44*, C. globosum* 38*, C. inaequalis* 84*, F. merismoides* 151*, M. vinacea* 74 and 82*, P. lilacinus* 127*, P. chrysogenum* 163 and *V. dahlia* 168 demonstrate the strong phytotoxic activity. Other strains showed a small phytotoxic activity and had small size of inhibition zones of the test culture (*A. parvulus* 31*, A. pullulans* 42*, B. cinerea* 55*, M. vinacea* 73 and other). Most strains with antibiotic activity did not show phytotoxic activity against test culture.

The strains of *B. bassiana, F. lactis, F. solani, Nectria* sp.*, N. oryzae, P. marquandii, P. clavigerum, P. ucrainicum, Phialophora* sp.*, P. boydii, S. brumptii, U. atrum* and *U. consortiale* did not display antibiotic and phytotoxic activity.

Discussion. The finding shows that micromycete strains have a large spectrum of biological active substances. The results demonstrated that representatives of the genus *Aspergillus* and genus *Penicillium* are characterized by high antibiotic activity against phytotoxic bacteria and middle antifungal activity. Only some strains of genus *Aspergillus* as *A. alliaceus* 16, *A. versicolor* 56, *A. parvulus* 30 and genus *Penicillium* as *P. aculeatum* 124, *P. chrysogenum* 163 and *P. ochrochloron* 156 have a remarkable phytotoxic activity against *C. vulgaris* 190. Our experiments are consistent with previous results [6, 7]. Tsyganenko shown that *Aspergillus* strains had a wide spectrum of antibiotic action and phytotoxic activity especially *A. parvulus* 3142 [6, 7]. Screening of antifungal activity against yeast-like fungi had shown only single displays of this activity [6]. Some authors have also suggested that all chloroform extracts of the tested isolates of *Aspergillus* and *Penicillium* species had antifungal effect against *Fusarium* spp. growth where those from *P. chrysogenum* and *A. flavus* had decreased their radial growth by 76% compared to their relative controls [8]. The literature review shows that extracts of cultural filtrates of *A. niveus* 2411 and *Penicillium* sp.1051 have been shown the activity concerning phytopathogenic bacteria *A. tumefaciens* 8464. Note should be taken that the traditional methods of controlling of phytopathogenic

bacteria such as metal-containing pesticides were not effective [9]. Therefore, the investigation of antibiotic activity of *Aspergillus* and *Penicillium* species against phytopathogenic bacteria and their practical use deserve attention.

Another promising finding was that species of the genus *Aureobasidium* and genus *Mortirella* are characterized by antibiotic and phytotoxic activity. It was reported in literature that a strain of *A. pullulans* is used to produce a cyclic peptide that has specific antifungal activity: aureobasidin A [10]. Due to its strong antagonistic activity against other microorganisms, *A. pullulans* is used as a biocontrol agent in agriculture [10]. Additionally, a recent study reported that some strains of *A. pullulans* can produce an antibacterial compound, exophilin A, as well as high yields of liamocins, and heavy oils. *A. pullulans* has a high importance in biotechnology to produce different enzymes, siderophores and pullulan [11]. Furthermore, *A. pullulans* is used in biological control of plant diseases, especially storage diseases [12, 13]. In recent years there has been considerable interest in strains of *A. pullulans* as biopesticides. A proprietary mixture of two strains of *A. pullulans* was recently registered in the U.S. under the trade name "Blossom Protect" as a biocontrol to prevent blossom infections by *Erwinia amylovora*, the fire blight pathogen. Blossom Protect has been used successfully both Europe and in the Pacific Northwest. The new Botector Fungicide contains yeast-like fungi, *A. pullulans* strains DSM 14940 and DSM 14941, as the active constituent [14]. Botector Fungicide is a biological fungicide intended for use as a preventative treatment in the control of botrytis bunch rot / grey mould (*B. cinerea*) in grapes.

There has been considerable interest in antibiotic potential of *M. vinacea* in recent years [15]. The known compound methyl 2,4-dihydroxy-3,5,6-trimethylbenzoate and three new related metabolites, which were named mortivinacins A, B and C, were identified as metabolites of the fungus *M. vinacea*. Nicotinic acid was also encountered. Methyl 2,4-dihydroxy-3,5,6-trimethylbenzoate, mortivinacins A and nicotinic acid were responsible for the antibacterial and antifungal activities of the extract. Our results suggest that cultural filtrates of strains of *A. pullulans* and *M. vinacea* possess marked antibacterial activity as well as phytotoxic activity. It must also be noted that it is no findings about phytotoxic effect of strains of *A. pullulans* and *M. vinacea.*

This study has gone some way towards enhancing our understanding of biologically active substances by micromycete strains. It will be important to carry on the further research of metabolites of *Aspergillus* and *Penicillium* strains, shown antibacterial or phytotoxic activity, which can afford a basis for obtaining of biopesticides.

