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Actinobacterial diversity of mangrove environment of the Bhitherkanika mangroves, east coast of Orissa, India

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Abstract- Studies on actinobacteria of marine habitats gaining international importance due to its proven abilities of novel compound production. Information on actinobacteria of the mangrove environments is less and hence the present study was carried out in the different mangrove environment of Bhitarkanikka, Orissa. A total of 116 actinobacterial colonies were recorded from 30 mangrove and marine sediment samples of Bhitherkanikka mangrove environment east coast of Orissa. Among them, 67 isolates of were morphologically distinct on the basis of colour of spore mass riverside colour, Arial and substrate mycelia format production of diffusible pigment sporophore morphology. Forty three isolates were assigned to the genus *Streptomyces*, *Saccharopolyspora* (5), *Nocardiopsis* (5), *Micromonospora* (3), *Actinomadura* (5), *Actinomycetes* (1), *Actinopolyspora* (5).

Index Terms- women entrepreneurs micro-enterprise, management styles, need for training.

I. INTRODUCTION

The term "Actinobacteria" gram-positive organisms that tend to grow slowly as branching filaments, resembling fungi, as their filamentous growth forms mycelial colonies (Kathiresan, 2008). Among all actinombacterial groups the streptomycetes represent as the dominant group and are best known for their ability to produce antibiotics. However ecological role of actinobacteria in the marine ecosystem is still understudied. However, the various marine resources such as mangroves, coral reefs, seaweeds and seagrasses and different marine environment like coastal, offshore and deep sea are presently considered as the best resources for isolating novel actinobacteria (Felseke et al., 1997, Greimon et al., 2003 and Rheims et al., 1999). Similarly, striking advances have been made in marine microbial ecology using molecular techniques and metagenomics that leads new insights into marine actinobacterial biodiversity and biogeography. Having with this in mind, present study was carried out in the mangrove ecosystem of Bhitarkanikka, Orissa to comprehend the diversity of actinobacteria.

II. MATERIALS AND METHODS

A. Isolation of actinobacteria, the samples were collected from 5 stations of Bhitarkanika mangroves

The area of Bhitarkanika mangrove forest spread over an area of 1712 ha divided into three major blocks Dangmal 636 ha., Kakranasi 310 ha., and Thakurdia 272 ha. (Chadha & Kar, 1999). Bhitarkanika and Dangmal Blocks constitute the core area. This area influenced by many rivers such as Dhamra, Hansua, Brahmani, Baitarani, and their tributaries. These sites experience tide of semi diurnal type with mean tidal amplitude of 1.66 M.
Inserting a pre sterilized (with alcohol) polyvinyl corer (10cm dia.) into the sediments.. The central portion of the top 2 cm sediment sample can be taken out with the help of a sterile spatula. This sample was transferred to a sterile polythene bag and transported immediately to the laboratory. The sediment samples thus collected are air dried aseptically for further use. Physico-chemical characteristics of the samples collected from the sites where samples collected for microbial analysis were tested. Various environmental parameters such as temperature, transparency, salinity, pH, dissolved oxygen etc. were recorded from the 5 stations Bhitarkanikka. Air and sediment temperatures were measured with a mercury thermometer with ± 0.02 ^{0}C accuracy. Sediment pH was measured by soil pH Tester (Model DM – 13, Takemura Electric Works Ltd., Tokyo, Japan). After taking sediment samples by using Peterson grab, they were transferred to clean polythene bags, then air-dried and used for the analysis of sediment composition and organic carbon. The textural analysis of the sediments was carried out by the combined method of sieving and pipette analysis after taking known quantity of sample by coning and quartering method. Dry sieving was done using Ro-Tap sieve shaker for 15 minutes (Folk, 1966). Fine particles were separated by pipette method, as proposed by Krumbein and Pettijohn (1938). The organic carbon content in the sediment samples was determined by using the standard method of el Wakeel and Riley (1956).

For the study of trace metals, known quantities of sediment samples were taken and were oven dried at 110^0 C for 24 hours and ground with the help of a mortar and pestle. The samples were digested with concentrated perchloric acid and nitric acid (1:3) based on the standard procedures of Topping (1973) and Watling (1981). The supernatant was analyzed in the Inductively Coupled Plasma Mass Spectrophotometer (ICP-MS). The amount of oil (petroleum hydrocarbon) present in the sea water was calculated using the method given in APHA (1971). The pesticide residue in the seawater was analysed by using the standard method of Grasshoff *et al.* (1999).

