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Phylogenetic Analysis and Characterization of Odorous Compound-Producing Actinomycetes in Sediments in the Sanbe Reservoir, A Drinking Water Reservoir in Japan

Shohei Hayashi[1,2] · Shingo Masuki[2] · Koichi Furuta[3] · Shinichi Doi[3] · Sangyeob Kim[2] · Yasushi Seike[2]

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Abstract
Odor caused by the presence of geosmin and 2-methylisoborneol (2-MIB) in aquatic ecosystems leads to considerable economic loss worldwide. The odorous compounds are primarily produced by cyanobacteria and actinomycetes. While the contribution of odorous compounds-producing cyanobacteria has been thoroughly investigated, the production of geosmin and 2-MIB by actinomycetes in aquatic ecosystems is poorly understood. In this study, we isolated geosmin and/or 2-MIB-producing actinomycetes in sediments collected from the Sanbe Reservoir, Japan, identified the biosynthetic gene of geosmin and 2-MIB, and investigated the production of the odorous compounds by the isolated strains. Partial sequence of 16S rRNA and the biosynthetic genes was determined to analyze the phylogenetic relationship among the strains. The geosmin and 2-MIB concentrations in the culture of the isolated strains were measured using gas chromatography mass spectrometry. Fifty-four strains of odorous compounds-producing and non-geosmin-producing actinomycetes were isolated from sediments from the Sanbe Reservoir. Diverse actinomycetes were identified and many of them produced geosmin and/or 2-MIB. Many odorous compounds-producing actinomycetes were phylogenetically different from previously reported producing actinomycetes. The producing ability of the odorous compounds of the isolated strains in this study was not significantly related with the phylogenetic groups of 16S rRNA and the biosynthetic genes. The findings suggest that the odorous compounds-producing actinomycetes in the sediments are diverse and different from previously reported strains.

Introduction

Geosmin and 2-methylisoborneol (2-MIB) are the odorous compounds responsible for the earthy and musty smell, respectively [1, 2]. They have been detected in drinking water reservoirs and fisheries worldwide [3–8]. Because human threshold for the detection of these odorous compounds is extremely low (ng L^{-1}) [9], even low concentrations make human uncomfortable and cause economic damages. The concentration of the odorous compounds in reservoirs and fisheries are regularly monitored. When the odorous compounds are detected, water sources and fishery products should be treated appropriately to remove them. The costs incurred for the removal in treatment plants increase the maintenance expenses and the final price of drinking water and products [10].

Geosmin and 2-MIB are produced by certain species of actinomycetes, cyanobacteria, myxobacteria, and fungi [5, 7, 11, 12]. Although there are several reports on geosmin and 2-MIB production by *Streptomyces* isolated from aquatic environments [13–19], there are fewer studies on odorous compound-producing aquatic actinomycetes than on cyanobacteria [5, 20–23]. Increased awareness of odor-producing cyanobacteria in surface water may be one of the reasons why investigations on odorous compound-producing aquatic actinomycetes present in sediments are infrequent. It is reported that several actinomyces strains from aquatic actinomycetes produced geosmin and/or 2-MIB of hundreds ng L^{-1}[10, 13]. Therefore, odorous compound-producing actinomyces should be evaluated to identify the source. While Auffret et al. (2011) detected *geoA* (geosmin synthase gene)

✉ Shohei Hayashi
shohaya@life.shimane-u.ac.jp

1 Faculty of Life and Environmental Science, Shimane University, 1060 Nishikawatsu, Matsue, Shimane 690-8504, Japan

2 Estuary Research Center, Shimane University, 1060 Nishikawatsu, Matsue, Shimane 690-8504, Japan

3 Shimane Environment & Health Public Corporation, 1-4-6 Koshibara, Matsue, Shimane 690-0012, Japan

gene from actinomycetes in recirculating aquaculture systems by qPCR assay, *geoA* was not PCR-amplified in water and sediments in Canada [24] and Australia [25]. It means that *geoA* gene in actinomycetes in aquatic ecosystems are diverse and information about *geoA* sequence is not enough to design suitable primers. Comprehensive information on strain identity, the sequence of geosmin and 2-MIB synthase genes (*geoA* and *tpc*), and odorous compound production by actinomycetes from aquatic ecosystems is not sufficient to understand their phylogenetic diversity and to monitor them by genetic techniques. This information would be useful for estimating the contribution of actinomycetes to odors in aquatic ecosystems.

This study was conducted using samples from the Sanbe Reservoir in south-western Honshu, Japan (Fig. 1). In the last 15 years, geosmin and 2-MIB has been repeatedly detected in early summer (from the end of June to July) and in fall (from September to October), respectively (at Shimane prefecture kenou prefectural land maintenance office, Personal communication). Activated charcoal is used at the water purification plant for the removal of geosmin and 2-MIB. This process is costly and increases the maintenance expenses of the plant. It is reported that cyanobacteria *Dolichospermum planctonicum* and *Dolichospermum crassum* were isolated from surface water and produced geosmin. *Pseudanabaena* sp. and *Aphanizomenon* cf. *flos-aquae* were found as 2-MIB-producing species [26]. On the other hand, odorous compound-producing actinomycetes in the Sanbe Reservoir was not investigated.

This study aimed to identify geosmin and/or 2-MIB-producing actinomycetes in sediments collected from the Sanbe Reservoir, as geosmin and 2-MIB have been detected in water close to the bottom of the reservoir. We also aimed to obtain comprehensive information on geosmin and/or 2-MIB-producing actinomycetes present in aquatic ecosystems to evaluate their phylogenetic diversity. To this end, we isolated and identified geosmin and/or 2-MIB-producing actinomycetes from sediments collected from the Sanbe Reservoir. We analyzed the geosmin and 2-MIB biosynthetic gene sequences, and measured geosmin and 2-MIB production of the isolated strains. The diversity of the isolated strains and the relationship between geosmin production and the phylogenetic grouping of the strains were discussed.

