Research Journal of
Soil Biology

ISSN 1819-3498

Academic
Journals Inc.

www.academicjournals.com

Research Journal of Soil Biology 4 (2): 32-41, 2012
ISSN 1819-3498 / DOI: 10.3923/rjsb.2012.32.41

Bacterial Isolates of the Mangrove Swamp Soils in Cross River Estuary, South-East Nigeria

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ABSTRACT

Six bacterial Genera were identified based on their morphological, biochemical and physiological characteristics for the mangrove swamp soils supporting tall mangrove, short mangrove and Nypa palms in the Cross River estuary, South-East Nigeria. The six genera of bacteria were *Streptomyces* sp., *Micrococcus* sp., *Bacillus* sp., *Pseudomonas* sp., *Staphylococcus* sp. and *Streptococcus* sp. The percentage distributions of the isolates were as follows: *Streptomyces* sp. (33-50%), *Micrococcus* sp. (17%), *Bacillus* sp. (17-33%), *Pseudomonas* sp. (17%), *Straphylococcus* sp. (17%) and *Streptococcus* sp. (17%). *Streptomyces* sp., *Micrococcus* sp., *Bacillus* sp. and *Pseudomonas* sp. were classified as indigenous (authochthonous) bacteria responsible for the decomposition of leaf litter in the mangrove swamp soils. The bacterial isolates, *Staphylococcus* sp. and *Streptococcus* sp. were regarded as foreign (allochthonous) and such bacteria lack potentials to degrade leaf litter and are contaminants introduced by human activities in the mangrove swamp forest area.

Key words: Bacterial isolates, mangrove swamp, estuary

INTRODUCTION

Mangrove swamps provide a unique ecological niche to different microbes which play various roles in nutrients recycling as well as various environmental activities (Sahoo and Dhal, 2009; Kannan and Vincent, 2011). The mangrove swamp is characterized by intertidal variation at intervals; at high tide the mud flat is submerged while at low tide the water flows away making the mud flat visible. Besides the intertidal variation, salinity level in the sea water could be considerably high, so most organisms inhabiting the swamps are therefore salt tolerant. The swamp soils are rich in nutrients due to the leaf litter fall. The litter supports the initial phase of the food chain through its decomposition by bacterial species and some fungi (Wafar *et al*., 1997). The microbial action on the litter mineralizes it, decreasing its carbon content and releasing mineral nutrients such as nitrogen, phosphorus and other nutrients. The nutritive/survival value indicator of the leaf litter/microorganisms is the ratio of carbon to nitrogen (C:N) content of the leaf litter. The plant residues with the C:N ratios of 20:1 or narrower have sufficient nitrogen to supply the decomposing microorganisms and also to release nitrogen for plant use (Miller and Donahue, 1995).

Mangrove swamp soils occupy about 12.6 million hectares worldwide. The mangrove ecosystem is extensive in South East Asia (Thailand, Vietnam, Indonesia and Malaysia), West Africa (Senegal, Gambia, Guniea Bissau and Cameroon); in Latin America (Venezuela, the Guyanas) and

in North America (Akpan-Idiok, 2002; Alongi, 2002). In Nigeria, mangrove forests occupy 973, 000 hectares, while in the Cross River estuary, about 70,400 ha of coastal swamp are vegetated by mangroves (Akpan-Idiok and Esu, 2003). In the Niger Delta of Nigeria, 6 species of mangroves have been identified namely, *Rhizophora racemosa*, *R. harrisonii* and *R. mangle*; others are *Avicennia africana*, *Leguncularia racemosa* and *Conocarpus erectus* (Akpan-Idiok, 2002; Anderson, 1966).

The nutritive enrichment of the mangrove swamp soils due to rich leaf litter supports microbial survival. The structure of the microbial communities may vary not only with substrate but also with variables such as season, water temperature, salinity, oxygen and duration of time that the leaf has been decomposed (Fell and Master, 1980). As the soil character is high in organic materials, the microbial abundance, distribution and species could be of interest to investigate.

