

# Spatial Shifts in Microbial Population Structure Within Poultry Litter Associated with Physicochemical Properties

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**ABSTRACT** Microbial populations within poultry litter have been largely ignored with the exception of potential human or livestock pathogens. A better understanding of the community structure and identity of the microbial populations within poultry litter could aid in the development of management practices that would reduce populations responsible for toxic air emissions and pathogen incidence. In this study, poultry litter air and physical properties were correlated to shifts in microbial community structure as analyzed by principal component analysis (PCA) and measured by denaturing gradient gel electrophoresis (DGGE). Litter samples were taken in a 36-point grid pattern at 5 m across and 12 m down a 146 m × 12.8 m chicken house. At each sample point, physical parameters such as litter moisture, pH, air and litter temperature, and relative humidity were recorded, and sam-

ples were taken for molecular analysis. The DGGE analysis showed that the banding pattern of samples from the back and water/feeder areas of poultry house were distinct from those of samples from other areas. There were distinct clusters of banding patterns corresponding to the front, middle front, middle back, back, and waterer/feeder areas. The PCA analysis showed similar cluster patterns, but with more distinct separation of the front and midhouse samples. The PCA analysis also showed that moisture content and litter temperature (accounting for 51.5 and 31.5% of the separation of samples, respectively) play a major role in spatial diversity of microbial community in the poultry house. Based on analysis of DGGE fingerprints and cloned DGGE band sequences, there appear to be differences in the types of microorganisms over the length of the house, which correspond to differences in the physical properties of the litter.

**Key words:** microbial diversity, denaturing gradient gel electrophoresis, poultry litter, principal component analysis

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## INTRODUCTION

The rapid growth of the poultry industry has resulted in the production of massive quantities of poultry wastes. These materials are alternatively viewed as essential soil fertilizers, energetic and nutritive substrates for feeds, or environmental contaminants. Many studies have been carried out to investigate these various aspects of poultry wastes (Smith, 1974; Lauer et al., 1976; El-Ashry et al., 1987; Pain et al., 1987; Tate, 1987; Chen et al., 1988; Diaz-Fierros et al., 1988; Hartung and Phillips, 1994; Martin et al., 1998; Stuvan and Bock, 2001). Poultry litters, a mixture of poultry manure and different bedding materials, are environmental ecosystems with a considerable range of characteristics. Their microbial diversity varies from one type of litter to the next, which makes their study interesting. Thus, many investigators have increased their efforts

in trying to understand the biotic properties of these poultry and other animal wastes (Lovett et al., 1971; Finstein and Morris, 1975; Corominas et al., 1987; De Bertoldi et al., 1987; Acea and Carballas, 1988a,b; Nodar et al., 1990; Martin et al., 1998; Lu et al., 2003b; Thaxton et al., 2003; Fries et al., 2005).

Poultry litter is a valuable fertilizer source for crop production. However, its value as a fertilizer is reduced over time due to the significant losses of nitrogen attributed to the volatilization of ammonia (Lauer et al., 1976; Pain et al., 1987; Hartung and Phillips, 1994). Ammonia emission and subsequent deposition can be a major source of pollution, causing nitrogen enrichment, acidification of soils and surface waters, and aerosol formation. In the poultry house, ammonia emissions can also adversely affect the health, performance, and welfare of animals and human operators (Donham et al., 1977; Donham and Gustafson, 1982; Donham, 1990).

In addition to its use as fertilizer, poultry litter has nutritional value as feeds for ruminants (Smith, 1974; Jeffrey et al., 1998). However, there are concerns regarding the safety of feeding poultry litter to cattle due to potential infection by pathogenic microorganisms that

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may be present in poultry litter. Many pathogenic strains such as *Listeria monocytogenes*, *Salmonella*, *Campylobacter* spp., *Clostridia* spp., and *Bordetella* spp. have been found in poultry litter samples (Martin et al., 1998; Lu et al., 2003b).

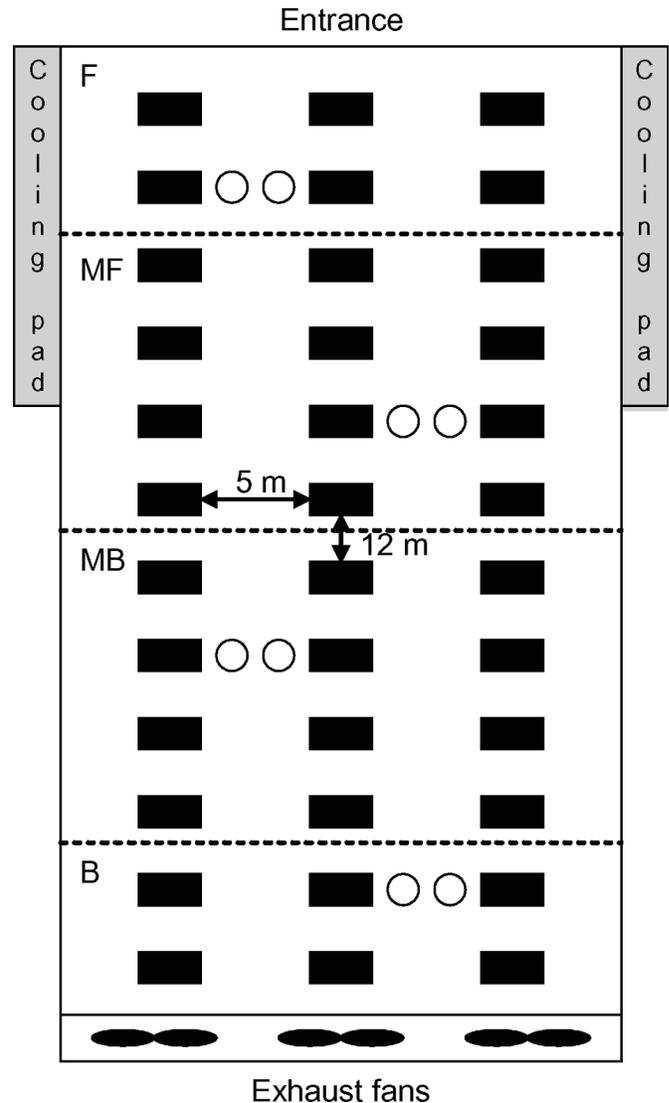
Microbial diversity in poultry litter plays an important role in shaping the quality of the poultry litter as a fertilizer and as a nutritional feedstock, and influences malodor production and potential health risks. It is, therefore, essential to understand how the structure of microbial populations within poultry litter is influenced by the physical environment of the poultry house. This knowledge, in turn, could aid in the development of management practices that would reduce populations responsible for toxic air emissions (especially ammonia emissions) and pathogen incidence. Even though many studies have been done to classify microbial composition in poultry litter (Lovett et al., 1971; Nodar et al., 1990; Martin et al., 1998; Lu et al., 2003b; Fries et al., 2005), these classifications were only carried out on different types of poultry litters (e.g., poultry manure with different bedding materials). Research on spatial shifts in microbial population structure within poultry litter associated with physicochemical properties is scarce and incomplete.

