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Abundance And Activity Of Soil Actinomycetes From Livingston Island, Antarctica

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Abstract

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The soils of Antarctica are severe environments inhabited by well adapted microorganisms, the knowledge of which is scarce. The study on their abundance, diversity and physiology will provide the investigators with new data on the mechanisms of their adaptation and gives new opportunities to isolate microorganisms with unique properties for practical use. The aim of the present work was to study bacterial abundance including actinomycetes and physiological diversity of Antarctic soils, by agar plate method and BIOLOG EcoPlates technique, respectively, and based on this actinomycete strains to be isolated, identified and screened for antimicrobial activity against different test bacteria. The objectives of our investigation were seven Antarctic soils taken from moss, *Deschampsia* vegetated and fell field habitats of Livingston Island. Some of the soil physicochemical parameters, like pH, moisture and humus, were determined. Bacterial physiological activity and diversity were assessed based on the average color development of EcoPlate wells, and community capacity to utilize different chemical categories of carbon sources, respectively. The results showed that highest physiological diversity and more intensive utilization of polyols, amino acids and phenolic compounds, and high abundance of actinomycetes characterized soil bacteria from habitats with cryptogam (moss) coverage. Most morphologically different actinomycete strains were isolated from the moss habitat denoted in the study as S6. The affiliation of the strains to genus *Streptomyces* was proved by the PCR amplification of 16S rDNA, using genus-specific primers. The screening of antimicrobial activity of the isolates by diffusion bioassay, using agar plugs showed that the strains synthesized antibacterial substances, active against both Gram-negative and Gram-positive bacteria. We conclude that the soils of moss habitats are favorable environments supporting high bacterial physiological activity, and high number and diversity of actinomycetes most of which are active producers of antibacterial metabolites.

Key words: Actinomycetes, antimicrobial activity, Biolog EcoPlates, PCR-amplification
Abbreviations: AWCD: average well color development; AWCDN*:* normalized average well color development

Introduction

Antarctica is a relatively unknown and not well studied area and is of significant interest for discovering of new species or unknown bioactive substances. The community structure and diversity of Antarctic bacteria have been studied by various methods (Ganzert et al., 2011; Yergeau et al., 2007, 2009; Villaescusa et al., 2010). Measurements of physiological diversity (metabolic capacity) and bacterial activity is an approach, allowing the study of different characteristics of bacterial communities. In the recent years using molecular genetic methods it was found that the majority of the isolated microorganisms are new species. Among microorganisms isolated from such areas actinomycetes are the least frequently met.

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The aim of the present work was to study bacterial abundance including actinomycetes and physiological diversity of Antarctic soils, by agar plate method and BIOLOG EcoPlates technique, respectively, and based on this actinomycete strains to be isolated, identified and screened for antimicrobial activity against different test bacteria.

Materials and Methods

Sampling sites. The soil samples were collected during the austral summer of 2010 from ice-free areas at a depth of 0–5 cm. Sites were chosen by different habitat characteristics and noted as follows: S1 and S9 (vegetated with *Deschampsia antarctica)*, S2 and S6 (cryptogam (moss) coverage), S3 (cryptogam coverage and individual vascular plants), S4 (vascular plant and moss coverage), S5 (non-vegetated soil among the rocks).

Soil analysis. Water and humus content as well pH of soil samples was determined as it was described by Kenarova et al. (2012).

Cell suspensions. Cell suspensions were prepared from 5 g soil samples suspended in 45 ml sterile 0.9% NaCl (Sigma-Aldrich), previously described by Kenarova et al. (2012).

Bacterial enumeration. The cultural method was chosen to plate decimal dilution series (10^{-1}–10^{-5}) of soil suspensions and to enumerate copiotrophic bacteria and actinomycetes. The inoculated agar plates were incubated in dark at 15ºC for 8 days.

Biolog test. A Biolog EcoPlate (Biolog Inc., Hayward CA, USA) containing 31 different carbon (C) sources was used to assess the rate (AWCD) of C source utilization by Antarctic soil bacterial communities according to the procedure, described by Kenarova et al. (2012). To reduce the influence of the inoculums density, the AWCD value of each plate was divided by the number of copiotrophic bacteria (N) and was noted as normalized average well color development (AWCDN x 10^{-7}).

DNA isolation. After cultivation of the actinomycete strains in YEME medium on a rotary shaker at 220 rpm, 28ºC, 48 h, DNA was isolated following the protocol of NucleoSpin® Tissue (MACHEREY-NAGEL) kit. The DNA from *Streptomyces hygroscopicus* 155 and *Streptomyces* sp. 54N were used as controls.

PCR amplification. The amplification of 16S rDNA was done by 2 pairs genus-specific primers: StrepB/StrepE and StrepB/StrepF. The amplification was performed in Techne TC-312 thermal cycler in a total volume of 25 µl, containing 0.5x *Taq* Master Mix RED, 4 pmol of each primer (STS Ltd.); 1 µl of the DNA sample in conditions described by Rintala et al. (2001).