The objectives of this investigation were to isolate, characterize and identify the various bacterial isolates of the mangrove swamp soils in the Cross River estuary, South-East Nigeria.

MATERIALS AND METHODS

Eighteen soil samples representing the Tall Mangrove (TM), Short Mangrove (SM) and Nypa Palm (NP) were collected at the depth of 0-20 cm from the Cross River estuary, South-East, Nigeria (Fig. 1). The wet soil samples were aseptically placed separately in sterile sample bags and transported immediately to the laboratory for analysis.

Physico-chemical analysis: Particle size distribution was determined by the hydrometer method (Bouyoucus, 1951). Soil pH was determined in 1:2 soil/water ratio using a glass electrode pH meter. Electrical Conductivity (EC) was measured in 1:2 soil-water extract using an electrical conductivity meter. Organic carbon was determined by Walkley and Black as outlined by Juo (1979). Total nitrogen was determined by the micro-kjeldahl digestion method (Jackson, 1962). Cation exchange capacity was determined by the ammonium ion displacement method (Black *et al.*, 1965). Soluble sulfate in extract was determined by the turbidity method as outlined by Juo (1979).

Isolation of bacteria: One gram of each soil sample was suspended in 9 mL of sterile distilled water. The mixture was well shaken to homogenize suspension (Jalal *et al.*, 2010). One milliliter of the supernatant was diluted serially (using physiological saline) in tenfold 10^{-1} to 10^{-6}. Using pipette, 0.1 mL aliquots of 10^{-4}, 10^{-5} and 10^{-6} were dispensed, respectively on triplicate plates of nutrient agar and spread on the surface (spread plate technique). Plates were incubated at 30°C for 48 h (APHA, 1998). Viable numbers of colonies on each plate were enumerated and multiplied by the reciprocal of dilution factor and recorded as colony forming unit per gram (CFU g^{-1}). Colonies were transferred aseptically on to fresh nutrient agar and incubated at 37°C for 48 h to obtain pure colonies. Pure colonies were then preserved in 15% glycerol solution and stored at -20°C to maintain viability for further studies. All 18 isolates were identified using Application Programming Interface (API) 20 identification system. Gram staining was also performed for all isolates. Biochemical properties were also determined.

Determination of growth on mineral salt-mangrove litter: Mineral salt medium was prepared with the following: NH_4Cl (4.0 g), K_2HPO_4 (1.8 g), KH_2PO_4 (1.2 g), $MgSO_4.7H_2O$ (0.2 g), NaCl (O.1 g), $FeSO_4$ (0.01 g) and 15 g Agar (Zajic and Supplisson, 1972). These were added up to

