Occurrence of cyanobacteria, actinomycetes, and geosmin in drinking water reservoir in Korea: a case study from an algal bloom in 2012
Jung Eun Lee, Seok-Jae Youn, Myeongseop Byeon and Soon-Ju Yu

ABSTRACT

In 2012, a large concentration of geosmin was found in the Paldang reservoir, which is the primary source of drinking water in Seoul, Korea. In June and September 2012, we measured the concentrations of cyanobacteria and actinomycetes, and geosmin, to identify the source of geosmin in the Paldang reservoir. A total of 68 water samples were collected from two sampling sites (Sambong, Paldang), and used to analyze the correlation between cyanobacteria, actinomycetes, and geosmin. The cell density attained a maximum of 24,722 cells/mL on August 11, 2012 and geosmin occurred at a high concentration of 3,934 ng/L on August 13 in Sambong. After July 31, 2012 a rapid increase in growth and cell density occurred with a peak value of 11,568 cells/mL on August 6, 2012. At the same time, the geosmin concentration increased to 3,157 ng/L in Paldang. The number of cyanobacteria positively correlated with geosmin concentration ($R^2 = 0.84$, $P < 0.0001$), while actinomycetes were not significantly correlated with geosmin ($R^2 = 0.01$, $P = 0.709$). In addition, the number of actinomycetes was associated with increased turbidity ($R = 0.507$). Among the various water quality constituents, temperature affected cyanobacteria in the Paldang reservoir ($R = 0.803$). These results suggest that cyanobacteria are the main source of geosmin in the Paldang reservoir, which might be providing useful information for managing the unpleasant taste of its drinking water.

Key words | actinomycetes, cyanobacteria, drinking water, geosmin, Paldang reservoir

HIGHLIGHTS

- A large amount of geosmin was found in a primary source of drinking water in Korea.
- We investigated the correlations among cyanobacteria, actinomycetes, and geosmin.
- Cyanobacteria positively correlated with geosmin, whereas actinomycete did not.
- The number of actinomycetes was associated with increased turbidity.

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INTRODUCTION

Geosmin is an odorous compound that contributes to the occurrence of the earthy and musty odor in water, which has been known to decrease the quality of drinking water. It is synthesized and secreted as a secondary metabolite by cyanobacteria, actinomycetes, and fungi (Wood et al. 2001; Jüttner & Watson 2007). Although geosmin is not associated with any serious health effects, consumers perceive it as being unsafe because of the unpleasant taste and odor that it causes in drinking water (Smith et al. 2005). The permissible limits for geosmin in drinking water in Japan and South Korea are 10 ng/L and 20 ng/L, respectively. However, the general population can recognize the taste and odor of geosmin at concentrations as low as 5–10 ng/L (Cook et al. 2001). Geosmin cannot be efficiently removed using conventional treatments such as chlorine, ozone, and activated carbon (Koch et al. 1992; Cook et al. 2001; Lin et al. 2005). Therefore, a better understanding of the source of geosmin will lead to better in-lake management and drinking water treatment strategies designed to reduce its prevalence (Zuo et al. 2010).

The Paldang reservoir is the largest and most important source of drinking water in Korea, located near the capital city of Seoul. Its water storage capacity is ~244 × 10⁶ ton and it has a watershed area of 23,800 km² (Kim et al. 2015). The average depth is 6.5 m and it has an average water retention time of 5.4 days. This large reservoir serves as the principal source of drinking water for the >24 million people (48% of the Korean population) who live in this region; however, its earthy and musty flavor is a major concern of the drinking water industry in Korea.

An estimated 40 genera of cyanobacteria are responsible for harmful algal blooms (HABs), although the primary genera are Anabaena, Aphanizomenon, Microcystis, Oscillatoria, Nostoc, Lyngbya and Cylindrospermopsis (Carmichael 2003). These cyanobacteria produce cyanotoxins, off-flavor compounds like geosmin, and other metabolites.

Among the main cyanobacteria genera, the Korea government monitors, by law, four cyanobacteria genera including Anabaena, Aphanizomenon, Microcystis and Oscillatoria. To reduce the harm caused by algae, the Korean Ministry of Environment has analyzed the algae and associated unpleasant flavor once a week since 1998. Despite this effort, large amounts of geosmin were measured during the 2012 summer season from the North Han River in the Paldang reservoir, resulting in an undesirable water flavor and an increasing number of complaints.