The aim of this work is to examine the spatial shifts in the microbial community structure in poultry litter using denaturing gradient gel electrophoresis (DGGE) and to evaluate how those shifts are associated with physical parameters. Principal component analysis (PCA) was used to determine the important factors that correlated with shifts in the microbial community structure. Understanding the contributing factors affecting microbial community structure may provide a rational basis for improving the design and optimizing the remediation options for toxic air and pathogenic reduction, whether these involve microscale biological treatment such as enzyme inhibition or physicochemical treatment such as alum amendment.

## MATERIALS AND METHODS

### Litter Sampling

At the end of a broiler flock (d 45), 44 litter samples were obtained from the upper 5 cm of the litter in a commercial house on a Mississippi farm. The house dimensions were 12.8 m × 146.3 m. The grid in Figure 1 illustrates the sampling locations, with 3 sites spaced at 5 m across the building and 12 locations (12 m between) along the length of the building to create a 36 sample grid. To consider areas of frequent bird activity, specifically near feeders and waterers, 8 supplementary samples were taken in a crisscross routine between the feeders/waterers. The feeder/waterer samples are shown as circles in Figure 1. Two feed lines were approximately 3.7 m from the center of the house, having a nipple water line 0.75 m on either side for a total of 4 water lines. A pocket meter (Kestrel 3000, Nielson Kellerman, Chester, PA) reported house air temperature and relative humidity at 1 m above the floor. At the laboratory, litter moisture was determined by drying for 48 h at 65°C. A litter to



**Figure 1.** Sampling schematic. The dashed lines mark the boundary of each region. F = front; MF = middle front; MB = middle back; B = back; circles represent waterer/feeder.

deionized water ratio of 1:5 was used to determine pH for the samples.

### DNA Extraction and PCR Optimization

The DNA was extracted from poultry litter samples (0.3 g, n = 27 samples) in duplicate using the Q-Biogene FastDNA Spin Kit for soil (Q-Biogene, Irvine, CA) according to manufacturers specifications. To determine the level of PCR inhibition in each extraction, dilutions of the DNA (1:50, 1:100, 1:200, 1:500, and 1:1,000) were made, and the samples were spiked with  $10^8$  copies of 16S rDNA standard. Spiked samples (5  $\mu$ L) were analyzed by quantitative, real-time PCR (QRT-PCR) to check for PCR inhibition. The QRT-PCR analysis of 16S rDNA copies was carried out as described by Harms et al. (2003) using the 1055f and 1392r primers at 600 nM each and the 16Staq1115-BHQ at a concentration of 200 nM. The amplification mixture contained 3.0 mM  $MgCl_2$ , 600 nM each

primer, 200 nM of probe and spiked sample DNA or standard (from  $10^2$  to  $10^8$  copies). The QRT-PCR program was 15 min at 95°C, 39 cycles at 95°C for 15 s and 58°C for 45 s. Baseline values were set as the lowest fluorescence signal measured in the well over all cycles. The baseline was subtracted from all values and the threshold was set to 1 standard deviation of the mean. All PCR runs included duplicates of standards and control reactions without template. Standard DNA consisted of plasmid PCR 2.1 vector (Invitrogen, Carlsbad, CA) carrying a 16S rDNA insert. DNA concentrations in each extraction were determined using the Hoechst 33258 nucleic acid stain (Invitrogen) and measured with a Hoefer DyNA Quant 200 fluorometer (Amersham Biosciences, San Francisco, CA) according to manufacturers instructions.

### **DGGE Analysis of Chicken Litter Microbial Populations**

Bacterial community 16S rDNA (2  $\mu$ L) from the 1:50 dilution was amplified with the bacterial specific primer set 341F-GC/907R, using the previously described PCR protocols (Casamayor et al., 2000) in a PTC-200 DNA thermal cycler (MJ Research, Las Vegas, NV). The guanine-cytosine (GC) designation on the 341F primer represents a 40-bp GC rich region on the 5' end of the primer necessary to prevent complete denaturation of the DNA strands during electrophoresis. Sequences were amplified using Ready-To-Go-PCR Beads (Amersham Pharmacia, Piscataway, NJ), with 800 nM each primer. Denaturing gradient gel electrophoresis was used to separate and characterize 16S rDNA by using a gradient of denaturants [100% denaturant solution consisting of a combination of 40% (vol/vol) formamide and 7 M urea] in a polyacrylamide gel (37.5:1) to separate DNA fragments according to melting behavior (i.e., sequence, melting domains). GelBond PAG Film (Cambrex BioSciences, Rockland, MA) was used during pouring of the DGGE gels to allow for easier manipulation of the polyacrylamide gel after electrophoresis. Then, 5  $\mu$ L of PCR product was electrophoresed through a 30 to 60% denaturing gradient according to Nubel et al. (1997) for 4 h at 200 V in a BioRad DCode universal mutation detection (BioRad Laboratories, Hercules, CA). The DGGE gels were stained with the BioRad Silver Stain kit according to the manufacturer's specifications, and the images were captured using an Epson Perfection 4990 Photo Scanner (Epson, Long Beach, CA). The DGGE fingerprint analysis was performed using the Fingerprint II software program (BioRad Laboratories) using the basic and clustering modules. The gel images were imported into the software and analyzed according to manufacturer's specifications, with unweighted pair group method with arithmetic mean (UPGMA) analyses being performed based upon the banding patterns present in each gel lane. The strength of the clusters obtained from the UPGMA analysis was based on cophenetic correlations, which are an estimate of the faithfulness of a grouping within a dendrogram,

with a score of 100 indicating that a grouping is extremely well supported.

### **DNA Sequencing and Phylogenetic Analysis**

Relevant DGGE bands were excised using a sterile scalpel and forceps and placed into 150  $\mu$ L of 10 mM Tris buffer. 0.1 mm Zirconia/Silica beads (BioSpec Products Inc., Bartlesville, OK) were added to each tube, and the samples were placed in a Fast Prep FP120 (Q-BIOgene) for 1 min at a speed of 5.5 m/s followed by overnight incubation at 4°C. Then, 2  $\mu$ L of the solution was PCR amplified using the primer set (substituting an identical forward primer without the 40-bp GC clamp), reaction mixture, and thermocycling conditions discussed above. The resultant PCR product was cloned into the pCR2.1-TOPO plasmid using a TA TOPO Cloning Kit (Invitrogen) according to manufacturer's specifications and sent to USDA-ARS MSA Genomics Laboratory (Stoneville, MS) for sequencing. The DGGE Band sequences were submitted to the BLASTn 2.2 search engine (Altschul et al., 1997) to obtain putative phylogenetic assignments for each band. The obtained sequences, combined with appropriate known 16S rDNA sequences from the GenBank database, were aligned using ClustalX 1.83 phylogenetic software package (Thompson et al., 1997). A 630-bp region of the alignment containing data from all sequences was selected for further phylogenetic studies. This alignment file was used to create bootstrapped ( $n = 1,000$ ) neighbor joining trees, which were visualized using Tree-View (Win32) 1.6.6 (<http://taxonomy.zoology.gla.ac.uk/rod/rod.html>).

