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ANTIBACTERIAL ACTIVITIES OF CULTURAL FILTRATES OF SOME STRAINS OF MICROMYCETE

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Annotation. *The purpose of this work was conducting study of the antibacterial activity of cultural filtrates of 125 micromycete strains isolated from different samples. Different Gram-negative and Gram-positive bacteria as test-organisms have been applicable. Activity of 64 strains has been established. Genus Aspergillus and Penicillium was characterized wide spectrum of activity. The strains of genus Aureobasidium and Paecilomyces as A. pullulans 41, P. variotii 68 have been shown antibacterial action against test-organisms. It is found that strains of M. vinacea exhibited antibacterial activity against Staphylococcus aureus 904 and E. coli 906. Most strains did not show antibacterial effect. The results obtained confirm that antibacterial potential of strains of A. pullulans, P. variotii and M. vinacea is the most potential for researches of biologically active substances.*

Keywords: *micromycetes, antibacterial activity, Gram-negative bacteria, Gram-positive bacteria*

Introduction.

The micromycetes have been famous as producers of antibiot.es and other secondary biologically active metabolites as vitamins, attractants, cytotoxins, insecticides, compounds that promote or inhibit growth, etc. [1, 2]. The strains of micromycetes have been traditionally used to produce a variety of important substances for the pharmaceutical and food industries.

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. Screening of novel strains arc bringing about microorganisms, not yet full assayed for their antibacterial activity that can produce useful templates with new antibiotics activity.

The purpose of our work was study of antibacterial activity of cultural filtrates of 125 micromycete strains as a starting point for father investigations of the most perspective strains for feasible obtaining of antibacterial substances.

Materials and methods.

In this work it was used 125 micromycete strains of the genus *Aspergillus*, *Alter-*

naria, *Aureobasidium*, *Beauveria*, *Botrytis*, *Chaetomium*, *Cladosporium*, *Curvularia*, *Endomyces*, *Fusarium*, *Gliocladium*, *Mortierella*, *Nigrospora*, *Paecilomyces*, *Penicillium*, *Pseudallescheria*, *Scopulariopsis*, *Trichoderma*, *Ulocladium* and separate strains *Nectria* sp., *Phialophora* sp. and *Verticillium dahlia*, 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 liquid filtrates were tested against Gram-positive bacteria as *Staphylococcus aureus* 904, *Bacillus licheniformis* 5 and Gram-negative bacteria as *Escherichia coli* 906, *Agrobacterium tumefaciens* 8464, *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.). *B. licheniformis* 5 is a bacterium that is commonly found in soil and bird feathers. These bacteria are known to cause food poisoning and food spoilage. *E. coli* lives in the intestines of humans and helps keep our guts healthy. But certain strains of *E. coli* can cause severe illness such as urinary tract infections and neonatal sepsis, represent a huge public health problem. Bacteria *S. aureus* is a human pathogen that causes a wide variety of different infections. Bacterial test organisms have beat incubated on the medium of agarose gel for 24 hours at 28 ° C.

The screening study of culture liquid filtrate of micromycete strains using standard agar well diffusion method was followed. Eight-millimeter 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 bacteria was measured after18-24 hours.

Bacterial 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 fable 1 that only 64 from 125 strains shown antibacterial activity and inhibit the growth of test organisms. Among them, strains such as *A. pullulons* 41, *P. variotii* 68. *P. brevicompactum* 144 have a broad spectrum of antibiotic activity against both phytopathogenic bacteria and non-phytopathogenic bacteria. The separate strains such as *A. ustus* 103, *A. terreus* 119, *T. viride* 120 and the strains of the genus *Penicillium* as *P. aculeatum* 121. *P. ochrochloron* 145 and *P. purpurogenum* 143 showed activity against four from live examined bacteria. The majority strains exhibited antibacterial activity against three different bacteria.

