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Characterization of the airborne bacteria community at different distances from the rotating brushes in a wastewater treatment plant by 16S rRNA gene clone libraries

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Abstract

Biological risks of bioaerosols emitted from wastewater treatment processes have attracted wide attention in the recent years. However, the culture-based analysis method has been mostly adopted for detecting the bacterial community in bioaerosols, which may result in the underestimation of total microorganism concentration as not all microorganisms are cultivable. In this study, oligonucleotide fingerprinting of 16S rRNA genes was applied to reveal the composition and structure of the bacterial community in bioaerosols from an Orbal oxidation ditch in a Beijing wastewater treatment plant (WWTP). Bioaerosols were collected at different distances from the aerosol source, rotating brushes, and the sampling height was 1.5 m which is the common respiratory height of a human being. The bacterial communities of bioaerosols were diverse, and the lowest bacterial diversity was found at the sampling site just after the rotating brush. A large proportion of bacteria in bioaerosols were affiliated with Proteobacteria and Bacteroidetes. Numerous bacteria present in the bioaerosols also emerged in water, indicating that the bacterial community in the bioaerosols was related to that of the aerosols’ sources. The forced aeration of rotating brushes brought about observably distinct bacterial communities between sampling sites situated before and after the rotating brush. Isolation sources of closest relatives in bioaerosols clone libraries were associated with the aqueous environment in the WWTP. Common potential pathogens in bioaerosols as well as those not reported in previous research were also analyzed in this study. Measures should be adopted to reduce the emission of bioaerosols and prevent their exposure to workers.

Key words: airborne bacteria; genetic structure; clone library; wastewater treatment plant; rotating brushes

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Introduction

Microorganisms with small aerodynamic diameters in water can be easily released into the atmosphere and become bioaerosols during wastewater treatment processes (Bauer et al., 2002; Sánchez-Monedero et al., 2008). Those portions containing moving mechanisms or forced aeration of wastewater are the stages with high bioaerosols emission (Brandi et al., 2000; Orsini et al., 2002). The bioaerosols may contain any pathogenic microorganism present in wastewater and constitute a potential danger to plant workers and nearby residents’ health (Carducci et al., 2000; Grisoli et al., 2009; Heinonen-Tanski et al., 2009).

Numerous studies have been carried out to estimate the level of bioaerosols present at each stage in wastewater treatment plants (WWTPs). Different types of WWTPs were investigated, and the ratio between cultivable bacteria and fungi in the aerosol during aeration was determined and compared (Bauer et al., 2002). The assessment of bioaerosols exposure risks to human health focused on identifying the hazards and possible human health associated with aerosolized pathogens produced in the processes of wastewater treatment. Klebsiella pneumoniae, Legionella sp. and Mycobacterium tuberculosis in bioaerosols generated in aeration basins from WWTP have been reported. Their potential hazards to on-site operators and adjacent communities were also evaluated (Brandi et al., 2000; Carducci et al., 2000; Sánchez-Monedero and Stentiford, 2003).

The bioaerosols detection methods in previous research usually depended on culture-based analysis, which provides the number of cultivable microorganisms in an air sample. However, the determination of only the cultivable airborne microorganism concentration may result in the...
underestimation of total microorganism concentration, as not all microorganisms are cultivable. The 16S rRNA gene clone library is a powerful tool in identifying bioaerosols (Brooks et al., 2010). It has been applied to examine the bacterial communities of aerosols and detect the size distribution of microorganisms in the atmosphere over the eastern Mediterranean during a storm (Polymenakou et al., 2008), as well as Asian dust events (Jeon et al., 2011), and to analyze the community composition and diversity of air samples collected from northern France (Maron et al., 2005). While this technique is widely used to assay microbial communities in the atmospheric environment, its application in determination of the genetic structure and diversity of airborne bacterial communities generated in WWTPs has been seldom reported up to now. The assessment of total bacteria in bioaerosols has profound significance for exploring the distribution and characteristics of potential pathogenic bacteria in bioaerosols from WWTPs.

