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Full length article

Use of a culture-independent approach to characterize aerosolized bacteria near an open-freestall dairy operation☆

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ABSTRACT

Animal manures are known to harbor a variety of zoonotic pathogens, which are suspected of being transported off-site as aerosols from confined feeding operations. In this study, aerosols were collected using a high-volume sampler downwind from a 10,000 cow open-freestall dairy and nearby fields being sprinkler irrigated with wastewater. DNA extracts were prepared from the aerosol samples, then a region of the16S ribosomal RNA gene was sequenced for bacterial identification and phylogenetic classification. At the dairy and irrigation sites, *Proteobacteria* (α-, β-, and γ-subdivisions) was the most abundant phylum, representing 78% and 69% of all sequences, respectively, while *Actinobacteria*, *Bacteroidetes* and *Firmicutes* represented only 10% or less of the sequences. Of the 191 clones sequenced from the dairy aerosol samples, 6 sequences were found to be homologous with uncultured bacteria from cow milk, rumen, and fecal samples. However, none of the sequence matches was affiliated with bacteria known to be pathogenic to otherwise healthy humans. Although our results do suggest a high diversity among the aerosolized bacteria, the sampling strategy employed in this study may not account for the variable nature of bioaerosol emissions.

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1. Introduction

The generation of bioaerosols at agricultural facilities with a high microbial load may cause a wide range of occupational and community health effects (Cole et al., 2000; Spaan et al., 2006). Exposed individuals can be affected by infectious disease, allergy, and acute toxic effects (Douwes et al., 2003). Concentrated animal feeding operations (CAFOs) are of particular concern from a bioaerosol-pathogen standpoint, as they produce large quantities of manure. At CAFOs, bioaerosols are generated during the handling and processing of manures (e.g. land application) or during animal movement and high wind events which dislodge particulate matter from feedlot surfaces (Dungan, 2010). Since a variety of zoonotic bacterial pathogens are associated with animal manures, their potential to be transported as aerosols is high. Bioaerosols containing pathogens or microbial byproducts may be inhaled or ingested after deposition on fomites or exposed fruit and vegetable crops. Although the common route of transmission for enteric bacterial pathogens is direct contact or ingestion, there is evidence from animal studies suggesting that aerogenic routes are possible (Cornick and VuKhac, 2008; Harbaugh et al., 2006; Wathes et al., 1988).

During the spray irrigation of swine slurry, a marker strain of *Escherichia coli* was detected 125 m downwind in aerosols, but not at 250 and 500 m downwind (Hutchinson et al., 2008). Boutin et al. (1988) found that airborne concentrations of indicator organisms decreased with downwind distance during the land application of swine and cattle slurries via tractor-pulled tanker and fixed high-pressure spray guns. A decreasing airborne microorganism concentration with increasing downwind distance is the general trend also documented by researchers at CAFOs (Chinivasagam et al., 2010; Dungan et al., 2010; Green et al., 2006). In recent study by Duan et al. (2009), airborne *E. coli* were recovered at distances up to 100 m from swine houses, which were closely related to isolates obtained from indoor air and fecal samples. While the above mentioned studies do suggest a decreased risk for exposure to bioaerosols as distance from the source is increased, some pathogenic microorganisms can be transported long distances (Brown and Hovmøller, 2002; Gloster et al., 1982). As a result, there is a need for studies that identify and characterize potential bioaerosol sources to allow for a more accurate assessment of associated animal and human health risks.

To date, relatively few PCR-based studies have been conducted to identify airborne bacteria near animal operations and wastewater spray irrigation sites (Murayama et al., 2010; Ravva et al., 2011). Compared to culture-dependent approaches, molecular techniques allow investigators to tentatively identify a wider range of organisms that could not normally be isolated using traditional growth mediums (Dungan and Leytem, 2009). In this study, aerosols were collected in the downwind environment of an open-lot freestall dairy and nearby fields being irrigated with dairy wastewater using a high-volume sampler. Nucleic acids were extracted from the samples, then a clone library was prepared using a PCR-amplified region of the 16S ribosomal RNA (rRNA) gene. The purpose was to provide information

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Table 1
Number of clones affiliated with the bacterial phyla at each site.

Phylum	Background	Dairy	Pivots
Actinobacteria		3 (2)	2 (4)
Bacteroidetes	2 (7[a])	11 (8)	4 (8)
Firmicutes	3 (10)	13 (9)	5 (10)
Proteobacteria	24 (83)	111 (78)	33 (69)
Unclassified		5 (3)	4 (8)

[a] Percentage of the total number of clones.

on the identity and diversity of airborne bacteria in the immediate vicinity of a large dairy operation.

