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Assessment of microbial pollution in the coastal environs of the Little Andaman island, India

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The qualitative and quantitative distribution of total heterotrophic bacteria and human pathogens from eight different marine locations along the east coast of Little Andaman island.had been examined. During the investigation, 82 bacterial strains were isolated and 12 genera were identified with the dominance of *Vibrio* (23%) and *Pseudomonas* (20%). THB population density recorded in the present study varied from 5.2 to 273×10^2 CFUml^{-1} in the water samples and from 6.2 to 40×10^3 CFUg^{-1} in the sediment samples. In the case of pathogenic forms, 10 species were recorded from the eight stations and their densities were within the optimal levels. The present study indicates the less polluted nature of the coastal environs of the Little Andaman island.

Key words: Little Andaman island, coral reef, THB, pathogenic bacteria

Introduction

Microorganisms distributed in the marine and brackish environments play an important role in the decomposition of organic matter and mineralization[1]. Since the last two or three decades, microbial water quality analysis was given more importance in the marine pollution monitoring programmes. These pathogenic bacteria invade into the marine environment through human and animal excreta through river run off, rain water, kitchen wastes, land runoff, sewage with organic and inorganic contents, agricultural wastes, industrial wastes, etc. Hence, the spatial and temporal distributions of the total and faecal coliforms as wells as the pathogenic bacteria in water, sediments and organisms is essential to assess the sanitary. The regular microbial monitoring in the coastal environment is an integral and essential part in predicting the microbial pollution of coastal waters.

The Little Andaman island is one of the unexplored areas for its biodiversity and for microbiological studies. Increasing human settlements, urbanization, tourism and marine transportation, warranted the coastal water quality monitoring in the Little Andaman Island. The present study is on microbial characteristics of the different marine location of the Little Andaman with special reference to human pathogens.

Materials and Methods

Study area

The Little Andaman forms the southern most part of the Andaman group of islands in the Bay of Bengal, lying between latitudes 10° 30′ and 10° 55′ N and longitudes, 92° 33′ and 92° 37′ E (Fig. 1). The island occupies a land area of 731.6 sq km, with a

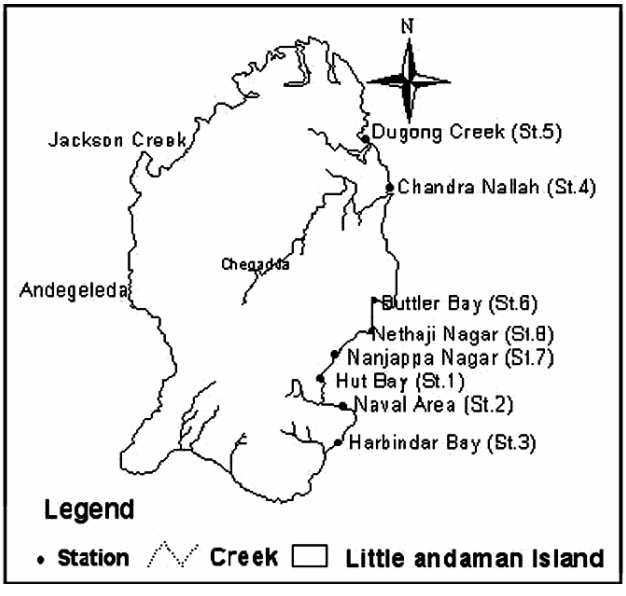

Fig. 1— Map showing the study areas in the Little Andaman island.

