MERCURY RESISTANT HALOPHILIC ACTINOMYCETES FROM THE SALT MARSH ENVIRONMENT OF VELLAR ESTUARY, SOUTHEAST COAST OF INDIA

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Abstract:
Occurrence of halophilic actinomycetes in the highly saline sediments of the salt marsh area of the Vellar estuary was studied. Halophilic actinomycetes were isolated and enumerated from four different locations of the estuary using different media viz. Starch casein agar (SCA) medium, SGA agar medium, KU agar medium and GAA agar medium and were supplemented with 20% sodium chloride. Population density of halophilic actinomycetes ranged from $0.1 \times 10^4$ to $8.5 \times 10^4$ CFU/g and the highest density was recorded in SCA medium than the other media used, signifying that the SCA can be best used for the isolation of halophilic actinomycetes. A halophilic strain SH-9 showed greater resistance towards mercuric chloride in agar diffusion assay. The isolate was classified as Actinopolyspora spp. by its morphological and chemotaxonomical characters.

Introduction:

Microbial halophiles have gained significance in recent years as these unusual microbes are believed to synthesize novel metabolites of unique properties (Ventosa et al., 1998). Halophilic actinomycetes hold a prominent position due to their diversity and ability to produce new compounds. Halophilic actinomycetes grow optimally in media containing 15 to 25% NaCl and also in saturated salt concentrations (Kushner and Kamekura, 1988; Valera, 1988; Kokare et al., 2004). These actinomycetes are widely distributed in different habitats such as hyper saline lakes, deserts, saline soils, saltern ponds, saltern mines, salted foods and mangroves (Ventosa, 1988 and Senthilkumar, 2003).

Mercury contamination in environment is arise from human activities, such as burning coal and petroleum products, use of microbial fungicides in paper making and agriculture and mercury catalysts in industry, which can increase local mercury levels several-fold above background (Ravel et al. 1998). Microorganisms in contaminated environments have developed resistance to mercury and are playing a major role in decontamination. However, the present study was carried out to screen the mercury resistant halophilic actinomycetes from the salt marsh environment of the Vellar estuary, lying along the southeast coast of India.

Materials and methods:

Sediment samples were collected from four different locations of the Vellar estuary viz. Dense Mangrove zone (Stn. 1), Salt marsh (Suaeda) zone (Stn. 2), Mouth region (Stn. 3) and Aquaculture pond zone (Stn. 4) at Parangipettai (Lat. 11° 24’ N; long. 79° 46’ E) during the month of August 2003. Sediment salinity was measured using a Hand-held refractometer following the method of Strickland and Parsons (1972).

(a) Isolation of halophilic actinomycetes:
Table 1 Population density of halophilic actinomycetes isolated from the sediments of the Vellar estuary using different media.

<table>
<thead>
<tr>
<th>Media</th>
<th>CFU X 10^7/g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Station 1</td>
</tr>
<tr>
<td>SCA</td>
<td>3.5</td>
</tr>
<tr>
<td>SGA</td>
<td>2.7</td>
</tr>
<tr>
<td>GAA</td>
<td>0.3</td>
</tr>
<tr>
<td>KUA</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Table 2 Comparison of the strain SH-9 with the genus *Actinopolyspora*.

<table>
<thead>
<tr>
<th>Character studied</th>
<th>SH-9</th>
<th><em>Actinopolyspora</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Halophilic nature</td>
<td>Truly halophilic</td>
<td>All the representatives are halophilic</td>
</tr>
<tr>
<td>NaCl (%)</td>
<td>Growth occurs in the presence of 15 – 25% NaCl. Optimal growth, between 17 &amp; 22% NaCl</td>
<td>Growth occurs in the presence of 10 – 30% NaCl. Optimal growth, between 15 &amp; 20% NaCl</td>
</tr>
<tr>
<td>Fragmentation of substrate mycelium</td>
<td>Occasionally found</td>
<td>Occasionally found</td>
</tr>
<tr>
<td>Sporophore</td>
<td>Straight, smooth surface, 25 or more spores (Fig. 1a &amp; b)</td>
<td>Straight or flexus, smooth surface, 20 or more spores</td>
</tr>
<tr>
<td>Spores in substrate mycelia</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Shape of the vegetative mycelium</td>
<td>Filamentous with several bends</td>
<td>Filamentous with several bends</td>
</tr>
<tr>
<td>Growth of the vegetative mycelium</td>
<td>Extensive mycelial formation before the production of aerial mycelium</td>
<td>Extensive mycelial formation before the production of aerial mycelium</td>
</tr>
</tbody>
</table>

