

Article

# Genetic Basis for Geosmin Production by the Water Bloom-Forming Cyanobacterium, *Anabaena ucrainica*

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**Abstract:** Geosmin is a common, musty-smelling sesquiterpene, principally produced by cyanobacteria. *Anabaena ucrainica* (Schhorb.) Watanabe, a water bloom-forming cyanobacterium, is the geosmin producer responsible for odor problems in Dianchi and Erhai lakes in China. In this study, the geosmin synthase gene (*geo*) of *A. ucrainica* and its flanking regions were identified and cloned by polymerase chain reaction (PCR) and genome walking. The *geo* gene was found to be located in a transcription unit with two cyclic nucleotide-binding protein genes (*cnb*). The two *cnb* genes were highly similar and were predicted members of the cyclic adenosine monophosphate (cAMP) receptor protein/fumarate nitrate reductase regulator (Crp–Fnr) family. Phylogenetic and evolutionary analyses implied that the evolution of the geosmin genes involved a horizontal gene transfer process in cyanobacteria. These genes showed a close relationship to 2-methylisoborneol genes in origin and evolution.

**Keywords:** geosmin; synthesis gene; cyanobacteria; *Anabaena ucrainica*; cyclic nucleotide-binding protein

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## 1. Introduction

Taste and odor (T & O) problems mainly caused by secondary metabolites of microorganisms in aquatic ecosystems have been frequently reported worldwide [1–4]. Geosmin (trans-1, 10-dimethyl-trans-9-decalol), a sesquiterpene derivative with an earthy/musty smell, is a common odor compound in surface water. Since its discovery in *Streptomyces griseus* LP-16 [5], geosmin has been regarded to cause frequent T & O incidents in water supplies, because of its strong earthy smell and extraordinarily low sensory threshold of 4–20 ng·L<sup>-1</sup> [6]. Cyanobacteria is the main phytoplankton group in many eutrophic waters; members of this group, along with myxobacteria, actinomycetes and fungi, are the principal producers of geosmin [4,7,8].

A 726-amino acid cyclase encoded by *sco6073* in *Streptomyces coelicolor* A3 (2) catalyzes the Mg<sup>2+</sup>-dependent cyclization of farnesyl diphosphate (FPP) to geosmin [9]. According to Jiang *et al.* [10], this type of cyclase is a bifunctional enzyme composed of two similar domains at the N-terminal and C-terminal positions, and FPP is converted to geosmin through the catalysis of these two domains. In cyanobacteria, two putative geosmin synthase genes (*geoA1* and *geoA2*) homologous to *sco6073* were identified from the geosmin-producing cyanobacterium, *Phormidium* sp., by using degenerating primers for PCR and reverse PCR [11]. Agger *et al.* [12] and Giglio *et al.* [13] cloned and successfully expressed the germacrene/germacradienol and geosmin synthase genes (*npunmod*) from the geosmin-producing cyanobacterium, *Nostoc punctiforme* PCC 73102, in *Escherichia coli*. Apart from the benthic/periphytic groups represented above, planktonic (bloom-forming) cyanobacterium species are also likely to produce geosmin [2,7]. However, only Giglio *et al.* [14] have analyzed the expression of geosmin synthase in *Anabaena circinalis*. Further in-depth studies on the genetics of geosmin synthesis from bloom-forming cyanobacteria have yet to be conducted.

Water blooms have become a frequent occurrence in Chinese waters, particularly in large shallow lakes. T & O episodes that result from these water blooms have become a major environmental problem, with the worst case recorded in Lake Taihu in May, 2007 [15]. Two plateau lakes (*i.e.*, Dianchi and Erhai) in Yunnan Province have experienced severe odor problems caused by water blooms in the past decade [3]. Our previous work found that the planktonic heterocystous species, *Anabaena ucrainica* (Schorb.) Watanabe, is involved in the odor problems of these two lakes (unpublished data). In the present study, two strains of *A. ucrainica* with high geosmin productivities were isolated from Dianchi and Erhai lakes and used to investigate the genes responsible for geosmin synthesis. The present study aimed to identify and characterize geosmin synthase genes from the bloom-forming *A. ucrainica* strains, to explore whether or not other genes are structurally or functionally related to this gene and to preliminarily investigate the origin and evolution of these genes.

## 2. Materials and Methods

### 2.1. Isolation of Cyanobacterial Strains

Two *A. ucrainica* strains, CHAB (Collection of Harmful Algal Biology) 1432 and CHAB 2154, were isolated from Dianchi and Erhai lakes, respectively, by using the Pasteur micropipette method and were maintained in screw-capped tubes that contained 5 mL of liquid CT medium [16].