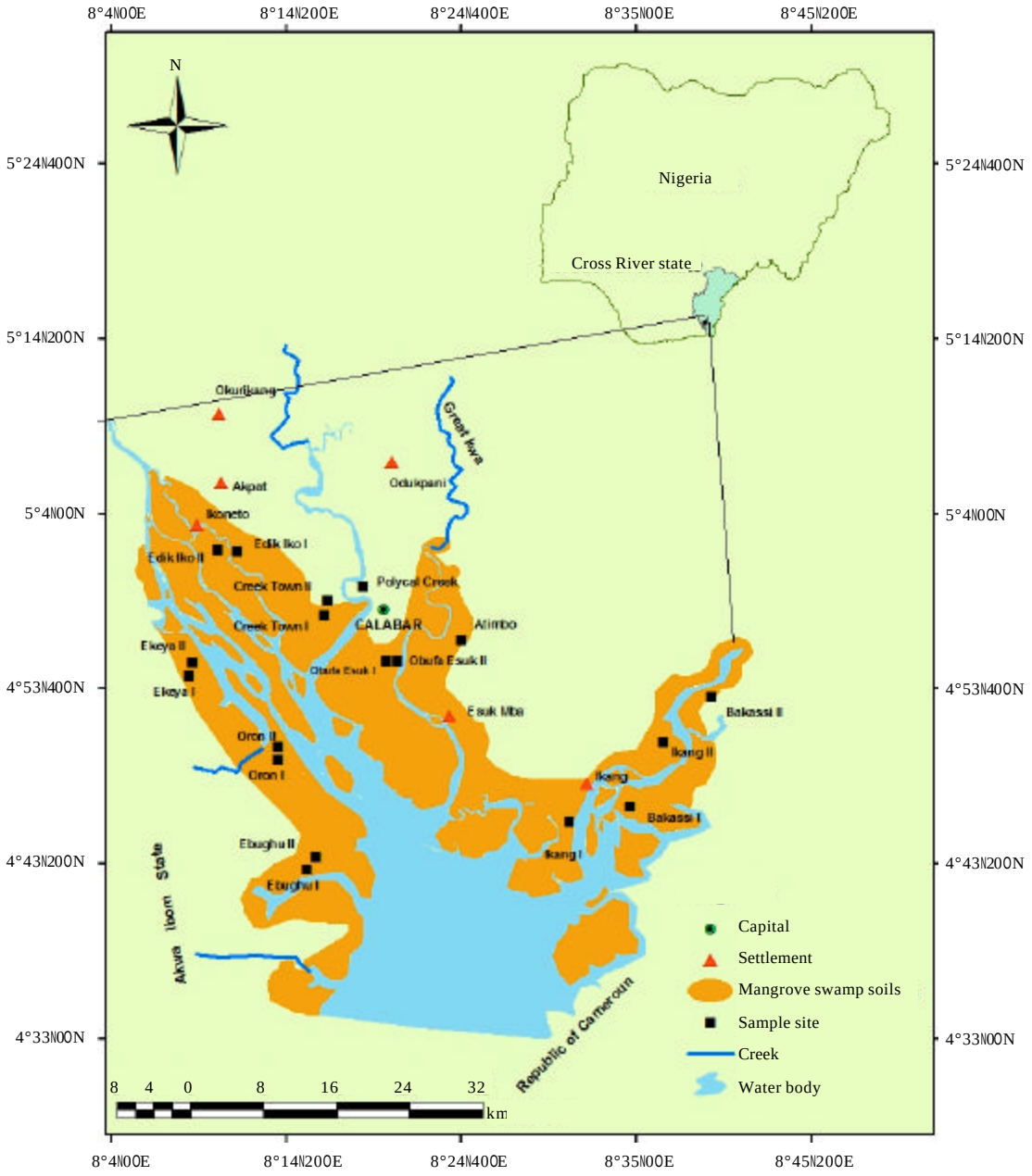

Fig. 1: Mangrove swamp soils of Cross River estuary showing soil sampling sites

750 mL of mangrove litter suspension and 250 mL of sterile distilled water to make up 1000 mL and then autoclaved at 121°C for 15 min. An aliquot of 0.1 mL of the 10^{-5} and 10^{-6} dilution of the soil suspension was seeded unto triplicate plates of the mineral salt-mangrove-litter agar medium using spread plate technique. Growth was determined as colony forming unit per gram of culture (CFU g^{-1}), after incubation at 37°C for 24 h.

RESULTS AND DISCUSSION

Soil analyses: Table 1 shows the selected properties of the soils studied. The texture of the soils varied from sandy loam to clay. The soils were slightly acid to neutral (pH 6.0-7.4) under wet condition and extremely acid (pH 2.0-4.6) in reaction under dry condition. The electrical conductivity values were high exceeding 4 dSm^{-1}, indicating that the soils were saline. Organic carbon content was high (range, 5.12-11.67%), indicating that the soils had high accumulation of organic matter. Correspondingly, total nitrogen was high (0.44-1.02%). The carbon-nitrogen (C/N) ratio was narrow (10-12), indicating a net mineralization of nitrogen in the soils. The Cation Exchange Capacity (CEC) was high (24.41 to 66.60 cmol kg^{-1}), indicating availability of basic nutrients for microbes in the soils. The soluble sulphate was considerable (0.002 to 0.015%), suggesting that the soils have potential to acidity upon aeration (Fitzpatrick *et al*., 2008).

