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Tracking temporal changes of bacterial community fingerprints during the initial stages of composting

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Abstract

The initial phase of composting is the most dynamic part of the process and is characterized by rapid increases in temperature, large swings in pH, and the degradation of simple organic compounds. DNA samples were taken from an active compost system to determine the microbial 16S rRNA gene sequences that were present during this phase. We observed two significant shifts in the composition of the microbial community, one between 12 and 24 h and the other between 60 and 72 h into the process using automated 16S–23S rRNA intergenic spacer amplification (ARISA). The 16S rRNA gene sequences adjoining the most common ARISA fragments at each time point were determined. We found that sequences related to lactic acid bacteria were most common during the first 60 h and *Bacillus*-type sequences were most common between 72 and 96 h. While the temperature increased steadily over the first 96 h, the pH dropped after 12 h and increased after 60 h correlating with the shift from *Bacillus* to lactic acid sequences and the later return to *Bacillus*-type sequences.

Keywords: Compost; 16S rRNA; rRNA intergenic spacer amplification; Microbial ecology; 16S–23S intergenic region

1. Introduction

Composting is one of the most complex biotechnologies that man has appropriated from nature. Although there have been many successful applications of this biotechnology, ranging from waste reduction to food production, the biocomplexity that is innate to composting has often yielded myriad physical and biological states that are unexpected and unwanted [1–6]. Odor formation, incomplete stabilization, disruption in the degradation processes, and poor experimental reproducibility are the manifestations of these unexpected states [5–9]. What is there about this old and familiar biotechnology that still leaves us with so much uncertainty?

For the last 10 years our research team has been seeking answers to this question. We have conducted an extensive set of experimental and modeling studies of high-solids aerobic decomposition at the bench and pilot scales. These studies have focused on identifying and modeling energy and mass transport mechanisms [10–12], assessing the influence of environmental conditions on the rates and extents of oxygen depletion and carbon dioxide evolution [13,14], assessing changes in the quantity and distribution of carbon sources [15], development of techniques for measuring free air space [16], analyzing and modeling water vapor and substrate moisture relationship [11], and measuring and analyzing odor generation [17]. We have also conducted extensive studies on how perturbations in the environmental conditions (temperature, moisture content and O_2 concentration) result in changes in rates of oxygen depletion and carbon dioxide evolution [14,18,19]. In many respects, we have taken a very traditional engineering approach to composting. Unfortunately, this approach has provided very limited insight into the dynamic changes in microbial communities and how these changes feedback into the process dynamics. Composting is a process driven by microbial community succession, but there

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is only a limited amount of qualitative data in this regard. We have just begun to ask the questions of 'who?' and 'how much?'.

Much of what we know about the microbial ecology of compost has come from studies of hot composts (i.e. 65°C) where culture-based techniques were used to obtain a snapshot of the microbial community at a single time point, which indicated limited bacterial diversity consisting primarily of thermophilic *Bacillus* [20–22]. Recent studies using molecular biology methods have revealed a more diverse microbial community, including lactic acid bacteria, *Bacillus* sp., actinomycetes, and some Gram-negative bacteria [23–29]. Methanogenic Archaea have been found in some composts and Eucarya, such as fungi and yeast, have also been detected [28,30,31]. Analysis of individual samples can provide extensive phylogenetic and metabolic information about that specific microbial community at a specific instance in time. Based on that knowledge alone however, we are unable to see how or if community change is associated with the rapid changes taking place in the compost environment. While two studies have focused on large-scale composting systems over a period of weeks, sampling only occurred every couple of days [24,27]. These studies focused on describing the general changes over the course of the process using sequence analysis combined with single strand conformational polymorphism [24] and denaturing gradient gel electrophoresis [27], but did not provide detailed information about the dynamic initial phase of composting.

While the literature has focused on the microbial diversity found in the thermophilic stage of composting [22,28], the initial phase provides an interesting system for examining the interaction between environmental variables and microbial ecology. This phase is characterized by rapid changes in temperature, pH, and O_2 and CO_2 concentration. Our past experience with composting food wastes has shown that during the first 96 h of composting we can expect an average 20°C rise in temperature, a pH change from 4.4 to 7.9, an average O_2 concentration of 17.5% ($O_{2,atmosphere} = 20.9\%$), and an average CO_2 concentration of 3% ($CO_{2,atmosphere} = 0.035\%$). Changes of a similar magnitude have been observed by other investigators during large-scale composting experiments [7,32,33].

