Bacterial Diversity in Livestock Manure Composts as Characterized by Terminal Restriction Fragment Length Polymorphisms (T-RFLP) of PCR-amplified 16s rRNA Gene Sequences

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Abstract. Composts contain a large and diverse community of microorganisms that play a central role in the decomposition of organic matter during the composting process. However, microbial communities active in composts have not been well described in the past. In the present study, the phylogenetic diversity of bacterial communities in livestock manure compost was determined based on terminal restriction fragment length polymorphisms (T-RFLP) of 16S rRNA genes. This technique uses a PCR in which one of the primers is fluorescently labeled. After amplification, the PCR product is then digested with restriction enzymes such as *Hha*I, *Msp*I, and *Rsa*I to generate T-RFLP fingerprints of bacterial communities. In the present study, a mixture of dairy and horse manure (dairy+horse manure; 1:1 ratio w/w) was composted in windrows and in-vessel to investigate compost bacterial diversity. The DNA was isolated from the feedstocks (day 0) and after 21 and 104 days of in-vessel and windrow composting, respectively, for T-RFLP analysis. A variety of techniques were then used to analyze T-RFLP data to gain insights about the structure of the bacterial community from these compost samples. Results of the T-RFLP analysis revealed high species diversity in the feedstocks sample. As many as 27 to 39 different terminal restriction fragments (T-RFs) were found in these samples, revealing high diversity in the livestock manure composts. After composting, an increase in the T-RFLP-based Shannon diversity index was observed in the in-vessel compost, while a decrease was found in the windrow compost. Differences in chemical properties were also observed in the windrow and in-vessel composts. The windrow compost had lower water, organic matter (OM) and C contents and higher C and OM loss than the in-vessel compost.

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Microbiology of Composting

Introduction

Composting is the biological conversion of organic material into a stabilized end product that can be used as a soil amendment. The process involves dynamic changes in temperature, oxygen concentration, moisture content, and nutrient availability. The active component mediating the composting process is the resident microbial community (Beffa et al. 1996). Defining the diversity and structure of microbial communities of composts through their constituent populations has been of considerable interest to compost researchers in order to address basic ecological questions such as how similar are microbial communities in mature compost that were made from different feedstocks and using different composting methods. It has been known that composts typically contain very high numbers of microorganisms (10^{10}-10^{12} viable cells g^{-1}) (Beffa et al. 1996; Tiquia et al. 1996), the majority of which are bacteria (Epstein 1997). Previous studies of bacterial populations in composts have relied largely on culture-based methods to isolate microorganisms in composts and assess microbial diversity in compost (Strom 1985a; Fujio and Kume 1991; Blanc et al. 1996).

Recently, attempts have been made to characterize bacterial communities from various environmental samples by molecular techniques utilizing the polymerase chain reaction (PCR) (Massol-Deya et al., 1995; Liu et al. 1997). The initial step of bacterial community analysis begins with the extraction of total community DNA from environmental samples. The DNAs present in the community are then PCR-amplified using universal primers targeted to conserved regions of the gene common to all bacteria. Fingerprinting techniques such as denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) (Heuer and Smalla 1997); single-strand-conformation polymorphism (SCCP) (Schwieger and Tebbe 1998); amplified rDNA restriction endonucleases analysis (ARDRA) (Massol-Deya et al. 1995), and terminal restriction fragment length polymorphism (T-RFLP) of the 16S rRNA genes (Liu et al. 1997) are then employed to understand the composition and diversity of the bacterial community. T-RFLP is a technique in which the PCR-amplified 16S rRNA genes are flourescently labeled on one end through the use of a single labeled primer during the PCR step (Liu et al. 1997). Therefore, only the sizes of the fluorescently labeled terminal restriction fragments (T-RF) are seen on polyacrylamide gels. The T-RFLP fingerprinting technique has been effectively used in the exploration of complex microbial environments and has provided a rapid means to assess community diversity in various environments (Liu et al. 1997; Kerkhof et al. 2000; Ludeman et al. 2000). However, this technique has not yet been employed in the characterization of bacterial diversity and community during composting of manure under different composting systems.

Several diversity indices that describe the species richness and evenness have been used to describe the assemblage of microbial populations within a community (Pielou 1969). A widely used measure of diversity is the Shannon index (Shannon-and Weaver 1949; Wiener 1948). This general diversity index is sensitive to both species richness and relative species abundace of the community.

In this study, the genetic diversity of the microbial community was assessed using T-RFLP based on the Shannon diversity index. The digested T-RFs separated by polyacrylamide electrophoresis were used to generate characteristic profile data for the estimation of diversity among different compost samples.

Principal components analysis (PCA) of T-RFLP patterns was also performed to detect statistical significance of changing composting conditions on microbial communities in the compost samples. PCA may provide a means to separate and group compost samples based on their complex T-RFLP patterns as this statistical analysis simultaneously considers many correlated variables, and then identifies the lowest number to accurately represent the structure of the data. In this study, the variables are the T-RFs or peak areas of a given T-RFLP.

This preliminary study was carried out to compare bacterial diversity of dairy+horse manure during the initial and final stage of composting in windrow and in-vessel composting systems using T-RFLP of the 16S rRNA genes. A variety of techniques were used to analyze T-RFLP data to gain insights about the structure of the bacterial communities in the dairy+horse manure compost.

