F-5.1.3 Studies on genetic diversity of corals (Final Report)

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Abstract The objective of this study is to investigate genetic diversity and species diversity of hermatypic corals and their intracellular symbionts, zooxanthellae. We analyzed internal transcribed spacer (ITS) region of rRNA gene of corals and zooxanthellae. The length of ITS region was different among species of corals studied. ITS region was useful for phylogenetic study of corals at least at generic level. ITS1 sequences of zooxanthellae, however, did not have enough information: most sequences formed an unresolved cluster. SSCP studies of LSUrRNA gene showed that a coral colony contained zooxanthellae of 2-3 genotypes and that genotypic composition of zooxanthellae might be influenced by light condition. An active form mariner with a complete open reading frame (ORF) for transposase was found in *Fungia* sp. The mariner-like sequence was very similar to the mariner found in the Emperor moth (Yonaguni –san).

1. Introduction

Corals of the same species may display different growth forms depending on their habitat. Colonies with different growth forms are considered to belong to the same species if there are colonies with intermediate growth forms. However, recent molecular studies sometimes showed that certain species are actually species complex and include cryptic species.

Zooxanthellae, intracellular symbionts of marine invertebrates, have been thought to be a single species. However, recent morphological, physiological, biochemical and molecular studies have revealed that zooxanthellae consist of many different species. Corals of the same species sometimes contain different genotypes of zooxanthellae depending on depth or light intensity. Even different part of a single colony may contain different genotypes of zooxanthellae. Zooxanthellae of different genotypes are considered to have different stress susceptibility. Some researchers consider bleaching as an opportunity for coral hosts to acquire zooxanthellae more fitted to their habitat.

2. Research Objectives

The length of ITS region of nuclear rRNA gene was different among some species[2), 3)]. Phylogenetic analyses of corals based on ITS sequences were performed only with *Porites*[4)] and *Acropora* [5)]. Recently Medina et al. (1999) claimed that the three recently distinguished species of *Montastrea annularis* complex belonged to the same species based on ITS sequence analysis[6)]. Coral specific primer for ITS region has been designed[7)], but the primer seems not applicable to all coral species.

The purpose of the present study was to examine whether ITS can be used for phylogenetic study of corals at various taxonomic levels and whether ITS can be used to distinguish closely related species of corals.

Darius et al. (1998) classified zooxanthellae derived from 7 species of corals into five types based on RFLP patterns of SSUrRNA[8)]. They found that five corals harbor only one type of zooxanthellae while other two species harbor at least two types of zooxanthellae. ITS and LSUrDNA may have more informative variation than SSUrDNA. However, only a few works have been done on LSUrDNA or ITS region of zooxanthellae [4), 9)].

In this study we performed RFLP analysis of SSUrRNA gene and SSCP analysis of LSUrRNA gene to examine genetic diversity of zooxanthellae in Okinawan corals and to examine whether light condition affects genotypic composition of zooxanthellae in coral hosts.

In some hermatypic corals, zooxanthellae are transmitted vertically to offspring from their parent. In other corals offspring have to acquire symbionts from the environment. To test that hypothesis that corals that vertically transmit symbionts from their mother colonies contain species-specific zooxanthellae, while corals that acquire symbionts from the environment contain locality-dependent zooxanthellae. We analyzed the internal transcribed spacer 1 (ITS1) region of algal nuclear ribosomal DNA to study the phylogenetic relation ships of zooxanthellae contained in shallow reef corals from Okinawa, Hawaii and Thailand.

We also attempted to find *mariner*-like elements in corals and zooxanthellae. *Mariners* are transposable elements. Phylogenetic relationships of *mariner*-like sequences may provide some interesting information on phylogeny of corals and zooxanthellae and on the evolution of symbiosis between them.

3. Research Methods

Coral ITS regions were amplified from genomic DNA extracted from coral sperm using universal ITS primers, ITS4 and ITS5. The PCR products were directly sequenced after purification using ALFexpress autosequencer. The primer sets ITS5/ITS3 and ITS3/ITS4 were used for cycle sequencing.

Algal 18S rDNA was amplified using the algae-specific primers (Rowan and Powers, 1991). RFLP analysis was performed on the amplified products by digestion with *Taq*I restriction

enzyme for 90-120 min at 65 °C.