Methods

Sampling Water and Sediments

This study was conducted in the Sanbe Reservoir, in south-western Honshu, Japan (35°10′11.5″N; 132°33′45.2″E) (Fig. 1). Water was collected from depths of 0.5, 3, 5, 7, 9, 11, 13, 22, 24, and 26 m at St. 1 using a water sampler (Kitahara 2-L type, Rigo Co., Ltd., Tokyo, Japan) on June 22 and September 1, 2017 (Fig. 1). The sediments from four sampling sites (St. 1, St. 2, St. 3, and St. 4) were collected using a grab type sediment sampler (Ekman-Brige bottom sampler Rigo Co., Ltd.) on June 22 and September 1, 2017. A 1 cm-thick layer was sliced and collected from the sediment surface. The water and sediments were transported to the laboratory in a cooler.

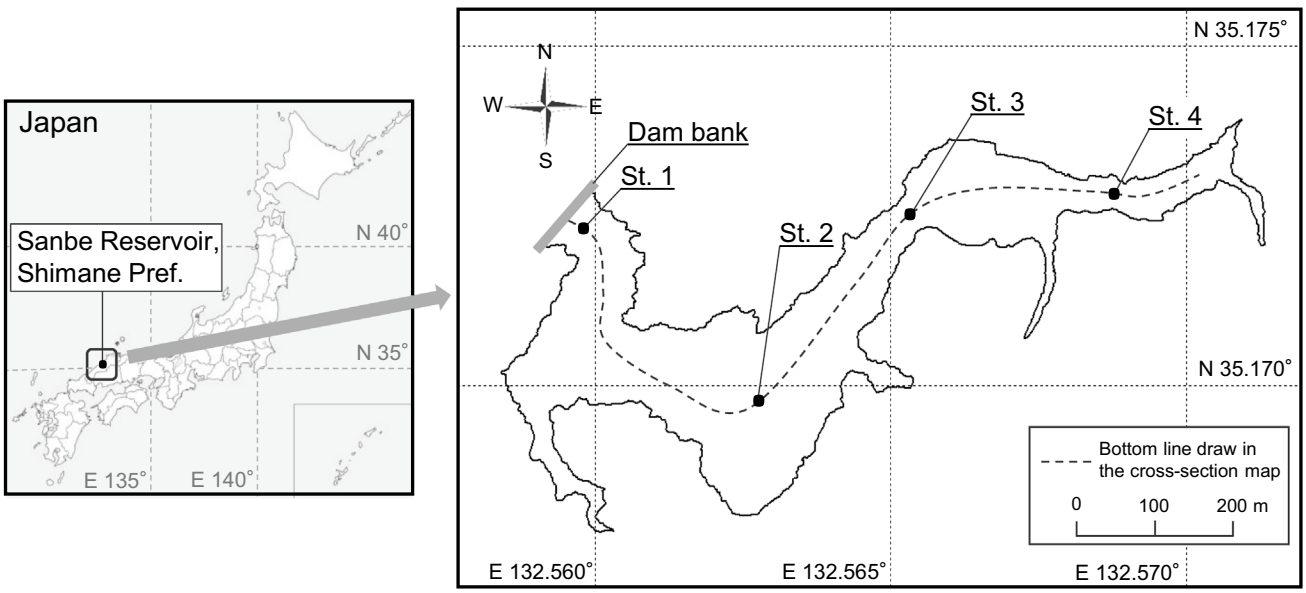


Fig. 1 Planimetric map of the sampling sites at the Sanbe Reservoir, Japan

Measurement of Geosmin and 2-MIB Concentrations in Water

The geosmin and 2-MIB concentrations in the water were measured using a gas chromatograph (GC-7890, column VF-5 ms, Agilent, CA, USA) equipped with a quadrupole mass spectrometer (5977B, Agilent) and a purge and trap autosampler (Atomx, Teledyne Tekmar, OH, USA), as described in the Ordinance of Ministry of Health, Labour and Welfare No. 261, 2003. The purge and trap conditions were as follows: purging, 60 °C for 15 min under 100 mL min^{-1}; desorption, 200 °C for 3 min under 300 mL min^{-1}. The column and oven temperature program was as follows: 40 °C hold for 5 min, increased to180 °C at 8 °C min^{-1}, increased to 255 °C at 15 °C min^{-1} and hold for 10 min. MS detector was 260 °C at 1 mL min^{-1} of helium gas. Each water sample (20 mL) was analyzed once. Gas chromatography mass spectrometry (GC/MS) analysis was performed in the ion monitoring mode (m/z 112 and 95 for geosmin and 2-MIB, respectively). As an internal standard substance, 2,4,6-Trichloroanisole-d_3 (m/z 213) was spiked in each sample (20 ng L^{-1}). The detection limit of geosmin and 2-MIB was 1 ng L^{-1}.

Isolation and Genetic Analysis of Actinomycetes

Following the method of Sugihara et al. [27], the sediments (1 g of each) collected on June 22 were inoculated into 9 mL of actinomycete medium (10 g L^{-1} soluble starch[1], 2 g L^{-1} KNO_3, 0.5 g L^{-1} KH_2PO_4, 0.2 g L^{-1} $MgSO_4$, 0.1 g L^{-1} $FeSO_4$) [27] in a test tube (20 cm × 2.5 cm dia.) and incubated at 26 °C for 1 week in a static culture. The culture fluids were inoculated on actinomycete agar medium (actinomycete medium and 15 g L^{-1} agar) and incubated at 26 °C for 1 week. The culture fluids were inoculated on Humic acid-vitamin (HV) agar medium (1.71 g L^{-1} KCl, 0.5 g L^{-1} Na_2HPO_4, 0.05 g L^{-1} $MgSO_4 \cdot 7H_2O$, 0.02 g L^{-1} $CaCO_3$, 0.01 g L^{-1} $FeSO_4 \cdot 7H_2O$, 1 g L^{-1} nitrohumic acid, 18 g L^{-1} agar, 5 ml L^{-1} vitamin drink [ALINAMIN V, Takeda Consumer Healthcare Co., Ltd.], 0.01 g L^{-1} nalidixic acid, 0.05 g L^{-1} cycloheximide, pH 7.2, prepared using tap water) [28]. HV agar medium is selective medium for actinomycetes. Nitrohumic acid is one of humic acid which is mainly degraded by actinomycetes. Sediments in the Sanbe Reservoir contain humic acid originated from plants and soils. The sediments collected on September 1 were diluted 1,000- and 10,000-fold using sterilized water, and the suspensions were inoculated on HV agar medium. After incubation at 26 °C for 1 week, the actinomycete colonies were isolated.

