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Case Study.

TROPICAL MANGROVE LEAF LITTER MICROBES AROUND PORT HARCOURT, NIGERIA

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Mangrove leaf litter samples were collected at two locations in Port Harcourt, namely Elechi creek and Tourist beach ecosystems to investigate associated microorganisms using appropriate laboratory media and other analytical procedures. Samples cut from each leaf were surface plated on Nutrient agar, MacConkey agar and Petroleum agar for bacteria while Potato dextrose agar, gari meal agar and estuarine gari meal agar were the microbiological media used for fungi. The major bacteria identified were both gram negative and gram positive microbes namely *Escherichia coli, Enterobacter, Klebsiella, Pseudomonas, Acinetobacter, Flavobacterium, Staphylo-coccus, Micrococcus,* and *Bacillus* species while the fungi include *Aspergillus, Cladosporium, Fusarium, Halophytophthora, Mucor, Nigrospora, Penicillium and Rhizopus* species. The abundance of these microbes is due to the turnover of nutrients from the decomposition of the leaf litters.

Keywords:Mangroves,Microbes,Decomposition,Leaflitter,Nutrients.

Introduction

Mangrove vegetation or 'mangal' is the tropical counterpart of tidal salt marshes of temperate regions (Walsh, 1974). Mangrove swamps are important because mangrove trees shed a great deal of organic matter in the form of litter into tropical and subtropical marine environments. The degradation of this litter, the bulk of which is leaves, constitutes an important flux in the energy budget of the ecosystem and, therefore, forms the basis of a detrital food chain (Oyun, 2006).

The degradation of plant litter in aquatic environments is totally dependent on the microbial biomass, its occurrence and diversity, as well as on environmental conditions (Steinke, 2000; Mumby *et al*., 2004)). Findlay *et al* (1986) and Rajendran and Kathiresan (2007) reported a high level of association of bacteria and fungi with leaf litter degradation. Studies on mangrove leaf litter degradation in the tropics showed that fungi are consequential decomposers of fallen, submerged leaves in mangrove ecosystems. These microbes play critical roles in the biogeochemical cycling and, hence, soil fertility. (Mackey and Smail, 1996; Gulis and Suberkropp, 2003a, b; Romero *et al*., 2005).

An appreciable body of information exists on microbes associated with mangrove leaf litter in various parts of the world (Findlay *et al*., 1986; Ho *et al*., 1991; 1992; Newell and Fell, 1992; Kathiresan and Bingham, 2001; Steinke, 2000; Dorothy *et a*l., 2003; Gulis and Suberkropp, 2003a and b; Mumby *et al*., 2004; Rajendran and Kathiresan, 1999; 2000; 2004; 2007). However, there is a dearth of information on microbes associated with leaf litter in the Nigeria mangrove - the third largest in the world and the largest in Africa (Moffat and Linden, 1995). The present study, therefore, was aimed at providing some data on the microbes associated with leaf litter in the mangrove around Port Harcourt, Nigeria.

Materials and Methods

Characteristics of the study area

The mangrove forest in Nigeria is the largest in Africa and the third largest in the world (9,730 kin2). Most of it is found in the delta of River Niger, the dominant river in Nigeria, and is estimated to cover between 5,400 km2 and 6, 000 km2 (Collins and Evans, 1986).Rivers State of Nigeria, with Port Harcourt as state capital , has a sizeable proportion (30%) of the mangrove forest of the Niger Delta (FAO, 1994).

Sample collection

Wet and dry season leaf litter samples were collected, in quadruplicate, at two locations, namely, Elechi creek and Tourist beach mangrove ecosystems in Port Harcourt. Leaves of the three species of mangrove available in the area, viz red (*Rhizophora racemosa*), black (*Laguncularia racemosa*) and white (*Avicenia africana*) were collected. These were yellow, freshly fallen, to brown submerged or floating leaves found along the mud bank or among the roots of mangrove trees at low tide, as well as blackened, disintegrating leaves. Leaves stiffened by extreme drying after senescence was avoided.Samples were aseptically washed free of adhering debris, placed in labeled, sterile polythene bags, and taken to the laboratory in ice-packed

coolers for microbiological analyses. Sampling lasted between April and November to cover wet and dry seasons.

