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Isolation, characterization and identification of actinobacteria of Mangrove ecosystem Ennoor, east coast of Tamil Nadu, India

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ABSTRACT

A study on marine actinobacteria and physicochemical characteristics of soil in marine environment of Ennoor, East Coast of Tamilnadu, India, was performed. The marine soil were selected for sampling and the following parameters were recorded at monthly intervals, The following Parameters like pH, Electrical conductivity, Organic carbon, available nitrogen, available phosphorus, available potassium, available zinc, available copper, available iron, available manganese, Cation exchange capacity, Calcium, Magnesium, Sodium, Potassium, were studied. Totally 21 actinobacteria strains were screened and identified as genus Actinokineospora, Actinopolyspora, Amycolata, Glycomyces, Microbispora, Microtetraspora, Micropolyspora, Nocardia, Nocardiopsis(2), Promicromonospora, Saccharothrix(2), Saccharopolyspora, Streptomyces microflavus, Streptomyces(4), Streptoverticillium, Spirillospora and Thermomonospora.

Keyword: Marine environment, Soil characteristics, Soil Actinomycetes.

INTRODUCTION

Mangrove are of great ecological, economic and social significance. Further, mangroves occurring along estuaries, back waters and the deltas function as the most important links between the land and sea. Such mangrove forests are estimated to cover an area of about 17 million hectares in world wide. The total area of mangroves in India is estimated to be 6,740 sq. km. In Tamil Nadu, mangrove coverage is about 150 sq. km at Pichavaram and Muthupet (Ajith,1998). Members of the actinobacteria, which live in Marine environment, are poorly understood and only few reports are available pertaining to actinobacteria from mangroves (Sivakumar, 2001).

Marine environments are largely untapped source for the isolation of new microorganisms with potentiality to produce active secondary metabolites (Baskaran *et al*., 2011). Among such Microorganisms, actinomycetes are of special interest, since they are known to produce chemically diverse compounds with a wide range of biological activities (Bredholt *et al.*, 2008).

Actinobacteria form part of the marine microbial community of sediment samples originated from terrestrial habitats and were simply carried out to sea in the form of resistant spores (Goodfellow and Haynes, 1984). Many commercially important bioactive compounds and antitumor agents in addition to enzymes of industrial interest have been produced from actinobacteria (Imasda, 2005). It has been estimated that approximately 203 of the naturally occurring antibiotics have been isolated from these organisms (Takizawa *et al*., 1993).

In recent years there has been a growing awareness of the potential value of marine water habitat as source of actinomycetes that produce useful metabolic products. Actinomycetes are the most economically and biotechnologically valuable prokaryotes. They are responsible for the production of about half of the discovered bioactive secondary metabolites **(**Berdy, 2005), antitumour agent (Cragg *et al.*, 2005), notably antibiotics (Strochl, 2004), immunosuppressive agents (Mann, 2001) and enzymes (Old field, 1998).

MATERIALS AND METHODS

Sample Collection

The marine soil samples were collected from mangrove environment of Ennoor, Tamil Nadu, India. The soil samples were collected in random in sterile polythene bags to avoid external contamination. The samples were collected from 6 inches from the soil surface, in order to avoid the contamination. The collected soil samples were brought to the laboratory and stored in refrigerator for further use.

Physico – chemical analysis of soil:

Moisture content was estimated for a known quantity of soil before and after drying in a hot air oven at 60°C for 6 hours. Soil samples after removing the debris were suspended in distilled water (1:2 w/v) and allowed to settle down the sand particles. The pH of the suspension was read using pH meter (Systronics, India), to find out the soil pH. Electrical conductivity of soil was determined in the filtrate of the water extract using conductivity bridge as described by Jackson (1973), Cation exchange capacity (CEC) of the soil was determined by using 1 N ammonium acetate solution as described by Jackson (1973).

