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Original Research Paper

Study on Biofouling Organisms Present on the Surface of Boats in Royapuram, Chennai

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ABSTRACT

Biofouling is a natural process of colonization of organisms on submerged surfaces, either living or artificial, by a wide range of microorganisms, plants, algae and animals. Biofilms on artificial structures create serious problems for industries worldwide, with effects such as increase in drag force and metal corrosion as well as reduction in heat transfer efficiency. For antifouling or preventing the attachment of fouling organisms, a knowledge of the microbial composition is of considerable importance. In the present study, biofouling samples were collected bimonthly from the boats docked at the Royapuram harbour, which is situated in northern Chennai. Culturable marine bacteria were isolated on Zobell's marine agar medium and identified by biochemical methods. The bacteria most frequently isolated were *Bacillus* spp., *Vibrio* spp., *Pseudomonas* spp., *Micrococcus luteus, Proteus mirabilis* and *Shigella* spp. The macrofouling community is dominated by barnacles, *Mytilus* spp.; green mussel, *Perna viridis*; polychaetes and other tubeworms. An analysis revealed that most of the marine bacteria are of anthropogenic origin. The stone crab *Menippe mercenaria* is reported as a macrofouler for the first time.

INTRODUCTION

Marine biofouling is an undesirable process of colonization of organisms on submerged surfaces, either living or artificial, by a wide range of microorganisms, plants, algae and animals. The initial biofilm is formed by motile bacteria and subsequent chemical cues promote further macrofouling (Bhattarai et al. 2007). On a ship's hull, the adverse effects caused by this biological settlement are high frictional resistance due to the generated roughness, which leads to an increase in weight and speed reduction, thereby causing additional fuel consumption and maintenance costs. Biofouling on ships have also been linked to the spread of invasive or non-indigenous species (NIS). This has been identified as a current threat to the environment. Ports and harbours are at a high risk due to the presence of invasive species as artificial substratum favour NIS over native species (Ralston & Swain 2014).

The marine biofouling challenges are greater for wooden-hulled boats because they spend more time near the shore and are exposed constantly to a wider array of fouling organisms compared to larger commercial fishing vessels that go farther out to sea. The fouling pressure is high, and significant amount of time and money is spent for manually clearing the hull of barnacles, mussels and algae fouling. Thus, the eradication of biofilms and inhibition of their growth are major concerns.

Costly mechanical processes coupled with toxic heavy-metal-based paint containing tin, copper, etc. have been used as antifouling agents. Due to the non-specific effects of metal leaching, such paints are environmentally hazardous and have been banned since 2003 and gradually removed from shipping fleets (IMO 2007). As a consequence, the need for the development of new environmentally compatible antifouling technologies is now the need of the hour. Subsequent studies largely concentrated on a few novel approaches such as natural product-based non-metallic and eco-friendly coatings (Kristensen et al. 2008), surface modification approaches such as engineered topographies (Magin et al. 2010), foul release polymer-based coatings (Chaudhury et al. 2005) and nanotechnological approaches (Gladis et al. 2010). But for any study on antifouling, knowledge of the microbial community constituting a target biofouling layer is of considerable importance. Most of the studies on the effect of natural antifouling have used standard one or few micro-or macrofoulers for analysis (Bazes et al. 2006, Qian et al. 2010, Manilal et al. 2010, Prabhu et al. 2014).

Therefore, the aim of this work is to investigate the seasonal variation in the biofouling community, isolate and identify culturable marine bacteria and macrofoulers that occupy the surfaces of boats, so that these common fouling communities can be used as test organisms against antifouling components in future.

MATERIALS AND METHODS

Biofouling samples were collected bimonthly from January 2014 to November 2014 from boats docked at the Royapuram harbour, Chennai Port, India. The small fishing boats travel to the Bay of Bengal, which remains tropical throughout the year with two short monsoons and is representative of conditions found in many other parts of Asia and Africa.

