Marine Pollution Bulletin 100 (2015) 710–718

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

Variations in the abundance and structural diversity of microbes forming biofilms in a thermally stressed coral reef system

Huda Mahmoud

Faculty of Science, Department of Biological Sciences, Kuwait University, P.O. Box (5969), Safat, Kuwait

ARTICLE INFO

Article history:
Received 20 January 2015
Received in revised form 10 October 2015
Accepted 16 October 2015
Available online 19 October 2015

Keywords:
Thermally stressed coral reefs
Arabian Gulf
Microbial succession
Microbial biofilms

ABSTRACT

Little information is known about biofilm formation in the thermally stressed coral reef systems north of the Arabian Gulf. The current study investigates the abundance and diversity of marine microbes involved in biofilm formation and their succession over a period of 14 weeks (May–August 2007) at temperatures exceeding 32 °C. The results showed variations in microbial numbers and the development of more stable biofilm communities as the biofilms aged. The culture-dependent technique and microscopic examination of the developed biofilms showed the dominance of key species known for their role in precipitating $CaCO_3$ such as *Vibrio* and in facilitating coral larvae settlement and metamorphosis such as *Pseudoalteromonas*, Bacillariophyceae and Rhodophyceae. The results revealed biofilm formations with microbial diversities that have the potential to support the larval settlement and metamorphism of marine organisms and to consolidate and stabilize biofilms via the process of calcification in the thermally stressed coral reef system considered herein.

1. Introduction

Microorganisms in marine environments inhabit animate and inanimate surfaces and form biofilms (Sutherland, 2001; Dunne, 2002). The formation of biofilms involves adsorption of organic and inorganic molecules to submerged surfaces prior to surface conditioning (Buscher and Mei, 2000) via the colonization of bacteria and microscopic eukaryotes (Dobretsov et al., 2006). This type of colonization may not be entirely random, in that specific bacterial species may have greater colonization abilities (Jackson et al., 2001). Although the role of the first colonizers is very important because they enable the resulting biofilm to remain intact or to detach as a result of shearing forces (Busscher et al., 1995), continuous growth of the biofilm results in a complex architecture that contains different microhabitats and, thus, facilitates the growth of a diverse microbial community and the settlement and subsequent growth of invertebrate larvae (Jackson et al., 2001; Dobretsov et al., 2006).

Studies have demonstrated the advantages of living in an aquatic biofilm, which provides its inhabitant with access to nutrients and protection against many external factors, including toxins and heavy metals. In addition, biofilms provide protection against dehydration and UV-radiation (Sutherland, 2001). The roles of microbial biofilms in the settlement and metamorphosis of invertebrate larvae are also well documented (see review by Wieczorek and Todd, 1998). It has been fully established that biofilms harbour bacteria and algae responsible for the production of molecular signals that can control the settlement and metamorphosis of coral larvae (Webster et al., 2004). For instance, Gamma-Proteobacteria isolated from biofilms have been found to produce chemical cues that facilitate induction in coral larvae (Webster et al., 2004), while crustose algae are able to produce or harbour bacterial biofilms that have the ability to produce morphogens (Negri et al., 2001). In addition, crustose coralline algae are able to precipitate calcium carbonate, which has implications for reef stability (Diaz-Pulido et al., 2014). Calcite precipitation could also occur via marine bacterioplankton in the vicinities of coral reef systems (Morita, 1980). Calcification, which, in this case, is a biogenic process in which precipitated crystals are used as solid substrates for attachment or protection against certain environmental conditions and predation (Lian et al., 2006), occurs widely throughout both heterotrophic and phototrophic microbial communities (Morita, 1980; Couradeau et al., 2013).

Successions of microbes in marine biofilms have been well described (Dang and Lovell, 2000; Webster et al., 2004; Lee et al., 2008; Sweet et al., 2011), and many studies have investigated this phenomenon in biofilms from tropical reef systems (Webster et al., 2004; Sweet et al., 2011; Sawall et al., 2012; Witt et al., 2012). Studies have shown that biofilm communities can be altered as a result of biotic and abiotic factors (Stoodley et al., 1999; Dahms and Qian, 2005). Due to recent concerns regarding global warming, researchers are particularly interested in understanding how increases in surface water temperatures may affect the structures and functions of biofilms in coral reef systems (Webster et al., 2011; Whalan and Webster, 2014). Increases in sea surface temperatures can alter the distribution, abundance, function and community dynamics of marine microbial systems (Webster and Hill, 2007; Rao, 2010) by positively or negatively affecting the biofilm

E-mail address: bsm8ham@yahoo.co.uk.

http://dx.doi.org/10.1016/j.marpolbul.2015.10.030
0025-326X/

recruitment and inductive capabilities of coral and other marine invertebrate larvae (Webster et al., 2011; Whalan and Webster, 2014). Webster et al. (2011) reported that maintaining the temperature at 32 °C for 7 days diminished the inductive capacities and, therefore, reduced the coral recruitment capabilities of crustose coralline algae (CCA) and associated bacterial biofilms. This high temperature caused a shift in the CCA microbial community from an Alpha-proteobacteria-dominant to a Bacteroidetes-dominant community. On the other hand, the findings of Whalan and Webster (2014) suggested that the most successful larval settlement for the sponge *Rhopaloeids odorabile* occurred in a biofilm developed over 10 days at 30–34 °C, suggesting that responses to elevated high temperatures may be taxa-specific. The findings of Lau et al. (2005) and Chiu et al. (2006) supported previous reports that biofilms developed under high temperatures (30 °C) induced higher rates of larval metamorphosis than biofilms developed at lower temperatures. All of these studies were conducted under controlled laboratory conditions and reported shifts in biofilm microbial community structures after incubating the respective biofilms at elevated water temperatures for specified periods of time.

