

DOMINANT MESOPHILIC ACTINOMYCETES IN OREDO SOILS

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ABSTRACT

Investigations were carried out on dominant mesophilic actinomycetes in soil samples from eight different farmlands in Oredo Local Government Area of Edo State, Nigeria. Actinomycetal isolates were characterized and identified using their morphological, biochemical traits and ability to utilize various carbon sources. The physical and chemical properties of the soil samples were also carried out, using qualitative and quantitative means of analysis. Total actinomycete counts ranged from 0.8×10^4 cfu/g to 3.8×10^4 cfu/g. Four genera of actinomycetes were isolated regularly. They included *Streptomyces*, *Nocardia*, *Micromonospora* and *Oerskovia* spp. of which *Streptomyces* and *Nocardia* spp. were the most abundant in all sampling sites. Particle size soil analysis showed a sand, silt and clay fraction of 82.6 – 92%, 1.2 - 10.8%, and 5.3-11% respectively. The pH of all soil samples ranged between 5.37 – 5.99. Percentage organic matter ranged from 0.51 – 1%. The nitrogen, phosphorus and potassium contents of the soil samples may influence the prevalence of actinomycetes in the soil.

Keywords: Actinomycetes, Oredo, mesophilic, soil, farmlands

INTRODUCTION

Actinomycetes are bacteria which form multicellular filaments, thus they resemble fungi. They are gram positive bacteria which produce branching mycelium which may be of two kinds; a substrate mycelium and aerial mycelium. These filamentous organisms have a high G+C, propagate themselves by spores and grow through the soil in the form of hyphae (Stackebrandt *et al.*, 1997). Actinomycetes are widely distributed and are next to bacteria in the order of abundance in soil (Pragya *et al.*, 2012). The majority of actinomycetes are free living, spore forming, saprophytic bacteria found widely distributed in soil, water and colonizing plants. Actinomycetes population has been identified as one of the major groups of soil population, which may vary with the soil type. As degradation agents, actinomycetes are important in the degradation of soil organic materials into humus (Jeffery *et al.*, 2007). Some actinomycetes secrete a range of enzymes that can completely degrade all the components of lignocellulose (lignin, hemicelluloses and cellulose) while other actinomycetes may secrete a narrower range that can only partially achieve this degradation (Mason *et al.*, 2001). With their ability to secrete these enzymes, they are effective at attacking tough raw plant tissues and softening them for other microorganisms (Jeffrey *et al.*, 2007).

The genera of actinomycetes isolated from compost and soil include *Nocardia*, *Streptomyces*, *Thermoactinomyces* and *Micromonospora* (Williams and Robinson, 1981). Incubation is usually at 25°C to 30°C for mesophiles and 45°C with colonies developing within 14 days. Actinomycetes are the most economically valuable prokaryotes, well known to produce chemically diverse metabolites with a wide range of biological activities (Radhakrishnan *et al.*, 2011). It has been estimated that about half of the microbial bioactive metabolites notably antibiotics, antitumor agents, immunosuppressives and enzyme inhibitors have been isolated from actinomycetes (Radhakrishnan *et al.*, 2011). *Streptomyces* spp. are especially prolific and synthesize a number of antibiotics like streptomycin, terramycin and aureomycin and other class of biologically active secondary metabolites (McCarthy, 1987). By means of their secondary metabolites, these microorganisms can influence plant development producing plant hormones, increasing the availability of mineral nutrients and also excreting antibiotics or toxins that act in the biological control of pathogens in the rhizosphere (Miyadoh, 1993; Hamdali, 2008).

The aim of this study is to identify dominant mesophilic soil actinomycetes in the Oredo Local Government area of Edo State, and to evaluate the influence of physical and chemical properties of the test soils on their prevalence.

MATERIAL AND METHODS

Study area

The study area comprised farmlands distributed along Oredo local Government area of Edo state (Fig. 1). The land types were nearly level to gently undulating slopes of 0-3°, which provided very stable physiographic environment for relatively uniform parent materials. The vegetation of the sampling locations comprised of mixed grasses, herbs, ferns, and cassava plants.



Figure 1a Map of Nigeria indicating Edo State where Oredo Local Government is situated.

Source (2011 Google – Map data).

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Table 2 showed the data on utilization of carbon sources. Isolate A (*Micromonospora* sp.) utilizes L-Arabinose, and D- glucose. D-fructose, sucrose, Mannitol and galactose, were not utilized. Isolate B (*Streptomyces* sp.), utilized L-Arabinose, D-glucose, fructose and galactose. Isolate C (*Nocardia* sp.), utilized all sugars; L-Arabinose, D-glucose, fructose, galactose, sucrose and Mannitol.

Isolate D (*Oerskovia* sp.), utilized D- glucose, mannitol and galactose. While fructose, sucrose and L-Arabinose, was not utilized. As seen in Table 2, the mass color of the mature sporulating aerial mycelium was observed following growth on the Starch casein agar plates. The aerial mass was classified according to the Bergey's manual of systemic bacteriology (Holt, 1989), (Tab. 2).

Table 2 Morphological, biochemical and utilization of carbon sources of four isolated actinomycetes colonies.

