JOURNAL OF PURE & APPLIED MICROBIOLOGY, Nov. 2014. Vol. **8**(Spl. Edn. 2), p. 103-113

Molecular Characterization of Actinomycetes Isolated from Terrestrial Environment and their Synthesis of Geosmin and 2-MIB

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(Received: 10 August 2014; accepted: 21 October 2014)

The occurrence of Actinomycetes, their molecular characteristics, and their release of synthesized odor compounds into soil and the water systems were investigated. The average numbers of Actinomycetes was 9.0 × 10^5 CFU/g in the soil and 4.8 CFU/mL in the water. The average numbers of Actinomycetes in the soil was highest at the Yangpyeong (YP) testing site (2.2 × 10^6 CFU/g), followed by the Gyeongan (GA), Paldang (PD), and Cheongpyeong (CP) sites. The *Streptomyces* genus, which can produce odor compounds such as geosmin and 2-Methylisoborneol (MIB), was dominant (67.6%). An enhanced proportion of *Streptomyces* with plant soil was also observed by independent pyrosequencing results of cultures. The bacterial diversity of GA and CP was greater than those of YP and PD, and pyrosequencing detected diverse genera of Actinobacteria. The rain leaching model experiment suggested that geosmin and 2-MIB produced by the Actinomycetes could be released into the water systems by rainfall. The concentrations of the geosmin and 2-MIB leached from the planted soils were 2.6 times and 2.8 times greater than those from the unplanted soil. Moreover, the concentration of *Streptomyces* positively correlated with the amount of geosmin and 2-MIB (R^2 = 0.809 and 0.847, respectively).

Key words: Actinomycetes, Geosmin, 2-MIB, 16S rDNA, pyrosequencing.

Actinomycetes are bacteria of the Actinobacteria order and inhabit various environments including soil, marine, freshwater, and it consist of morphologically diverse Gram-positive bacteria[1–3]. They play an important role in the decomposition of organic compounds and organic matter turnover, including the carbon cycle. In addition, Actinomycetes are well studied as secondary metabolite producers; therefore, they have been used to generate various natural antibiotics in the pharmacological and commercial fields[4–6]. Although various genera within Actinomycetes have been isolated from terrestrial environments, there is little known about the Actinomycetes genera in aquatic environments.

Musty and earthy odor compounds reduce the quality of surface water for drinking in many countries. One of the most common causes of odor problems in drinking water recorded by water utilities was chlorine contamination[7–9]. Actinomycetes have long been associated with musty/earthy odor compounds in water and fisheries, however their role in generating odor in freshwater remains unclear[10–12]. Although Actinomycetes are involved in multiple taste and odor (T&O) episodes[13–15], however, little is known about the Actinomycetes odor-producing compounds in Korea.

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In Korea, T&O events have linked to geosmin and 2-Methylisoborneol (MIB) produced by cyanobacteria such as *Anabaena* and *Oscillatoria* [16–18]. Interestingly, many T&O events have occurred with no increase in the number of algal species or blooms (Unpublished data). In such cases, odor compounds produced by Actinomycetes might have leached from the soil into the water as a result of rainfall or during the thaw period from winter to spring. Precipitation, especially in areas with a high basin surface area-to-volume (SA:V) ratio, such as the Paldang (PD) study area (618), can easily affect the water quality of the lake[18].

In this study, the occurrence of Actinomycetes in soil from four sampling locations and the bacterial communities in sampling sites were investigated by 16S rRNA gene sequencing of isolates and pyrosequencing analysis of metagenomic DNA. The effect of plants in soil on exposure odor products to lake was investigated using a previously designed model system. In addition, by using model experiments, we examined the possibilities of odor compounds produced by Actinomycetes in the soil surrounding drinking water resources entering the water system.

MATERIALS AND METHODS

Ethics Statement

All necessary permits for the collection of water and soil samples were obtained from K-water, a Korea public enterprise, and this study was granted by K-water (KIWE-WARC-2011). K-water has been implementing national water resources management policies regarding water supply dams and regional water supply systems. These field studies did not involve endangered or protected species.

Sampling sites, sampling, and preparation

Paldang (PD) and Cheongpyeong (CP) are artificial lakes and Gyeongan (GA) and Yangpyeong (YP) are streams, located in Gyeonggi-do of the Republic of Korea. Water from CP, GA, and YP flows into the PD Lake. PD lakewater is used as a water source for water treatment plants in the Seoul metropolitan area (Fig. 1a). Surface soil and water from the four sampling sites were collected in March, June, and September 2011 and Actinomycetes were isolated. The soil samples were collected within 0 - 10 cm below the ground of four random spots at each site. Water samples were collected in a sterilized water collection bottle without any pretreatment, and kept at 4 °C until use. The soil was air dried for one to two weeks at room temperature. After air-drying, the 1 g of soil was diluted 10-fold in saline solution (0.85% NaCl) and processed for culture.

