Journal of Experimental Marine Biology and Ecology 497 (2017) 86–98

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Microbial dynamics in a tropical monsoon influenced estuary: Elucidation through field observations and microcosm experiments on biofilms

Lidita Khandeparker, Niyati Hede, Ranjith Eswaran, Akshaya Usgaonkar, Arga Chandrashekar Anil*

CSIR, National Institute of Oceanography, Dona Paula, 403 004 Goa, India

ARTICLE INFO

Keywords:
Biofilms
Periphyton
Bacteria
Microcosms
Vibrio spp.
Estuary

ABSTRACT

The changes that occur in biofilms reflect the influence of the environment. In a case study, the biofilms developed along a salinity gradient from marine to freshwater in a tropical monsoon influenced estuary (Zuari, west coast of India) were evaluated through in situ observations, and effect of translocation through microcosm experiments. The bacterial abundance was tide driven and high at the mouth of the estuary, whereas periphyton biomass, in terms of chlorophyll *a* was higher in the freshwater to brackish zone. These results point out decoupling of bacteria and phototrophic organisms in estuarine early stage biofilms. *Vibrio* spp. and faecal indicator bacteria were abundant in marine and brackish biofilms. The translocation experiments revealed that at times when marine biofilms with high total bacterial abundance were transferred to either brackish or freshwater, the abundance decreased. However, a significant increase in the bacterial abundance was observed when freshwater biofilms were transferred to either brackish or marine conditions. The microcosm experiments revealed that the influence of grazers differed with the type of biofilm and played an important role in modulating the composition of biofilms. The diagnostic pigments provided considerable insights into spatial and seasonal dynamics of periphyton groups in different biofilms and were determined by the surrounding water column communities and the availability of nutrients. Decay of freshwater periphyton in biofilms when translocated to marine and brackish conditions lead to remineralisation and higher bacterial population.

1. Introduction

Biofilms are complex structured communities of autotrophic and heterotrophic organisms surrounded by the matrix of exopolysaccharides. Bacteria and diatoms are dominant components of biofilms and their interplay, influence the composition of biofilms (Khandeparker et al., 2014). The biofilm community composition is determined by its ecosystem, mainly the seasonal, and geographical variations including biogeochemical and physical interactions (Chiu et al., 2005; Lau et al., 2005; Bellou et al., 2012; Briand et al., 2012; Battin et al., 2016; Oberbeckmann et al., 2014, 2016). In coastal areas, research on the biofilm communities is vast but very little is known about biofilm communities in the estuarine environments. They are regarded as environmentally naturally stressed areas because of variability in physicochemical properties (Elliott and Quintino, 2007; Elliott and Whitfield, 2011). Many of these parameters differ over daily light and tidal cycles, rainfall events and seasons (Moss et al., 2006).

Along the subcontinent of India, majority of the peninsular rivers originating in the Western Ghats along the west coast of India experience maximal rainfall during the south-west monsoon. During this period, freshwater influx from land runoff and precipitation is added to the estuary resulting in large fluctuations in the physicochemical characteristics of the water (Devassy and Goes, 1988). The physical processes in some estuarine environments differ significantly between the monsoon season, when runoff is high, and the dry season when runoff is negligible, besides the tide dominates circulation and mixing in the estuaries. Therefore, such estuaries are referred to as monsoonal estuaries (Shetye et al., 2007). They are highly unique and do not reach steady state at any time when compared to the temperate estuarine systems (Vijith et al., 2009). Zuari is one of the tropical monsoon influenced estuaries of Goa located along the central west coast, where runoff, rainfall, tides and salinity play an important role in determining its environment in comparison to the other estuaries in different parts of the world (Vijith et al., 2009).

Pollution of coastal waters due to anthropogenic activity has been a global issue (Nogales et al., 2011; Malham et al., 2014). The bacterial load, especially potential pathogens in the coastal waters are generally higher than the open sea (Brettar et al., 2007). Monitoring tropical

* Corresponding author.
E-mail address: acanil@nio.org (A.C. Anil).

http://dx.doi.org/10.1016/j.jembe.2017.09.014
Received 6 June 2017; Accepted 23 September 2017
Available online 30 September 2017
0022-0981/

monsoon influenced environment for pathogenic bacteria and sewage indicators have focused on the water column (Nagvenkar and Ramaiah, 2009; Rodrigues et al., 2011; Khandeparker et al., 2015). Biofilms on submerged surfaces also act as a reservoir for pathogens such as *Escherichia coli* and *Vibrio cholerae* (Shikuma and Hadfield, 2010). Studies relevant to bacterial pathogens associated with biofilms in estuaries is least studied except for a recent work (Khandeparker et al., 2014), which addressed their importance in marine biofilms.

Estuaries are dynamic environments, with a regular physical exchange of water with different properties. In this context, floating or moving objects experience different environmental ambience. Biofilms in such environments will also experience such a change and insights to such influence provide an opportunity to study the response of different biofilm communities. The response studies are sometimes difficult to evaluate by only using field based studies (Sabater and Borrego, 2015). The use of microcosms allows for investigation of the responses of the biofilms to specific environmental conditions such as salinity, nutrients under controlled conditions when compared to in situ observations (Craig et al., 2004; Kästner and Richnow, 2010). Other than environmental variables, protozoan grazing is also one of the important factors in controlling and altering the biofilm composition in aquatic ecosystems, which can influence marine invertebrate larval settlement (Pederson, 1990; Skov et al., 2010; Shimeta et al., 2012). It is often prey-selective, wherein the protozoans exhibit species-specific responses and thus significantly alter the biofilm population dynamics (Huws et al., 2005; Pernthaler, 2005; Dopheide et al., 2011; Yang et al., 2013). Protozoans have been demonstrated as important components of biofilms, which can rapidly colonise new substrata and occur in high abundances on biofilms (Arndt et al., 2003).

