

Full Length Research Paper

Clade identification of symbiotic zooxanthellae of dominant scleractinian coral species of intertidal pools in Hengam Island

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Zooxanthellae of reef-building corals are unicellular dinoflagellates of the *Symbiodinium* genus, which has an important role in bleaching phenomenon. *Symbiodinium* and their coral hosts are sensitive to environmental stresses that include salinity, high temperatures, low temperatures, extreme light levels and turbidity. Tidal pools have harsh conditions due to lack of nutrients, food and pronounced changes in physical conditions such as pH, salinity and temperature, hence the study of symbiotic zooxanthellae on coral reefs of tidal pool seems to be necessary. Samples of five coral species that include *Siderastrea savignyana*, *Coscinaraea columna*, *Anomastrea irregulariis*, *Cyphastrea serailia*, *Psammocora superficialis* were collected at intertidal pool of Hengam Island in the northern Persian Gulf. Partial 28S nuclear ribosomal (nr) DNA of *Symbiodinium* were amplified by polymerase chain reaction (PCR) and then PCR products were analyzed by the phylogenetic analyses of the LSU DNA sequences based on PAUP and Clustal X software. The results showed that there are at least two clades of *Symbiodinium* from Hengam Island. Clade D was detected from 3 of the coral species while clade C was found in 2 species only. This study showed dominance of clade D at intertidal pool in Hengam Island and the dominance of clade D might be explained by the high environmental stresses for the Persian Gulf.

Key words: Persian Gulf, clade D, tides, *Symbiodinium* and Hengam Island.

INTRODUCTION

Zooxanthellae is an algae essential to the survival of coral reefs which belongs to the genus *Symbiodinium* (Birkland, 1996) and provide oxygen and other nutrients

to the coral polyps and the polyps give the algae the carbon dioxide they need to survive (Trench, 1979). There are eight genetics clades of *Symbiodinium* (A to H) (Rowan and Power, 1991a; LaJeunesse, 2001; Pochon et al., 2004; Baker, 2001), which their ecology and evolution have been, improved (LaJeunesse et al., 2010). *Symbiodinium* and their coral hosts are sensitive to environmental stresses that include high and low temperatures (Hoegh-Guldberg and Smith, 1989; Steen and Muscatine, 1987), extreme light levels (Hoegh-Guldberg and Smith, 1989), salinity (Reimer, 1971) and turbidity. Scleractinian corals are among the most flexible hosts identified to date, containing symbionts from clades

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Abbreviations: PCR, Polymerase chain reaction; DMSO, dimethyl sulfoxide; EDTA, ethylenediaminetetraacetic acid; nr, nuclear ribosomal; MP, maximum parsimony; BLAST, basic local alignment search tool.



Figure 1. Situation of Hengam Island.

A, B, C, D, and F (Baker, 2003).

Considerable flexibility in the relationship between hosts and symbionts occurs and coral species may have more than one type of *Symbiodinium* simultaneously specially on distributed colonies (Trench, 1988; Thornhill et al., 2006).

Diverse *Symbiodinium* types will presumably have various physiology (Tchernov et al., 2004), and this may play an important role in survival of corals (Baker, 2003). Therefore any physiological response of *Symbiodinium* to stressful environments may have a profound effect on its host (Hoegh-Guldberg et al., 2002).

Few studies have been undertaken in the Indian Ocean. These studies were from corals of Eastern Africa (clade C and D; Baker et al., 2004; Visram et al., 2006), the Red Sea (clade A and C; Baker et al., 2004); the Saudi Arabian coast of the southern Persian Gulf (clade A, C and D; Baker et al., 2004) and Kish and Larak Islands of the northern part of the Persian Gulf (clade A and C; Mostafavi et al., 2007). There are 17 islands of the Iranian coastline with fringing coral reefs in the Persian Gulf (Shokri et al., 2005). Of these, Hengam Island is located near the Qeshm Island in the more saline and oligotrophic waters of the inner Persian Gulf. We focused on corals of the tidal pools of Hengam Island. Tidal pools that are high on the shore and infrequently flushed by wave action can become stagnant, resulting in harsh conditions due to lack of nutrients and food, and pronounced changes in physical conditions. The coral reefs in tidal pools are exposed to high environmental stresses due to

long period of air exposure and high irradiance in daytime (Knox, 2001).

