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Original Research Article

Actinobacterial diversity of mangrove environment of the Palaverkadu mangroves, east coast of Tamil Nadu, India

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A B S T R A C T

Twenty one actinomycetes strains were isolated from mangrove environment of Palaverkadu,Tamil Nadu,India.The physico-chemical characteristics of soil samples were analysed. Morphological studies indicated that the strains belonged to the genera. *Actinomadura*(2), *Actinokineospora, Catellospora, Kitasatosporia, Nocardia, Nocardiopsis(4), Planobispora, Planomospora, Terrabactor, Saccharothrix, Streptomyces*(3), *Streptomyces albus, Steptomyces annulatus, Streptosporangium, Streptoverticillium* were screened. Many natural products have been isolated from marine environments. However, only a small fraction of them was derived from marine microorganisms. Marine actinomycetes are antibiotic producers with diverse biological activities. In the present investigation, an effort was made to screen different marine sediments which is a large unscreened and diverse ecosystem for the isolation of potent antibiotic producing Actinomycetes.

K e y w o r d s

Mangrove environment; Soil characteristics; Marine Actinomycetes.

Introduction

Marine actinomycetes constitute an important and potential source of novel bioactive compounds. Since environmental conditions of the sea are extremely different from terrestrial conditions, they produce different types of antibiotics. Almost 80% of the world's antibiotics are known to come from actinomycetes, mostly from the genera *Streptomyces* and *Micromonospora* (Pandey *et al.*,2004). Actinomycetes have gained prominence in recent years because of their potential for producing antibiotics (Kumar *et al.*, 2005). Streptomycin, gentamicin, rifamycin are some of the antibiotics which are in use presently and erythromycin are the product of actinomycetes. The actinomycetes are important in the field of pharmaceutical industries and also the agriculture. Previous study showed that actinomycetes isolated from Malaysia soil have the potential to inhibit the growth of several plant pathogens (Jeffrey *et al.*, 2007). Actinomycetes, isolate from marine source are of terrestrial origin. Sixty-three of the

isolated actinomycetes were tested for the effects of seawater on growth. *Streptomycetes* growth in nonsaline media was reduced by 39% compared with that in sea water. The actinoplanetes had a near obligate requirement of seawater of growth, and this is presented as evidence that actinomycetes can be physiologically active in the marine environment. Problems encountered with the enumeration of actinomycetes in marine sediments are also discussed. (Jensen, *et al.,2005*). Most of the microbial antibiotics discovered so far are originated from actinomycete bacteria, only a few of them from soil derived genera (*Streptomyces* and *Micromonospora*). Actinomycetes produce a wide range of secondary metabolites and more than 70% of the naturally derived antibiotics that are currently in clinical use are derived from marine actinomycetes (Pimentel-Elardo *et al*., 2009).

Since marine sediments represent an environment which is markedly different from that associated with soil samples, it is not clear how effective the pre-treatment of such sediments would be for the recovery of bioactive actinomycetesMarine sediment is an inexhaustible resource that has not been properly exploited. Few reports from the East Coast of India, suggests that soil is a major source of Actinomycetes (Sivakumar *et al.*, 2005; Vijayakumar *et al.*, 2007; Dhanasekaran *et al.*, 2008).

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 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 Palaverkadu, 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, 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 isolated from palaverkadu. Morphological studies indicated that the strains belonged to the genera, *Actinomadura* (2), *Actinokineospora, Catellospora, Kitasatosporia, Nocardia, Nocardiopsis* (4), *Planobispora, Planomospora, Terrabactor, Saccharothrix, Streptomyces*

(3), *Streptomyces albus, Steptomyces annulatus, Streptosporangium, Streptoverticillium*.

The diversity of actinomycetes isolated from different location in forest research Institute Malaysia (FRIM) and its various sub-stations, and to establish a collection of diverse actinomycete groups to form the basis for future research and development in drug discovery. A total of 273 soil samples were collected from natural forest areas in FRIM, kepong and its sub-stations. More than 2000 isolates of actinomycetes were isolated and they were classified into five genera *(Streptomyces, Micromonospora, Actinoplanes, Nocardia* and *Digosparic type)* (Getha *et al.*, 2004).

In the present study a total of 21 actinomycetes isolates recorded including different locations in marine soils of Palaverkadu, Tamilnadu. Mean population density of actinomycetes varied from 9.7 to 14.71 × 10^6 CFU/g. Most of the actinomycetes strains belonging to the genera *Planobispora* sp 30 X 10^{-6} CFU. g-1(7.37%) *Nocardiopsis* sp 25 X 10^{-6} CFU . g-1 (6.14 %) *Saccharothrix* sp 18 X 10^{-6} CFU. g-1 (4.42 %); *Nocardia* sp and the minimum level of *Jonesia* sp were recorded (12 X 10^{-6} CFU. g-1 (2.94 %) (Table -1).