Fig. 1 Mercury resistance activity of strain SH-9 by agar diffusion assay.
Halophilic actinomycetes

The sediment samples were air-dried aseptically. Heat treatments were performed by holding sediment samples in a water bath at 50°C for 60 min. Then, the samples were diluted and shaken at 2000 rpm for 30 min. Then, 10-fold serial dilutions were prepared using filtered and sterilized 50% sea water and 0.1 ml of each of the serially diluted samples were plated in the following four media viz. Starch Casein Agar (SCA) (Ruan et al. 1994), Sehgal and Gibbons Agar (SGA) (Al-Tai, 1994), Glycerol Asparagine Agar (GAA) (Pridham and Lyons, 1961) and Kuster’s Agar (KUA) (Kuster and Williams, 1964). NaCl was added to each medium at a concentration of 20% (wt/vol), and cultures were incubated for 15 days at 37°C.

(b) Assay for mercury resistance: ISP2 medium was used to screen for mercury resistant halophilic actinomycetes by trough assay and the activity was determined by agar diffusion method (Ravel et al., 1998). Sensitive strains showed zones of inhibition of > 10 mm, whereas zones of inhibition of resistant strains were < 7 mm at 100 nmol HgCl₂. Growth of strain was compared to the Escherichia coli TP 001 control strain (mercury sensitive).

(c) Chemotaxonomy: Isomers of diaminopimelic acid (DAP) and the sugars in whole-cell hydrolysates of strain SH-9 were determined using the methods of Becker et al. (1965) and Lechevalier and Lechevalier (1970). The cell wall amino acid and sugars were separated on thin layer plates following the methods of Stanek and Roberts (1974).

(d) Morphological properties: The key of Nonomura (1974) and Bergey’s Manual of Determinative bacteriology (1972) were followed to determine the morphological properties. Aerial mass colour of mature sporulating aerial mycelium was examined using oatmeal agar (ISP3).

Spore chain morphology: Characteristics of the spore-bearing hyphae and spore chains were determined by using direct microscopic examinations.

Assimilation of carbon source: The ability of different halophilic actinomycete strains in utilizing various carbon compounds as source of energy was studied following the method recommended by International Streptomyces Project (Shirling and Gottlieb, 1966) and Treser et al. (1968).

Results and discussion:

In the present study, sediment salinity recorded at four stations ranged from 35 to 70 %o. The minimum salinity was recorded at station 3 (Mouth region 35%o) and the highest, at station 2 (Salt marsh zone 70%), Station 1 and 4 were recorded salinity of 40%o and 36%o respectively.

Occurrence of halophilic actinomycetes at four stations of the Vellar estuary is given in Table 1. After 15 days of incubation, whitish leathery colonies were observed. Population density of halophilic actinomycetes varied with different culture media and collection sites. In the present study, it could be noted that the population density of halophilic actinomycetes varied broadly according to the salinity conditions (35%o to 70%o) of the study sites. Among the four stations, the salt marsh environment (station 2) registered comparably higher density of halophilic actinomycetes population (8.5 x 10⁴ CFU/g), were the salinity was also reported high (70%). It is worth mentioning here that the higher salinity and salt deposited regions would enrich the growth of halophilic actinomycetes (Okazaki and Okami 1972; Ruan, 1994; Al-Tai, 1994) and they can grow optimally in media containing 15 to 25% NaCl and in saturated salt concentrations (Kushner and Kamekura, 1988; Valera, 1998; Senthilkumar, 2003). Obviously, Station 2 which has dense salt marsh vegetation and where salt deposition occurs often could have provided with the suitable environment for the good growth of halophilic actinomycetes due to which a higher population density of halophilic actinomycetes could be recorded from here in the present study. The mangrove zone (station 1) exhibited the next higher density (0.1 X 10⁴ to 3.5 X 10⁴ CFU/g) of halophilic actinomycetes due probable to its moderate salinity as explained by Weyland (1986).
lower density of halophilic actinomycetes was recorded at both stations 3 and 4 (mouth region and aquaculture pond zone respectively) due to their comparably lower salinity (35% and 36%).