The study of antibiotic effect of micromycete strains against *B. licheniformis* 5 indicated that *A. alleaceus* 118, *A. versicolor* 56, *A pullulans* 43-46, *C. cladosporiodes* 8, *G. virens* 39, *C. inaequalis* 84, *F. merismoides* 151, *M. vinacea* 62, 81-82, *P. variotii* 67, 69, *T. harzianum* 157 and separate strains of the genus *Penicillium* as *P. funiculosum* 2, *P. chrysogenum* 163 and *P. rubrum* 91 didn't visualize antibacterial effect. Other strains inhibited growth of *B. licheniformis* 5 (inhibition zone from 24 to 8,7 mm).

The result of study indicated that strains as *A. versicolor* 56, *F. solani* 128, *M. vinacea* 81-82 inhibited the growth of human pathogen strain *E. coli* 906 only (zone of inhibition from 25 to 11 mm).

The separate strains such as *A. alleaceus* 118, *A. pullulans* 43-46, *P. rubrum* 91 displayed antibiotic activity against *P. carotovorum* only. These strains didn't show antibiotic affect against tested *E. coli, S. aureus* and *B. licheniformis*. The strain *G. virens* 39 shown antibiotic activity against *A. tumefaciens* (inhibition zone is 18 mm).

The strains of *B. bassiana*, *C. globosum*, *F. culmorum*, *Fusarium sp*., *Nectria sp*., *N. oryzae*, *P. ucrainicum*, S. *brumptii*, *T. brevicompacium*, *Trichoderma sp*. and *U. atrum* did not display antibiotic activity.

Discussion.

The finding shows that micromycete strains produce many bioactive compounds as secondary metabolites including antibiotics and toxins. The results obtained demonstrated that representatives of the genus *Aspergillus* and genus *Penicillium* arc characterized by high antibiotic activity against phytopathogen bacteria and *B. licheniformis* 5. Only some strains of genus *Aspergillus* as *A. parvulus* 30, *A. terreus* 119, *A versicolor* 56, *A. ustus* 103 and genus *Penicillium* as *P. aculeatum* 123, *P. brevicompactum* 144, *P. purpurogenum* 143 and *P. ochrochloron* 145 have antibiotic activity against *S. aureus* 904 and *E. coli* 906 or one of them.

Our experiments are consistent with previous results [6, 7]. Tsyganenko shown that *Aspergillus* strains demonstrated antibiotic activity especially *A. parvulus* 3142 [6, 7]. Some authors have also suggested that most of the *Aspergillus* strains displayed antimicrobial activity against methicillin-resistant *S. aureus*, extended-spectrum beta-lactamase-producing *E. coli* [8]. A large number of fungal extracts and/or extracellular products have been found to have antimicrobial activity, mainly from the filamentous fungus *Penicillium* sp. [9,10]. The *Penicillium* species shown distinguished antimicrobial activities towards *Candida albicans*, *B. subtillis*, *S. aureus, Salmonella typhi* and *E. coli* [11]. Metabolites as penicillanthone and penicillidic acids A-C from strain of

P. aculeatum PSU-RSPG105 showed moderate antibacterial activity against *E. coli* and *S. aureus* [12].

The 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 [13]. The fact of antibacterial activity of fungal culture filtrates against *A. tumefaciens* 8464 and *P. carotovorum* 8636 has been established previously [14]. Of great interest for the researches is the subsequent investigation of antibiotic activity of *Aspergillus* and *Penicillium* species as *A. alleaceus* 118, *A. pullulans* 43-46, *P. rubrum* 91 shown phytopathogenic activity against *P. carotovorum* 8636 and *A. versicolor* 56 against *E. coli* 906.