This is the first study on *Symbiodinium* diversity of the tide pool's corals of the Iranian Island. Before now, clade D *Symbiodinium* from the corals near the Arabian coastline of Persian Gulf (Baker et al., 2004) and clade D and C *Symbiodinium* from Kish and Larak Islands in the northern part of Persian Gulf have been prevalent. Since *Symbiodinium* clade D is more tolerant to stressful conditions than other clades of *Symbiodinium* (Rowan, 2004), it may be expected that corals of tidal pools of Hengam Island contain this clade of *Symbiodinium*. Therefore, the aim is to survey the genetic diversity of *Symbiodinium* from coral species of Hengam Island for comparison with *Symbiodinium* from other coral communities in the Persian Gulf and worldwide.

MATERIALS AND METHODS

Sample collection, DNA extraction and polymerase chain reaction (PCR)

Prior to this experiment, the scleractinian corals which harbored zooxanthellae were identified in the intertidal pools of Hengam Island (Persian Gulf, Iran; Figure 1), then the 5 colonies of *Siderastrea savignyana* Milne Edwards and Haime, 1850; *Coscinaraea columna* Dana, 1846; *Anomastrea irregularis* Von Marenzeller, 1901, *Cyphastrea serailia* Forskål, 1775 and *Psammocora superficialis* Gardiner, 1868 at intertidal pool of the northern coastline of Hengam Island were collected in 3 replicates during January 2009. Coral fragments were preserved by dipping in