Bacterial isolates of the mangrove swamp soils: Six bacterial genera namely *Streptomyces* sp., *Micrococcus* sp., *Bacillus* sp., *Pseudomonas* sp., *Staphylococcus* and *Streptococcus* sp. were identified in the three mangrove swamp types (Table 2). Figure 2 shows the distribution of gram positive and gram negative bacteria identified from the three mangrove swamp types. Relatively, tall mangrove had the highest number of gram positive bacterial isolates, followed by the short mangrove and Nypa palm swamp soils. Also, the Gram negative bacteria were at par in the short mangrove and Nypa palm, while the tall mangrove swamp soils had none (Fig. 2). The Gram negative bacteria found in short mangrove and Nypa palm were identified as *Pseudomonas* sp.

Table 1: Selected physico-chemical characteristics of the mangrove swamp soils of Cross River estuary, South-East Nigeria

Sample location	Depth (cm)	pH (wet)	pH (dry)	EC (dS m^{-1})	Org. C (%)	N (%)	C:N ratio	CEC (cmol kg^{-1})	BS (%)	SO_4^- (%)	Particle size (%)			Texture
											Sand	Silt	Clay	
Tall mangrove swamp soils														
Bakassi I	0-20	7.4	4.6	16.16	5.12	0.44	12	66.60	64	0.007	33.2	22.0	44.8	c
Oron	0-20	6.6	4.2	14.59	6.01	0.52	12	56.45	67	0.015	21.2	32.0	46.8	c
Edik Iko	0-20	6.8	4.1	12.54	11.47	1.02	11	26.36	54	0.002	11.2	31.4	57.4	c
Polycal Creek	0-20	6.2	3.6	0.88	9.58	0.83	12	37.99	66	0.004	67.2	19.4	13.4	sl
Edik Iko II	0-20	6.9	3.8	15.66	6.58	0.60	11	31.59	63	0.003	29.2	37.4	33.4	cl
Ekeya I	0-20	7.3	3.1	30.75	8.65	0.76	11	40.50	64	0.003	51.2	29.4	19.4	l
Short mangrove swamp soils														
Bakassi II	0-20	6.1	2.8	8.19	8.32	0.72	12	49.50	37	0.003	57.2	28.0	14.8	sl
Ebughu I	0-20	6.0	2.0	29.24	7.84	0.68	12	46.70	65	0.003	39.2	28.0	32.8	cl
Ekeya II	0-20	6.9	3.4	43.77	11.67	1.01	12	26.68	51	0.004	51.2	27.4	21.4	scl
Obufa Esuk I	0-20	7.1	3.0	18.63	6.48	0.60	11	44.03	69	0.002	51.2	27.4	21.4	scl
Atimbo	0-20	6.5	2.8	41.58	7.48	0.70	11	34.53	59	0.002	74.6	13.4	12.0	sl
Ikang I	0-20	6.8	2.9	42.68	7.66	0.65	12	39.28	64	0.002	62.9	20.4	16.7	sl
Nypa palm swamp soils														
Obufa Esuk	0-20	6.7	4.2	42.81	8.78	0.76	12	30.36	64	0.004	23.2	33.4	43.4	c
Creek town	0-20	6.9	2.5	49.40	11.67	1.02	11	36.86	65	0.005	44.6	31.4	24.0	l
Ikang II	0-20	6.8	4.1	27.00	8.99	0.79	11	26.72	76	0.004	15.2	39.4	45.4	c
Oron	0-20	6.0	4.4	34.91	6.54	0.68	10	24.41	68	0.006	17.2	35.4	47.1	c
Ebughu II	0-20	6.4	3.5	38.00	7.20	0.66	11	35.82	69	0.005	18.2	36.4	45.4	c
Creek Town II	0-20	6.5	3.4	40.00	7.10	0.61	12	32.10	68	0.005	19.2	35.4	45.4	c

c: Clay, sl: Sandy loam, cl: Clay loam, scl: Sandy clay loam, l: Loam, NB: Some locations had tall mangrove, short mangrove and Nypa palm vegetation in patches on contiguous landscape, BS: Base saturation