For microbial analyze, after a week, the sediment samples were incubated at 55^0 C for 5 min (Balagurunathan, 1992). Then, 10-fold serial dilutions of the sediments were prepared, using filtered and sterilized 50% seawater. One ml of the serially diluted samples was plated in the Kuster's Agar (Siva Kumar, 2001) in triplicate petriplates. To minimize fungal contamination, all agar plates were supplemented with 50 ug/ml of nystatin. The actinobacterial colonies that appear on the petriplates were counted from 5th day onwards, upto 28th day. All the colonies that are growing on the petriplates was separately streaked in petriplates, subcultured, ensured for their axenicity and

maintained in slants. The correlation and co-effcient analysis between physic-chemical parameters of sediment and actinobacterial population was also made using SPSS package.

III. IDENTIFICATIONS

Classical approaches for classification make use of morphological, physiological, and biochemical characters. The classical method described in the identification key by Nonomura (1974) and Bergey's Manual of Determinative Bacteriology (Buchanan & Gibbons, 1974) is very much useful in the identification of streptomycetes. These characteristics have been commonly employed in taxonomy of streptomycetes for many years. They are quite useful in routine identification. Chemotaxonomy is the study of chemical variation in organisms and the use of chemical characters in the classification and identification. It is one of the valuable methods to identify the genus of actinobacteria.

IV. CULTURAL AND MORPHOLOGICAL CHARACTERISTICS

The growth and colony characters Chromogenecity of the aerial mycelium is considered to be an important character for the grouping and identification of actinobacteria Tresner, (1974) and Lakshmanaperumalswamy, (1974). (Colour of aerial mycelium, riverside pigment, melanoid pigment. Soluble pigment and diffusible pigment) in all strains (BKM-1 to BKM-68). ISP-I, ISP-II and ISP-VII Shrilling and Gottlieb (1966), Sivakumar (2000) and inoculated 37° C were studied. The shape of mycelium and spore chains of strains grown ISP-II medium were observed by using light Photomicroscope.

A. Analysis of whole cell hydrolysates

The isomers of diaminopimilic acids and sugar type were determined as methods described by Sivakumar (2001). Diaminopimilic acid (a mixture of LL, DD and meso isomers, sigma chemical Co). Were used as standards. A mixture of amino acids (glycine, asparagines,cystein, leucine and alanine) and a mixture of sugars (gulucose, galactose, mannose and xylose) were used to determine the type of amino acids and type of sugars respectively.

V. BIOCHEMICAL CHARACTERISTICS

The media and procedures used for determination of biochemical characteristics were those described by Sivakumar (2001), Shirling and gottlieb (1966), Tresnar *et al* (1968), Williams *et al* (1969).

VI. RESULT AND DISCUSSION

The Bhitarkanika sanctuary is bounded by river Dhamra in the north, the river Hansua to the west and Bay of Bengal on the eastern and southern sides. The sanctuary encompasses 35 km sea coast known as 'Gohirmatha Coast' from Dhamra mouth to Barunei, the mouth of river Hansua. The area has about 200 km. of water body inside the sanctuary and falls in the deltaic region of the river Brahmani, Baitarani, and their tributaries. The estuarine rivers- Brahmani, Baitarani, Kharasrota, Dhamra, Pathasala, Maipura, Hansua, and Hansina during their course flow into the Bay of Bengal are further crises crossed by numerous creeks, channels, and nallahs, thus providing the peculiar ecological niche for the growth, development of rich and varied mangrove life forms, both flora and fauna along with their associates (Mishra et al.,2005). Our study revealed that the microbial ecology of the sampling sites of the Bhitarkanika is influenced by the chemical nature of the soil. A very typical pattern of soil sediment characteristics of almost all of the samples were observed, that is low in organic carbon and moderately high in salinity. The soil pH was ranging from slightly acidic to slightly alkaline (pH range 6.5 to 7.0). So, this nature of the sediment nutrients and also plenty of microbial population especially with actinobacteria.

In the present study, a total of 116 microbial strains were isolated from five stations of the Bhitarkanikka mangrove environment. Of the 67 isolates 43 strains are identified as the genus *Streptomyces* followed by *Sacharopolyspora* (5), *Nocardiopsis* (5), *Micromonospora* (3), *Actinomadura* (5), *Actinomycetes* (1), *and Actinopolyspora* (5). The maximum number of actinobacteria isolates (from sediment samples) was recorded in Kola creek (nearest terrestrial, starting in the mangrove area) (13) followed by Bhiterkkanika (14), Baguludia (estuarine canal and dens mangrove) (11), Kalibanjidia (Island of dharma river and dens mangrove) (17) and Thanidia (Mangrove) (13) (Table 1). The recognition and isolation of streptomycetes colonies were greatly facilitated by their spore forming property and pigmented aerial growth on the surface spread plats (Table 2). Of the 116 colonies belongs to 67 colonies were isolated, maximum number of actinobacterial colonies was higher population density in dens mangrove area sediment (30), mangrove area sediment (25) and near terrestrial and mangrove area (12) (Table 1).