Several studies have shown that geosmin, cyanobacteria, and actinomycetes are present in reservoirs. However, these studies focused on the relationship between geosmin and a single geosmin-producing microorganism (Sherr & Sherr 1993; Jüttner & Watson 2007; Kutovaya & Watson 2014). Geosmin is produced by a range of microorganisms, notably cyanobacteria and actinomycetes (Kutovaya & Watson 2014), and these geosmin-producing microorganisms may be present in the same water resource. However, it remains
unknown which microorganism primarily contributes to the odor problem in the Paldang reservoir, and whether geosmin is the main source of the problem. Thus, it was necessary to analyze the parameters of all microorganisms. The objectives of this study were: (i) to identify the main source of geosmin in the Paldang reservoir, which is an important drinking water reserve in Korea; and (ii) to analyze correlations between the numbers of geosmin and geosmin-producing microorganisms such as cyanobacteria and actinomycetes.

MATERIALS AND METHODS

Water sampling

The Paldang reservoir has two major tributaries: the North Han River and the South Han River. Two water sampling sites in the Paldang reservoir, Sambong and Paldang, were used in the current study (Figure 1). The water was sampled 3–4 times a week from June 29 to September 25, 2012 and 68 water samples were obtained. The water samples were collected in sterilized polyethylene bottles and stored at 4 °C until subsequent analysis. Water quality constituents, including water temperature, pH, dissolved oxygen (DO), and turbidity, were measured immediately at the sampling site using multiprobe (YSI 6600, USA).

Quantification of cyanobacteria and actinomycetes

To quantify the number of cyanobacteria, samples were preserved using Lugol’s iodine at a final concentration of 5% (Sherr & Sherr 1993), and kept in the dark until counting. The cyanobacteria cell density was determined using a Sedgewick-Rafter counting chamber under a microscope (Eclipse 80i, Nikon Corporation, Sendai, Japan) using phase-contrast and bright field illumination. The number of cells in 50 of the 1,000 grids in the chamber was counted. To identity the relationship between cyanobacteria and geosmin, four cyanobacteria genera (Anabaena sp., Aphanizomenon sp., Microcystis sp., and Oscillatoria sp.) were selected.

To determine the cell density of actinomycetes, a 1 L of water sample was filtered through a Sartorius bottle top vacuum filter (polyethersulfone membrane, 0.2 μm). The membranes were cut into pieces, and placed on humic acid-vitamin (HA) agar (catalog no. H0663; MB Cell, CA, USA). Pure sterilized water (1 mL) was placed on the HA agar and spread using a glass triangle spreader; the cut filter was then detached from the HA agar. The samples were incubated at 28 °C for 5–7 days followed by counting of colony-forming units (CFU) (Hirsch & Christensen 1983; Lee & Hwang 2002).

Geosmin analysis

All analyses were conducted by the Korean Ministry of Environment using headspace solid-phase microextraction (HS-SPME) and gas chromatography/mass spectrometry (GC/MS; 450-GC, 320-MS, Bruker, Billerica, MA, USA). A Polydimethylsiloxane (PDMS) fiber (57329-U, Supelco, Sigma-Aldrich, MO, USA) was used for the SPME, helium was the carrier gas, and a VF-5MS column 30 m in length and 0.25 mm in diameter was used for separation. Geosmin concentrations <1 ng/L were considered not detectable (ND).
Environmental parameters were calculated to explore correlations among cyanobacteria, actinomycetes, and geosmin levels. SPSS (ver. 19.0; Armonk, NY, USA) was used to perform correlation analyses and statistical tests.

RESULTS AND DISCUSSION

Abundance of cyanobacteria and actinomycetes

Variations in cyanobacterial cell density and geosmin levels in Sambong and Paldang are shown in Figure 2(a). In Sambong, cyanobacterial numbers ranged from 32 cells/mL to 24,722 cells/mL (median = 1,105 cells/mL). The cell density in Sambong increased gradually from >500 cells/mL on July 4, 2012 to reach the maximum value of 24,722 cells/mL on August 11, 2012 and rapidly declined to <500 cells/mL after August 18. A previous study described a cyanobacterial bloom that occurred in 1994, peaking at a cell density of 10⁶ cells/mL in the Nakdong River of Korea (Ha et al. 1999). Geosmin reached the peak value of 4,384 ng/L on July 24, 2012 in Sambong, and declined rapidly to reach a minimum concentration of 7 ng/L on August 18. The highest geosmin levels reported in natural waters were

Figure 2 | Comparison between (a) cyanobacteria and geosmin levels and (b) actinomycetes levels, turbidity, and rainfall in Sambong and Paldang. (Continued.)
7,100 ng/L in North China (Li et al. 2010), 4,000 ng/L in Australia (Jones & Korth 1995) and 3,170 ng/L in South Africa (Wnorowski & Scott 1992). Thus, our maximum geosmin concentration could be one of the largest values reported to date in Korea.