Rain model experiments

To investigate the odor compounds synthesized by the Actinomycetes in the soil released and entered into the water system during rainfall, we conducted a rain model experiment. Soil from a depth of 15 cm below the surface was collected and placed in a flowerpot without mixing layers. Each location of sample collection had its own pot, namely CP, GA, YP, and PD. An environment for optimal plant growth with water and sunlight was provided, and water samples were taken from each flowerpot by pouring 500 mL water into each pot and allowing it to filter through the soil in the pot after 2 months. The sampled water and soil were subjected to separation and identification; furthermore, we analyzed the presence of geosmin and 2-MIB (Fig. 1b).

Actinomycetes culture and their identification by sequencing of 16S rDNA

Humic acid-vitamin (HV) agar was used to culture Actinomycetes. Bennet's agar was used as a medium for transferring the isolated Actinomycetes. Protocols published by Lee *et al*. (2011) were used to generate the media and culture the Actinomycetes[19]. To identify the suspicious Acitnomycete colonies, the colonies that were cultured on the HV agar were picked, transferred into 100 µL distilled water, and heated to 100 °C for 10 min. After cooling to room temperature, the tube was centrifuged at 12,000 × g for 5 min, and 1 µL of supernatant was used as a template for 16S rDNA PCR amplification. The primers and PCR conditions used were previously published[19]. The amplified PCR products were purified using the QIAquick Gel Extraction Kit (Qiagen, Valencia, CA) and were sequenced via automated DNA sequencing by Solgent. BLASTN search was used to identify Actinomycetes sequences.

DNA extraction and pyrosequencing

Genomic DNA was extracted from 1 g of soil sample using the FastDNA SPIN kit for soil (MPbio, Solon, OH). Bacterial 16S rRNA genes

targeting V1-V3 variable regions were amplified using V1-9F and V3-541R[20]. PCR reactions were carried out in a final volume 50 µL with 10× Taq buffer, dNTP mixture (Takara, Shiga, Japan), 10 µM of each primer, and 2 U of Taq polymerase (Ex Taq; Takara) using a C1000 Touch thermal PCR cycler (BioRad, Hercules, CA). The PCR amplification were performed using the following cycling conditions: initial denaturation at 94 °C for 5 min, 30 cycles of denaturation (30 sec, 94 °C), primer annealing (30 sec, 55 °C), and extension (30 sec, 72 °C), with a final extension step of 7 min at 72 °C. The PCR products were confirmed using gel electrophoresis and Gel-Doc system (BioRad) and purified with the QIAquick PCR purification kit (Qiagen). Purified PCR products from different samples were pooled together and short fragments were removed by AMPure bead kit (Agencourt Bioscience, Beverly, MA). After discarding short fragments, the purified product was checked for quality and size using a Bioanalyzer 2100 (Agilent, Palo Alto, CA) with a DNA 7500 chip. Checked products were sequenced with a 454 GS Junior system (Roche, Brandford, CT) following the manufacturer's instructions.

Analysis of pyrosequencing reads

The analysis of pyrosequencing reads was performed as previously described[20, 21]. Sequence reads obtained from each sample were sorted by their unique barcode, and once the barcode was removed, by their primer sequences. Any sequences containing two or more ambiguous nucleotides, had a low quality score (average score < 25), or contained reads shorter than 300 bp, were discarded. Chimeric DNA sequences were checked using the UCHIME and chimera sequences were trimmed for further analyses[22]. The taxonomic assignment of each read was performed using the EzTaxon-e database (http://eztaxon-e.ezbiocloud.net)[23]. The richness and diversity of the community originating from each sample was calculated by the Chao1 estimation and Shannon index using 3% dissimilarity and the Mothur program[24]. To compare different obtained reads from the pyrosequencing among samples, random subsampling was conducted using CLcommunity software (Chunlab, Inc., Seoul, Korea).

Geosmin and 2-MIB analysis by SBSE-TDU-GC-MS

In order to identify and quantify geosmin and 2-MIB, gas chromatograph (GC)/mass spectrometry (MS) analysis was conducted using a thermal desorption unit (TDU)-mass selective detector interfaced to a HP6890 series GC. The stir bar sorptive extraction (SBSE) method and the GC-MS analysis method were used to extract target compounds and analyze geosmin and 2-MIB in the samples[19, 25].

