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RESEARCH ARTICLE

Isolation and characterization of a new reported cyanobacterium *Leptolyngbya bijugata* coproducing odorous geosmin and 2-methylisoborneol

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Abstract The earthy-musty compounds geosmin and 2-methylisoborneol (MIB) produced by cyanobacteria are considered as the main biological causes of off-flavor events, especially in aquatic ecosystems. More than 50 filamentous cyanobacteria species have been documented as geosmin or MIB producers; however, little is known about the species coproducing these two metabolites. In this study, an epiphytic sample was collected from a river in Hubei, China. Three isolated strains (A2, B2, and B4) producing earthy odors were successfully isolated and identified as the cyanobacterium *Leptolyngbya bijugata* Anagnostidis et Komárek 1988 based on morphology and 16S rDNA sequences. Gas chromatography analysis confirmed that the isolated *L. bijugata* strains were geosmin and MIB coproducers, with accumulation ranging from 13.6 to 22.4 and 12.3 to 57.5 μg L^{-1}, respectively. The partial fragments of geosmin and MIB synthesis genes in the *L. bijugata* strains were cloned and sequenced. Further sequences and phylogenetic analysis indicated the high conservation and a common origin of these genes in cyanobacteria. This study is the first to report and characterize the coproduction of geosmin and MIB by *L. bijugata*, representing a new source for potential risk of off-flavor events.

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Introduction

Cyanobacteria are well-known to be the most ancient photoautotrophic life on earth. They are recognized worldwide for their ability to produce diverse secondary metabolites. Among these metabolites, odorous terpenoids, such as geosmin (*trans*-1, 10-dimethyl-*trans*-9-decalol) and 2-methylisoborneol (MIB), attract considerable research for their significant negative effects on water quality (Jüttner and Watson 2007; Smith et al. 2008; Merel et al. 2013). More than 200 compounds that cause off-flavor have been identified from various algal groups to date; geosmin and MIB are the best known and most widespread saturated cyclic terpenoids with earthy-musty odors produced mainly by cyanobacteria and actinomycetes (Act) (Izaguirre et al. 1982; Watson 2003; Izaguirre and Taylor 2004; Smith et al. 2008). Although no direct evidence suggests that geosmin and MIB have health risks for humans or aquatic animals (Dionigi et al. 1993; Jüttner and Watson 2007), these two terpenoids have been reported to be responsible for the majority of off-flavor episodes in water ecosystems, mainly because of their extraordinary low detection thresholds (2–10 ng L^{-1}) (Lloyd et al. 1998; Cook et al. 2001; Izaguirre and Taylor 2004).

Initially identified in *Streptomyces* (Gerber and Lecheval 1965), geosmin and MIB have been documented to be produced by many cyanobacterial taxa (Izaguirre and Taylor 2004; Jüttner and Watson 2007). In contrast to Act, in which most odor producers are *Streptomyces*, diverse cyanobacterial groups are reported to produce geosmin or MIB including the genera *Anabaena*, *Nostoc*, *Lyngbya*, *Oscillatoria*, *Phormidium*, *Planktothrix*, *Pseudanabaena*, and

Aphanizomenon (Izaguirre et al. 1982; Izaguirre and Taylor 2004; Smith et al. 2008; Wang et al. 2011; Su et al. 2013). Surface waters are generally considered as the primary habitats of odor-producing cyanobacteria because of their suitable growth conditions; however, many benthic or epiphytic producers isolated from soil or aquatic plants are also reported (Jüttner and Watson 2007; Smith et al. 2008; Zhang et al. 2014). A large number of studies on the morphological identification and ecology of odor-producing cyanobacteria expanded the knowledge on diversity, and more than 50 geosmin/MIB-producing species have been identified (Jüttner and Watson 2007; Smith et al. 2008). Along with the development of modern biological technologies, the geosmin/MIB synthesis genes and the biochemical mechanisms in cyanobacteria have been found in a few filamentous species, i.e., geosmin-producing *Phormidium* sp. (Ludwig et al. 2007), *Nostoc punctiforme* PCC 73102 (Agger et al. 2008; Giglio et al. 2008), and MIB-producing *Pseudanabaena* sp. and *Planktothricoides raciborskii* (Giglio et al. 2011; Wang et al. 2011). Upon the detection of the geosmin/MIB synthesis genes, more cyanobacterial taxa responsible for such odor production are expected to be explored.

Understanding the diversity of geosmin and MIB-producing groups is essential for the control and management of off-flavor events. The most reported earthy-musty odorants are either geosmin or MIB, but the species producing both geosmin and MIB are rarely identified. In China, off-flavor events caused by cyanobacterial odor compounds have been reported frequently in recent years (Li et al. 2007; Yu et al. 2009; Sun et al. 2013; Zhao et al. 2013). The worst drinking water crisis occurred in Lake Taihu on May 2007 (Guo 2007), and several geosmin or MIB producing species were identified (Wang et al. 2011, 2015; Su et al. 2013; Zhang et al. 2014). In the present study, three epiphytic *Leptolyngbya* (cyanobacteria) strains with earthy-musty odors were isolated from a river in Hubei, China. This study aimed to describe isolated *Leptolyngbya* strains and characterize their geosmin and MIB synthesis gene to provide novel and useful knowledge for geosmin/MIB-coproducing groups.

