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Studies on Diversity of Marine Actinobacteria from Tamilnadu Part of Bay of Bengal, India

P. Manivasagan, S. Gnanam, K. Sivakumar, T. Thangaradjou,
S. Vijayalakshmi and T. Balasubramanian

Centre of Advanced Study in Marine Biology Annamalai University, Parangipettai - 608 502 Tamil Nadu, India

Abstract: A study on marine actinobacteria and physicochemical characteristics of water and sediment in marine environment of Tamilnadu part of Bay of Bengal, India was carried out during January to December 2008. Six stations at different parts of the marine sites were selected for sampling and the following parameters were recorded at monthly intervals temperature, pH, salinity, dissolved oxygen, nitrite, nitrate, total phosphorus, ammonia, silicate, total organic carbon, sediment total nitrogen and sediment total phosphate. Totally 125 strains were isolated from marine sediment samples of Tamilnadu part of Bay of Bengal, India. Among them, 125 isolates were morphologically distinct on the basis of colour of spore mass, melanin pigment, reverse side pigment, soluble pigment, aerial and substrate mycelium formation and sporophore morphology. Ninety isolates were identified as genus *Streptomyces*, *Actinopolyspora* (10), *Actinomadura* (5), *Nocardiopsis* (7), *Micromonospora* (8) and *Actinomyces* (5).

Key words: Diversity % Actinobacteria % Physicochemical characteristics % Tamilnadu

INTRODUCTION

Actinobacteria are gram positive bacteria frequently filamentous and sporulating with DNA rich in G+C from 57-75%. Some of their secondary metabolites have employed as useful microbial compounds [1]. Actinobacteia are primarily saprophytic microorganisms of the soil, where they contribute significantly to the turnover of complex biopolymers, such as lignocellulose, hemicelulose, pectin, keratin and Chitin [2]. The actinomycetes have provided many important bioactive compounds of high commercial value and are being routinely screened for new bioactive substances. These searches have been remarkably successful and approximately two-thirds of naturally occurring antibiotics, have been isolated from actinomycetes [3]. About 61% of the bioactive all microbial metabolites were isolated from actinomycetes especially from streptomycetes and also from some rare actinomycetes (non streptomycetes) [4]. It has been emphasized that actinomycetes from marine sediments may be valuable for the isolation of novel strains of actinomycetes. Which could potentially yield useful new products. However, it has been resolved whether actinomycetes from part of the autochthonous marine microbial community of sediment samples originated from terrestrial habitats and were simply carried out to sea in the form of resistant spores [5].

Actinomycetes are also well known as a rich source of antibiotics and bioactive molecules and are of considerable importance in industry. When conventional isolation techniques were applied, most of the isolates recovered on agar plates have been identified as genus *Streptomyces*, which are the dominant actinomycetes in soil [6-8].

The oceans cover more than 70% of the earth's surface and little is known about the microbial diversity of marine sediments. Which is an inexhaustible resource that has not been properly exploited. However, the full potential of this domain as the basis for biotechnology, particularly in India, remains largely unexplored. India with a long coastal line of over 7,500 km an area of 2.02 million sq km in our exclusive economic zone, with very rich biodiversity, gives us an opportunity to investigate the mankind and ultimately for the economic uplift of India. The Tamil Nadu coastal region has diverse marine habitats such as seashore, hyper saline lakes, estuaries, saltpans and a variety of soil habitats. This paper deals with the actinomycetes isolated from the marine sediments of Tamil Nadu coastal of Bay of Bengal their distribution pattern and taxonomy.

Corresponding Author: P. Manivasagan, Centre of Advanced Study in Marine Biology Annamalai University, Parangipettai - 608 502 Tamil Nadu, India, Mob: +91-9942185018.

MATERIALS AND METHODS

Collection of Samples: Marine samples were collected from six stations, Chennai harbour (Lat. 13°7' N and Long. 80°23'E), Cuddalore harbour (Lat. 11°42' N and long. 79°52'E), Nagapattinam harbour (Lat. 10°45' N and long. 79°56' E), Mandapam fishing harbour (Lat. 9°22' N and Long. 79°8' E), Tuticorin new harbour (Lat. 8°44' N and Long. 78°19'E) and Kanyakumari fishing harbour (Lat. 8°1' N and long. 77°39' E) were selected for the present study (Fig. 1).

Field collection of samples was made during January to December 2008 while cruising in the Sagar Paschimi coastal research vessel from depths of 10-30 m at six stations in the Bay of Bengal (Tamilnadu) in order to record various physico-chemical parameters from water and sediment samples and microbial analysis from sediment samples and transported to the laboratory by keeping them in ice box and processed within 24 hours and microbial analysis were carried within 4 hours.

Physico-chemical Parameters: Initial measurements on temperature (mercury glass thermometer), pH (pH Scan 1 Tester-Eutech Instruments) and salinity (Refractometer Atago F/mill 8901) of the water samples were made onboard and dissolved oxygen was estimated by the modified Winkler's method [9]. Concentration of water nutrients such as nitrite (NO_2), nitrate (NO_3), total phosphorus (PO_4), ammonia (NH_4) and silicate (SiO_3) were analyzed by following the methods [9, 10].

The total organic carbon was determined using potassium chromate as an oxidizing reagent [11]. Total nitrogen and total phosphorus in sediment samples were extracted according to [12] and the analysis was done by the method [10]. Parsons correlation co-efficient was carried out for understanding the interrelationships between various physico-chemical parameters using SPSS-10.

Microbiological Analysis

Sediment Samples Treatment: Heat treatment was performed by holding the sediment samples in a water bath at 50°C for 60 min for prevention of other bacterial flora. All samples were diluted (up to $10G^5$) with sterile 0.5% saline prior to inoculation into the isolation plates [21].

