JOURNAL OF CULTURE COLLECTIONS
Volume 4, 2004-2005, pp. 29-35

SCREENING THE ANTIMICROBIAL ACTIVITY OF ACTINOMYCETES STRAINS ISOLATED FROM ANTARCTICA

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Summary

A total of 40 actinomycete strains, isolated from Antarctica, were tested for antagonistic activity against 7 Gram-positive and Gram negative bacteria and yeasts, 16 phytopathogenic fungi and bacteria. During the initial screening 60 % of the strains showed inhibition potential against test-microorganisms. Ten of them had a broader spectrum of antibacterial activity and could be used in the development of new substances for pharmaceutical or agricultural purposes.

Introduction

During the last 20-30 years, the interest in the glacier microflora increased due to the investigation of novel bioactive compounds, especially antibiotics and enzymes, active in low temperatures. Because of the severe environmental conditions (low temperature, low humidity, high radiation etc.) Antarctica could be used in modeling the life on other planets [1, 16, 17], investigating the intercontinental contacts and the results of the changes between glacial and post-glacial periods as well as their effect on the organisms.

Exploring the continental glacial samples and those of shelves and icebergs, a lot of sporulating and non-sporulating bacteria, some yeasts species, fungi and Actinomycetes were found [1-5, 7, 15]. The members of the last group were not only less than other microorganisms but the number of the species was also small. Mainly, representatives of the genera *Nocardia, Streptomyces* and *Nocardiopsis* were established [2, 3, 15]. Some new Actinomyces species were isolated too, like *Friedmanella antarctica* gen. nov., sp. nov., *Friedmanella lacustris* sp. nov., *Nocardiopsis aurantiacus, Modestobacterium multiseptatum* gen. nov., sp.nov. [11, 12, 18, 20].

Parallel with the investigations of the microbial diversity of the permanently frozen continent, the biochemical characteristics of the microorganisms were examined too. It was found that most of the actinomycete isolates possessed high proteolytic, cellulase and chitinase activity [8] and it was also mentioned about an antibacterial one [13]. That points the possible directions in which the local microflora researches have to be developed.

The aim of this study was to investigate the antibacterial activity of actinomycete isolates from Antarctic soils, taken from some consecutive expeditions from 1995 to 1998.

Materials and methods

Microorganisms. Forty actinomycete strains isolated from Antarctic soils were used in screening procedure (Table 1). For testing the antibiotic activity of the investigated strains the following test-microorganisms were used: *Bacillus subtilis* ATCC 6633 (NBIMCC 1709), *Staphylococcus aureus* NBIMCC 3703, *Pseudomonas putida* NBIMCC 1090, *Ps. aeruginosa* NBIMCC 3700, *Micrococcus luteus* NBIMCC 159, *Saccharomyces cerevisiae* NBIMCC 537, *Enterococcus faecalis* NBIMCC 3915, *Escherichia coli* NBIMCC 3398 [14]. Microorganisms studied in the collaboration research work with the Plant Protection Institute, Kostinbrod were also used: phytopathogenic bacteria – *Clavibacter michiganense p.v. michiganense*, *P. syringae p.v. syringae*, *P. syringae p.v. tabaci*, *Xanthomonas campestris p.v. vesicatoriae*; phytopathogenic fungi - *Alternaria sp.* (cucumber isolate), *Ascophyta melonis, Cladosporium fulvum, Cladosporium sp., Fusarium avenaceum, F. culmorum, F. moniliforme, F. oxysporum, Helmintosporium gramineum, Penicillium exspansum, Verticillium dahliae, Verticillium sp.*

Media and cultivation conditions. The actinomycete isolates were cultivated on mineral agar I [6], ISP-2 and ISP-3 [19] at 28 °C. The culture media applied for test-microorganisms were Nutrient agar I, Nutrient agar II, MRS, YPD, Potato's agar and Saborought agar [14] and the strains were grown at 26 °C, 30 °C and 37 °C. The dynamic batch cultivation of the actinomycetes was carried out in medium – liquid YEME in 500 ml flasks with 100 ml media at 28 °C and 240 rpm for 120 h. The fermentation medium was sown with 10 % of inoculum, taken on the 48th hour (the inoculation media had the same composition as the fermentation one). Ethanol extracts of the mycelium were prepared.

Two variants of the agar plate diffusion method were applied.

1. Method of agar blocks. Cylindrical pieces were cut out from well grown and sporulated culture of the actinomycete strain on solid nutrition medium. The blocks were placed on the Petri dishes deep inoculated with a fixed amount of test-microorganisms (10^8 cells/ml). The cultures stayed for 14 – 18 hours at 2 - 8 °C for the antibacterial substance diffusion and thereafter they were cultivated at the appropriate for the test-microorganisms temperature and duration. The antibacterial activity was measured in mm sterile zone on the 48^{th} hour for bacteria and yeasts and on the 7^{th} day for phytopathogenic fungi.

