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MICROBIAL COMMUNITY DYNAMICS IN MANURE COMPOSTS BASED ON 16S AND 18S rDNA T-RFLP PROFILES

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ABSTRACT

Compost processing is assumed to be related to the microbial communities present. However, methods that will evaluate these relationships are not well understood. In this study, terminal restriction fragment length polymorphism (T-RFLP) analysis was used to evaluate the diversity of PCR-amplified bacterial 16S and fungal 18S rDNA communities from manure composts at different stages of composting (initial [day 0], thermophilic [day 24], and mature [day 104]). Results showed that the bacterial and fungal community profiles changed over the composting process, with bacterial communities showing a higher diversity compared with the fungal communities. During the thermophilic stage (day 24), the diversity of the bacterial communities increased, while the fungal communities decreased. As the compost reached maturity (day 104), a reverse pattern was observed between the diversity of bacterial and fungal communities. That is, the 18S rDNA T-RFLP-based diversity indices increased, while the 16S rDNA T-RFLP-based diversity decreased. Differences in temperature profiles at different stages of composting impacted the chemical properties and the diversity of the microbial communities. The day 104 compost (mature) had lower water, organic matter and C contents and higher C and OM loss compared with the day 0 (initial) and day 24 (thermophilic) composts, which affected the diversity of the microbial communities. The results presented here demonstrated that distinctive community patterns from manure composts could be rapidly generated using T-RFLP analysis. The succession of peaks in combination of increasing and decreasing peak heights at different stage of composting indicates the high potential of T-RFLP technique to monitor the dynamics of microbial communities, and their variation qualitatively and quantitatively.

Keywords: T-RFLP, genetic diversity, principal components analysis, manure composting, compost maturity, organic matter

INTRODUCTION

Characterizing and understanding of microbial community structure and diversity may provide information needed to improve and evaluate compost processing and quality. While changes in physico-chemical properties (temperature, bulk density, cation-exchange capacity, C:N ratio, pH and organic C) during the composting process have been extensively studied [1–7], information on microbial communities during composting is underexplored. Moreoever, there is a need for a rapid method of assessment of microbial diversity during composting.

Many early studies on the microbiology of composting dealt with the isolation and description of various microbes in compost using classical culture method [8-14]. Since this method cannot detect non-culturable species, there is a possibility that organisms which are difficult to isolate are predominant. Recently, molecular tools have been used to detect compost microbes independent of culture [15-17]. Blanc et al. [15] used the amplified ribosomal DNA restriction analysis (ARDRA) to characterize thermotolerant populations such as *Thermus* strains and *Bacillus*-related bacteria isolated from composts. Kowalchuk et al. [16] characterized ammonia-oxidising bacteria in compost using denaturing gradient gel electrophoresis (DGGE) from reverse transcriptase-PCR (RT-PCR) amplified 16S rRNA genes. Peters et al. [17] analyzed microbial community succession during composting at a broader phylogenetic level using single-strand-conformation polymorphisms (SSCP). Much of these works, however, were based on the organisms that could be found and utilized to speed up decomposition. Attempts have not been made to assess the diversity of the microbial communities at different stages of composting.

In this study, terminal restriction fragment length polymorphisms (T-RFLP) of bacterial 16S and fungal 18S rDNA genes were used to characterize microbial community structure and diversity in manure composts at different stages of composting. This analysis is based on the restriction endonuclease digestion of fluorescently end-labelled PCR products. The digested product is mixed with a DNA size standard, itself labelled with a distinct fluorescent dye, and the fragments are then separated by gel electrophoresis using

an automated sequencer [18]. Upon analysis, only the terminal end-labelled restrictrion fragments are detected. An electropherogram is produced, which shows a profile of compost microbial community as a series of peaks of varying height. This technique has been effectively used in the exploration of complex microbial environments [19-22]. Bruce [19] exploited this technique to analyse the diversity of mercury resistance genes in polluted soils. T-RFLP has also been used to study bacterial [20], archeal [21] and eukaryal [22] populations in natural habitats.

This paper addresses an area of applied microbiology that has growing importance. There is a need for a rapid method to gain insights about the structure and diversity of microbial communities during composting. The use of 16S and 18S rRNA T-RFLP profiles may provide one such route. In this study, T-RFLP was found to be useful in investigating the diversity of complex compost communities. The T-RFLP profiles showed distinct characteristics of the microbial communities under different composting conditions and may, therefore, be useful in evaluating the progress of the composting process.

MATERIALS AND METHODS

Composting Set-up

Cow and horse manures were mixed homogeneously at a ratio of 1:1 (cow manure:horse manure, wet w/w) using a mixer wagon. These manure composts were stacked in three windrows. Each windrow was 2.7 m wide, 1.2 m high, and 12 m long. The windrows were turned every 2 days during the first 14 days of composting, using a windrow turner and then weekly until the end of the composting trial. The composting process lasted for 104 days. Samples were collected from the left, middle, and right side of the windrows at 60 cm depth. These samples were combined and mixed to generate a single composite sample. The resulting three composite samples from each windrow were collected on day 0 (initial stage), day 21 (thermophilic stage), and day 104 (mature stage). Air and windrow temperatures were monitored using a temperature probe during the composting trial. Windrow temperatures were taken at the same location the samples were taken.

Characterization of Compost Physico-chemical Properties

The compost samples were characterized for water content (105 ^{o}C for 24 h), pH (1:10 w/v compost:water extract) using a pH electrode; organic matter and C contents by loss on ignition (550 ^{o}C for 5 h) [23]; total N using Kjeldahl distillation method [24]; NH_4^+-N using KCL extraction method [25]; and NO_3^--N using a NO_3^- electrode.

