

Actinomycetes from Protected Forest Ecosystems of Assam, India: Diversity and Antagonistic Activity

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Abstract : Background: Actinomycetes are the richest source of novel bioactive secondary metabolites such as antibiotics, enzymes and other therapeutically useful metabolites with diverse biological activities. The present study aims at the antimicrobial potential and genetic diversity of culturable Actinomycetes isolated from protected forest ecosystems of Assam which includes Kaziranga National Park (26°30"-26°45"N and 93°08"-93°36"E), Pobitora Wildlife Sanctuary (26°12"-26°16"N and 91°58"-92°05"E) and Gibbon Wildlife Sanctuary (26°40"-26°45"N and 94°20"-94°25"E) which are located in the North-eastern part of India. Northeast India is a part of the Indo-Burma mega biodiversity hotspot and most of the protected forests of this region are still unexplored for the isolation of effective antibiotic-producing Actinomycetes. Thus, there is tremendous possibility that these virgin forests could be a potential storehouse of novel microorganisms, particularly Actinomycetes, exhibiting diverse biological properties. Methodology: Soil samples were collected from different ecological niches of the protected forest ecosystems of Assam and Actinomycetes were isolated by serial dilution spread plate technique using five selective isolation media. Preliminary screening of Actinomycetes for an antimicrobial activity was done by spot inoculation method and the secondary screening by disc diffusion method against several test pathogens, including multidrug resistant *Staphylococcus aureus* (MRSA). The strains were further screened for the presence of antibiotic synthetic genes such as type I polyketide synthases (PKS-I), type II polyketide synthases (PKS-II) and non-ribosomal peptide synthetases (NRPS) genes. Genetic diversity of the Actinomycetes producing antimicrobial metabolites was analyzed through 16S rDNA-RFLP using *Hinf*I restriction endonuclease. Results: Based on the phenotypic characterization, a total of 172 morphologically distinct Actinomycetes were isolated and screened for antimicrobial activity by spot inoculation method on agar medium. Among the strains tested, 102 (59.3%) strains showed activity against Gram-positive bacteria, 98 (56.97%) against Gram-negative bacteria, 92 (53.48%) against *Candida albicans* MTCC 227 and 130 (75.58%) strains showed activity against at least one of the test pathogens. Twelve Actinomycetes exhibited broad spectrum antimicrobial activity in the secondary screening. The taxonomic identification of these twelve strains by 16S rDNA sequencing revealed that *Streptomyces* was found to be the predominant genus. The PKS-I, PKS-II and NRPS genes detection indicated diverse bioactive products of these twelve Actinomycetes. Genetic diversity by 16S rDNA-RFLP indicated that *Streptomyces* was the dominant genus amongst the antimicrobial metabolite producing Actinomycetes. Conclusion: These findings imply that Actinomycetes from the protected forest ecosystems of Assam, India, are a potential source of bioactive secondary metabolites. These areas are as yet poorly studied and represent diverse and largely unscreened ecosystem for the isolation of potent Actinomycetes producing antimicrobial secondary metabolites. Detailed characterization of the bioactive Actinomycetes as well as purification and structure elucidation of the bioactive compounds from the potent Actinomycetes is the subject of ongoing investigation. Thus, to exploit Actinomycetes from such unexplored forest ecosystems is a way to develop bioactive products.

Keywords : Actinomycetes, antimicrobial activity, forest ecosystems, RFLP

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