2. Materials and methods

2.1. Dairy operation

Aerosols were collected downwind from an open-freestall dairy and center pivot irrigation systems that sprayed wastewater from

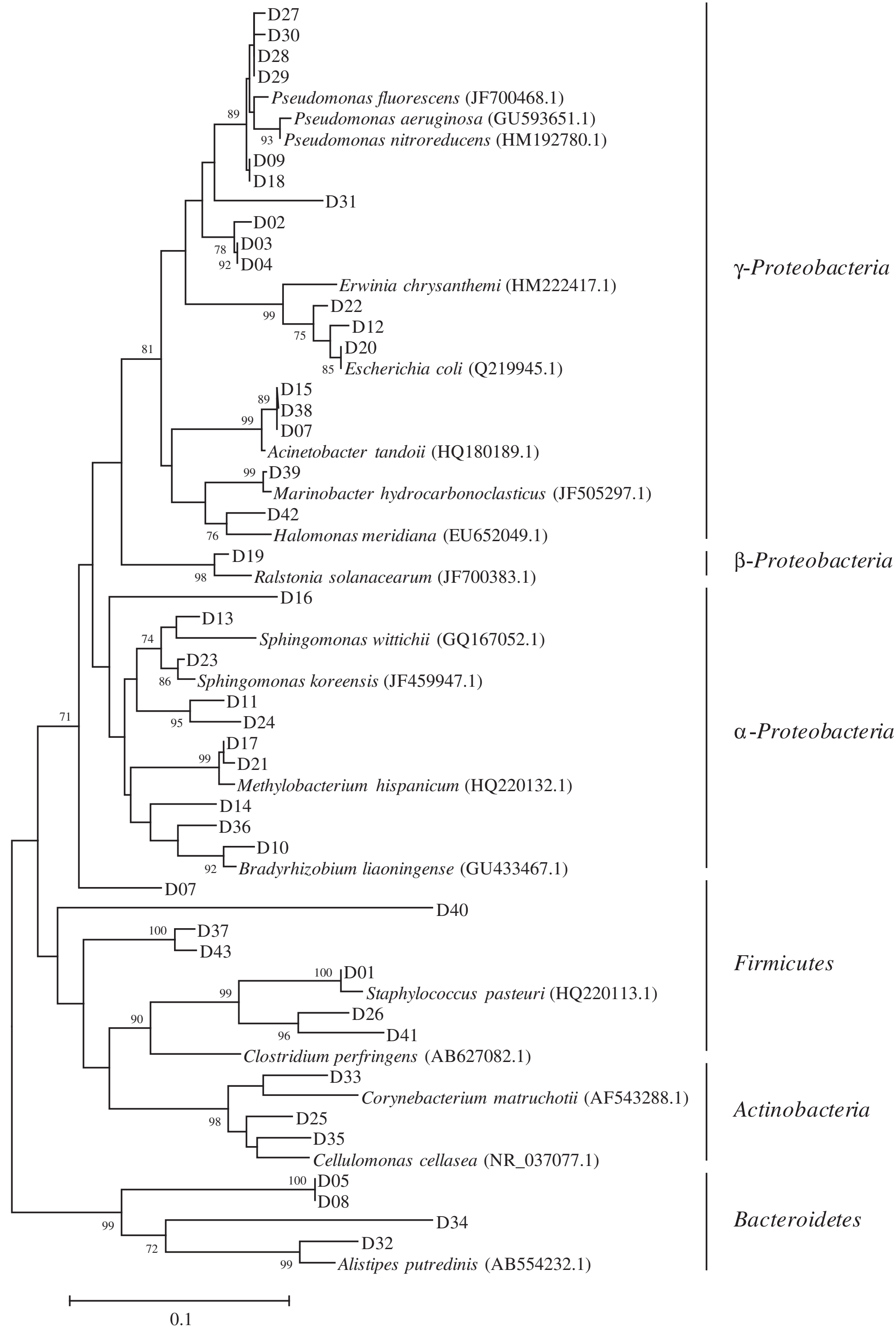

Fig. 1. Phylogenetic tree displaying relationships between the clone sequences collected downwind from the open-freestall dairy and reference species. Bootstrap values > 70% are noted at the branch junctions. The bar indicates a 10% estimated sequence divergence.

the dairy. There were six barns at the freestall dairy containing a total of 10,000 milking cows. Four of the barns were approximately 670 m long and the remaining two barns were half the size of the longest barns. The barns were oriented lengthwise in an east–west direction and contained side curtains that were maintained in the open position during the time of sampling. Between each set of barns was an exercise yard, which was harrowed on a regular basis when in use by the cows. Manure in the alleys was flushed daily and then sent to a solids separator. The pivot irrigated fields (0.45 km^2) of silage corn were located adjacent to the freestall dairy. Irrigation water was blended with approximately 25% (v/v) dairy wastewater and discharged through drop-down sprinklers (~2 m above ground level) and end guns. Background samples were collected at the USDA-ARS NWISRL and at an upwind site about 850 m from the freestall dairy.