Materials and Methods

Composting and Determination of Compost Physico-Chemical Properties

The manures used in this study were (1) non-separated dairy manure from a free stall barn, and (2) horse manure+wood shavings bedding. The manures were mixed homogeneously at a ratio of 1:1 (dairy manure:horse manure, wet w/w) using a mixer wagon. Table 1 shows the general properties of dairy manure, horse manure, and compost feedstock (dairy+horse manure) at the beginning and end of the composting process. The dairy+horse manure was then composted using two methods: in-vessel (for 21 days) and windrow (outdoor; for 104 days). For the in-vessel composting method, three vessels (205 l) were filled with dairy+horse manure compost. For the windrow composting, the dairy+horse manure was stacked at 2.7 m wide, 1.2 m high, and 12 m long. Temperatures in the vessels and windrows were recorded during composting. The day 0 (feedstock), in-vessel, and windrow composts were characterized for water content (105 °C for 24 h), pH, organic matter, C, total N, NH_4^+-N, and NO_3^-N.

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, USA). 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 rDNA) DNAs present in the community were PCR-amplified using the universal bacterial primers: 8F forward (5'-AGAGTTTGATCCTGGCTCAG-3') and 1406r (5'-ACGGGCGGTGTG TRC-3') reverse, with the 8F forward primer labeled with HEX (5-hexachloro-flourescein) (Operon, Inc., California, USA). Each PCR reaction mixture contained 50 ng DNA template, 2.5 mM $MgCl_2$, 2.5 units *Taq* polymerase [Boehringer Mannheim Biochemicals (BMB), Indiana, USA], 1X PCR reaction buffer, 0.2 mM PCR nucleotide mix (BMB, Indiana, USA), 0.5 µM DNA primers, and 0.6 µl bovine serum albumin (BMB, Indiana, USA) in a final volume of 50 µl. Reaction mixtures were heated at 94 °C for 9 min, and cycled 30 times through three steps: denaturing (94 °C; 60 s); annealing (58 °C; 45 s); and primer extension (72 °C; 90s); in a PTC-100 thermal cycler (MJ Research, Inc., Massachusetts, USA). 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, California, USA), and eluted in a final volume of 50 µl.

Positive Controls

Arthrobacter globiformis (a Gram-positive bacterium) and *Xanthomonas campestris* (a Gram-negative bacterium) were used as positive controls in this study. Both bacteria were obtained from the Department of Plant Pathology culture collection at The Ohio State University. Genomic DNA of each bacterium was isolated using the same kit (Soil DNA extraction kit, Mobiolab, California) used for extracting total DNAs from dairy+horse manure compost samples.

T-RFLP of 16 S rRNA Genes

Aliquots (10 µl) of amplified 16S rRNA genes obtained from the dairy+horse manure compost and the two pure cultures (*A. globiformis* and *X. campestris*) were digested separately with restriction endonucleases, *Hha*I (for 5 h), *Msp*I (for 3 h) and *Rsa*I (for 3 h) (BMB, Indiana, USA), to produce a mixture of variable length end-labeled 16S rRNA fragments. The labeled fragments were separated electrophoretically on a polyacrylamide gel (5.5%) in an ABI model 373 automated sequencer (Perkin Elmer, California, USA). Thereafter, the lengths of flourescently labeled terminal restriction fragments (T-RFs) were determined, by comparison with internal standards, and were analyzed using Genescan software (Perkin Elmer, California, USA) with a peak height detection of 50.

Similarity and Diversity of T-RFLP Patterns

The levels of similarity between T-RFLP fingerprints were determined using the T-RFLP profile matrix analysis program from the Ribosomal Database Project

(RDP) II web site (http://www.cme.edu/RDP/html). T-RFLP data generated from *Hha*I, *Msp*I, and *Rsa*I digestions from each compost sample were combined to produce a similarity matrix for the pattern fragments in the sample.

The T-RFLP-based Shannon diversity index and equitability index as per Atlas and Bartha (1997) was used as a measure of diversity, which was derived on Shannon and Weaver's formula based on the information theory (Wiener 1948; Shannon and Weaver 1949).

Principal Components Analysis of T-RFLP Patterns

Principal components analysis (PCA) of T-RFLPs was employed to cluster samples based on the presence or absence of T-RFs or relative abundance of the T-RFS (% peak area) from each T-RFLP pattern. For PCA based on presence or absence of T-RFs, the sample data were arranged based on Boolean character sets (1 or 0) (Hammer and Rudeanu 1968), which correspond to the absence and presence of a given T-RFLP pattern. Each T-RF size found from each T-RFLP fingerprint was scored 0 (if the T-RF size in the list was absent in a given T-RFLP fingerprint) or 1 (if the T-RF size in the list was present in the given T-RFLP fingerprint). For PCA based on the relative abundance of T-RFLP patterns, the peak area of each T-RF was standardized by calculating the percentage peak area from the total peak area of all T-RFs of each T-RFLP fingerprint. PCA was performed using SYSTAT statistical computing package (SYSTAT version 9.0) using the Boolean or % peak area datasets from T-RFLPs generated from *Hha*I, *Msp*I, and *Rsa*I digestions. PCA was also carried out from merged digest data by stacking the T-RFLP data derived from *Hha*I, *Msp*I, and *Rsa*I digestions of the same compost sample, to combine information from three different digests and provide accurate community characterization.

