論文の内容の要旨

論文題目　**A Study on the Microbial Community Structure, Diversity and Function in the Sea Surface Microlayer**

（海表面マイクロレイヤーにおける微生物群集構造・多様性・機能に関する研究）

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The sea surface microlayer (SML) is defined as the uppermost first millimeter of the surface water. Being at the air-sea interface, the SML serves as a critical boundary for different chemical, biological and physical processes. Bacterial communities found in the SML were collectively termed as bacterioneuston while their counterparts from UW were known as bacterioplankton. The SML is well known to be concentrated with a lot of biological matters, pollutants as well as with high UV radiation, which might exert stressful conditions to the microbial community in this layer. However, in some cases, it has been shown that the bacterial abundance in the SML can be higher than the UW suggesting that the microbial community might have developed adaptations strategies to thrive in the SML.

As there was a lack of data on the SML samplers to be used for molecular microbiological studies using more sensitive techniques such as the deep sequencing methods (454 pyrosequencing), three most commonly used SML samplers were compared for their suitability and efficiency in sampling the microbial community for molecular microbiology studies. Furthermore, this thesis also aims to increase the knowledge and understanding on the dynamics and microbial community structure in the SML, which is to date, still remains poorly characterized. Lastly, this study will be the first to shed a light on the functions of these microbial communities in the SML, with regards to biogeochemical cycling.

The selection of appropriate sampling techniques and strategies to sample the thin SML is especially crucial but the best sampling practice has yet been resolved, at least for the molecular microbiology techniques using sensitive analytical methods (e.g. 454 pyrosequencing) to characterize the microbial community structures. From microbiological viewpoint, it is ideal to sample the ‘true’ SML with the least contamination with the underlying water and within the shortest sampling time frame possible to preserve the quality of the sample and to reduce the introduction in temporal changes of microbial community structures. In order to address this issue, three different common SML samplers, the polycarbonate membrane, glass plate and drum

sampler; which was shown in order studies for their ability to sample the thinnest SML depths (< 60 μm) were compared. Sampling was carried out three events each in summer and winter at the pier of Misaki Marine Biological Station within the Aburatsubo Inlet. DNA was extracted from the water samples and the bacterial 16S rRNA gene was amplified and sequenced using 454 pyrosequencing. Pyrosequencing was chosen because it has better resolution and higher sequence number per sample, compared to most of the commonly used molecular methods to reveal the SML microbial community structure. The polycarbonate membrane was found to be able to sample a different microbial community from the UW, regardless of the wind and wave activity. As the volume of water sample obtained using this sampler is limited, the use of this sampler coupled with either the drum sampler or the glass plate sampler was recommended. At class level, the bacterial communities sampled by the drum sampler and glass plate were almost similar but the glass plate sampler tends to show an underrepresentation when the concentrations of Chl-a and transparent exopolymer particles (TEP) were high. When the wind speed during sampling was low (< 5 ms^{-1}) and the SML was enriched with biological matters, the bacterioneuston community in the SML was different from the UW. Members from the *Gammaproteobacteria*, *Alphaproteobacteria*, *Betaproteobacteria* and *Chloroflexi* groups were generally more enriched in the SML while the SAR 11, SAR 324 and SAR 406 clades were generally more abundant in the UW, regardless the season. Groups such as *Burkholderiales* and *Planctomycetes*, which were usually from sediment origin, were also found in the SML, suggesting that bacteria from the sediment that could be introduced into water column during resuspension events could also colonize the SML. Interestingly, the proportion of bacterial groups that were enriched in the SML increases in winter and when the enrichment of organic matters was high in the SML. During these conditions, the particle-associated bacterial groups and anaerobic bacteria (*Chloroflexi*, *Planctomycetes*) could possibly use the enriched particles in the SML as microniches. The bacterial community in the SML could also use other adaptive mechanisms such as buoyancy (*Cyanobacteria*) to move up to the SML.

