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ORIGINAL ARTICLE

Diversity of aerosolized bacteria during land application of biosolids

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Abstract

Aims: The purpose of this study was to determine the diversity of bacterial communities associated with bioaerosols generated during land application of biosolids using 16S ribosomal RNA (16S rRNA) PCR.
Methods and Results: Anaerobically digested Class B biosolids were land applied to an agricultural site located in South Central Arizona. Aerosol samples were collected downwind of the biosolids operations and were collected via the use of SKC Biosamplers and subsequently extracted for the presence of bacterial community DNA. All DNA was amplified using 16S rRNA primers, cloned and sequenced. All sequences were aligned and phylogenetic trees were developed to generate community profiles. The majority of aerosolized bacterial clone sequences belonged to the *Actinobacteria* and *alpha-* and *beta-proteobacterial* taxa. Aerosol samples collected downwind of soil aerosolization produced similar profiles. These profiles differed from upwind and background samples.
Conclusions: No one clone sequence isolated from the aerosol samples could be solely attributed to biosolids; on the contrary, the majority appeared to have arisen from soil.
Significance and Impact of the Study: This study demonstrates that in dry, arid climates the majority of aerosols associated with biosolids land application appear to be associated with the onsite soil.

Introduction

It is widely accepted that biological aerosols are created during agricultural operations. Aerosols are created during routine agricultural operations such as the ploughing of a field, the mechanical collection of crops and the cutting of grass (Swan and Crook 1998; Stetzenbach 2002). It is also accepted that biological aerosols are generated from the land application of biological wastes such as animal manure or biosolids (Pillai *et al.* 1996; Brooks *et al.* 2005). Biosolids are the by-product of sewage wastewater treatment and result from the physical/chemical treatment of sewage sludge (National Research Council 2002). Over the past several years, national (USA.) concern has centred on the quantity and quality of aerosols generated during the land application of biosolids.

Many studies associated with aerosols generated by the land application of biosolids are specifically targeted at the aerosolization of pathogens or indicators of faecal contamination (Sorber *et al.* 1984; Pillai *et al.* 1996; Tanner 2004; Brooks *et al.* 2005). However, even the most comprehensive studies have only been able to detect relatively few pathogenic or indicator bacteria or viruses arising from biosolids material. Sorber *et al.* (1984) was able to detect increased levels of faecal coliforms downwind of biosolids operations; however, no pathogenic viruses were detected even when large sample volumes were analysed. In a similar study, Pillai *et al.* (1996) was only able to detect increased numbers of faecal indicators, specifically *Clostridium perfringens*, from directly downwind of biosolids piles and during biosolids 'loading' events, in which the material was placed into a field applicator using a front-end loader. In a follow-up study, Dowd *et al.*

(1997) was able to detect aerosolized *Salmonella* downwind of the biosolids 'loading' event, but only directly downwind of this event, suggesting that significant mechanical disruption of the biosolids was necessary to create bioaerosols. In two similar studies, total coliforms, *Escherichia coli* and *Cl. perfringens,* were detected at significant levels downwind of 'loading' events, and it was noted that these events were increasingly associated with large amounts of aerosolized dust or soil particles (Tanner 2004; Brooks *et al.* 2005).

While indicator bacteria were detected infrequently in these studies, aerosolized heterotrophic plate count bacteria (HPC) were readily detected downwind of these sites. During biosolids application events, maximum aerosolized HPC levels have been reported to reach as high as 10^5 m^{-3} (Pillai *et al.* 1996; Dowd *et al.* 1997; Tanner 2004; Brooks *et al.* 2005). On the contrary, ambient background agricultural air HPC numbers were typically around 10^3 m^{-3}, at low to moderate wind speeds. Therefore, there is some evidence of bacterial aerosolization taking place during land application operations. However, it is likely that these values represent only a small per cent (0·1–1%) of the total aerosolized bacterial levels because of the potential for the larger viable but nonculturable (VBNC) bacterial populations that may have been present (Roszak and Colwell 1987).

The advent of noncultural (culture-independent) molecular techniques such as those used in the study of microbial ecology (Olsen *et al.* 1986; Pace *et al.* 1986; Head *et al.* 1998; Gentry *et al.* 2004) has now enabled researchers to detect VBNC bacteria. Amplification of the 16S ribosomal RNA (16S rRNA) gene enables the identification of noncultured bacteria. Using this technology, the identity of aerosolized bacterial communities during biosolids land application can be determined. When land application occurs on dry soil, aerosolized HPC numbers are frequently at least two orders of magnitude greater than aerosolized indicator values. Therefore, it could be inferred that HPC bacteria make up the vast majority of aerosolized bacteria during biosolids land application, at least at sites where soil is readily aerosolized (Brooks *et al.* 2005). However, when bacterial counts are elevated, the question remains as to whether these aerosolized bacteria are related to biosolids or other sources such as dry soil or plant material.

The purpose of this study was to determine the diversity of the majority of bacteria aerosolized during the land application of biosolids. To accomplish this, aerosol samples were collected downwind of a biosolids application site in Southern Arizona, prior to and during application. In addition, aerosol samples were collected during tractor operations without land application of biosolids to serve as an additional control. Microbial diversity from all samples was determined using 16S rRNA PCR of extracted DNA (culture-independent). In addition, Class B biosolids and soil were assayed to determine microbial diversity within potential sources of aerosols.

Materials and methods

Sample site

Class B biosolids land application operations located within Maricopa County (South Central Arizona) were selected for this study during the months of November and December of 2004. Class B biosolids is sewage sludge which has been physically or chemically treated to reduce thermotolerant coliform counts to $<2 \times 10^6$ MPN g^{-1} (National Research Council 2002). The biosolids used in this study had been mesophillically digested under anaerobic conditions for 15–20 days. The soil at this site was characterized as a sandy loam and was used for the growth of cotton. The site had not previously received biosolids within the past 5 years.