Antimicrobial activity. The antimicrobial activity of the actinomycetes was assayed by the diffusion agar plug method using different Gram-positive and Gram-negative test-microorganisms, measuring the diameter of the inhibition zone around the plugs. All experiments were performed in triplicate.

Results and Discussion

Soil characteristics

The soils were analyzed for pH, moisture content and humus content. The moisture content of the soils was high at 11–14%, and even higher for plot S5 at 50%. pH of the soils was slightly acidic and varied in a relatively narrow range. Humus content was low, from undetectable to 0.28%.

Soil bacterial and actinomycete abundance

The number of the copiotrophic bacteria and the actinomycetes as a part of the soil microbial community was almost the same for the plots with the moss coverage, except plot S3 where actinomycetes were not found (Figure 1). The fell field plot S5 showed highest number of microorganisms the reason for which is unclear but the same tendency has also been recorded for soils taken in 2009 (unpublished data). The proliferation of copiotrophs at plot S5 may be supported by the extracellular metabolites of terrestrial cyanobacteria (Zakhia et al., 2008) and from the high level of humidity 50% which is the main factor determining bacterial number, activity and diversity. Plots S1, S4 and S9 from the vascular habitats showed similarity in the number of bacteria and actinomycetes but in

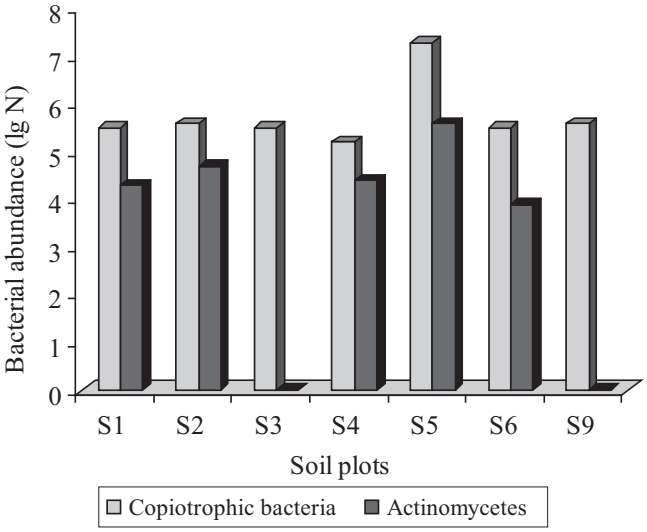

Fig. 1. Bacterial and actinomycete abundance (Lg N) in different soil plots on Livingston Island

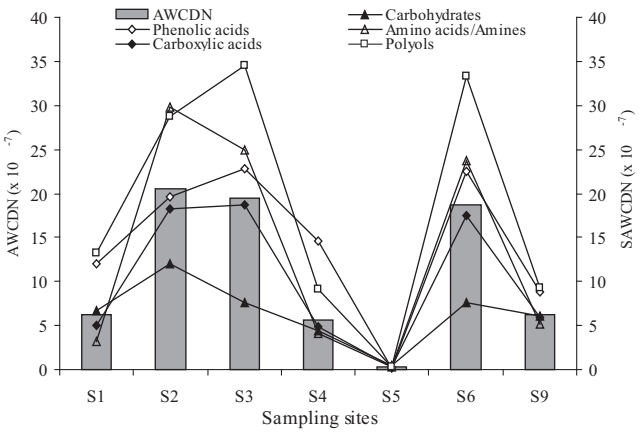

Fig. 2. Average well color development normalized (AWCDN) and substrate groups average well color development (SAWCDN) of soil bacteria from Livingston Island

plot S9 no actinomycetes were registered. The reason for the lack of actinomycetes in plots S3 and S9 might be in the presence of extremely uncultivable species or in the unsuitability of the media used for enumeration, which did not support their growth. On the base of morphology of actinomycete colonies, plot S6 was characterized with the highest diversity and was used for the isolation of pure actinomycete cultures.

Rates of substrate utilization (AWCDN). The bacterial communities (copiotrophs including actinomycetes) from plots S2, S3, and S6 (soil habitats with cryptogam coverage) were more active on the EcoPlates which was based mainly on the utilization of polyols, amino acids/amines, and phenolic compounds (Figure 2). The lowest substrate utilization rate was indicated for plot S5 – skeletal fell field soils of maritime Antarctica inhabited mainly by bacteria and algae. Bacterial community from the vascular plots (S1, S4 and S9) utilized with an insignificant preference polyols and phenolic compounds. In our previous work, we demonstrated that these communities had the widest metabolic capacity utilizing the EcoPlate's C-sources at relatively equal rates (Kenarova et al., 2012). Bacterial communities from all of the studied plots most intensively used polyols – widespread antifreeze agents among cold-adapted organisms. Amino acids were one of the most intensively utilized C sources in soils of moss plots, but not in the vascular plots. The higher competition of *D. antarctica* for N likely reduced the importance of amino acids as nutrients for bacteria in the vascular plots but not in the moss plots.