Microbiological analyses

Four discs (1 cm x 1 cm) cut from each leaf were surface plated on nutrient agar (Oxoid), MacConkey agar (Oxoid) and petroleum agar (IPS,1988) for bacteria and potato dextrose agar, gari meal agar and estuarine gari meal agar (Sokari *et al*., 1998) for fungi. The inoculated plates were incubated at 37^0C for 18 – 24h (bacteria) and at ambient temperatures of 28 $\pm$ 2^0C for 48 – 72h (fungi). Visible and discrete colonies were purified by repeated subculture onto appropriate media for each type of microorganism (Sanni, 1989; Sokari *et al*., 1998). The pure isolates were identified on the basis of their cultural, morphological and physiological characteristics in accordance with the methods described by Cruickshank *et al* (1975) and Cheesebrough (1993).

Result and Discussion

The relative occurrence of specific genera of microbes especially bacteria and fungi could be used as an index of biodegradation potential of an environment (Dorothy *et al*., 2003; Rajendran and Kathiresan, 2007).In the present study, it was observed that the major bacterial genera implicated in the decomposition of mangrove leaf litters were *Pseudomonas, Flavobacterium, Staphylococcus, Acinetobacter,Enterobacter,Micrococcus,Bacillus,Escherichia coli and Klebsiella species.*These bacteria were isolated both in the nutrient agar made in estuarine salt and in distilled water (Table 1).The study revealed that constitution of media in estuarine salt solution optimized detection of all microbes of decaying mangrove leaves. *Pseudomonas sp* was observed to be a predominant bacterium of submerged decaying leaves at a frequency of 56% while *Bacillus, E.coli* and *Staphylococcus* species has percentage frequencies of 43%, 35% and 25% respectively (fig.1). The relatively high proportions of *E.coli* could be due to the water bodies receiving direct inputs of human wastes at the two sampling stations. Studies on bacteria and fungi as microbial communities of mangrove ecosystems are widely reported (Benner *et al.,* 1988; Robertson, 1988; Peduzzi and Herndl, 1991; Dorothy *et al*., 2003)

Apart from the characteristics ascribed to these bacterial genera, their presence especially *Pseudomonas* species in the mangrove leaf litters play a substantial role in the turn over of essential nutrients like carbon, nitrogen and phosphorus contained in the leaf biomass (Robertson and Daniel, 1989; Steinke, 2000; Mumby *et al.,* 2004; Romero *et al*., 2005). Accordingly, eutrophication can directly influence microbial activity through elevated nutrient concentrations (especially N and P) and consequently affect plant litter decomposition (Suberkropp and Chauvet, 1995; Sridhar and Barlocher, 2000; Grattan and Suberkropp, 2001; Gulis and Suberkropp, 2003a)

Microorganisms play important ecological roles in decomposing organic matter and producing protein- rich detritus that serves as food for fishes (Dickinson and Pugh, 1974; Steinke, 2000; Mumby et al., 2004) and serve as intermediaries of energy flow from submerged decaying plant litter to higher trophic levels (Barlocher and Kendrick, 1981; Gulis and Suberkropp, 2003a) especially in marine ecosystems like the mangroves. These microbes through their saprophytic nutrition can make nutrients available which can be utilized from the soil by retranslocation of nutrients via fungal mycelia (Lambert *et al*., 1980).However, in tidal and irregularly flooded systems such as mangrove forests, there are sediment accretion processes, driven by input of allochthonous mineral sediments and autochthonous plant litter (Cahoon and Hynch, 1997; Gulis and Suberkropp, 2003) so that the decaying leaves on the soil surfaces often becomes buried and thus subject to decomposition under different environmental conditions.

Microbial decomposition of leaf litter of poor quality (i.e. low nutrient content) must utilize nutrients in the soil to raise the nitrogen content of the decomposing material to that of their own mass (Lambert *et al*., 1980; Zimmerman *et al*., 1995).Studies in various ecosystems have also shown net accumulation of nitrogen during the decomposition of forest derived litter. This relationship between nitrogen content and decay rate only applies for cases in which the physical removal of material is minimized, the nitrogen content of the litter material limits microbial activity, and in sites where there is continuous source of nitrogen to the decomposers (Aber and Melillo, 1980). Consequently, nitrogen content alone or in combination with lignin content (i.e. lignin: N or C: N) is often used as a measure of litter quality and thus a predictor of decomposition rates (Aber *et al*., 1990; Dehairs *et al.,* 2000; Rajendran and Kathiresan, 2000; Kathiresan and Bingham, 2001).