2.2. Aerosol sampling

A wetted-wall cyclone (SASS 2300, Smart Air Sampler System, Research International, Monroe, WA, USA) with an air flow rate of 325 l min^{-1} was used to collect the aerosols. The samples were collected at various intervals over a 4 month period (July to October), during daylight hours, in 2009 and 2010. The SASS unit was mounted 1.5 m above ground level and placed about 60 m away from a freestall barn and near the edge of the pivot irrigated fields, but out of range of the end guns. Depending upon the meteorological conditions during the time of sampling, the unit was operated for up to 10 h via battery power. Sterile deionized (DI) water was used as the collection liquid as recommended by the manufacturer. While in the field, the liquid sample was transferred to a sterile 50-ml conical tube. To enhance recovery of bacteria from the SASS, the cyclone was subsequently rinsed twice with sterile DI (by temporarily operating the system for 1 min) and the rinsate was also added to the sterile tube. The sample (~15 ml) was transferred to the laboratory in a cooler and stored at −20 °C until processed.

2.3. DNA extraction

To process the SASS samples, they were initially thawed at room temperature and then centrifuged at 4500×g for 2 min to settle out larger particles. The supernatant was then passed through a sterile 25-mm 0.4 μm polycarbonate track-etch membrane (Whatman Inc., Piscataway, NJ, USA). The membrane was added to a bead beating tube from an UltraClean soil DNA isolation kit (MoBio Laboratories, Inc., Carlsbad, CA, USA), rapidly vortexed for 1 min, and then the membrane was aseptically removed using sterile forceps. The remaining pellet (~100 μl) from the SASS sample was then added to the tubes, which

Table 2
Phylogenetic affiliations of bacterial 16S rRNA gene sequences obtained from aerosol samples collected downwind from the open-freestall dairy.

Clone	Closet match in GenBank database	Phylogenetic group	Accession no.	% Similarity
D01	*Staphylococcus sciuri* H9B-25	*Firmicutes*	HQ238860.1	100
D02	*Pseudomonas rhizosphaerae* GAPP71	γ *-Proteobacteria*	GU396285.1	92
D03	*Pseudomonas* sp. CC-CY503	γ *-Proteobacteria*	JF432053.1	99
D04	*Pseudomonas pertucinogena* mol25	γ *-Proteobacteria*	HM031486.1	99
D05	*Chryseobacterium* sp. TB2-6-I	*Bacteroidetes*	AY599654.1	98
D06	Uncultured Clostridiales bacterium clone M9-76	*Firmicutes*	EU530505.1	91
D07	*Acinetobacter lwoffii* MYL-1	γ *-Proteobacteria*	HQ738472.1	99
D08	*Chryseobacterium bovis* G55-3	*Bacteroidetes*	HM217959.1	97
D09	*Pseudomonas mendocina* SORA1	γ *-Proteobacteria*	HM171902.1	99
D10	*Bradyrhizobium japonicum* OS3-81	α*-Proteobacteria*	FN178436.1	100
D11	*Skermanella* sp. Py-2-1	α*-Proteobacteria*	GU195651.1	99
D12	*Pantoea agglomerans* TAC 93.XII.8	γ *-Proteobacteria*	AY616175.1	99
D13	*Sphingomonas rhizogenes* BW59UT1570	α*-Proteobacteria*	JF276901.1	99
D14	*Candidatus Reyranella massiliensis* URTM1	α*-Proteobacteria*	EF394922.1	99
D15	*Acinetobacter baumannii* 1656-2	γ *-Proteobacteria*	CP001921.1	99
D16	*Caulobacter* sp. CCGE4014	α*-Proteobacteria*	GU980220.1	100
D17	*Methylobacterium radiotolerans* GF7	α*-Proteobacteria*	FN796863.1	99
D18	*Pseudomonas alcaliphila* RCT11	γ *-Proteobacteria*	HM805114.1	99
D19	*Ralstonia pickettii* L2	β*-Proteobacteria*	GQ906999.1	100
D20	*Escherichia coli* UMN026	γ *-Proteobacteria*	CU928163.2	99
D21	*Methylobacterium tardum* Sco-A27	α*-Proteobacteria*	FN386716.1	97
D22	*Escherichia coli* KO11	γ *-Proteobacteria*	CP002516.1	100
D23	*Sphingomonas* sp. BR5-22	α*-Proteobacteria*	EU423303.1	99
D24	*Skermanella aerolata* 5416T-32	α*-Proteobacteria*	DQ672568.1	98
D25	*Arthrobacter agilis*	*Actinobacteria*	AJ577725.1	97
D26	*Planococcus* sp. IP20B	*Firmicutes*	GU726560.1	99
D27	*Pseudomonas pseudoalcaligenes* MHF ENV 11	γ *-Proteobacteria*	GU055765.1	99
D28	*Pseudomonas putida* IPPBC-E11	γ *-Proteobacteria*	HQ840763.1	100
D29	*Pseudomonas fluorescens* RHH45	γ *-Proteobacteria*	HQ143617.1	99
D30	*Pseudomonas rhizosphaerae* OW-2	γ *-Proteobacteria*	AY866408.1	97
D31	*Pseudomonas tuomuerensis* 78-123	γ *-Proteobacteria*	DQ868767.1	96
D32	Uncultured *Alistipes* sp. clone EMP_AA14	*Bacteroidetes*	EU794075.1	96
D33	*Corynebacterium amycolatum* GN-04-9d	*Actinobacteria*	DQ872448.1	91
D34	Uncultured Bacteroidetes bacterium clone edNE64	*Bacteroidetes*	DQ886234.1	100
D35	*Cellulomonas bogoriaensis* 69B4	*Actinobacteria*	AJ863164.1	95
D36	*Sphingomonas astaxanthinifaciens*	α*-Proteobacteria*	AB277583.1	94
D37	*Clostridium* sp. enrichment culture clone NHT38	*Firmicutes*	JF312678.1	97
D38	*Acinetobacter radioresistens* F71005	γ *-Proteobacteria*	HQ908727.1	99
D39	*Marinobacter vinifirmus* D7035	γ *-Proteobacteria*	FJ161339.1	98
D40	Uncultured rumen bacterium clone 17-P3		AM884077.1	91
D41	*Jeotgalicoccus* sp. A76(2010)	*Firmicutes*	HQ433462.3	98
D42	*Halomonas salina* GSP23	γ *-Proteobacteria*	AY553073.1	96
D43	Uncultured *Clostridium* sp. clone MS159A1_E02	*Firmicutes*	EF705259.1	99

were subjected to bead beating for 2 min and processed according to the manufacturer's instructions. DNA extracts were stored at −70 °C until PCR was performed.

2.4. PCR amplification

The V1–V3 region of the 16S rRNA gene was amplified with the forward primer 63F (5′ CAG GCC TAA CAC ATG CAA GTC 3′) and reverse primer BA518R (5′ CGT ATT ACC GCG GCT GCT GG 3′) (Muyzer et al., 1993; Marchesi et al., 1998). In brief, reaction mixtures were prepared with 5 μl of DNA template, 15 μl of AmpliTaq Gold® PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 0.3 μM of each primer, and molecular-grade water to a final volume of 30 μl. PCR amplification was performed using the following temperature protocol: 95 °C for 5 min, then 30 cycles of 92 °C for 1 min, 55 °C for 30 s, and 72 °C for 1 min, and a single final extension at 72 °C for 5 min. Both negative (water only) and positive (*E. coli*, ATCC 13706) controls were used during each PCR run. The PCR products (~450 bp) were visualized by UV transillumination on 1.5% agarose gels after staining with ethidium bromide.

2.5. Clone library construction

The PCR products were cloned using pGEM-T Easy Vector (Promega Corp., Madison, WI, USA) according to the manufacturer's instructions. After transformation of the ligated plasmids into *E. coli* JM109, the clones were plated onto duplicate Luria-Bertani (LB) agar plates containing ampicillin (0.1 g l^{-1}), IPTG (0.5 mM), and X-Gal (80 μg l^{-1}) and incubated overnight at 37 °C. A total of 25 white colonies were randomly selected from each plate, which were then grown overnight at 37 °C with shaking in 10 ml of LB medium containing 0.1 g ampicillin l^{-1}. Plasmids from the clones were isolated using