Organic carbon content was determined by adopting chromic acid wet digestion method as described by Walkley and Black (1934); available nitrogen was estimated by alkaline permanganate method as described by Subbiah and Asija (1956) and available phosphorus by Brayl method as described by Bray and Kutz (1945). Available potassium was extracted from soil with neutral 1 N ammonium acetate (1:5) and the potassium content in the extract was determined by using flame photometer (Standfold and English, 1949). Calcium (Neutral 1 N NH4 OAC extractable 1:5) was extracted with neutral 1 N ammonium acetate and the available calcium in the extract was determined by versenate method (Jackson, 1973). Available micronutrients such as Zn, Cu and Mn were determined in the diethylene triamine pentaacetic extract of soil using Perkin-Elmer (model 2280) Atomic Absorption Spectrophotometer (Lindsay and Norvell, 1978). Other nutrients such as magnesium, sodium and available iron were analysed following the method of Barnes (1959) and Muthuvel and Udayasoorian (1999).

Isolation of Actinomycetes

Isolation of actinomycetes was performed by plating technique using starch casein agar (Kuster and Williams, 1964) medium. The medium was prepared and sterilized at 121°C in 15 lbs pressure for 15 minutes. Then it was supplemented with Griseofulvin and streptomycin to prevent the bacterial and fungal growth. The medium was poured into the sterile petriplates. The collected soil samples were diluted upto 10^{-6} and 0.1ml of the diluted samples was spread over the agar plates. The inoculated plates were incubated at 28 ± 2°C for 7 – 10 days. After incubation actinomycetes colonies were observed, and used for further investigation**(**Porter *et al.*, 1960). Streak plate method was used to purify the culture of actinomycetes. After inoculation, the plates were incubated at 28 ± 2°C for 7 – 10 days and were maintained in starch casein agar medium and stored at 4°C for further investigation.

Characterization of Actinomycetes (Coverslip Culture Technique)

Actinomycetes culture plates was prepared and 2-4 sterile coverslips were inserted at an angle of 45°C. The actinomycetes culture was slowly released at the intersection of medium and coverslip. The plates were incubated at 28 ± 2°C for 4-8 days. The cover slips were removed and observed under the high power magnification. The photomicrography was taken using Nikon Microscope. The morphological features of spores, sporangia and aerial and substrate mycelium were observed and recorded. Among the isolate, predominate organisms were selected for further studies (Pridham *et al.,* 1958).

Colony characteristics

Colony morphology was recorded with respect to colour, aerial mycelium, size and nature of colony, reverse side colour and pigmentation. The isolates were observed under the Nikon Binocular microscope **(**Burholder *et al.,* 1954).

RESULTS AND DISCUSSION

Isolation and Identification of Actinomycetes

A total of 21 actinomycetes were isolated from Ennoor. Morphological studies indicated that the strains belonged to the genera, *Actinokineospora*, *Actinopolyspora*, *Amycolata*, *Glycomyces*, *Microbispora*, *Microtetraspora*, *Micropolyspora*, *Nocardia*, *Nocardiopsis*(2), *Promicromonospora*, *Saccharothrix*(2), *Saccharopolyspora*, *Streptomyces microflavus*, *Streptomyces*(4), *Streptoverticillium*, *Spirillospora* and *Thermomonospora.* Balagurunathan *et al.,* (1996) reported most of the genus identified from this study from south Indian soil. The similar type of work has been reported by many workers including Dhanasekaran *et al*.,2008.