Materials and methods

Isolation and cultivation of cyanobacterial strains

Cyanobacterial samples with earthy-musty odors were collected from a river in Xiaogan, Hubei Province, China in 2012. Uni-algal strains were isolated by the Pasteur capillary pipette method. In brief, single filaments were selected using a Pastuer capillary pipette under a dissecting microscope (Nikon, SMZ1500, Japan), and filaments were washed seven to eight times with sterile double distilled water (ddH_2O) to remove adherent microorganisms. The isolates were then placed into a 24-well plate, which contained liquid CT medium (Watanabe and Hiroki 1997) and cultured under 30 μmol photons m^{-2} s^{-1}, 25 °C for 30 to 60 days. Isolates were initially checked by olfaction, and cultures with odors were selected for gas chromatographic (GC) analysis and morphological and molecular characterization. All obtained strains were maintained in the Collection of Harmful Algae Biology Lab, Institute of Hydrobiology of the Chinese Academy of Sciences.

For odor (geosmin and MIB) analysis, strains were cultured in 250-mL flasks containing 100 mL of CT liquid medium with shaking (60 rpm). Biomass in exponential phase was used for the qualitative and quantitative analyses of the corresponding odor compounds. The observation and characterization of algal cells were performed using Olympus BX 51 microscope (Olympus, Japan) and Image-pro Express Iplite Application 5.1 (Media Cybenetics Inc., USA).

Analysis of odorous compounds

The extraction of odor compounds in cyanobacteria cultures was performed employing the headspace solid phase microextraction method based on Lloyd et al.'s (1998) protocols with minor modification. A 65 μm PDMS/DVB fiber (57310-U, Supelco, USA) was placed into the headspace of a 25 mL glass extraction vial through a polytetrafluoroethylene-coated silicone pad. The reaction vial contained 2 mL of fresh algae culture, 8 mL of ddH_2O, and 3 g of NaCl. The vial was heated to 60 °C and then baked for 45 min to extract the odor. GC (GC-2014C; Shimadzu, Japan) equipped with an FID detector and a capillary column (TC series, WondaCap 5, 0.25 mm× 30 m×0.25 μm; Shimadzu, Japan) was used for the separation and analysis of extracted odorous compounds. GC temperature program was as follows: 60 °C for 2 min, increased to 200 °C at a rate of 5 °C min^{-1} and maintained for 2 min, then increased to 250 °C at 20 °C min^{-1} and maintained for 2 min. High-purity N_2 (≥99.99 %) was used as the carrier gas.

The geosmin and MIB solutions (100 ng μL^{-1}; Supelco, USA) were used as standards for GC analysis. Peaks corresponding to geosmin or MIB were confirmed by GC–MS (HP6890GC-5973MSD; HP, USA) according to Wang et al. (2011). To determine odor productivity, we analyzed the cyanobacteria cultures as described previously and quantified using the external standard method.

DNA extraction and amplification of 16S rRNA gene

Cyanobacteria cells were collected by centrifugation (12, 000*g*, 5 min) from 10 mL unialgal cultures, and the genomic DNA was prepared using a DNA Mini Spin kit (Tiangen, China). Isolated genomic DNA was dissolved in 30 μL of sterile ddH_2O and stored at −20 °C.

For amplification of 16S rDNA fragments, primers Cya106F (5′-CGGACGGGTGAGTAACGCGTGA-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) were used. The 20 μL PCR reaction systems containing 10 μL of 2× Taq DNA polymerase contained buffer (Takara, Japan), 100 μM dNTP, 10 pmol of primers, and 20 to 30 ng of genomic DNA. The systems were performed in a GeneAmp PCR System 9700 (ABI, USA) using the following program: 3 min at 94 °C for denaturalization, followed by 35 cycles, with a cycle of 30 s at 94 °C, 30 s at 55 °C, and 45 s at 72 °C. The target products were cloned into the PMD18-T vector (Takara, Japan) and sequenced.

Cloning and sequencing the geosmin and MIB synthesis genes

Based on the reported cyanobacterial geosmin and MIB genes, we designed two primer sets targeting the geosmin and MIB synthesis genes. A set involved GsyF (5′-ATGC AACCMTTTRAACTGCC-3′) and GsyR (5′-TTCAAATT CYTTRTADCGGTCTAC-3′) for the geosmin synthase gene, and another set involved Mibf (5′-ATGCCCCAAAMTATC ACTGCC-3′) and Mibr (5′-GCCGCAATCTGTAGCACC AT-3′) for the MIB methyltransferase and cyclase genes. These primer sets are designed based on the reported cyanobacterial geosmin/MIB genes in geosmin-producing *Phormidium* sp. (Ludwig et al. 2007), *N. punctiforme* PCC 73102 (Agger et al. 2008; Giglio et al. 2008), *Lyngbya kuetzingii* UTEX 1547 (Zhang et al. 2014), *Anabaena ucrainica* (Wang et al. 2015), and MIB-producing *Pseudanabaena* sp. dqh15 (Wang et al. 2011), *Pseudanabaena limnetica* (Giglio et al. 2011), to amplify the corresponding sequences from the isolated cyanobacteria strains. PCR amplifications were performed as described previously, but the extension time was modified to 1 min. All PCR products were recombined into PMD-18T vector (Takara, Japan) through TA clone and sequenced with ABI 3730XL (Invitrogen, Shanghai, China).

