Appl Microbiol Biotechnol (2005) 67: 412–419
DOI 10.1007/s00253-004-1788-y

ENVIRONMENTAL BIOTECHNOLOGY

S. M. Tiquia · J. M. Ichida · H. M. Keener · D. L. Elwell · E. H. Burtt Jr. · F. C. Michel Jr.

Bacterial community profiles on feathers during composting as determined by terminal restriction fragment length polymorphism analysis of 16S rDNA genes

Received: 13 May 2004 / Revised: 10 September 2004 / Accepted: 2 October 2004 / Published online: 22 December 2004
© Springer-Verlag 2004

Abstract Composting is one of the more economical and environmentally safe methods of recycling feather waste generated by the poultry industry, since 90% of the feather weight consists of crude keratin protein, and feathers contain 15% N. However, the keratin in waste feathers is resistant to biodegradation and may require the addition of bacterial inocula to enhance the degradation process during composting. Two keratin-degrading bacteria isolated from plumage of wild songbirds and identified as *Bacillus licheneformis* (OWU 1411T) and *Streptomyces* sp. (OWU 1441) were inoculated into poultry feather composts (1.13×10^8 cfu g^{-1} feathers) and co-composted with poultry litter and straw in 200-l compost vessels. Composting temperatures, as well as CO_2 and NH_3 evolution, were measured in these vessels to determine the effects of inoculation on the rate and extent of poultry feather decomposition during composting. Terminal restriction fragment length polymorphisms of 16S rRNA genes were used to follow changes in microbial community structure during composting. The results indicated that extensive carbon conversion occurred in both treatments (55.5 and 56.1%). The addition of the bacterial inocula did not enhance the rate of waste feather composting. The microbial community structure over time was very similar in inoculated and uninoculated waste feather composts.

S. M. Tiquia (✉)
Department of Natural Sciences,
The University of Michigan,
Dearborn, Michigan, 481281, USA
e-mail: smtiquia@umd.umich.edu
Tel.: +1-313-5935148
Fax: +1-313-5934937

J. M. Ichida
Department of Botany/Microbiology,
Ohio Wesleyan University,
Delaware, Ohio, 43015, USA

H. M. Keener · D. L. Elwell · F. C. Michel Jr.
Department of Food, Agricultural,
and Biological Engineering,
Ohio Agricultural Research and Development Center,
The Ohio State University,
Wooster, Ohio, 44691, USA

E. H. Burtt Jr.
Department of Zoology,
Ohio Wesleyan University,
Delaware, Ohio, 43015, USA

Introduction

The poultry industry produces an enormous quantity of wastes, particularly litter. Each year, poultry processors produce thousands of tons of feather waste, which accounts for 5–7% of the total weight of mature chickens (D. Jensen, Animal Welfare Information Center, USDA-ARS, Beltsville, Md., personal communication in 1995). Feather waste is largely β-keratin protein (Fisher et al. 1981). In its native state, β-keratin is not degradable by common proteolytic enzymes such as trypsin, pepsin, and papain (Kim et al. 2001). Nonetheless, β-keratin does not accumulate in nature. Moreover, keratinolytic activity has been reported in a variety of *Bacillus* (Kim et al. 2001) and *Streptomyces* species (Ichida et al. 2001).

At present, feathers generated from the poultry industry are used on a limited basis as a dietary protein supplement for animal feed, and feather meal (Papadopoulos 1985). Prior to being used as feather meal, feathers are steam pressure-cooked or chemically treated to modify their polypeptide and disulfide bond structure to make them more digestible (Papadopoulos 1985). These treatment methods, however, require significant amounts of energy. Composting is one of the more economical and environmentally safe methods of recycling feather wastes. Feathers contain approximately 15% N (Ichida et al. 2001) and can be utilized as N fertilizer. Animal waste composts have been used as a nutrient source in crop production (Dick and McCoy 1993). Over the last three decades, research has been conducted to improve the agronomic utilization of animal wastes, including poultry wastes, via composting (Tiquia 2002). During composting, organic materials are mixed to create a moist, aerobic environment where organic matter decomposition and humification occur at rapid rates. The nutrients

are also biologically and chemically stabilized to more stable organic forms before application to agricultural soils (Tiquia 2002). These dynamics could help degrade and convert waste feathers into a useful resource (compost), which can help improve soil fertility.