The biofilms were scraped off from five different locations and divided into two parts. The first part for bacterial isolation was placed in a sterile container; an additional 100 mL of sterilized seawater was added and placed in an icebox. The other part for macrofouler analysis was kept in a sterile container and transferred to the laboratory. The sample was subjected to vigorous vortexing for 5 minutes and serially diluted using sterilized seawater. A volume of 100 µL of the diluents were spread on sterile Zobell's Marine Agar 2216 (HiMedia, Mumbai). The plates were incubated at room temperature (27°C-30°C) for 5 days, and isolation of bacteria with different colony characteristics was carried out from the third day onwards up to the fifth day. Day 5 counts were used for the calculation of colony forming units (CFU). The isolated colonies showing different morphological characteristics were identified using minimum biochemical tests (Das et al., 2007) and confirmed using *Bergey's Manual*. The purified isolates were then cultured on Zobell's Marine Slant and stored at 4°C. Macrofouling organisms including both soft and hard foulers were separated, washed, identified and stored in 5% formalin.

RESULTS

Culturable marine bacteria and macrofouling organisms isolated from boats during this analysis are given in Tables 1(a)-1(f). The total bacterial count was maximum during September and least during March as given in Table 2. The diversity of microorganisms varied depending on the nutritive status of water.

The diversity of micro and macrofoulers was also maximum during September and least during March 2014. With the exception of mussels, the settlement of various macrofoulers was found to be maximum during summer. *Balanus* sp. and *Mytilus* sp. were always recorded as major macrofoulers. Hydroides, tube worms and bryozoans were also present in large numbers. Green algae, *Enterophora* and *Ulva* sp., were not observed in the January and March sampling. Oyster, *Crassostrea madrasensis*, and limpet, *Patella sp*., were observed in the January sample. A burrowing bivalve, *Abra sp*., and a bryozoan, *Bugula sp*., were present in the May sample. A stone crab species, *Menippe mercenaria*, was present in the July sample.

RESULTS AND DISCUSSION

It is clear from this work that bacterial biofilms on boat surfaces harbour a diverse group of culturable marine bacteria. The biofilms contained the spore-forming *Bacillus* sp., which was the most common and dominant in all samples (Vardhan et al. 2011), non-spore forming halophilic bacteria like *Halomonas* sp. (Sass et al. 2001) followed by gram-positive cocci like *Micrococcus luteus* (Madigan et al. 2005), gram-negative bacteria like *Vibrio, Pseudomonas* and *Pseudoalteromonas.* Uncommon and pathogenic organisms like *Proteus mirabilis* (Aiassa et al. 2010)*, Shigella, Staphylococcus, Aeromonas* and *Aerococcus* were also isolated in the biofilm sample.

The presence of pathogenic bacteria and anthropogenic microbial invaders like *Proteus* and *Shigella* in the marine environment has been previously reported (Shikuma & Hadfield 2010). Biofilm formation might be one of the survival strategies possessed by bacteria such as *Proteus* entering the marine environment from land run off in which the bacterial genome is equipped with adhesive-like proteins, which may form biofilms better than others (Aiassa et al. 2010).

Studies also have shown that Zobell's Marine Agar 2216 selectively isolate marine bacteria that fall predominantly within the gamma subclass of the Proteobacteria clade. An earlier report on phylogenetic analysis using 16s rDNA sequences of marine biofilm bacteria from a ship's hull in Ennore Harbour, Chennai Port, indicated that *Firmicutes* were dominant (56.25%) compared to Gram-positive bacteria (18.75%), G-proteobacteria (12.5%), CFB group bacteria (6.25%) and Enterobacteria (6.25%), and a majority of the marine bacterial species are of anthropogenic origin (Inbakandan et al. 2010).

Both cyprid larvae and adult acorn barnacles were noted in large numbers. Several environmental- and substratum related factors, especially surface biochemistry, play vital roles in inducing the settlement and metamorphosis of barnacles (Daniel et al. 2014). The most common mollusc was *Perna viridis* followed by green mussel, *Mytilus edulus*. Biofilm ageing is commonly assumed to improve mussel settlement on artificial substrata. As biofilms can constitute a consistent food resource for larvae, the lipid quality may be a selection criterion for settlement (Nicolas et al. 2012).

