Composition and distribution of bacteria in an operating rainwater harvesting tank

Mikyeong Kim and Mooyoung Han

ABSTRACT

In this study, we investigated the phylogenetic distribution of the bacteria present in an operating rainwater tank by denaturing gradient gel electrophoresis (DGGE), and compared the bacterial composition in rainwater and biofilm from the inlet and outlet of the tank. Seventeen species were identified, the DGGE profiles of which showed a clear difference between the planktonic bacterial community and the community in the biofilm. Most of the bacteria were closely related to fresh water, soil, and biofilm bacteria found in natural environments. The high proportion of Proteobacteria indicates the generally clean oligotrophic nature of the tank water. Biofilm formation is an advantage for bacteria that exist in oligotrophic environments. The groups identified in the biofilm, such as *Sphingomonas*, *Bacillus*, and *Sphingophyxis*, have been demonstrated to degrade certain contaminants and to act as bio-control agents. Thus, biofilm formation in rainwater tanks not only represents a survival strategy for bacteria, but also serves as a natural filter by removing contaminants and bacteria from rainwater.

Key words | bacterial composition, biofilm, DGGE, rainwater, rainwater tank

INTRODUCTION

The management of rainwater has become more common recently, and is expected to become an essential element of urban water management in the future. However, rainwater use is limited by uncertainty about rainwater quality, and especially its microbial quality (Krampitz et al. 1998; 1999; Evans et al. 2009).

Microbiological research on rainwater has focused on the presence or absence of pathogens and indicator species, but the results differ with the research method and the design and maintenance of the facilities being examined (Dillaha & Zolan 1985; Pinfold et al. 1993; Simmons et al. 2001; Handia et al. 2003; Ahmed et al. 2008). There is also a conflict of opinion over the relationship between tank water quality and health risk (Gould 1999; Lye 2002; Heyworth et al. 2006).

About 13% of all Australian households use rainwater tanks as a source of drinking water (Cunilffe 1998). Krampitz et al. (1998; 1999) showed that after tank cleansing the water quality was poorer, and Lye (1991) found that heterotrophic bacterial levels were lower in rainwater cisterns that did not receive any type of maintenance or cleaning. Treating a rainwater tank as a unique ecosystem and understanding its ecological function could thus lead to improvements in rainwater quality.

In this study, we investigated the kinds of bacteria that inhabit rainwater tanks and compared the bacterial composition in rainwater and biofilm, and in samples from the inlet and outlet of the tank.

METHODS AND MATERIALS

Study sites

The study was carried out on a rainwater system at Seoul National University, South Korea. The system was constructed in November 2003, and consists of a concrete storage tank about 200 m³ in size that is located underground and a roof catchment area comprising 2,098 m² of concrete surface. The rainwater collected therein supplies the toilets of 167 households and a garden (Han et al. 2006) (Figure 1). The rainwater from the roof flows through a filter (VF6 type with a mesh size of 0.65 mm and a capacity of 70.5 L/sec) at first,
and then enters the main tank through a calm inlet. Inside the tank, the $W \times L \times H$ ratio changes from $7.4 \times 15.4 \times 2$ to $3.7 \times 30.8 \times 2$ due to the installation of a baffle.

**Physicochemical characteristics**

The samples for physicochemical monitoring were collected at a depth of 50 cm from the outlet of the tank. Various physicochemical parameters of the rainwater, such as temperature, $pH$, dissolved oxygen (DO), electric conductivity (EC), and turbidity, were measured for one year from November 2007 to September 2008 to monitor the variation in rainwater quality. The temperature, $pH$, DO, EC, turbidity, suspended solid content (SS), volatile suspended solid content (VSS), total nitrogen (TN), total phosphate (TP), chemical oxygen demand (COD), and total organic carbon (TOC) were measured at the same location when the sampling for PCR-DGGE was performed.