Bioaerosol sampling

Bioaerosol samples were collected in triplicate via the use of three SKC Biosamplers (SKC Inc., Valencia, CA, USA) operating simultaneously for 20 min and impinged into 0·1% peptone buffer with antifoam agent B. All samples were collected at a height of 1·5 m, thus simulating the average human breathing height (American Society for Testing and Materials 1993). Vacuum pressure was provided by SKC Vac-U-Go sampling pumps (SKC Inc.), which were operated at a constant 12·5 l min^{-1}. Approximately 40 downwind aerosol samples were collected. Downwind samples were collected 5 m downwind of the biosolids operation site (200×400 m), during periods when both biosolids land application 'loading' and 'slinging' operations were occurring. Sample collection began approx. 10 min following operation commencement. In addition to these samples, ambient aerosol control samples were also collected. Here, background (upwind) samples were collected 20 min prior to biosolids exposure and without any mechanical disturbance of the site. In addition, aerosol samples were also collected 5 m downwind of an operation in which soil was aerosolized via the operation of a single tractor without land application of biosolids. These samples were used to ascertain the extent of soil aerosolization and any contributing bacterial sequences from soil. All aerosol samples were only collected during wind speeds of between 2·0 and 3·0 m s^{-1}, with a nominal direction of approx. 90° perpendicular to the aerosol samplers. All aerosol samples were placed on ice, followed by transport back to the laboratory for

analysis. All samples were frozen upon arrival at the laboratory until assay.

Soil and biosolids sampling

Composite soil samples were collected approx. 15 cm below the surface, using a disinfected (70% ethanol) sampling trowel. All samples were placed in sterile soil sample bags (Nasco, Fort Atkinson, WI, USA) and transported back to the laboratory on ice. Prior to DNA extraction and analysis, soil samples were sieved through a 2·0 mm sieve and subsamples were removed and dried to ascertain moisture content. Composite biosolids samples were collected directly from the preapplied biosolids loads, stored in sterile sampling bags and transported to the laboratory on ice. All biosolids were characterized as anaerobically digested Class B biosolids with a dry weight content of 15–20%.

Soil, biosolids and aerosol community DNA extraction

Soil and biosolids community extracted for DNA using the Mo Bio UltraClean Soil DNA Isolation kit (Mo Bio Laboratories, Carlsbad, CA, USA) using the recommended extraction protocol provided by the manufacturer. Briefly, a total of 1·0 g was extracted from soil or biosolids samples by first placing 0·25 g into an extraction tube. This was repeated four times (e.g. four separate extractions = 1·0 g); extracted DNA was subsequently combined into one tube to comprise a single composite sample of 200 μl DNA solution. This single composite volume was concentrated and further purified using a Qiagen PCR purification kit (Qiagen Inc., Valencia, CA, USA).

Aerosol samples were defrosted in a 25°C water bath prior to extraction of DNA. Of the approximate 40 biosolids slinging and loading downwind bioaerosol samples collected, a random selection of three replicate aerosol samples were combined and concentrated using a Centriprep 50 liquid concentrator (Millipore, Billerica, MA, USA) to yield one composite aerosol sample. Briefly, 15 ml from each replicate aerosol sample was centrifuged at 1000 ***g*** for 5 min, followed by a second centrifugation step at 1000 ***g*** for 5 min and a third centrifugation step at 1000 ***g*** for 1 min. This was repeated for each replicate sample using the same concentrator. Approximately 45 ml was concentrated (15 ml from each separate sample) and following this concentration step, a total of 1·0 ml was available from each composite aerosol sample. This was repeated for both ambient and soil aerosolization bioaerosol samples.

Composite samples were subsequently extracted for DNA using the Mo Bio UltraClean Soil DNA Isolation kit. The soil DNA extraction protocol was modified for DNA extraction of aerosol collection fluid, by decanting the lysis buffer provided in tube 1 of the extraction kit and leaving only the lysis beads. At this point, 0·25 ml of composite aerosol sample was placed into the tube and extracted according to the manufacturer's protocol. A total of 50 μl of extracted DNA solution was available following each extraction. This process was repeated four times to extract the entire amount (1 ml) of the composite aerosol sample. Each 50 μl volume was then recombined to a volume of 200 μl and was further concentrated and purified using the Qiagen PCR purification kit to a final volume of 50 μl.

Initial 16S PCR amplification of community DNA

Following purification, 1 μl of total DNA was used as template in a 16S rRNA gene PCR reaction using the following conditions: 1 × Gold Buffer (Applied Biosystems, Foster City, CA, USA), 2·5 mmol l^{-1} $MgCl_2$ (Applied Bioystems), 0·2 mmol l^{-1} DNTP (Applied Biosystems), 1·5 U Taq Gold (Applied Biosystems), 16S rRNA primers 45 F (5′ GCCTAACACATGCAAGTCGA 3′) and 784R (5′ GGACTACCAGGGTATCTAATC 3′) (J. McQuaid, Pers. Communication) (0·5 μmol l^{-1} each), dH_2O (Integrated DNA Technologies, Coralville, IA, USA) to 50 μl volume. All reactions were carried out in an Applied Biosystems Geneamp PCR system 2700 (Applied Biosystems). Cycling conditions were: initial denaturation, 95°C (10 min); 36 cycles at denaturation 95°C (40 s), annealing 51°C (45 s); and extension, 72°C (70 s), followed by two holds at 72°C (5 min) and 4°C infinite hold. This PCR reaction resulted in a 739-bp product.

Following 16S PCR amplification, the PCR product was purified using the Qiagen PCR purification kit to a final volume of 50 μl. This entire volume was then placed in a 1·6% agarose gel, operated at 120 V for 60 min and stained with ethidium bromide. This was followed by visualization using UV light box operated at approx. 365 nm with a glass plate placed between the UV source and the gel. The product was then excised with a sterile scalpel blade and purified using a Qiagen PCR Gel Purification kit following the manufacturer's recommended protocol.