The micro morphologically observations of the strains BKM-1 to BKM-67 reveal that all these belongs to the genus *streptomyces.* The predominance of streptomyces in any actinobacterial population is a well known fact (Alexader, 1961). The analysis all compounds in the present study has revealed the dominance of streptomyces in the sediments of the Bhiterkanika mangrove environment. Inspite of the little attention to the diversity of actinomycetes in marine habitats even though these organisms have been studied in more detail than members of other groups of prokaryotes due to their biotechnological importance (Goodfellow *et al*., 1988; Veigh *et al.,*1994; Bull *et al*., 2000). Though the marine environment has been proved as one of the potent sources for actinobacteria, very less attention has been paid to explore the mangrove environment. Occurrence and distribution of bacteria and fungi have been well studied in the mangrove environment. However, such studies on actinobacteria in the mangrove area are much lacking (Rathnakala and Chanrika 1993; Balagurunathan, 1992; Laksmanaperumalswamy, 1978). Hence the present investigation has been carried out to preuxilouer the lacuna similar to this investigation (Sivakumar *et al*., 2005), Also isolated 91 stations of actinobacteria from the sediments of pichavaram mangrove environment. This study also adds to the support that for the predominance of streptomyces in the isolated actinobacterial population.

The occurrences of actinobacteria in water and sediments of marine environment has been reported by Grein and Mayers (1958), Weyland (1969) and Walker and Colwell (1975). Grein and Meyers (1958) have also reported that Streptomycetes was the dominant genus collected, while species of Nocardia and Micromonospora constitute about 20% of the total number of

isolates. Similarly, Streptomycetes accounted 90 -95% of the total actinomycetes found in various soil types (Lechevalier, 1964; Lechivalier and Lechivalier, 1967; Rangaswamy et al., 1967, Lakshmanaperumalswamy, 1952 and Sivakumar, 2001). This is clearly supported in the present study and 80% Streptomycetes genus and 20% other genus (*Sacchropolyspora Nocardiopsis* , and *Micromonospora)* were recorded.

Even though a number of reports are available on the occurrence and distribution of actinobacteria by depends upon the pigment formation (Lakshmanaperumalswamy, 1974), this is the first study has been carried out in the Bhitarkanika mangrove ecosystem. Hence, there are 67 species isolates of actinobacteria belong to different genera recorded from Bhitarkanika mangrove ecosystem, though it is not depicted a complete picture of actinobacteria diversity from this environ. So, further study will provide the more information about this actinobacterial diversity.

Table 1: Actinobacterial diversity recorded from the Bhitarkanika mangroves environment

Sampling environment	Location	No. of samples		No. of actinobacteria isolated						
				Streptomycetes	Saccharopolyspora	Nocardiopsis	Micromonospora	Actinomadura	Actinomycetes	actinopolyspora
Near terrestrial and mangrove area	Kola creek	5	15/12	7	1	1	-	-	-	3
Mangrove area	Bhiterkkanika	6	23/14	9	1	1	1	2	-	-
	Baguludia	5	27/11	7	2	1	-	-	-	-
Dense mangrove area	Kalibhanchidia (island)	8	25/17	13	-	2	1	2	-	2
	Thanidia (new island)	6	26/13	7	1	-	1	1	1	-
Total			116/67	43	5	5	3	5	1	5

Table.2. Streptomycetes species recorded from the Bhitarkanika mangroves

Strain Name	Aerial mycelium colour	Reverse said colour	Diffusible pigment	Colony size (mm)	Actinomycetes Species Name
Near terrestrial and mangrove area					
BKM1	White	Brown	-	2.5	streptomyces sp.
BKM 2	White	Light brown	-	3.0	Streptomyces sp.
BKM 3	White	yellow	-	4.0	Streptomyces sp.
BKM 4	Light green	Yellow	-	3.5	Actinopolyspora sp.
BKM 5	Dull white	White	-	2.5	Nocadiopsis sp.
BKM 6	Pale white	Yellowish white	-	6.5	Streptomyces sp.
BKM 7	white	Dark brown	-	5.0	Actinopolyspora sp.
BKM 8	Dull	Dark yellow	-	5.5	Streptomyces sp.