The degradation of feathers is slow due to their resistance to proteolytic enzymes. The use of microbial inocula may represent a method to enhance the degradation of keratin during composting. It has already been demonstrated that *Bacillus licheniformis* (OWU 1411T) and *Streptomyces* strain (OWU 1441) enhance keratin degradation and biofilm formation during co-composting with poultry manure and straw (Ichida et al. 2001). Ichida et al. (2001) reported that degradation of feathers without bacterial inocula showed some degradation but resisted breaking, retained some resilience, and were still well defined in shape based on scanning electron microscopy (SEM) analyses, whereas inoculated feathers were difficult to identify after 28 days of composting. The feather residue in inoculated reactors had a putty-like consistency and any recognizable feather structures were extremely pliable. Examination of inoculated feathers by SEM, showed extensive degradation of keratin, and a feather surface that was covered with a complex microbial matrix (Ichida et al. 2001). The diversity of microbial communities on these feathers has not been explored at a broader phylogenetic range in the past. Moreover, very little is known about the microbial community structure on feathers at different stages of composting, which may be important in the degradation process.

A variety of methods (Liu et al. 1997; Kowalchuk et al. 1999; Peters et al. 2000) have been developed that allow rapid profiling of microbial communities without cultivation, and can provide information about the specific phylogenetic groups present in a microbial community. In this paper, we used terminal restriction fragment length polymorphisms (TRFLP) of PCR-amplified 16S rRNA genes to follow microbial community changes on feathers during composting.

Materials and methods

Microbial inocula

Bacillus licheniformis (OWU 1411T) and *Streptomyces* sp. (OWU 1441) were used as inocula in this study. These two thermotolerant organisms (Ichida et al. 2001) were selected from over 400 feather-degrading strains maintained in the Ohio Wesleyan culture collection. *B. licheniformis* was grown on tryptic soy agar (TSA) at 50°C for 24 h, while *Streptomyces* sp. was grown on yeast malt agar (YMA) at 28°C for 72 h. Saline suspensions were prepared to match a 1.0 McFarland turbidity and used to inoculate the seed batch. Basal feather medium was prepared as described previously (Ichida et al. 2001). Clean goose feathers (15 g) were added to each flask containing the medium before autoclaving. The flasks were inoculated with 1.5 ml bacterial suspension. One flask of each medium was inoculated with *B. licheniformis* and incubated at 36°C in an orbital shaker (75 rpm) for 5 days. Another set was inoculated with *Streptomyces* sp. and incubated at 28°C in an orbital shaker (75 rpm) for 5 days. Two flasks of each were left uninoculated and used as controls.

Composting set-up and sampling

White chicken feathers from a local broiler processing plant were co-composted with baled wheat straw and poultry litter obtained from poultry facilities at the Ohio Agricultural Research and Development Center (OARDC). The C, N, and C:N ratio, moisture and mass of these feedstocks are listed in Table 1. Four 200-l reaction vessels were layered with straw, poultry litter, and water. A duplicate series of compost vessels contained inoculated feathers, and a second set of duplicates contained control (uninoculated) feathers. The feedstocks (feather wastes, straw, and poultry litter) were stacked in layers (Ichida et al. 2001). During composting, the air in the vessels was blown through a plenum and up through the compost based on temperature feedback control at a continuous rate of 0.21 or 0.07 kg_{air} $kg^{-1}{}_{compost}$ h^{-1}. The temperature set point was 60°C. Temperatures in the vessels were recorded every 10 min using type K thermocouples, inserted into the compost at heights of 24, 48, and 73 cm. Carbon dioxide, oxygen, ammonia, C and N concentrations as well as pH (1:10 feather:water extract) were measured as described by Elwell et al. (1994). Feather samples were collected at days 0, 5, 12, 21, and 28 for molecular analyses.

DNA extraction and PCR amplification

Feather samples from each replicate treatment were ground in liquid N_2 as described previously (Tiquia et al. 2002), prior to DNA extraction. The total community DNA from each replicate compost sample was extracted and purified using an UltraClean Soil DNA Isolation Kit (MoBio Laboratories, Solana Beach, Calif.). Bacterial (16S rDNA) DNAs present in the community were PCR-amplified using the universal eubacterial primers: 8F forward (3′-AGAGTT TGATCCTGGCTCAG-5′) and 1406r (3′ ACGGGCGGTG TGTRC-5′) reverse, with the 8F forward primer labeled

Table 1 Nitrogen, carbon and C:N ratio, moisture and mass of uninoculated and inoculated feathers, straw, and poultry litter