Another promising finding was that species of the genus *Aureobasidium* and *Paecilomyces* genus are characterized by antibacterial activity. Two strains such as *A. pullulans* 41, P. *variotii* 68 shown antibacterial effect against all test bacterial strains. It was reported in literature that strains of *A. pullulans* produce a wide range of substances such as antimicrobial chemicals, siderophores, enzymes, polysaccharides, polyesters and heavy oils [15]. In addition, an *A. pullulans* strain can produce antimicrobial compounds towards the Gram-negative *Pseudomonas fluorescens* and Gram-positive *S. au-*

reus bacteria [16]. The antibacterial activity of *A. pullulans* strains can be associated with 2-propylacrylic acid, 8,9-dihydroxy-2-methyl-4H,5H-pyrano [3,2-c]-chromon-4-one, 2-methylene-succinic acid and hexane- 1,2,3.5,6- hexol [17]. Equally important, some strains of *A. pullulans* are used in biological control of plant and storage diseases [18, 19]. 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 Botecior Fungicide contains yeast-like fungi, *A. pullulans* strains DSM 14940 and DSM 14941, as the active constituent [20].

The authors of more studies of secondary metabolites of *P. variotii* have exhibited that Gram-positive bacteria *Enterococcus faecalis* were inhibited by fungal extract [21]. Paeciloketals (1-3), new benzannulated spiroketal derivatives, were isolated from the marine fungus *P. variotii* derived from the giant jellyfish *Nemopilema nomurai*. Compound 1 showed modest antibacterial activity against the marine pathogen *Vibrio ichthyoenteri* [22].

There have been several reports of antibiotic potential of *M. vinacea*. The cultural filtrates certain strains *M. vinacea* have been shown antibacterial activity against phytopathogenic bacteria *A. tumefaciens* and *P. carotovorum* [14]. The known compound from *M. vinacea* methyl 2,4-dthydroxy-3,5,6-trimethyl-benzoate, mortivinacins A and nicotinic acid were responsible for the antibacterial activities of the extract [23].

The results obtained confirm that cultural filtrates of strains of *A. pullulans*, *P. variotii* and *M. vinacea* possess marked antibacterial activity. It must also be noted that it is very little data on antibiotic effect of strains of *A. pullulans*, *M. vinacea* and *P. variotii*. The evidence suggests that these strains are potentially important set of targets for farther investigation.

1. Antimicrobial activity of cultural liquid of micromycetes

No п/п	Species	Strains	Diameter of inhibition zones of test, mm				
			Bacterium				
			Gram positive		Gram negative		
			S. aureus 904	B. licheniformis 5	E. coli 906	Agrobacterium tumefaciens 8464	Pectobacterium carotovorum 8636
1	2	3	4	5	6	7	8
1	Altemaria alternata	116	0	0	0	16,0±0,7	11,0±0,9
2	Aspergillus alliaceus	118	0	0	0	0	15,0±1,0
3	A. parvulus	30	0	14,7±0,3	10,3±0,3	30,0±0,7	0
4		31	0	29,0±1,5	0	0	24,0±2,0
5	A. terreus	119	30±1,5	29,5±1,0	28,0±1,5	0	15,0±2,0

