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Full Length Research Paper

Epiphytic organisms on the pneumatophores of the mangrove A*vicennia marina*: occurrence and possible function

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The nature of the epiphytism of red algae and also the occurrence and possible role of other epiphytic micro-organisms within the superficial tissues of pneumatophores of *Avicennia marina* (Forssk.) Vierh. were investigated. Transmission electron microscopy (TEM) studies revealed that bacteria and holdfasts of red algae were present in damaged tissue of the periderm. Culture studies indicated that some bacteria were diazotrophic. A mutualistic relationship between epiphytes and mangrove pneumatophores was postulated.

Keywords: Algae, bacteria, epiphytism, mangrove, *Avicennia marina*.

INTRODUCTION

The pneumatophores of *Avicennia marina* (Forssk.) Vierh. have been found to support a rich flora of algae and bacteria. In South Africa the algae epiphytic on the pneumatophores of this mangrove have been the subject of several taxonomic (Lambert et al.1987, 1989), physiological (Mann and Steinke, 1988; 1989; 1993; Steinke and Naidoo, 1990) and ecological (Phillips et al., 1994; 1996; Steinke et al., 2003; Yasuyuki, 2005) investtigations. However, these studies raised questions on the nature of the algal epiphytism and also on the role of the micro-organisms on the pneumatophores. To address these considerations a study was undertaken to obtain information on this issue. This paper has arisen from that study and reports the presence of micro-organisms within the superficial tissues of the pneumatophores and indicates a possible role for some of these organisms.

MATERIALS AND METHODS

Collection and preparation of material

Pneumatophores of *A. marina,* with epiphytic algae attached, were collected from the intertidal zone of the channel in the Beachwood Mangroves Nature Reserve in the Mgeni estuary. The pneumatophores were cut off at the base with a sharp knife and, within 20 min, were transported to the laboratory in a bucket of channel water at ambient temperature. The temperature of the water was approximately 25ºC and did not increase by more than 1ºC during transport. Pneumatophores were washed carefully in sterilized seawater, then adjusted with distilled water to 25% which was the mean salinity recorded in the channel at the site of collection.

For uniformity, 2 cm segments were excised from the middle portion of each pneumatophore. Epiphytic algae were carefully removed from the surface with a sterile blade so as not to damage the bark, after which the segments were washed in sterile seawater (25%).

This material was used for two main lines of investigation, viz. transmission electron microscopy to elucidate the nature of the epiphytism and the presence of organisms other than algae and, secondly, characterisation of the role of other organisms.

Light microscopy (LM)

For light microscope studies, segments of pneumatophores embedded in paraffin wax were used. Material was fixed in FAA, dehydrated in a tertiary butyl alcohol series and embedded in paraffin wax. Sections 10 - 15 µm thick were cut on a base sledge microtome, mounted on glass slides, de-paraffinised and photographed with a Zeiss Axiophot photomicroscope (Oberkochen, Germany).

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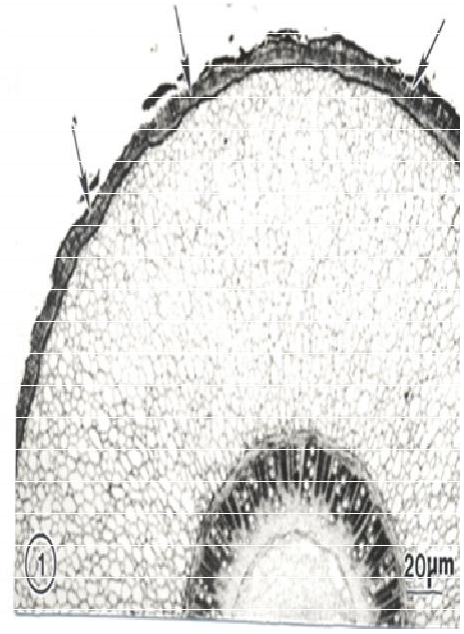

Figure 1. Transverse section of segment of pneumatophore of *A. marina*, showing damage to outer wall layers of cork sheath (arrows).

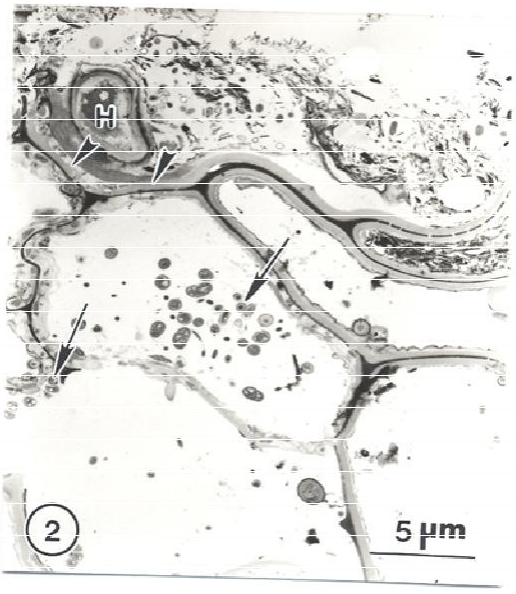

Figure 2. Damaged and underlying cells invaded by bacteria (arrows). Note algal holdfast (H) closely appressed to thick cell wall (arrowheads).

