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### Microbial communities in the eelgrass bed of the Izu Peninsula, Japan

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The eelgrass, *Zostera marina*, is a flowering plant and forms the eelgrass bed in shallow bottoms throughout the Atlantic, eastern Pacific and Mediterranean. Since the eelgrass bed can temper the tidal current, it plays an important role as breeding and nursing habitats for fish and shellfish. This study was performed to investigate the microbial communities of the thallus of eelgrass specimens, and seawater and sand samples using the clone library method.

The eelgrass specimens, and seawater and sand samples were collected in June and September 2008 at the Tanoura Inlet in Shimoda, Shizuoka, Japan. Microbial communities attached to the thallus of eelgrass specimens were recovered by swabbing with sterile cotton swabs. The DNA was extracted from each sample and 16S rDNAs were amplified by PCR to construct clone libraries. The 16S rDNA sequence of each clone was analyzed by a model ABI 3130xl Genetic Analyzer. The similarity in 16S rDNA sequences between a given isolate and other organisms was compared with all sequence data in the DDBJ/EMBL/GenBank databases using the BLAST algorithm and identification to species was confirmed by phylogenetic analysis using Clustal W software package. Direct counts were determined by the epifluorescent staining method after fixation with Lugol iodine solutions.

The total count of microbial communities ranged from  $3.1 \times 10^6$  to  $1.6 \times 10^7$  cells/mL in seawater samples, from  $1.2 \times 10^8$  to  $2.4 \times 10^8$  cells/g in sand samples, and from  $1.1 \times 10^7$  to  $1.8 \times 10^7$  cells/cm<sup>2</sup> on the thallus of eelgrass specimens. A total of 315 clones were classified into 14 classes, 62 families, 126 genera and 158 species. Alphaproteobacteria and Gammaproteobacteria accounted for 30 and 35% of clones, respectively. Clones obtained from seawater, sand and thallus samples were 50 to 61, 50 to 60 and 44 to 50 species, respectively, suggesting that the microbial communities on the thallus of eelgrass consisted in a diverse of bacterial species.