2. Well diffusion method [9]. Culture media filtrates and ethanol mycelium extracts were dropped in prepared holes of the solid nutrition medium deep inoculated with the test-microorganisms. The Petri dishes were incubated and cultivated as it was above described. The antibacterial activity was measured in mm sterile zone. The activity from 7 to 15 mm inhibition zone was accepted as low, 16 to 15 mm - as medium, more than 25 mm – as high activity.

Results and discussion

The initial antibacterial activity screening of Antarctic isolates was made against four test microorganisms in agar medium. 40 % of the investigated strains did not possess any activity against the tests. Nine strains showed high inhibition potential against *B. subtilis* 1709 – 30 and over mm sterile zone. It should be pointed out that only one of the studied strains suppressed the growth of *E. coli* 3398 and two strains were effective against *S. cerevisiae* 537 but the zone diameter was smaller than 25 mm. None of the Antarctic isolates exhibited activity against *P. putida* 1090 (Table 1).

Based on the results of the mentioned above screening 15 actinomycete strains with high inhibition potential were selected. Their activity range was checked out against large number of test-microorganisms (Table 2).

Table 1. Antibiotic activity of the actinomycete strains determined by the agar block method.

Strains	Test-microorganisms (inhibition zone in mm)				Strains	Test-microorganisms (inhibition zone in mm)			
	E. coli	*B. subtilis*	*Ps. putida*	*Sacch. cerevisiae*		*E. coli*	*B. subtilis*	*Ps. putida*	*Sacch. cerevisiae*
3725	22	42	0	0	3726	0	10	0	0
3717	0	36	0	0	8016	0	10	0	0
8007	0	34	0	0	3719	0	0	0	0
8022	0	32	0	0	3720	0	0	0	0
3789	0	31	0	0	3721	0	0	0	0
3718	0	30	0	0	3723	0	0	0	0
3784	0	30	0	0	3724	0	0	0	0
8013	0	30	0	0	3786	0	0	0	0
3787	0	30	0	0	8003	0	0	0	0
8010	0	30	0	0	8004	0	0	0	0
8018	0	29	0	0	8005	0	0	0	0
3715	0	28	0	0	8006	0	0	0	0
3783	0	28	0	0	8008	0	0	0	0
8009	0	22	0	0	8011	0	0	0	12
3716	0	18	0	0	8012	0	0	0	0
3714	0	16	0	0	8014	0	0	0	0
3788	0	16	0	0	8015	0	0	0	0
8019	0	14	0	0	8017	0	0	0	18
3722	0	12	0	0	8020	0	0	0	0
3785	0	11	0	0	3790	0	0	0	0

Table 2. Antibiotic activity of the actinomycete strains after cultivation in fermentation media.

Strains	Test-microorganisms (inhibition zone in mm)							
	E. coli	*B. subtilis*	*M. luteus*	*Ps. aeruginosa*	*Ps. putida*	*St. aureus*	*E. faecalis*	*Sacch. cerevisiae*
3784	0	30/30[1]	22/22	0	0	16/16	15/15	0
3718	0	28/30	19/21	0	0	10/12	14/14	0
8018	0	18/0	10/10	0	0	0	0	0
8016	0	32/32	24/24	0	0	34/22	16/16	0
3717	0	23/24	16/16	0	0	0/12	12/14	0
8013	0	26/25	17/19	0	0	10/10	12/0	0
8010	0	26/30	19/16	0	0	10/0	12/14	0
3787	0	27/30	28/28	0	0	20/15	18/16	0
8009	0	22/22	15/15	0	0	0	0	0
8007	0	34/26	25/18	0	0	18/0	16/10	0

[1]filtrate from liquid culture/ethanol extract of the mycelium

For the purposes of that experiment the actinonmycete isolates were cultivated in fermentation media on shaker for 120 h. The obtained ethanol extracts of the mycelium and the filtrates from the liquid culture were used for the antibiotic activity determination by the well diffusion method.

During the tests it was found that 30 % of the strains had lost their inhibition potential perhaps due to the inconvenient liquid growth medium. Such results had been reported from other scientists too, which had found the activity reducing in comparison with that showed by the method of agar blocks [13]. None of the antarctic isolates suppressed *S. cerevisiae* 537, *P. putida* 1090, *P. aeruginosa* 3700, *E. coli* 3398 but they had a significant activity against Gram-positive microorganisms. The highest inhibition was shown against *B. subtilis* 1709 and in the most of the cases both the mycelium extracts and the liquid culture filtrates were active. The suppression of the *M. luteus* 159 was weaker as from the 10 active strains only 50 % produced sterile zones larger than 20 mm and none of them caused more than 30 mm in diameter zones. *E. coli* 3398 and *S. aureus* 3703 were even less inhibited - only 2 actinomycete isolates had higher potential against them.

Comparing the obtained results with those of other scientists [13], it could be said that exhibited activity against *B. subtilis* 1709 varied in the same range between 18 and 34 mm, as the last one was the highest obtained by us result.

Table 3. Antibiotic activity of the actinomycete strains against phytopathogenic bacteria.