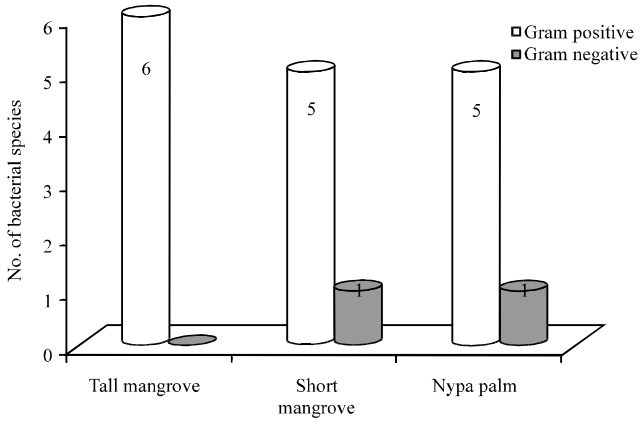

Fig. 2: The distribution of gram positive and gram negative bacteria in different mangrove swamp types

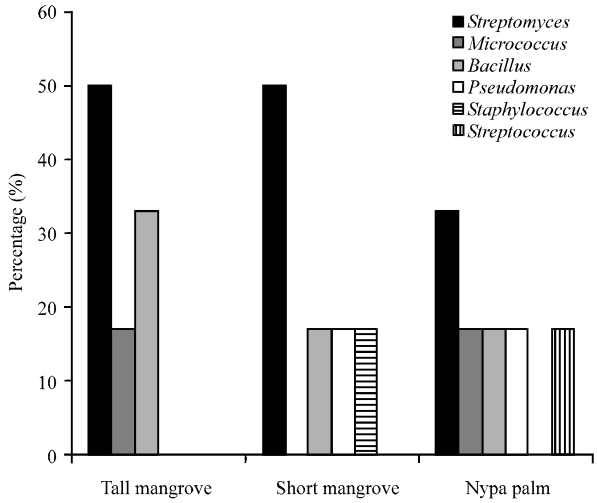

Fig. 3: Distribution of bacterial isolates in different mangrove swamp types

Distribution pattern confirmed that *Streptomyces* sp. displayed the highest population density followed by *Micrococcus* sp., *Pseudomonas* sp. and *Bacillus* sp. in descending order. Two genera *Staphylococcus* and *Streptococcus* sp. which grew on nutrient agar did not grow on salt-mangrove-litter agar. This shows that *Streptomyces*, *Micrococcus*, *Pseudomonas* and *Bacillus* were original residence microbes (autochthonous) of the mangrove-swamp soils, while *Staphylococcus* and *Streptococcus* spp. are described as invaders (allochthonous) and transmitted from surrounding environment.

***Streptomyces* sp.:** The percentage distribution of the bacterial isolates is shown in Fig. 3. *Streptomyces* spp. was more abundant in the tall and short mangroves than the Nypa pam soils. The species has enormous capacity to decompose organic materials, so it thrives well in near neutral and alkaline condition high in leaf litter such as the mangrove soil environment (Atlas, 1981). It

is one of the primary oxidizers of inorganic sulphur in neutral and alkaline waterlogged mangrove soils until a low pH is obtained when the soils are under aeration (Paul and Clark, 1989). *Streptomyces* isolated from a mangrove soil and other soil types were found to show a wide-range of anti-microbial activity and also produced cellulase that enzyme able to degrade cellulolytic waste materials (Chandramohan *et al*., 1972; Kumari *et al*., 2006; Sahoo and Dhal, 2009). Also, a vast array of fibre hydrolytic enzymes such as pectinase, xylanase and cellulotytic enzymes have been reported by many researchers to be produced by different species of *Streptomyces* isolated from different soil types (Saadoun *et al*., 2007; Kannan and Vincent, 2011). Commercial production and utilization of these enzymes have been reported also (Alam *et al*., 2004; Arunachalam *et al*., 2010; Chiani *et al*., 2010; Boroujeni *et al*., 2012).

