Marine Pollution Bulletin xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

Molecular phylogeny and community fingerprinting of coral-associated *Symbiodinium* north of the Arabian Gulf

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ARTICLE INFO

Article history:
Received 18 January 2015
Received in revised form 17 April 2016
Accepted 25 April 2016
Available online xxxx

Keywords:
Symbiodinium
North Arabian Gulf corals
Temporal variation
Spatial variation
RFLP
DGGE

ABSTRACT

Understanding coral–*Symbiodinium* relationships including changes in the genotypes and the numbers of *Symbiodinium* can explain the ability of Kuwait coral to survive high fluctuations in water temperature. In the current study, the diversity of *Symbiodinium* associated with fourteen coral species from six reef systems south of Kuwait was investigated. The results proved the predominance of clade C members in all corals tested, which reflects the importance of this type in helping corals thrive in the Gulf's harsh conditions. *Platygyra daedalea* was the only coral found that harbored clades A, B and C in their tissue but it is the most vulnerable coral for bleaching. The total number of *Symbiodinium*-like cells in the seawater was 10^4 cell ml^{-1} while in coral tissue and mucus 10^7 cell g^{-1} and 10^7 cell ml^{-1} were found, respectively, and a strong positive correlation with the seawater temperature, salinity and conductivity was found.

1. Introduction

Coral reefs are usually surrounded by oligotrophic, nutrient poor, water systems where productivity rarely exceeds 0.01 g of fixed carbon m^{-2} day^{-1} (Hatcher, 1988). However, productivity within coral tissue can reach 40 g of fixed carbon m^{-2} day^{-1} (Hatcher, 1988; Hoegh-Guldberg, 1999). This makes coral reefs one of the most productive systems on Earth, due to the unique relationship between the corals and their algal symbionts, commonly known as zooxanthellae, which are responsible for 50–70% of the total primary production in most reefs (D'Angelo and Wiedenmann, 2014). The zooxanthellae are unicellular dinoflagellates from the genus *Symbiodinium* (Rowan and Powers, 1991a; Baker, 1999; Carlos et al., 1999; Goulet, 2006) and provide the coral host with up to 95% of their own metabolites such as glycerol, glucose, alanine and organic acids necessary for coral productivity and growth (Rowan and Powers, 1991a, 1991b; Pochon et al., 2004). In return, the coral provides the *Symbiodinium* with urea, amino acids, glycerophosphate and CO_2 needed by the algae for photosynthesis (Hackett et al., 2004). Furthermore, the hard coral structure provides the endosymbionts with protection against grazing and sedimentation, while the mucus production and sloughing process carried by the coral themselves prevent sediments from accumulating on the coral surface, hence hindering light from reaching the endosymbionts (Rogers, 1990). Historically, the symbiotic dinoflagellates in corals probably evolved from nonsymbiotic dinoflagellates (Rowan, 1998). The symbiotic relationship starts when the newly formed coral colony acquires the photosynthetic symbiont from the surrounding environment or from the eggs, depending entirely on the reproduction mode of each coral species (Thornhill et al., 2006).

Recent studies have shown that five genetic clades of *Symbiodinium* are associated with corals: A, B, C, D and G (Stat et al., 2008). Baker and Romanski (2007) reported that more than 50% of scleractinian corals (250 species) can host more than one *Symbiodinium* clade and that diverse symbionts can be found within the same clade; this is contrary to the conclusion of Goulet (2006) who suggested that 77% of the 442 scleractinian and octocorals species host a single *Symbiodinium* clade and only 23% host multiple clades. The findings of Baker and Romanski (2007) are widely accepted, and it is believed that the corals harboring multiple clades can change the symbiont type according to the prevailing conditions. Different environmental factors both regulate the distribution and determine the type of *Symbiodinium* clades inhabiting the coral tissue (Van Oppen et al., 2001; Goulet and Coffroth, 2003). Therefore, sclerectinian corals can flexibly associate with assorted symbionts with diverse physiologies which can confer better resistance to extreme environmental conditions (Baker, 2003; Baker et al., 2004; Berkelmans and Van Oppen, 2006).

