STUDIES ON MANGROVE SWAMPS OF GOA:
II. MICROORGANISMS DEGRADING PHENOLIC COMPOUNDS

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ABSTRACT

Sediment samples from three stations in mangrove swamps along the Mandovi-Zuari estuarine complex showed the presence of phenolic compounds varying from 0.26 to 1.01 mg/kg. Phenolic acids such as ferulic, p-coumaric, p-hydroxy benzoic and gentisic were identified. Most of the heterotrophic bacterial and yeast isolates from the swamps could tolerate and grow at 0.50% concentration of phenol. Amongst the 144 cultures screened, 35 could grow on phenolic concentration as high as 1%. These cultures were capable of growing on phenolic compounds such as p-hydroxy benzoic acid, protocatechuic, ferulic acid, gallic acid and orcinol, but showed poor growth on cresols.

Key words: Mangrove swamps, sediment, phenolic compounds, microorganisms.

INTRODUCTION

The mangrove swamps in Goa are rich in dense evergreen vegetation which shed the leaves throughout the year. As a result, large amounts of plant polymers like cellulose, amylose, pectin and lignin are available for microbial degradation. Lignin, although resistant to bacterial attack, is decomposed mainly by white rot fungi to phenolic subunits (Burges, 1967). The latter are converted into polymeric forms and humus, which also serves as food material for the fungi (Burges, 1967; Doetsch and Cook, 1973). Thus, phenolic compounds may mainly be formed during the degradation of organic matter derived from plants (Stout, Tate and Molloy, 1976). Effluents may also be a factor for increasing the concentrations of phenol in such ecosystem (Grob, 1975). The mangrove sediments of Goa shows the presence of various phenolic acids (Karanth, Lokabharati and Nair, 1975).

Microorganisms possess a remarkable adaptive capacity and may develop resistance and/or degradative ability to any given organic compounds. The mangrove sediments are known to harbour a heterogenous microflora exhibiting degradative enzymes, such as cellulases, amylases and pectinases (Matondkar, Mahtani and Mavinkurve, 1980b) and may also possess the phenol degrading activities.

The present paper reports the qualitative and quantitative analysis of mangrove sediments for phenolic compounds, and the ability of microorganisms isolated from mangrove swamps situated along the Mandovi-Zuari estuarine complex of Goa, to utilise various phenolic compounds.
MATERIAL AND METHODS

Estimation of phenolic compounds in sediments

Two kilograms of sediment samples were collected from three stations (Stn. 1 - S. Pedro; Stn. 2 - Bhanastari; Stn. 3 - Borim) in plastic bags and analysed within 2 hours (Matondkar, Mahtani and Mavinkurve, 1980a). Phenolic compounds from sediments were extracted by methods described earlier (Karanth, Lokabharati and Nair, 1975) and were estimated quantitatively by colorimetric method (Stone, 1956). The extracts were also analysed by paper chromatography using solvent systems (i) Isopropanol-ammonia-water (20:1:2 V/V), (ii) Benzene-acetic acid-water (10:7:3 V/V, organic phase) and (iii) Formic acid-water (2:98 V/V).

Assay of phenolic degradation by microflora

A total of 144 cultures isolated from three mangrove stations in Goa (Matondkar, Mahtani and Mavinkurve, 1980b) were screened for their phenol degrading ability. Phenol-agar was prepared by adding distilled phenol to mineral agar (Aarsonson, 1970) at concentrations ranging from 0.05 to 1.0%. Mineral media plates without phenol served as control. Each of the microbial isolates was spot-inoculated initially on 0.05% phenol-agar and subsequently those showing growth were subcultured on increasing concentrations of phenol media.

The cultures which could tolerate 1% phenol in the media and showed consistent growth in three subcultures in this medium, were further screened for growth on various phenolic compounds such as p-hydroxy benzoic acid, ferulic acid, protocatechuate etc. used in the media at a concentration of 0.05%.

The mode of aromatic ring cleavage of di-hydroxy benzoate by cultures showing good growth on p-hydroxy benzoic acid was studied by a modified Rothera test (Ottow and Zolg, 1974).

