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Characteristics of actinomycetes producing geosmin in Paldang Lake, Korea

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ABSTRACT

Geosmin and 2-methylisoborneol (2-MIB) are organic compounds known to cause earthy and musty odors in drinking water. In Korea, during the summer of 2012, a geosmin outbreak in the drinking water supply prompted further research to identify the source of the odorous materials, since these occurrences were not well understood or monitored. The purpose of this study was to investigate how important a role actinomycetes play in the production of geosmin and 2-MIB in Paldang Lake by measurement of microbial density (the number of colony-forming units), pyrosequencing of 16S rRNA genes, and determination of the prevalence of the geosmin synthase gene. Three sampling sites (P1, P2, and P3) were selected. The increase in geosmin concentration was paralleled by an increase in actinomycetes (*Streptomyces* spp.) and cyanobacteria (*Anabaena* spp.) populations. The bacterial communities in Paldang Lake were characterized by 454 pyrosequencing based on 16S rRNA gene sequences. P1 (North Han River) showed the highest density of actinomycetes, followed by P2 (Paldang Dam), and P3 (South Han River). The density of *Streptomyces* spp. increased at P1, while that of cyanobacteria increased at P3. No significant density change was noted in those two species at P2, a confluence of two rivers. The genes producing geosmin or 2-MIB were detected in 60 single strains that were isolated from water samples in Paldang Lake. This study confirmed that *Streptomyces* spp. significantly affect the production of geosmin that causes odors in Paldang Lake.

Keywords: Actinomycetes; *Streptomyces* spp.; Geosmin; 2-MIB

1. Introduction

In 2012, a nationwide geosmin outbreak in drinking water supply occurred in Korea. Since Paldang Lake is the largest drinking water supply reservoir in Korea, the presence of geosmin and 2-methylisoborneol (2-MIB) in this lake received widespread attention. Geosmin is a well-known odoriferous metabolite, produced by a wide variety of micro-organisms, including *Streptomyces*, cyanobacteria, myxobacteria, and

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various fungi [1,2]. Geosmin and 2-MIB have long been known to be associated with actinomycetes and cyanobacteria [3]. Compared with cyanobacteria, relatively little was known about the contribution of actinomycetes to taste and odor in drinking water. Actinomycetes are very common gram-positive filamentous bacteria, and are major producers of odorous compounds like geosmin and 2-MIB in aquatic environments [3]. Bacteria within the order *Actinomycetales*, such as *Actinomycetaceae* and *Streptomycetaceae* (also referred to as actinomycetes), are believed to be among the major geosmin- and MIB-producing bacteria in nature [4]. Geosmin outbreaks occur frequently, and consumers who think that water with these odors is unsafe to drink make many complaints about musty and earthy odors, although the water supplies are adequately treated [5]. It is tricky and complicated to operate and manage water treatment processes, and the cost of the treatment of odorous water is high [6]. Since geosmin outbreaks are not well understood or monitored, more research to find the source of the objectionable compounds needs to be conducted to precisely determine the source of the unpalatable compounds and to prevent nationwide geosmin outbreaks. In order to establish an early warning system for algal bloom, we are continuously monitoring water quality in Paldang Lake, conducting weekly measurements of parameters such as biological oxygen demand, chemical oxygen demand, contents of total nitrogen, total phosphorus, chlorophyll a, and other pollutants, and the density of blue-green algae. The goal of this study was to investigate the role actinomycetes play in the production of geosmin and 2-MIB in Paldang Lake, by analyzing actinomycetes density, pyrosequencing, and by determining the prevalence of the geosmin synthase gene.

2. Materials and methods

2.1. Study area

Paldang Lake is the largest drinking water supply reservoir in Korea, with a water storage capacity of 244×10^6 m^3 and a surface area of 20,085 km^2. This lake has two major tributaries, the North and South Han Rivers, and one minor tributary, the Gyung-An Stream, which provide 57.0, 40.5, and 2.5% of the water inflow, respectively. The Paldang dam, located in Yangsuri where the two main branches merge, is the main reservoir supplying drinking water for downstream metropolitan areas.

2.2. Sampling locations

Samples were collected on 35 different dates from Paldang Lake at three representative points during the summer of 2012 (from 29 June to 25 September 2012). Fig. 1 shows the sampling locations: P1 in the North Han River, P3 in the South Han River, and P2 at the confluence of the North and South Han Rivers. River water samples were taken from 30 cm below the surfacewater and collected in 2-L sterilized polyethylene containers kept below 4˚C in a cool bag, and then transferred to the laboratory at the Han River Environment Research Center.

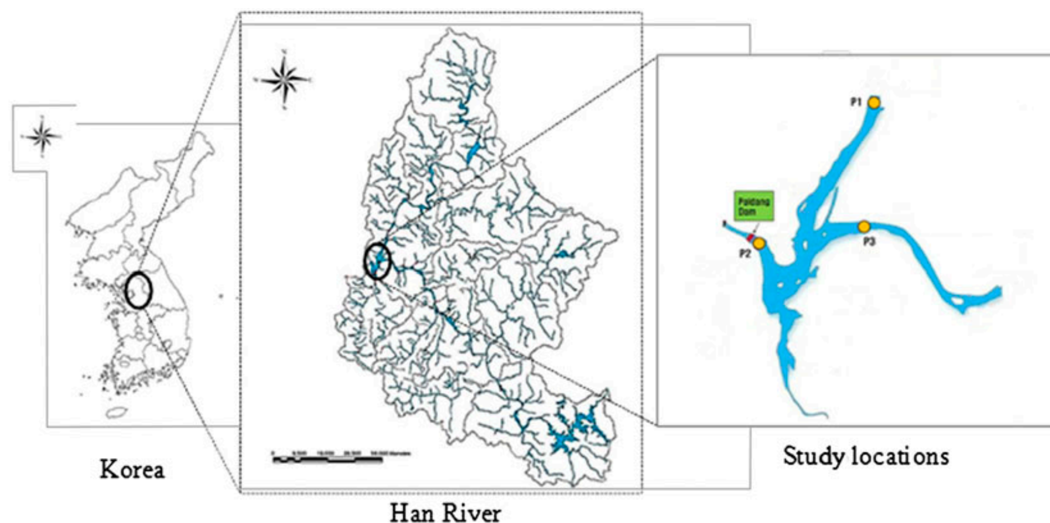

Fig. 1. Study area and locations of sample collection sites.