Table:1 Number of Colonies, Mean Density (CFU/g) and Percentage Contribution of Actinomycetes Recorded in Ennoor

S. No.	Name of the Actinomycetes	Oct 2011 to Sep 2012																						**Total no. Of Colonies**	**% concentration**
		OCT		NOV		DEC		JAN		FEB		MAR		APR		MAY		JUNE		JULY		AUG		SEP			
		TNC	**MD**	**TNC**	**MD**	**TNC**	**MD**	**TNC**	**MD**	**TNC**	**MD**	**TNC**	**MD**	**TNC**	**MD**	**TNC**	**MD**	**TNC**	**MD**	**TNC**	**MD**	**TNC**	**MD**	**TNC**	**MD**		
1.	*Actinokineospora* sp	2	0.67	0	0	3	1.00	2	0.67	2	0.67	2	0.67	2	0.67	0	0	0	0	3	1.00	2	0.67	0	0	18	4.45
2.	*Actinopolyspora* sp	0	0	3	1.00	0	0	2	067	0	0	2	0.67	3	1.00	0	0	2	0.67	0	0	0	0	2	0.67	14	2.62
3.	*Amycolata* sp	3	1.00	0	0	2	0.67	0	0	2	0.67	3	1.00	0	0	0	0	2	0.67	0	0	2	0.67	3	1.00	17	3.18
4.	*Glycomyces* sp	3	1.00	2	0.67	3	1.00	2	0.67	0	0	2	0.67	3	1.00	0	0	2	0.67	0	0	2	0.67	0	0	19	3.55
5.	*Microbispora* sp	0	0	2	0.67	2	0.67	3	1.00	0	0	2	0.67	2	0.67	0	0	0	0	2	0.67	2	0.67	2	0.67	17	3.18
6..	*Microtetraspora* sp	2	0.67	3	1.00	2	0.67	0	0	0	0	0	0	2	0.67	2	0.67	2	0.67	0	0	3	1.00	2	0.67	18	3.37
7.	*Micropolyspora* sp	4	1.33	0	0	2	0.67	0	0	2	0.67	0	0	3	1.00	2	0.67	2	0.67	2	0.67	2	0.67	0	0	19	3.55
8.	*Nocardia* sp	0	0	0	0	0	0	0	0	0	0	4	1.33	2	0.67	3	1.00	0	0	3	1.00	2	0.67	2	0.67	16	2.99
9.	*Nocardiopsis* sp	4	1.33	3	1.00	2	0.67	2	0.67	0	0	3	1.00	2	0.67	3	1.00	2	0.67	3	1.00	0	0	2	0.67	26	6.43
10.	*Nocardiopsis* sp	2	0.67	2	0.67	0	0	2	0.67	2	0.67	0	0	0	0	0	0	2	0.67	3	1.00	2	0.67	0	0	15	3.71
11.	*Promicromonospora* sp	2	0.67	0	0	0	0	3	1.00	2	0.67	2	0.67	3	1.00	0	0	2	0.67	3	1.00	0	0	0	0	15	3.71
12.	*Saccharothrix* sp	2	0.67	0	0	2	0.67	2	0.67	2	0.67	0	0	0	0	2	0.67	0	0	2	0.67	0	0	2	0.67	14	3.46
13.	*Saccharothrix* sp	2	0.67	2	0.67	0	0	0	0	3	1.00	2	0.67	2	0.67	0	0	0	0	2	0.67	0	0	3	1.00	16	3.96
14.	*Saccharopolyspora* sp	2	0.67	0	0	0	0	2	0.67	3	1.00	0	0	2	0.67	0	0	2	0.67	0	0	2	0.67	2	0.67	15	3.71
15.	*Streptomyces microflvus*	0	0	2	0.67	4	1.33	3	1.00	2	0.67	2	0.67	3	1.00	2	0.67	3	1.00	2	0.67	2	0.67	2	0.67	27	6.68
16.	*Streptomyces* sp	2	0.67	3	1.00	3	1.00	2	0.67	3	1.00	2	0.67	4	1.33	0	0	2	0.67	3	1.00	2	0.67	0	0	26	6.43
17.	*Streptomyces* sp	0	0	2	0.67	3	1.00	0	0	0	0	2	0.67	3	1.00	3	1.00	0	0	0	0	2	0.67	3	1.00	18	4.45
18.	*Streptomyces* sp	2	0.67	2	0.67	2	0.67	0	0	0	0	2	0.67	2	0.67	0	0	0	0	2	0.67	3	1.00	2	0.67	17	4.20
19.	*Streptoverticillium* sp	2	0.67	2	0.67	3	1.00	2	0.67	2	0.67	2	0.67	3	1.00	2	0.67	2	0.67	2	0.67	3	1.00	2	0.67	27	6.68
20.	*Spirillospora* sp	0	0	2	0.67	0	0	2	0.67	3	1.00	0	0	0	0	2	0.67	4	1.33	0	0	2	0.67	3	1.00	18	4.45
21.	*Thermomonospora* sp	4	1.33	2	0.67	2	0.67	3	1.00	2	0.67	2	0.67	3	1.00	3	1.00	3	1.00	3	1.00	3	1.00	2	0.67	32	7.92
	Total	38	12.69	32	10.7	35	11.69	32	10.7	30	10.03	34	11.37	44	14.69	24	8.02	32	10.7	35	11.69	36	11.37	36	11.37	404	

