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Genetik Uniformity of Widely Separated Population of Coral *Acropora aspera* from Karimunjawa and Panjang Island Waters Revealed by Partial Sequence of Internal Transcribed Spacer-4 Regions

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Abstract

It is well known that coral species are broadly dispersed across the tropical and subtropical Indo Pacific. Unfortunately, there is little information about the genetic connectivity between coral populations separated by large distance. Variability in the nucleotide sequence of the internal transcribed spacer-4 (ITS-4) of the nuclear ribosomal gene in coral Acropora aspera was analyzed. Two populations of corals from Karimunjawa and Jepara were investigated. Sequencing analysis of ITS region of rDNA gene showed that there is closely related between parental of A. aspera Karimunjawa and Jepara. This relation suggest that presumably A. aspera population in Jepara was originated from Karimunjawa through genetic flow.

Key words: *Acropora aspera, gene flow, ITS-4*

Abstrak

Telah kita ketahui dengan baik bahwa kaarang hidup tersebar dari daerah tropis sampai subtropis di Indo Pasifik. Namun malangnya informasi tentang hubungan secara genetic antar populasi terumbu karang yang dipisahkan oleh jarak tersebut sangat kurang. Variasi genetic karang Acropora aspera telah dianalisa dengan internal transcribed spancer-4 (ITS-4). Analisa dilakukan terhadap dua pupulasi karang yang berasal dari Karimunjawa dan Jepara. Hasil sequencing dengan ITS-4 menunjukan bahwa diantara meraka terdapat hubungan yang dekat baik yang ada di Karimunjawa dan Jepara. Dari hubungan tersebut dapat diasumsikan bahwa populasi A. aspera yang ada di Jepara berasal dari Karimunjawa bila dillihat sebaran genetis.

Kata kunci: *Acropora aspera, sebaran genetis, ITS-4*

Introduction

Coral reefs are the most species-rich environments in the oceans. Reefs cover 0.2% of the ocean's area and yet they provide home to one-third of marine fishes and to tens of thousands of other species. Coral reefs provide essential fish habitat, support endangered and threatened species, and harbor protected marine mammals. Despite the obvious ecological value of these habitats, most coral reefs around the world, including Indonesia's, are threatened or already being destroyed by human activities. It has been estimated that more than 50% of the world's reefs are affected by human activities, which include coastal development, destructive and over-fishing practices, over-exploitation of marine resources, pollution and terrestrial run-off due to the deforestation associated with extensive agricultural exploitation. Ridgway et al. (2001) stated that to recover of coral populations from disturbance depend on a number of factors, for example larval supply and recruitment. Therefore, the science of gene flow and larval connectivity between neighboring and distant population is urgently needed to know how coral reefs might deal with the disturbance.The main determinants of the genetic connectivity between populations of several marine organisms is the extent to which larvae from two or more populations are transported or exchanged. The distribution of larvae is influenced by the biological and physical characteristics, for example larval behaviour, length of larval life and oceanic current (Palumbi, 1994). Recently, several studies have focused on the relationship between the length of larval competency period and the distance that marine invertebrate larvae distributed. Takabayashi et al. (2003) conducted the study of genetic variation on coral *Stylophora pistillata* across the Western Pasific Ocean showed that high levels of connectivity across

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the species' latitudinal distribution range in the western Pacific, as is seen in many marine invertebrates.

Schleractinian corals are physiologically and morphologically various within species (Takabayashi and Hoegh-Guldberg, 1995). They have complex population structures (Veron, 1995). Genetic analyses of marine population structure often find only slight geographic differentiation in species with high dispersal potential (Palumbi, 2003). In addition, examination of genetic isolation by distance, in which close populations are more similar than distant ones, has the potential to increase confidence in the significance of slight genetic differentiation. DNA-based techniques which have the potential to further our understanding of the genetic structure of coral populations develop so fast recently (Avise, 1994). The polymerase chain reaction (PCR) of coral DNA using specific primers has the potential to overwhelm the symbiont's nucleic acid. Romano and Palumbi (1996) stated that coral-specific primers have been used successfully in amplification of mitochondrial DNA, also the ribosomal intergenic spacer (ITS) region (Smith et al., 1997).

