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Antimicrobial and Antioxidant Activities of Actinobacteria Isolated from the Pollen of Pinus sylvestris Grown on the Lake Baikal Shore

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Abstract : Isolated ecosystems existing under specific environmental conditions have been shown to be promising sources of new strains of actinobacteria. The taiga forest of Baikal Siberia has not been well studied, and its actinobacterial population remains uncharacterized. The proximity between the huge water mass of Lake Baikal and high mountain ranges influences the structure and diversity of the plant world in Siberia. Here, we report the isolation of eighteen actinobacterial strains from male cones of Pinus sylvestris trees growing on the shore of the ancient Lake Baikal in Siberia. The actinobacterial strains were isolated on solid nutrient MS media and Czapek agar supplemented with cycloheximide and phosphomycin. Identification of actinobacteria was carried out by 16S rRNA gene sequencing and further analysis of the evolutionary history. Four different liquid and solid media (NL19, DNPM, SG and ISP) were tested for metabolite production. The metabolite extracts produced by the isolated strains were tested for antibacterial and antifungal activities. Also, antiradical activity of crude extracts was carried out. Strain Streptomyces sp. IB 2014 I 74-3 that active against Gram-negative bacteria was selected for dereplication analysis with using the high-yield liquid chromatography with mass-spectrometry. Mass detection was performed in both positive and negative modes, with the detection range set to 160–2500 m/z. Data were collected and analyzed using Bruker Compass Data Analysis software, version 4.1. Dereplication was performed using the Dictionary of Natural Products (DNP) database version 6.1 with the following search parameters: accurate molecular mass, absorption spectra and source of compound isolation. Thus, in addition to more common representative strains of Streptomyces, several species belonging to the genera Rhodococcus, Amycolatopsis, and Micromonospora were isolated. Several of the selected strains were deposited in the Russian Collection of Agricultural Microorganisms (RCAM), St. Petersburg, Russia. All isolated strains exhibited antibacterial and antifungal activities. We identified several strains that inhibited the growth of the pathogen Candida albicans but did not hinder the growth of Saccharomyces cerevisiae. Several isolates were active against Gram-positive and Gram-negative bacteria. Moreover, extracts of several strains demonstrated high antioxidant activity. The high proportion of biologically active strains producing antibacterial and specific antifungal compounds may reflect their role in protecting pollen against phytopathogens. Dereplication of the secondary metabolites of the strain Streptomyces sp. IB 2014 I 74-3 was resulted in the fact that a total of 59 major compounds were detected in the culture liquid extract of strain cultivated in ISP medium. Eight compounds were preliminarily identified based on characteristics described in the Dictionary of Natural Products database, using the search parameters Streptomyces sp. IB 2014 I 74-3 was found to produce saframycin A, Y3 and S; 2-amino-3-oxo-3H-phenoxazine-1,8-dicarboxylic acid; galtamycinone; platencin A4-13R and A4-4S; ganefromycin d1; the antibiotic SS 8201B; and streptothricin D, 40-decarbamoyl, 60-carbamoyl. Moreover, forty-nine of the 59 compounds detected in the extract examined in the present study did not result in any positive hits when searching within the DNP database and could not be identified based on available mass-spec data. Thus, these compounds might represent new findings.

Keywords : actinobacteria, Baikal Lake, biodiversity, male cones, Pinus sylvestris
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