Short Communication

Journal of Bacteriology and Infectious diseases

2020
Vol. 4, Iss. 2

Bacteriology and Infectious Diseases 2018- Characterization of antiproliferative red-like pigments produced by actinomycete soil strains identified as streptomyces coelicoflavus

Mouslim Assia

University of Hassan II Casablanca, Morocco

Keywords: Actinomycetes, Metabolites , Osteosarcoma

Actinomycetes are filamentous bacteria, widely distributed in soil, water and plants rhisosphere. They are able to produce secondary metabolites with diverse chemical structures and biological activities. Among these bioactive metabolites, anthracycline antibiotics such as prodigiosin are known to exhibit antitumor, antioxidant, and immunosuppressive activities. In this study, five strains of actinomycetes isolated from soil were revealed to produce red-like pigments. Phenotypic and RNA gene coding sequence analysis allowed identification of the all strains as Streptomyces coelicoflavus, which reported here for the first time to produce an antiproliferative red-like pigment. The pigments are intracellular, hydrophobic and photosensitive. Their extraction could be performed with organic solvent from which, ethanol showed the most effective extracting ability. Like prodigiosin and undecylprodigiosin, UV-Vis absorbance of the pigments present sharp peak at nearby 534 nm at neutral or acid conditions, where the pigment color is red. In basic condition the pigment color turn yellow with a λmax of 458 nm. However, TLC analysis, antibacterial assay and production media suggest highly that these red-like pigments are undecylprodigiosin analogues or probably other similar anthracyclines. Cytotoxicity of crude extract and two fractions (FA, soluble in petroleum ether and FB, soluble in chloroform) of two strains were performed by MTT assay on mice P3 myeloid cancer cells and human osteosarcoma cancer cells (U-2 OS (ATCC HTB-96). Results on mice P3 cell line showed that the crude extract of one from two tested strains have the highest antiproliferative activity at low dose. At 100 µg/ml, both fractions A and B of the two strains showed high antiproliferative effect on mice P3 cell line. In human U2OS osteosarcoma cell line, 3 fractions showed more antiproliferative effect than on mice P3 cell line. FACs analysis suggests a cell phase cycle arrest at G1 and S according to the fractions. Five new strains MFB11, MFB20, MFB21, MFB23 and MFB24 of actinomycetes showed an intracellular hydrophobic pink red-like pigment production. These pigments present similar physico-chemical characteristics with anthracycline antibiotics of prodigiosin family. Nevertheless, negative antibacterial assay, Thin-layer chromatography (TLC) and interaction with organic solvents analysis of these pigments revealed their difference from known anthracycline antibiotics. Morphological, biochemical and gene coding 16S RNA sequence analysis allowed identification of the producer strains as Streptomyces coelicoflavus; known to produce important aminoglycoside antibiotics and other bioactive compounds but not anthracyclines red-like pigments. The identification of the five strains and physico-chemical properties of the produced pink red-like pigments are presented in this report. Five new strains MFB11, MFB20, MFB21, MFB23 and MFB24 of actinomycetes showed an intracellular hydrophobic pink red-like pigment production. These pigments present similar physico-chemical characteristics with anthracycline antibiotics of prodigiosin family. Nevertheless, negative antibacterial assay, Thin-layer chromatography (TLC) and interaction with organic solvents analysis of these pigments revealed their difference from known anthracycline antibiotics. Morphological, biochemical and gene cod-

Note: This work is partly presented at 7th International Conference on Bacteriology and Infectious Diseases June 04-05, 2018 Osaka, Japan

