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Genetic Identification of Symbiodinium in Genus *Acropora* off Farur Island, Persian Gulf

[1]*S. Rahmani;* [1]**P. Ghavam Mostafavi;* [2]*M. H. Shahhosseiny;* [3]*Gh. Vosoughi;* [4]*A. Faraji*

[1]*Department of Marine Biology, Graduate school of Marine Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran*
[2]*Department of Microbiology, Shahr-e- Qods Branch, Islamic Azad University, Iran*
[3]*Department of Veterinary , Tehran University, Iran*
[4]*Biotechnology Incubator, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran*

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ABSTRACT: Coral reefs which form some of the most diverse ecosystems on Earth support many symbiotic relationships. Symbiodinium can provide up to 90% of a coral's energy requirements. Temperature rise, turbid water and high salinity in the Persian Gulf were among the factors separating zooxanthellae from corals and result in bleaching phenomenon. Therefore, it is crucial to identify Symbiodinium of the Persian Gulf corals. Since zooxanthellae identification according to morphological characteristics is not precise enough, today, Symbiodinium different clades are identified using molecular techniques. In this study, Symbiodinium of genus Acropora as a dominant scleractinian coral in Farur Island, northern Persian Gulf were identified using molecular techniques. For this reason, sampling from depth of 7-10 meters was carried out. After DNA extraction, the target gene which was large subunit ribosomal RNA (28S rDNA) gene was amplified, using Polymerase Chain Reaction (PCR). Then the amplified fragments analyzed, by sequencing, and Symbiodinium clade was identified, using phylogenetic tree, and the situation of considered clade and its phylogenetic association with other studied samples in Iran and other parts of the world were defined. The results showed that clade D was the only symbiont of Acropora off Farur Island. Since clade D is the most resistant clade against environmental changes, it is natural to identify this clade from Symbiodinium in Persian Gulf specific conditions.

Keywords: *Persian Gulf, Farur Island, Acropora, Clade D, Symbiodinium, 28S rDNA*

INTRODUCTION

Coral reefs which are the shallow water ecosystems of tropical regions are resulted from symbiosis of corals and zooxanthellae (Birkland, 1996; Nybakken, 2000). Carrying out the act of photosynthesis, zooxanthellae play a significant role in providing energy (Muscatine et al., 1984), nutrients, oxygen and calcium carbonate (Birkland, 1996) for corals and therefore their calcification, so that some corals are dependent on primary products of these symbionts for their survival (Trench, 1986). When coral is under stress, Zooxanthella separates from its host and bleaching occurs, which if continued, results in coral death (Baker, 2003; Baker et al., 2004). Bleaching in corals is caused by changes in some environmental factors such as temperature (Baker, 2003; Baker et al., 2004), salinity (Reimer, 1971) light level (Hoegh-Guldberg and Smith, 1989) and turbidity (Ulstrup and van Oppen, 2003). It also occurs by changes in atmosphere factors such as El Nino (Hoegh-Guldberg, 1999). Bleaching is a threat for all coral reefs of the world (Hoegh-Guldberg, 1999).

Persian Gulf is a marginal and semi-closed sea in northwest region of Indian Ocean, and its connection to open seas is limited to Strait of Hormuz. Its depth is low with an average of about 35 meters (Hunter, 1985; Wilkinson, 2008). These are reasons why Persian Gulf is highly affected by atmosphere variants and the regional corals become vulnerable to bleaching (Hunter, 1985). During ENSO (EL Nino-Southern Oscillation) event in 1996 and 1998, Persian Gulf coral reefs along with other coral reefs all over the world underwent bleaching (Glynn et al., 2001; Goreau et al., 2000; Wilkinson, 2000), so that significant amounts of the region corals (about 25%) annihilated (Wilkinson, 2000). Sudden temperature increase in August 2007,

*Corresponding Author Email: *mostafavi_pa@srbiau.ac.ir*
Tel: +98 9124435190

and occurrence of Red Tide event in 2008 and 2009 in Persian Gulf resulted in bleaching of most coral reefs of the region (Wilkinson, 2008).

Up to now, 8 different genetic clades including A, B, C (Rowan and Powers, 1991a), D, E (Baker, 2003), F, G and H (Pochon et al., 2004) have been identified all over the world. It has been determined that different clades of Symbiodinium have different physiological resistance against environmental conditions and stresses, therefore different survival rates of corals have been observed (Baker, 2003). Beside different types of Symbiodinium, the difference might be due to variation in host coral species (Huang et al., 2006).