This study reported the genetic connectivity of reef-building corals using the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) to analyze the genetic uniformity of coral *A. aspera* at distances that span tens of kilometers (Karimunjawa - Jepara).

Materials and Methods

Collection of samples

Colonies of the coral Acropora millepora were collected at Karimunjawa and Panjang Island waters. A small fragment approximately 15 cm in diameter for parent colony and 3 cm in diameter for filial colony from 3 colonies of *A. millepora* was collected at each reef. Coral colonies collected were at least 20 m away from each other to prevent the likelihood.

Extraction, PCR amplification and Sequencing of DNA

Tissues of corals were immersed into Ethanol-chaos (4M Guanidin thiocyanate 100 gr, 0.5% sarkosyl 1 gr, 2.5 mM Tris, pH 8 1 M stock, 0.1 M 2-mercaptoethanol 1.5 ml, diluted water until 200 ml). DNA from the samples was extracted by incubating the Ethanol-chaos into CIAA for 3 minute in room temperature. DNA was then precipitated in cold alcohol 95% twice of volume, incubated on -20^0 C for 30 minute. Alcohol 70% was used to precipitated DNA, put on laminar airflow for 40 minute to dry up DNA. Then, the DNA was resuspended on TE buffer, stored at -20^0 C. All PCRs consisted of 1 µl DNA, 1.5 µl primer ad 20 µl ddH2O. The complementary universal primer ITS-4 (TCCTCCGCTTATTGATATGC) and ITS-5 (GGAAGTAAAAGTCGTAACAAG) were used to amplify DNA coral. Amplifications were performed in Biorad Thermal Cycler as follow: 30 cyclus of 4 min at 94^{0}C, 1 min 94^{0}C, 2 min at 55 ^{0}C, 30s at 72 ^{0}C, 10 min 72^{0}C. Amplified DNA was purified using Wizard PCR Preps DNA Purification System and directly sequenced with 310 Genetic Analyzer.

Sequen data Analysis

Sequences spanning the complete ITS-4 region were aligned using CLUSTAL W, and resulting alignment. Phylogenetic analyses were performed by Tree-Con Program.

Resuls and Discussion

The DNA PCR-amplification of parental and filial branching coral *A. aspera* were presented in Figure 1

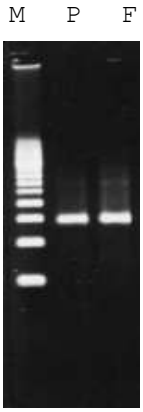

Figure 1. PCR-DNA Amplification of ITS-4 region (M: DNA marker; P: Parent; F: Filial)

A PCR primer that specifically amplifies the ITS region of coral rDNA from the tissues of adult colonies (parental) and young colonies (filial) when paired with universal primer. Takabayashi *et al.* (1998) stated that ITS sequence analysis can be used not only interspecific but also intraspecific genetic comparison for some coral species.

