1. Introduction

Marine environmental conditions are extremely different from terrestrial ones. They are excellent sources for novel microbes including actinobacteria, many of which produce bioactive compounds. However, studies on the diversity, distribution and ecology of actinobacteria in the oceans are not many (Stach et al., 2003a, b), though biological diversity in the coastal and marine ecosystems, such as deep seas, mangroves, coral reefs and other ecosystems, is higher than that of the tropical rain forests (Lam, 2006). Though the ocean covers more than 71% of the earth’s surface, only little is known about the microbial diversity of marine habitats especially sediments that contain limited amounts of readily available organic matter, with most sources of carbon being present in the complex forms (Magarvey et al., 2004).

Actinomycetes are best known as soil bacteria and are generally believed to occur in the oceans largely as dormant spores that are washed into the sea (Goodfellow and Heynes, 1984). Marine microbiology is developing strongly in several countries with a distinct focus on bioactive compounds. Analysis of the geographical origins of compounds, extracts and bioactivities of marine actinobacteria up to 2003, indicates that 67% of marine natural products have been sourced from Australia, the Caribbean, Indian Oceans, Japan, the Mediterranean, and Western Pacific Ocean sites (Blunt et al., 2007).

Molecular methods have been well established now for the characterization of complex actinobacterial communities associated with various environments and are revealing the extent of their diversity. Further, no information is available on the actinobacteria of the ecologically sensitive areas such as Neil island of the Andamans
with regard to their diversity, occurrence and distribution which are largely dependent on the physico-chemical parameters prevailing in their immediate environment. In this direction, to gain a better understanding of the marine actinobacterial diversity, culture-dependent and culture-independent study was undertaken, using sediment samples collected from different coastal habitats of the Neil island, the Andamans, with the following objectives.

- Selective isolation of actinobacteria from the different coastal habitats (Mangrove, Coral reef and Beach) of the Neil island

- Enumeration of the actinobacterial density and its interactions with the physico-chemical parameters of the different coastal habitats (Mangrove, Coral reef and Beach) of the Neil island

- Isolation and identification of culture-dependent actinobacteria to find out their diversity in the different coastal habitats (Mangrove, Coral reef and Beach) of the Neil island

- Exploration of the culture-independent actinobacterial diversity in the different coastal habitats (Mangrove, Coral reef and Beach) of the Neil island, using pyrosequencing

2. Study area

Neil Island

The Neil island is the most southerly inhabited island of Richie’s archipelago, a couple of hours ferry ride northeast of Port Blair. The Neil island is a good place for eco-tourism, located 36 Km from the Port Blair by sea (Lat. 11° 49’ N, Long. 93° 01’E). It occupies an area of 18.9 square kilometers. This island is tiny and triangular in shape and dotted with tropical trees, green paddies and banana plantations. The island of Neil consists of five villages and has a lone jetty at Bharathpur, which serves as the single
entry/exit point. Agriculture is the primary occupation of the villagers, and the island supplies vegetables to the rest of the Andamans. In the island, marine life is abundant, endowed with very important coastal ecosystems of mangroves, coral reefs and seagrasses. The beaches at Lakshmanpur, Bharatpur and Sitapur are the best for scientific studies.

3. **Selective isolation of actinobacteria**

In the present study, actinobacteria were isolated using three different media: Kuster’s Agar (KUA), Glycerol Glycine Agar (GGA) and Starch Casein Agar (SCA).

In KUA, actinobacterial population density was higher ($19 \times 10^2$ CFU gm$^{-1}$) at station 4 (Mangrove) and lower ($8 \times 10^2$ CFU gm$^{-1}$) at station 6 (Beach).

In GGA, actinobacterial population density was lower ($3 \times 10^2$ CFU gm$^{-1}$) at stations 3, 5 and 6 (Beach, Coral reef and Beach respectively) and higher ($8 \times 10^2$ CFU gm$^{-1}$) at station 4 (Mangrove).