**PCR-DGGE analysis**

**Sampling and sample preparation**

The sampling points were S1 (biofilm at the inlet), S2 (biofilm at the outlet), S3 (rainwater at the inlet), and S4 (rainwater at outlet), as indicated in Figure 1. One and a half litres of rainwater was sampled at a depth of 50 cm at the inlet and outlet of the rainwater tank and was carried directly to the lab in a sterile water bottle (see Figure 1). Biofilm was collected from 0.04 m$^2$ of the wall surface in the rainwater tank (see Figure 1) and placed in a sterile tube containing 20 mL of distilled water. In the laboratory, 1 L of rainwater was concentrated to 100 mL by using a refrigerated centrifuge at 8,000 g for 20 min at 4°C.

**DNA extraction**

The concentrated rainwater and biofilm were passed through a 0.2 um filter, and genomic DNA was isolated with a water RNA/DNA purification kit (Norgen, Canada) according to the manufacturer’s instructions. The DNA concentration was measured with a Nanodrop Spectrophotometer (ND-1000, Thermo Fisher Scientific).

**Polymerase chain reaction (PCR)**

The primer pair EUB 341F-GC and PRUN518R, which are universal primers specific to bacteria, was used (Muyzer et al. 1993). PCR was performed with a thermal cycler (GeneAmp PCR System 9700, Perkin Elmer) using h-Taq DNA polymerase (Solgent, Korea) mixed with 2 µL of 10 pmole/µL for each primer and 2 µL of the eluted template DNA. Negative
controls were run for each reaction. The PCR conditions are described in Table 1.

**Denaturing gradient gel electrophoresis (DGGE)**

DGGE analysis was performed by using a D-Code system (Bio-rad, USA). The gel contained 8% (wt./vol.) of polyacrylamide and a series of denaturant concentrations ranging from 30% to 60% (formamide and urea). The gels were run at 70 V for 11 h in 1× TAE buffer at 60°C. After electrophoresis, the gels were stained with ethidium bromide in 1× TAE for 15 min and then destained in DDW for 20 min. The DGGE gels were visualised with a UV transilluminator (302 nm) mounted with a digital camera to capture photographs of the gels.

**Re-amplification of the DGGE bands and sequencing**

The DNA bands on the DGGE gels were excised under UV trans-illumination using sterile scalpels and then soaked in 50 μL of sterile DDW at 4°C overnight. Two microlitres of DNA solution was used for re-amplification using the same primer pair without a GC clamp. The reaction conditions for the PCR were the same as those described in Table 1. The PCR products were purified using a purification kit (Accu-Prep PCR purification kit, Bioneer, Korea) and then sequenced using EUB341F (for bacteria) and F984 (for actinomycetes) in an automatic DNA sequencer (ABI Prism 3730 XL DNA Analyzer, PE Applied Biosystems) at the National Instrumentation Center for Environmental Management of Seoul National University. The DGGE band sequences were compared with 16S rDNA sequences obtained through a BLAST search from the database of the DNA Data Bank of Japan (http://blast.ddbj.nig.ac.jp/top-e.html).

**Statistical analysis**

To compare the DGGE band patterns across the samples, the similarities among the lanes were calculated statistically using the dice coefficient method. The similarity matrix included every band in the similarity comparison for the samples, and both band position and intensity calculated with the Gaussian model were used when comparing samples. A dendrogram for the DGGE band patterns was proposed based on a similarity matrix derived using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) method.

**RESULTS**

**Physicochemical characteristics**

The physicochemical characteristics, including temperature, pH, DO, EC, and turbidity, were monitored from November 2007 to October 2008 (see Figure 2). The temperature ranged from 11 to 26°C and tended to decrease during winter and to increase during summer. The pH and DO ranged from 6.9 to 8.2 and from 6.3 to 6.9 mg/L, respectively, and the values remained relatively stable across the year. The electric conductivity was greater during summer rainfall, and the turbidity was greater in summer and winter when precipitation was high.

Table 2 shows the physicochemical characteristics of the rainwater at the inlet and outlet of the rainwater tank. The turbidity, EC, SS, and VSS were lower at the outlet than at the inlet, and the DO was slightly lower at the inlet than at the outlet. The TN and TP were 4.9±0.4 mg/L and 0.08±0.04 mg/L, respectively, at the inlet but decreased at the outlet to 4.4±0.2 mg/L and 0.05±0.01 mg/L, respectively. The COD was 1.9±1.12 mg/L and 0.9±0.01 mg/L, and the TOC 0.78±0.03 mg/L and 0.26±0.15 mg/L at the inlet and outlet, respectively. Thus, the values of most of the parameters were better at the outlet than at the inlet.

**Phylogenetic distribution**

The bacterial composition in the rainwater and biofilm samples showed different tendencies. Seventeen species
were identified from the sequence of the selected DGGE bands (Figure 2). According to the standard phylogenetic classification of prokaryotes, the species belonged to 13 genera, 10 families, eight orders, five classes, and three phyla. Proteobacteria accounted for 88% of the species identified, with the remainder being Bacteriodetes and Firmicutes.

**Comparison of bacterial compositions**

The DGGE profiles showed a clear difference between the planktonic bacterial community and the community in the biofilm (Figure 3). The bacterial composition tended to differ across the biofilm samples, but was similar across the rainwater samples. *Limonohabitans* sp., *Aquaspirillum* sp., *Rubrivivax gelatinosus*, *Roseovirga ehrenbergii*, and *Rhodobacter gluconicum* were found only in the rainwater samples, whereas *Ralstonia insidiosa*, *Blastochloris sulfoviridis*, *Bacillus* sp., *Sphingomonas* sp., *Sphingobium* sp., and *Beijerinckiaceae bacterium* were identified in the biofilm. Some species, such as *Novosphingobium resinovorum*, *Sphingopyxis* sp., *Sphingomonas* sp., and *Sphingobium yanoikuyae*, were detected in both planktonic and biofilm communities.

The bacterial composition in the biofilm differed with location. *Sphingopyxis* sp. (band no. 7) and *Blastochloris sulfoviridis* (band no. 9) were detected in the inlet samples, whereas *Ralstonia insidiosa* (band no. 4), *Novosphingobium resinovorum* (band no. 6), *Sphingomonas* sp. (band no. 8), and *Sphingobium* sp. (band no. 15) were found in the outlet samples. *Sphingomonas* sp. (band no. 14), *Bacillus* sp. (band no. 13), *Sphingobium yanoikuyae* (band no. 12) and *Beijerinckiaceae bacterium* (band no. 17) were detected in both locations. The rainwater showed a similar bacterial composition at the inlet and outlet.

The similarities among the DGGE lanes were quantified using cluster analysis, and a dendrogram was constructed using the UPGMA method (see Figure 4). The analysis revealed a relatively greater similarity between lanes S3 and S4. In other words, the DGGE band patterns of the rainwater from the inlet and outlet were more similar than those of the biofilm samples from the two locations. There was a clear difference in the band patterns of the rainwater and biofilm overall.

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**Table 2** Physicochemical characteristics at the inlet and outlet of the rainwater tank

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Inlet</th>
<th>Outlet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turbidity (NTU)</td>
<td>5.4 ± 0.02</td>
<td>4.2 ± 0.01</td>
</tr>
<tr>
<td>Electric conductivity (μS/cm)</td>
<td>63.7 ± 0.15</td>
<td>55.6 ± 0.35</td>
</tr>
<tr>
<td>pH</td>
<td>6.7 ± 0.06</td>
<td>6.5 ± 0.03</td>
</tr>
<tr>
<td>Dissolved oxygen (mg/L)</td>
<td>5.8 ± 0.05</td>
<td>6.5 ± 0.06</td>
</tr>
<tr>
<td>Temp. (°C)</td>
<td>18.5 ± 0.25</td>
<td>17.9 ± 0.31</td>
</tr>
<tr>
<td>Suspended solids (mg/L)</td>
<td>2.0 ± 0.01</td>
<td>1.0 ± 0.00</td>
</tr>
<tr>
<td>Volatile suspended solids (mg/L)</td>
<td>1.5 ± 0.00</td>
<td>0.9 ± 0.00</td>
</tr>
<tr>
<td>Total phosphorus (mg/L)</td>
<td>0.08 ± 0.04</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>Total nitrogen (mg/L)</td>
<td>4.9 ± 0.4</td>
<td>4.4 ± 0.2</td>
</tr>
<tr>
<td>COD (mg/L)</td>
<td>1.9 ± 1.12</td>
<td>0.9 ± 0.01</td>
</tr>
<tr>
<td>Total organic carbon (mg/L)</td>
<td>0.78 ± 0.03</td>
<td>0.26 ± 0.15</td>
</tr>
</tbody>
</table>