A total of 12 sequences were submitted to the GenBank database and were assigned the accession numbers of EF158011-EF158022.

### **Principal Component Analysis**

Factor analysis as a multivariate statistical method is used to find a small number of factors from a data set of many correlated variables. Factor analysis is a useful tool for extracting latent information or variables (principal components) such as underlying, but not directly observable relationships between variables. Thus, PCA allows for the identification of groups of variables that are inter-related via phenomena that cannot be directly observed. This is accomplished by assuming that any observed (manifest) variables are correlated with a small number of underlying phenomena, which cannot be measured directly (latent variables). Factor analysis is based on the mathematical model of the reduced factor analytical solution (Pearson, 1901). The original data matrix is decomposed into the product of a matrix of factor loadings and a matrix of factor scores plus a residual matrix. The residual matrix contains the part of variance of the data set that cannot be explained by common factors (e.g., analytical uncertainties or feature-own variances). On the basis of the correlation matrix, orthogonal factors are ex-

**Table 1.** Average values of physical parameters in different regions of a poultry house<sup>1</sup>

Region	Litter temperature (°C)	pH	Moisture (%)	Air temperature (°C)	RH (%)
Front	32.2 ± 1.8	8.5 ± 0.1	35.1 ± 6.3	27.9 ± 0.3	86.8 ± 5.0
Midfront	31.5 ± 0.9	8.6 ± 0.1	31.6 ± 6.3	28.4 ± 0.2	88.0 ± 2.7
Midback	32.0 ± 0.8	8.6 ± 0.1	32.8 ± 9.8	29.6 ± 0.8	87.3 ± 1.3
Back	33.7 ± 1.0	8.4 ± 0.2	38.8 ± 4.8	29.8 ± 0.2	87.3 ± 0.5
Waterer	32.1 ± 1.2	8.2 ± 0.2	51.0 ± 4.7	29.3 ± 0.6	87.9 ± 1.8

<sup>1</sup>Means ± SD of at least 4 samples.

tracted solving an eigenvalue problem. In general, the number of extracted factors is less than the number of measured parameters. The dimensionality of the original data space can be decreased by means of factor analysis. After rotation of the factor-loading matrix, the factors can often be interpreted as origins or common sources (Harman, 1976; Kleinbaum et al., 1988). In this work, PCA were carried out on physicochemical factors (i.e., pH, air and litter temperature, litter moisture, and relative humidity) to determine the most important factor(s) affecting the spatial diversity of microbial community in poultry litters.

## RESULTS AND DISCUSSION

### Physical Parameters

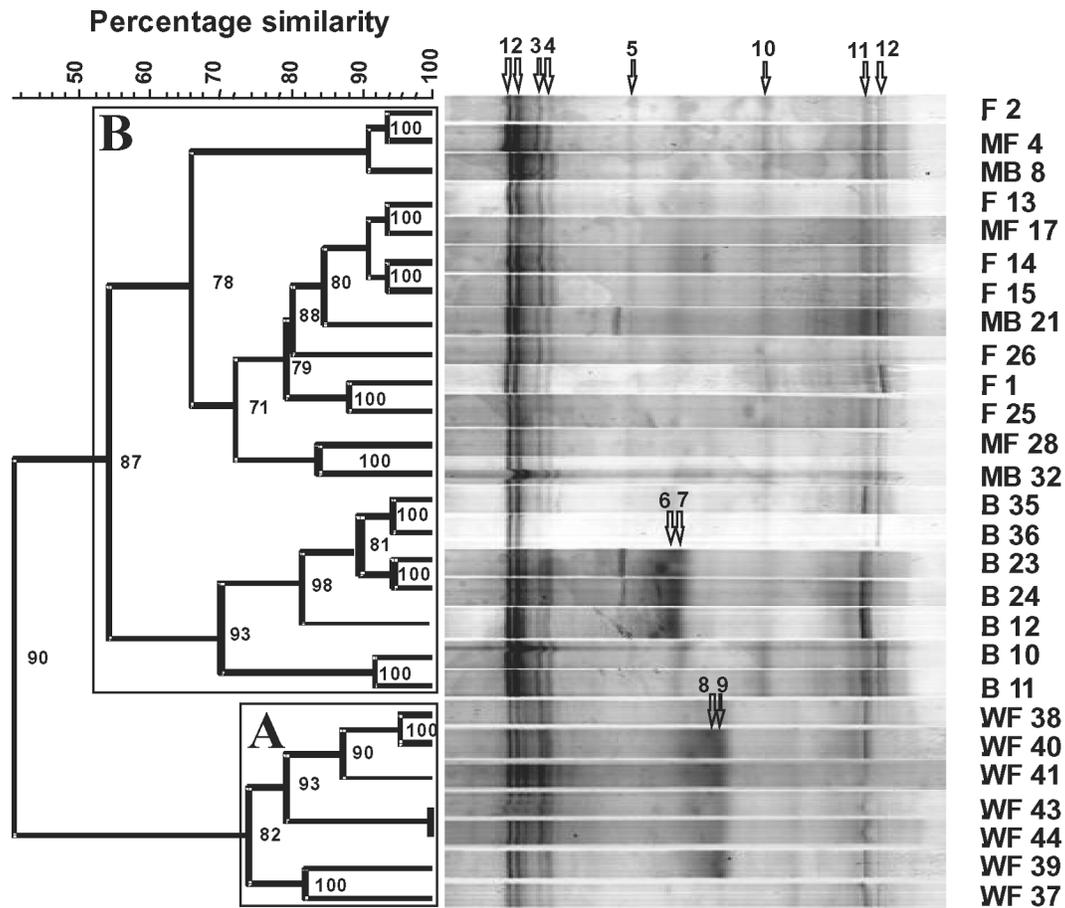
Physical parameters of litter samples (n = 44) from a commercial broiler house were determined and analyzed. These physical parameters consisted of litter temperature, pH, moisture, air temperature, and relative humidity. The results showed that the litter temperature ranged from 29.7 to 35.8°C, pH from 7.8 to 8.8, moisture from 25.5 to 58.4%, air temperature from 27.4 to 30.8°C, and relative humidity of 79.0 to 91.0%. These data were further broken down and categorized based on different regions (Figure 1) of the poultry house to see if any of these physical parameters would be factors affecting microbial diversity in poultry litter. We found that the litter temperature of the back areas (average temperature = 33.7°C;  $P < 0.05$ ) was higher than the other areas of the poultry house (Table 1). The waterer/feeder areas had the lowest average pH value of 8.2 ( $P < 0.05$ ) and the highest average moisture content at 51.0% ( $P < 0.05$ ; Table 1). All regions of the poultry house showed similar values for air temperature and relative humidity. The values for these physical parameters were similar to other findings (Gay et al., 2006; Miles et al., 2006).

### Bacterial 16S rDNA Analyses

Fingerprint analyses of DGGE banding patterns (Figure 2) revealed distinct poultry litter bacterial community structures within different areas of the poultry house. Using UPGMA analysis, the DGGE fingerprint patterns formed 2 major, well-supported (cophenetic correlation values  $\geq 82$ ) clusters, one containing all of the waterer/feeder samples (Figure 2, cluster A) and the second con-

taining the grid sampling sites (Figure 2, cluster B). Cluster A contained 2 unique DGGE bands (bands 8 and 9) not found in the rest of the poultry house, whereas 3 bands that were present in the DGGE fingerprints from the rest of the house (bands 5, 10, and 12), were absent. The waterer/feeder samples of cluster A also contained the highest number of distinctive bands as compared with fingerprints from the rest of the poultry house (cluster B), suggesting a more diverse bacterial community in the higher moisture and higher traffic areas. Cluster B DGGE fingerprints, which belong to the grid samples, consisted of 2 distinct subclusters. The first subcluster, which included samples only found at the back of the poultry house next to the exhaust fans (Figure 2, B samples), formed a very well supported cluster (cophenetic correlation value of 93). The second subcluster included samples taken from the front, middle-front, and middle-back areas of the poultry house (Figure 2), with no discernible clustering pattern based on position within the house between these grid samples. The DGGE analyses have been used previously to monitor the change in aquatic microbial communities affected by poultry litter runoff (Ringbauer et al., 2006) and to monitor changes in the poultry litter microbial communities during the fertilization process (Enticknap et al., 2006), but to our knowledge, this is the first study using DGGE to determine the spatial variability of the poultry litter microbial community within a single poultry house.

Sequences from prominent DGGE bands (Figure 2) were cloned and subjected to phylogenetic analysis. Alignment of cloned DGGE band sequences with those of organisms submitted to the GenBank database (Benson et al., 2005) showed that the DGGE band sequences clustered with 2 major phylogenetic groups, encompassing both low-GC (*Bacillales* and *Lactobacillales*) and high-GC (*Actinomycetales*) gram-positive bacteria (Figure 3). Our sequence analyses data are similar to those of Lu et al. (2003b), in which they reported that low-GC gram positive bacterial groups made up of about 62% of total 16S rDNA clones from wood-shaving poultry litter samples compared with 67% for our findings. In addition, the authors found that high-GC gram-positive bacteria comprised about 25% of the total clones compared with 33% for our study. The 3 major phylogenetic groups (*Bacillales*, *Lactobacillales*, and *Actinomycetes*) found in this study also corroborated well with other broiler chicken intestinal (Lu et al., 2003b) and litter (Martin et al., 1998; Fries et al., 2005, Enticknap et al., 2006) studies. No sequences



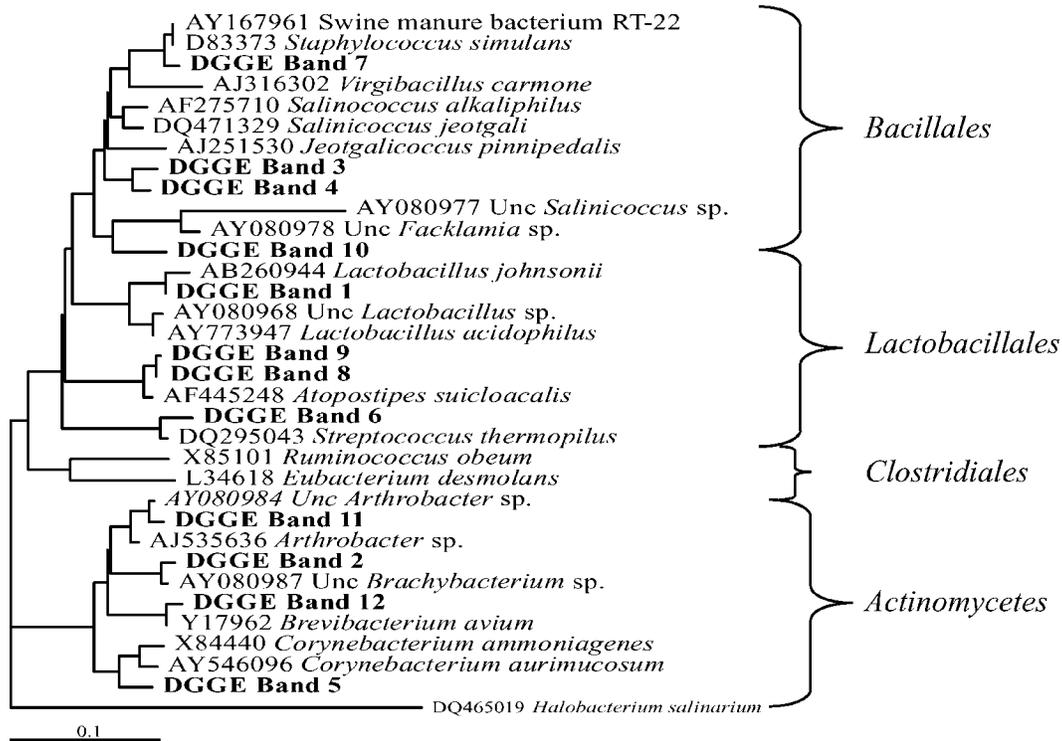
**Figure 2.** The 16S ribosomal DNA denaturing gradient gel electrophoresis (DGGE) bacterial community fingerprint analyses. A dendrogram representing the percent similarity of banding patterns based on unweighted pair group method with arithmetic mean cluster analysis are shown to the left of the DGGE image. The numbers at the nodes of the dendrogram represent the cophenetic correlations, which is an estimate of the reproducibility of each subcluster. The DGGE image represents a 30 to 60% denaturant gradient with sample ID shown to the right of the DGGE image. The arrows represent DGGE bands that were excised from the gel and sequenced for identification. F = front; MF = middle front; MB = middle back; B = back; WF = waterer/feeder.

from prominent DGGE bands were found to be similar to gram-negative bacteria, which was not surprising because gram-positive bacteria are known to dominant poultry litter communities, with gram-negative bacteria accounting for between 13% (Lu et al., 2003b, Fries et al., 2005) to 0% (Martin et al., 1998; Enticknap et al., 2006) of the bacterial community from fresh litter. Pathogenic strains of bacteria found in previous studies of broiler litter, including *Bordetella* sp., *Clostridium* sp. (Lu et al., 2003b), and *Campylobacter* spp. (Bull et al., 2006), were not found in the sequence analysis of our DGGE bands.

