

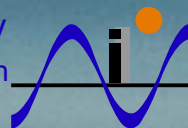
Studies on phytoplankton-bacterial interactions

Thesis submitted to the Goa University
for the degree of Doctor of Philosophy in
Marine Sciences

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Dedicated to my parents

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Statement

As required under the University ordinance 0.19.8 (vi), I state that the present thesis titled “*Studies on phytoplankton-bacterial interactions*” is my original contribution and the same has not been submitted on any previous occasion. To the best of my knowledge the present study is the first comprehensive work of its kind from the area mentioned.

The literature related to the problem investigated has been cited. Due acknowledgements have been made wherever facilities and suggestions have been availed of.

(Priya M. D’Costa)

Certificate

This is to certify that the thesis entitled “*Studies on phytoplankton-bacterial interactions*” submitted by *Ms. Priya M. D’Costa* for the award of the degree of Doctor of Philosophy in Marine Sciences is based on her original studies carried out by her under my supervision. The thesis or any part thereof has not been previously submitted for any other degree or diploma in any Universities or Institutions.

Dr. A. C. Anil

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Chapter 1
General introduction

Phytoplankton are single-celled microscopic algae that form the base of the food web in the open sea. They contribute significantly to climatic processes (Jeffrey and Vesk 1997) and play an important role in determining ecosystem functioning and trophic dynamics. The other group of interest - bacteria are ubiquitous in nature and occur abundantly in marine environments. They contribute significantly to decomposition processes and are important in the microbial food web. Phytoplankton and bacteria differ from each other in many respects. Phytoplankton are the simplest eukaryotes and function as producers in the food web whereas heterotrophic bacteria are prokaryotic organisms that participate mainly as decomposers.

Phytoplankton and bacteria are closely associated in the natural environment (Armbrust 2009); their interactions are numerous and wide-ranging. They could be classified into 2 broad categories: (1) cell-level interactions, and (2) system-level interactions.

1.1. Cell-level interactions

Bacteria are closely associated with both, diatoms and dinoflagellates. Bacteria have also been noticed in frustule crevices in diatoms, and appear to be inaccessible to flushing methods and treatment with antibiotics (Kaczmarska et al. 2005). These are termed 'satellite' bacteria (Schafer et al. 2002), and are most abundant in areas of the frustule where they have greater access to organic nutrient molecules (Kaczmarska et al. 2005). Diatoms rely on bacteria for vitamins (Croft et al. 2005, Droop 2007). The necessity of these bacteria to diatoms is evident in the enhanced exopolymer production, increased metal tolerance and higher growth rate of non-

axenic diatoms compared to axenic diatoms (Thomas and Robinson 1987, Patil and Anil 2005).

Intracellular bacteria have also been observed in the pennate diatom *Pinnularia* (Schmid 2000), and in dinoflagellates - *Alexandrium catenella* and *Protoceratium reticulatum* (Cordova et al. 2003). Intracytoplasmic bacteria have been observed in hypnocysts of *Alexandrium tamarense* (Schweikert 2003). These cyst-associated bacteria could seed germinating *A. tamarense* populations, maintaining a bacterial association even without the need for endocytobiosis after each dormancy period (Schweikert 2003).

Further evidence for the close association between phytoplankton and bacteria is provided by the detection of a large number of bacterial genes in the genome of the diatom *Phaeodactylum tricorutum* (Bowler et al. 2008). In fact, a high level of horizontal gene transfer has been observed between *P. tricorutum* and bacteria, at rates similar to that observed between bacteria (Keeling and Palmer 2008). These appear to be recent acquisitions (Bowler et al. 2008), pointing to the constantly evolving nature of these interactions. In a similar vein, a pronounced shift in gene expression patterns in the dinoflagellate *A. tamarense* was observed in response to the presence of bacteria (Moustafa et al. 2010).

1.2. System-level interactions

Considering the system level, phytoplankton-bacterial interactions extend from symbiotic to pure parasitic relationships (Cole 1982). Traditionally, bacteria were thought to play an important role in degradation of organic matter produced by phytoplankton (Grossart et al. 2005), i.e., from particulate to dissolved form. The

reverse is also true. Bacteria also increase aggregation of phytoplankton or other particles and stabilize already existing aggregates (Allison and Sutherland 1987, Decho 1990, Heissenberger and Herndl 1994). The precise nature of these relationships depends on environmental conditions, to a large extent, and presumably on the function of bacteria present (Grossart 1999). This is clearly illustrated in the shift in phytoplankton-bacterial dynamics during the progression of phytoplankton blooms.

Phytoplankton blooms are associated with specific bacterial assemblages that change in abundance, species composition and enzyme activities as the bloom progresses (Riemann et al. 2000). The species succession in bacterial communities determines the fate of the organic matter produced during the bloom. For e.g. dominance of particle-colonizing bacteria would lead to enhanced remineralization of detritus. Similarly, free-living bacteria adapted for efficiently utilizing dissolved organic matter (DOM) for production of cell biomass could prevent large scale accumulation of DOM despite its high production rates typical in blooms (Koike et al. 1990, Smith et al. 1995). The biomass produced is transferred to higher trophic levels of the food web (Grossart et al. 2005), through the microbial loop (bacteria-micro-mesozooplankton).