2.3. Isolation and culture of Streptomyces spp.

Since this study aimed to culture as many actinomycetes (mainly *Streptomyces* spp.) as possible in order to determine their distribution density, and perform molecular characterization with a culture-based approach, we selected humic acid-vitamin (HV) agar, a medium widely used for culturing this species, as the growth medium [7]. One liter of sample was filtered with a Sartolab disposable sterile filter (polyethersulfone membrane, 0.2 μm; Fisher Scientific, USA) to isolate *Streptomyces* spp. In order to isolate, spreading was performed in two steps. In the first step, the filter paper was divided into four pieces by using a sterilized knife, and placed on HV agar medium. One milliliter of sterilized water was added to each membrane piece on the plate, and the first spreading was carried out. After turning the membrane face down, an additional second spreading was performed. After incubation at 28˚C for five d, some colonies were formed. From these, *Streptomyces* spp. colonies were identified based on spore color in the aerial mycelium and the color of the substrate mycelium, and were isolated and cultured.

HV agar was prepared as follows: humic acid (1.0 g), Na_2HPO_4 (0.5 g), KCl (1.71 g), $MgSO_4{\cdot}7H_2O$ (0.05 g), $FeSO_4{\cdot}7H_2O$ (0.01 g), $CaCO_3$ (0.02 g), and agar (20 g) per 1 L of distilled water were melted together and the pH of the solution adjusted at 7.2. It was then sterilized at 121˚C for 15 min. When the solution had cooled, but before the agar solidified, 0.5 mg inositol, 0.5 mg Ca-pantothenate, 0.5 mg thiamine-HCl, 0.25 mg biotin, 50 mg cycloheximide, and 20 mg nalidixic acid were added. The mixture was then poured into culturing plates and allowed to solidify.

Isolated *Streptomyces* spp. were maintained on Bennett's agar, prepared as follows: 10 g glucose, 1 g yeast extract, 2 g bacto peptone, and 20 g agar per 1 L of distilled water were melted together, the pH was adjusted to 7.2, and the solution was sterilized [7].

2.4. Microbial density analysis of Streptomyces spp.

In order to analyze the numbers of the *Streptomyces* spp. by culture-dependent approaches, viable cells were counted on HV agar medium after days d of incubation at 28˚C. Samples obtained from 1 and 250 mL of lake water were incubated as follows: for 1×, a 1-mL aliquot water sample was plated and for 250×, 1 L of water sample was filtered, and then a quarter of the filter paper used, as described above colony-forming units (CFUs) were calculated as the average number of colonies in 1 L of water sample.

2.5. DNA extraction, polymerase chain reaction and sequencing

Amplification of the 16S rRNA, geosmin synthase and 2-MIB genes, and sequencing of 16S rRNA of isolates were carried out. A single colony of an isolate grown on Bennett's agar was resuspended in 100 mL of 5% (w/v) Chelex-100 solution (Bio-Rad, USA) and boiled for 10 min to prepare crude genomic DNA lysates. The genes were amplified by polymerase chain reaction (PCR) using the primers in Table 1. The following thermal profiles were used for PCR: denaturation at 94˚C for 5 min, primer annealing for 1 min at various temperatures for actinomycetes (Table 1), and extension at 72˚C for 1 min. The final cycle included an extension at 72˚C for 10 min to ensure full extension of the products. The PCR products were assayed by electrophoresis on a 0.8% (w/v) agarose gel, stained with RedSafeTM (iNtRON Biotech, Korea), and visualized with a UV transilluminator. The PCR products were purified using the QIAquick PCR Purification kit (QIAGEN, Germany).

Sequencing of the purified 16S rRNA gene was performed using an ABI PRISM BigDye Terminator cycle sequencing kit (Applied Biosystems, USA) as recommended by the manufacturer's instructions. The purified reaction mixtures were electrophoresed automatically using an Applied Biosystems model 3730XL automatic DNA sequencer. The resultant 16S rRNA gene sequence of the isolates were compared with available sequences from GenBank using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/; [12]) to determine an approximate phylogenetic affiliation, and sequence similarity values were computed using the EzTaxon server (http://www.eztaxon-e.ezcloud.net/; [13]).

2.6. Phylogenetic tree

To determine the phylogenetic location of the isolates, the 16S rRNA gene sequence was aligned with the published sequences of closely related bacteria with CLUSTAL W 2.1 software [14]. Phylogenetic trees were constructed using the neighbor-joining [15] algorithms within the MEGA5 program [16]. Evolutionary distance matrices for the neighbor-joining method were calculated using the algorithm of the Kimura 2-parameter model [17]. To evaluate the stability of the phylogenetic tree, a bootstrap analysis (1,000 replications) was performed [18].