The 16S rRNA gene sequences of the isolated actinomycetes were PCR-amplified using KOD plus polymerase (Toyobo Co., Ltd., Osaka, Japan) with the primers fD1 (5′-AGAGTTTGATCCTGGCTCAG-3′) and rP2 (5′-ACGGCTACCTTGTTACGACTT-3′) [29], as described by Godo et al. (2017). The PCR amplification mixture was prepared according to the manufacturer's instructions. A part of the colony of an isolated strain was added to the mixture as a template. The PCR cycle comprised a pre-run at 94 °C for 2 min, 30 cycles of denaturation at 94 °C for 15 s, annealing at 50 °C for 30 s, and extension at 68 °C for 2.5 min, followed by a final extension at 68 °C for 10 min. The nucleotide sequencing of amplified fragments of most strains was performed at Eurofins Genomics K.K., and that of the remaining strains was performed using a BigDye Terminator Cycle Sequencing Ready Reaction Kit and an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems, CA, USA). The primer fD1 was used to determine the nucleotide sequence of all strains. The primers F338 (5′-ACTCCTACGGGAGGCAGCAG-3′) and 518r (5′-ATTACCGCGGCTGCTGG-3′) [30] were used to determine the nucleotide sequence of some of the strains because the length of the sequence determined using fD1 was short. The nucleotide sequences were compared with those reported in the National Center for Biotechnology Information (NCBI) using the BLAST algorithm (https://www.ncbi.nlm.nih.gov/).

The fragment of *geoA* (geosmin synthase gene) in the isolated actinomycetes strains was PCR-amplified using KOD FX Neo polymerase (Toyobo Co., Ltd., Osaka, Japan) with the primers 248F (5′-TCTTCTTCGACGACCACTTCC-3′) and 1832R (5′-CCCTCGTACTCGATCTCCTTCTT-3′) [31]. The PCR mixture prepared according to the manufacturer's instructions. The PCR comprised a pre-run at 94 °C for 2 min, 30 cycles of denaturation at 98 °C for 10 s, annealing at 63 °C for 30 s, and extension at 68 °C for 1 min, followed by a final extension at 68 °C for 5 min. The PCR amplification of the fragment of *tpc* (2-MIB synthase gene) was conducted with the primers St-mib-F1 (5′-TSGACRRCTGCTACTGCGAGG-3′) and St-mib-R3 (5′-TTCCTTSGTGWASGAGTASAGGTCG-3′). The PCR comprised a pre-run at 94 °C for 2 min, 30 cycles of denaturation at 98 °C for 10 s, annealing at 60 °C for 30 s, and extension at 68 °C for 10 s. The nucleotide sequence was determined as described above using the primers 248F and St-mib-F1 for *geoA* and *tpc*, respectively. A phylogenetic tree was constructed based on the partial sequences of the 16S rRNA gene (733 bp), *geoA* (555 bp), and *tpc* (360 bp) using the neighbor-joining method in the software program MEGA7 [32].

The nucleotide sequences determined in this study have been submitted to the DNA Data Bank of Japan, European Nucleotide Archive, and GenBank databases under the accession numbers LC510398-LC510451, LC511813-LC511858 and LC719157-LC719187 (Table S1).

Geosmin and 2-MIB Productions by Actinomycetes

The isolated actinomycete strains were cultured for a week in 10 mL of HV liquid medium at 26 °C with shaking at 120 rpm. HV liquid media were used for the production of geosmin and 2-MIB because the actinomycete strains were isolated on HV agar medium and containing nitrohumic acid make it similar to sediment conditions. The geosmin and 2-MIB concentrations in the culture fluid were measured using GC/MS analysis, as described above. The culture fluids were diluted 10- or 100-fold using distilled water before analysis because the culture fluids contained high concentration of medium components. Even if geosmin and 2-MIB in the culture fluid was not detected, it is possible that the strain produces them at lower concentration (< 10 ng L^{-1}).

Results

Vertical Distribution of Geosmin and 2-MIB in the Sanbe Reservoir

Geosmin was detected in the water collected from St. 1 on June 22 and September 1 (Fig. 2a). The highest concentration was 133 ng L^{-1} at a depth of 0.5 m on June 22. The concentration on June 22 decreased with depth till 13 m (Fig. 2a), whereas it increased close to the bottom of the reservoir and reached 7 ng L^{-1} at a depth of 26 m. On September 1, the geosmin concentration between depths of 0.5 and 13 m was < 2 ng L^{-1}, whereas it was 15 ng L^{-1} in water close to the bottom. The geosmin concentrations in the water intake at the water purification plant on June 13 was 18 ng L^{-1}.

2-MIB was detected on September 1 (Fig. 2b). The highest concentration was 645 ng L^{-1} at a depth of 3 m. The concentration decreased with depth till 13 m, whereas it increased close to the bottom of the reservoir and reached 13 ng L^{-1} at a depth of 26 m. The 2-MIB concentrations in the water intake on October 3 was 449 ng L^{-1}. Geosmin and 2-MIB were not detected in the inflow water in the eastern side of the reservoir.

Isolation and Genetic Identification of the Actinomycetes from the Sanbe Reservoir

HV agar medium was used for the isolation of actinomycetes because no actinomycete colony was formed on the actinomycete agar medium owing to the growth of other bacterial and fungal colonies. A total of 23 and 31 strains of actinomycetes were isolated from the sediments collected on June 22 and September 1, respectively (Fig. 3; Table S1). 16S rRNA sequences of 750 to 1197 bp were determined in this study. Based on the analysis of the partial sequences

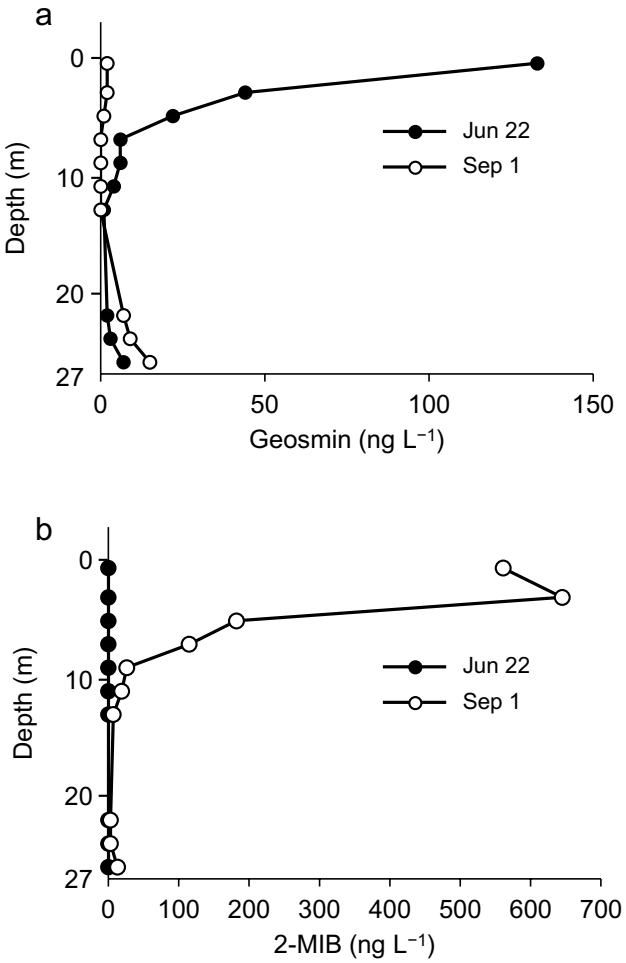


Fig. 2 Geosmin (**a**) and 2-MIB (**b**) concentrations at the sampling sites St. 1 in the Sanbe Reservoir on June 22 and September 1, 2017

of 16S rRNA, 49 of the 54 isolated strains belonged to the genus *Streptomyces* (Fig. 3a; Table S1). The strains in genus *Streptomyces* were diverse at the species level. Twenty-eight different species in genus *Streptomyces* were identified as closely related species of the isolated strains. Three and two strains belonged to the genera *Kitasatospora* and *Nocardia*, respectively. Three strains of *Kitasatospora* were different at the species level. Two strains of *Nocardia* were different at the species level. The identity of the strains to the most closely related strain was more than 99% (Table S1). There were numerous branches in the phylogenetic tree, including some representing isolated strains (Fig. 3a).

Genetic Analysis of Geosmin and 2-MIB Synthase in Isolated Actinomycetes

Fragments *of geoA* of 46 of the 54 isolated strains were PCR-amplified and *geoA* sequences of 571 to 901 bp were determined. Most *geoA* sequences from the isolated strains