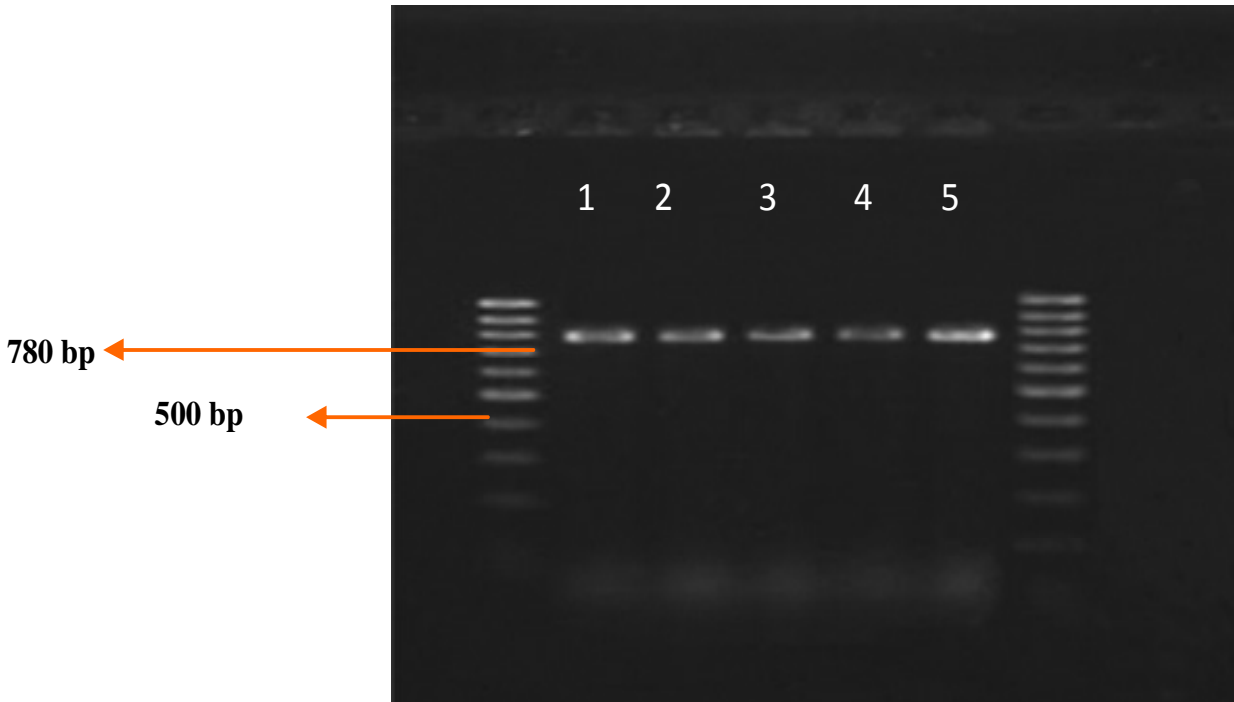


Figure 2. PCR products on agarose. (1) 100 bp DNA ladder (ready to use); (2) *P. superficialis*; (3) *S. savignya* (4); *C. serailia* (5); *A. irregulariis* (6); *C. columna*; 7: 100 bp DNA ladder (ready to use).

dimethyl sulfoxide (DMSO) buffer (20% DMSO, 250 mM ethylenediaminetetraacetic acid (EDTA), saturated with NaCl, pH 8.0, (Seutin et al., 1991) and then airbrushed with DNAB buffer (0.4 M NaCl, 50 mM EDTA, pH 8.0). DNA was extracted from the slurries using the cetyl trimethylammonium bromide (CTAB)/chloroform method of Baker (1999). The partial 28S nuclear ribosomal (nr) DNA of *Symbiodinium* (D1/D2 domains) was amplified using *Symbiodinium* specific primers. These primers were MOS Forward (ATA TAA GTAAGC GGA GGA GGA AAAG) and MOS Reverse (CTT TCG GGT CCT AAC ACA CAT G) (Mostafavi et al., 2007). All PCR contained 1 ng of template DNA, 1 mM total dNTP, 7.5 pmol of each primer and 1.5 U of *Taq* DNA polymerase (Ampli-Taq, Cinnagen) in a total volume of 25 ml. Amplification was performed using a Corbett PCR Thermal Cycler with the following thermal profile: 30 cycles of 30 s at 94°C, 1 min at 64°C, 30 s at 72°C and a final extension for 5 min at 72°C. The PCR products were analyzed by electrophoresis in 1.5% agarose gels (100 V, 40 mA), and stained with ethidium bromide.

Sequencing and phylogenetic analyses

DNA sequences were determined in the forward direction using the dideoxy chain termination method. All of *Symbiodinium* sequences obtained from 5 corals samples were used for phylogenetic analyses. All sequences were aligned by using Clustal X (Thompson et al., 1994).

The reference symbiont sequences included here are clade A (accession numbers (AF279914, DQ060763, AJ621128, AJ620944, AJ620935, AF396628*, AJ308902, AF349547, AY588448, AF170149), clade E (AY684264, *S. varians* AF060899), clade F (AJ621142, AJ621144, AJ621146), clade G (AJ291536), clade H (AJ621129, AJ621149, AJ621131, AJ621132). The tree was rooted using *Gymnodinium beii* (AF060900). Trees were constructed using

maximum parsimony (MP) methods. MP analyses were conducted using the PAUP beta version 4.0b10 (Swofford, 2002). All characters were given equal weight and were unordered. The MP analysis was carried out by the heuristic search method, with 100 random additions of taxa, each followed by TBR branch swapping. Starting-trees were obtained by stepwise-addition using simple addition sequence. MP clades were assessed with 1,000 bootstrap replicates (excluding uninformative characters), with 100 random additions of taxa for each replicate. The phylogenetic trees generated in all analyses were visualized using TREEVIEW version 1.6.5 (Page 1996).

