博士論文（要約）

Studies on diversity, community structure and functional potential of particle-associated and free-living bacteria in the Pacific Ocean

（太平洋における粒子付着性及び自由生活性細菌の多様性・群集構造・機能に関する研究）

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1. Introduction

Bacteria play fundamental roles in marine ecosystems and biogeochemical cycles. They sustain photosynthetic production supplying inorganic nutrients via degradation and remineralization of organic materials and also involve in microbial food webs. Many key biogeochemical processes including nitrogen fixation, nitrification and denitrification are known to be mediated by bacteria. Hence, diversity, community structure and functional potential of bacteria are fundamental to understand ecosystem dynamics and biogeochemical cycles in the ocean.

Basic questions about the ecology of bacteria in the Pacific Ocean were addressed in this study. How many species of bacteria are present in the Pacific Ocean? How do diversity and community structures of bacteria change along with latitude and depth? What factors do determine their diversity and community structures? Previous studies have already addressed these questions. However, most of them have been conducted in particular local areas. There has been few studies describing a large scale patterns of both particle-associated (PA) and free-living (FL) bacteria. Recent development of high-throughput sequencing technologies has enabled us to analyze huge number of samples like global ocean collections, which is a strong motive force to investigate large scale patterns of microbial diversity and functions.

The objectives of this study were (1) to describe latitudinal and vertical patterns of bacterial diversity and community structures in the Pacific Ocean, (2) to determine factors of controlling their diversity and community structures, (3) to determine functional differences of bacteria between PA and FL fractions or among different oceanic regions and (4) to determine distribution and functional characteristics of marine *Verrucomicrobia*, a taxa specialized to particle association. To achieve these objectives, I used 254 samples systematically collected during 4 transpacific cruises of R/V Hakuhomaru covering large area of the Pacific Ocean. These include vertical profile collections from 0 to 5000 m of both PA and FL fractions and horizontal collections along with a latitudinal transect from 40° S to 68° N. I believe that this is one of the largest data sets for analyzing bacterial diversity and community structures in the Pacific Ocean. I also characterized functional differences in PA and FL assemblages of bacteria by means of metagenomic analysis. To my knowledge, this is the first study of comparative metagenomics of PA and FL bacteria in the tropical and subtropical Pacific Ocean.

2. Diversity and community structure of PA and FL bacteria in the Pacific Ocean

Following the general introduction in the Chapter 1, patterns of diversity and community structures of PA and FL bacteria in the Pacific Ocean were described in the Chapter 2. During the 4 cruises of R/V Hakuho-maru from 2011 to 2014, totally 254 seawater samples were collected from surface to the bottom at 33 locations along a meridional transect in the Pacific Ocean. Seawater was serially filtered through 3.0 μm and 0.22 μm pore-size filters to have PA and FL bacteria respectively. After the extraction of DNA, bacterial 16S rRNA gene was amplified by PCR targeting to the V1-V3 hypervariable region. PCR amplicons were sequenced with the use of the 454 pyrosequencer. Obtained sequences were clustered into OTUs with 97 % similarity and assigned to taxonomic groups in the SILVA database.

Totally 18,103 OTUs were obtained from 1.4 million sequence reads. An estimate of species richness suggested that 37,356 OTUs (20,894 PA and 16,462 FL) of bacteria were present in the water column of the Pacific Ocean, which was consistent with other global estimates. PA assemblages often show higher species diversity than FL ones probably because of richness and diversity of organic

molecules in marine particles. This trend was applicable to the samples covering a large spatial scale of the Pacific Ocean. Vertical profiles of diversity (Inverse-Simpson index) of PA and FL bacteria were similar to each other from the surface to the mesopelagic layers showing steep increase down to several hundred meters. However, the profiles were different from each other in the bathypelagic layer showing slight decrease of diversity for PA bacteria and constant diversity for FL bacteria. Such difference in diversity profiles suggested the difference in factors or mechanisms to determine the diversity between PA and FL assemblages of bacteria. Also, community structures were largely different between shallow (0-200m) and deep (250-5000m) water samples. The difference between PA and FL assemblages was larger in the deep water than in the shallow water. Major taxonomic groups of PA bacteria were *Alphaproteobacteria*, *Bacteroidetes*, and *Cyanobacteria* in the surface seawater and *Alphaproteobacteria*, *Deltaproteobacteria*, *Gammaproteobacteria* and *Planctomycetes* in the deep, whereas those of FL bacteria were *Alphaproteobacteria* and *Cyanobacteria* in the surface and *Alphaproteobacteria*, *Deltaproteobacteria,* and *Defferibacteres* in the deep.

3. Environmental and spatial factors structuring bacterial community in the Pacific Ocean

In the Chapter 3, relationship between bacterial diversity and community structures and environmental variables was described to reveal determinative factors structuring bacterial community in the Pacific Ocean. Also, the metacommunity concept was discussed to explain the patterns found in this study. Spatial variability of diversity found in surface water samples was fitted by the linear regression model, and the diversity of PA bacteria showed significant correlation with temperature and latitude respectively. It was higher at higher temperature or at lower latitude. High diversity in the mesopelagic layer could be explained by the niche hypothesis (high diversity of resources). Interestingly, FL bacteria kept constantly high diversity down to the bathypelagic layer, which was explained by the area effect hypothesis. I proposed the hypothesis that huge and uniform space of bathypelagic layer allows more diversification of FL bacteria than small space of the epipelagic layer. Since the community structure was clearly different between shallow and deep samples, correlation test with environmental variables by the redundancy analysis was done at each depth. Variability of temperature, nutrient concentration and partly chl *a* concentration significantly correlated with variability of PA and FL community structures in the shallow (0 and 100 m) samples, whereas salinity and nitrate concentration correlated in the bottom-50 m samples. Additionally I found that the dissimilarity of community structures positively correlated with distance between the sampling locations, suggesting the contribution of immigration as another determinative factor. Fitting to the metacommunity concept based on the partial Mantel test, the species sorting and the mass effect were possible mechanistic processes to determine both PA and FL bacterial communities in the shallow water. It was also suggested that the neutral model or the patch dynamics possibly determined the FL community in 1000 m water and the species sorting possibly determined both PA and FL community in the bottom water.

