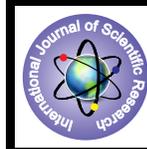


Isolation And Enumeration of Marine Actinobacteria Diversity from Kovalam Mangrove Soil, East Coast of Tamil Nadu, India



Medical Science

KEYWORDS : Mangrove environment, Physico-chemical, Marine Actinobacteria.

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ABSTRACT

The marine soil sample were collected from marine mangrove environment of Kovalam east coast of Tamil Nadu India. A total of 23 different actinobacteria were isolated by serial dilution plate technique on starch casein agar medium. Physico-chemical analysis of the soil revealed the following parameters like pH, Electrical conductivity, Organic carbon, nitrogen, phosphorus, potassium, salinity, zinc, copper, iron, manganese, nitrate, nitrite, Cation exchange capacity, Calcium, Magnesium, Sodium, Potassium, were studied. Actinobacteria strains were chosen using selective isolation approaches, and then morphological properties of the isolates were determined. The isolates belonged to the following genus, *Jonesia*, *Planomonospora*, *Pseudonocardia* (2), *Kineospora*, *Micromonospora*, *Nocardia*(2), *Nocardioopsis*, *Saccharopolyspora hirsuta*, *Saccharopolyspora*, *Streptovorticillium cinnamomeum*, *Streptomyces cyaneus*, *Streptomyces* (7), *Terrabacter* (2) and *Thermoactinomyces*.

INTRODUCTION

Mangrove forests are among the world's most productive ecosystem that enriches coastal waters, yields commercial forest products, protect coastlines and support coastal fisheries. However, mangroves exist under condition of high salinity, extreme tides, strong winds, high temperature and muddy, anaerobic soils. There may be no other group of plants with such highly developed morphological, biological, ecological and physiological adaptations to extreme conditions. Mangroves are woody plants that grow at the interface between land and sea in tropical and subtropical latitudes. These plants, and the associated microbes, fungi, plants and animals, constitute the mangrove forest community or mangal. (Kathiresan and Bingham, 2001).

Around 23,000 bioactive secondary metabolites produced by microorganisms have been reported and over 10,000 of these compounds are produced by actinomycetes, representing 45% of all bioactive microbial metabolites discovered (Vimal *et al.*, 2009). Among actinomycetes, around 7600 compounds are produced by *Streptomyces* species. Many of these secondary metabolites are potent antibiotics, which has made *Streptomyces* the primary antibiotic-producing organisms exploited by the pharmaceutical industry (Ramesh 2009., Jensen PR., 2007). 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.

Actinomycetes from marine sample have rarely undergone screening for novel metabolites and there is evidence that actinomycetes usually make up only a small portion of a bacterial flora of marine habitats, with absolute number of actinomycetes much lower than in terrestrial habitats. (Good fellow and Haynes, 1984). *Streptomyces* is the most common actinomycetes genus in the soils, which forms 90% of the population.

Natural organic compounds produced by microorganisms are an important screening target for a variety of bioactive compounds of actinomycetal origin in particular, have been valuable in the field of antibiotic production. Isolated actinomycetes from a marine environment, requiring sea water for growth, and these strains were designated marine actinomycetes (Mincer *et al.*, 2002).

MATERIALS AND METHODS

Sample Collection

The marine soil samples were collected from mangrove environment of Kovalam, 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 Bray 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 (Standford 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 penta acetic 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 Actinobacteria

Isolation of actinobacteria 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 pres-

sure 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 10⁶ 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 actinobacteria colonies were observed, and used for further investigation (Porter *et al.*, 1960). Streak plate method was used to purify the culture of actinobacteria. 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 Actinobacteria (Coverslip Culture Technique)

Actinobacteria culture plates was prepared and 2-4 sterile coverslips were inserted at an angle of 45°C. The actinobacteria 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 Actinobacteria

A total of 21 actinobacteria were isolated from Kovalam. Morphological studies indicated that the strains belonged to the genera, *Jonesia*, *Planomonospora*, *Pseudonocardia* (2), *Kineospora*, *Micromonospora*, *Nocardia*(2), *Nocardioopsis*, *Saccharopolyspora hirsuta*, *Saccharopolyspora*, *Streptovorticillium cinnamoneum*, *Streptomyces cyaneus*, *Streptomyces* (7), *Terrabacter* (2) and *Thermoactinomyces*. Sixty five actinomycetes were isolated from 32 soil samples collected from cuddalore east coast region of Tamilnadu (Dhanasekaran *et al.*,2005).

