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Investigating Actinomycetes for Antimicrobial Activities

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Prolific production of bio-active secondary metabolites has made actinomycetes an important group of microorganisms to humans,because they contribute to nearly 80% of clinically important antibiotics. This study aims at investigating actinomycetes isolated from terrestrial and fresh water habitats for their antimicrobial potential towards a range of microorganisms and identifying active isolates using morphological characteristics and molecular techniques. A total of 108 actinomycetes were isolated using starch casein agar as the selective medium upon different pretreatments such as air drying, chemical, and heat pretreatment. Their identity was primarily confirmed based on Gram reaction and morphological features, especially of spores and spore arrangement. Crude extracts in ethyl acetate of 19 selected isolates (based on rapid growth), grown in starch casein agar broth for 5 days, were subjected to screening against *Bacillus subtilis* ATCC®11778™, *Escherichia coli* ATCC®25922™, *Pseudomonas aeruginosa* ATCC®25353™, *Staphylococcus aureus* ATCC®25923™, and *Candida albicans* ATCC®90028™. The disc diffusion technique was conducted in triplicate using Ciprofloxacin (antibacterial) and Ketoconazole (antifungal) as positive controls (concentrations of antibiotics and crude extract as per 1 mg/disc). Diameters of inhibition zones were measured and 12 isolates showed antifungal activity against *C. albicans* and broad spectrum antibacterial activity against Gram positive and negative bacteria (except *E. coli*). Three of these isolates showed strong inhibitory activity, importantly a higher growth inhibition compared to that of Ketoconazole. None of the isolates were antagonistic towards *E. coli*. Nine isolates having considerable inhibitory activity were selected for further identification using molecular techniques. Actinomycetes were grown in starch casein agar broth for 4-5 days for DNA extraction and 16s rRNA gene (16s rDNA sequence) from extracted DNA was amplified using the universal primer pair 27F and 1492R, by PCR. Three of the actinomycete Isolates identified by using molecular techniques are*Actinobacteria* bacterium strain nenu_DS_12, *Streptomyces pseudogriseolus* strain 173735, and *Nocardiopsis flavescens* strain BF10 (Accession numbers are KY009689.1, EU570428.1 and KU973973.1, respectively). Respective sequences were deposited in the NCBI repository. In conclusion it can be mentioned that 12 isolates tested above have the potential to be developed as sources of novel antimicrobial compound/s of clinical importance.

Keywords: secondary metabolites, antibiotics, actinomycetes, antimicrobial compounds, disc diffusion technique.

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