Effects of the 2010 coral bleaching on phylogenetic clades and diversity of zooxanthellae (*Symbiodinium* spp.) in soft corals of the genus *Sinularia*

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Abstract: The mass coral bleaching event in South East Asian countries in 2010 caused severe damage to reef dwellers on the coast of the Andaman Sea and the Gulf of Thailand. Bleaching is a process where the symbiosis between corals and zooxanthellae (dinoflagellates of the genus *Symbiodinium*) breaks down. The symbiosis between *Symbiodinium* and corals is essential to the productivity, survival and success of corals. *Symbiodinium* associated with coral hosts is classified into six genetically distinct clades based on nuclear ribosomal DNA and partial chloroplast large subunit rDNA. Each clade possesses unique environmental, ecological and geographic variations, influencing the resilience of corals to elevated temperatures and bleaching. This study was the first field observation of changes in *Symbiodinium* communities in the Gulf of Thailand over two years including a natural bleaching event from late April to June, 2010. Phylogenetic clades and diversity of *Symbiodinium* inhabiting soft corals of the genus *Sinularia* were investigated using the analysis of chloroplast gene sequence variation at the ribosomal large subunit 23S Domain V. A total of 69 *Sinularia* spp. samples were obtained from two sampling sites off Jorake and Juang Islands (Sattahip, Chonburi Province) during two sampling periods: before (November 2008–January 2010, n=45) and after (November 2010, n=24) the 2010 coral bleaching event. The results revealed a significant change in the *Symbiodinium* community after the bleaching event. Before bleaching, most of *Symbiodinium* clade D was detected from Jorake Island while clade C was dominant in the Juang Islands. In contrast, after bleaching, only clade D was identified from both sampling sites.

Key words: chloroplast gene, coral bleaching, *Sinularia*, *Symbiodinium*, the Gulf of Thailand

Introduction

During late April to June 2010, mass coral bleaching events in South East Asian countries caused severe damage to reef dwellers along the coasts of the Andaman Sea and the Gulf of Thailand as sea surface temperatures anomalies in the range of 30–34°C were recorded. Several reef surveys conducted by the Department of Marine and Coastal Resources in collaboration with a network of Thai universities and NGOs revealed widespread (over 80%) and severe damage on most reef sites (Yeemin et al. 2012). In the Gulf of Thailand, coral bleaching was observed in Kood Island, Chang Island, Man Islands, Samae-San Is-

lands and nearby, but was less severe and less extensive compared with the Andaman Sea (Yeemin et al. 2010). Not only hard corals suffered from the bleaching event, other marine invertebrates forming mutualistic symbioses with zooxanthellae (e.g. soft corals, zoanthids and giant clams) were also bleached (Tun et al. 2010, Yeemin 2010).

The symbiosis between *Symbiodinium* (a genus of unicellular dinoflagellates also referred to as zooxanthellae) and corals is essential to the productivity, survival and success of the coral hosts (Muscattine & Porter 1977). Bleaching is a process whereby this symbiosis breaks down. Advanced molecular studies reveal that the genus *Symbiodinium* is highly diverse and is classified into 9 genetically distinct clades (clades A to I; Pochon & Gates 2010) based on nuclear ribosomal DNA (rDNA) and partial chloroplast large subunit rDNA sequence variations. Among these, six clades (clades A to D, F and G) were reported to form mutualistic symbiosis with coral hosts (LaJeunesse et al. 2010). Each clade possesses unique environmental, ecolog-