DNA Extraction and PCR Amplification

The total community DNA from each replicate compost sample was extracted and purified using the UltraClean Soil DNA Isolation Kit (MoBio Laboratories, Inc., California, U.S.A.). A PCR inhibitor removal solution (UltraClean IRS solution; MoBio Laboratories) was added to reduce humic acid contamination in compost and produce a PCR-quality DNA. Bacterial 16S rDNAs present in the community were PCR-amplified using the universal bacterial primers: 8F forward (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1406r (5′ ACGGGCGGTGTGTRC-3′) reverse [18], with the 8F forward primer labeled with HEX (5-hexachloroflourescein) (Operon, Inc., California, U.S.A.). Each PCR reaction mixture contained 50 ng DNA template, 2.5 mM $MgCl_2$, 2.5 units *Taq* polymerase (Roche Biochemicals, Indiana, U.S.A.), 1X PCR reaction buffer (Roche Biochemicals), 0.2 mM PCR nucleotide mix (Roche Biochemicals), 0.5 μM DNA primers, and 0.6 μl bovine serum albumin (BMB, Indiana, U.S.A.) in a final volume of 50 μl. Reaction mixtures were heated at 94^{o}C for 9 min, and cycled 30 times through three steps: denaturing (94 ^{o}C; 60 s); annealing (58 ^{o}C; 45 s); and primer extension (72 ^{o}C; 90s); in a PTC-100 thermal cycler (MJ Research, Inc., Massachusetts, U.S.A.). Fungal 18S rDNA from the compost community DNA were PCR-amplified using primers EF4 (5′ –GGAAGGG[G/A]TGTATTTA-TTAG- 3′; 5′ HEX labeled) and EF3 (5′TCCTCTAAATGACCAAGTTTG- 3′) [Smit et al., 1999] using the following thermal cycling program: 1x (94 ^{o}C, 3 min); 40x (94 ^{o}C, 1 min; 48 ^{o}C, 1 min; 72 ^{o}C, 3 min); 1x (72 ^{o}C, 10 min). Amplified DNAs were verified by electrophoresis of aliquots of PCR mixtures (5 μl) in 2.0% agarose and 1x TAE buffer. To minimize PCR bias, amplicons from three PCR runs were combined and then purified, using a PCR purification kit (PCR Clean-up Kit; MoBio Laboratories, Inc.), and eluted in a final volume of 50 μl.

T-RFLPs Analysis of 16S and 18S rDNAs Amplified from Manure Composts

Aliquots (10 μl) of amplified 16S or 18S rRNA genes obtained from the manure composts were digested separately with restriction endonucleases, *Hha*I (for 5 h), *Msp*I (for 3 h) and *Rsa*I (for 3 h) (Roche Biochemicals, Indiana, USA), to produce a mixture of variable length end-labeled 16S or 18S rDNA fragments. The labeled fragments were separated electrophoretically on a polyacrylamide gel (5.5%) in an ABI model 373 automated sequencer (Applied Biosystems, Inc., California, U.S.A.). Thereafter, the lengths of fluorescently labeled terminal restriction fragments (T-RFs) were determined, by comparison with internal standards (ROX 2500; Applied Biosystems, Inc) and were analyzed using Genescan software (Applied Biosystems, Inc.) with a peak height detection of 50 fluorescent units.

Diversity of T-RFLP Patterns

Four different measures of diversity were used for each T-RFLP pattern. The Shannon index ($\hat{H}$), equitability index (J), richness (d), and evenness (e) were calculated using the

formula suggested by Atlas and Bartha [26]. The T-RFLP-based diversity statistics were calculated as follows:

$$\text{Shannon index (H)} = \frac{C}{N}(N \log_{10} N - \sum ni \log_{10} ni)$$

where C = 2.3; N= sum of peak areas in a given T-RFLP; n_i = area of T-RF i; and i = number of T-RFs of each T-RFLP pattern. This calculation was derived on Shannon and Weaver's formula based on the

$$\text{Equitability index (J)} = \frac{H}{H_{max}}$$

where H= Shannon-diversity index, and H_{max} = theoretical maximal Shannon index for the T-RFLP examined assuming that each peak represents only one member.

$$\text{Richness (d)} = \frac{S-1}{\log N}$$

where S= number of T-RFs, N= sum of all peak areas in a given T-RFLP pattern

$$\text{Evenness (e)} = \frac{H}{\log S}$$

where H = Shannon index, S= total number of T-RFs

PRINCIPAL COMPONENTS ANALYSIS OF T-RFLP PATTERNS

Principal components analysis (PCA) of T-RFLPs was employed to group or separate samples based on the presence or absence of T-RFs from each T-RFLP pattern. The sample data were arranged based on Boolean character sets (1 or 0) [27], which correspond to the absence and presence of a given T-RFLP pattern. PCA was performed using SYSTAT statistical computing package (SYSTAT version 9.0) using the Boolean or % peak area data sets from T-RFLPs generated from *Hha*I, *Msp*I, and *Rsa*I digestions.

Cluster Analysis

Cluster analysis is another statistical technique that can be used to identify natural groupings among individuals, and then present these groupings in the form of a hierarchical tree or dendograms, which shows the similarities between individuals. In this study, the similarity of the communities was estimated by numerically analyzing the pattern of T-RFLPs in the gel images. Each community was represented by stacking three individual 5′ T-RFLP patterns corresponding to three restriction enzymes (*Hha*I, *Msp*I, and *Rsa*I) using Bionumerics Software (Applied Maths, Kortrijk, Belgium). T-RFLP community profiles were digitized to generate patterns that could be analyzed with the software. The Jaccard coefficient was used to determine the presence or absence of bands, the number of T-RFs common in the communities, and the total number of T-RFs observed. The unweighed pair group method using average linkages (UPGMA), single linkage, and complete linkage were performed to cluster patterns and obtain similarity dendogram for each coefficient using Bionumerics software.

RESULTS

Temperature profiles and compost chemical properties

The windrow temperature was 37ºC right after piling and rose to thermophilic temperature (55ºC) after 24 h (Table 1). Compost temperatures then peaked at 67 ºC by day two. After peaking, the temperature declined, due to windrow turning, but then rose to nearly 65 ºC by day 6. Windrow temperatures fluctuated between 55 and 70º C

Table 1. Temperature characteristics during composting.

