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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 x 10^4 cfu/g to 3.8 x 10^4cfu/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° 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).

Figure 1b Sampling location map of a section of Oredo Local Government Area. Source (2011 Google – Map data).

Sources of sample

Eight soil samples labeled A to H were collected in UV sterilized cellophane bags that have been previously exposed to ultraviolet radiation for 1 hr. Triplicates soil samples were obtained from each location, using soil auger from a depth of 15cm upwards (**Ekundayo, 2004**). They were spread out and air dried on a laboratory bench for 3 days. It was then oven dried at 55^0C for 30 min **(Vijayakumar *et al*., 2007).**

Isolation and enumeration of actinomycetes

Isolation was performed using the pour plate technique (**Sharma, 2009**). One gram of dried soil was added in 9 ml of distilled water, and properly hand shaken. One ml of different aqueous dilutions, 10^2, 10^3 and 10^4 of the suspension were applied onto plates, and then 20 ml of melted medium at ca 50^0C was added to it. This was done in triplicate for each soil sample. After gently swirling, the plates were incubated at room temperature for 7 to10 days. Total actinomycetes count was carried out by counting the visible colonies using a Digital colony counter (LABTECH, U.S.A) with a magnifying glass lens. Morphological observations were made with a light microscope (Model SE; NICON), for each pure isolate. and photographed.

Colonies of actinomycetes were transferred from mixed culture of the plates onto respective Starch casein agar plates and incubated at room temperature for 7 days. Plates containing pure cultures were stored at 4^0C until further examinations**.**

Subculturing of actinomycetes

This was carried out using the streak and the seeding methods (**Sharma, 2009**). An identified colony was picked from the mixed population on the water agar, and streaked on five plates to prevent completely losing these colonies, since they are slow growers. Also using the seeding method, a portion of an identified colony was cut out with a needle, and placed on a fresh starch casein agar plate. This was also done five times for a particular colony. The plates were then incubated at room temperature for 7 to 10 days. It was checked frequently, taking note of changes in the media, like pigmentation produced. The frequency of occurrence of each actinomycetes isolate was determined during the sub culturing of these isolates. This was done by taking note of the particular soil in which a given isolate was found. Their cultural characteristics and morphology were criteria for this identification and was further confirmed via biochemical tests performed as suggested by Bergey's manual of determinative bacteriology (**Holt, 1989**).

Microscopic examination

Colony morphology was noted with respect to colour, aerial mycelium, size and nature of the colony, reverse side colour and pigmentation.

Taxonomic grouping of active actinomycete isolates

Actinomycetes colonies were characterized morphologically and physiologically following the directions given by the Bergey's Manual of Determinative Bacteriology (**Holt, 1989**). Cultural characteristics of pure isolates in various media were recorded after incubation for 7 to 14 days at room temperature. Active purified isolates of actinomycetes were identified up to the species level by comparing their morphology of spore bearing hyphae with entire spore chain and structure of spore chain with the actinomycetes morphologies, as described in Bergey's manual (**Lechevalier *et al*., 1980**). A small portion of the culture was taken and placed on a clean, flame- sterilized glass slide. The slide was then gently pressed examined at a magnification of 100 × in immersion oil with a light microscope (Model SE; NICON) and photographed. Also, physiological and biochemical tests which included; Gram staining, Casein hydrolysis, Starch hydrolysis, H_2S production, Citrate utilization and acid and gas production from several sugars (Fructose, Galactose, Glucose, Lactose, Maltose, Mannitol and Sucrose) incorporated respectively into Basal medium consisting of peptone, sodium chloride, and phenol red (**Harley and Prescott, 2002**) were also conducted to further identify the respective actinomycetal isolates.

Physiochemical analysis of soil samples

The temperature of the entire sample collected, was measured by dipping PT -2 digital thermometers with model number ST-3 into the soil at site, before collection at each soil sampling points. The pH for the respective soil samples was determined with the aid of a calibrated JENWAY pH meter (ADVENTURER OHAUS Company, USA). Particle size analysis was done using the Bouyoucous hydrometer method (**Bouyoucous, 1962**). The total nitrogen content of the respective soil samples were determined using micro Kjeldahl digestion and colorimetric method (**Bremmer and Mulvaney, 1982**). The available Phosphorus and Potassium content of the respective soil samples were evaluated using methods described by Onyeonwu (2000). Total organic carbon content of each sample was determined using method as described by **Walkley (1947).** Also, Water Holding Capacity (WHC) and Total Organic Matter (TOM) of the soil samples were determined according to methods described by **Kalra and Maynard, (1991).**

Statistical anaylsis

Analysis of variance (ANOVA) was carried out on the data using SPSS version 16. Mean comparison was carried out using Duncan Least Significant Difference (Sokal and Rolf, 1981). The microbial counts obtained for each sample was regressed against the physical and chemical property of the soil sample (α=0.05) to ascertain their relationship with each other. This was conducted using Microsoft Excel 2007 version.

