Isolation and Screening of Antimicrobial Activity of Marine Sediment Bacteria from Bay of Bengal coast, Visakhapatnam

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ABSTRACT

A total of 28 marine sediment samples were collected from different locations of Visakhapatnam coast of Bay of Bengal, Andhra Pradesh, India. Twenty three isolates of marine sediment bacteria were isolated using nutrient agar medium. Among 23 isolates, six exhibited antimicrobial activity against human pathogens such as Escherichia coli MTCC443, Pseudomonas aeruginosa MTCC424, Proteus vulgaris MTCC1771, Bacillus cereus MTCC430, Bacillus subtilis MTCC441 and Staphylococcus aureus MTCC3160. Resulting mean diameter of inhibitory zones revealed isolate MSB-6 was the most potent among all the isolates. It has shown zone of inhibition 19 mm. The current investigation results of diversity and antimicrobial activity have increased the scope of finding industrially important marine bacteria from the Bay of Bengal and these organisms could be vital sources for the discovery of industrially useful bioactive molecules.

Key words: Antimicrobial activity, Marine sediment bacteria, Bioactive compounds.

INTRODUCTION

Microorganisms are developing resistance rapidly against antibiotics available in the market so there is an urgent need to identify newer antibiotics to fight with those resistant microbes. The most propitious source of antibiotics and drugs was microorganisms (Fenical, 1993; Alanis, 2005) and natural products (Bull & Stach, 2007). In the last decade several bioactive compounds have been isolated from marine bacteria and are new resources for the development of medically useful compounds (Donia & Hamann, 2003; Anand et al., 2006). There are approximately 32,500 natural products reported from microbial sources (Antibase data base) including about 1000 derived from marine microbes (Singh & Pelaez, 2008).

Marine sediments are well known to harbor diverse microbes which are the significant source for well potent natural bioactive compounds (Hill, 2004). The screening of microbial natural products continues to represent an important route to the discovery of novel chemicals, for development of new therapeutic agents (Kurtboke & Wildman, 1998) and the Streptomyces species produce about 75% of commercially and medically useful antibiotics (Miyadoh, 1993). Taking into consideration the extensive applications of the antibiotics, microorganisms producing them has been isolated from marine sediments in the current investigation.

MATERIALS AND METHODS

Sample collection and treatment: Marine sediment samples were obtained from different locations at the Visakhapatnam coast, Bay of Bengal, Andhra Pradesh, India. From each location, 15 g of sample was collected at 50 to 100 cm depth from the surface of shore. These samples were placed in small pre-labeled plastic bags and tightly sealed. It was pretreated with CaCO₃ (10:1 w/w) and incubated at 37°C for 4 days and subjected to serial dilution (up to 10⁶ dilution) by adding 1 g of soil sample in 10 mL of distilled water. Bacteria, fungi, actinomycetes and yeast were isolated from marine sediment by serial dilution technique. 1mL of sample was serially diluted using sterile distilled water as diluents and 0.1 mL of the sample was spread over media and incubated (Cruickshank et al., 1995).

Isolation of bacteria from marine sediment: Bacterial isolation was done according to the method of Crawford et al. 1993. To obtain pure culture of bacteria about 0.1 mL of each dilution was placed onto nutrient agar medium by spread plate technique. Pure cultures were inoculated in 10mL of media and incubated for 24 to 48 h prior to screening. Morphological studies were conducted on isolated pure cultures.

Primary screening: Antimicrobial activity of the pure cultures was primarily studied by perpendicular streak method (Egorov, 1985) on Nutrient Agar (NA) by in vitro screening of isolates, NA plates were prepared and inoculated with isolates by a single streak of inoculum in the center of the Petri dish. After 24 h of incubation at 37°C the plates were seeded with test organisms by a single streak at a 90° angle. The microbial interactions were analyzed by the determination of the diameter of the zone of inhibition (Figure 1).

