RESEARCH ARTICLE

A STUDY ON ACTINOMYCETES POPULATION AND PHYSICO CHEMICAL PROPERTIES OF SHORE LINE SOIL FROM SELECTED COASTAL AREAS OF TAMIL NADU

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ARTICLE INFO	ABSTRACT
	Shore line soil samples were collected from twelve different coastal areas of Tamil Nadu and tested by dilution plating technique on Starch casein and Kuster's agar medium to assess the actinomycetes diversity. Physico chemical characteristics of the soil such as pH, temperature, electrical conductivity, salinity, total dissolved solids, moisture content, water holding capacity, carbon, nitrogen, hydrogen and sulfur content were analyzed to find out the impact on actinomycetes population. A total number of 376 actinomycetes were recorded from all the shoreline soil samples. Physico chemical analysis revealed the variations of parameters in each sampling sites. Correlation analysis made between physico chemical properties of shore line soil and actinomycetes population revealed no factor as accountable for the actinomycetes population changes in different sampling sites. Multivariate cluster analysis statistical technique was applied to evaluate the variations in shore line soil quality of different sampling sites.

INTRODUCTION

India has a coastline stretches about 7500 km in which Tamil Nadu, the southernmost state of the Indian Peninsula, has a length of about 1076 km constitutes about 15% of the total coastal length of India and stretches along the Bay of Bengal, Indian Ocean and Arabian Sea and lies between 8° 5" to 13° 35" N and 76° 15" to 80° 20" E (Muthukrishnan and Aruchamy, 2012). Tamil Nadu coastal environment plays an important role in country's economy by natural resources and rich biodiversity (Muthukrishnan and Aruchamy, 2012). Currently, Tamil Nadu coastline is facing increasing human and natural pressures like overexploitation of marine resources, dumping of industrial and toxic wastes, oil spills, tsunami which has resulted in substantial damage to its ecosystems including microbes. Environmental conditions such as pH, electrical conductivity, salinity, carbon, nitrogen, temperature and nutrients influence the composition, distribution and growth of its biota (Swami et al., 2000; Satheesh Kumar and Anisa Khan, 2009). Actinomycetes are gram positive, filamentous bacteria which are excellent secondary metabolite producers (Olano et al., 2009). At present actinomycetes from terrestrial origin produce hundreds of antibiotics which are used commercially as antibiotics, anti cancer drugs, immunosuppressive agents etc (Sathish Kumar and Kokati Venkata Bhaskara Rao, 2012). Some differences could be expected among actinomycetes prevailing in marine and coastal environments due to variation in the physical, chemical and biological factors (Kumar Saurav and

Kannabiran, 2010; Sathish Kumar and Kokati Venkata Bhaskara Rao, 2012). In this present study the physico-chemical parameters of shoreline soil of selected locations of Tamil Nadu coast of India with respect to actinomycetes population was reported.

MATERIALS AND METHODS

Soil sample collection

The shore line soil samples were collected from twelve different locations of Tamil Nadu, India namely, Chennai ($13^0 03'02.05''$N and $80^0 16'58.81''$E), Mahabalipuram ($12^0 36'43.64''$N and $80^0 11'48.92''$E), Nagore ($10^0 48'49.23''$N and $79^0 51'04.98''$E), Velankanni ($10^0 40'52.03''$N and $79^0 51'12.85''$E), Kodiyakarai ($10^0 16'59.55''$N and $79^0 49'56.15''$E), Tuticorin ($8^0 43'31.97''$N and $78^0 09'21.70''$E), Tiruchendur ($8^0 29'46.36''$N and $78^0 07'46.24''$E), Kulasekarapattinam ($8^0 23'44.97''$N and $78^0 03'30.95''$E), Uvari ($8^0 16'31.67''$N and $77^0 53'26.22''$E), Kudankulam ($8^0 09'26.59''$N and $77^0 38'28.44''$E), Kanyakumari ($8^0 04'38.73''$N and $77^0 33'04.35''$E) and Medalam ($8^0 12'09.69''$N and $77^0 12'54.95''$E) , during the period of June and July 2012. The location of the sampling sites was in figure 1. The soil samples were collected from 15 cm depth using sterile spatula, and brought to the laboratory in sterile polythene containers and used for physico chemical and microbiological analysis. *In situ* observations of the sampling site are tabulated in Table 1.

