Ecological factors and their influence on actinobacterial density from different coastal habitats of Neil island, Andamans

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Sediment and water samples were collected from three different coastal habitats (Mangrove, Coral reef and Beach) of the Neil island, the Andamans, to estimate various physico-chemical parameters viz: temperature, salinity, pH, DO, EC, Total organic carbon, nutrients and soil texture. Principal Component Analysis (PCA) and Cluster analysis (CA) were used to identify the trends between the highly correlating physico-chemical parameters and the actinobacterial density and it revealed the distinct relationship between the coastal habitats and their physico-chemical parameters with the actinobacterial population density. Positive significant correlation between the actinobacterial density and clay, TOC, sediment pH, potassium (K), nitrogen content, phosphorus content, and EC was noticed in the mangrove habitats, implying that the mangrove environment is worth studying for isolation of actinobacteria that might prove to be novel and potential, for commercial applications.

[Keywords: Ecological factors, microbial ecology, Principal Component Analysis (PCA), Actinobacterial density].

Introduction

Marine environment, a complex system, is mainly influenced by various physical and chemical processes. One of the basic goals for the study of the physico-chemical parameters is to understand the factors that play an important role in the distribution pattern of organisms in the marine environment. Physico-chemical properties in a coastal environment, particularly in the nearshore waters and sediments, exhibit considerable variations depending upon the regional environmental set up such as rainfall, quantum of fresh water inflow, tidal incursion and also biological activities. Microbial density is largely influenced by nutrients and physiochemical parameters.

Considering the importance of the physico-chemical parameters on the productivity potential of coastal waters and sediments, many studies have been made in coastal waters and sediments and their impacts on the occurrence and abundance of actinomycetes density. In the present study, influence of the environmental factors such as temperature, salinity, pH, DO, EC, Total organic carbon, nutrients and soil texture on the actinobacterial density of the coastal habitats of the Neil island, the Andamans, was studied.

Materials and Methods

Samples for the estimation of various parameters were collected from the Neil Island, the Andamans, in the month of November, 2011. Surface water and sediment samples were collected from six stations (Table 1), covering the mangrove, coral reef and beach habitats of the Neil island. Air, water and sediment temperatures were measured with a mercury thermometer. With the use of a hand held refractometer (Erma company, Japan), salinity of the samples was measured. pH was measured by pH Tester Model DM – 13, Takemura Electric Works Ltd Tokyo, Japan.

<table>
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<th>Station No.</th>
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<td>6</td>
<td>11°49'04.3&quot;N</td>
<td>93°01' 48.4&quot;E</td>
<td>Beach</td>
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</table>

Water samples collected for dissolved oxygen estimation were transferred carefully to BOD bottles. The dissolved oxygen was immediately fixed and the samples were brought to the field laboratory for further analysis. Modified
Winkler’s method described by Strickland and Parsons\textsuperscript{12} was adopted for the estimation of dissolved oxygen. In the powdered digested samples of the sediments, levels of Nitrogen, Phosphorus and Potassium were analyzed using Kjeldahl method\textsuperscript{13} and colorimetric methods\textsuperscript{14}, in the Sugarcane Breeding Research Institute, Cuddalore. The values obtained are expressed in mg g\textsuperscript{-1}.

Sample weighing 100 g was taken and sieved through a mesh (62 µ) for ten minutes in a sieve shaker. The sample remaining in the sieve was weighed and treated as sand. The sediment samples which passed through the 62 µ sieve were the silt and clay. The silt and clay were then separated by means of pipette method, described by Ramanathan et al.\textsuperscript{15}.

For the actinobacterial assessment, sediment samples were collected by a spatula sterilized with alcohol before sampling. Sample was then transferred to a sterilized sample collector. Sediments were aseptically air dried to increase the isolation of overall actinobacteria and the dried samples were pretreated and mixed with equal weight of CaCO\textsubscript{3} (1:1) and incubated at 55°C for 5 min\textsuperscript{16}.