coastline of about 110 km. Hut Bay (Station 1) is situated in the latitude of 10° 35` 33.7`` N and longitude of 92° 32` 49.6`` E. The dead coral reefs extend from the Hut bay jetty towards north of the island for about 1.5 km. The reefs and coral rocks are exposed in about 500 to 1 km from the shore towards the sea. Station 2 (Naval area) is situated 1 km away from the Hut Bay boat jetty breakwater towards southern side. It is fully covered by the dead coral rocks to a stretch of more than 2 km further south. The width of the reef flat varies between 0.8 km in the north and 10 m in the south. This area is fully dominated by dense seaweed vegetation with higher diversity. There are live corals along the seaward side of the reef flat. Station 3 (Harbindar Bay) is situated in the south (5 km) of the Hut Bay. This coast also has dead coral rocks for more than 2 km length and the seaward side of these rocks is supporting good growth of live massive corals. Station 4 (Chandra nallah coast) is situated 22 km north of Hut. Chandra nallah is a perennial deep water canal discharging fresh water into the sea and a distance of 1 km from the sea inside it forms estuarine condition. This mangrove creek discharges large quantities of turbid water into the sea during low tides. Samples were collected in the open coast along the northern bank of the creek, where dead coral rocks and rocks exist. Station 5 (Dugong creek) is covered by long stretches of dead coral rocks and live corals along the seaward side. This creak also discharges turbid water into the sea during the low tides. Station 6 (Buttler Bay) is situated in the latitude of 10° 40` 45.5`` N and longitude of 92° 35` 36.8`` E. Samples were collected on the shore, 2 km north of the Buttler Bay. This station consists of dead coral rocks, extending 2 km towards north. During low tides, more than 700 m to 1 km width of dead coral area from the shore is seen exposed. Both massive and branching live corals are present in isolated patches towards the seaward side. Station 7 (Nanjappa nagar) is situated in the latitude of 10° 35` 55.2`` N and longitude of 92° 32` 29.7`` E. This station is fully sandy in nature and devoid of any rocks and coral blocks. Station 8 (Nethaji nagar) is situated in the latitude of 10° 37` 27.5`` N and longitude of 92° 32` 35.9`` E. This is another sandy coast situated between Nanjappa nagar and Buttler Bay. This station is also dumped with remnants of tsunami and devoid of coral rocks and rocks.

Sampling

Field collections were carried out during January 2006 at eight different stations vize. Hut Bay (Station-1); Navel Area (Station-2); Harbindra Bay (Station-3); Chandra Nallah (Station-4); Dugong Creek (Station-5); Buttler Bay (Station-6); Nethaji Nagar (Station-7) and Nanjappa Nagar (Station-8) along east coast of the Little Andaman island (Fig. 1). Surface water samples were collected in 100 ml sterile screw capped bottles for bacteriological assessment. The sediment samples were collected by employing an alcohol rinsed and air-dried small Peterson's grab. The central portion of the collected sediment was aseptically transferred into sterile polyethylene bags using sterile spatula. All samples were brought to the field laboratory at Hut Bay (Little Andaman) in portable icebox with in 4 hours. Immediately after arrival, inoculations were made using suitable specific media with necessary dilutions and pure cultures were established.

Bacteriological analysis

THB population was enumerated by adopting the spread plate method using ZoBell's Marine (M384) Agar medium (Hi-Media, Mumbai). The plates after inoculation were incubated in an inverted position at a temperature of 28±2°C for 24 to 48 h. The bacterial colonies were picked up from the petridishes and restreaked in appropriate nutrient agar plates thrice before a pure culture was established in agar slants for further identification. Specific media, TCBS (M870S, Hi-Media, Mumbai) agar for *Vibrio chloreae* and *V. parahemolyticus* like organisms, MacConkey (M008, Hi-Media, Mumbai) and M-FC (M1122, Hi-Media, Mumbai) agar for *Escherichia coli* and faecal coliforms respectively, XLD (031, Hi-Media, Mumbai) agar for *Salmonella, Shigella* and *Klebsiella* like organisms, Cetrimide (MM024, Hi-Media, Mumbai) agar for *Pseudomonas aeruginosa* like organisms and M-enterococcus agar for S*treptococcus fecalis* like organisms were used for isolation of pathogenic bacteria.

For enumeration of *Vibrio* spp., *E. coli* and enteric pathogens, spread plate method was employed while *Streptococcus* spp. and *Pseudomonas* spp. were enumerated by following membrane filter technique using 0.45 µm Whatman filter paper. All these agar plates were incubated at 37°C for 24 to 48 h. Triplicate samples were maintained in all the experiments and average values were expressed as colony forming units (CFU/ml) in case of water and

CFU/g in case of sediment. Biochemical analysis *viz.* IMViC (Indole, Methylred, Voges Proskauer, Citrate test), H_2S production test, Cytochrome oxidase test, ONPG test, motility of bacteria, Gram staining and fermentation of carbohydrates (Acid and gas production) tests were carried out by following the methods of Simidu and Aiso[2] and the strains were identified by following Bergeys manual of bacteriology[3].

