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PCR- T-RFLP Analyses of Bacterial Communities in Activated Sludges in the Aeration Tanks of Domestic and Industrial Wastewater Treatment Plants

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Abstract In order to compare bacterial community structure and diversity in activated sludges, terminal restriction fragment length polymorphism (T-RFLP) of PCR-amplified 16s rDNAs was analyzed for 31 domestic and industrial wastewater treatment plants (WTPs). Regardless of the characteristics of the wastewaters, the bacterial community structures of activated sludges appeared diverse and complex. In particular, activated sludges in domestic WTPs contained higher bacterial diversity than those in industrial WTPs. It was also found that terminal restriction fragment (T-RF) profiles derived from domestic WTPs were very similar with each other, although activated sludges were collected from different plants at different locations. Interestingly, activated sludges of a WTP where restaurant and toilet sewages of a company were managed showed a bacterial community structure similar to that of domestic WTPs. Activated sludges in leather industrial WTPs also showed a high similarity. However, other wastewaters possessed different bacterial communities, so that overall similarity was as low as about 30%. Since activated sludges from WTPs for domestic wastewaters and a company sewage appeared to hold similar bacterial communities, it was necessary to confirm if similar wastewaters induce a similar bacterial community. To answer this question, analysis of T-RFs for activated sludges, taken from another 12 domestic WTPs, was conducted by using a 6-FAM™-labeled primer and an automated DNA sequencer for higher sensitivity. Among 12 samples, it was again found that T-RF profiles of activated sludges from Yongin, Sungnam, Suwon, and Tancheon domestic WTPs in Kyonggi-do were very similar with each other. On the other hand, T-RF profiles of activated sludges from Shihwa and Ansan WTPs were quite different from each other. It was thought that this deviation was caused by wastewaters, since Ansan and Shihwa WTPs receive both domestic and industrial wastewaters. From these results, it was tentatively concluded that similar bacterial communities might be developed in activated sludges, if WTPs treat similar wastewaters.

Key words: Bacterial community, terminal restriction fragment length polymorphism, activated sludge

Today, the activated sludge system is being widely used for biological treatments of municipal and industrial wastewaters. Communities of microorganisms present in activated sludge are responsible for most of the carbon and nutrient removal from wastewater and thus represent the core component of WTPs [31]. Nevertheless, it is largely unknown which bacterial communities in activated sludge are responsible for enhanced biological wastewater treatment [9]. Moreover, certain bacterial species can also be detrimental for wastewater treatment by negatively influencing the settling properties of activated sludge, by contributing to the formation of foam, or by outcompeting microorganisms for nutrient removal [19]. Therefore, the knowledge of ecology of the bacterial communities is required to understand the efficiency and stability of biological wastewater treatment [31]. For instance, information about the numbers, locations, and activities of microorganisms are needed for characterizing bacterial community structure and diversity.

Various approaches, both culture-dependent and independent, have been applied to analyze and compare the bacterial community structures of activated sludges [3, 5, 26]. However, most microorganisms have not been cultivated, and only a small fraction (0.1- 10%) of bacteria in nature can be cultured by conventional culture-dependent methods [11]. Recently, culture-independent molecular techniques, such as fluorescence in situ hybridization (FISH) or polymerase chain reaction (PCR), have been applied to analyze bacterial communities. These approaches rely on sequence determination

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of 16S rRNA and/or hybridization of probes to specific 16S rRNA fragments [5, 14, 15, 28]. Although such techniques have been shown to be more sensitive than culture-dependent methods, they are difficult to apply for microbial ecology with many samples, due to consuming time and effort. Furthermore, they are still limited by rRNA databases that have been established mainly through the traditional culture-dependent method.

Alternative approaches, such as denaturing gradient gel electrophoresis (DGGE) [21, 24, 29], thermal gradient gel electrophoresis (TGGE) [8, 10], restriction fragment length polymorphism (RFLP) [23], single-strand conformation polymorphism (SSCP) [17], and terminal restriction fragment length polymorphism (T-RFLP) [1, 6, 12, 21, 22], have been used for characterizing complex bacterial communities. One of the methods well suited for studying complex bacterial communities is the analysis of electrophoretic patterns of terminal restriction fragments (T-RFs), which can be used for quantitative and qualitative analyses of community structure in a complex ecosystem. The T-RFLP method has been used mainly for analyzing 16S rRNA genes. Recently, the usage of this method has been extended to functional genes, such as nitrous oxide reductase (*nos*Z) [27] and ammonia monooxygenase (*amo*A) genes [13].

In T-RFLP analysis, only the labeled terminal (5' or 3') fragments generated by a restriction digestion are examined on the basis of size (length). Extracted community DNA is amplified by PCR with fluorescence-labeled or biotin-labeled primer at the 5' end. The PCR products are then digested with one or more restriction enzymes, and the labeled terminal restriction fragments are electrophoretically separated and detected [22]. Each T-RF is regarded as an individual bacterial species in a complex community.

In this study, we investigated bacterial community structures of activated sludges collected from WTPs in Korea by using the T-RFLP method. In particular, it was aimed to investigate if different wastewaters lead to establishment of different bacterial communities. For this scope, domestic and various industrial wastewaters were collected from all over Korea, and their bacterial community structures were

Table 1. Activated sludges collected from the aerobic tanks of 31 different wastewater treatment plants in Korea.

No.	Name of company	Location	Category
1	Gayang domestic wastewater treatment plant	Seoul/Gayang	Domestic wastewater treatment plants
2	Kongju domestic wastewater treatment plant	Chungnam/Kongju	Domestic wastewater treatment plants
3	Suwon domestic wastewater treatment plant	Kyonggi/Suwon	Domestic wastewater treatment plants
4	Pohang domestic wastewater treatment plant	Kyoungbuk/Pohang	Domestic wastewater treatment plants
5	Bucheon domestic wastewater treatment plant	Kyonggi/Bucheon	Domestic wastewater treatment plants
6	Yongin domestic wastewater treatment plant	Kyonggi/Yongin	Domestic wastewater treatment plants
7	Gumi domestic wastewater treatment plant	Kyoungbuk/Gumi	Domestic wastewater treatment plants
8	Chungnang domestic wastewater treatment plant	Seoul/Chungnang	Domestic wastewater treatment plants
9	Koyang domestic wastewater treatment plant	Kyonggi/Koyang	Domestic wastewater treatment plants
10	Gapyong domestic wastewater treatment plant	Kyonggi/Gapyong	Domestic wastewater treatment plants
11	Komex Latex	Kyungbuk/Daegu	Chemical industry
12	Valeo Pyeong Haw	Kyungbuk/Daegu	Chemical industry
13	Songwon Industrial	Kyonggi/Suwon	Chemical industry
14	Hyundal-Motor	Chungnam/Asan	Chemical industry
15	LG Chemical	Kyungnam/Onsan	Chemical industry
16	Kyung-in Synthetic Corporation	Kyonggi/Shihwa	Textile/dye industry
17	Uksung Chemical	Kyungnam/Pusan	Textile/dye industry
18	Kapeul Textile	Kyungbuk/Daegu	Textile/dye industry
19	Songwon Color	Kyungnam/Onsan	Textile/dye industry
20	Hyosung	Kyonggi/Anyang	Textile/dye industry
21	Korea Tobacco & Ginseng Corporation	Kangwon/Wonju	Textile/dye industry
22	Samjeong Pulp	Chungnam/Chonan	Paper industry
23	Poongman Paper	Chungnam/Yongigun	Paper industry
24	Samyang Genex	Kyonggi/Inchon	Food industry
25	Sinwoo Industry	Kyonggi/Yongin	Food industry
26	Namyang Firm	Kyonggi/Uijeonbu	Leather industry
27	Jihyoun Product	Kyonggi/Ansan	Leather industry
28	Dukchon Industry	Kyonggi/Ansan	Leather industry
29	LG Silton	Kyoungbuk/Gumi	Electrical machinery/electronics industry
30	Samyoung Electronics	Kyonggi/Sungnam	Electrical machinery/electronics industry
31	Hysung Wastewater Treatment Plant	Kyonggi/Anyang	Sewage treatment plant

compared with each other. Since conventional environmental analyses, such as BOD (biological oxygen demand), COD (chemical oxygen demand), and SS (suspended solid), can not distinguish features of wastewater, it would be interesting if similar community structures were seen for similar wastewaters, regardless of the locations of the WTPs. For this question, activated sludges from 12 domestic WTPs at different locations were analyzed. In addition, results of this study would provide fundamental data for constructing advanced analyses tools such as a gene chip.

MATERIALS AND METHODS

Sludge Samples

Activated sludge was collected from the main aerobic tanks of 31 domestic and industrial WTPs in Korea (Table 1). To compare bacterial communities in domestic wastewater treatments, additional activated sludge samples were collected from 12 domestic WTPs (Table 2). Upon receiving, suspended solids in mixed liquors were harvested by centrifugation (300 ×*g* at 4°C for 10 min) and kept at -20°C until used.

DNA Extraction from Sludge

Genomic DNA from the sludge was extracted according to the modified method of Miller *et al.* [20]. Sludge (0.2 g wet weight), 600 µl of extraction buffer (50 mM NaH_2PO_4, 50 mM NaCl, 500 mM Tris-HCl, pH 8.0, 5% SDS), and 300 µl of chloroform/isoamylalchohol (24:1) were mixed in a 2-ml tube, containing 0.5 g of 0.1-mm zirconia glass beads (BioSpec Products, U.S.A.). The tube was shaken, using a Mini Bead Beater (BioSpec Products, U.S.A.) at 4,200 rpm for 2 min. Supernatant obtained from homogenized suspension by centrifugation (12,000 rpm at 4°C for 10 min) was extracted with equal volume of the mixture of phenol, chloroform, and isoamylalcohol (25:24:1), re-extracted with an equal volume of chloroform and isoamylalcohol (24:1), precipitated with cold 0.3 M isopropanol, washed with 70% ethanol, and dissolved in 100 µl of TE buffer (10 mM Tris-Cl, 1 mM EDTA, pH 8.0). For further purification of DNA, UltraClean™15 (Mo Bio, U.S.A.) was used, according to the manufacturer's instruction. Obtained genomic DNA extracts were stored at -20°C until used.

PCR Amplification of 16S rDNA for T-RFLP Analysis

For amplifying the 16S rDNA gene from the genomic DNA extract by using PCR, biotin-labeled forward primer 27F (*E. coli* numbering 8-27: 5'-AGA GTT TGA TCC TGG CTC AG-3') and nonlabeled reverse primer 785R (*E. coli* numbering 785-804: 5'-ACTACCRGGGTATCTAATCC-3') [16] were custom synthesized (Takara, Japan). PCR was carried out in 50 µl, containing 100-150 ng of extracted DNA from activated sludge as a template, 1× reaction buffer (100 mM Tris HCl, 400 mM KCl, 1.5 mM $MgCl_2$, 500 µg/ml BSA, pH 8.3), 200 µM dNTPs, 0.1 µM primer, and 2.5 unit of Taq polymerase (Takara, Japan). Temperature cycles for the reaction were: 95°C for 5°Cmin (hot start), 30 cycles of 95°C for 30 sec, 58°C for 30 sec, and 72°C for 1 min, followed by 72°C for 10 min. Primers used for PCR of additional activated sludges, collected from 12 domestic WTPs, were 27F and 1492R (5'-GGT TAC CTT GTT ACG ACT T-3') [21]. The forward primer 27F was 5'-end labeled with phosphoramidite fluorochrome 5-carboxyfluorescein (6-FAM) by the metabion (metabion GmbH, Germany). Temperature cycles for the reaction were: initial 95°C for 3 min, 30 cycles of 95°C for 30 sec, 49°C for 30 sec, and 72°C for 1 min 30 sec, followed by 72°C for 10 min. PCR products were confirmed by electrophoresis in a 0.7% agarose gel followed by ethidium bromide staining.

T-RFLP Analysis of Amplified 16S rDNA

The biotin-labeled PCR products were digested with the restriction enzyme *Hae*III (Promega, U.S.A.) at 37°C for 2 h. Digested DNAs were reacted with streptavidin paramagnetic particles (Promega, U.S.A.) at room temperature for 10 min in 1× SSC buffer (0.3 M sodium citrate, 3 M NaCl, pH

Table 2. Twelve domestic wastewater treatment plants examined in this study.

No.	Name of wastewater treatment plant	Location	Property of wastewater
32	Ansan domestic wastewater treatment plant	Kyonggi/Ansan	Domestic wastewater+Industrial wastewater
33	Sungnam domestic wastewater treatment plant	Kyonggi/Sungnam	Domestic wastewater
34	Suwon domestic wastewater treatment plant	Kyonggi/Suwon	Domestic wastewater
35	Yongin domestic wastewater treatment plant	Kyonggi/Yongin	Domestic wastewater
36	Tancheon domestic wastewater treatment plant	Seoul/Tancheon	Domestic wastewater
37	Shihwa wastewater treatment plant	Kyonggi/Shihwa	Domestic wastewater+Industrial wastewater
38	Cheju domestic wastewater treatment plant	Cheju/Cheju	Domestic wastewater
39	Chochiwon domestic wastewater treatment plant	Chungnam/Chochiwon	Domestic wastewater
40	Jincheon domestic wastewater treatment plant	Chungbuk/Jincheon	Domestic wastewater
41	Sincheon domestic wastewater treatment plant	Kyungbuk/Sincheon	Domestic wastewater
42	Kongju domestic wastewater treatment plant	Chungnam/Kongju	Domestic wastewater
43	Nangi domestic wastewater treatment plant	Seoul/Nangi	Domestic wastewater

7.0), separated with a magnetic stand, and washed with 0.1× SSC buffer. To separate biotinylated ssT-RFs, DNAs obtained were treated with 0.2 M NaOH for 5 min at room temperature and separated from streptavidin by adding 25% NH_4OH at 65°C for 15 min. The supernatants (containing biotinylated ssT-RFs) were collected by centrifugation (12,000 rpm, 5 min), vacuum dried to eliminate remaining ammonia, and dissolved in 10 µl of TE buffer (pH 8.0). To separate T-RFs, electrophoresis was conducted on a 6% polyacrylamide gel, containing 8 M urea and 1× TBE (200 mM Tris, 100 mM boric acid, and 100 mM EDTA) as a running buffer. T-RF solution (2.5 µl) and 1.5 µl of loading dye (95% formamide, 10 mM NaOH, 20 mM EDTA, 0.02% bromophenol blue, 0.02% xylene cyanol FF) were mixed, denatured at 95°C for 3 min, and immediately chilled on ice. Two-and-a-half µl of this mixture was loaded onto the gel (20 cm×45 cm, S4S, Owl, U.S.A.) and run at 55 W and 1,900 V for 160 min. After electrophoresis, the gel was washed with 10% acetic acid for 30 min, rinsed with distilled water, and stained in the silver-containing solution (0.1% $AgNO_3$, 0.055% formaldehyde) for 30 min. For visualizing bands, the developing solution (3% Na_2CO_3, 2 mg/ml of $Na_2S_2O_3$, 0.055% formaldehyde) was added for 3-5 min and was replaced with 10% acetic acid. In order to survey the similarity of community structures, the *Hae*III-digested T-RF profiles were analyzed by Gel Compar II software (Applied Maths, Kortrijk, Belgium) through cluster analysis, using a multi-line screener program. Dendrograms were created by using a UPGMA (unweighted pair group with mathematical averages) algorithm by the Pearson correlation method, by means of the Gel Compar II software.

For T-RFLP analyses of activated sludges from 12 domestic WTPs, PCR was performed using a FAM™-labeled broad-range primer 27F in a combination with a broad-range primer 1492R. The amplified PCR products were purified, using the QIAquick PCR Purification Kit (QIAGEN, U.S.A.). The FAM™-labeled PCR products were digested with *Hha*I (Promega, U.S.A.) at 37°C for 1 h 30 min. Three µl of the digest were mixed with 2 µl of loading buffer and 0.5 µl of internal length standard (Rox™ Size Standard, Applied Biosystems, U.S.A.), and analyzed on the ABI PRISM-System 3770 automated sequencer (Applied Biosystems, U.S.A.). After electroporesis, the sizes of the generated fluorescence-labeled 5'-terminal fragments were determined by comparing with the internal length standard, using GeneScan 3.7 analysis software (Applied Biosystems, U.S.A.).

Calculation of Diversity Indices

The similarity between communities was estimated by numerically analyzing the profile of T-RFs in gel images with Gel Compare II (Applied Maths, Kortrijk, Belgium) and GeneScan 3.7 analysis software (Applied Biosystems). The UPGMA (unweighted pair group with mathematical averages) method was applied together with the site distance matrix method to determine similarities between T-RF fingerprints [7]. Various diversity indices were calculated from T-RFLP by using the Shannon-Weaver index (H), Pielou's evenness index (e), and Simpsons dominance index (c) [4]. Species richness, which represents the total number of species or operational taxonomic units (OTUs), was calculated by rarefaction with the online calculator (http://www.biology.ualberta.ca/jbrzusto/rarefact.php).

Results and Discussion

T-RFLP Analysis of Activated Sludges in 31 Wastewater Treatment Plants

T-RFLP analyses were performed for activated sludges from the aerobic tanks of 31 domestic and industrial WTPs. As seen in Fig. 1, electropherograms of 31 activated sludge samples (Table 1) showed different or similar T-RF profiles that could be used to differentiate bacterial communities. From 16-79 bands appeared for each samples, with sizes ranging between 120 and 500 bp (mostly 200-300 bp), and these results seemed to be similar with a previous study of Wagner and Loy [31] who reported that OTUs of

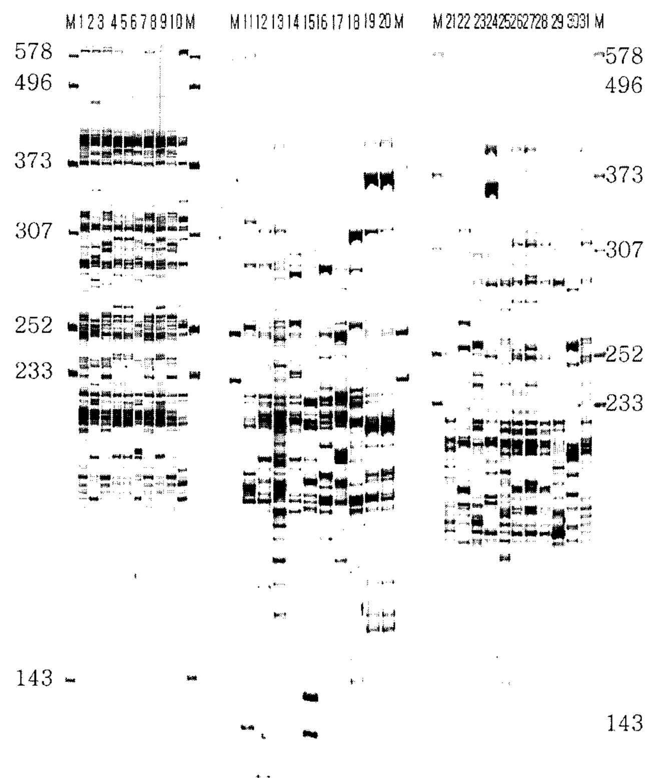

Fig. 1. T-RF profiles of the activated sludges generated by *Hae*III digestion.
(M: size marker, Lanes 1-31: listed on Table 1). Number on both sides represent sizes (number of bases) of the bands.

Table 3. The commonly appeared T-RFs found in the electropherogram.

Source of activated sludge	Size of commonly appeared T-RFs (bases)
Domestic wastewater treatment plant	342, 278, 235, 197, 191
Chemical industry	239, 236, 194, 191
Textile/dye industry	236, 193, 191, 187
Tobacco/paper industry	413, 251, 198, 185
Food industry	392, 341, 314, 297, 290, 269, 223, 179
Leather industry	415, 413, 369, 341, 338, 330, 308, 305,301, 286, 275, 256, 252, 240, 234, 229, 225, 223, 197, 195, 156
Electrical machinery/electronics industry	412, 345, 324, 318, 313, 307, 275, 233, 231, 211, 196, 189

16S rRNA-based diversity surveys of wastewater treatment plants and reactors were 16-75.

Sludge samples from the domestic WTPs (No. 1-10) and leather-processing industrial WTPs (No. 26-28) showed very similar T-RF profiles, although they were collected from different locations with different plant operation conditions. However, T-RF profiles of the rest of the samples appeared to be quite different. It was apparent from this electropherogram that no T-RF was common for all 31 samples, but some bands were commonly present according to the type of activated sludge (Table 3). For these T-RFs, putative bacterial species were assigned to each band by matching the sizes of appeared bands with *Hae*III-digestion simulated T-RFs of the 16S rRNA sequence of the RDP database. However, it should be noted that more than one species was matched for each T-RF (data not shown). Meanwhile, some T-RFs did not match with any of the species in the database, possibly indicating that these strains have never been cultured or found until now. As a matter of fact, bacterial 16S rDNAs have been reported for about 8,000 species [25], which estimated to be less than 10% of total organisms existing in the environment [2].

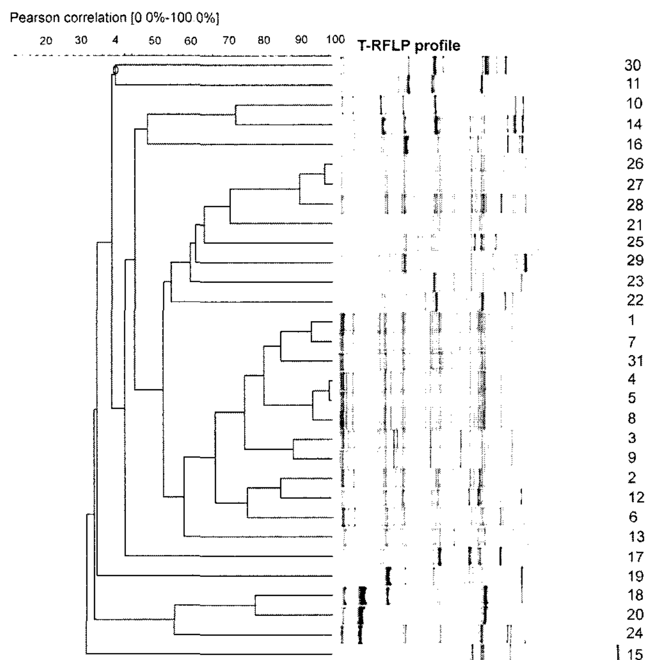

Fig. 2. Dendrogram of the T-RFs of *Hae*III-digested 16S rDNAs amplified from 31 different activated sludges.
Lane numbers are identical to those in Table 1.

The *Hae*III-digested T-RF profiles, representing fingerprints of bacterial communities and the degrees of similarity among the communities, were examined through cluster analysis by using Gel Compar II software that generates a dendrogram by the Pearson correlation method (Fig. 2) [18]. Total similarity among the 31 samples was as low as 10%. However, similarity between activated sludges from domestic WTPs (No. 1-10) appeared very high. Except for Gapyong (No. 10), activated sludges from domestic WTPs showed total similarity of as high as 65%. It was also found that the sewage (mainly from restaurants and rest rooms in the company) treatment plant at Hyosung (No. 31) seemed to contain a bacterial community similar to those of domestic WTPs (No. 1-9). However, except for the leather industry samples (90%), the similarity of activated sludges from other industrial WTPs was lower than those of domestic WTPs.

Since the classification of the industry is based on its product (not on the characteristics of wastewater), it was not surprising that similarity in the class was somewhat low. However, it was of interest that sludges from 9 domestic WTPs showed high degree of similarity, regardless of locations of the plants. Hiraishi *et al.* [12] also reported that the variation in T-RFLP profiles among activated sludges from domestic WTPs was relatively small, independent of the location of plants.

T-RFLP Analysis of Activated Sludges from 12 Additional Domestic Wastewater Treatment Plants

Since 9 domestic WTPs showed high similarity in terms of bacterial community structure, 12 activated sludge samples were additionally collected from different domestic WTPs. In this case, T-RFs were labeled with 6-FAM™, separated, and detected by using an automatic DNA sequencer for higher sensitivity. As shown in Fig. 3, digestion with *Hha*I of 16S rDNA fragments amplified from the sludges generated