TNC – Total number of colonies, MD – Mean density

Table-2 Analysis of physico - chemical parameters of marine soil samples from Ennoor

		October 2011 to September 2012											
S. No	Name of the Parameters	Oct	Nov	Dec	Jan	Feb	Mar	Apr	Mar	Jun	July	Aug	Sep
1.	pH	7.63	7.56	7.69	7.53	7.58	7.45	7.62	7.51	7.44	7.41	7.22	7.43
2.	Electrical conductivity (dsm-1)	0.52	0.48	0.51	0.49	0.48	0.41	0.50	0.49	0.45	0.46	0.46	0.54
3.	Organic carbon (%)	0.60	0.44	0.36	0.56	0.51	0.58	0.52	0.58	0.50	0.44	0.49	0.59
4.	Available nitrogen (mg/kg)	2.93	2.74	3.08	2.90	3.29	2.90	2.93	2.52	2.84	2.91	2.77	2.90
5.	Avaible phosphorus (mg/kg)	1.18	1.12	1.2	1.28	1.20	1.08	1.11	1.1	1.11	1.19	1.25	1.22
6.	Available potassium (mg/kg)	5.00	4.82	5.03	4.86	5.34	5.31	5.06	4.55	4.13	3.86	4.31	5.34
7.	Available zinc (ppm)	0.85	0.89	0.68	0.53	0.67	0.68	0.64	0.61	0.63	0.59	0.68	0.71
8.	Available copper (ppm)	1.05	1.06	1.09	1.97	0.83	0.85	1.08	0.76	0.53	0.57	0.68	1.15
9.	Available iron (ppm)	8.23	8.23	2.69	3.48	2.46	4.34	8.54	8.37	7.36	8.54	8.16	8.17
10.	Available manganese(ppm)	3.69	3.10	3.29	3.15	3.65	3.62	3.35	3.26	3.57	3.61	3.43	3.51
11.	Cation exchange capacity (C.Mole proton/kg)	21.5	23.8	23.4	22.5	21.6	20.5	21.6	20.9	19.9	18.2	16.3	21.4
12.	Calcium (mg/kg)	11.2	11.6	10.8	11.5	11.2	11.5	12.3	12.8	11.1	10.6	10.5	12.6
13.	Magnesium(mg/kg)	8.7	8.5	9.2	8.9	8.4	8.6	8.6	7.6	7.9	7.2	6.7	8.9
14.	Sodium(mg/kg)	2.25	2.14	2.49	2.98	2.57	2.23	2.36	1.69	1.89	1.72	1.33	2.19
15.	Potassium(mg/kg)	0.15	0.18	0.23	0.16	0.25	0.26	0.28	0.16	0.14	0.16	0.14	0.14
	TNC	38	32	35	32	30	34	44	24	32	35	36	36

Table:3 Percentage frequency and frequency class of different species of Actinomycetes recorded in Ennoor (n=4)