Bioinformatics analysis

The search of homologous genes was performed using the BLAST algorithm (www.blast.ncbi.nlm.nih.gov/Blast.cgi). All reported sequences of cyanobacterial geosmin and MIB synthesis genes and partial from representing Act and myxobacteria (Myx) species were collected. For 16S rDNA, the sequences of representative species in Oscillatoriales were selected and downloaded from the NCBI database. Obtained sequences and reference sequences were edited and aligned using BioEdit (http://www.mbio.ncsu.edu/bioedit/bioedit.html). Neighbor-joining (NJ) and maximum likelihood (ML) phylogenetic trees based on the 16S rDNA, geosmin synthase gene, MIB methyltransferase, and cyclase genes were constructed using Mega 6 (Tamura et al. 2013) with a bootstrap value of 1000.

The geosmin and MIB gene sequences of each strain have been deposited in the GenBank, and the accession numbers are as follows: 16S rDNA (KP013057, KP013058, and KP013059); geosmin synthase gene (KP013060, KP013061, and KP013062); MIB cyclase gene (KP013063, KP013064, and KP013065); and MIB methyltransferase gene (KP013066, KP013067, and KP013068).

Results and discussion

Identification of the *Leptolyngbya bijugata* strains based on morphological and phylogenetic analyses

From the collected samples, more than 20 filamentous epiphytic cyanobacterial strains were isolated. From the cultures, three strains (A2, B2, and B4) determined as odor producers by olfaction were selected and further purified, and their morphological characters were observed under light microscopy. The features of the three strains are shown in Fig. 1. The cultures have similar features, namely, trichomes blue-green or yellow-green in color, trichomes and cells surrounded by visible uncolored sheaths, cells 1.6–2.0 μm in width, usually longer than wide, 1.8–2.7 μm in length, and without false-branching in life cycle. Similar morphological features of strains A2, B2, and B4 suggest that they belonged to the same species in taxonomy. These features were in accordance with the description of species *Leptolyngbya bijugata* by Komárek and Anagnostidis (2005).

Since established by Anagnostidis and Komárek (1988), the heterogeneity of *Leptolyngbya* has been questioned (Komárek and Anagnostidis 2005; Bruno et al. 2009). *Leptolyngbya* species are very difficult to identify because of their controversial position in cyanobacteria and simple morphology without significant discrimination. A polyphasic approach for characterizing *Leptolyngbya* species should be used. Using primers Cya106F and 1492R, the 16S rDNA sequences (~1330 bp) of strains A2, B2, and B4 were obtained. The 16S rDNA from the three strains showed identical sequences, confirming the aforementioned morphological identification as the same species. The phylogenetic analysis of these *L. bijugata* strains was conducted based on a 16S rDNA data set of Oscillatoriales, including the reported representative *Leptolyngbya* species, and inferred using NJ and ML methods. As shown in Fig. 2, the three *L. bijugata* strains formed a monophyletic branch in the phylogenetic tree with other *Leptolyngbya* species isolated from different habitats, e.g., *Leptolyngbya boryana* (type species of this genus), *Leptolyngbya laminosa*, *Leptolyngbya tenuis*, and *Leptolyngbya frigida*. This branch was supported by the high bootstrap values of 0.95/0.87 (NJ/ML). Based on the