The D1/D2 domains of algal LSUrDNA were amplified using an algal specific primer and the complimentary primer. SSCP analysis was performed using 20 ul of PCR product mixed with an equal volume of loading buffer (95% formamide, 10 mM NaOH, 0.25% bromophenol blue and 0.25 % xylene cyanol) and denatured for 5 min at 95°C. After denaturing the mixed aliquots were immediately chilled on crushed ice and electrophoresed. The SSCP patterns were stained with ethidium bromide and visualized with UV transillumination.

Algal ITS1 regions were amplified by PCR using zooxanthellae-specific primers [4], and PCR products were directly sequenced after purification. In each case, one, presumably dominant, symbiont genotype was analyzed for each colony.

Genomic DNA was isolated from a Fungia sp. A *mariner*-like sequence was amplified by PCR using degenerate primers designed from consensus amino acid sequence of *mariners*. Then *mariner*-like sequence was amplified by long PCR using the inverted terminal repeat of *H. cecropia mariner*-like elements. The PCR product corresponding to 1.2 kb was purified, subcloned and sequenced.

4. Results and Discussion

The length of ITS region was different among species of corals studied. ITS sequences were determined for 7 coral species. We constructed phylogenetic trees using our data together with the sequences registered in DNA database. ITS was useful for phylogenetic study of corals at least at generic level.

RFLP studies of SSUrDNAS showed that zooxanthellae from *Stylophora pistillata* (n=3), *Seriatopora hystrix* (n=1), *Seriatopora caliendrum* (n=4) and *Pocillopora damicornis* (n=1) were all type C. A branch of *S. hystrix* acclimated to 8% PAR for three months contained type A in addition to smaller amount of type C. The hydrocoral *Millepora intricata* contained type C zooxanthellae in addition to smaller amount of type A. Cultured zooxanthellae from two bivalves, *Fragum fragum* and *Hippopus hippopus* were type A.

At least fifteen distinct SSCP patterns were derived from denatured SD PCR products from the nine coral species studied (Fig. 1). A single genotype of zooxanthellae would produce two bands corresponding to each of double strands in SSCP analysis. Each sample produced 3-5 bands, suggesting that each coral colony contained zooxanthellae of 2-3 genotypes. Variations of the SSCP patterns observed between coral colonies could be attributed to genetic polymorphism of rDNA within the same genotype. However, variations of the SSCP patterns were also detected between branches isolated from the same colony and kept at different light conditions. This suggests that some colonies contained zooxanthellae of distinct genotypes and that genotypic composition of zooxanthellae was influenced by light condition.

ITS1 sequences of zooxanthellae did not have enough information for phylogenetic analysis: most sequences formed an unresolved cluster (Fig. 2). However, zooxanthellae from

Thai corals were included in one cluster. In *Galaxea* and *Pavona*, zooxanthellae from Okinawan colonies and those from Thai colonies did not form a cluster, suggesting that they are locality-dependent. Phylogenetic relationship of zooxanthellae did not reflect the phylogeny of host corals even in corals that transmit symbionts from their mother colonies. Symbiont identity appeared to depend on the locality where host corals were collected rather than the specific identity of the coral host or its mode of acquisition. This suggests that corals that receive zooxanthellae from their mother colonies may also acquire symbionts from the environment.

A single band of the expected 500 bp size was obtained from *Fungia* spp using degenerate PCR primer derived from *mariner* consensus amino acids sequence[11] . When PCR were performed using the terminal inverted repeats of *H. cecropia mariner* as primers, a single band about 1.2 kb was obtained. The *mariner*-like sequence isolated from the coral *Fungia* sp. was very similar to the *mariner* found in the Emperor moth (Yonaguni –san). Both *mariner*-like sequences had a complete open reading frame (ORF) for transposase (Fig. 3), hence are considered as active form *mariner*. *Mariner*-like elements were also amplified by PCR from DNA extracted from *Montipora* sperm. This suggests that the *mariner*-like element was derived from corals and not from zooxanthellae. The *mariner*-like sequences of several Okinawan corals were electrophretically indistinguishable, while *mariner*-like sequence from Hawaii corals were different from that of Okinawan corals. It is likely that *mariners* of corals are locality-dependent.