Feedstock[a]	Nitrogen (%)	Carbon (%)	C:N ratio	Moisture (%)[b]	Mass (kg)
Feathers (−)	14.17	48.14	4:1	55	7.14
Feathers (+)	14.17	48.14	4:1	55	7.05
Straw	0.60	36.10	15:1	55	1.52
Poultry litter	2.49	43.51	72:1	55	26.22

[a](−) uninoculated, (+) inoculated

[b]Moisture content of the feedstocks was adjusted by adding water before layering

with HEX (5-hexachloroflourescein) (Liu et al. 1997). Each 50 µl PCR reaction mixture contained 50 ng DNA template, 2.5 mM $MgCl_2$, 2.5 units *Taq* polymerase (Roche, Indianapolis, Ind.), 1× PCR reaction buffer, 0.2 mM PCR nucleotide mix (Roche), 0.5 µM DNA primers, and 0.6 µl bovine serum albumin (Roche). 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; 90 s) in a PTC-100 thermal cycler (MJ Research, Waltham, Mass.). The amplified DNAs were verified by electrophoresis of aliquots of PCR mixtures (5 µl) in 2.0% agarose and 1× TAE buffer. To minimize PCR bias, fluorescently labeled amplicons from three PCR runs were combined and then purified using a PCR purification kit (PCR Clean-up Kit; MoBio Laboratories). Purified DNAs were then eluted in a final volume of 50 µl. Genomic DNAs of *B. licheniformis* (OWU 1411T) and *Streptomyces* sp. (OWU 1441) were isolated using the same kit used for extracting total community DNAs from feather samples. The DNA of the resuspended pellet was then extracted. The DNAs from these two pure cultures were PCR-amplified and digested using the same procedure employed for the feather samples.

TRFLP analysis

Aliquots (10 µl) of amplified 16S rDNAs of feather samples compost and the two pure cultures (*B. licheniformis* and *Streptomyces* sp.) were separately digested with restriction endonucleases *Hha*I, *Msp*I, and *Rsa*I (Roche), for 5, 3 and 5 h, respectively to produce a mixture of variable length end-labeled 16S rDNA fragments. The labeled fragments were electrophoretically separated on a polyacrylamide gel (5.5%) in an ABI model 377 automated sequencer (Applied Biosystems, Foster City, Calif.). Thereafter, the lengths of fluorescently labeled terminal restriction fragments (TRFs) were determined by comparison with internal standards, using Genescan software (Applied Biosystems) with a peak height detection of 50. The size, in base pairs, of the terminal restriction fragments (TRFs) was estimated with reference to the internal standard using Local Southern method.