In SCA, actinobacterial population density was higher ($5 \times 10^2$ CFU gm$^{-1}$) at station 4 (Mangrove) and lower ($2 \times 10^2$ CFU gm$^{-1}$) at stations 3, 5 & 6 (Beach, Coral and Beach respectively).

Among the three media, Kuster’s agar (KUA) yielded higher counts of actinobacteria at all the stations, as compared to other two media. Moreover, it supported the isolation of different types of actinobacteria, with distinct morphological characters. Hence, KUA medium is recommended as a suitable medium for the study of actinobacteria from different coastal habitats.
4. Ecology: Physio-chemical parameters and their influence on the actinobacterial population density

Air temperature varied from 25 to 29°C; Surface water temperature varied from 25 to 27°C and the sediment temperature varied from 26 to 28°C. Higher air temperature (29°C) at station 3 (Beach), higher surface temperature (27°C) at stations 2 and 3 (Coral reef and Beach respectively) and higher sediment temperature (28°C) at stations 2 and 3 (Coral reef and Beach respectively) were observed. Sunshine, evaporation, cooling, fresh water influx and admixture of ebb and flow from the adjoining neritic waters could have influenced the temperature.

Water salinity varied from 14 to 35 psu; it was higher (34 psu) at station 5 (Coral reef). Sediment salinity was higher (35 psu) at station 5 (Coral reef) and it was lower (28 psu) at station 6 (Beach). Such higher salinity values could be ascribed to the exposure to the solar radiation resulting in the evaporation and precipitation of salts in the sand environment.

Water pH varied from 7.1 to 8 and sediment pH varied from 7.4 to 8.2. Both water and sediment pH was higher at stations 3 and 5 (Beach and Coral reef respectively). Higher pH could have occurred due to the removal of CO$_2$ by the photosynthesizing communities in the environment.

DO content ranged from 2.4 to 4.5 ml $1^{-1}$; lower at station 1 (Mangrove) and this could be due to the microbial utilization of O$_2$ for decomposition of biota and uptake of oxygen.

EC varied from 1.3 to 4.9 dSm$^{-1}$, recording the higher value at station 3 (Beach).
Higher nitrogen content (12.72 mg g\(^{-1}\)) was recorded at station 1 (Mangrove); higher content of phosphorus was recorded at station 2 (Coral reef) and higher content of potassium was noticed at station 1 (Mangrove). These are responsible for promoting the microbial growth and diversity in the marine environment. This was evidenced from the positive correlation obtained between the sediment nutrients (Nitrate and Potassium) with the actinobacterial density (Nitrate \(r = 0.822 < 0.05\) and Potassium: \(r = 0.829 < 0.05\)). This clearly indicates that the nutrients are important and are capable of influencing the actinobacterial growth.

TOC ranged from 1.5 to 4.6 mg g\(^{-1}\), registering the higher value (4.6 mg g\(^{-1}\)) at station 4 (mangrove), where the actinobacterial population density was also higher (19 x 10\(^2\) CFU gm\(^{-1}\)). This is evidenced by the significant positive correlation obtained between the total organic carbon and actinobacterial density (\(r = 0.927 < 0.01\)) in the sediments.

Sand content was higher at station 2 (Coral reef) where actinobacterial population density was lower; silt content was higher at station 1 (Mangrove) and the clay content was higher at station 4 (Mangrove) where the actinobacterial population density was higher. This is supported by the positive correlation obtained between the actinobacterial population density and clay (\(r = 0.952, < 0.01\)) and negative correlation with sand (\(r = 0.953, < 0.01\)).

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 has provided with a visual summary of the clustering process. Four clusters were observed in
the dendrogram. Cluster 1 corresponded to stations 1 and 4. Cluster 2 corresponded to station 6. Cluster 3 corresponded to stations 3 and Cluster 4 corresponded to stations 2 and 5.

Cluster 1 (stations 1 and 4) representing a dense mangrove habitat with the favorable physico-chemical conditions, supported higher actinobacterial density.

