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Contributed Paper

Earthy-musty Odour and Off-flavour Taints in Phayao Lake, Thailand

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ABSTRACT

This study aimed to determine and monitor the levels of earthy-musty odour in Phayao Lake water and the microbiota responsible for the production of odorous compounds, geosmin and 2-methylisoboneol. Geosmin was identified as the main odorous substance in both water and lake sediments. It was significantly related (P<0.05) to the density of cyanobacteria in lake water. Five genera of cyanobacteria were identified as the most frequently encountered groups, namely: *Microcystis* sp., *Lyngbya* sp., *Phormidium* sp., *Anabaena* sp. *and Oscillatoria* sp. Furthermore, three cyanobacterial genera, were found to be the most dominant and are well known producers of odorous compounds. Geosmin levels were significantly but weakly correlated (r=0.36; P<0.05) to actinomycetes count. *Streptomyces* spp. were found to be significantly present in the sediment. Analytical results by GC/MS showed that the majority (63%) of *Streptomyces* spp. isolated from the sediments were geosmin-producers. Consequently, it was suggested that filamentous cyanobacteria, rather than actinomycetes, were the major contributor of earthy-musty odour in Phayao Lake.

Keywords: geosmin, MIB, Phayao Lake, musty odour, actinomycetes, cyanobacteria

1. INTRODUCTION

Phayao Lake (Kwan Phayao, in Thai) is one of popular tourist destinations in Phayao Province. The lake covers an area of 2.3 square the kilometers and located at an altitude of 380 metres above sea level. It is one of the largest artificial lakes in northern

Thailand [1]. This lake is a raw water source for water supply and a fishing ground for the local fishery. However, the water quality is deteriorating and suffers from eutrophication due to anthropogenic activities such as farming and urbanisation. The livestock effluent from the basin and household wastewater from Phayao city are the major pollution sources [2]. Nutrients from these sources accumulate in the lake and promote cyanobacterial blooms which can adversely affect the water quality.

Besides the toxins (microcystins), another major consequence of blooms dominated by cyanobacteria, are secondary metabolites that impart taste and odour in drinking water. Most frequently reported odours in the surface water are earthy, muddy and musty smell commonly caused by geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol) and 2-methylisoborneol (MIB) which are terpenoid metabolites produced by certain members of cyanobacteria and actinomycetes (filamentous actinobacteria) [3]. Cyanobacteria and actinomycetes have long been known to be associated with geosmin and MIB in water [4]. However, it was claimed that cyanobacteria were the more frequent source of these earthy-musty odour in the water than actinomycetes [5]. *Anabaena, Oscillatoria, Phormidium* and *Lyngbya* are the major genera of filamentous cyanobacteria that produce geosmin and MIB in freshwaters such as lakes and rivers including pond water used for aquaculture. Toxin-producing *Microcystis* was also linked to odorous compounds in many studies [6-8]. However, actinomycetes was the first microorganism reported as source of geosmin and MIB [9,10]. They are routinely found in freshwater environments especially in lakes, but they are primarily soil microorganisms whose main function is to degrade organic matter. Sugiura and Nakano [11] isolated 40 strains of actinomycetes from sediments in Lake Kasumigaura and all of them could produce both geosmin and MIB. Similarly, Zuo et al. [12] found that actinomycetes were able to produce geosmin and MIB from the sediments in Lake Lotus, China. Due to the present status of Phayao Lake, it is highly likely that the lake is contaminated with earthy-musty odour compounds.

There were reports on the water quality and algal diversity in Phayao Lake [2, 22] but reports on the levels of earthy-musty odour and its source in this Lake are lacking. Therefore, this study aimed to determine and monitor the levels of geosmin and MIB in Phayao Lake, including the genera of phytoplankton and actinomycetes responsible for the production of these odorous compounds, and explore the correlation between these compounds and their potential sources.