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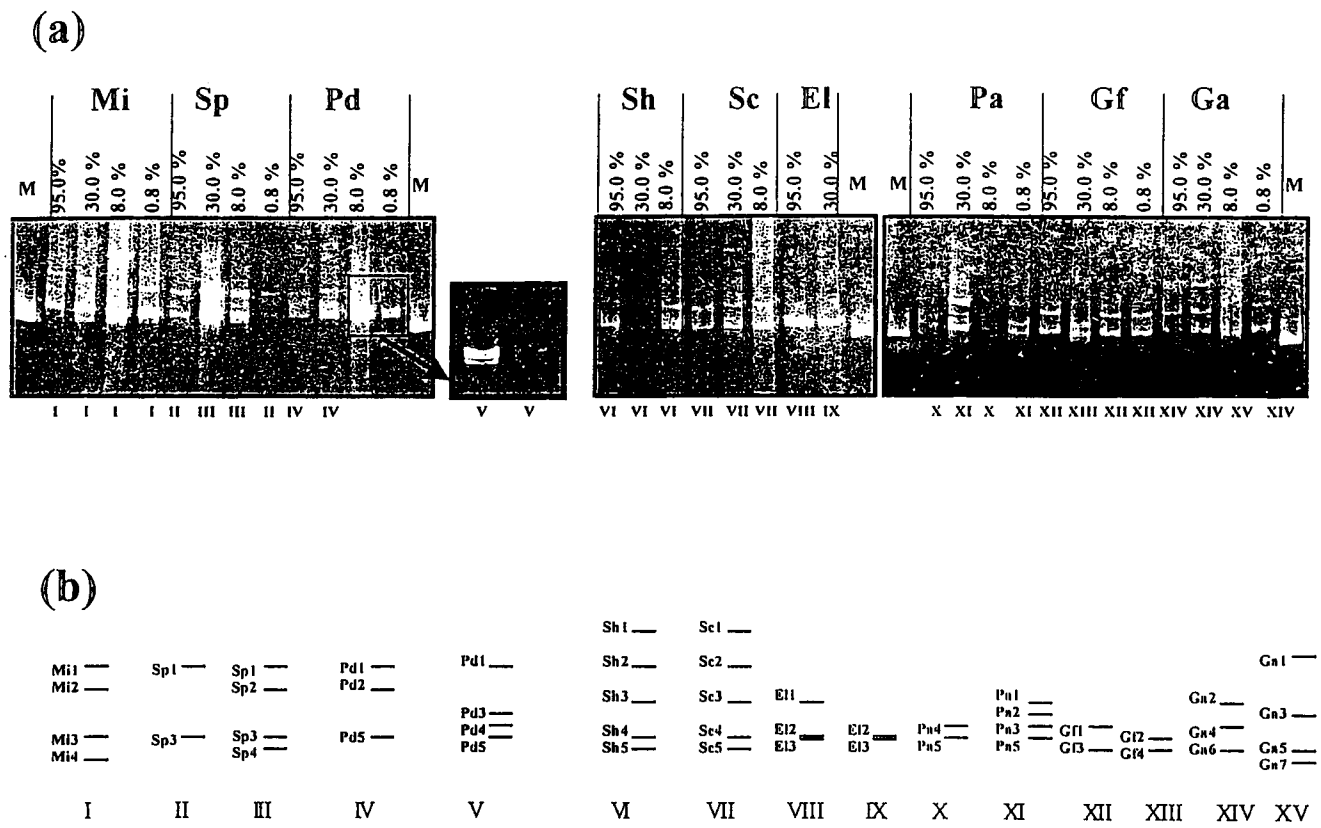

Fig. 1. (a) SSCP patterns of LSUrDNA of zooxanthellae from eight scleractinian coral and one hydrocoral species. Branches of the same colony of each species were kept at 8, 30, and 95% PAR for two months. (b) Diagrammatic representation of the SSCP patterns. Mi, *Millepora intricata*; Sp, *Stylophora pistillata*; Pd, *Pocillopora damicornis*; Sh, *Seriatopora hystrix*; Sc, *Seriatopora caliendrum*; El, *Echinopora lamellina*; Pa, *Porites attenuata*; Gf, *Galaxea fascicularis*; Ga, *Goniastrea aspera*.