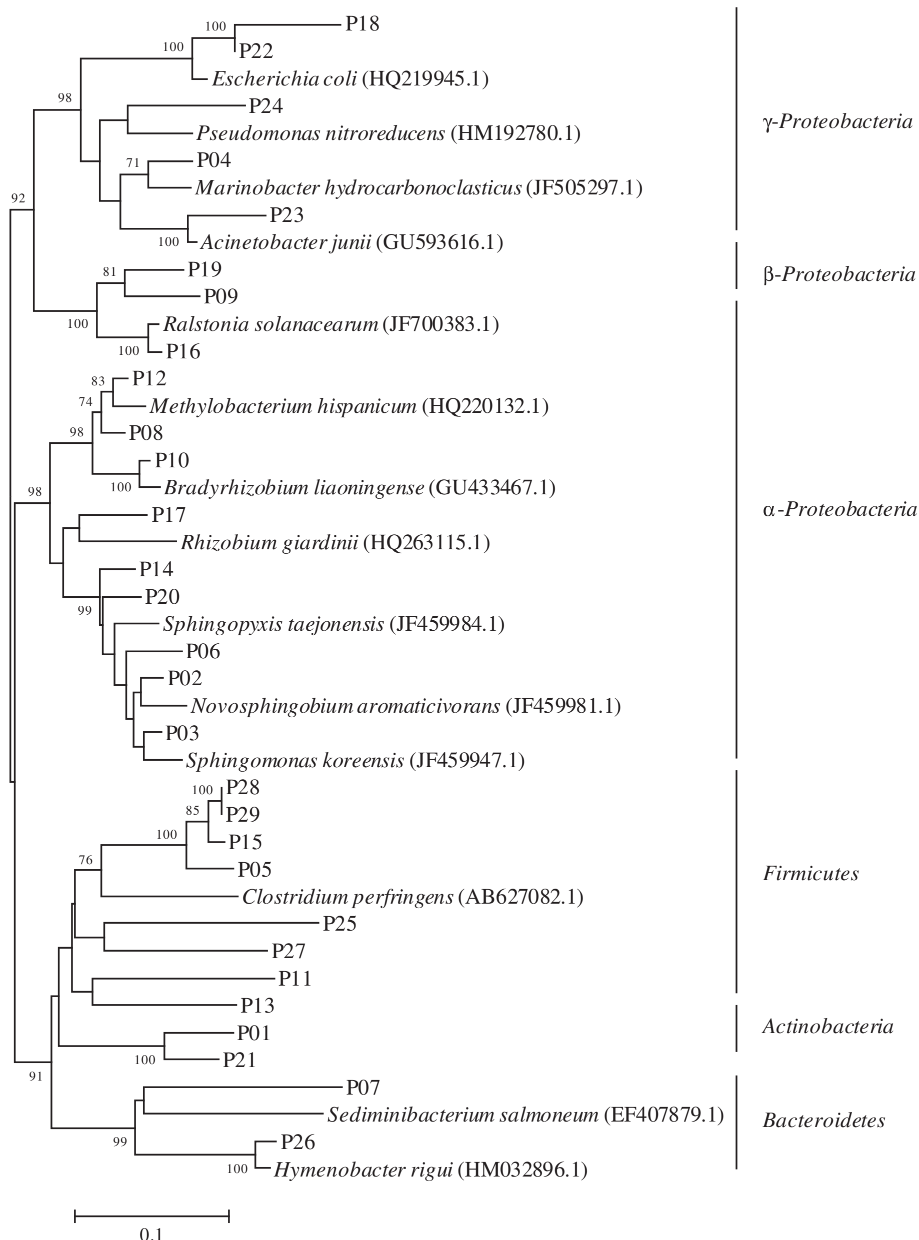

Fig. 2. Phylogenetic tree displaying relationships between the clone sequences collected downwind from the pivot irrigation sites and reference species. Bootstrap values >70% are noted at each node. The bar indicates a 10% estimated sequence divergence.

the UltraClean Mini Plasmid Prep (MoBio Laboratories, Inc.) and QIAprep Spin Miniprep (Qiagen, Inc., Valencia, CA, USA) kits.

2.6. Sequence and phylogenetic analysis

Plasmid inserts from clones were amplified with the 63F primer and sequenced using an ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Raw sequences were handled using BioEdit Sequence Alignment Editor (Hall, 1999) and checked for putative chimeric sequences using Bellerophon (Huber et al., 2004). Clone sequences were aligned and classified using tools available on the Greengenes web site (DeSantis et al., 2006). Sequence identification was performed using the Basic Local Alignment Search Tool (BLAST) at the National Center for Biotechnology Information (NCBI). Only clones with ≥90% 16S rRNA sequence similarities were selected for phylogenetic analysis. References sequences were obtained from the Nucleotide database at NCBI. Evolutionary distances were calculated using the Jukes–Cantor method and phylogenetic trees were constructed using the neighbor-joining method with MEGA5 (Tamura et al., 2011). Bootstrap analyses of the neighbor-joining data were conducted based on 1000 iterations to assess the stability of the phylogenetic relationships.

3. Results and discussion

Dairy operations, like other animal operations, produce large quantities of manure that are generally stored on site or processed until they are land applied. Because manures are a potential source of pathogenic microorganisms, activities that create aerosols within the dairy environment are cause for concern from a human health standpoint. At a dairy, animal movement and manure management, such as composting and land application of liquid and solid manures, have the potential to generate bioaerosols (Millner, 2009). In this study a total of 220 clones, representing bacteria in aerosols near the freestall dairy, center pivots spraying dairy wastewater and background sites, were analyzed. The clones were putatively classified as belonging to the following phyla: *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* (Table 1). At the dairy and pivot irrigation sites, *Proteobacteria* was the most abundant phylum, representing 78% and 69% of all sequences from the aerosols, respectively. Similar results were obtained from the background sites, with *Proteobacteria* representing 83% of all sequences. *Proteobacteria* is the largest phylum of *Bacteria*, which are all Gram-negative and include a wide variety of zoonotic pathogens, such as *Escherichia*, *Salmonella*, *Brucella*, and *Campylobacter*. Whether at the dairy, irrigation sites, or background sites, *Actinobacteria*, *Bacteroidetes* and *Firmicutes* represented only 10% or less of the sequences. Some important zoonotic pathogens from *Actinobacteria* and *Firmicutes* are *Mycobacterium* and *Listeria* and *Clostridium*, respectively. The distribution of sequences among the phyla was markedly similar to results obtained in a study of aerosols at an open-lot dairy in the Central Valley of California (Ravva et al., 2011). Interestingly, none of the aerosol sequences from the Central Valley dairy was found to originate from either fresh or dry manure collected on site, although a few sequence matches with fresh manure were obtained at a freestall dairy in Sonoma, CA. Downwind from a biosolids land-application site, Baertsch et al. (2007) reported that *Proteobacteria* represented 81% of the bacterial sequences obtained in aerosol samples.