Isolation of actinomycetes

We isolated 14 strains on the base of the morphology of the actinomycete colonies, eight of which were from plot S6 characterized with highest diversity of actinomycetes.

Screening of antibacterial activity of actinomycetes strains

All actinomycete strains were screened for antibacterial activity against eight test-bacteria. Nine of the actinomycetes showed such kind of activity (Table 1). Most of the strains demonstrated a broad spectrum of activity and affected both Gram-positive and Gram-negative bacteria.

Identification of the actinomycete isolates

DNA from the actinomycetes with antibacterial activity was extracted and amplified using two pairs of primers specific for the genus *Streptomyces*. All of the strains formed an amplification product of 519 bp with the primers StrepB/StrepE, and of 1074 bp with the pair StrepB/StrepF (Figure 3A, B) as expected (Rintala et al., 2001). The results proved their belonging to the genus *Streptomyces*.

Table 1

Antibacterial activity of actinomycete from Antarctica

Test-bacteria	Antimicrobial activity (mm inhibition zone)								
	Number of actinomycete strains								
	1	**2**	**4**	**5**	**6**	**8**	**9**	**10**	**11**
Bacillus subtilis ATCC* 6633	–	24	34	26	29	30	25	37	10
Xanthomonas euvesicatoria	14	13	20	18	15	10	10	20	11
Staphylococcus aureus NBIMCC** 3359	11	11	24	23	8	18	20	17	–
Staphylococcus epidermidis NBIMCC 1093	11	8	22	23	8	18	15	18	–
Burkholderia gladioli 33	12	15	30	31	18	23	22	25	–
Pseudomonas sp. 45	–	–	13	20	–	13	–	15	–
Erwinia amylovora NBIMCC 8509	15	12	–	11	10	–	–	–	–
Erwinia amylovora NBIMCC 8490	15	–	–	–	–	–	–	–	–

*ATCC – American Type Culture Collection;
**NBIMCC – National Bank for Industrial Microorganisms and Cell Cultures

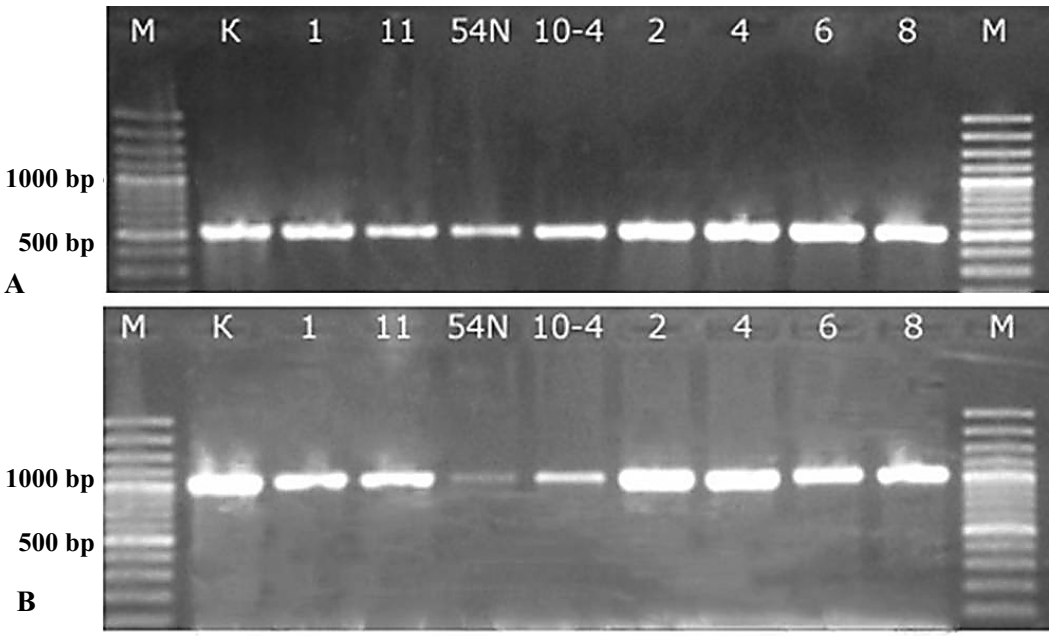

Fig. 3. PCR-amplification of selected actinomycetes DNA with the genus specific primer pairs StrepB/StrepE (A) and StrepB/StrepF (B) M- DNA Ladder 100 bp (Fermentas); K – *Streptomyces hygroscopicus* 155; 54N – *Streptomyces* sp. 54N; The number above each start corresponds to the number of each of the strains.

Conclusion

The results confirm that bacteria, including actinomycetes play an important role in nutrient cycling in soils of Antarctica and indicate a lack of site specific distribution of bacterial abundance. Soil bacteria intensively use polyols, which are widespread antifreeze agents among cold adapted organisms. The site-specificity of bacterial metabolic activity depends on the presence/absence and the type of vegetation. Actinomycetes, most of which possess antibacterial activity are found almost in all soil plots. All of the active strains were proved to belong to the genus *Streptomyces*.

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