In the present study a total of 23actinobacteria isolates recorded including different locations in marine soils of Kovalm,Tamilnadu. Mean population density of actinomycetes varied from 10.02 to 14.02× 10⁶ CFU/g. Most of the actinobacteria strains belonging to the genera *Streptomyces* sp., 29 x 10⁶ CFU g⁻¹(6.44%), *Kineospora* sp., 28 x 10⁶CFU g⁻¹ (6.22%), *Miromo-*

nopora sp., 26 x 10⁶ CFU g⁻¹ (5.77%), *Streptomyces* sp., 26 x 10⁶ CFU g⁻¹ (5.77%) and the minimum level of *Saccharopolyspora* sp., 10 x 10⁶ CFU g⁻¹ (2.22%), *Streptomyces* sp., 10x 10⁶ CFU g⁻¹ (2.22%) were recorded (Table – 1).

Percentage contribution of the individual species to the total actinobacteria population at all the seasons showed variation. The maximum percentage contribution of 6.44% was found with *Streptomyces* sp. KSPK16. This was followed by *Micromonospora* KSPK6(6.22%) *Streptomyces* sp. KSPK17 (6%), *Nocardia* sp. KSPK7 (5.77% each) *Terrabacter* sp. KSPK21 (5.55%) *Streptovorticillium cinnamoneum* KSPK12 (5.33%) *Pseudonocardia* sp. KSPK4 (4.88%) *Nocardia* sp. KSPK8 (4.44% each); *Thermoactinomyces* sp. KSPK23 *Pseudonocardia* sp. KSPK3 (4.22% each); *Kineospora* sp KSPK5, *Planomonospora* sp. KSPK3 (4% each); *Nocardioopsis* sp. KSPK9, *Streptomyces* sp. KSPK14 *Streptomyces* sp. KSPK15 (3.77%) *Terrabacter* sp. KSPK22 (3.55%) *Jonesia* sp. KSPK1 (3.33% each); *Saccharopolyspora hirsuta* KSPK10, *Streptomyces cyaneus* KSPK13 *Streptomyces* sp.KSPK20 (2.88%) *Saccharopolyspora* sp. KSPK11 (2.22% each) and *Streptomyces* sp.KSPK19 respectively (Table -1).

Soil characteristics such as pH 7.56 to 7.31, electrical conductivity 0.23 to 0.33 dSm⁻¹, cation exchange capacity 22.7 to 22.0c.mol proton+/kg, organic carbon 0.39 to 0.42%, nitrogen 3.15 to 3.18 (g / kg), phosphorus 1.05 to 1.18 (g / kg), potassium 4.5 to 4.6 (g / kg), Salinity 30.1 to 32.6(g/kg), zinc 0.82 to 0.87 ppm, copper 0.93 to 0.98 ppm, iron 4.81 to 4.86ppm, manganese 3.23 to 3.27ppm,nitrate 21 to 31ppm,nitrite0.03 to 0.08 ppm, calcium 10.3to 10.9 (C. Mole Proton+ / kg), magnesium 9.2 to 9.6 (C. Mole Proton+ / kg), sodium 2.12 to 2.18(C. Mole Proton+ / kg) and potassium 0.15 to 0.19 (C. Mole Proton+ / kg), during different seasons(Table-2). Five actinomycetes strains were isolated from soil collected in two different regions of Parangipettai. The physico-chemical characteristics of soil samples are analysed (Sathiyaseelan and Stella, 2011).

Physico-chemical properties of sediment and total actinobacterial population (TAP). It revealed that the significant positive correlation between available Sodium and pH (r = 0.592;P< 0.05%), cation exchange capacity and available potassium (r = 0.614; P < 0.05%), potassium and available iron (r =0.638;P < 0.05%), magnesium and calcium (r = 0.626; P < 0.05%). Similar type of study was reported by cho *et al.*, 2008, soil temperature is found to have positive correlation with actinomycetes load. The correlation between salinity, pH and organic content of marine sediments and actinomycetes population has been reported by several workers Ndonde *et al.*, 2000 and Jensen *et al.*,1991. The work concluded that the study area showed good diversity of actinobacteria from Kovalm marine environment (Table-3).