We found DGGE band sequences matching *Actinomyces* from sampling sites throughout the poultry house. The nearest match in the GenBank database to sequences from DGGE band 2, band 5, band 11, and band 12 were similar to *Brachybacterium* sp. (99% similar), *Corynebacterium* sp. (96% similar), *Arthrobacter* sp. (98% similar), and *Brevibacterium* sp. (99% similar), respectively (Figure 3). Of these 4 DGGE bands, *Brachybacterium* sp. and *Arthrobacter* sp. were found to be dominant community members in all poultry litter samples (Figure 2, band 2 and band 11). This is understandable because *Actinomycetes* are ubiquitous, especially in soil systems (Madigan et al.,

1997) and have been found to be prevalent components in poultry litter (Martin et al., 1998, Lu et al., 2003b, Fries et al., 2005). These bacteria are rod-shaped to filamentous, aerobic, and generally nonmotile in the vegetative phase. They have been known to decompose woods (Fu and Thayer, 1975; Thayer, 1976), chitins (Reguera and Leschine, 2001), herbicides, caffeine, nicotine, phenol, and other unusual organic compounds (Atlas and Bartha, 1981; Madigan et al., 1997). The main genera of these *Actinomycetes* are *Corynebacterium* and *Arthrobacter*, both of which are known to be resistant to desiccation and starvation even though they do not form spores or other resting cells (Madigan et al., 1997). Specifically, *Arthrobacter* is a heterogeneous group that has considerable nutritional versatility. Thus, it is not surprising that these *Actinomycetes* should survive well in wood-shaving poultry litter environment.

Three of the 5 dominant bands found in all of the poultry samples contained sequences matching low-GC gram-positive bacteria (*Lactobacillales* and *Bacillales*) with 1 matching 99% to *Lactobacillus* sp. (Figure 2, band 1) and the other 2 matching 97% to *Salinococcus* sp. (Figure 2, band 3 and band 4). These 2 low-GC genera have been



**Figure 3.** Neighbor-joining tree showing the phylogenetic relationship between denaturing gradient gel electrophoresis (DGGE) band sequences (red text) to 16S ribosomal DNA sequences retrieved from the GenBank database (accession numbers in parentheses). The tree represents the alignment of a 630-bp region present in all DGGE band and GenBank sequences. The major phylogenetic groups found in this study are indicated along the right side of the figure. An archaea was used as an outgroup [*Halobacterium salinarium* (DQ465019)].

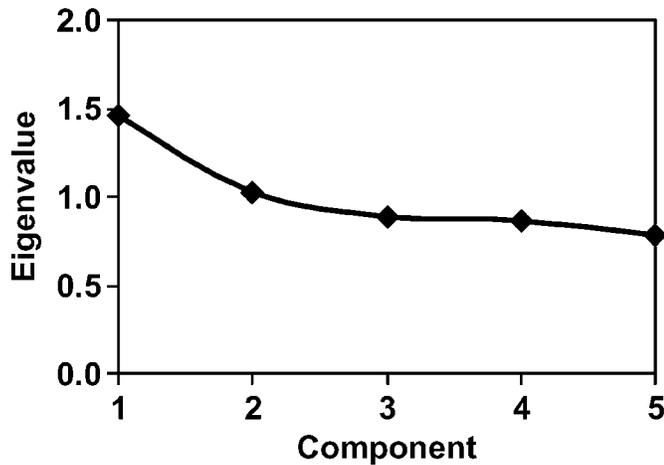
previously found to dominate poultry ileum (Lu et al., 2003a) and litter (Lu et al., 2003b) bacterial communities. *Lactobacillales* tended to predominate in the waterer/feeder areas, possessing 2 unique bands (Figure 2, band 8 and 9) that were not found throughout the rest of the poultry house. Sequences from these 2 bands were found to be 99% similar to *Atopostipes suicloacalis* strains (Figure 3), a nonspore-forming facultatively anaerobic organism isolated from an underground swine manure storage pit (Cotta et al., 2004). These waterer/feeder areas are considered to have high moisture content (average about 50 vs. 30% for the rest of the poultry house) and low pH (average 8.2 vs. 8.4 to 8.6 for the rest of the house; Table 1). *Lactobacillales*, also called lactic acid bacteria based on their ability to produce lactic acid via fermentation (Madigan et al., 1997), are typically more resistant to acidic conditions and being able to grow well at low pH (around 4 to 5). Even though the pH of the waterer/feeder area litter was much higher than the optimum pH for growth of *Lactobacillales*, it has been reported that acidic conditions (pH = 5.5) can occur in waterer/feeder areas (Miles et al., 2006), which would be conducive to the dominance of these lactic acid bacteria within the litter bacterial communities.

A second unique set of DGGE bands (Figure 2, band 6 and 7) were present only in grid samples located at the back of the poultry house, where poultry litters are more dry and compact. Sequences from these bands identified them as low-GC gram positive bacteria, matching 97% to

*Streptococcus thermophilus* (band 6) and 99% to *Staphylococcus* sp. (band 7) strain isolated from swine manure (Whitehead and Cotta, 2004; Figure 3). The presence of sequences matching these 2 organisms was not unexpected, given the fact that they have both been shown to constitute a minor portion of the poultry ileum (Lu et al., 2003a) and litter (Lu et al., 2003b) bacterial communities. The back areas of the poultry house appear to have the highest litter temperatures (average 33.5 vs. 31.7°C for the rest of the house) and lower moisture content, roughly 35% (Table 1). Both streptococci and staphylococci are facultative anaerobes that are able to produce lactic acid from lactose. In particular, staphylococci are relatively resistant to reduced water potential and tolerate dry and high salt conditions fairly well (Madigan et al., 1997). These conditions were observed in this study where the sodium concentrations from the poultry litters range from 11,000 to 16,000 mg per kg of poultry litter.