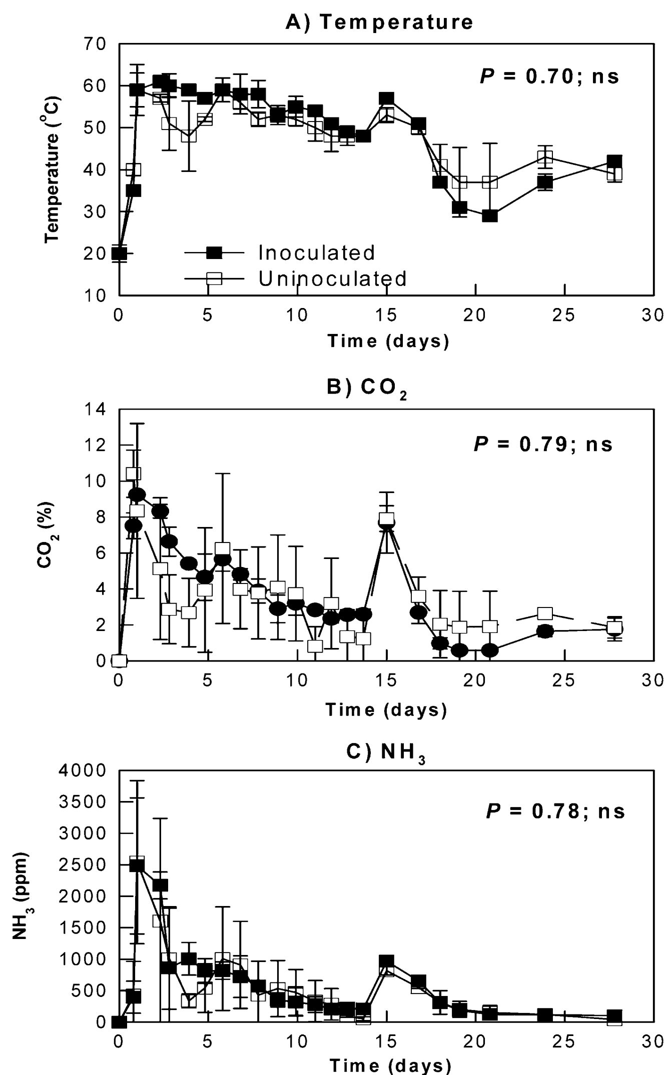

Fig. 1 Changes in **a** temperature and concentrations of **b** CO_2, and **c** NH_3 during composting. *Values* Mean of two replicates, *error bars* standard deviation, *ns* not significant at $P \leq 0.05$ probability level

Results

The composting process

The composting process was characterized by an early period of self-heating due to rapid microbial metabolism. Temperatures in the compost vessels reached thermophilic levels (55°C) by day two. Thereafter, vessel temperatures declined to 50°C by day 14. After the compost was mixed at day 14, vessel temperatures increased to 55–59°C, declined rapidly at day 16, and then leveled off at the end of composting (Fig. 1a).

Carbon dioxide and NH_3 were not detected in the compost on day 0 (Fig. 1b, c) but peaked on day 2. As composting proceeded, CO_2 and NH_3 concentrations decreased continuously until day 14, then increased dramatically on day 15, when the compost was turned. At the end of composting, CO_2 concentrations in the vessels declined to as little as 1.8 and 2.0%, respectively (Fig. 1b), whereas NH_3 concentrations dropped to non-detectable levels (Fig. 1c). The temporal changes in temperature, CO_2, and NH_3 in the uninoculated and inoculated vessels were similar (Fig. 1a–c). The chemical properties of the inoculated and uninoculated feathers, including N, C, C:N ratio, ash, and pH, as well as other important composting parameters such as dry matter loss, C loss, and N loss were also very similar in both treatments at the end of composting (Table 2), indicating that composting in the uninoculated and inoculated vessels proceeded at similar rates.

Table 2 Chemical properties of the uninoculated and inoculated composts at the end of the composting trial. Values indicate the mean and standard deviation of two replicates

Chemical properties	Uninoculated vessel	Inoculated vessel	*P* value	Significance
N (%)	4.00±0.48	3.38±0.15	0.45	ns[a]
C (%)	38.60±1.84	37.45±1.20	0.13	ns
C:N ratio	10:1±0.71	11:1±0.14	0.63	ns
Ash (%)	21.38±0.35	23.30±1.56	0.23	ns
pH	7.74±0.45	7.96±0.04	0.56	ns
Dry matter loss (% of initial)	54.75±1.34	52.75±4.03	0.57	ns
Carbon loss (% of initial)[b]	55.46±0.00	56.08±3.18	0.81	ns
N loss (% of initial)[b]	67.91±4.08	70.36±0.18	0.49	ns

[a]Not significant at $P\leq 0.05$ probability level

[b]$\text{Mass loss}_{\text{N or C}}\ (\%\text{ of initial}) = \frac{\text{initial mass}_{\text{N or C}} - \text{final mass}_{\text{N or C}}}{\text{initial mass}_{\text{N or C}}} \times 100$

Feather bacterial community TRFLP profiles

Analysis of *Hha*I, *Msp*I, and *Rsa*I-TRFLP profiles of microbial communities on feathers revealed extensive bacterial diversity. The relative diversity of the samples was estimated by counting the number of TRFs present in each profile. About 31–52 *Hha*I-, *Msp*I-, or *Rsa*I-TRFs were observed in the feedstocks during composting. At day 5 (when temperatures were around 55–58°C; see Fig. 1a), the number of TRFs had increased to 48–56. As compost temperatures started to decline, the number of TRFs remained essentially the same (50–56).

Comparison of TRFs with those predicted by computer simulation using TAP-TOOL

In theory, individual TRFs sizes from eubacterial 16S rRNA TRFLP profiles can be compared with data predicted from sequence databases tools such as the Ribosomal Database Project (RDP) TAP-TRFLP (http://www.rdp.cme.msu.edu/index.jsp) to infer the potential bacterial composition of samples. Having determined the sizes of the *Hha*I, *Msp*I, and *Rsa*I TRFs that were consistent at all stages of composting both in the uninoculated and inoculated feathers, we attempted to identify the bacterial species giving rise to the common TRFs. We used the TRFLP-TAP tool to generate a list of bacterial genera that could have given rise to the observed TRFs. In total 16, primarily low-GC, Gram-positive bacteria belonging to six different genera were consistent with the three different TRFLP patterns generated by multiple restriction digestions of the uninoculated and inoculated feather TRFLP profiles (Table 3). These included TRFs similar to some well-known feather and livestock-inhabiting genera such as *Bacillus*, *Lactobacillus*, *Alicyclobacillus*, *Erysipelothrix*, *Planococcus,* and *Caryophanon* (Kim et al. 2001). One high-GC Gram-positive