Isolation of Actinobacteria: Dilutions ($10G^1$ - $10G^5$) of one gram of sediments in sterile 50% aged seawater were prepared and plated on starch-casein agar medium

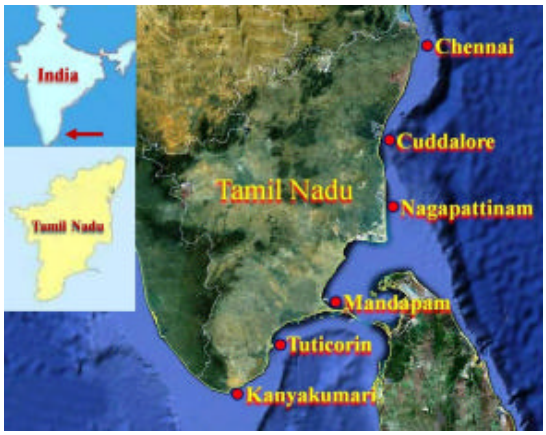

Fig. 1: Shows the study area map of Tamil Nadu coast

(starch, 10.0g; vitamin free casamino acids, 0.3g; $CaCO_3$, 0.02g; $Fe_3SO_4.7H_2O$, 0.01g; KNO_3, 2.0g; $MgSO_4.7H_2O$, 0.05g; NaCl, 2.0g; agar, 18.0g; pH, 7.2; 50% aged seawater) to isolate the actinobacteria. The medium was supplemented with 20 mg/l of nystatin and cycloheximide (100 mg/l) respectively [13](Kathiresan *et al.* 2005) to eliminate bacterial and fungal contaminations. All experiments were carried out in triplicates. The strains were sub-cultured onto starch casein agar slant (medium with 50% sea water), incubated at 28° C for 2-4 weeks to achieve good sporulation then they were preserved in 20% glycerol at -80°.

Identification of Actinobacteria: Purified isolates of actinobacteria were identified using morphological and cultural characteristics by the methods as described in the international Streptomyces Project (ISP) [14]. The morphology of the spore bearing hyphae with the entire spore chain, the structure and arrangement of the spore chain with the substrate and aerial mycelium of the actinobacteria were examined using slide culture technique and identified [15]. After growth, the slide cultures were examined under light microscope. Colour of spore mass was visually estimated by using the colour chart [16].

Cell wall composition (DL- and LL-Diaminopimielic acid isomer, A2 pm) was determined by the method [17]. One of two colonies were placed in a cryogenic vial with 0.1 ml of 6 M HCl. The vial was heated by autoclaving at 121°C for 15 min. After cooling 1 µl of the hydrolysate was placed on a thin cellulose plate. One µl of 0.01 M L-Diaminopimielic acid, meso-Diaminopimielic acid and aspartic acid were spotted on the same plate a standard. The plate was developed on the solvent system methonal-distilled water 6 M HCl-pyridine (80:26:4:10, v/v)

for 3-4 h. After the plate had been dried, it was sprayed with Ninhydrin Spray Reagent and was heated at 100°C for 5 min. The spots of A2pm appeared yellowish-green in colour. The same procedure for A2pm was used to analyse the whole-cell sugar, except that the hydrolysis and development solvents were 0.25 M HCl and n-butanol-distilled water –pyridine-toluene (10:6:6:1, v/v), respectively and the spraying reagent was acid aniline phthalate. The standard sugar solution contained galactose, glucose, mannose, arabinose, xylose and ribose each at 1% concentration.

RESULTS

Atmospheric temperature, water temperature, pH, salinity, dissolved oxygen, nitrite, nitrate, total phosphorus, ammonia and silicate values are shown in Fig. 2 - 11 and sediment total organic carbon, total nitrogen and total phosphorus values are shown in Fig. 12 - 14. In the sediment samples (Fig. 15), microbial load of the actinobacteria enumerated from the six stations varied from 12 to 38 $\times 10^5$ CFU gG^1 dry wt with the minimum (12 $\times 10^5$ CFU gG^1 dry wt) at station 5 during May and the maximum (38 $\times 10^5$ CFU gG^1 dry wt) at station 4 during October.

In general there is only very little spatial variation in most of the physical-chemical parameters recorded during this study outing to their closer geographical location. However, there is a cleat temporal variations in most of these parameters. Specifically speaking increasing amount of water nutrients recorded during the monsoon season correlating with land run off and higher rain water inflow.

Regarding correlation study between the parameters of water, station 1 water temperature showed a significant positive correlation with dissolved oxygen (r =857). Nitrate showed a significant positive correlation with total phosphorus at p =0.01 level. In station 2 atmospheric temperature showed a significant positive correlation with salinity (r = 885). Total phosphate showed a significant positive correlation with sediment nitrogen at p =0.01 level. In station 3 pH showed a significant negative correlation with nitrite (r = -842). While silicate exhibited a positive correlation with sediment total phosphate at p = 0.01 level. In station 4 total organic carbon showed a significant positive correlation with sediment total phosphate (r = 995). Silicate showed a significant positive correlation with sediment total nitrogen at p = 0.01 level. In station 5 dissolved oxygen showed a significant negative correlation with nitrite (r = -974) and sediment total nitrogen showed a negative correlation with population of actinobacteria at p = 0.01 level. In station 6 salinity showed a significant positive correlation with pH (r = 884) and dissolved oxygen showed a positive correlation with population of actinobacteria at p = 0.01 level (Table 1-6).

A total of 125 strains of actinobacteria were isolated based on colonies morphological and cultural characteristics for identification (Table 7). The majority of the isolated strains from station 5 were identified. The lowest number of isolates was identified from station 4. Among them, 125 isolates produced aerial and substrate mycelium and *Streptomyces* sp., *Actinopolyspora* sp., *Actinomadura* sp., *Nocardiopsis* sp., *Micromonospora* sp. and *Actinomyces* sp. groups (Table 8).

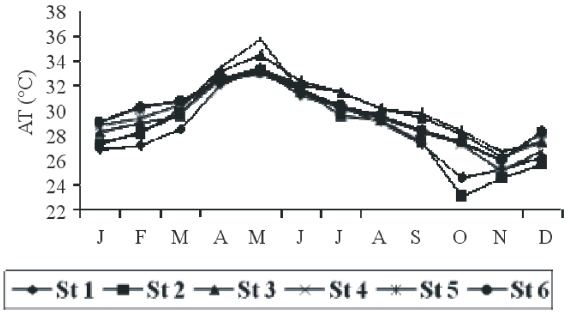

Fig. 2: Variations in atmospheric temperature recorded at six different stations

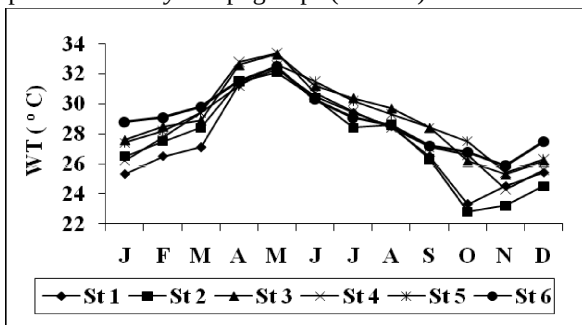

Fig. 3: Variations in surface water temperature recorded at six different stations