Taking the above points into consideration, we examined (1) impact of monsoon influenced estuarine conditions on the microbial dynamics of biofilms along a salinity gradient, (2) the response of biofilms developed under specific habitats to changes in the environmental conditions (survival in marine, brackish and freshwater habitats), and inoculum therein through laboratory microcosm experiments. The influence of grazing was indirectly evaluated by filtering the water used for translocation, which enabled removal of fresh inoculum and unfiltered water provided fresh inoculum and grazers.

2. Materials and methods

2.1. Description of study sites

This study was carried out in the Zuari estuary (15°27.5′ N, 73°48′ E) located in Goa, west coast of India (Fig. 1). The main channel of the Zuari estuary is about 50 km long and 5 km wide with a depth of ~5 m. The cross section area decreases towards the riverine end (0.5 km) (Shetye et al., 1995; Sundar and Shetye, 2005). Many streams and rivers are freshwater sources to this estuary (Shetye et al., 1995). During the monsoon season (June to September) freshwater influence is comparatively higher than the summer season (March to May), wherein there is less river discharge and is vertically mixed throughout the estuary till riverine region (Shetye et al., 1995). In this study, the stations were chosen along the estuary experiencing different tidal influence. Tides in this estuary are of the mixed semidiurnal type normally occurring twice a day and tide ranges are approximately 2.3 and 1.5 m during the spring and neap tide respectively (Shetye et al., 2007). The tidal influence is more at the mouth of the estuary when compared to the upstream end of the estuary. These tides as well as monsoon runoff are responsible for the mixing and circulation of water and hence are seasonal in nature (Shetye et al., 2007). Based on physico-chemical characteristics, three different stations were selected for developing biofilms, which are (1) Dona Paula (15°27.5′ N, 73°48′ E) which is located at the mouth of the estuary with salinity ranging from 15 to 35 psu representing marine condition. (2) Cortalim (15°24′32.0′ N, 73°54′50.2′ E) located at 13.7 km from the mouth of the estuary with salinity ranging from 2 to 32 psu representing brackish condition and (3) Sanvordem (15°16′01.1′ N, 74°06′36.0′ E) station which, is located at the upstream of the estuary with 0 psu salinity representing freshwater condition. These stations are hereafter referred as marine (Dona Paula), brackish (Cortalim) and freshwater (Sanvordem) stations.

2.2. Simultaneous sampling of biofilms from the study sites

Before deployment, glass slides (25.4 mm × 76.2 mm) were cleaned as described by Bhosle et al. (2005). For each season, the treated glass slides were deployed at marine, brackish and freshwater stations simultaneously during spring (n = 210) and neap tide (n = 210), for the development of marine, brackish and freshwater biofilms for 24 h. The slides were suspended from trawlers that were anchored midstream in the Zuari estuary at each station. These stations were sampled simultaneously for physicochemical parameters and biofilm samples twice during the premonsoon (PreM, 19th May 2011 and 27th April 2011) followed by monsoon (MoN, 1st August 2011 and 9th August 2011) and post monsoon season (PostM, 29th October 2011 and 23rd October 2011) over spring and neap cycles respectively. For control samples, set of thirty slides were collected for microbiological analysis during each season and tide. Three slides were sampled (n = 1 × 3) and each one was scraped separately into 10 ml of 0.22 μm filtered autoclaved seawater for microbial counts. 1 ml of subsample was used for the analysis of viable and pathogenic bacterial abundance while 5 ml of subsample was fixed with formalin for enumeration of total bacterial counts. For pigment analysis, twenty seven slides were sampled (n = 9 × 3) and nine slides were scraped separately in 25 ml filtered seawater. The remaining six sets of thirty slides (n = 180) with the biofilms developed at different stations were used to carry out microcosm experiments as described in Section 2.4.

2.3. Physicochemical parameters

All the physicochemical parameters (temperature, salinity, and dissolved nutrients) were collected in duplicates each time (seasons and tides) during the sampling period. Water temperature and salinity were recorded at the sampling stations by using conductivity-temperature-depth (CTD) probe (Sea bird, USA). Tide and flow were recorded using a current meter. Nutrients in the water column, namely nitrate (NO_3), nitrite (NO_2), phosphate (PO_4) and silicate (SiO_4) were analysed using autoanalyser (Skalar SAN PLUS 8505 Interface v3.31, Netherland). The nutrient samples were analysed within 30 days of sampling date.

2.4. Microcosm experiments

The microcosm experiments were carried out to assess the response of biofilm communities to changes in the environmental conditions. The experimental protocol used for performing the microcosm experiment relied on an earlier investigation used to study bacteria-diatom interactions using an antibiotic approach (Khandeparker et al., 2014). Briefly, biofilms were developed at three different stations for 24 h in the field. After this, one set of slides (n = 30) from each station was kept as control as described above in Section 2.2 and remaining six sets of thirty slides (n = 180) were translocated to the thirty six microcosm units (5 slides per microcosm) in the laboratory, containing unfiltered and filtered water from (marine/brackish/freshwater) stations. For example, the slides with marine biofilms were translocated to unfiltered marine (n = 30), brackish (n = 30), freshwater (n = 30), and filtered (0.22 μ pore size filters) marine (n = 30), brackish (n = 30) and freshwater (n = 30). Experiments with filtered seawater enabled the removal of fresh inoculum and grazers whereas unfiltered seawater conditions provided fresh inoculum and grazers. The glass slides were placed in each of the sterile polycarbonate square jars (75 × 74 × 138 mm, 450 ml, Himedia). All the jars (or microcosms) were closed and static during the incubation. The experimental units