Community 16S PCR product analysis

Following gel DNA purification, each DNA sample was ligated into a transport plasmid vector using the Promega pGEM-T Vector System and cloned using Promega JM109 high efficiency competent cells (Promega, Madison, WI, USA) following the manufacturer's recommended protocol. Positive (i.e. white) colonies with inserts were selected and subjected to colony PCR for product

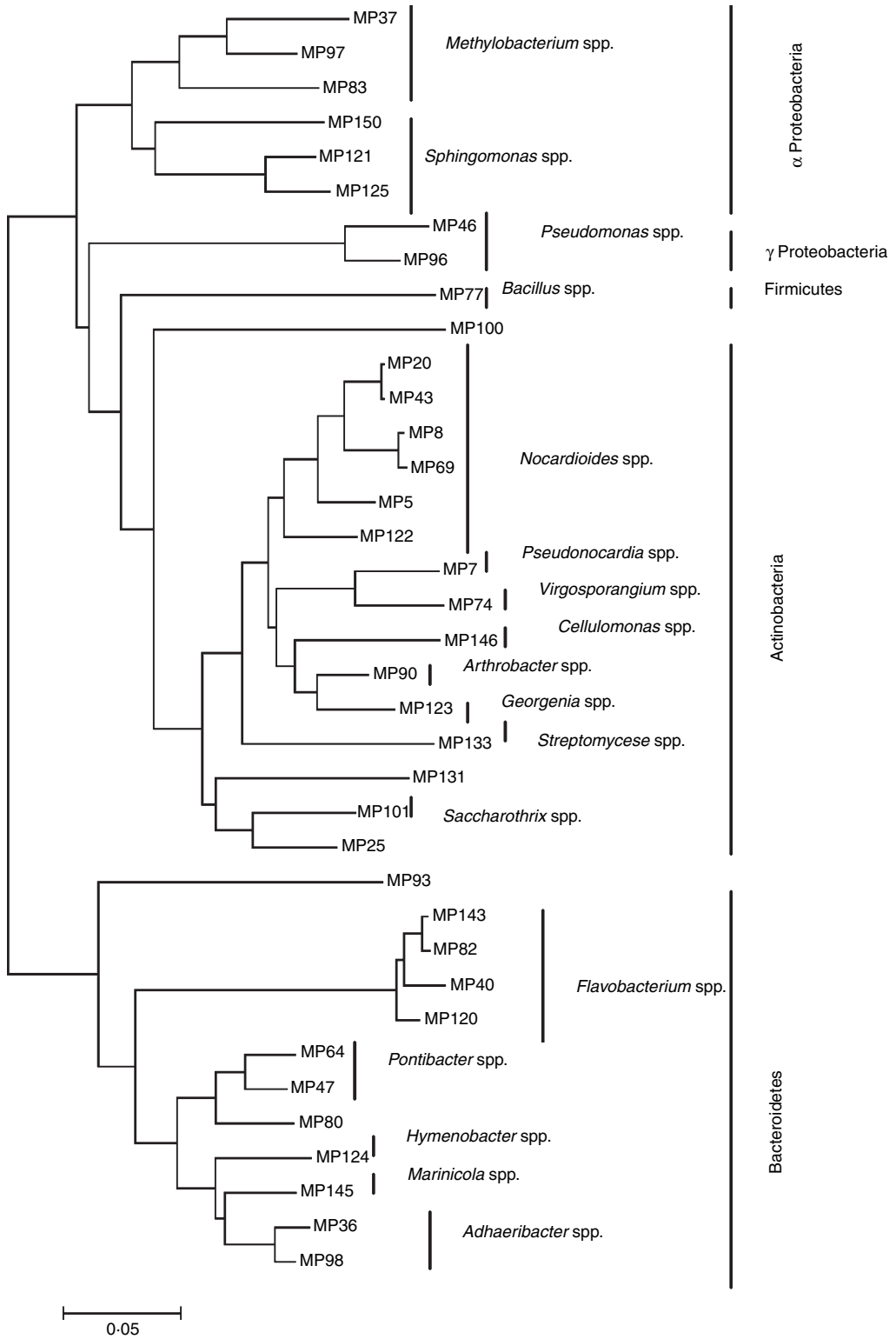

Figure 1 Phylogenetic tree displaying relationships between the isolated clone sequences collected from soil sampled on site with no prior biosolids application. Genus identification level is noted to the side of each unique clone sequence followed by bacterial class/phylum identification. No mark next to the clone sequence represents below 97% homology to available sequences. All branches represent at least a 50% bootstrapping value, when bootstrapped with a level of 500 replications.

insert confirmation. Each white colony was removed with a sterile inoculation loop or needle and placed into 100 μl sterile Tris buffer (pH 7·4), followed by pulse vortexing at high speed for 10 s. Colony DNA was removed by heating the extracted colony to 98°C for 10 min. The resulting solution was subsequently centrifuged at 10 000 ***g*** for 4 min. A total of 10 μl of the supernatant was then used in a PCR assay for insert verification, in which identical PCR conditions were followed as previously stated, with the exception that primers T7 and SP6 (Integrated DNA Technologies) were used in lieu of the previously used 16S specific primers (45 F, 784R). Specifically, these primers were complementary for the two vector promoter sequences, which flank the product insertion site. A 900 bp PCR product was then visualized on an ethidium bromide stained 1·6% agarose gel.

DNA restriction digest to delineate unique clones

Following colony PCR, the 900-bp product was subject to restriction enzyme digestion, to identify unique clone inserts (i.e. nonredundant clone inserts) and avoid redundancy in DNA sequencing. A total of 20 μl was added to a 5 μl enzyme mix. The enzyme mix was comprised of enzyme Mse I (3 U) (New England Biolabs, Ipswich, MA, USA), Rsa I (3 U) (New England Biolabs), NE Buffer II (1×) (New England Biolabs), BSA (0·05 μg) (New England Biolabs) and nuclease free H_2O (to 5 μl) (New England Biolabs). Digestions were incubated at 37°C for 2 h. All digestion products were visualized using ethidium bromide on a 3% Metaphor Gel (Cambrex, East Rutherford, NJ, USA) operated at 120 V for 3 h. Digest profiles were screened visually to identify unique profiles

Table 1 Grouping of bacterial sequence clone isolates from a biosolids land application site located in Maricopa County, AZ