For geosmin analysis and further molecular experiments, the strains were cultured in 150 mL of CT medium in 300 mL flasks under a light:dark regime of 12:12 at a photon density of approximately  $25 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  and a temperature of  $25 \pm 1$  °C. Other geosmin producers used in this study were cultured in CT medium and then deposited in the CHAB, Institute of Hydrobiology, Chinese Academy of Sciences. For the determination of geosmin productivities, experiments were performed for 16 days in 250-mL flasks that contained 120 mL of *A. ucrainica* cultures with an initial concentration of approximately  $1.6 \times 10^6$  cells·mL<sup>-1</sup>. All experiments were performed in triplicate.

## 2.2. Analysis of Geosmin

The geosmin produced by *A. ucrainica* strains was analyzed by headspace solid phase micro-extraction coupled with gas chromatography (GC), as described by Watson *et al.* [6] and Li *et al.* [3]. Solid phase micro-extraction (SPME) fiber (Polydimethylsiloxane/Divinylbenzene (PDMS/DVB), 65  $\mu\text{m}$ , Supelco, Sigma-Aldrich, St. Louis, MO, USA), GC with an flame ionization detector (FID) detector (GC-2014C, Shimadzu, Tokyo, Japan) and a capillary column (TC series, WondaCap 5, 0.25 mm  $\times$  30 m  $\times$  0.25  $\mu\text{m}$ , Shimadzu) were used in the analyses. The oven temperature program was set as described by Li *et al.* [3]. The geosmin standard solution (100 ng· $\mu\text{L}^{-1}$ , Supelco) was used to verify the analysis results of samples through the external standard method. Peaks corresponding to the geosmin standard were also confirmed by gas chromatography-mass spectrometer (GC-MS) (HP6890GC-5973MSD, HP, Palo Alto, CA, USA).

## 2.3. General Molecular Techniques and Genome Walking

The genomic DNAs of the two strains were prepared using a DNA Mini Spin kit (Tiangen, Beijing, China). Two primers, G2f (5'-GCAACGACCTCTTCTCCTAC-3') and G2r (5'-CACCCAACTGTCAGTCATATCCT-3'), were designed on the basis of the geosmin synthase genes of *N. punctiforme* PCC 73102 and *Phormidium* sp. to amplify the corresponding sequence (943 bp) of *A. ucrainica* strains. PCR was carried out using the LA *Taq* polymerase kit (Takara, Dalian, China) and performed in a Bio-Rad MJ mini personal thermal cycler (MJ Research, Foster, CA, USA) with the following program: 3 min at 94 °C for denaturation, followed by 35 cycles of 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 60 s.

Genome walking experiments were performed to amplify the flanking regions by using a genome walking kit (Takara). The primers used in the genome walking are listed in Table S1. All of the PCR and genome walking products were cloned to the PMD18-T vector (Japan) and then sequenced.

Primer sets GTC1F(R) and GTC2F(R) (listed in Table S1) covering the intergenic spacers between neighboring genes were designed in this study and used to confirm the gene composition and arrangement of the geosmin operon in seven geosmin-producing species by using the same PCR program as mentioned above.

## 2.4. RNA Extraction and Reverse Transcriptional PCR

Fresh cells of the two *A. ucrainica* strains were collected by centrifugation (12,000 $\times$  g, 4 °C, 5 min) and then transferred into 2-mL centrifuge tubes. Mini-beadbeater (0.5 mL) and TRIzol reagent (1 mL,

Invitrogen, Carlsbad, CA, USA) were added into tubes for cell resuspension. Total RNA was extracted using TRIzol reagent in accordance with the manufacturer's instructions, and the RNA sample was treated with DNase I (Promega, Madison, WI, USA) to remove the mixed genomic DNA. Reverse transcription to cDNA using a transcriptase kit (Generay, Shanghai, China) was carried out.

Primers RNC1F with RNC1R and RNC2F with RNC2R (Table S1) were used to examine the integrity of the geosmin synthase gene with flanking genes by PCR using cDNA as a template. Control PCR was conducted using the RNA that underwent DNase digestion as the template. The elongation time was modified to 30 s, and the other steps were identical to the above description.

## 2.5. Bioinformatics Analysis

Homologous genes of the *geo* gene in *A. ucrainica* CHAB 1432 were searched by BLAST [17]. The Conserved Domain Database (CDD; [www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml](http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml)) [18] was used to identify the conserved motifs and functional sites. Neighbor-joining (NJ) and maximum parsimony (MP) phylogenetic trees of the geosmin gene were constructed by Mega 4.0 [19] with a bootstrap value of 1000.