Statistical analysis

The SPSS 12.0 software for Windows and Paint Shop Pro version 7.04 (Jasc Software Inc.) were used for conducting linear regression analysis and generating graphs.

RESULTS

Detection of Actinomycetes in South Korea's terrestrial and aquatic environments

The number of Actinomycetes was investigated from soil and water at four sampling sites. The number of Actinomycetes in the soil was 9.0×10^5 colony forming units (CFU)/g and 4.8 CFU/mL in water. There were higher numbers in soil samples collected at June (1.4×10^6 CFU/g) than March (1.9×10^4 CFU/g) or September (2.1×10^5 CFU/g) (Table 1). The number of Actinomycetes was highest in the soil from the YP site (2.2×10^6 CFU/g), followed by GA (1.0×10^6 CFU/g), PD (3.1×10^5 CFU/g), and CP (8.3×10^4 CFU/g) (Table 1). The average numbers of Actinomycetes in the water was 4.8 CFU/mL.

Identification and molecular characterization via 16S rDNA sequencing

The 16S rDNA nucleotide sequences of 355 suspicious Actinomycete colonies cultured on Actinomycetes selective agar media were analyzed by BLASTN search. A total of 253 colonies were identified as Actinobacteria (71.3%), seven colonies as Alpha proteobacteria (2.0%), six as Firmicutes (1.7%), and thirteen as Gamma proteobacteria (3.7%) (Fig. 2a). The bacteria that were identified as Actinobacteria were analyzed at the genus level. The genus of *Streptomyces* (171) was dominant (67.6%) in this phylum, followed by *Arthrobacter* (56 colonies, (22.1%), *Rhodococcus* (5.5%), and *Norcadiopsis* (4.7%) (Fig. 2b).

Geosmin and 2-MIB leaching simulation using a rain model experiment

A model experiment was conducted to confirm whether odor compounds synthesized by

Actinomycetes could be leached by rainwater and filtered through the soil (Fig. 1b). Concentrations of Geosmin and 2-MIB in the water were measured to check for leaching after 500 mL of water was sprinkled over the soil in the flowerpots and allowed to pass through the soil (Fig. 1b and Table 2). Geosmin concentration was found to be 2.6 times higher in the water samples filtered through out from the soil with plants (the average concentration of 4 sites, 176.8 ± 108.6 ng/L) than the soils without plants (68.5 ± 54.2 ng/L). 2-MIB concentration also was 2.8 times higher in the water samples filtered through out from the soils with plants (average concentration of 4 sites, 277.5 ± 201.4 ng/L) than the soils without plants (97.6 ± 74.2 ng/L). The average Actinomycete concentrations of the 4 sites with and without plants were 2.1×10^7 CFU/g and 9.0×10^5 CFU/g, respectively, and the number of Actinomycete in the soil with plants was approximately 24.2 times more than that in the soil without plants (Table 2).

Next, we compared Actinomycete concentration in the soil with plants and the quantity of odor compounds in the water, and found that Geosmin (339.5 ± 19.1 ng/L) and 2-MIB (579.0 ± 8.5 ng/L) levels were highest at the CP site and lowest at the YP site (7.8×10^7 CFU/g). This was not consistent with the earlier studies that show a positive correlation between Actinomycete

Table 1. Number of *Actinomycetes* in terrestrial and aquatic environments.

Sites	Samples	(CFU/g or CFU/mL) Date			Average
		Mar. 2011	Jun. 2011	Sep. 2011	
GA	Soil	2.0×10^4	3.0×10^6	9.6×10^4	1.0×10^6
	Water	0	0	0	0
YP	Soil	9.9×10^2	1.7×10^6	5.2×10^5	2.2×10^6
	Water	0	4	0	1.3
CP	Soil	5.0×10^4	1.0×10^5	1.0×10^5	8.3×10^4
	Water	0	0	3	1
PD	Soil	3.3×10^3	8.0×10^5	1.4×10^5	3.1×10^5
	Water	0	4.6×10	5	1.7×10
Average	Soil	1.9×10^4	1.4×10^6	2.1×10^5	9.0×10^5
	Water	0	1.3×10	2	4.8

Table 2. Distribution of Actinomycetes and *Streptomyces* and geosmin and 2-MIB synthesis by rain leaching simulation