T-RFLP Analysis from 16s rRNA Gene Sequence Database

Phylogenetic groups of bacteria from each T-RFLP fingerprint were theoretically evaluated and matched with T-RF lengths of 16S rRNA gene sequences deposited at the RDP II web site. To access the database, the T-RFLP analysis program (TAP) from the RDP II web site was used. TAP is a web-based research tool that facilitates microbial community analysis using T-RFLPs of 16S rRNA genes. At present, TAP contains 16, 277 aligned 16S rRNA gene sequences (Maidak 2000). This program permits the user to perform simulated restriction digestions of the entire 16S sequence database and derive terminal restriction fragment sizes (T-RFs), measured in base pairs, from the 5' terminus of the user-specified primer to the 3' terminus of the restriction endonuclease target site (Marsh et al. 2000). Each sequence that is successfully recognized by the primer sequence is digested by the specified enzyme(s). The program displays each resulting T-RF size and enzyme with the organism's name.

Results and Discussion

Temperature Profiles and Chemical Properties

Temperatures of the in-vessel compost reached thermophilic levels (>55°C) by day 1 and then peaked at 70 °C ± 3 °C by day 2 (Fig. 1A). Vessel temperatures ranged between 56 and 65 °C until sampling. At the end of 21 days of in-vessel composting, compost temperatures were 54 °C ± 2 °C (Fig. 1A). In windrows, compost temperatures peaked at 73 °C. After 2 days, the temperature declined, due to windrow turning, but then rose to nearly 70 °C by day 6. Windrow temperatures fluctuated between 46 and 70 °C during the composting process and by the end of 109 days of composting, temperature in the windrow was 67 °C (Fig. 1B). Differences in chemical properties were observed in final composts (Table 1), probably due to differences in temperature profiles and duration of composting in the two trials (Fig. 1). Windrow compost had lower water content, organic matter (OM) and C concentrations but higher C and OM loss than the in-vessel compost (Table 1). This could be attributed to the fact that the windrow compost had been through a much longer (five times longer) composting process than the in-vessel compost. During composting, a major part of the process is the loss C, H, O, and N (Golueke 1972). As composting progressed, the amount of these elements further decreased (Table 1). Ammonium levels in the windrow compost samples were lower but yielded higher NO_3^-N, suggesting a much greater nitrification process than in the in-vessel compost.

Table 1. General properties of the dairy manure, horse manure, feedstock (day 0), and composted manure

Properties	Dairy manure[a]	Horse manure[b]	Feedstock (day 0)[b]	In-vessel compost[b]	Windrow compost[b]
pH	ND	8.2 ± 0.70	8.61 ± 0.02	7.77 ±0.02	8.04±0.04
Water content (%)	91	57 ± 2.10	73 ± 0.50	67 ± 1.10	30.0 ± 2.20
Dry matter content (%)	9	43 ± 2.13	26.6 ± 0.50	32.9 ± 1.10	70.0 ± 2.20
Ash content (%)	47.5	5.6 ± 1.10	15.5 ± 0.43	22.0 ± 2.97	42.31 ± 1.86
OM content (%)	52.5	94.4 ± 1.14	84.5 ± 0.40	78.0 ± 3.0	57.7 ± 1.90
Carbon (%)	25.5	46.9 ± 1.20	41.3 ± 2.60	41.1 ± 1.20	34.5 ± 0.70
Nitrogen (%)	4.9	1.0 ± 0.10	1.38 ± 0.02	2.11 ± 0.13	2.66 ± 0.30
NH_4^+-N (mg kg^{-1})	ND	ND	4258 ± 43.0	414 ± 12.0	109 ± 8.0
NO_3^-N (mg kg^{-1})	ND	ND	<50.0± 0.00	<50.0± 0.00	224.5 ± 35.1

Table 1. (cont.)

C:N ratio	5:1	47:1 ± 4.50	30:1 ± 1.50	19:1 ± 1.70	13:1 ± 0.30
C:OM ratio	0.49	0.50 ± 0.02	0.49 ± 0.03	0.53 ± 0.01	0.60 ± 0.01
Final temp. (°C)	-	-	-	54 ± 2.00	67
C loss (% of initial)	-	-	-	0.7 ± 0.03	16.5 ± 1.60
O.M. loss (%)	-	-	-	7.6 ± 0.04	31.7 ± 2.20

OM= organic matter; ND= not determined.
a Data obtained from Keener et al. (2000)
b Data obtained from this study. Mean and standard deviation of three replicates are shown.

Bacterial Community T-RFLPs from Dairy+horse Manure Compost

Figure 2A revealed diversity among three different samples. An increase in diversity, as indicated by the T-RFLP based Shannon diversity index, was observed in dairy+horse manure after 21 days of in-vessel composting, while a decrease in Shannon diversity (from 2.8 to 2.6) was found in the manure composted in windrows for 104 days (Table 2). The Shannon diversity value derived from the windrow compost was lower than that derived from the initial feedstock (day 0) (Table 2). The windrow compost had been subjected to prolonged high temperatures (Fig. 1B), which may have reduced the diversity of the biological community.