Temperature profile	Windrow compost
Initial temperature (º C)	36
Time to reach >55º C (days)	1
Peak temperature (º C)	67
Time to reach peak temperature (days)	2
Range of thermophilic temperature (º C)	55–67
Temperature at day 24	65
Duration of thermophilic stage (days)	33
Final temperature (º C)	30
Duration of composting (days)	104

the thermophilic stage of composting (days 2 to 35) and then dropped continuously thereafter. Temperatures in the windrows did not approach ambient level until day 104 (Table 1).

Differences in chemical properties were observed during composting (Table 2). The initial compost contained high concentrations of organic matter and nutrients. As composting progressed, the concentrations of these elements further decreased (Table 2). Ammonium levels in the final compost were lower but yielded higher NO_3^-N, due to nitrification. The C:N ratio also declined from 30:1 to 13:1, indicating that manure compost reached maturity.

Diversity of 16S and 18S rDNA T-RFLPs

The bacterial and fungal diversity was determined using various indices based on the number of T-RF and the relative abundance (peak area) of the T-RFs found in each sample (Figs. 1-3). The digested T-RF fragments separated by polyacrylamide electrophoresis generated a characteristic profile. Electropherogram generated by the Genescan program indicated marked differences between 16S and 18S rDNA T-RFLPs derived from *Hha*I, *Msp*I, and *Rsa*I digestions (Figs. 1–3). The number of T-RFs found in 16S rDNA T-RFLP was greater (29-44) than the 18S rDNA (7-31) (Table 3). The composting process also affected the number of T-RFs. For the 16S rDNA T-RFLP profiles, the number of T-RFs increased after 21 days of composting, and decreased after compostinng (day 104). That of 18S rDNA, on the other hand, had mixed results (Table 3). In general, the 18S rDNA T-RFLP profiles of the day 0 and day 21 composts were similar and then declined by the end of composting.

The diversity of bacterial (16S rDNA) diversity, as indicated by the T-RFLP-based Shannon and equitability indices, richness, and evenness was observed in manure, increased after 21 days of composting, and then decreased at 104 days of composting (Table 3). A reverse trend of change was found in T-RFLP from amplified 18S rDNA. Fungal (18S rDNA) diversity decreased after 21 days and then increased by the end of composting (Table 3). Overall, the bacterial (16S rDNA) diversity in the manure compost was higher than fungal (18S rDNA) diversity based on the diversity indices used in this study. Visual observation of the T-RFLP profiles also revealed a much higher bacterial (16S rDNA) diversity than fungal (18S rDNA) diversity (Figs. 1–3).

Principal Components Analysis of Bacterial and Fungal T-RFLP

Figure 4 shows the PCA results based on the presence or absence (Boolean data) of T-RF peaks. T-RFLPs generated using *Hha*I, *Msp*I, and *Rsa*I digests, which accounted for 89% of the total variance for the 16S rDNA T-RFLP profiles (Fig. 4A), and 77% the total variance for the 18S rDNA T-RFLP profiles (Fig. 4B). The scree plot for these PCAs indicated that the variation in *Hha*1, *Msp*1, and *Rsa*I data could be explained by principal components 1 (PC1) and PC2 (data not shown). PCA of 16S rDNA T-RFLP profiles derived from *Hha*I, *Msp*I, and *Rsa*I digests yields three major groups (Fig. 4A). While these groups are based on the 16S rRNA profiles of microbial communities, they are separated by the age of compost from which they were sampled. Group I consists of samples taken at the beginning of composting (day 0); Group II consists of samples taken during the thermophilic stage (day 24); and Group III consists of samples taken during the mature stage (day 104). The thermophilic (day 24) and mature composts (day 104) were closer to each other and were distant from the initial composts (day 0), indicating that the T-RFLP profiles from these two composts were similar and were different

Table 2. Physico-chemical properties of manure composts at different stages of composting.

Properties	Day 0	Day 21	Day 104
pH	8.61 ± 0.02	7.77 ± 0.02	8.04 ± 0.04
Water content (%)	73 ± 0.50	67 ± 1.10	30.0 ± 2.20
Dry matter content (%)	26.6 ± 0.50	32.9 ± 1.10	70.0 ± 2.20
Ash content (%)	15.5 ± 0.43	22.0 ± 2.97	42.31 ± 1.86
OM content (%)	84.5 ± 0.40	78.0 ± 3.0	57.7 ± 1.90
Carbon (%)	41.3 ± 2.60	41.1 ± 1.20	34.5 ± 0.70
Nitrogen (%)	1.38 ± 0.02	2.11 ± 0.13	2.66 ± 0.30
NH_4^+-N (mg kg^{-1})	4258 ± 43.0	414 ± 12.0	109 ± 8.0
NO_3^-N (mg kg^{-1})	<50.0± 0.00	<50.0± 0.00	224.5 ± 35.1
C:N ratio	30:1 ± 1.50	19:1 ± 1.70	13:1 ± 0.30
C:OM ratio	0.49 ± 0.03	0.53 ± 0.01	0.60 ± 0.01
C loss (% of initial)	-	0.7 ± 0.03	16.5 ± 1.60
O.M. loss (% of initial)	-	7.6 ± 0.04	31.7 ± 2.20

OM= organic matter. Mean and standard deviation are shown.

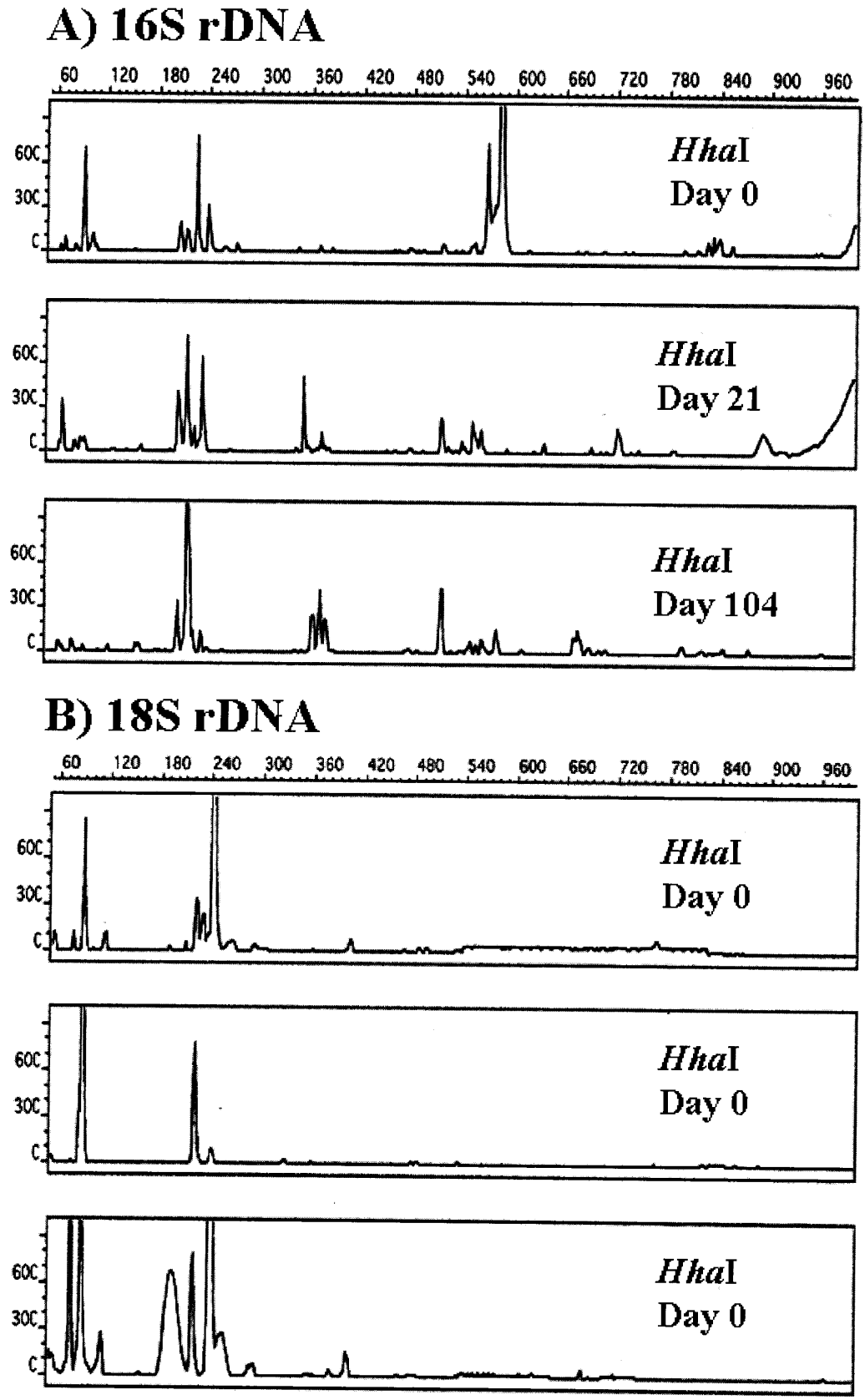

Figure 1. Electropherograms of the 5′ T-RFLP of *Hha*I-digested (A) 16S and (B) 18S rDNAs amplified from manure composts.

