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Original paper

DIVERSITY OF CULTURABLE BACTERIAL COMMUNITY ASSOCIATED WITH THE CORAL *Galaxea fascicularis* FROM UJUNG KULON, INDONESIA

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ABSTRACT

Coral reefs are the most diverse marine ecosystems; however, little is known about their microbial diversity in these ecosystems. The present study is aimed at investigating the general insights into the diversity of the bacterial community associated with the coral Galaxea fascicularis. A culture collection of 45 bacteria associated with coral G. fascicularis from Ujung Kulon, Indonesia was established by plating on Zobell's 2214E. Isolates were screened by means of RLFP and sequencing of representative 16S rDNAs. Using the restriction enzyme HaeIII, isolates were classified into 8 pattern group. The sequence results indicated that a high diversity of bacterial phylotypes was present within the coral Galaxea fascicularis. In general, there are three major groups of bacteria: (i) members of the division Firmicutes , (ii) Actinobacteria, and (iii) γ-proteobacteria. Phylogenetic data on microbial community composition in coral G. fascicularis will help in the rational selection of culture conditions to improve the diversity of bacteria and the knowledge on the physiological, biochemical, genetic, and molecular properties of coral bacteria.

Keywords: *Galaxea fascicularis,* 16S rDNA, RFLP, diversity, Ujung Kulon

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INTRODUCTION

Coral reefs are among the most ancient structures on earth built by living organisms. Reefs are scatterred over an area of 190 million km^2 wherever a suitable substratum lies within the lighted waters of tropics beyond the influence of continental sediments. These are deterministic phenomena of sedentary organisms with high metabolism living in warm marine waters within the zone of strong illumination.

Coral *Galaxea fascicularis* is usually ball or dome shaped in captivity. Shapes that include spires, plates, encrustations, and branches are sometimes found. *G. fascicularis* can be green, grey, pink or brown, but always with contrasting colored tips. This type of coral has clear to translucent sweeper tentacles, most often with white tips. The tentacles will be out during the day (Lukan, 2005). There are two reasons why this coral was chosen,

first, resistance in high sedimentation, and second, in the previous study found that this culturable bacterial community associated with *G. fascicularis* possesses antibacterial property against shrimp pathogenic bacterium *Vibrio harveyi*.

It is well understood that corals harbor diverse microbial communities (Pascal and Vacelet, 1981). Their surface is covered by muco-polysaccharides, which provides a matrix for bacterial colonization leading to the formation of biofilm-forming microbial communities. Mucus-covered coral surfaces are often colonized by bacteria and other micro-organisms (Kim, 1994). However, a few studies have suggested that corals may be associated with specific bacteria. Rohwer et al. (2001) stated that differences in the composition of the surface mucus produced by specific corals resulted in different populations of associated microbes. Furthermore, Ritchie and Smith (1995) showed that mucus-associated bacteria had specific carbon source utilization patterns that were consistently associated with certain coral species and varied among different species of coral. However, still many questions evoke about the specificity and dynamics of association between bacteria and corals, even Frias-Lopez et al (2002) stated that corals harbor unique microbes inconsistencies across studies. Fuhrman and Campbell (1998) stated that the majority of microbes cannot be cultured using standard methodologies. In addition, Rohwer et al. (2001) showed that the microbial diversity associated with corals can be greatly underestimated when relying on culture-based methods.

The understanding of population structure in natural bacterial communities has been significantly improved by the availability of molecular tools for community analyses in microbial ecology. The use of rDNA sequence analyses for the investigation of microbial community composition offers new perspectives on the traditional phenotypic classification systems. The differences of 16S rDNA sequences provide more complete understanding of microbial phylogeny and the identification of bacteria (Gray and Herwig, 1996). However, analysis of microbial communities based on 16S rDNA is restricted to the phylogenetic nature of grouping, and is not appropriate to investigate a functional group of different species in the bacterial community (Goebel and Stackebrandt, 1994). Variation in the 16S rDNA genes as reflected in RFLP were used to classify isolates. This article describes the characterization of bacteria associated with coral *G. fascicularis* using culture-based methods. Our results show that microbial diversity on coral *G. fascicularis* is very high.

MATERIALS AND METHODS

Sampling and isolation of bacteria associated with coral

G. fascicularis is found on open reef bottoms and form morphologically similar colonies of individual, upright tubes. Specimens of the hard corals *G. fascicularis* were collected by scuba diving at depths of 5 to 10 m off the Peucang Island, Ujung Kulon, Indonesia, in July 2004 (S 06° 45,149' E 105° 15,298' and S 06° 45,150' E 105° 15,671'). **Fig. 1** shows the sampling sites. Individual specimens were placed separately into plastic bags to avoid contact with air and brought to the surface. The samples were kept individually in plastic bags containing natural seawater until processing within a few hours after collection. Tissue samples were removed from the sceleton with a sterilized scrapper, and the exposed surface tissues were removed with a sterile scalpel blade. The resultant tissues were serially diluted, spread on ½ strength ZoBell 2216E marine agar medium and incubated

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at room temperature for 48 hours. On the basis of morphological features, colonies were randomly picked and purified by making streak plates (Madigan *et al*. 2000).

