

[SIV-2]

Diversity of Bacterial Communities Distributed in Sea Water of Mass Bleaching Areas of Jeju Coast

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In this study, the bacterial communities distributed in sea water of the mass bleaching areas of Gangjeong and Seongsan coasts, Jeju-do have been analyzed using the PCR amplification of 16S rRNA to obtain fundamental data or information on a relationship between the bleaching phenomenon and bacterial ecosystem. In Gangjeong, diverse bacteria such as *Alcanivorax*, *Paracoccus*, *Damselae*, *Pseudomonas*, *Rhodovulum*, *Silicibacter*, *Sulfitobacter*, and *Roseobacter* have been found, and *Alcanivorax* was the most abundant clone. The most abundant clone from Seongsan was *Pseudomonas*, of which *Pseudomonas tolaasii* and *Pseudomonas mandeli* were most abundantly occurred in the frequency of approx 44 % and 24 %, respectively. Approx 4 % of the bacterial clones closest to *Verrucomicrobiales* and other unidentified clones were also found in Seongsan, suggesting there is a great discrepancy between bacterial communities from the bleaching areas of Seongsan and Gangjeong coasts. The mean temperature, chlorine concentration, pH, and dissolved oxygen (DO) of the sea water of Gangjeong and Seongsan in August of 2001 (sampling period) was 27 °C ~ 27.5 °C, 30.24 ~ 30.60 %, 8.23 ~ 8.36, 7.20 ~ 7.28 mg/L, suggesting other environmental factors except for the factors mentioned above might result in difference of bacterial communities distributed in both areas.

Introduction

It has been considered that recent mass bleaching observed in the bottom of the sea might result from calcification of corals, loss of symbionts or pigments, shell abnormalities in large foraminifers, and increasing water temperature, low pH, inflow of excess nitrogen and phosphorus, water pollution. It is well known that bleaching affects symbioses of hosts ranging from cnidarians, sponges, mollusks to foraminifers associated with symbionts which are either dinoflagellates, diatoms or cyanobacteria. Even coasts of Jeju, Korea have been observed to experience similar phenomena. Many Korean ocean scientists have assumed that large algae like sea tangles, brown seaweeds, agar-agars have been eaten in large quantities by turban shells, abalones, sea urchins, etc being cultivated in coasts and diminished. Instead, *Melobesioideae* included into *Corallinaceae* dominate while large algae mentioned above lost their territories to live in. *Corallinaceae* is composed of *Corallinoideae* and *Melobesioideae*. Especially, *Melobesioideae* including *Lithophyllum*, *Lithothamnion*, and *Fosliella* almost have not been used as foods by turban shells, abalones, sea urchins, etc. As a result, the number of turban shells, abalones, sea urchins, etc might greatly decrease because of a food shortage.

Ultimately, these problems might result in decrease of biodiversities and destruction of earth ecosystems. The striking feature of recent mass bleaching is that it has been being observed in broad coast areas all over the seasons as well as spring when the surface water temperature rise. According to NFRDI (National Fisheries Research and Development Institute) report, as of 1999, approx 20% of Jeju coast has been suffered from the mass bleaching phenomena, and the bleaching is probably increasing in magnitude and becoming chronic. This finding is different from the past observations that beginning and end of bleaching event repeat according to the water temperature resulting from seasonal change. In particular, approx 36% of Seogwipo coast and 31% of Namjeju-gun coast have been reported to experience bleaching phenomena, giving an alarming threat on coast ecosystems of Jeju. In this respect, it is urgent and significant to understand microbial populations in the bleaching area in that we can understand how mass bleaching affects the microbial ecosystems. This study put an emphasis on analysis of bacterial communities distributed in surface seawater of the bleaching areas of Gangjeong and Seongsan coasts, Jeju-do to obtain fundamental data on microbial diversity in the bleaching areas.