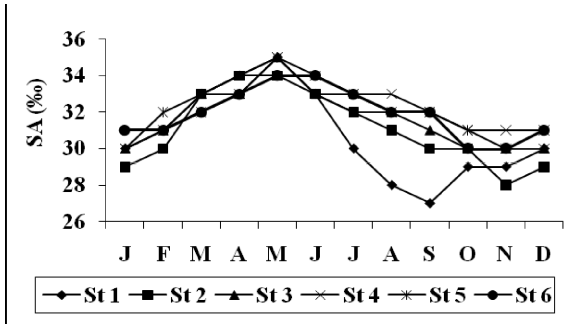

Fig. 4: Variations in salinity recorded at six different stations

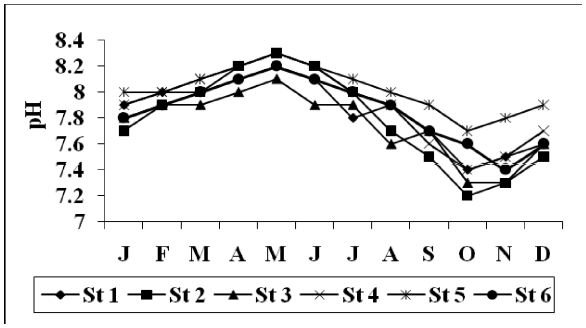

Fig. 5: Variations in pH recorded at six different stations

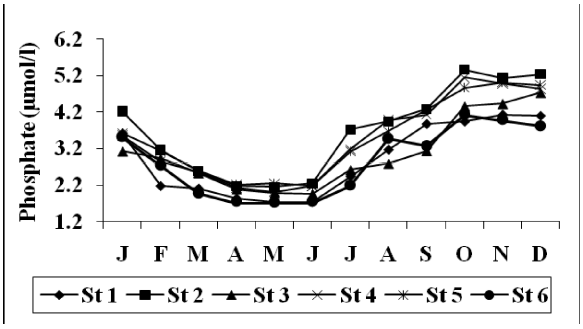

Fig. 9: Variations in phosphate recorded at six different stations

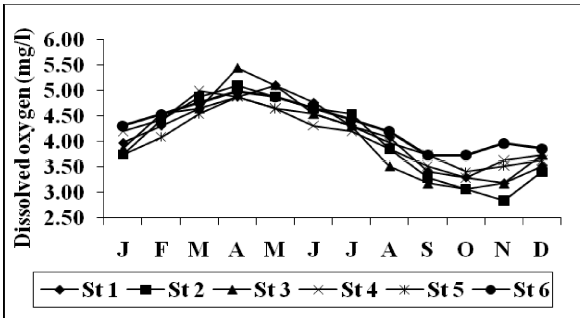

Fig. 6: Variations in dissolved oxygen recorded at six different stations

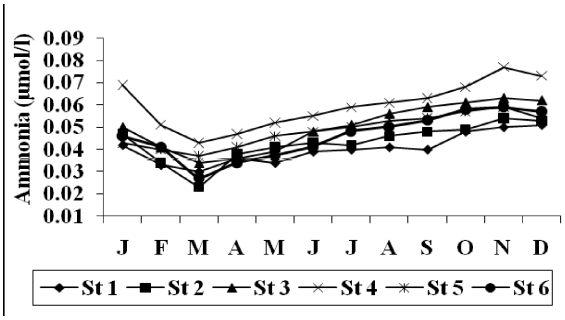

Fig. 10: Variations in ammonia recorded at six different stations

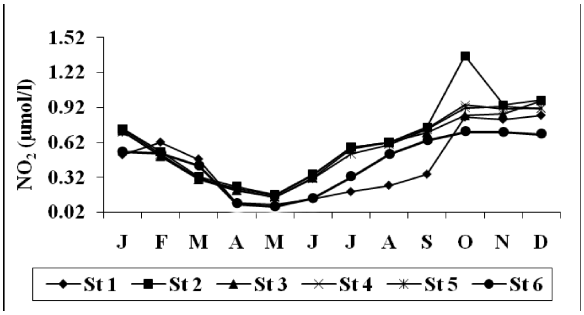

Fig. 7: Variations in nitrite recorded at six different stations

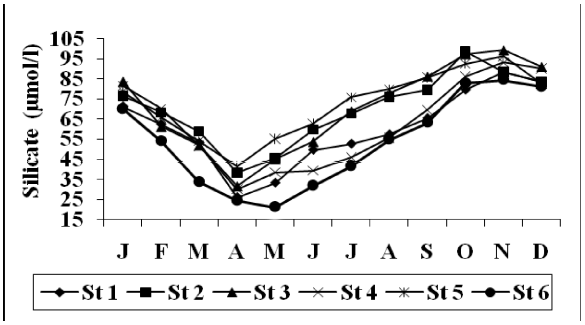

Fig. 11: Variations in silicate recorded at six different stations

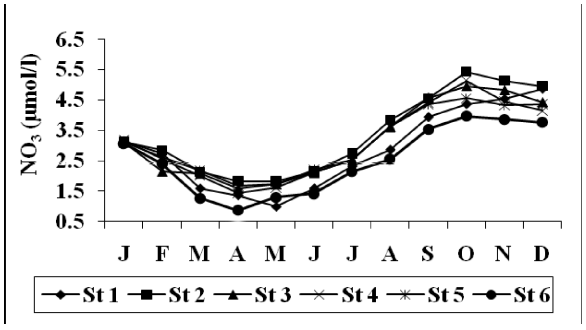

Fig. 8: Variations in nitrate recorded at six different stations

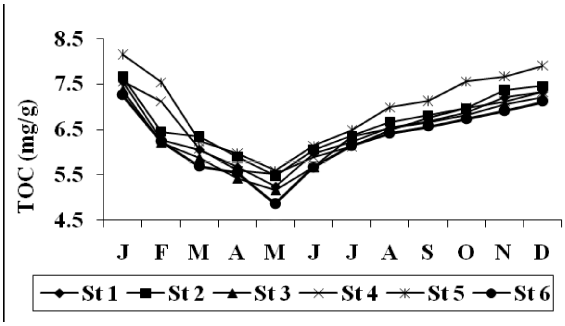

Fig. 12: Variations in total organic carbon recorded at six different stations

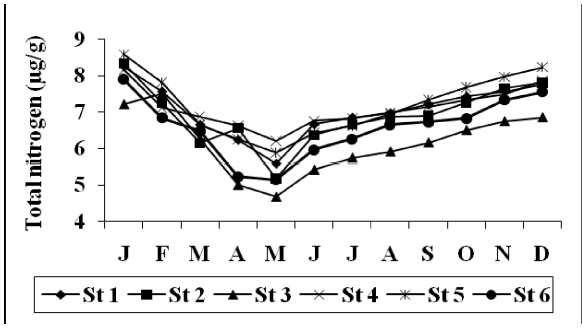

Fig. 13: Variations in sediment nitrogen recorded at six different stations

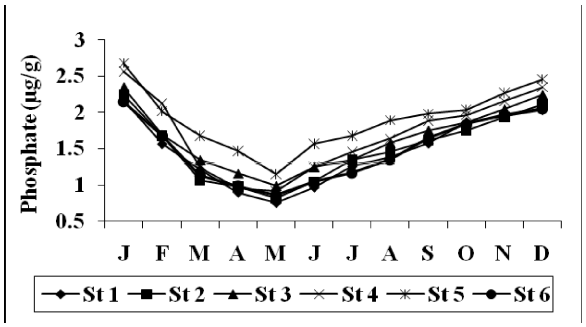

Fig. 14: Variations in sediment phosphate recorded at six different stations

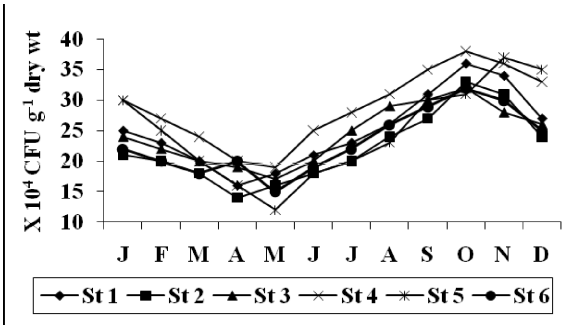

Fig. 15: Variations in population of actinobacteria recorded at six different stations

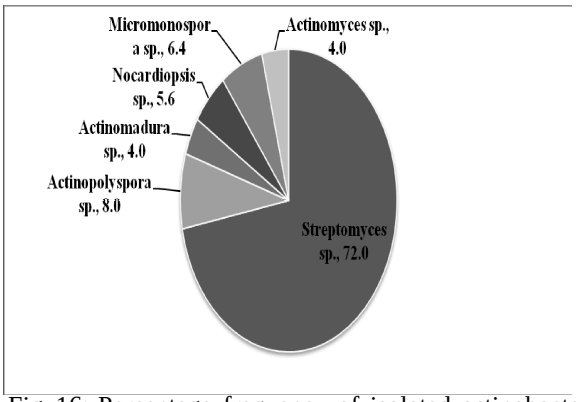

Fig. 16: Percentage frequency of isolated actinobacteria genera

The occurrence and distribution of different genera of actinobacteria in different marine sediment are presented. Out of 125 isolates of actinobacteria, 90 isolates were identified as genus *Streptomyces* sp. (spore chain with rectiflexibiles (RF), retinaculiaperti (RA) and spiral (S), 10 as *Actinopolyspora* sp. (long chains of spores on aerial hyphae), 5 as *Actinomadura* sp. (spore chains are straight and open hooked), 7 as *Nocardiopsis* sp. (aerial mycelium totally sporulated), 8 as *Micromonospora* sp. (clusters of single conidia on substrate mycelium) and 5 as *Actinomyces* sp. (branching vegetative mycelium).