Sample group	Type	Clones sequenced	Unique clones*	Bacterial group†
M4	Background aerosol	62	6	Proteobacteria (Alpha)
			2	Proteobacteria (Beta)
			6	Proteobacteria (Gamma)
			1	Actinobacteria
			1	Firmicutes
			16	
M5	Downwind biosolids operation aerosol	77	5	Proteobacteria (Alpha)
			7	Proteobacteria (Beta)
			1	Proteobacteria (Gamma)
			7	Actinobacteria
			2	Bacteroidetes
			3	Firmicutes
			2	Unclassified
			27	
M8	Downwind soil operation aerosol	74	11	Proteobacteria (Alpha)
			3	Proteobacteria (Beta)
			7	Actinobacteria
			3	Bacteroidetes
			1	Firmicutes
			1	Unclassified
			26	
MP	Soil	83	6	Proteobacteria (Alpha)
			2	Proteobacteria (Gamma)
			15	Actinobacteria
			11	Bacteroidetes
			1	Firmicutes
			2	Unclassified
			37	
MBS	Class B biosolids	77	2	Proteobacteria (Alpha)
			2	Proteobacteria (Beta)
			11	Proteobacteria (Gamma)
			7	Chloroflexi
			22	
Total		373	129	

*Unique clones (i.e. noredundant 16s rRNA sequences) used to prepare phylogenetic trees.
†Phylum designation (Class).

and identify redundant digest profiles. All unique inserts were then subject to DNA sequencing.

Phylogenetic analysis of unique clone isolates

White colonies which were screened and determined to contain unique 16S PCR products were subject to another round of colony PCR, from which the total 50 μl volume was purified using the Qiagen PCR Purification kit following manufacturer instructions. Following purification, samples were sent to the University of Arizona DNA Sequencing Facility. An end-terminus dye ABI Prism 7000 (Applied Biosystems) sequencer was used for DNA sequencing, followed by sequence query submission to the NCBI Blast Program available on the World Wide Web (Altschul *et al.* 1990). Only sequences with at least 500 bp of readable sequence were used, with most sequences containing at least 650 bp of readable sequence. In addition, only sequences which showed at least 98% sequence similarity to available accession sequences were used for identification to the genus level. Unidentifiable, unique sequences were only identified to the bacterial phylum level. Blast sequence identities were aligned with the Clustal X 1·83 program (Thompson *et al.* 1997), from which phylogenetic relation-

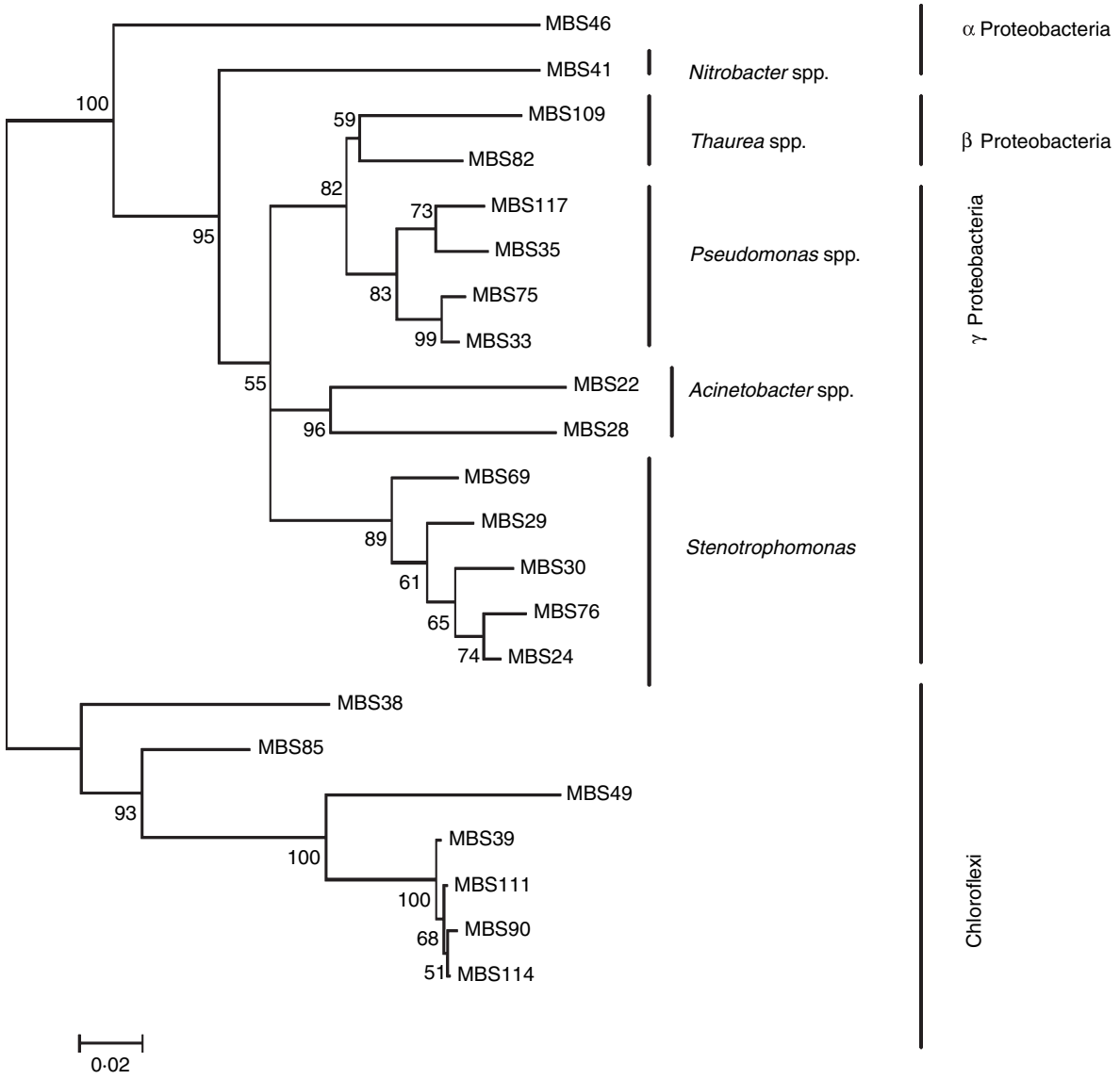

Figure 2 Phylogenetic tree displaying relationships between the isolated clone sequences collected from Class B anaerobically digested biosolids prior to land application. Genus identification level is noted to the side of each unique clone sequence followed by bacterial class/phylum identification. No mark next to the clone sequence represents below 97% homology to available sequences. All branches represent at least a 50% bootstrapping value, when bootstrapped with a level of 500 replications.

ships were identified. The Neighbour Joining distance correction method of Jukes Cantor was used to construct phylogenetic trees using Mega 3·1 (Kumar *et al.* 2004) with a bootstrapping value of 500. All tree branches demonstrating less than 50% when bootstrapped were omitted from the presented trees. Tree labels were fixed using Microsoft Word XP (Microsoft Corp., Redmond, WA, USA).