Characteristics	Isolate			
	A ^a	B	C	D
Morphological				
Spore chain Morphology	Long sporophores	Rectiflexibles spores.	Long chain conidia	–
Motility	Non motile	Motile		Motile
Pigmentation	Blackish	White	Brown	Yellowish
Aerial mycelium	–	+	+	–
Biochemical properties				
Gram Reaction	+	+	+/-	+
Starch Hydrolysis	+	+	–	+
Casein Hydrolysis	+ ^b	+	+	+
Catalase Test	+	–	+	+
Hydrogen sulphide production	+	+	+	+
Utilization of carbon sources				
L-Arabinose	+	+	+	–
D-Glucose	+	+	+	+
D-Fructose	–	+	+	–
D-Sucrose	–	–	+	–
D-Mannitol	–	–	+	+
D-Galactose	–	+	+	+

Legend: ^a Isolates identified were A (*Micromonospora* sp.), B (*Streptomyces* sp.), C (*Nocardia* sp.) D (*Oerskovia* sp.) ^b + and – represents positive and negative results respectively.

Dominant actinomycetes isolated were four. They included *Streptomyces*, *Nocardia*, *Micromonospora* and *Oerskovia*. spp. (Fig. 1). *Streptomyces* sp. was the most frequently isolated in all soil samples, while *Oerskovia* the least occurring on starch casein agar (Fig. 1).

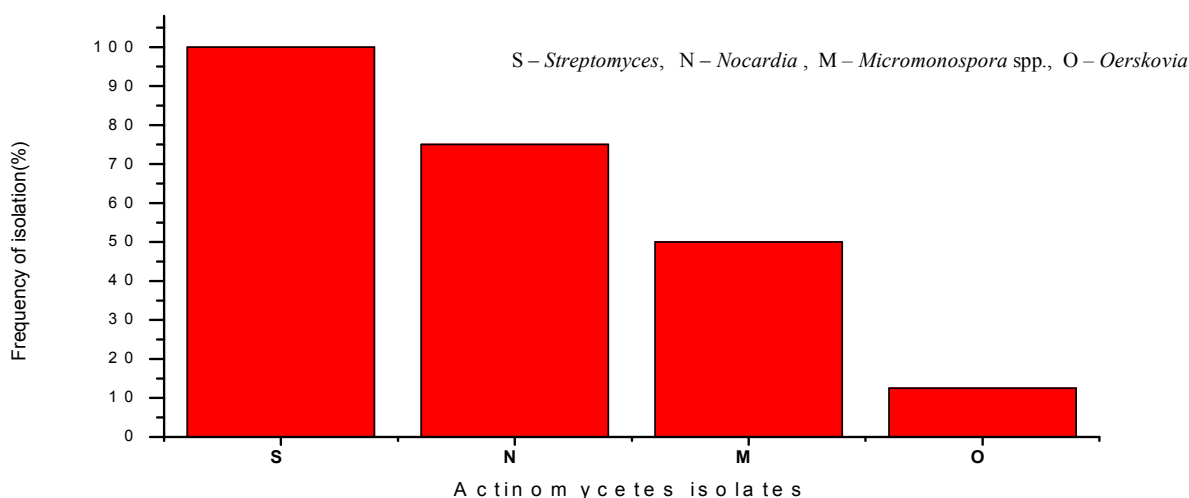


Figure 1 Frequency of isolation of four genera of actinomycetes isolated from soil samples on starch casein agar.

The particle sizes data of the soils is as shown on Table 3. These revealed the texture of the soils as varying from sandy to sandy loamy, with the sand, clay and silt fractions that ranged between 82.6 -92%, 1.2 - 10.8%, and 5.3 -11%,

respectively. The water holding capacity of the various samples ranged from 13.3% to 20%. With soil sample H at 13.3%, and soil samples E, F, G, at 20% respectively. The soil samples had moderate acid pH values, ranging from 5.37 to 5.99. Sample A, had a pH value of 5.99 (the highest) and soil sample H had a pH value of 5.37 (the lowest) (Tab. 3).

Table 3 Physical properties of test soils

SOIL SAMPLES	Properties			
	1 ^a	2	3	4
A	18.3 ^b	83 ^c	10.8 ^d	5.3 ^e
B	16.6	90.6	1.2	8
C	17.7	86	3.7	9.3
D	18.3	89.8	1.4	8.8
E	20	85	4	11
F	19.3	92	2	6
G	20	85.7	3.3	11
H	13.3	82.6	6.9	10.5

Legend: ^a Properties tested: Water holding capacity(%) (1), Percent Sand (2), Percent Silt (3), Percent Clay (4)

Total organic carbon and Total Organic matter content, ranged from 0.29 to 0.59 % and 0.51 to 1% respectively (Table 4). Table 4, showed that soil sample D, had the highest percentage of both organic carbon and organic matter content, while soil sample F, had the lowest organic carbon and organic matter content. Nitrogen content in the soil samples, ranged from 3 to 19.5%. Soil sample G, had the lowest nitrogen content from all soil samples while soil sample H, had the

highest nitrogen content as shown in Table 4. Phosphorus was more in soil sample H, with 0.85% content in the soil sample while soil sample B, had the lowest phosphorus content of 0.13%. Percentage potassium content in all soil samples was determined. Soil sample A, had the lowest percentage of 0.26. Soil sample H, had a percentage of 1.80, which was the highest (Tab. 4).