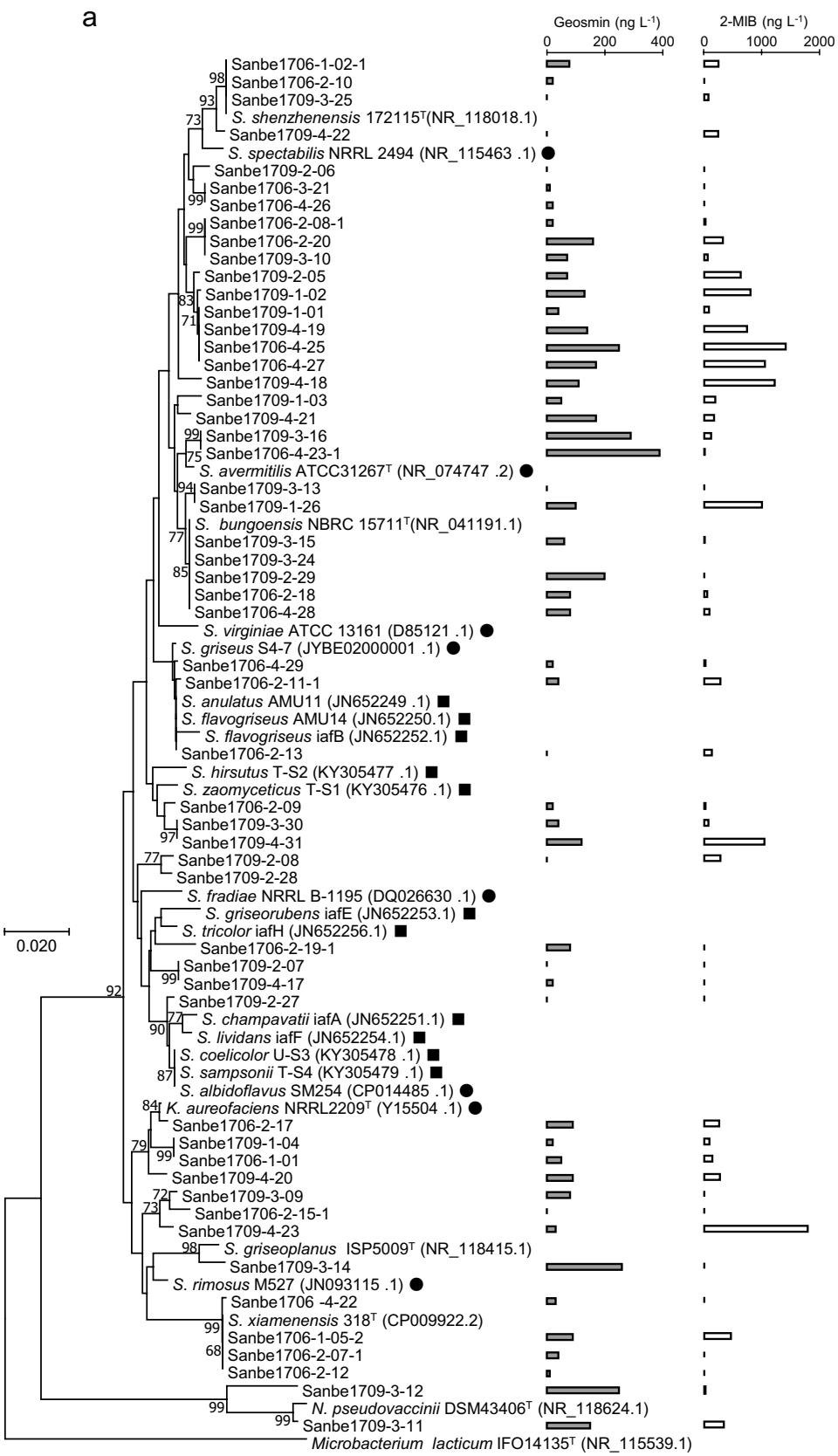
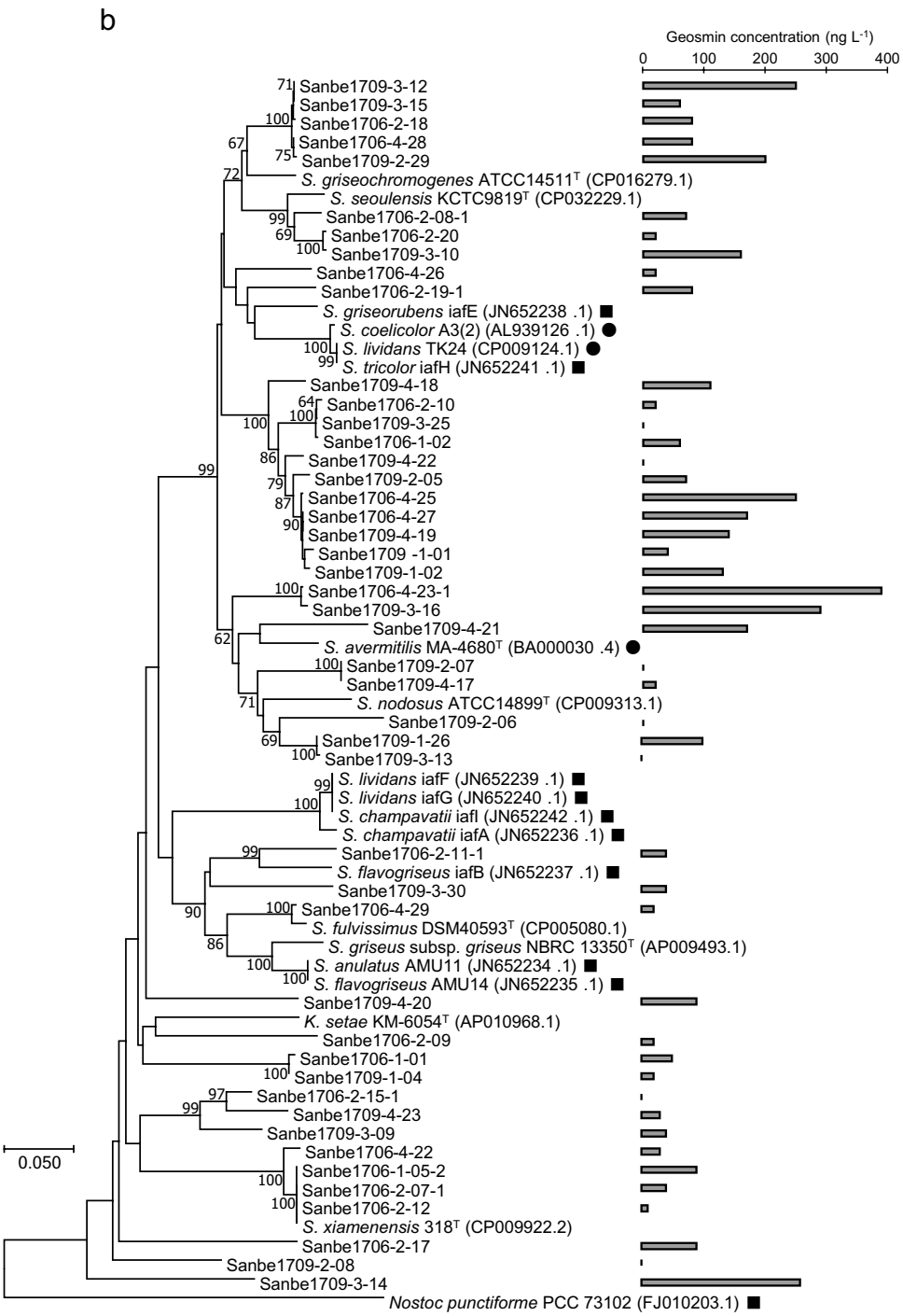


Fig. 3 Phylogenetic trees based on the partial 16S rRNA gene (**a**), *geoA* (**b**), and *tpc* (**c**) sequences of actinomycete isolates and related strains. The phylogenetic trees were constructed using the neighbor-joining method with 1000 bootstrap replicates in the software program MEGA7. Bootstrap values above 60% are shown at the nodes. The sequences of the isolated strains are boldfaced. The accession numbers are indicated to the right of the strain name. The black squares and circles indicate previously reported geosmin and 2-MIB-producing strains from aquatic ecosystems and other environments, respectively. The scale bar indicates the substitutions per site. *Microbacterium lacticum* IFO14135 (NR_115539.1), *Nostoc punctiforme* PCC 73102 (FJ010203.1), and *Planktothricoides raciborskii* CHAB3331 (HQ830029) constituted an outgroup. The gray and white bars to the right of the strain name indicate the geosmin and 2-MIB concentration in the culture, respectively