In this context, Rowan and Knowlton (1995) reported that in the same *Montastraea annularis* individual, variation in the distribution of *Symbiodinium* was linked primarily to light exposure. The tissue exposed to high light intensity harbored clade A and B, while coral tissue exposed to lower light intensity harbored clade C. *Symbiodinium* clade

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http://dx.doi.org/10.1016/j.marpolbul.2016.04.053
0025-326X/

D has been linked to temperature conditions and is known to be thermotolerant (Toller et al., 2001; Rowan, 2004) with the ability to tolerate a water temperature variation from 14 to 34 °C (Mostafavi et al., 2007). Mostafavi et al. (2007) also reported that clade D was predominant in *Acropora clathrata*, *Cyphastrea microphthalma*, *Favia pallida*, *Tubinaria reniformis*, *Pavona decussata*, *Platygyra daedalea*, and *Psammocora contigua*, sampled from the coasts of Iran, north of the Arabian Gulf, while clade C was found only in *Porites compressa* and *P. contigua*.

It is well documented that the Arabian Gulf is one of the hottest water bodies on Earth (Riegl and Purkis, 2012; Hume et al., 2015; Bento et al., 2016). Coral reefs found in the Arabian Gulf are regularly exposed to large variations in water temperature, ranging from 11 °C to 37 °C (Coles and Riegl, 2012). There have been some key research results concerning adaptation strategies associated with coral reefs in the warm waters of the Arabian Gulf (see Riegl and Purkis, 2012). Coral studies from Saudi Arabia (Baker et al., 2004), Iran (Mostafavi et al., 2007; Shahhosseiny et al., 2011) and Abu Dhabi (Hume et al., 2013) suggest that *Symbiodinium* clades A, C and D are present in the Gulf corals. While Baker et al. (2004), Mostafavi et al. (2007) and Shahhosseiny et al. (2011) showed the predominance of the thermotolerant clade D in the corals they investigated, Hume et al. (2013) showed the predominance of clade C3 in 7 thermotolerant reef builders in Abu Dhabi corals. Hume et al. (2013) suggested that clade D may be partially responsible for the resilience of the Gulf corals. Recently, *Symbiodinium thermophilum* clade C3 was identified as the most prevalent clade found in Abu Dhabi corals (Hume et al., 2015).

So far, all of the studies done on the Gulf coral *Symbiodinium* were conducted either south of the Gulf or north of the Gulf near the coast of Iran; there are no regional-based studies on the Kuwaiti Reefs, the most northern coral reefs in the Arabian Gulf. No information is available about the type of *Symbiodinium* that is present in these coral reefs, which regularly experience temperatures above 30 °C. Coral reefs are found south of Kuwait across an inshore to offshore gradient. Inshore coral reefs are relatively small in size and occur in shallow waters of depths between 3 and 8 m, while offshore coral reefs can be found at depths reaching 30 m. Corals in Kuwait reefs are exposed to unique conditions; they experience one of the highest annual temperature variations with temperatures of more than 36 °C in the summer (Al-Yamani et al., 2004) and low temperatures of 9 °C in the winter (Spalding et al., 2001). Studies performed on corals from other parts of the world have shown that no other coral system on Earth could survive the prolonged high temperatures that the Kuwaiti and the Gulf corals experience regularly. Reports showed that the Great Barrier Reef corals bleached when water temperature exceeded a 10 day threshold of 30.8 °C (Berkelmans et al., 2004), while Caribbean corals (for example, *Montastrea annulari*) bleached when the temperature exceeded 32 °C (Fitt and Warner, 1995). Therefore, understanding the coral–*Symbiodinium* relationship may be useful to further clarify how Kuwait corals can adapt to the high variation in water temperature. The current study provides information about *Symbiodinium* diversity and abundance in Kuwait corals, and the results show both the predominance of clade C in 14 coral species sampled from inshore and offshore reef systems south of Kuwait and spatial and temporal variation in the *Symbiodinium* population.