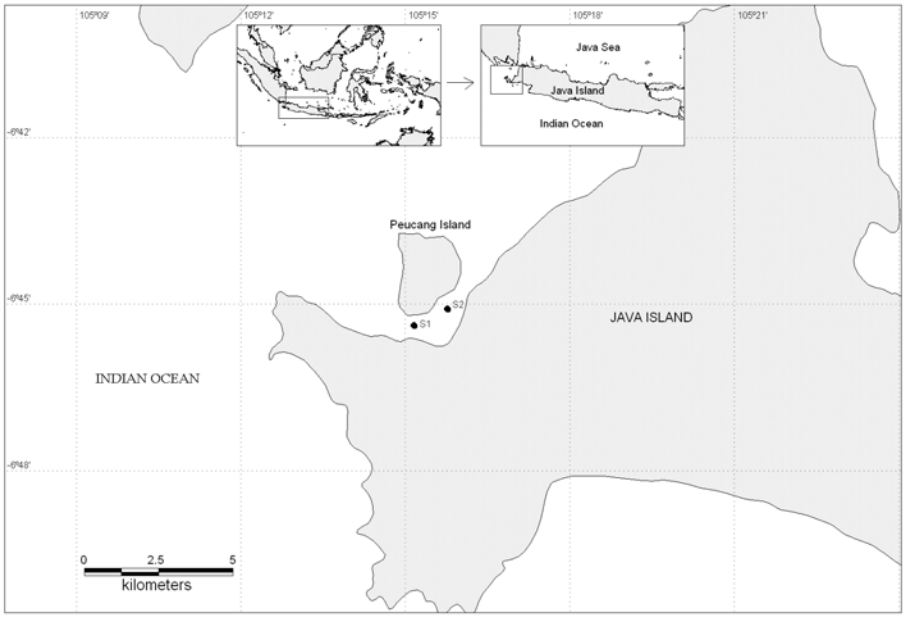

Fig. 1 Map of sampling sites

PCR amplification and RFLP analysis

Bacterial strains were cultured at room temperature on half-strength ZoBell 2216E agar plates. After harvest, DNA was extracted by five cycles freeze (-80^{0}C) and thaw (95^{0}C). PCR was performed with an Eppendorf Mastercycler (Eppendorf Inc., Germany) as follows: 2 µl template DNA, 40 pmol of each of the appropriate primers, 125 µmol of each deoxyribonucleoside triphosphate, 5 µl of 10 x RedTaq^{TM} PCR buffer (Sigma, Germany), 1.2 mg ml^{-1} (final concentration) bovine serum albumin (Sigma) and 0.75 unit RedTaq^{TM} DNA polymerase (Sigma) were adjusted to a final volume of 50 µl with sterile water (Sigma). A PCR run comprised 40 cycles with denaturing conditions for one minute at 95°C, annealing for one minute at 70 °C and extension for two minutes at 72 °C, respectively. The PCR products were separately digested with restriction endonucleases *Hae*III (Ulrich and Wirth., 1999). The gels were stained with ethidium bromide and finally documented with camera. Isolates with identical restriction patterns were designated as a single 16S rDNA genotype group.

Sequencing and phylogenetic analysis

The PCR product was purified and concentrated with Microcon-100 microconcentrators (Amicon, Beverly, MA, USA) according to manufacturer's instructions. Sequencing was carried out with a SequiTherm Long-Read Cycle Sequencing Kit (Epicentre Technologies, Madison, WI, USA) and an automated sequencer (the ALF DNA sequences: Pharmacia LKB Biotech, Uppsala, Sweden). Representative of groups were used for sequencing. The resulting 16S

rRNA sequences corresponding to the genotype, respectively, were analyzed for homologies with sequences in the data base using Fasta searching. The determined 16S rRNA sequences and various of 16S rRNA of reference strains were also aligned using Clustal W. Neighbour-joining method was used to calculate the distant matrix and to construct the phylogenetic tree.

RESULTS AND DISCUSSION

Coral bacterial isolates displayed a wide variability of morphological features after growth on Zobell's2216E marine agar. Selected bacterial colonies were subcultured and purified. The rDNA of these isolates could be amplified resulting in a characteristic single band of about 1500 bp. Restriction analysis of the PCR products of the 45 isolates using the restriction endonuclease *Hae*III revealed 8 restriction patterns as presented in **Fig. 2**.