Table 1
Primers used in PCR

Primer	Target gene	Sequence (5´–3´)	Amplicon size (base pairs)	Annealing temp. (℃)	Reference
27F	16S rRNA	AGAGTTTGATCCTGGCTCAG	1,500	50	[8]
1492R		GGTTACCTTGTTACGACTT			
CycFW	*geoA*	TGGTAYGTITGGGTITTYTTYTTYGAYGAYCAYTT	730	52	[9]
CycRW		CATRTGCCAYTCRTGICCICCISWYTGCCARTCYTG			
250F	*geoA*	TTCTTCGACGAYCACTTCC	743	60	[10]
971R		CCCTYGTTCATGTARCGGC			
AMmib-F	*tpc*	TGGACGACTGCTACTGCGAG	592	58	[11]
AMmib-R		AAGGCGTGCTGTAGTTCGTTGTG			
AMgeo-F	QPCR primers *geoA*	GAGTACATCGAGATGCGCCGCAA	167	66	[11]
AMgeo-R		GAGAAGAGGTCGTTGCGCAGGTG			
FuB27F	Fusion primers (pyrosequencing)	CCTATCCCCTGTGTGCCTTGGCAGTC TCAGACGAGTTTGATCMTGGCTCAG	491	56–60	
FuB518R	16S rRNA	CCATCTCATCCCTGCGTGTCTCCGACTCA GATCAGCACACWTTACCGCGGCTGCTGG			

2.7. Pyrosequencing

In order to analyze the bacterial community in Paldang Lake by a culture-independent approach, pyrosequencing reactions based on the 16S rRNA gene sequence were performed using a sequence analyzer (454 FLX pyrosequencer; Life Science, Roche USA) (Table 3). Water samples were extracted using UltraClean® Soil DNA Isolation Kit (MO BIO Laboratories Inc. USA). Extracted DNA was examined by electrophoresis on an agarose gel, and quantified using a NanoDrop 1000TM UV–VIS spectrophotometer (Thermo Scientific, USA). Variable regions, namely, V1–V3, of the 16S rRNA gene were amplified with barcoded bacterial universal primers FuB27F and FuB518R (Table 1). PCR was performed as follows: 5 μL 10× buffer, 1 μL 10 mM dNTP, and 2 μL primers (20 pmol/μL; forward/reverse) were mixed, and then, 0.25 μL *Taq* Polymerase (5 unit/μL; Roche, USA) and 1 μL DNA (100 μg/mL) were added. The total volume was made up to 50 μL by addition of distilled water, after which PCR was performed. Amplifications were performed at 94℃ for 5 min, followed by 25 cycles of denaturation at 94℃ for 45 s, primer annealing at 56℃ for 45 s, and extension at 72℃ for 60 s, with a final extension at 72℃ for 6 min. The PCR products were purified with Prep-A-Gene (Bio-Rad, USA) according to the manufacturer's instructions. After sequencing was completed, all sequence reads were quality checked using Mothur software [19]. Any poor quality reads less than 200 bp were removed. Mothur software was also used to conduct rarefaction analysis, and assign sequences to operational taxonomic units (OTUs, 97% similarity).

2.8. Analysis of geosmin and 2-MIB

Each water sample was put in a 20-mL glass tube and 3 g NaCl was added. Solid-phase microextraction (SPME) was used with the Combi PAL autosampler (CTC Analytics, Switzerland), purchased from Supelco (Sigma-Aldrich, Bellefonte, PA, USA). Using SPME, geosmin was analyzed by GC–MS–MS (450-GC/320-MS; Bruker USA). The analytical conditions for GC/MS are described in Table 2. GC–MS–MS was performed in the selected ion monitoring mode. After using the full scan mode, geosmin was quantified with its main characteristic ion, *m*/*z* 97, while 2-MIB was quantified with its *m*/*z* 91 ion.

3. Results and discussion

3.1. Microbial density analysis

In order to investigate the relationship between the density distribution of actinomycetes and the concentration of geosmin and 2-MIB in Paldang Lake, 35

Table 2
Analytical conditions for GC and GC–MS–MS

GC run conditions	
Analytical column	30 m × 0.25 mm, 0.25 μm, VF-5 ms
Injection mode	Split mode, ratio 50:1
Inlet temperature	270℃
Flow mode	Constant flow, 1 mL/L, helium
	35℃ for 5 min
	8℃/min to 180℃, hold for 1 min
Oven temperature	40℃/min to 295℃, hold for 1 min
Carrier gas	Helium in constant flow mode, 1 mL/min
Transfer line temperature	230℃
MS–MS conditions	
Scan mode	Electron impact, 50 m/z through 350 m/z
MRM mode	Electron impact, multiple reaction monitoring (MRM)
CID gas pressure	1.5 mTorr, He
MS temperature	Source 230℃

water samples from 29 June to 25 September 2012, were tested. We also tested for Anabaena, which is known to cause odors. Geosmin concentration in the water increased to >4,000 ppt during 30 d from the mid-July to mid-August, and decreased dramatically after mid-August, because of the monsoon and typhoons (Fig. 2(B)). 2-MIB concentration rapidly increased to >20 ppt in a short period at the end of July and subsequently decreased (Fig. 2(C)). The number of *Anabaena* spiroides increased until mid-August, and then clearly decreased because of the monsoon and typhoons. It is noted that the increase in

Table 3
Identification of actinomycetes isolates from Paldang Lake (16S rRNA gene sequence similarity results by using EzTaxon-e server)

