Journal of Occupational and Environmental Hygiene
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713657996

Culture-Independent Characterization of Bacteria and Fungi in a Poultry Bioaerosol Using Pyrosequencing: A New Approach

M. W. Nonnenmann[a]; B. Bextine[b]; S. E. Dowd[c]; K. Gilmore[a]; J. L. Levin[a]
[a] Department of Occupational Health Sciences, University of Texas Health Science Center at Tyler, Tyler, Texas [b] Department of Biology, University of Texas at Tyler, Tyler, Texas [c] Pathogen Research Laboratory, Lubbock, Texas

First published on: 05 November 2010

To cite this Article Nonnenmann, M. W. , Bextine, B. , Dowd, S. E. , Gilmore, K. and Levin, J. L.(2010) 'Culture-Independent Characterization of Bacteria and Fungi in a Poultry Bioaerosol Using Pyrosequencing: A New Approach', Journal of Occupational and Environmental Hygiene, 7: 12, 693 — 699, First published on: 05 November 2010 (iFirst)
To link to this Article: DOI: 10.1080/15459624.2010.526893
URL: http://dx.doi.org/10.1080/15459624.2010.526893

Journal of Occupational and Environmental Hygiene, 7: 693–699
ISSN: 1545-9624 print / 1545-9632 online

DOI: 10.1080/15459624.2010.526893

Culture-Independent Characterization of Bacteria and Fungi in a Poultry Bioaerosol Using Pyrosequencing: A New Approach

M.W. Nonnenmann,[1] B. Bextine,[2] S.E. Dowd,[3] K. Gilmore,[1] and J.L. Levin[1]

[1]Department of Occupational Health Sciences, University of Texas Health Science Center at Tyler, Tyler, Texas
[2]Department of Biology, University of Texas at Tyler, Tyler, Texas
[3]Pathogen Research Laboratory, Lubbock, Texas

Work in animal production facilities often results in exposure to organic dusts. Previous studies have documented decreases in pulmonary function and lung inflammation among workers exposed to organic dust in the poultry industry. Bacteria and fungi have been reported as components of the organic dust produced in poultry facilities. To date, little is known about the diversity and concentration of bacteria and fungi inside poultry buildings. All previous investigations have utilized culture-based methods for analysis that identify only biota cultured on selected media. The bacterial tag-encoded flexible (FLX) amplicon pyrosequencing (bTEFAP) and fungal tag-encoded flexible (FLX) amplicon pyrosequencing (fTEFAP) are modern and comprehensive approaches for determining biodiversity of microorganisms and have not previously been used to provide characterization of exposure to microorganisms in an occupational environment. This article illustrates the potential application of this novel technique in occupational exposure assessment as well as other settings. An 8-hr area sample was collected using an Institute of Medicine inhalable sampler attached to a mannequin in a poultry confinement building. The sample was analyzed using bTEFAP and fTEFAP. Of the bacteria and fungi detected, 116 and 39 genera were identified, respectively. Among bacteria, Staphylococcus cohnii *was present in the highest proportion (23%). The total inhalable bacteria concentration was estimated to be 7503 cells/m^3. Among the fungi identified,* Sagenomella sclerotialis *was present in the highest proportion (37%).* Aspergillus ochraceus *and* Penicillium janthinellum *were also present in high proportions. The total inhalable fungi concentration was estimated to be 1810 cells/m^3. These estimates are lower than what has been reported by others using standard epifluorescence microscope methods. However, no study has used non-culture-based techniques, such as bTEFAP and fTEFAP, to evaluate bacteria and fungi in the inhalable fraction of a bioaerosol in a broiler production environment. Furthermore, the impact of this bTEFAP and fTEFAP technology has yet to be realized by the scientific community dedicated to evaluating occupational and environmental bioaerosol exposure.*

Keywords *Aspergillus ochraceus*, bioaerosol, organic dust, poultry, pyrosequencing

Address correspondence to: M.W. Nonnenmann, University of Texas Health Science Center at Tyler, Department of Occupational Health Sciences, 11837 U.S. Highway 271, Tyler, TX 75708-3154.