Not all blooms support increased abundance of bacteria. Blooms of *Asterionellopsis glacialis*, a surf-zone diatom were strongly decoupled with bacterial abundance (Abreu et al. 2003). Several reasons were put forth to explain this phenomenon and included viral infection, bacterial grazing, quality of dissolved organic carbon, nutrient competition and antibiotic production. Consequently, a large fraction of *A. glacialis* primary productivity (in the form of DOC) was not channeled

through the microbial food web but was available for consumption by grazers (Abreu et al. 2003). Therefore, the microbial loop regulates the proportion of primary productivity that passes through the conventional phytoplankton-zooplankton food web.

This has important implications in studies involving sequestration of anthropogenic carbon, mainly the ocean fertilization experiments carried out in High Nutrient Low Chlorophyll (HNCL) regions as a means of mitigating the rise in atmospheric carbon dioxide. These experiments rely on the fact that a majority of the Particulate Organic Carbon (POC) produced during an iron-induced bloom will sink to the bottom where the fixed carbon will be sequestered for long time periods. However, Boyd et al. (2004) have reported that a significant fraction of the POC produced during the Subarctic Ecosystem Response to Iron Enrichment Study (SERIES) iron-induced bloom in the Gulf of Alaska did not sink to deep waters as expected but was remineralized by bacterial activity in the surface waters.

There are still many more examples of phytoplankton-bacterial interactions at the system-level. Bacteria are capable of promoting as well as terminating Harmful Algal Blooms (HABs) (Fukami et al. 1997), and are involved in modification of algal toxins (Kodama 1990, Hold et al. 2001a and b). Bacteria are also involved in the dissolution of diatom frustules and silica cycling (Bidle and Azam 2001). At the other end of the spectrum, bacteria influence formation of diatom biofilms (Cooksey et al. 1980), as well as sediment stabilization (Wigglesworth-Cooksey et al. 2001).

Therefore, the ecology of both groups is closely linked. Phytoplankton can regulate bacterial communities through variations in the Dissolved Organic Matter

(DOM) produced during different stages of their life-cycle (Grossart et al. 2005), and through bioactive polyunsaturated aldehydes (PUAs) (Ribalet et al. 2008). Does the reverse hold true? Bacteria have been shown to have many effects on phytoplankton, including algicidal activity (Mitsutani et al. 1992, Yoshinaga et al. 1997), stimulation of phytoplankton growth (Ferrier et al. 2002), production or modulation of toxicity (Gallacher et al. 1996, 1997, Kopp et al. 1997), and inhibition or promotion of cyst formation (Adachi et al. 2003, Fukami et al. 1991). Bacteria may stimulate phytoplankton growth via the production of vitamins (Croft et al. 2005, Droop 2007), iron chelators (siderophores) (Soria-Dengg et al. 2001), and cytokinins (Maruyama et al. 1986). But what about changes at the community level? Will changes in bacterial communities be reflected in phytoplankton communities?

Phytoplankton community structure is conventionally regulated by the interactive effects of ‘bottom-up’ factors (light and nutrient supply), loss factors (sinking and mortality), ‘top-down’ control by organisms feeding on microalgae like copepods (Assmy et al. 2007) and interactions with other trophic levels (Reynolds 1980). These categories of regulating factors play important but different roles in structuring phytoplankton communities, especially in tropical, monsoon-influenced environments. In view of this and since phytoplankton are never free of bacterial influences (Armbrust 2009), the present study focuses on the factors regulating phytoplankton community structure and analyzes the relevance of bacteria in the suite of regulating factors.

The species composition-level links between phytoplankton and free-living and attached bacterial communities have been studied in the pelagic coastal system by Rooney-Varga et al. (2005). However, not much is known about these community-

level interactions in benthic environments. Therefore, subsequent efforts were directed towards the effect of bacteria on microphytobenthic diatom communities, which serve as significant regulators of energy flow in intertidal foodwebs (Kuipers et al. 1981), and interact with bacteria to mediate sediment stabilization (Guerrini et al. 1998, Wigglesworth-Cooksey et al. 2001, Gerbersdorf et al. 2008). In addition to monitoring changes in diatom community structure following treatment with specific antibiotics, the changes in the bacterial fraction were analyzed. The effect of antibiotics on diatom monocultures, isolated from the study area, was also studied.

To determine whether the potential of bacteria to modulate microphytobenthic diatom community structure varies on a spatial scale, 3 sites with differing habitat characteristics were selected. In view of the stochastic nature of the monsoons that is the main source of climatic variation at these sites, and the resulting effects on diatom and bacterial communities, these sites were sampled on a seasonal basis. This provided an idea of the temporal variations in the ability of bacteria to modulate diatom community structure in benthic environments.

The studies carried out are presented in the following chapters:

- *Phytoplankton community structure in a tropical, monsoon-influenced environment*
- *Diatom-bacterial interplay in benthic environments*
- *Spatial and temporal variations in diatom-bacterial interactions*