Isolate	Type strain name	Strain numbers	Accession no.	Similarity (%)
HR001	*Streptomyces antibioticus*	NBRC 12838(T)	AB184184	99.57
HR002	*Streptomyces scabrisporus*	NBRC 100760(T)	AB249946	99.36
HR003	*Streptomyces cyaneofuscatus*	JCM 4364(T)	AY999770	99.57
HR005	*Streptomyces thermolineatus*	DSM41451(T)	Z68097	99.36
HR006	*Micromonospora haikouensis* (Invalid name)	232617(T)	GU130129	98.99
HR007	*Streptomyces griseorubens*	NBRC 12780(T)	AB184139	99.71
HR008	*Streptomyces cacaoi subsp. Asoensis*	NRRL B-16592(T)	DQ026644	99.35
HR009	*Streptomyces coelicoflavus*	NBRC 15399(T)	AB184650	99.15
HR012A	*Streptomyces rishiriensis*	NBRC 13407(T)	AB184383	98.79
HR012B	*Streptomyces caeruleatus*	GIMN4(T)	GQ329712	98.92
HR013	*Streptomyces yanii*	NBRC 14669(T)	AB006159	99.63
HR014	*Streptomyces griseoplanus*	AS.4.1868(T)	AY999894	99.86
HR015	*Kribbella karoonensis*	Q41(T)	AY995146	99.06
HR016	*Streptomyces olivochromogenes*	NBRC 3178(T)	AB184737	99.21
HR018	*Streptomyces anulatus*	NRRL B-2000(T)	DQ026637	99.43
HR020	*Streptomyces aurantiogriseus*	NBRC 12842(T)	AB184188	98.29
HR021	*Streptomyces glauciniger*	NBRC 100913(T)	AB249964	99.57
HR022	*Streptomyces exfoliates*	NBRC 13191(T)	AB184324	99.22
HR023	*Streptomyces exfoliates*	NBRC 13191(T)	AB184324	99.50
HR024	*Rhodococcus jostii*	IFO16295(T)	AB046357	98.85
HR025	*Nocardioides fonticola*	NAA-13(T)	EF626689	98.48
HR026	*Streptomyces cirratus*	NRRL B-3250(T)	AY999794	99.22
HR027	*Streptomyces viridobrunneus*	LMG 20317(T)	AJ781372	98.00

(*Continued*)

Table 3
(*Continued*)

Isolate	Type strain name	Strain numbers	Accession no.	Similarity (%)
HR028	*Streptomyces olivochromogenes*	NBRC 3178(T)	AB184737	99.43
HR029	*Streptomyces phaeoluteichromatogenes*	NRRL 5799(T)	AJ391814	99.07
HR030	*Streptomyces rubiginosohelvolus*	NBRC 12912(T)	AB184240	99.15
HR031	*Streptomyces brevispora*	BK160(T)	FR692104	98.51
HR032	*Streptomyces toxytricini*	NBRC 12823(T)	AB184173	99.22
HR033	*Streptomyces caeruleatus*	GIMN4(T)	GQ329712	98.71
HR036	*Streptomyces griseorubiginosus*	NBRC 13047(T)	AB184276	99.28
HR040	*Duganella phyllosphaerae* (Invalid name)	T54(T)	FR852575	97.93
HR041	*Streptomyces phaeoluteichromatogenes*	NRRL 5799(T)	AJ391814	98.93
HR043	*Streptomyces griseoplanus*	AS 4.1868	AY999894	99.43
HR044	*Streptomyces brevispora*	BK160(T)	FR692104	98.86
HR045	*Streptomyces anthocyanicus*	NBRC 14892(T)	AB184631	99.43
HR046	*Streptomyces viridochromogenes*	NBRC 3113(T)	AB184728	99.21
HR047	*Streptomyces rishiriensis*	NBRC 13407(T)	AB184383	99.28
HR048	*Streptomyces rishiriensis*	NBRC 13407(T)	AB184383	99.00
HR049	*Streptomyces rishiriensis*	NBRC 13407(T)	AB184383	99.43
HR054	*Streptomyces caeruleatus*	GIMN4(T)	GQ329712	98.92
HR055	*Streptomyces glauciniger*	NBRC 100913(T)	AB249964	99.57
HR056	*Streptomyces griseorubiginosus*	NBRC 13047(T)	AB184276	99.50
HR057	*Streptomyces europaeiscabiei*	KACC 20186(T)	AY207598	99.00
HR058	*Streptomyces celluloflavus*	NBRC 13780(T)	AB184476	99.08
HR059	*Streptomyces chromofuscus*	NBRC 12851(T)	AB184194	98.50
HR060	*Streptomyces coelicoflavus*	NBRC 15399(T)	AB184650	99.01
HR061	*Micromonospora siamensis*	TT2-4(T)	AB193565	98.55
HR062A	*Streptomyces ciscaucasicus*	NBRC 12872(T)	AB184208	99.07
HR062B	*Streptomyces canus*	NRRL B-1989(T)	AY999775	99.22
HR063	*Phycicoccus ginsenosidimutans*	BXN5-13(T)	EU332824	97.55
HR064	*Streptomyces glauciniger*	NBRC 100913(T)	AB249964	99.14
HR065A	*Streptomyces faradiae*	NBRC 3439(T)	AB184776	99.64
HR065B	*Streptomyces faradiae*	NBRC 3439(T)	AB184776	99.22
HR065C	*Streptomyces faradiae*	NBRC 3439(T)	AB184776	99.22
HR066	*Streptomyces spororaveus*	LMG 20313(T)	AJ781370	99.29

the density of actinomycetes followed the profile of geosmin concentration over 30 d, achieving a cell density of over 10^5 CFU/L. In particular, when the geosmin and 2-MIB concentrations dramatically increased after 18 July, the densities of both actinomycetes and *Anabaena* spp. also increased (Fig. 2(A) and (D)). When the actinomycete populations at the three sampling sites were compared, P1 showed the highest concentration, followed by P2 and P3. Fig. 2 shows that densities of actinomycetes and *Anabaena* spp. changed accordingly due to monsoon and typhoons. The concentration of geosmin continuously increased from 4 July before a typhoon came to Korea, and then it decreased dramatically after the typhoon went out. However, the concentration of geosmin fluctuated. This can be explained by the fact that the inflow of soil into the Lake led to the increase of actinomycetes, while outflow of the upper part of the Lake from the dam lowered the concentration of actinomycetes. Therefore, it can be assumed that there is a correlation between actinomycetes and geosmin in Paldang Lake. Fig. 2 shows the number of CFUs of *Anabaena* spp. and the concentration of geosmin and 2-MIB over the same time period. It shows that when the concentration of geosmin increased, the CFUs of *Anabaena* spp. also increased. It suggests that geosmin was produced not only by actinomycetes, but also by *Anabaena* spp. Additionally, the overall concentration of 2-MIB, which produces odorous compounds like geosmin in *Anabaena* spp. or actinomycetes, is low. This implies that the microbes that affect geosmin production are mainly associated with both actinomycetes and *Anabaena* spp.

