Antibacterial Activity of Actinomycetes from Pichavaram Mangrove of Tamil Nadu

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Abstract: In this study the potency of mangrove actinomycetes to produce antimicrobial substances has been studied in 38 strains isolated from different samples of pichavaram mangrove. Antibacterial activity of all the isolated actinomycete strains were checked by cross streak method against Gram positive bacteria; Staphylococcus sp and Bacillus and Gram negative bacteria; E. coli, Salmonella sp, Klebsiella sp and Proteus sp. Among 38 isolates tested, 17 isolates were found to be antibacterial compound producers. KMA02 showed the maximum activity against all pathogens and it was identified as Streptomyces sp.

Key words: Mangrove • Actinomycetes • Streptomyces and pathogens

INTRODUCTION

The actinomycetes are noteworthy as antibiotic producers, making three quarters of all known products. Streptomyces are especially prolific and can produce a great many antibiotics and other class of biologically active secondary metabolites [1]. Actinomycetes thus represent an important group of microbes found in environment and plays a significant role not only in therapeutic applications but also on recycling of organic matter [2]. Actinomycetes constitute a considerable proportion of the population of soil, lakes and river muds. Traditionally actinomycetes have been isolated from terrestrial sources, although the first report of mycelium forming actinomycetes being recovered from marine sediments appeared several decades ago [3]. Marine sediments are potential sources for isolation of novel actinomycetes yielding new products and are recognized as source of novel antibiotic and anticancer agents [4, 5].

The mangrove ecosystem is a largely unexplored source for actinomycetes with the potential to produce biologically active secondary metabolites. Mangrove ecosystems are well known potent areas for distribution and occurrence of microbes [6]. It is also encouraging that bioactive compounds have been obtained from mangrove plants, fungi and bacteria, including actinomycetes. Antibiotics have been used in many fields including agriculture, veterinary and pharmaceutical industry. Actinomycetes have the capability to synthesize many different biologically active secondary metabolites such as antibiotics, herbicides, pesticides, anti-parasitic and enzymes like cellulase and xylanase used in waste treatment [7].

Screening and isolation of promising strains of actinomycetes with potential antibiotics is still a thrust area of search by scientific group from many years. Our investigation was aimed to screen actinomycetes from Mangrove soil for antibacterial compounds against some Gram-negative and Gram positive pathogenic bacteria.

MATERIALS AND METHODS

Collection and Pre-Treatment of Mangrove Soil: Soil samples were collected from four different niche habitats of Pichavaram mangrove, Tamilnadu, India. Each collection was made from 10-15 cm depth of the soil [8]. Samples were collected in sterile polythene bags and transferred immediately to the laboratory. They were air-dried for 1 week. The air dried and sieved samples were kept at 55°C for 60 min in a glass container for pre-treatment [9]. The pre-treated soil samples were then used for actinomycete isolation.

Isolation of Actinomycetes from Mangrove Soil: Five g air dried soil sample was suspended in 50 ml of sterilized water in a 250 ml-Erlenmeyer flask and then shaken at 150 rpm for 30 min. The soil suspension (0.5 ml) was spread on the starch casein agar (1g casein powder, 10g
starch, 37ml sea water, 15g agar and 1L Water with final pH 7.2±0.2). The plates were incubated for 4 weeks at 30°C. After 5 days, the actinomycetes colonies grown on Petri plates were counted at regular intervals. All the morphologically different actinomycete colonies were sub-cultured on yeast extract malt extract agar (ISP No. 2) g/L: yeast extract 4, malt extract 10, dextrose 4, 50% sea water, agar 20 and pH 7.3 [10] by streak plate technique.

