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Full Length Research Paper

Survey of bacterial pathogens on leaves and seeds of red mangrove (*Rhizophora mangle*)

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Bacterial pathogens of red mangrove *(Rhizophora mangle)* were investigated. 50 samples each of leaves and seeds (healthy and diseased) were randomly collected and used for the analysis. Mean bacterial counts obtained were: healthy and diseased leaves; 8.26 x 10^3 and 5.9 l x $l0^3$ cfu/ml respectively; healthy seeds 7.96 x $l0^3$ cfu/ml and diseased seeds 1.1 4x 10^4 cfu/ml. The bacterial populations of the healthy and diseased leaves and seeds showed no significant difference at P > 0.05 (F – cal = 4.69; F – tab = 4.76). Nine bacterial genera isolated include *Acinetobacter* (7.69%), *Aerococcus* and *Alcaligenes* (3.84%), *Bacillus* (19.23%), *Corynebacterium* (34.61%), *Flavobacterium* and *Pseudomonas* (3.84%), *Staphylococcus* (7.69%) and *Streptomyces* (15.38%). *Corynebacterium* had the highest percentage occurrence and was isolated from both healthy and diseased samples. Some of the bacterial species such *Corynebacterium*, *Bacillus, Pseudomonas* are known pathogens of plants and were implicated as pathogens of red mangrove.

Key word: Bacterial pathogens, red mangrove, diseased leaves, healthy leaves.

INTRODUCTION

Bacteria species colonise trees endophyically without causing apparent damage, but as facultative parasites by utilizing tissues altered by abiotic factors. Bacteria, especially anaerobic species can cause wet wood and act as pioneer micro-organisms that prepare substrates for decay of fungi. *Xylella fastidiosa*, a xylem-limited bacterium acts as a primary pathogen inciting leaf scorch and dieback in some trees (Housten, 1987a and 1978b). Bacteria also, can colonise trees endophytically without causing apparent damage but some species have the potential to react with tree tissues that have been altered physiologically. Bacteria can cause permanent changes in heart wood inciting wet wood with slime flux on the bark of the host plant as well as discoloration of heart wood (Lund, 1982). Many bacteria species, most of them anaerobic have been isolated from forest trees showing symptoms of wet wood. Examples of such trees are, *Abies alba, Populus deltoids, Ulmus americana* and *Quorums* spp. Bacteria species such as *Clostidium, Erwinia, Latobacillus, Xanthomonas, Agrobacterium* and some species of Coryneform have been isolated most frequently on the tree species mentioned earlier (Scortichini et al., 19991; Schink et al.,1981 and Tiedemmann et al.,1977). Ukoima et al. (2007), reported on some bacteria which occur on *Avicennia africana* in the Niger of Nigeria. Other authors have reported on fungi associated with mangrove trees in Nigeria and elsewhere (Ukoima et al., 1996, 2000, 2002; Fell et al., 1975; Follosco et al., 1984; Teas, 1982; Kohlmeyer and Kohlmeyer, 1979). The fungal pathogens isolated by these workers include *Cytospora rhizophorae, Didymosphaeria rhizophorae, Keissleriella blepharespora, Mycosphaerella pneumatophorae, Rhabdespora avicenniae, Alternaria solani, Rhoma* sp, *Colletotrichum gloesporoides, Phomopsis* sp*, Pestalotia diachaeta* and *Fusarium moniliforme* on mangrove plants. The Niger Delta mangrove ecosystem of Nigeria is largely muddy and uninhabitable. The widest area stretches from Sapele to Warri and from Abonnema to Port Harcourt (Ekundayo, 1985). In West Africa, of the 55 recognized species of true mangroves, only 7 species are known and belong to 5 families namely, Rhizophoraceae, Meliaceae, Combretaceae, Avicenniaceae and Kubiaceae. Only 3 of these families occur in Nigeria and they are Rhizophoraceae, Combretaceae and Avicenniaceae. In Nigeria, the

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mangrove forest is made up of 2 main genera, *Rhizophora and Avicennia (Wilcox*, 1985). Mangrove forest serves as a link between terrestrial and marine ecosystem. There is an import of nutrients from the land to the mangrove and export of organic matter from the mangrove to the sea (Jayewardene, 1984). Mangrove trees also serve as habitats for some commercially exploited marine organisms like fin fish, oysters and crabs (Ong et al., 1984). Firewood and charcoal from mangrove plant species are utilized for domestic purposes such as cooking, heating and cloth ironing especially in rural area. On the potential basis, studies have been conducted on the suitability of mangrove timber for pulp and paper manufacture (Unesco, 1983). Little or no empirical data are available on the bacterial pathogens of red mangrove plants in the Niger Delta area of Nigeria. This investigation therefore was initiated to survey the bacterial pathogens of red mangrove *(Rhizophara mangle)* in Port Harcourt, Southern Nigeria.

