Marine Genomics 29 (2016) 69–80

Contents lists available at ScienceDirect

Marine Genomics

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

Comparison of archaeal and bacterial communities in two sponge species and seawater from an Indonesian coral reef environment

Ana Rita Moura Polónia [a,*], Daniel Francis Richard Cleary [a], Rossana Freitas [a], Francisco José Riso da Costa Coelho [a], Nicole Joy de Voogd [b], Newton Carlos Marcial Gomes [a]

[a] Department of Biology, CESAM, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal
[b] Naturalis Biodiversity Center, P.O. Box 9517, 2300 RA Leiden, The Netherlands

ARTICLE INFO

Article history:
Received 15 January 2016
Received in revised form 28 April 2016
Accepted 28 April 2016
Available online 22 May 2016

Keywords:
16S rRNA gene
Pyrosequencing
Procrustes
Stylissa carteri
Stylissa massa
Berau reef system

ABSTRACT

Most existing coral reef studies have focused on a single biotope and a single domain (Archaea or Bacteria). Few coral reef studies have explored the archaeal and bacterial community simultaneously. In this study, we compare the diversity and composition of archaeal and bacterial communities in seawater and two closely related sponge species (*Stylissa carteri* and *Stylissa massa*) in the Berau reef system, Indonesia. A 16S rRNA gene barcoded pyrosequencing approach was used to test to what extent seawater, *S. carteri* and *S. massa* host compositionally distinct communities of Archaea and Bacteria. Proteobacteria dominated the bacterial communities of all three studied biotopes whereas Euryarchaeota was the most abundant archaeal phylum in seawater and Crenarchaeota the most abundant archaeal phylum in both *Stylissa* species. Biotopes explained 56% and 53% of the variation in archaeal and bacterial composition respectively and there was significant congruence between the composition of archaeal and bacterial communities. These results suggest that the processes that drive bacterial composition within the studied biotopes may be fundamentally similar to those that drive archaeal composition.

1. Introduction

Coral reefs provide habitat for a highly diverse community of organisms, including a diverse microbial community. These microbial communities can be planktonic (living in the water column), benthic (living in sediment) or symbiotic (living in hosts such as sponges or corals). All of these biotopes have specific characteristics and properties, which may influence their microbial communities. Seawater, for example, is a nutrient-irregular habitat subjected to environmental fluctuations such as upwelling and currents (physical mixing) while marine sponges are a relatively stable, nutrient-rich habitat (Hentschel et al., 2012) protected, more or less, from environmental fluctuations. Although different, these two habitats are connected through the pumping activity of marine sponges. Coral reef sponges have been shown to harbor exceptional microbial densities that contribute to the sponge metabolism (carbon and nitrogen nutrition) and chemical defense (Hentschel et al., 2006; Taylor et al., 2007). However, the abundance of sponge microbial communities varies from species to species (Kamke et al., 2010) and seems, at least in part, to be related to their life strategies (Polónia et al., 2014). Low microbial abundance sponges (LMA) have a microbial concentration close to that of seawater (10^5–10^6 bacteria/g of tissue; Hentschel et al., 2006) and rely on their high pumping rates to acquire energy (Weisz et al., 2007) while high microbial abundance (HMA) sponges have lower pumping rates but higher microbial concentrations (around 10^8–10^{10} bacteria/g of tissue; Hentschel et al., 2006) on which they rely to acquire energy (Weisz et al., 2007). According to some studies (Moitinho-Silva et al., 2013; Thacker and Freeman 2012), LMA sponges host microbial communities with low diversity and specificity and compositionally similar to bacterioplankton communities. Previous studies (Bayer et al., 2014; Blanquer et al., 2013; Polónia et al., 2013, 2014) have shown considerable overlap in terms of spatial ordinations and abundant OTUs between microbial communities of LMA sponges and seawater.

Some sponge species are dominated by Archaea (e.g., *Dragmacidon mexicanum*; Preston et al., 1996 and *Inflatella pellicula*; Jackson et al., 2013) while others are dominated by bacteria (e.g., *Cymbastela concentrica*; Thomas et al., 2010). Most sponge studies have, however, focused on a single domain (Archaea or Bacteria) and a single biotope. Although there have been an increasing number of studies that have compared archaeal and bacterial communities in recent years (Bayer et al. 2008; Easson and Thacker, 2014; Hardoim and Costa, 2014; Hoffmann et al. 2009; Lee et al. 2011), there is still a scarcity of comparative studies of both prokaryotic domains in different habitats.