To further our research, we intend to study the phytotoxic cultural filtrates and extracts of micromycetes using a wide spectrum of crops and weeds. More broadly, research is also needed to determine the phytotoxic potential of strains of *A. pullulans* and *M. vinacea.* The literature on phytotoxic activity of strains of the genus *Aureobasidium* and *Mortirella* is absent. It is interesting to test the strains of *A. pullulans* and *M. vinacea* against a range of negative and positive bacteria also.

Table 1

Antibiotic and phytotoxic activities of cultural filtrate of micromycetes

No п/п	Species	Strains	Diameter of inhibition zones of test, mm			
			A. tumefaciens 8464	*P. carotovorum 8636*	*T. cutaneum 1502*	*C. vulgaris 190*
1	2	3	7	8	9	10
1	*Alternaria alternata*	116	16,0±0,7	11,0±0,9	0	0
2	*Aspergillus alliaceus*	16	0	0	0	22,0±2,7
3		118	0	15,0±1,0	0	0
4	*A. parvulus*	30	30,0±0,7	0	0	16,0±0,7
5		31	0	24,0±2,0	15,0±0,7	12,0±3,0
6	*A. terreus*	119	0	15,0±2,0	0	0
7	*A. versicolor*	56	0	0	0	20,0±1,0
8		58	0	17,5±0,5	0	0
9		60	13,0±1,0	0	12,0±2,0	9,0±1,0
10		61	0	19,0±3,0	0	0
11	*A. ustus*	101	0	13,0±1,0	0	0
12		102	0	15,0±2,0	0	0
13		103	0	15,0±1,0	0	0
14	*Aureobasidium pullulans*	41	30,0±0,8	25,0±1,0	0	17,3±0,9
15		42	0	0	0	13,0±1,0
16		43	0	15,7±0,4	0	0
17		44	0	10,8±0,4	0	19,0±0,7
18		45	0	20,0±0,7	0	0
19		46	0	20,0±0,7	0	0
20	*Botrytis cinerea*	55	0	0	0	11,0±1,0
21	*Chaetomium globosum*	38	0	0	0	22,0±3,0
22	*Cladosporium cladosporiodes*	8	30,0±1,0	10,0±1,0	0	0
23		9	10,7±0,3	12,0±2,0	0	0
24		10	0	0	7,0±0,3	0
25		11	0	0	9,0±0,3	0
26	*Gliocladium virens*	39	18,0±1,0	0	0	0
27	*Curvularia inaequalis*	84	0	0	0	19,0±2,0
28	*Fusarium merismoides f. merismoides*	151	15,0±0,3	16,0±1,0	0	20,0±2,0
29	*Mortirella vinacea*	62	0	22,0±1,0	0	0
30		63	0	24,0±1,0	0	0
31		73	0	0	0	13,0±0,7
32		74	0	0	0	25,0±0,7
33		82	0	0	0	15,0±0,1
34	*Paecilomyces variotii*	67	12,7±0,1	10,0±1,0	0	0
35		68	30,0±1,0	20,0±0,7	0	0
36		69	0	10,7±0,8	0	0
37	*P. lilacinus*	127	0	0	0	21,0±1,0
38	*Penicillium aculeatum*	123	12,0±0,7	14,0±1,0	0	0
39		124	20,0±2,0	11,0±0,7	0	14±0,7
40	*P. brevicompactum*	144	13,0±2,0	17,0±2,0	10,0±1,3	0

41	*P. funiculosum*	1	15,0±2,0	12,0±0,7	0	0
42		2	32,0±1,0	9,0±0,3	0	0
43		3	18±0,7	19,3±0,7	0	0
44		4	16,7±0,4	16,3±0,4	0	0
45		5	16,3±0,4	15,3±0,4	0	0
46		6	17,0±0,7	17,7±1,1	0	0
47		7	0	11,0±0,7	0	0
48	*P. chrysogenum*	163	39,0±0,7	14,0±0,7	0	24,3±1,1
49	*P. ochrochloron*	145	12,0±0,7	13,7±0,1	0	0
50		156	20,0±0,7	11,0±1,3	0	13±0,7
51	*P. purpurogenum*	143	12,0±1,3	11,7±0,4	0	0
52	*P. rubrum*	91	0	20,0±1,3	13,0±0,7	0
53		93	0	0	11,0±0,7	0
54	*Trichoderma harzianum*	157	23,0±0,7	17,7±0,4	0	12,3±0,9
55	*T. viride*	120	0	15,0±0,7	0	0
56	*Trichoderma* sp.	147	0	0	0	0
57	*Verticillium dahliae*	168	11,0±0,7	17,7±0,4	0	19,0±0,7

