Seafoam bacteria from the Havelock island of the Andamans, India

Rajagopal Gobalakrishnan, Kannan Sivakumar*, Gunasekaran Vijayabaskara Sethubathi & Lakshmanan Kannan
CAS in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai - 608 502, Tamilnadu, India
*[E-mail: oceanactino@gmail.com]

Present investigation was carried out to study the total heterotrophic and pathogenic bacteria from the seafoam samples of the Havelock coast, Andamans. In this study, 38 strains were isolated and 9 genera viz. Escherichia, Enterococcus, Aeromonas, Vibrio, Salmonella, Klebsella, Streptococcus, Shigella and Pseudomonas were identified. Among the 38 isolates, 11 isolates were selected based on the colony morphology and identified up to species level. Population density of THB was higher in Zobell Marine agar (86 × 10^5 CFU mL⁻¹) and among the THB, E. coli recorded higher density (57 × 10^5 CFU mL⁻¹). This study reveals the presence of THB and pathogenic bacteria in the seafoam; the latter being a signal to the coastal pollution of the island. Moreover, this study is the first of its kind in the Andaman waters of India and it would pave way for future workers to elucidate the role of THB and pathogenic bacteria in the seafoam formation.

[Keywords: Havelock Island, pathogenic bacteria, seafoam, total heterotrophic bacteria]

Microorganisms are cosmopolitan, diverse and distributed in open sea waters, sediments, estuaries, and hydrothermal vents. Most marine ecosystems are fueled by the regeneration and recycling of nutrients, mediated by the microorganisms, which are responsible to sustain all the living things in the oceans. Generally, the oceanic environment is characterized by the hostile parameters such as high pressure, salinity, low temperature, absence of light, etc. and the marine heterotrophic bacteria have adapted themselves to survive in this environment and serve as the major agents, shaping the organic composition of the ocean. Their distribution, diversity and activities are controlled by various hydro-biological factors and nutrient levels present in the ambient environment and have been well studied in different marine environmental niches except seafoam which offers living space for unicellular organisms.

Wissmar and Simenstad explained that foams act as important sites for nutrient cycling and potential food reservoirs for estuarine consumers. Release of great amounts of dissolved organic substances may cause extensive sea foam formation. Production of surface active molecules through bacteria could be an important factor for foam formation. Recently, Baba et al. have reported unusual foam formation in Thiruvananthapuram coast, caused by more deposition of jellyfish. However, mechanism for foam formation is still unclear and there are no reports on seafoam bacteria. Hence, the present study was carried out to enumerate the total heterotrophic and pathogenic bacteria from the seafoam samples of the Havelock island of the Andamans, hitherto not reported from here.

Surface seafoam samples were collected from Radhanagar beach (11° 59'04.2" N & 92° 57' 04.0" E) of the Havelock island, which is an open sandy beach, in August 2011. Foam samples were carefully transferred into sterile plastic bags with a sterile spoon without taking sand grains from the sea surface water as suggested by Schlichting. Collected foam samples were transported to the field laboratory at Havelock Island, by keeping them in an icebox within four hours. Immediately after arrival, inoculants were made for bacterial assay, using ZoBell’s Marine Agar 2216 (Himedia) and various specific media with proper dilutions and the cultures were established.

Total heterotrophic bacterial (THB) population was enumerated using the spread plate method with Marine Agar medium. The plates after inoculation were incubated in an inverted position at a temperature of 28 ± 2°C for 24 to 48 hours. After incubation, colonies in the triplicate samples were counted and expressed as colony forming units (CFU mL⁻¹) in the foam samples. Bacterial colonies were than picked out from the pertidishes and restreaked in appropriate nutrient agar plates and
pure cultures were stored in agar slants for further identification. Specific media were used for *V. cholerae* and *V. parahaemolyticus* (TCBS agar), *E. coli* (MacConkey agar), species of *Salmonella*, *Shigella* and *Klebsella* (XLD agar), *Pseudomonas aeruginosa* (Cetrimide agar), *Enterococcus* (M-enterococcus agar) and *Aeromonas hydrophila* (AMB agar). Different morphological and biochemical characteristics of the isolates were studied according to the Bergey’s Manual of Determinative Bacteriology. All the chemicals and media were purchased from Hi-media, Mumbai.

Totally, 38 THB strains were isolated from the seafoam samples of the Havelock island and identified as belonging to 9 genera: *Escherichia*, *Enterococcus*, *Aeromonas*, *Vibrio*, *Salmonella*, *Klebsella*, *Streptococcus*, *Shigella* and *Pseudomonas*. Of these, *Vibrio* contributed higher percentage (19%) followed by *Salmonella* (18%). *Escherichia*, *Pseudomonas*, *Aeromonas*, *Klebsella*, *Streptococcus*, *Enterococcus* and *Shigella* contributed 9% each (Fig. 1). Among the 9 genera, 8 genera (*Escherichia*, *Enterococcus*, *Aeromonas*, *Vibrio*, *Salmonella*, *Klebsella*, *Shigella* and *Pseudomonas*) belong to the gram negative group and the remaining genus *Streptococcus* belongs to the gram positive group.