Results

Generic composition of total heterotrophic bacteria

A total of 82 strains were isolated from water and sediment samples of eight stations. Based on colony morphology, 42 strains were selected, sub-cultured and identified as 12 genera viz. *Flavobacterium, Bacillus, Shigella, Klebsiella, Corynebacterium, Pseudomonas, Aeromonas, Vibrio, Streptococcus, Esherichia, Proteus* and *Salmonella*. Of these, *Vibrio* contributed more (23%) followed by *Pseudomonas* (20%), *Esherichia coli* (17%) and *Streptococcus* (15%) (Fig. 2). Among the 12 genera, 9 viz. genera *Vibrio, Pseudomonas, Shigella, Klebsiella, Escherchia, Flavobacterium, Streptococcus, Proteus* and *Salmonella* were belong to the gram-negative group and 3 genera (*Corynebacterium, Aeromonas* and *Bacillus*) were belong to the gram positive group. In the case of pathogenic bacteria a total 10 species (*V. cholerae* and *V. parahaemolyticus, Shigella dysenteriae, Klebsiella pneumoniae, Salmonella typhimerium, S. paratyphi, Proteus vulgaris, E. coli, S. faecalis, P. aeruginosa*) were recorded.

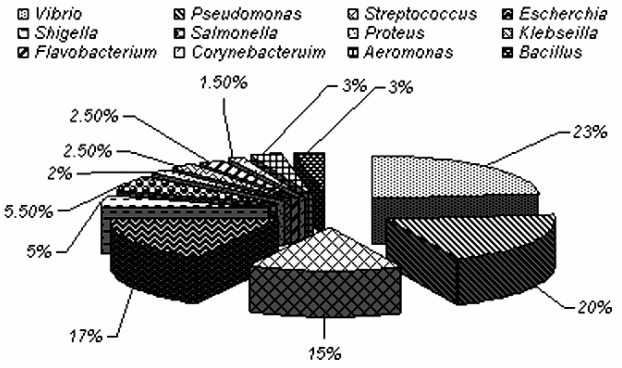

Fig. 2— Percentage composition of bacterial genera recorded from the Little Andaman island.

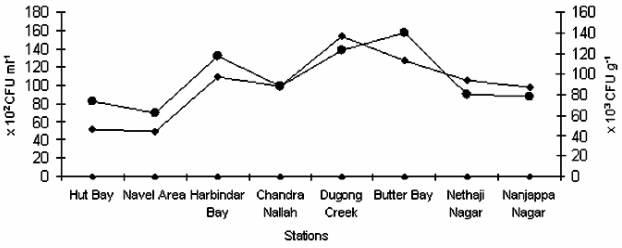

Fig. 3— Population density of THB in the water and sediment samples collected from the Little Andaman island.

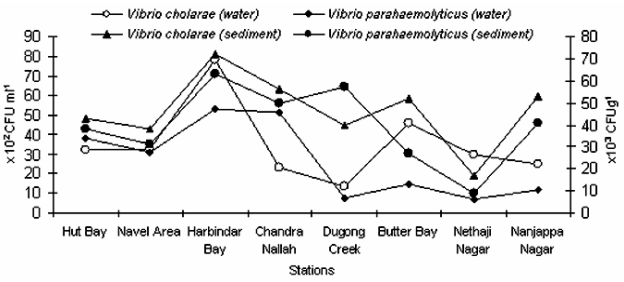

Fig. 4— Population density of *Vibrio* spp. in the water and sediment samples collected from the Little Andaman island

Population density

Population density of THB in the water samples varied from 52×10^2 to 273×10^2 CFU ml^{-1} registering the minimum at Hut Bay and maximum at Dugong creek. In case of the sediment samples, it varies from 62×10^3 CFU g^{-1} at Naval area to 140×10^3 CFU g^{-1} at Buttler Bay (Fig. 3).

Population density of both the *V. cholerae* and *V. parahaemolyticus* were recorded in water and sediment samples in all the eight stations, *V. cholerae* showed lower density (14×10^2 CFU ml^{-1}) in Dugong Creek and higher density (78×10^2 CFU ml^{-1}) in Harbindhar Bay while, *V. parahaemolyticus* registered lower population density (7×10^2 CFU ml^{-1}) in Nathaji Nagar and higher density (53×10^2CFU ml^{-1}) in Harbindhar Bay. In the sediment samples, both *V. cholarae* (17×10^3 CFU g^{-1}) and *V. parahaemolyticus* (9×10^3 CFU g^{-1}) population densities recorded the minimum values in Nethaji Nagar and the maximum of 72×10^3 CFU g^{-1} for *V. cholarae* and 63×10^3 CFU g^{-1} for *V. parahaemolyticus* was recorded at in Harabindhar Bay (Fig. 4).