Sampling sites	Plants	Actinomycetes (CFU/g)	Streptomyces (CFU/g)	Geosmin (ng/L)	2-MIB (ng/L)
GA	w	3.0×10^6	3.0×10^6	115.0 ± 7.1	184.0 ± 22.6
	w/o	6.0×10^4	2.0×10^4	148.5 ± 2.1	204.0 ± 1.4
YP	w	7.8×10^7	2.0×10^5	128.0 ± 7.1	159.5 ± 13.4
	w/o	1.6×10^6	1.6×10^6	34.0 ± 1.4	40.5 ± 3.5
CP	w	5.6×10^6	5.6×10^6	339.5 ± 19.1	579.0 ± 8.5
	w/o	1.3×10^5	1.3×10^5	36.0 ± 1.4	92.0 ± 1.4
PD	w	3.4×10^5	3.0×10^4	124.5 ± 13.4	187.5 ± 20.5
	w/o	1.8×10^6	1.8×10^5	55.5 ± 0.7	54.0 ± 1.4
Average	w	2.1×10^7	2.2×10^6	176.8 ± 108.6	277.5 ± 201.4
	w/o	9.0×10^5	4.8×10^5	68.5 ± 54.2	97.6 ± 74.2

Table 3. Linear regression analysis between Actinomycetes or Streptomyces and odorous compounds

R^2 values	Geosmin	2-MIB
Actnimycetes	0.393	0.357
Streptomyces	0.809	0.847

concentrations and odor compound concentrations. Thus, the number of *Streptomyces*, an Actinomycete genus known for releasing odor compounds, was compared. Our results indicate that the concentration of *Streptomyces* at CP (5.6 × 10^6 CFU/g) was higher than at YP (2.0 × 10^5 CFU/g). A linear regression analysis yielded $R^2 = 0.393$ between the Actinomycetes concentration and

Table 4. Summary of pyrosequence reads and estimated values after normalization

	Plants	Total reads	Normalized reads	Average length (bp)	Observed OTUs	Estimated OTUs (Chao1)	Shannon index
GA	w	3,978	2,250	464.5	1,986	29,554.5	7.48
	w/o	4,388	2,250	464.4	1,787	16,520.4	7.23
YP	w	2,949	2,250	542.9	1,553	9,186.5	6.93
	w/o	2,261	2,250	453.9	1,564	9,671.8	6.89
CP	w	1,048	1,048	458.2	834	6,829.1	6.42
	w/o	6,993	2,250	466.4	1,953	25,991.5	7.43
PD	w	3,441	2,250	452.4	1,539	10,585.7	6.69
	w/o	8,744	2,250	452.4	1,447	8,941.0	6.68
CP_rn	w	1,048	1,040		831	6,919.1	6.41
	w/o	6,993	1,040		954	12,034.1	6.79

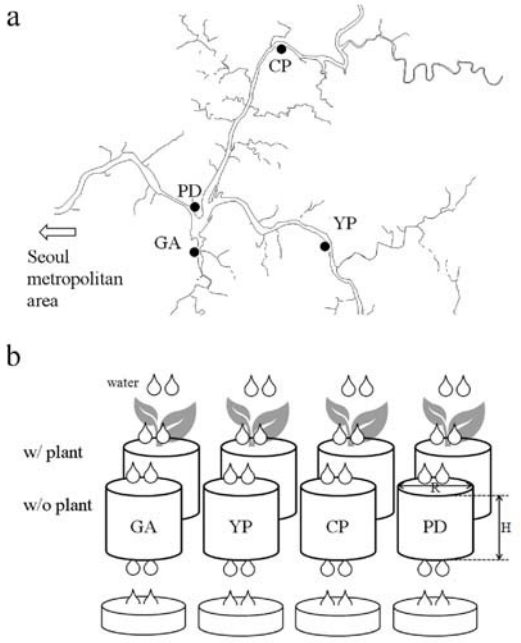

Fig. 1. Sampling locations and schematic diagram of the model experiments.

(a), Four sampling sites, Paldang (PD) and Cheongpyeong (CP) are artificial lakes and Gyeongan (GA) and Yangpyeong (YP) are streams. The water of CP, GA, and YP flow into PD, and the lakewater of PD is used as a water source for the Seoul metropolitan area.(b), The rain model experiments were designed to determine whether odor compounds synthesized by Actinomycetes could be leached by rainwater filtered through the soil.

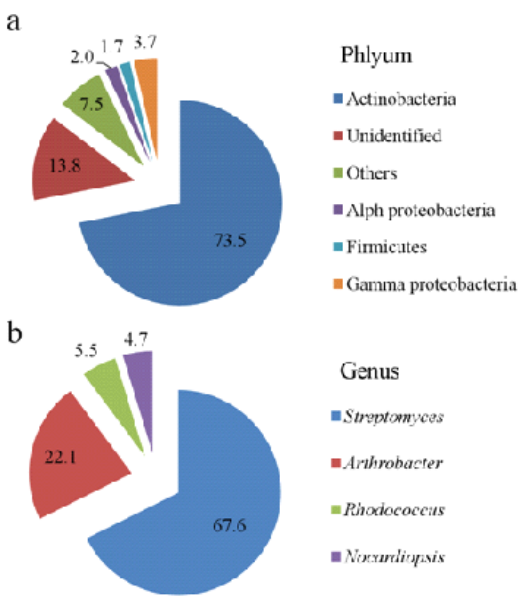

Fig. 2. Distribution of Actinobacteria based on 16S rRNA sequencing.