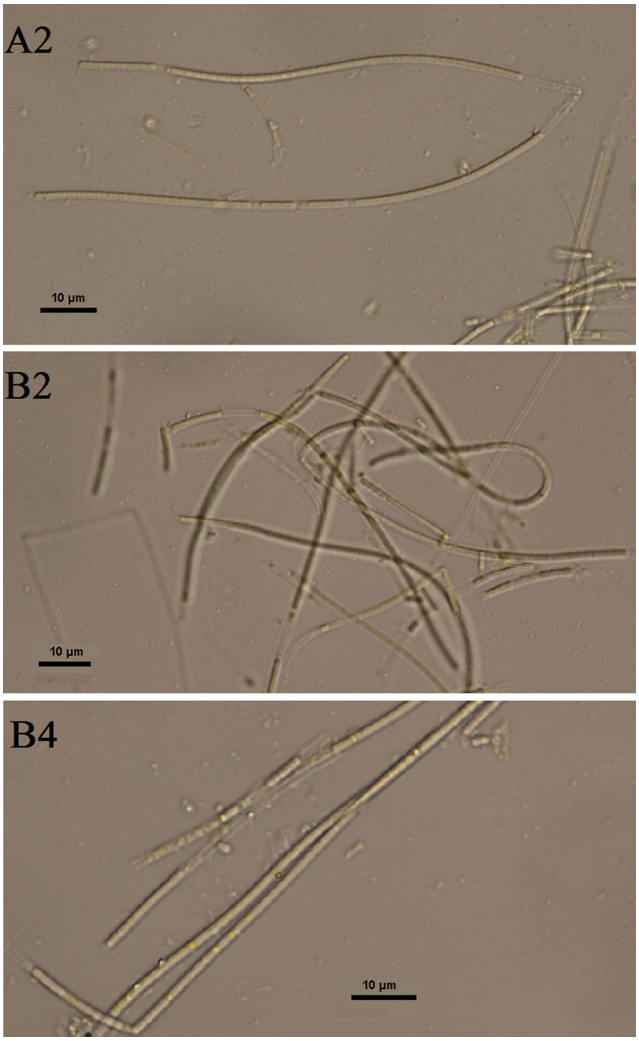

Fig. 1 Morphological features of the three isolated *L. bijugata* strains. As shown in imagines, these strains are coded as A2, B2, and B4

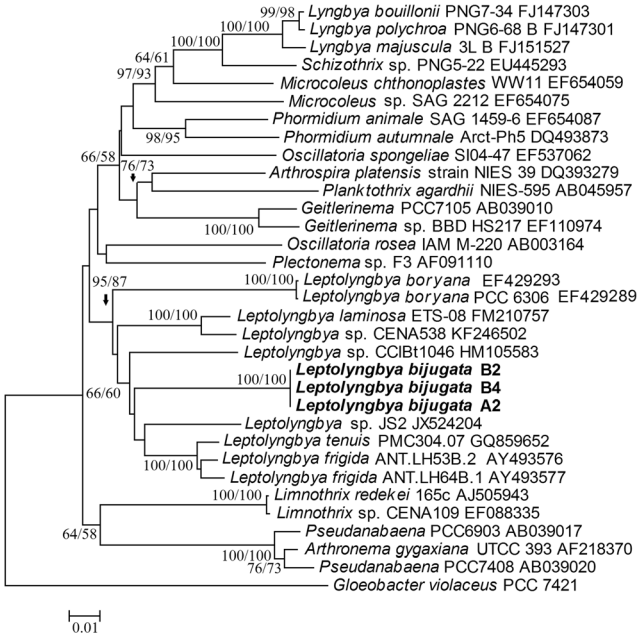

Fig. 2 Phylogenetic tree of three *L. bijugata* strains and their closely related groups based on 16S rDNA sequences (1330 bp). NJ/ML algorithms were used, and only the bootstrap values greater than 60 % were indicated. The unicellular cyanobacterium *Gloeobacter violaceus* PCC 7421 was used as outgroup

evidences from morphology and phylogeny, the strains A2, B2, and B4 were identified as *L. bijugata*.

Odors produced by isolated *L. bijugata* strains

The odorous compounds produced by the *L. bijugata* A2, B2, and B4 strains were chemically analyzed using SPME-GC. Figure 3 shows the two main components of strain A2 extracted compounds (with retention times of 14.77 and 21.32 min), which matched the peaks of MIB and geosmin standards. The strains B2 and B4 have similar components with A2, but with different productivities (data not shown). In addition, the odor productivities of the three *L. bijugata* strains were evaluated using the SPME-GC analysis and external standard method. The average MIB contents at late logarithmic growth phase were as high as 57.5, 36.4, and 12.3 μg L^{-1} for the strains A2, B2, and B4, respectively. For geosmin, the productivities were 22.4, 19.7, and 13.6 μg L^{-1} for A2, B2, and B4, respectively.

Increasing cyanobacterial geosmin and MIB-producing species has been identified and reported worldwide accompanying the emphasis on off-flavor events. Statistics by Izaguirre and Taylor (2004), Jüttner and Watson (2007), and Smith et al. (2008) indicated that nearly 50 species of geosmin and MIB-producing cyanobacteria exist, which are represented in four orders (Chroococcales, Nostocales, Oscillatoriales, and Pleurocapsales). Planktonic taxa are significantly less than benthic/epiphytic ones (about 32 versus 68 % in proportion). Meanwhile, the species that could coproduce geosmin and MIB are rarely documented, and less than ten species have been reported up to date (Jüttner and Watson 2007). The geosmin and MIB coproducing *L. bijugata* identified in the present study expanded our understanding on the diversity of odor-producing cyanobacteria. To our knowledge, this study is the first to report the coproduction of geosmin/MIB by *Leptolyngbya* species.

Most of the off-flavor studies focus on aquatic ecosystems, especially those associated with cyanobacterial blooms (Li et al. 2007, 2010; Wang et al. 2011; Su et al. 2013). The role of benthic/epiphytic cyanobacterial groups in off-flavor episodes has been constantly ignored because of water bottom or epiphytic aquatic plants. Considering the low detection thresholds (2–10 ng L^{-1}) of geosmin/MIB (Cook et al. 2001; Izaguirre and Taylor 2004; Jüttner and Watson 2007) and the high geosmin/MIB productivities by isolated epiphytic *L. bijugata* strains in this study, the presence of this species in the water bottom or rocky shores implied a potential off-

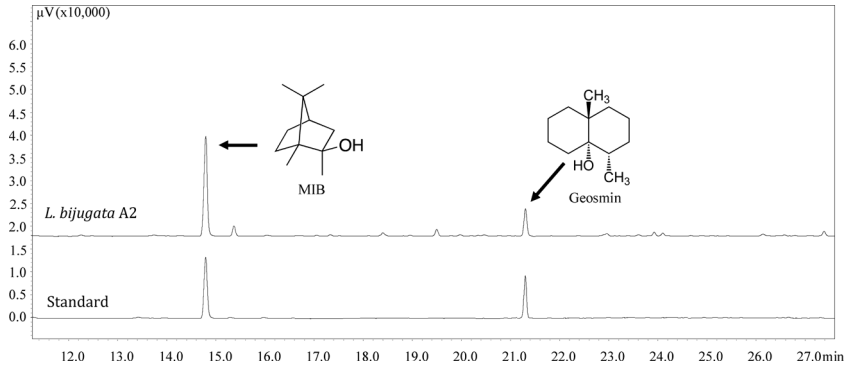

Fig. 3 Analysis of the volatile compounds produced by *L. bijugata* strain A2 using SPME-GC-FID

flavor risk for water quality. Several studies have reported taste and odor problems in waters and fishery products caused by epiphytic *Lyngbya* (Brown and Boyd 1982; Schrader and Blevins 1993; Sugiura et al. 1998).

Clone and sequence analysis of geosmin and MIB synthesis gene

Along with the studies of geosmin and MIB synthesis in *Streptomyces* (Jiang et al. 2007; Komatsu et al. 2008), the corresponding synthesis genes and biochemical mechanisms in cyanobacteria have been determined in some geosmin/MIB-producing species. The cloned partial geosmin synthase gene (2142 bp) of the strains A2, B2, and B4 had the same sequences and are homologous to the elucidated geosmin genes in *N. punctiforme* PCC 73102 (GenBank accession number: FJ010203), *Calothrix* sp. PCC 7507 (CP003943), *Cylindrospermum stagnale* PCC 7417 (CP003642), *L. kuetzingii* UTEX 1547 (JX962775), and *Phormidium* sp. (*geo*A) (EF619621) with DNA identities of 0.739, 0.74, 0.75, 0.71, and 0.67, respectively. For MIB, the cloned whole sequences of th MIB methyltransferase gene (864 bp) and partial cyclase gene (956 bp) from *L. bijugata* strains have 0.91/0.84, 0.91/0.85, 0.91/0.84, and 0.96/0.91 DNA homologies with *Pseudanabaena* sp. dqh15, *P. limnetica*, *P. galeata* NIES-512, and *P. raciborskii* CHAB 3331, respectively.

The amino acid sequences of the geosmin synthase, MIB methyltransferase, and cyclase were analyzed using CDD of the NCBI, and typical Mg^{2+}-binding motifs were annotated. As shown in Fig. 4, the geosmin synthase of *L. bijugata* has four Mg^{2+}-binding motifs, namely, **DD**HFL**E** and R**N**DLF**S**YQR**E** in N-terminal and **DD**YF**P** and **ND**VF**S**YQK**E** in C-terminal. Two Mg^{2+}-binding motifs, **DD**YYA**D** and **ND**LL**S**VAK**D**, were also identified in MIB cyclase. These motifs are essential for catalysis of geosmin/MIB by the Mg^{2+}-dependent synthase gene (Giglio et al. 2008, 2011; Wang et al. 2011). The alignment showed that these motifs are highly conserved in cyanobacterial groups (Fig. 4).

The analysis in sequences and motifs indicated a high conservation of geosmin and MIB synthesis genes in cyanobacteria, and this conservation provided a potential possibility of molecular detection technique. Although the cyanobacteria are responsible for most off-flavor events, not all strains within the same species produce off-flavors (Jüttner and Watson 2007). Thus, fast and reliable assays to distinguish odor producers are urgently needed for off-flavor research and management. Several protocols for the detection of geosmin have been reported along with the elucidated genetic