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DNASIS     Translation  Editor  [sanngoÅ[TP(  ORF  )]                2000.06.12
         1 ATGGAAAACTTGAAATATCGCGTTATTTACGAGTATGAGTTCCACCGTGGCACCAGTGCT       60
       1 M  E  N  L  K  Y  R  V  I  Y  E  Y  E  F  H  R  G  T  S  A       20

        61 GCAGAAACGGCTCGAAGGATTAATGATGTGTATGGCGGCGGTGTGACAAAAGAAAACACA      120
      21 A  E  T  A  R  R  I  N  D  V  Y  G  G  G  V  T  K  E  N  T       40

       121 GTGCGTTTTTGGTTCCAACGTTTTCGTTCTGGAAATTTCGACCTCCGAAACAAGCCCCGT      180
      41 V  R  F  W  F  Q  R  F  R  S  G  N  F  D  L  R  N  K  P  R       60

       181 GGACGGCCGGATACCAAAGTTGATAATGAAGAATTGAAGGCTATTGTGGAAGCGGATCCA      240
      61 G  R  P  D  T  K  V  D  N  E  E  L  K  A  I  V  E  A  D  P       80

       241 TCGCAAACCACGCCCGAGTTAGCTGCAGGCTGCGGTGTTAGTGACAAAATTGTTTTAATC      300
      81 S  Q  T  T  P  E  L  A  A  G  C  G  V  S  D  K  I  V  L  I      100

       301 CATTTGAAGCAAACTGGGAAGGTTAAAAAGCTCGAAAGGTGGGTACCTCATGAATTAAGT      360
     101 H  L  K  Q  T  G  K  V  K  K  L  E  R  W  V  P  H  E  L  S      120

       361 GAAACAAACCGACAAACGCGTGTCGACTGTTGCGTTACATTACTCAACCGGCACAACAAT      420
     121 E  T  N  R  Q  T  R  V  D  C  C  V  T  L  L  N  R  H  N  N      140

       421 GAAGGGATTTTAAATCGAATCATTACGTGTGATGAAAAATGGATCCTGTACGATAATCGG      480
     141 E  G  I  L  N  R  I  I  T  C  D  E  K  W  I  L  Y  D  N  R      160

       481 AAACGCTCATCGCAATGGCTGAACCCTGGCGAACCAGCCAAATCCTGCCCTAAGCGAAAA      540
     161 K  R  S  S  Q  W  L  N  P  G  E  P  A  K  S  C  P  K  R  K      180

       541 TTGACTCAAAAAAAGTTACTTGTGAGCGTTTGGTGGACTAGTGCCGGTGTCGTCCACTAC      600
     181 L  T  Q  K  K  L  L  V  S  V  W  W  T  S  A  G  V  V  H  Y      200

       601 AGCTTTCTTAAATCCGGCCTGACAATTACGGCAGATGTCTATTGTCAACAACTGCAAGTC      660
     201 S  F  L  K  S  G  L  T  I  T  A  D  V  Y  C  Q  Q  L  Q  V      220

       661 ATGATGGAAAAGCTAGCTGCTAAACAACCGAGGCTGATCAATCGCTCTAGGCCACTACTG      720
     221 M  M  E  K  L  A  A  K  Q  P  R  L  I  N  R  S  R  P  L  L      240

       721 CTTCATGACAATGCTAGACCACACACTGCACAACAGACGGCTACTAAATTAGGGGAGCTT      780
     241 L  H  D  N  A  R  P  H  T  A  Q  Q  T  A  T  K  L  G  E  L      260

       781 CAATTGGAATGTCTAAGACATCCACCGTATTCCCCGGATCTTGCTCCAACTGATTACCAC      840
     261 Q  L  E  C  L  R  H  P  P  Y  S  P  D  L  A  P  T  D  Y  H      280

       841 TTTTTTCGTAATTTGGATAACTTTTTGCAAGGGAAAAAATTCAACTCCGATGGGGCAGTC      900
     281 F  F  R  N  L  D  N  F  L  Q  G  K  K  F  N  S  D  G  A  V      300

       901 CAAACCGCCTTCAAAGATTTTATTGATTCCCGTCCGAATAGTTTTTTTTAG               951
     301 Q  T  A  F  K  D  F  I  D  S  R  P  N  S  F  F  *                 317
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Fig. 3. Nucleotide and amino acid sequences of a *Fungia* sp. *mariner*-like element. The open reading frame from ATG (M) to TAG (*) contains 317 amino acid residues.