2. MATERIALS AND METHODS

2.1 Sampling

The study was carried out for a period of 8 months from June 2012 to February 2013 at six collection points (KP 1-3, adjacent to the city; and KP 4-6, adjacent to the agricultural areas) across Phayao Lake (Figure 1). Surface water and sediment samples were collected monthly from June 2012 to February 2013. The sampling sites were accessed using the Department of Fisheries (DoF) motorized boat. All samples for physico-chemical (1.5L) and earthy-musty odour analyses (120 mL) were collected directly from the water surface (20-40 cm depth) using plastic containers. Sediment samples (about 500 g) for earthy-musty odour analysis were collected using a fabricated sediment sampler (a hard plastic dipper attached to a PVC pole) by scraping the surface layer of the lake's floor and were placed in wide-mouthed 200-mL plastic bottles. Phytoplankton was sampled by filtering 5L of lake water with plankton net of 25 μm mesh. Samples were concentrated in a 30-mL plastic bottle and preserved with 3 drops of Lugol's solution. Each sample of water, sediment and phytoplankton was immediately placed on ice in sample coolers and transported to the laboratory within 24 hours.

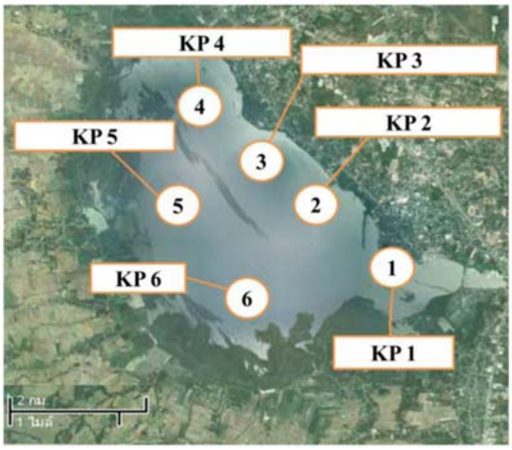

Figure 1. Collection points in Phayao Lake: KP1-3 city areas, KP4-6 agricultural areas.

2.2 Analysis of Geosmin and MIB

Dissolved and particulate geosmin and MIB were extracted from water and sediment samples using solid phase microextraction (SPME) and quantified with gas chromatography-mass spectrometry (GC/MS) according to Gutierrez et al. [13]. The limit of detection was 0.01 μgL^{-1} for both geosmin and MIB.

2.3 Water Quality and Nutrient Analysis

Physico-chemical parameters (pH, temperature dissolved oxygen turbidity and conductivity) were measured *in situ* using a multimeter (TOA-DKK WQC-22A Model, Japan). Standard methods [14] were used for the analysis of total ammonia-nitrogen (TAN), nitrate-nitrogen, nitrite-nitrogen, orthophosphate-phosphorus and total suspended solids (TSS) in the laboratory.

2.4 Hydro-biological Analysis

Chlorophyll-*a* in the water samples was extracted with 10 mL of hot methanol (60°C in water bath) and quantified with a spectrometer (Hach D9000, USA) [14]. Chlorophyll-*a* concentration in the extract was calculated as described by Wintermans and de Mots [15] and Saijo [16]. Phytoplankton genera/species and numbers were determined using a light microscope (Olympus BH2, Japan). The identification of phytoplankton was carried out using picture database and related texts such as Peerapornpisal [17].

2.5 Isolation and Screening of Earthy-Musty Odour-producing Streptomycetes in Sediments

Ten grams of sediment sample from six collection points in Phayao Lake were mixed with 90 mL of 0.85 % NaCl solution. The suspension was shaken at 150 rpm for 30 minutes at room temperature and serially diluted ten folds to 10^{-5}. Soil suspensions (100 μL) from 10^{-3}, 10^{-4} and 10^{-5} dilution were spread onto the surface of starch casein agar (SCA) plates, and incubated at 30°C for 7-14 days. Actinomycetes were assigned into streptomycetes (filamentous and fungus-like sporulation structures) and non-streptomycetes groups. Colonies from both groups were counted and recorded as CFU g^{-1}. Earthy-musty odour-producing streptomycetes were purified by re-streaking on SCA, sealed with paraffin film and incubated at 30°C for 7-14 days or until complete sporulation was observed. The isolates were subjected to qualitative testing and verified whether they were geosmin or MIB-producers or both by GC/MS [18].