Transmission electron microscopy (TEM)

Small segments of samples (2 mm^2), taken from the mid-region of the pneumatophores were diced in 0.05 M sodium cacodylate buffer (pH 7.2) in a Petri dish. The material was fixed under vacuum in cold 6 % glutaraldehyde buffered with 0.05 M sodium cacodylate for approximately 6 h, washed 3 times for 20 min each in 0.05M cacodylate buffer, and post-fixed in 2% osmium tetroxide made up in the same buffer and stored overnight in the refrigerator at 4^oC. The material was then washed twice in 0.05 M cacodylate buffer before being dehydrated through a graded series of ethanol (that is, 20 min each in 50, 70 and 80% ethanol, 30 min in 90% and 2 changes of 30 min each in 100% ethanol). The samples were washed in two changes of propylene oxide for 20 min each and taken through increasing concentrations of Spurr's (1969) low viscosity resin, diluted with propylene oxide. Material was embedded in 100% Spurr resin. Ultrathin sections were cut with a Diatome diamond knife, collected on uncoated 200-square mesh copper grids and post-stained with 2% aqueous uranyl acetate for 20 min, followed by Reynold's (1963) lead citrate for approximately 5 - 10 min. The sections were examined and photographed with a Philips 301 TEM at 60 kV.

Characterisation of the role of epiphytic bacteria

Before attempting to culture bacteria from the outer cork cells of the pneumatophores, a reliable surface-sterilization technique had to be developed to eliminate interference from surface organisms. A number of treatments for surface-sterilization were investigated (Table 1) . In order to determine the success of each treatment, that is, if any surface contaminants remained, segments were swabbed with sterile swabs which were then streaked over the surface of agar plates. The heterotrophic medium used was trypticase soy agar made up with synthetic seawater according to Atlas (1993).
Five replications were used for each treatment. Plates were incubated for 48 h at 30^oC.

Under sterile conditions, surface-sterilized (95% ethyl alcohol for 5 min, then flamed) pneumatophore segments were either cut in half longitudinally and placed on agar plates or pieces of bark were peeled off and placed on agar. Material was placed on;

a) Nitrogen-free medium to test for diazotrophs (Centifanto and Silver, 1964).
b) Heterotrophic medium.

Five replications of each treatment were incubated under anaerobic (Merck Anaerocult) and aerobic conditions for 48 h at 30^oC. As growth of bacteria occurred in all cases, colonies were re-plated by streaking on to fresh nitrogen-free medium under anaerobic and aerobic conditions and again five replications were incubated for 48 h at 30^oC. The results of these treatments were assessed visually for growth of bacteria which were Gram-stained and examined microscopically.

RESULTS

Transmission electron microscopy

A light microscope cross-section of a pneumatophore of *A. marina* revealed a damaged periderm (Figure 1). The cells of the outermost phellem or cork layers have broken walls (Figure 1) . This damaged tissue showed the presence of algal holdfasts which appeared to be closely appressed to the cell walls. The cells of the holdfasts possess thick walls (Figure 2). The damaged cells and underlying intact (Figure 2) tissue have been invaded by

Table 1. Surface sterilization treatments to control surface contaminants.

Treatments		Sodium hypochlorite, then rinsed	95% ethyl alcohol, then rinsed	95% ethyl alcohol, then flamed	U.V. light
	15 Sec	+	+	+	+
Period	30 Sec	+	+	+	+
	1 Min	+	+	+	+
Time	2 Min	+	+	+	+
	4 Min	+	+	+	+
	5 Min	+	+	-	+

\+ = growth of bacteria
\- = no growth

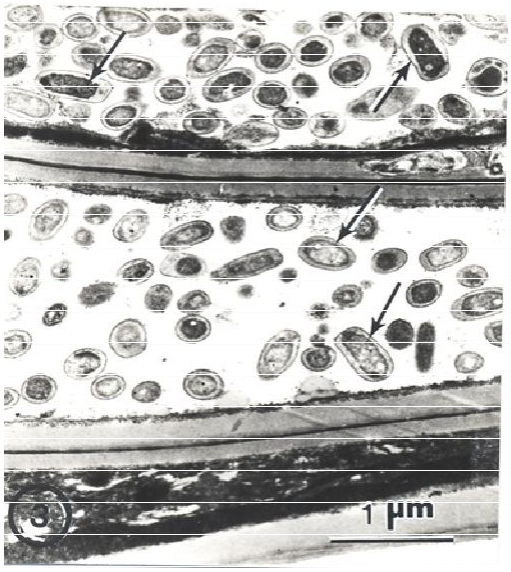

Figure 3. Underlying cells of cork sheath, colonized by bacteria possessing thick walls (arrows).