Microbial diversity has been shown to be markedly lower in habitats under conditions of stress or disturbance, and environmental fluctuations (Atlas and Bartha 1997). The Shannon diversity of the in-vessel compost was higher than the windrow compost due to the fact that the process was stopped at a lower temperature. The temperature of the in-vessel compost was 54 °C at the end of composting while that of the windrow compost was 67 °C (Fig. 1A and Table 1). Strom (1985b) showed that species diversity of culturable bacteria dropped markedly from 0.65 to 0.07 when compost temperature rose to 60 °C and above during a laboratory composting. In his study, the highest diversity was found when temperatures are between 50 and 57 °C.

It is interesting to note that in the present study, the decline in Shannon diversity index of the windrow compost bacterial communities also corresponded with a decrease the equitability index (a measure of dominance) (Table 2). Moreover, the in-vessel compost had a higher diversity index and showed an increase in the equitability index value, which indicated the presence of dominant T-RF peaks in this sample (Table 2). In these diversity calculations, both the number of peaks and peak areas were considered.

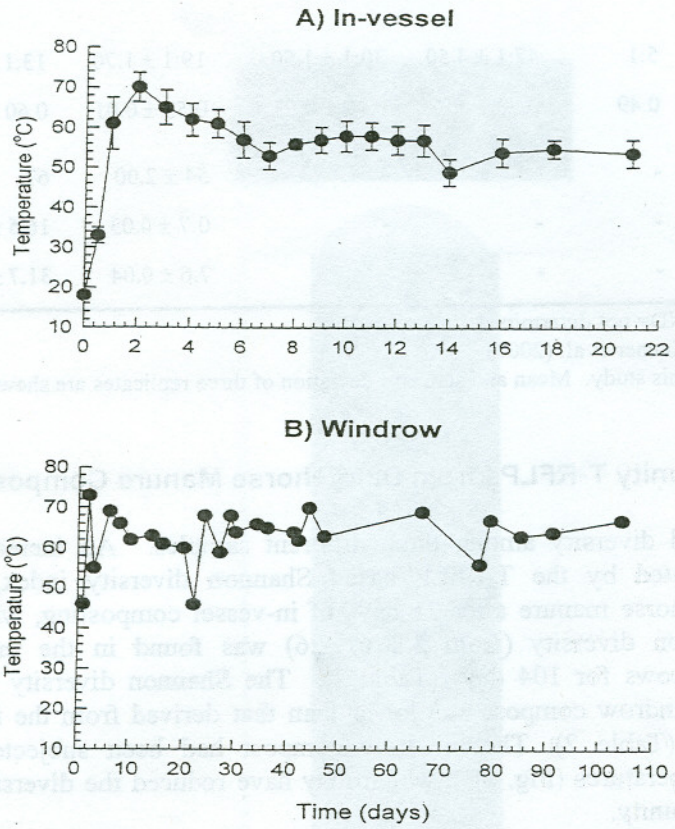
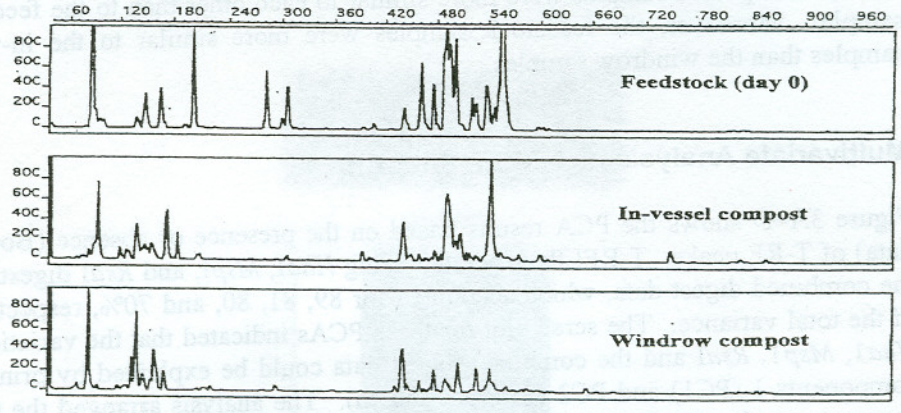


Fig. 1A,B. Temperature change during composting of dairy+ horse manure

However, there are ongoing debates as to whether peak areas should be included in PCR-based analyses, due to PCR amplification rates of difference templates (Suzuki and Giovanni 1996). Therefore the genetic diversity of the microbial community was determined, based on the number of T-RF fragments found in each T-RFLP profile. The diversity and overall similarities between samples were estimated by the number of bands found per sample. Therefore, the greater the number of fragments found, the greater the diversity. The number of T-RF peaks found in day 0 (feedstock) sample was about 27 to 39 (Table 2). This number increased after in-vessel composting to 31–44 peaks, and decreased after windrow composting (21–28). This pattern change in diversity was similar to the Shannon diversity and equitability index, where both the number of peaks and relative abundance of peaks were considered (Table 2). The T-RFLP similarity matrix (Table 2) showed that 59% (0.59) of the T-RF peaks in the in-vessel compost could be found in the initial feedstock (day 0 sample), while 44% (0.44) of the T-RF peaks in the windrow sample could be found in the day 0 sample (Table 2).