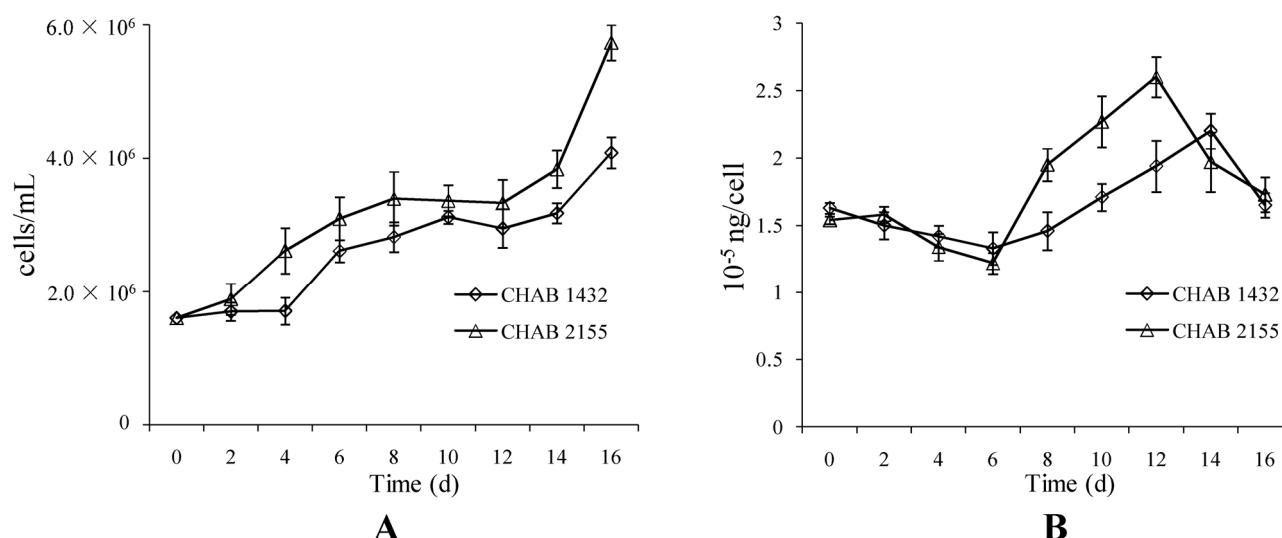
Geosmin synthesis operons and 16S rDNA sequences of *A. ucrainica* CHAB 1432 and CHAB2155 were deposited in the NCBI nucleotide sequence database. The accession numbers are HQ404996 and HQ404997 for the geosmin operons and GU197649 and GU197642 for the 16S rDNA.

## 3. Results and Discussion

### 3.1. Isolation of a Bloom-Forming Cyanobacterium Responsible for Geosmin Production

Two *A. ucrainica* strains that exude an earthy/musty odor were obtained from Dianchi and Erhai lakes, and chemical analysis showed geosmin as their main volatile component (Figure S1). The features of geosmin production in *A. ucrainica* strains were studied (Figure 1). Strains CHAB 1432 and 2155 similarly showed the following pattern in geosmin production: A slight decrease in rapid growth period (0–6 days), a rapid increase in stagnant period (approximately 14 days) and a significant decrease in the last period of cultivation. The maximum total geosmin production of these two strains were  $2.20 \times 10^{-5} \text{ ng} \cdot \text{cell}^{-1}$  and  $2.60 \times 10^{-5} \text{ ng} \cdot \text{cell}^{-1}$ , and the total production was more than  $1.20 \times 10^{-5} \text{ ng} \cdot \text{cell}^{-1}$  in the entire growth cycle.