2. Materials and methods

2.1. Sampling sites and sample collection

Samples were collected from three offshore and three inshore reef systems (Fig. 1) on April, May, July and October 2008. The samples were utilized to investigate spatial and temporal variation in *Symbiodinium* diversity and abundance. All reef systems investigated were located south of Kuwait, and in total, 14 different coral species were investigated (Table 1). Three different coral colonies of each coral species were sampled in triplicate from various parts of the colony. From each coral colony one-centimeter size coral nubbins were collected underwater in sterile plastic bags and coral mucus was harvested in the field using sterile 20 ml sterile syringes (Cervino et al., 2008). Seawater samples from 1 m away from the sampled coral colonies were collected in 1 l sterile polyethylene bottles to investigate the free-living *Symbiodinium*. All samples were packed on ice and returned to the laboratory for immediate processing. At all sites, water temperature, pH, salinity, turbidity and conductivity were measured using a Multi-Parameter Water Quality Checker (Horiba, USA) (Table S1, Supplementary data).

2.2. Sample processing

The seawater samples were filtered through a 0.45 μm membrane filter (Whatman, England). The filter was cut with a sterile blade, and the pieces were transferred to sterile 15 ml tubes containing 2 ml of sterile phosphate buffer saline (PBS; 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, 0.24 g KH_2PO_4 in 1 l of water, pH adjusted to 7.4, Sambrook et al., 1989) before storing at −20 °C for molecular analysis. In addition, 10 ml aliquots of fresh water samples were fixed in 2% *v*/*v* formaldehyde and stored at 4 °C for a period of 1 day to 1 week before being examined under an epifluorescent microscope in order to determine the abundance of the *Symbiodinium*-like cells (Mahmoud et al., 2005). Each coral nubbin was washed in a sterile bag with 10 ml of sterile 3% NaCl water and vigorously shaken for 5 min to release the epiphytic zooxanthellae. The nubbins were then weighed and turned into a slurry using a sterile mortar and pestle (Thurber et al., 2009). Five milliliters of the coral slurry, now treated as a tissue sample throughout the study, was fixed with 2% *v*/*v* formaldehyde and used for a direct count. An additional 5 ml of the suspension was pipetted into 5 sterile Eppendorf tubes containing 0.5 ml aliquots of sterile PBS and stored at −20 °C for molecular analysis (Al-Sarraf, 2009).

Mucus samples were carefully injected into 15 ml sterile tubes and separated from the water by centrifugation at 4000 rpm for 5 min. The mucus volume was estimated, and 10 ml of sterile 3% NaCl water was added to the mucus followed by vigorous shaking to form a homogeneous suspension. Each 1 ml of the suspension was transferred into 8 Eppendorf tubes containing 0.5 ml PBS and stored at −20 °C for later use in molecular analysis. Another 1 ml of the suspension was transferred to 2 Eppendorf tubes containing 2% *v*/*v* formaldehyde and examined under the epiflourescent microscope (Zeiss, Germany) (Al-Sarraf, 2009).

2.3. Direct counting of Symbiodinium-like cells using an epifluorescent microscope

One milliliter of formaldehyde fixed samples was filtered on 0.22 μm nitrocellulose membrane filters (Millipore, Ireland). The membrane filters were examined under a Zeiss Epiflourescent Microscope (Zeiss, Germany) equipped with a green excitation filter (510–560 nm) and barrier filter LP 590. Cells with the expected size and shape were counted in 20 fields. Then, the total number of cells in the sample was determined.

2.4. DNA extraction and polymerase chain reaction

DNA from the coral tissue and mucus was extracted using Soil Spin Kit (Qbiogene, USA). The extraction procedure was done following the manufacturer's protocol. Modified versions of the protocols of Schafer and Muyzer (2001) and Baker (1999) were followed to extract the DNA from the seawater samples.

Different target sites were amplified using different primers (Table S2, Supplementary data) (Rowan and Powers, 1991a, 1991b; Baker, 1999; Pochon et al., 2001; Mostafavi et al., 2007). The purpose was to investigate different nuclear regions in order to obtain the

Please cite this article as: Mahmoud, H.M., Al-Sarraf, M., Molecular phylogeny and community fingerprinting of coral-associated *Symbiodinium* north of the Arabian Gulf, Marine Pollution Bulletin (2016), http://dx.doi.org/10.1016/j.marpolbul.2016.04.053