# 南大洋インド洋区における 16S rRNA を用いた海洋細菌群集の生物多様性評価

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# Vertical and horizontal profiles on biodiversity of the active bacterial communities using 16S rRNA molecules in the Pacific sector of the Southern Ocean

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The prokaryotes play an important role in global $CO_2$ fixation as their total carbon on earth is enormous, approximately 60-100% of the total carbon found in plants and phytoplankton. It is important to focus on prokaryotes to understand the whole pictures of complex marine ecosystem. However, little is known about prokaryotic abundance, distribution, diversity, function and process in the Southern Ocean where was pronounced the noticeable change on ocean acidification. Most experiments of them used a short-term perturbation approach by cultured organisms; few were conducted in mesocosms and none *in situ*. It has been noted that only a small fraction (<1%) of the microorganisms found in nature can be cultured using traditional cultivation methods. Thus, it is effective to use culture-independent molecular approaches such as 16S rDNA phylogenetic analysis for revolutionizing our perspective on microbial diversity and distribution. However, the DNA pool extracted from seawater would be consisted of nucleic acids in various physiological states; living, dead and dormant microbial cells and as well as of extracellular free DNA. As RNA molecules possess a much shorter lifetime compared with that of DNA, an environmental RNA was used through recent studies as an indicator of metabolically active microbial populations. Therefore, the 16S rRNA analysis can be used to reveal the active microbial community by the sequencing of its clone library. In this study, we evaluated the current vertical and horizontal profiles on the active bacterial community structures and their biodiversity based on phylogenetic analysis of 16S rRNA molecules at four stations and five depth on longitude 110° E in the Indian sector of the Southern Ocean.

Oceanographic observations were conducted in the Indian sector of the Southern Ocean during cruses of the TR/V *Umitaka-maru* (Tokyo University of Marine Science and Technology) in the austral summers of 2015. Sampling was carried out four stations (KC3, KC4, KC5, and KC6, Fig. 1) and the bulk seawater was collected from five depths (surface 0, 50, 100, 300 and 500 m) at each station. Bacterial samples in the seawater were collected on 0.2-μm Millipore Isopore membrane filter from the 1 L of the <2.0-μm filtrates. Each filter was permeated into 10 mL RNA later® (Ambion, Inc., USA) for stabilizing and protecting RNA and stored at -25°C until further analysis. Total RNA was extracted using an automated nucleic acid extraction instrument with a mechanism of separating magnetic beads. Complementary DNA (cDNA) was synthesized from the total RNA extract using the universal primer, U1492R. Bacterial 16S rRNA gene was amplified using specific primers for the domain Bacteria (24F, U1492R, Lane, 1991) and purified. These products were applied to 16S rRNA phylogenetic analysis. The biodiversity index scores including the nucleotide diversity and the Shannon-Wiener index were calculated as genetic and species diversity, respectively.

The bacterial 16S rRNA clone libraries were successfully constructed using total RNA extracted from 20 seawater samples. A total of 984 clones of the bacterial 16S rRNA was sequenced. An approximately 65% of the sequences were highly similar to those of uncultured species. Many phylotypes more over 11 at least were detected from each sampling site. However, the Evenness values were very high because of large number of phylotypes consisting of one clone. The higher species diversity was shown in surface seawater at St. KC3, KC4, and KC6, whereas the lowest value was shown in that of KC5. On the other hand, the genetic diversity was higher in below 300 m seawater at all stations. A variety of uncultured bacterial lineages were found and the different bacterial genetic and species diversity scores were evaluated in the Indian sector of the Southern Ocean in this study. Although the results that be brought in this study showed the current value at the present time, we could provide

the standard values contributing to future research regarding the effects on complex marine ecosystem caused by ocean acidification that has been expected to progress in the future.

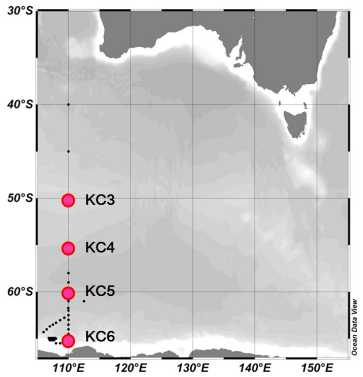


Fig. 1. Location of sampling stations in the Indian sector of the Southern Ocean.