One g of pretreated sample was serially diluted and plated by spread plate method using Kuster’s agar. The plates were incubated at 30°C in inverted position for 7 to 15 days. Colonies from the isolation plate were sub-cultured in nutrient agar prepared in 50% sea water. Slants of the above media were stored and used for further studies. Leathery colonies of actinobacteria that appeared on the petriplates were counted from the 5\textsuperscript{th} day onwards up to 28\textsuperscript{th} day. Population density of actinobacteria has been expressed as Colony Forming Units (CFUs) per gm of the sample. All the colonies that grew on the petriplates were separately streaked for sub-culturing so as to ensure axenicity and maintained in the slants.

Graphical representation of the physico-chemical parameters of the water and sediments were prepared using ORIGIN 6 software. Statistical analysis was performed to find out the relationship between the physico-chemical parameters of the water and sediments with actinobacterial population density, using SPSS version 11.5. Principal Component Analysis (PCA) and Cluster Analysis were used to identify the trends between the highly correlating physico-chemical parameters and actinobacterial population density, using PRIMER 6.1 software (Table 2).

### Results

Air temperature varied from 25 to 29°C; lower value was recorded at station 5 (Coral reef) and higher value was recorded at stations 3 (Beach) (Fig. 1). Surface water temperature varied from 25 to 27°C, recording the lower value at stations 4 and 6 (Mangrove and Beach respectively) and higher value at stations 2 and 3 (Coral reef and Beach respectively). Sediment temperature varied from 26 to 28°C, recording the lower value at station 5 (Coral reef) and higher value at stations 2 and 3 (Coral reef and Beach respectively).

Water salinity fluctuated from 14 to 34 psu, registering the lower value (14 psu), at station 6 (Beach) and higher value at station 5 (Coral reef) (Fig. 2). Sediment salinity varied between 28 to 35 psu, registering the lower value (28 psu) at station 6 (Beach) and higher value (35 psu) at station 5 (Coral reef).

Water pH fluctuated between 7.1 to 8.0 and registered the higher value (8.0 pH) at stations 3 and 5 (Beach and Coral reef respectively) and the lower value (7.1 pH), at station 6 (Beach) (Fig. 3). Sediment pH exhibited variations, registering the lower value (7.4 pH) at stations 2 and 6 (Coral reef and Beach respectively) and higher value (8.2 pH) at station 3 (Beach).
Table 2. Simple correlation co-efficient (r) between actinobacterial density in KUA with physico-chemical parameters

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<th>EC</th>
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<th>P</th>
<th>K</th>
<th>TOC</th>
<th>Sand</th>
<th>Silt</th>
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<td>0.952&lt;sup&gt;**&lt;/sup&gt;</td>
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* Correlation is significant at the 0.05 level (2-tailed); **. Correlation is significant at the 0.01 level (2-tailed).
DO content of water ranged between 2.4 to 4.5 ml l⁻¹. At station 5 (Coral reef), higher DO concentration was recorded and the lower value was recorded at station 1 (Mangrove) (Fig. 4).

Electrical conductivity of the sediments fluctuated widely at all the stations investigated. It ranged from 1.3 to 4.9 dS m⁻¹, registering the lower value (1.3 dS m⁻¹) at station 6 (Beach) and higher value (4.9 dS m⁻¹) at station 3 (Beach) (Fig. 5).

Sediment nitrogen content varied from 7.38 to 12.72 mg g⁻¹ recording the lower value at station 6 (Beach) and higher value at station 1 (Mangrove) (Fig. 6). Sediment phosphorus content varied from 3.7 to 5.6 mg g⁻¹, recording the lower value at station 5 (Coral reef) and higher value at station 2 (Coral reef) (Fig. 7).

Sediment potassium content showed fluctuations, registering higher value (13.75 mg g⁻¹) at station 1 (Mangrove) and lower value (8.09 mg g⁻¹) at station 6 (Beach) (Fig. 8).
Fig. 7. Sediment phosphorus content recorded at six stations of the Neil island.

Fig. 8. Sediment potassium content recorded at six stations of the Neil island.

Sediment TOC content exhibited wide variations at all the stations investigated, registering the lower value (1.5 mg g⁻¹) at station 6 (Beach) and higher value (4.6 mg g⁻¹) at station 4 (Mangrove) (Fig. 9).