S. No.	Name of the actinomycetes	No. of seasons in which the Actinomycetes occured	Percentage Frequency	Frequency Class
1.	*Actinokineospora* sp	8	66.66	F
2.	*Actinopolyspora* sp	6	50.00	O
3.	*Amycolata* sp	7	58.33	F
4.	*Glycomyces* sp	8	66.66	F
5.	*Microbispora* sp	8	66.66	F
6.	*Microtetraspora* sp	8	66.66	F
7.	*Micropolyspora* sp	8	66.66	F
8.	*Nocardia* sp	6	50.00	O
9.	*Nocardiopsis* sp	10	83.33	C
10.	*Nocardiopsis* sp	7	58.33	F
11.	*Promicromonospora* sp	7	58.33	F
12.	*Saccharothrix* sp	7	58.33	F
13.	*Saccharothrix* sp	7	58.33	F
14.	*Saccharopolyspora* sp	7	58.33	F
15.	*Streptomyces microflvus*	11	91.66	C
16.	*Streptomyces* sp	10	83.33	C
17.	*Streptomyces* sp	7	58.33	C
18.	*Streptomyces* sp	8	66.66	C
19.	*Streptoverticillium* sp	12	100.00	C
20.	*Spirillospora* sp	7	58.33	F
21.	*Thermomonospora* sp	12	100.00	O

R - Rare (0-25%); O - Occasional (26-50%); F – Frequent (51-75%); C – Common (76-100%)

Table: 4 The correlation coefficient between the physico-chemical characters and total number of colonies at Ennoor

	PH	EC	OC	AN	APH	APO	AZ	AC	AI	AM	CATION	CALCIUM	MAGNE	SODIUM	POTASS	TNC
PH	1															
EC	.457	1														
OC	-.142	.085	1													
AN	.385	.109	-.252	1												
APH	-.213	.388	-.063	.330	1											
APO	.506	.341	.329	.451	.000	1										
AZ	.233	.230	-.036	-.026	-.197	.302	1									
AC	.389	.436	.207	.123	.469	.468	-.100	1								
AI	-.362	.141	.199	-.667*	-.259	-.467	.273	-.373	1							
AM	-.195	-.212	.282	.426	-.044	.011	.040	-.533	.017	1						
CATION	.853**	.451	-.081	.219	-.160	.616*	.317	.591*	-.382	-.463	1					
CALCIUM	.220	.394	.571	-.393	-.366	.422	-.019	.249	.272	-.294	.388	1				
MAGNE	.765**	.449	.089	.435	-.008	.783**	.228	.655*	-.488	-.169	.872**	.315	1			
SODIUM	.700*	.304	.077	.582*	.214	.662*	-.075	.785**	-.682(*)	-.194	.762**	.145	.864**	1		
POTASS	.497	-.197	-.165	.509	-.375	.560	-.048	.044	-.476	.033	.344	.091	.406	.443	1	
TNC	.096	.213	-.072	.307	.121	.168	.181	.113	.231	.205	-.079	-.146	.205	.113	.286	1

** Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed).*

In the present study a total of 21 actinomycetes isolates recorded including different locations in marine soils of Ennoor, Tamilnadu. Mean population density of actinomycetes varied from 8.02 to 14.69 × 10^6 CFU/g. Most of the actinomycetes strains belonging to the genera *Thermomonospora*, 32 X 10^{-6} CFU. g-1(7.92%) *Streptomyces microflavus*, 27 X 10^{-6} CFU . g-1 (6.68%) *Streptoverticillium*, 27 X 10^{-6} CFU. g-1 (6.68%), *Streptomyces* sp, 22 X 10^{-6} CFU. g-1 (6.43%), *Nocardiopsis* sp, 26 X 10^{-6} CFU. g-1 (6.43%) and the minimum level of *Actinopolyspora* sp, 14 X 10^{-6} CFU. g-1 (2.62%), *Saccharothrix* sp 14 X 10^{-6} CFU. g-1 (3.46%) were recorded (Table – 1).