In the present investigation, the rich halophilic actinomycete population was observed in Starch Casein Agar medium (SCA) (Table 1). In the SCA medium, mean population density ranged from \(1.2 \times 10^4\) to \(8.5 \times 10^4\) CFU/g. The maximum was observed at station 2 \((8.5 \times 10^4\) CFU/g). This was followed by station 1 \((3.5 \times 10^4\) CFU/g), station 4 \((2.3 \times 10^4\) CFU/g) and Station 3 \((1.2 \times 10^4\) CFU/g). In SGA medium, the mean population density ranged from \(0.2 \times 10^4\) CFU/g to \(7.8 \times 10^4\) CFU/g in sediments. The maximum density \((7.8 \times 10^4\) CFU/g) was observed at station 2 followed by station 1 \((2.7 \times 10^4\) CFU/g), station 4 \((1.7 \times 10^4\) CFU/g) and station 3 \((0.2 \times 10^4\) CFU/g). In GAA medium, the mean density varied from \(0.2 \times 10^4\) CFU/g to \(2.2 \times 10^4\) CFU/g recording the higher density \((2.2 \times 10^4\) CFU/g) at station 2, followed by station 4 \((0.4 \times 10^4\) CFU/g). Stations 1 and 3 registered the least population density \((0.3 \times 10^4\) and \(0.2 \times 10^4\) CFU/g respectively). In KUA medium, station 2 was found to have the maximum density \((0.9 \times 10^4\) CFU/g) of halophilic actinomycetes followed by station 4 \((0.3 \times 10^4\) CFU/g). Stations 1 and 3 had the least population density \((0.1 \times 10^4\) CFU/g).

Among the 41 different isolates, strain SH-9 isolated from salt marsh (Suaeda) environ, was found to be significantly resistant to mercuric chloride (HgCl₂), using trough assay (Ravel et al., 1998). The mercury resistant strain (SH-9) was further characterized by agar diffusion assay. The strain SH-9 showed greater resistance (4 mm) towards HgCl₂ at the concentration of 100 - 150 nmole whereas, the control strain E.coli TP 001 exhibited higher inhibition zone as it is sensitive to mercuric chloride (Fig. 2).

The broad spectrum mercury resistance of SH-9 was substantiated and the mechanism for resistance has been investigated earlier by Brunker et al. (1996). According to him organomercural lyase encoded by merB is required to cleave C-Hg bonds and Hg²⁺ ions released are reduced by the product called merB, a mercury reductase. Hence, the strain SH-9 was assured to contain homologous merA and merB genes conferring broad spectrum mercury resistance.

Chemotaxonomically, the strain SH-9 possessed the cell wall type IV. It contained meso-DAP in the peptidoglycan layer and type ‘A’ whole cell sugar pattern with arabinose and galactose. According to Embley (1992), the family Pseudonocardiales is characterized with cell wall type IV. The genus belonging to the wall type IV are Actinopolyspora, Thermomonospora, Micropolyspora, Pseudonocardia, Nocardia and Saccharopolyspora (Lechevalier and Lechevalier, 1970). The development of spore chains on aerial mycelium and the unfragmented vegetative mycelium was observed. Morphological characters of the strain SH-9 were compared with the general morphological properties of Actinopolyspora (Table 2). All the carbon sources viz. fructose, galactose, glucose, lactose, maltose, mannitol, raffinose, arabinose, sucrose and sorbitol tested were utilized, except rhamnose, which was weakly utilized. On the basis of the properties described above, the strain SH-9 should be placed in the genus Actinopolyspora. From the present study, it can be concluded that SCA agar medium is the best among the various media tested to isolate the halophilic actinomycetes from the sediments of Vellar estuary. And among the different marine locations studied the salt marsh regions harbors comparably more population density and potential mercury resistant halophilic actinomycetes. Hence, from the above work the way has been paved for researchers to explore the Suaeda zone to isolate new halophilic actinomycetes in view of their potential application in heavy metal resistant properties.

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Reference:


