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PCR-TRFLP and PCR-RFLP Analyses of Activated Sludges from Wastewater Treatment Plants in Korea

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Rapid analysis of complex microbial communities has been one of major objects in microbial ecology (Dunbar et al., 2000). Changes of community structure caused by pollution, climate change, physical disturbances, etc., need to be identified through analysis. In addition, capability of an ecological community to recover from disturbances and its efficiency of resource utilization could be investigated through ecological studies.

In this study, a culture-independent molecular technique using terminal restriction fragment polymorphism (TRFLP) of 16S rDNA genes (Silva and Francisco, 1997) was applied for rapid comparison of bacterial communities of activated sludges taken from 31 wastewater (domestic and industrial) treatment plants (WTPs). As shown Fig. 1, 20-79 terminal restriction fragments (T-RFs) were found in these environmental samples, and community structure of these activated sludges appeared diverse and complex. Sludges from domestic WTPs (No. 1-10 in Fig. 1) and leather-processing industrial WTP (No. 26-28) showed a high degree of similarity in T-RF patterns regardless of locations and operational conditions of the plants. For instances, similarity calculated by the Pearson correlation method between Pohang (No. 4) and Bucheon (No. 5) domestic WTPs was almost 100%. Except the sludge from Gayang (No.1), activated sludges from 9 domestic WTPs showed the total similarity was as high as 65%. Ecological parameters including richness and evenness were also calculated using T-RF band patterns.

In order to examine community structures of activated sludges in detail, 3 samples in Fig. 1 were randomly chosen for restriction fragment polymorphism (RFLP) analysis. Using genomic DNAs (Gayang domestic WTP, Songwon chemical industry WTP, and Hyosung textile industry WTP) as templates, bacterial 16S rDNA were amplified through PCR with universal primers, 27F and 1492R, and cloned into *E. coli* DH5α. Among transformants obtained, 852, 1088, and 1079 clones were analyzed for Gayang, Songwon, and Hyosung, respectively. Unique phylotypes estimated based on RFLP patterns appeared to be 75-77 % o f total number of clones. It was also found that the most dominant phylotype included 1.9%, 2.8%, and 7.1% of clones for Gayang, Songwon, and Hyosung, respectively.