MATERIALS AND METHODS

Study area

The study area was Bundu water front in Port Harcourt, Rivers State. The area is made up of matured mangrove forest vegetation in the Niger Delta area of southern Nigeria. This site was chosen because of its accessibility and hydrographic conditions having the qualities of other mangrove vegetation around the area.

Collection of samples

Leaves' and seeds' samples of red mangrove *(R. mangle)* were collected from the study site. 50 samples of healthy and unhealthy leaves and seeds each were randomly collected into fresh unused polythene bags and transported to the laboratory for analysis within 2 h of collection.

Treatment and preparation of samples

Both the leaves' and seeds' samples (healthy and unhealthy) were treated by first washing with tap water to remove all debris and dirt on the surface. These were washed with alcohol (70%) to sterilize the surface in order to prevent contamination from external sources. The samples were then rinsed in sterile distilled water to reduce the inhibitory effect of the alcohol on the bacteria being isolated. The samples were mashed in a mortar previously sterilized with alcohol, and 50 ml of sterile distilled water was introduced into the mashed samples and mixed thoroughly. The mixture was put into sterile conical flask, allowed to stand for 5 – 10 min before being used for serial dilution.

Bacterial growth medium

The medium used for cultivation and enumeration of bacteria was nutrient agar. This was prepared according to manufacturer's specifications: 28 g of the nutrient agar was dissolved in 1 litre of distilled water in a conical flask and then shaken thoroughly for proper mixing. The set up was autoclaved at 15 psi and allowed to cool at 40 - 50°C before use.

Microbiological analysis of the samples

For the purpose of cultivation and enumerationof the bacteria, serial dilution (Ofunne, 1999) was employed. This was done by taking 1.0 ml of the supernatant (10° dilution) of each prepared sample and adding into 9.0 ml of sterile normal saline (diluents) in different test tubes to give 10^{-1} dilution. From the 10^{-1} dilution, further dilutions were made up to 10^{2}. About 0.l ml aliquot of the appropriate dilutions was inoculated onto freshly prepared nutrient agar plates in triplicate. The inoculum was spread evenly on the surface of the medium using a sterile bent glass rod. The inoculated plates were incubated at 37°C for 24 to 48 h. Discrete colonies that developed were counted and recorded. These were taken as the total number of bacteria enumerated. Also, colonial morphology of representative colonies were observed and recorded.

Isolation, characterization and identification of bacterial isolates

Pure cultures of bacteria were obtained by aseptically transferring representative colonies of different morphological types which developed onto freshly prepared nutrient agar plates and incubated at 28°C for 24 h. Isolated colonies, which developed were sub-cultured onto nutrient agar slants and incubated at 28°C for 24 h. These served as pure stock cultures for biochemical test which included gram reaction, motility, methyl red, Voges proskauer, catalase, coagulase, indole, citrate utilization and sugar fermentation tests (Cruickshank et al., 1975). The characterized bacteria were identified by reference to Buchanan and Gubbons (1974) and Cowan (1974).

Statistical analysis

Anova (Analysis of variance) was used to test significant differences of data obtained in this study

RESULTS AND DISCUSSION

Results of the bacterial counts of seeds and leaves (healthy and unhealthy) samples of *Rhizophora mangle* are shown in Table 1. Mean counts of healthy and diseased leaves were 8.26 x 10^{3} cfu/ml and 5.9l x $l0^{3}$ cfu/ml respectively. Mean counts for healthy seeds were 7.96 x $l0^{3}$ cfü/ml and counts of diseased seeds were 1 .l4 x $l0^{4}$ cfü/ml. The mean bacterial populations of the healthy leaves were higher than the counts for diseased leaves whereas the reverse was the case for healthy and diseased seeds. However, there was no significant difference at $P > 0.05$($F - cal = 4.69$; $F - tab = 4.76$) between the bacterial counts of healthy and unhealthy samples in general. The replicate counts ranged from 3.8 x 10^{2} to 2.2 x $l0^{4}$ cfu/ml for healthy leaves; 1 .34 x 10^{3} to 1.2 x 10^{4} cfu/ml for diseased leaves; 2.7 x 10^{2} to 2.2 x $l0^{4}$ cfu/ml and 1 .58 x 10^{3} to 2.1 x $l0^{4}$ cfu/ml for healthy seeds and diseased seeds respectively.