### Principal Component Analysis of Physicochemical Parameters

Based on the fingerprint analysis (Figure 2), different bandings were observed for different areas of the poultry house. Therefore, PCA was carried out to extract the most important physical parameters affecting the diversity of the microbial community as observed from the DGGE bands. These physical parameters include the relative humidity, air temperature, litter temperature, pH, and mois-



**Figure 4.** Eigenvalues scree plot for determining principal components for further analysis.

ture content of the poultry litters ( $n = 27$ ). Statistica 7.0 (Statsoft, Tulsa, OK) was used to carry out principal component analysis to determine the main principal components from the original variables (Muller et al., 2001; Ogino et al., 2001; Van Der Gucht et al., 2001; Yang et al., 2001). Based on the eigenvalues scree plot (Figure 4), the original 5 physical parameters were reduced to 2 main factors (factor 1 and factor 2) from the leveling-off point(s) in the scree plot as suggested by Cattell (1966). The factor corresponding to the largest eigen value (1.46) accounts for approximately 51.5% of the total variance. The second factor corresponding to the second eigenvalue (1.02) accounts for approximately 31.5% of the total variance. The remaining 3 factors have eigenvalues of less than unity. The scree plot agrees well with the Kaiser criterion (Kaiser, 1960) where factors with an eigenvalue greater than unity would be retained for further analysis (in this case, 2 principal components were retained). Further analysis of factor loadings showed that moisture content and litter temperature were the 2 major factors affecting the diversity of the microbial community (Table 2). For factor 1, moisture has the highest factor loading value (0.903), which shows that moisture is the most influential variable for the first factor or principal component. For factor 2, litter temperature has the highest factor loading value (0.784), and pH is a second influential variable with factor loading value of 0.521. Factor loadings can be interpreted as the correlation between the factors and the variables (physical parameters).

**Table 2.** Results of factor loading analysis to determine correlation between factors and variables

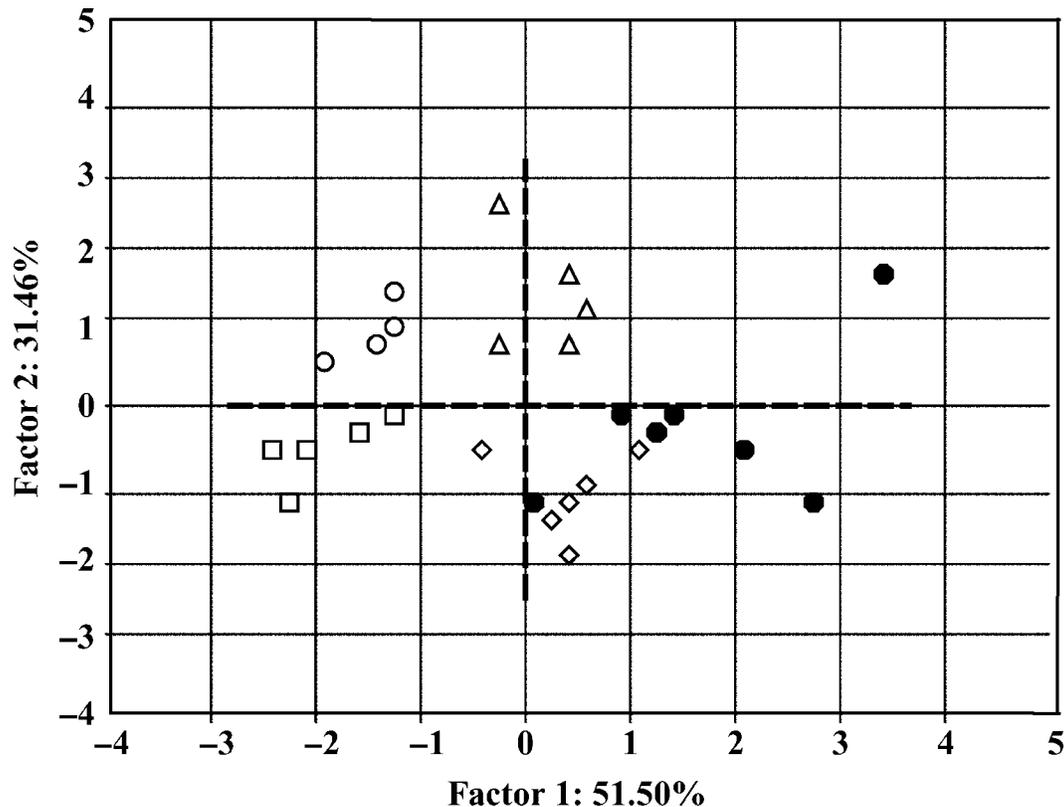
Variable	Factor 1	Factor 2
Litter temperature, °C	-0.582	0.784
RH, %	-0.142	0.326
Air temperature, °C	-0.068	-0.035
pH	-0.405	0.521
Moisture, %	0.903	-0.321

To determine which sampling points were closely related, a plot of factor coordinates for all observations (cases) was constructed using the factors obtained from factor loading analysis. Figure 5 shows the cluster of sampling points (as affected by all 5 physical parameters). The cases (i.e., sampling points) that are clustered near each other have similar characteristics with respect to the factors. As can be seen from Figure 5, there are 5 distinct clusters when projecting all the cases ( $n = 27$  sampling points) onto the factor plane. The middle front and middle back clusters are located toward the negative (left) side of this dimension—toward litter temperature, whereas the back and waterer/feeder clusters are located on the positive (right) side of the dimension—toward moisture content. Thus, it appears that litter temperature plays a major role in shaping the diversity of the microbial community toward the middle of the poultry house. Moisture, on the other hand, appears to play a major role in the microbial community structure in the back and waterer/feeder areas. The front cluster is more affected by moisture than litter temperature (e.g., sampling points from the front cluster are located on both sides of the dimension). There seems to be an overlap between the waterer/feeder cluster and the back cluster. Previous studies have shown that these areas exhibit similar physical properties (e.g., high moisture content, compacted litter layer that leads to caking; Gates et al., 1997; Miles et al., 2006). These areas contain the most diverse microbial community as demonstrated by the DNA fingerprinting analysis.

## Conclusions

At the end of a growout during summer flock, poultry litter samples ( $n = 44$ ) were collected and DNA was extracted from a subset of these samples ( $n = 27$ ). The DGGE and PCA were carried out to learn more about microbial diversity and factors that affect their spatial diversity in the poultry house. Litter properties and microbial diversity were observed to vary from 1 region of the poultry house to the next. Based on the DNA fingerprinting analysis, 3 distinct regions exhibit similar banding patterns. The waterer/feeder areas tend to be more diverse (more bands in the DGGE analysis) than the other regions of the house. Similar clustering was also observed from PCA analyses. However, the waterer/feeder cluster appeared to overlap with the cluster in the back of the poultry house. This overlap was readily explained by their similarity in physical conditions (e.g., more moisture and more compacted litter). The major environmental factors affecting microbial diversity in this particular poultry house appear to be moisture content, pH, and litter temperature based on PCA analyses. Based on the PCA and sequencing analyses, the environmental conditions (i.e., pH, temperature, and moisture content) dictate the proliferation of distinct microbial communities within different areas of the poultry house.