The goal of this study was to measure changes in key environmental variables and to correlate these changes with changes in microbial ecology. Our hypothesis is that correlating changes in key environmental variables with changes in microbial community structure will enable us to establish a foundation that will lead to a better understanding of compost microbial dynamics. More specifically, by using culture-independent polymerase chain reaction (PCR) and cloning techniques, we can observe changes in microbial community structure and correlate these changes to changes in environmental variables.

2. Materials and methods

2.1. Composting methodology

Substrate preparation, reactor operation, and data acquisition have been described in detail elsewhere [18,34]. Briefly, dog food and wood chips were mixed to obtain a carbon to nitrogen ratio (C/N) of 18:1 and tap water was used to increase the moisture content of reactors to 55% (w.b.). The C/N ratio and moisture content used represent the 'base' condition for our previous studies [10–19] and are consistent with other experimental studies conducted with the pilot- and full-scale composting reactors [5,35–37]. Dog food (Big Red Puppy Food, Pro-Pet, Syracuse, NY, USA) was chosen as the substrate because it has a fiber, fat and protein content similar to food waste and because its physical and chemical composition is fairly uniform from batch to batch and year to year. Wet substrate (240 g) was loaded into 500-ml micro-reactors and aerated at a flow rate of 150 ml min^{-1} with humidified air. The reactors were housed in an upright incubator (model 1560, Sheldon Manufacturing, Cornelius, OR, USA) with the thermostat disabled so the reactors could auto-heat.

The experiment was repeated three times over a period of 4 months. For each run, the compost samples were collected by sacrificing reactors after 12, 24, 36, 48, 60, 72, 84, and 96 h. For each time point, nine reactors were operated simultaneously. For this report, the group of nine reactors is considered an experimental trial. Therefore, a total of 27 reactors were used to evaluate the microbial community structure for each of the eight time points. In addition to the material collected from the sacrificed reactors, samples were obtained from the initial material for further analysis.

DNA was extracted from three aliquots of compost from each reactor using a bead-beating technique and the purified DNA was pooled for further analysis. A 5-g compost sample was combined with 5 g of glass beads, 10 ml of TE buffer (10 mM Tris–HCl, pH 8.0, 1 mM EDTA, pH 8.0), and 500 μl of 10% sodium dodecyl sulfate. The samples were incubated for 60 min at 70°C. The samples were vortexed thoroughly, and centrifuged at 4500×*g* for 10 min. The compost debris was washed twice more and the washes were pooled. DNA was purified and precipitated using hexadecyltrimethyl ammonium bromide extraction and polyethylene glycol precipitation [38]. The resulting DNA was further purified using a glass milk (Express Matrix, Bio101, Vista, CA, USA).

2.2. Automated 16S–23S rRNA intergenic spacer amplification (ARISA)

All PCRs were performed using 2×*Taq* PCR Master Mix (Qiagen, Valencia, CA, USA). The final concentrations of the master mix constituents were 0.05 U μl^{-1} of *Taq* DNA polymerase, 1×PCR buffer, 1.5 mM $MgCl_2$,

and 200 μM of each dNTP. DNA template and 500 nM of each primer were added to each reaction. ARISA was performed on compost DNA using PCR to amplify the 16S–23S rRNA intergenic spacer region using primers 1406f (5′-TGYACACACCGCCCGT-3′) and 23Sr (5′-GGGTTBCCCCATTCRG-3′) as described by Fisher and Triplett [39]. The sizes of ARISA fragments were determined using an internal rhodamine-labeled GeneScan-2500 size standard on an ABI Prism 377 sequencer (Perkin-Elmer, Foster City, CA, USA). The computer programs GeneScan and GenoTyper (Perkin-Elmer) were used to identify ARISA fragment lengths differing by one nucleotide, which were designated separate ARISA operational taxonomic units (A-OTUs). Comparisons between community ARISA fingerprints were made using distance values (*D*-values) calculated with the Jaccard distance coefficient [40]:

$$D(i,j) = 1 - \frac{a}{a+b+c}$$

where a = number of fragments contained in both samples, b = number of fragments contained in sample i, but not in sample j, c = number of fragments contained in sample j, but not in sample i. This allowed for comparison of multiple fingerprints based on the presence or absence of defined A-OTUs. The PHYLIP software package was used to construct dendrograms using the UPGMA cluster analysis method (http://evolution.genetics.washington.edu/phylip.html).