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**Figure 2** Physicochemical characteristics between November 2007 and October 2008.
DISCUSSION

Physicochemical conditions in rainwater tanks create a distinct microbial habitat

In the underground rainwater tank under study, the lack of sunlight and the average water temperature of as low as 17°C led to the absence of photosynthetic microbes such as algae. The nutrient input thus depended on rainfall. Rainwater tanks are an oligotrophic environment, as the concentration of dissolved organic matter in these habitats is commonly less than 10 mgL⁻¹ (Wahl 1989). The inflow and outflow of rainwater in such tanks changes according to precipitation characteristics and rainwater usage. Thus, rainwater tanks constitute a unique habitat for microbes.

Bacterial composition and distribution

The samples contained mostly nonpathogenic proteobacterial species. Many of the bacteria identified were closely related to fresh water, soil, and biofilm bacteria found in natural environments (Takeuchi et al. 1995; White et al. 1996; Coenye et al. 2003; Williams et al. 2004; Janssen 2006; Okano et al. 2009; Kasalick et al. 2010). Over 88% of the bacteria identified were Proteobacteria. Agogue et al. (2005) reported that Proteobacteria are consistently more abundant at pristine sampling sites, whereas Firmicutes and Actinobacteria are dominant at polluted sites. Although in this study estimates were made in terms of detection ratio only and the species were not quantified, the results still indicate the generally clean oligotrophic nature of the tank water.

The bacterial composition in the biofilm samples was different from that in the rainwater samples. It is believed that biofilm formation provides an advantage for bacteria that exist in oligotrophic environments (Kjelleberg et al. 1982; Kjelleberg & Hermansson 1984). Some of the species identified in the biofilm in this study, such as Bacillus sp., Sphingomonas sp., and Sphingobium sp., have been demonstrated to degrade certain contaminants and to act as bio-control agents (White et al. 1996; Li et al. 2005; Okano et al. 2009). These species may be relatively sensitive to nutrients in oligotrophic conditions, and thus tend to develop a
biofilm for survival. Thus, in oligotrophic rainwater tanks, microbial species remain constant in rainwater through biofilm formation.

The bacterial composition in the biofilm differed at the inlet and the outlet, whereas the bacterial composition in the rainwater samples from the two locations was similar. The different nutrient concentrations at the inlet and outlet may have influenced the bacterial composition in the biofilm, and the biofilm bacteria seemed to have site specificity due to less mobility conferred by living in a surface habitat.

**Possibility of self-purifying rainwater tanks and implications for rainwater quality**

Bacterial communities in nature play a key role in the production and degradation of organic matter, the degradation of many types of environmental contamination, and the cycling of nitrogen, sulfur, and many metals (Davey & O’Toole 2000). In addition, the sorptive capacity of biofilm for dissolved organic matter and metals has been widely demonstrated in sewage and marine systems (Brown & Lester 1982; Lion et al. 1988). Thus, biofilm formation in rainwater tanks seems not only to promote the survival of bacteria, but also serves as a natural filter by removing contaminants and bacteria from rainwater.

**CONCLUSION**

The phylogenetic distribution of the bacteria present in an operating rainwater tank was investigated by denaturing gradient gel electrophoresis (DGGE), and the bacterial composition was compared in rainwater and biofilm from the inlet and outlet of the tank. Seventeen species were identified, the DGGE profiles of which showed a clear difference between the planktonic bacterial community and the community in the biofilm. Most of the bacteria were closely related to fresh water, soil, and biofilm bacteria found in natural environments. The high proportion of Proteobacteria indicates the generally clean oligotrophic nature of the tank water. Biofilm formation is an advantage for bacteria that exist in oligotrophic environments. The groups identified in the biofilm, such as *Sphingomonas, Bacillus,* and *Sphingophyxis,* have been demonstrated to degrade certain contaminants and to act as bio-control agents. Thus, biofilm formation in rainwater tanks not only represents a survival strategy for bacteria, but also serves as a natural filter by removing contaminants and bacteria from rainwater.

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