3.2. Pyrosequencing

In order to understand the microbial community structure of Paldang Lake, culture-independent molecular approach was used for two samples: one sampled

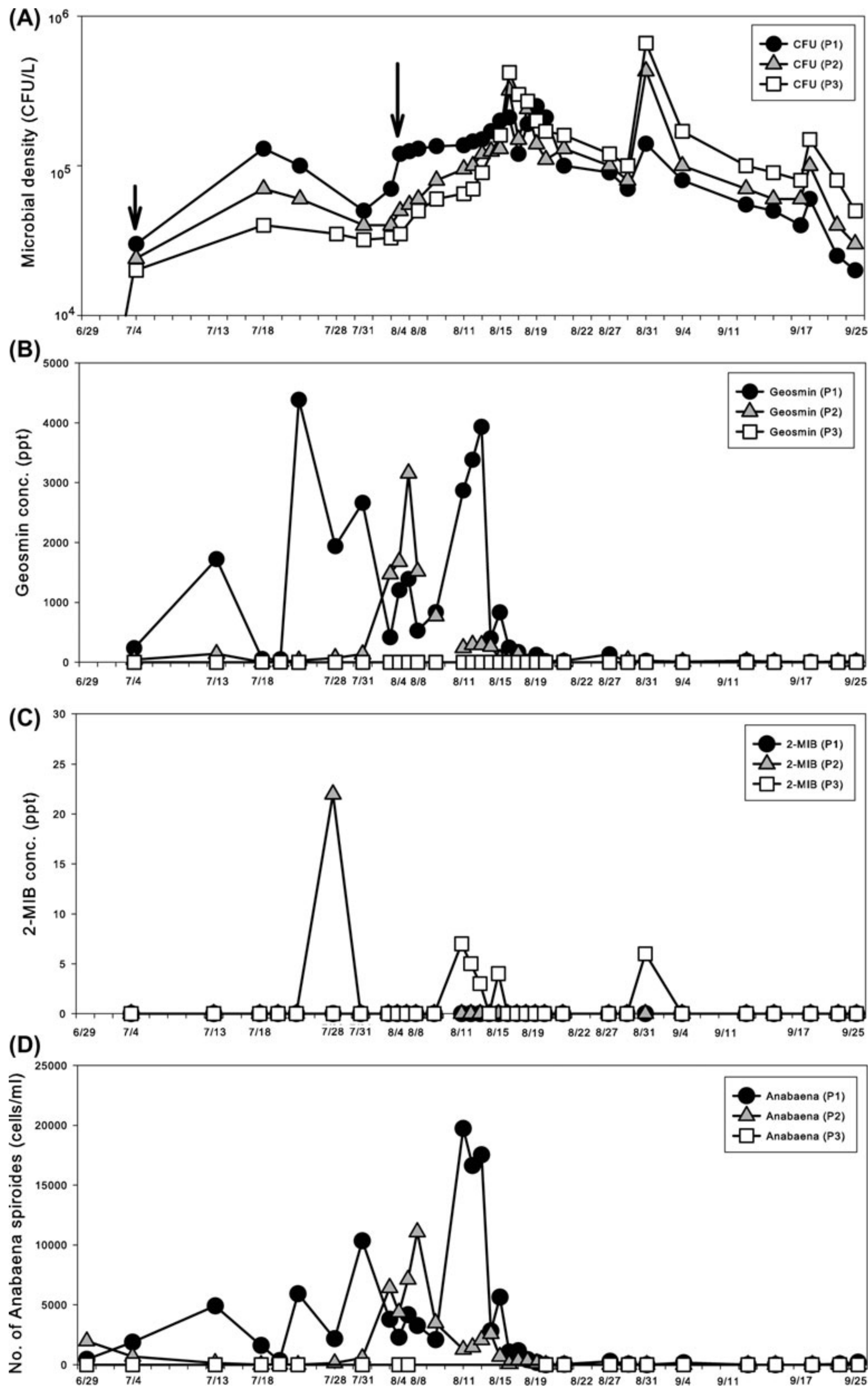

Fig. 2. The changes in the number of CFUs of actinomycetes (A) and *Anabaena* spp. (D) and the concentrations of geosmin (B) and 2-MIB (C) in Paldang Lake from late June through mid-September 2012.

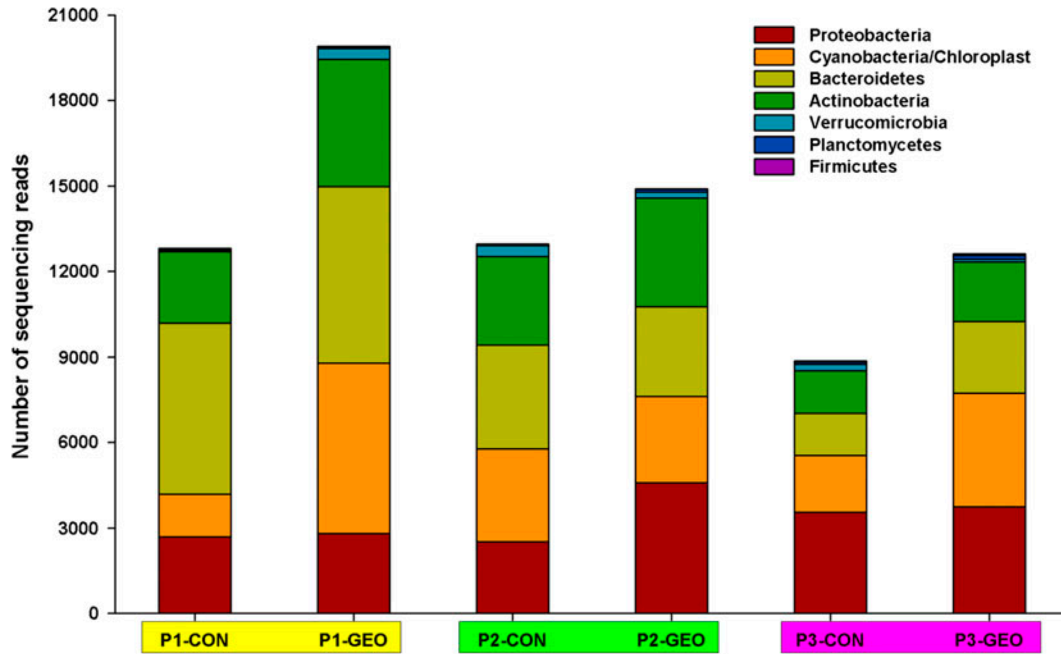

Fig. 3. Rarefaction curves of OTUs identified at the 3% cutoff for water samples from Paldang Lake.