INTRODUCTION

Organic dusts are often present in confined poultry production work environments.[1–3] Components of organic dust are highly diverse and generally depend on the type of agricultural production present on the farm. A few studies have begun to look at the characteristics of these dusts in poultry production.[3–6]

As biotechnology continues to advance, new tools emerge to better characterize components of organic dust. For example, determining the concentration of bacteria and fungi present in organic dusts has largely been limited to culture-based techniques.[7,8] However, culture-based techniques contain an inherent bias, as only the viable microorganisms that can be grown in culture are identified. Furthermore, the majority of microorganisms cannot be cultured using standard techniques.[9–11] This culture bias may overestimate the importance of microorganisms that are easily cultured through standard techniques (e.g., *Escherichia coli*). Furthermore, non-culturable methods have shown airborne bacterial counts to be much higher than those estimated by culture-based methods.[12,13]

Ribosomal DNA (16s) is a universal molecule that is part of the bacterial or prokaryotic ribosome.[14] A similar ribosomal structure (18s) has also been used for phylogenetic analysis of eukaryotic organisms (e.g., fungi).[15] The genes have areas of evolutionary conservation as well as regions of hypervariability that can be utilized to identify bacteria, archaea, and fungi.[16] Genetic analysis of these molecules has become a standard technique for bacterial phylogenetic analysis.[17] Therefore, these stable or "highly conserved"

structures can be used to identify individual genera or species of bacteria, archaea, and fungi from an environmental sample that may contain a diverse mixture of these organisms.

To date, one study[6] has utilized non-culture-based methods such as real-time quantitative polymerase chain reaction (Q-PCR), targeting ribosomal DNA to genetically identify and/or calculate total airborne concentrations of microorganisms from a specific genus (e.g., *Staphylococcus*). Specifically, Oppliger and colleagues evaluated the inhalation exposure (i.e., cells/m^3) to the *Staphylococcus* genus using non-culture-based Q-PCR methods. Clearly, additional genetic work is needed to characterize the biodiversity of microorganisms in these environments.

A bioaerosol exposure assessment working group has reported on the strengths of Q-PCR to identify microorganisms in bioaerosols. This group also identified an inherent weakness of using Q-PCR for analyzing the biodiversity of inhalation exposure of bioaerosols. Specifically, Q-PCR is typically limited by the use of highly specialized primers to identify specific bacteria in the sampled air.[18] Therefore, a sequencing method that can better characterize and quantify diverse bioaerosols may prove useful for measuring occupational and environmental bioaerosol exposure.

Recently a new, fast, low-cost pyrosequencing technology has emerged that has revolutionized DNA sequencing. This pyrosequencing technology has expanded sequencing capabilities and reduced the time of analysis and cost compared with previous Sanger methods.[19] The bacterial and fungal tag-encoded flexible (FLX) amplicon pyrosequencing methods (bTEFAP and fTEFAP) are pyrosequencing methods developed to classify microorganisms in complex environments[20–29] and have quickly become a useful method for determining microbial ecology. The DNA in the sample is sequenced using universal primers to target the specific ribosomal DNA of bacteria or fungi.

Furthermore, pyrosequencing allows for the characterization of the relative percentages and species of bacteria or fungi present in the environmental sample. This technology allows both the identification of bacteria and fungi with a high level of precision (i.e., $\geq$95%) at the genus level as well as quantification of a proportion at the species level. Furthermore, with this technology, airborne concentrations for both the culturable and non-culturable microorganisms can be determined simultaneously, as each microorganism contains only one copy of the ribosomal DNA gene sequence. This capability has not been used to evaluate inhalable bioaerosols in the community or work environment. Therefore, this study will demonstrate the utility of this pyrosequencing technology to identify and estimate concentrations of bacteria and fungi in the inhalable fraction of a bioaerosol in a broiler chicken production facility by sequencing ribosomal DNA. This facility was chosen to demonstrate the pyrosequencing method, as limited information exists describing bioaerosols present in poultry production. In addition, inhalation exposure to bioaerosols in a poultry production facility may have implications for worker health.

MATERIALS AND METHODS

Sampling Site, Collection, and Preparation

Sampling was completed at the Broiler Research Center at the Walter C. Todd Agricultural Research Center of Stephen F. Austin State University, Nacogdoches, Texas. The room where the sampling took place had the following dimensions: 13.1 m by 152.4 m and 3.7 m high. The broiler chickens were 14 days old, and the room contained approximately 27,000 chickens, or 13.5 birds/m^2. Twelve ventilation fans, each with a diameter of 1.32 m, were located in this room. Of these 12 fans, 10 were exhaust fans and two were supply air fans. The 10 exhaust fans were located at the west end of the room (6 on the end wall and 4 on the side walls). The two remaining supply air fans were located on the east wall. Twenty-nine fresh air inlets, each measuring 1.2 m by 0.18 m, were located on the side walls. The supply air vents had hinged internal flaps that opened when exhaust fans were operating due to the negative air pressure in the room. The ventilation system was designed to maintain the room temperature and relative humidity at 28°C, and 74%, respectively, for this age of broiler chicken. The floor was covered with pine shavings and was tilled and leveled prior to the introduction of the current flock.