BKM 9	White	Dark yellow	-	6.0	Streptomyces sp.
BKM 10	Ash	yellowish	-	4.5	Actinopolyspora sp.
BKM 11	White	Dark brown	-	3.5	Streptomyces sp.
BKM 12	White	Dark brown	-	3.0	Saccarapolyspora sp
Dens mangrove area					
BKM 13	Dark ash	Light ash	-	6.0	Actinomadura sp.
BKM 14	White	Brown	-	7.0	Streptomyces sp.
BKM 15	White	Light brown	-	6.5	Sacaropolyspora sp.
BKM 16	Ash	Dull yellow	-	5.0	Streptomyces sp.
BKM 17	White	Yellow	-	4.0	Streptomyces sp.
BKM 18	White	Dark brown	Brown	4.5	Streptomyces sp.
BKM 19	Milky white	Light yellow	-	3.5	Sacaropolyspora sp.
BKM 20	Powdery Light	Yellow	-	3.0	Streptomyces sp.
BKM 21	ash	Yellow	-	5.0	Nocardiopsis sp.
BKM 22	Light yellow	Brown yellow	-	5.0	Streptomyces sp.
BKM 23	Yellowish	Brown	-	6.5	Actinomadura sp.
BKM 24	white	Yellowish brown	-	4.5	Streptomyces sp.
BKM 25	White	Yellow	-	4.0	Streptomyces sp.
BKM 26	Yellowish	Dark ash	-	2.5	Micromonospora sp.
BKM 27	white	Yellow	-	2.0	Streptomyces sp.
BKM 28	White	Brown yellow	-	3.5	Streptomyces sp.
BKM 29	White	Brown	-	6.0	Streptomyces sp.
BKM 30	White	Brown	-	7.0	Streptomyces sp.
BKM 31	Yellowish	Light yellow	-	5.0]	Sacaropolyspora sp.
BKM 32	white	Yellow	-	5.5	Streptomyces sp.
BKM 33	White	Yellow	-	6.5	Streptomyces sp.
BKM 34	White	Peal yellow	-	3.5	Streptomyces sp.
RBM 35	White	Light ash	-	7.5	Streptomyces sp.
BKM 36	Light green	Yellow	-	6.0	Nocardiopsis sp.
BKM 37	Peal white	White	-	5.0	Streptomyces sp.
Estuarine mouth and mangrove area					
BKM 38					
BKM 39	Dull white	Light yellow	-	4.5	Streptomyces sp.
BKM 40	Yellow white	Yellow	-	3.5	Micromonospora sp.
BKM 41	Pale white	Yellowish	-	3.5	Streptomyces sp.
BKM 42	Pale white	Yellow	-	4.0	Actinomadura sp.
BKM 43	White	Brown	-	5.0	Streptomyces sp.
BKM 44	Ash	Light brown	-	6.5	Actinomycetes sp.
RBM45	White	Brown	-	6.0	Streptomyces sp.
BKM 46	Dark ash	Dull ash	-	5.5	Streptomyces sp.
BKM 47	Milky white	Yellow	-	2.5	Nocardiopsis sp
BKM 48	White	Light yellow	-	3.5	Streptomyces sp.
BKM 49	Gray	Yellowish white	-	5.0	Streptomyces sp.
BKM 50	White	Dark brown	-	6.5	Actinopolyspora sp.
BKM 51	White	Light black	-	5.5	Streptomyces sp.
BKM 52	Yellowish	Dark yellow	-	6.0	Streptomyces sp.
BKM 53	white	yellowish	-	3.5	Actinopolyspora sp.
BKM 54	Light ash	Yellow	-	3.0	Streptomyces sp.
BKM 55	White	Light yellow	-	3.0	Streptomyces sp.
BKM 56	Yellowish	Brown	-	4.0	Actinomadura sp.
BKM 57	white	Light brown	-	4.5	Streptomyces sp.
BKM 58	White	Dark yellow	-	6.0	Streptomyces sp.
BKM 59	Milky white	Light Yellow	-	6.5	Micromonospora sp

BKM 60	White	Light brown	-	5.0	Streptomyces sp.
BKM 61	Milky white	Dark yellow	-	5.5	Streptomyces sp.
BKM 62	Milky white	Light white	-	2.5	Sacaropolyspora sp.
BKM 63	White	Yellowish	-	2.0	Streptomyces sp.
BKM 64	White	Brown	-	3.5	Actinomadura sp.
BKM 65	Peal white	White	-	6.5	Streptomyces sp.
BKM 66	Light ash	Dark yellow	-	4.5	Streptomyces sp.
BKM 67	White	White	-	4.0	Nocardipsis sp.
	White	White	-	3.5	Streptomyces sp.