In the water sample, population density of the pathogenic bacterium, *S. dysenteriae* was minimum (3×10^2 CFU ml^{-1}) in Chandra Nullah and maximum (36×10^2 CFU ml^{-1}) in the Buttler Bay (Fig. 5) while in sediments, it ranged from 15×10^3 CFU g^{-1} (Dugong Creek) to 115×10^3CFU g^{-1} (Hut Bay) (Fig. 6). Population density of *K. pneumoniae*, in the water sample was lower (7×10^2 CFU ml^{-1}) in Chandra Nullah and higher (32×10^2 CFU ml^{-1}) at Naval area (Fig. 5). In the sediments, it varied from

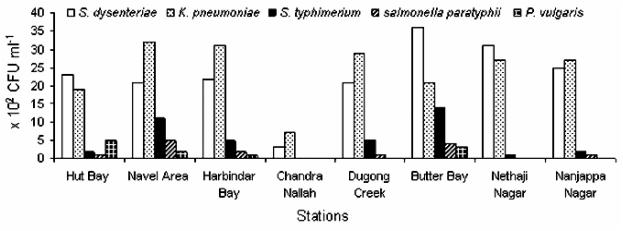

Fig. 5— Population density of *Shigella*, *Klebsiella*, *Salmonella* and *Proteus spp*. in the water samples collected from the Little Andaman island.

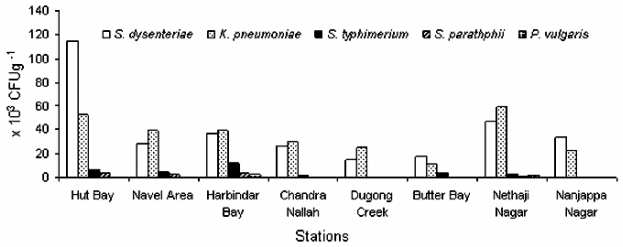

Fig. 6— Population density of *Shigella*, *Klebsiella*, *Salmonella* and *Proteus* spp. in the sediment samples collected from the Little Andaman island.

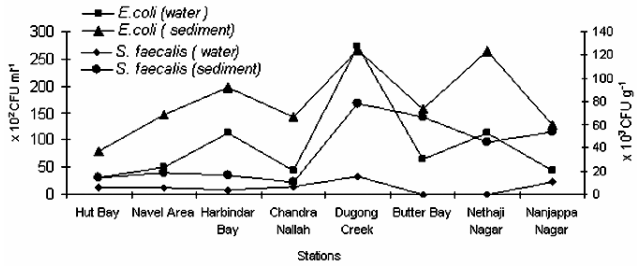

Fig. 7— Population density of *E. coli* and *S. faecalis* in the water and sediment samples collected from the Little Andaman island

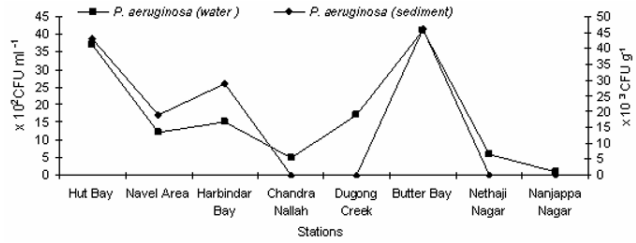

Fig. 8— Population density of *Pseudomonas aeruginosa* in the water and sediment samples collected from the Little Andaman island

11×10^3 CFU g^{-1} (Buttler Bay) to 59×10^3 CFU g^{-1} (Nethaji Nagar) (Fig. 6). In general, *S. dysenteriae* and *K. pneumoniae* were recorded both in water and sediment samples in all the stations.

Population density of *Salmonella typhimerium* in the water sample was lower (1×10^2 CFU ml^{-1}) in Nethaji Nagar and higher (14×10^2 CFU ml^{-1}) at Buttler Bay (Fig. 5) where as it was not recorded at Chandra Nallah. In case of sediments, the lower density (2×10^3 CFU g^{-1}) was recorded in Chandra Nallah, and higher density (12×10^3 CFU g^{-1}) in Harbindhar Bay and no population density were recorded in Dugong Creek and Nanjappa Nagar (Fig. 6).