The sample was collected in December, during the day, over an 8-hr period, at the center of the building away from possible ventilation sources. An Institute of Occupational Medicine (IOM; SKC Inc., Eighty Four, Pa.) inhalable sampler was loaded with a 25-mm, sterile, gelatin membrane filter with a pore size of 3 μm (SKC Inc.) and attached to a mannequin at a height of 1.5 m above the floor to simulate an occupational exposure. The mannequin was rotated 90° every 30 min to reduce the effect of incomplete mixing of replacement air. The sampling apparatus was connected to a personal sampling pump (model 210-5000, SKC Inc.). The flow rate was set at 2 L/min and calibrated using a primary standard airflow device (DryCal Lite; Bios International, Butler, N.J.) before and after sampling. After collection, the sample was stored on ice packs and transported to the lab. The filter was removed from the IOM cassette and placed in a 50-mL sterile polypropylene centrifuge tube with 10 mL of filtered water and vortexed for 30 sec. The sample was then stored at –20° C for 3 days until being shipped overnight to a pyrosequencing laboratory. A blank sample of filtered water was also sent for background analysis.

DNA Extraction

The sample was thawed and centrifuged at 14,000 rpm for 30 sec and resuspended in 500 μL Buffer RLT (Qiagen, Valencia, Calif.) (with β-mercaptoethanol). A sterile 500-μL volume of 0.1-mm glass beads (Scientific Industries, Inc., Bohemia, N.Y.) and a 5-mm sterile steel bead (Qiagen) was added for complete bacterial lysis in a Qiagen Tissue Lyser run at 30 Hz for 5 min.

The sample was centrifuged briefly, and 100 μL of 100% ethanol was added to a 100-μL aliquot of the supernatant. This mixture was added to a DNA spin column, and DNA

recovery protocols were followed as instructed in the QIAamp DNA Mini Kit (Qiagen) starting at Step 5 of the tissue protocol. DNA was eluted from the column with 30 μL of water, and the sample was diluted accordingly to a final concentration of 20 ng/μL. The DNA was quantified using a Nanodrop spectrophotometer (Nyxor Biotech, Paris, France) at a wavelength of 260–280 nm and a detection limit of 2 ng/μL of DNA.

Pyrosequencing: Massively Parallel bTEFAP, bTEFAP Titanium, and fTEFAP Titanium

Bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) and fungal tag-encoded FLX amplicon pyrosequencing (fTEFAP) were performed as described previously.[24,26] The new bacterial tag-encoded FLX-Titanium amplicon pyrosequencing (bTETAP) approach is based on similar principles to bTEFAP but utilizes Titanium reagents and Titanium procedures and a one-step PCR, mixture of Hot Start and HotStar high-fidelity taq polymerases and amplicons originating from the 27F region numbered in relation to *Escherichia coli* rRNA. The bTEFAP procedures were performed at the same pyrosequencing laboratory that analyzed the 8-hr area samples.

Bacteria and Fungal Diversity Analysis

Following sequencing, all failed sequence reads and low-quality sequence ends and tags were removed, and sequences were depleted of any non-bacterial or fungal ribosomal DNA sequences and chimeras using custom software and Black Box Chimera Check software B2C2 (Research and Testing Laboratories, LLC, Lubbock, Texas), both of which have been used previously to classify ribosomal DNA from microorganisms.[20,21,25,28–30] Sequences less than 300 bp were also removed. To determine the identity of bacteria and fungi in the remaining sequences, sequences were first queried using a distributed Basic Local Alignment Search Tool (BLASTn) .NET algorithm[31] against a database of high-quality bacterial ribosomal DNA sequences and 18S fungal ribosomal DNA sequences derived from the National Center for Biotechnology Information (NCBI) database. Database sequences were characterized as high quality based on the criteria of the Ribosomal Database Project (version 9).[32] Using a .NET and C# analysis pipeline, the resulting BLASTn outputs were compiled, validated using taxonomic distance methods, and data reduction analysis performed as described previously.[22,25,29]

Bacteria and Fungi Identification

Based on the above BLASTn derived sequence identity (percent of total length query sequence that aligns with a given database sequence) and validated using taxonomic distance methods, the bacteria and fungi were classified at the genus and species taxonomic levels based on the following criteria. Sequences with identity scores were compared with known or well-characterized ribosomal DNA sequences. Sequence identities greater than 97% (<3% divergence) were resolved at the species level, between 95% and 97% at the genus level, between 90% and 95% at the family level, and between 80% and 90% at the order level. After resolution based on these parameters, the percentage of each bacterial or fungal identity was individually analyzed for the sample providing relative abundance information based on relative numbers of ribosomal DNA sequences within a given sample. Evaluations presented at a given taxonomic level, except species level, represent all sequences resolved to their primary genera identification or their closest relative (where indicated).

Data Analysis

Once identified, bacteria and fungi were summarized at the genus and species levels. Due to the magnitude of information generated from pyrosequencing, if a particular genus has less than 70 sequences, those data were not reported. Of the genera reported, inhalable concentrations were calculated by sequences identified and divided by the volume of air sampled to report cells/m^3. Individual species that accounted for the majority of a particular genus were also reported. Fungi were summarized using the same method; however, if a particular genus had fewer than 10 sequences, those data were not reported. Estimates of total bacteria and fungi were calculated by adding the total number of sequences identified for the bTEFAP and fTEFAP, respectively.

RESULTS

Of the bacteria and fungi detected, 116 and 39 genera were identified, respectively (Figures 1 and 2). Among bacteria, *Staphylococcus cohnii* was present in the highest proportion (23%), followed by *Staphylococcaceae* (family) at 14%, a grouping of *Bacillales* (order) and *Lacotbacillus crispatus* at 7%. *Staphylococci* were detected at a concentration of 2187 cells/m^3, followed by *Salinicocci* at 1452 cells/m^3 and *Lactobacillus* at 1130 cells/m^3. The total inhalable bacteria concentration was estimated to be 7503 cells/m^3 (Table I).

Sagenomella sclerotialis was present in the highest proportion (37%) among the fungi identified. *Aspergillus ochraceus* and *Penicillium janthinellum* also represented a large proportion of the fungi identified at 14% and 13%, respectively. Additional fungi were identified and reported in Figure 2. Concentrations of various genera of fungi were reported in Table I. *Sagenomella* were identified in the highest concentration at 689 cells/m^3. *Aspergillus* and *Penicillium* were present at 255 cells/m^3 and 248 cells/m^3, respectively. The total inhalable fungi concentration was estimated to be 1810 cells/m^3. The blank sample submitted to the lab for analysis identified 103 ribosomal DNA sequences, which were removed from the reported information.

DISCUSSION

In this study, inhalable distribution of genera and species of bacteria and fungi in the air of a broiler chicken confinement facility were identified using pyrosequencing. The air in the

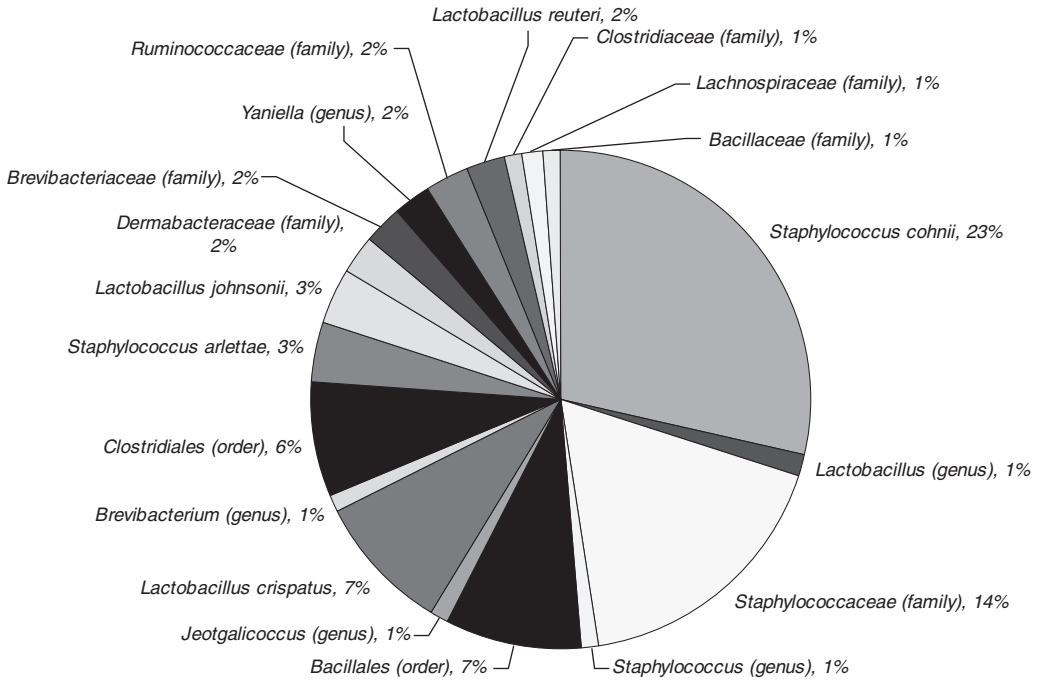

FIGURE 1. Percent distribution of bacteria present in the inhalable fraction of a chicken bioaerosol

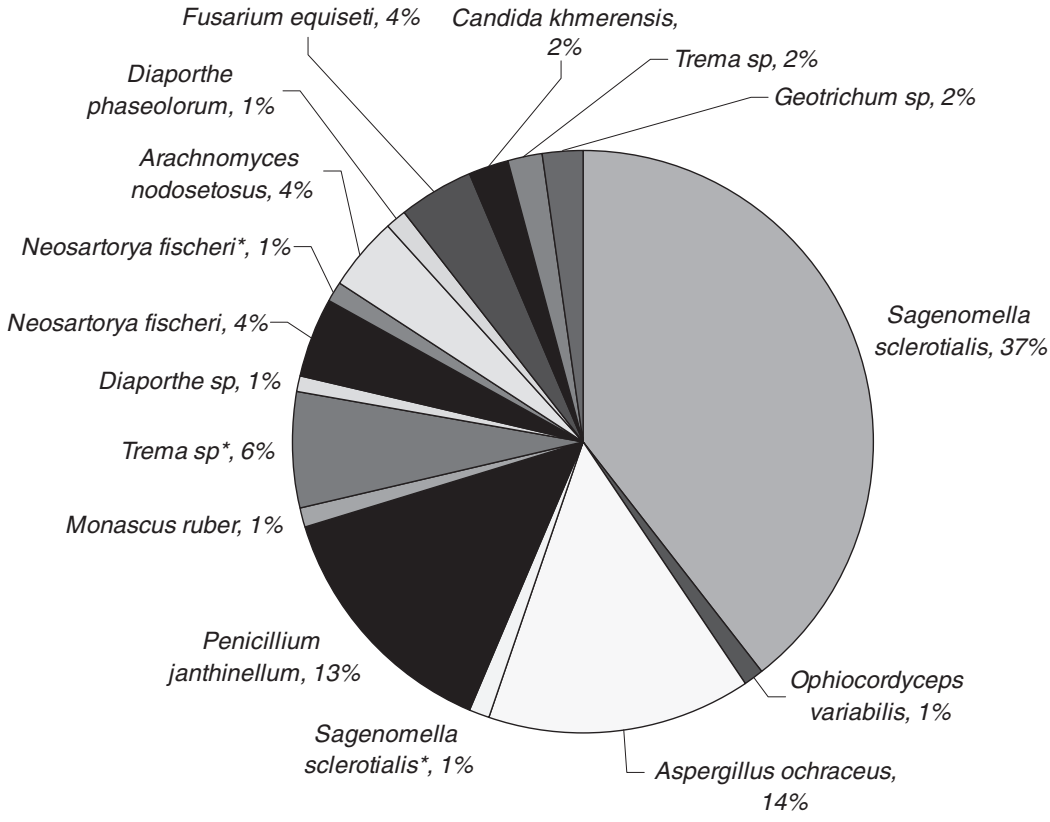

FIGURE 2. Percent distribution of fungi present in the inhalable fraction of a chicken bioaerosol

TABLE I. Concentrations of Bacteria and Fungi in the Inhalable Faction of a Chicken Bioaerosol

Bacteria (Genus)	Cells/m^3	Fungi (Genus)	Cells/m^3
Staphylococcus	2187	*Sagenomella*	698
Salinicoccus	1452	*Aspergillus*	255
Lactobacillus	1130	*Penicillium*	248
Ruminococcus	277	*Trematosphaeria*	142
Brevibacterium	269	*Neosartorya*	103
Clostridium	247	*Arachnomyces*	68
Roseburia	211	*Fusarium*	68
Brachybacterium	194	*Diaporthe*	54
Yaniella	181	*Candida*	45
Jeotgalicoccus	135	*Geotrichum*	38
Nocardiopsis	105	*Monascus*	24
Faecalibacterium	92	*Ophiocordyceps*	18
Turicibacter	90	*Antrodiella*	17
Enterococcus	74	*Valsa*	16
Other	697	Other	15
Total Bacteria	7503	Total Fungi	1810

confinement facility was dominated primarily by the presence of *Staphylococci, Salinicocci, Lactobacillus, Sagenomella, Aspergillus, and Penicillium.*

The inhalable fraction of bacteria and fungi were estimated to be 7503 cells/m^3 and 1810 cells/m^3. These estimates are lower than what has been reported by others using a standard epifluorescence microscope method,[3] who reported total bacteria at 4.7×10^9 cells/m^3 and total fungi 2.0×10^7 cells/m^3. The reason for this discrepancy is unclear; however, among confined animal feeding operations, temperature, humidity, animal size, density, type of bedding, and animal activity levels impact the concentration and distribution of bacterial and fungal genera.[33] Furthermore, previous studies have evaluated personal exposure rather than the area sampling method used in this study. The presence of a mobile worker may impact animal activity and generate a greater concentration of bioaerosol.

Staphylococci have been identified previously as being present in high concentrations in chicken facilities. Oppliger[6] reported inhalable geometric mean concentrations of *Staphylococci* to be 88×10^3 cells/m^3 (GSD 5.9) using Q-PCR methods in 12 chicken confinement buildings. *Staphylococcus cohnii* (a coagulase-negative staphylococci) was identified and represented a large proportion of the *Staphylococcus* genera present in this facility. Further, the *Staphylococcus* genera were expected to be present in a large proportion, as this genera is often present commensally on the skin.[34]

A large proportion of fungi in the inhalable fraction of this bioaerosol included *Sagenomella sclerotialis, Penicillium janthinellum,* and *Aspergillus ochraceus*. *Aspergillus ochraceus* has been associated with the production of the mycotoxin, ochratoxin A.[35] A previous biomonitoring study found no difference in serum levels of ochratoxin A when comparing farm workers with those who did not work on a farm.[36] The cohort of 106 farm workers included poultry, dairy, and swine workers; ochratoxin A levels did not differ among the types of production. To date, no study has evaluated occupational exposure to airborne concentrations of ochratoxin among poultry workers. Additional research is needed to evaluate the extent to which ochratoxin-producing fungi are present in the environment surrounding poultry production facilities.

Few guidelines exist to allow interpretation of risk of inhalation exposure of bacteria and fungi. Some published guidelines in Europe suggest that airborne exposure limits not exceed 10,000 CFU/m^3 for total bacteria and 1000 CFU/m^3 for gram negative bacteria.[37] ACGIH® recommends that fungi exposure be limited to concentrations found in the outside air.[38] Currently, no standards exist for inhalation exposure to bacteria or fungi. Furthermore, assigning a level of risk to concentrations of bacteria and fungi measured in this study is problematic, as guidelines are typically reported using a culture-based method (CFU/m^3).

The ability to identify and quantify the distributions of bacteria and fungi from a single air sample is an exciting strength of using pyrosequencing for occupational and environmental exposure assessment. Additional strengths of pyrosequencing include the relative ease of sampling, fast sample processing time (i.e., 8 hr), and low cost (i.e., $110 per sample U.S. dollars in 2010).

Limitations

There are several limitations with this study. For example, only one environmental sample was taken. One sample may not represent the indoor environment of this facility. However, measures were taken to minimize indoor variables that may impact the validity of our sample. For example, the mannequin was rotated to reduce the effect of incomplete mixing of replacement air. The sampler was also centrally located, away from sources replacement air.

The distribution of bacteria and fungi inside the broiler facility are likely dependent on environmental variables, such as temperature, humidity, light level, and ventilation, as well as the number and size of the chickens. Our sample was collected from only one broiler facility; therefore, the type and distribution of bacteria and fungi observed inside this facility may not represent other broiler production facilities. Nonetheless, the sample was collected from a modern broiler production facility that adheres to current broiler production standards. Therefore, the characteristics of this facility such as size, feed used, and bedding are similar to other facilities in the broiler production industry.

An additional limitation of the pyrosequencing technology is that the sequences identified may be from non-viable organisms. However, little is known about bioaerosols in poultry production or other animal production facilties, so identifying all organisms both non-culturable as well as non-viable organisms will contribute to characterizing bioaerosols in animal production facilities.[18] As inhalation exposure to

non-viable components of microorganisms (e.g., endotoxin) may place workers at risk, evaluating non-viable components of microorganisms may prove useful for assessing risk for pulmonary disease.

Another limitation may be that the identified sequences may not be 100% matched to the NCBI database. We accounted for this by reporting only the taxonomic level that resulted in a high proportion of match (90% or above), as all sequences were "forced" to a match in the NCBI database. There may be limitations with the NCBI database as well. Specifically, the NCBI database has an inherent bias to human pathogens, as these organisms are more likely to be fully sequenced and added to the database. This bias may overrepresent human pathogens in environmental samples. However, the NCBI microbial genomic BLAST database currently contains 1272 bacterial, 72 archaeal, and 211 eukaryotic (including fungi) genomes.[39] The NCBI databases will continue to grow as additional microorganisms are identified and sequenced and may become a powerful tool for characterizing microorganisms in the environment.

Future Research

A limited amount of information exists on the bioaerosols present in a poultry production environment. Future work should include an expanded sampling plan and additional production sites for enhanced generalizability of the results. Additional work in other areas of animal production is needed to better characterize bioaerosols utilizing this pyrosequencing methodology. Specifically, future research should further evaluate the strengths and weaknesses of pyrosequencing by comparison studies of bioaerosols analyzed using pyrosequencing to other standard total bioaerosol techniques (e.g., fluorescence and other culture-based techniques). Future work might evaluate minimum sampling times and additional types of bioaerosol sampling media (e.g., liquid). This future work would allow for better interpretation of results obtained using non-culture-based techniques for the evaluation of occupational and environmental exposure to bioaerosols.