4. Functional potential of PA and FL bacteria in the tropical and subtropical Pacific Ocean

In the Chapter 4, microbial community genomes or metagenomes were compared between PA and FL assemblages or different oceanic regions representing the distinctive functional potentials. In addition to whole metagenomic comparison, I especially focused on the genomes of *Prochlorococcus* of *Cyanobacteria*, a major taxonomic group of bacteria in the open ocean. The key functions to determine

PA and FL lifestyles or regional distribution in the Pacific Ocean were discussed.

Seawater samples used in this study were collected from the surface at 9 locations, 3 of each located in the North Pacific subtropical gyre (NPSG), the South Pacific subtropical gyre (SPSG), and an equatorial region (EQ). After the extraction of DNA, MiSeq 250 bp paired-end sequences was performed to have about 10-20 million reads per sample. KEGG orthology was assigned to each predicted gene. We evaluated metabolic potential of PA and FL bacterial communities by using the MAPLE system, an automatic system for mapping genes to the KEGG modules. Metagenomes as well as community structures of PA and FL bacteria showed distinctive repertories of functional gene sets. The similarity percentage analysis suggested that higher abundance of some transporters (amino acids, sugar, iron complex, phosphate and metals) largely contributed to the functional dissimilarity between them. Comparison between regions also suggested that transporters contributed the difference between regions. Especially, the urea transport system was enriched in the subtropical region. Metagenome mapping to *Prochlorococcus* suggested again that the urea transporter system was more abundant in the NPSG and SPSG than the EQ and also the cyanate transporter and the cyanate hydratase were more abundant in the SPSG than the other regions. Surface seawater in a subtropical gyre is extremely oligotrophic with depletion of nitrate and nitrite, which might require the use of alternative sources of nitrogen such as urea and cyanate. Other metagenomic characteristics, enrichment of the amino-acid and the peptide transport systems, supported this idea. These results implied that the distribution and dynamics of alternative nitrogen sources especially urea and cyanate should be the key to understand ecosystem dynamics in the subtropical Pacific Ocean.

5. Distribution of marine *Verrucomicrobia* in the Pacific Ocean

Since marine *Verrucomicrobia* have reportedly associated with particles and been able to decompose polysaccharide, they may play a significant role in degrading particulate organic matter. Thus, the distribution of *Verrucomicrobia* is important information to understand the fate of particulate organic matter. In the Chapter 5, relative abundance of *Verrucomicrobia* was compared between PA and FL assemblages, and distribution patterns of several subgroups along a latitudinal transect were described. Also, functional difference between representative genomes of *Verrucomicrobia* subgroups was described based on genome and metagenome analyses. Community structure analysis represented much higher percentages (27 % at maximum) of *Verrucomicrobia* in the PA assemblage than the FL one. This is the first systematic evidence clearly showing niche preference of *Verrucomicrobia* on marine particles. Also, OTUs belonging to two subgroups, *Opitutae* and *Verrucomicrobiae*, were the majority and showed distinctive distribution along the transect. Comparison of 4 representative genomes of *Verrucomicrobia* showed that four there were functional modules not possessed by the *Verrucomicrobiae* strain (*Akkermansia muciniphila*) but possessed by 3 *Opitutae* strains. Three out of 4 modules were related to nitrogen metabolism such as assimilatory nitrate reduction, urea transport system and nitrate assimilation. When the genes related to these modules were sorted out from metagenome mapping data, relative abundance of the urea ABC transporter gene showed high abundance in the NPSG and the SPSG and almost nothing in the EQ. Again, nitrogen metabolisms including the urea transport system was suggested to be important to understand distinctive distribution of marine *Verrucomicrobia* subgroups in the Pacific Ocean.

6. Concluding remarks

A large scale analysis of bacterial diversity and community structures along a meridional transect in the central Pacific Ocean revealed distinctive features of bacterial distribution on and off marine particles. The numbers of OTUs found in this study (20,894 PA and 16,462 FL) provided the estimate of bacterial species numbers in the Pacific Ocean. Other findings listed below also provide new insights into understanding bacterial dynamics in the Pacific Ocean. (1) Vertical changes of bacterial diversity were explained by the niche hypothesis and the area effect hypothesis. (2) The community structure of bacteria was clearly different in between shallow and deep waters, and determinative environmental variables also varied at different depths. (3) Distance between the sampling locations or the effect of immigration as well as environmental variables was important to determine bacterial community structure. (4) Marine *Verrucomicrobia* is a taxa relatively abundant on particles and can be a major degrader of organic particles in the Pacific Ocean. In addition, comparison of functional potential of PA and FL community genomes showed distinctive repertories of functional gene sets and especially the difference of membrane transport systems. One of the striking findings was that functional potential of utilizing alternative nitrogen sources particularly urea and cyanate could be the key to determine ecotype distribution of bacteria in the subtropical Pacific Ocean. This would provide an important insight into the future study of nitrogen cycles and ecosystem responses in the ocean.