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ical and geographic variations, influencing the resilience of coral hosts to elevated temperatures and bleaching. Few studies (e.g. LaJeunesse et al. 2010, Panithanarak 2012) have described compositions of *Symbiodinium* communities in coral hosts from the Andaman Sea and the Gulf of Thailand. *Symbiodinium* clades C and D were reported from the Andaman Sea (Cape Panwa, Koh Hae, Koh Phi Phi Don, Koh Phi Phi Lae, Koh Racha and the Similand Islands) and the Gulf of Thailand (Nang-Rong Beach, Jorake, Juang and Jan Islands) with the thermally resistant clade D being the most common type in the Andaman Sea, especially at inshore reef sites (LaJeunesse et al. 2010). Similar to the Andaman Sea, clade D was abundant at inshore reef sites (Nang-Rong Beach and Jorake Island) in the Gulf of Thailand (Panithanarak 2012). Clade D *Symbiodinium* was more diverse and occurred frequently in coral hosts from inshore locations compared with clade C, most likely as a result of high temperature and turbid conditions of the inshore relative to offshore locations (LaJeunesse et al. 2010, Panithanarak 2012). However, none of the studies so far has examined the impact of high temperature or coral bleaching on changes in clades of *Symbiodinium* communities in Thailand. As coral bleaching is predicted to increase in frequency and severity owing to climate change (Burke et al. 2011, Hoegh-Guldberg 2011), understanding and monitoring the response of *Symbiodinium* to elevated temperature and bleaching is required for developing strategies and to minimize the effects of climate change on coral reef ecosystems.

The present study is the first report monitoring changes in the *Symbiodinium* community within the Gulf of Thailand covering a two-year period, which included the mass coral bleaching event during late April to June, 2010. Phylogenetic clades and diversity of *Symbiodinium* (genus *Sinularia*, Family Alcyoniidae) inhabiting an abundant soft coral in the Gulf of Thailand were examined from two reef sites of Jorake and Juang Islands (coral reefs affected by bleaching in the nearby Samae-San Islands, Sattahip, Chonburi Province) by analyzing partial chloroplast large subunit rDNA sequence variations. *Symbiodinium* communities from two sampling periods, pre and post-bleaching, were monitored and compared. The aims are to investigate any changes in *Symbiodinium* community assemblages as a consequence of the bleaching event, and to identify *Symbiodinium* clades resistant to coral bleaching.

Materials and Methods

Soft coral sampling

Soft coral colonies of *Sinularia* spp. were collected randomly by scuba diving from two sampling sites off Jorake and Juang Islands (Sattahip, Chonburi Province, the Gulf of Thailand; Fig. 1) during two sampling periods: before the coral bleaching (November 2008 to January 2010, n=45) and seven months after the coral bleaching (November 2010, n=24). Pieces of colony lobes (0.5–1.0 cm, 2–3 pieces/colony) were removed and preserved in absolute

Fig. 1. Map showing *Symbiodinium* monitoring sites of Jorake and Juang Islands (Sattahip, Chonburi Province) modified from a map of Amphur Sattahip by Esri Map.

ethanol for molecular analysis. Pre-bleaching specimens were obtained from the study by Panithanarak et al. (2011).

DNA extraction, amplification and sequencing

DNA was extracted from ethanol-preserved tissues using QIAamp DNA Minikits (Qiagen, Hilden, Germany). PCR amplification of chloroplast ribosomal large subunit 23S Domain V (cp23S rDNA) used the specific *Symbiodinium* primers cp23s1 (forward primer): 5'-GCTGTAAC-TATAACGGTCC-3' and cp23s2 (reverse primer): 5'-CCATCGTATTGAACCCAGC-3' (Oliver & Palumbi 2011). The PCR protocols were modified from Zhang et al. (2000) with a 50 μ L reaction volume containing 0.2 mM dNTP (Promega, Madison, WI, USA), 1xPCR Buffer, 50 nmol primer (Macrogen Inc., Seoul, Korea), 2.0 mM $MgCl_2$, 1.5 unit platinum *Taq* DNA polymerase (Invitrogen, Carlsbad, CA, USA) and 0.1–1.0 ng μ L⁻¹ template DNA. The PCR products were further purified using QIAquick PCR purification kits (Qiagen). Direct sequencing of purified PCR products was carried out by the sequencing service, Macrogen (Seoul, Korea) using both primers (cp23s1 and cp23s2).