The population density of *Salmonella paratyphi* in the water sample was lower (1×10^2 CFU g^{-1}) in Hut Bay and higher (5×10^2 CFU g^{-1}) in Naval Area (Fig. 5) and no density was recorded in Chandra Nallah and Nethaji Nagar. In the case of sediment samples, lower density was recorded (1×10^3 CFU g^{-1}) in Nethaji Nagar and higher density (4×10^3 CFU g^{-1}), in the Harbindhar Bay (Fig. 6) and no density was recorded in stations Chandra Nallah, Dugong Creek, Buttler Bay and Nanjappa Nagar.

In the water sample minimum (1×10^2 CFU ml^{-1}), population density of the pathogenic bacterium *P. vulgaris* was observed Harbindhar Bay while the maximum (5×10^2 CFU ml^{-1}) was recorded at in Hut Bay (Fig. 5) and no density was recorded in stations Chandra Nallah, Dugong Creek, Nethaji Nagar and Nanjappa Nagar. In the sediments, it was recorded only at Nethaji Nagar (1×10^3 CFU g^{-1}) and Harbindhar Bay (5×10^3CFU g^{-1}) and no population density was recorded in other stations (Fig. 6). The lesser values of density of *Salmonell*, *Shigella* and *Proteus* were recorded in sediment than the water samples, which indicate that these pathogens are entered into the coastal environment by terrestrial washed-off by tsunami.

Population density of *E. coli* was lower in water (32×10^2 CFU ml^{-1}) and sediments (37×10^3 CFU g^{-1}) in Hut Bay while Dugong creek recorded higher both in water (153×10^2 CFU ml^{-1}) and sediment (124×10^3 CFU g^{-1}) samples (Fig.7).

In the water sample, population density of *S. faecalis* was found to be lower (9×10^2 CFU ml^{-1}) in Harabindhar Bay and the population was high (34×10^2 CFU ml^{-1}) in Dugong Creek (Fig. 7). In the sediments, the lowest population density (11×10^3 CFU g^{-1}) was observed in Chandra Nullah and the higher population density (78×10^3CFU g^{-1}) was found in Dugong Creek (Fig. 7).

Population density of *P. aeruginosa* in water was found to be lower (1×10^2 CFU ml^{-1}) in Nanjappa Nagar and higher (41×10^2 CFU ml^{-1}) in Buttler Bay (Fig. 8). In the sediment samples, minimum

population density of 19×10^3 CFU g^{-1} was recorded in Naval Area and the maximum population density of 46×10^3 CFU g^{-1} was noticed in Buttler Bay (Fig. 8) while stations 4,5,7 and 8 were not registered any *P. aeruginosa* population in sediments.

Discussion

Present investigation highlights the ubiquitous distribution of the Total Heterotrophic Bacteria (THB) and pathogenic bacteria in the water and sediment samples collected from the eight stations along the east coast of the Little Andaman island. THB and pathogenic bacterial densities were higher in the sediments than in water samples. This could be ascribed to the fact that the coastal and shelf sediments play a significant role in the demineralization of organic[4] which supports the growth of microbes. Higher bacterial population density in the sediments than water is generally due to the rich organic content of the former and the lesser residence time of the microorganisms in the water column than the sediments[5]. ANOVA worked out for the THB and other pathogenic bacterial load recorded at different stations indicated there is a significant variation in THB population ($F = 0.036$, $P < 0.005$) in the sediment samples collected from different locations of the Little Andaman. However no such significant relations were obtained for any of the pathogenic bacteria studied.

Specific indicator organisms are used as monitoring tools for assessing the potential presence of pathogenic organisms in the marine environment. Pathogenic bacteria such as *Vibrio, Pseudomonas, Streptococcus, Esherichia, Shigella, Salmonella, Proteus* and *Klebsiella* have been recorded in the present study. *Vibrio* contributed more (23.0%), followed by *Pseudomonas* (20%), *Escherchia* (17.0%) and *Streptococcus* (15%). Even higher pathogenic bacterial load were recorded from the Great Nicobar island than the present study, which indicates the comparatively less polluted environment of the Little Andaman island. Tsunami waters might have contributed to the THB[6], pathogenic and other indicator bacteria of the coastal environment of the Little Andaman considerably as storm water runoff and tsunami waters[6] can bring more indicator bacteria. However, continuous water exchanges with oceanic waters might have reduced the pathogenic bacterial population of this island in due course of time.