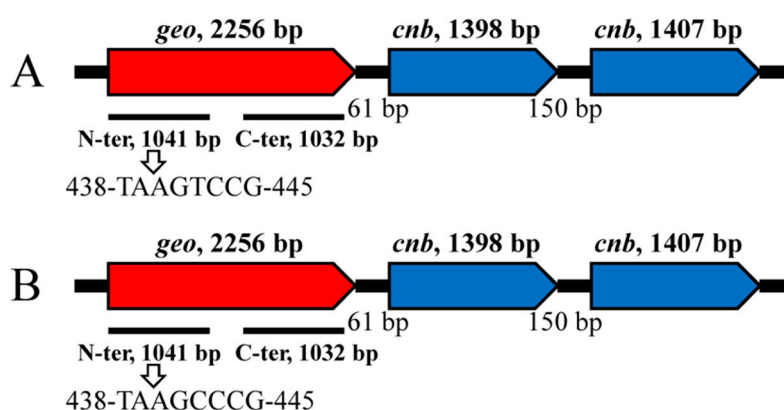
Among documented cyanobacterial geosmin/2-methylisoborneol (MIB) producers, more than 13 planktonic species were identified [2,7]. These planktonic taxa can form unsightly or highly visible surface blooms, and numerous T & O episodes have been attributed to bloom-forming genera, such as *Anabaena*, *Aphanizomenon* and *Planktothrix* [4,20–22]. *A. ucrainica* has been historically implicated as the main source of geosmin in a reservoir in Japan [21]. Results of the chemical analysis in the present study showed *A. ucrainica* as a geosmin producer and probably the principal producer of geosmin in Dianchi and Erhai lakes. On the basis of the total geosmin productivity determined in these experiments ( $1.20\text{--}2.60 \times 10^{-5} \text{ ng} \cdot \text{cell}^{-1}$ ) and the sensory threshold of geosmin, water bodies are at risk for T & O events when the abundance of geosmin-producing cyanobacteria is greater than  $5 \times 10^5 \text{ cells/L}$  ( $10 \text{ ng} \cdot \text{L}^{-1}$  total geosmin according to the average productivity).



**Figure 1.** Cell growth (A) and geosmin productions (B) of *A. ucrainica* CHAB 1432 and 2155 in the test cycle.

### 3.2. Identification and Characterization of the Geosmin Synthase Genes of *A. ucrainica*

Two 943-bp identical fragments were amplified from *A. ucrainica* strains CHAB 1432 and CHAB 2155 by using primers G2f and G2r. Subsequently, the whole geosmin synthase gene (*geo* gene) in *A. ucrainica* was successfully cloned through genome walking-PCR (Figure 2). The *geo* gene of CHAB 1432 and CHAB 2155 is 2256 bp in length. Only one base pair difference at the 442 bp site (T for 1432 and C for 2155) was found between these two strains. The BLAST search revealed that the *geo* genes from these two strains were 76.9%, 65.4%, 81.0% and 80.6% identical in DNA sequences and 82.9%, 61.4%, 86.8% and 87.1% identical in deduced amino acid sequences with the well-elucidated geosmin synthase genes, *npunmod* (*N. punctiforme* PCC 73102), *geoA1* (*Phormidium* sp.), *geoA2* (*Phormidium* sp.) and that from *Oscillatoria* sp., respectively. The high conservation of the geosmin gene in cyanobacteria could provide essential information for the development of molecular monitoring methods of geosmin-producing cyanobacteria. A recent study has reported a quantification method for potential geosmin-producing *Anabaena* in freshwater on the basis of geosmin gene sequences [20,23].

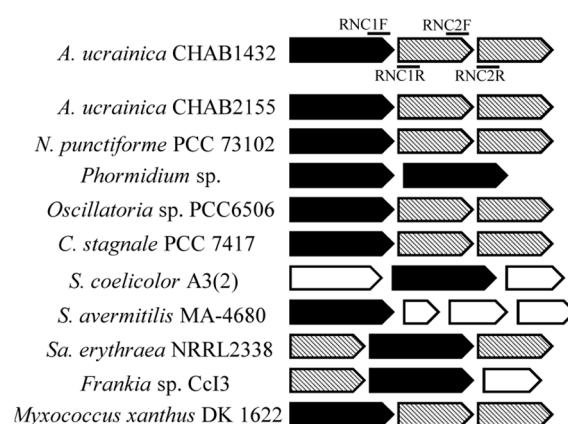


**Figure 2.** Geosmin synthesis genes of *A. ucrainica*. (A) Strain CHAB 1432; (B) strain CHAB 2155.

The functional sites of Geo were identified by CDD search (specific hits with terpene cyclase non-plant C1) and amino acid alignment; these sites include NPUNMOD (*N. punctiforme* PCC 73102), GeoA1 (*Phormidium* sp.), GeoA2 (*Phormidium* sp.), SAV2163 (*Streptomyces avermitilis* MA-4680) and SCO6073 (*S. coelicolor* A3 (2)) (Figure S2). In general, the N-terminal domains of all of the analyzed proteins are more conserved than the C-terminal domains and contain two typical  $Mg^{2+}$ -binding motifs, namely, **DDHFLE** and **RNDLFSYQRE** sequences (Figure S2) [9,10]. Two less conserved  $Mg^{2+}$ -binding motifs, namely, **DDY(F/Y)(P/H)** and **ND(I/V)(F/V)SY(Q/R)KE**, were also found in the C-terminus. Interestingly, the N-domains and C-domains of Geo and NPUNMOD were homologous to each other and shared identical motifs, such as the  $Mg^{2+}$ -binding sites (Figure S3). The DNA sequences of *geo* and *npunmod* in the N- and C-domains shared 50.9% and 44.2% similarities, respectively.