2.3. Construction of 16S–23S rRNA intergenic spacer clone libraries

Identification of A-OTUs using 16S rRNA gene-based sequence information was facilitated by PCR amplifying fragments that contained a 420-bp DNA fragment of the 16S rRNA gene and the contiguous 16S–23S intergenic region from the same template used for the ARISA analysis. This was accomplished using primers 1055f (5′-ATGGCTGTCGTCAGCT-3′) and 23Sr, followed by visualization on an agarose gel. A modified touchdown PCR protocol was used for amplification, which included an initial denaturing step at 94°C for 3 min, followed by 10 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C. The annealing temperature, originally 55°C, was decreased by 1°C each cycle. Further amplification was obtained by 25 cycles of 1 min at 94°C, 1 min at 45°C, and 1 min at 72°C. The final extension was for 10 min at 72°C (PTC-100, MJ Research, Waltham, MA, USA).

Reaction products were separated by electrophoresis on agarose gels, sections of gels containing bands of interest were excised, and the DNA was extracted using a gel purification kit (QIAquick gel extraction kit, Qiagen). The purified DNA was re-amplified using the 1055f/23Sr primers and the amplification products were ligated into the pGEM-T Easy vector (Promega, Madison, WI, USA). Ligation products were electroporated into competent *Escherichia coli* JM109 cells and blue/white screening was used to identify transformants containing inserts. Plasmid DNA was obtained from transformants using the Express Matrix Mini-prep kit (Bio101). Cloned inserts were amplified using the universal T7f and M13r primers for restriction digest analysis using *Hae*III and *Dpn*II to distinguish unique OTUs (R-OTU).

2.4. Sequence and fragment analysis

Clones representing unique R-OTUs were amplified from purified plasmid DNA using primers 1055f and 1492r (5′-TACGGYTACCTTGTTACGACTT-3′) with the same PCR protocol used for the 1055f/23Sr amplification except that the initial and final annealing temperatures were 65 and 55°C, respectively. The 16S rRNA gene from each uniquely cloned R-OTU was sequenced using the 1492r primer at the Cornell University Bioresource Center. ARISA was performed on each clone as described above for total compost DNA in order to determine the adjoining RISA fragment size. This allowed for a correlation between sequenced R-OTUs and the unsequenced A-OTUs from the compost ARISA fingerprints. In order to facilitate the reporting of our results, we refer to the identity of an R-OTU based on the sequence of the 16S rRNA gene fragment from the cloned DNA.

Phylogenetic identity was determined by comparing the partial 16S rRNA gene sequences of the clones to sequences found in GenBank (http://www.ncbi.nlm.nih.gov/BLAST/) using the *blastn* database. Chimeric sequences were detected using the CHECK_CHIMERA program at the Ribosomal Database Project website (http://rdp.cme.msu.edu/html/). The sequences of the 99 partial 16S rRNA gene sequences and the length of the adjoining RISA fragment have been deposited in GenBank under accession numbers AF437514 to AF437612.

3. Results

3.1. Temporal changes in microbial community composition

During the first 24 h, there was an average temperature increase of nearly 10°C and a drop in pH of 0.5 units (Fig. 1). Additionally, the average O_2 concentration decreased linearly by more than one percentage point during the first 24 h and the average CO_2 concentration increased linearly by more than half a percentage point over the course of 96 h. Analysis of Jaccard distances of the community fingerprints of samples collected initially and at 12 h revealed them to be more similar to those from samples collected between 72 and 96 h than they were to samples collected between 24 and 60 h (Fig. 2). In a previous analysis we found that when samples were compared to the starting material (the 0-h samples), the time point samples all clus-

tered separately from the initial samples indicating that the microbial community structure actually differentiated from the initial material after only 12 h [41]. The only measured variable that was the same at 12 and 72 h was the matrix pH, although it had changed dramatically between those time points (Fig. 1B).