CONCLUSION

To our knowledge, this is the first study to report the distribution of genera and species of the inhalable fraction of bacteria and fungi in the air of a poultry confinement operation using a non-culture-based method or, more specifically, pyrosequencing. The low cost and the fast processing speed of this pyrosequencing technology may revolutionize the ability to identify the distribution and concentration of bioaerosols. The impact of this technology has yet to be realized by the scientific community dedicated to evaluating occupational and environmental bioaerosol exposure.

ACKNOWLEDGMENTS

This study would not have been possible without the assistance of Dr. Michael Pangburn and Ms. Aika Hussain. Grant support was provided for this project from the Southwest Center for Agricultural Health, Injury Prevention and Education (CDC/NIOSH U50 OH07541) at the University of Texas Health Science Center at Tyler.

REFERENCES

1. **Donham, K.J., D. Cumro, S.J. Reynolds, et al.:** Dose-response relationships between occupational aerosol exposures and cross-shift declines of lung function in poultry workers: Recommendations for exposure limits. *J. Occup. Environ. Med. 42(3)*:260–269 (2000).
2. **Pedersen, S., M. Nonnenmann, R. Rautiainen, et al.:** Dust in pig buildings. *J. Agric. Saf. Health 6(4)*:261–274 (2000).
3. **Radon, K., C. Weber, M. Iversen, et al.:** Exposure assessment and lung function in pig and poultry farmers. *Occup. Environ. Med. 58*:405–410 (2001).
4. **Donham, K.J.,D. Cumro, and S.J. Reynolds:** Synergistic effects of dust and ammonia on the occupational health effects of poultry production workers. *J. Agromedicine 8(2)*:57–76 (2002).
5. **Kirychuk, S.P., J.A. Dosman, S.J. Reynolds, et al.:** Total dust and endotoxin in poultry operations: Comparison between cage and floor housing and respiratory effects in workers. *J. Occup. Environ. Hyg. 48*:741–748 (2006).
6. **Oppliger, A., N. Charrière, P.O. Droz, et al.:** Exposure to bioaerosols in poultry houses at different stages of fattening; use of real-time PCR for airborne bacterial quantification. *Ann. Occup. Hyg. 52(5)*:405–412 (2008).
7. **Lee, S., A. Adhikari, S.A. Grinshpun, et al.:** Personal exposure to airborne dust and microorganisms in agricultural environments. *J. Occup. Environ. Hyg. 3*:118–130 (2006).
8. **Clark, S., R. Rylander, and L. Larsson:** Airborne bacteria, endotoxin and fungi in the dust of poultry and swine confinement buildings. *AIHA J. 44*:537–541 (1983).
9. **Torsvik, V., R. Sørheim, and J. Goksøyr:** Total bacterial diversity in soil and sediment communities—A review. *J. Ind. Microbiol. 17*:170–178 (1996).
10. **Torsvik, V., F.L. Daae, R.A. Sandaa, et al.:** Novel techniques for analysing microbial diversity in natural and perturbed environments. *J. Biotechnol. 64(1)*:53–62 (1998).
11. **DeLong, E.F., and N.R. Pace:** Environmental diversity of bacteria and archaea. *Syst. Biol. 50(4)*:470–478 (2001).
12. **Nehmé, B., V. Letourneau, R.J. Forster, et al.:** Culture-independent approach of the bacterial bioaerosol diversity in the standard swine confinement buildings, and assessment of the seasonal effect. *Environ. Microbiol. 10(3)*:665–675 (2008).
13. **Nehmé B., Y. Gilbert, V. Letourneau, et al.:** Culture-independent characterization of archaeal biodiversity in swine confinement building bioaerosols. *Appl. Environ. Microbiol. 75(17)*:5445–5450 (2009).
14. **Woese, C.:** Bacterial evolution. *Microbiol. Rev. 51(2)*:221–271 (1987).
15. **Solits, D.E., P.S. Solits, M.W. Chase, et al.:** Angiosperm phylogeny inferred from 18S rDNA, vbcL, and atpB sequences. *Bot. J. Linn. Soc. 133(4)*:381–461 (2000).
16. **Stackebrandt, E., and B.M. Goebel:** Taxonomic note: A place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int. J. Syst. Bacteriol. 44:*846–849 (1994).
17. **Coenye, T., and P. Vandamme:** Intragenomic heterogeneity between multiple 16S ribosomal RNA operons in sequenced bacterial genomes. *FEMS Microbiol. Lett. 228(1)*:45–49 (2003).
18. **Thorne, P.S., C. Duchaine, J. Douwes, et al.:** Working Group Report 4: Exposure assessment for biological agents. *AJIM 46(4)*:419–422 (2004).
19. **Margulies, M., M. Egholm, W.E. Altman, et al.:** Genome sequencing in open microfabricated high density picoliter reactors. *Nature 437(7057)*:376–380 (2005).
20. **Bailey, M.T., S.E. Dowd, N.M. Parry, et al.:** Stressor exposure disrupts commensal microbial populations in the intestines and leads to increased