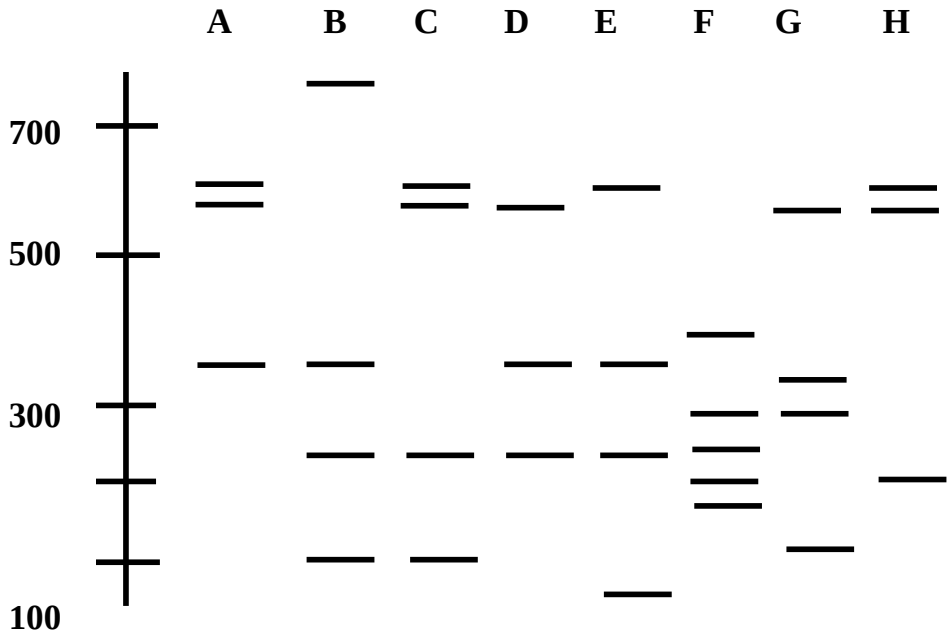

Fig. 2. RFLP patterns among coral bacteria as digested by *Hae*III enzyme

The 16S rDNA characterization and distribution of each phylotipe is summarized in **Table 1**. As shown in the phylogenetic tree, the position of representative phylotypes reflected the different groups identified by RFLP (**Fig. 3**)

Table 1. Phylogenetic characterization and distribution of phylotype

Phylotype	No. of strain	Percentage	Closest species	Homology (%)
A	20	44.4	*Bacillus sp.*	98.0
B	3	6.66	*Agroccoccus jenensis*	95.0
C	6	13.33	*Arthrobacter nicotianae*	99.0
D	2	4.44	*Kocuria sp 221635.31*	97.0
E	1	2.22	*Microbacterium sp CME1*	98.0
F	7	15.55	*Acinetobacter radioreistence*	97.0
G	3	6.66	*Halomonas spSA-Hm1*	98.0
H	3	6.66	*Pseudomonas RDPT5*	98.0

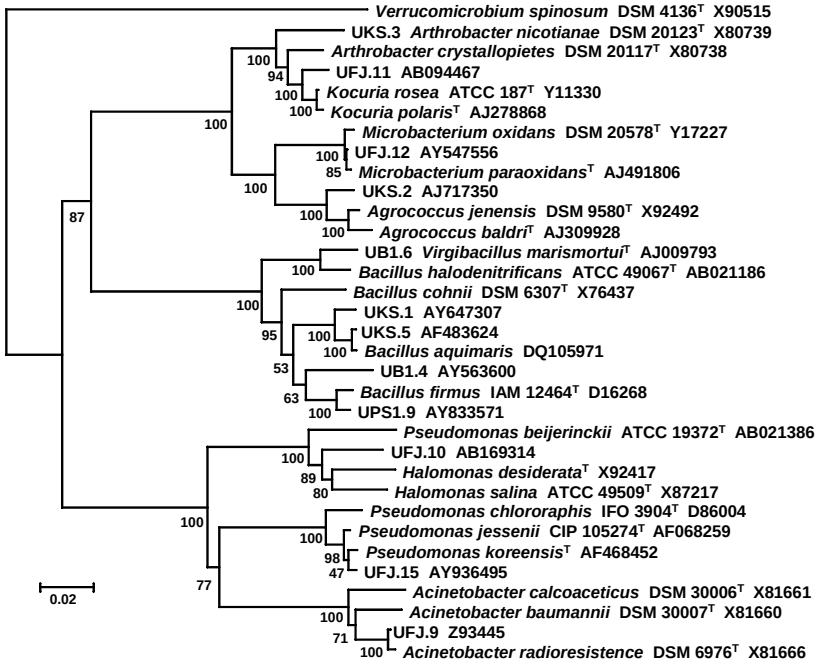

Fig. 3. Phylogenetic tree of bacteria associated with coral *G. fascicularis* isolated from Ujung Kulon Indonesia. *Verucomicrobium spinosum* was used as out group. Bar indicated 2% dissimilarity of sequences.