* Corresponding author at: CESAM and Department of Biology, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal.
E-mail addresses: ritapolonia@ua.pt, ritapolonia@gmail.com (A.R.M. Polónia), cleary@ua.pt (D.F.R. Cleary), rossana.freitas@ua.pt (R. Freitas), franciscorcoelho@ua.pt (F.J.R.C. Coelho), nicole.devoogd@naturalis.nl (N.J. de Voogd), gomesncm@ua.pt (N.C.M. Gomes).

http://dx.doi.org/10.1016/j.margen.2016.04.014
1874-7787/

Here, we investigated communities of Archaea and Bacteria in three distinct biotopes (two host biotopes and one non-host biotope), namely, the closely related LMA sponge species *Stylissa carteri* and *Stylissa massa* and seawater in the Berau reef system, Indonesia. Our main goals were to (1) study the effect of different biotopes on higher taxon abundance and diversity of prokaryote communities, (2) compare archaeal and bacterial community structure patterns among biotopes and (3) test the concordance in composition between archaeal and bacterial communities.

2. Material and methods

2.1. Study site

Sampling for the present study took place in the barrier reef system of Berau, East Kalimantan, Indonesia (E 118° 04′ 37.6″–E 118° 36′ 17.5″; N 02° 08′ 08.7″–N 02° 17′ 31.9″—Fig. 1). The barrier reef system is located eastward of the delta front of the Berau River basin and extends to the offshore islands of Kakaban and Maratua and the oceanic reefs that border the Makassar Strait (de Voogd et al., 2009; Tomascik et al., 1997). The coral reefs located in this area are influenced by the Berau River, which is amplified during the rainy season when river plumes can extend 15 to 30 km offshore. The inshore reefs are dominated by a diverse community of filter feeders such as soft corals and sponges while the barrier reefs are dominated by corals (de Voogd et al., 2009; Renema, 2006).

2.2. Sampling

The sponge species *Stylissa carteri* and *Stylissa massa* are considered LMA sponges and are fast growers with a loose collagen-rich skeleton containing relatively large spicules (Van Soest et al., 2002). *Stylissa massa* is a medium-sized orange colored sponge that only occurs in very shallow water (0.5–3 m) while *S. carteri* is a red-orange flabellate sponge that can occur between 3 and 35 m depth.

Four *S. massa* and seawater samples and five *S. carteri* samples were collected at six different reef sites along an on-to-offshore gradient surveyed using SCUBA diving or snorkeling in August 2012 (Fig. 1). The closest reef to the river Berau is a shallow submerged reef (reef top at 5 m depth) with a lighthouse on top of it (RvL); here a single individual of *S. carteri* (Sc01–12 m depth) and a seawater sample were collected (Wt01). At the western side of the uninhabited island of Samama (SmW), one individual of *S. carteri* (Sc02–10 m depth) and a seawater sample (Wt02) were collected; at the eastern side (SmE) two individuals of *S. massa* (Sm01 and Sm02 - <2 m depth) and a single *S. carteri* (Sc03–13 m depth) sample were collected. At the reef of the island of Sangalaki (SgE) one *S. carteri* (Sc04–12 m depth) and one seawater sample (Wt03) were collected. At the southern part of the Maratua atoll, one individual of *S. carteri* (Sc05–25 m depth) and one seawater sample (Wt04) were collected near the island of Nunukan (MrN). During snorkeling inside the large lagoon of Maratua and near the small resort island of Nabucco (MrNA) two individuals of *S. massa* were collected (Sm03 and Sm04 - <2 m depth).

Surface seawater samples were collected by filtering 1 l (Bowen et al., 2012) of water through a Millipore® White Isopore Membrane Filter (0.22 μm pore size). Cores of *S. massa* and *S. carteri* were sampled including segments of surface and interior in order to sample, as much as possible, the whole archaeal and bacterial community (Cleary et al., 2013; Polónia et al., 2014). All samples were stored in 96% EtOH (Cleary et al., 2013) and kept at temperatures lower than 4 °C immediately after collection. Once in the laboratory, samples were stored at −20 °C until DNA extraction.

