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Comparison of *Symbiodinium* populations in corals from subtidal region and tidal pools of northern coasts of Hengam Island, Iran

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ABSTRACT

Coral reefs are integral part of tropical ecosystems and obligatory symbiosis with the genus *Symbiodinium* has a vital role in resistance and survival of the holobiont. Nine different genetic clades (A to I) have been discovered by molecular techniques. Members of *Symbiodinium* are ecologically and biogeographically diverse and their distribution patterns are determined by temperature, irradiance, depth, season and latitude however stress-tolerant species might be detected within each clade. Samples of four coral species from subtidal region in the north of Hengam Island, Persian Gulf (PG) and its tidal pools were analyzed by internal transcribed spacer of ribosomal DNA and by the aid of Denaturing Gradient Gel Electrophoresis (DGGE) to compare the differences in populations of their symbionts. The results showed that some subtidal colonies of one species harbored less tolerant subtypes of *Symbiodinium* subclade C7. Nonetheless, clade D *Symbiodinium* was the most dominant symbiotic clade in both tidal pool and subtidal coral colonies. A novel subtype of subclade D1 *Symbiodinium* was also detected in some coral colonies and was inferred as subclade D1.PG *Symbiodinium*. The prevalence of clade D *Symbiodinium* might reflect corals response to extreme environmental stresses of tidal pools and the northern PG itself that coral inhabitants have to cope with.

1. Introduction

Coral reefs are unique in building three dimension living structures providing habitat for a vast variety of tropical organisms. They also play significant role in primary production of marine tropical ecosystems (Castro and Huber, 2008; Polovina, 1984). Their success as primary producers in nutrient depleted tropical waters is entirely due to their obligatory symbiosis with dinoflagellate algae of the genus *Symbiodinium*. These dinoflagellates densely populate the coral tissues (greater than 10^6 per cm^2) and provide up to 90% of corals energy demand (Davies, 1984; Muscatine and Porter, 1977). Nevertheless, increase in temperature could affect *Symbiodinium* light processing ability and in case of exceeding solar energy, its photosynthetic apparatus will be damaged, leading to the production of reactive oxygen species, which further damage cellular structures. Eventually, coral hosts expel *Symbiodinium*, which is known as coral bleaching (Baird et al., 2009; Marshall and Schuttenberg, 2006).

Identification of *Symbiodinium* species and subspecies by using morphological characteristics could not practically classify this divergent group. Nonetheless, molecular techniques have been profoundly successful and hitherto nine phylogenetical clades (A–I) (Pochon and Gates, 2010), each including subclades or subtypes (Baker et al., 2004), have been identified based on SSU-LSU rDNA (Loh et al., 2001; Rowan and Powers, 1991), ITS regions (Pawlowski et al., 2001; Pochon et al., 2001), 23S chloroplast large subunit (Santos et al., 2002) and mitochondrial DNA (Takabayashi et al., 2004). Each *Symbiodinium* lineage has specific physiological features (Little et al., 2004; Tchernov et al., 2004) which has ecological importance by affecting the holobiont responses to environmental stresses (Fisher et al., 2011).

Reef corals have been heavily disturbed by anthropogenic and natural sources in recent decades (Adjeroud et al., 2009; Selkoe et al., 2009). The main natural influencing factors are temperature fluctuations, high salinities, extreme low tides, winter macroalgal blooms and marine pollution (Burt et al., 2008; Coles and Fadlallah, 1991; Kabiri et al., 2013; Shinn, 1976). Frequency and scale of mass coral bleaching events have also escalated since the early 1980s (Baker et al., 2008; Glynn, 1993; Hoegh-Guldberg et al., 2007) due to rapid increases in the atmospheric carbon dioxide concentration leading to global warming and ocean acidification (Hoegh-Guldberg et al., 2007).

The Persian Gulf (PG) is a shallow semi-enclosed marginal water body in the northwest region of Indian Ocean (Mostafavi et al., 2007) affected mainly by the weather systems from the northwest of the Indian Ocean (Reynolds, 1993; Wilkinson, 2000). Shallowness of the PG, high evaporation and low water exchange with Oman sea and the Indian Ocean (merely via Hormuz strait) result in higher sea surface temperatures and salinities compared to adjacent water columns (Reynolds, 1993) with highest record of 38 °C and 39.73 ppt, respectively (Baker et al., 2004; Forouzan et al., 2014; Kavousi et al., 2011). The tides in the PG are complex standing waves which co-oscillate with those in the narrow Strait of Hormuz and the dominant pattern varies

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from being primarily semi-diurnal to diurnal. The tidal range is large, with values greater than 2 m everywhere (Purkis et al., 2005; Reynolds, 1993).

Coral reefs flourish best in the range 25–29 °C within salinity range of 34–36 part per thousand. Nevertheless, scleractinian corals inhabiting the tidal pools are exposed to extreme desiccation, high temperature and salinity increases during low tide, irradiance and osmotic stress due to problems in gas exchange and accumulation of metabolic wastes during emersion (Castro and Huber, 2008; Grant and McDonald, 1979; Rawlings, 1999). Dealing with these hostile conditions could not be achieved without a tolerant symbiont. Different *Symbiodinium* clades show different levels of tolerance during thermal and other disturbances (Kemp et al., 2015). In this study, in hospite *Symbiodinium* populations from four scleractinian species including *Siderastrea savignyana*, *Cyphastrea microphthalma*, *Platygyra daedalea* and *Leptastrea transversa* in three sites along the northern coast of Hengam Island, where is mostly dominated by rocky shores and experience temperature anomalies, have been analyzed by using ITS2 rDNA molecular marker in order to understand the diversity of symbionts both in extreme condition of tidal pools and sub-intertidal region. This analysis could provide important insights into the high resilience of the PG tidal colonies.