The 16S rDNA nucleotide sequences of 355 suspicious Actinomycetes colonies were analyzed by BLASTN search. (a), A total of 253 colonies were identified as Actinobacteria (71.3%). (b), Bacteria that were identified as Actinobacteria were analyzed at the genus level. The *Streptomyces* genus (171) was dominant (67.6%) in this phylum

Geosmin concentration and $R^2 = 0.357$ with 2-MIB, whereas the R^2 values between the *Streptomyces* concentration and Geosmin and 2-MIB concentrations were 0.809 and 0.847, respectively (Table 3). These results suggest that the *Streptomyces* concentration more highly correlates with Geosmin or 2-MIB concentrations than all Actinomycetes. Table 3

In addition, this indicates that these odor compounds could be retained in the soil and leached into the water reservoirs during rainfall.

Analysis of bacterial communities obtained from pyrosequencing data

Summary of pyrosequencing results, including the numbers of observed OTUs and estimated values after normalized read sizes, are shown in Table 4. Typically, bacterial diversities from GA and CP were higher than those from YP and PD. The diversity indices from plants were higher than without plants, with the exception of the sample from CP. Since the read number from CP soil with plants (1,048 reads) was smaller than the normalized read size (2,250), another normalized step was conducted to compare CP samples with plants and without plants (subsampled size of CP_rn was 1,040). A significant difference of bacterial communities was observed with planting treatment from the CP samples (0.38 change in Shannon index and 2-fold higher than the estimated OTUs in CP samples without plants by Chao1 analysis). Similar trends and richness were observed in the rarefaction curve slopes (Fig. 3). The differences of phyla composition was investigated and compared with one another (Fig. 4). Four phyla were commonly dominant in all samples, namely Proteobacteria, Actinobacteria, Acidobacteria, and Chloroflexi, while Firmicutes, Thermobaculum_p, and Bacteroidetes were only abundant in few samples. The relative abundance of Chloroflexi was higher in soil from the PD site (15.4% with plants and 15.0% without plants) than other sites (less than 10.5% of total reads). Proteobacteria was most abundant in CP soil (42.6% with plants and 44.9% without plant) as compared to other soil (less than 37.9%). The

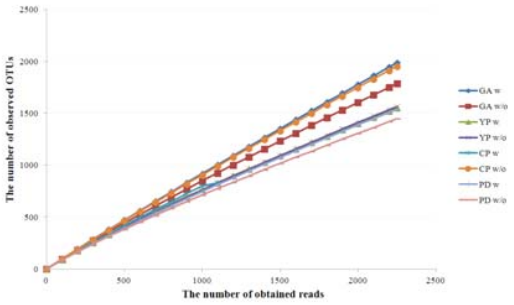

Fig. 3. Rarefaction curves of samples were compared with normalized read number. GA indicates Gyeongan; YP, Yangpyeong; CP, Cheongpyeong; PD, Paldang; w indicates with plant treatment; w/o indicates without plant.

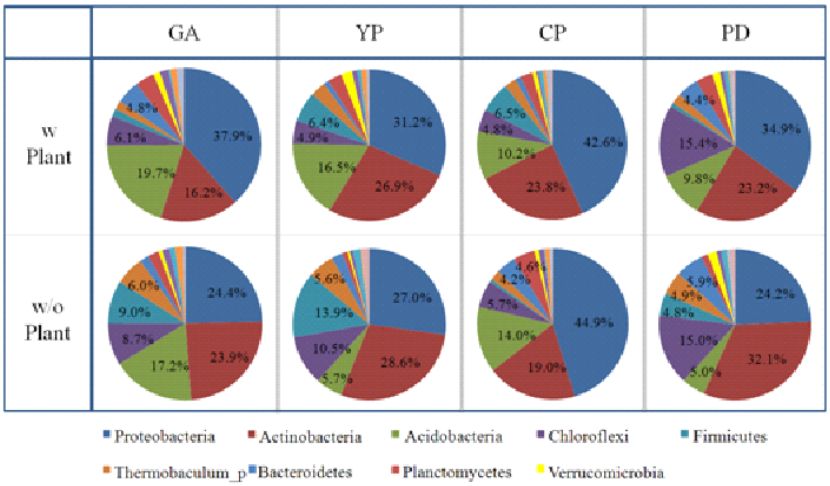

Fig. 4. The compositions of phyla were compared among each site soil with plant and without plants. The proportions over 4% of total reads were present at each pie, and their names are indicated below the figure. GA indicates Gyeongan; YP, Yangpyeong; CP, Cheongpyeong; PD, Paldang; w Plant, with plant; w/o Plant, without plant.