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Fig. 1: Map of the study area showing sample location points

Table 1. *In situ* observations for each sampling site

S.No	Sampling site	Observation
1	Medalam	Soil has a brownish black color, fine granular structure, sandy beach
2	Kanyakumari	Soil has a pale brown color, fine granular and crumb structure, rocky beach
3	Kudankulam	Soil has a pale brown color, fine granular structure, sandy beach
4	Uvari	Soil has a reddish brown color, very fine granular structure, sandy beach
5	Kulasekarapattinam	Soil has a light brown color, very fine granular structure, sandy beach
6	Tiruchendur	Soil has a light brown color, very fine granular structure, sandy beach
7	Tuticorin	Soil has a dark brown color, very fine granular structure, sandy beach
8	Kodiyakarai	Soil has a brown color, very fine granular structure, sandy beach
9	Velankanni	Soil has a light brown color, very fine granular structure, sandy beach
10	Nagore	Soil has a dark brown color, very fine granular structure, sandy beach
11	Mahabalipuram	Soil has a pale brown color, fine granular and crumb structure, rocky beach
12	Chennai	Soil has a light brown color, fine granular and crumb structure, sandy beach

Analysis of Physico-Chemical Characteristics of Soil

Initial measurements on pH, temperature, electrical conductivity, salinity, total dissolved solids were done by Multi-Parameter PCS Testr TM series (Eutech Instruments, Oakton) by suspending soil in distilled water. Ten ml of distilled water was taken in a beaker and the soil was added till the volume became 15ml and again 15 ml of distilled water was added. It was stirred well using glass rod and the soil was allowed to settle. Moisture content was estimated by finding the weight difference of known quantity of soil before and after keeping the soil in hot air oven at 60^0C for 6 hours. Water holding capacity of the soil was done by adding water to the known quantity of dried soil in a beaker and incubated it over night. Drain water and note down the weight of the soil. The difference between the weight of moist and dried soil gave the result of water holding capacity. Carbon, Nitrogen, Sulfur and Hydrogen contents were analyzed by Elementar analyzer. Vario EL Elementar analyzer is a fully automatic instrument which enables a speedy quantitative analysis of CHNS. The soil samples were grinded using Pestle and Mortar and, sieved through fine mesh sieve. The homogenized soil sample was packed in Tin foil weighed and placed into the carousel of the automatic sample feeder. Carrier gas was Helium and Combustion gas was oxygen. The soils were heated to 1200^0C and then processed. Sulphanilic acid was used as standard. The results were recorded in computer by WinVar software.

Sample Treatment

Soil samples were dried in room temperature for 24 hours. All the soil samples were diluted up to 10^{-7} in sterile saline solution prior to inoculation in the petriplates.

Isolation of Actinomycetes

Isolation of actinomycetes was carried out by spread plate technique (Collins et al., 1989). Starch Casein agar and Kuster's agar were used for the isolation and enumeration of actinomycetes. The medium was supplemented with 100µg/ml cyclohexamide. Using conventional dilution plate technique, 1gm of marine soil sample was suspended in 10 ml of sterile saline and serially diluted to 10^{-7} dilution. 0.1 ml of suspension from 10^{-3} to 10^{-7} dilutions were poured over the agar medium in separate plates and spread by sterile L rod. These plates were incubated for 15 days at 25^0C.
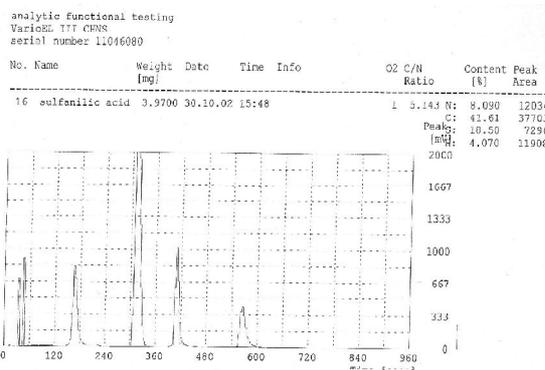
Statistical Analysis

Correlation was done to understand the relationship between the various parameters and to test the significance of the shore line soil with actinomycetes population. It was considered to be significant when the value of the probability of significance (P) was greater than 0.05 (Senthil kumar et al., 2011; Satheeshkumar and Anisa Khan, 2009). All these statistical analyses were performed using Microsoft Excel 2010 (Microsoft Corporation, USA). Bray-Curtis similarity was analyzed using PAST 2.16.
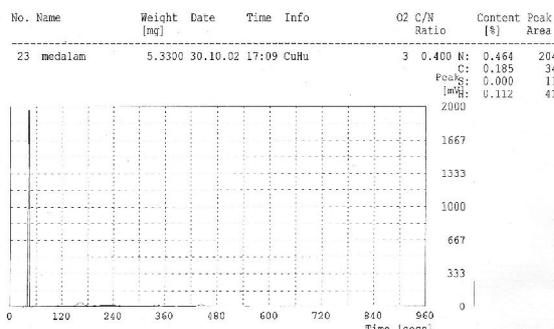
RESULTS AND DISCUSSION