In this regard the 16S rDNA sequencing assist resolve the exact taxonomic position of coral bacteria, and provided more detail information on their phylogenetic position among their closest relatives. Although restriction patterns based genotype on a single endonuclease were not adequate to estimate the genetic distance of 8 16S rDNA genotype groups, a high similarity of the predominant genotype A was revealed (44.4%). In contrast, phylotype E represented a small proportion of total strains (2.22%). The application of a single restriction enzyme (*Hae*III) to characterize bacterial communities has recently been demonstrated by Ulrich and Wirth (1999) and Ravenschlag et al. (1999), and *Hha*I

(Radjasa, et. al. 2001). Urakawa et al. (1999) reported that among 4 base specific restriction endonucleases (*Hha*I, *Dde*I, *Rsa*I, and *Sau*3AI), *Hha*I gave the clearest RFLP patterns, and our result also clearly demonstrated its differentiation. Other parameters like season, time interval, and phytoplankton were not observed, since the primary objective was to provide a first assessment on the diversity of culturable coral bacteria in the marine national park from the time of sampling. However, this is likely to have a significant impact on the types of culturable bacteria obtained, just as would different types of culture media.

CONCLUSION

The present study indicates that using a culture dependent 16S rDNA–based approach it is possible to explore the diversity of culturable coral bacteria and their phenotypic traits. The implementation of the 16S rDNA approach has developed the field of microbial ecology. The phylogenetic position of environmental bacterial populations in the evolutionary tree could be determined and traced precisely by using this approach even in those complex ecosystems. This approach will be useful to increase the knowledge on the physiological, biochemical, genetic, and molecular properties of coral bacteria.

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REFERENCES

Frias-Lopez J, Zerkle AL, Bonheyo GT, Fouke BW 2002. Partitioning of bacterial communities between seawater and healthy, black band diseased, and dead coral surfaces. *Appl. Environ. Microbiol.* 68:2214–2228

Fuhrman, J.A. and L. Campbell. 1998. Microbial microdiversity. *Nature* 393:410-411

Pascal H, and Vacelet E 1981. Bacterial utilization of mucus on the coral reef of Aquaba (Red Sea). *Proc 4th Int. Coral. Reef. Symp* 1:669–677

Goebel, B.M. and E. Stackebrandt 1994. Cultural and phylogenetic analysis of mixed microbial populations found in natural and commercial bioleaching environments. *Appl. Environ. Microbiol.* 60: 1614-1621

Gray, J.P. and R.P. Herwig 1996. Phylogenetic analysis of the bacterial communities in marine sediments. *Appl. Environ. Microbiol*. 62: 4049-4059

Radjasa, O.K., H. Urakawa, K. Kita-Tsukamoto and K. Ohwada 2001. Characterization of Psychro-trophic Bacteria in the Surface and Deep-Sea Waters from the North western Pacific Ocean Based on16S Ribosomal DNA Analysis. *Mar.Biotechnol*. 3: 454–462

Rohwer, F., M. Breitbart, J. Jara, F. Azam and N. Knowlton 2001. Diversity of bacteria associated with the Caribbean coral *Montastrea franksi. Coral. Reefs* 20: 85-91

Ravenschlag, K., Sahm, K., Pernthaler, J., and Amann, R. 1999. High bacterial diversity in permanently cold

marine sediments. *Appl. Environ. Microbiol.* 65:3982–3989.

Ritchie KB, Smith GW. 1995. Preferential carbon utilization by surface bacterial communities from water mass, normal, and white-band diseased *Acropora cervicornis*. *Mol. Mar. Biol. Biotechnol.* 4:345–354

Ulrich, A., and Wirth, S. 1999. Phylogenetic diversity and populations densities of culturable cellulolytic soil bacteria across an agricultural encatchment. *Microb. Ecol.* 37:238–247.

Urakawa, H., Kita-Tsukamoto, K., and Ohwada, K. 1999. Restriction fragment length polymorphism analysis of psychrophilic and psychrotrophic *Vibrio* and *Photobacterium* from the northwestern Pacific Ocean, and Otsuchi Bay, Japan. *Can. J. Microbiol.* 45:67–76.