Table 3 Bacteria corresponding to three different observed terminal restriction fragment (TRF) sizes in samples collected on all five sampling days (days 0, 5, 12, 21 and 28). Identifications were based on TRFs after *Hha*I, *Msp*I, and *Rsa*I digestion

Species	Phylogenetic group	*Msp*I TRF	*Rsa*I TRF	*Hha*I TRF
Alicyclobacillus acidoterrestris	*Bacillus/Clostridium*	149	489	242
Erysipelothrix rhusiopathiae	*Bacillus/Clostridium*	169	485	239
Bacillus fusiformis	*Bacillus/Clostridium*	149	459	242
Bacillus pasteurii	*Bacillus/Clostridium*	150	459	242
Bacillus popilliae	*Bacillus/Clostridium*	147	487	240
Planococcus citreus	*Bacillus/Clostridium*	148	458	241
Caryophanon latum	*Bacillus/Clostridium*	147	487	241
Bacillus licheniformis	*Bacillus/Clostridium*	147	458	241
Bacillus polymyxa	*Bacillus/Clostridium*	152	488	242
Bacillus licheniformis	*Bacillus/Clostridium*	147	458	241
Bacillus amyloliquefaciens	*Bacillus/Clostridium*	147	487	240
Bacillus firmus	*Bacillus/Clostridium*	148	459	242
Bacillus lentimorbus	*Bacillus/Clostridium*	147	487	240
Bacillus macquariensis	*Bacillus/Clostridium*	150	487	241
Bacillus lautus	*Bacillus/Clostridium*	147	458	241
Lactobacillus thermophilus	*Bacillus/Clostridium*	149	488	242
Streptomyces sp	Streptomyces			
Unidentified TRFs (bp)	109		490; 487; 480; 361; 286; 270; 186; 109; 94; 440	760; 586; 536; 361; 237; 233; 222; 186; 172; 116; 109
% of unidentified TRFs		7%	91%	85%

Table 4 Unique TRF peaks from *Hha*I, *Msp*I and *Rsa*I TRF length polymorphisms (TRFLP) profiles of 16S rRNA genes amplified from uninoculated feathers and microorganisms/phylogenetic groups corresponding to observed TRFs from the ribosomal database project (RDP) (TAP-TRFLP tool). Values in *bold* are genera consistent with observed TRF sizes from at least two restriction digestions

Feather samples[a]	*Hha*I TRFs (bp)/ corresponding organisms	*Msp*I TRFs (bp)/ corresponding organisms	*Rsa*I TRFs (bp)/ corresponding organisms
Day 0	***578Bacillus megaterium*** Clone AJ009486 785 None	320 Clone AJ131819	310 Clone AF068798 *Sphingobacterium spiritovorum* *Persibacter diffluens* ***415Bacillus alvei*** 446 *Fervibacterium islandum*
	Number of TRFs = 46	Number of TRFs = 52	Number of TRFs = 48
Day 5	157 Clone AB01553	***461Vibrio metschikovii*** 473 *Peptostreptococcus* sp *Sulfobacillus desulfooxidans* *Campylobacter hyointestinalis* Clone AB015582	**223***Vibrio* sp.
	Number of TRFs = 48	Number of TRFs = 56	Number of TRFs = 56
Day 12	325 *Pseudonocardia saturnea* 430 *Mycoplasma spermatophilum* *Nocardioides luteus* ***698Clostridium butyricum***	***316Clostridium aminovalerium***	***205Clostridium sphenoides*** 213 Clone U65915 *Kibdelosporangium avidum* *Leptospirillum* sp
	Number of TRFs = 51	Number of TRFs = 56	Number of TRFs = 54
Day 21	480 Clone AB015586	***485Eubacterium brachy*** Clone AB013836 Clone AB015534 625 None	***560Eubacterium brachy*** 571 Clone AB015150 Clone AB015572 Clone AB015521
	Number of TRFs = 49	Number of TRFs = 55	Number of TRFs = 55
Day 28	458 *Abiotrophia elegans* ***461Lactobacillus vitulinus***	415 None ***570Lactobacillus aviarus***	328 Clone AF029050
	Number of TRFs = 53	Number of TRFs = 56	Number of TRFs = 55

[a] Uninoculated feather samples

genus, *Streptomyces,* was predicted to be present in the all TRFLP profiles.