### 3.3. Structure and Features of the Geosmin Operon

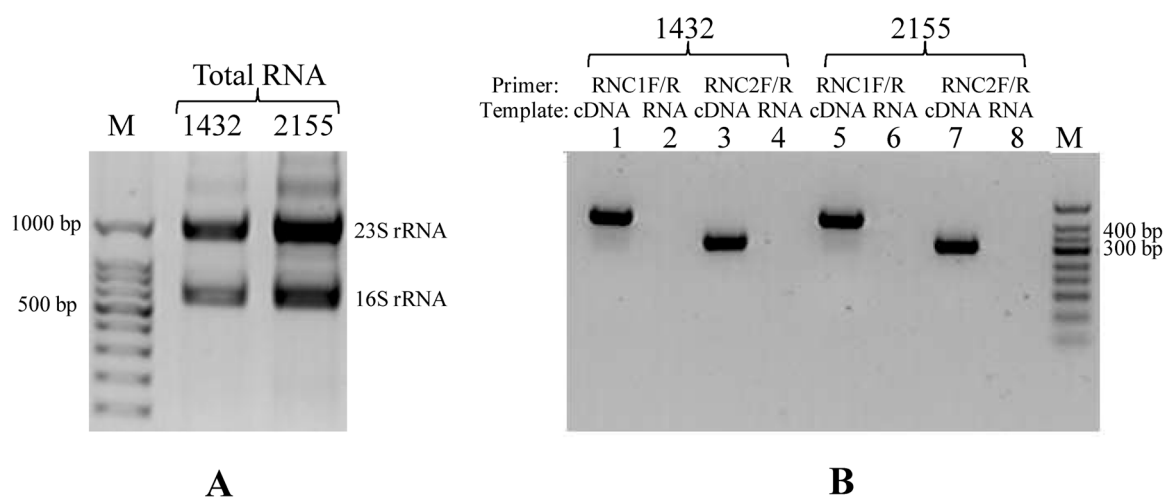
Two putative cyclic nucleotide-binding protein genes (*cnb*) that are 1398 and 1407 bp in length were identified downstream of the *geo* gene through genome walking (Figure 2). These two *cnb* genes have the same transcriptional orientation and are homologous, with 73% DNA similarity. Further BLAST and CDD searches suggest that this type of cyclic nucleotide-binding gene belongs to the Crp–Fnr regulator family. The flanking regions of the geosmin synthase gene were also analyzed in the released genomic data (from NCBI) of *N. punctiforme* PCC 73102, *Oscillatoria* sp. PCC 6506, *Cylindrospermum stagnale* PCC 7417, four representative species of Actinomycetes and *Myxococcus xanthus* DK 1622. As shown in Figure 3, the organization of the geosmin synthase genes in *N. punctiforme* PCC 73102, *Oscillatoria* sp. PCC 6506, *C. stagnale* PCC 7417 and *M. xanthus* DK 1622 are similar to that of the *A. ucrainica* strains. No *cnb* genes were found in adjacent regions of the geosmin synthases of *S. coelicolor* A3 (2) and *S. avermitilis* MA-4680. The array of the geosmin synthase with *cnb* genes in *Saccharopolyspora erythraea* NRRL2338 is different from that in *A. ucrainica*, and only one *cnb* gene is upstream of the geosmin synthase gene in *Frankia* sp. CcI3.



**Figure 3.** Organization of genes that encode predicted geosmin/germacradienol synthases and flanking genes in different microorganisms. The filled, oblique-lined and unfilled arrows indicate the geosmin/germacradienol synthase gene, cyclic nucleotide-binding protein gene (*cnb*) and other unrelated genes, respectively. Line segments located in *A. ucrainica* CHAB 1432 show the positions of primers RNC1F(R) and RNC2F(R).

