



Short Communication

‡Diversity of marine actinomycetes in the Bay of Bengal and their antibacterial activity against human pathogens

Mannagatti Krishnaraj and *Narayanasamy Mathivanan

Biocontrol and Microbial Metabolites Lab, Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai - 600 025, Tamil Nadu, India. *E-mail: prabhamathi@yahoo.com

Abstract

A total of 137 different marine actinomycetes were isolated from deep sea sediment samples collected from the Bay of Bengal. Among them, 85 isolates were tested for antibacterial activity against two human pathogenic bacteria, *Staphylococcus aureus* (methicillin resistant) and *Pseudomonas aeruginosa* as well as an antibiotic sensitive bacterial strain *Bacillus pumilus*. All the 85 isolates exhibited antibacterial activity. Based on the screening results, 10 marine actinomycetes were selected and tested against methicillin resistant *S. aureus*. Out of these five isolates showed good antibacterial activity and the nine among 10 isolates obtained were from estuarine sediments.

Keywords: Bay of Bengal, marine actinomycetes, antibacterial activity, estuary

Introduction

Discovery of novel bioactive secondary metabolites from marine organisms is gaining importance in recent years. Indian marine environment is believed to have rich microbial diversity. However, the wealth of indigenous Indian marine microflora has not been fully explored. Most of the studies on marine microorganisms have been limited to isolation, identification and maintenance of these organisms in different culture media. Further, attention has been focused on studying their antagonistic properties against different pathogens. Importantly, their biotechnological potentials are yet to be fully explored (Sivakumar *et al.*, 2007). Therefore, there is tremendous scope to identify new or rare marine microorganisms and also to discover novel microbial metabolites with diverse biological activities (Dhanasekaran *et al.*, 2005; Ramesh, 2009). Among marine organisms, actinomycetes are considered as vital source for diverse bioactive compounds (Jenson *et al.*, 1991; Ramesh, 2009). The secondary metabolites

especially antibiotics derived from actinomycetes are being used as therapeutic drugs for the treatments of various ailments in humans and animals and as agrochemicals for the management of insect pests, diseases and weeds in agriculture (Lange and Lopez, 1996; Prabavathy *et al.*, 2009). In this scenario, it is worthwhile to isolate and screen marine actinomycetes for the discovery of novel bioactive compounds as only limited work has been done in this group compared to soil actinomycetes. Therefore, the present research was carried out to discover novel metabolites from marine actinomycetes with the aim of developing new bioactive compound(s) as antimicrobial agents.

Material and Methods

Collection of marine sediments and isolation of marine actinomycetes: Deep sea sediment samples were collected from the Bay of Bengal during the cruise programmes organized by the National Institute of Ocean Technology (NIOT), Chennai. The vessel was equipped with a corer as well as a sediment collector which were utilized for the

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sediment collection. Collected sediment samples were pre-treated with 60°C in a water bath for 5 minutes (Goodfellow and Haynes, 1984). The resultant sediment suspension was serially diluted. From the required dilutions, 100 µl of suspension was poured into petriplates containing aged seawater amended starch casein agar (SCA) medium and incubated for 21 days at room temperature (28±2°C). The developments of powdery, chalky and leathery marine actinomycetes colonies were observed. Prominent marine actinomycetes colonies were picked and subcultured on SCA slants. After 7 days of growth, pure colonies were selected and maintained in SCA slants as well as glycerol stocks. All the marine actinomycetes were designated with three letters MML followed by four digit Arabic numerical. The culture characteristics of all the marine actinomycetes were documented.

Extraction of extracellular and intracellular metabolites: The marine actinomycetes were grown in antibiotic production medium at room temperature for 10 days. The cultures were harvested and filtered through cheesecloth to separate the mycelial biomass. The culture broths were centrifuged at 12000 g for 15 min and the supernatants were collected. The cell-free supernatants and mycelial biomass were used for the extraction of active principles. Extraction of extracellular metabolites was done by adding ethyl acetate to the cell-free supernatant in the ratio of 1:1 (v/v) using separating funnels. The solvent layer (upper) was collected and concentrated using a rotoevaporator. In addition, cold acetone was added to the cell-free supernatants in the ratio of 1:1 (v/v) and kept overnight at 4°C. The suspension was centrifuged at 10000 g for 15 min at 4°C and the pellets were dissolved separately in 0.2 M phosphate buffer.

The mycelial biomasses were used for extraction of intracellular bioactive metabolites. Acidified acetone was added to the mycelial biomass in the ratio of 2:1 (v/w) and kept under shaking at 200 rpm in a rotary shaker. After 12 h, the acetone extracts were separated by vacuum filtration and concentrated using a rotoevaporator.

Screening the crude metabolites against human pathogens: The concentrated crude extracts of extra

and intracellular metabolites were dissolved in ethyl acetate and double distilled water at 1:9 (v/v) ratio and filter sterilized using 0.2 µ filter. The antibacterial activity was determined according to the method of Peela *et al.* (2005) using Muller Hinton agar (MHA). In each sterile petriplate, 20 ml of MHA was poured and allowed to solidify under aseptic condition. Two human pathogenic bacteria, *Staphylococcus aureus* (methicillin resistant) and *Pseudomonas aeruginosa* as well as the antibiotic sensitive bacterial strain of *Bacillus pumilus* were spread inoculated onto MHA in separate plates using sterile cotton buds. After this, wells were made in each plate using sterile 5 mm diameter cork borer. The filter sterilized extra and intracellular metabolites were added separately (100 µl) in each well and incubated at 37°C. Whole culture filtrates and heat killed culture filtrates were also tested in this study along with streptomycin as control. After 24 h, the bacterial growth was observed and the zone of inhibition was measured.