Symbiodinium molecular identification is predominantly carried out, using nuclear ribosomal RNA genes (nrDNA), for the purpose of which different genetic markers might be studied. These markers are as following: small subunit ribosomal RNA gene (SSU) (Brown et al., 2002; Rowan and Powers, 1992; Sadler et al., 1992), large subunit ribosomal RNA gene (LSU) (Baker, 1999; Loh et al., 2000; Pawlowski et al., 2001; Pochon et al., 2001; Savage et al., 2002; van Oppen et al., 2001), internal transcribed spacers (ITS1, ITS2), and 5.8S regions (Brown et al., 2000, 2002; Hunter et al., 1997; LaJeunesse, 2001, 2002; LaJeunesse et al., 2003; Pochon et al., 2004; Wilkinson, 2000). Chloroplast rDNA sequence of ribosomal large subunit gene is also recently used to interpret from nrDNA as well (Santos et al., 2003).

Most studies on Symbiodinium molecular identification has been related to the Pacific Ocean (Glynn et al., 2001; LaJeunesse et al., 2003; Loh et al., 1998) and Carrabin Sea (Baker and Rowan, 1997; Diekmann et al., 2003; Rowan et al., 1997; Visram et al., 2006), and then Indian Ocean. There have been reports of clades D and C (Baker et al., 2004; Wilson et al., 2002) in eastern Africa, as well as clades A and C (Baker et al., 2004) in Red Sea. Clades D, C and A (Baker et al., 2004) with dominance of clade D have been identified in southern seashores of the Persian Gulf in Saudi Arabian region. There has been a report of clades D and C90 (Mostafavi et al., 2007) in Kish Island, northern region of Persian Gulf, clade D (Mostafavi et al., 2007; Azadbadi, 2009) in Larak Island, as well as clades D and C (Shahhosseiny et al., 2011) in Hengam Island. Clade D was introduced as dominant clade in all studies performed in Persian Gulf.

MATERIALS AND METHODS

Sample collection and DNA extraction:

Sampling was carried out from Acropora , the dominant genus of coral reefs around the Farur Island, from depth of 7-10 meters through scuba diving. Five separate colonies were chosen and three replicates (3 corals) from each colony were collected. Samples were preserved in salt saturated 20% dimethyl sulfoxide buffer (DMSO 20%, EDTA 250 mM, pH=8) (Posada and Crandall, 1998) and transferred to laboratory.

Coral pieces were washed by air brush and DNAB buffer (NaCl 0.4 M, EDTA 0.25 M, pH=8) and zooxanthellae were preserved in -20 °C, then DNA was extracted using CTAB-chloroform method (Baker, 1999, 2001).

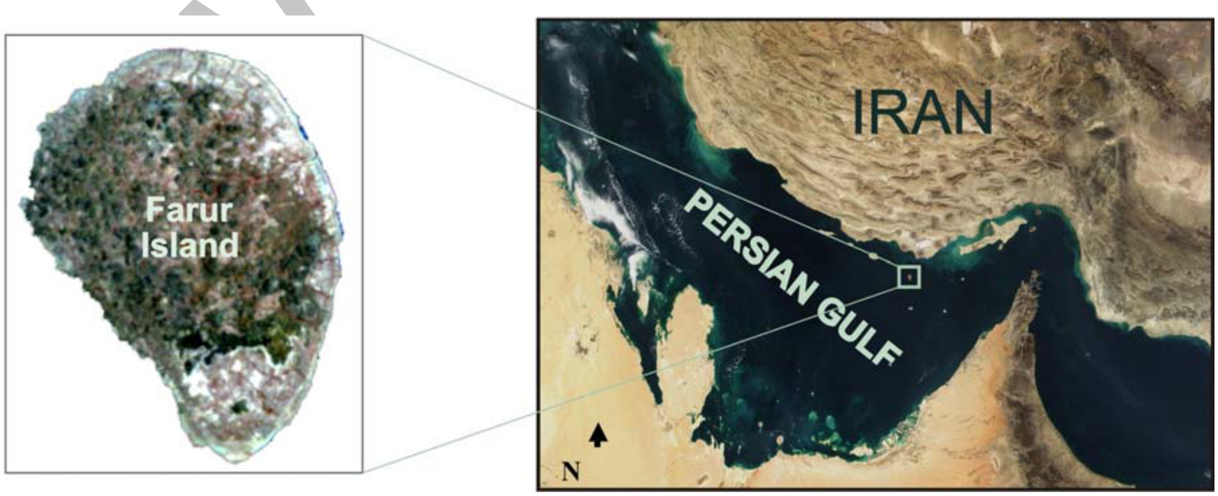

Fig. 1: Situation of Farur Island, Persian Gulf.