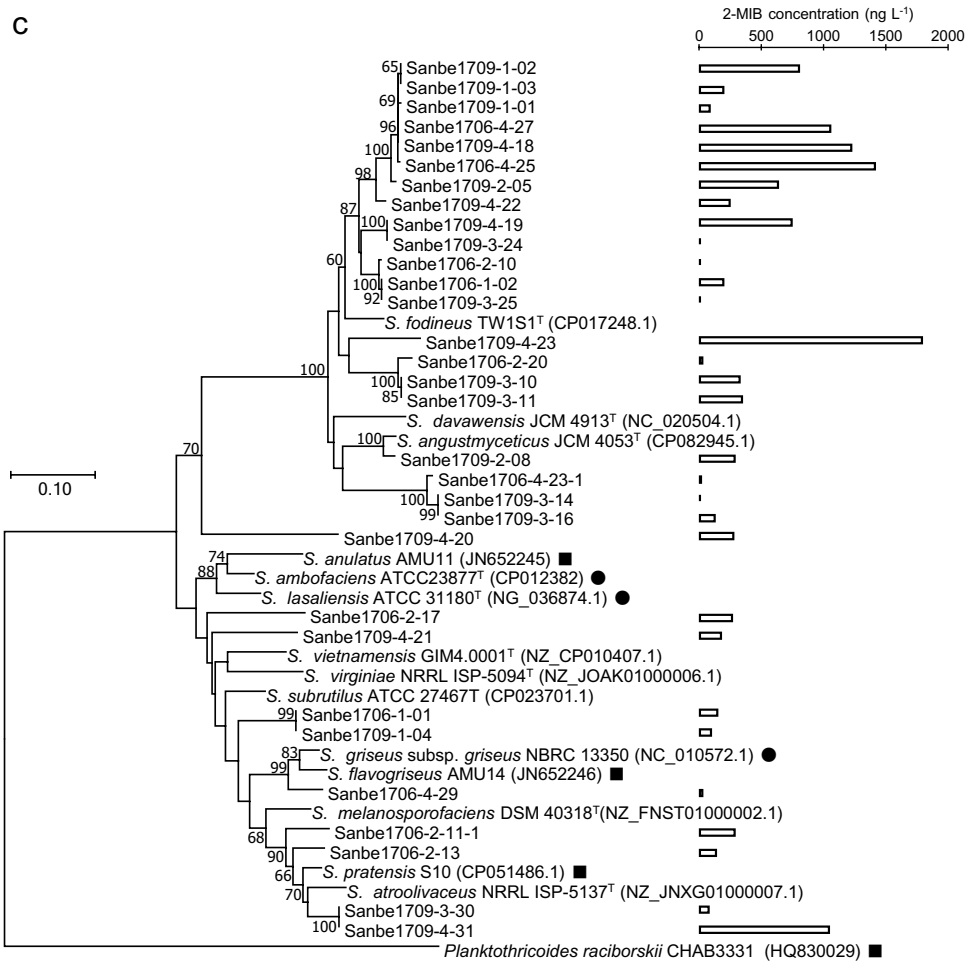
Fig. 3 (continued)



were related to the terpene cyclase gene sequence of *Streptomyces* (Fig. 3b; Table S1). The similarities of the *geoA* sequences with the related genes ranged from 80 to 95%, except for *geoA* sequences from five strains (Sanbe1706-1-05-2, Sanbe1706-2-07-1, Sanbe1706-2-12, Sanbe1706-4-22, and Sanbe1706-4-29). In the phylogenetic tree, the *geoA* sequences from the isolated strains formed several branches. Additionally, the *geoA* sequences of most isolated strains did not group with the *geoA* sequence reported in the database.

The deduced amino acid sequence (193–300 aa) shared 85% to 100% similarity with the sequences of germacradienol/geosmin synthase and terpene synthase family proteins from the genera *Streptomyces* and *Kitasatospora* (Table S1). Based on a BLAST search of the NCBI conserved domain database, most of the deduced amino acid sequences of *geoA* were part of the N-terminal regions of the terpene cyclase sequence. The N-terminal region contained a conserved (N/D)DXX(S/T)XX(K/R)(D/E) motif, which is a Mg^{2+}-binding motif [33]

Fig. 3 (continued)



(Fig. S1). The deduced amino acid sequence of *geoA* from almost all strains had a conserved NDLFSYQRE motif, which is identical to the geosmin synthase *Streptomyces coelicolor* A3(2).

Fragments of *tpc* of the 31 strains were PCR-amplified and *tpc* sequences of 368 to 381 bp were determined. Most *tpc* sequences from the isolated strains were related to 2-MIB synthase gene of *Streptomyces* (Fig. 3c; Table S1). The similarities of the *tpc* sequences with the related genes ranged from 77 to 94%, except for eight strains (Sanbe1706-2-13, Sanbe1706-4-23-1, Sanbe1706-4-29, Sanbe1709-2-08, Sanbe1709-3-10, Sanbe1709-3-11, Sanbe1709-3-14, and Sanbe1709-3-16). In the phylogenetic tree, the *tpc* sequences from the isolated strains were separated by several branches. The *tpc* sequences of most isolated strains and the *tpc* sequence reported in the database formed separate branches.

Geosmin and 2-MIB Production by Isolated Actinomycetes

Geosmin was detected in the culture of 43 of the 52 isolated strains. The concentration of geosmin in the culture differed widely depending on the strains (Fig. 3 and S1; Table S1). The highest and the second highest geosmin concentrations were 390 ng L^{-1} in Sanbe1706-4-23-1 and 290 ng L^{-1} in Sanbe1709-3-16, respectively. Their most closely related species was *Streptomyces olivochronogenes* (Fig. 3a; Table S1), and their *geoA* sequences were located on the same branch in the phylogenetic tree (Fig. 3b).

2-MIB was produced by 36 of the 52 isolated strains. The concentration of 2-MIB in in the culture differed widely depending on the strains (Fig. 3 and S1; Table S1). The highest 2-MIB concentration was 1790 ng L^{-1} in Sanbe1709-4-23. The 2-MIB production in the isolated strains was not related with the geosmin production (Fig. 4).

