論 文 の 内 容 の 要 旨

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論文題目

Dynamics of major phylogenetic groups of marine bacteria in response to phytoplankton blooms, as revealed by novel single-cell based technique

(新規細胞レベルでの解析手法を用いた、植物プランクトンブルームに応答する海洋細菌主要系統群の動態に関する研究)

Phytoplankton bloom is the most important event to stimulate the productivity of ocean ecosystems. This event largely contributes to a process known as biological pump, a major natural process of carbon dioxide sequestration in the ocean. About a half of the photosynthetically produced organic matters are consumed by heterotrophic microorganisms in marine surface layers. Active growth of these heterotrophs facilitates organic matters flux to higher trophic levels via microbial loop and controls sinking flux of organic matters as well as their remineralization for recycling inorganic nutrients. Therefore, the interactions between phytoplankton and heterotrophic bacteria lie at the heart of the carbon cycle in the oceans.

During the last three decades, microbial communities in the ocean have been described in regard to phylogenetically diverse microbial taxa, due to the developments of

culture-independent molecular ecological approaches such as nucleic acid sequencing of phylogenetic marker genes directly retrieved from environmental DNA samples. In addition, total bacterial production has been routinely measured by the incorporation of radiolabeled substrates. The fundamental questions are “Which phylogenetic groups account for total bacterial productivity?” and “What is the relative contribution of each?” These are substantially important to our understanding of the food web dynamics and biogeochemical cycles in the ocean but remain unknown very well.

To answer these questions, I have firstly developed the novel single-cell based method, named bromodeoxyuridine immunocytochemistry-fluorescence *in situ* hybridization (BIC-FISH). This is a combined method of two different techniques that can measure single-cell activity or growth rates identifying their phylotypes. Bromodeoxyuridine (BrdU) immunocytochemistry is a technique to detect BrdU-incorporating (presumably growing) cells with the use of fluorescently labeled antibody. Fluorescence *in situ* hybridization (FISH) is a technique to detect specific phylogenetic groups of microorganisms with the use of fluorescently labeled oligonucleotide probes targeting to 16S ribosomal RNA.

In Chapter 2, the newly developed method has successfully applied to seawater bacterial assemblages in order to compare phylotype-specific growth rates. Also the method worked well with eight bacterial isolates tested in the study. Significant positive correlation between average cellular fluorescence intensity and cell-specific BrdU contents indicated the potential of the method to calculate the growth rate at single-cell levels identifying the phylotype of each cell. When the method was applied to a eutrophic seawater, analysis of single-cell immunofluorescence intensity revealed the differences of cellular growth rates and percentage in actively growing cells among various phylotypes, in which SAR86 and *Vibrio* group bacteria were growing more rapidly than others.

In Chapter 3, I applied the BIC-FISH method to study year-round changes of phylotype-specific bacterial production in a eutrophic seawater and explored the environmental factors controlling these changes. The monitoring by BIC-FISH throughout the year revealed the importance of the *Roseobacter/Rhodobacter* group bacteria as a constantly proliferating basic population (27% of BrdU-positive cells), although their contribution was not significantly correlated with water temperature or with chlorophyll *a* or organic matter concentrations. Also, the Bacteroidetes group bacteria was another important group, as they greatly increased in abundance after the end of phytoplankton blooms. Two other phylotypes tested in this study, the SAR86 and *Vibrio* group bacteria, changed their activities corresponding with water temperature.