Table 1. The homology analyses of parental sequence coral *A. aspera*

```
>>EM_INV:AF538514 AF538514.1 Acropora aspera isolate   (346 nt)
rev-comp initn: 1091 init1: 727 opt: 1203  Z-score: 742.7  bits: 145.9
E(): 2.7e-33
banded Smith-Waterman score: 1203;  89.873% identity (93.115% ungapped)
in 316 nt overlap (362-48:11-319)

                360       350       340       330       320
Seque-          CNNTTGTGAGAATGGTAGCACACTCCCNTCTATCTCGCCCTATACATACT
                :  ::::: ::::::::: :::::::: :  :: :::  : :::::::::
EM_INV CATCATCGATCGCTTGTGTGAATGGTAGTACACTCCCATTGATATCGAACGATACATACT
             10        20        30        40        50        60

       310       300       290       280       270       260
Seque- ATAGACACACGAGAGAGACTCTGCACGGTGAATCTCTCGGCTCGCGCATCGATGAAGAAC
       ::::: ::::::::::::::::::::::::::::::::::::::::::::::::::::
EM_INV ATAGAAACACGAGAGAGACTCTGCACGGTGAATCTCTCGGCTCGCGCATCGATGAAGAAC
              70        80        90       100       110       120

       250       240       230       220       210       200
Seque- GCAGCCAACTGCGACAGACGTAGTGTGAATTGCAGATCCGATCGTCGATTCTTTGAACGC
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
EM_INV GCAGCCAACTGCGACAGACGTAGTGTGAATTGCAGATCCGATCGTCGATTCTTTGAACG-
             130       140       150       160       170

       190       180       170       160       150       140
Seque- CAAAAGGCGCTCGTCTCTTGCGAGGCGAGCAAGGCTGTCCGAGCGTCCCTTTGCTTCTAC
       :::: ::::::::::::::::::::::::::::::::::::::::::::::::::::::
EM_INV CAAATGGCGCTCGTCTCTTGCGAGGCGAGCAAGGCTGTCCGAGCGTCCCTTTGCTTCTA-
     180       190       200       210       220       230

       130       120       110       100        90        80
Seque- CCCAGTTACCACGGGTGAGTTGGAGTAGTCGCCGGCCCTGCCCTGCGAATCGCTAGGCCC
       :::::::: ::::::::::::::::: :::::  : :::: :::::::::::  :: ::
EM_INV CCCAGTTA-CACGGGTGAGTTGGAGTGGTCGC--GGCCTG-CCTGCGAATCGCGTGG-CC
      240       250       260         270       280       290

        70         60        50        40        30        20
Seque- GCTTTTTCT-AATAGAGAGACCGGATGCGCCACCCCCCCCCNCCCNCCCNNCNCAGNTTN
       :: :  ::: :: :::  ::::: ::
EM_INV GCGTCCTCTAAAGAGAAGGACCGAATTTTACAAAACTATAACGTAGTTTATGA
          300       310       320       330       340
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The ITS4/ITS5 primer pair produced a single fragment from young and adult coral tissues. These did not vary in size between young and adult colonies (346-349 nucleotide). The length of the ITS-4 sequence of the coral *A. aspera* obtained (submitted to Genbank; accession numbers: AF538514 and AF538503) varied between 346 and 349 bp. The homology analyses of sequence were presented in Table 1 and 2. Both adult colonies from Karimunjawa and young colonies from Jepara are closely related with *A. aspera*. Chen *et al*. (1996) reported that the ITS region is highly variable and thus suitable for comparative studies of closely related species and population. In this study, the coral *A. aspera* of Karimunjawa's parents is almost similar to that of Panjang Island's young colonies in the length of DNA.

Table 2. The homology analyses of parental sequence coral *A. aspera*

```
>>EM_INV:AF538503 AF538503.1 Acropora aspera isolate   (349 nt)
rev-comp initn: 1227 init1: 1227 opt: 1292  Z-score: 884.4  bits: 172.0
E(): 3.5e-41
banded Smith-Waterman score: 1292;  97.794% identity (98.155% ungapped)
in 272 nt overlap (319-48:71-341)

                                    320       310       300
Seque-                                 GAGAGAGACTATACACGGTGAATCTCTCGG
                                       :::::::::: : ::::::::::::::::
EM_INV GATATCGAACTATTTATACTATAGAAACACGAGAGAGACTCTGCACGGTGAATCTCTCGG
               50        60        70        80        90       100

    290       280       270       260       250       240
Seque- CTCGCGCATCGATGAAGAACGCAGCCAACTGCGACAGACGTAGTGTGAATTGCAGATCCG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
EM_INV CTCGCGCATCGATGAAGAACGCAGCCAACTGCGACAGACGTAGTGTGAATTGCAGATCCG
              110       120       130       140       150       160

    230       220       210       200       190       180
Seque- ATCGTCGATTCTTTGAACGCAAATGGCGCTCGTCTCTTGCGAGGCGAGCAAGGCTGTCCG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
EM_INV ATCGTCGATTCTTTGAACGCAAATGGCGCTCGTCTCTTGCGAGGCGAGCAAGGCTGTCCG
              170       180       190       200       210       220

    170       160       150       140       130       120
Seque- AGCGTCCCTTTGCTTCTACCCAGTTACATGGGTGAGTTGGAGTGGTCGCGGCCTGCCTGC
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
EM_INV AGCGTCCCTTTGCTTCTACCCAGTTACATGGGTGAGTTGGAGTGGTCGCGGCCTGCCTGC
              230       240       250       260       270       280