Results

Community DNA samples from soil with no prior exposure to biosolids were analysed for the presence of unique 16S rRNA gene sequences and demonstrated the presence of bacterial community members typically found in an arid sandy loam soil (Fig. 1). Bacteria from the *Actinobacteria* phylum (15/37), the *alpha-proteobacteria* (class level taxonomy) (6/37) and *Bacteroidetes* (11/37) phyla of bacteria were found to dominate the unique clone sequences analysed for this study. Sequences belonging to the *gamma-proteobacteria* class of bacteria such as *Pseudomonas* spp. were among the least represented unique clone sequences isolated from the pristine (no previous biosolids application) soil (Table 1).

Biosolids (Class B, anaerobically digested) collected from the application site were found to contain a much less diverse community profile than that from the 'pristine' soil (Fig. 2). Members of the *gamma-proteobacteria* class comprised 11/22 of the clones. The profiles revealed a dominating presence of members of the *Stenotrophomonas* (5/22) and *Pseudomonas* (4/22) genera relative to other members within this class. Members of the *Chloroflexi* phylum were found to be abundant and represented one in three of all clone sequences analysed (7/22). Members of the *Chloroflexi* phylum were unique only to biosolids.

Air samples collected from the site during background operations when no biosolids were being loaded or applied were amongst the least bacterially diverse of all samples analysed. These sequences represent the typical background aerosolized bacterial diversity found during nonbiosolids operations, nonagricultural operations with little to no soil disturbance. Here, 14 of 16 unique clones were members of the *alpha-*, *beta-* or *gamma-proteobacteria* class. Amongst the 16 unique clone sequences found (Fig. 3), the majority of these sequences were shown to be members of the *Pseudomonas*, *Stenotrophomonas*, *Methylobacterium* and *Agrobacterium* genera. It is import-

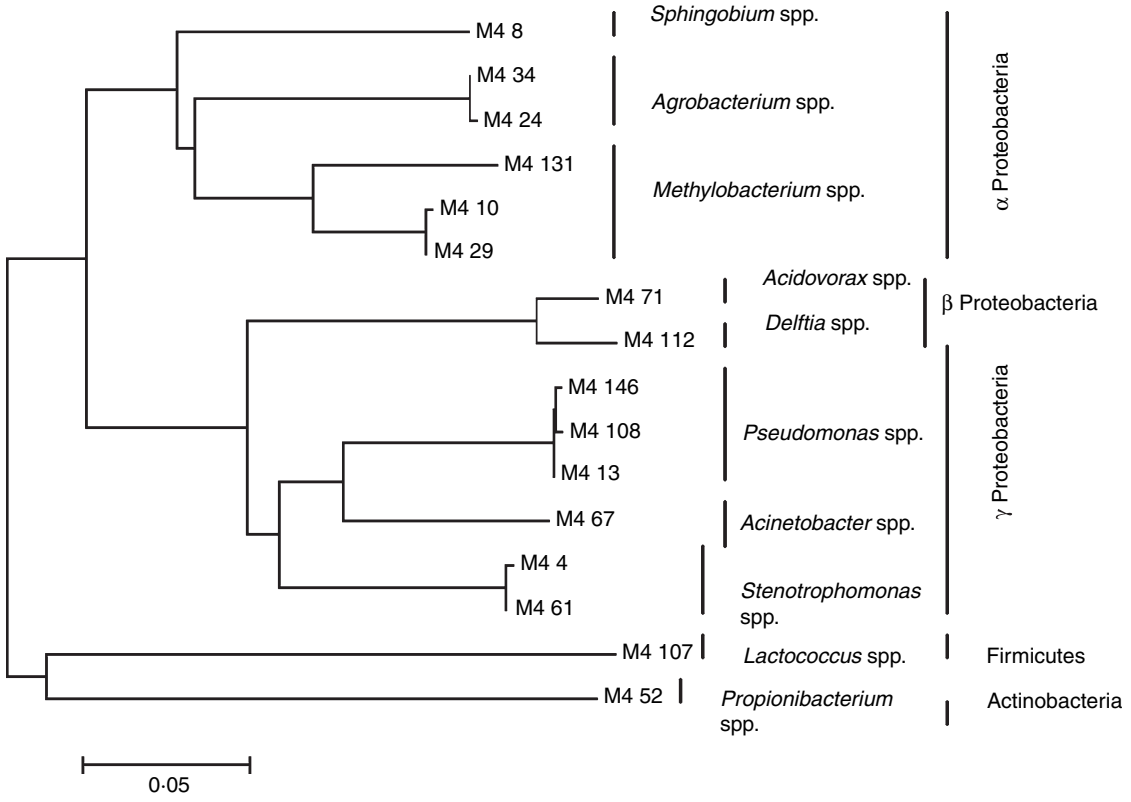

Figure 3 Phylogenetic tree displaying relationships between the isolated clone sequences collected during background aerosol sample collection (ie. no biosolids application, no field disturbances). Genus identification level is noted to the side of each unique clone sequence followed by bacterial class/phylum identification. All branches represent at least a 50% bootstrapping value, when bootstrapped with a level of 500 replications.

ant to note that although only 16 sequences were represented in the phylogenetic tree, 62 background clone isolates were analysed either to the restriction digest or sequencing level.

