Molecular analysis of bacterial population inhabiting coconut husk retting area

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As part of the biodegradation of the phenolic compounds, a microbiological survey was conducted in the Kadinamkulam estuary during 2003. The aim of this study was to screen the bacterial population and to elucidate their plasmid profile in the context of coconut husk retting. Species of Pseudomonas, Escherichia, Enterobacter, Micrococcus, Shigella, Salmonella, and Klebsiella were found to be present in the area. Of these the most dominant species was Pseudomonas. The study was also planned to delineate the antimicrobial susceptibility pattern prevalent in the area. Of the various antibiotics tested, only gentamycin and ofloxacin were found to be sensitive to all the bacterial strains. During the present study nineteen isolates showed the presence of plasmids of varying sizes as their extrachromosomal genetic material.

Keywords: husk retting, estuary, bacteriology, antibiogram, plasmid profile

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Retting is the basic process involved in the manufacture of coir. It is a biological process which involves the pectinolytic activity of microorganisms especially bacteria and fungi present in the retting grounds that liberate large quantities of organic substances into the medium. Although many studies have been undertaken to elucidate the ecobiological status of the backwaters of Kerala, no major effort has hitherto been made to elucidate the bacterial population and its molecular nature in the context of pollution abatement in these biotopes. Estuarine microbes are the less studied living resources of the backwaters, although reports available indicate their high potential in bioremediation. In this context, a study was conducted on the biochemical and molecular nature of bacterial population in the Kadinamkulam estuary, a major retting zone in Kerala.

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waterbodies through land drainage, during the present investigation the most dominant species found was that of *Pseudomonas*. Among these water samples, *Vibrio* sp. was spotted only in sample 3. Anaerobic condition conjoined with the production of hydrogen sulphide is a general phenomenon noticed in the retting area. Although *vibrios* are facultative anaerobes, the high concentration of hydrogen sulphide produced in the area may be lethal to this community. The dominant *Pseudomonas* sp. inhabiting the retting area could inhibit the growth of other organisms in the region. The *Pseudomonas* sp. during the long course of time might have got acclimatized and, hence, the physico-chemical nature may be highly suitable for its succession in the environment. In any aquatic biotope as a whole, the combined activities of bacterial and other microorganisms tend to create environmental conditions, suitable for the growth of its living resources. But on certain occasions, due to anthropogenic activities like coconut husk retting, the activities of bacteria act as a limiting factor by changing the equilibrium of physico-chemical characteristics of the medium.

Resistance of a particular bacterial strain to a particular antibiotic may be due to speciality of its plasmids, nucleus or may be due to cytoplasm. Sensitivity of bacterial strains against nine antibiotics was tested in this study. All the bacterial strains showed resistance to penicillin, vancomycin and erythromycin. It was also revealed that all the bacterial strains were sensitive to gentamycin and ofloxacin. Only two strains showed resistance to cotrimaxazole. Three strains showed multi-drug resistance to chloroamphenicol, clindamycin, erythromycin, penicillin, vancomycin, and ampicillin. Whereas other two strains showed clindamycin, cotrimaxazole, erythromycin, penicillin, vancomycin and ampicillin pattern of multi-drug resistance (MDR). 'Amp' gene in some particular plasmids encoding the enzyme β-lactamase has a capacity to degrade penicillin antibiotics such as ampicillin. In this study, majority of bacterial strains showed ampicillin resistance. Percentage resistance showed by bacterial strains against different antibiotics are represented as erythromycin (100%), penicillin (100%), vancomycin (100%), clindamycin (76.47%), ampicillin (70.51%), chloroamphenicol (41.18%), cotrimaxazole (11.76%), gentamycin (Nil) and ofloxacin (Nil).

Seventy eight bacterial colonies isolated were screened for plasmids. Of these, 19 isolates showed plasmids of varying sizes as their extra-chromosomal genetic material (Figs 1 & 2). Plasmids seem to be ubiquitous in bacteria, many encode genetic information for properties such as resistance to antibiotics, bacteriocin production, resistance to toxic metal ions, enterotoxin production, enhanced pathogenicity, reduced sensitivity to mutagens and the ability to degrade complex organic molecules. The

<table>
<thead>
<tr>
<th>S No</th>
<th>Genus</th>
<th>Indole</th>
<th>MR</th>
<th>VP</th>
<th>Citrate</th>
<th>Nitrate</th>
<th>Oxidase</th>
<th>Catalase</th>
<th>G. Staining</th>
<th>Motility</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Pseudomonas</em> sp.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-ve rods</td>
<td>Motile</td>
</tr>
<tr>
<td>2</td>
<td><em>Escherichia</em> sp.</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-ve rods</td>
<td>Non-motile</td>
</tr>
<tr>
<td>3</td>
<td><em>Shigella</em> sp.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-ve rods</td>
<td>Non-motile</td>
</tr>
<tr>
<td>4</td>
<td><em>Salmonella</em> sp.</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-ve rods</td>
<td>Motile</td>
</tr>
<tr>
<td>5</td>
<td><em>Klebsiella</em> sp.</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-ve rods</td>
<td>Motile</td>
</tr>
<tr>
<td>6</td>
<td><em>Enterobacter</em> sp.</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-ve rods</td>
<td>Motile</td>
</tr>
<tr>
<td>7</td>
<td><em>Micrococcus</em> sp.</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+ve diplococci in chains</td>
<td>Non-motile</td>
</tr>
</tbody>
</table>

+ indicates positive results; - indicates negative results
The present investigation suggests that these plasmids may contain genes encoding for degradation of phenolic compounds in the area. Genes coding for some enzymes essential for biodegradation of organic compounds are plasmid borne. Several workers have reported the plasmid-mediated degradation of complex compounds by many bacterial species. Further studies on plasmid-mediated biodegradation of phenol by these bacterial isolates are in progress.

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