Strains	Test-microorganisms (inhibition zone in mm)			
	P. syringae p.v. tabaci	C. michig. p.v. michiganense	X. campestris p.v. vesicatoria	P. syringae p.v. syringae
8022	0	0	0	0
3783	0	0	0	0
3784	15	16	14	18
8019	0	0	0	0
3788	0	0	0	0
8014	0	0	0	0
8018	0	10	10	13
8016	0	17	16	20
8013	0	27	26	26
8010	0	12	0	15
3789	10	12	16	20
8003	0	10	10	0
3787	14	12	0	10
8009	12	16	10	12
8007	40	35	28	36
3785	14	12	0	12
3726	12	12	14	12
3725	14	15	14	13
3718	20	20	20	18
3717	12	10	12	12
3714	10	10	12	10
3715	10	10	0	10
3719	12	12	0	0
3721	0	10	0	10
3723	0	0	0	0

Table 4. Antibiotic activity of the actinomycete strains against phytopathogenic fungi.

Strains	Test-fungi (inhibition zone in mm)											
	A. melonis	*H. sporium gramineum*	*Cladosporium sp.*	*C. fulvum*	*P. expansum*	*F. avenaceum*	*F. moniliforme*	*F. oxysporum*	*F. culmorum*	*Verticillium sp.*	*Verticillium dahliae*	*Alternaria sp.*
8019	0	0	0	0	0	0	0	0	0	0	0	16
3788	10	20	12	15	12	25	12	0	16	0	15	15
8014	0	12	12	15	12	30	16	0	16	0	15	14
8010	0	0	12	0	0	0	0	0	0	0	0	0
3790	0	0	0	15	0	0	0	0	0	0	0	0
3787	14	0	0	0	0	0	0	0	0	0	0	0
8008	16	0	0	12	0	0	0	0	0	0	12	0
8009	0	0	0	10	0	0	0	0	0	0	0	0
8007	25	25	18	30	20	12	15	0	20	12	20	28
3785	15	0	0	0	0	0	0	0	0	0	14	0
3718	0	10	0	0	0	0	0	0	0	0	0	0
3717	0	10	0	0	0	0	0	0	0	0	0	0
3714	0	10	0	0	0	0	0	0	0	0	0	0
3719	0	12	0	0	0	0	0	0	0	0	0	0

Strain 8016 possessed a higher activity against *S. aureus* 3703 than the described ones till that moment in literature but in all of the cases the obtained average activities were lower.

The antibiotic activity spectrum of the 40 strains was extended by testing their suppression potential against four phytopathogenic Gram-positive and negative bacteria (Table 3). The active strains against all the tests were 25.2 % of them and only two actinomycete isolates showed higher results. *P. syringae p.v. tabaci* was the most resistant to the antibiotic treatment. The inhibition potential against *C. michiganense* varied between 10 to 35 mm sterile zone and the most often was between 10 to 12 mm. The results were much worse than those obtained by the other scientists [18], according which the activity ranged between 30 and 40 mm in diameter. The inhibition potential shown against *X. campestris* varied between 10 to 28 mm and correlated with the data published until now. In conclusion, it could be said that 20 % of the actinomycete isolates possessed antibacterial activity against Gram - positive and negative bacteria, while the obtained data from other scientists were mainly about Gram-negative ones [13].

The inhibition potential of the antarctic isolates against phytopathogenic fungi was much more different from the given above (Table 4). The active actinomycetes were only 14 or 35% of the tested strains. Most of them (9 strains) inhibited one test and only three suppressed more than 9 phytopathogenic fungi. The widest activity range possessed strain 8007 and its inhibition zones were the biggest ones - 28 to 30 mm against *C. fulvum* and *F. culmorim.* There were no actinomycetes active against *F. oxysporum*.

Considering the mentioned above results, it could be seen that ten from the investigated strains exhibited higher activity against pathogenic and phytopathogenic bacteria and fungi. The widest activity spectrum and the largest inhibition zones were shown by strains 8013, 3787, 3718 and 8007, and the last one possessed the best properties. Probably, the antibacterial activity of the strains is due to an antibacterial complex active against pro- and eukaryotic organisms. These four actinomycetes have a potential to be included in researches of new preparations with antibacterial action or for plant protection.

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СКРИНИНГ НА АНТИМИКРОБНАТА АКТИВНОСТ ПРИ ЩАМОВЕ АКТИНОМИЦЕТИ, ИЗОЛАТИ ОТ АНТАРКТИДА

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Резюме

40 актиномицетни щама, изолати от Антарктида, са тестирани за наличие на антагонистична активност спрямо 7 Грам-положителни и Грам-отрицателни бактерии и дрожди, и 16 фитопатогенни плесени и бактерии. При първичния скрининг 60 % от щамовете показаха инхибиращо действие спрямо тест – микроорганизми. Десет от тях имат по-широк спектър на антибактериална активност и могат да намерят приложение при разработването на фармацевтични препарати или за растителна защита.