TRFs consistent with both of the bacterial inocula used in this study, *Bacillus licheniformis* (H241, M144, and R460), and *Streptomyces* sp. TRFs (H240, M80, and R487) were present on inoculated and uninoculated feathers on days 0, 5, 12, 21, and 28 of composting. However, the relative abundance (% peak area) of the TRFs changed substantially during composting. The normalized TRF peak areas of both *B. licheniformis* and *Streptomyces* sp. were greatest at the beginning of composting. As composting proceeded, the relative abundance of these TRFs decreased. As expected, the initial percent peak areas of TRFs similar to those of *B. licheniformis* and *Streptomyces* sp. were higher in the inoculated than the uninoculated feathers. However, as composting proceeded, no noticeable difference was observed in the percent peak areas of TRFs between inoculated and uninoculated feathers.

Evaluation of unique TRFs

Unique TRFs from uninoculated (Table 4) and inoculated (Table 5) feathers on days 0, 5, 12, 21, and 28 of composting were also identified. Unique peaks were those that appeared at only one sampling point. The set of unique TRF peaks from the three restriction digestions were consistent with only one or two) TRF genera level fragment sizes predicted by the TRFL-TAP tool (see bacterial species highlighted in bold in Tables 4 and 5). Fragments H578 and R415 TRFs unique to day 0 inoculated TRFLP profiles corresponded with TRF sizes of *Bacillus* spp. (Table 4). *Hha*I (605 bp) and *Msp*I (80 bp) TRFs unique to day 0 inoculated TRFLP profiles also corresponded to TRF sizes of *Bacillus* spp. (Table 5). Some unique peaks that were predicted to be potentially present in the inoculated and uninoculated feathers generally fell into the same genera, including *Bacillus* and *Clostridium*. TRFs consistent with bacterial species such as *Vibrio* spp., *Eubacterium brachy,* and *Lactobacillus* spp. were found only in uninoculated feathers (Table 5), while TRFs consistent with *Actinomyces* and *Streptomyces* spp. were unique to inoculated feathers (Table 5).

Table 5 Unique TRF peaks from *Hha*I, *Rsa*I and *Msp*I TRFLP profiles of 16S rRNA genes amplified from inoculated feathers and microorganisms/phylogenetic groups corresponding to observed TRFs from the RDP (TAP-TRFLP). Values in *bold* are genera consistent with observed TRF sizes from at least two restriction digestions

Feather samples[a]	*Hha*I TRFs (bp)/Correspond organisms	*Msp*I TRFs (bp)/ Corresponding organisms	*Rsa*I TRFs (bp)/ Corresponding organisms
Day 0	380 *Aerococcus urinae*	78 *Chondromyces crocatus*	143 *Eubacterium halii*
	Clone AB015540	*Chondromyces perfringens*	638 *Mycobacterium tuberculosis*
	Desulfotomaculum thermosapovora	*Polyangium cellulosum*	*Mycobacterium bovis*
	605*Bacillus piliformis*	*Polyangium* sp	783 None
		80*Bacillus pumilis*	
		612 *Nitrospira* sp	
Day 5	Number of TRFs = 31	Number of TRFs = 46	Number of TRFs = 49
	172*Enterococcus saccharolyticus*	375 None	398 *Acholeplasma laidwaii*
	Streptomyces neyagawaensis	453 *Rickettsia typhi*	**409*Streptomyces salmonis***
	***Streptomyces*sp.**	*Rickettsia rickettssi*	
	563 *Desulfotomaculum halophilum*	*Rickettsia bellii*	
	Spiroplasma citri	*Rickettsia* sp	
	215 *Xanthomonas* sp		
	Number of TRFs = 55	Number of TRFs = 56	Number of TRFs = 56
Day 12	**223***Lactobacillus delbruecki*	**523*Clostridium argentinense***	506 *Actinomyces viscosus*
	Clostridium spiriforme	***Clostridium perfringens***	Clone AF018564
		Clostridium carnis	
		Clostridium tetani	
	Number of TRFs = 56	Number of TRFs = 56	Number of TRFs = 52
Day 21	530 *Zymophilis paucivorans*	360 None	
		655 None	**607*Actinomyces*sp.**
		90*Actinomyces viscosus*	
	Number of TRFs = 45	Number of TRFs = 50	Number of TRFs = 57
Day 28	570 *Nitrosomonas* sp	128 *Streptomyces mobaraensis*	178 *Clostridium butyricum*
	Azoarcus sp	**134*Bacillus megaterium***	*Clostridium beijerinckii*
	Shewanella sp	***Bacillus methanolicus***	*Clostridium kainantoi*
		417 *Streptococcus* sp	*Spirochaeta halophila*
		490 *Pseudomonas* sp	**371*Bacillus brevis***
			708 None
			809 *Mycobacterium xenopi*
			Spiroplasma ixodetis
	Number of TRFs = 56	Number of TRFs = 56	Number of TRFs = 55