Discussion

Diverse geosmin- and 2-MIB-producing actinomycetes were isolated and identified from the sediments collected from the Sanbe Reservoir. HV medium was suitable for the isolation and identification of actinomycete strains in this study. Determination of more than 750 bp of 16S rRNA sequences

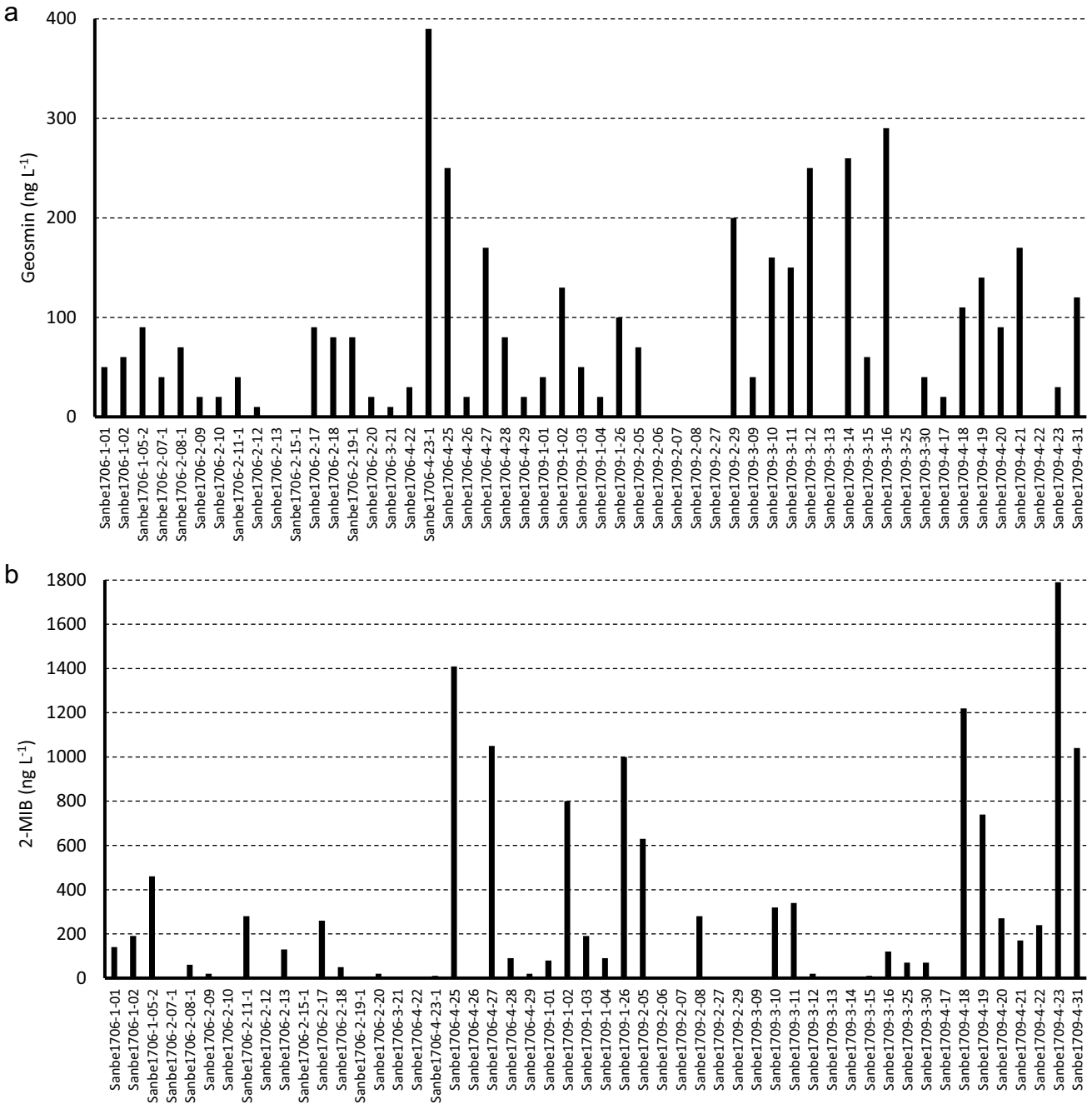


Fig. 4 Geosmin (**a**) and 2-MIB (**b**) concentrations in the culture of the actinomycete isolates

was enough for phylogenetical identification and comparison with previously reported strains. Most odorous compounds-producing *Streptomyces* species reported in previous reports [8, 13, 15, 17–19, 31, 34–36] were not identified in this study (Fig. 3a). Sanbe1706-2-13, which was closely related to the geosmin- and 2-MIB-producing *S. flacogriseus* AMU14 and *S. anulatus* AMU11 from recirculating aquaculture systems [34], produced 2-MIB but not geosmin. Although four species (*S. chromofuscus*, *S. glauciniger*, *S. griseoplanus*, and *S. olivochromogenes*) were reported to be closely related to strains isolated from Paldang Lake, Korea [19], most of the species isolated from Paldang Lake were not identified in our study. The geosmin and 2-MIB-producing ability of several species in genus *Streptomyces* was reported for the first time.

In this study, a new group of odorous compounds-producing *Kitasatospora* and *Nocardia* strains was isolated and identified. Geosmin-producing *K. setae* was isolated from

soil samples in Japan [37, 38]. Geosmin- and 2-MIB-producing *Nocardia cummidelens* and *Nocardia fluminea* were isolated from biosolids obtained from a recirculating aquaculture system [35, 39]. The strains isolated in this study were not closely related to *K. setae*, *N. cummidelens*, or *N. fluminea*. The geosmin and 2-MIB producing ability of three species in genus *Kitasatospora* and two species in genus *Nocardia* was shown for the first time. Although *Kitasatospora* and *Nocardia* strains were not a dominant group in the Sanbe Reservoir, the finding indicates that the genera *Kitasatospora* and *Nocardia* are also geosmin and 2-MIB sources in aquatic ecosystems. These two genera should be further studied as geosmin- and 2-MIB-producing actinomycetes. Based on these results, it is concluded that various kinds of odorous compounds-producing actinomycetes are present in the sediments in the Sanbe Reservoir. It indicates that a wide range of species needs to be considered to estimate the effect of actinomycetes on the concentration of odorous compounds. Our findings provide new information on odorous compound-producing actinomycetes in aquatic ecosystems.