The reports of Ukoima (1995) and Rajendran and Kathiresan (2007) stated that some species of *Phytophthora* and *Deuteromycetes* can occur on submerged decaying leaves of vascular plants in coastal marine environment from at least latitudes 5 – 50^o. The dominant fungal decomposers of the mangrove leaf litters isolated in this study include *Penicillium, Aspergillus, Cladosporium, Fusarium, Mucor, Halophytophthora, Rhizopus* and *Nigrospora* species (Table 2) from cultures made onto Potato dextrose gar and Gelled Gari medium. The use of these media reveals that fungi organisms are variously distributed in different mangrove plants. This results is similar to those reported by Rajendran and Kathiresan (2007) who stated that marine fungi especially *Halophytophthora* species can inhabit senescent leaves of red mangrove at 75 – 100% frequency within 24h after submergence. Similarly, *Pencillium, Aspergillus* and

Halophytophthora species are the predominant fungi of submerged decaying leaves of mangroves occurring consistently at frequencies of 72%, 64% and 31% respectively in PDA estuarine medium and 75%, 70% and 33% respectively in gelled gari – estuarine medium (fig.2).These results were observed due to the presence of some accessory factors in the media based on the estuarine salt solution (Sokari *et al*., 1998).

However, the fungal species were further identified based on their shape and colour of spores as well as the colours on the various media used for the study. The fungi isolated from the mangrove leaf litter were found to be widely distributed probably because of the rich detritus of the mangrove environment. This may further be attributed to large scale transport of fungal spores from the land through freshwater inflow into mangrove ecosystems. This therefore could account for a single fungal species being inconsistent as domineering organisms in the various mangrove zones.This result corroborates with the report of Ukoima (1995) which shows that among the classes, Deuteromyces, Ascomyces and Oomycetes, the majority of fungi isolated from mangrove leaves are ubiquitous saprobes found on terrestrial plant material.

It was further observed that the population of the fungi was higher at the upper and middle zones, than at the shoreline zones in the two study centres used for the study. This report is in consonance with the reports of several workers who observed that the distribution, occurrence and population density of fungi varied according to the zones of mangroves from seaward to landward (Ukoima, 1995; Venkateswara Sama *et al.,* 2001; Maria and Sridhar, 2002)

As mangrove forests are located along a tidal range, tidal influence could have a considerable effect on decomposition processes, therefore it is worthwhile to understand the spatial variability in leaf litter decomposition rates and in the nutrient pool of decaying leaf.

Table 1: Morphological and biochemical characteristics of bacteria isolated from mangrove leaf litter

Colonial and cell morphology	Gram reaction	Motility	Indole	Urea	Oxidase	Catalase	Citrate	Glucose	Sucrose	Lactose	Maltose	Mannitol	Probable identity
Yellow colonies; straight rods, singly in chains	-	+	+	-	-	-	-	AG	AG	A	AG	AG	*Escherichia coli*
Large mucoid colonies; straight rods in chains	-	+	-	-	-	-	+	AG	AG	AG	-	-	*Enterobacter*
Large, flat, milky colonies; entire edge, smooth surface; slightly curved rods	-	-	-	-	-	+	+	AG	AG	AG	AG	-	*Kiebsiela sp*
Rods are spherical but occur in pairs occasionally in chains	-	+	-	+	+	+	-	A	-	-	A	A	*Pseudomonas sp*
Colonies are translucent, occasionally opaque. circular, smooth and shiny with entire edges, rods with parallel sides but rounded ends	-	-	-	-	-	+	+	A	-	-	A	-	*Acinetobacter sp*
Large, creamy white, smooth, opaque colonies; cocci, singly or in chains	-	-	+	-	+	-	+	A	A	A	-	-	*Flavobacterium sp*
Yellow colonies, cocci in clusters and chains	+	-	-	-	-	+	+	A	A	-	-	A	*Staphylococcus sp*
Milky white colonies, entire edge; cocci in pairs and chains	+	-	-	-	A	A	A	-	A	-	A	-	*Micrococcus sp*
Large gray or milky white colonies; straight rods; in chains	+	+	-	-	-	-	+	-	-	A	-	A	*Bacillus sp*