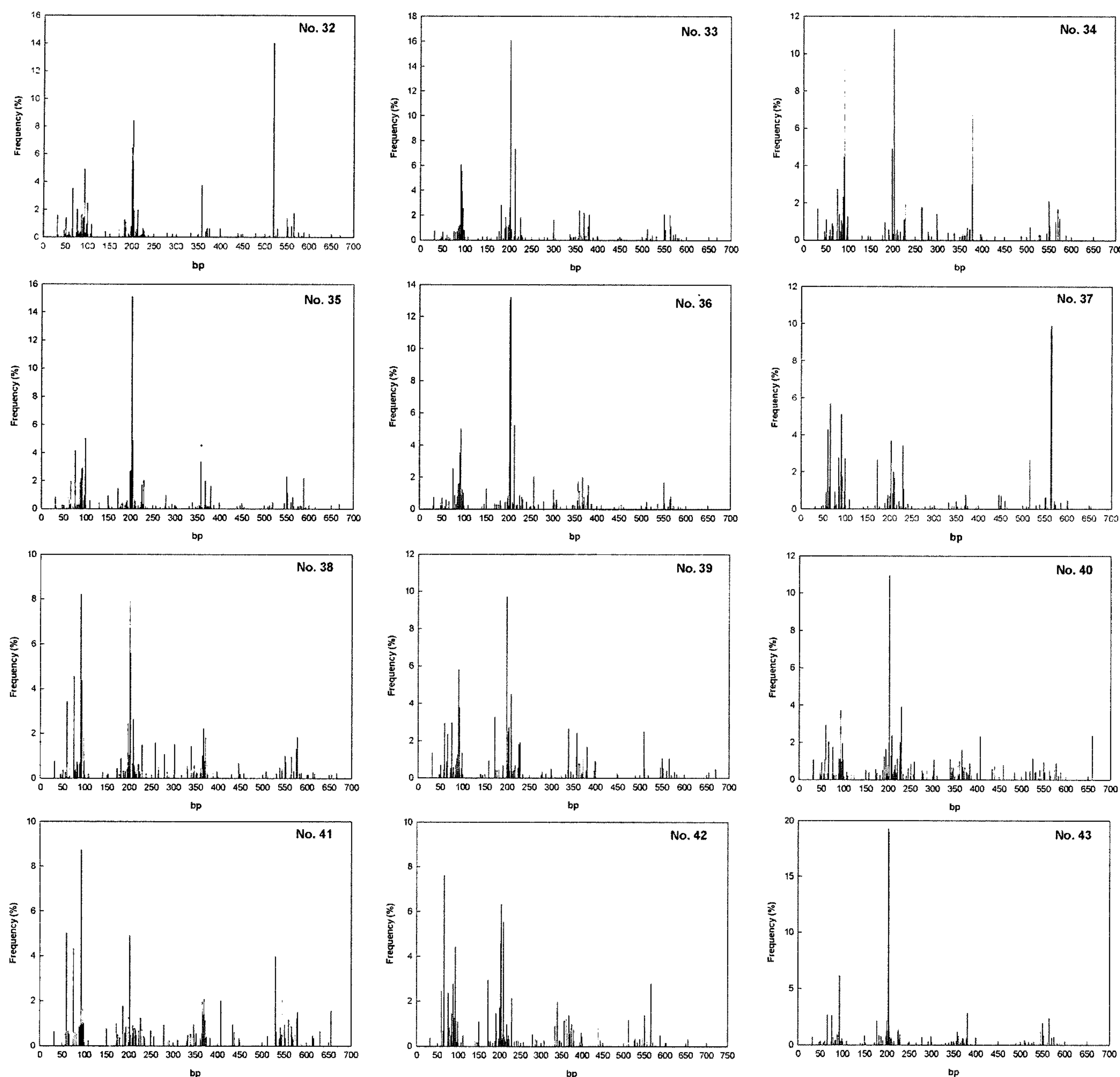

Fig. 3. 5' T-RF profiles of *Hha*I-digested 16S rDNAs amplified from activated sludges of 12 wastewater treatment plants.

at least 80 T-RFs which were much more than shown in Fig. 1. The difference in the number of T-RFs might be due to differences in primers, restriction enzymes, PCR conditions, sites of wastewater treatment plants and T-RFLP analysis methods. In particular, this result might have been obtained because more T-RF bands were detected due to the FAM™-labeled primer and the automatic sequencer with higher resolution [12].

Based on the size and frequency of each band, a distance matrix was calculated, followed by construction of a dendrogram (Fig. 4), by using the UPGMA (unweighted pair group with mathematical averages) method. The correlation coefficient between activated sludges of Yongin and Tancheon was 0.768, which represented the closest similarity among the 12 samples. Bacterial community in the activated sludge of Sungnam also showed a close similarity with those of Tancheon and Yongin.

Except for samples from Ansan and Shihwa WTPs, the farthest distance (0.707) was found between Tancheon and Chochiwon. Therefore, the overall similarity (Pearson correlation) of bacterial community structures for 10 activated sludges was about 30%, which was significantly lower

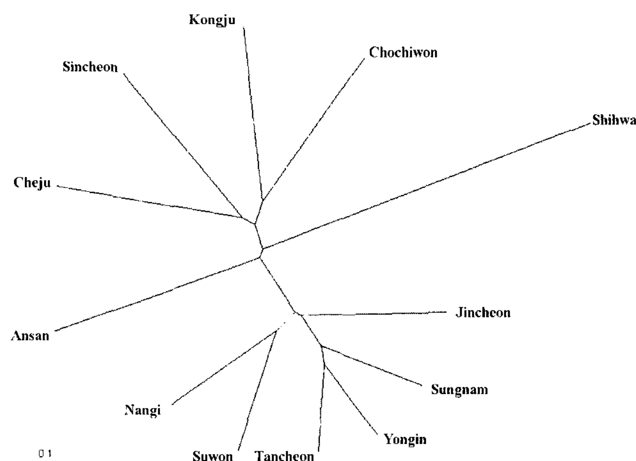

Fig. 4. Similarities between bacterial community structures of activated sludges.

An unrooted neighbor-joining tree was constructed, based on correlation coefficients of T-RF patterns from 12 different domestic wastewater treatment plants. Scale bar indicates correlation coefficient of 0.1.

than about 55% found in Fig. 2. However, 6 activated sludges from Nangi, Suwon, Tancheon, Yongin, Sungnam, and Jincheon showed about 50% of overall similarity for bacterial community structure. Activated sludges of Ansan and, particularly, Shihwa deviated far from the other sludges. This indicated that influent wastewaters for these two treatment plants might be significantly different from the others. Interestingly, Shihwa and Ansan WTPs are receiving both domestic and industrial wastewaters. Industrial wastewaters are effluents from the primary treatment facilities of nearby Shihwa and Panwol industrial complexes. The basic question raised in this study was: Are the bacterial community structures similar if characteristics of wastewaters are similar, or vice versa? From the results in Fig. 2 and Fig. 4, it could tentatively be concluded that two bacterial community structures would be similar in cases where wastewaters are similar.

Diversity Indices

The degrees of diversity among the communities were quantified by numerically calculating diversity indices (richness, diversity, evenness, and dominance) from T-RF profiles (Fig. 2) assessed with Microsoft Excel software (Microsoft, U.S.A.) (Table 3). Species richness (the number of species within a community) and species evenness (the size of species population within a community) are two essential parameters for defining microbial community structure and diversity. According to T-RF analyses of the 31 WTPs, activated sludges from domestic wastewater plants (No. 1-9), except for Gapyong (No. 10), showed higher richness than those of industrial WTPs. In particular, the richness of activated sludges from chemical and textile/dye industrial WTPs was lower than that of other industrial WTPs. This

Table 4. Diversity indices obtained, based on T-RFLP profiles of 31 activated sludges.