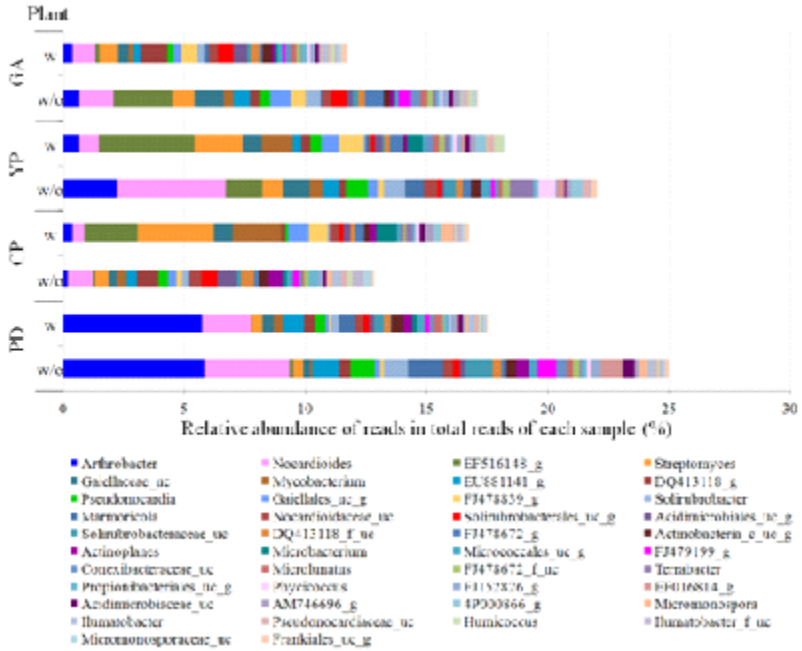

Fig. 5. The relative abundances of genera within Actinobacteria phylum of each site were compared. The name of each color is shown below the figure. The nomenclatures of phylotypes are based on the EzTaxon-e database (Kim *et al.*[23], http://eztaxon-e.ezbiocloud.net/).

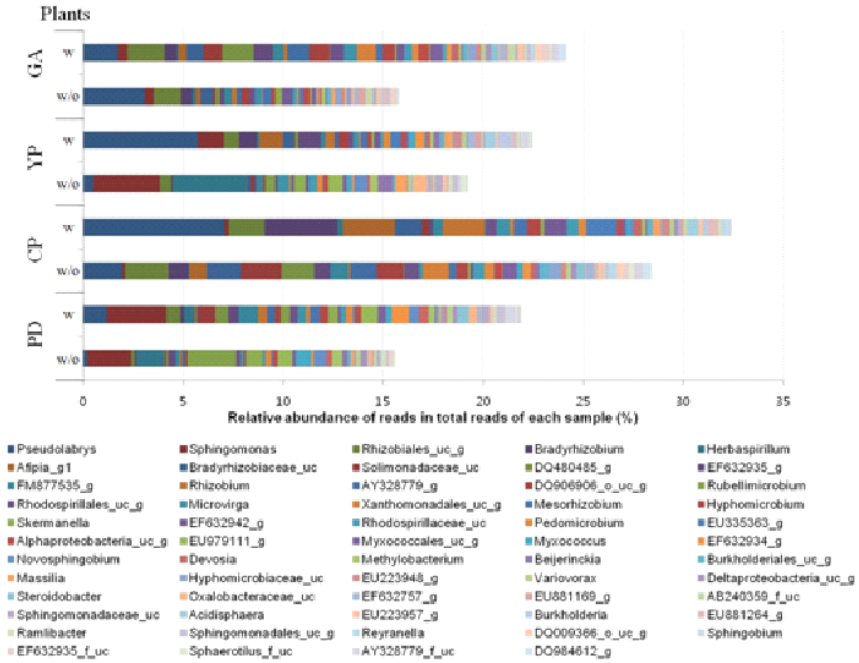

Fig. 6. The comparisons of genera within Proteobacteria obtained from each site. The name of each genus appears below the figure. The nomenclatures of genera are based on the EzTaxon-e database (Kim *et al.*[23], http://eztaxon-e.ezbiocloud.net/).