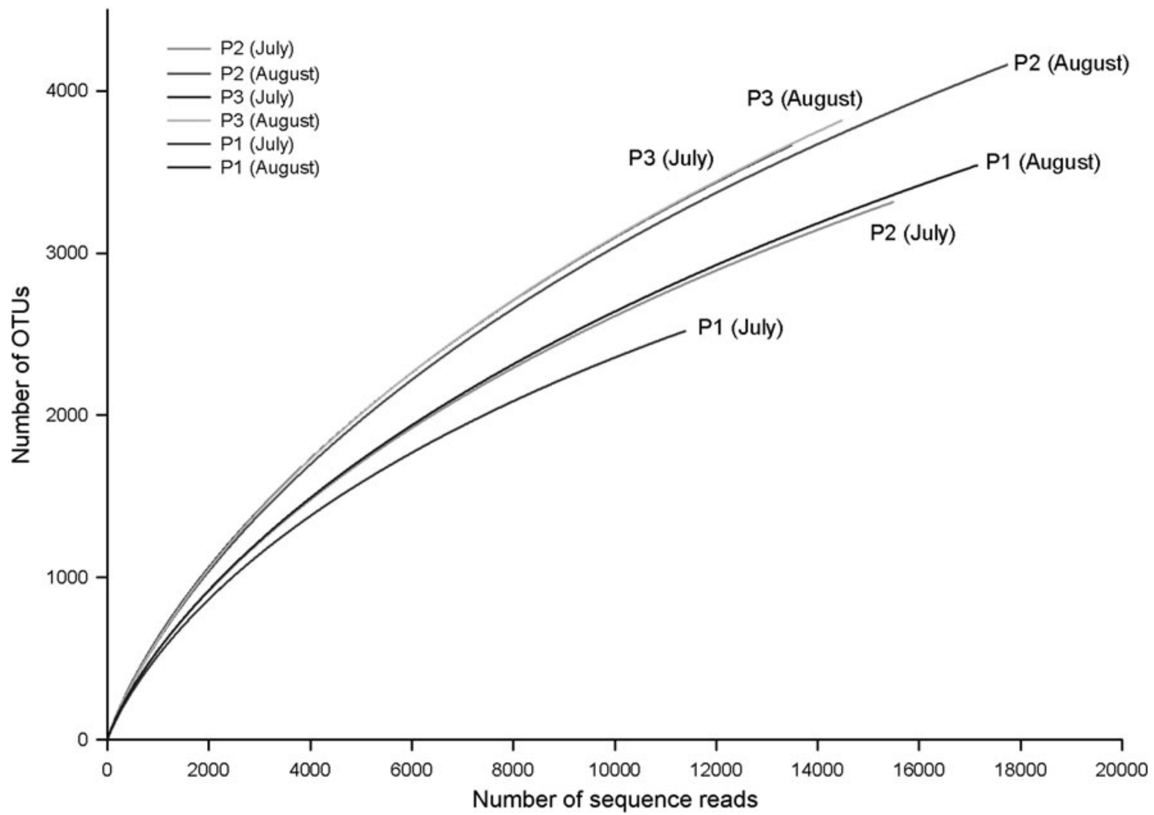

Fig. 4. Taxonomic distributions at the phylum level determined by pyrosequencing.