2.6 Data Analysis

Analysis of variance (ANOVA) was used to test for difference between means of observed parameters and each treatment.

Duncan Multiple Range Test (DMRT) at 95% confidence level was used for treatment comparison. Relationships between water quality variables and earthy-musty odour compounds were analysed using Pearson correlation analysis. Significant correlation was assumed when $p<0.05$ in either positive or negative correlations.

3. RESULTS AND DISCUSSION

3.1 Earthy-musty Odour in Lake Water

Total geosmin and MIB concentrations measured in the water samples collected from Phayao Lake are shown in Figure 2. Geosmin concentration was highest in October 2012 with peak concentration of 11.95 μgL^{-1}, collected at KP3 near the water pumping station. Geosmin concentrations detected at other sites were also relatively high though some samples collected during June and August 2012 gave non-detectable (ND) geosmin values. MIB was detected only during the latter part (November 2012 to February 2013) of the monitoring period which ranged from 0.05 to 1.08 μgL^{-1}. MIB was less frequently detected and its concentration was much lower than that of geosmin suggesting that geosmin is the most prevalent odorous compound in Phayao Lake. The amount of geosmin in water was significantly correlated ($p<0.05$) with the density of cyanobacteria and chlorophyll-*a* concentration. These relationships suggest that chlorophyll-*a* levels may indicate increasing cyanobacterial abundance, which may result in increased geosmin concentrations. The MIB concentrations were low or not detected in many samples therefore geosmin is probably the main cause of odour during the study period.

The concentration of geosmin in Phayao Lake was higher than those in eutrophic lakes with cyanobacterial population such as Yanghe reservoir (max. concentration detected: 7.1 μgL^{-1} total geosmin) [19], Lake Dianchi (max. concentration detected: 0.13 μgL^{-1}) [20] and Lake Zurich (max. concentration detected: 2.71 μgL^{-1}) [21]. In fact the maximum total geosmin concentration of 11.95 μgL^{-1} recorded in this study is the highest geosmin level ever reported in natural water. It is assumed that Phayao Lake is generally more polluted by human activities as are lakes in China and Zurich. Moreover, the lake's shallow depth (1.9 m average) with its very slow water flow and Thailand's tropical climate could have contributed to the pronounced levels of geosmin in the lake.

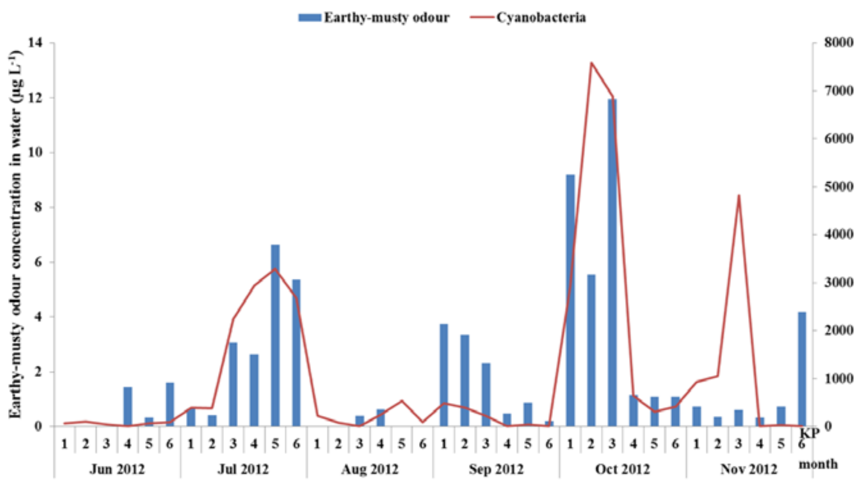

Figure 2. Earthy-musty odour levels and cyanobacterial abundance in water.

