

SL324

## Molecular Ecological Study on the Structure of Bacterial Communities in Lake Soyang

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It is essential to research the structure and function of bacterial communities which play an important role of producers as well as decomposers in order to understand the primary properties of the system, dynamics and stability in the aquatic ecosystem. The difficulties in the research of the structure and species diversity of whole bacterial communities lie in the methodological limitation in isolation and identification of natural bacterial populations of the ecosystem. The purpose of this study was to develop a new method for the research of the structure and diversity of bacterial communities in the aquatic ecosystem. Using the new method, monthly variation of bacterial communities was analyzed according to the change of physico-chemical parameters in Lake Soyang.

The database (EMBL) which contains 16S rRNA sequences of 1,262 bacterial strains was searched, which confirmed that the variable V3 region agreed with the purpose of this study and then a set of eubacterial universal primers was established for amplification of the V3 region. To verify the fitness of the established primers, five Gram positive and seven Gram negative strains were used for optimizing the reaction conditions of polymerase chain reaction(PCR) and single strand conformation polymorphism(SSCP)/heteroduplex pattern analysis, and a laser beam densitometer was used for a quantitative measurement.

The SSCP/heteroduplex pattern analysis showed that each bacterial strain had its own band pattern and the more the strain number the more band pattern, and accordingly, the measurement of relative diversity in bacterial communities could be possible. In addition, an experiment of variable mixed ratios of two strains, *Acinetobacter calcoaceticus* and *Zoogloea ramigera*, from 0.5 to 50%, showed that the bacterial population with a share of even 1% could be detected. In the field study for Lake Soyang and an eutrophic pond, the distinctive differences between bacterial populations and their temporal variations were observed. The newly developed method with PCR amplification and SSCP/heteroduplex pattern analyses of 16S rRNA genes was, therefore, found to be able to apply into the study of temporal and spatial changes in bacterial community structures of various aquatic ecosystems.

When the structure of bacterial communities and its seasonal variation were monitored using the newly developed method, the variations of 33 groups of free-living and 36 groups of attached bacteria in surface water, and 44 groups and 37 groups for each case in deep water were observed. Especially, the proteobacteria alpha group predominated in Lake Soyang with the mean density of 64.4% for free-living and 55.8% for attached populations in surface water, and deep water populations also contained them in high levels, 52.1% and 13.3% in each case. Dominance of this group was previously reported in marine ecosystems and concurrence of it in a freshwater ecosystem in this investigation advocates the general dominance of the group in various aquatic habitats. On the contrary, the increase of the density of proteobacteria gamma group occurred concomitantly with the growth of phytoplankton. As the relationships between the seasonal variation of each 16S rDNA fragment group and various environmental factors were analyzed, the provision of organic matters by the increase of water temperature and that of the activity of phytoplankton affected the structure of bacterial community in surface water, and the sedimentation of particulate organic matters and mixing of water body did in deep water.

For the further characterization of each 16S rDNA fragment group, the sequences and phylogenetic relationships of several purified PCR products were analyzed. Two of them showed close relationship with *Z. ramigera* and *A. lwoffii*. Other one had 93% homology with an unknown alpha proteobacterium (GenBank No. M63812) found in Sargasso Sea, and another product which has identical sequence with *Cyanobacterium* (S. R7) was also identified. Thus, it was approved that the members of bacterial population in Lake Soyang, a freshwater ecosystem, had close relation with those of an oligotrophic marine ecosystem.