    110       100        90        80        70        60
Seque- GAATCGCTTGGCCGCGTCCTCTAAAGAGAAGGACCGAATCTTACGAAACTTATGACGTAG
       :::::::::::::::::::::::::::::::::::: :::: :::: ::: ::::::
EM_INV GAATCGCTTGGCCGCGTCCTCTAAAGAGAAGGACCGAATTTTACAAAAC-TATAACGTAG
              290       300       310       320        330

     50        40        30        20        10
Seque- TTGTGTTCGGGACTTCGGGTTCGTGCCTCGGGTCATCCTGTCGTCCCTA
       ::
EM_INV TTTATGT
     340
```

Veron (1995) stated that species of reef-building corals typically have very broad distribution ranges. It is important to interpret the population ecology of these species ranges for understanding coral reef ecosystems, even difficult given the highly variable local environments spanning the large distances and complex underlying factor. Prior studies on *Pocillopora verrucosa* in South Africa (Ridgway *et al.*, 2001) and *Pocillopora damicornis* in southwestern Australia (Stoddart,1984) have found high recent connectivity and low genetic variance between sites located across relatively small geographic scales. Lack of genetic divergence over large distances while each population is divergent is a common biogeographic pattern in marine invertebrates (Takabayashi *et al.*, 2003). They showed that gene flow among coral populations separated by thousands of kilometers is sufficient to prevent the accumulation of a substantial number of fixed genetic difference.

Conclusion

Sequencing analysis of ITS region of rDNA gene showed that there is closely related between parental (adult) colonies of *A. aspera* Karimunjawa and filial (young) colonies of Jepara. This relation suggest that presumably *A. aspera* population in Jepara was originated from Karimunjawa through genetic flow.

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References

Avies, J.C. 1994. Molecular markers, natural history and evolution. Chapman & Hall, London.

Chen, C.A., B.L. Willis and D.J. Miller 1996. Systematic relationships between tropical corallimorpharians (Cnidaria : Anthozoa : Corallimorpharia) utility of the 5.8S and internal transcribed spacer (ITS) regions of the rRNA transcription unit. *Bulletin of Marine Science*, 59 (1): 196-208

Palumbi, S.R. 1994. Genetic divergence, reproductive isolation and marine speciation. *Annu. Rev. Ecol Syst*, 25: 547-572

Palumbi, S.R. 2003. Population genetics, demographic connectivity, and the design of marine reserves. *Ecol. Appli*, 13 (1): S146-S148

Ridgway, T., O. Hoegh-Guldberg and D.J. Ayre 2001. Panmixia in *Pocillopora verrucosa* from South Africa. *Mar. Biol*, 139 (1): 175-182

Romano, S.L. and S.R. Palumbi 1996. Evolution of scleractinion corals inferred from molecular systematics. *Science*, 271: 640-642

Smith, C., C.A. Chen, H-P. Yang and D.J. Miller 1997. A PCR-based method of assaying molecular variation in corals based on RFLP analysis of the ribosomal intergenic spacer region. *Mol. Ecol.* 6: 683-685

Stoddart, J.A. 1984. Genetic differentiation amongst populations of the coral *Pocillopora damicornis* of southwestern Australia. *Coral Reefs.* 3:149-156

Takabayashi, M. and O. Hoegh-Guldberg 1995. Ecological and physiological differences between two colour morphs of the coral *Pocillopora damicornis*. *Mar. Biol* 123: 705-714

Takabayashi, M. D.A. Carter, W.K.W. Loh and O. Hoegh-Guldberg 1998. A coral-specific primer for PCR amplification of the internal transcribed spacer region in ribosomal DNA. *Mol. Ecol*. 7: 928-930.

Takabayashi, M., D. Carter, S. Ward and O. Hoegh-Guldberg 2003. Inter- and intra-specific variability in ribosomal DNA sequence in the internal transcribed spacer region of corals. In Proc. Of the Australian Coral Reef Society 75th Anniversary Conference. The University of Queensland, Brisbane: 241-248.

Veron, J.E.N. 1995. Coral in space and time: The biologeography and evolution of the scleractinia. University of New South Wales Press, Sydney.