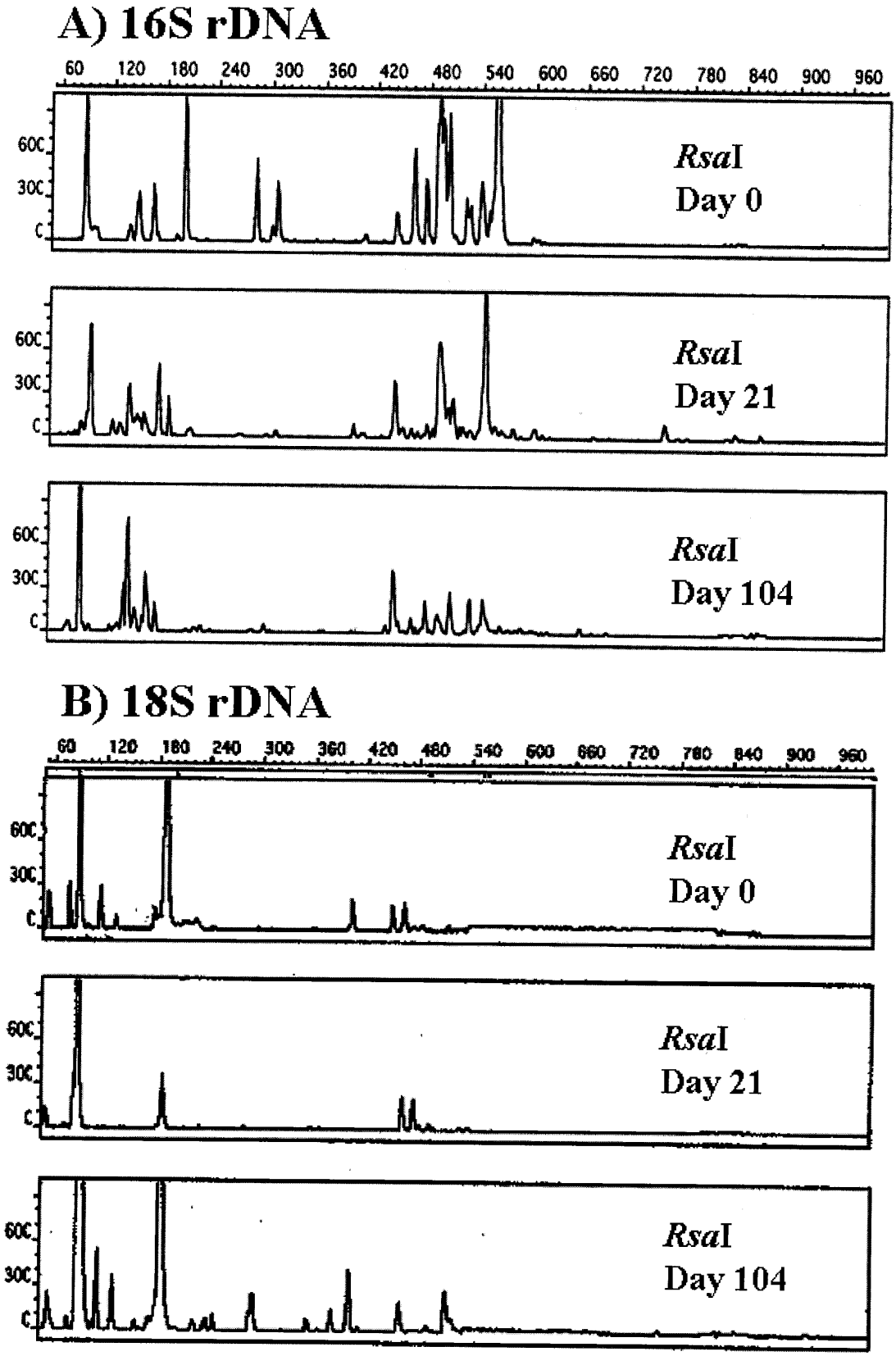

Figure 2. Electropherograms of the 5′ T-RFLP of *Msp*I-digested (A) 16S and (B) 18S rDNAs amplified from manure composts.

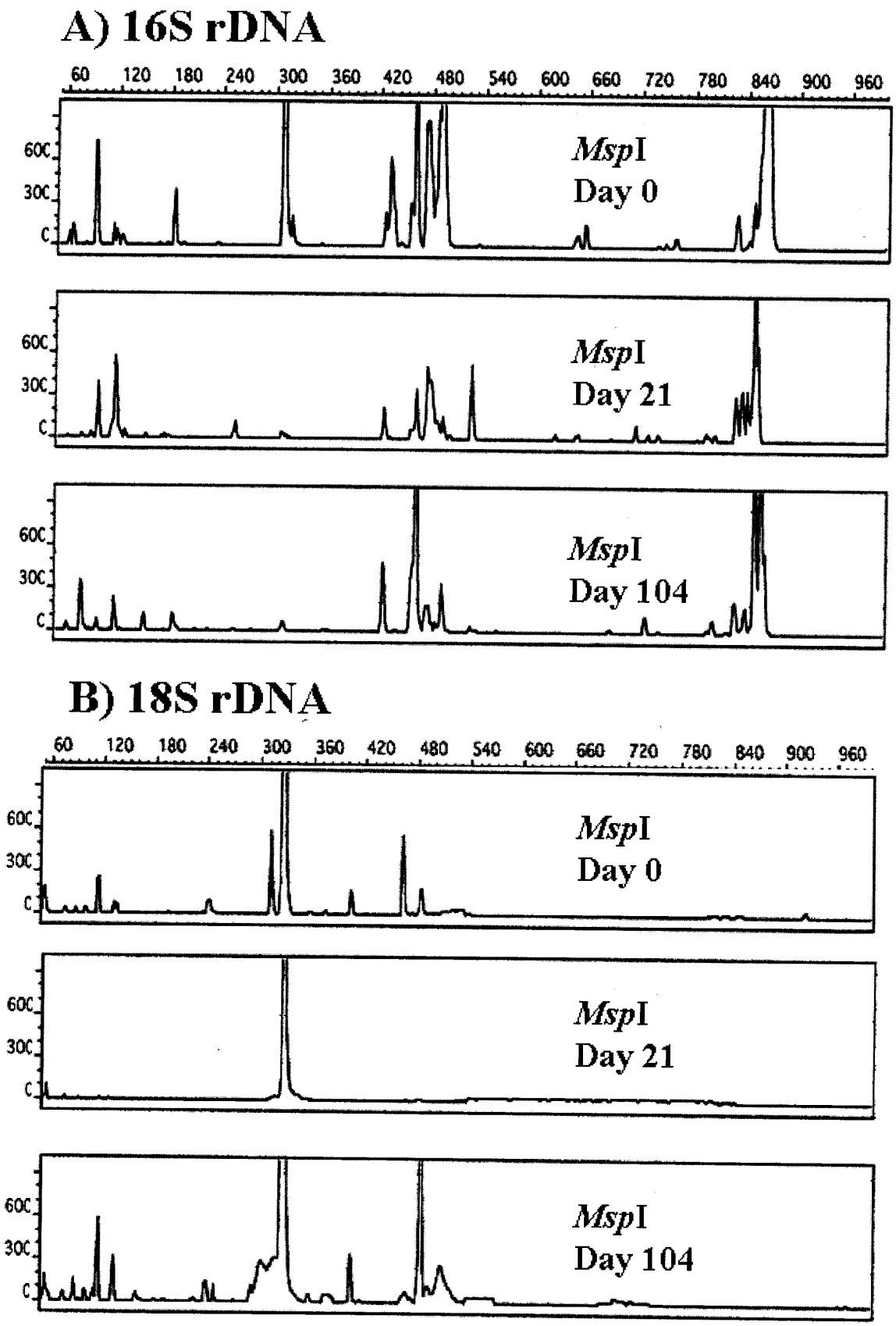

Figure 3. Electropherograms of the 5′ T-RFLP of *Rsa*I-digested (A) 16S and (B) 18S rDNAs amplified from manure composts.

Table 3. Diversity indices calculated from T-RFLP profiles of 16S and 18S rDNAs amplified from manure composts at days 0, 21, and 104.