АНТИБІОТИЧНА ТА ФІТОТОКСИЧНА АКТИВНОСТІ КУЛЬТУРАЛЬНИХ ФІЛЬТРАТІВ ДЕЯКИХ ШТАМІВ МІКРОМІЦЕТІВ

І.Г. Рубежняк

Національний університет біоресурсів та природокористування України, вул. Героїв Оборони, 15, Київ, 03041, Україна

Резюме

Метою роботи був скринінг 125 штамів мікроміцетів, виділених з різних екологічних ніш, на антибіотичну та фітотоксичну активності. **Методи.** Активність вивчалася з використанням різних тест-організмів − грамнегативних фітопатогенних бактерій, дріжджів і зелених водоростей. **Результати.** Показано, що тільки 57 досліджуваних штамів проявили біологічну активність. Широким спектром антибіотичної дії і помірною антифунгальною активністю характеризувалися досліджувані штами роду *Aspergillus* та *Penicillium.* Фунгістатичну дію проявляли штами *A. parvulus* 31*, A. versicolor* 60, *P. brevicompactum* 144, *P. rubrum* 91 і 93. Значну групу складали штами з фітотоксичною активністю − *A. alliaceus* 16, *A. parvulus* 30, *A. versicolor* 56, *Aureobasidium pullulans* 41 і 44, *Chaetomium globosum* 38, *Curvularia inaequalis* 84, *Fusarium merismoides* 151, *Mortirella vinacea* 74 і 82, *Paecilomyces lilacinus* 127, *P. chrysogenum* 163. Більшість штамів з антибіотичною активністю не показали фітотоксичної дії. **Висновки.** Найбільш перспективними для подальших досліджень є штами *A. pullulans* та *M. vinacea,* які проявляють фітотоксичну активність, що раніше не досліджувалось.

Ключові слова: мікроміцети, антибіотична активність, фітотоксична активність, тест-організми.

АНТИБИОТИЧЕСКАЯ И ФИТОТОКСИЧЕСКАЯ АКТИВНОСТИ КУЛЬТУРАЛЬНЫХ ФИЛЬТРАТОВ НЕКОТОРЫХ ШТАММОВ МИКРОМИЦЕТОВ

И.Г. Рубежняк

Национальный университет биоресурсов и природопользования Украины, ул. Героев Обороны, 15, Киев, 03041, Украина

Резюме

Целью работы был скрининг 125 штаммов микромицетов, выделенных из разных экологических ниш, по антибиотической и фитотоксической активности. **Методы.** Активность изучалась с использованием различных тест-организмов − грамотрицательных фитопатогенных бактерий, дрожжей и зеленых водорослей. **Результаты.** Показано, что только 57 исследуемых штаммов проявили биологическую активность. Широким спектром антибиотического действия и умеренной антифунгальной активностью характеризовались исследуемые штаммы рода *Aspergillus* and *Penicillium.* Фунгистатическое действие проявляли только штаммы *A. parvulus* 31, *A. versicolor* 60, *P. brevicompactum* 144, P. *rubrum* 91 и 93. Значительную группу составляли штаммы с фитотоксической активностью – *A. alliaceus* 16*, A. parvulus* 30*, A. versicolor* 56*, Aureobasidium pullulans* 41 и 44*, Chaetomium globosum* 38*, Curvularia inaequalis* 84*, Fusarium merismoides* 151*, Mortirella vinacea* 74 и 82*, Paecilomyces lilacinus* 127*, P. chrysogenum* 163. Большинство штаммов с антибиотической активностью не проявляли фитотоксическое действие. **Выводы.** Наиболее перспективными для дальнейших исследований являются штаммы *A. pullulans* и *M. vinacea*, которые проявляют фитотоксическую активность, что раньше не исследовалось.

Ключевые слова: микромицеты, антибиотическая активность, фитотоксическая активность, тест-организмы.

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