3.2 Diversity and Composition of Phytoplankton in Phayao Lake

The phytoplankton found in Phayao Lake were categorised into 40 genera and 6 divisions, namely Cyanophyta (8; 20.0%), Chlorophyta (15; 37.5%), Chromophyta (2; 5.0%), Bacillariophyta (4; 10.0%), Euglenophyta (7; 17.5%) and Cryptophyta (4; 10.0%). Chlorophyta was the most abundant in terms of composition but Cyanophyta (cyanobacteria) dominated the lake water (Figure 3). The dominant cyanobacteria were *Oscillatoria*, followed by *Microcystis* and *Anabaena*. These cyanobacteria are known earthy-musty odour-producers including *Phormidium* and *Lyngbya* (Figure 4). *Microcystis* was included in the odour-producing list because there were claims that some species have the potential to produce geosmin apart from hepatotoxins (microcystins) [6-8]. These cyanobacteria except *Phormidium* were also found in a previous study [2] conducted in Phayao Lake. However, there was no record of which cyanobacterial species or genus dominated the lake during the time of their study. Prommana [22] on the other hand reported that *Microcystis aeruginosa* Kutz and *Microcystis wesenbergii* Kom. dominated Phayao Lake during a survey conducted from 1999 to 2000. However, the study mainly focused on the survey of microcystin-producing species present in the lake rather than cyanobacterial diversity in general.

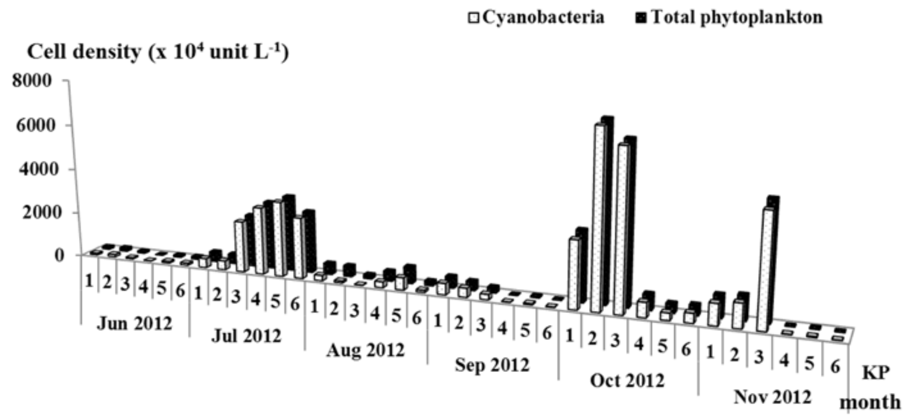

Figure 3. Density of phytoplankton and cyanobacteria in Phayao Lake.

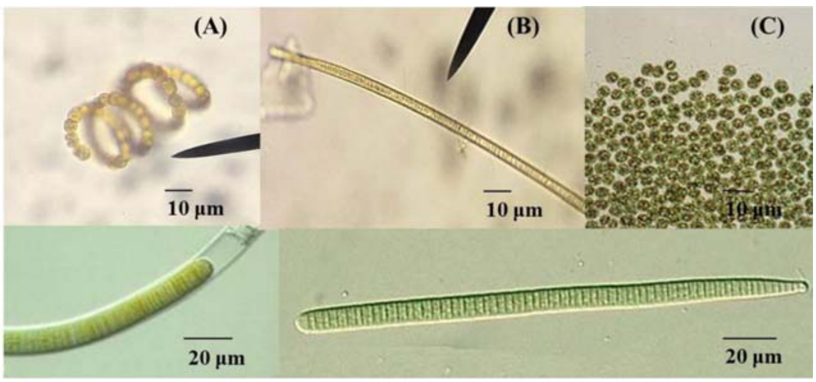

Figure 4. Photomicrographs of the dominant cyanobacteria in Phayao Lake June 2012 to February 2013. (A) *Anabaena* sp., (B) *Oscillatoria* sp., (C) *Microcystis* sp., (D) *Lyngbya* sp. and (E) *Phormidium* sp.

Cyanobacteria are generally believed to be the main source of the odours in Phayao Lake. They were found to be abundant at the sites adjacent to the city (KP 1-3) especially in KP-3 (Figure 5). This part of the lake (southeast to eastern portion) is polluted with large cyanobacterial blooms due to nutrient enrichment brought about by the wastewater released from the city and from the livestock effluents. This finding is consistent with the levels of geosmin in water especially in October 2012 when geosmin concentration was highest. Species and biomass of cyanobacteria are the factors that affect the MIB and geosmin concentrations in water [23]. It is possible that geosmin-producing *Oscillatoria* along with *Anabaena*, [24] contributed significantly to geosmin's prevalence over MIB in the lake.