KEY: - negative; + positive reaction; A acidic; AG acid and gas produced

Table 2: Characteristics and Identification of mangrove leaf litter fungi

Colonial morphology	**Mycelial structure**	**Spore and associated structures**	**Probable identity**
Whitish mass initially, turning black or grayish green with age	Conidiophores upright, terminating in a clavate swelling	Conidia globose in dry, basipedal chains	*Aspergillu spp*
Restricted thick velvety colour, varying from deep, rich green to dark grey to green or greenish black	Conidiophores tall and upright, branched variously near the apex, all parts dark coloured	Conidia dark ovoid to cylindrical	*Cladosporium spp*
Mycelium extensive and cottony some tinge of yellow/pink coloration in the medium	Conidiophores slender and simple, branched irregularly	Conidia hyaline, held in small, moist heads	*Fusarium spp*
White petalloid, cottony mycelia with definite margin and aerial hyphae (rosette colonies)	Conidiophores branched, non septate (or septate with age)	Conidia hyaline and smooth walled	*Halophythophthora spp*
White to grey cottony mass with black or brown spores	Conidiophore non septate and branched	Conidia round, cylindrical or oval and dark	*Mucor spp*
Pale, yellowish brown colonies	Conidiophores short and simple	Conidia hyaline and globose at end of conidiophore	*Nigrospora spp*
Velvety, grayish-green to blue-green mass growing in concentric circles	Conidiophores arising from mycelium	Conidia hyaline and globose or ovoid in dry basipetal chains	*Penicillium spp*
Rapidly growing, dense, whitish, cottony mass turning grey to brown with age	Stolons and rhizoids clearly visible; rhizoids emerging from where stolons touch substratum	Globose sporangia on erect sporangiophores	*Rhizopus spp*

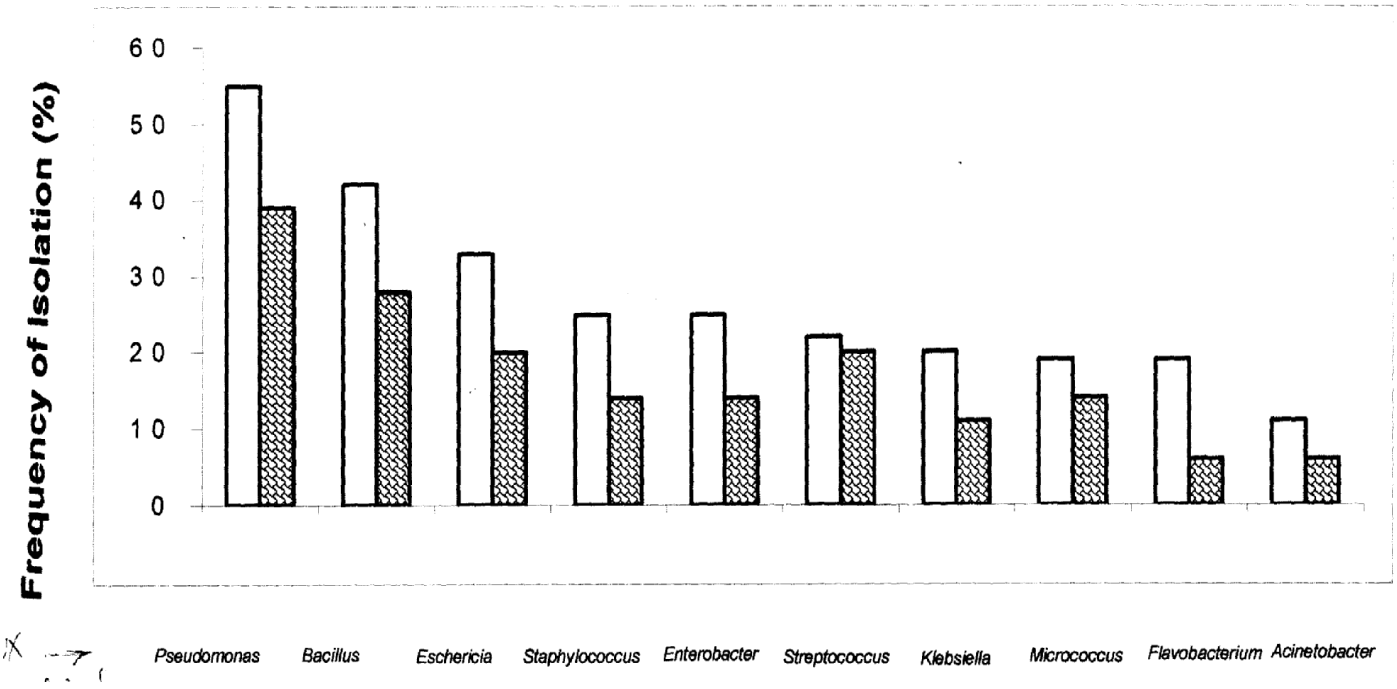

Fig. 1 Frequency of isolation of Bacteria from mangrove leaf disc from nutrient agar medium