Based on these analyses, it is clear that microbial diversity does exist at a microscale (i.e., within different regions



**Figure 5.** Principal component analysis of physical parameters affecting microbial community structure using Varimax raw option in Statistica, along with projection of cases (sampling points) onto the factor plane. Note: Cases or sampling points that are close to one another exhibit similar attributes.  $\Delta$ - Front samples;  $\circ$  - Middle front samples;  $\square$ - Middle back samples;  $\diamond$  - Back samples;  $\bullet$ - waterer/feeder samples.

of a poultry house). Environmental conditions vary at each location within the poultry house. These changes in environmental conditions do have a major effect on microbial dynamics, even at the microscale level. Many studies have shown that spatial scaling of microbial diversity does exist at the scale of a few centimeters (Harte et al., 1999; Green et al., 2004; Horner-Devine et al., 2004). For instance, Horner-Devine et al. (2004) found the existence of a taxa-area relationship for microorganisms in salt marsh sediments over an area of a few centimeters.

Although PCA may be a good tool in determining microbial diversity based on factor coordinates analysis, DGGE analysis is also necessary to effectively study spatial shifts in microbial diversity at the microscale level in a poultry house. The results from these microscale levels of analyses (e.g., understanding the major factors affecting microbial diversity) are a necessary first step in applying macroscale remediation options to reduce toxic air emissions and pathogenic incidence, whether on zone litter treatment or whole house treatment. Therefore, more experiments are needed to determine what fraction, if any, of these microorganisms is responsible for toxic air emission such as ammonia production or pathogenic incidence such as *Salmonella* or *Campylobacter*.

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## REFERENCES

- Acea, M. J., and T. Carballas. 1988a. Effects of cattle-slurry treatment on microorganisms of the carbon and sulphur cycles in the soil. *Biol. Wastes* 24:251–258.
- Acea, M. J., and T. Carballas. 1988b. The influence of cattle slurry on soil microbial population and nitrogen cycle microorganisms. *Biol. Wastes* 23:229–241.
- Altschul, S. F., T. L. Madden, A. A. Schaffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman. 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res.* 25:3389–3402.
- Atlas, R. M., and R. Bartha. 1981. *Microbial Ecology: Fundamentals and Applications*. Addison-Wesley Publ. Co., London, UK.
- Benson, D. A., I. Karsch-Mizrachi, D. J. Lipman, J. Ostell, and D. L. Wheeler. 2005. GenBank. *Nucleic Acids Res.* 33:D34–D37.
- Bull, S. A., V. M. Allen, G. Domingue, F. Jørgensen, J. A. Frost, R. Ure, R. Whyte, D. Tinker, J. E. L. Corry, J. Gillard-King, and T. J. Humphrey. 2006. Sources of *Campylobacter* spp. Colonizing housed broiler flocks during rearing. *Appl. Environ. Microbiol.* 72:645–652.
- Casamayor, E. O., H. Schäfer, L. Bañeras, C. Pedrós-Alió, and G. Muyzer. 2000. Identification of and spatio-temporal differ-