Fig. 1 shows the phylogenetic distribution of unique aerosol clone sequences obtained downwind from the open-freestall dairy. Of the 43 clones, the majority (i.e. 29 of 43) were related to the α-, β-, and γ-subdivisions of *Proteobacteria*. Within γ-*Proteobacteria*, 10 sequences were ≥92% homologous with pseudomonad reference sequences from the GenBank database, including *P. alcaliphia*, *P. fluorescens*, *P. mendocina*, *P. pertucinogena*, *P. pseudoalcaligenes*, *P. putida*, *P. rhizosphaerae*, and *P. Tuomuerensis* (Table 2). Of these reference sequences, some were associated with isolates from soil (*P. fluorescens*, *pertucinogena*, *rhizosphaerae*) and cow manure (*P. pseudoalcaligenes*). Other abundant clone matches were with genera of *Acinetobacter* (3), *Chryseobacterium* (2), *Escherichia* (2), *Methylobacterium* (2), *Skermanella* (2), and *Sphingomonas* (3). Clones D34 and D40, were 96% and 91% homologous with uncultured clones from cow fecal and rumen samples, respectively.

The phylogenetic distribution of aerosol clone sequences from the pivot irrigation sites is presented in Fig. 2. Of the 29 unique clone sequences, 17 were related to the α-, β-, and γ-subdivisions of *Proteobacteria*. However, unlike the dairy aerosol samples, more than one-half of the sequences related to *Proteobacteria* clustered with the α, not γ subdivision. At both the dairy and irrigation sites, the fewest number of clones clustered with the β subdivision of *Proteobacteria*. Sequence matches from the pivot irrigation sites are presented in Table 3, most of which were associated with isolates from environmental origins. Four clones in particular (i.e. P11, P13, P15, and P28) were associated with uncultured bacteria from the rumen and raw cow milk. All sequence matches from the background sites (Table 4) matched genera at the pivot and dairy sites, except those from *Aquamicrobium*, *Sediminibacterium*, *Azospirillum*, and *Stenotrophomonas*. A phylogenetic distribution of clone sequences from the background sites is presented in Fig. 3. While species from these genera are mainly from environmental origins and are not pathogenic to humans, *Stenotrophomonas maltophilia* has been identified as an opportunistic pathogen responsible nosocomial infections (Denton and Kerr, 1988).

Among the sample sites, some members of the same genera were commonly present, including *Clostridium*, *Sphingomonas*, *Pseudomonas*, and *Methylobacterium*. *Clostridium* in particular contains species that can cause foodborne illnesses in humans and are present in aerosols at dairy, swine, and poultry operations (Brooks et al., 2010; Nehme et al., 2008;

Table 3
Phylogenetic affiliations of bacterial 16S rRNA gene sequences obtained from aerosol samples collected downwind from the center pivot irrigation systems using dairy wastewater.