2. Materials and methods

2.1. Sample collection

Samples of four scleractinian coral species (n = 38), including three replicate of each species (tidal pools replicates were sampled according to their availability), were collected from two sites (26° 40′ 53.39″ N, 55° 53′ 43.49″ E) on the northern shores of Hengam Island during low tide in March 2012 (Fig. 1). In order to compare perpetual submerged colonies with intertidal denizens, coral colonies from both subtidal zone tidal pools were sampled. Tidal pools in both sites were chosen by distinct presence of coral colonies, Table 1. Tiny fragments of coral colonies, up to 3 cm, were sampled to minimize the impact on remaining community and then preserved in DMSO buffer (20% DMSO, 250 mM EDTA, saturated with NaCl, pH 8.0) in the field. After transportation to the lab, coral tissues, containing in hospite *Symbiodinium*, were removed from of corals skeleton by using DNAB buffer airbrush (0.4 NaCl, 50 mM EDTA, pH 8.0). This was followed by preserving acquired slurry at −20 °C for further analysis.

2.2. DNA extraction, PCR amplification and sequencing

DNA extraction from melted slurry was performed by CTAB/chloroform method (Baker, 1999). Dinoflagellate ribosomal DNA sequences, consisting of merely internal transcribed spacer 2 (ITS2), were amplified by using touchdown thermal cycle with the ITSintfor2 (5′-GAA TTG CAG AAC TCC GTG-3′) as forward primer and ITS2CLAMP (5′-CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CCC GGG ATC CAT ATG CTT AAG TTC AGC GGG T-3′), with a 39 pair guanine and cytosine clamp (underlined) as reverse primer (LaJeunesse and Trench, 2000).

The resulted amplicons were electrophoresed on 1% agarose gel in order to ensure their quality and quantity and then analyzed via denaturing gradient gel electrophoresis (DGGE) (LaJeunesse et al., 2003). Amplified sequences were then separated by electrophoresis on 8% polyacrylamide denaturing gradient gel (45–80% urea–formamide gradient: 100% consists of 7 mol L^{-1} urea and 40% deionized formamide) for 14 h at 100 V, 60 °C (LaJeunesse and Trench, 2000). Gel was stained with ethidium bromide for 30 min. Then, noticeable bands of unique profiles were excised, purified (eluted) by DNA Gel Extraction Kit (Fermentas, K0513) and reamplified using the same primer set (GC clamp excluded) following the same PCR conditions. Reamplified PCR products finally were sent for forward direction sequencing using the dideoxy chain termination method in Macrogen Company, South Korea.

2.3. Phylogenetic analysis

Functionality and stability of ITS2 secondary structures were predicted as described in Stat et al. (2009) and Pochon and Gates (2010). Unstable ITS2 sequences were excluded from downstream analysis. Geneious v. 8.1.4 Mac (Kearse et al., 2012) were used as a main platform

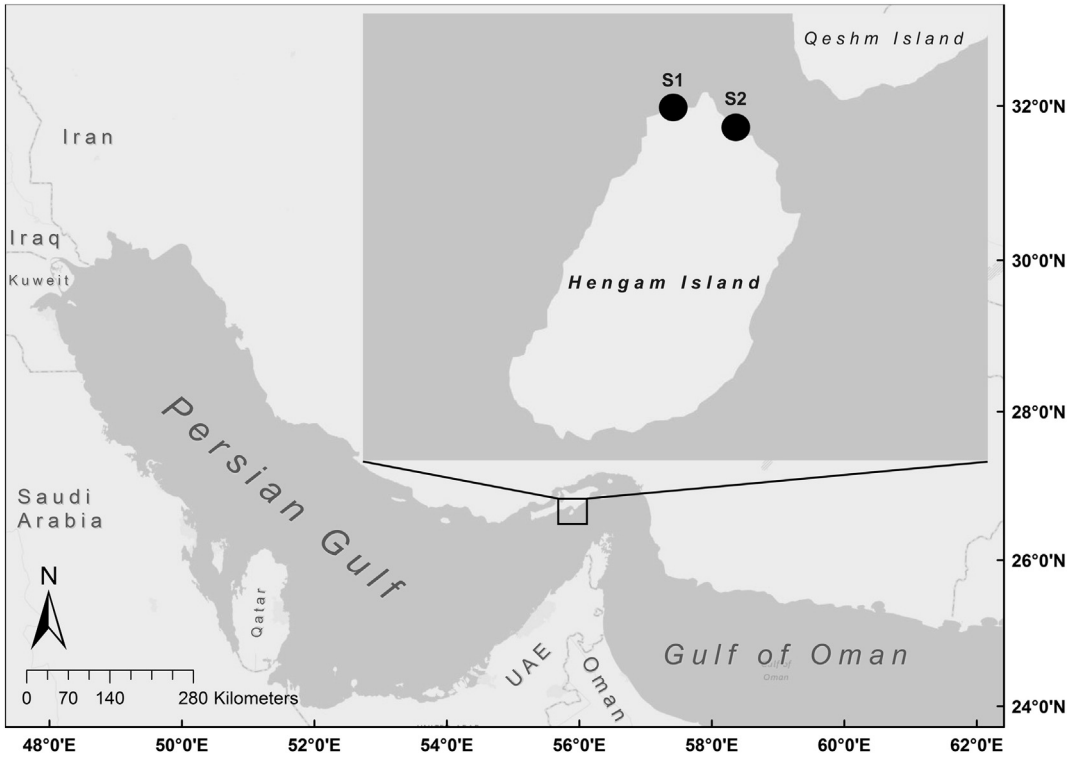

Fig. 1. Map of the Persian Gulf showing sampling sites on the northern shores of Hengam Island.