Table 4 Chemical properties of test soils

Soil samples	Properties					
	a ⁺	b	c	d	e	f
A	5.99	1.00±0.004	0.59±0.02	6.2±0.2	0.22±0.004	0.26±0.004
B	5.98	1.00±0.004	0.59±0.02	4.5±0	0.13±0	0.58±0.008
C	5.59	1.16±0.03	0.67±0.03	14.7±0	0.28±0.004	0.4±0.02
D	5.81	1.19±0.00	0.69±0.01	4.8±0.2	0.7±0.02	1.56±0.01
E	5.40	1.05±0.00	0.61±0.02	18.3±0.6	0.52±0.008	0.97±0.004
F	5.68	0.51±0.04	0.29±0.06	10.5±0.3	0.35±0.01	0.82±0.008
G	5.58	0.65±0.00	0.38±0.03	3±0.2	0.41±0.02	1.05±0.02
H	5.37	1.09±0.02	0.56±0.02	19.5±0.2	0.85±0.02	1.8±0.004

Legend: a⁺, pH ; b, Total organic matter (%); c, Total organic carbon(%); d, Nitrogen(%); e, Phosphorus(%); f, Potassium(%), ^ mean value ± std error

There was a significant regression of the actinomycetal counts on the water holding capacity, pH, clay, phosphorus and potassium content of the respective soil samples (P<0.05) (Table 5). The regression of the actinomycetal counts on sand, silt, total organic matter, total organic carbon and nitrogen values was insignificant (P>0.05) (Tab. 5).

Table 5 Summary of the regression analysis

Parameters	Microbial Count (cfu/g)
Microbial Counts (cfu/g)	1.000
Water holding capacity (%)	^a 3.008732
Sand (%)	0.118151
Clay (%)	^a 1.421058
Silt (%)	0.284124
pH	^a 1.985816
Total Organic Matter(%)	0.000685
Total Organic Carbon (%)	0.129003
Nitrogen (%)	0.298393
Phosphorus (%)	^a 5.322647
Potassium (%)	^a 5.494389

Legend: ^aF values preceded by alphabet "a" indicate a significant regression of Y on X

DISCUSSION

The population density of actinomycetes in soil sample H, which was the highest in all soil samples, maybe due to soil nutrients like the total organic carbon, nitrogen and phosphorus (Table 4). This is in accordance with **Baby et al., (2002)**. *Streptomyces* spp. which was the most frequently isolated of the various sampling sites, tend to grow in fertile soils, this is an important characteristic feature of *Streptomyces* spp. (**Stackebrandt et al., 1981**), and adequate source of carbon and nitrogen, present in the soil, enhances the rate of degradation (**Tien et al., 1987**). Among the various carbon sources tested L-arabinose, was proved to be suitable for the growth of *Streptomyces* spp., *Nocardia* spp. and *Micromonospora* sp. while glucose was proved to be suitable for the growth of all four isolates (Table 2). Actinomycetes are nutritionally versatile being also to grow in rich substrate and on those containing a minimum or even an apparently lack of nutrients (**Wellington et al., 1994**). Based on morphological, physiological and biochemical characteristics, the dominant isolates of actinomycetes, found belonged to *Streptomyces* spp., *Nocardia* spp. *Micromonospora* sp. and *Oerskovia* sp. They were found to be gram positive in accordance with **Holt (1989)**. Pigment production, was also observed on the

isolates, and all the actinomycetal isolates hydrolyzed casein (Table 2). The optimum temperature for their growth was found to be 25°C the pH was 6.5 for their maximal growth. The range of mean actinomycete counts obtained in the present study were at variance with a report by **Jeffrey et al., (2007)** which stated a range of comparative lower counts (4.40 ×10² cfu/g – 1.57×10² cfu/g) for soils collected from several districts in Malaysia. The isolation of *Streptomyces* sp. and *Micromonospora* sp. from soils collected from several farmlands in Oredo Local Government Area, is in line with a report by **Gurung et al., (2009)** which isolated these actinomycete species from soils in Kalapattar, Nepal. Actinomycetes are useful to man, and the environment as a whole. Their involvement in degradation of recalcitrant and their ability to produce potential antibiotics are qualities that make them stand out from the microbial biomass. The search for novel metabolites from actinomycetes, require a large number of isolates, in order to discover a novel compound, which could be of pharmaceutical interest. Soils from farmlands should be used, as it is based on the hypothesis that actinomycetes diversity may be influenced by the diversity of cultivated plant species, as these actinomycetes grow profusely in humus and leaf litter layer.

CONCLUSION

In view of the important role of actinomycetes in humus formation (**Goodfellow and Williams, 1983**), which is required for a sustainable agriculture, further work on Oredo soils in particular and edaphic regions across Nigeria in general, should be done to isolate more actinomycetes which could be of scientific and economic importance to both urban and rural population living in these regions.

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