Data analysis

Nucleotide sequences of cp23S rDNA were manually checked for accuracy. In each sample, two sequences (forward and reverse) were obtained and assembled using CAP3 (Huang & Madan 1999; <http://pbil.univ-lyon1.fr/cap3.php>). All sequences were compared with existing databases of *Symbiodinium* cp23S rDNA deposited in GenBank using BLASTN (<http://blast.ncbi.nlm.nih.gov>) and aligned using Clustal X 1.81.msw (Thompson et al. 1997). They were assigned to major clade identities C or D on the basis of showing highest identity with the respective database sequences. Haplotype frequencies of *Symbiodinium* clades C and D collected before and after the 2010 coral bleaching were calculated and compared using Arlequin 3.5 (Excoffier et al. 2005). Levels of genetic differentiation between pre and post-bleaching *Symbiodinium* communities was also estimated with F_{ST} (molecular distance: pairwise difference) using Arlequin 3.5.

Phylogenetic analyses

The sequence data used for phylogenetic analyses included all haplotypes identified from this study and 16 cp23S rDNA sequences of clade C and D symbionts from various coral hosts deposited in GenBank and/or as published (Santos et al. 2002, Pochon et al. 2006, Cantin et al. 2009, Stat et al. 2009, Pochon et al. 2010, Oliver & Palumbi 2011, Pochon et al. 2012, Jeong et al. 2014). A neighbor-joining (NJ) phylogenetic tree (Saitou & Nei 1987) based on the cp23S rDNA gene was constructed using the distance method based on the Kimura two parameter substitution model (Kimura 1980). NJ trees were constructed using PAUP* 4.0 beta 10 (Swofford 2002) and rooted using the midpoint method. To assess the statistical significance of

internal branching, a thousand bootstrap replicates were performed.

Results

As cp23S rDNA shows length variation across *Symbiodinium* clades A, C and D (Oliver & Palumbi 2011), clades were first identified by visualization of band lengths on the agarose gels (compared with a standard 100 bp DNA ladder). In general, both clades C and D were detected from 45 *Sinularia* host samples collected before the 2010 coral bleaching event. Among 45 samples, three (6.67%) showed multiple bands on a gel, indicating mixed symbiont clades C and D within a single sample. Clade D was unique for Jorake Island ($n=16$, $C=0$, $D=16$) while clade C was dominant in offshore reefs of the Juang Islands ($n=29$, $C=20$, $D=6$, $C+D=3$, Fig. 2). Seven months after the bleaching event, only clade D was detected from both sampling sites off Jorake and Juang Islands (Fig. 2). When clade identities and frequencies were compared between pre and post-bleaching *Symbiodinium* communities, a significant change in composition of *Symbiodinium* clades from Juang Islands after the bleaching event was obvious (see Fig. 2).

Following analysis of partial cp23S rDNA sequence variations (66 sequences analyzed excluding 3 mixed clade samples), *Symbiodinium* clades C and D were assigned on the basis of highest identity with the respective cp23S rDNA *Symbiodinium* sequences in Genbank. *Symbiodinium* clade identities were consistent with the previous clade identification by cp23S length variation. Among the 66 cp23S rDNA sequences, 661 nucleotides in length (pre-bleaching $n=42$, post-bleaching $n=24$), 4 haplotypes were identified with 3 and 1 haplotypes belonging to clade D and clade C, respectively. The sequences of clade D and C haplotypes in this study were deposited in GenBank (accession numbers KJ801955–KJ801958). One clade C haplotype obviously differed from other haplotypes of clade D in 168 nucleotide substitutions mostly derived from insertions or deletions (indels). In contrast, nucleotide variation within clade D was low, with indels of only 1 bp. Before the bleaching event, clade C and D haplotypes were detected at comparable frequencies (relative frequencies: clade C haplotype=0.452; clade D haplotype=0.500), although after the bleaching event only clade D haplotypes were apparent. In addition, the *Symbiodinium* population of before the bleaching event was genetically different from the post-bleaching population ($F_{ST}=0.37$, $P<0.00001$; no. of permutations=1,023).