The bloom event that was observed in Paldang was relatively short-lived, at about two weeks. After July 31, 2012, rapid growth of cyanobacteria commenced and the cell density increased to the maximum of 11,568 cells/mL on August 6 in Paldang (range, 16–11,568 cells; median, 124 cells/mL). During this time, the geosmin concentration increased to 3,157 ng/L. Cell numbers decreased gradually to <500 cells/mL after August 16 and the decline in geosmin slightly preceded the cell decline in Paldang. Cyanobacteria and geosmin increased and decreased in a similar temporal pattern at both sites, indicating that cyanobacteria likely were the dominant producers of geosmin in the reservoir at this time. To identify the primary geosmin-producing cyanobacteria, we assessed Anabaena, Aphanizomenon, Microcystis, and Oscillatoria levels; these are the four most harmful cyanobacteria. The variation in geosmin levels was closely related to the cell concentration of...
Anabaena (Figure 2(a) inset box). When Anabaena began to decline and Microcystis became the dominant species for a short time, the geosmin concentration was not increased by Microcystis in Paldang (Figure 2(a) inset box). Another reservoir study in North China showed a similar link between total geosmin concentration and A. spiroides density (Li et al. 2010). Anabaena, a well-known bloom-forming cyanobacterial genus, has been reported to synthesize geosmin (Saadoun et al. 2013; Wang et al. 2005).

The cyanobacterial bloom occurred earlier in Sambong than in Paldang, and its concentration was higher. The Sambong site is located in the North Han River; this tributary combines with the South Han River, and the two tributaries flow into the Paldang reservoir (Figure 1). Cyanobacteria might have appeared first in the Sambong area, and then flowed into Paldang (Figure 2(a)).

Actinomycetes were present at a concentration of $1.1 \times 10^2$ CFU/mL ($\pm$ 64) in Sambong, and $1 \times 10^2$ CFU/mL ($\pm$ 89) in Paldang (Figure 2(b)). A previous study showed that the mean concentration of actinomycetes in Paldang was $8.3 \times 10^1$ CFU/mL between March and April of 2011 (Lee et al. 2011). In addition, after rainfall on August 12 and 15 of 2012, the concentration of actinomycetes and turbidity increased, and both the cyanobacteria and geosmin disappeared. A previous study found that increased actinomycete levels were associated with turbidity (Uhnáková et al. 2002).

Rainfall events cause the erosion of soil and sand from the local watershed into aquatic water bodies. This sand, which may contain large numbers of actinomycetes, flows into water reservoirs. Therefore, turbidity increases with an increased number of actinomycetes after rainfall (Figure 2(b)). Previous studies have reported that the water surface is greatly influenced by both rainfall and runoff during rainfall (Kim et al. 2013). With heavy rainfall, the microbial loads of runoff increased and reached water reservoirs quickly (Kistemann et al. 2001). Runoff events corresponded well with rapid increases in turbidity and the concentrations of bacteria were increased. Significant associations of bacterial load with turbidity were observed during heavy rainfall and runoff events (Kistemann et al. 2002). Therefore, runoff and rainfall events are important sources of water pollution (Noble et al. 2003).

**Correlations among cyanobacteria, actinomycetes, and geosmin**

Figure 3(a) shows the correlation between cyanobacterial cells and geosmin concentrations. When geosmin levels increased, cyanobacteria were at high cell densities. The number of cyanobacteria positively correlated with geosmin levels ($R^2 = 0.84; P < 0.0001$). Previous studies have reported that cyanobacteria are major producers of geosmin (Watson 2003), and that cyanobacterial blooms frequently occur in water reservoirs worldwide. These blooms are accompanied by odor problems caused by cyanobacterial metabolites such as geosmin (Battocchi et al. 2010). The antecedents of geosmin in lake water have been studied since the 1970s. Tabachek & Yurkowski (1976) reported that in several Canadian lakes cyanobacteria were primarily responsible for the formation of geosmin. Other studies have shown that in most cases geosmin formation can be attributed to the mass development of cyanobacteria (Izaguirre...
et al. 1982; Izaguirre & Taylor 1995). Recent studies reported that geosmin was mainly produced by cyanobacteria, in particular by *Anabaena* spp. in the Laurentian Great Lake (Kutovaya & Watson 2014). Furthermore, in Diamond Valley Lake, a large drinking water reservoir in western Riverside Country, an intense geosmin event occurred in association with cyanobacteria (Izaguirre & Taylor 2007).