= Nutrient agar (Estuarine) = Nutrient agar (Distilled water)

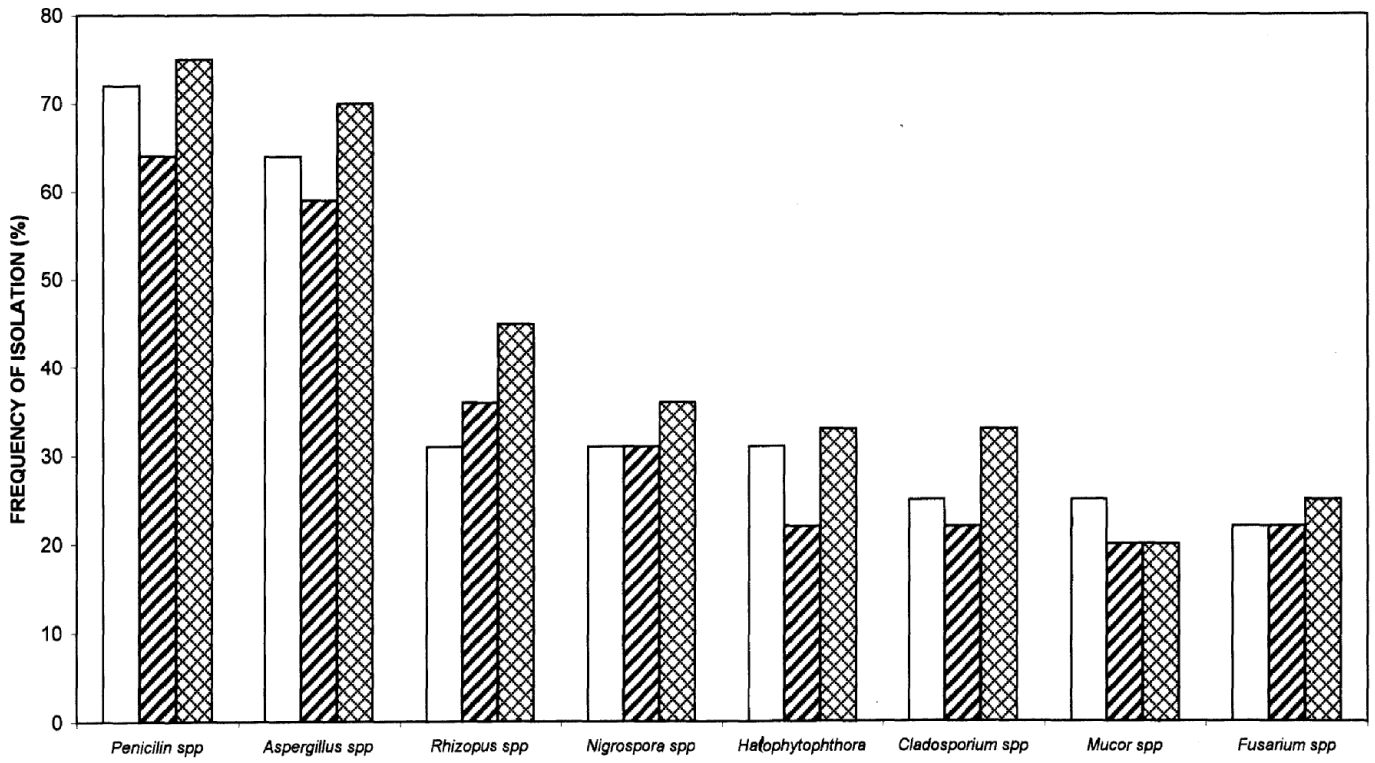

Fig. 2 Frequency of isolation of fungi from mangrove leaf disc from different media:

☐ PDA(Estuarine) ▨ Gari - Estuarine ⊠ Gari Peanut (Estuarine)

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