[a] Inoculated feather samples

Discussion

Composting is a microbial process in which organic matter progresses through stages of decomposition and stabilization over a period of time. In this process, the temperature within a composting mass determines the rate at which many biological activities take place (Stentiford 1996). The temperature change in the present study followed a pattern similar to that of other composting systems (Stentiford 1996). The temperature of the compost mass rose immediately after piling and maintained a level between 60–70°C. Thereafter, the temperature slowly decreased to close to ambient level. The maturation process was accompanied by a decline in temperature to close to ambient level, and decreases in concentrations of C, N, CO_2, NH_3, and the C:N ratio. The rapidly changing physico-chemical conditions in this composting process 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 (Strom 1985; Peters et al. 2000). In this study, TRFLP analysis of 16S rDNA genes amplified directly from feather DNA can be used to visualize microbial community profiles at different stages of composting. The TRF peaks were useful in investigating the diversity of complex compost communities, which may in turn be useful in evaluating the dynamics of

the composting process. The microbial diversity (based on the number of TRFs) in the feathers increased as vessel temperatures reached thermophilic temperatures (>55°C) and remained unchanged as vessel temperatures dropped at the end of composting. While the number of bacterial TRFs did not decrease at the end of composting, the peak areas of some of the large individual TRFs decreased substantially (data not shown), indicating a relative decrease in the abundance of these individual TRFs.

The composting process in uninoculated and inoculated vessels proceeded at similar rates, indicating that the inoculation of β-keratin degrading strains *B. licheneformis* (OWU 1411T) and *Streptomyces* sp., (OWU 1441) did not enhance the rate of composting. Golueke et al. (1954) reported that the addition of bacterial inoculum is of value in composting only if the bacterial population in the compost piles is unable to develop rapidly enough to take full advantage of the compost's capacity to support bacterial growth. They found that the inocula failed in terms of temperature pattern and chemical analyses, due to the adequacy of the microbial population already existing on the material. TRFLP analysis indicated that TRFs consistent with both *Bacillus licheniformis* (H241, M144, and R460) and *Streptomyces* sp. TRFs (H240, M80, and R487) were also present on the uninoculated feathers at the beginning of composting. Such a finding is of remarkable importance to the composting of waste feathers, since it means that the whole process could be run without addition of inocula.

TRFs in *Hha*I, *Msp*I, and *Rsa*I TRFLPs that are common to both inoculated and uninoculated samples correspond to several known organisms in the RDP. Many of these bacterial groups are thermophilic (Holt et al. 1994), and the majority belong to the *Bacillus*/*Clostridium* phylogenetic subdivision. In the present study, TRFs that correspond to the *Bacillus*/*Clostridium* phylogenetic subdivision were found at temperatures ranging between 40 and 60°C (Fig. 1a). *Bacillus*/*Clostridium* and *Streptomyces* microbes belong to very large groups of organisms (Holt et al. 1994). It is anticipated that only certain strains of these organisms are involved in the degradation process. Furthermore, these organisms are widely distributed in nature, and many of these organisms (Table 3) have been detected in manure composts (Peters et al. 2000; Tiquia et al. 2002) and chicken feathers (Burtt and Ichida 1999; Ichida et al. 2001; Kim et al. 2001). It is important to note that many TRFs in the profiles did not correspond to any TRFs predicted using the TRFLP-TAP tool. Interestingly, but not surprisingly, an average of 7, 91, and 85% of the TRFs in the *Msp*I, *Rsa*I and *Hha*I profiles, respectively, remained unidentified (Table 3). It is possible that these peaks may correspond to TRFs of prokaryotic bacteria that have not yet been sequenced. This result is typical of the high level of novel microbes found in environmental samples using nucleic-acid-based methods (Hugenholtz et al. 1998).