1. Antimicrobial activity of cultural liquid of micromycetes

1	2	3	4	5	6	7	8
6	A. versicolor	56	0	0	25,0±1,5	0	0
7		58	0	23,0±1,0	0	0	0
8		59	0	14,0±0,6	0	0	0
9		60	0	17,3±0,4	0	13,0±1,0	0
10		61	0	14,3±0,4	0	0	19,0±3,0
11	A. ustus	101	0	15,2±0,4	0	0	13,0±1,0
12		102	0	12,0±1,0	0	0	15,0±2,0
13		103	15,0±0,7	16,0±0,7	19,8±0,4	0	15,0±1,0
14	Aureobasidium pullulans	41	10,0±0,7	15,0±0,7	11,7±0,9	30,0±0,8	25,0±1,0
15		43	0	0	0	0	15,7±0,4
16		44	0	0	0	0	10,8±0,4
17		45	0	0	0	0	20,0±0,7
18		46	0	0	0	0	20,0±0,7
19		150	0	25±2,0	0	0	0
20	Botrytis sp.	15	15,0±0,3	10,0±0,7	0	0	0
21	Botrytis cinerea	55	12,0±1,0	11,0±0,6	33,7±1,1	0	0
22	Cladosporium cladosporiodes	8	0	0	0	23,5±4,0	14,5±4,5
23		9	0	11,0±0,3	0	10,7±0,3	12,0±2,0
24		12	0	18,7±0,6	0	0	0
25	Gliocladium virens	39	0	0	0	18,0±1,0	0
26	Curvularia inaequalis	84	0	0	11,7±0,4	0	0
27	Fusarium lactis	130	0	18,3±0,3	0	0	0
28	F. merismoides f. merismoides	151	0	0	0	15,0±0,3	16,0±1,0
29	F. solani	128	0	14,0±1.0	0	0	0
30		129	0	0	19±0,7	0	0
31	Mortierella vinacea	62	0	0	20,0±0,7	0	22,0±1,0
32		63	12,0±1,0	14±1,0	0	0	24,0± 1.0
33		81	0	0	11,3±0,4	0	0
34		82	0	0	11,7±0,4	0	0
35	Paecilomyces variotii	67	0	0	0	12,7±0,1	10,0±1,0
36		68	14,3±0,6	17,6±0,4	12,0±2,0	30,0±1,0	20,0±0,7
37		69	11,0±0,7	0	0	0	10,7±0,8
38	P. lilacinus	127	0	24,0±1,5	20,0±1,0	0	0
39	P. marquandii	126	0	16,7±0,4	16,3±0,4	0	0
40		132	0	17,7±0,4	0	0	0
41	Penicillium aculeatum	121	0	20,3±0.4	0	0	0
42		123	12,0±0,7	19,0±1,0	0	12,0±0,7	14,0± 1,0

1. Antimicrobial activity of cultural liquid of micromycetes							
1	2	3	4	5	6	7	8
43		124	0	13,5±0,5	0	20,0±2,0	11,0±0,7
44	P. brevicompactum	144	12,7±0,4	14,0±1,0	10,7±0,4	13,0±2,0	17,0±2,0
45	P. clavigerum	112	0	11,0±0,7	0	0	0
46	P. funiculosum	1	0	12,7±0,4	0	15,0±2,0	12,0±0,7
47		2	0	0	0	32,0±1,0	9,0±0,3
48		3	0	24,0±1,0	0	18,0±0,7	19,3±0,7
49		4	0	18,3±0,7	0	16,7±0,4	16,3±0,4
50		5	0	14,0±0	0	16,3±0,4	15,3±0,4
51		6	0	20,3±0,4	0	17,0±0,7	17,7±1,1
52		7	0	12,0±2,0	0	0	11,0±0,7
53	P. chrysogenum	163	0	0	0	39,0±0,7	14,0±0,7
54	P. ochrochloron	145	11,7±0,4	19,0±1,0	0	12,0±0,7	13,7±0,1
55		156	0	14,3±0,4		20,0±0,7	11,0± 1,3
56	P. purpurogenum	143	0	21,0±0,5	16,7±0,4	12,0±1,3	11,7±0.4
57	P. rubrum	91	0	0	0	0	20,0±1,3
58	Phialophora sp.	135	0	22,0 ±2,0	13,3±0,4	0	0
59	Pseudallescheria boydii	131	0	17,3±0,6	12,5±0,5	0	0
60	T. harzianum	157	0	0	0	23,0±0,7	17,7±0,4
61	T. viride	120	12,0± 1,0	15,7±0,4	11,3±0,7	0	15,0±0,7
62	Verticillium dahliaee	168	0	14,3±0,9	0	11,0± 0,7	19, 0±0,7
63	U. consortiale	106	0	8,7 ±0,4	0	0	15,0 ±1,0
64		107	0	14,3±0,4	0	0	15,0± 0,7