Sand content varied from 80% to 95%, recording the lower value at stations 1 and 4 (Mangrove), and higher value at station 2 (Coral) (Fig. 10). Silt content exhibited variations, registering lower value (3.05%) at station 3 (Beach), and higher value (3.65%) at station 1 (Mangrove). Clay content recorded higher value (16.6%) at station 4 (Mangrove) and lower value (1.8%) at station 2 (Coral reef).

Fig. 10. Sediment texture recorded at six stations of the Neil island.

Actinobacterial population density varied from 8 to 19 x 10² CFU gm⁻¹. Minimum (8 x 10² CFU gm⁻¹) was recorded at 6 (Beach) and the maximum (19 x 10² CFU gm⁻¹) was recorded at station 4 (Mangrove) (Fig. 11).

Fig. 11. Sediment actinobacterial population density on Kuster’s agar recorded at six stations of the Neil island
Discussion

Ecological parameters are mainly constituted by the bioclimatic and edaphic criteria and this general assumption is valid for terrestrial as well as the coastal ecosystems. This would mean that whenever we want to establish the ecological relationships, we have to quantify the values of environmental parameters that are responsible for the presence of the biological systems like microbes including actinobacteria. Hence, the present study was made to analyse the physico-chemical properties of the coastal environment in relation to the actinobacterial population density.

Physical and chemical parameters investigated in the present study includes air temperature, surface water temperature, sediment temperature, sediment salinity, water salinity, water pH, sediment pH, dissolved oxygen, electrical conductivity, macronutrients viz. nitrogen, phosphorous and potassium and soil texture of six stations.

Temperature has a prominent role to play among all the environmental factors. Because, it influences all aspects of life as a result of fundamental thermodynamics. The relationship between temperature and individual performance is reasonably well understood. In general, sea water temperature is influenced by sunshine, evaporation, cooling fresh water influx and admixture of ebb and flow from the adjoining neritic waters. In the present study, beach temperature was always higher than the coral reef and mangrove environments. However, all of them showed close similarity, due to the influence of the air temperature on the other two. Similarly, Sethubathi has reported that the air temperature ranged from 27°C to 32.5°C; surface water temperature from 27°C to 30°C and sediment temperature, from 28°C to 33.5°C in the coastal waters of the Andamans.

Salinity can play a significant role in the growth and size of aquatic and marine organisms. Fluctuation in salinity would definitely affect the biological system of the marine environment. In the present investigation, higher salinity was recorded in the coral reef habitat. Corals flourish in the salinity range of approximately 35 psu. However, many coral species can be found in localities that show significant variation in mean or extreme conditions and Karuppiah has reported that the salinity ranged from 31 to 36 psu in the coral reef environment of the Gulf of Mannar.

Hydrogen-ion concentration (pH) plays an important role in the biological processes of almost all the aquatic organisms and pH is the factor which is controlling all the other parameters like dissolved oxygen. The stations showed alkaline nature (pH 7.2 to 8.2). Generally, fluctuation in pH occurs due to the removal of CO₂ by photosynthesis through bicarbonate degradation, dilution of seawater by freshwater influx, reduction of salinity. Swarnakumar et al. have found that the alkaline and monomesohaline situations prevailed in the coastal waters of the Great Nicobar island of the Andamans.

Dissolved oxygen is vital for aquatic life and is needed to keep the organisms alive. It is the most significant parameter affecting the productivity of aquatic systems. The two main sources of dissolved oxygen in seawater are diffusion of oxygen from atmosphere and photosynthetic activity of aquatic flora. Gnanam reported that the observed dissolved oxygen level was between 3.7 ml 1⁻¹ to 5.33 ml 1⁻¹ in the Bay of Bengal. In the present study, lower value of dissolved oxygen (2.4 ml 1⁻¹) was observed at station 1 (Mangrove) which could be due to the microbial utilization of O₂ for the decomposition of biota and uptake of oxygen by the marine organisms. Higher value of dissolved oxygen (4.5 ml 1⁻¹) was observed at station 5 (Coral reef) and this could be correlated to the fresh water input and mixing, at this station.