Actinomycetes load varied between 4.1×102 and 26×103 CFU/g in post monsoons, from 17×103 to 44×103 CFU/g in pre monsoon and from 3.0×102 to17×103 CFU/g in monsoon (Varghese *et al.*, 2012). Percentage contribution of the individual species to the total actinomycetes population at all the seasons showed variation. The maximum percentage contribution of 7.367% was found with *Planobispora* sp. This was followed by *Nocardiopsis* sp (6.14%); *Catellospora* sp (5.89%) and *Nocardiopsis* sp (5.40% each *); Streptomyces* sp and *Streptoverticillium* sp (5.94 % each); *Kitasatosporia* sp and *Planonobispora* sp (4.91% each *); Steptomyces annulatus* and *Actinokineospora* sp (4.66% each); *Nocardiopsis* sp, *Streptomyces albus*, *Streptomyces* and *Streptosporangium*, *Nocardia* sp (4.24% each); *Saccharothrix* sp and *Nocardiopsis* sp(4.17%) *and Steptomyces* sp(3.68%) and *Actinomadura* sp (3.43%) and *Planomospora* sp (3.19%) and *Actinomadura* sp (2.94%) (Table: 1).

Soil characteristics such as pH 7.21 to 7.56, electrical conductivity 0.21 to 0.42 dSm^{-1}, cation exchange capacity 19.7 to 23.8 c.mol proton+/kg, organic carbon 0.31 to 0.52%, nitrogen 76.1 to 84.2 (Kg / ac), phosphorus 3.25 to 3.76(Kg / ac), potassium 112 to 148 (Kg / ac), zinc 0.61 to 1.06 ppm, copper 0.47 to 1.83 ppm, iron 7.23 to 8.23 ppm, manganese 2.26 to 3.68 ppm, calcium 9.4 to 12.5 (C. Mole Proton+ / kg), magnesium 7.3 to 8.9 (C. Mole Proton+ / kg), sodium 1.17 to 2.43 (C. Mole Proton+ / kg) and potassium 0.16 to 0.25 (C. Mole Proton+ / kg), showed variation during different seasons. Similar type of study was reported by Sivakumar (2001) the correlation between salinity, pH and organic content of mangrove sediments and actinobacterial population has been reported (Table-2)

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Physico-chemical properties of sediment and total Actinobacterial population (TAP), revealed that the significant positive correlation between available manganese and pH ($r = 0.619$;$P < 0.05\%$) organic carbon and electrical conductivity ($r = 0.628$; $P < 0.05\%$), available copper and electrical conductivity ($r = 0.704$;$P < 0.05\%$),available zinc and organic carbon ($r = 0.583$; $P < 0.05\%$), available

Table.1 Number of Colonies, Mean Density (CFU/g) and Percentage Contribution of Actinomycetes recorded in Palaverkadu