RESULTS AND DISCUSSION

In the present study, mangrove sediment samples showed very wide stationwise variations in phenol concentration which ranged from 0.26 (stn. 1) - 1.01 (stn. 2)

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Total number</th>
<th>Protocatechuate acid.</th>
<th>p-OH Benzoic acid.</th>
<th>Quinol</th>
<th>Oresinol</th>
<th>Ferulic acid</th>
<th>Catechol</th>
<th>Phloroglucinol</th>
<th>o-Cresol</th>
<th>Gallic Acid</th>
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<tbody>
<tr>
<td>Staphylococcus</td>
<td>14</td>
<td>100</td>
<td>92.8</td>
<td>0</td>
<td>78.5</td>
<td>85.7</td>
<td>14.2</td>
<td>42.8</td>
<td>14.2</td>
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<tr>
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<td>6</td>
<td>100</td>
<td>100</td>
<td>33.3</td>
<td>83.3</td>
<td>100</td>
<td>33.3</td>
<td>16.6</td>
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<td>Micrococcus</td>
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<td>0</td>
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<tr>
<td>Planococcus</td>
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<tr>
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<td>Coryneform</td>
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<td>100</td>
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<td>50</td>
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<tr>
<td>Yeast</td>
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mg/kg (Table I). The concentration in Borim sediments (stn. 3) was found to be 0.53 mg/kg. Bhanastari (stn. 2), which showed highest phenol concentration also showed the highest carbon content (unpublished results), perhaps indicating the accumulation of lignin and humus known to be resistant to bacterial attack. It is of further interest to note that the extract of sediment from this station resolved on chromatographic analysis into a single fairly polar spot which could not be identified with the methodology used. Stn. 1 and 3 showed the presence of phenolic acids—p-hydroxy benzoic, p-coumaric, ferulic and gentisic acids.

The present study indicates that a large number of microorganisms exist in this ecosystem which can tolerate high phenol concentrations and can utilise these as sole source of carbon and energy. Amongst a total of 144 bacterial and yeast cultures isolated from mangrove sediments, 104 cultures could grow in the presence of 0.05% phenol concentration. These cultures were sub-cultured on to plates containing increasingly higher phenol concentrations. Fig. 1 indicates percent viability of

![Graph showing viability of microorganisms in increasing concentrations of phenol in growth media](image)

*Fig. 1. Viability of microorganisms in increasing concentrations of phenol in growth media*
various groups of microorganisms in increasing phenol concentrations. Gram positive bacterial cultures were seen to be more tolerant to phenol than Gram negative organisms. As phenol concentrations increased, the viability of cultures from all the groups was reduced. It was however surprising that even at a concentration of phenol as high as 1%, a total of 35 cultures showed visible and persistent growth. These 35 isolates also utilised various other phenolic compounds (Table I). Each of these cultures could grow on protocatechuic acid, which is a key intermediate in the metabolic pathway of various aromatic substrates (Rogoff, 1961). Most of the cultures growing on protocatechuic acid were also able to grow on p-hydroxy benzoic acid. Good growth was observed on ferulic acid, which is detected in mangrove sediments of S. Pedro and Borim. A moderately good growth was observed on Gallic acid (Table I). In the present study response to cresols was poor with all the cultures. The o- and p-cresols are usually found as contaminants in polluted water (Grob, 1975). In general, a poor growth was also observed with catechol and quinol as substrates (Table I). Amongst the cultures, 3 strains of Bacillus, 2 strains of Coryneforms and 1 strain of Pseudomonas, examined for mode of aromatic ring cleavage, showed the presence of ortho fission pathway with β-keto adipate as one of the key intermediate products. The meta-pathway is known to serve as a general mechanism for catabolism of various alkyl derivatives of catechol, derived from substituted phenol compounds whereas ortho-pathway is more specific and serves primarily in the catabolism of catechol and precursors of catechol (Fiest and Hegeman, 1969).

The influence of phenolic acids in the mangrove ecosystem is not clearly understood. These compounds are known to affect the seed germination and inhibit root elongation, thus being harmful to vegetation. A high concentration of phenol in the sediment is known to have a toxic effect on the heterotrophic microbial population, thereby reducing the productivity of the region. Phenol-degrading bacteria may thus play a crucial role, removing the block in the food chain in the mangrove ecosystem by degradation of accumulated phenolic compounds resulting from biodegradation of plant macromolecules.

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REFERENCES