RESULTS AND DISCUSSION

The total actinomycete colony counts obtained from the different soil samples are shown in Table 1. The counts ranged from 0.8×10^3 to 3.8×10^4 cfu/g. Soil sample H, had the highest count at 3.8×10^4cfu/g, while soil sample E, had the lowest count at 0.8×10^3cfu/g (Tab. 1).

Table 1 Actinomycete counts (cfu/g) after 7 days of incubation

Soil Sample	Plate replication			Total	M±SEM
	1	2**	3		
A	1.6*	1.3	1.1	40	$1.3\pm1.45^{a,b,c}$
B	0.7	0.9	1.2	28	$0.9\pm1.45^{a,b}$
C	1.9	1.3	1.8	50	$1.7\pm1.86^{b,c}$
D	1.9	2.2	1.6	57	$1.9.\pm1.73^{c}$
E	0.7	0.9	0.8	24	0.8 ± 0.58^{a}
F	1.4	1.1	1.6	41	$1.8\pm1.45^{a,b,c}$
G	2.4	3.0	2.7	81	2.7 ± 1.73^{d}
H	3.8	4.1	3.5	114	3.8 ± 1.73^{e}

Legend: * Colony forming units ; ** dilution of $\times10^3$,Means followed by the same alphabet are not significantly different at (P > 0.05) from each other, using Duncan's LSD Test.

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.

	Isolate			
Characteristics	**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	+	+	+/v	+
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). *Streptomycetes* sp. was the most frequently isolated in all soil samples, while *Oerskovia* the least occurring on starch casein agar (Fig. 1).

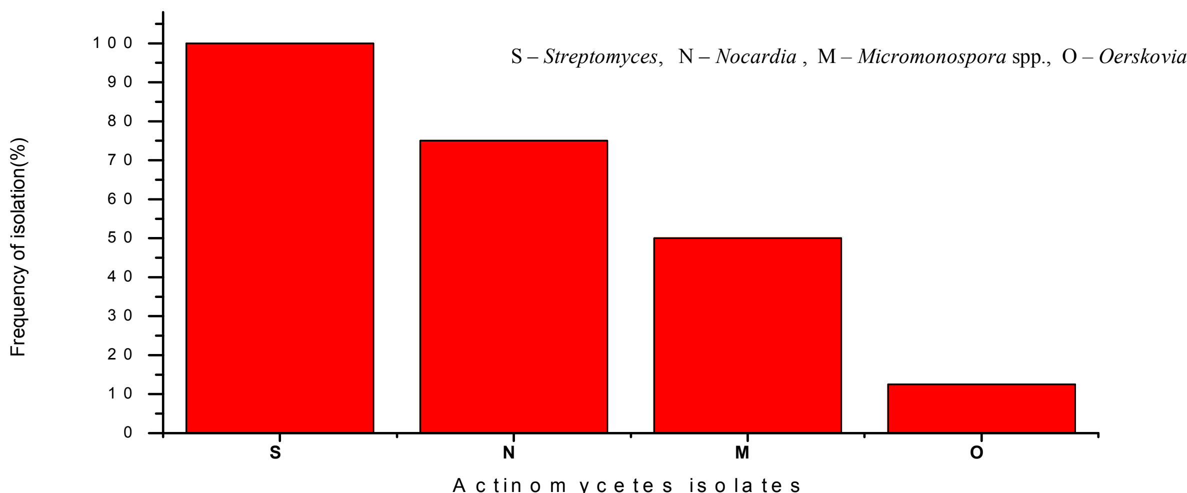

Figure 1 Frequency of isolation of four genera of actinomycetes isolated from soil samples on starch casein agar.

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 from13.3% 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: [*]F values preceeded 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 *Streptomycetes* 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 couts obtained in the present study were at variance with a report by **Jeffrey *et al*., (2007)** which stated a range of comparative lower counts (4.40×10^2 cfu/g – 1.57×10^2 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 Kalapatthar, 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 standout 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.

REFERENCES

BABY, U.I. - TENSINGH-BALIAH, N. - PONMURUGAN, P. - PREMKUMAR, R. 2002. Population level of certain beneficial microorganisms in tea soils In *Tea Resorts Foundation Newsletter* vol.12,2002, p. 3-10.

BOUYOUCOUS, G.J. 1962. Improved hydrometer method for making particle size analysis. In *Soil Agronomy Journal* vol. 54, 1962, p. 464-465.

BREMMER, I. M. - MULVANEY, C. S. 1982. Total Nitrogen. In *Methods of soil analysis*. *Agronomy monograph 9*, American Society of Agronomy and soil science of America (Ed). Madison, Wisconsin, p. 595-627.

EKUNDAYO, E.O. 2004. Fungi in the rhizosphere and non-rhizosphere soil of okra cultivated in Southern Nigeria. *Nigerian Journal of Soil Science,* 14, 48-55.

GOODFELLOW, M., WILLIAMS, S. T. 1983. Ecology of actinomycetes. *Annual Review of Microbiology*, 37, 189-216.