Analysis of Jaccard distances revealed that a second phase of community fingerprint change occurred between 60 and 72 h (Fig. 2). The bottom box in Fig. 2 primarily contains samples collected at 0 and 12 h and those collected at 72 h and after, while the top box contains those samples collected between 24 and 60 h. While several of the representative samples included in Fig. 2 cluster outside the appropriate box, the overall trend is clear – between 12 and 24 h and between 60 and 72 h there is a shift in the composition of the microbial communities. Again, the changes in community structure composition correlated with the changes in pH (Fig. 1B). Between 60 and 72 h, the pH of the matrix starts to become more basic. Throughout the process the temperature of the reactors

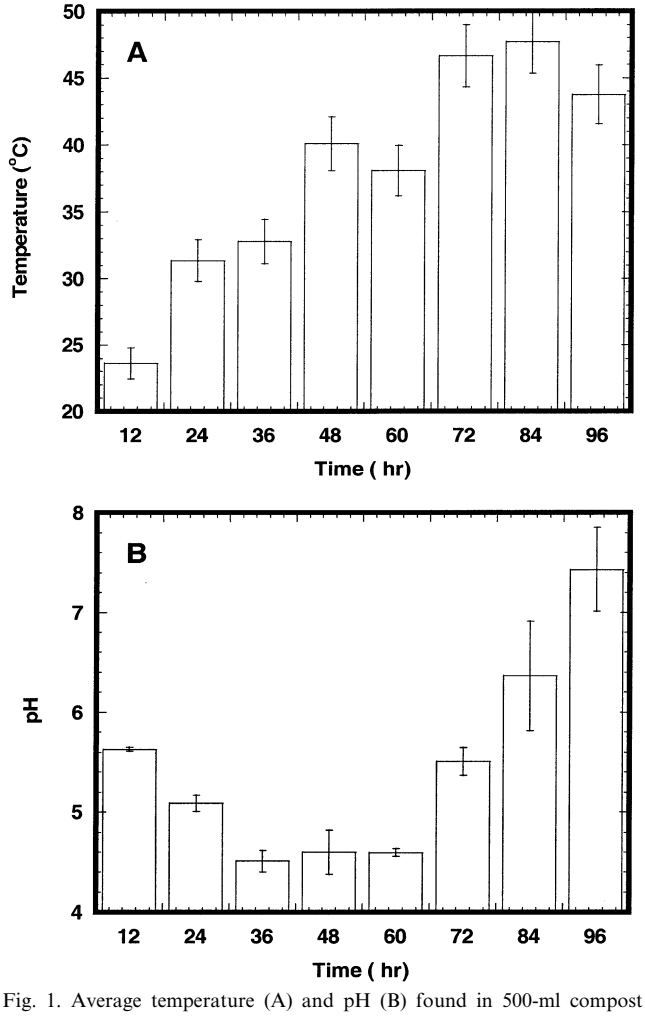

Fig. 1. Average temperature (A) and pH (B) found in 500-ml compost reactors during the first 96 h of the process. Each bar represents the average of nine reactors and error bars correspond to the standard deviation.

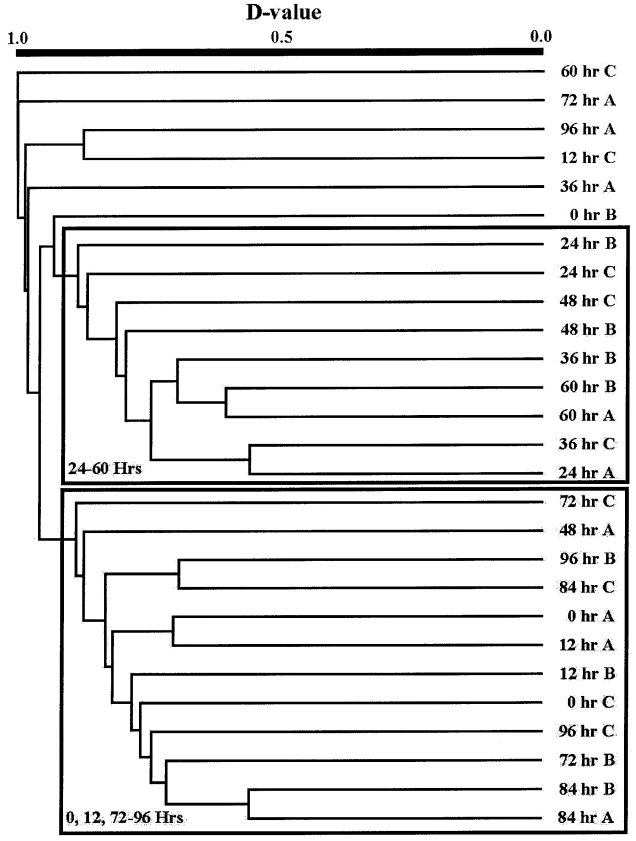

Fig. 2. Differences between community structure compositions found between 12 and 96 h. A representative sample is presented for each experimental trial and time. The top box groups most of the samples collected at 12 h and between 72 and 96 h while the bottom box groups those samples collected between 24 and 60 h. A, B, and C to the right of each time denote the first, second, and third representative sample for each time point. The definition of the *D*-value used to construct the dendrogram is given in Section 2.2.

gradually rises to nearly 50°C. However, there do not appear to be any points in the temperature profile that correlate with the changes in the community fingerprint.