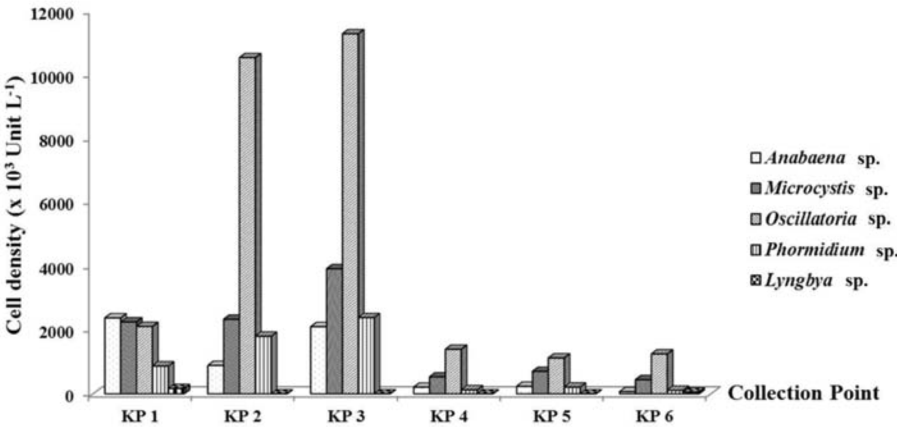

Figure 5. Mean distribution of cyanobacterial density from 6 stations.

3.3 Earthy-musty Odour in Lake Sediment

Geosmin and MIB levels in sediment samples collected from Phayao Lake are presented in Figure 6. Geosmin and MIB in the lake sediments ranged from ND to 0.92 and from ND to 0.85 $\mu g kg^{-1}$ respectively. Similarly, geosmin is prevalent over MIB in the sediments as observed in the lake water. Concentrations of both compounds were mostly non-detectable in the sediments during the early months of monitoring (June to August 2012) but high geosmin concentrations were observed later in December 2012 (0.78 $\mu g kg^{-1}$) and February 2013 (0.92 $\mu g kg^{-1}$). MIB was sparsely detected throughout the period but very high identical concentration (0.85 $\mu g kg^{-1}$) was recorded at KP-1 in December 2012 and February 2013. KP-1 site is located downstream in the east near the outflow canal to Ing River. This site is similar to KP-3 where cyanobacterial blooms are visible at the surface but with a mucky bottom. The sediment in this part of the lake floor is mixed with decomposed plant materials (lotus) and has black color with noxious odour possibly due to sulfides that indicates anoxic condition. This condition reduces the rate of organic decomposition, allowing organic matter to accumulate at the lake bottom. However, the sediment is not an absolute anoxic environment. Its top layer is generally an oxic zone so it is common to find streptomycetes (odour-producing actinomycetes) in the sediments as dormant spores and active cells. This fact was supported by convincing evidence emerged from culture independent work in freshwater habitats

[25-26]. Moreover, in freshwater systems, odour-producing actinomycetes have been found in association with aquatic plants [27-29]. Geosmin concentration in the sediment was positively correlated (r=0.36; $P<0.05$) with actinomycetes. This finding confirms the results obtained by Sugiura and Nakano [11], and Zuo et al. [12] that actinomycetes were able to produce geosmin and MIB from the sediments. This suggests that the variation in earthy-musty odour compounds, particularly geosmin, is weakly related to the concentration of actinomycetes in the sediment. However, even if actinomycetes are present in low numbers across the Phayao Lake, we cannot disregard the role of actinomycetes as source of earthy-musty odour in the lake.