We have investigated the distribution of cultivable airborne bacteria and fungi at different treatment units in a Beijing municipal WWTP and high levels of cultivable airborne bacteria and fungi were observed from the aeration system in the Orbal oxidation ditch (Li et al., 2011). However, the total bacterial structure and their migration have not been previously revealed. Therefore, the aims of the present study were to: (1) assess the genetic structure and diversity of total bacteria in bioaerosols generated from a WWTP that uses an oxidation ditch process by 16S rRNA; (2) determine the variation of bacterial communities in bioaerosols to find out their relationship with the distance from the aerosol source; (3) characterize their resources; (4) analyze the presence of potential pathogens associated with bioaerosols from the oxidation ditch.

1 Materials and methods

1.1 WWTP description and sampling locations

The study to assess the genetic structure and diversity of airborne bacterial communities was conducted in a municipal WWTP that uses an Orbal oxidation ditch process (Li and Liu, 2011). This plant with 80,000 m$^3$/day of design capacity is located in the southern part of Beijing, China. Several sets of rotating brushes with rotating speed of 50–60 t/min were installed across the channel of the Orbal oxidation ditch to aerate the water and push the flow.

Aerosol samples were collected at different distances from the rotating brushes in the oxidation ditch, the source of bioaerosols (e.g. 0.5 m before, 0.5 m after, and 40 m downwind from the rotating brush). All samples were collected 1.5 m above the ground level. The sampling sites established at the WWTP monitored airflow from the aerosol source (rotating brushes in the oxidation ditch) and are shown in Fig. 1. Water (AW) collected from the oxidation ditch was used as a control measurement.

Parallel to the microbiological assays, meteorological parameters, e.g. temperature, relative humidity, wind speed and irradiance, were also observed. The wind speed and irradiance were measured by a portable anemometer (HD2303, Delta OHM, Italy) and irradiance meter (HD2302.0, Delta OHM, Italy), respectively. Temperature and relative humidity were recorded by a Dewpoint Thermohygrometer (WD-35612, OAKTON, Germany). During bioaerosols sampling, the ranges of wind speed, irradiance, temperature and relative humidity were 0.15–0.42 m/sec, 419.2–437.6 W/m², 28.5–31.3°C and 47.3%–50.2%, respectively.

1.2 Air sample collection

A glass impingement airborne microorganism sampler (SKC BioSampler, SKC Inc., USA) was applied for bioaerosols collection. The SKC BioSampler was designed for sampling airborne particulates of biological origin, e.g., bacteria, fungi, pollen, and viruses. It consists of an inlet, a nozzle section with three tangential sonic nozzles, and a collection vessel. In cases of low bacterial concentrations, the impingement method is preferable to the impaction method (Brandi et al., 2000). In an impinger the separation of the particulates from the air stream takes place by collision with a liquid surface. A 20-mL of sterile phosphate buffered saline was filled in the collection vessel as the collection medium. Aerosol samples were transferred into the collection medium by drawing the gas at a flow rate of 12.5 L/min. The volume of sampling for each sample was 1.5 m$^3$. Once the required volume of air had been drawn through, the sampler was removed and sealed to prevent contamination. Cells in liquid were concentrated according to previous research (Moletta et al., 2007). All samplers were sterilized in advance in an autoclave for 120 min and the inside surfaces were maintained in a sterile condition until sampling.

Airborne microorganisms from each sampling site described above and an outdoor control (OC, sampled upwind 80 m from the oxidation ditch) were captured to calculate the concentration of culturable bacteria in bioaerosols with a six-stage impacting airborne microorganism sampler (FA-1, China) as described in our previous report (Li and Liu, 2011). Results were calculated as the
geometric mean of the replicates and expressed as colony forming units per cubic meter of air (CFU/m$^3$).

1.3 DNA extraction, PCR amplification and sequencing

The isolation of total DNA was accomplished with Magnetic System-16 (TanBead, Taiwan). Universal primers (F16S-27/R16S-1492) were used to amplify the segment of eubacterial 16S rRNA (Martin-Laurent et al., 2001). The PCR amplification reaction was performed using the Gene AmpR PCR System (9700, AB, USA) at a final volume of 50 μL and followed by 25 cycles of 94°C for 30 sec, 56°C for 30 sec, and 72°C for 90 sec. After purification by an Agarose-Gel Extraction Kit (Dinggguo, China), the PCR products were ligated and transformed into E. coli DH5α competent cells. The positive clones were selected and cultured on LB medium with X-gal, IPTG and Amp to submit for sequencing using the ABI 3730D XL DNA sequencer (AB, USA).