3.2. Clone library construction

In order to determine which bacterial groups were present throughout the process and which could be correlated with the shift observed in Fig. 2, we cloned the 16S–23S rRNA intergenic region and the adjoining 3′-end of the 16S rRNA gene from the most commonly observed peaks from the automated RISA electropherograms of samples from all time points. We also targeted the 10 most common A-OTUs that were observed in the initial samples for cloning. Among these, six were found in at least 50% of the initial samples. In addition, we targeted the five most common A-OTUs at each time point for cloning. Since there was some overlap between the most common A-OTUs observed at the different time points and the initial material, there were a total of 23 A-OTUs for which we were specifically interested in obtaining sequencing information. Table 1 summarizes infor-

Table 1
A-OTU size, frequency of occurrence, and partial 16S rRNA gene sequence information for the 10 most common A-OTUs obtained in the initial material (24 total initial samples)

Clone ID	A-OTU size (bp)	Closest Blast match	Sequence similarity	Frequency of detection ($n = 72$)
	406			40.3
PDSB048	**464**	***Bacillus subtilis***	**97.6**	**61.1**
PDSB026	**470**	***Bacillus subtilis***	**99.5**	**68.1**
PDSB042	492	*Bacillus subtilis*	99.5	41.7
PDSB037	493	*Klebsiella pneumoniae*	100.0	66.7
PDSB044	**516**	***Pediococcus acidilactici***	**99.3**	**59.7**
PDSB079	**543**	***Weissella confusa***	**99.3**	**65.3**
PDSB115	638	*Bacillus* TGS650	99.0	47.2
PDSB067	641	*Lactococcus garvieae*	100.0	45.8
PDSB075	**648**	***Weissella confusa***	**99.5**	**61.1**

Boldfaced A-OTUs were also common later in the process (see Table 2). Blank cells are those A-OTUs that could not be cloned.

mation about the 10 most common A-OTUs found in the initial samples. Five of those were also common later in the process (Tables 1 and 2, bold entries). Table 2 summarizes information regarding the five most common A-OTUs identified at each time point and the percentage of the samples at a given time point that contained one of the 18 A-OTUs.

Sequences approximately 420 bp in length from the 3′-terminus of the 16S RNA in the cloned fragments were obtained for 110 clones that had been identified as unique R-OTUs. Among the 110 R-OTUs, there were 67 unique sequences and 74 unique fragment lengths, suggesting that multiple rRNA operons from either the same or closely related organisms had been cloned. A combination of ARISA fragment size and the partial 16S rRNA gene sequence analysis revealed 99 uniquely cloned R-OTUs. Based solely on length determination, 14 of these R-OTUs had at least two possible phylogenetic-based identities. The automated RISA fragment size of 12 R-OTUs did not correspond to any of the A-OTUs identified in the ARISA community fingerprint.

3.3. Phylogenetic analysis of community fingerprint change

Sequence analysis of the 420-bp 16S RNA gene fragments from the 99 cloned R-OTUs showed the presence of five main bacterial groups in compost. These included *Klebsiella* ($n = 6$), *Pseudomonas* ($n = 14$), thermophilic *Bacillus* ($n = 15$), mesophilic *Bacillus* ($n = 24$), *Weissella* ($n = 24$), and *Lactobacillus*/*Lactococcus* ($n = 16$). The most common A-OTUs were represented within each of these groups and typically had highly similar sequences to those

Table 2
A-OTU size, frequency of occurrence, and partial 16S rRNA sequence information for the five most common A-OTUs obtained at each time point (nine total samples at each time point)