Downwind aerosol samples collected during biosolids operations yielded no one genus that could be attributed to biosolids exclusively. It was noted that a shift in the 16S profiles, relative to the upwind background aerosol samples, did occur. Alignments demonstrated the presence of multiple aerosolized unique clone sequences (27). Among the 27 clone sequences represented in the tree (Fig. 4) members of the *Proteobacteria* phylum were

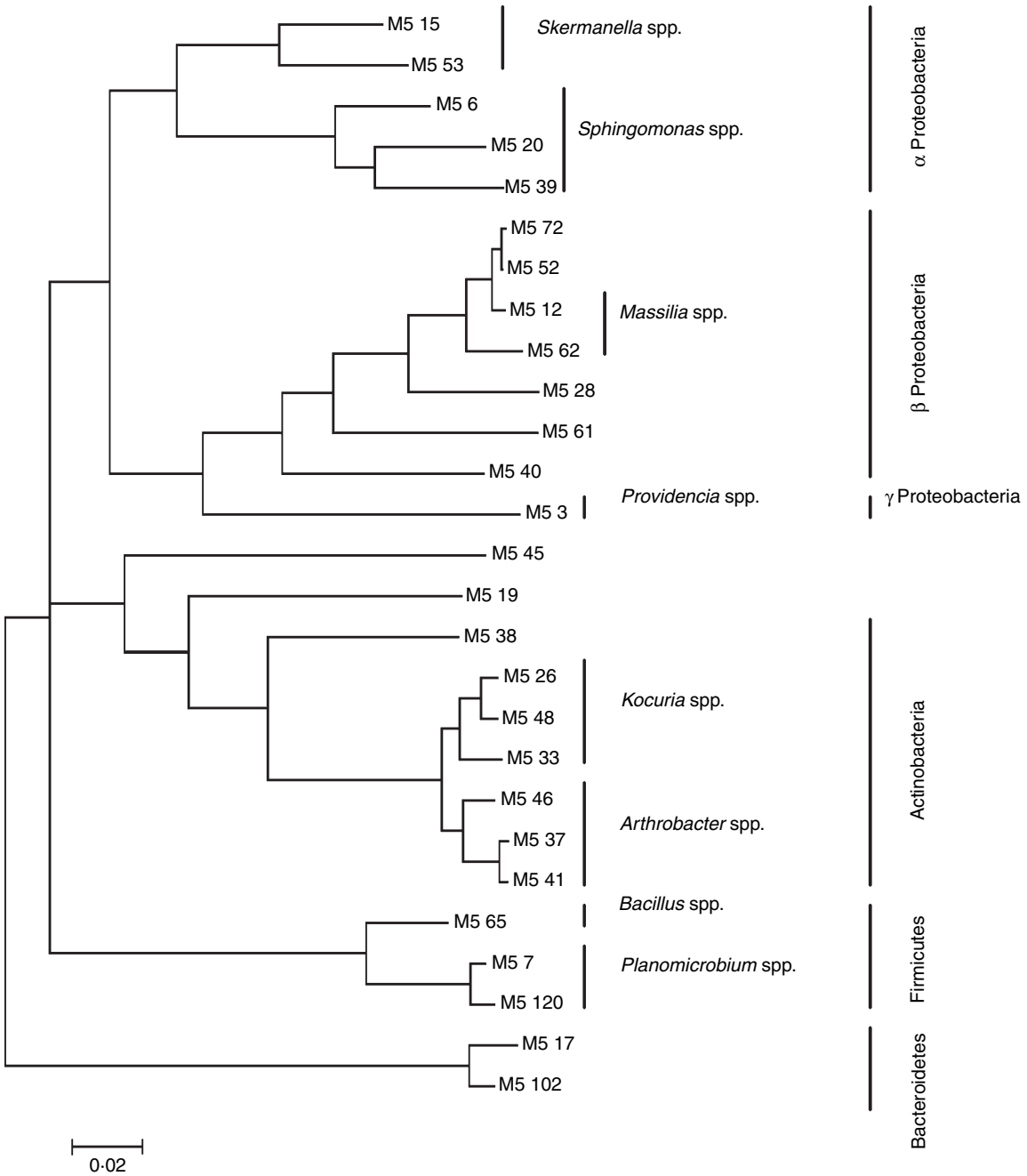

Figure 4 Phylogenetic tree displaying relationships between the isolated clone sequences collected from downwind aerosol samples during biosolids operations. Genus identification level is noted to the side of each unique clone sequence followed by bacterial class/phylum identification. No mark next to the clone sequence represents below 97% homology to available sequences. All branches represent at least a 50% bootstrapping value, when bootstrapped with a level of 500 replications.

amongst the dominant isolates, with the *alpha-* (5/27) and *beta-proteobacteria* (7/27) classes represented. The *Actinobacteria* phylum also represented a large portion of the unique sequences (7/27). Members of the *Firmicutes* and *Bacteroidetes* phyla were also represented, however, only a few clone sequences were represented (5/27). A single clone sequence representing the *Providencia* genus was also detected from these aerosols.

Aerosolization of pristine soil borne bacteria (no biosolids land application) were amongst the most diverse 16S profiles isolated. The samples were collected during tractor operation on a near-by upwind site with a similar soil profile as that of the experimental site used for land application (Fig. 5). The site had no previous biosolids land application and no biosolids were applied to the field, before or during sample collection. The aerosolized