Clone	Closet match in GenBank database	Phylogenetic group	Accession no.	% Similarity
P01	*Georgenia* sp. T04-04	*Actinobacteria*	AY880044.1	98
P02	*Sphingomonas melonis* PR-3	α-*Proteobacteria*	FJ605424.1	99
P03	*Sphingomonas rhizogenes* BW59UT1570	α-*Proteobacteria*	JF276901.1	100
P04	*Marinobacter vinifirmus D7035*	γ -*Proteobacteria*	FJ161339.1	98
P05	Uncultured bacterium clone BARB_aaa04d05		EU475675.1	92
P06	*Sphingomonas pituitosa* EDIV	α-*Proteobacteria*	NR_025363.1	99
P07	*Bacteroides* sp. enrichment culture clone dylF13	*Bacteroidetes*	EU834833.1	97
P08	*Methylobacterium rhodesianum* H13	α-*Proteobacteria*	HM245434.1	99
P09	*Variovorax* sp. WPCB174	β-*Proteobacteria*	FJ006917.1	100
P10	*Bradyrhizobium elkanii* USDA 117	α-*Proteobacteria*	HQ233241.1	100
P11	Uncultured rumen bacterium clone P5_I07		EU382029.1	90
P12	*Methylobacterium fujisawaense* 6L3	α-*Proteobacteria*	HQ284832.1	100
P13	Uncultured rumen bacterium clone CARS2E08		GQ327334.1	96
P14	*Novosphingobium pentaromativorans* 17-34	α-*Proteobacteria*	EU167958.1	97
P15	Uncultured rumen bacterium F23-F06		AB185550.1	99
P16	*Rhizobium* sp. T24(2010)	α-*Proteobacteria*	GU998817.1	99
P17	*Paracoccus aminophilus* Zw-11	α-*Proteobacteria*	GU201846.1	94
P18	Enterobacteriaceae bacterium 19NS2	γ -*Proteobacteria*	HQ284948.1	90
P19	*Pseudomonas saccharophila* MG63	β-*Proteobacteria*	AJ746120.1	99
P20	*Sphingopyxis chilensis*	α-*Proteobacteria*	JF459974.1	99
P21	*Arthrobacter* sp. TP-Snow-C29	*Actinobacteria*	HQ327138.1	99
P22	*Pseudomonas flectens* ATCC 12775	γ -*Proteobacteria*	NR_024706.1	95
P23	*Acinetobacter calcoaceticus BHUPSB11*	γ -*Proteobacteria*	GU124831.1	94
P24	*Pseudomonas fluorescens* MS300	γ -*Proteobacteria*	HQ589333.1	90
P25	Clostridiales bacterium JN18_A24_M	*Firmicutes*	DQ168655.1	98
P26	*Hymenobacter* sp. MJ532	*Bacteroidetes*	GU933568.1	100
P27	Uncultured Firmicutes bacterium clone F13_4A_FF	*Firmicutes*	EF682875.1	100
P28	Uncultured *Clostridium* sp. clone M1103	*Firmicutes*	EU029330.1	99
P29	*Clostridium lituseburense*	*Firmicutes*	M59107.1	96

Table 4
Phylogenetic affiliations of bacterial 16S rRNA gene sequences obtained from aerosol samples from the background sites.

Clone	Closet match in GenBank database	Phylogenetic group	Accession no.	% Similarity
B01	*Aquamicrobium aerolatum* Sa14T	α-*Proteobacteria*	FM210786.1	100
B02	*Clostridium lituseburense*	*Firmicutes*	M59107.1	97
B03	Uncultured *Clostridium* sp. clone MS183A1_G06	*Firmicutes*	EF708258.1	99
B04	*Methylobacterium tardum* Sco-A27	α-*Proteobacteria*	FN386716.1	99
B05	*Sphingomonas kaistensis* PB229	α-*Proteobacteria*	AY785128.1	96
B06	*Sediminibacterium* sp. *I-32*	*Bacteroidetes*	AM990455.1	99
B07	*Azospirillum* sp. AKB-2008-TE21	α-*Proteobacteria*	AM988978.1	90
B08	*Candidatus Reyranella massiliensis* TKU11	α-*Proteobacteria*	FR666713.1	90
B09	*Sphingomonas paucimobilis* V18	α-*Proteobacteria*	AM882688.1	99
B10	*Agrobacterium albertimagni*	α-*Proteobacteria*	AF316615.1	99
B11	*Ralstonia pickettii L2*	β-*Proteobacteria*	GQ906999.1	99
B12	*Rhizobium gallicum* S81	α-*Proteobacteria*	AY972457.1	92
B13	*Pseudomonas* sp. SAJ4	γ -*Proteobacteria*	HQ876747.1	99
B14	*Pseudomonas putida* CT363	γ -*Proteobacteria*	GU124699.1	99
B15	*Stenotrophomonas* sp. 3C_5	γ -*Proteobacteria*	AY689032.1	94
B16	*Pseudomonas pseudoalcaligenes* MHF ENV 11	γ -*Proteobacteria*	GU055765.1	99

Ravva et al., 2011). While the clones from the dairy and pivot irrigation sites were not affiliated with bacterial species known to be pathogenic to healthy humans, *Acinetobacter baumannii*, *Corynebacterium amycolatum*, *Pantoea agglomerans*, and *Ralstonia pickettii* were detected in aerosols (Table 2) and have been associated with life-threatening infections in compromised patients (Forbes et al., 1998). Other zoonotic bacterial pathogens commonly associated with cattle and manures, such as *Salmonella* spp. and enterohemorrhagic *E. coli*, were not detected in the aerosols downwind of the dairy and pivots. Two clones from the dairy (D20, D22) though matched with *E. coli*, which are