A neighbor-joining phylogenetic tree based on 4 haplotypes of symbiont clades C and D in this study and 16 cp23S rDNA sequences of symbiont clades C and D from various coral hosts deposited in GenBank is shown in Figure 3. Phylogenetic analysis revealed differentiation of 2 genetically distinct clades C and D. Each haplotype identified from this study was clustered within its own clade. The clade D cluster was strongly supported by bootstrap

Fig. 2. Frequencies of *Symbiodinium* clades (C, D and a mixture of C and D) inhabiting a soft coral host, genus *Sinularia* from Jorake and Juang Islands (Sattahip, Chonburi Province). The frequencies are compared between the two monitoring periods: (a) before the 2010 coral bleaching event and (b) 7 months after the bleaching event. Size of pie charts is in proportion to the sample size.

values (see Fig. 3).

Discussion

The present study revealed a significant change in composition of *Symbiodinium* clades inhabiting *Sinularia* hosts

from the Juang Islands after the 2010 coral bleaching event. Before the bleaching event, 68.97% of the randomly obtained samples harbored *Symbiodinium* clade C, while the remainder harbored clade D and a mixture of clades C and D (Fig. 2). After the bleaching event, surviving samples retained only heat-resistant clade D *Symbiodinium*.

Fig. 3. A neighbor-joining cladogram of the relationships of 4 *Symbiodinium* clade C and D haplotypes in this study and 16 cp23S rDNA sequences of symbiont clades C and D from various coral hosts deposited in GenBank. The tree was rooted using the midpoint method. Numbers above internal branches are bootstrap values based on 1,000 replicates. Data from this study are in bold and provided with species names of the coral hosts (*Sinularia* spp.) followed by symbiont clades and GenBank accession numbers (KJ801955-KJ801958). For the reference data, species names of the coral hosts are followed by symbiont clades and GenBank accession numbers.

Loss of clade C or an increase of clade D after bleaching could possibly be explained by differential mortality of hosts harboring sensitive symbiont types (clade C) or symbiont changes within soft coral colonies (*Symbiodinium* shuffling or switching). Symbiont shuffling refers to changes in the relative abundance of each *Symbiodinium* clade/subclade under the influence of the environment. This change in symbiont community supports the Adaptive Bleaching Hypothesis (ABH) of Buddemeier & Fautin (1993) and Buddemeier et al. (2004), in which coral hosts switch or shuffle their thermally sensitive *Symbiodinium* for more tolerant clades/subclades during bleaching. How-

ever, this change may come with coral fitness trade-offs such as lower skeletal growth (Jones & Berkelmans 2010) and loss of photosynthetic efficiency (Rowan 2004).

Several other studies have also shown increases in the abundance of *Symbiodinium* clade D in coral hosts following bleaching events (Baker et al. 2004, Rowan 2004, Berkelmans & van Oppen 2006, LaJeunesse et al. 2007, Jones et al. 2008). The field evidence of changes in *Symbiodinium* communities in tagged colonies of *Acropora millepora* before and after a natural bleaching event in 2006 in the Keppel Islands on the southern Great Barrier Reef (sGBR) suggested both symbiont shuffling and differential

mortality as explanations for the changes in symbiont communities (Jones et al. 2008). However, another field observation in tagged colonies of *Stylophora pistillata* at Heron Island (sGBR) supported differential mortality of hosts harboring thermally sensitive symbionts rather than symbiont shuffling within a single host colony (Sampayo et al. 2008). In the present study, none of the colonies of *Sinularia* was tagged, so sampling before and after the 2010 coral bleaching event was random. As a consequence of this random sampling of host colonies, neither differential mortality nor symbiont shuffling explanations of the change in *Symbiodinium* communities after bleaching can be ruled out.

There was no change in *Symbiodinium* community from Jorake Island after the bleaching event as *Sinularia* hosts normally harbored only heat-tolerant *Symbiodinium* clade D at inshore locations (Panithanarak 2012). Jorake Island is close to Nang-Rong Beach (Fig. 1) while the Juang Islands are farther out at approximately 7–8 nautical miles (13–15 km) from the coast. Therefore, stressful environments in Jorake Island (e.g. turbid sea water and large tidal exchanges) influence the abundance and evolutionary success of clade D over clade C. Several studies indicate that clade D *Symbiodinium* is most common in coral hosts from inshore locations with turbid environments (van Oppen et al. 2005, Mostafavi et al. 2007, LaJeunesse et al. 2010).

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