Antibacterial activity of extracellular metabolites of selected marine actinomycetes against methicillin resistant *Staphylococcus aureus*: Acetone precipitated extracellular metabolites of 10 selected marine actinomycetes were used to determine the antibacterial activity against methicillin resistant *S. aureus* as mentioned above. Methicillin was used as control. The plates were incubated at 37°C and the zone of inhibition was measured after 24 h.

Results

Out of a total of 137 marine actinomycetes that were isolated from 40 different marine sediment samples, 85 were tested for antibacterial activity against two human pathogenic bacteria, *S. aureus* (methicillin resistant) and *P. aeruginosa* as well as antibiotic sensitive bacterial strain of *B. pumilus*. All the 85 isolated marine actinomycetes were active against all the three test organisms (Fig. 1). Intra and extracellular, live culture filtrates and crude extracts of marine actinomycetes exhibited antibacterial activity against the test organisms. Based on the screening results, 10 marine actinomycetes were selected for further studies. Interestingly, nine out of these 10 isolates were

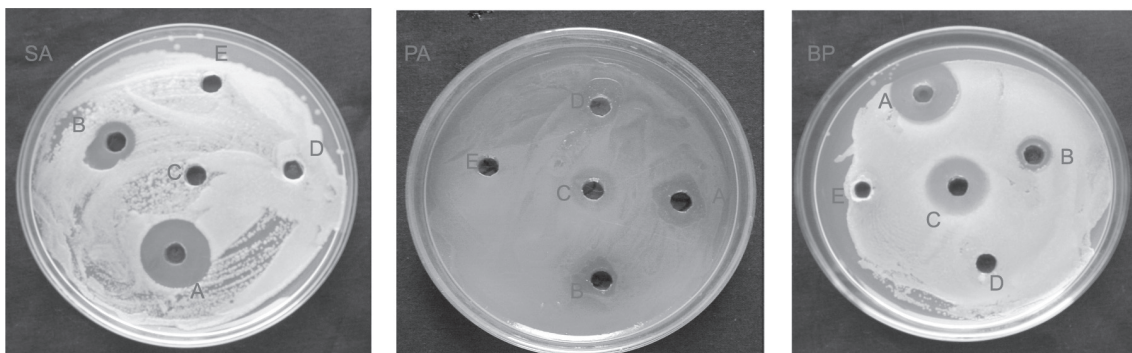


Fig. 1. Antibacterial effect of culture filtrates of marine actinomycetes in well assay SA: *S. aureus*; PA: *P. aeruginosa*; BP: *B. pumilus*
 A: Extracellular; B: Intracellular; C: Control (Streptomycin); D: Culture filtrate (Live); E : Culture filtrate (Heat killed)

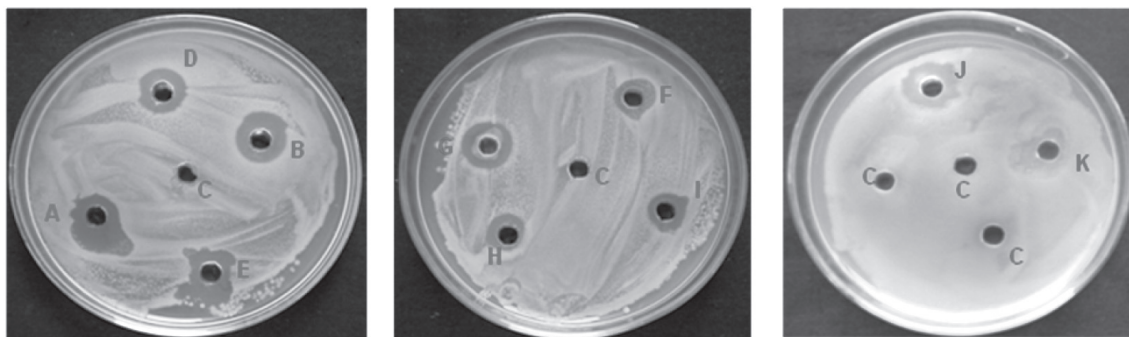


Fig. 2. Antibacterial activity of extracellular acetone fraction of selected marine actinomycetes against methicillin resistant *S. aureus* in well assay
 A: MML1703; B: MML1715; C: Control (Methicillin); D: MML1723;
 E: MML1729; F: MML1735; G: MML1749; H: MML1756; I: MML1760; J: MML1767;
 K: MML1778

isolated from estuarine sediments.

The acetone extracts of 10 isolates showed inhibitory effect against methicillin resistant *S. aureus* (Fig. 2).

Discussion

Many new secondary metabolites have been reported from actinomycetes of marine origin and we have limited knowledge as to whether their production is a direct result of adaptation in the marine environment (Goodfellow and Williams, 1983). The cruise programme organized by the NIOT, Chennai enabled us to collect 40 deep sea sediment

samples from different locations of the Bay of Bengal. Isolation of 137 marine actinomycetes from these samples indicates the richness of microbial diversity in the Bay of Bengal.

All the 85 isolates showed good antibacterial activity against test organisms. Further, the marine isolates obtained from the estuary sediment samples were more active than the others that were isolated from the normal sea bed. The selected 10 strains were more prominent for producing peptide based antibiotic as determined in the present study using protein and heat killed protein fractions. Sponga *et al.* (1999) assumed that the actinomycetes inhibiting

the growth of antibiotic-resistant microorganisms produce more active antimicrobial substances than the known antibacterial preparations. Further, our previous studies revealed that the marine actinomycetes were highly active than terrestrial counterpart. Based on the present study, we conclude that the actinomycetes from the Bay of Bengal have enormous potential for producing various biologically active substances. Further study is in progress for isolating the antimicrobial compounds.

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