***Micrococcus* sp.:** *Micrococcus* sp. was identified based on the characteristics shown in Table 2. The percentage distribution of the bacterium is shown in Fig. 3, indicating its presence in tall mangrove and Nypa palm soils. *Micrococcus* sp. has the ability to degrade not only the leaf litter but also spilled crude oil and petroleum products in the Niger Delta of Nigeria (Atlas and Bartha, 1972; Sahoo and Dhal, 2009). A species of *Micrococcus* identified as a non-spore forming gram positive bacterium was isolated from *Ferula galbanum* plant and terpene soaked soil (Kashi *et al*., 2008). It is reported to have effectively converted β-pinene to α-pinene which is used in the flavor and fragrance industry.

***Bacillus* sp.:** The characteristics for identification of *Bacillus* sp. are shown in Table 2. The percentage dominance is shown in Fig. 3. Equal number of *Bacillus* sp. was found in short mangrove and Nypa palm soils which was lesser than its occurrence in tall mangrove soils. *Bacillus* sp. is a good candidate in hydrocarbon degradation especially hydrocarbons associated with crude oil and petroleum products in Niger Delta region of Nigeria (Antai, 1990). *Bacillus* sp. isolated from Australian mangrove showed insecticidal activity against larvae of *Anopheles maculatatus*. Also, a *Bacillus* sp. such as *B. cereus* isolated from Pichavaram mangrove, South East, India exhibited magnetic behavior which classified it as one of the magnetobacteria (Sahoo and Dhal, 2009; Saravanan, 1995).

***Pseudomonas* sp.:** Table 2 shows the characteristics used in the identification of *Pseudomonas* sp. It had the same percentage distribution in short mangrove and Nypa palm soils (Fig. 3). *Pseudomonas* sp. is a Gram negative bacterium and a good degrader of leaf litter as well as spilled crude oil and petroleum products. The genuse has been found to be associated with white mangrove (*Languncularia racemosa*) roots and may be responsible for the fixation of atmospheric nitrogen in the mangrove soils (Sahoo and Dhal, 2009). It is also reported to be involved in the phosphate solubilization through the production of organic acids which can act as chelators displacing metals from phosphate complexes (Sahoo and Dhal, 2009; Vazquez *et al*., 2000). Since it can exhibit magnetic bahaviour, it is one of the magnetobacteria (Sahoo and Dhal, 2009). As one of the active decomposers of organic materials, it degrades organic materials in mangrove swamp soils. *Pseudomonas* sp. has been found to degrade 20.54% of polyethene and 8.16% of plastics in one month period (Sahoo and Dhal, 2009). Also, some strains of *Pseudomonas* sp. have been isolated and implicated to effectively bioaccumulate heavy metals in polluted agricultural soils (Zolgharnein *et al*., 2010; Ahemad and Malik, 2012).

Table 2: Physiological reactions of isolated microbs in mangrove swamp soils of Cross River estuary, South-East Nigeria