- ences between microbial assemblages from two neighboring sulfurous lakes: Comparison by microscopy and denaturing gradient gel electrophoresis. *Appl. Environ. Microbiol.* 66:499–508.
- Cattell, R. B. 1966. The scree test for the number of factors. *Multivariate Behav. Res.* 1:245–276.
- Chen, T. H., D. L. Day, and M. P. Steinberg. 1988. Methane production from fresh versus dry dairy manure. *Biol. Wastes* 24:297–306.
- Corominas, S., F. Perestelo, M. L. Perez, and M. A. Falcon. 1987. Microorganisms and environmental factors in composting of agricultural waste of the Canary Islands. Pages 127–134 in *Compost: Production, Quality and Use*. M. De Bertoldi, M. P. Ferranti, P. L'Hermite, and F. Zucchini, ed. Elsevier Appl. Sci., London, UK.
- Cotta, M. A., T. R. Whitehead, M. D. Collins, and P. A. Lawson. 2004. *Atopostipes suicloacale* gen. nov., sp. nov., isolated from an underground swine manure storage pit. *Anaerobe* 10:191–195.
- De Bertoldi, M., M. P. Ferranti, P. L'Hermite, and F. Zucchini, ed. 1987. *Compost: Production, Quality and Use*. Elsevier Appl. Sci., London, UK.
- Diaz-Fierros, F., M. C. Villar, M. Carballas, M. C. Leiros, T. Carballas, and A. Cabaneiro. 1988. Effect of cattle slurry fractions on nitrogen mineralization in soil. *J. Agric. Sci. Camb.* 110:491–497.
- Donham, K. J. 1990. Relationships of air quality and productivity in intensive swine housing. *J. Agric. Practice* 10:15–18.
- Donham, K. J., and K. E. Gustafson. 1982. Human occupational hazards from swine confinement. *Ann. Am. Conf. Gov. Hyg.* 2:137–142.
- Donham, K. J., M. J. Rubino, T. D. Thedell, and J. Kammermeyer. 1977. Potential health hazards to agricultural workers in swine confinement buildings. *J. Occup. Med.* 19:383–387.
- El-Ashry, M. A., H. M. Khattab, A. El-Serafy, H. Solimam, and S. M. Abd Elmoula. 1987. Nutritive value of poultry wastes for sheep. *Biol. Wastes* 19:287–298.
- Enticknap, J. J., H. Nonogaki, A. R. Place, and R. T. Hill. 2006. Microbial diversity associated with odor modification for production of fertilizers from chicken litter. *Appl. Environ. Microbiol.* 72:4105–4114.
- Finstein, M. S., and M. L. Morris. 1975. Microbiology of municipal solid wastes composting. *Adv. Appl. Microbiol.* 19:113–151.
- Fries, R., M. Akcan, N. Bandick, and A. Kobe. 2005. Microflora of two different types of poultry litter. *Br. Poult. Sci.* 46:668–672.
- Fu, T. T., and D. W. Thayer. 1975. Comparison of batch and semicontinuous cultures for production of protein from mesquite wood by *Brevibacterium* sp. JM98A. *Biotechnol. Bioeng.* 17:1749–1760.
- Gates, R. S., J. L. Taraba, N. S. Ferguson, and L. W. Turner. 1997. A technique for determining ammonia equilibrium and volatilization from broiler litter. Presented August 10–14 at the 1997 ASAE Annu. Int. Meet. Paper no. 974074. ASAE, St. Joseph, MI.
- Gay, S. W., E. F. Wheeler, J. L. Zajackowski, and P. A. Topper. 2006. Ammonia emissions from U.S. tom turkey growout and brooder houses under cold weather minimum ventilation. *Appl. Eng. Agric.* 22:127–134.
- Green, J. L., A. J. Holmes, M. Westoby, I. Oliver, D. Briscoe, M. Dangerfield, M. Gillings, and A. J. Beattie. 2004. Spatial scaling of microbial eukaryote diversity. *Nature* 432:747–750.
- Harman, H. H. 1976. *Modern factor analysis*. Univ. Chicago Press, Chicago, IL.
- Harms, G., A. C. Layton, H. M. Dionisi, I. R. Gregory, V. M. Garrett, S. A. Hawkins, K. G. Robinson, and G. S. Sayler. 2003. Real-time PCR quantification of nitrifying bacteria in a municipal wastewater treatment plant. *Environ. Sci. Technol.* 37:343–351.
- Harte, J., S. McCarthy, K. Taylor, A. Kinzig, and M. L. Fischer. 1999. Estimating species-area relationships from plot to landscape scale using species spatial-turnover data. *Oikos* 86:45–54.
- Hartung, J., and V. R. Phillips. 1994. Control of gaseous emissions from livestock buildings and manure stores. *J. Agric. Eng. Res.* 57:173–189.
- Horner-Devine, M. C., M. Lage, J. B. Hughes, and B. J. M. Bohannan. 2004. A taxa-area relationship for bacteria. *Nature* 432:750–753.
- Jeffrey, J. S., J. H. Kirk, E. R. Atwill, and J. S. Cullor. 1998. Prevalence of selected microbial pathogens in processed poultry waste used as dairy cattle feed. *Poult. Sci.* 77:808–811.
- Kaiser, H. F. 1960. The application of electronic computers to factor analysis. *Educ. Psychol. Meas.* 20:141–151.
- Kleinbaum, D. G., L. Kupper, and K. E. Muller. 1988. Variable reduction and factor analysis. Pages 595–641 in *Applied Regression Analysis and Other Multivariable Methods*. M. Payne, ed. Duxbury Press, Belmont, CA.
- Lauer, D. A., D. R. Bouldin, and S. D. Klaususner. 1976. Ammonia volatilization from dairy manure spread on the soil surface. *J. Environ. Qual.* 5:131–141.
- Lovett, J., J. W. Messer, and R. B. Read Jr. 1971. The microflora of southern Ohio poultry litter. *Poult. Sci.* 50:746–751.
- Lu, J., U. Idris, B. Harmon, C. Hofacre, J. J. Maurer, and M. D. Lee. 2003a. Diversity and succession of the intestinal bacterial community of the maturing broiler chicken. *Appl. Environ. Microbiol.* 69:6816–6824.
- Lu, J., S. Sanchez, C. Hofacre, J. J. Maurer, B. G. Harmon, and M. D. Lee. 2003b. Evaluation of broiler litter with reference to the microbial composition as assessed by using 16S rRNA and functional gene markers. *Appl. Environ. Microbiol.* 69:901–908.
- Madigan, M. T., J. M. Martinko, and J. Parker. 1997. *Brock Biology of Microorganisms*. 8th ed. Prentice Hall, Upper Saddle River, NJ.
- Martin, S. A., M. A. McCann, and W. D. Waltman II. 1998. Microbiological survey of Georgia poultry litter. *J. Appl. Poult. Res.* 7:90–98.
- Miles, D. M., D. E. Rowe, and P. R. Owens. 2006. Winter broiler litter gases and nitrogen compounds. Temporal and spatial trends. *Environ.* doi:10.1016/j.atmosenv.2006.11.056
- Muller, A. K., K. Westergaard, S. Christensen, and S. J. Sorensen. 2001. The effect of long-term mercury pollution on the soil microbial community. *FEMS Microbiol. Ecol.* 36:11–19.
- Nodar, R., M. J. Acea, and T. Carballas. 1990. Microbial populations of poultry pine-sawdust litter. *Biol. Wastes* 33:295–306.
- Nubel, U., F. Garcia-Pichel, and G. Muyzer. 1997. PCR primers to amplify 16S rRNA genes from Cyanobacteria. *Appl. Environ. Microbiol.* 63:3327–3332.
- Ogino, A., H. Koshikawa, T. Nakahara, and H. Uchiyama. 2001. Succession of microbial communities during a biostimulation process as evaluated by DGGE and clone library analysis. *J. Appl. Microbiol.* 91:625–635.
- Pain, B. F., R. B. Thompson, L. C. N. De La Cremer, and L. Ten Holte. 1987. The use of additives in livestock slurries to improve their flow properties, conserve nitrogen and reduce odours. Pages 229–246 in *Development in Plant Soil Sciences. Animal Manure on Grassland and Fodder Crops. Fertiliser or Waste? H. G. Van der Meer, R. J. Unwin, T. A. Van Dijk, and G. C. Ennik, ed. Martius Nijhoff Publ., Dordrecht, the Netherlands.*
- Pearson, K. 1901. On lines and planes of closest fit to systems of points in space. *Philos. Mag.* 2:559–572.
- Reguera, G., and S. B. Leschine. 2001. Chitin degradation by cellulolytic anaerobes and facultative aerobes from soils and sediments. *FEMS Microbiol. Lett.* 204:367–374.
- Ringbauer, J. A., Jr., J. B. James, and F. J. Genthner. 2006. Effects of large-scale poultry farms on aquatic microbial communities: A molecular investigation. *J. Water Health* 4:77–86.
- Smith, L. W. 1974. Dehydrated poultry excreta as a crude protein supplement for ruminants. *World Anim. Rev* 1974:6–11.

- Stuven, R., and E. Bock. 2001. Nitrification and denitrification as a source for NO and NO<sub>2</sub> production in high-strength wastewater. *Water Res.* 35:1905–1914.
- Tate, R. L., III. 1987. Ecosystem management and soil organic matter nivel. Pages 260–280 in *Soil Organic Matter*. John Wiley, New York, NY.
- Thaxton, Y. V., C. L. Balzli, and J. D. Tankson. 2003. Relationship of broiler flock numbers to litter microflora. *J. Appl. Poult. Res.* 12:81–84.
- Thayer, D. W. 1976. Facultative wood-digesting bacteria from the hind-gut of the termite *Reticulitermes hesperus*. *J. Gen. Microbiol.* 95:287–296.
- Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins. 1997. The ClustalX windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 24:4876–4882.
- Van Der Gucht, K., K. Sabbe, L. de Meester, N. Vloemans, G. Zwart, M. Gillis, and W. Vyverman. 2001. Contrasting bacterioplankton community composition and seasonal dynamics in two neighbouring hypertrophic freshwater lakes. *Environ. Microbiol.* 3:680–690.
- Whitehead, T. R., and M. A. Cotta. 2004. Isolation and identification of hyper-ammonia producing bacteria from swine manure storage pits. *Curr. Microbiol.* 48:20–26.
- Yang, C. H., D. E. Crowley, and J. A. Menge. 2001. 16S rDNA fingerprinting of rhizosphere bacterial communities associated with healthy and *Phytophthora* infected avocado roots. *FEMS Microbiol. Ecol.* 35:129–136.