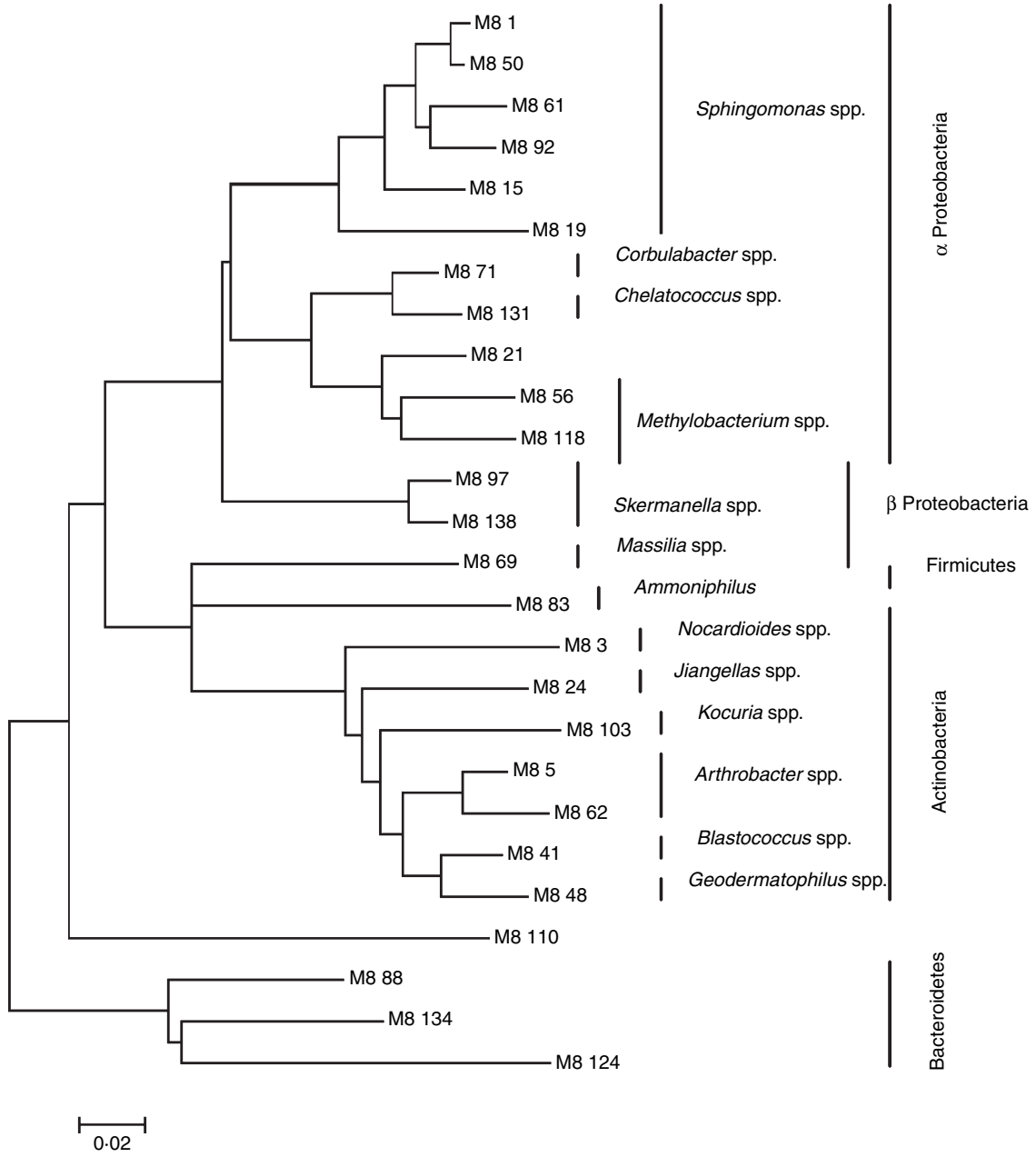

Figure 5 Phylogenetic tree displaying relationships between the isolated clone sequences collected from downwind aerosol samples during a tractor operation involving only soil aerosolization (ie. no land application of biosolids). Genus identification level is noted to the side of each unique clone sequence followed by bacterial class/phylum identification. No mark next to the clone sequence represents below 97% homology to available sequences. All branches represent at least a 50% bootstrapping value, when bootstrapped with a level of 500 replications.

community profile revealed the presence of aerosolized bacterial identities which were typical of the site soil bacterial profile such as those found in the *alpha-proteobacteria* and *Actinobacteria* class and phylum. Like the aerosols collected downwind of the biosolids operations, these aerosolized bacterial communities were amongst the most diverse of the samples collected. A total of 26 unique clone sequences were found (Fig. 5). Amongst these sequences a total of 13 different genera were represented.

Discussion

It appears that amongst the aerosolized 16S rRNA community profiles present during biosolids land application operations, most appeared to have originated from the onsite soil, as aerosol samples collected downwind of biosolids application and soil aerosolization (no biosolids) yielded little differences at the class level of classification (Fig. 6). At the very least, no bacterial 16S rRNA gene sequence (as determined by NCBI Blastn) found in these downwind bioaerosol samples were found exclusively in the biosolids. The major dominant phyla or class for both populations (soil or biosolids aerosolization samples) were *Actinobacteria*, *beta-proteobacteria*, *alpha-proteobacteria* and *Bacteroidetes*, while *Actinobacteria* and *alpha-proteobacteria* were detected more often. Both these latter taxa can have soil origins; however, it is important to note that *alpha-proteobacteria* and