Physico chemical properties of the shore line soil showed variations in different sampling sites. The pH of the soils ranged from 6.9 to 8.4 indicating that all the shore line soils were alkaline in nature except Uvari soil (pH 6.9). The maximum pH was observed in Medalam and Nagore shore line soils. When the hydrogen ion in the sample increased, pH got decreased. Alkaline condition was explained as the characteristic feature of marine and marine influenced habitats which were well adopted to grow in alkaline pH (Nadimuthu, 1998; Madhanraj et al., 2010). Hence, the actinomycetes population did not seem to be influenced by it. The temperature of the sample sites ranged from 27^0C to 31^0C. Actinomycetes grew well between 25^0C to 35^0C, so the change in temperature in different sampling site did not affect the actinomycetes population. Electrical conductivity revealed variations from 2.15 to 14.87. The shore line soils of Kanyakumari, Mahabalipuram and Chennai showed comparatively lesser electrical conductivity than other locations. Total dissolved solids ranged from 1.55 to 9.68. Salinity ranged from 1.12 to 8.89. Tiruchendur shoreline soil sample had more salt content (8.89 ppt) than others. Moisture Content was ranged from 2.01% to 24.86%, while the water holding capacity was found to be from 9.67 % to 16.72% in shore line soil collected from different sampling site. The results obtained using elementar analyzer revealed the carbon, nitrogen, sulfur and hydrogen content in the shore line soil samples. The results were recorded in the table 2. Tiruchendur shore line soil showed maximum carbon content
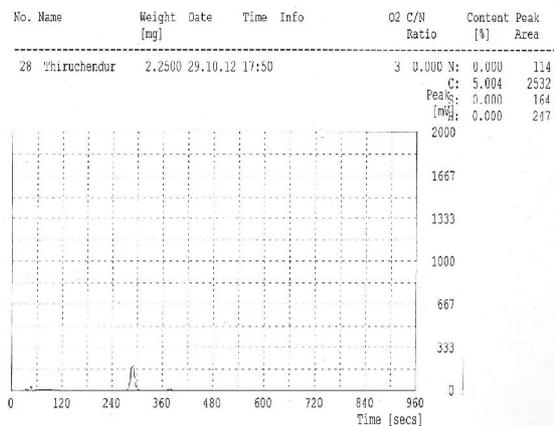
(5.004%) while Velankanni shore line soil showed maximum nitrogen content (0.658%). Sulfur (0 to 0.072%) and hydrogen content (0 to 0.081) were very low and absent in few sampling site. From correlation analysis it could be inferred that, change in electrical conductivity, significantly affected the total dissolved solids in the shore line soil samples, Change in temperature did not bring about any significant change in total dissolved solids. Change in the electrical conductivity significantly affected the salinity and moisture content of the shore line soil. Alteration in total dissolved solids significantly affected the salinity and moisture content of the shore line soil. Variation in pH significantly affected the hydrogen, nitrogen and sulfur content in the shore line soil samples. Change in hydrogen content significantly affected the sulfur and nitrogen content. Variation in sulfur content did not bring about significant changes in the carbon content in the shore line soil samples. Variations in nitrogen content significantly did not affect the carbon content of the shore line soil samples. The correlation analysis revealed that the physico chemical properties did not have any relationship with actinomycetes population. Dendrogram indicated the Bray-Curtis similarity for physico-chemical parameters of shore line soil and actinomycetes population (Fig. 4).
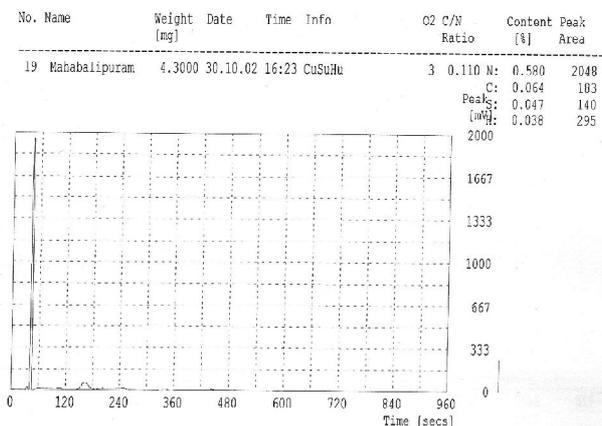
2a) CHNS content in Tiruchendur soil

2b) CHNS content in Mahabalipuram soil

2c) CHNS content in Sulphanilic acid

2d) CHNS content in Medalam soil

Figure: 2 Results of elementar analysis

Actinomycetes colonies grown in Starch casein agar in Kudankulam and Medalam soil