In Chapter 4, the BIC-FISH method was used in a mesotrophic seawater to explore whether I can find a similar pattern of phylotype-specific growth response as described in the Chapter 3 in other environmental settings. The method was improved to increase the sensitivity of FISH and applied to less productive oceanic region than that studied in the Chapter 3. The improved protocol was successfully applied to the samples collected during the spring phytoplankton bloom in the western North Pacific. The growth pattern of *Roseobacter/Rhodobacter* and Bacteroidetes group bacteria was in agreement with the results as described in the Chapter 3. *Roseobacter/Rhodobacter* group bacteria were highly active regardless of the presence and absence of phytoplankton bloom, and the activity and production of Bacteroidetes group bacteria were especially high at the aged phytoplankton bloom stations. The dominance of these subgroups in total bacterial production indicated their importance as key bacterial groups in this season and area. The SAR86 group bacteria were more abundant in active cells at the bloom stations than other stations, although their activity was not correlated with several environmental factors including water temperature as indicated by the Chapter 3. The activities of SAR86 and SAR11 group bacteria (percentage of BrdU-positive cells) were high in the bloom stations. However they were not significantly correlated with chlorophyll *a* and organic matter concentrations. Phytoplankton bloom conditions, e.g. fresh and aged, might be affected their activity. The study also showed that *Alteromonas* group bacteria known as numerically minor group in *in situ* seawater were abundant in actively proliferating cells in the bloom stations. Their abundance and activity were strongly correlated with chlorophyll *a* and organic carbon concentrations.

In Chapter 5, I described succession patterns in biomass and productivity of major phylogenetic groups of bacteria during an artificial phytoplankton bloom of mesocosm experiment using the BIC-FISH method, and revealed phylotype-specific contribution to total bacterial production and decomposition of the phytoplankton bloom. The biomass and activity of *Roseobacter/Rhodobacter* and Bacteroidetes group bacteria were maintained at high levels of contribution to total bacterial biomass and production through the experiment and further increased following the peak of the phytoplankton bloom. Especially, the activity of Bacteroidetes group bacteria increased earlier than other bacteria just after the peak of the phytoplankton bloom, indicating that they triggered to decompose the phytoplankton bloom. *Alteromonas* group bacteria were only appeared as a major group in bacterial biomass and production at the peak of the phytoplankton bloom, showing their rapid response and high growth rate. The biomass and activity of SAR11 group bacteria increased with increase of phytoplankton. However, SAR86 group bacteria didn’t respond to the phytoplankton bloom

in this experiment, implying their limited response to some specific organic matters derived from phytoplankton under high temperature conditions.

This study revealed growth performance of each phylogenetic group of marine bacteria in response to phytoplankton blooms, their patterns of response and relative contribution to total bacterial biomass and production by the use of newly developed single-cell method. *Roseobacter/Rhodobacter*, Bacteroidetes, *Alteromonas*, SAR11 group bacteria were suggested to be important subgroups as prominent consumers of the photosynthetically produced organic matters. Based on my spatiotemporal and experimental study of phytoplankton blooms, their relative contributions to total bacterial production were estimated to be 20~40% for *Roseobacter/Rhodobacter* group bacteria, 20~40% for Bacteroidetes group bacteria, 0~30% for *Alteromonas*group bacteria, and 5~15% for SAR11 group bacteria. The sequential growth response of these subgroups to a phytoplankton bloom also illustrated a degradation mechanism of phytoplankton derived organic matters. I have hypothesized that Bacteroidetes group bacteria are firstly proliferated and trigger the collapse of phytoplankton bloom, then *Roseobacter/Rhodobacter* and SAR11 group bacteria actively proliferate and facilitate degradation. Finally, the activity of *Alteromonas* group bacteria contributes the degradation. I found that some phylogenetic groups of bacteria (*Roseobacter/Rhodobacter*, Bacteroidetes, and *Alteromonas*) enlarged their cell sizes during the phytoplankton bloom. In addition to the change of bacterial community structure, up-shift of averaged cell volumes due to the increase of large phylotype abundance may drastically change the organic matter flux from bacteria to their grazers flagellates. The exponential increase of bacterial biomass should stimulate a predator-prey interaction between protozoa and bacteria, which may lead to the change of ecosystem structures through a microbial “trophic cascade”.

The BrdU approach described in this study would be one of the powerful tools for better understanding of the linkage between organic matters and bacterial communities. The diversity and function of microorganisms in the ocean ecosystems will be revealed combining this approach with some other culture-independent molecular approaches and also culture-dependent approaches.