Isolate code	Gram reaction	Citrate	Indole	Methyl red	VP	Coagulase	Oxidase	Catalase	Motility	Sucrose	Lactose	Mannitol	Glucose	Maltose	Probable genus
Tall mangrove swamp soils															
Bakassi I	G^+rods in chain	+	-	-	-	-	-	+	-	A	A	-	A	A	*Streptomyces* sp.
Oron	G^+rods in chain	+	-	-	-	-	+	+	-	A	A	-	A	A	*Streptomyces* sp.
Edik Iko	G^+rods	+	-	-	-	-	+	+	-	A	A	A	A	A	*Streptomyces* sp.
Polycal Creek	G^+cocci	+	-	-	-	-	+	-	-	A	A/G	S	A	A	*Micrococcus* sp.
Edik Iko II	G^+rods	-	-	+	-	-	-	+	+	A/G	A	A/G	A/G	A/G	*Bacillus* sp.
Ekeya I	G^+rods	+	-	+	+	-	-	+	+	A/G	A	A/G	A/G	A/G	*Bacillus* sp.
Short mangrove swamp soils															
Bakassi II	G^+rods in chain	+	-	-	-	-	+	-	-	A	-	A	A	A	*Streptomyces* sp.
Ebughu I	G^+rods	-	-	-	-	-	+	+	+	A	A	A	A	A	*Streptomyces* sp.
Ekeya II	G^-rods	-	-	+	+	-	+	+	+	A	A	A/G	A/G	A	*Pseudomonas* sp.
Obufa Esuk I	G^+cocci in cluster	+	-	-	-	-	+	-	-	A	A	-	A	A	*Staphyloccus* sp.
Atimbo	G^+rods	-	-	-	+	+	+	+	+	A	A/G	A	-	A	*Streptomyces* sp.
Ikang I	G^+rods	+	-	+	+	+	-	+	+	A/G	A	A/G	A/G	A/G	*Bacillus* sp.
Nypa palm swamp soils															
Obufa Esuk	G^+rods	+	-	-	-	-	+	+	-	A	A	A	A/G	A	*Streptomyces* sp.
Creek town	G^+rods	+	-	-	-	-	+	+	+	A	A	-	A	A	*Streptomyces* sp.
Ikang II	G^-rods	-	-	+	+	-	+	+	+	A	A	A/G	A/G	A	*Pseudomonas* sp.
Oron	G^+cocci	+	-	-	-	-	-	+	-	A	A/G	-	-	A	*Micrococcus* sp.
Ebughu II	G^+cocci in chain	+	-	+	+	-	-	-	-	A	A	A	A	A	*Streptococcus* sp.
Creek Town II	G^+rods	+	-	+	+	+	-	+	+	A/G	A	A/G	A/G	A/G	*Bacillus* sp.

-: Negative, +: Positive, A: Acid produced, A/G: Acid and gas produced. Some locations had tall mangrove, short mangrove and Nypa palm vegetation in patches on contiguous landscape

***Staphylococcus* sp. and *Streptococcus* sp.:** These bacteria were classified based on characteristics presented in Table 2. *Staphylococcus* sp. was found only in short mangrove soils, while *Streptococcus* was only identified in Nypa palm soils (Fig. 3). They are gram positive bacteria and have no potential to degrade organic materials and are considered foreign (allochthnous) as well as contaminants introduced by human activities into the mangrove forest (Akpan-Idiok, 2002; Benka-Coker and Olumagin, 1995). *Staphylococcus* sp. isolated from mangrove roots by Holguin and Bashan (1996) was considered as non-N_2 fixer but rather promoted N_2 fixation by *Azospirillum brasilence*.

CONCLUSION

The studies of bacterial isolates in the mangrove swamp soils of Cross River estuary, South-East, Nigeria, indicate that *Streptomyces* sp. is predominant followed by *Bacillus*, *Micrococcus* and *Pseudomonas* species. Their presence in such soils indicates that they have ability to degrade organic materials. The *Staphylococcus* sp. and *Streptococcus* sp. found in short mangrove and Nypa palm, respectively were regarded as invaders and contaminants introduced by human activities into the mangrove swamp forest. Mangrove swamp soils constitute a biological entity and therefore require conservation measures that would maintain their productivity.

ACKNOWLEDGEMENT

The authors acknowledge the efforts of the staff of Soil Testing Laboratory, Department of Soil Science, University of Calabar, Nigeria for analyzing some of the soil physic-chemical properties.

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