Frequencies of identified genera of actinobacteria, in different sites, were fluctuated. The frequency of the genus *Streptomyces* was 72.0% followed by *Actinopolyspora* (8.0%), *Actinomadura* (4.0%), *Nocardiopsis* (5.6%), *Micromonospora* (6.4%) and *Actinomyces* (4.0%) (Fig. 16).

Table 1: Simple correlation co-efficient (r) values obtained between physico-chemical parameters and actinobacteria isolated from station 1

	AT	WT	SA	pH	DO	NO_2	NO_3	PO_4	NH_4	Silicate	TOC	S-Nitrogen	S-Phosphate	Actino bacteria
AT	1.000													
WT	0.994**	1.000												
SA	0.727**	0.689*	1.000											
pH	0.851**	0.834**	0.754**	1.000										
DO	0.901**	0.875**	0.865**	0.947**	1.000									
NO_2	-0.926**	-0.923**	-0.475	-0.787**	-0.781**	1.000								
NO_3	-0.894**	-0.864**	-0.806**	-0.922**	-0.975**	0.833**	1.000							
PO_4	-0.845**	-0.833**	-0.829**	-0.891**	-0.962**	0. 738**	0.955**	1.000						
NH_4	-0.632*	-0.604*	-0.596*	-0.836**	-0.810**	0.631*	0.851**	0.838**	1.000					
Silicate	-0.940**	-0.926**	-0.727**	-0.860**	-0.911**	0.898**	0.928**	0.881**	0.770**	1.000				
TOC	-0.861**	-0.860**	-0.741**	-0.751**	-0.851**	0.758**	0.871**	0.897**	0.761**	0.902**	1.000			
S-Nitrogen	-0.863**	-0.859**	-0.688*	-0.628*	-0.752**	0.751**	0.781**	0.748**	0.579*	0.850**	0.938**	1.000		
S-Phosphate	-0.918**	-0.921**	-0.706*	-0.767**	-0.857**	0.851**	0.886**	0.885**	0.710**	0.923**	0.972**	0.949**	1.000	
Actino bacteia	-0.856**	-0.835**	-0.825**	-0.930**	-0.953**	0.727**	0.895**	0.886**	0.763**	0.867**	0.734**	0.637*	0.753**	1.000

** Correlation is significant at the 0.01 level

* Correlation is significant at the 0.05 level

Table 2: Simple correlation co-efficient (r) values obtained between physico-chemical parameters and actinobacteria isolated from station 2