The geosmin and 2-MIB concentrations in the cultures differed depending on the strains in this study, even when the strains belonged to a same branch in the phylogenic tree of 16S rRNA, *geoA* and *tpc* (Fig. 3a–c). Although the phylogenetic trees were constructed based on the partial sequences of 16S rRNA, *geoA*, and *tpc*, the trees showed phylogenetic relationships and differences between the isolated strains and previously reported strains. Especially, in the phylogenetic trees of *geoA* and *tpc* (Fig. 3b, c), it was shown phylogenetic differences between the isolated strains and odorous compounds-producing-actinomycetes in the database. Many isolated strains produced geosmin and/or 2-MIB in HV liquid medium. The productivity of the odorous compounds in HV medium is one of the characteristics of the isolated strains. Although the producing ability of the isolated strains was evaluated under one condition, the producing ability is not significantly related to phylogenetic grouping based on the 16S rRNA gene and biosynthetic gene sequences. Several *Streptomyces* species are reportedly able to produce geosmin and 2-MIB, and the producing ability differed even among taxa from a phylogenetically related lineage [13, 15, 16]. In the report by Anuar et al. [13], three isolated strains which were similar with *S. coelicolor* DSM 40233 had the different productivity of geosmin. Similarly, it was reported that the difference in the production of geosmin and 2-MIB of three phylogenetically related strains [15]. Therefore, geosmin and 2-MIB productivity of actinomycetes present in aquatic ecosystems considerably differ despite similarities in phylogenetic group and biosynthetic gene sequences. Additionally, several strains which have the biosynthetic genes did not produce geosmin and 2-MIB. It was reported that *S. flavogriseus* iafB and *S. tricolor* iafH did not produce 2-MIB despite they had 2-MIB synthase gene [34]. It indicates that the detection of certain group of actinomycetes and specific lineage of biosynthetic gene would underestimate the whole of odorous compounds-producing actinomycetes and would not predict the outbreak of odor in aquatic ecosystems.

Diverse geosmin and 2-MIB synthase genes (*geoA* and *tpc*) were identified from the isolated strains. It is found that no specific phylogenic group of biosynthetic gene dominated in the Sanbe Reservoir. While the similarities of the 16S rRNA gene sequences from the isolated strains with sequences from the database were more than 99%, almost all *geoA* and *tpc* sequences from the isolated strains showed low similarity to the related genes from the database (Table S1). Additionally, *geoA* and *tpc* from the isolated strains formed different branches from previously reported *geoA* and *tpc* genes, respectively, identified from terrestrial actinomycetes (Fig. 3b, c). Therefore, the biosynthetic genes of the odorous compounds in the isolated actinomycetes are different from terrestrial Streptomyces. Although the phylogenic trees were constructed based on the partial sequences of *geoA* and *tpc*, they showed phylogenetic differences between the isolated strains and terrestrial Streptomyces. Geosmin is a sesquiterpene and geosmin biosynthetic pathway in *S. coelicolor* A3(2) and *S. avermitilis* has been thoroughly studied [40–43]. All deduced amino acid sequences of *geoA* determined in this study had the conserved motif (Fig. S1), indicating that the *geoA* gene in the isolated strains was responsible for the geosmin production by these strains and that the isolated strains which amplified a *geoA* fragment harbor similar geosmin synthase with *S. coelicolor* A3(2). Therefore, the biosynthetic pathway of geosmin in actinomycetes in sediments is the same as that of *S. coelicolor* A3(2), although the phylogenetic group of *geoA* is different from terrestrial.

The 16S rRNA gene and *geoA* sequences of some strains (Sanbe1706-1-05-2, Sanbe1706-2-07-1, Sanbe1706-2-12, and Sanbe1706-4-22) completely matched those of *Streptomyces xiamenensis* 318 (=DSM 41903^T) and showed a high similarity (99%) with the germacradienol/germacrene D synthase gene sequence of *S. xiamenensis* 318. As *S. xiamenensis* 318 was isolated from a mangrove sediment in Fujian Province, China [44], this group may be a common aquatic *Streptomyces* strain that harbors *geoA*. The *tpc* sequences of the isolated strains was not similar with that of 2-MIB-producing *S. flacogriseus* AMU14 and *S. anulatus* AMU11 [34]. These results indicate that the *geoA* and *tpc* sequences of actinomycetes from aquatic environments are diverse and that *geoA* and *tpc* sequences in the database might not be enough to design a primer to estimate actinomycetes in aquatic environments. In fact, *geoA* and *tpc* genes from several geosmin or 2-MIB producing strains were not amplified by the primers used in this study.

Geosmin and 2-MIB was detected close to the bottom of the Sanbe Reservoir (Fig. 2). While the results of our previous research indicated that geosmin and 2-MIB were originated from cyanobacteria[26], the geosmin- and/or 2-MIB-producing actinomycetes were isolated from the sediments in this study. It is suggested that geosmin detected close to the bottom may have been released from actinomycetes present in the sediment. This is supported by a previous report showing geosmin in sediments could be released into overlying water [45].

In conclusion, this study provides comprehensive information on strain identification, geosmin and 2-MIB synthase gene sequence similarities, and the production of odorous compounds for multiple actinomycetes isolated from the Sanbe Reservoir.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00284-022-03052-8.

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Author Contributions SH, SM, SK and YS designed the project and wrote the manuscript. SH designed and performed the isolation and genetic analysis. SH and SM collected water and sediment samples. KF and DS contributed to the analysis of geosmin and 2-MIB. All authors reviewed and approved the final manuscript.

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Declarations

Conflict of interest The authors declare that they have no competing interests.

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