RESULTS

PCR amplification

PCR amplification of *Symbiodinium* 28S rRNA genes yielded products of approximately 780 bp from all 5 coral species (Figure 2).

DNA sequencing

Direct sequencing of PCR products was done from 5 coral species. Basic Local Alignment Search Tool (BLAST)N analysis of the resulting sequences showed highest identity (>99%) with clade C or D. The results showed that there are at least two clades of *Symbiodinium* from Hengam Island. Clade D was detected from three coral species (*S. savignyana* and *P. superficialis*), while clade C was just found in two species. (*C. columna*, *A. irregulariis* and *C. serailia*).

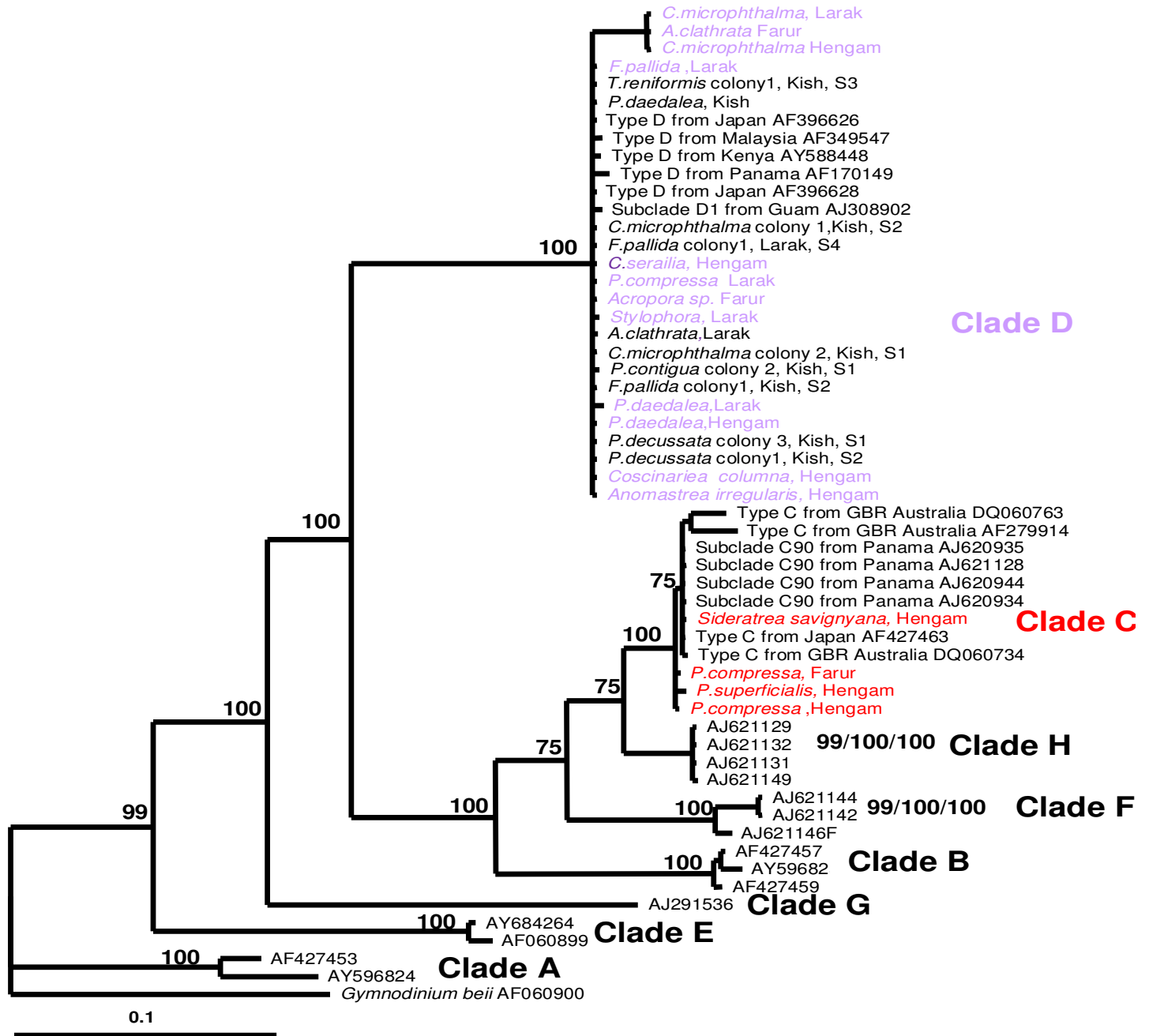


Figure 3. MP tree of the *Symbiodinium* 28S nuclear ribosomal (*nr*) DNA genotypes from coral colonies at sites of Hengam Island. Clade controls (A, B, C, D, E, F and G) and an out-group organism (*G. beii*) were included in the analysis (accession codes shown in figure). Maximum parsimony bootstrap percentages from 1,000 trees is shown at nodes. Distance represents the number of substitutions per 100 bases.

Phylogenetic analyses

MP analysis resulted in 1,000 equally-parsimonious trees well-supported clades C and D were recovered. As the tree topologies were similar in all analyses, the bootstrap values for MP analyses are shown on the tree (Figure 3). Hengam Island supported clades C and D groups. The clade D *Symbiodinium* from *C. columna*, *A. irregularis*

and *C. serailia* and our clade C *Symbiodinium* clustered closely with the C genotypes.

DISCUSSION

Symbiodinium clade D is dominance clade on the tidal pool's corals of Hengam Island. *C. columna*, *A.*

irregulariis, *C. serailia* hosted *Symbiodinium* clade D. The previous survey of *Symbiodinium* clades by Baker et al. (2004) covered relatively few colonies of each coral species (1 to 4 colonies from 13 species) from several Saudi Arabian reefs in the southern Persian Gulf and *Symbiodinium* clade D reported on some coral