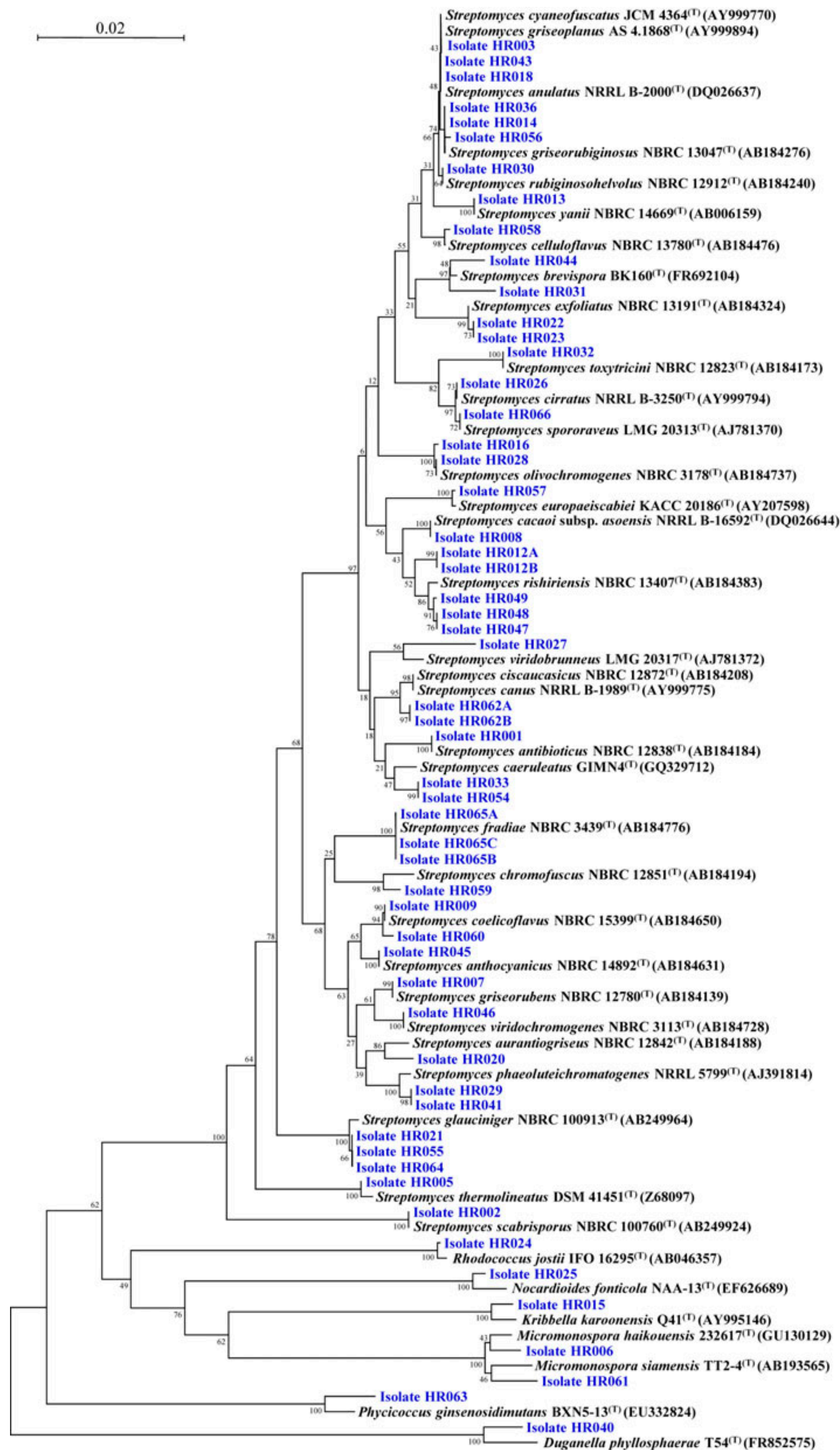

Fig. 5. Phylogenetic tree obtained by distance matrix analysis of 16S rRNA gene sequences, showing the position of isolates. Neighbor-joining tree using Kimura-2 parameter model of Actinomycete isolate sequences. Numbers at the branch nodes are bootstrap values, expressed as a percentage of 1,000 replicates. Bar, 0.02 substitutions per nucleotide position.

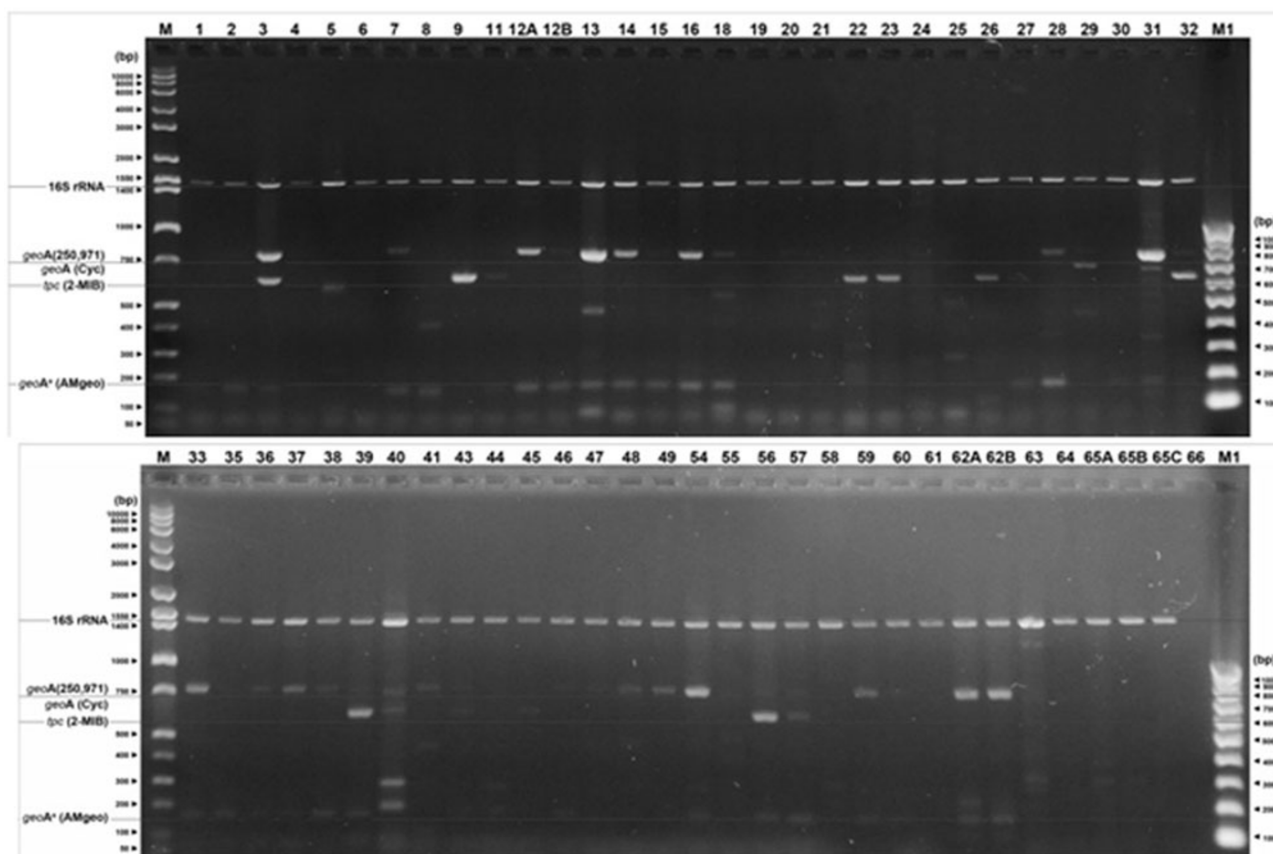

Fig. 6. PCR products from actinomycete isolates amplified by geosmin synthase and 2-MIB synthase gene primers.