The differences in bacterial community structure using the 454-pyrosequencing techniques had prompted further research into the viable portion of bacterioneuston community and if there were any bacteria that were specific to the SML can be isolated. In order to answer these questions, the bacterial communities from the inlet opening and from the pier of Misaki Biological Station were sampled using culture-dependent methods. Most of the culturable and viable bacterial fraction was found to be higher in the SML than UW. The dominant bacterial groups were variable between the two samplings but generally, the dominance of isolates from the family *Pseudoalteromonadaceae* were found in the SML. Comparisons of all the 127 isolated strains with 16S rRNA bacterial gene sequences have shown that most of the isolated strains were ubiquitous in both SML and UW. However, some strains such as those from genus *Mesoflavibacter*, *Vibrio* and *Pseudoalteromonas* were generally more abundant in the SML. Strains that were only specific to the SML and had low similarity with already isolated and

described species were also found suggesting that these could be putative neustonic species that might have adapted to thrive in the SML.

Unlike the bacteria, the archaeal community structure in the SML is even less known. Furthermore, quantification of functional genes abundances in the SML has never been carried out. Again, summer and winter samples from the pier of Misaki were analyzed using 454 pyrosequencing. Since the proportion of Marine Group-I (MG-I) Thaumarchaeota, which most of the ammonia oxidizing archaea (AOA) belonged to, were high in some of our samples, the abundance of MG-I 16S rRNA gene and the ammonia monoxygenase subunit (*amoA*) gene used in ammonia oxidation were quantified. The diversity of *amoA* genes was also investigated using cloning methods. From the results, it was found that the archaeal communities in the SML were different from the UW when the wind speed was low and the enrichment of organic matters was high in the SML, a pattern that is very similar to the bacterial community structure shown earlier. In general, the abundance of the marine group II (MG-II) Euryarchaeota, which was frequently associated to particles, was higher in the SML. Quantification of the Thaumarchaeotal Marine Group I (MG-I) and ammonia-oxidizing gene (*amoA*) related to the group have shown that the abundances of this group in the SML were low. This again suggested that enrichments of particles were every important in shaping the microbial community structure in the SML and subsequently their functions. However when the wind speed was high and the abundance of the *amoA* gene increases and was higher than the UW, indicating that the abundance of these genes could increase in the SML at times of mixing. Despite being present in such low abundances and SML being a harsh environment for the archaea with *amoA* genes (e.g. high organic matter content and high light intensity), the gene diversity of archaeal groups carrying the *amoA* gene was surprisingly high in the SML at all times and this could explain a pattern in SML-adapted ammonia oxidizing archaea species. Like the sediment-originated bacteria, a large proportion of *amoA* gene in the SML was also found to be closest to clones obtained from sediment.

The functional potential of the microbial communities in the SML remains unknown till today. This is the first study to elucidate the functional potential of the SML microbial community (bacteria, archaea, eukaryote), in-depth, using the comprehensive microarray, GeoChip 5.0M that is able to target functional genes that are responsible in key biogeochemical cycles using oligonucleotide probes. At glance at the functional gene content of the bacterial, archaeal and eukaryal communities using 16S rRNA have shown that the genes in the SML and UW were also different. Genes that were significantly abundant in the SML was found to be involved in the 3-hydroxypropionate bicycle, dicarboxylate/4-hydroxybutyrate cycle, reductive tricarboxylic acid cycle in carbon fixation; ammonification and anammox in nitrogen cycling as well as DMSP demethylation. On the other hand, sulfur and methane cycling genes were generally more enriched in the UW compared to the SML. Since the probes for GeoChip were derived from known organisms, clones and metagenome data, the lower number of probes detected in all SML samples

using this microarray implied that there could be novel functions in the SML that remained unknown to date.

Most of the research topics highlighted in this study were pilot researches, adding new insights into the microbial community structure and functions in the SML. It has been shown that the SML can be a very dynamic environment compared to the relatively stable UW. Fluctuations in the microbial community was highly affected by enrichment in organic matter that, a phenomenon that is induced in the SML during low wind speed. These enrichments may act as microniches, providing an alternative habitats for microbes that are efficient colonizer, degraders and even anaerobic microbes to thrive, thus, increasing the microbial diversity in the SML. Furthermore, resuspension of sediment as well as motile bacteria could introduce new communities into the microlayer. These communities, in turn, were related to the enrichments of functional genes involved biogeochemical cycling such as carbon fixation cycles, carbon degradation especially in the form of labile carbon, nitrogen cycling as well as in the DMSP demethylation pathways.