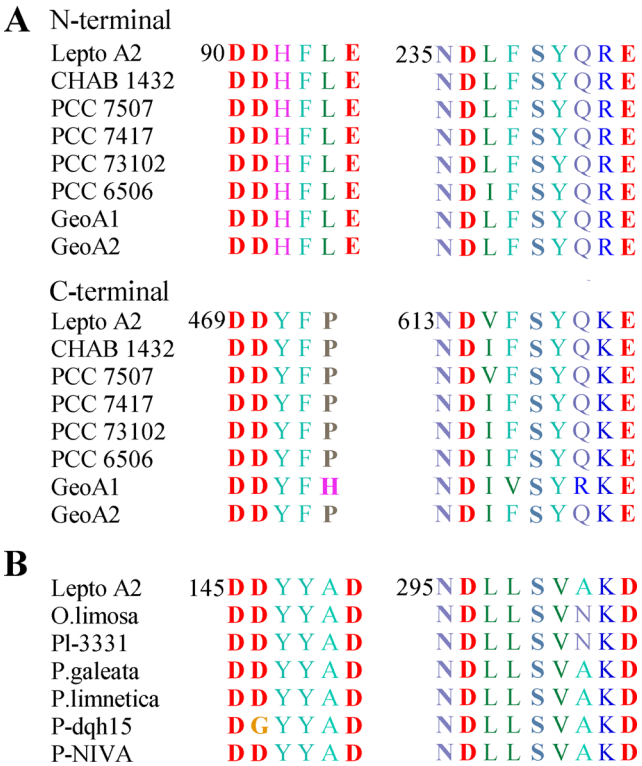

Fig. 4 The conserved Mg^{2+}-binding motifs identified from geosmin and MIB synthase genes of *L. bijugata*. **a** geosmin; **b** MIB. Lepto A2: *L. bijugata*; CHAB 1432: *Anabaena ucrainica*; PCC 7507: *Calothrix* sp.; PCC7417: *Cylindrospermum stagnale*; PCC 73102: *Nostoc punctiforme*; PCC 6506: *Oscillatoria* sp.; GeoA1/A2: *Phormidium* sp.; PI-3331: *Planktothricoids raciborskii* CHAB 3331; P-dqh15 and P-NIVA: *Pseudanabaena* sp.

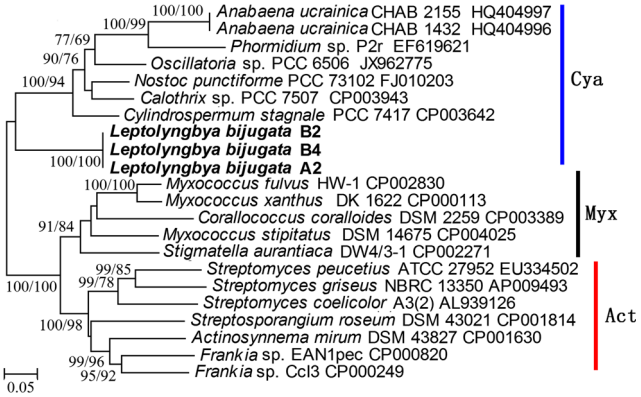

Fig. 5 Unrooted phylogenetic tree based on the geosmin synthase gene (2145 bp) of cyanobacteria species and other microorganisms. NJ/ML algorithms were used, and only the bootstrap values greater than 60 % were indicated. The Cya, Myx, and Act are abbreviations of cyanobacteria, myxobacteria, and actinomycetes, respectively

background. Auffret et al. (2011), Su et al. (2013), and Tsao et al. (2014) established real-time PCR assays for *Streptomyces* and *Anabaena* species. However, no molecular technique for MIB-producing cyanobacteria has been established.

Phylogenetic analysis of geosmin and MIB synthesis gene

The phylogenetic relationships of geosmin synthase gene in different microorganism groups were analyzed using NJ/ML algorithms and are shown in Fig. 5. The *L. bijugata* branch was the sister to the branch containing all other cyanobacterial geosmin producers, forming a monophyletic clade (Cya) in the tree. The other two groups were clusters into their independent branches, including Myx and Act, and formed another clade with well-supported bootstrap values. In NJ/ML trees based on a MIB cyclase gene (Fig. 6a), the monophyletic cyanobacteria branch formed a clade with two Myx species and, apart from another clade, consisted of Act. Similar topologies were also observed in the methyltransferase gene tree, in which the cyanobacteria species formed a monophyletic branch (Fig. 6b).

The evidences described above suggest a common gene origin in cyanobacteria, whether the geosmin or MIB synthesis genes. Compared with other cyanobacterial secondary metabolites, such as microcystins, that are usually widespread only in cyanobacteria (Rantala et al. 2004), geosmin and MIB are synthesized by cyanobacteria and diverse microorganism groups, i.e., fungi, Act, and Myx (Izaguirre and Taylor 2004; Jüttner and Watson 2007). The previous studies by Giglio et al. (2008, 2011), Wang et al. (2011), and Zhang et al. (2014) indicated relatively high similarities between geosmin/MIB genes and conserved functional motifs in Act, Myx, and cyanobacteria. Therefore, all geosmin/MIB genes are postulated to have the same origin and formed different lineages in microorganisms during evolution history.