Actinobacteria phylum was second most abundant group in the collected samples. The planting effect on each soil was different due to their unique bacterial community. Overall, Proteobacteria was the most abundant group in the samples with plants, while Actinobacteria was enhanced in soil samples without plants. However, the relative abundance of Actinobacteria lower at CP sites without plants. At the GA site, the relative abundance of Actinobacteria was higher without plants (23.9% of total reads) than with plants (16.2%), and Firmicutes (9.0% without planting and 1.6% with plants) and Thermobaculum_p (6.0% at without and 1.8% at with planting) were more abundant in samples without plants. However, of the Proteobacteria phyla, Acidobacteria and Bacteroidetes were more abundant in samples with plants than without plants. As compared to the YP soil, the relative abundance of Actinobacteria was higher in without plants (28.6% of total reads) than with plants (26.9%), and Chloroflexi, Firmicutes Thermobaculum_p were most abundant in samples without plants. The Proteobacteria phylum was abundant in YP samples with plants. Originating CP samples contained more Actinobacteria with plants (23.8%) than without plants (19.0%). The relative abundance of Firmicutes in CP samples with plants was higher than without plants, while Proteobacteria, Acidobacteria, Chloroflexi, Bacteroidetes, and Planctomycetes were higher in samples without plants as compared to samples with planning soil. The proportions of Actinobacteria was also higher in PD samples without plants (32.1%) than with plants (23.2%), while Firmicutes, Thermobaculum_p, and Bacteroidetes were higher in samples without plants. The proportions of Proteobacteria, Acidobacteria, and Chloroflexi were higher in samples with plants.

The differences of genera within Actinobacteria originating from each soil sample were then compared with one another (Fig. 5). The compositions of genera within Actinobacteria were different from each site in the samples when plants were or were not present. The *Arthrobacter* genus was the most abundant genus in PD soil, and this was not influenced by plant (5.7% of total reads from plant soil and 5.8% without plant soil). The relative abundances of *Nocardioides* were higher without plants than in with plants (higher difference obtained from YP soil; 0.8% at with plants and 4.4% without plants). Sequences similar to uncultured sequences, the EF516148 composition was different for each soil type. The relative

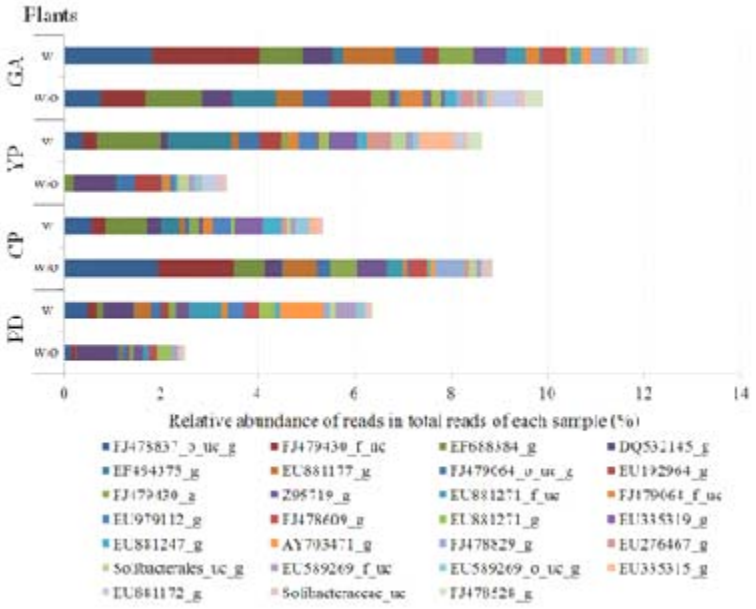

Fig. 7. The compositions of genera within Acidobacteria were compared. The names of genera are shown below the figure. The nomenclatures of genera are based on the EzTaxon-e database (Kim *et al.*[23], http://eztaxon-e.ezbiocloud.net/). All of genera are uncultured Acidobacteria.

abundances of *Streptomyces* were higher with CP (3.2% with plant and 0.5% without plant) and YP (2.0% with plant and 0.9% without plant) plants, while *Streptomyces* was found to have similar abundances in soil from GA and PD despite plants. The relative abundances of *Mycobacterium* were higher in samples with plants than without plants from the PD, CP, and YP sites. A total of 277 genera within Actinobacteria were detected from all soil samples with and without plants. Most of genera were uncultured actinobacteria, since there was a large diversity of species from the soil. A total of 588 genera within Proteobacteria were detected from all samples, and the *Pseudolabrys*, *Sphingomonas*, and *Bradyrhizobium* genera were dominant in most samples (Fig. 6). Most of genera were related with rhizosphere bacteria. Acidobacteria was also one of the dominant phyla in all the samples, and their genera composition was investigated by pyrosequencing reads (Fig. 7). A total of 232 genera were detected from all of samples, and all each were assigned to uncultured Acidobacteria.