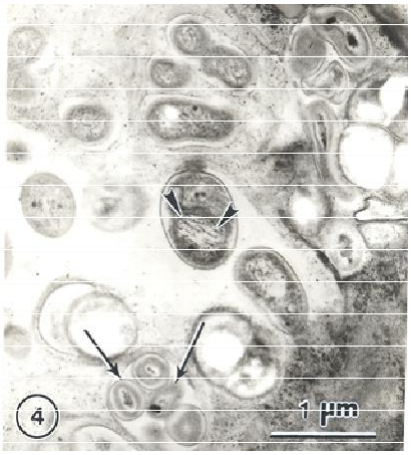

Figure 4. Adhesion of bacteria, probably a result of mucilage surrounding the cells (arrows). Note spiral arrangement of membranes within some bacterial cells (arrowheads).

bacteria, which occur either singly or in colonies.

Some of these bacteria have thick, resistant walls, while those occurring in colonies appear to be surrounded by a mucilaginous sheath (Figures 3 and 4). Transmission electron microscopy of the bacteria revealed the presence of spiraling membranes in the cytomatrix, a feature typical of a diazotroph (Figure 4).

Characterization of the role of epiphytic bacteria

The surface-sterilization technique adopted was the treatment which did not allow growth of bacteria, that is, 95% ethyl alcohol for 5 min, then flamed (Table 1). In all cases growth of bacteria occurred on the nitrogen-free medium, suggesting that some of the bacteria were diazotrophic and confirming the observation above (Figure 4). No identification was possible from microscopic examination of the Gram-stained material.

DISCUSSION

It has been shown that there is a dense growth of epiphytic algae and bacteria on pneumatophores of *A. marina* (Phillips et al., 1996; Steinke and Naidoo, 1990; Mann and Steinke, 1993; Proches and Marshall, 2002). Among the algae the Rhodophyta (the "bostrychietum") and Cyanophyta (cyanobacteria) are usually abundant (Post, 1936; Lambert et al., 1987; 1989). Although the pneumatophores contain chlorophyll and are potentially autotrophic, the dense growth of epiphytes and thick coating of sediment probably restricts their photosynthesis (Steinke and Naidoo, 1990). The presence of algal holdfasts, within both the damaged and intact periderm of pneumatophores, suggests that these structures are not used simply as a substrate for the attachment of the red algae. Previous research has shown that the epiphytic red algae may be responsible for high rates of photosynthesis (Mann and Steinke, 1988) and it is postulated that some photosynthates may

reach the mangrove tissues via the holdfasts. Similarly, the blue-green algae (cyanobacteria) are capable of high levels of nitrogen fixation, some of the nitrogenous products which might also be available, if not immediately, then through the estuarine food chain, to the mangroves (Mann and Steinke, 1993). These cyanobacteria have been shown to fix atmospheric nitrogen (Toledo et al., 1995; Kyaruzi, 2003). Clearly, the diazotrophic bacteria found within the periderm may also have a role in augmenting the nitrogen nutrition of their host.

It is suggested, therefore, that there could be a mutualistic relationship between mangroves and epiphytes. The epiphytes clearly benefit from having a substrate for attachment and from protection provided by the mangrove, and may also derive nutritional advantages from the association. On the other hand, the carbon and nitrogen metabolism of the pneumatophores could benefit through photosynthesis and nitrogen fixation by the epiphytes. Previous work has shown that biological nitrogen fixation occurs on *A. marina* pneumatophores (Lugomela and Bergman, 2002). Although previous research did not establish the presence of diazotrophs within the mangrove tissues, there is clear evidence of the advantages of nitrogen fixation by bacteria and blue-green algae (cyanobacteria) to the mangrove ecosystem (Potts, 1979; Van der Valk and Attiwill, 1984; Hicks and Sylvester, 1985; Toledo et al., 1995; Sheridan, 2001).

The results reported here appear significant although it is clear that the research has posed questions about the possible role of the association between mangroves and epiphytes and these can only be addressed with further investigations. It is hoped that the diverse issues raised by this study will lead to research which will provide further evidence of an association of mutual benefit to all partners.

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