Parameter	Sample number										
	1	2	3	4	5	6	7	8	9	10	11
Richness (S)[a]	76	60	70	59	60	53	59	66	59	38	38
Diversity (H)[b]	5.84	5.32	5.83	5.46	5.26	5.35	5.6	5.43	5.62	4.96	4.85
Evenness (e)[c]	0.934	0.902	0.951	0.929	0.892	0.937	0.952	0.899	0.956	0.945	0.924
Dominance (D)[d]	0.023	0.027	0.021	0.032	0.035	0.03	0.025	0.031	0.023	0.039	0.046
Parameter	Sample number										
	12	13	14	15	16	17	18	19	20	21	22
Richness (S)[a]	48	62	39	35	35	34	34	20	27	48	50
Diversity (H)[b]	5.22	5.75	4.83	4.63	4.68	4.58	4.33	3.54	3.88	5.41	5.27
Evenness (e)[c]	0.935	0.966	0.914	0.903	0.912	0.899	0.852	0.819	0.816	0.969	0.934
Dominance (D)[d]	0.034	0.021	0.045	0.056	0.056	0.059	0.083	0.124	0.113	0.027	0.032
Parameter	Sample number										
	23	24	25	26	27	28	29	30	31		
Richness (S)[a]	48	52	60	47	54	50	66	35	56		
Diversity (H)[b]	5.39	5.37	5.65	5.33	5.49	5.44	5.75	4.73	5.62		
Evenness (e)[c]	0.966	0.942	0.957	0.96	0.954	0.965	0.951	0.923	0.967		
Dominance (D)[d]	0.028	0.031	0.024	0.029	0.026	0.026	0.023	0.049	0.023		

[a]Calculated as $S=\sum$(number of distinct T-RF in a profile).
[b]Calculated as Shannon-Weaver diversity $(H)=\sum(P_i)(\log_2 P_i)$, where P_i is the proportion of an individual T-RF.
[c]Calculated from H as follows: $e=H/H_{max}$, where $H_{max}=\log_2 S$ and S is the total number of T-RF.
[d]Calculated as Simpson index $(D)=\sum(P_i)^2$, where P_i is the proportion of an individual T-RF.

is probably because toxicity of industrial wastewaters (chemical and textile/dye industrial wastewaters) was higher than that of the domestic wastewater. Turpeinen *et al.* [30] reported that a decrease in microbial diversity was observed at the wood impregnating plant with the highest level of chromated copper arsenate (CCA) contamination, and the total number of T-RFs was reduced at a site contaminated by heavy metals with high solubility and bioavailability.

Simpson's dominance index values for No. 19 (Songwon color) and No. 20 (Hyosung) were higher than most of the other samples, which meant that bacterial communities of the Songwon and Hyosung activated sludges were dominated by a few T-RFs. Cho *et al.* [4] analyzed community structures of groundwaters and showed that the community structure of groundwater contaminated with a livestock wastewater was more diverse than that of uncontaminated groundwater. They also calculated diversity indices from RFLP patterns of 16S rDNA clone libraries: For clean groundwaters, values for diversity, evenness, and dominance were 2.538-2.589, 0.679-0.712, and 0.147-0.152, respectively, whereas values of diversity (4.207-4.585), evenness (0.930-0.947), and dominance (0.014-0.021) for contaminated groundwater were higher. However, these values were lower than those of the activated sludges in this study (Table 4), indicating that the community structure of activated sludges was as diverse as that of groundwater contaminated with livestock wastewaters.

CONCLUSION

Regardless of the characteristics of wastewaters, bacterial community structures of activated sludges from 31 WTPS appeared diverse and complex. It was found that T-RF profiles derived from domestic WTPs and leather industrial WTPs were very similar with each other, although activated sludges were collected from different plants at different locations. Since activated sludges from WTPs for domestic wastewaters and a company sewage appeared to have similar bacterial communities, it was necessary to confirm if similar wastewaters induce a similar bacterial community. To answer this question, an analysis of T-RFs for activated sludges, taken from another 12 domestic WTPs, was conducted by using a 6-FAM™-labeled primer and an automated DNA sequencer for higher sensitivity. Among the 12 samples, it was again found that T-RF profiles of activated sludges from Yongin, Sungnam, Suwon, and Tancheon domestic WTPs in Kyonggi-do were very similar with each other. But, T-RF profiles of activated sludges of the Shihwa and Ansan WTPs were quite different from the rest of the samples. This deviation might have been due to the wastewaters, since Ansan and Shihwa WTPs receive both domestic and industrial wastewaters. These results lead us to tentatively conclude that similar bacterial communities might be developed in activated sludges if WTPs treated similar wastewaters.

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