The Arabian Gulf, one of the hottest bodies of water on the earth, is characterized by the highest variabilities in annual temperatures (Kinsman, 1964; Sheppard et al., 1992). Corals in this system are thriving under conditions close to their thermal distribution limits (Baker et al., 2008). Corals suffer dramatically from thermal stress when water temperatures exceed 32 °C or drop below 18 °C (Jokiel and Coles, 1977; Glynn and D'croz, 1990). However, Gulf corals are able to survive extreme temperature fluctuations exceeding 35 °C in summer and falling below 11 °C in winter (Riegl and Purkis, 2012; Coles and Fadlallah, 1991; Spalding et al., 2001). The uniqueness of the environmental conditions provides a landscape that investigates the mechanisms that corals use in this system under conditions of elevated temperatures. This will provide important information in managing coral reefs in a changing climate world. Notably, the coral-spawning season north of the Arabian Gulf occurs between May and August, exposing the larvae produced to high thermal stress. Given that microbial biofilms provide the necessary cues for larvae to settle and metamorphose, it is important to understand how biofilms develop under such harsh conditions. Thus far, all studies regarding the succession of microbes in coral reef systems have investigated biofilms that develop in tropical reefs. Thus, despite our need to develop models to enable us to understand how these animals thrive under thermal stress, no information is available regarding the succession of microbial biofilms in the thermally stressed coral reef systems of the Arabian Gulf. Although a few studies from this region have investigated microbial biofilms developed on natural substrata from coastal areas north of the Arabian Gulf (Mahmoud et al., 2010; Radwan et al., 2011), these studies fail to report any data concerning succession, making it difficult to ascertain what happens to these biofilms under conditions of naturally elevated water temperatures.

In the present study, we investigated succession in microbial biofilms in a coral reef system north of the Arabian Gulf under natural conditions of elevated sea temperatures reaching 33 °C. Thus, the current study is critical with regard to understanding one of the most important stages in coral survival at high temperatures. Therefore, the goals of this study were to investigate the effects of temperature and age, under natural conditions, on biofilm composition and to further investigate the abilities of the most dominant bacterial species to consolidate and stabilize biofilms via the process of calcification.

2. Materials and methods

2.1. Sampling site

The experiment was conducted in the inshore reef system of Qit'at Benayah (Fig. 1A), north of the Arabian Gulf [N28 37.021 E48 25.702]. Qit'at Benayah is an oval-shaped reef 196 m long and 108 m wide, with a circumference of 530 m. The depth of its seabed at low tide varies from approximately 5 m at the east end to 4.5 m at the west and south ends to approximately 3.6 m at the north end. The highest vertex in Qit'at Benayah is approximately 4 m above the seabed. The highest underwater point is approximately 1 m, and, at low tide in some areas, the coral reef reaches approximately 30 cm under the water surface. Complete records of the Qit'at Benayah coral species and their spawning behaviours are available (Carpenter et al., 1997). In addition, information is available regarding the microbial groups associated with some of the corals that dominate this system. *Porites horrisoni*, *Platygyra daedalea*, *Cyphastrea serailia* and *Acropora downingi* mucus samples were all rich in Gamma-Proteobacteria, Alpha-Proteobacteria, Firmicutes, Flavobacteria and Actinobacteria (Ashkanani, 2008).

2.2. Producing the microbial biofilms

Glass slides (90 × 50 × 2 mm, LXWXD) were deployed for three months during the spawning season of the corals considered herein. The substrata were deployed east of Qit'at Benayah on 14 May, 2007, where they were fitted onto stands made of anti-corrosion stainless steel and designed with dimensions and geometric angles that facilitate the formation of biofilms while minimizing the risks of losing these films to strong currents and/or protecting the films from being blanketed by precipitated particles (Fig. 1B).

Replicas of the deployed substrata (n = 5) were recovered 1, 2, 4, 6, 8, 10, 12 and 14 weeks after deployment. Substrata were collected via

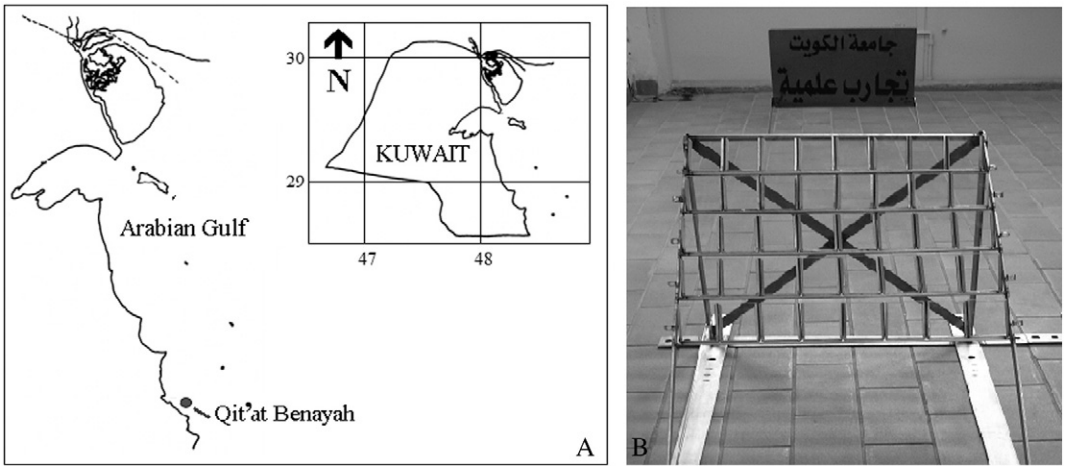

Fig. 1. A) Kuwait map showing the position of Qit'at Benayah inshore reef B) anticorrosion metal stand designed to contain glass substrata in designed pockets.