Menippe mercenaria, the stone crab is a non-indigenous species (Fig. 1) and has been reported for the first time from this area. It inhabits sub-tidal regions; they burrow under emergent hard substrate or in seagrass beds. The stone crab larvae travel with the zooplankton, upon which they feed in the near-shore marine environment (Bert & Stevely 1999, Gulf Shores Marine Fisheries Commission 2001).

Table 1(a): Micro and macrofouling organisms isolated in January 2014.

Sl.	Microorganisms	Macrofoulers
1.	*Bacillus* sp.	Adult and cyprid larvae Barnacle, *Balanus amphitrite Balanus* sp.
2.	*Pseudomonas putida*	*Mytilus edulus*
3.	*Micrococcus luteus*	Green mussel, *Perna viridis*
4.	*Vibrio parahemolyticus*	*Crassostrea madrasensis*
5.	*Aeromonas* sp.	Polychaete worms, *Hydroides elegans* and tube worms
6.	*Serratia marsescens*	Limpet, *Patella* sp.
7.	*Pseudoalteromonas* sp.	
8.	*Vibrio harveyi*	
9.	*Pseudomonas* sp.	

Table 1(b): Micro and macrofouling organisms isolated in March 2014.

Sl.	Microorganisms	Macrofoulers
1.	*Bacillus* sp.	Adult and cyprid larvae Barnacle, *Balanus amphitrite*
2.	*Vibrio parahemolyticus*	*Mytilus edulus*
3.	*Vibrio* sp.	Green mussel, *Perna viridis*
4.	*Micrococcus luteus*	Polychaete worms, *Hydroides*
5.	*Pseudomonas* sp.	*elegans* and tube worms
6.	*Proteus mirabilis*	
7.	*Serratia marcescens*	

Table 1(c): Micro and macrofouling organisms isolated in May 2014.

Sl.	Microorganisms	Macrofoulers
1.	*Bacillus* sp.	Adult and cyprid larvae Barnacle, *Balanus amphitrite*
2.	*Vibrio* sp.	*Mytilus edulus*
3.	*Vibrio harveyi*	Green mussel, *Perna viridis*
4.	*Vibrio parahemolyticus*	Polychaete worms, *Hydroides elegans* and tube worms
5.	*Pseudomonas putida*	Burrowing bivalve, *Abra* spp.
6.	*Micrococcus luteus*	Bryozoan, *Bugula* spp.
7.	*Halomonas* sp.	Green algae, *Enteromorpha, Ulva* sp.
8.	*Pseudoalteromonas* sp.	
9.	*Pseudomonas* sp.	
10.	*Staphylococcus* sp.	

Table 1(d): Micro and macrofouling organisms isolated in July 2014.

Sl.	Microorganisms	Macrofoulers
1.	*Bacillus* sp.	Adult and cyprid larvae
2.	*Vibrio harveyi*	Barnacle, *Balanus amphitrite*
3.	*Vibrio marinus*	*Mytilus edulus*
4.	*Vibrio parahemolyticus*	Green mussel, *Perna viridis*
5.	*Proteus mirabilis*	Polychaete worms, *Hydroides elegans* and tube worms
6.	*Pseudomonas aeruginosa*	Green algae, *Enteromorpha, Ulva* sp.
7.	*Pseudomonas putida*	Stone crab, *Menippe mercenaria*
8.	*Aeromonas* sp.	
9.	*Aerococcus* sp.	
10.	*Pseudoalteromonas* sp.	
11.	*Staphylococcus* sp.	
12	*Halomonas* sp.	

Table 1(e): Micro and macrofouling organisms isolated in September 2014.