Table 1. Number of colonies, mean density (CFU/g) and percentage contribution of actinobacteria recorded in Kovalam

Oct 2011 to Sep 2012																						Total no. Of Colonies	% concentration						
S. No	Name of the Actinobacteria	OCT		NOV		DEC		JAN		FEB		MAR		APR		MAY		JUNE		JULY				AUG		SEP			
		TNC	MD	TNC	MD	TNC	MD			TNC	MD	TNC	MD																
1.	<i>Jonesia</i> sp. KSPK1	2	0.67	0	0	0	0	2	0.67	0	0	3	1.00	0	0	2	0.67	2	0.67	0	0	2	0.67	2	0.67	2	0.67	15	3.33
2.	<i>Planomonospora</i> sp. KSPK2	2	0.67	0	0	2	0.67	4	1.33	2	0.67	0	0	0	0	0	0	0	0	0	3	1.00	2	0.67	3	1.00	18	4.00	
3.	<i>Pseudonocardia</i> sp. KSPK3	3	1.00	2	0.67	2	0.67	0	0	0	0	2	0.67	0	0	2	0.67	0	0	3	1.00	2	0.67	3	1.00	19	4.22		
4.	<i>Pseudonocardia</i> sp. KSPK4	0	0	4	1.33	3	1.00	2	0.67	0	0	0	0	4	1.33	3	1.00	0	0	3	1.00	0	0	3	1.00	22	4.88		
5.	<i>Kineospora</i> sp. KSPK5	3	1.00	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	0	0	0	19	4.22	

6.	Micromonospora sp. KSPK6	2	0.67	2	0.67	3	1.00	3	1.00	4	1.33	2	0.67	2	0.67	2	0.67	2	0.67	2	0.67	2	0.67	2	0.67	2	0.67	2	0.67	2	0.67	2	0.67	2	0.67	28	6.22
7.	Nocardia sp. KSPK7	3	1.00	2	0.67	2	0.67	2	0.67	3	1.00	0	0	2	0.67	2	0.67	3	1.00	3	1.00	2	0.67	2	0.67	2	0.67	0	0	20	4.44						
8.	Nocardia sp. KSPK8	0	0	2	0.67	2	0.67	0	0	0	0	4	1.33	3	1.00	3	1.00	2	0.67	2	0.67	2	0.67	2	0.67	0	0	20	4.44								
9.	Nocardiopsis sp. KSPK9	3	1.00	2		2	0.67	0	0	0	0	2	0.67	2	0.67	3	1.00	0	0	0	0	2	0.67	2	0.67	2	0.67	18	4.00								
10.	Saccharopolyspora hirsuta KSPK10	3	1.00	0	0	0	0	0	0	2	0.67	0	0	2	0.67	2	0.67	4	1.33	0	0	2	0.67	0	0	0	0	15	3.33								
11.	Saccharopolyspora sp. KSPK11	0	0	0	0	2	0.67	2	0.67	2	0.67	0	0	0	0	2	0.67	2	0.67	0	0	0	0	0	0	0	0	10	2.22								
12.	Streptoverticillium cinnamomeum KSPK12	2	0.67	0	0	2	0.67	3	1.00	2	0.67	0	0	0	0	2	0.67	4	1.33	3	1.00	4	1.33	2	0.67	2	0.67	24	5.33								
13.	Streptomyces cyaneus KSPK13	0	0	2	0.67	2	0.67	0	0	0		3	1.00	0	0	0	0	2	0.67	2	0.67	2	0.67	2	0.67	2	0.67	15	3.33								
14.	Streptomyces sp. KSPK14	0	0	2	0.67	0	0	2	0.67	2	0.67	3	1.00	0	0	0	0	2	0.67	3	1.00	2	0.67	2	0.67	2	0.67	18	4.00								
15.	Streptomyces sp. KSPK15	3	1.00	0	0	2	0.67	0	0	2	0.67	2	0.67	0	0	4	1.33	2	0.67	0	0	2	0.67	0	0	2	0.67	17	3.77								
16.	Streptomyces sp. KSPK16	2	0.67	3	1.00	0	0	2	0.67	4	1.33	2	0.67	3	1.00	2	0.67	2	0.67	4	1.33	3	1.00	2	0.67	2	0.67	29	6.44								
17.	Streptomyces sp. KSPK17	2	0.67	3	1.00	3	1.00	2	0.67	3	1.00	4	1.33	0	0	3	1.00	2	0.67	2	0.67	0	0	3	1.00	27	6.00										
18.	Streptomyces sp. KSPK18	2	0.67	3	1.00	3	1.00	2	0.67	2	0.67	3	1.00	2	0.67	2	0.67	2	0.67	0	0	2	0.67	3	1.00	26	5.77										
19.	Streptomyces sp. KSPK19	0	0	0	0	0	0	0	0	4	1.33	0	0	2	0.67	0	0	2	0.67	0	0	2	0.67	0	0	10	2.22										
20.	Streptomyces sp. KSPK20	0	0	2	0.67	0	0	0	0	3	1.00	3	1.00	0	0	0	0	3	1.00	0	0	2	0.67	0	0	13	2.88										
21.	Terrabacter sp. KSPK21	2	0.67	3	1.00	2	0.67	4	1.33	2	0.67	3	1.00	2	0.67	3	1.00	0	0	0	0	2	0.67	2	0.67	2	0.67	25	5.55								
22.	Terrabacter sp. KSPK22	0	0	2	0.67	2	0.67	3	1.00	3	1.00	2	0.67	0	0	0	0	0	0	0	0	2	0.67	2	0.67	2	0.67	16	3.55								
23.	Thermoactinomyces sp. KSPK23	3	1.00	2	0.67	2	0.67	0	0	0	0	3	1.00	3	1.00	2	0.67	0	0	3	1.00	0	0	2	0.67	2	0.67	20	4.44								
		37	12.36	39	12.36	36	12.04	36	12.02	41	14.02	44	14.68	30	10.02	39	13.03	38	13.37	33	11.07	39	13.05	38	12.37	450											