Clone ID	A-OTU size (bp)	Closest Blast match	Sequence similarity	Frequency of occurrence ($n = 27$ per time)							
				12 h	24 h	36 h	48 h	60 h	72 h	84 h	96 h
	459			0.0	0.0	0.0	7.4	0.0	40.7	66.7	29.6
PDSB048	**464**	***Bacillus subtilis***	**97.6**	**51.9**	**0.0**	**0.0**	**18.5**	**3.7**	**44.4**	**40.7**	**18.5**
PDSB060	465	*Bacillus subtilis*	99.3	55.6	0.0	0.0	0.0	0.0	51.9	44.4	48.1
PDSB043	467	*Bacillus coagulans*	98.1	3.7	0.0	0.0	0.0	0.0	18.5	63.0	33.3
PDSB026	**470**	***Bacillus subtilis***	**99.5**	**55.6**	**0.0**	**0.0**	**29.6**	**14.8**	**55.6**	**22.2**	**18.5**
PDSB063	471	*Bacillus subtilis*	100.0	37.0	0.0	0.0	0.0	0.0	55.6	33.3	48.1
	481			11.1	0.0	0.0	3.7	0.0	29.6	55.6	59.3
PDSB006	488	*Pseudomonas putida*	100.0	33.3	37.0	40.7	44.4	37.0	18.5	33.3	11.1
PDSB044	**516**	***Pediococcus acidilactici***	**99.3**	**51.9**	**0.0**	**3.7**	**7.4**	**7.4**	**37.0**	**81.5**	**55.6**
PDSB079	**543**	***Weissella confusa***	**99.3**	**59.3**	**96.3**	**88.9**	**63.0**	**77.8**	**55.6**	**40.7**	**0.0**
PDSB076	563	*Weissella confusa*	99.5	7.4	59.3	33.3	25.9	51.9	3.7	14.8	0.0
PDSB019	575	*Pseudomonas putida*	100.0	22.2	3.7	51.9	29.6	14.8	3.7	0.0	0.0
PDSB075	**648**	***Weissella confusa***	**99.3**	**44.4**	**33.3**	**48.1**	**59.3**	**77.8**	**51.9**	**51.9**	**48.1**
	669			25.9	77.8	59.3	55.6	74.1	11.1	3.7	11.1
PDSB038	706	*Lactobacillus manihotivorans*	99.0	7.4	11.1	55.6	0.0	14.8	33.3	3.7	22.2
	738			14.8	11.1	0.0	29.6	0.0	0.0	0.0	59.3
	740			29.6	74.1	22.2	18.5	51.9	33.3	37.0	29.6
PDSB072	761	*Weissella confusa*	99.5	0.0	48.1	3.7	18.5	3.7	0.0	11.1	18.5

Boldfaced A-OTUs were also common among the initial samples (see Table 1). Blank cells are those A-OTUs that were not represented in the clone library.

in the GenBank database (Tables 1 and 2). Based solely on size comparisons using ARISA, we were able to associate 17 of the 23 targeted A-OTUs with specific partial 16S rRNA gene sequences from R-OTUs.

While dendrograms, such as Fig. 2, may cluster samples according to the presence or absence of A-OTUs, they can also be used to cluster A-OTUs according to how frequently two OTUs are found together in the same sample. This analysis, shown in Fig. 3, suggests that bacteria from two phylogenetic groups were present at separate times. Those A-OTUs which form a cluster within Fig. 3A are primarily related to lactic acid bacteria while those in Fig. 3B are related to *Bacillus* sp. As shown in Table 2, mesophilic *Bacillus*-type A-OTUs were commonly found at 12 h and again when the pH and temperature began to rise (Fig. 1). *Weissella* and *Lactobacillus*-type A-OTUs were most common during the acidic phase between 24 and 60 h. Sequences related to *Pseudomonas* were found throughout the time course, but were most common during the acidic phase.

The temporal changes in the presence or absence of the 18 A-OTUs described in Table 2 were typical of the other 81 A-OTUs and were consistent among phylogenetic groupings (data not shown). While sequences similar to those from *Klebsiella* sp. were not as common as the five most frequently observed A-OTUs at any time point, several A-OTUs found in the compost DNA ARISA fingerprint during the acidic phase corresponded unambiguously to R-OTUs having significant sequences similarity (greater than 98%) with *Klebsiella* sp.