Polymerase chain reaction (PCR)

A fragment of large subunit ribosomal RNA gene of Symbiodinium (28S rDNA) was amplified using following specific primers: Mos Forward (*ATA TAA GTA AGC GGA GGA AAA G*) and Mos Reverse (*CTT TCG GGT CCT AAC ACA CAT G*) (Mostafavi et al., 2007).

PCR reaction includes 25 ng of target DNA, 2.5 µl of 10X PCR Buffer, 1.5 mM of $Mgcl_2$, 0.2 mM of dNTP, 0.2 µM of each primer, 1.5 U of *Taq* DNA Polymerase, in total volume of 25 µl and PCR reaction was carried out in Corbett thermal cycler under following temperature profile: 5 minutes in 94 °C for 1 cycle and then 30 seconds in 94 °C, 1 minute in 60 °C, 30 seconds in 72 °C for 30 cycles and a final extension, 5 minutes in 72 °C was carried out for 1 cycle (Mostafavi et al., 2007). Then the PCR products were analyzed by electrophoresis in 2% agarose gel. Agarose gel was stained by Ethidium Bromide and observed by UV transilluminator.

Sequencing and Phylogenetic analyses

DNA sequencing in forward and reverse directions was carried out via Dideoxy-Chain Termination method using ABI 3730 XL apparatus American company of Applied Biosystem. Resulted sequences were blasted in NCBI gene bank and compared with reference sequences. Phylogenetic analysis was carried out using Clustal X software (Thompson et al., 1994; Thornhill et al., 2006). Symbiodinium clades sequences were considered according to Baker study in 2003 (Baker, 2003).

Then Neighbor Joining phylogeny tree was drawn on basis of Gymnodinium beii (AF0620900). After searching in BLASTN (Altschul et al., 1997), clades D references were chosen on basis of highest identity to obtained sequences of this study. The phylogenetic tree was observed using 1.6.5 version of TREE VIEW program (Page, 1996).

RESULTS AND DISCUSSION

DNA extraction

Extracted DNA had enough concentration and purity in order to amplified.

Polymerase Chain Reaction (PCR)

The size of PCR products on Symbiodinium was 780 bp with suitable quality and quantity for sequencing.

Sequencing and Phylogenetic analyses

The resulted sequences through direct sequencing operation for PCR products were completely definite and clear. BLASTN analysis of sequences in gene bank and comparison of obtained sequences with reference sequences in NCBI gene bank indicated highest identity (more than 99%) to registered sequences of Symbiodinium clade D in NCBI.

All obtained sequences were registered in American NCBI gene bank for the first time.

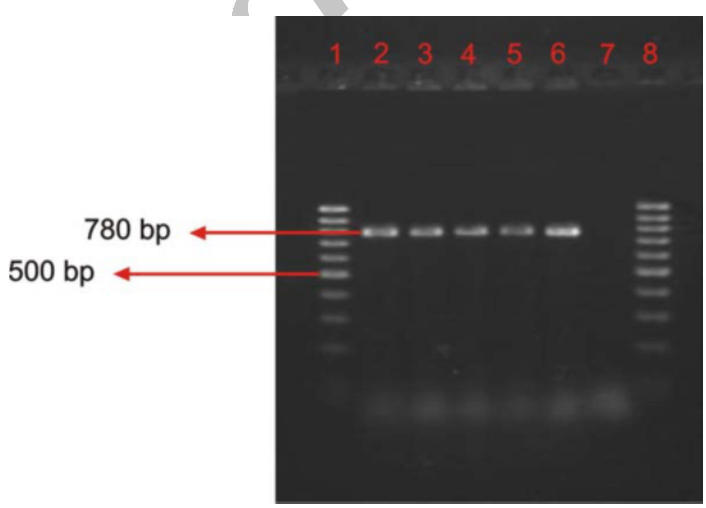

Fig. 2: PCR products on agarose gel. 1. 100 bp DNA ladder (ready to use); 2. Acropora colony1; 3. Acropora colony2; 4. Acropora colony3; 5. Acropora colony4; 6. Acropora colony5; 7. C-; 8. 100 bp DNA ladder (ready to use).

Coral reefs and their symbiont diversity are affected by environmental conditions such as day length, salinity, turbidity and temperature (Nybakken, 2000; Rowan et al., 1997; Savage et al., 2002; Tchernov et al., 2004).

Persian Gulf has created some limitations for growth and diversity of coral population. Persian Gulf coral reefs are always under stress and exposed to bleaching due to environmental specific conditions such as intense temperature oscillations, turbidity, and high salinity (Baker et al., 2004). Coral different survivals might be associated to coral colony resistance (Bhagooli and Hidaka, 2004; Brown et al., 2002), Symbiodinium resistance or both of them (Rowan et al., 1997).