As shown in Figure 3(b), actinomycetes and geosmin were not correlated ($R^2 = 0.01; P = 0.709$), suggesting that actinomycetes were not a major contributor to the observed geosmin event. Alternatively, the resident actinomycetes simply were not producing geosmin during this bloom. Although actinomycetes have been associated with odorous compounds, adverse flavors and odors might be attributable to these bacteria (Zaitlin & Watson 2006) as the highest amounts of actinomycetes are in the sediment. A previous *in vitro* study suggest that off-flavor compounds could be produced by actinomycetes in sediment (Zuo et al. 2010). Surface soil and sediment exhibited the greatest concentration of actinomycetes, whereas water samples contained concentrations that were 100-fold lower (Lee et al. 2011). Further study is needed to investigate whether geosmin could be detected at high concentrations, together with actinomycetes, in sediment of the Paldang reservoir.

The results of the correlation coefficients of the four parameters analyzed in the current study are shown in Table 1 as a matrix. According to the matrix, geosmin only correlated with cyanobacteria ($R = 0.8188$). The actinomycete parameters were dependent on turbidity, and the correlation coefficient was 0.5072 (Table 1). A previous study reported that the number of actinomycetes in the water source correlated with increased turbidity (Jensen et al. 1994).

Previous studies reported that water temperature, DO, nutrient availability, and water transparency play important roles in the occurrence of cyanobacterial blooms (Chirico et al. 2020). In this study, water temperature, pH, and DO were monitored over a period of 3 months (Figure 4). The temperature ranged from 19–29 °C, and 19–32 °C, at Sambong and Paldang, respectively (Figure 4). At the two sampling sites, the temperature was maintained at $\sim 20$ °C, but increased to $> 25$ °C when the cyanobacterial concentration was high ($> 500$ cells/mL). With regard to the number of cyanobacterial cells, the Korean Ministry of Environment has established an algae alert system based on the cyanobacterial cell count. This system includes three categories: caution, warning and outbreak. An alert

| Table 1 | Matrix of the correlation coefficient ($r$) comparing geosmin, cyanobacteria, actinomycetes and turbidity |
|---------|---------------------------------------------------------------|
| Geosmin | 1.0000                                                        |
| Cyanobacteria | 0.8188                                                  |
| Actinomycetes | $-0.1154$                                           |
| Turbidity | 0.0169                                                  |

Figure 4 | Water quality constituents (temperature (°C), pH and DO (mg/L)) in (a) Sambong and (b) Paldang.
could be triggered when the number of cyanobacteria cells exceeds 500 per milliliter.

An analysis of the water quality constituents revealed that temperature was correlated with cyanobacteria concentrations ($R = 0.803; P < 0.0001$, data not shown). A previous study reported that the cyanobacteria growth rate was temperature-dependent, and was optimum at $\geq 25 ^\circ C$ (Robarts & Zohary 1987). Therefore, a high temperature during the summer could increase the development of cyanobacterial blooms (Joehnk et al. 2008). Additionally, recent climate change could affect cyanobacteria growth; a previous study reported that global warming caused a significant increase in the chlorophyll concentration in Paldang (Park et al. 2013).

**CONCLUSIONS**

The current study investigated the occurrence of and correlations among cyanobacterial, actinomycetes, and geosmin levels during the summer and fall of 2012 in Paldang, a drinking water reservoir in South Korea. The densities of cyanobacteria were positively correlated with geosmin levels and were associated with high concentrations of geosmin. Also, the geosmin concentration was closely related to the cell density of *Anabaena*, which is a known geosmin-producing cyanobacterium. Actinomycetes were associated with increased turbidity, but not geosmin concentration. In addition, among water quality constituents, a water temperature increase was related to cyanobacterial concentration.

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