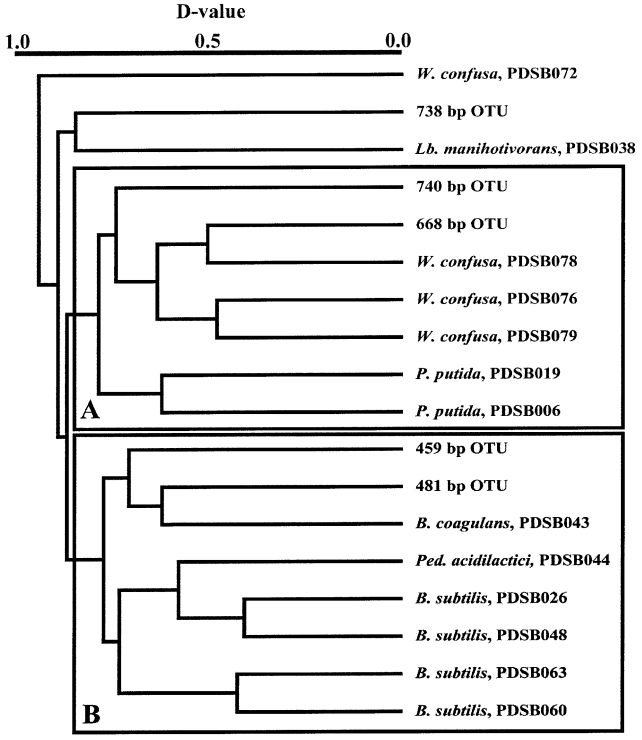

Fig. 3. Dendrogram showing clustering of the A-OTUs listed in Table 2 on whether or not two A-OTUs were present together in a sample. The A-OTUs within box A were predominant during the acidic phase while the A-OTUs within box B were common at the end of the process.

4. Discussion

After performing in silico analysis of the ribosomal intergenic region, Fisher and Triplett [39] suggested the possibility that a single A-OTU may represent more than one organism and that a single organism may be identified by multiple A-OTUs because a single bacterial genome may have numerous copies of the *rrn* operon. Evidence for both possibilities was found in this study. Among the 99 unique R-OTUs, 35 were represented by a total of 14 intergenic fragment lengths or A-OTUs. This suggests that analysis based on size comparisons alone could underestimate the number of R-OTUs present by approximately 21% (78 A-OTUs/99 R-OTUs), thereby possibly underestimating diversity in this sample. Correlation of unique phylogenetic groups, based solely on sequence analysis, with A-OTUs revealed that size analysis alone could overestimate diversity by approximately 10% (7 redundant A-OTUs/67 sequences). While these inaccuracies are not trivial and are at best estimates, they suggest that ARISA is still a useful tool for looking at a large proportion of the community.

Although no attempt was made to identify the majority of the A-OTUs via 16S rRNA sequences, analysis of the *D*-value matrix constructed in the same manner as the matrix used to construct Fig. 3 showed a number of A-OTUs that always clustered with one another (data not shown). This would suggest either that the A-OTUs are from the same organism, or that multiple organisms were always found together.

Previous studies by our research group and others have indicated that composting is a highly variable process [5,42]. For example, after performing a statistical power analysis with data collected using a similar composting system, we found that it was not possible to reliably detect a 15°C temperature difference that actually existed between two treatments [42]. Because of this large variability, we have continually attempted to maximize process reproducibility by controlling the reactor environment (i.e. housing in an insulated incubator), substrate (i.e. use of dog food), and reactor design (i.e. small, highly controllable reactors). In addition to controlling process variability, these modifications also enabled us to have a large number of replicates that would not have been possible at the field scale. Similar strategies have been employed throughout environmental microbiology to ascertain salient variables for degradation of environmental pollutants [43,44].

The use of our 'idealized' compost reaction system and substrate has been extremely useful for generating substrate-specific kinetics [18,19]. These kinetics have been successfully incorporated into dynamic process models

and partially validated at the pilot-scale level [12,14]. A clear weakness in our kinetic model framework is our failure to explicitly incorporate microbial biomass levels and composition into our modeling effort. Despite significant efforts taken to minimize variability in this study, our results (see Figs. 1–3) underscore the challenges in deducing linkages between physical state variables, such as temperature and pH, and community composition. This is even more of a challenge for undefined substrates and poorly characterized compost reactor systems. While real-world reactor systems and substrates are ultimately of more interest, such systems are subject to both temporal and spatial dynamics that have proven difficult to characterize even with respect to physical state variables [5,8,12]. By focusing on controlled systems, we have identified potential linkages between changes in community structure and state variables that might have been difficult to detect in a less controlled setting. This information can now be used as a starting point for examining more complex systems and may ultimately provide for more explicit linkages between microbial biomass levels and composition in our kinetic framework.