A) Electropherograms of the 5' T-RFLP of *Msp*I-digested 16S rRNA genes



B) 5' T-RFLP of *Hha*I-, *Msp*I-, and *Rsa*I-digested 16S rRNA genes amplified from *A. globiformis* and *X. campestris*

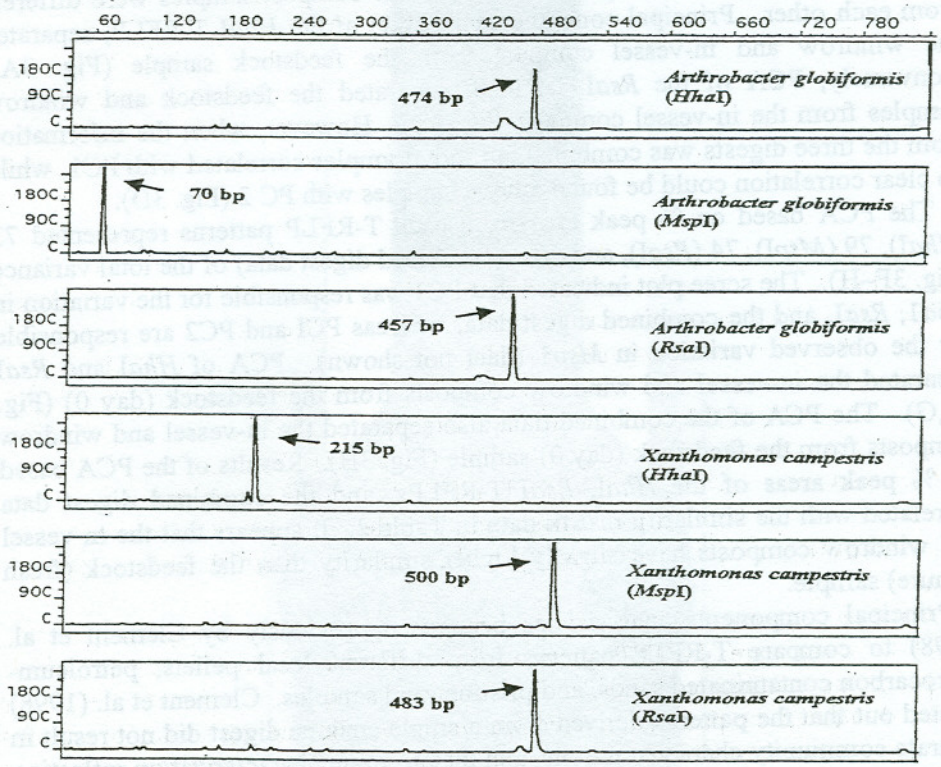


Fig. 2A,B. Electropherograms of the **A** 5' T-RFLP of *Msp*I-digested 16S rRNA genes amplified from dairy+horse manure composts and **B** 5' T-RFLP of *Hha*I-, *Msp*I-, and *Rsa*I-digested 16S rRNA genes amplified from *A. globiformis* and *X. campestris*

The final compost samples had a similarity of 61% (0.61) (Table 2), suggesting that the composted samples were more similar to each other than to the feedstock sample. Moreover, the feedstock samples were more similar to the in-vessel samples than the windrow samples.

Multivariate Analysis of T-RFLP Patterns

Figure 3A–D 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 and the combined digest data, which accounted for 89, 81, 80, and 70%, respectively of the total variance. The scree plot for these PCAs indicated that the variation in *Hha*1, *Msp*1, *Rsa*I and the combined digest data could be explained by principal components 1 (PC1) and PC2 (data not shown). The analysis arranged the three compost samples (feedstock, in-vessel compost, and windrow compost) differently depending on the restriction enzyme used to create the T-RFLP pattern (Fig. 3A–D). PCA of *Hha*I, *Msp*I, and *Rsa*I failed to produce significant pairing among samples (Fig. 3A–C), suggesting that the Boolean data (presence or absence of T-RFs) derived from T-RFLP patterns of the three compost samples were different from each other. Principal components 1 (PC1) of the *Hha*I T-RFLP, separated the windrow and in-vessel compost from the feedstock sample (Fig. 3A). Conversely, PCA of the *Rsa*I T-RFLP, separated the feedstock and windrow samples from the in-vessel compost (Fig. 3C). However, when the information from the three digests was combined, all three samples correlated with PC1, while no clear correlation could be found among samples with PC 2 (Fig. 3D).

The PCA based on % peak area from each T-RFLP patterns represented 73 (*Hha*I), 79 (*Msp*I), 74 (*Rsa*I), and 70% (combined digest data) of the total variance (Fig. 3F–H). The scree plot indicated that PC1 was responsible for the variation in *Hha*1, *Rsa*I, and the combined digest data, whereas PCI and PC2 are responsible for the observed variation in *Msp*1 (data not shown). PCA of *Hha*I and *Rsa*I separated the in-vessel and windrow composts from the feedstock (day 0) (Fig. 3E,G). The PCA of the combined data also separated the in-vessel and windrow composts from the feedstock (day 0) sample (Fig. 3H). Results of the PCA based on % peak areas of the *Hha*I, *Rsa*I T-RFLPs, and the combined digest data correlated with the similarity matrix data in Table 2. It appears that the in-vessel and windrow composts have slightly higher similarity than the feedstock (fresh manure) sample.