2.3. DNA extraction and pyrosequencing

We isolated PCR-ready genomic DNA from *S. massa*, *S. carteri* and seawater using the FastDNA® SPIN Kit (MPbiomedicals) following manufacturer's instructions. This is an extraction method frequently used for this purpose (Cleary et al., 2013; Polónia et al., 2014). Briefly, 500 mg of *S. massa* and *S. carteri* sponges and the whole membrane filter were

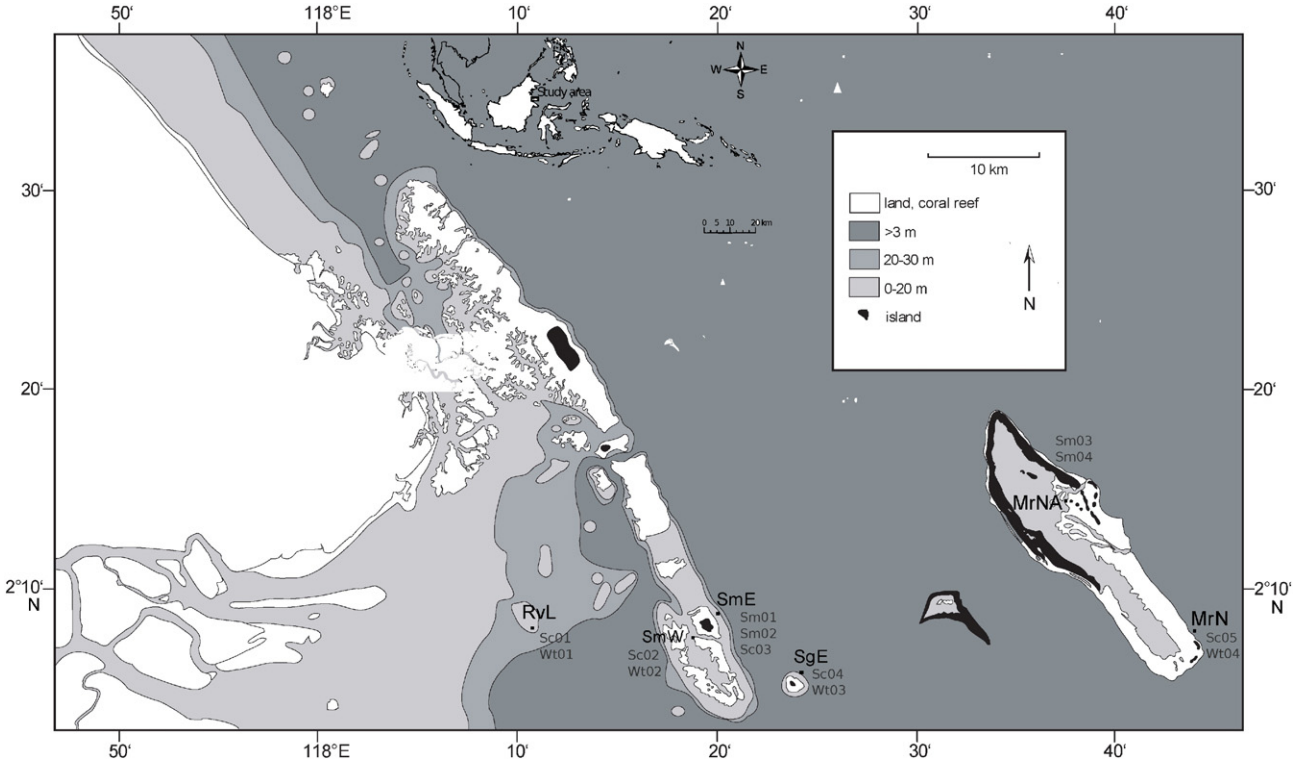

Fig. 1. Map of the study area (Berau Reef System) showing the study sites. River Berau-Lighthouse (RvL); Samama West (SmW); Samama East (SmE); Sangalaki East (SgE); Nunukan (MrN); Lagoon of Maratua-Nabucco (MrNA). The inset in the top shows the location of the Berau Reef System in Southeast Asia—Indonesia.