1.4 Clone library construction and phylogenetic analysis of sequences in clone libraries

All sequences were manually checked and trimmed to exclude vector sequences, then checked for chimeras using Bellerophon from the Greengenes website (http://greengenes.lbl.gov/). Seventy clones were picked up from each sample. The excluded chimeras and false positive clones were 5 and 5 for AFB, 5 and 5 for ABB, 4 and 5 for 40m.ABB and 5 and 6 for AW, respectively. Sequences which had a threshold of over 97% similarity were aligned and classified into universal operational taxonomic units (OTUs), and individual sequences were also assigned into OTUs as individual OTUs. The representative OTU sequences obtained in this study were deposited in the NCBI database under the accession numbers (JQ624242-JQ624361).

1.5 Diversity and richness estimation of clone libraries

To determine whether the number of a clone library was large enough to represent the diversity of an original community, the coverage (C) of each clone library was calculated according to the following equation:

\[
C = 1 - \frac{n}{N}
\]

where, \(n\) is the number of unique clones and \(N\) is the total number of sequences examined (Good, 1953). Rarefaction curves were created using the species diversity function of the Analytic Rarefaction 1.3 statistical software (Holland, 2003). The Shannon Wiener index was used to estimate the diversity of each clone library (Ding et al., 2008).

1.6 Accession numbers for nucleotide sequences

The representative OTU sequences obtained in this study (OTU-1-AW to OTU-34-40m.ABB) were deposited in the NCBI database under the accession numbers (JQ624242-JQ624361).

2 Results and discussion

2.1 Culturable airborne bacteria at each sampling site

Concentrations of airborne bacteria at each WWTP sampling site are shown in Fig. 2. Among the sampling sites, ABB had the highest concentration of culturable airborne bacteria with 2049 CFU/m$^3$. Mean concentrations of heterotrophic bacteria in OC were only 512 CFU/m$^3$, approximately one quarter of that detected at ABB. The concentration of bacteria at ABB was higher than that at AFB which was situated before the rotating brush, due to the mechanical rotation of the rotating brush. With the extension of distance from the rotating brush, the culturable bacteria concentration decreased gradually. The concentration of bacteria for 40m.ABB was 1378 CFU/m$^3$. During sampling periods, the wind speed was 0.15–0.42 m/sec. Wind dilution might the reason for the low bacterial concentration at 40m.ABB.

The particle size distributions of the total culturable bacteria from different sampling sites are shown in Fig. 2. The bacterial distribution in OC was more even in particle size compared to the other samples. A similar phenomenon was found in the distribution of AFB. The bacterial content in bioaerosols with particle size within 0.65–2.1 μm increased significantly after the rotating brush (at ABB) compared to before the rotating brush (at AFB). The bacterial content in 0.65–2.1 μm bioaerosols decreased 56.4% 40 m away from the rotating bush (at 40m.ABB), for small particles could be easily carried away by the wind.

The analysis of the culturable bacterial distribution was
based on culture-dependent methods which do not reflect all of the bacteria in the bioaerosols because only a small proportion (less than 10%) of microorganisms can be cultivated (Amann et al., 1995). The clone library method was also applied in this present study.

### 2.2 Clone libraries construction

Oligonucleotide fingerprinting of 16S rRNA genes was applied to assay the bacterial genetic structure and diversity of bioaerosols collected from different sampling sites as well as mixed liquid in the oxidation ditch. A total of 242 positive clones from bioaerosols samples were submitted to sequence and construct the clone libraries. There were 33 different OTUs of 62 positive clones from the sample of AFB, whereas 19 of 60, 34 of 61 and 34 of 59 were included for ABB, 40m.ABB, and AW, respectively. Coverage analysis suggested that the bioaerosols libraries represented approximately 74.20% (AFB), 86.70% (ABB), 67.20% (40m.ABB) and 72.90% (AW) of the total number of clones examined, providing a dependable inventory of the bacterial 16S rRNA gene sequences present in the bioaerosols. Rarefaction curves generated for the 16S rRNA clone libraries are shown in **Fig. 3**. The Shannon index is commonly used to characterize species diversity in microbial communities. The indexes were 3.34 for AFB, 2.60 for ABB, 3.34 for 40m.ABB, and 3.45 for AW, indicating that the bacterial community in the water had the highest diversity. Bioaerosols from ABB presented the lowest bacterial diversity whereas similar diversity was found for AFB and 40m.ABB.