Oct 2011 to Sep 2012																										Total no. Of Colonies	%Contribution
S. No.	Name of the Actinomycetes	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.	*Actinomadura* sp	2	0.67	0	0	2	0.67	0	0	2	0.67	2	0.67	0	0	2	0.67	0	0	2	0.67	0	0	2	0.67	14	3.43
2.	*Actinomadura* sp	2	0.67	0	0	3	1.00	0	0	2	0.67	0	0	3	1.00	2	0.67	0	0	0	0	0	0	0	0	12	2.94
3.	*Actinokineospora* sp	0	0	0	0	4	1.33	2	0.67	2	0.67	2	0.67	2	0.67	3	1.00	2	0.67	0	0	0	0	2	0.67	19	4.66
4.	*Catellospora* sp	3	1.00	2	0.67	0	0	2	0.67	2	0.67	2	0.67	2	0.67	0	0	2	0.67	3	1.00	4	1.33	2	0.67	24	5.89
5.	*Kitasatospori*a sp	3	1.00	2	0.67	0	0	0	0	2	0.67	3	1.00	2	0.67	0	0	2	0.67	2	0.67	3	1.00	2	0.67	21	5.15
6..	*Nocardia* sp	2	0.67	0	0	0	0	0	0	2	0.67	3	1.00	2	0.67	3	1.00	2	0.67	2	0.67	0	0	2	0.67	18	4.42
7.	*Nocardiopsis* sp	2	0.67	3	1.00	2	0.67	0	0	2	0.67	4	1.33	2	0.67	0	0	2	0.67	2	0.67	2	0.67	4	1.33	25	6.14
8.	*Nocardiopsis* sp	2	0.67	3	1.00	2	0.67	3	1.00	0	0	2	0.67	2	0.67	0	0	2	0.67	2	0.67	2	0.67	2	0.67	22	5.40
9.	*Nocardiopsis* sp	0	0	2	0.67	0	0	2	0.67	0	0	2	0.67	3	1.00	2	0.67	2	0.67	2	0.67	2	0.67	0	0	17	4.17
10.	*Nocardiopsis* sp	2	0.67	0	0	4	1.33	3	1.00	0	0	0	0	2	0.67	0	0	4	1.33	2	0.67	0	0	2	0.67	19	4.66
11.	*Planobispora* sp	3	1.00	2	0.67	2	0.67	2	0.67	3	1.00	3	1.00	3	1.00	3	1.00	2	0.67	3	1.00	2	0.67	2	0.67	30	7.37
12.	*Planomonospora* sp	2	0.67	2	0.67	0	0	4	1.33	0	0	2	0.67	4	1.33	2	0.67	0	0	0	0	2	0.67	2	0.67	20	4.91
13.	*Terrabacter* sp	2	0.67	0	0	0	0	3	1.00	0	0	2	0.67	2	0.67	0	0	0	0	0	0	2	0.67	2	0.67	13	3.19
14.	*Saccharothrix* sp	2	0.67	4	1.33	2	0.67	2	0.67	0	0	0	0	2	0.67	0	0	2	0.67	2	0.67	0	0	2	0.67	18	4.42
15.	*Streptomyces* albus	2	0.67	2	0.67	0	0	3	1.00	2	0.67	0	0	0	0	4	1.33	2	0.67	2	0.67	0	0	2	0.67	19	4.66
16.	*Streptomyces* annulatus	2	0.67	2	0.67	3	1.00	0	0	3	1.00	2	0.67	3	1.00	3	1.00	0	0	2	0.67	0	0	0	0	20	4.91
17.	*Streptomyces* sp	2	0.67	2	0.67	3	1.00	0	0	0	0	2	0.67	2	0.67	2	0.67	2	0.67	0	0	2	0.67	2	0.67	19	4.66
18.	*Streptomyces* sp	2	0.67	3	1.00	3	1.00	3	1.00	0	0	3	1.00	2	0.67	2	0.67	2	0.67	0	0	2	0.67	0	0	22	5.40
19.	*Streptomyces* sp	0	0	3	1.00	2	0.67	2	0.67	2	0.67	0	0	2	0.67	2	0.67	0	0	0	0	2	0.67	0	0	15	3.68
20.	*Streptosporangium* sp	3	1.00	2	0.67	0	0	2	0.67	2	0.67	0	0	2	0.67	0	0	2	0.67	2	0.67	2	0.67	2	0.67	19	4.66
21	*Streptoverticillium* sp	3	1.00	2	0.67	0	0	2	0.67	2	0.67	2	0.67	2	0.67	0	0	2	0.67	2	0.67	2	0.67	2	0.67	21	5.15
	TNC	41	13.71	36	13.03	32	10.68	35	11.34	28	9.37	36	12.03	44	14.71	30	10.02	32	10.71	30	10.04	29	9.7	34	11.38	407	

TNC – Total number of colonies, MD – Mean density

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

October 2011		**to**						**September 2012**					
S.No	Name of the Parameters	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep
1.	pH	7.50	7.42	7.50	7.47	7.56	7.42	7.31	7.21	7.45	7.33	7.45	7.32
2.	Electrical conductivity (dsm-1)	0.36	0.32	0.29	0.22	0.31	0.30	0.31	0.38	0.26	0.35	0.21	0.42
3.	Organic carbon (%)	0.52	0.49	0.39	0.36	0.31	0.40	0.42	0.49	0.50	0.51	0.33	0.48
4.	Available nitrogen (Kg/ac)	84.2	84.1	83.1	83.5	79.3	77.1	78.2	76.1	77.3	78.5	81.4	83.6
5.	Avaible phosphorus (Kg/ac)	3.50	3.76	3.38	3.25	3.46	3.76	3.65	3.64	3.42	3.58	3.47	3.53
6.	Available potassium (Kg/ac)	140	146	135	142	148	136	144	146	132	124	112	127
7.	Available zinc (ppm)	0.89	0.85	0.62	0.68	0.73	0.69	0.61	0.74	1.06	0.74	0.63	0.67
8.	Available copper (ppm)	0.96	0.98	0.86	0.53	0.56	0.67	0.79	0.85	0.79	0.54	0.47	1.83
9.	Available iron (ppm)	7.62	8.23	7.96	7.23	7.46	7.24	7.27	7.62	7.22	7.34	7.58	7.51
10.	Available manganesh(ppm)	3.15	3.68	3.65	3.25	3.56	3.21	3.02	2.26	2.45	2.73	3.12	3.27
11.	Cation exchange capacity (C.Mole proton/kg)	23.8	21.5	27.6	22.3	22.2	21.8	21.1	19.7	20.6	19.9	20.4	22.5
12.	Calcium (mg/kg)	11.2	11.5	11.6	12.3	11.7	10.1	12.5	11.8	10.5	9.4	9.6	11.9
13.	Magnesium(mg/kg)	8.9	8.7	8.9	8.2	7.9	8.1	7.3	8.2	8.7	7.5	7.4	8.1
14.	Sodium(mg/kg)	2.35	2.25	2.18	2.22	2.43	2.13	2.31	1.17	2.26	2.21	2.11	2.41
15.	Potassium(mg/kg)	0.19	0.21	0.23	0.18	0.25	0.25	0.25	0.19	0.15	0.16	0.18	0.22
	TNC	41	36	32	35	28	36	44	30	32	30	29	34