ing 16S RNA sequence analysis allowed identification of the producer strains as Streptomyces coelicoflavus; known to produce important aminoglycoside antibiotics and other bioactive compounds but not anthracyclines red-like pigments. The identification of the five strains and physico-chemical properties of the produced pink red-like pigments are presented in this report. Among 29 soil isolated actinomycetes, five new strains MFB11, MFB20, MFB21, MFB23 and MFB24 showed an intracellular hydrophobic pink red-like pigment production. These pigments present similar physio-chemical characteristics with anthracycline antibiotics of prodigiosin family. Crud extract and prepared fractions were tested by MTT on mice cancer cell line as well on human cancer cell line. The results indicated an important antiproliferative effect of the different strain pigments on the two organism cell types. Human cells were more sensitive to the pigments and presented different antiproliferative effect profiles. FACs analysis of this antiproliferative effect on cancer human cells line showed a cell cycle phase arrests at G1 and S. Nevertheless, negative antibacterial assay, Thin-layer chromatography (TLC) and interaction with organic solvents analysis of these pigments revealed their difference from known anthracycline antibiotics. Morphological, biochemical and gene coding 16S RNA sequence analysis allowed identification of the producer strains as Streptomyces coelicoflavus; known to produce important aminoglycoside antibiotics and other bioactive compounds but not anthracycline red-like pigments. Otherwise, two other strains produced water soluble Gram positive antibiotics and chloroform soluble bioactive compounds with strong and dramatic apoptotic antiproliferative activity as indicated by MTT and their cell cycle phase arrests at G0/G1 and G2. Actinomycetes are Gram-positive, facultative anaerobic fungus-like filamentous bacteria which remain on the top of the natural antibiotic producers. Due to the climatic and geographical diversity of Nepal, a wide range of microorganisms with potent source of antimicrobials are available. The objective of this study was to isolate, identify, and screen the potential antimicrobial-producing actinomycetes from soils covering different altitude range of Nepal. Forty-one isolates of actinomycetes were isolated from 11 soil samples collected from different locations in Nepal with altitude ranging from 1500 to 4380 meters. The isolates were identified on the basis of morphological study, different sugar utilization, protein utilization, and hydrolysis tests. They were also characterized on the basis of temperature and pH. Primary screening for antimicrobial activity was carried out against several test organisms: Staphylococcus aureus (ATCC 25923), Escherichia coli (ATCC 25922), Klebsiella pneumoniae (ATCC 700603), and Pseudomonas aeruginosa (ATCC 27853) by the perpendicular streaking method, and secondary screening was carried out by the agar well diffusion method using ethyl acetate for solvent extraction. 70.7% of the isolates were identified as Streptomyces spp., 19.5% as Nocardia spp., and 9.5% as Micromonospora spp. 43.34% of actinomycete isolates was found to be potent antimicrobial producers from the primary screening among which 46.34% were effective against Gram-positive and 12.19% against Gram-negative test organisms. Isolate C7 (Micromonospora spp.) showed the best broad-spectrum antimicrobial activity during secondary screening. A total of 11 different types of pigments were observed to be produced by different isolates, of which, the yellow pigment was the most prominent. The association between elevation, pH, and pigment with the antimicrobial production was found to be insignificant. This finding can be of importance for further investigation towards obtaining broad-spectrum antibiotics for therapeutic purpose. A highly active actinobacterial strain isolated from untapped areas of Northwestern Himalayas and characterised as Streptomyces puniceus strain AS13 by 16S rRNA gene sequencing was selected for production of bioactive metabolites. The bioassay-guided fractionation of microbial cultured

Note: This work is partly presented at 7th International Conference on Bacteriology and Infectious Diseases June 04-05, 2018 Osaka, Japan

ethyl acetate extract of the strain, led to isolation of macrotetrolide compound 1 (Dinactin) and compound 2 (1-(2,4-dihydroxy-6-methylphenyl)-ethanone). Structures of the isolated compounds were elucidated interpretation of NMR and other spectroscopic data including HR-ESI–MS, FT-IR. These compounds are reported for first time from Streptomyces Puniceus. Compound 1 exhibited strong anti-microbial activity against all tested bacterial pathogens including Mycobacterium tuberculosis. The MIC values of compound 1 against Gram negative and Gram positive bacterial pathogens ranged between 0.019 – 0.156 µg ml−1 and 1 µg ml−1 against Mycobacterium tuberculosis H37Rv. Dinactin exhibited marked anti-tumor potential with IC50 of 1.1- 9.7 µM in various human cancerous cell lines and showed least cytotoxicity (IC50 □ 80 µM) in normal cells (HEK-293). Dinactin inhabited cellular proliferation in cancer cells, reduced their clonogenic survival as validated by clonogenic assay and also inhabited cell migration and invasion characteristics in colon cancer (HCT-116) cells. Our results expressed the antimicrobial potential of dinactin and also spotted its prospective as an antitumor antibiotic.

Note: This work is partly presented at 7th International Conference on Bacteriology and Infectious Diseases June 04-05, 2018 Osaka, Japan