		Diversity indices				
Compost Samples	Restriction enzyme	Number of T-RFs	Richness	Evenness	Shannon Diversity index	Equitability index
			T-RFLP from amplified 16 S rDNA			
Day 0	*Hha* I	29.0 ± 1.73	5.51 ± 0.70	1.85 ± 0.15	2.7 ± 0.21	0.81 ± 0.06
	Msp I	39.3 ± 1.15	6.01 ± 1.37	2.08 ± 0.12	3.1 ± 0.21	0.89 ± 0.06
	Rsa I	27.6 ± 1.15	5.49 ± 0.62	1.80 ± 0.18	2.6 ± 0.06	0.79 ± 0.07
Day 21	*Hha* I	31.6 ± 1.53	7.11 ± 0.40	1.95 ± 0.02	3.1 ± 0.21	0.84 ± 0.02
	Msp I	44.3 ± 1.15	8.44 ± 1.18	1.95 ± 0.10	3.2 ± 0.12	0.86 ± 0.06
	Rsa I	34.0 ± 0.00	5.57 ± 1.18	2.00 ± 0.12	2.9 ± 0.29	0.88 ± 0.06
Day 104	*Hha* I	28.3 ± 2.08	4.88 ± 0.75	1.82 ± 0.17	2.6 ± 0.10	0.79 ± 0.07
	Msp I	28.3 ± 1.15	6.39 ± 0.35	1.89 ± 0.06	2.9 ± 0.20	0.82 ± 0.04
	Rsa I	21.0 ± 1.00	4.10 ± 0.30	1.66 ± 0.26	2.2 ± 0.06	0.74 ± 0.10
			T-RFLP from amplified 18S rDNA			
Day 0	*Hha* I	18.6 ± 1.53	3.43 ± 0.30	1.67 ± 0.04	2.1 ± 0.06	0.73 ± 0.02
	Msp I	30.6 ± 1.53	5.25 ± 0.30	1.67 ± 0.04	2.5 ± 0.70	0.73 ± 0.03
	Rsa I	5.3 ± 1.15	0.85 ± 0.23	1.48 ± 0.03	1.0 ± 0.90	0.64 ± 0.01
Day 21	*Hha* I	7.6 ± 2.05	1.46 ± 0.38	2.09 ± 0.02	1.8 ± 0.64	0.91 ± 0.01
	Msp I	13.6 ± 1.15	2.40 ± 0.28	1.12 ± 0.10	1.2 ± 0.06	0.48 ± 0.04
	Rsa I	7.0 ± 0.00	1.24 ± 0.05	0.55 ± 0.43	0.5 ± 0.35	0.24 ± 0.19
Day 104	*Hha* I	18.6 ± 1.53	3.39 ± 0.27	1.55 ± 0.01	2.0 ± 0.06	0.67 ± 0.00
	Msp I	28.0 ± 1.73	5.02 ± 0.32	1.55 ± 0.03	2.2 ± 0.10	0.67 ± 0.01
	Rsa I	25.0 ± 0.00	5.25 ± 0.00	1.65 ± 0.00	2.5 ± 0.00	0.72 ± 0.00

Mean and standard deviation of three replicates are shown.

from the day 0 composts. With respect to the 18S rDNA T-RFLP profiles, PCA yields two groups (Fig. 4B). PCA of 18S rDNA T-RFLP profiles derived from *Hha*I and *Rsa*I digests separated the days 24 and 104 composts (Group I) from the day 0 composts (Group II), suggesting that the 18S T-RFs in samples collected during the thermophilic stage of composting were different from that collected at the beginning and final stages of composting.

Cluster Analysis of Fungal and Bacterial T-RFLPs

Cluster analysis was used to group T-RFLPs of compost samples similarity pattern. The stability of the clustering was tested based on three different algorithms (UPGMA, Single linkage, and complete linkage) (Figs. 5 and 6). The T-RFLP pattern of 16S rDNA derived from *Hha*I, *Msp*I and *Rsa*I digestion indicated grouping between the day 21 and day 104 composts, indicating that the 16S rDNA bacterial community patterns in these samples were similar and were different from the day 0 composts (Fig. 5). However, when the T-RFLP of 18S rDNA were analyzed, similarities were found between the day 0 and day 104 composts based on three separate digestions (Fig. 6). Results of the cluster analysis correlated with PCA (Figure 4).

DISCUSSION

The composting process is a biochemical decomposition of organic matter of the starting material by microorganisms [28–29]. During composting, the starting material is modified by decomposition and humification though a wide variety of biological and biochemical processes [30]. The rapidly changing physico-chemical conditions in the composting process, as described in this study, are likely to select for a succession of different microbial communities. It is evident that temperature and available substrates are the key factors in the selection of microbial communities [17, 31–35]. In this study, T-RFLP analysis of 16S and 18S rDNA genes amplified directly from compost DNA can be used to visualize microbial community profiles at different stages of