Sl.	Microorganisms	Macrofoulers
1.	*Bacillus* sp.	Adult and cyprid larvae
2.	*Bacillus pumilus*	Barnacle, *Balanus amphitrite*
3.	*Pseudoalteromonas* sp.	
4.	*Vibrio marinus*	*Mytilus edulus*
5.	*Vibrio parahemolyticus*	Green mussel*, Perna viridis*
6.	*Vibrio harveyi*	
7.	*Micrococcus luteus*	Polychaete worms, *Hydroides elegans* and tube worms
8.	*Proteus mirabilis*	Green algae, *Enteromorpha, Ulva* sp.
9.	*Pseudomonas putida*	
10.	*Aerococcus*	
11.	*Staphylococcus aureus*	
12.	*Serratia marcescens*	
13.	*Shigella* sp.	
14.	*Halomonas* sp.	

Table 1(f): Micro and macrofouling organisms isolated in November 2014.

Sl.	Microorganisms	Macrofoulers
1.	*Bacillus* sp.	Adult and cyprid larvae Barnacle
2.	*Vibrio marinus*	*Balanus amphitrite*
3.	*Vibrio parahemolyticus*	
4.	*Vibrio harveyi*	*Mytilus edulus*
5.	*Micrococcus luteus*	Green mussel, *Perna viridis*
6.	*Staphylococcus aureus*	
7.	*Serratia marcescens*	Polychaete worms, *Hydroides elegans* and tube worms
8.	*Pseudomonas* spp.	Green algae, *Enteromorpha*, *Ulva* sp.
9.	*Pseudoalteromonas*	

Table 2: Total count of bacteria on Zobell's Marine Agar.

Sl.	Months	CFU/mL
1.	January	3.6×10^8
2.	March	3.5×10^8
3.	May	4.8×10^8
4.	July	5.1×10^8
5.	September	7.2×10^9
6.	November	5.9×10^8

The other common type of shell foulers, polychaete worms, can destroy unprotected wooden hulls in a short period of time. The polychaete, *Hydroides elegans*, a tube-building worm has been reported as a dominant fouling species to be widely distributed in tropical and subtropical seas and thus a major target organism in antifouling research (Zhang et al. 2014).

Among the soft foulers, green algae, *Enteromorpha*, *Ulva*, and bryozoans were noted. The green algae, *Entero- morpha*, is the most important macroalga that fouls ships, submarines and underwater structures. Major factors in its success in colonising new substrata are the production of enormous numbers of swimming spores and their ability to locate surfaces on which to settle. The level of gregarious zoospore

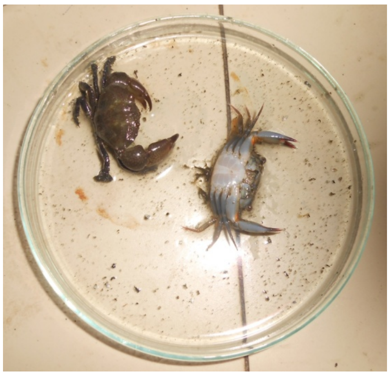

Fig. 1: Stone crab, *Menippe mercenaria.*

settlement is related to spore density and may be mediated by a number of external cues including fatty acids and 'detritus' (Callow & Callow 2000).

Thus, marine biofilms contain different species of heterotrophic bacteria (mainly Proteobacteria), while the densities of Sarcodines and Ciliates remain low (reviewed by Dobretsov 2010, Wahl et al. 2012).

CONCLUSION AND FUTURE DIRECTIONS

This research leads to a better understanding of common micro and macrofoulers of boats in Royapuram. The extent to which marine biofilms on boat surfaces serve as a reservoir and means of dissemination for bacteria of anthropogenic origin has been reported here. The selection of active molecules or coatings to prevent fouling of man-made structures requires the development of bioassays that target multiple groups of biofouling organisms, both micro and macrofoulers, prevalent in a particular area. This may aid in the future development of novel antifouling strategies that deter settlement using chemical signatures rather than the biocidal mode of action.

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