	AT	WT	SA	pH	DO	NO_2	NO_3	PO_4	NH_4	Silicate	TOC	S-Nitrogen	S-Phosphate	Actino bacteria
AT	1.000													
WT	0.994**	1.000												
SA	0.885**	0.890**	1.000											
pH	0.965**	0.958**	0.868**	1.000										
DO	0.907**	0.909**	0.891**	0.965**	1.000									
NO_2	-0.962**	-0.944**	-0.809**	-0.948**	-0.906**	1.000								
NO_3	-0.920**	-0.919**	-0.832**	-0.973**	-0.968**	0.938**	1.000							
PO_4	-0.944**	-0.941**	-0.886**	-0.956**	-0.943**	0.955**	0.957**	1.000						
NH_4	-0.557	-0.560	-0.639*	-0.646*	-0.787**	0.672*	0.741**	0.735**	1.000					
Silicate	-0.950**	-0.942**	-0.871**	-0.946**	-0.932**	0.949**	0.929**	0.942**	0.634*	1.000				
TOC	-0.847**	-0.856**	-0.924**	-0.829**	-0.826**	0.786**	0.764**	0.869**	0.590*	0.822**	1.000			
S-Nitrogen	-0.759**	-0.751**	-0.878**	-0.709**	-0.694*	0.706*	0.637*	0.756**	0.536	0.700*	0.930**	1.000		
S-Phosphate	-0.837**	-0.832**	-0.956**	-0.794**	-0.805**	0.784**	0.744	0.856**	0.615*	0.804**	0.961**	0.949**	1.000	
Actino bacteia	-0.911**	-0.905**	-0.777**	-0.957**	-0.946**	0.919**	0.952**	0.896**	0.656*	0.940**	0.683*	0.541	0.649*	1.000

** Correlation is significant at the 0.01 level

* Correlation is significant at the 0.05 level

Table 3: Simple correlation co-efficient (r) values obtained between physico-chemical parameters and actinobacteria isolated from station 3

	AT	WT	SA	pH	DO	NO_2	NO_3	PO_4	NH_4	Silicate	TOC	S-Nitrogen	S-Phosphate	Actino bacteria
AT	1.000													
WT	0.987**	1.000												
SA	0.913**	0.915**	1.000											
pH	0.798**	0.843*	0.765**	1.000										
DO	0.777**	0.815**	0.779**	0.890**	1.000									
NO_2	-0.872**	-0.903**	-0.925**	-0.842**	-0.882**	1.000								
NO_3	-0.774**	-0.829**	-0.784**	-0.923**	-0.953**	0.913**	1.000							
PO_4	-0.875**	-0.920**	-0.852**	-0.863**	-0.770**	0.928**	0.878**	1.000						
NH_4	-0.651*	-0.712**	-0.736**	-0.848**	-0.909**	0.900**	0.932**	0.791**	1.000					
Silicate	-0.847**	-0.888**	-0.859**	-0.881**	-0.960**	0.960**	0.945**	0.876**	0.921**	1.000				
TOC	-0.887**	-0.887**	-0.938**	-0.735**	-0.830**	0.949**	0.806**	0.825**	0.787**	0.915**	1.000			
S-Nitrogen	-0.882**	-0.838**	-0.843**	-0.491	-0.544	0.698*	0.480	0.660*	0.383	0.643*	0.783**	1.000		
S-Phosphate	-0.893**	-0.885**	-0.931**	-0.668*	-0.725**	0.896**	0.734**	0.826**	0.683*	0.838**	0.960**	0.839**	1.000	
Actino bacteia	-0.685*	-0.724**	-0.757**	-0.865**	-0.946**	0.847**	0.918**	0.726**	0.876**	0.886**	0.763**	0.458	0.623*	1.000

** Correlation is significant at the 0.01 level

* Correlation is significant at the 0.05 level

Table 4: Simple correlation co-efficient (r) values obtained between physico-chemical parameters and actinobacteria isolated from station 4

	AT	WT	SA	pH	DO	NO_2	NO_3	PO_4	NH_4	Silicate	TOC	S-Nitrogen	S-Phosphate	Actino bacteria
AT	1.000													
WT	0.987**	1.000												
SA	0.875**	0.854**	1.000											
pH	0.841**	0.859**	0.678*	1.000										
DO	0.695*	0.736**	0.427	0.901**	1.000									
NO_2	-0.906**	-0.930**	-0.748**	-0.940**	-0.894**	1.000								
NO_3	-0.813**	-0.848**	-0.608*	-0.976**	-0.956**	0.946**	1.000							
PO_4	-0.842**	-0.876**	-0.655*	-0.962**	-0.904**	0.969**	0.970**	1.000						
NH_4	-0.744**	-0.810**	-0.546	-0.799**	-0.848**	0.901**	0.834**	0.873**	1.000					
Silicate	-0.912**	-0.951**	-0.850**	-0.863**	-0.731**	0.917**	0.851**	0.879**	0.823**	1.000				
TOC	-0.907**	-0.929**	-0.911**	-0.730**	-0.629*	0.857**	0.735**	0.757**	0.739**	0.935**	1.000			
S-Nitrogen	-0.845**	-0.865**	-0.894**	-0.637*	-0.523	0.800**	0.639*	0.691*	0.743**	0.852**	0.930**	1.000		
S-Phosphate	-0.892	-0.917**	-0.887**	-0.707*	-0.633*	0.855**	0.718**	0.741**	0.765**	0.918**	0.995**	0.937**	1.000	
Actino bacteia	-0.881**	-0.896**	-0.664*	-0.969**	-0.937**	0.962**	0.981**	0.955**	0.827**	0.853**	0.772**	0.682*	0.759**	1.000

** Correlation is significant at the 0.01 level.

* Correlation is significant at the 0.05 level.

Table 5: Simple correlation co-efficient (r) values obtained between physico-chemical parameters and actinobacteria isolated from station 5

	AT	WT	SA	pH	DO	NO_2	NO_3	PO_4	NH_4	Silicate	TOC	S-Nitrogen	S-Phosphate	Actino bacteria
AT	1.000													
WT	0.993**	1.000												
SA	0.953**	0.947**	1.000											
pH	0.896**	0.884**	0.828**	1.000										
DO	0.904**	0.886**	0.894**	0.937**	1.000									
NO_2	-0.925**	-0.917**	-0.917**	-0.943**	-0.974**	1.000								
NO_3	-0.843**	-0.825**	-0.808**	-0.930**	-0.949**	0.956**	1.000							
PO_4	-0.903**	-0.895**	-0.850**	-0.938**	-0.955**	0.980**	0.971**	1.000						
NH_4	-0.512	-0.476	0-.490	-0.661*	-0.705*	0.735**	0.811**	0.775**	1.000					
Silicate	-0.801**	-0.778**	-0.799**	-0.866**	-0.946**	0.933**	0.924**	0.912**	0.835**	1.000				
TOC	-0.914**	-0.919**	-0.957**	-0.815**	-0.895**	0.885**	0.772**	0.818**	0.394	0.762**	1.000			
S-Nitrogen	-0.907**	-0.916**	-0.952**	-0.766**	-0.852**	0.844**	0.714**	0.773**	0.310	0.699*	0.989**	1.000		
S-Phosphate	-0.885**	-0.898**	-0.966**	-0.724**	-0.822**	0.835**	0.705*	0.753**	0.315	0.692*	0.968**	0.978**	1.000	
Actino bacteia	-0.973**	-0.974**	-0.952**	-0.907**	-0.934**	0.963**	0.903**	0.941**	0.607*	0.851**	0.913**	0.903**	0.891**	1.000

** Correlation is significant at the 0.01 level.

* Correlation is significant at the 0.05 level.