Principal components analysis has been used previously by Clement et al. (1998) to compare T-RFLP patterns from different fecal pellets, petroleum-hydrocarbon contaminated sands, and pristine sand samples. Clement et al. (1998) pointed out that the patterns derived from a single enzyme digest did not result in accurate community characterizations, and that accurate characterization reflecting the expected bacterial community biology, were achieved by combining T-RFLP data derived from different enzyme digestions. In their study, PCA of the combined data resulted in a clear separation of the two fecal samples from two petroleum-contaminated sands, and pristine sand.

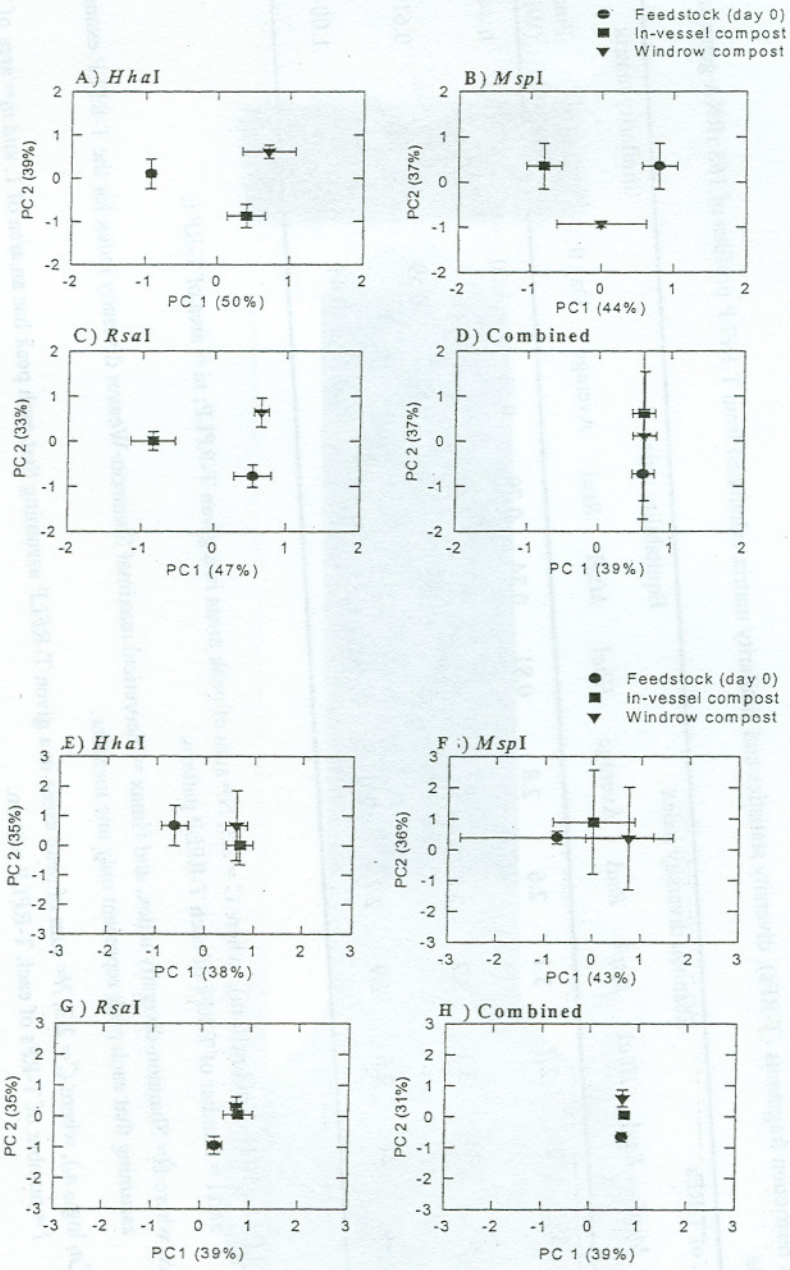


Fig. 3A–H. Principal components analyses of T-RFLP data sets based A–D on presence or absence of T-RFs and E–H % peak area from HhaI, MspI, RsaI, and D combined digests data. Error bars indicate standard error for each eigen vector element. Values in parentheses indicate variances of PCA derived from HhaI, MspI, RsaI, and combined digests data

Table 2. Number of terminal restriction fragments (T-RFs), diversity statistics and similarity matrix calculated from T-RFLP profiles of 16S rRNA genes amplified from dairy+horse manure composts

	No. of T-RFs			Shannon diversity index				Equitability index				Similarity matrix		
Sample	*Hha*I	*Msp*I	*Rsa*I	*Hha*I	*Msp*I	*Rsa*I	Average	*Hha*I	*Msp*I	*Rsa*I	Average	Day 0	Final (Bio-reactor)	Final (Windrow)
Feedstock (day 0)	29	39	27	2.7	3.1	2.6	2.8	0.81	0.84	0.79	0.81	1.00	0.59	0.44
In-vessel compost	31	44	34	3.1	3.2	2.9	3.1	0.89	0.86	0.82	0.85	0.59	1.00	0.61
Windrow compost	28	28	21	2.6	2.9	2.2	2.6	0.79	0.88	0.74	0.80	0.44	0.61	1.00

Calculations:

Shannon index ($\hat{H}$) = C/N (N log10 N- $\sum$ni log10 ni), where C = 2.3; N= sum of peak areas in a given T-RFLP; ni = area of T-RF i; and i = number of T-RFs of each T-RFLP pattern.