### 2.3 Bacterial community characteristics of each sampling site

Bioaerosols collected at different distances from the rotating brush in the oxidation ditch were analyzed for bacterial community by 16S rRNA clone library. Bacteria affiliated with Proteobacteria and Bacteroidetes dominated in all bioaerosols samples (**Figs. 4–6**). A number of bacteria belonging to these two phyla could also be observed in the library of the water (AW).

The bacterial communities of the bioaerosols from each sampling site had their own characteristics. As shown in **Fig. 4**, Proteobacteria (48.39%), unclassified bacterium (25.81%) and Bacteroidetes (11.29%) were dominant bacteria in the bioaerosols of AFB situated before the rotating brush. The percentages of Actinobacteria, Verrucomicrobia, Planctomycetes and Spirochaetes were less than 5%. For ABB, the widespread bacterial groups were assigned to Proteobacteria (70.00%) and Bacteroidetes (23.33%). The Proteobacteria were distributed in $\alpha$- (1.67%), $\beta$- (16.67%) and $\gamma$- (53.33%) subgroups, with no $\delta$-subgroups. Only 3.33% of Firmicutes and 1.67% of unclassified bacteria were detected from this sampling site (**Fig. 5**). Compared with the other samples, the bacterial community of bioaerosols sampled from ABB had less richness. Proteobacteria (67.21%) was still the majority phylum in bioaerosols at the sampling site of 40m.ABB (**Fig. 6**). Bacteroidetes, Actinobacteria and unclassified bacteria were 6.56%, 14.75% and 11.48%, respectively.

The composition and concentration of microbial populations comprising the bioaerosols vary with their sources (Pillai and Ricke, 2002). The bacterial community of the water in the oxidation ditch was analyzed in order to explore the relationship between the microorganisms in water and ambient air. It was observed that the representative phyla in water were Proteobacteria, Bacteroidetes, Actinobacteria, Spirochaetes and Deferrribacteres (**Fig. 7**). Similarly, bacteria affiliated with Proteobacteria and Bacteroidetes accounted for a large proportion in all bacteria, which is in agreement with previous reports. The research of Liu et al. (2007) analyzed bacterial community structures in two sewage treatment plants in Beijing with different treatment processes. They found that Proteobacteria, Bacteroidetes, Actinobacteria, Spirochaetes and Deferrribacteres were major components of the bacterial communities.

### Table 1 OTUs in each Clone library of all samples

<table>
<thead>
<tr>
<th>Sampling sites</th>
<th>Universal OTUs number</th>
<th>Sequences number</th>
<th>Individual OTUs number</th>
<th>Sequences number</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFB</td>
<td>OTU-1-AFB to OTU-17-AFB</td>
<td>46</td>
<td>OTU-18-AFB to OTU-33-AFB</td>
<td>16</td>
</tr>
<tr>
<td>ABB</td>
<td>OTU-1-AABB to OTU-11-ABB</td>
<td>52</td>
<td>OTU-12-AABB to OTU-19-AABB</td>
<td>8</td>
</tr>
<tr>
<td>40m.ABB</td>
<td>OTU-1-40m.ABB to OTU-14-40m.ABB</td>
<td>41</td>
<td>OTU-15-40m.ABB to OTU-34-40m.ABB</td>
<td>20</td>
</tr>
<tr>
<td>AW</td>
<td>OTU-1-AW to OTU-18-AW</td>
<td>43</td>
<td>OTU-19-AW to OTU-34-AW</td>
<td>16</td>
</tr>
</tbody>
</table>

Universal OTUs number: the number of universal OTUs composed of sequences having a threshold of over 97% similarity. Individual OTUs number: the number of individual OTUs.
Characterization of the airborne bacteria community at different distances from the rotating brushes

2.4 Distribution and variation of bacterial communities

The distribution of bacterial communities in bioaerosols presented site-related characteristics (Fig. 8). A total of four classes (Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria and Deltaproteobacteria) in Proteobacteria were detected both in the sampling sites of AFB and 40m.ABB (e.g. OTU-3-AFB and OTU-10-40m.ABB), whereas Deltaproteobacteria could not be found in ABB (e.g. OTU-24-AFB and OTU-16-40m.ABB). The content of each sub-group of Proteobacteria varied significantly from 9.68% in AFB to 1.67% in ABB and 22.95% in 40m.ABB with a significant increase occurring in Gammaproteobacteria. The distribution of Bacteroidetes, another important phylum in bioaerosols, was uneven among all the bioaerosols sampling sites. Approximately twice and quadruple the amount of Bacteroidetes was observed in AFB and ABB compared to that in 40m.ABB. Other bacteria, e.g. Spirochaetes, Planctomycetes and Verrucomicrobia, could be detected only in the AFB clone library.