This study has demonstrated that distinctive community patterns from waste feathers could be rapidly generated using TRFLP analysis. TRFLP analysis of feather microbial communities during composting indicated extensive bacterial diversity. The changing compost physico-chemical conditions in this study probably selected for a succession of microbial communities on feathers. Temporal changes in temperature, concentrations of CO_2 and NH_3, and the forms of C and N available to the microbes are among the factors probably affecting microbial community structure. With TRFLP profiles, we were able to identify TRFs in feather bacterial communities that were present consistently during composting, and to use multiple TRFLP patterns to infer the potential identities of members of the communities. This approach also allowed tentative classification of unique TRFs found in only a few of the compost samples. Organic matter, CO_2, temperature data, pH, C:N ratio, N and ash content, also indicated that the addition of bacterial inocula did not enhance the rate of waste feather composting.

Acknowledgements This work was financially supported by funds from The University of Michigan-Dearborn Campus Grants, The Ohio State University, Agricultural Research and Development Center Seed Grant Program and National Science Foundation C-RUI grant DBI 9978805

References

Burtt EH Jr, Ichida JM (1999) Occurrence of feather-degrading bacilli in the plumage of birds. Auk 116:364–372

Dick WA, McCoy EL (1993) Enhancing soil fertility by addition of compost. In: Hoitink HAJ, Keener HM (eds) Science and engineering of composting: design, environmental, microbiological, and utilization aspects. Renaissance Publications, Worthington, Ohio, pp 622–644.

Elwell DL, Keener HM, Hoitink HAJ, Hansen RC, Hoff J (1994) Pilot and full-scale evaluations of leaves as an amendment in sewage sludge composting. Compost Sci Util 4:6–15

Fisher ML, Leeson S, Morrison WD, Summers JD (1981) Feather growth and feather composition of broiler chickens. Can J Anim Sci 61:768–773

Golueke CG, Card BJ, McGauley PH (1954) A critical evaluation of inocula in composting. Appl Microbiol 2:45–63

Holt JG, Krieg NK, Sneath PHA, Bergy D (1994) Bergey's manual of determinative bacteriology, 9th edn. Williams and Wilkins, Baltimore

Hugenholtz P, Goebel BM, Pace NR (1998) Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. J Bacteriol 180:4765–4774

Ichida JM, Krizova L, LeFevre CA, Keener HM, Elwell DL, Burtt EH Jr (2001) Bacterial inoculum enhances keratin degradation and biofilm formation in poultry compost. J Microbiol Methods 47: 199–208

Kim JM, Lim WJ, Suh HJ (2001) Feather-degrading *Bacillus* species from poultry wastes. Process Biochem 37:287–291

Kowalchuk GA, Naoumenko ZA, Derikx JL, Felske A, Stephen JR, Arkhipchenko IA (1999) Molecular analysis of ammonia-oxidizing bacteria of the beta subdivision of the class *Proteobacteria* in compost and composted materials. Appl Environ Microbiol 65:396–403

Liu WT, Marsh T, Cheng H, Forney LJ (1997) Characterization of microbial community by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. Appl Environ Microbiol 63:4516–4522

Papadopoulos MC (1985) Processed chicken feathers as feed stuff for poultry and swine. A review. Agric Wastes 14:275–290

Peters S, Koschinsky S, Schweiger F, Tebbe CC (2000). Succession of microbial communities during hot composting as detected by PCR-single-strand-conformation polymorphism-based genetic profiles of small-subunit rRNA genes. Appl Environ Microbiol 66:930–936

Stentiford EI (1996) Composting control: principles and practice. In: DeBertoldi M, Sequi P, Lemmes B, Papi T (eds) The science of composting. Part I. Chapman and Hall, London, pp 49–59

Strom PF (1985) Effect of temperature on bacterial species diversity during thermophilic solid waste composting. Appl Environ Microbiol 50:899–905

Tiquia SM (2002) Evaluation of organic matter and nutrient composition of partially decomposed and composted spent pig litter. Environ Technol 24:97–107

Tiquia SM, Michel FC Jr (2002) Bacterial diversity in livestock manure composts as characterized by terminal restriction fragment length polymorphisms (TRFLP) of PCR-amplified 16S rRNA gene sequences. In: Insam H, Riddech N, Klammer S (eds) Microbiology of composting. Springer, Berlin Heidelberg New York, pp 64–82