Population density of *Vibrio* spp. in the marine environment is usually more because Vibrios can occur in a wide range of aquatic environments including estuaries, marine and coastal waters and sediments[7-12]. Halophilic Vibrios can represent as much as 40% of the total microbiota of the subtropical coastal waters[13]. But, in the present investigation, Vibrios recorded only 23% in the coastal areas of the Little Andaman, which indicates lesser microbial pollution due to *Vibrio* in the coastal environment of the Little Andaman. It is also important that among all the eight stations 6 and 7 were not recorded much *Vibrio* population, this should due to the sandy coast prevailing in this station while the rest of the stations were bestowed with corals, dead corals, mangrove, and seaweeds all contributing much more organic load to the immediate environment which intern support the growth of bacteria.

In the past, the fecal *Streptococci* were used as indicators of presence of fecal source, the health effects related to beach water pollution in Hong Kong was reported[23] with *Streptococcus* spp. population of $31 - 248 \times 10^3$ CFU ml^{-1} and the *E. coli* density ranging from $69 - 1714 \times 10^3$ CFU ml^{-1}. But, in the Little Andaman coastal waters the lower population densities ($9 - 34$ CFU $\times 10^3$ ml^{-1} of *Streptococcus* spp. and $32 - 153 \times 10^3$ CFU ml^{-1} of *E. coli*) of these fecal coliforms indicated the less polluted nature of the coastal waters of this pristine island.

The other pathogens studied as a part of this study were recorded very less population in all the stations and are not capable causing any immediate human health problems in the coastal waters. Due to the growth of modern society and also the developmental activities after the tsunami the anthropogenic inputs have become unavoidable especially in this small island. Due to the above in the small islands like this disposal of solid and liquid wastes become a challenging task which ultimately knockout the adjoining natural system especially critical habitats like coral reefs and mangroves, when such wastes exist for a longer period without any check, would lead to the degradation of critical habitats.

Conclusions

The present study elucidates those eight coastal stations along the east coast of the Little Andaman island supports high total heterotrophic bacterial population. The above might have supported in degradation and recycling of organic and inorganic

materials. The study also indicates that eight coastal stations along the east coast of the island are less polluted by the human pathogens. The studies on the west coast of the island will provide the total microbial pollution status of the island.

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References

1 Hollibaugh J T, Carruthers A B, Fuhrman J A & Azam F Cycling of organic nitrogen in marine Plankton communities studied in enclosed water columns. *Mar. Biol*., 59 (1980) 15-19.

2 Simidu U & Aiso K Occurrence and distribution of heterotrophic bacteria in seawater from the Kamogawa Bay, *Bul. Jap. Soc. Scient. Fish*, 28 (1962) 1133-1141.

3 David R Boone & Richard W, Castenholz. Bergey's Manual of Systematic Bacteriology, 2nd edition Volume one. *The Archae and the deeply branching and Photptrophic bacteria, Springer, Verlag* (1974) 721 pp.

4 Wollast R '*The coastal organic carbon cycle: fluxes, sources and sinks*. In: Ocean margin process in global change', edited by Mantoura, R.F.C, Martin J M and Wollast R, (John Wiley and Sons, New York), 1991, pp.365-381.

5 Anon J '*Ecological, toxicological and environmental impacts* assessment studies of the effluent discharge from MRL - CHR in marine environs of Nagapattinam', Tamil Nadu, Technology Reference Number NIO, 12/97, (1997) 86.

6 Surjit Das, P S Lyla & Ajmal Khan S, Variation in Microbial Population in a Mangrove Ecosystem in Parangipettai Coast (Southeast Coast of India): Effect of Tsunami?, Res. *J. Chem Environ*, 9 (2005) 46-49.

7 Urakawa H, Yoshida, T, Nishimura M & Ohwada K Characterization of depth-related population variation in microbial communities of a coastal marine sediment using 16S rDNA-based approaches and quinone profiling, *Environ. Microbiol*, 2 (2000) 542-554.

8 Suantika G, Dhert P, Rombaut G, Vandenberghe J, De Wolf T & Sorgeloos P. The use of ozone in a high density recirculation system for rotifers, *Aquaculture*, 201 (2001) 35-49.