According to the previous research, many factors were believed to affect the microbial communities in bioaerosols, such as source, operational activities, volume of treated water and season, type and performance of aerator, sampling time, and distance from bioaerosols source (Pillai and Ricke, 2002). Proteobacteria, Bacteroidetes and other bacteria existing in bioaerosols also could be detected in the clone library of AW, e.g. OTU-3-AFB, OTU-4-AFB, OTU-19-AFB, OTU-21-AFB,
Fig. 5 Phylogenetic tree of the representative sequences of OTUs in ABB and related organisms aligned based on 16S rRNA sequences (neighbor-joining tree). Scale bar: number of nucleotide changes per sequence position.

Fig. 6 Phylogenetic tree of the representative sequences of OTUs in 40m.ABB and related organisms aligned based on 16S rRNA sequences (neighbor-joining tree). Scale bar: number of nucleotide changes per sequence position.
OTU-1-40m.ABB and OTU-10-40m.ABB in bioaerosols were the same bacteria as OTU-5-AW, OTU-7-AW, OTU-9-AW, OTU-18-AW and OTU-22-AW in AW, respectively. Rotating brushes for aeration and pushing of water were installed across the channel of the oxidation ditch. The mechanical action of the rotating brush paddles caused a spray of water and increased bacterial emission from the water to the atmosphere. Numerous bacteria belonging to the clone library of ABB could also be observed in the library of water (AW), indicating that the water in the oxidation ditch was the source of the bioaerosols. Bacteria originally in AW, e.g. 6.67% uncultured γ-Proteobacteria bacterium (OTU-21-AW), also emerged in ABB (OTU-10-ABB), which possibly transferred into the atmosphere by the rotation of the rotating brush. The sampling sites of AFB and ABB were located 0.5 m before and after the rotating brush, respectively. The analysis of the bacterial communities also indicated that the bacterial diversity in ABB was less than for AFB. This distinction between AFB and ABB was brought about by the air disturbance of the rotating brush. Noticeable changes could be observed in the bacteria communities of the bioaerosols with the extension of distance downwind from the bioaerosols source (rotating brushes). Significant decreases occurred in Bacteroidetes, Gammaproteobacteria and Firmicutes from the ABB clone library to that of 40m.ABB. During sampling periods, the ranges of wind speed, irradiance, temperature and relative humidity were 0.15–0.42 m/sec, 419.2–437.6 W/m, 28.5–31.3°C and 47.3%–50.2%, respectively. The ultraviolet radiation, wind dilution and gravity sedimentation might be the reasons for variation of the bacterial communities between the sampling sites close to and far from the rotating brushes.

The bacterial community in the bioaerosols of AFB was similar to that of 40m.ABB (Fig. 8), which was in agreement with their Shannon indexes. Rhodobacteraceae (OTU-32-AFB. OTU-27-40m.ABB and OTU-33-40m.ABB) and Actinobacteria (OTU-13-AFB and OTU-2-40m.ABB) were found both in AFB and 40m.ABB. They could survive in the sampling site far from the source because Rhodobacteraceae had the photosynthetic feature and Actinobacteria could breed through spores in air.

2.5 Isolated resources characteristics of closest relatives

The composition and concentration of bacterial communities in bioaerosols were affected by the environmental conditions prevailing at the particular site (Pillai and Riche, 2002). The isolated resources of closest relatives by BLAST in NCBI databases can provide information on their associated environments (Polymenakou et al., 2008; Jeon et al., 2011) (Fig. 9). These associated environments
can point out the sources of bacteria in bioaerosols. For the clone libraries in AFB, ABB and 40m.ABB, more than 50% of the identified bacteria were associated with the aqueous environment, in which 67.74%–70.00% were related to the activated sludge in the WWTPs. This result also indicated that the bacterial structure of bioaerosols in the WWTP mainly derived from local water sources, especially the WWTP.

