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# Picophytoplankton community from tropical marine biofilms

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ABSTRACT

Although the importance of picophytoplankton (<3 μm) as the major primary producers is well established in the oceanic and coastal marine environment, occurrence of this community in the marine biofilms is not explored. Considering their small size and high abundance which is next to bacteria, the initial heterotrophic colonizers, it was hypothesized that picophytoplankton are the first autotrophic colonizers in marine biofilms. In this study biofilms were developed by immersing glass slides in the tropical waters of the Dona Paula Bay up to a period of 192 h. The presence of picophytoplankton and the temporal variations in community structure was investigated flow cytometrically after every 24 h. Biofilms were also developed in the laboratory by immersing the glass slides in fresh natural seawater and the sequence of appearance of the microorganisms was assessed with high frequency samplings. Field observations showed the presence of three groups of picophytoplankton, two prokaryotes, *Prochlorococcus*-like organisms (*PRO*), *Synechococcus* (*SYN*) and the picoeukaryotes (PEUK). Nanoeukaryotes, which are mostly represented by diatoms were also monitored. In the total biofilm community, prokaryotes were dominant throughout the study period wherein contribution of *SYN* was highest (50%) in the earlier stages which were later overtaken by *PRO*-like cells. The contribution of PEUK and nanoeukaryotes was always below 20%. Picophytoplankton contribution to the total photosynthetic biomass was >60% in the initial period of biofilm formation, both in terms of numbers and biomass with PEUK as the major contributors. However, after 2 days of incubation, their contribution to total chlorophyll declined thus revealing that although their numbers were increasing, picophytoplankton were succeeded by nanoeukaryotes in terms of biomass. Laboratory experiments revealed that heterotrophic bacteria and picophytoplankton appeared within 5 min in the biofilms which were followed by the nanoeukaryotes after 5 h. This study shows that picophytoplankton are the pioneer autotrophic colonizers in the tropical marine biofilms, suggesting an essential role in the biofilm food web dynamics, especially in the initial stages.



## 1. Introduction

Diatoms and dinoflagellates are more commonly regarded as the dominant phytoplankters in estuaries and lagoons, reaching bloom proportions in regions with requisite bioavailable nutrients and light (Kennish, 1990; Paerl, 1988). Diatoms are also recognized for their vital role in marine biofilms where they form a major part of the biomass and are considered to be the initial autotrophic colonizers in coastal regions (Callow, 2000; Cooksey et al., 1980). Diatom metabolic activities provide the sole carbon and energy source for heterotrophic bacteria in biofilms where both types of organisms are present (Murray et al., 1986). Both these organisms, due to their higher numbers in the ambient waters have a higher surface encountering probability as a result of which they form important components in the initial phases of biofilm formation (Cooksey et al., 1984). Biofilms have at least three clearly defined roles in marine ecology: i) as major sources of primary production on the shore for microphagous herbivores (Hawkins et al., 1992; Sommer, 1999); ii) as primary attachment sites for macroalgal propagules which settle and germinate on it (Steinberg et al., 2002; Wahl, 1989); and iii) as sources of settlement cues for a variety of marine invertebrates and algae and may promote cellular metamorphosis in the process of biofouling (Dobretsov and Qian, 2006). All these aspects highlight the importance of biofilms in the marine environment.

With epifluorescence microscopy and flow cytometry, unicellular picophytoplankton (cells <3 μm; Chen et al., 2011), which includes cyanobacteria (*Prochlorococcus* and *Synechococcus*) and eukaryotic algae (picoeukaryotes) have been recognized as vital components of the phytoplankton community in oceanic (Campbell et al., 1998; Vaulot et al., 1990) and estuarine ecosystems (Ray et al., 1989; Shang et al., 2007). Their contribution to the total photosynthetic biomass is known to be significantly high in the open oceans and also in the coastal regions (Campbell et al., 1998; Morán, 2007). The higher abundance of picophytoplankton increases their surface encountering capabilities as compared to diatoms. Although, picocyanobacteria have also been observed in mats and biofilms in hot springs (Ferris et al., 1996; Miller and Castenholz, 2000; Ward et al., 1998) and hypersaline ponds (Garcia-Pichel et al., 1998), the role of picophytoplankton community in marine biofilms is not well addressed.

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In the process of biofilm formation, within minutes of immersing a clean surface in water it adsorbs a molecular 'conditioning' film consisting of organic material (Bakker et al., 2003). For some years, it was held that there was an obligatory order in the succession of organisms that followed the adsorption of the conditioning film (Marzalek et al., 1979). Bacteria were considered to be the initial colonizers (Bhosle et al., 1989; Corpe, 1970; Sieburth, 1979), followed by diatoms, other algae, and invertebrate larvae. However, some studies have shown that although the presence of the bacterial film may facilitate the attachment of diatoms, it is not a prerequisite (Cooksey et al., 1980; Horbund and Freiberger, 1970). These initial biofilm organisms are known to produce exopolymers that assist the process of adhesion and movement (Molino and Wetherbee, 2008). So, the observed pattern of biofilm community development in natural systems (bacteria preceding algae) is more likely to be a reflection of the average abundance and the availability of the different colonizing forms at the moment of immersion of a new substratum (Gawne et al., 1998; Wahl, 1989). In view of this, considering the cell size, cell availability and cell abundance, since picophytoplankton are next to bacteria, it was hypothesized that picophytoplankton are the first autotrophic colonizers in biofilms contributing significantly to the biofilm photosynthetic biomass in the initial stages thereby playing an important role in the biofilm food web dynamics and probably in the production of exopolymers similar to bacteria and diatoms (Patil and Anil, 2005c). This investigation was carried out at the Dona Paula Bay where extensive studies on biofilm diatom communities have been conducted (Mitbavkar and Anil, 2007, 2008; Patil and Anil, 2005a,b) but reports on picophytoplankton from the biofilms are lacking. The study addressed the following (1) the succession of organisms in the early phases of biofilm formation and (2) their contribution to the photosynthetic biomass so as to better understand the base of the food web in biofilms which support the higher trophic levels.