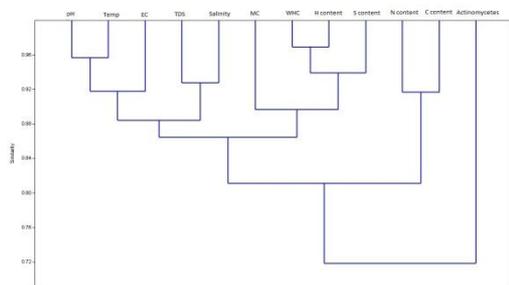**Figure: 3 Actinomycetes isolated from different shore line samples**

Figure: 4 Dendrogram of Physico-chemical parameters of shore line soil characteristics and actinomycetes population

The dendrogram (Fig. 4) showed the cluster being combined and the values of the coefficient at each step. The above given results clearly showed that the groupings could be well known that apparently revealed the differences in shore line factors and actinomycetes population within coastal areas of Tamil Nadu.

Table:2 Physico-Chemical Parameter of the shore line soil samples

Sampling site	pH	Temperature (°C)	EC (mS)	TDS (ppt)	Salinity (ppt)	MC (%)	WHC (%)	Carbon content	Nitrogen Content	Sulphur Content	Hydrogen Content	Actinomycetes
S₁	8.4	31.4	7.24	4.95	3.85	13.53	12.80	0.185	0.464	-	0.112	35
S₂	7.2	29.2	2.15	1.55	1.12	2.01	13.15	2.987	-	-	-	14
S₃	7.5	27.6	13.62	9.91	8.11	17.70	11.22	1.540	-	-	-	34
S₄	6.9	27.3	13.59	9.68	7.90	18.95	9.67	1.009	-	-	-	41
S₅	7.3	28.1	8.5	6.00	4.70	11.10	13.27	3.478	-	-	-	25
S₆	7.4	27.2	14.87	7.44	8.89	20.20	12.21	5.004	-	-	-	19
S₇	7.4	26.9	13.42	9.50	7.74	24.86	15.41	4.287	-	-	-	31
S₈	8.3	28.4	13.70	9.69	7.91	17.74	11.12	0.792	0.610	0.052	0.081	18
S₉	8.3	28.5	9.93	7.02	5.58	16.07	11.27	0.110	0.658	0.061	0.055	32
S₁₀	8.4	28.7	8.91	6.26	5.01	14.87	10.15	0.059	0.525	-	0.009	34
S₁₁	7.8	30.5	3.65	2.55	1.90	13.47	13.06	0.064	0.580	0.047	0.038	52
S₁₂	8.0	30.5	3.18	2.25	1.66	18.01	16.72	0.218	0.500	0.072	0.140	41

S1 – Medalam, S2 – Kanyakumari, S3 – Kudankulam, S4-Uvari, S5-Kulasekarapattinam, S6- Tiruchendur, S7-Tuticorin, S8-Kodiyakarai, S9-Velankanni, S10- Nagore, S11-Mahabalipuram, S12-Chennai, EC – Electrical Conductivity, MC- Moisture Content, WHC – Water Holding Capacity, TDS- Total Dissolved Solids, CHNS contents are expressed in terms of µg/g

Table: 3 Correlation values for physico-chemical properties of soil and actinomycetes

	pH	Temp	EC	TDS	Salinity	MC	WHC	H Content	S Content	N Content	C Content	Actinomycetes
pH	1											
Temp	0.389	1										
EC	-0.220	-0.522	1									
TDS	-0.134	-0.66*	0.952*	1								
Salinity	-0.190	-0.493	0.995*	0.954*	1							
MC	0.0430	-0.258	0.646*	0.678*	0.701*	1						
WHC	-0.049	0.278	-0.480	-0.486	-0.461	0.061	1					
H Content	0.771*	0.539	-0.554	-0.449	-0.503	-0.036	0.138	1				
S Content	0.446	0.285	-0.395	-0.313	-0.350	0.044	0.201	0.805*	1			
N Content	0.892*	0.388	-0.376	-0.266	-0.339	-0.007	-0.049	0.933*	0.751*	1		
C Content	-0.655*	-0.182	0.404	0.207	0.370	0.117	0.243	-0.776*	-0.545	-0.796*	1	
Actinomycetes	0.135	0.114	-0.317	-0.150	-0.246	0.272	0.083	0.533	0.399	0.345	-0.585	1

*P>0.05   EC – Electrical Conductivity, MC- Moisture Content, WHC – Water Holding Capacity, TDS- Total Dissolved Solids

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