

THESIS SUMMARY

論文の内容の要旨

論文題目 **Mechanisms Controlling Bacterial Community Structure in Coastal Marine Environments**

(沿岸域における微生物群集構造の形成メカニズム)

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Bacteria are widely distributed in aquatic and terrestrial environments and their community structure is one of the most fundamental information to understand the characteristics of the environments they live. The bacteria of the marine environments require special adaptations as the marine habitats are diversified, vast and some are fluctuating such as coastal marine environments. This thesis combined the studies conducted to evaluate the mechanisms controlling bacterial community structure of the coastal habitat, one of the most important but inconsistent marine environments. Specific attention was given to clarify the bacterial community structure in coastal environments considering local, seasonal, and fractional (PA or FL) similarities or dissimilarities and uniqueness and to clarify how the major phylogenetic groups such as the Flavobacteriia, Alphaproteobacteria and Gammaproteobacteria are maintained to the coastal marine environments considering the dominancy of the members belonging to these three major classes, their species richness and evenness, habitability and utilization ability of different organic materials. For the seasonal and locational, samples were collected from two closely located coastal stations, the port side, and the sea side, at 5 times samplings from March 2013 to July 2014. The community structure was observed from broader to a finer scale and tracked the presence of any location specific or unique group in the community after pyrosequencing by using 454 GS Junior sequencer. The community structures analysis of bacteria showed that common phylogenetic groups appeared at both stations; at phylum level Bacteroidetes and Proteobacteria, and at class level Flavobacteriia, Alphaproteobacteria, and Gammaproteobacteria were consistently dominant. Class Flavobacteriia, Alphaproteobacteria,

Gammaproteobacteria contributed about 78 to 98% of the relative abundance in the port side station while about 62 to 92% of the relative abundance in sea side station. Top 25 most abundant OTUs contributed about 24 to 73% of the relative abundance in different samples at port side and about 12 to 56% of the relative abundance at sea side station; 23 of these most abundance OTUs belonged to any of these three major classes. At higher phylogenetic level, the difference between two stations and between two consecutive sampling periods was generally small but, marked differences in community structure and diversity were seen at lower phylogenetic levels. At phylum and class level, the difference between PA and FL were generally small, i.e. the major contributing groups appeared in both the fractions. Some locations specific OTUs or genera appeared uniquely to one of the two stations and most of them are members of the Gammaproteobacteria, some condition-specific unique groups were also identified. Species richness was similar for these three classes but the evenness of Flavobacteriia was significantly higher than those of Gammaproteobacteria and Alphaproteobacteria. Average number of OTUs was higher for the Flavobacteriia and lower for the Gammaproteobacteria while the average number of genera, families and orders were higher for the Gammaproteobacteria and lower for Flavobacteriia. But, the average contributions to the relative abundance by the Flavobacteriia and Alphaproteobacteria were similar; about 27 to 35% while by the Gammaproteobacteria almost their half, about 15 to 17%. These findings are quite interesting, inspired further investigations on their adaptation ability/ mechanisms or habitability as well as response to nutritional factors.

Habitability is defined as the ability of any organism to inhabit different environments. The studies described in chapter 3 of this thesis are to evaluate the habitability of the bacteria to assess their probable distribution and adaptation mechanisms using a recently developed database, the MetaMetaDB. The database gives us information from what kind of environments one particular 16S rRNA sequence data has ever been obtained and thus enables us to infer the habitability of the bacterium in question. Initially, the applicability of MetaMetaDB was assessed by using different samples, from a series of locations with different salinities. MetaMetaDB reasonably showed that the lower saline stations were dominated by the sequences with “freshwater-groundwater”, “human” and “wastewater” habitabilities while the higher saline stations by those with “marine” habitability. The phylotypes of lower saline stations with a particular habitability were absent or rare in the higher saline stations and vice-versa. The database was

then used to get the habitability information of the seasonally obtained phylotypes from the coastal areas of Oarai, Ibaraki, Japan in order to assess the possible distribution and adaptation mechanisms of the coastal bacteria. Analyses at both 97% (species) and 85% (order) level of identity showed that most of the phylotypes are assigned to “marine”, indicating the identified bacterial groups are mostly distributed to marine environments and their distribution is driven by the salinity. MetaMetaDB also indicated that a particular phylum may contain members with different habitabilities which are overlooked by ordinary community structure analysis. Habitability analysis showed that among the three major classes, the members of Flavobacteriia may be adapted to specific environments and thus they are relatively stable. It may explain the reasons of their higher evenness compare to the others. For Alpha and Gammaproteobacteria, however, habitability did not clearly indicate the differences in the adaptive ability of different members. These findings then raised another question, what are the important factors to control the community structures of Gammaproteobacteria as well as Flavobacteriia and Alphaproteobacteria? In order to have the answer, seawater samples were collected from different coastal and oceanic habitats and culture experiment was conducted with the amendments of different monomeric and polymeric organic substances, described in chapter 4 of this thesis.

In chapter 4, bacterial response to the qualitative and quantitative supply of organic materials and their pattern of utilizations was considered to clarify the differences in the formations of populations especially by the major bacterial groups, the Flavobacteriia, Alphaproteobacteria, and Gammaproteobacteria. Several sets of seawater culture experiments were conducted in laboratory condition resembled an environmental spot with a higher concentration of nutrients, to evaluate the influences in a relatively shorter period of time. Sea water samples were collected from different locations, ranging from coastal (Oarai, Ibaraki, Japan) to oceanic (Kuroshio Current) and eutrophic (north station of the Pacific Ocean, NBD) to oligotrophic (south station of the Pacific, SBD) habitats and treated with different monomeric and polymeric substances. The subsamples were taken and community structure was evaluated by pyrosequencing using 454 GS Junior. The findings of the culture experiments were even more surprising. The degree of influences was varied with the types of environment, temperature and types of nutritional substances added. Some members of the class Gammaproteobacteria, more specifically the genera *Vibrio* and *Alteromonas* were quickly reacted and used the low molecular weight

substances and proliferated massively which ultimately changed the community structure; some others e.g. *Pseudoalteromonas* guessed to involve in degradation of high molecular weight substances. The members in Alphaproteobacteria showed a similar tendency in utilizing monomeric substances become abundant at the end in some conditions and sometimes replaced the Gammaproteobacteria. This shifting in proteobacterial classes may answer us the question, why the relative abundance Alphaproteobacteria is usually higher than the Gammaproteobacteria in marine environments. Members in Flavobacteria maintained a minimum but steady growth rate but did not show any specific response like the members in Alphaproteobacteria and Gammaproteobacteria. They were also found relatively abundant in some polymer treated tanks suggesting their involvement in polymer degradations. The overall finding suggested that Gammaproteobacteria is more sensitive to changes, which make them uneven and sometimes unique. Moreover, they are probably more affected by the top-down factors such as the predation and viral lysis compare to the Alphaproteobacteria, may be another reason of their relatively less abundance in marine environments.