Table 6: Simple correlation co-efficient (r) values obtained between physico-chemical parameters and actinobacteria isolated from station 6

	AT	WT	SA	pH	DO	NO_2	NO_3	PO_4	NH_4	Silicate	TOC	S-Nitrogen	S-Phosphate	Actino bacteria
AT	1.000													
WT	0.984**	1.000												
SA	0.875**	0.831**	1.000											
pH	0.981**	0.951**	0.884**	1.000										
DO	0.900**	0.919**	0.699*	0.878**	1.000									
NO_2	-0.939**	-0.939**	-0.910**	-0.920**	-0.890**	1.000								
NO_3	-0.939	-0.923**	-0.817**	-0.938**	-0.961**	0.922**	1.000							
PO_4	-0.927**	-0.892**	-0.878**	-0.921**	-0.903**	0.928**	0.957**	1.000						
NH_4	-0.840**	-0.838**	-0.610*	-0.833**	-0.914**	0.742**	0.918**	0.861**	1.000					
Silicate	-0.956**	-0.928**	-0.899**	-0.960**	-0.904**	0.941**	0.974**	0.974**	0.864**	1.000				
TOC	-0.874**	-0.859**	-0.817**	-0.852**	-0.813**	0.875**	0.877**	0.895**	0.762**	0.928**	1.000			
S-Nitrogen	-0.815**	-0.794**	-0.809**	-0.787**	-0.708**	0.867**	0.799**	0.820**	0.604*	0.870**	0.938**	1.000		
S-Phosphate	-0.852**	-0.809**	-0.879**	-0.871**	-0.771**	0.879**	0.892**	0.895**	0.714**	0.943**	0.945**	0.934**	1.000	
Actino bacteia	-0.898**	-0.908**	-0.726**	-0.870**	-0.910**	0.816**	0.872**	0.867**	0.869**	0.843**	0.739**	0.559	0.661*	1.000

** Correlation is significant at the 0.01 level.

* Correlation is significant at the 0.05 level.

Table 7: Occurrence and distribution of actinobacteria in different marine sediment samples

		Stations						
S. No	Genus	1	2	3	4	5	6	Total
1	*Streptomyces* sp.	13	10	16	9	28	14	90
2	*Actinopolyspora* sp.	2	0	1	2	5	0	10
3	*Actinomadura* sp.	1	0	1	0	1	2	5
4	*Nocardiopsis* sp.	1	0	2	1	3	0	7
5	*Micromonospora* sp.	1	3	0	2	2	0	8
6	*Actinomyces* sp.	0	2	0	0	2	1	5
	Total	18	15	20	14	41	17	125

Table 8: Cultural characteristics of actinobacteria isolates

Name of actinobacteria	Strains No.	Aerial Mass colour	Melanoid Pigment	Reverse side pigment	Soluble pigment	Colony size (mm)
Station 1						
Streptomyces sp.	MACH 1	White	Greenish brown	-	-	1.2
Streptomyces sp.	MACH 2	Gray	-	-	-	2.0
Actinopolyspora sp.	MACH 3	White	-	Light yellow	-	1.3
Streptomyces sp.	MACH 4	Light white	-	-	-	1.0
Actinomadura sp.	MACH 5	White	-	Dark yellow	-	1.5
Streptomyces sp.	MACH 6	Light white	Brownish black	Yellow	Yellow	2.1
Streptomyces sp.	MACH 7	Grey	-	-	-	2.3
Streptomyces sp.	MACH 8	White	Brownish black	Yellow	Yellowish brown	1.5
Micromonospora sp.	MACH 9	Ash	-	Yellowish	-	1.0
Streptomyces sp.	MACH 10	Red	-	-	-	1.5
Streptomyces sp.	MACH 11	Grey	-	-	-	2.0
Nocardiopsis sp.	MACH 12	Light green	-	Yellow	-	1.5
Streptomyces sp.	MACH 13	White	Brown	Yellowish	Blue	1.0
Streptomyces sp.	MACH 14	Dark ash	-	-	-	1.5
Streptomyces sp.	MACH 15	Greenish ash	Black	Yellow	-	2.0
Streptomyces sp.	MACH 16	Red	-	-	-	1.5
Actinopolyspora sp.	MACH 17	Dull shite	-	Yellow	-	1.0
Streptomyces sp.	MACH 18	Grey	Greenish brown	-	-	2.5
Station 2						
Streptomyces sp.	MACU 1	Grey	Brown	Yellow	Orange	3.0
Streptomyces sp.	MACU 2	Green	-	Yellowish brown		2.5
Micromonospora sp.	MACU 3	Yellow	Brownish black	Yellow	Yellow	1.5
Streptomyces sp.	MACU 4	Red	-	Light yellow	-	2.0
Actinomyces sp.	MACU 5	Rose	-	Light yellow	Orange	2.5
Streptomyces sp.	MACU 6	Grey	Black	Pale yellow	-	3.0
Micromonospora sp.	MACU 7	Ash		Yellow		3.5
Streptomyces sp.	MACU 8	Light blue	Brownish black	Yellow	Blue	3.0
Streptomyces sp.	MACU 9	Grey	Distinct brown	Light yellow	-	2.0
Streptomyces sp.	MACU 10	Grey	-	Dark yellow	-	1.5
Micromonospora sp.	MACU 11	Dark ash	Brown	Light ash	-	2.0
Actinomyces sp.	MACU 12	Light rose	-	Violet	-	1.0
Streptomyces sp.	MACU 13	White	-	Dark yellow	-	3.5
Streptomyces sp.	MACU 14	Grey	-	Yellowish	-	2.0
Streptomyces sp.	MACU 15	Light white	Distinct brown	Light yellow	-	1.0
Station 3						
Streptomyces sp.	MANA 1	Light white	-	Light brown	-	2.5
Streptomyces sp.	MANA 2	Dark ash	-	Light yellow	-	1.5
Streptomyces sp.	MANA 3	Ash	Brownish black	Black	-	2.0
Nocardiopsis sp.	MANA 4	Ash	-	Yellowish	-	2.5
Streptomyces sp.	MANA 5	Grey	-	Dark yellow	-	3.0
Streptomyces sp.	MANA 6	Light grey	-	Light yellow	-	1.0
Actinopolyspora sp.	MANA 7	Dull white	-	Yellow	-	2.5
Streptomyces sp.	MANA 8	Red	-	Pale yellow	-	3.5
Streptomyces sp.	MANA 9	Light red	Black	Light black	-	2.0
Streptomyces sp.	MANA 10	White	Greenish brown	Olive	Yellow	1.5
Streptomyces sp.	MANA 11	Pure white	Black	Light black	-	2.5
Streptomyces sp.	MANA 12	Dull white	-	Light yellow	-	1.0
Streptomyces sp.	MANA 13	Red	-	Yellow	-	2.5
Nocardiopsis sp.	MANA 14	Green	-	Yellowish	-	3.0