Only 15.25% of related bacteria in the AW clone library were isolated from soils and other nonaqueous environments. They increased to 32.26% for AFB, 30.00% for ABB and 31.15% for 40m.ABB, when aerosolized into the air. Particularly, there were 3.23% of related bacteria in AFB, 1.67% in ABB and 9.84% in 40m.ABB from biogas or urban aerosols. The isolation sources of bioaerosols from WWTP are very different to those from a nonaqueous environment. Maron et al. (2005) investigated bacterial structure in aerosols from a rural environment to the south of Paris on two dates. They found that most of the identified bacteria in aerosols were known to be commonly associated with the soil and plant environment. The assessment of the bacterial community in aerosols over the eastern Mediterranean region during an African storm showed that all of the bacteria were commonly found in soil and marine ecosystems (Polymenakou et al., 2008). Similar results were obtained by Jeon et al. (2011), when they applied a molecular method to analyze the bacterial structure in aerosols from an atmospheric environment affected by Asian dust events. Different from those emitted from soil or non-aqueous surfaces, bioaerosols generated from water sources (such as WWTPs) usually form with a thin layer of moisture surrounding the microorganisms (Wickman, 1994). In addition, airborne bacteria often appear attached to sludge particles while airborne fungi appear mainly as single organisms. Therefore, the composition of microorganisms in the water was found to be one of the most important sources of bacterial communities in bioaerosols. The genetic structures of bioaerosols from wastewater treatment processes were very different with those from nonaqueous environments.

2.6 Pathogenic bacteria in bioaerosols

In this study, the sampling height was designed to be 1.5 m above the ground level, which is the common respiratory height of employees in the WWTP. Bioaerosols with fungal spores, fungal hyphal fragments and pathogenic bacteria from wastewater can be vehicles for the dissemination of human and animal pathogens (National Research Council, 2002). The potential pathogenic bacteria detected in this study are listed in Table 2. The pathogenic bacteria were detected more frequently from the ABB clone library than the others. Enterobacteriaceae (33.34%) with six species of *Proteus* sp. (6.67%), *Proteus mirabilis* (11.67%), *Escherichia coli* (8.33%), *Enterobacter aerogenes* (3.33%), *Providencia alcalifaciens* (1.67%) and *Morganella* sp. (1.67%) as well as *Aeromonadaceae* (13.34%) with two species of *Aeromonas simiae* (6.67%) and *Aeromonas hydrophila* (6.67%) occupied a large proportion in the bacterial community of the ABB clone library. Among them, *Proteus* sp., *Proteus mirabilis*, *Escherichia coli*, *Enterobacter aerogenes*, *Providencia alcalifaciens* and *Morganella* sp. as the normal bacteria of human being might cause sepsis, meningitis, and urinary infections in humans who already have suppressed host immunity defenses. *Aeromonas simiae* and *Aeromonas hydrophila* are potential waterborne pathogen. With a
higher exposure to these bacteria, skin problems, diarrhea and other gastrointestinal symptoms occurred frequently among employees of WWTP (Lundholm and Rylander, 1983; Thorn and Kerekes, 2001; Prazmo et al., 2003). *Alcaligenes faecalis* (15.00%) and *Kerstersia gyiorum* (1.67%) belonging to Alcaligenaceae, a main family of parasitic bacteria, emerged in the ABB clone library. They often cause conditional infections of human beings (e.g. sepsis, meningitis, urinary infections). Von Graevenitz (1985) exactly described the Alcaligenes faecalis infections including bacteraemias, infected wounds, otitis, and infected cerebrospinal fluid. Being one of the main phyla in the ABB clone library, the potential pathogenicity of bacteria affiliated with Bacteroidetes should be noted. For example, the pathogenic features of *Bacteroides* sp. (23.33% in ABB) were not obvious when it existed alone. However, their serious lethality would emerge when they infected synergistically with *Escherichia coli* (Rodloff and Hahn, 1984). The sampling site of ABB was located just after the rotating brushes, which suggested that bioaerosols from after the rotating brush could be the main source of biological risks in the oxidation ditch. In addition to ABB, pathogenic bacteria in bioaerosols from other sampling sites were found. *Burkholderia fungorum* (3.28%) with resistance to antibiotics was detected in the 40m.ABB clone library, and it could be recovered from the respiratory tracts of cystic fibrosis patients (Gilligan, 1995; Ulrich et al., 2006; Patton et al., 2009).