References

1. Mérillon, J-M. & Ramawat, K.G. (Eds). (2017). Fungal Metabolites. Switzerland: Springer International Publishing.
2. Rubezhniak, I.G. (2011) [Some features of secondary metabolism of microorganisms]. In: Akutina, S.P. (Eds). Selected issues of modern science. Part 3. Monography (pp. 283–310). In: Moscow: Pero. Russian.
3. Tsyhanenko, K.S., Zaichenko, O. M. (2004). Antybiotychni vlastyvosti deiakykh vydiv rodu Aspergillus Mich. [Antibiotic properties of some species of genus Aspergillus Mich.] Mikrobiol. Z., 66 (4), 56–61.
4. Tsyhanenko, K. S. (2004). Otsinka antybiotychnoho potentsialu Aspergillus parvulus Smith. [Evolution of biotic potential of Aspergillus parvulus Smith.] Naukovyi visnyk Chernivetskoho un-tu. Ser. Biolohiia., 194, 33–36.
5. Bilay, V.I. (Eds). (1982). [Methods of experimental mycology]. Kiev: Naukova Dumka.
6. Tsyganenko, K.S. The evaluation of antibiotic and toxigenic potential of some species micromycetes of genera Aspergillus Mich. (2005). [author's abstract]. Danilo Zabolotny Institute of Microbiology and Virology of National Academy of Sciences of Ukraine, Kyiv.
7. Tsyhanenko, K.S., Zaichenko, O.M. (2004). Kharakterystyka fitotoksychnykh vlastyvostei Aspergillus parvulus Smith. [Characteristics of phytotoxic properties of Aspergillus parvulus Smith.] Ahroekol. Z., 4, 42–45.
8. Svahn, K. S., Goransson, U., El-Seedi, H., Bohlin, L., Larsson, D.G. J., Olsen, B., Chryssanthou, E. (2012). Antimicrobial activity of

filamentous fungi isolated from highly antibiotic-contaminated river sediment. Infection Ecology and Epidemiology, 2, l-6. DOI: 10.3402/iee.v2i0.11591