Distribution of nutrients in the marine environment is mainly based on the tidal conditions and river flow. Nutrients such as N, P and K are responsible for promoting the microbial growth and diversity in the marine environments. In the present study, this was evidenced from the positive correlation obtained between the sediment nutrients (Nitrogen and Potassium) with the actinobacterial density (Nitrogen r = 0.822 < 0.05 and Potassium: r = 0.829 < 0.05). This clearly indicates that the nutrients are important and are capable of influencing the actinobacterial growth.

Distribution of total organic carbon is closely related with the sediment type. For example, if the clay content is lower in the sediments, the total organic carbon will also be low. In the present study, higher amount of clay was
observed in the mangrove sediments and as a result, its total organic carbon content was also higher. Similarly, studies show that the increase of clay content would lead to the increase in total organic carbon level\textsuperscript{29,30}. In the present study, higher amount of total organic carbon (4.6 mg g\textsuperscript{-1}) was recorded at station 4 (Mangrove) and lower amount (1.5 mg g\textsuperscript{-1}) was noticed at station 6 in the beach environment; the former supporting higher actinobacterial population density, as compared to other stations. This is evidenced by the significant positive correlation obtained between the total organic carbon and the actinobacterial density ($r = 0.927 < 0.01$) in the sediments.

Soil texture plays an important role in the distribution and abundance of microorganisms, as revealed by the positive correlation obtained between the actinobacterial population density and clay ($r = 0.952, < 0.01$) and negative correlation, in sand ($r = 0.953, < 0.01$). Studies of Lakshmanaperumalsamy\textsuperscript{31} further confirm this. Vasanth,\textsuperscript{32} have also reported that the clay sediments containing sufficient nutrients promote good propagation of the microbes than the sandy sediments.

In the present study, hierarchical cluster analysis was performed to investigate the similarities or dissimilarities between the stations and physico-chemical parameters. Dendrogram of the analyzed stations and physico-chemical parameters provides with a visual summary of the clustering process (Figs. 12 and 13). Four clusters were observed in the dendrogram (Fig. 12).

Cluster 1 corresponds to stations 1 and 4. Cluster 2 corresponds to station 6, Cluster 3 corresponds to station 3 and Cluster 4 corresponds to stations 2 and 5.

Cluster 1 (stations 1 and 4) represents the dense mangrove habitat with favorable physico-chemical conditions, supporting higher actinobacterial density. In the Cluster 4 (stations 2 and 5), station 2 clade showed a very close distance similarity with the station 5 (4.951) and these two coral habitats were having moderate actinobacterial density in relation to the physico-chemical features. In the Cluster 3, station 3 (beach habitat) comprising sandy area is under the influence of anthropogenic activity. Similarly, station 6 in the Cluster 2 is also a sandy area with coral rubbles. Evidently these stations of the clusters 2 and 3 had lower actinobacterial density.

Though the dendrogram can reveal the dataset structure, it does not allow the interpretation of the observed patterns in terms of the original parameters. Therefore, the target physico-chemical parameters dataset was subjected to Principal Component Analysis (PCA) in order to determine which parameter influences the variation in the actinobacterial density, in the six stations of the Neil island. PCA was used to identify the trends between the highly correlating physico-chemical parameters and actinobacterial density and it revealed the distinct relationship between the stations and the physico-chemical parameters with the actinobacterial population density (Fig. 13).
In PCA, higher correlation component score was obtained between the actinobacterial population density and the physico-chemical parameters at stations 1 and 4 (76.2 and 79.5, respectively). In the PC1 axis, these two stations had positive significant correlation with clay, TOC, sediment pH, potassium (K), actinobacterial population density, nitrogen content, phosphorus content, air temperature and EC. It could be due to the fact that these stations are occupied by mangroves (stations 1 and 4). Whereas, stations 5, 2, 3 and 6 had negative correlation component scores (-33.1, -34, -34.6, and -53.9, respectively). It could be ascribed to the fact that these four stations are coral reef and sandy beach environments. In addition, in these stations, very poor vegetation exists, as compared to stations 1 and 4 which are mangrove habitats. Hence, it can be concluded that the mangrove environment can be intensively studied for the isolation of actinobacteria that might prove to be the novel and potential organisms, for commercial application.

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Reference