Materials and Methods

Samples used in this study were the surface seawater of Ganjeong and Seongsan coasts where mass bleaching phenomena have been observed. Several sterile slide glasses were prepared and submerged providing bacteria with opportunities to attach for a couple of weeks. Also, sea samples have been collected periodically and deposited in a refrigerator. At the last day samples were extracted, all the samples (sea water plus the slide-attached) from the same location were mixed and centrifuged to collect solid samples. The collected samples were used to analyze the compositions of the bacterial communities from a 16S rDNA clonal library. For structural analyses of bacterial communities from Ganjeong and Seongsan, DNA was extracted directly from the collected samples and 16S rDNA genes were amplified using 27f and 1522r primers. PCR products were cloned and 50 randomly selected clones from each location were sequenced. Sequence results were comparatively analyzed based on database homology searches using the Blast algorithm, and a phylogenetic tree was generated with the 50 partial 16S rDNA sequences.

Results and Discussion

Comparative sequence analyses of 16S rRNA clonal library indicated that in the mass bleaching area of Seongsan coast, *Pseudomonas* was the dominant genus, and *Pseudomonas tolaasii* or *Pseudomonas mandeli* were most abundantly occurred in the frequency of approx 44 % and 24 %, respectively. *Pseudomonas extremorientalis* and *Verrucomicrobia bacterium* have been occurred in the frequency of approx 4%. In contrast to the bacterial clones distributed in Seongsan coasts, clones obtained from Gangjeongl were distributed among the genera *Alcanivorax*, *Damselae*, *Paracoccus*, *Pseudomonas*, *Rhodovulum*, *Silicibacter*, *Sulfitobacter*, *Roseobacter*, etc (Table 1). The most abundant clones from this location were *Alcanivorax* (20%) and *Pseudomonas* (16%). However, approx 48% of the analyzed clones have been unidentified, warranting elaborate and further sequencing. Comparison of bacterial communities from Seongsan and Gangjeong coasts

showed that there is a great discrepancy between bacterial communities from both areas, suggesting bleaching phenomena made no coherent effect on bacterial distribution. An examination of seawater quality revealed that the mean temperature, chlorine concentration, pH, and dissolved oxygen (DO) of the sea water of Gangjeong and Seongsan in August of 2001 (sampling period) was 27 °C ~ 27.5 °C, 30.24 ~ 30.60 %, 8.23 ~ 8.36, 7.20 ~ 7.28 mg/L. These similar conditions at both areas suggested that other environmental factors except for the factors mentioned above might result in difference of bacterial communities distributed in both areas.

The mechanisms of recent mass and chronic bleaching phenomenon observed in the bottom of the sea have not been identified due to several possibilities that it may be caused by diverse environmental factors like the global warming, weather pattern change in tropics, excess amount of calcium in sea water, low pH, water pollution, increasing freshwater inflow, depositing large amounts of sediment, etc. Particularly, bleaching observed in Jeju coasts gives rise to a serious problem to sea breeding industry encouraged in Jeju-do. It is potentially dangerous than every other disturbance such as sea level rise (or fall). Lack of explanation of the phenomenon makes it even more alarming than impact of ozone depletion in polar and temperate regions or acid rain on temperate forests, for which remedies are known. These obscure hypotheses made us begin to study on bacterial communities in bleaching areas of Jeju coasts, because we thought the possibility that bacteria might provide a cause to bleaching phenomenon (and/ or adapt most adequately to the new environment). The present work could not provide satisfactory information or a clue to solve a bleaching phenomenon, because seawater of bleaching areas was used. However, it is significant firstly to attempt to study on bacterial communities in bleaching areas of Jeju coasts. Future study need to be intensively conducted using bleaching samples.

Table 1. Bacteria occurred in seawater of mass bleaching areas of Ganjeong and Seongsan

Bacteria	% Occurrence	
	Gangjeong	Seongsan
<i>Alcanivorax</i> sp.	20	0
<i>Paracoccus carotinifaciens</i>	4	0
<i>Photobacterium damsela</i>	4	0
<i>Pseudomonas extremorientalis</i>	0	4
<i>Pseudomonas mandeli</i>	0	24
<i>Pseudomonas tolaasii</i>	8	44
<i>Roseobacter</i> sp.	8	0
<i>Silicibacter</i> sp.	4	0
<i>Sulfitobacter</i> sp.	8	0

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