on 4 August had the highest concentration of geosmin, while the other, sampled on 4 July, had the lowest, and was used as a control. Fig. 3 displays the rarefaction curves that can determine the diversity of the microbial community with the number of sequence reads and OTUs at the species level. On 4 July, P1, P2, and P3 produced 11,400, 15,500, and 13,500 16S rRNA sequence reads, and 2,500, 3,300, and 3,700 OTUs, respectively. On 4 August, P1, P2, and P3 produced 17,150, 17,730, and 14,500 sequence reads and 3,500, 4,200, and 3,800 OTUs, respectively. This shows that the diversity of bacteria in P1 and P2 increased in the August sample in which the concentration of geosmin was high, while diversity in P3 was not changed. Fig. 4 shows that proteobacteria, cyanobacteria, bacteroidetes, and actinomycetes are the dominant phyla at the three sites (P1, P2, and P3), followed by verrucomicrobia, planctomycetes, and firmicutes. As the concentration of geosmin increases, the population of cyanobacteria and actinomycetes increases at P1 and P3. There is no similar change in the microbial community at P2 which has a mixture of communities from the other two sampling sites (P1 and P3). Many studies have shown that cyanobacteria and actinomycetes contribute to the production of geosmin [2,20,21]. It is therefore, likely that these two phyla are responsible for producing geosmin in Paldang Lake. P1 in the North Han River is considered to be the main area in which algal bloom occurs, followed by P3, with P2 showing a mixture of communities from the other two sampling sites. This implies that Actinomycetes play an important role in producing geosmin, like cyanobacteria, and that the two phyla, cyanobacteria and actinomycetes, equally affect the production of geosmin in Paldang Lake.

3.3. Isolation of actinomycetes and prevalence of the geosmin synthase gene

A total of 68 different species of actinomycetes were isolated from HV agar and identified, based on 16S rRNA gene sequences. These isolates were used to investigate the prevalence of geosmin synthase and 2-MIB genes. By the culture-dependent approach, the diversity of actinomycetes, which are assumed to cause geosmin production, was analyzed. As shown

in Table 2, the identification of phylogenetic neighbors, and calculation of pairwise 16S rRNA gene sequence similarity were achieved using the EzTaxon-e server (http://www.eztaxon-e.ezcloud.net/; [13]). Table 2 and a phylogenetic tree (Fig. 5) show that 61 of the 68 species were confirmed to be *Streptomyces* spp., accounting for >98% of the 16S rRNA sequence similarity. Other identified species were *Kribbella* spp., *Rhodococcus* spp., *Nocardioides* spp., *Duganella* spp., *Micromonospora* spp., and *Phycicoccus* spp., taking up >98% of the sequence similarity. To confirm the presence of genes related to geosmin synthase genes, PCR was performed, resulting in the agarose gel bands shown in Fig. 6. Currently, actinomycetes in which the sequence of the geosmin synthase gene has been determined are *Streptomyces scabiei* 87.22 (FN554889), *Streptomyces* sp. SA3_actG (NZ_ADXA01000019), *Streptomyces griseus* subsp. *griseus* NBRC 1335 (AP009493), *Streptomyces flabogriseus* ATCC 33331 (CP002476.1), *Streptomyces bingchenggensis* BCW-1 (CP002047), *Streptomyces acermitilis* MA-4680 (NC_003155), *Streptomyces peucetius* ATCC 27952 (EU334502), *Streptomyces albus* J1074 (NZ_ABYC01000374), and *Stretomyces violaceusniger* Tu 4113 (NZ_AEDI01000041). The DNA sequences from the nine species described above can be found in GenBank. In our study, none of the actinomycetes species were identical to the nine described above. However, PCR amplification with geosmin synthase primers was successful, implying that diverse species of actinomycetes may produce geosmin, and this warrants further investigation.

Additionally, a PCR band was obtained in a test of whether the actinomycetes had a 2-MIB synthase gene. 2-MIB is a terpenoid, also known as an isoprenoid. Isopentenyl diphosphate (IPP) is a precursor, and it has the same biosynthetic pathway as geosmin. After IPP is transformed into dimethylallyl diphosphate, it is converted into two different materials. When it is changed into farnesyl diphosphate, geosmin is synthesized; when it is transmuted into geranyl diphosphate, 2-MIB is synthesized [22]. It could be explained by the fact that having the band of geosmin synthase gene and 2-MIB synthase gene from the actinomycetes indicates most of actinomycetes have not only geosmin genes, but also 2-MIB genes causing the odors. It is clear, therefore, that actinomycetes in Paldang Lake have geosmin synthase genes, and play a crucial role in producing geosmin.

4. Conclusions

In order to investigate the effect of actinomycetes, cultivation-based and independent approaches were used at three sites in Paldang Lake. A microbial community structure was examined by population density analysis by isolating actinomycetes, including *Streptomyces* spp. (which are known to be responsible for producing an earthy odor in drinking water), and pyrosequencing of water samples. In addition, related genes were confirmed by PCR amplification. When the concentration of geosmin increased, the density of actinomycetes (*Streptomyces* spp.) and cyanobacteria (*Anabaena* spp.) also increased. In a microbial community structure analysis using pyrosequencing, the P1 location had the highest density of actinomycetes, followed by P2 and P3. The density of *Streptomyces* spp. increased at P1 and P3. There was no density change at P2, which is at the confluence of two rivers. This would seem to suggest that *Streptomyces* spp. significantly affect the increase in geosmin, along with *Anabaena* spp. Genes responsible for geosmin and 2-MIB production were confirmed in 60 *Streptomyces* spp. isolated from water samples in Paldang Lake. As a consequence, this study confirms that *Streptomyces* spp. significantly affect the production of geosmin in Paldang Lake.

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