Table 8: Continued

Streptomyces sp.	MANA 15	Light white	Distinct brown	Light yellow	-	2.0
Actinomadura sp.	MANA 16	Pure white	-	Light yellow	-	2.5
Streptomyces sp.	MANA 17	Ash	Brownish black	Light black	Blue	3.0
Streptomyces sp.	MANA 18	Pure white	-	Light yellow	Orange	2.5
Streptomyces sp.	MANA 19	Grey	Black	Light back	-	3.5
Streptomyces sp.	MANA 20	Red	-	Light red	-	2.0
Station 4						
Actinopolyspora sp.	MAMA 1	Ash	-	Light blue	-	2.5
Nocardiopsis sp.	MAMA 2	Light ash	-	Light yellow	-	3.0
Streptomyces sp.	MAMA 3	Pure white	-	Light black	-	3.0
Actinopolyspora sp.	MAMA 4	Dull ash	-	Light yellow	-	2.5
Streptomyces sp.	MAMA 5	Grey	Brownish black	Light black	Yellow	3.0
Micromonospora sp.	MAMA 6	Yellow	-	Light yellow	-	2.5
Streptomyces sp.	MAMA 7	Pure white	-	-	-	3.0
Streptomyces sp.	MAMA 8	White	Brownish black	Yellow	-	2.0
Streptomyces sp.	MAMA 9	Pure white	-	Pure yellow	-	2.5
Micromonospora sp.	MAMA 10	Dark ash	-	Light brown	-	2.0
Streptomyces sp.	MAMA 11	Light grey	-	Light black	-	3.0
Streptomyces sp.	MAMA 12	Pure white	Black	Light black	blue	3.5
Streptomyces sp.	MAMA 13	White	Brownish black	Light yellow	Orange	2.0
Streptomyces sp.	MAMA 14	White	-	Yellow	-	2.0
Station 5						
Actinopolyspora sp.	MATU 1	Dull ash	Brownish black	Brown	-	3.0
Nocardiopsis sp.	MATU 2	Green	-	Light yellow	-	2.5
Streptomyces sp.	MATU 3	Grey	Brown	Light black	-	3.0
Actinomyces sp.	MATU 4	Red	-	Light yellow	Orange	1.0
Streptomyces sp.	MATU 5	Pure white	-	Yellow	-	1.5
Streptomyces sp.	MATU 6	White	-	Yellowish	-	2.0
Streptomyces sp.	MATU 7	Grey	Black	Light yellow	Blue	3.0
Streptomyces sp.	MATU 8	Light grey	Brownish black	Light yellow	Yellow	2.0
Actinopolyspora sp.	MATU 9	White	-	Light yellow	-	2.5
Streptomyces sp.	MATU 10	Light white	-	Yellow	-	3.0
Streptomyces sp.	MATU 11	Pure white	-	Yellowish brown		3.5
Streptomyces sp.	MATU 12	White	-	Yellow	-	2.5
Streptomyces sp.	MATU 13	Light grey	-	Light brown	-	3.0
Micromonospora sp.	MATU 14	Yellow	-	Light yellow	-	2.0
Streptomyces sp.	MATU 15	Grey	Brown	Light brown	-	2.5
Streptomyces sp.	MATU 16	Pure white	-	Light yellow	-	2.0
Streptomyces sp.	MATU 17	White	-	Pale yellow	-	2.5
Streptomyces sp.	MATU 18	White	Brownish black	Light yellow	Green	2.0
Streptomyces sp.	MATU 19	Red	-	Light red	-	2.5
Actinopolyspora sp.	MATU 20	ASh	-	Light ash	-	3.0
Streptomyces sp.	MATU 21	Pure white	-	yellow	-	3.5
Actinomyces sp.	MATU 22	Red	Brownish	Light yellow	Orange	3.0
Streptomyces sp.	MATU 23	Light grey	-	Yellow	-	2.5
Streptomyces sp.	MATU 24	Grey	-	Yellowish	-	2.0
Actinomadura sp.	MATU 25	Dark ash	-	Dark yellow	-	2.0
Streptomyces sp.	MATU 26	Red	-	Light yellow	-	2.5
Streptomyces sp.	MATU 27	Light red	-	Light yellow	-	2.0
Actinopolyspora sp.	MATU 28	White	-	Yellow	-	2.0
Nocardiopsis sp.	MATU 29	Light ash	-	Yellow	-	2.0

Table 8: Continued

Streptomyces sp.	MATU 30	Grey	-	Light yellow	-	3.5
Streptomyces sp.	MATU 31	White	-	Pale yellow	-	2.5
Streptomyces sp.	MATU 32	Pure white	Black	Light black	Blue	2.0
Actinopolyspora sp.	MATU 33	Dull ash	-	Light yellow	-	2.5
Micromonospora sp.	MATU 34	Ash	-	Yellow	-	2.0
Streptomyces sp.	MATU 35	Light grey	-	Light yellow	-	2.5
Streptomyces sp.	MATU 36	Grey	-	Dark yellow	-	1.5
Nocardiopsis sp.	MATU 37	Light green	-	Yellow	-	2.0
Streptomyces sp.	MATU 38	White	-	Light yellow	-	2.5
Streptomyces sp.	MATU 39	Light white	-	Pale yellow	-	2.0
Streptomyces sp.	MATU 40	Grey	Brownish black	Light yellow	-	3.0
Streptomyces sp.	MATU 41	White	Black	Yellow	Blue	2.0
Station 6						
Actinomadura sp.	MAKA 1	Pure white	Brownish black	Light yellow	Blue	2.5
Streptomyces sp.	MAKA 2	White	-	Yellowish	-	2.0
Streptomyces sp.	MAKA 3	Light grey	-	Pale yellow	-	2.5
Streptomyces sp.	MAKA 4	Light green	-	Light yellow	-	3.0
Actinomadura sp.	MAKA 5	Light white	-	Light yellow	-	2.5
Actinomyces sp.	MAKA 6	White	-	Pale yellow	-	3.5
Streptomyces sp.	MAKA 7	Red	-	Yellow	-	3.0
Streptomyces sp.	MAKA 8	Pure white	-	Light yellow	-	2.0
Streptomyces sp.	MAKA 9	Light white	-	Yellow	-	2.5
Streptomyces sp.	MAKA 10	White	-	Light yellow	-	3.0
Streptomyces sp.	MAKA 11	Grey	-	Pale yellow	-	3.5
Streptomyces sp.	MAKA 12	Pure white	Brownish black	Light yellow	-	3.0
Streptomyces sp.	MAKA 13	Red	Greenish brown	yellow	Orange	2.0
Streptomyces sp.	MAKA 14	Light grey	-	Light yellow	-	2.5
Streptomyces sp.	MAKA 15	Grey	Black	Yellow	-	2.0
Streptomyces sp.	MAKA 16	White	-	Light yellow	-	2.5
Streptomyces sp.	MAKA 17	Pure white	Brownish black	Yellow	Blue	3.0