Conclusions

The three epiphytic cyanobacterial strains (A2, B2, and B4) isolated from Hubei Province, China that accumulated MIB and geosmin were identified as *L. bijugata* based on morphology and 16S rDNA sequences. These strains were the geosmin and MIB coproducers with high odor accumulation ranging from 13.6 to 22.4 μg L^{-1} for geosmin and 12.3 to 57.5 μg L^{-1} for MIB. This study is the first to report the coproduction of geosmin/MIB by cyanobacterium *L. bijugata*, a taxa widely distributed in aquatic environment, that poses a risk for off-flavor events globally. The geosmin and MIB synthesis genes, including geosmin synthase gene,

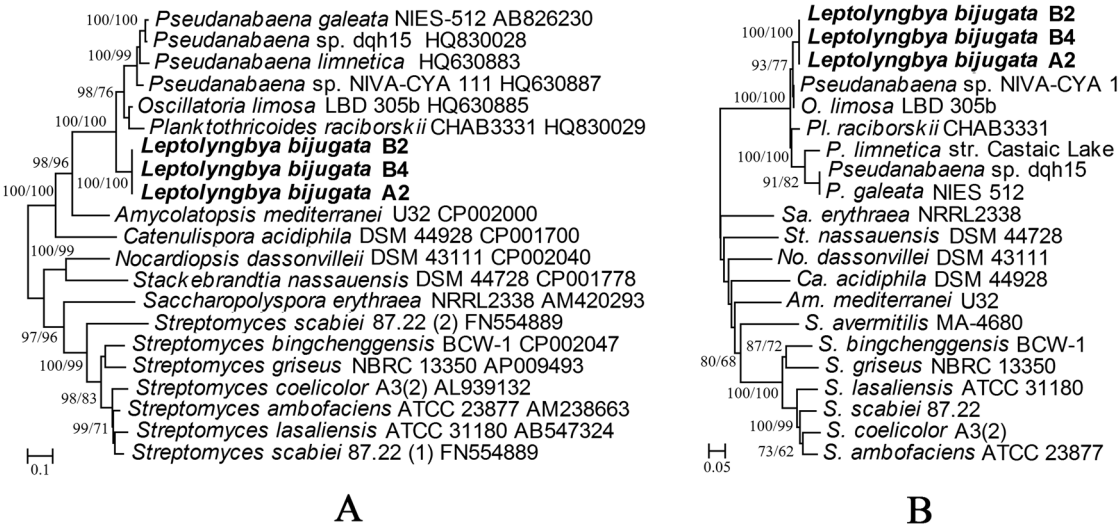

Fig. 6 Unrooted phylogenetic tree based on the MIB synthesis genes of cyanobacteria species and other microorganisms. **a** Based on cyclase gene (956 bp); **b** based on methyltransferase gene (864 bp). NJ/ML algorithms were used, and only the bootstrap values greater than 60 % were indicated

MIB methyltransferase, and cyclase genes, in *L. bijugata* were cloned and sequenced. Sequence similarities (more than 0.67, 0.81, and 0.84 for the three genes, respectively) between *L. bijugata* and other elucidated cyanobacteria suggested high conservation in cyanobacteria. Further phylogenetic analysis revealed a common origin of geosmin/MIB genes in cyanobacteria.

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Conflict of interest The authors declare that there is no conflict of interest.

References

Agger SA, Lopez-Gallego F, Hoye TR, Schmidt-Dannert C (2008) Identification of sesquiterpene synthases from *Nostoc punctiforme* PCC 73102 and *Nostoc* sp. strain PCC 7120. J Bacteriol 190:6084–6096

Anagnostidis K, Komárek J (1988) Modern approach to the classification system of cyanophytes, 3: Oscillatoriales. Algol Stud 50–53:327–472

Auffret M, Pilote A, Proulx E, Proulx D, Vandenberg G, Villemur R (2011) Establishment of a real-time PCR method for quantification of geosmin-producing *Streptomyces* spp. in recirculating aquaculture systems. Water Res 45:6753–6762

Brown SW, Boyd CE (1982) Off-flavor in channel catfish from commercial ponds. Trans Am Fish Soc 111:379–383

Bruno L, Billi D, Bellezza S, Albertano P (2009) Cytomorphological and genetic characterization of troglobitic *Leptolyngbya* strains isolated from Roman Hypogea. Appl Environ Microbiol 75:608–617

Cook D, Newcombe G, Sztajnbok P (2001) The application of powdered activated carbon for 2-MIB and geosmin removal: predicting PAC doses in four raw waters. Water Res 35:1325–1333

Dionigi CP, Lawlor TE, McFarland JE, Johnsen PB (1993) Evaluation of geosmin and 2-methylisoborneol on the histidine dependence of TA98 and TA100 *Salmonella typhimurium* tester strains. Water Res 27:1615–1618

Gerber NN, Lecheval HA (1965) Geosmin an earthy-smelling substance isolated from actinomycetes. Appl Microbiol 13:935–938

Giglio S, Jiang JY, Saint CP, Cane DE, Monis PT (2008) Isolation and characterization of the gene associated with geosmin production in cyanobacteria. Environ Sci Technol 42:8027–8032

Giglio S, Chou WKW, Ikeda H, Cane DE, Monis PT (2011) Biosynthesis of 2-methylisoborneol in cyanobacteria. Environ Sci Technol 45:992–998

Guo L (2007) Ecology—doing battle with the green monster of Taihu Lake. Science 317:1166

Izaguirre G, Taylor WD (2004) A guide to geosmin- and MIB-producing cyanobacteria in the United States. Water Sci Technol 49:19–24

Izaguirre G, Hwang CJ, Krasner SW, McGuire MJ (1982) Geosmin and 2-methylisoborneol from cyanobacteria in three water supply systems. Appl Environ Microbiol 43:708–714