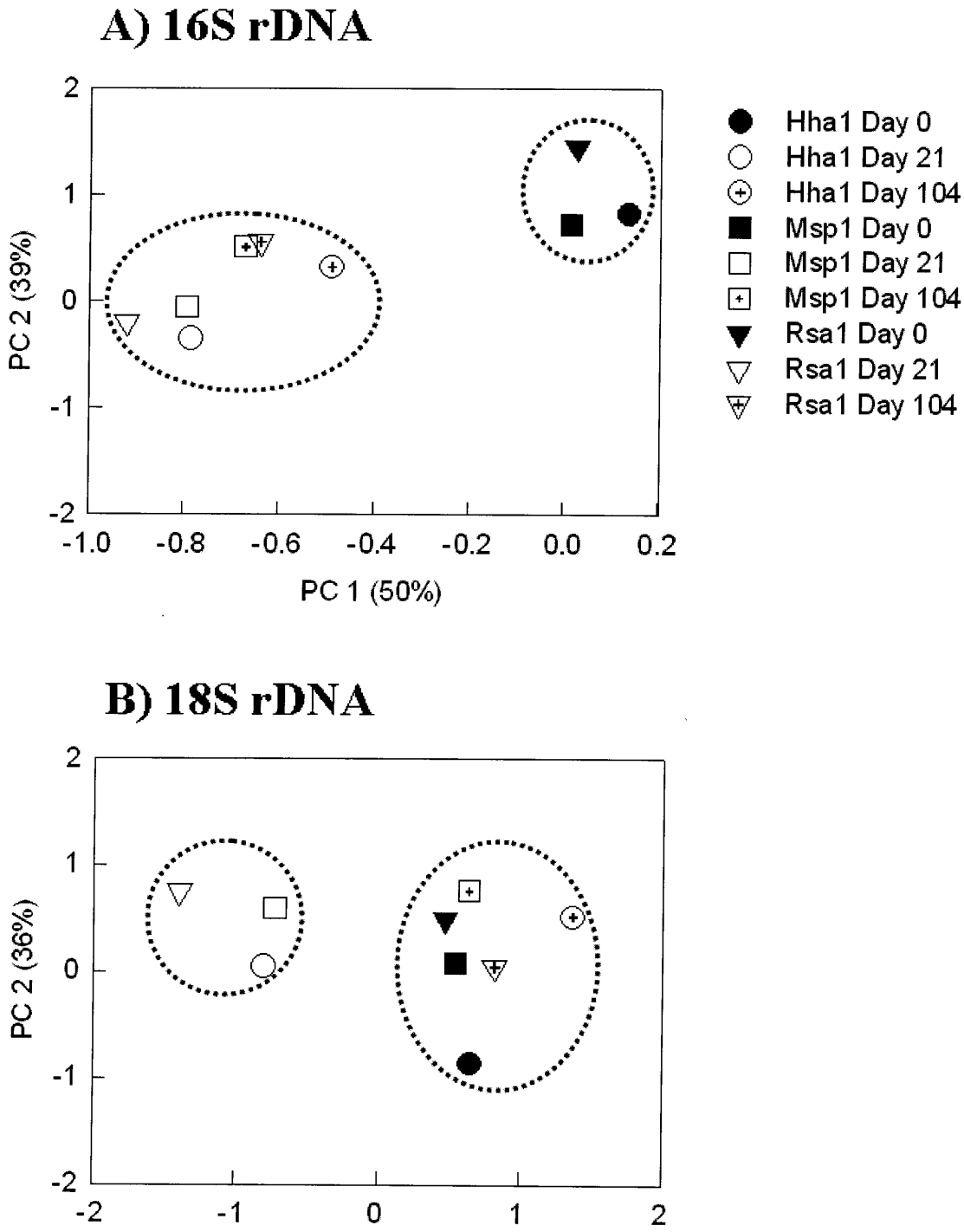

Figure 4. Principal component analyses of T-RFLP data sets (based on presence or absence of T-RFs) from *Hha*I, *Msp*I, and *Rsa*I. Values in parentheses indicate variances of PCA derived from *Hha*I, *Msp*I, and *Rsa*I digestions.

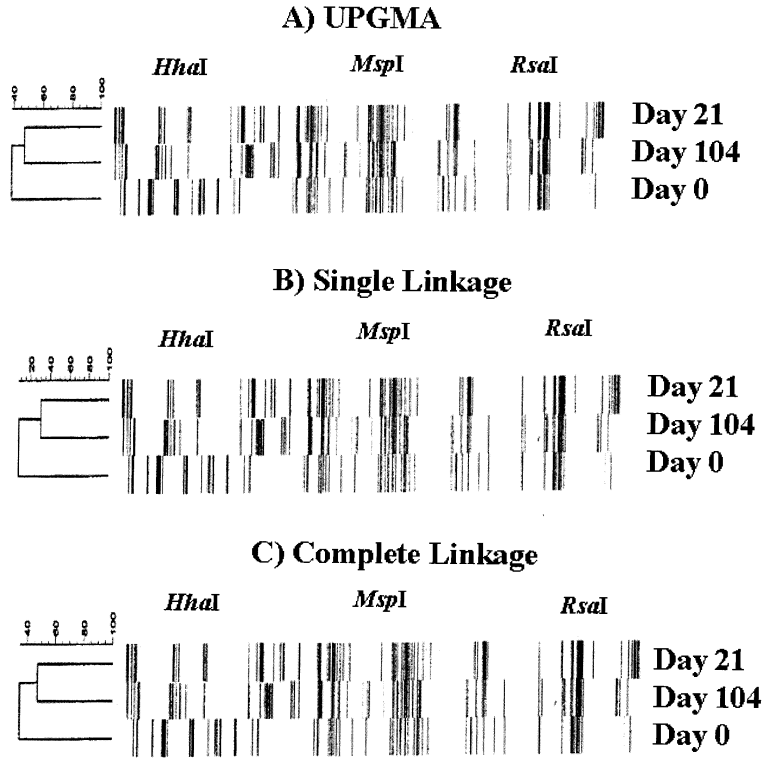

Figure 5. Dendograms showing the relatedness of T-RFLP profiles of *Hha*I-, *Msp*I- and *Rsa*I-digested 16S rDNA from manure composts.

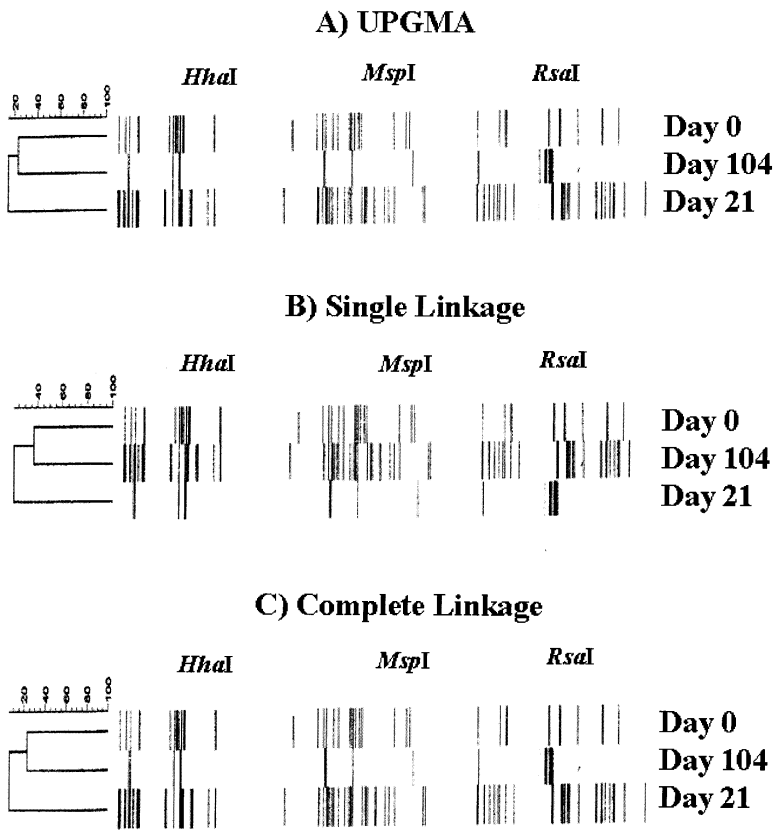

Figure 6. Dendograms showing the relatedness of T-RFLP profiles of *Hha*I-, *Msp*I- and *Rsa*I-digested of 18S rDNA from manure composts.