## 2. Materials and methods

### 2.1. Field experiments

#### 2.1.1. Environmental parameters

During each of the experiments, water temperature was monitored at the study site. Salinity was measured every day with an autosal. Samples (500 ml) were collected for nutrient (nitrate, phosphate and silicate) analysis.

#### 2.1.2. Experimental protocol

This study was carried out three times, (1) from 2 May 2009 to 10 May 2009, (2) 15 December 2010 to 22 December 2010 and (3) 3 May to 10 May 2011 at the Dona Paula Bay located at the mouth of the Zuari estuary, Goa, (15° 27.5′ N, 73° 48′ E), on the west coast of India. For biofilm development, 100 glass slides (7.6×2.2 cm) were fixed vertically to wooden frames and suspended at the sub-surface level (~1 m below lowest low tide level) for a maximum period of eight days. Initially after 6 h and thereafter every 24 h till the eighth day, nine slides were removed. The retrieved slides were transferred separately into 0.2 μm membrane filtered autoclaved seawater and brought to the laboratory. Each of the slides was then rinsed and scraped separately with a sterile scraper (BD Falcon™) into 15 ml of 0.2 μm membrane filtered autoclaved seawater. Out of nine scraped materials, three were fixed in paraformaldehyde (PFA; 0.2% final concentration) for picophytoplankton analysis in triplicate. These samples were then transferred to liquid nitrogen and subsequently stored at −80 °C and analyzed within a week. Other three scraped materials (triplicate) were used for *in-vivo* fluorometric measurement of chlorophyll *a* using Turner fluorometer (Triology) from total sample. Subsequently, this sample was filtered through 3 μm Millipore filter paper and the filtrate (<3 μm) was used to measure the chlorophyll *a* in order to estimate the relative contribution of picophytoplankton to total photosynthetic biomass. Total chlorophyll *a* concentration was also estimated through spectrofluorometric method from the remaining three scraped materials (Parsons et al., 1984).

### 2.2. Laboratory experiments

High frequency observations were carried out in the laboratory in order to ascertain the time of arrival of picoplankton in the marine biofilms. This experiment was conducted in June 2009, January 2011 and July 2011. Sixty glass slides were incubated in nutrient enriched seawater (f/2 media; Guillard and Rhyther, 1962) under 12 h:12 h light:dark condition at 27 °C. After incubation, three slides were retrieved at every five minute interval from the incubation tub for the first half-an-hour. For the next 1 h, three slides were retrieved after 15 min interval. Subsequently, the slides were removed after 30 min, followed by one hour and two hour intervals until the completion of 5 h incubation. The initial duration was changed to 15 min in the second experiment. This experiment duration was 48 h and that of the third experiment was 72 h. The retrieved slides were first rinsed with 0.2 μm membrane filtered autoclaved seawater so as to remove any unattached material and separately scraped in petriplates by adding 15 ml of autoclaved filtered seawater. The scraped material was preserved in PFA for picophytoplankton and heterotrophic bacterial analysis. Similarly, water samples from the incubation tub were also preserved at the same time.

### 2.3. Flow cytometric analysis

A BD FACSAria™ II flow cytometer equipped with a laser emitting at 488 nm and a 70 μm nozzle was used for picoplankton analysis. Emitted light was collected through the following set of filters: 488/10 band pass (BP) for side scatter, 575/26 BP for orange fluorescence,

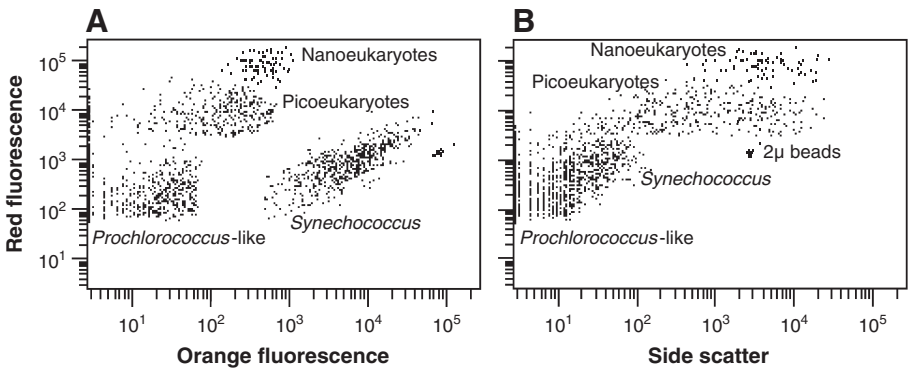


**Fig. 1.** Examples of flow cytometry cytograms. Data of samples taken from 5 day old biofilm in the field. (A) Phycoerythrin orange fluorescence vs. chlorophyll red fluorescence (B) side scatter (a proxy for cell size) vs. chlorophyll red fluorescence.