A *rho*-independent transcriptional terminator was not identified in the interspacers of *geo* and two *cnb* genes (61 bp and 150 bp). This result suggests that these three genes are located in the same transcription unit and probably form an operon structure. Both reverse transcriptional-PCR and PCR using two pairs of primers (RNC1F with RNC1R, and RNC2F with RNC2R; see the positions in Figure 3) with cDNA as a template confirm that *geo* and *cnb* genes are located in one transcription unit (Figure 4). Furthermore, seven geosmin-producing cyanobacterial species that represent seven genera in Nostocales and Oscillatoriales were used to verify the organization of the geosmin operon in cyanobacteria. The results of regular-PCR with primer sets GTC1F(R) and GTC2F(R), gel electrophoresis and sequencing indicate a universal gene composition and arrangement of the geosmin operon (*geo/cnb/cnb*) in all investigated strains (Table 1).

The above results revealed the pattern of geosmin synthase genes in an operon with two *cnb* genes that were predicted members of the Crp–Fnr regulator family, and this pattern was proven as the gene structure in most investigated cyanobacteria (except the *Phormidium* sp.) (Table 1 and Figure 3). In the MIB biosynthesis genes identified in cyanobacteria, homologous *cnb* genes form an operon structure with MIB cyclase [24,25]. The present study also showed that the Crp–Fnr family was highly conserved in different groups of microorganisms and was only found around terpene (geosmin/germacradienol and MIB) cyclase genes. This result suggests that this family of regulators is related to the corresponding cyclase genes, both in location and function. The Crp–Fnr family is a universally positive regulator that plays key roles in nitrogen fixation, photosynthesis and respiration in various microorganisms [26]. The functions of *cnb* genes in the synthesis and evolution of sesquiterpenes (geosmin) and monoterpenes (MIB) are interesting topics worthy of further study.



**Figure 4.** Electrophoretic results of total RNA and reverse transcriptional-PCR. (A) Total RNA extracted from *A. ucrainica* CHAB1432 and CHAB 2155 (2% agarose; the two bands, 23S and 16S rRNA, indicate a high quality of extracted total RNA); (B) Lanes 1, 3, 5 and 7, amplification products (1, 5: partial *geo*, *cnb1* and their internal spacer; 3, 7: partial *cnb1*, *cnb2* and their internal spacer) from RT-PCR products of CHAB1432 and CHAB2155, used two pairs of primers, RNC1F/R and RNC2F/R, respectively. Lanes 2, 4, 6 and 8 were PCRs conducted on the same RNA used to make the cDNA that served as a template for the PCRs in Lanes 1, 3, 5 and 7 used the same primers. M, marker.

**Table 1.** Organization of the operon in other geosmin-producing cyanobacteria.

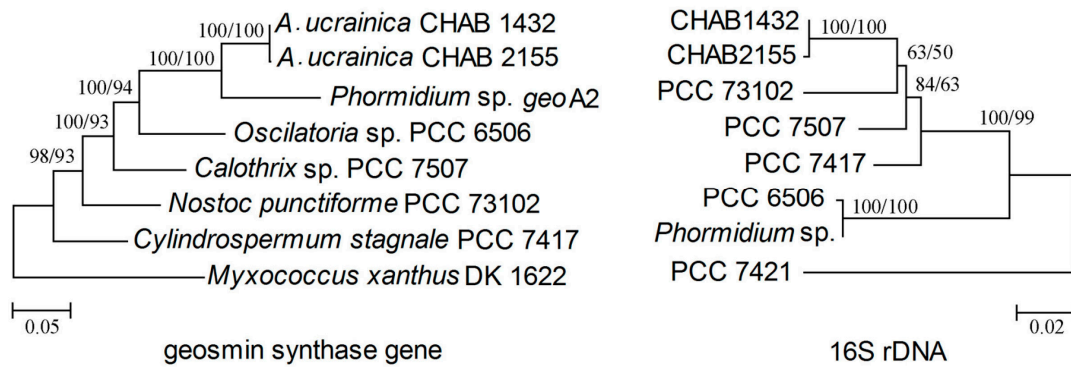
Species	Taxonomy	Primers GTC1F/R	Primers GTC2F/R	Homology	Organization of genes
<i>Anabaena</i> sp. 3585	Nostocales	+ a/– b	+/–	+ c	<i>geo/cnb/cnb</i>
<i>Calothrix</i> sp. 2384	Nostocales	+/–	+/–	+	<i>geo/cnb/cnb</i>
<i>Nostoc</i> sp. 261	Nostocales	+/–	+/–	+	<i>geo/cnb/cnb</i>
<i>Aphanizomenon gracile</i> 2417	Nostocales	+/–	+/–	+	<i>geo/cnb/cnb</i>
<i>Phormidium</i> sp. D6	Oscillatoriales	+/–	+/–	+	<i>geo/cnb/cnb</i>
<i>Lyngbya kuetzingii</i> 388	Oscillatoriales	+/–	+/–	+	<i>geo/cnb/cnb</i>
<i>Tychonema bourrellyi</i> 663	Oscillatoriales	+/–	+/–	+	<i>geo/cnb/cnb</i>

Notes: a, target fragments; b, non-specific products were not observed; c, DNA similarity of obtained sequences (excluding the intergenic region) is more than 60% with corresponding fragments of CHAB 1432.