species. In the studies of Mostafavi et al. (2007), the most common species of scleractinians from Iranian coral reefs strongly suggested that subclade D1 is the dominant symbiont population. The coral reefs of Kish and Larak Islands harbored mostly clade D from wide range of coral families that include Acroporidae, Agariciidae, Dendrophylliidae, Faviidae, Poritidae and Siderastreidae (Mostafavi et al., 2007). Clade D is also reported on *Sinularia erecta*, *Sinularia sp.* and *Sarcophyton*, the most common soft coral species off Larak Island (Mostafavi et al., 2007). The present survey of up to five colonies each from three of the most common species of scleractinians from tidal pool's coral reefs have *Symbiodinium* clade D which is the more tolerant clade of *Symbiodinium* (Baker, 2003). The corals in the tidal pools of Hengam Island belong to family of Siderastreidae and Faviidae. In this survey, only two species of the family Siderastreidae, *P. superficialis* and *S. savignyana* were found to host *Symbiodinium* clade C. In the Indo-Pacific most coral surveys showed a predominance of *Symbiodinium* clade C (Baker et al., 2004). The previous studies by Mostafavi et al. (2007) showed the presence of clade C identified as subclade C90 (>99% identity) in the *P. compressa* and *P. contigua* off Kish Island. Clade C90 is the type of clade C that has been found only in East Pacific foraminiferans (*Sorites* spp., Pochon et al., 2004); however, *Symbiodinium*-coral surveys off Kish and Hengam Islands showed the presence of the mentioned subclade in cnidarians. The C15 subclade common to *Porites* corals are thought to be evolved from C90 which represents an "ancestral" type (Pochon et al., 2004).

In the most common coral species of the Iranian and Saudi Arabian coral reefs of the Persian Gulf, *Symbiodinium* clade D is dominant, which may lead to some protection from future heat stresses and coral bleaching.

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