DISCUSSION

In this study, we have determined that *Streptomyces*, which is known for producing the odor compounds Geosmin and 2-MIB, is a dominant organism. It is well known that Actinomycetes always exists in soil and the odor compounds produced by *Streptomyces* are predominately 2-MIB and Geosmin[10-15]. Furthermore, using a rain leaching model, this study showed that Geosmin and 2-MIB produced by *Streptomyces* can be leached through the soil because of rainfall and revealed a positive correlation between the number of *Streptomyces*, but not Actinomycetes, and leached odor compound concentration. The higher proportion of *Streptomyces* in soil with plants was also detected through culture-independent pyrosequencing data. Although most of detected genera were identified as uncultured bacteria, a diverse population was observed by this technique. The relative abundances of each genera within Actinobacteria differed depending on the culturing methods (Fig. 2b and Fig. 5). This is due to the selectivity of the culture media and the detection of various genera using culture independent pyrosequencing. The relative abundance of each phylotype obtained from our pyrosequencing results was not quantitative, but it does reveal the comparative proportions of each bacterial community. As such, we could not determine the increased cell number for each phylotype with planting treatment by pyrosequencing. However, the shift of whole bacterial community caused by planting treatment is important for future studies that investigate drinking water quality.

In Korea, the total tap water production was approximately 59 ton in 2010; the tap water distribution rate was 97.7%, and the population using tap water was approximately 50 million. The volume of tap water used per person in a day is 333 L[26]. There were 16,393 civil complaints in 2010 regarding water quality, including tap water odor. The management of the T&O of tap water is one of the most important issues for the tap water producing business. In Korea, the cause of T&O in tap water is due to blue-green algae contamination[16–18]. One study identified Cyanophyceae as the algal family releasing the odor compound into the water system at the PD site[18], which is a site included in this study. However, occasionally T&O occurred in tap water with a low number of algae, thus suggesting that Actinomycetes might be the cause of the odor[3, 27]. However, unlike algae that are found in large quantities in the water resulting in T&O, Actinomycetes do not exist much in water. Therefore, it is hard to detect a significant difference in the Actinomycetes concentrations during a T&O event. Thus, it is difficult to show the correlation between the numbers of Actinomycetes in the water and concentration of the odor compounds.

One possibility for this T&O event is that odor compounds produced by Actinomycetes enter drinking water resources. This is thought to occur because high levels of Actinomycetes exist in the soil around drinking water resources. Therefore, the results of this study show that the odor compounds could be produced by the soil Actinomycetes and that compounds released in the soil then leach into the drinking water resources. It was reported that the water quality of the lake and the ecosystem could be greatly affected by the pollutants that leach out from the soil as a result of rainfall and the PD site's basin

SA:V ratio of 618 is much larger than that of Biwa, Japan and Lake Michigan, USA[18, 28, 29].

This study also shows that Actinomycetes is more present in soil with plants than without plants; and 16S rDNA sequencing and pyrosequencing analysis identified various groups. Furthermore, increased quantities of odor compounds were produced and leached from the soil with plants. The plant rhizosphere contains a variety of exudates from plant roots and thus can support a highly diverse soil microbial biota including Actinomycetes and rhizobacteria[30–32]. The abundance and diversity of microorganisms in the soil, especially the rhizosphere, could be affected by the soil physicochemical properties, the organic matter quality, plant nutrient uptake characteristics, and so on[33, 34]. Therefore, it is thought that *Streptomyces* thrives more in soil around the water system where plant distribution is high, thus producing large quantities of odor compounds. From the regression analysis of this study, R^2 values between the number of Actinomycetes and concentration of Geosmin or 2-MIB were 0.393 and 0.357 respectively, whereas R^2 values between the number of *Streptomyces* and concentration of Geosmin or 2-MIB were 0.809 and 0.847, respectively, showing a strong positive correlation between the number of *Streptomyces* and known odor causing compounds. Future studies are required to show whether odor compounds enter water sources during rainfall or a thaw period, and whether a correlation exists between the Actinomycetes concentrations in the water and the concentration of the odor compounds as more sensitive techniques are required to test Actinomycetes concentrations in water.

ACKNOWLEDGEMENTS

This work was supported by K-water (KIWE-WARC-2011).

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