TNC-Total number of colonies

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

S.No.	Name of the actinomycetes	No. of seasons in which the Actinomycetes occured	Percentage Frequency	Frequency Class
1.	*Actinomadura* sp	7	58.33	F
2.	*Actinomadura* sp	5	41.66	O
3.	*Actinokineospora* sp	8	66.66	F
4.	*Catellospora* sp	10	83.33	C
5.	*Kitasatospori*a sp	9	75.00	C
6.	*Nocardia* sp	8	66.66	F
7.	*Nocardiopsis* sp	10	83.33	C
8.	*Nocardiopsis* sp	10	83.33	C
9.	*Nocardiopsis* sp	8	66.66	F
10.	*Nocardiopsis* sp	7	58.33	F
11.	*Planobispora* sp	12	100.00	C
12.	*Planomonospora* sp	8	66.66	F
13.	*Terrabacter* sp	6	50.00	O
14.	*Saccharothrix* sp	8	66.66	F
15.	*Streptomyces* albus	8	66.66	F
16.	*Streptomyces* annulatus	8	66.66	F
17.	*Streptomyces* sp	9	75.00	F
18.	*Streptomyces* sp	9	75.00	F
19.	*Streptomyces* sp	7	58.33	F
20.	*Streptosporangium* sp	9	75.00	F
21.	*Streptoverticillium* sp	10	83.33	C

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 Palaverkadu

	PH	EC	OC	AN	APH	APO	AZ	AC	AI	AM	CATION	CALCIUM	MAGNE	SODIUM	POTASS	TNC
PH	1															
EC	-.525	1														
OC	-.495	.628(*)	1													
AN	.432	-.021	-.032	1												
APH	-.498	.459	.366	-.342	1											
APO	.032	.219	.042	-.064	.174	1										
AZ	.178	.046	.583(*)	-.090	.002	.146	1									
AC	-.318	.704(*)	.471	.371	.167	.003	.045	1								
AI	.108	.187	.142	.538	.222	.152	.006	.262	1							
AM	.619(*)	-.138	-.467	.692(*)	-.067	.149	-.349	.117	.492	1						
CATION	.536	-.008	-.194	.576	-.380	.139	-.211	.228	.400	.644(*)	1					
CALCIUM	-.080	.212	-.090	.271	-.162	.719(**)	-.198	.377	.145	.251	.323	1				
MAGNE	.368	.113	.365	.405	-.170	.340	.558	.304	.482	.195	.578(*)	.207	1			
SODIUM	.610(*)	-.155	-.173	.477	-.229	-.141	.066	.135	-.101	.604(*)	.334	-.009	.007	1		
POTASS	.108	.193	-.436	-.002	.356	.428	-.540	.177	.125	.585(*)	.381	.440	-.121	.205	1	
TNC	-.095	.139	.283	.218	.274	.348	.003	.243	-.068	.138	.162	.415	.095	.293	.305	1

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

manganese and available nitrogen (r = 0.692; P < 0.05%) available potassium and calcium (r = 0.719; P < 0.01%), available manganese and cation ion exchange capacity (r = 0.644; P < 0.05%), available manganese and sodium (r = 0.604; P < 0.05%), available manganese and potassium (r = 0. 585; P < 0.05%), magnesium and cation ion exchange capacity (r = 0.578; P < 0.05%).Similar type of study was reported by Lakshmanaperumalsamy *et al*., (1986); Jiang and Xu, (1996) ; Saadoun and Al-Momoni, (1997) studied the pH, moisture, organic matter, nitrogen and phosphorous content of the soils and correlated with actinomycetes population(Table-4).

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