### 3.4. Analysis of Geosmin-Associated Genes: Origin and Evolution

#### 3.4.1. Inconsistent Topologies between Geosmin Gene and 16S rDNA Phylogenetic Trees

To investigate the evolutionary history in the distribution of the geosmin/germacradienol synthase gene among cyanobacteria, the reported sequences of this gene were collected and phylogenetically analyzed with the housekeeping 16S rRNA gene from the same taxa. As shown in Figure 5, the NJ/MP topologies of these phylogenetic trees generated from the geosmin/germacradienol synthase gene and 16S rRNA gene were not congruent. In the geosmin/germacradienol gene tree, *A. ucrainica* strains were separated from other heterocystous cyanobacterial species, but clustered with non-heterocystous *Phormidium* sp. (Geo A) and *Oscillatoria* sp. PCC 6506. These relationships differed from the recognized relationships revealed by the 16S rRNA gene-based tree.



**Figure 5.** Comparison between the topologies of the geosmin synthase gene (2248 bp) and 16S rDNA (1235 bp) trees. Neighbor-joining (NJ) and maximum parsimony (MP) methods were used in the construction of trees. The accession numbers of sequences used in the trees are listed in Table S2. PCC 7421: *Gloeobacter violaceus* PCC 7421.

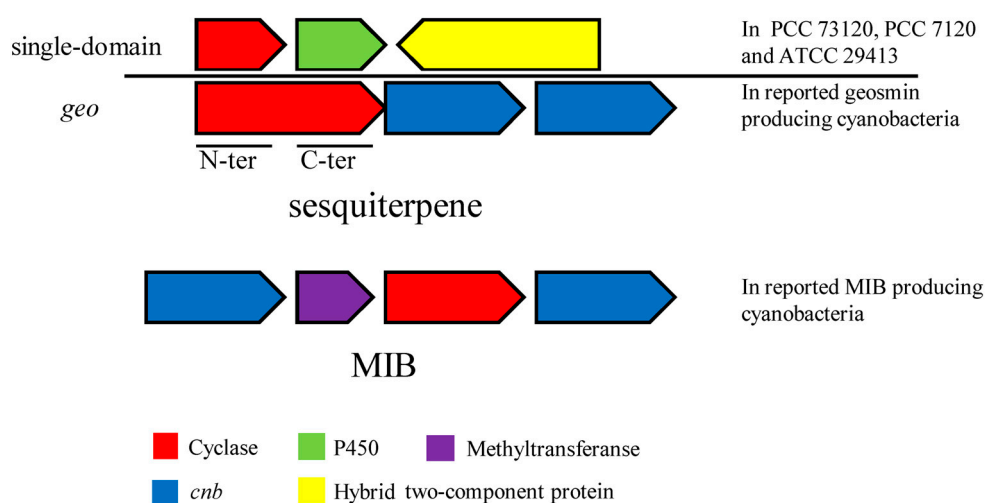
The inconsistent phylogenetic topologies of the geosmin gene and 16S rRNA gene (Figure 5) implied that the geosmin synthesis genes did not co-evolve with the corresponding genomes during the evolutionary history of cyanobacteria, suggesting that these genes were acquired by horizontal gene transfer (HGT) among different groups. HGT is an important evolutionary force in bacteria, and the



genes responsible for cyanobactin and saxitoxin synthesis in cyanobacteria have a history of HGT [27,28]. Sequences from various cyanobacterial taxa are necessary to ascertain the occurrence of HGT in geosmin evolution and the role of HGT in the distribution of the geosmin synthase gene in cyanobacteria. However, geosmin-associated genes were only elucidated in a few strains, including *N. punctiforme* PCC 73102, *Phormidium* sp., and *Lyngbya kuetzingii* UTEX 1547 [11,13,29].