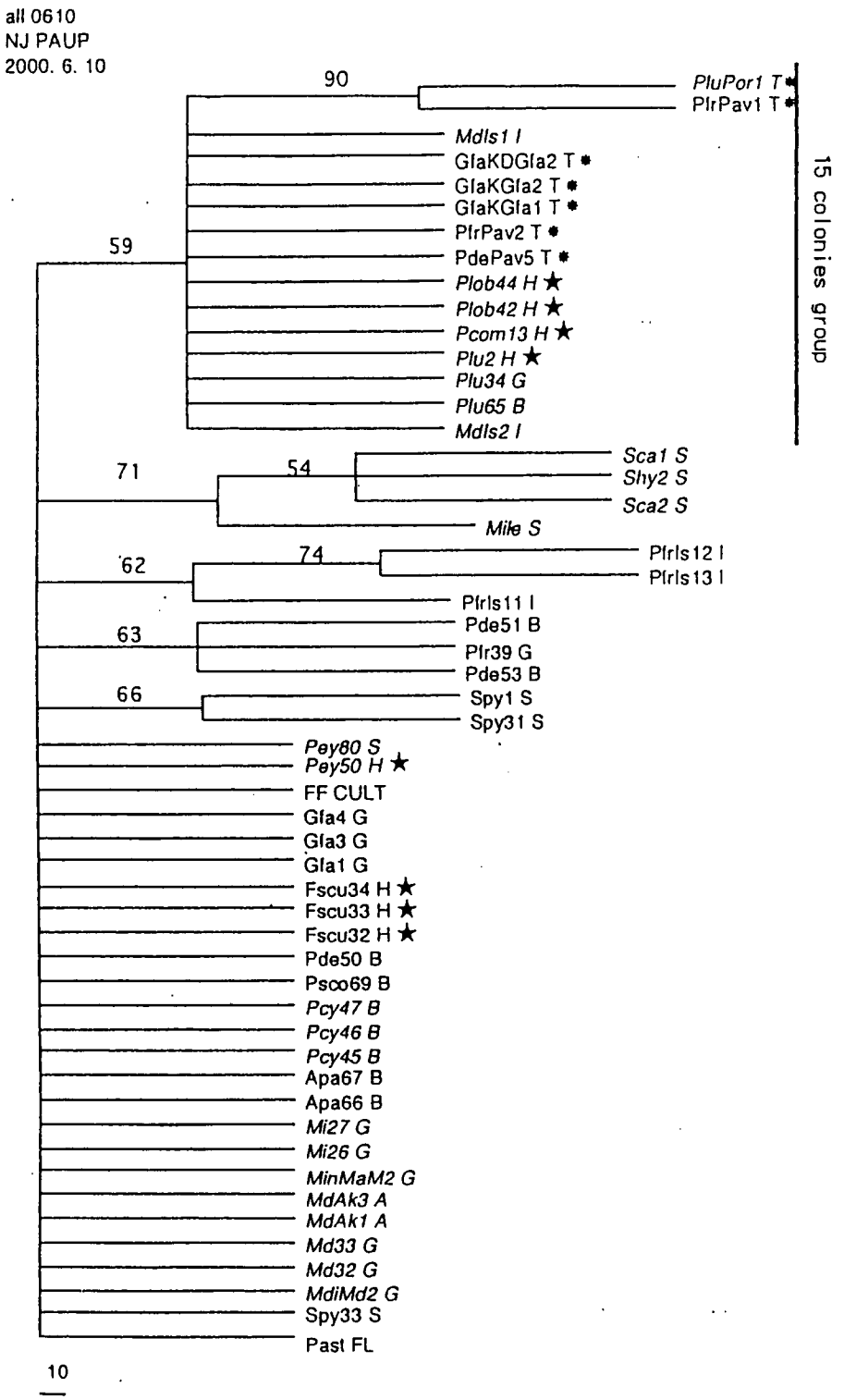

Fig. 2. Neighbor-joining (NJ) dendrogram of zooxanthellae derived from various hosts collected from Okinawa, Hawaii and Thailand based on zooxanthella ITS1 sequence data. Numbers above branches are bootstrap proportions of the 1,000 pseudoreplications. Letters represent host species and site of collection. H, Hawaii, T, Thai, I, Ishaki island, others are Okinawa island. Apa, *Acropora palifera*; Fscu, *Fungia scutaria*; Gfa, *Galaxea fascicularis*; Md, *Montipora digitata*; Mile, *Millepora intricata*; Min, *Montipora informis*; Past, *Porites astreata*; Pcom, *Porites compressa*; Pcy, *Porites cylindrica*; Pde, *Pavona decussata*; Pey, *Pocillopora eydouxi*; Pfr, *Pavona frondifera*; Plo, *Porites lobata*; Plu, *Porites lutea*; Psco, *Psammocora contigua*; Sca, *Seriatopora caliendrum*; Shy, *Seriatopora hystrix*; Spy, *Stylophora pistillata*.