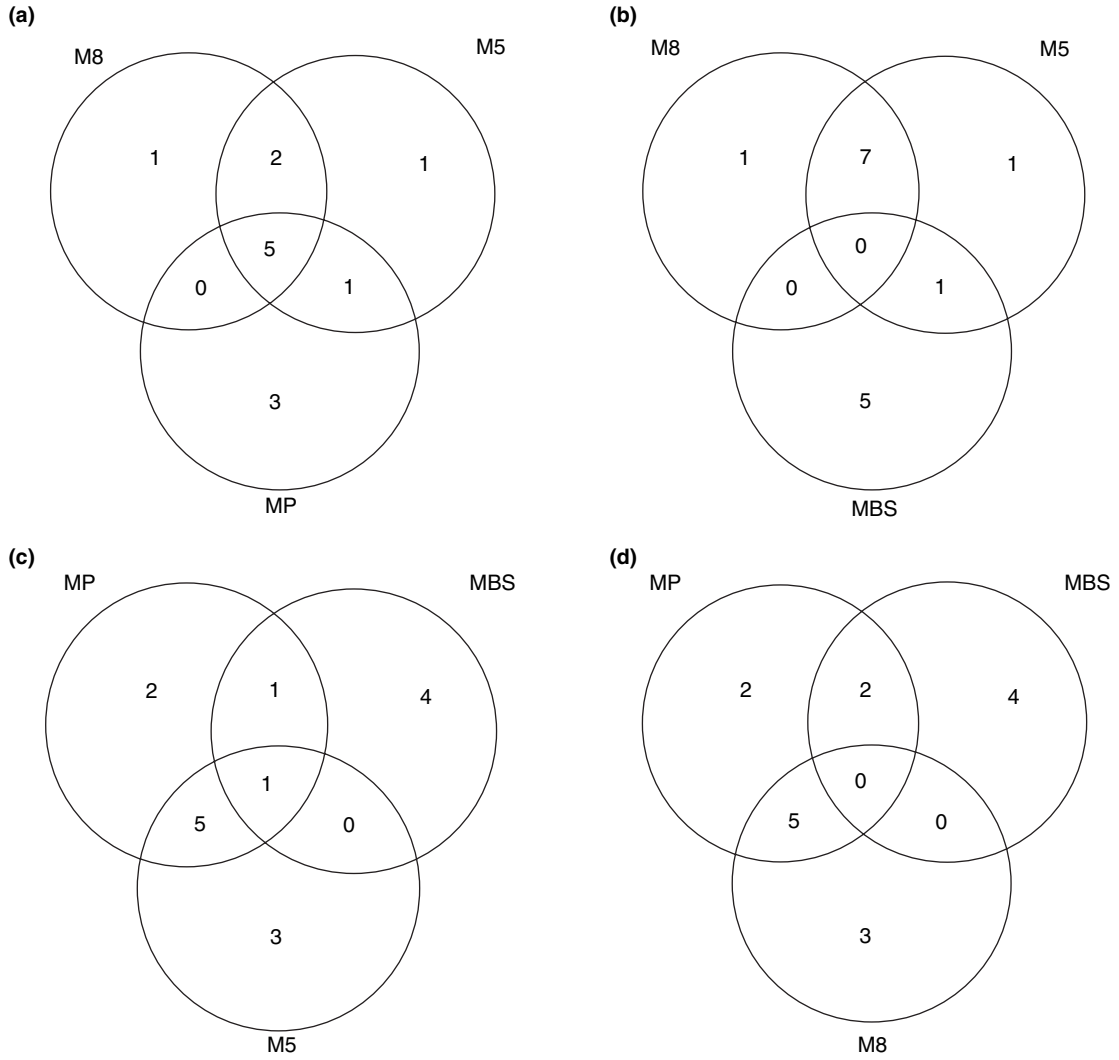

Figure 6 (a) Number of shared orders associated with unique aerosolized clone isolates detected from downwind of aerosolized soil without biosolids (M8), from downwind of biosolids land application operations (M5) when compared to onsite soil (MP); (b) when compared to Class B biosolids (MBS); (c) Number of shared orders associated with unique clone isolates detected from onsite soil (MP), Class B biosolids (MBS) when compared to aerosols collected during biosolids land application operations (M5); (d) when compared to aerosolized soil without biosolids (M8).

Actinobacteria can be found in activated sludge (Sekiguchi *et al.* 1998). The latter was not found in the biosolids samples analysed for this study. On the contrary, members of the phylum *Chloroflexi* were found to be exclusively present in the biosolids and in a large portion of the isolated clone sequences. As such, members of this phylum could prove to be a useful model for tracking bioaerosols from biosolids (Baertsch *et al.* 2006; Rubio *et al.* 2006). However, in this study, no members of that phylum were found in aerosol samples collected during land application operations.

Many biosolids application sites, particularly those in dry, arid environments result in the aerosolization of dust or dry soil (Pillai *et al.* 1996; Brooks *et al.* 2005). As soil or plant debris is present in such a dry state, it is more easily aerosolized. Aerosolized soil can contain culturable bacteria at levels as high as 10^8 g^{-1}. In addition, it is likely that there are at least two orders of magnitude greater numbers of nonculturable bacteria; therefore, a large number of aerosolized clone sequences potentially belonging to soil-borne bacteria were not unexpected. However, anaerobically digested Class B biosolids can also contain upwards of 10^8 g^{-1} culturable bacteria and could likely contribute to aerosolized bacteria. As such it can be difficult to state with certainty whether soil or biosolids are the major source of aerosolized micro-organisms during land application of biosolids, without the use of more complex bacterial source tracking methods such as pulse field gel electrophoresis (PFGE) of selected cultured isolates (Tenover *et al.* 1995).

It is important to note that while a relatively large volume of air was analysed for the presence of these sequences, it only represented a small portion of the available downwind air, and as such it is possible that some unique sequences may not have been detected. In addition, although these techniques are quite useful, their ultimate success depends not only on the capabilities of the aerosol sampler but also on the ability of the PCR amplification step to capture the diversity of the available 16S gene sequences. It should also be noted that while this type of analysis may be able to suggest relative aerosolized bacterial levels, it cannot be used as a quantitative approach for overall microbial aerosolized densities. It has been shown that PCR amplification reactions can favour a specific 16S sequence and bias results, giving the illusion of abundance with regard to a specific sequence and vice versa (Becker *et al.* 2000; Kanagawa 2003; Kurata *et al.* 2004). New approaches are now available for the isolation and quantification of previously 'unculturable' bacteria, and these approaches could be used for more quantitative measures (Joseph *et al.* 2003).

Results presented here may be typical of this type of operation in a dry arid environment, where soil can be aerosolized more easily. When soils are moist, the levels of aerosolized culturable micro-organisms present during the land application of biosolids have been shown to be greatly reduced, and it has been demonstrated that without the incorporation of large amounts of dry easily aerosolized soil, the amount of detectable culturable aerosolized micro-organisms greatly decrease (Brooks *et al.* 2005). Hence, in this current study, the use of molecular methods to ascertain aerosolized bacterial populations suggest that soil may be a large contributor of the bacterial sequences found in the downwind air environment associated with the land application of anaerobically digested Class B biosolids in arid climates; however, further studies appear to be warranted involving more complex source tracking methods.

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