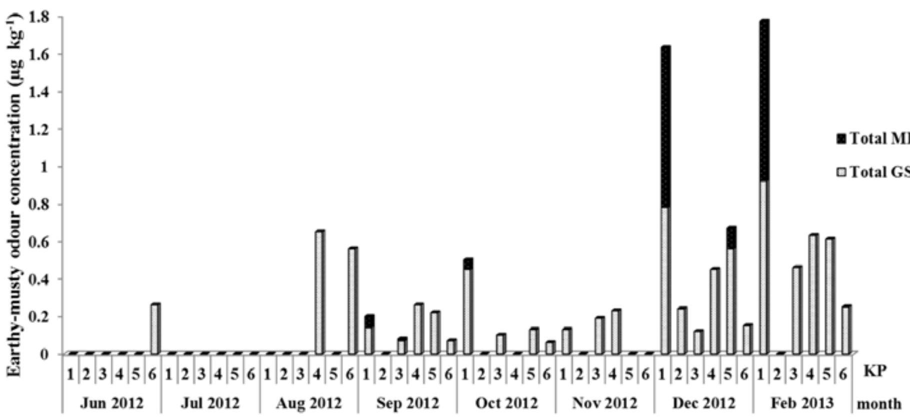

Figure 6. Concentration of geosmin and MIB in sediment from Phayao Lake.

3.4 Occurrence of Actinomycetes in Lake Sediment

The numbers of actinomycetes in the sediment of Phayao Lake (Figure 7) were between 0.80 and 15.47×10^4 CFU g^{-1}. Most of the isolated were in the genus *Streptomyces* with counts ranging from 0.30 to 15.43×10^4 CFU g^{-1}. The abundance of odour-producing actinomycetes in the sediments further indicates that cyanobacteria may not be the only producers of odour in the lake. Pan et al. [30] reported that the concentration of MIB was found to be closely correlated with the gross biomass of actinomycetes in the water. Zaitlin and Watson [29] also reported that *Streptomyces* are the major producers of the geosmin and MIB in terrestrial soil environments. High concentrations of geosmin in lake water were recorded in certain sampling points from several collection periods (KP4 in June 2012; KP1-6 in September 2012 and; KP4-6 in November 2012) particularly at KP1-6 (up to 3.74 μgL^{-1}) and MIB at KP4 (0.086 μgL^{-1}) in September 2012 in the lake despite low abundance of potential odour-producing cyanobacteria during these periods (Figure 2).

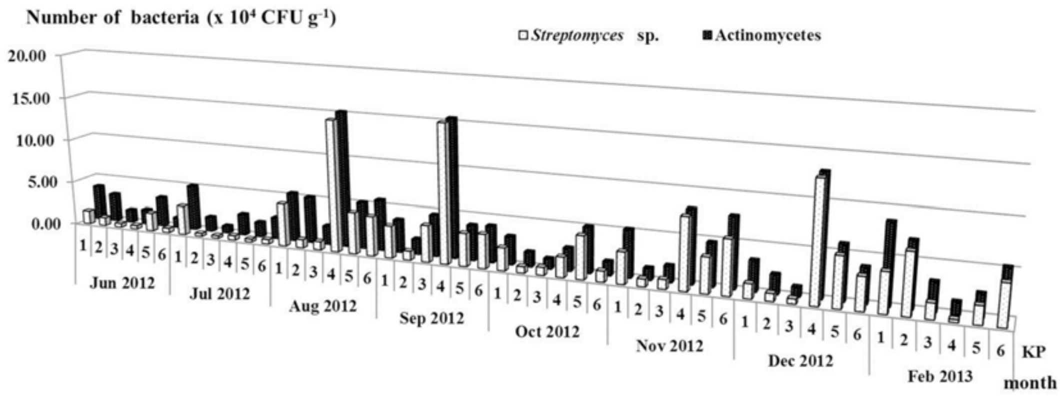

Figure 7. Number of actinomycetes and *Streptomyces* spp. in sediments (CFU g^{-1}).