Secondary screening: Selected isolates from primary screening were screened for antimicrobial property using the agar-well diffusion method (Schillinger, 1989; Howell & Stipanovic, 1995) on NA plates. Pure cultures of 14 pathogenic bacterial strains and 2 fungal strains were used for present investigation (Table 1). Fresh and pure culture of each isolate from the primary screening was inoculated in broth and incubated at appropriate temperature conditions for 24 h in an incubator. Growth of the organism in the flask was confirmed by turbidity in the broth. Contents of flasks were separated by centrifuging for 15 min at 7000 rpm. Pellets and supernatants were prepared on the day of use. The supernatant was used for the determination of antimicrobial activity against the standard test microbes. The respective wells (5 mm) generated by the cork borer were loaded by 50μL of supernatant. The plates were incubated at 37°C for 24 h whereas for actinomycetes and fungi are incubated at room temperature. Antimicrobial activity was estimated by measuring the inhibition zone diameters (Haque, Sen & Pal, 1996; Antal, Fiedler, Stackebrand & Beil, 2005). Zone of inhibition was measured by using scale. The isolates exhibiting zones of inhibition against test bacteria were chosen. The above tests were repeated 3 times in time and in space.
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Table 1 Results of antimicrobial activity of six bacterial isolates from marine sediment against pathogenic bacteria and fungi.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Zones of inhibition (Mean ± SD) (mm)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extracts from bacterial isolates isolated from marine sediments</td>
<td></td>
</tr>
<tr>
<td>Gram -ve Bacteria</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa MTCC-424</td>
<td>6±0.4, 8±0.3, 8±0.4, 6±0.2 -</td>
</tr>
<tr>
<td>Proteus vulgaris MTCC1771</td>
<td>18±0.2, 19±0.3, 14±0.5, 7±0.3 10±0.2 -</td>
</tr>
<tr>
<td>E. coli MTCC443</td>
<td>15±0.3, 18±0.2, 9±0.4, 18±0.2 11±0.4 5±0.5</td>
</tr>
<tr>
<td>Aeromonas hydrophila</td>
<td>8±0.2, 10±0.3, 8±0.4, 8±0.5 -</td>
</tr>
<tr>
<td>Aeromonas veronii</td>
<td>7±0.4, 19±0.2, 16±0.3, 7±0.6 13±0.6 15±0.5</td>
</tr>
<tr>
<td>Pseudomonas putida</td>
<td>8±0.4, 17±1.1, 13±0.5, 8±0.5 16±0.5 12±0.2 11±0.3</td>
</tr>
<tr>
<td>Pseudomonas luteola</td>
<td>5±0.7, 13±0.2, 11±0.3, 5±0.6 12±0.2 11±0.3</td>
</tr>
<tr>
<td>Vibrio fischer</td>
<td>6±0.5, 13±0.4, - 6±0.5 12±0.5 9±0.6</td>
</tr>
<tr>
<td>E. coli</td>
<td>3±0.7, 8±0.3, 8±0.6 3±0.8 -</td>
</tr>
<tr>
<td>Gram +ve Bacteria</td>
<td></td>
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<tr>
<td>Staphylococcus aureus</td>
<td>- 18±0.2, 15±0.4, - 15±0.3 12±0.4</td>
</tr>
<tr>
<td>Micrococcus luteus</td>
<td>4±0.6, 15±0.3, 11±0.5, 4±0.7 14±0.4 13±0.3</td>
</tr>
<tr>
<td>Bacillus cereus MTCC430</td>
<td>3±0.5, - - 5±0.5 12±0.3 9±0.4</td>
</tr>
<tr>
<td>Bacillus subtilis MTCC441</td>
<td>15±0.3, 19±0.2, 13±0.2, 10±0.4 5±0.6 8±0.5</td>
</tr>
<tr>
<td>Staphylococcus aureus MTCC3160</td>
<td>- 14±0.4, 16±0.2 - 13±0.3 15±0.2</td>
</tr>
<tr>
<td>Fungal strains</td>
<td></td>
</tr>
<tr>
<td>Candida albicans</td>
<td>8±0.2, 5±0.6, 7±0.3 9±0.4 -</td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td>10±0.3, - 8±0.2 6±0.6 - 3±0.5</td>
</tr>
</tbody>
</table>

aValues are mean of three replicates ± SD
bMSB- Marine Sediment Bacteria

RESULTS AND DISCUSSION
Among the 28 marine sediment samples the total heterotrophic bacterial load ranged from 1.7x10^6 to 4.5x10^6 CFU/g of sediment. Twenty three isolates of marine sediment bacteria were isolated and primarily screened based on the zone of inhibition. Six bacterial isolates (Table 1) selected for secondary screening and further analysis. Out of the screened 6 bacterial strains MSB-6 showed the best anti-microbial activity (zone of inhibition 19±0.2mm) against Bacillus subtilis, Aeromonas veronii and Proteus vulgaris whereas MSB-2 (Marine Sediment Bacteria) and MSB-11 also showed activity (zone of inhibition 18±0.2mm).

The first detailed study of antibiotic producing marine bacteria was done by Rosenfeld and Zobell in 1947, since then there were several reports showed their antagonistic effect against human pathogens (James et al., 1996; Jensen et al., 1994). In the present study marine sediments from coastal region of Bay of Bengal were obtained and the bacteria present in sediments were screened for antimicrobial activity. Many members of the group continue to be dominant bacterial workhorses in microbial fermentation for the production of novel proteins (Schallamay, 2004).

From all these observations the bacterial isolates from the marine sediments are found to be the most prolific marine producers of novel compounds. In this paper we have investigated the possibility of isolating marine sediment microorganisms and their ability of producing medically and industrially useful compounds. Further studies of purification and characterization are underway.

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