Principal Component Analysis (PCA) was used to identify the trends between the highly correlating physico-chemical parameters and the actinobacterial density and it revealed the distinct relationship between the stations and the physico-chemical parameters with the actinobacterial population density.

In the 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 a positive significant correlation with clay, TOC, sediment pH, potassium (K), actinobacterial population density, nitrogen content, phosphorus content, and EC. It could be due to the fact that these stations are occupied by mangroves.

Whereas, stations 5, 2, 3 and 6 had negative correlation component scores (-33.1, -34, -34.6, and -53.9, respectively). It could be due 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 the mangrove habitats.
5. Culturable diversity: Identification of actinobacteria

During the present study, a total of 21 morphologically distinct strains of actinobacteria (AUNIA-1 to AUNIA-21), isolated from the sediments of the coastal habitats (Mangrove, Coral reef and Beach) of the Neil island, were subjected to taxonomic studies.

Out of the 21 morphologically distinct strains, majority of them (11) (AUNIA-2, AUNIA-4, AUNIA-5, AUNIA-8, AUNIA-11, AUNIA-12, AUNIA-13, AUNIA-14, AUNIA-15, AUNIA-17 and AUNIA-18) showed the presence of LL-DAP along with glycine of the peptidoglycan layer with no other characteristic sugar pattern, indicating that these strains belong to the cell wall chemotype I. Presence of spores in a long chain occurring on the aerial mycelium and branched nature of the substrate mycelium clearly indicated that these strains belong to the genus, *Streptomyces*.

Whole cell hydrolysates of 7 strains (AUNIA-1, AUNIA-3, AUNIA-6, AUNIA-7, AUNIA-9, AUNIA-19 and AUNIA-20) possessed meso-diaminopimelic acid as the only amino acid of peptidoglycan, but no diagnostic sugar pattern was present, indicating that these strains belong to the cell wall chemotype III. Based on the micromorphological and molecular characteristics, these strains were assigned to the genus, *Nocardiopsis*.

Remaining three strains, AUNIA-10, AUNIA-16 and AUNIA-21 contained meso-DAP with arabinose and galactose as the characteristic sugar pattern, indicating that these strains belong to the cell wall chemotype IV. Based on the micromorphological and molecular characteristics, these strains were assigned to the genus, *Nocardia*.

Cultural and morphological characters and their carbon sources utilization were also studied to identify the isolates.
Results of identification of all the strains (AUNIA-1 to AUNIA-21) were as follows and all the strains closely resembled their representative species.

- **AUNIA-1** - *Nocardiopsis dassonovillei* subsp. *dassonovillei*,
- **AUNIA-2** - *Streptomyces misawanensis*,
- **AUNIA-3** - *Nocardiopsis dassonvillei*,
- **AUNIA-4** - *Streptomyces puniceus*,
- **AUNIA-5** - *Streptomyces griseobrunneus*,
- **AUNIA-6** - *Nocardiopsis dassonovillei* subsp. *dassonovillei*,
- **AUNIA-7** - *Nocardiopsis terrae*,
- **AUNIA-8** - *Streptomyces tendae*,
- **AUNIA-9** - *Nocardiopsis alba*,
- **AUNIA-10** - *Nocardia rhamnosiphila*,
- **AUNIA-11** - *Streptomyces collinus* subsp. *albescens*,
- **AUNIA-12** - *Streptomyces misawanensis*,
- **AUNIA-13** - *Streptomyces albiflaviniger*,
- **AUNIA-14** - *Streptomyces fradiae*,
- **AUNIA-15** - *Streptomyces puniceus*,
- **AUNIA-16** - *Nocardia lijiangensis*,
- **AUNIA-17** - *Streptomyces globisporus*,
- **AUNIA-18** - *Streptomyces pactum*,
- **AUNIA-19** - *Nocardiopsis alba*,
- **AUNIA-20** - *Nocardiopsis metallicus*, and
- **AUNIA-21** - *Nocardia miyunensis*. 
6. Culture-independent microbial diversity with special reference to actinobacteria

Metagenomic DNA was isolated from the coastal samples (Mangrove, Coral reef and Beach). Amplification and bacterial Tag encoded FLX titanium amplicon pyrosequencing were also carried out.