DISCUSSION

Atmospheric temperature is one of the most important factors controlling the physiological activities of tropical marine organisms. In nature, each species has its maximal, optimal and minimal temperature requirements for growth and development. In the present study, the higher atmospheric temperature of 35.8°C was recorded at station 4 during May and it could be attributed to the solar radiation with clear sky. The surface water temperature is influenced by sunshine, evaporation, cooled freshwater influx and admixture ebb flow from the adjoining neritic waters. The maximum surface water temperature of 33.4°C was recorded at station 4 during May.

In the present study salinity was minimum during the monsoon due to mixing of fresh water through rain fall and precipitation. Salinity is one of the most important key factors which determine the composition of biological component in the marine environment. The fluctuations in salinity affect the biological characteristics of the marine environment. All the stations recorded lower (28°/$_{oo}$) salinity during the monsoon season than the other seasons reaching the maximum (35°/$_{oo}$) during the summer when there is no rain water flow in the rivers. Higher pH recorded (8.3) during the summer in the present study due to the removal of CO_2 by the photosynthetic organisms and the lower pH observed (7.2) during the monsoon season. The first two factors encourage a heating of the water during the day, provoking evaporation and an increase in the salinity values and rain reduces the temperature, pH and salinity during monsoon period. Low dissolved oxygen concentration observed during the summer season might be due to the shallow nature of the water column of the study areas coupled with the biological oxidation of detritus and respiration of bottom communities along with slow diffusion of dissolved gases. Higher dissolved oxygen were recorded at summer (5.47 mg/l) season. This similar observation was made earlier by [18, 19] from this Parangaipettai and Cuddalore coast and coral reef environment as well.

The present study observed that the nitrite, nitrate and phosphate concentrations in water in higher ranges (1.362µmol/l, 5.436µmol/l and 5.361µmol/l) respectively. Regarding concentration of ammonia in water higher range in (0.077µmol/l) monsoon season. The maximum concentration of reactive silicate (99.4µmol/l) was observed during the monsoon period and the minimum recorded during summer. The results were highly correlate with the results of [20]. The increased level of nutrients in water and sediment during monsoon period due to land run off and rainwater inflow in the rivers through leaching from manured and fertilized agricultural soils, aquaculture discharge and sewage effluents from the surrounding environment. High amounts of organic carbon (8.16 mg/g) were observed during monsoon and post monsoon periods might have been brought by the external inputs. Higher nitrogen and phosphate (8.59 and 2.679 µg/g) were recorded during post monsoon.

Microbial diversity comprises a wide range of microbes than any other living group of organisms of the world. This rich diversity is due to existence of microbes in all niches where life is possible. Actinobacteria populations in the estuarine and marine sediments vary in density with varying regions and even among sites within an ecosystem and actinobacteria are being reported from the marine sub habitats such as marine sediments [21, 22, 23] and marine soils [24, 25] of almost all parts of the world. Thus, they have worldwide distribution which indicates their plasticity and adaptability to extremely varied environmental conditions.

In the present study, the highest population density in sediment samples was recorded at station 4 during October (38 $X10^5$ CFU g^{-1} dry wt) and the lowest in the station 5 during May (12 $X10^5$ CFU g^{-1} dry wt). These ranges are in agreement with the ranges reported by previous workers [26, 27, 28, 29]. It is quit natural and also proved that the population of actinobacteria would decrease in numbers as distance from the shore increases [30, 31, 32].

A total of 125 strains of actinobacteria were isolated based on colonies morphological and cultural characteristics for identification. The majority of the isolated strains from station 5 were identified. The lowest number of isolates was identified from station 4. Among them, 125 isolates produced aerial and substrate mycelium and *Streptomyces* sp., *Actinopolyspora* sp., *Actinomadura* sp., *Nocardiopsis* sp., *Micromonospora* sp. and *Actinomyces* sp. groups. The similar results were observed [26, 33].

The occurrence and distribution of different genera of actinobacteria in different marine sediment are presented. Out of 125 isolates of actinobacteria, 90 isolates were identified as genus *Streptomyces* sp., 10 as *Actinopolyspora* sp., 5 as *Actinomadura* sp., 7 as *Nocardiopsis* sp., 8 as *Micromonospora* sp. and 5 as *Actinomyces* sp. Actinobacteria, especially *Strepomyces*, have been reported from the marine sub habitts such as marine sediments [5, 21, 22, 24].

Frequencies of identified genera of actinobacteria, in different sites, were fluctuated. The frequency of the genus *Streptomyces* was 72.0% followed by *Actinopolyspora* (8.0%), *Actinomadura* (4.0%), *Nocardiopsis* (5.6%), *Micromonospora* (6.4%) and *Actinomyces* (4.0%). Among the genera recorded, in the present study, *Streptomyces* was the most predominant when compared to other genera. The dominance of *Streptomyces* among the actinobacteria especially in soils has also been reported by many workers [4, 34-37]. Besides *Streptomyces*, the genera most frequently appeared on media were *Actinopolyspora*, *Actinomadura*, *Nocardiopsis*, *Micromonospora* and *Actinomyces*.

Thought there are 125 isolates of actinobacteria belonged to different genera recorded during the course of study, it does not give a complete picture of actinobacteria diversity. It needs frequent visits to the field, isolation from different substrates collected from the habitat and the usage of different media.

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