In current study on 15 replicates of genus Acropora, which are among most common genus of scleractinian coral reefs all over the world as well as Iranian coral reefs, molecular identification of Symbiodinium clades was carried out. Clade D was identified as a symbiont of Acropora in this study.

In the study of Mostafavi et al., (2007), on zooxanthellae diversity on 8 dominant coral species including Acropora off Kish and Larak Islands, clades D and C90 were observed, with the dominance of clade D. Acropora clathrata species studied by Mostafavi in Kish and Larak Islands was reported to have clade D as well as this study (Mostafavi et al., 2007).

Baker in 2004 studied clades of zooxanthellae, in southern marginal corals of Persian Gulf in Saudi Arabia and reported clades D, C and A, clade D was dominant in studied coral reefs of the Arabian region too. Clade D covers about 63% of all coral reefs of the southern coastline of the Persian Gulf (Baker et al., 2004).

Symbiodinium different clades have different resistance against environmental stresses and therefore bleaching. According to many studies all over the world, it has been defined that clade D would have most resistance among clades and therefore could be more frequent after bleaching and become dominant in environment (Baker et al., 2004; Chen et al., 2003; Coles and Brown, 2003; Glynn, 2006; Glynn et al., 2001; Rowan, 2004; Thornhill et al., 2006; van Oppen et al., 2000). Studies on photosynthesis mechanism of Symbiodinium clade D in coral reefs all over the world has demonstrated that clade D is typically seen in corals of high temperature environments such as Kenya coral reefs (Visram et al., 2006), high salinity such as Palau salt marsh (Fabricius et al., 2004), high temperature degree and weak light (van Oppen, 2004) and high effects of land on sea such as high turbidity (Chen et al., 2003; Loh et al., 1998) and human activity on seashore (van Oppen et al., 2001).

Clade D predominance in the Persian Gulf might be due to the clades adjustment against inappropriate environmental conditions of Persian Gulf waters. In studies on zooxanthellae of 3 species of dominant soft corals off Larak Island, only clade D was identified (Azadbadi, 2009). In the study of Shahhosseiny et al. in 2011, on 5 reef-building coral species of Hengam Island tidal pools, clades D and C were reported with the dominance of clade D (Shahhosseiny et al., 2011).

Iranian coral reefs have undergone intense bleaching through 1996 and 1998 after occurrence of global El Nino (Glynn et al., 2001; Goreau et al., 2000; Wilkinson, 2000). Mass bleaching in northern waters of Persian Gulf has also occurred in August, 2007, due to sudden temperature increase as well as red tide in 2008 and 2009 (Wilkinson, 2008). According to Adaptive Bleaching Hypothesis (ABH), the phenomenon makes it possible for coral to accept more appropriate zooxanthellae for encountered stress including new environmental situation (Buddemeier and Fautin, 1993). It could be concluded that corals might adjust themselves with environment, through changing symbiotic populations in inappropriate environmental situations (Rowan, 1998).

Therefore, it could be stated in terms of clade D dominancy in reef-building corals of Persian Gulf that recovery after occurrence of bleaching might cause clade D replacement and expression as dominant clade of Iranian coral reefs, or it might be stated that before El Nino in 1996, clade D has been the constant and permanent symbiont of the area due to Persian Gulf specific situation. However, nothing is certainly stated in this area due to lack of studies on Persian Gulf reef-building corals before 2004.

CONCLUSION

According to results of Acropora study in 3 Islands of Farur, Kish and Larak in Persian Gulf region (Mostafavi et al., 2007), it might be concluded that this genus of hard corals in Persian Gulf tend more to be symbiotic with clade D Symbiodinium. Altogether, it could be stated that clade D might be a favorable symbiont for stressful habitats which is expressed in situations that other clades do not prefer symbiosis and might play a critical role in recovery process of constant societies of bleached corals (Baker, 1999, 2001; Toller et al., 2001; van Oppen et al., 2001).

In this study, it was defined that genus Acropora off Farur Island has clade D Symbiodinium and all resulted sequences were submitted in American Gene Bank for the first time. Since clade D is commonly found in marginal waters and inappropriate environmental

Fig. 3: Neighbor Joining tree of the Symbiodinium 28S nuclear ribosomal (nr) DNA genotypes from coral colonies at sites of Farur Island. Clade controls (A, B, C, D, E, F, G and H) and an out-group organism (G. beii) were included in the analysis (accession numbers shown in figure).

conditions, in fluctuating condition of Persian Gulf, it is natural to identify this clade from Symbiodinium in coral reefs of the Persian Gulf.

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