Equitability (J) = $\hat{H}$/Hmax, where $\hat{H}$= Shannon-diversity index, and Hmax = theoretical maximal Shannon-Weaver diversity index for the T-RFLP examined assuming that each peak represent only one member.

$H_{max} = C/N\ (N \log_{10} N - \sum n_i \log_{10} n_i)$, where $C = 2.3$, N= sum of peak areas in a given T-RFLP assuming that each peak has an area of 1, and n_i = area of T-RF i (1); i = number of T-RFs of each T-RFLP pattern.

Thus, the PCA results, correlated with the expected bacterial community biology of each pattern when the data from multiple sample enyme digestions were combined in their study. However, in the present study the separation among samples was not clear when the *Hha*I, *Msp*I, and *Rsa*I digest data were combined (Fig. 3D). There seems to be a complete overlap of variances for PC1 in spite of the combined variance for PC1 given as 39% in Table 2. Therefore, the PCA of the combined of digest data did not have much meaning in this study.

Fragment Length and Phylogeny

Since the current 16S rRNA gene sequence database in the TAP-TRFLP program of the RDP II can be distinguished based on T-RFLP analysis, an attempt has been made to identify phylogenetic groups or species from dairy+horse manure compost samples. Prior to this, efforts were also made to assure that the restriction digestions are specific. To do this, amplified products of two known microorganisms (*A. globiformis* and *X. campestris*) were subjected to T-RFLP analysis. The T-RFs of these two known organisms were then compared and matched with the aligned 16S rRNA gene sequences *A. globiformis and X. campestris* deposited in the TAP-TRFLP program. The observed sizes of the T-RF of *A. globiformis* were 474, 70, and 457 bp for *Hha*I, *Msp*I, and *Rsa*I, respectively (Figure 2B) while the predicted sizes found in the database were 472, 69, and 457 bp. For *X. campestris,* the observed T-RFs (*Hha*I=215 bp; *Msp*I= 500 bp; *Rsa*I=483 bp), which was very close to the predicted T-RFs (*Hha*I=215 bp; *Msp*I= 498 bp; *Rsa*I=481 bp) from the database. The accuracy for the three different digests was around 2± bp. This result therefore demonstrated that the digestions were specific and sizing of fragments was accurate for both of the two known organisms, and compost samples processed in the same way.

The ten T-RFs with the greatest peak area from *Hha*1, *Msp*I, and *Rsa*I digestions were determined based on % peak area percentage for each T-RFLP pattern and were matched with the aligned 16S rRNA gene sequences from the database. The fragments in the database were sorted first by the T-RF length from *Hha*I digestion, and then by T-RF lengths from *Msp*I and *Rsa*I digestions, respectively. Tables 4 to 6 present the putative organisms that correspond to T-RFs from the dairy+horse manure samples. It has been known that different organisms, even unrelated species, may produce the same T-RF length with a given restriction enzyme (Liu et al. 1997; Marsh et al. 2000). This information can be mitigated by the use of multiple enzymes so that organisms that produce identical T-RF lengths with one restriction enzyme may produce different T-RF length when digested with another enzyme. Although three different enzymes were used in the present study, the T-RFs produced from these digestions matched with more than one organism in the 16S rRNA sequence database. This made it difficult to identify a single organism that corresponds to the three different T-RF lengths derived from three different digestions. In most cases, the T-RFs from the dairy+horse manure samples could only be correctly matched with two digestions from the 16S rRNA gene sequence database (Table 3). Fragment sizes common to

all compost samples corresponded to bacterial groups including *Bacillus, Mycoplasma,* and *Eubacterium.* These bacterial groups were resolved with at least two digestions. In some cases, T-RF sizes from all three digestions were out of range and could not be resolved from the database (Table 3). It could be possible that the specific phylogenetic groups/species that correspond to these T-RF sizes are unknown. These results, however, underscore the need to identify dominant T-RF peaks of specific importance by DNA sequencing in order to dissect complex compost microbial communities using T-RFLP technique.

The fact that the *in silico* digestions of the RDP indicate that a significant fraction of the TAP-TRFLP database can be distinguished on the basis of T-RF length does not imply that one can positively identify phylogenetic groups or species based on T-RF length. Therefore, one cannot be certain that all putative species identified in Tables 3 are indeed present in the samples. Marsh et al. (2000) pointed out that T-RF sizes greater than 600 bp are resolved poorly by gel systems. Hence, the T-RFLP profile may not be able to reveal or track all potentially resolvable populations of the community. To reveal the true identity of T-RF peaks, and confirm the putative organisms found in the compost samples, cloning and complete sequencing of the 16S rRNA genes obtained from the compost samples are necessary.