Table 2 showed the bacterial genera isolated from the leave and seed samples (healthy and unhealthy) during this study. *Corynebacterium* species occurred in all the healthy and diseased samples. *Bacillus* occurred in healthy leaves, healthy and a diseased seed while *Streptomyces* occurred in diseased leaves, healthy and diseased seeds. *Acinetobacter* occurred in healthy and diseased leaves and not in seeds. *Aerococcus, Flavobacterium, Pseudomonas* and *Staphylococcus* were isolated

Table 1. Bacterial populations of leaves and seeds (healthy and unhealthy) *of R. mangle*.

Types of sample	Bacterial counts (cfu/ml) Replicate samples			
	I	II	III	Average
Healthy leaves	3.8×10^2	2.4 x 103	2.2 x 104	8.2 x 103
Diseased leaves	1.34×10^3	4.4 x 103	1.2 x 104	5.91 x 103
Healthy seeds	2.7×10^2	1.6 x 103	2.2 x 104	7.96 x 103
Diseased seeds	1.58×10^3	1.7 x 104	2.1 x 104	1.14 x 104

Cfu/ml: Colony forming units per milliliter of distilled water extract.

Table 2. Bacterial types isolated from leaves and seeds (healthy and diseased) of R. *mangle.*

Bacterial Type	Leaves		Seeds	
	healthy	Diseased	healthy	Diseased
Acinetobacter	+	+	-	-
Aerococcus		-	-	+
Alcaligenes	+	-	-	-
Bacillus	+	-	+	+
Corynebacterium	+	+	+	+
Flavobacteium	-	-	-	+
Pseudomonas	-	-	-	+
Staphylococcus	-	-	-	+
Streptomyces	-	+	+	+

KEY
+ = Bacterial type isolated
- = Bacterial type not isolated.

Table 3. Percentage frequency of bacteria isolated from healthy and diseased leaves and seeds of *R. mangle.*

Bacteria type	Frequency	% Frequency
Acinetobacter	2	7.69
Aerococcus	1	3.84
Alcaligenes	1	3.84
Bacillus	*5*	19.23
Coryebacterium	9	34.61
Flavobacterium	1	3.84
Pseudomonas	1	3.84
Staphylococcus	2	7.69
Streptomyces	*4*	15.38

from diseased seeds only while *Alcaligenes* occurred in healthy leaves only. The bacterial organisms were invaried frequency (Table 3). *Conynebacterium* was more predominant having percentage occurrence rate of 34.6%, followed by *Bacillus* (19.2%), *Streptomyces* (15.4%).

Acinetobacter and *Staphylococcus* species (7.7%) each, *Aerococcus, Alcaligenes, Flavobacterium* and *Pseudomonas* species were the least predominant all having percentage occurrence rate of 3.8%. Of the 9 bacterial genera isolated, only *Corynebacterium, Pseudomonas* and 2 species of *Streptomyces* had been isolated as plant pathogens by previous workers (Onuegbu, 1993). These organisms may have been confirmed as pathogens of mangrove trees since they were isolated from either diseased samples or both. *Corynebacterium* is a known pathogen of plants (Buchanan and Gibbons, 1974). Most of the other organisms are saprophytes and are widely distributed in nature. However, some species of *Staphylococcus* and *Bacillus* are pathogenic to human and animals (Baker and Silverton, 2001). It should be noted that the saprophytic bacteria isolated in this study, may likely turn out to become pathogenic where predisposing conditions occur. Housten (1987a) showed that bacteria can colonise trees endophytically without causing apparent damage but some species have the potential to react with tree tissues that have been altered physiologically and cause damage. *Aeroceccus* for instance was isolated from diseased seeds only. In conclusion, the mean bacterial counts of the healthy and diseased samples were not significantly different. Of the 9 bacterial genera isolated, 3 were implicated as pathogens of red mangrove trees, *Corynebacterium*, *Bacillus* and *Pseudomonas*. Some of these bacteria have been implicated as pathogenic on some forest trees (Scortichini et el., 1991). Even though pathogenicity test was not carried out, it is likely that these bacteria pathogens may pose as a potential threat to red mangrove trees.

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