Table.3. Correlation co-efficient between Physico-chemical properties of mangrove sediments and total actinobacerial population

	pH	Sand	Silt	Clay	POC	TSS	Cd	Cr	Cu	Fe	Mg	Mn	Ni	*Pb*	*Zn*	*TAP*
pH	1															
Sand	-0.3406	1														
Silt	0.3688	-0.9423	1													
Clay	-0.5.117	-0.4577	0.1392	1												
POC	0.0865	-0.8770	0.9497	0.1093	1											
TSS	-0.9270	0.3361	-0.2460	-0.3314	0.0249	1										
Cd	0.4511	-0.3417	0.4152	-0.2375	0.2128	-0.2257	1									
Cr	0.3261	0.4828	-0.4338	-0.4109	-0.6307	-0.1954	0.6074	1								
Cu	0.115	0.2626	-0.5132	0.4896	-0.6708	-0.3297	0.1572	0.5551	1							
Fe	0.2343	-0.8650	0.8938	0.2663	0.9249	-0.2101	-0.0336	-0.7586	-0.6332	1						
Mg	0.2725	0.5215	-0.4250	-0.5429	-0.5956	-0.0967	0.6043	0.9880	0.4324	-0.7502	1					
Mn	-0.2700	-0.4496	0.6482	-0.3294	0.8229	0.4709	0.0050	-0.5966	-0.9227	0.6844	-0.4859	1				
Ni	0.1701	0.6153	-0.5152	-0.5737	-0.6548	-0.0026	0.5209	0.9722	0.4221	-0.8116	0.9922	-0.4838	1			
Pb	-0.0940	0.9675	-0.9093	-0.4590	-0.9188	0.0991	-0.2413	0.6043	0.3383	-0.8644	0.6264	-0.5727	0.6980	1		
Zn	0.6008	0.0622	-0.1473	0.0702	-0.4310	-0.5878	0.6755	0.8379	0.7477	-0.4681	0.7534	-0.7247	0.6872	0.2430	1	
TAP	0.2295	-0.4800	0.7446	-0.5489	0.7547	0.0879	0.4300	-0.1735	-0.8195	0.6049	-0.0704	0.8276	-0.1168	-0.4736	-0.2730	1

*Significant p<0.05

VII. FREQUENCY

Frequencies of identified genera of actinobacteria, in various mangrove environments. The frequency of the genus Streptomyces was (43) followed by Actinopolyspora (5), Saccharopolyspora (5), Nocardiopsis (5), Micromonospora (3), Actinomadura (5), Actinomycetes (1) (Table 1). Among the genera recorded, in the present study, Streptomyces was the most predominant when compared to other genera. The dominance of Streptomyces among the actinomycetes especially in soils has also been reported by many workers (Moncheva et al., 2002; Jensen et al 1991; Balagurunathan et al., 1996; You et al., 2005). Besides Streptomyces, the genera most frequently appeared on media were Actinopolyspora, Saccaharopolyspora, Nocardiopsis, Micromonospora, Actinomadura, Actinomycetes occurred very rarely and also as a pathogen Waksman, 1967.

Inspite of the fact that the actinobacteria have wide distribution they show variation in their population dynamics. In the present investigation it was found that there was correlation co-efficient between physico-chemical properties of sediment and total Actinobacterial population (TAP). It revealed that the significant positive correlation between Zing and pH (r = 0.600; P < 0.05%) sand and phosphorus (r = 0. 967; P < 0.05%), particulate organic carbon and silt (r = 0.949; P < 0.05%), ferrous and particulate organic carbon (r = 0.924; P < 0.05%), Zing and cadmium (r = 0.675; P < 0.05%) magnesium nickel and magnesium (r = 0.992; P < 0.05%), total actinobacterial population and manganese (r = 0.827; P < 0.05%), magnesium and chromium (r = 0.988; P < 0.05%), ing an copper (r = 0. 747; P < 0.05%), ferrous and manganese (r = 0.684; P < 0.05%), phosphorus and nickel (0.698; P < 0.05%) (Table 3). Similar type of study was reported by Mansour 2003; Lakshmanaperumalsamy et al.1986; Jiang and Xu1996; Saadoun and Al-Momoni1996 has studied the pH, moisture, organic matter, nitrogen and phosphorous content of the soils and correlated with actinomycetes population. The correlation between salinity, pH and organic content of mangrove sediments and actinobacterial population has been reported by Sivakumar 2001. That the variation in temperature, pH and dissolved phosphate showed insignificant values, but variation in total nitrogen and organic matter was significant in the population in Alexandria. Hence it could be concluded that though actinomycetes are ubiquitous, their population dynamics are often influenced by the available nutrients and the physico-chemical conditions of the ecosystem.

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