composting (Figs. 1–3). Distinctive community patterns from manure composts could be rapidly generated using T-RFLP analysis. The T-RF peaks were useful in investigating the diversity of complex compost communities, which may be useful in evaluating the dynamics of the composting process.

Temperature reflects the rate of microbial acitivity during composting [36]. The temperature increase during composting involves a rapid transition from mesophilic to a thermophilic microflora. The final phase of composting is a cooling and maturation stage [28]. In this phase, the amount of readily available nutrients becomes a limiting factor that causes a decline in microbial activity and heat output. In this study, differences in temperature patterns of the thermophilic (day 24) and mature (day 104) composts impacted the chemical properties, (Table 2), diversity of the microbial communities based on the number of T-RFs (Table 3), and the maturity of manure compost. The maturation of manure compost was accompanied by changes in chemical properties such as pH, total C, organic matter, ash, various forms of N and C:N ratio (Table 2). These parameters showed various correlations with each other [37] and with changes in microbial populations [36] during composting of swine manure. High temperatures during composting have been considered as consequences of microbial activity, whereby heat is liberated through respiration of microorganisms [38]. In the present study, the diversity of the bacterial and fungal communities was affected by temperature. During the thermophilic stage (day 24), the diversity of the bacterial communities increased, while the fungal communities decreased (Table 3). As the temperature dropped to ambient level, a reverse pattern was observed between the diversity of bacterial and fungal communities (Table 3). It has been known that bacteria are involved in the self-heating during the initial stage of composting, because of their availability to grow rapidly on soluble proteins and other readily available nutrients [28, 39]. Fungi also play a part in bringing the initial rise in compost temperature [38]. However, most fungi are eliminated by high temperature (>55ºC). Fungi are commonly recovered when temperatures are moderate (>45ºC) [35]. This is probably why the diversity of fungal communities increased towards the end of composting (Table 3).

In laboratory composting systems, temperatures above 60 ºC cause a significant decrease in species diversity determined by plate counts [13]. McKinley and Vestal [12] concluded that significantly greater rates of microbial activity are found when average tempratures do not exceed 58 ºC. Strom [13] showed that species diversity of culturable bacteria dropped markedly from 0.65 to 0.07 when compost temperature rose to 60 ºC and above during a laboratory composting. In the present study, the diversity of bacteria increased even when compost temperatures were higher than 60ºC. Microbial diversity measured using a phenotypic approach has several limitations. One limitation in this approach is that the microbial strain has to be isolated from the biotype and cultivated in the laboratory. Also, only a restricted part of the genetic information is revealed using this method. Therefore, a diversity measure based on the genetic composition of the organisms, as described in this study, is important as this method also covers the nonculturable members of the microbial community.

Several diversity statistics that describe the species richness and evenness have been used to describe the assemblage of microbial populations within a community [39]. A widely used measure of diversity is the Shannon index [42-43]. This general index is sensitive to both species richness and relative species abundance of the community. In this study, the genetic diversity of the microbial communities among different composts was assessed based on the number and relative abundance (peak area) of T-RFs from each T-RFLPs derived from *Hha*I-, *Msp*I-, and *Rsa*I-digestions. The number of T-RFs of the compost sample, varied depending on the restriction enzyme used (Table 3), indicating the inability of T-RFLP profiles to provide a reliable measure of phylotype richness and community structure. This result was not surprising since a single peak (T-RF) in a T-RFLP profile may represent multiple phylotypes. This would tend to obscure differences in phylotype abundance (richness) and evenness that might be detected by other methods with higher phylogenetic resolution. Despite this caveat, differences in the diversity between different restriction enzymes used, the 16S and 18S T-RFLPs in the manure composts, both yielded complementary results that demonstrate an important aspect of microbial community dynamics.

Principal components analysis and cluster analysis are very useful tools in ecosystems studies. In this study PCA was used to separate and group compost samples based on their complex T-RFLP patterns. Because of the high diversity of the investigated samples, cluster analysis was also carried out to check whether a stable separation of the samples can be supported by dendograms calculated from the data. Results of the PCA based on % peak area (relative abundance of T-RF peaks) correlated with cluster analysis. It appears that the thermophilic and mature composts (days 24 and 104) have a similar bacterial community structure, since these composts have similar chemical properties which are different from the uncomposted manure (fresh manure) (Table 1). On the other hand, the uncomposted manure (day 0) has a similar fungal community structure to the mature compost (day 104). This result is expected since the temperature of these composts are similar (Table 2). Clement et al. [41] used PCA to test for similarities among different microbial communities using T-RFLP data. In their study, accurate characterizations reflecting the expected bacterial community biology from different environmental samples were resolved using PCA. That is, PCA separated fecal samples from different sand samples; and the pristine sands from two petroleum-contaminated soils.

In summary, this study also demonstrated that distinctive community patterns from livestock composts could be rapidly generated using T-RFLP analysis. The T-RF peaks were useful in investigating the diversity of complex compost communities. The succession of peaks in

combination of increasing and decreasing peak heights at different stages of composting indicates the high potential of T-RFLP technique to monitor microbial communities. The data clearly show that both composting methods strongly influence the development of highly differing microbial community structures. The research presented here begins to address the questions of critical factors controlling microbial diversity at different stages of composting, and may provide important baseline information critical for the design and optimization of microbial-based composting systems in the future.

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