9 Vandenberghe J, Thompson F L, Gomez-Gil, B. & Swings, J. Phenotypic diversity amongst Vibrio isolates from marine aquaculture systems, *Aquaculture*, 219 (2003) 9-20.

10 Thompson F L, Iida T & Swings J. *Biodiversity of Vibrios, Microbiol. Mol. Biol. Rev*, (2004) 403-431.

11 Venter J C, Remington K, Heidelberg J F, Halpern A L, Rusch D, Eisen J A, Wu D, Paulsen I, Nelson K E, Nelson W, Fouts D E, Levy S, Knap A H, Lomas M W, Nealson K, White O, Peterson J, Hoffman J, Parsons R, Baden-Tillson H, Pfannkoch C, Rogers Y H & Smith, H.O. *Environmental genome shotgun sequencing of the Sargasso Sea, Science*, 304 (2004) 66-74.

12 Chan K Y, Woo M L, Lo K W & French G L. Occurrence and distribution of halophilic Vibrios in subtropical coastal waters of Hong Kong, *Appl. Environ. Microbiol*., 52 (1986)1407-1411.

13 Cheung W H S, Chang K C K & Hung R P S. Health effects of beach water pollution in Hong Kong, *Epidem. Infec*., 105 (1990) 139-162.

Appendix-1

Media composition

Thiosulphate Citrate Bile Sucrose Agar

Yeast Extract-5.0 g
Peptone, Protease-10.0 g
Sodium thiosulphate-10.0 g
Sodium citrate-10.0 g
Ox bile -8.0 g
Sucrose-20.0 g
Sodium chloride-10.0 g
Ferric chloride-1.0 g
Bromothymol blue-0.04 g
Thymol blue-0.04 g
Agar-15.0 g
*p*H-8.6±0.2
50% seawater-1000 ml

Cetrimide Agar

Pancreatic digest of gelatin-20 g
Magnesium chloride-1.4 g
Potassium sulphate-10 g
Cetrimide-0.3 g
Agar-13.60
*p*H-7 ± 0.1
50% seawater-1000 ml

MacConkey Agar

Peptic digest of animal tissue-17 g
Proteose peptone-3 g
Lactose-10 g
Bile salts-1.5 g
Sodium chloride-5 g
Neutral red-0.03 g
Agar-15 g
*p*H-7 ± 0.1
50% seawater-1000 ml

M- Enterococcus Agar

Trptone-15.0 g
Soya extract-5.0 g
Yeast extract-5.0 g
Dextrose-2.0 g
Dipotassium phosphate-4.0 g

Sodium azide-0.4 g
Agar-10. 0 g
2,3 - Triphenyl tetrazolium chloride-0.1 g
pH-7.2± 0.2
50% seawater-1000 ml

M-FC Agar

Tryptose-10 g
Proteose peptone-5 g
Yeast extract-3 g
Lactose -12.5 g
Bile salt mixture-1.5 g
Sodium chloride-5 g
Aniline blue-0.1 g
Agar-15 g
*p*H-7 ± 0.1
50% seawater-1000 ml

Xylose Lysine Deoxycholate Agar

Xylose -3.5 g
L- Lysine -5.0 g
Lactose -7.5 g
Sucrose-7.5 g
Sodium chloride-5.0 g
Yeast Extract-3.0 g
Phenol Red -0.08 g
Sodium dioxy cholate-2.5 g
Sodium thiosulphate-6.8 g
Ferric ammonium citrate-0.8 g
Agar-15.0 g
*p*H-7.4 ± 0.2
50% seawater-1000 ml

Zobell Marine Agar

Peptic digest of animal tissue-5 g
Yeast extract-1 g
Ferric citrate-0.1 g
Sodium chloride-19.45 g
Magnesium chloride-8.8 g
Sodium sulphate-3.24 g
Calcium chloride-1.8 g
Potassium chloride-0.55 g
Sodium bicarbonate-0.16 g
Potassium bromide-0.08 g
Strontium chloride-0.034 g
Boric acid-0.022 g
Sodium silicate-0.004 g
Sodium fluorate-0.0024 g
Ammonium nitrate-0.0016 g
Disodium phosphate-0.008 g
Agar-15 g
H-7 ± 0.1
Distilled water-1000 ml