A total of 6,120 sequence reads obtained from the mangrove sample were screened for the quality reads and the primer sequences were trimmed by using MOTHUR (http://www.mothur.org) which gave 5,150 high sequence reads with the average read length of 560 bp.

The sequences revealed nineteen phyla, where the majority of the sequences were assigned to the Phylum Proteobacteria (39%), Actinobacteria (36%), Gemmatimonadetes (21%), Planctomycetes (6%), Acidobacteria (4%), Firmicutes (4%) and Nitrospirae (2%). Verrucomicrobia, Nitrospinae, Thermodesulfobacteria, Lentisphaerae, Deinococcus-Thermus, Cyanobacteria, Bacteroidetes, Aquificae, Dictyoglombi, Chlorobi, Thermatogae and Chloroflexi were the other phyla identified with relatively low abundance (<1%).

In the RDP data analysis, 90 different abundant genera were found in the mangrove sediments. In that, there were 64 bacterial genera were found: Akkermansia, Alkalilimnicola, Alkaliphilus, Anaerocellum, Anaeromyxobacter, Anaplasma, Bacillus, Bradyrhizobium, Brevibacillus, Campylobacter, Carboxydotermus, Caulobacter, Chlorobium, Chromohalobacter, Clostridium, Coprothermobacter, Coxiella, Dehalococcoides, Desulfitibacillus, Desulfotalea, Desulfotomaculum, Desulfovibrio, Dictyoglomus, Ehrlichia, Gemmatimonas, Geobacillus, Geobacter, Halorhodospira, Halothermothrix, Magnetococcus, Magnetospirillum, Maricaulis, Mesorhizobium,
Methylacidiphilum, Methylobacterium, Moorella, Myxococcus, Nitriruptor, Nitrobacter, Novosphingobium, Oceanobacillus, Oligotropha, Paenibacillus, Parvibaculum, Pelobacter, Pelotomaculum, Persephonella, Pseudoalteromonas, Pseudomonas, Rhodopirellula, Shigella, Sorangium, Sphingomonas, Sphingopyxis, Staphylococcus, Sulfurihydrogenibium, Syntrophus, Thermoanaerobacter, Thermosipho, Thermosynechococcus, Thioalkalivibrio, Vibrio, Wolinella and Zymomonas.

In addition, 26 actinobacterial genera were found in the mangrove sediments: Acidothermus, Actinomadura, Actinomyces, Arthrobacter, Beutenbergia, Clavibacter, Corynebacterium, Frankia, Iamia, Ilumatobacter, Kineococcus, Kocuria, Leifsonia, Micrococcus, Micromonospora, Mycobacterium, Nocardia, Nocardioides, Propionibacterium, Rhodococcus, Rubrobacter, Saccharopolyspora, Salinispora, Streptomyces, Thermobifida and Tropheryma.

A total of 2,580 sequence reads obtained from the coral reef sample were screened for the quality reads and the primer sequences were trimmed by using MOTHUR (http://www.mothur.org), which gave 2,339 high sequence reads with the average read length of 480 bp.