Jiang J, He X, Cane DE (2007) Biosynthesis of the earthy odorant geosmin by a bifunctional *Streptomyces coelicolor* enzyme. Nat Chem Biol 3:711–715

Jüttner F, Watson SB (2007) Biochemical and ecological control of geosmin and 2-methylisoborneol in source waters. Appl Environ Microbiol 73:4395–4406

Komárek J, Anagnostidis K (2005) Cyanoprokaryota 2. Teil/ 2nd Part: oscillatoriales. In: Büdel B, Krienitz L, Gärtner G, Schagerl M (eds) Süsswasserflora von Mitteleuropa 19/2. Elsevier/Spektrum, Heidelberg

Komatsu M, Tsuda M, Omura S, Oikawa H, Ikeda H (2008) Identification and functional analysis of genes controlling biosynthesis of 2-methylisobornrol. Proc Natl Acad Sci U S A 105:7422–7427

Li L, Wan N, Gan N, Xiao BD, Song LR (2007) Annual dynamics and origins of the odorous compounds in the pilot experimental area of Lake Dianchi, China. Water Sci Technol 55:43–50

Li Z, Yu J, Yang M, Zhang J, Burch MD, Han W (2010) Cyanobacterial population and harmful metabolites dynamics during a bloom in Yanghe Reservoir, North China. Harmful Algae 9:481–488

Lloyd SW, Lea JM, Zimba PV, Grimm CC (1998) Rapid analysis of geosmin and 2-methylisoborneol in water using solid phase micro extraction procedures. Water Res 32:2140–2146

Ludwig F, Medger A, Börnick H, Opitz M, Lang K, Göttfert M, Röskel I (2007) Identification and expression analyses of putative sesquiterpene synthase genes in *Phormidium* sp. and prevalence of *geoA*-like genes in a drinking water reservoir. Appl Environ Microbiol 73:6988–6993

Merel S, Walker D, Chicana R, Snyder S, Baures E, Thomas O (2013) State of knowledge and concerns on cyanobacterial blooms and cyanotoxins. Environ Int 59:303–327

Rantala A, Fewer DP, Hisbergues M, Rouhiainen L, Vaitomaa J, Borner T, Sivonen K (2004) Phylogenetic evidence for the early evolution of microcystin synthesis. Proc Natl Acad Sci U S A 101:568–573

Schrader KK, Blevins WT (1993) Geosmin-producing species of *Streptomyces* and *Lyngbya* from aquaculture ponds. Can J Microbiol 39:834–840

Smith JL, Boyer GL, Zimba PV (2008) A review of cyanobacterial odorous and bioactive metabolites: Impacts and management alternatives in aquaculture. Aquaculture 280:5–20

Su M, Gaget V, Giglio S, Burch M, An W, Yang M (2013) Establishment of quantitative PCR methods for the quantification of geosmin-producing potential and *Anabaena* sp. in freshwater systems. Water Res 47:3444–3454

Sugiura N, Iwami N, Inamori Y, Nishimura O, Sudo R (1998) Significance of attached cyanobacteria relevant to the occurrence of musty odor in Lake Kasumigaura. Water Res 32:3549–3554

Sun D, Yu J, An W, Yang M, Chen G, Zhang S (2013) Identification of causative compounds and microorganisms for musty odor occurrence in the Huangpu River, China. J Environ Sci (China) 25:460–465

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729

Tsao HW, Michinaka A, Yen HK, Giglio S, Hobson P, Monis P, Lin TF (2014) Monitoring of geosmin producing *Anabaena circinalis* using quantitative PCR. Water Res 49:416–425

Wang Z, Xu Y, Shao J, Wang J, Li R (2011) Genes associated with 2-methylisoborneol biosynthesis in cyanobacteria: Isolation, characterization, and expression in response to light. PLoS One 6, e18665

Wang Z, Shao J, Xu Y, Yan B, Li R (2015) Genetic basis for geosmin production by the water bloom-forming cyanobacterium, *Anabaena ucrainica*. Water 7:175–187

Watanabe MM, Hiroki M (1997) NIES-collection. List of strains, algae and protozoa. National Institute for Environmental Studies, Environment Agency, Japan, p 140

Watson SB (2003) Cyanobacterial and eukaryotic algal odour compounds: signals or by-products? A review of their biological activity. Phycologia 42:332–350

Yu WJ, Zhao YM, Yang M, Lin T-F, Guo ZH, Gu NJ, Li S, Han W (2009) Occurrence of odour-causing compounds in different source waters of China. J Water Supply Res Technol AQUA 58:587–594

Zhang T, Li D, Wang G, Song L, Li L (2014) Identification and expression analysis of the gene associated with geosmin production in *Lyngbya kuetzingii* UTEX 1547 (cyanobacteria). Harmful Algae 39:127–133

Zhao Y, Yu J, Su M, An W, Yang M (2013) A fishy odor episode in a north China reservoir: occurrence, origin, and possible odor causing compounds. J Environ Sci (China) 25:2361–2366