In the seafoam samples, THB population density was higher in ZMA medium (86 × 10⁵ CFU mL⁻¹), followed by the pathogenic bacteria, enumerated in TCBS agar (61 × 10⁵ CFU mL⁻¹), MacConkey agar (57 × 10⁵ CFU mL⁻¹), XLD agar (44 × 10⁵ CFU mL⁻¹), M-enterococcus agar (39 × 10⁵ CFU mL⁻¹), Cetrimide agar (21 × 10⁵ CFU mL⁻¹) and AMB agar (18× 10⁵ CFU mL⁻¹) (Fig. 2).

Among the seafoam bacteria, *E. coli* recorded higher density (57 × 10⁵ CFU mL⁻¹), followed by *Enterococcus* sp. (39 × 10⁵ CFU mL⁻¹), *V. parahaemolyticus* (33 × 10⁵ CFU mL⁻¹), *V. cholerae* (28 × 10⁵ CFU mL⁻¹), *P. aeruginosa* (21 × 10⁵ CFU mL⁻¹), *Salmonella* sp. (21 × 10⁵ CFU mL⁻¹), *A. hydrophila* (18 × 10⁵ CFU mL⁻¹), *Klebsella* sp. (14 × 10⁵ CFU mL⁻¹) and *S. dysenteriae* (9 × 10⁵ CFU mL⁻¹) (Fig. 3).

Among the 38 isolates, 11 different strains were selected based on their colony morphology and these isolates were subjected to biochemical characterization such as IMViC (indole, Methyl Red, Voges Proskauer, citrate utilization), Triple Sugar Iron, oxidase, urease, carbohydrate fermentation (lactose, maltose, sucrose, xylose, Inositol), starch and catalase tests. They were also categorized as Gram Positive and Gram Negative forms (Table 1). These were identified as *V. cholerae* SFHA1, *P. aeruginosa* SFHA2, *E. coli* SFHA3, *S. paratyphi* SFHA4, *A. hydrophila* SFHA5, *S. typhimurium* SFHA6, *S. dysenteriae* SFHA7, *V. parahaemolyticus* SFHA8.

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Fig. 2—Population density of seafoam THB and pathogenic bacteria in different media.

Fig. 1—Generic composition of The THB in the seafoam samples of the Havelock island.

Fig. 3—Population density of pathogenic bacteria, isolated from the seafoam sample.
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1564

S. faecalis SFHA9, Klebsella sp. SFHA10 and Enterococcus sp. SFHA11, following the Bergey’s Manual of Systematic Bacteriology.

In the Havelock island, the coast comprises mangroves, seagrass beds, coral reefs and sandy beaches, influenced by different biotic and abiotic features. Present study site, Radhanagar beach of the Havelock island is a sandy beach with anthropogenic activities. In general, marine THB and pathogens are often found in association with the surfaces of the marine animals, phytoplankton, sediments and suspended detritus. Druzhkov et al. discussed seafoam as an object for sea surface film studies. In fact, many researchers have reported about the foam formation from various parts of the world. Pope found that protein debris from damaged planktonic organisms and alginates from seaweeds are responsible for foam formation. Velimirov opined that most of the sugars which are present in the mucilage released during the fragmentation of macrophytes are used up by a fast-growing bacterial population in the foam. Thus, the foam reflects the end product of micro-heterotrophic colonization.

The present investigation, oriented towards seafoam forming bacteria, 38 strains belonging to 9 genera (Escherichia, Enterococcus, Aeromonas, Vibrio, Salmonella, Klebsella, Streptococcus, Shigella and Pseudomonas) were recorded from the seafoam samples. This includes 8 genera of gram negative and 1 strain of gram positive bacteria. Previous studies from the Great Nicobar island and Little Andaman island reported 10 and 12 genera of THB respectively from marine water and sediment samples. Though the seafoam THB generic diversity is low, this report is first of its kind from the Indian marine environment. Further, this type of study revealing the presence of diverse THB in the seafoam samples can give a clue to the seafoam formation, in the light of the observation that great amounts of dissolved organic substances can cause extensive seafoam formation. A recent study has shown that, as particles sink, activity of the bacterial extracellular enzymes leads to leaching of a plume of dissolved organic material, which provides with a concentrated nutrient source for suspended planktonic bacteria that are responsible for the utilization of bulk of organic carbon and respiration in the sea and shaping the organic composition of the ocean.

Pathogenic microbes in the coastal waters could occur at any time due to the disposal of sewage (human or animal origin) along the coast; especially fecal bacteria may come from natural products of weathering and many man-made materials as well as sewage sludge and septic tank wastes. As Pai has opined, marine resources become the ultimate dumping grounds for the mounting burden of human waste materials. Present study area is a sandy open beach, with anthropogenic activity that might contribute to fecal coliforms such as E.coli and

Table 1—Biochemical characterization of seafoam isolates, from the Havelock island

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Positive result (+); Negative result (-); Doubtful (±).

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S. faecalis, recorded now from the seafoam samples. Those pathogens such as E.coli, when binding to mannosylated surfaces via the adhesive protein FimH, may adhere weakly or strongly. So, there is every possibility for the pathogens, derived from the anthropogenic sources, to adhere to the seafoam. In conclusion, it can be stated that the seafoam of the Havelock island is impregnated with bacteria, including pathogens. This study, to our knowledge is the first of its kind in the Andaman waters of India and it would pave way for future workers to elucidate the role of THB and pathogenic bacteria in the seafoam formation.

Acknowledgments

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References