3.5 Screening of Earthy-musty Odour Producing Streptomycetes in Sediments

Thirty-five isolates of *Streptomyces* spp. were tested (using SPME-GC/MS) for earthy-musty odour production and the morphology of the positive isolates was analysed using scanning electron microscopy (SEM) (Figure 8). It was found that 85.71% (30) of the isolates were odour-producers. Out of the 30 isolates, 22 were exclusive geosmin-producers, 2 produced MIB only and 6 produced both geosmin and MIB (Table 1). The above result was consistent with the results obtained for water and sediment where geosmin was the more prevalent odorous compound than MIB during the period of study. Geosmin was detected in 75.0% and 54.17% of the water and sediment samples collected respectively whilst MIB was only detected in 12.5% of both samples. This finding suggests that the presence of a large number of geosmin-producing *Streptomyces* spp. could have significantly contributed to the prevalence of geosmin over MIB in both water and sediments of Phayao Lake. Zuo et al. [12] observed *Streptomyces* was the main species responsible for the earthy-musty odour and produce geosmin more than MIB in Lake Lotus, China [23].

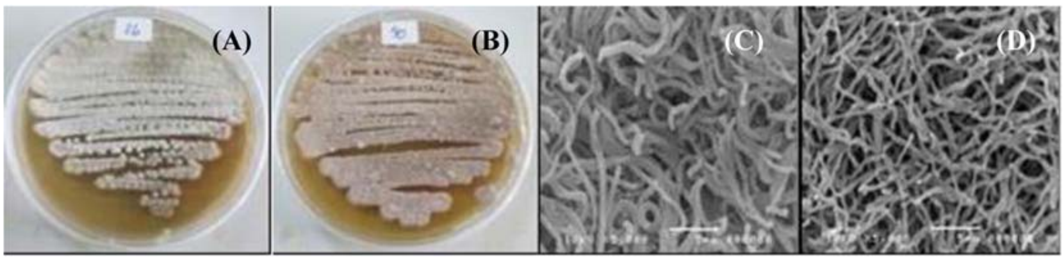

Figure 8. Morphological characteristics of *Streptomyces* spp. isolated from lake sediments, KP-1 (A) and KP-5 (B), on starch casein agar, incubated at 30°C for 7-14 days. SEM images of *Streptomyces* spp. isolated from KP-1 (C) and KP-5 (D) sediments.

Table 1. Odour production of selected *Streptomyces* spp. (n=35).

Odour production characteristics	Number of *Streptomyces* spp.	Percentage of total *Streptomyces* spp.
Odour producer	30	85.71
Geosmin only	22	62.86
MIB only	2	5.71
Both geosmin and MIB	6	17.14
Non-odour producer	5	14.29

3.6 Water Quality in Phayao Lake

The water quality parameters monitored at 6 stations in the lake (such as pH, temperature, DO, conductivity, turbidity, total ammonia-nitrogen, nitrite-nitrogen, nitrate-nitrogen, orthophosphate-phosphorus and TSS) were not significantly different. They were generally within the acceptable ranges for fish culture. Mean chlorophyll-*a* concentration in KP-1, KP-2 and KP-3 (adjacent to the city) were significantly ($p<0.05$) higher compared to those in other collection points (data not shown).

4. Conclusion

Geosmin was the main substance found to cause off-flavour taint in water and sediment in Phayao Lake. Earthy odour concentration (geosmin) in the water was positively correlated with the numbers of cyanobacteria and amount of chlorophyll-a ($p<0.05$) whereas earthy-musty odour concentration in the sediments was significantly but weakly correlated with actinomycetes (r=0.36; $P<0.05$). Determination of species diversity and composition of phytoplankton in Phayao Lake revealed 3 dominant genera of cyanobacteria: *Microcystis*, *Anabaena* and *Oscillatoria*. The two latter genera are well known sources of geosmin and MIB. The dominant species of *Streptomyces* spp. in the lake sediment were odour-producers. Qualitative tests confirmed that geosmin-producing *Streptomyces* sp. were more common in the lake than their MIB-producing counterparts. However, the abundances of all actinomycetes in Phayao Lake may have little relation to earthy-musty odour, they should not be disregarded as potential sources of these odourous metabolites. Due to the lack of wastewater treatment facilities within the catchment area, the lake is subjected to nutrient inputs from point and non-point sources which significantly promote the growth of cyanobacteria including those genera that produce musty odour. Aside from the establishment of a wastewater treatment facility, the application of an aerated lagoon purification to the household wastewater from Phayao city could be helpful to minimize nutrient build up.

Acknowledgements

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