TNC – Total number of colonies, MD – Mean density

Table 2. Physico - chemical parameters of marine soil samples From Kovalam

October 2011 to September 2012													
S.No	Name of the Parameters	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sep
1.	Ph	7.56	7.31	7.33	7.47	7.28	7.54	7.43	7.41	7.47	7.51	7.53	7.52
2.	Electrical conductivity (dsm ⁻¹)	0.26	0.25	0.28	0.23	0.33	0.31	0.29	0.26	0.29	0.25	0.26	0.29
3.	Organic carbon (%)	0.42	0.41	0.45	0.39	0.41	0.43	0.4	0.42	0.42	0.44	0.41	0.42
4.	Available nitrogen (g/kg)	3.18	3.18	3.19	3.18	3.17	3.16	3.18	3.17	3.16	3.16	3.15	3.18
5.	Available phosphorus (g/kg)	1.16	1.17	1.16	1.18	1.17	1.17	1.16	1.17	1.18	1.17	1.16	1.05
6.	Available potassium (g/kg)	4.5	4.56	4.43	4.53	4.63	4.56	4.40	4.5	4.43	4.56	4.5	4.53
7.	Available zinc (ppm)	0.85	0.85	0.83	0.85	0.82	0.86	0.83	0.87	0.83	0.82	0.85	0.82
8.	Available copper (ppm)	0.98	0.97	0.93	0.93	0.98	0.95	0.93	0.98	0.98	0.96	0.95	0.97
9.	Available iron (ppm)	4.85	4.85	4.83	4.81	4.84	4.83	4.86	4.85	4.82	4.83	4.85	4.82
10.	Available manganese(ppm)	3.26	3.23	3.26	3.25	3.23	3.27	3.25	3.25	3.27	3.26	3.23	3.25
11.	Cation exchange capacity (C.Mole proton/kg)	22.5	22.6	22.3	22.3	22.7	22.6	22.3	22.0	22.4	22.5	22.3	22.6
12.	Calcium (mg/g)	10.8	10.8	10.5	10.3	10.3	10.6	10.9	10.5	10.8	10.8	10.7	10.6