The phase of composting addressed in this study provides an interesting system for the study of microbial ecology. Based on data regarding the presence or absence of unique A-OTUs it is probable that there were no or few (i.e. PDSB044, PDSB075, PDSB079, and the unidentified 658-bp A-OTU; Tables 1 and 2) A-OTUs essential to the process during this initial phase. The variable community fingerprint observed in different replicates at the same time may be related to the significant variability found in process parameters, such as temperature and pH [45,46]. Although phylogeny is not always a good measure of function, the presence of related but distinct A-OTUs from well characterized phylogenetic groups such as lactic acid bacteria and *Bacillus* in this study also suggests significant functional redundancy among composting microorganisms. To be designated an A-OTU, only peaks from the electropherogram which gave a fluorescence signal greater than 5% of the maximum signal within a sample were selected. This may have caused an artificial increase in the apparent *D*-values between samples observed in Fig. 2. For example, A and B in Fig. 4 show electropherograms of samples collected at 36 and 48 h. These electropherograms appeared identical, yet the analysis showed them to differ by about 70% due to the presence of numerous small peaks. This finding, however, did not obscure our ability to detect more obvious differences as seen in the electropherogram from 72 h in Fig. 4C, which differed from the other two electropherograms by more than 90% according to the Jaccard distance analysis. This demonstrates that although there was variability between samples among the less intense peaks, the differences between time groupings shown in Fig. 2 are meaningful.

Problems with using PCR to draw quantitative conclusions have been well documented in the environmental microbiology literature [47–51]. Despite the existence of these biases, PCR-based analyses have been shown to approximate results obtained using more quantitative techniques [52,53]. The most significant source of PCR bias in this study was due to the initial automated RISA analysis. Since a low relative abundance threshold was used (5%) to identify fragments of interest, the probability of missing an abundant fragment was reduced.

The overall goal of this study was to expand the general knowledge of compost microbial ecology and to gain insight into some of the variables that may effect changes in the composition of microbial communities throughout the process. The more immediate purpose of the study was to evaluate the changes in microbial community structure during the initial phase of the process using molecular methods. Furthermore, our study complements two previous fingerprinting-based studies that addressed changes in compost by incorporating more frequent sampling points during the dynamic rise to thermophilic temperatures and by providing substantial sequence-based information regarding the phylogenetic association of individual fingerprint components [24,27]. To our knowledge, our

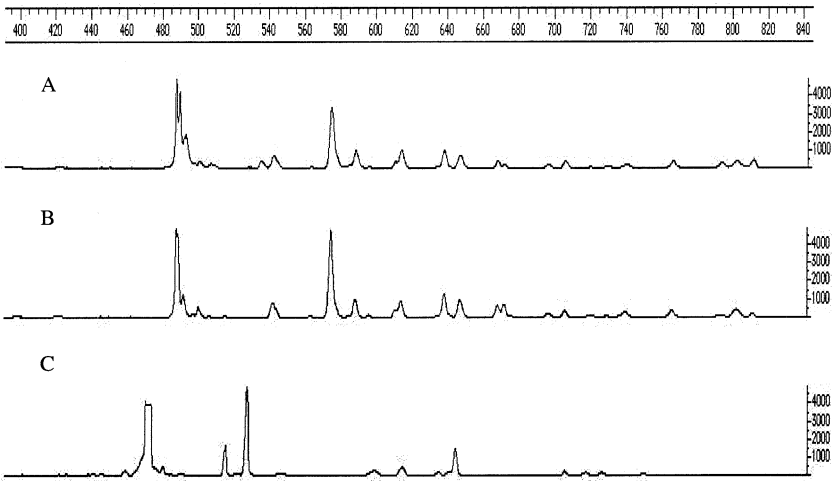

Fig. 4. Electropherograms representing typical ARISA profiles from samples of reactors after 36 (A), 48 (B), and 72 (C) h.

study is the first to detail the temporal shifts in compost communities during the initial rise to thermophilic conditions. It presents data suggesting a possible correlation between pH, a key process variable, and the presence of a specific guild of bacteria, namely mesophilic *Bacillus* sp. Future work using more quantitative methods is required before possible links between environmental factors, such as pH and community structure changes, can be more fully understood.

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