**In Vitro Screening of Isolates for Antagonism:** All the isolated actinomycetes were tested for their antibacterial activity against bacterial pathogens namely *Staphylococcus* sp, *Bacillus*, *Salmonella* sp, *Klebsiella* sp and *Proteus* sp. The antibacterial activity was carried out by cross streak method [11]. Single streak of the actinomycetes were made on starch casein agar. After observing a good ribbon like growth of the actinomycetes, the bacterial pathogens were streaked at right angle to the original streak of actinomycetes and incubated at 28 ± 2°C. The inhibition zone (mm) was measured after 24 and 48 h. Control plates were also maintained without inoculating actinomycetes to assess the normal growth of the pathogenic bacteria.

**RESULTS AND DISCUSSION**

**Isolation of Actinomycetes:** A total of 38 different actinomycete strains was recovered from mangrove soil samples collected from Pichavaram, Tamilnadu using starch casein agar. This medium seems to be specific and sensitive for actinomycetes, since it contains starch that most actinomycetes use as a carbohydrate source and casein as nitrogen source. The salts of seawater provide complex ionic sources that make the medium suitable for marine microbial flora and also buffer the medium [17].

**Antibacterial Activities of Isolates:** Thirty eight actinomycetes isolates were screened for the antibacterial activity against six strains of Gram positive and Gram negative pathogens namely *E. coli*, *Staphylococcus* sp, *Salmonella* sp, *Klebsiella* sp, *Bacillus* sp and *Proteus* sp. No growth of the test organisms after 24h adjacent to the streaking of actinomycetes was detected indicating good antimicrobial activity of the isolates. If growth of the test organisms occurred in the entire streak line, then antimicrobial activity of the isolate was recorded as negative [18].

In the present study, the antibacterial activity of the test isolates was varied. Among the 38 isolates tested, seventeen (44.7%) showed antimicrobial activities against more than one genus of test pathogens. Isolate no. KMA02 showed antibacterial activity against all the test pathogens. It showed highest activity against *Salmonella* sp. Two isolates KMA09 and KMA12 were found to have similar activity against test pathogens but ineffective against *Klebsiella* sp and *Proteus*. Isolate KMA04 showed antibacterial activity to three genera of the test pathogens *Staphylococcus*, *Bacillus* and *E. coli*. KMA08 and KMA13 were found to be effective against *Staphylococcus*, *E. coli* and *Klebsiella* (Table 1). By observing the antibacterial activity of the all isolates, KMA02 showed the highest effect on all the pathogens. Hence this strain has been taken for further character analysis.

Alexander [19] reported that about 20-45% of marine actinomycetes exhibit antimicrobial activity; whereas actinomycetes isolated from marine sediments of Visakapatnam, exhibited only 18% of antimicrobial activity as stated by Ellaiah and Reddy [20]. Remya and Vijayakumar [21] also determined that out of 64 strains, 12 isolates (18.8%) showed antibacterial activity, 13 isolates (20.3%) showed antifungal activity (against *C. albicans*) and 9 isolates (14.1%) showed both antibacterial and antifungal activity.

Actinomycetes isolated from mangrove sediments of Pichavaram southeast coast of India exhibiting prominent antibiotic activity against *C. albicans* were detected [22]. Nevine [23] reported that marine actinomycetes are useful and suitable source of new bioactive natural products;
Table 1: Antibacterial activity of selected actinomycetes isolates against pathogenic bacteria

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Staphylococcus</th>
<th>Bacillus</th>
<th>E. coli</th>
<th>Salmonella</th>
<th>Klebsiella</th>
<th>Proteus</th>
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<td>KMA02</td>
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<td>+++</td>
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<td>KMA04</td>
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<td>KMA08</td>
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<td>KMA09</td>
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<td>KMA12</td>
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<tr>
<td>KMA13</td>
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<td>KMA17</td>
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<td>KMA18</td>
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</tr>
</tbody>
</table>

+ = Fair, ++ = potent, +++ = highly potent

- Isolate KMA02: Three isolates (KMA02, KMA17 and KMA18) from the mangrove region southeast coast of India are rich with Actinomycetes. These isolates exhibited high activity against the tested pathogenic bacteria. Further investigations are needed in order to determine the active metabolites of these isolates.

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REFERENCES