Percentage contribution of the individual species to the total actinomycetes population at all the seasons showed variation. The maximum percentage contribution of 7.92% was found with *Thermomonospora* sp. This was followed by *Streptomyces microflavus* (6.68% each); *Streptoverticillium; Nocardiopsis* sp (6.23% each)*; Streptomyces* sp; *Actinokineospora* sp, (4.45% each)*; Streptomyces* sp, *Spirillospora* sp; *Streptomyces* sp (4.20%); *Saccharothrix* sp (3.96%); *Nocardiopsis* sp (3.71% each)*; Promicromonospora* sp, *Saccharopolyspora* sp ; *Micropolyspora* sp (3.55); *Saccharothrix* sp (3.46%); *Amycolata* sp (3.18% each)*; Microbispora* sp; *Nocardia* sp (2.99%) and *Actinopolyspora* sp (2.62%) (Table -1).

Soil characteristics such as pH 7.22 to 7.69, electrical conductivity 0.41 to 0.54 dSm^{-1}, cation exchange capacity 16.3 to 23.8 c.mol proton+/kg, organic carbon 0.36 to 0.60%, nitrogen 2.74 to 3.29 (mg / kg), phosphorus 1.1 to 1.28 (mg / kg), potassium 4.13 to 5.34 (mg / kg), zinc 0.53 to 0.89 ppm, copper 0.53 to 1.97 ppm, iron 2.46 to 8.54 ppm, manganese 3.10 to 3.69 ppm, calcium 10.5 to 12.8 (C. Mole Proton+ / kg), magnesium 6.7 to 9.2 (C. Mole Proton+ / kg), sodium 1.33 to 2.98 (C. Mole Proton+ / kg) and potassium 0.14 to 0.28 (C. Mole Proton+ / kg), showed variation during different seasons. Relationship between load of *Actinomycetes* and soil physicochemical properties like soil temperature, pH, organic carbon, available nitrogen, phosphorous and potassium etc. were calculated. The distribution of soil microbial population is determined by a number of environmental factors like pH, moisture content and soil organic matter etc. Kennedy *et al*, 2005 (Table-2).

Physico-chemical properties of sediment and total Actinobacterial population (TAP). It revealed that the significant positive correlation between available cation ion exchange capacity and pH ($r = 0.853$;$P < 0.01\%$), magnesium and pH($r = 0.765$; $P < 0.01\%$), sodium and pH($r = 0.700$;$P < 0.05\%$), available iron and organic nitrogen ($r = 0.667$; $P < 0.05\%$), available manganese and avilable nitrogen ($r = 0.692$; $P < 0.05\%$), sodium and available nitrogen ($r = 0.582$; $P < 0.01\%$), cation ion exchange capacity and available potassium ($r = 0.616$; $P < 0.05\%$), cation ion exchange capacity and available potassium($r = 0.783$; $P < 0.01\%$), cation ion exchange capacity and available potassium ($r = 0.662$; $P < 0.05\%$), cation ion exchange capacity and available copper ($r = 0.591$; $P < 0.05\%$), magnesium and available copper ($r = 0.655$; $P < 0.05\%$), sodium and available copper($r = 0.785$; $P < 0.01\%$), sodium and available iron($r = 0.682$; $P < 0.05\%$), magnesium and cation ion exchange capacity ($r = 0.872$; $P < 0.01\%$), sodium and cation ion exchange capacity($r = 0.762$; $P < 0.01\%$), sodium and magnesium ($r = 0.864$; $P < 0.01\%$),(Table-4) Similar type of study was reported by Lakshmanaperumalsamy *et al*.,1986; Jiang and Xu,1990, correlated with actinomycetes population. The correlation between salinity, pH and organic content of marine sediments and actinomycetes population has been reported by several workers Ndonde, 2000 and Jensen, 1991. 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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