13.	Magnesium(mg/g)	9.5	9.2	9.5	9.0	9.3	9.3	9.5	9.2	9.6	9.6	9.4	9.5
14.	Sodium(mg/g)	2.16	2.13	2.13	2.15	2.12	2.16	2.18	2.13	2.17	2.12	2.16	2.17
15.	Potassium(mg/g)	0.18	0.18	0.15	0.17	0.18	0.16	0.18	0.19	0.14	0.19	0.18	0.15
	TNC	37	39	36	36	41	44	30	39	38	33	39	38

TNC-Total number of colonies

Table 2. Percentage frequency and frequency class of different species of actinobacteria recorded in Kovalam-October 2011 to September 2012

S.No.	Name of the actinobacteria	No. of seasons in which the actinobacteria occurred	Percentage Frequency	Frequency Class
1.	Jonesia sp. KSPK1	7	58.33	F
2.	Planomonospora sp. KSPK2	7	58.33	C
3.	Pseudonocardia sp. KSPK3	8	66.66	F
4.	Pseudonocardia sp. KSPK4	7	58.33	F
5.	Kineospora sp. KSPK5	7	58.33	F
6.	Micromonospora sp. KSPK6	12	100.00	C
7.	Nocardia sp. KSPK7	11	91.66	C
8.	Nocardia sp. KSPK8	8	66.66	F
9.	Nocardiopsis sp. KSPK9	8	66.66	F
10.	Saccharopolyspora hirsuta KSPK10	6	50.00	O
11.	Saccharopolyspora sp. KSPK11	5	41.60	O
12.	Streptovercillium cinnamomeum KSPK12	9	75.00	C
13.	Streptomyces cyaneus KSPK13	7	58.33	F
14.	Streptomyces sp. KSPK14	8	66.66	F
15.	Streptomyces sp. KSPK15	7	58.33	F
16.	Streptomyces sp. KSPK16	11	91.66	C
17.	Streptomyces sp. KSPK17	10	83.33	C
18.	Streptomyces sp. KSPK18	11	91.66	C
19.	Streptomyces sp. KSPK19	4	33.33	O
20.	Streptomyces sp. KSPK20	5	41.66	O
21.	Terrabacter sp. KSPK21	10	83.33	C
22.	Terrabacter sp. KSPK22	7	58.33	F
23.	Thermoactinomyces sp.KSPK23	8	66.66	F

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

Table 3. The correlation coefficient between the physico-chemical characters and total number of actinobacteria colonies at Kovalam

	pH	EC	OC	AN	AP	APO	Zn	AC	Fe	Mn	Cation	Ca	Mg	Na	K	TNC
pH	1															
EC	-.231	1														
OC	.031	.175	1													
AN	-.184	-.146	-.073	1												
AP	-.199	-.205	-.063	-.187	1											
APO	-.138	.119	-.059	-.355	-.050	1										
Zn	.184	-.375	-.195	-.050	.362	.000	1									
AC	-.020	.193	.076	-.341	-.146	.379	.026	1								

Fe	-.191	.041	-.130	-.098	.163	-.154	.274	.132	1							
Mn	.483	.077	.505	.190	.093	-.387	.000	-.089	-.431	1						
Cation	-.044	.448	.071	-.150	-.253	.614(*)	-.462	.275	-.179	-.104	1					
Ca	.351	-.143	.128	-.194	.007	-.439	-.079	.095	.444	.208	.046	1				
Mg	.284	.294	.558	-.126	-.245	-.417	-.605	.139	.074	.414	.163	626(*)	1			
Na	.592(*)	.135	-.347	.119	-.308	-.554	.025	-.190	-.018	.301	-.055	.418	.270	1		
K	-.083	-.353	-.259	-.195	.299	.338	.286	.107	.638(*)	-.492	-.173	.072	-.288	-.430	1	
TNC	-.037	.372	.092	-.385	-.005	.555	.414	.433	-.146	-.096	.328	-.413	-.368	-.154	-.203	1