9. Rančić , A., Soković , M., Karioti, A., Vukojević, J., Skaltsa, H. (2006). Isolation and structural elucidation of two secondary metabolites from the filamentous fungus Penicillium ochrochloron with antimicrobial activity. Environ. Toxicol. and Pharmacol., 22 (1), 80–84. DOI:10.1016/j.etap.2005.12.003
10. Petita, K.E., Mondeguerb, F., Roquebcrtc, M.F., Biarda, J.F., Pouchusa, Y.F. (2004). Detection of griseofulvin in a marine strain of Penicillium waksmanii by ion trap mass spectrometry. J Microbiol. Methods, 58, 59-65.
11. Ciharaci-Fathahm, E., Tajick-Ghanbary, M.A., Shahrokhi, N. (2014). Antimicrobial properties of Penicillium species isolated from agricultural soils of Northern Iran. Research Journal of Toxins, 6 (1), 1–7.
12. Daengrot, C., Rukachaisinkul, V., Tadpetch, K., Phongpaichi,t S., Bowornwiriyapan, K., Sakayarojc, J., Shend, X. (2016). Penicillanthone and penicillidic acids A - C from the soil-derived fungus Penicillium aculeatum PSU -RSPG105. RSC Advances, 46, 39530 – 40625.
13. Savchuk, Ya.I., Zaichenko, O.M., Tsiganenko, K.S. (2013). Antibiotic activity of some fungi. Mikrobiol. Z., 75 (5), 52– 61.
14. Rubezhniak, I.G. Antibiotic and phytotoxic activities of cultural filtrates of some micromycete strains. (2018). Mikrobiol. Z., 80 (5), 90 –97. DOI: https://doi.org/10.15407/microbiolj80.05.090
15. Bozoudi, D., Tsaltas, D. (2018). The multiple and versatile roles of Aureobasidium pullulans in the vitivinicultural sector. Fermentation, 4 (85), 1–15. DOI:10.3390/fermentation4040085
16. Zain, M.E., Awaad, A.S., Razak, A.A., Maitland, D.J., Khaims, N.E., Sakhawy, M.A. (2009). Secondary metabolites of Aureobasidium pullulans isolated front Egyptian soil and their biological activity. J. Appl. Sci. Res., 5, 1582– 1591.
17. McCormack, P.J., Wildman, H.G., Jeffries, P. (1994). Production of antibacterial compounds by phylloplane-inhabiting yeasts and yeast like fungi. Appl Environ. Microbiol., 60, 927 – 931.
18. Ferreira-Pinto, M.M., Moura-Guedes, M.C., Barreiro, M.G., Pais, I., Santos, M.R., Silva, M.J. (2006). Aureobasidium pullulans a biocontrol agent of blue mold in “Rocha” pear”. Communications in agricultural and applied biological sciences, 71(3 Pt B), 973–978.
19. Zhang, D., Spadaro, D., Valente, S., Garibaldi, A., Gullino, M.L. (2012). Cloning, characterization, expression and antifungal activity of an alkaline serine protease of Aureobasidium pullulans PL5 involved in the biological control of postharvest pathogens. Int. J. of Food Microbiology, 153 (3), 453–464. DOI: 10.1016/j.ijfoodmicro.2011.12.016
20. Australian pesticides and veterinary medicines authority. (2017). Public release summary on the evaluation of the new active Aureobasidium pullulans (strains DSM 14940 and DSM 14941) in the product Botector Fungicide. Retrieved from URL: https://apvma.gov.au/sites/default/files/publication/27401-82495_105881_-_botector_fungicide_-_prs_0.docx
21. Oliveira, S.M., Sena, K.X., Gusmio, N.B. (2009). Secondary metabolites produced by endophytic fungus Paecilomyces variotii Bainier with antimicrobial activity against Enterococcus faecalis. In: Proceedings of the II Intonational Conference on Environmental, Industrial and Applied Microbiology (BioMicroWorld2007), 2007 Nov 28 - Dec 1; University of Seville, Spain. Formatex Research Center, Spain, 519 – 22.
22. Wang, H., Hong, J., Yin, J., Moon, H.R., Liu, Y., Wei, X., Oh, D.C., Jung, J.H. (2015). Dimeric octaketide spiroketals from the jellyfish-derived fungus Paecilomyces variotii J08NF-1. J. Nat. Prod., 78 (11), 2832–2836.
23. Soman, A.G., Gloer, J.B., Wicklow, D.T. (1999). Antifungal and Antibacterial Metabolites from a Sclerotium-Colonizing Isolate of Mortierella vinacea. J. Nat. Prod., 62 (2), 386–388.

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Анотація. *Метою роботи був скринінг серед 125 штамів мікроміцетів, виділених із різних екологічних ніш, на антибактеріальну активність. Активність вивчалася з використанням різних тест-організмів – грамнегативних та грампозитивних бактерій. Показано, що тільки 64 досліджуваних штамів проявили антибактеріальну активність. Широким спектром антибіотичної дії характеризувалися досліджувані штами роду Asp ergillus та Penicillium. Штами роду Aureobasidium та Paecilomyces, такі як A. pullulans 41, P. variotii 68, також проявили антибактеріальний ефект на досліджувані бактерії. Встановлено, що штами M. vinacea проявляють антибактеріальний ефект на Staphylococcus aureus 904 та E. coli 906. Більшість штамів не показали антибактеріальної дії. Найбільш перспективними для подальших досліджень є штами A. pullulans, P. variotii та M. vinacea, які проявляють високу антибактеріальну активність та мало досліджені.*

Ключові слова: *мікроміцети, антибактеріальна активність, грамнегативні та грампозитивні бактерії.*