In this study, *Clostridium sporogenes* was detected in the clone libraries of ABB. In previous reports, pathogenicity of *Clostridium* species with other bacteria in mixed infections was studied by means of a subcutaneous abscess model in mice (Brook and Walker, 1986). In recent years, *Clostridium* species as responsible for a wide range of diseases raised high concerns again. Janvilisri et al. (2010) studied the population and concentration of *Clostridium* species in samples from centers for disease control and prevention, hospital, department of pathology and so on. The results suggested that a large number of *Clostridi-

<table>
<thead>
<tr>
<th>Species</th>
<th>Genus</th>
<th>Family</th>
<th>References</th>
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</thead>
<tbody>
<tr>
<td>Proteus sp.</td>
<td>Proteus</td>
<td>Enterobacteriaceae</td>
<td>Lundholm and Rylander, 1983; Fannin et al., 1985; Thorn and Kerekes, 2001; Prazmo et al., 2003</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
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<tr>
<td><em>Escherichia coli</em></td>
<td><em>Escherichia</em></td>
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<td><em>Enterobacter aerogenes</em></td>
<td><em>Enterobacter</em></td>
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<tr>
<td><em>Providencia alcalifaciens</em></td>
<td><em>Providencia</em></td>
<td></td>
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<tr>
<td><em>Morganella</em> sp.</td>
<td><em>Morganella</em></td>
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<tr>
<td><em>Aeromonas simiae</em></td>
<td><em>Aeromonas</em></td>
<td>Aeromonadaceae</td>
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<td><em>Aeromonas hydrophila</em></td>
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<tr>
<td><em>Kerstersia gyiorum</em></td>
<td><em>Kerstersia</em></td>
<td>Alcaligenaceae</td>
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</tr>
<tr>
<td><em>Alcaligenes faecalis</em></td>
<td><em>Alcaligenes</em></td>
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<tr>
<td><em>Burkholderia fungorum</em></td>
<td><em>Burkholderia</em></td>
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<tr>
<td><em>Bacteroides</em> sp. and</td>
<td><em>Bacteroides</em> and</td>
<td>Bacteroidaceae and</td>
<td>Rodloff and Hahn, 1984; Dungan, 2012</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td><em>Escherichia</em></td>
<td>Enterobacteriaceae</td>
<td></td>
</tr>
<tr>
<td><em>Clostridium sporogenes</em></td>
<td><em>Clostridium</em></td>
<td>Clostridiaceae</td>
<td>This study</td>
</tr>
</tbody>
</table>

**Fig. 9** Scale drawing of isolated resources of closest relatives in the NCBI database corresponding to representative sequences in OTUs.

*um sporogenes* presented in samples of centers for disease control and prevention. However, it did not be detected in bioaerosols from the WWTP atmosphere in previous reports. It is necessary to focus more attention on the potential pathogenicity of this bacterium for producing toxins.

**3 Conclusions**

The oligonucleotide fingerprinting of 16S rRNA genes applied in this study has provided insights on the bacterial communities in bioaerosols from an Orbal oxidation ditch in a WWTP. The majority of bacteria in the bioaerosols were Proteobacteria and Bacteroidetes in the atmosphere surrounding the oxidation ditch. The bacterial structure in the bioaerosols was associated with those in water. The isolation sources of clone libraries in bioaerosols from WWTP were very different with those from nonaqueous environments. Commonly pathogenic bacteria e.g. *Proteus* sp., *Proteus mirabilis*, *Escherichia coli*, *Enterobacter aerogenes*, *Providencia alcalifaciens* and *Morganella* sp. as well as those not reported in previous research e.g. *Clostridium sporogenes* was detected from bioaerosols in WWTP using an oxidation ditch treatment process.
As rotating brush aeration was the main source of bioaerosols in the oxidation ditch, the rotating brush could be covered to reduce bioaerosols emission on the condition of meeting the demand of aeration. Ingestion, skin or mucosal contact is the main pathways for transmission of pathogenic bacteria to humans. On-site employees in the WWTP should wear gloves, face mask and protective clothing to prevent exposure to bioaerosols in the surrounding atmosphere.

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