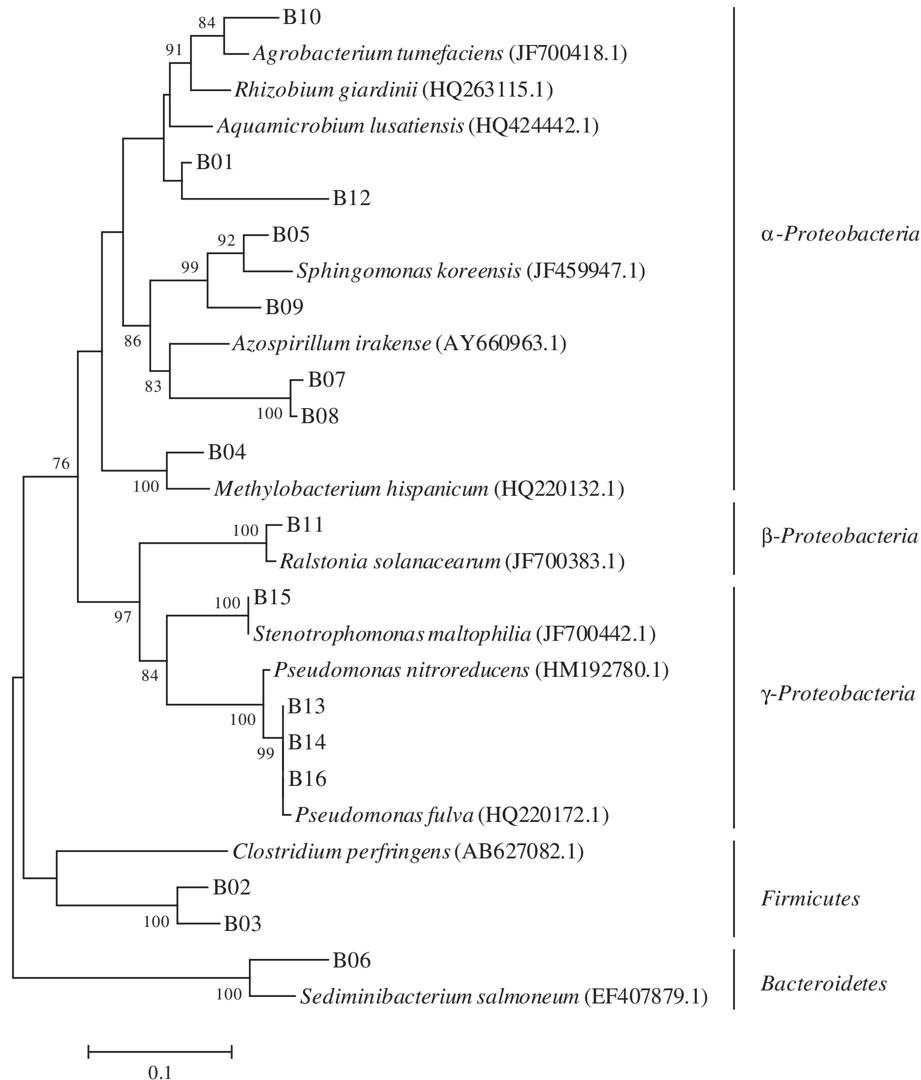

Fig. 3. Phylogenetic tree displaying relationships between the clone sequences collected downwind from the background sites and reference species. Bootstrap values >70% are noted at each node. The bar indicates a 10% estimated sequence divergence.

recognized as being native inhabitants of the gastrointestinal tract of cattle and other mammals (Muirhead et al., 2006; Weaver et al., 2005). *E. coli*, as well as other pathogenic bacteria, have been shown to survive for extended periods in the environment (Gagliardi and Karns, 2002; Nicholson et al., 2005; Sjogren, 1994), therefore, the direct source of aerosolized *E. coli* in this study may not be cattle manure.

Overall, results from this study suggest that there was a low incidence of airborne bacterial pathogens immediately downwind from the dairy and pivot irrigation sites. This result is similar to recent PCR-based aerosol studies conducted at dairies and during the aerial spreading of dairy slurry (Murayama et al., 2010; Ravva et al., 2011). In both cases, the authors did not detect either foodborne pathogens or pathogens associated with an inhalation route of transmission. Although a relatively large volume of air was sampled at each site (dairy, 6.2×10^5 l; pivot, 4.1×10^5 l; background, 3.2×10^5 l), the results should be used cautiously, as the variable nature of aerosol emissions may not have been accounted for during this study. In addition, the specificity of the universal primer sets among a broad range of pathogens is unknown, as well as the DNA extraction efficiencies and PCR detection limits. Thus, viable pathogens may have been present in the aerosols but not detected or conversely, the bacteria identified may represent non-viable organisms as a result of high inactivation rates of some airborne bacteria (Ko et al., 2000; Paez-Rubio and Peccia, 2005; Walter et al., 1990). The uncertainty of these results is further compounded, as there is no way to know if the bacteria identified represent a single cell or a larger number of microorganisms in the aerosol. Despite these limitations, the use of a PCR-based approach allowed for the identification and phylogenetic analysis of a diverse number of aerosolized bacteria near a large dairy operation, which would not have been possible when using a culture-dependent approach. However, if human health risks are to be determined for studies such as these, a quantitative component should be included.

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