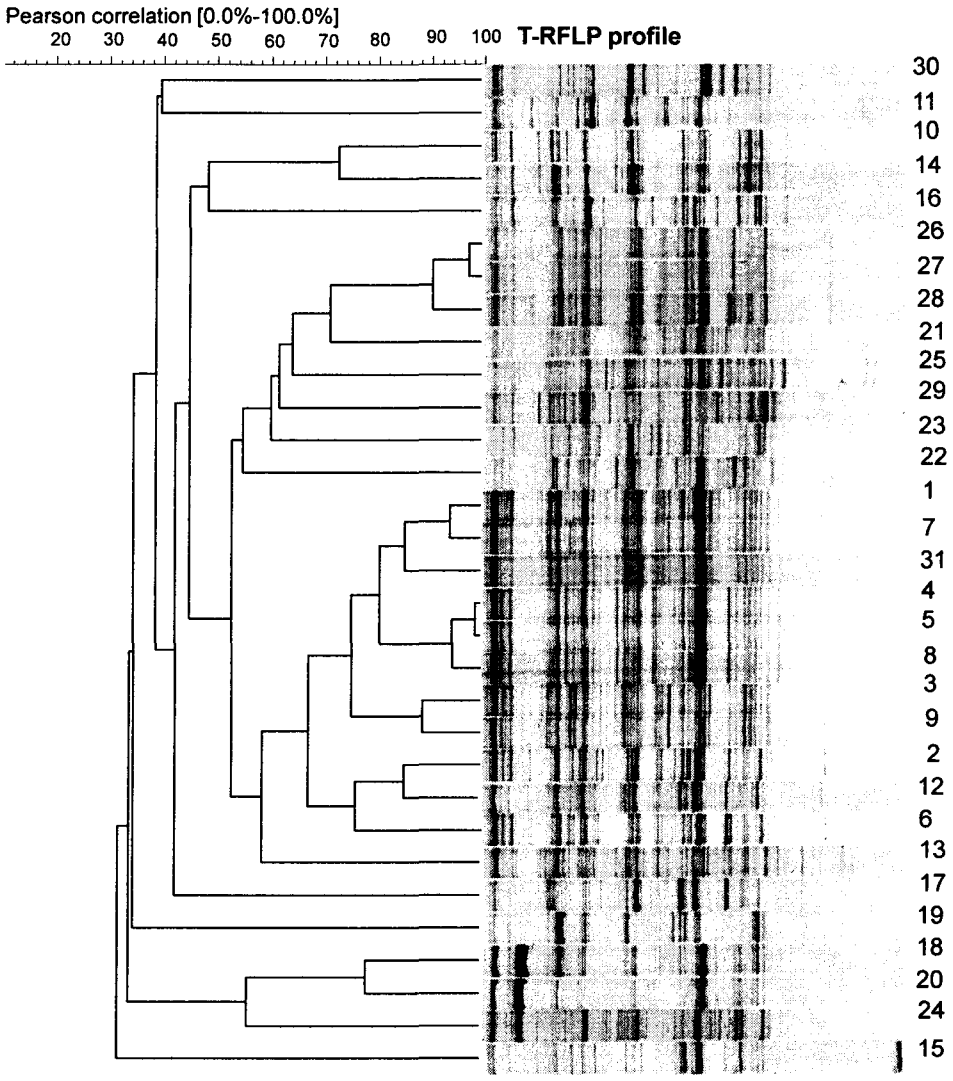

Fig. 1. Dendrogram of the T-RFs of *Hae*III-digested 16S rDNAs amplified from 31 activated sludges. No. 1-10: domestic WTPs, No. 11-15: chemical industry WTPs, No. 16-20: textile and dye industry WTPs, No. 21-23: paper-processing industry WTPs, No. 24-25: food industry WTPs, No. 26-28: leather processing industry WTPs, No. 29-30: electrical industry WTPs, No. 31: sewage treatment plant.

Based on these results, a richness curve was calculated by rarefaction, which showed that these 3 activated sludges contained very diverse bacterial communities. 132 clones were chosen from the most dominant phylotypes for 16S rDNA sequence determination. Obtained sequences (about 500 nucleotides each) were compared with those in the RDP II database. The sequence information indicated that bacteria contained in activated sludges were classified mainly to three divisions of the *Proteobacteria*, the Gram-positive group, and the *Cytophage-Flexibacter-Bacteriroides* groups (Fig. 2).

For clones from Gayang, the *Planctomycetes* and the *Acidobacteria* were also found. Many clones were not assigned to known bacterial division and remained unclassified. This has been a typical result of cloning studies for environmental samples (Liesack and Stackebrant, 1992) and indicates that most bacteria in nature including WTPs have not yet been cultured and characterized.

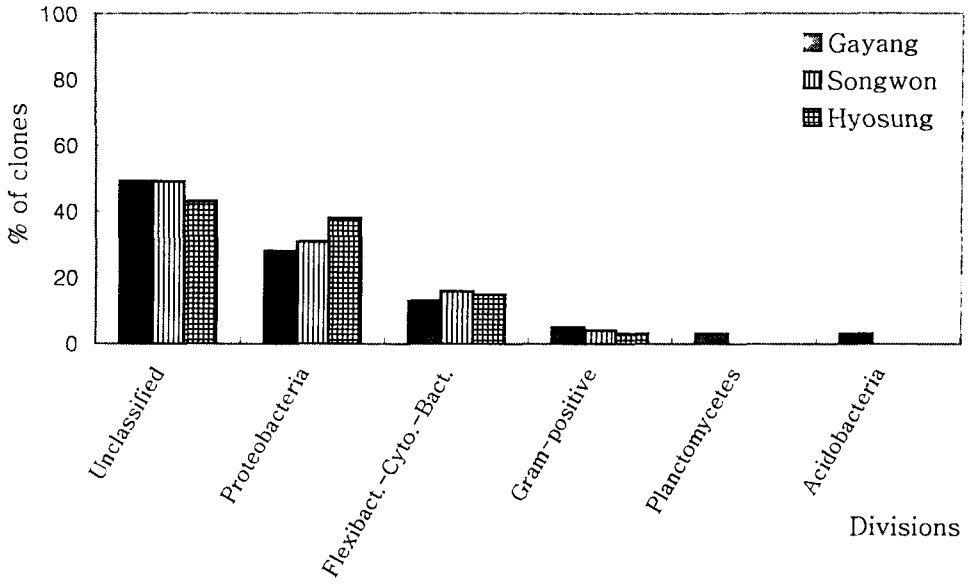

Fig.2. Distribution of phylogenetic groups of clones from activated sludges. Division level affiliations were identified by partial sequences of 16S rDNA. *Flexibact.-Cyto.-Bact., Flexibacter-Cytophaga-Bacteriodes.*

References

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