Table 3. Determination of putative of phylogenic groups based on 10 T-RF peaks with the greatest peak areas found in the day 0, in-vessel, and windrow composts.

Dominant T-RF	T-RF lengths (bp)			Putative phylogenetic groups/species
	*Hha*I	*Msp*I	*Rsa*I	
1	**583 (581-583)**	**557 (557-589)**	490	*Aerococcus viridans, Streptococcus bovis, S. salivarius, S. sanguis, Globicatella sanguis, Lactococcus garvieae,*
2	**567 (568)**	**491 (492)**	309	*Bordetella bronchiseptica, Bordetella parapertussis,*
	567	**491 (489)**	**309 (311)**	*Flexibacter tractuosus*
3	**226 (225)**	**495 (497)**	460	*Vibrio* sp.
	226 (227)	495	**460 (458)**	*Clostridium* sp.
4	**92 (90)**	**87 (87)**	486	*Capnocytophaga gingivalis, C. succinicans, C. aquatilis, C. flevensis, C. johnsonae, C. saccharophila, Flavobacterium aquatile*
	92	**87 (86)**	**486 (486-488)**	*Bacillus* sp.
	93 (90)	87	**486 (484)**	*Desulfovibrio* sp.
5	588	502	475	Unknown

Table 3. (cont.)

6	580	202	473	Unknown
7	239	**462 (458-461)**	**431 (429)**	*Clostridium* sp.
8	576	**489 (492)**	**93 (94)**	*Flavobacterium* sp.
9	572	**283 (283)**	**484 (485)**	*Eubacterium* sp.
10	**840 (841)**	561	**478 (476-478)**	*Mycoplasma* sp.
	(In-vessel compost)			
1	**234 (235)**	**545 (544)**	850	*Mycoplasma* sp.
2	215	**492 (490)**	**854 (857)**	*Eubacterium* sp.
3	**206 (207)**	497	**475 (474)**	*Nitrosolobus multiformis*
	206 (205)	**497 (496)**	475	*Oceanospirilum* sp.
4	352	**95 (95)**	**118 (117)**	*Flavobacterium* sp.
5	515	495	526	Unknown
6	217	**509 (508)**	**478 (476)**	*Bacillus* sp.
7	561	442	481	Unknown
8	893	174	836	Unknown
9	723	543	829	Unknown
10	552	140	842	Unknown
	(Windrow compost)			
1	188	**85 (86)**	**462 (461-464)**	*Bacillus brevis* , *Bacillus* sp.
2	**190 (189-192)**	**140 (141)**	426	*Eubacterium* sp.; *Clostridium innocuum*
3	385	**442 (442)**	**459 (460)**	*Eubacterium* sp.
4	293	**507 (508)**	**493 (490-492)**	*Bacillus* sp.
5	181	**161 (161)**	**477 (475-478)**	*Mycoplasma* sp.
	181 (180-183)	**161 (161-163)**	477	*Mycobacterium* sp., *Clostridium* sp.
6	**297 (294)**	162	**476 (478)**	*Flexibacter* sp.
	297	**162 (161)**	**476 (478)**	*Mycoplasma pulmonis*
7	287	545	79	Unknown
8	286	**493 (494)**	**186 (183)**	*Flexibacter* sp.
9	473	**148 (145)**	**118 (117-119)**	*Clostridium* sp.
10	423	530	727	Unknown

Values in bold are observed fragment sizes that correspond with sizes found in the RDP (TAP-TRFLP) database (RDP release 7.1). Non-bold values are observed fragment sizes that are not found in the TAP-TRFLP database. Values in parentheses are predicted values for the listed phylogenetic groups. Observed T-RF lengths are within ±2 bp of those T-RF lengths found in the TAP-TRFLP database.

Conclusions

Differences in chemical properties and bacterial community patterns were observed between in-vessel and windrow composts. An increase in diversity was observed in in-vessel compost after 21 days of composting, while a decrease in diversity was observed in the windrow compost after 109 days of composting. The in-vessel compost also showed an increase in equitability index, which indicated the presence of dominant T-RF peaks in this sample. Results of the PCA based on Boolean character sets and peak areas of the combined digest data correlated with the similarity matrix data. It appears that the in-vessel and windrow composts have similar bacterial community structure, which are different from the feedstock (fresh manure) sample in the present study. Although this study was rather preliminary, the research presented here begins to address the questions of critical factors controlling microbial diversity under different composting systems, and may provide important baseline information critical for the design and optimization of microbial-based composting systems in the future.

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 using composting methods indicates the high potential of T-RFLP technique to monitor microbial communities and their variation qualitatively and quantitatively. The use of T-RFLP fingerprinting profile reduces the number of product that needs to be identified by DNA sequencing to those that are assumed to be of specific importance.

Acknowledgements. The authors thank T. Meulia of the Molecular and Cellular Imaging Center for the Genescan analysis, and W. A. Dick (Department of Natural Sciences) and H.A.J. Hoitink (Department of Plant Pathology) for providing laboratory space. The authors also thank the two anonymous reviewers for their improvements to the earlier draft of this chapter. This work was financially supported by funds from The Ohio State University, Agricultural Research and Development Center Seed Grant Program.

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