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INTRIGUING BIOLOGICAL PERSPECTIVES OF ACTINOMYCETES FROM TWO VARIOUS COMMODITIES

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ABSTRACT

The actinomycetes from two different marine commodities were isolated, screened, and characterised in the current investigation. Twelve or so distinct bacterial isolates were obtained and tested using morphological, microscopic, culture, and biochemical characterisation techniques. The chosen bacterial isolates exhibit strong antagonistic activity against pathogenic pathogens and food spoilage microorganisms. The Kirby-Bauer disc diffusion method was used to measure the antagonistic properties of crude extracts. After being selected for additional research, the isolate AMWM showed good antagonistic activity against the test organisms in comparison to the other isolates. 16S rRNA was used to identify the active strains, and a phylogenetic tree was created. The isolate AMWM's gene sequence showed complete homology to *Streptomyces lincolnensis.*Characterization of isolated bioactive metabolite are structurally elucidated through FTIR and HPTLC.

Keywords: Marine Actinomycetes, Sequencing, FTIR, HPTLC

INTRODUCTION

Biotechnology in marine environments has led to the discovery of novel microorganisms in marine sediments, including Actinomycetes—Gram-positive bacteria with high G+C content—significant for organic matter recycling, pharmaceutical production, and vitamin synthesis (Ravenschlay et al., 1999; Stach et al., 2003; Lacey et al., 1976). Among Actinomycetes, Streptomyces are particularly notable, producing up to 80% of antibiotics (Williams et al., 1984) and contributing to various applications such as disease treatment, biocontrol, industrial enzymes, agrochemicals, and soil fertility (Abdelhalem Hamza et al., 2013; Abdelhalem Hamza et al., 2015). As the largest

genus within Actinobacteria, Streptomyces are also responsible for approximately 70% of antibiotic production (Adil A El Hussein et al., 2014).

For precise bacterial identification, genotypic methods like 16S rRNA gene sequence analysis are preferred over phenotypic methods, especially for rare or novel strains. High-performance thin-layer chromatography (HPTLC) enhances thin-layer chromatography (TLC) by automating processes and improving resolution and quantitative accuracy, overcoming manual application issues (Morlock et al., 2010). Two-dimensional chromatography further increases analytical capacity by rotating plates with different solvents (Nurok and David, 1989). Additionally, Fourier-transform infrared (FTIR) spectroscopy offers a non-invasive, label-free approach to rapidly characterize microbial strains by analyzing the content, structure, and chemical modifications of biomolecules (Ami et al., 2012).

MATERIALS AND METHODS

ISOLATION AND ENRICHMENT OF ACTINOMYCETES

Isolation of actinomycetes was carried out using Actinomycetes Isolation Agar media through serial dilution methods (Nonomura and Ohara, 1969) and the spread plate technique (Collins and Lyne, 1989). All pure colonies of marine actinomycetes were subcultured on agar plates and stored at 4°C.

CHARACTERIZATION OF ACTINOMYCETES ISOLATES

i)Gram staining

For Gram staining, a smear of the isolate was prepared, air-dried, and heat-fixed. It was then stained with crystal violet, washed with water, treated with iodine, decolorized with alcohol, counter-stained with safranin, and washed again with water. Finally, the slide was dried and examined under an oil immersion microscope.

ii):- Biochemical Analysis of Actinomycetes isolates

Various biochemical tests were conducted following the guidelines provided in Bergey's Manual of Systematic Bacteriology (Buchannan and Gibbons, 1974; Bergey et al., 2000).

ANTAGONISTIC ACTIVITY

The antagonistic activity of actinomycetes against food spoilage bacterial flora was assessed using the Kirby-Bauer disc diffusion method.

MOLECULAR CHARACTERIZATION

The molecular characterisation was determined by 16S rRNA sequencing.

HPTLC ANALYSIS

Chromatography based purification of the antibacterial compound.

FTIR ANALYSIS:- Spectroscopic technique for functional group identification of bioactive compound.

RESULT AND CONCLUSION

In this work , twelve different actinomycetes have been isolated from the marine environment. In the present study chalky white coloured and gray coloured isolates were noted. Such a dominance of members of gray series has already been reported in different marine environments (Pridham and Tresner,1974; Kim et al.,1999; Ndonde and Semu,2000).

Various biochemical parameters of the Streptomyces were used for their identification (Gottlieb,1961; Jones and Bradley,1964; Mafino et al.,2003). The production of citrase , urease, catalase , and oxidase are considered for characterizing Streptomyces (Nitsh and Kutzner, 1969; Gotoh et al.,1982).The hydrogen sulphide and melanin production has been considered as the other important characters for the identification of actinomycetes (Shirling and Gottlieb,1966).

Actinomycetes are the good source of antagonistic activity. The Actinomycetes isolates were tested for their antagonistic activity against the Food spoilage microorganisms. The food spoilage microorganisms are *Bacillus megaterium, Bacillus licheniformis, Bacillus subtilis, Lactobacillus sps., Lactobacillus lactis.* They show a high antagonistic activity against the food spoilage microorganisms. Apart from these two biologically active metabolites produced from actinomycetes tested against the organisms like *Vibrio haemolyticus, Enterococcus sps.,*

Based on the 16S rRNA analysis, the isolate AMWM Showed 100% similarity with Streptomyces lincolensis. Analysis of 16S rRNA began by using the polymerase chain reaction (Hopwood, 1985) and the amplification of the gene coding for 16S rRNA using polymerase chain reaction.

HPTLC analysis was used to identify biologically active metabolites produced by marine water and soil actinomycetes. From marine water, Loboporin was detected, while Heronamide and Desotamide were identified as secondary metabolites from marine soil. FTIR analysis

complemented HPTLC by determining the functional groups present in these secondary metabolites.

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