*Correlation is significant at the 0.05 level

REFERENCE

Barnes, H., 1959. Apparatus and methods of Oceanography, Part I Chemical, Allen and Unwin Ltd., London. | Bray, R.H. and Kutz, L.T., 1945. Determination of total, organic and available forms of phosphorus in soils. *Soil Sci.*, 59: 39-45. | Burholder, P.R., Sun, S.H., Ehrlich, J. and Anderson, L., 1954. Criteria of speciation in the genus *Streptomyces*. *Ann. New York Acad. Sci.*, 60:102-103. | Cho, S.T., Tsai, S.H., Ravindran, A., Selvam, A., and Yang, S.S., 2008. Seasonal variation of Microbial | population and biomass in *Tatchia* grassland soils of Taiwan. *Environ. Geochem. Health*, 30(3), pp. 255-272. | Dhanasekaran, D., Rajakumar, G., Sivamani, P., Panneerselvam A. and Thajuddin, N., 2005. Screening of saltpan actinomycetes for antibacterial agents. *Internet J. Microbial.*, 1(2). | Goodfellow, M. and J.A. Haynes, 1984. *Actinomycetes in Marine Sediments*. In: *Biological, Biochemical | and Biomedical Aspects of Actinomycetes*, Oritz-Oritz, L., C.F. Bojali and V. Yakoleff (Eds.). Academic Press, New York, London, pp: 453. | Jackson, M.L., 1973. *Soil chemical analysis*. Prentice Hall of India Pvt. Ltd., New Delhi. | Jensen, P. R.R. Dwight & W. Fenical., 1991. Distribution of actinomycetes in near shore tropical marine | sediments. *Appl. Environ. Microbiol.* 57: 1102-1108. | Jensen, P.R., Williams, P.G., Oh D.C, Zeigler, L., Fenical, W., 2007. Species-specific secondary Metabolite | production in marine actinomycetes of the genus *Salinospora*. *Appl Environ Microbiol* ; 73(4); 1146-1152. | Kathiresan, N.K and Bingham, B.L, 2001, Biology of Mangroves and Mangrove ecosystems. *Advances | in Marine Biology*. 40:81-251. | Kuster, E. and Williams, S.T., 1964. Production of hydrogen sulphide by *Streptomyces*. *Appl Microbiol.* 12(4):335-336. | Lindsay, W.C. and Norvell, A., 1978. Development of a DTPA soil test for zinc, iron, manganese, and | copper. *Proc Sci. Soc. AM.*, 42: 421-428 | Mincer TJ, Jensen PR, Kauffman CA, Fenical W, 2002. Widespread and persistent Populations of a major | new marine actinomycete taxon in ocean sediments. *Appl Environ Microbiol*, 68: 5005-5011. | Muthuvel, P. and Udayasorian, C., 1999. Soil, plant, water and agrochemical analysis, Tamil Nadu | Agricultural University, Coimbatore, India. | | Ndonde, M. J. M. & E. Semu, 2000. Preliminary characterization of some *Streptomyces* species from four | Tanzanian soils and their antimicrobial potential against selected plant and animal pathogenic bacteria. *World J. Microbiol. Biotechnol.* 16: 595-599. | Porter, J.N., Wilhelm, J.J., and Tresner, M.D., 1960. Method for preferential isolation of actinomycetes from soils. *Applications of Microbiology*, 8: 174-178. | Pridham, T.G., Hesselstine, C.W. and Penedit, R.G., 1958. A guide for the classification of *Streptomyces* | according to selected group. *Applications of Microbiology*, 6:52-79. | Ramesh S, Rajesh M, Mathivanan N., 2009. Characterization of athermostable alkaline protease produced | by marine *Streptomyces fungicidicus* MML1614. *Bioprocess Bio Syst Eng*. 32: 791-800. | Sathiyaseelan, K and Stella Isolation, D., 2011. Identification and Antimicrobial Activity of Marine | Actinomycetes Isolated from Parangipettai. *Recent Research in Science and Technology*, 3(9): 74-77 | Standfold, S., and L. English., 1949. Use of flame photometer in rapid soil test for K and Ca. *J of Agronomy*. | 41: 446-447. | Subbiah, B.V. and Asija, G.L., 1956. A rapid method for estimation of available nitrogen in soil. *Curr. Sci.*, 25: 258-260. Tamil Nadu Agricultural University, Coimbatore, India. | Vimal V, Rajan BM, Kannabiran K, 2009. Antimicrobial activity of marine actinomycete, *Nocardopsis* sp. *VITSVK 5 (FJ973467)*. *Asian J Med Sci*; 1(2): 57-63 | Walkley, A. and Black, I.A., 1934. An Examination of the Degtjareff Method for Determining Soil Organic Matter, and A Proposed Modification of the Chromic Acid Titration Method. *Soil. Sci.*, 37: 29-38. |