GURUNG, T. D., SHERPA, C., AGRAWAL, V. P., LEKHAK, B. 2009. Isolation and characterization of antibacterial actinomycetes from soil samples of

Kalapatthar, Mount Everest region. *Nepal Journal of Science and Technology* , 10, 173-182
HAMDALI, H., BOUIZGARNE, B., HAFIDI, M., LEBRIHI, A., VIROLLE, M. J., OUHDOUCH, Y. 2008. Screening for rock phosphate solubilizing Actinomycetes from Moroccan phosphate mines. *Applied Soil Ecology*, 38, 12-19.
HARLEY, J. P., PRESCOTT, L. M. 2002. Laboratory Exercises in Microbiology. Fifth edit. New York: Mac Graw Hill, 449 p.
HOLT, J. G. 1989. Bergey's Manual of Systemic Bacteriology. Volume four. London: Cambridge University Press, 1989, 2493 p.
JEFFREY, I. S. I., SAHILAH, A. M., TOSIAH, S. 2007. Isolation and screening of actinomycetes from Malaysian soil for their enzymatic and antimicrobial activities. *Journal of Tropical Agriculture and Fadama Science*, 35(1), 159 – 164.
KALRA, Y. P., MAYNARD, D. G. 1991. Methods manual for forest soil and plant analysis. Edmonton: Minster of supply and services, 125 p. ISBN 0-662-18665-6.
LE CHEVALIER, M. W., SEIDLER, R. J., EVANS, T. M. 1980. The influence of increasing numbers of non-indicator organisms. *Applied Environmental Microbiology*, 12, 922-930.
MASON, M. G., BALL, A. S., REEDER, B. J., SILKSTONE, P. N., WILSON, M. T. 2001. Extracellular heme peroxidases in Actinomycetes: A case of mistaken identity. *Applied Environmental Microbiology,* 67(10), 4512 - 4519
Mc CARTHY, A. J. 1987. Lignocellulose-degrading actinomycetes. *FEMS Microbiology Review*, 46, 145-163.
MIYADOH, S. 1993. Research on antibiotic screening in Japan over the last decade: A producing microorganisms approach. *Actinomycetologica*, 7,100-106.
ONYEONWU, R.O. 2000. Manual for Water/Wastewater, Soil/Sediment, Plant and Fish Analysis. Benin City: Macgill Environmental Research Laboratory Manual. 81 p.
PRAGYA, R., YASMIN, A. - ANSHULA, J. 2012. An insight into agricultural properties of actinomycetes. *International Journal of Research in Biosciences*, 1(1), 7-12.
RADHAKRISHNAN, M. - BALAGURUATHAN, R. - SELVAKUMAR, N. - DOBLE, M. - KUMAR V. 2011. Bioprospecting of marine derived actinomycetes with special reference to antimycobacterial activity. *Indian Journal of Geo-Marine Sciences,* 40(3), 407-410.
SHARMA, P. 2009. Manual of Microbiology, tools and techniques. Second edit. New Delhi: Ane books, 405 p. ISBN 81-8052-143-5.
SOKAL, R. - ROLF, R. J. 1981. Biometry: The Principle and Practice of Statistics in Biological Research. Second edit. New York: Freeman Company, 556 p.
STACKEBRANDT, E., WITT, D., KEMMERLING, C., KROPPENSTED, M ., LIESACK, W. 1981. Designation of *Streptomyces* 16S and 23S rRNA based target regions of oligonucleotide probes. *Applied Environmental Microbiology,* 57, 1468-1477.
STACKEBRANDT, E., RAINEYF, A., WARD-RAINEY, N. L. 1997. Proposal for a new hierarchic classification system. *Actinobacteria* classes nov. *International Journal of Systematic Bacteriology*, 47, 479- 491.
TIEN, M., KERSTEN, P. J., KIRK, T. K. 1987. Selection and improvement of lignin degrading microorganisms: Potential strategy based on lignin model-amino acid adjucts. In *Applied Environmental Microbiology*, 53, 242-245.
VIJAYAKUMAR, R., MUTHUKUMAR, C., THAJUDDIN, N., PANNEEVSELVAN, A., SARAVANAMUTHU, R. 2007.Studies on the diversity of actinomycetes in the Palk Strait region of Bay of Bengal, India. *Actinomycetologica,* 21, 65 -59.
WALKLEY, A. 1947. A critical examination of a rapid method for determining organic carbon in soils: effect of variations in digestion conditions and of inorganic soil constituents. *Soil Science,* 63, 251-263.
WELLINGTON, E. M. H., STACKEBRANDT, E., SANDERS, D., WOLSTRUP, N., JORGENSEN, O.G. 1994. Taxonomic status of Kitasatosporia and proposed unification with *Streptomyces* on the basis of phenotypic and 16S rRNA analysis and emendation of *Streptomyces*. *International Journal of Systematic Bacteriol*ogy, 42, 156-160.
WILLIAMS, S. T., ROBINSON, C. S. 1981. The role of *Streptomyces* in decomposition of chitin in acidic soils. *Journal of General Microbiology* , 127, 55-63.