The sequences revealed fourteen phyla, where the majority of the sequences were assigned to the Phylum Actinobacteria (58%), Proteobacteria (20%), Planctomycetes (8%), Firmicutes (4%), Nitrospirae (4%); Gammatimonadetes (3%). Acidobacteria, Verrucomicrobia, Thermodesulfobacteria, Lentisphaerae, Thermotogae, Chlorobi, Chloroflexi and Aquificae were the other phyla identified with relatively low abundance (<1%).
In the RDP data analysis, 55 different abundant genera were found in the coral reef sediments. In this, 30 bacterial genera were found: *Alkalilimnicola*, *Alkaliphilus*, *Anaerocellum*, *Anaeromyxobacter*, *Bartonella*, *Brucella*, *Caldicellulosiruptor*, *Carboxydothermus*, *Chlorobaculum*, *Chlorobium*, *Chromohalobacter*, *Coxiella*, *Desulfatibacillum*, *Desulfotalea*, *Desulfovibrio*, *Gemmatimonas*, *Geobacillus*, *Magnetospirillum*, *Methylobacterium*, *Moorella*, *Myxococcus*, *Nitrosococcus*, *Paenibacillus*, *Pelobacter*, *Sorangium*, *Syntrophobacter*, *Thermoanaerobacter*, *Thermosipho*, *Thermotoga* and *Thioalkalivibrio*.

In addition, 22 actinobacterial genera were found in the coral reef sediments: *Acidothermus*, *Actinomadura*, *Arthrobacter*, *Beutenbergia*, *Bifidobacterium*, *Clavibacter*, *Corynebacterium*, *Kineococcus*, *Kocuria*, *Leifsonia*, *Illumatobacter*, *Iamia*, *Micrococcus*, *Mycobacterium*, *Nocardia*, *Nocardioides*, *Propionibacterium*, *Rhodococcus*, *Saccharopolyspora*, *Streptomyces*, *Thermobifida* and *Tropheryma*.

A total of 2,215 sequence reads obtained from the beach sample were screened for the quality reads and the primer sequences were trimmed by using MOTHUR ([http://www.mothur.org](http://www.mothur.org)) which gave 2,014 high sequence reads with the average read length of 440 bp.

The sequences revealed ten phyla, where the majority of the sequences were assigned to the Phylum Actinobacteria (23%), Proteobacteria (22%), Planctomycetes (11%), Firmicutes (10%) Nitrospirae (8%) and Acidobacteria (2%). Cyanobacteria, Thermodesulfobacteria, Lentisphaerae and Deinococcus-Thermus were the other phyla identified with relatively low abundance (<1%).
In the RDP data analysis, 49 different abundant genera were found in the beach sediments. Among them, 29 bacterial genera were found: *Alkaliphilus*, *Anaerocellum*, *Anaeromyxobacter*, *Aquifex*, *Bacillus*, *Caldicellulosiruptor*, *Carboxydyothermus*, *Caulobacter*, *Desulfotalea*, *Desulfovibrio*, *Dictyoglomus*, *Ehrlichia*, *Gemmatimonas*, *Geobacter*, *Gloeobacter*, *Methylacidiphilum*, *Myxococcus*, *Natranaerobius*, *Novosphingobium*, *Paenibacillus*, *Pelobacter*, *Persephonella*, *Rhodopirellula*, *Sorangium*, *Syntrophobacter*, *Thermoanaerobacter*, *Thermosipho*, *Thermosynechococcus* and *Thioalkalivibrio*.

Further, 20 actinobacterial genera were found in the beach sediments: *Acidothermus*, *Actinomadura*, *Arthrobacter*, *Corynebacterium*, *Kineococcus*, *Kocuria*, *Leifsonia*, *Illumatobacter*, *Iamia*, *Micrococcus*, *Mycobacterium*, *Nocardia*, *Nocardioides*, *Propionibacterium*, *Rhodococcus*, *Rubrobacter*, *Saccharopolyspora*, *Streptomyces*, *Thermobifida* and *Tropheryma*.

Proteobacteria and Actinobacteria were dominant in the coastal habitats of the Neil island and the mangrove environment showed higher actinobacterial diversity.

Thus, the conventional and molecular information of the marine actinobacteria, obtained from the present study would help facilitate the rational selection of culture media to target specific groups and species of marine actinobacteria for culturing them in the laboratory and screening them for bioactive natural products for drug discovery and other uses.

Additionally, information pertaining to the water quality parameters (physiochemical and microbial features) would help understand the present status of the coast of the Neil island, which is now open for tourism.