### 3.4.2. Comparison of *geo*, Single-Domain Sesquiterpene Gene, MIB Synthesis Gene and Their Related Gene Clusters in Cyanobacteria

Another typical sesquiterpene synthase gene with a single domain in cyanobacteria was revealed through the BLAST search and analysis on the homologous genes of *geo* (Figure 6). The single-domain genes are highly homologous with *geo* in functional sites, such as  $Mg^{2+}$ -binding sites, but different in several other sites (Figure S3), and could catalyze the FPP to sesquiterpenes, such as germacrene [12]. As shown in Figure 6, the single-domain gene clustered with a cytochrome P450 gene and a hybrid two-component protein gene, and the gene array and constitution were universal in the published genomes of cyanobacteria, including *N. punctiforme* PCC 73102, *Nostoc* sp. PCC 7120 and *Anabaena variabilis* ATCC 29413. As elucidated in the present study, the two-domain *geo* gene and two *cnb* genes formed an operon structure, and this operon structure was proven to be consistent in cyanobacteria. Interestingly, Wang *et al.* [25] revealed that the *cnb* genes are also closely linked to MIB synthesis genes and form an operon structure in cyanobacteria (Figure 6).



**Figure 6.** Clustering of cyanobacterial *geo*, single-domain sesquiterpene gene and MIB-synthesis-associated open reading frames.

The *cnb* genes of the geosmin operon are highly homologous with those of the MIB gene cluster in cyanobacteria. Table 2 shows the similarities in DNA sequences among these *cnb* genes from geosmin and MIB gene clusters. The similarities among six *cnb* genes from representative cyanobacterial species, including *A. ucrainica* CHAB 1432 (geosmin), *Pseudanabaena* sp. dqh15 (MIB) and *Planktothricoides raciborskii* CHAB 3331 (MIB), are between 60% and 88.5%. These *cnb* genes associated with MIB synthesis have higher similarities (77.1% to 88.5%) to those between geosmin and MIB operon (60% to 62.8%).

**Table 2.** DNA similarity matrix of geosmin and MIB-related *cnb* genes in cyanobacteria (1398 bp).

Sequences	A	B	C	D	E
A	-	-	-	-	-
B	0.773	-	-	-	-
C	0.874	0.791	-	-	-
D	0.771	0.885	0.803	-	-
E	0.615	0.612	0.604	0.606	-
F	0.628	0.612	0.605	0.601	0.73

Notes: A and B, MIB synthesis-associated *cnb* genes of *Pseudanabaena* sp. dqh15; C and D, MIB synthesis-associated *cnb* genes of *Planktothricoides raciborskii* CHAB 3331; E and F, geosmin synthesis-associated *cnb* genes of *A. ucrainica* CHAB 1432.

The gene array and constitution of two sesquiterpene synthase operons or open reading frames were significantly different, representative of the *geo* with two *cnb* genes and the single-domain one with cytochrome P450 and hybrid two-component protein genes (Figure 6). The P450 gene plays an important role in the modification of sesquiterpene products in fungi and cyanobacteria [12,30]. The large difference in the structure of the gene cluster and gene function of these two types of sesquiterpene genes suggests that they have independent origins and evolutionary lineages. Although they have the same substrate (FPP) and similar catalytic mechanism of the N-domain and single-domain synthase [10,12], the low homology of their sequences (Figure S3) implies an independent evolutionary lineage of geosmin from other sesquiterpene in cyanobacteria. Interestingly, the high homologies between the *cnb* genes of geosmin and MIB operon in cyanobacteria (Table 2) elucidated that geosmin/germacradienol might be closely related to MIB in origin and evolution in cyanobacteria. However, this presumption needs further investigation.

#### 4. Conclusions

Two water bloom-forming *A. ucrainica* strains, CHAB 1432 and 2155, were isolated and identified as geosmin producers. This cyanobacterium has a total geosmin production of over  $1.20 \times 10^{-5}$  ng·cell<sup>-1</sup> and could be a primary biological source of odor episodes in two Chinese lakes, namely Dianchi and Erhai.

The geosmin synthase gene of *A. ucrainica* is located in an operon with two cyclic *cnb* genes that were predicted members of the Crp–Fnr regulator family. This pattern was proven universal in investigated cyanobacteria for the first time.

The evolution of the geosmin synthesis gene probably involves HGT in cyanobacteria, and a close relationship to MIB genes in origin and evolution is suggested; however, this presumption needs further investigation.

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## Author Contributions

Zhongjie Wang and Renhui Li designed and carried out this experiment and prepared the manuscript. Jihai Shao, Yao Xu and Biao Yan partially contributed to the experiment and analysis of the data.

## Supplementary Materials

Supplementary materials can be accessed at: <http://www.mdpi.com/2073-4441/7/1/175/s1>.

## Conflicts of Interest

The authors declare no conflict of interest.

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