colonization by *Citrobacter rodentium*. *Infect. Immun. 78(4)*:1509–1519 (2010).
21. **Bailey, M.T., J.C. Walton, S.E. Dowd, et al.:** Photoperiod modulates gut bacteria composition in male Siberian hamsters (*Phodopus sungorus*). *Brain Behav. Immun. 24(4)*:577–584 (2010).
22. **Dowd, S.E., R.D. Wolcott, Y. Sun, et al.:** Polymicrobial nature of chronic diabetic foot ulcer biofilm infections determined using bacterial tag encoded FLX amplicon pyrosequencing (bTEFAP). *PLoS. One 3(10)*:e3326 (2008).
23. **Dowd, S.E., Y. Sun, R.D. Wolcott, et al.:** Bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) for microbiome studies: bacterial diversity in the ileum of newly weaned Salmonella-infected pigs. *Foodborne Pathog. Dis. 5*:459–472 (2008).
24. **Dowd, S.E., T.R. Callaway, R.D. Wolcott, et al.:** Evaluation of the bacterial diversity in the feces of cattle using 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP). *BMC Microbiol. 8*:125 (2008).
25. **Dowd, S.E., Y. Sun, P.R. Secor, et al.:** Survey of bacterial diversity in chronic wounds using pyrosequencing, DGGE, and full ribosome shotgun sequencing. *BMC Microbiol. 8*:43 (2008).
26. **Leake, J.L., S.E. Dowd, R.D. Wolcott, et al.:** Identification of yeast in chronic wounds using new pathogen-detection technologies. *J. Wound Care 18*:103–108 (2009).
27. **Pitta, D.W., W.E. Pinchak, S.E. Dowd, et al.:** Rumen bacterial diversity dynamics associated with changing from Bermuda grass hay to grazed winter wheat diets. *Microb. Ecol. 59(3)*:511–522 (2009).
28. **Suchodolski, J.S., S.E. Dowd, E. Westermarck, et al.:** The effect of the macrolide antibiotic tylosin on microbial diversity in the canine small intestine as demonstrated by massive parallel 16S rRNA gene sequencing. *BMC Microbiol. 9*:210 (2009).
29. **Wolcott, R.D., V. Gontcharova, Y. Sun, et al.:** Bacterial diversity in surgical site infections: Not just aerobic cocci any more. *J. Wound Care 18*:317–323 (2009).
30. **Gontcharova, V., E. Youn, R. D. Wolcott, E.B. et al.:** Black box chimera check (B2C2): A Windows-based software for batch depletion of chimeras from bacterial 16S rRNA gene datasets. *Open Micro J. 4*:47–52 (2010).
31. **Dowd, S.E., J. Zaragoza, J.R. Rodriguez, et al.:** Windows.NET network distributed basic local alignment search toolkit (W.ND-BLAST). *BMC Bioinformatics 6*:93 (2005).
32. **Cole, J.R., Q. Wang, E. Cardenas, et al.:** The Ribosomal Database Project: Improved alignments and new tools for rRNA analysis. *Nucleic Acids Res. 37*(database issue):D141–D145 (2009).
33. **Gustafsson, G.:** Factors affecting the release and concentration of dust in pig houses. *J. Agr. Eng. Res. 74(4)*:379–390 (1999).
34. **Black, J.G.:** *Microbiology: Principles and Applications*. Upper Saddle River, N.J.: Prentice Hall, 1993.
35. **Van der Merwe, K.J., P.S. Steyn, and L. Fourie:** Ochratoxin A, a toxic metabolite produced by *Aspergillus ochraceus*. *Nature 205*:1112–1113 (1965).
36. **Skaug, M.A.:** Levels of ochratoxin A and IgG against conidia of *Penicillium verrucosum* in blood samples from healthy farm workers. *Ann. Agric. Environ. Med. 10*:73–77 (2003).
37. **Nielsen, E.M., B.H. Nielsen, and N.O. Breum:** Occupational bioaerosol exposure during collection of household waste. *Ann. Agric. Environ. Med. 2*:53–59 (1995).
38. **Macher, J.:** *Bioaerosols Assessment and Control.* Cincinnati, Ohio: American Conference of Governmental Industrial Hygienists, 1999.
39. "BLAST with Microbial Genomes." [Online] Available at http://www.ncbi.nlm.nih.gov/sutils/genom_table.cgi (Accessed June 1, 2010).