

Isolation, identification and odour-producing abilities of geosmin/2-MIB in actinomycetes from sediments in Lake Lotus, China

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ABSTRACT

This study aimed to determine whether actinomycetes could contribute to the odours occurring in Lake Lotus, China. In total, 55 actinomycete strains were isolated from sediments in Lake Lotus and their odorous metabolites, geosmin and 2-methylisoborneol (2-MIB), were identified using HSPME-GC-MS. Results showed that 24, 23 and 34 strains produced geosmin and/or 2-MIB in Gause, TSB and M liquid media, respectively. Of odour-producing actinomycetes, most could produce geosmin and some produced both metabolites, while few of them produced only 2-MIB. Six isolates with high-level odour were selected for further investigation. Their biomass and odour-producing abilities were monitored in both the slants and liquid media. The results suggest that TSB was the most suitable medium for the growth of mycelium; Gause and M slant supported good production of spores, while M liquid medium was the most favourable for the production of both geosmin and 2-MIB. Those strains that produced geosmin only were less influenced than those that produced both geosmin and 2-MIB under shaking conditions. The results indicate that actinomycetes from sediments should be taken into consideration when off-flavours occur in water columns. According to the 16S rRNA sequences, six actinomycetes were classified in the *Streptomyces*.

Key words | actinomycete, geosmin, off-flavour, 2-methylisoborneol

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INTRODUCTION

Geosmin and 2-MIB (2-methylisoborneol) are receiving widespread attention as they can impart earthy-musty tastes and odours to water and aquatic products. They can compromise the quality of drinking water, render fish unmarketable and also reduce scenic value (Jüttner & Watson 2007; Guttman & van Rijn 2008). They are tertiary alcohols produced as odorous secondary metabolites by microorganisms, including several genera of cyanobacteria, fungi, myxobacteria and various actinomycetes (Gerber & Lechevalier 1965; Sugiura *et al.* 1998; Sugiura & Nakano 2000; Dickschat *et al.* 2004, 2005; La Guerche *et al.* 2005). Both odorous compounds present exceptionally low threshold concentrations for human detection at ng l^{-1}

levels (Young *et al.* 1996; Watson *et al.* 2000) and cannot be efficiently removed by conventional water treatments, such as ozone, chlorine and activated carbon adsorption (Saito *et al.* 1999; Lawton *et al.* 2003; Ho *et al.* 2007; Song & O'Shea 2007). Moreover, these treatment processes greatly increase the cost of water treatment. In order to decrease the occurrence of geosmin and 2-MIB, it is desirable to better understand their sources. Cyanobacteria and actinomycetes have long been known to be associated with geosmin and 2-MIB occurrence in water (Henatsch & Jüttner 1986; Jensen *et al.* 1994; Lanciotti *et al.* 2003; Westerhoff *et al.* 2005), while the exact contribution of actinomycetes to odours in fresh water still lacks sufficient direct evidence.

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In fact, early evidence indicated that the two compounds originated from actinomycetes (Gerber & Lechevalier 1965; Gerber 1979). Actinomycetes are very common Gram-positive filamentous bacteria and are the major producers of the characteristic odorous compounds geosmin and 2-MIB in terrestrial soil environments. Most actinomycetes are able to produce spores, which can survive under extreme conditions and are distributed widely by wind and water flow (Goodfellow & Williams 1983). Reports have shown that episodes of high terrestrial runoff may introduce actinomycetes and/or their secondary metabolites into surface waters, resulting in odours (Zaitlin *et al.* 2003). Actinomycetes, through waterborne spores, can germinate and favour the production of unpleasant odorous compounds under appropriate environmental conditions. Actinomycetes are also detected in many aquatic systems, such as aquatic plants (Zaitlin *et al.* 2003) and drinking water pipeline deposits (Zacheus *et al.* 2001). However, it is unclear how long the actinomycetes can keep metabolically active and produce odorous compounds in the water column. Furthermore, there is still debate over whether these odorous compounds are produced in terrestrial soils and transported into the water or if actinomycetes produce the musty odours in water environments. In addition to those sources of geosmin and 2-MIB, actinomycetes have also been found to be capable of producing musty odours in sediments (Johnston & Cross 1976; Schrader & Blevins 1993). However, systematic investigations on the abundance and taxonomy of actinomycetes that are responsible for geosmin and 2-MIB production in sediments are still lacking.

Lake Lotus, located in the Park Lianhuahu, Wuhan, mainly serves as a public recreation landscape, with an area of about 64,000 m². In recent years, great damage to the scenic value and economic revenue of Lianhuahu Park has been caused by the emission of strong odours from the lake, where *Anabaena* has been documented to be closely correlated with concentrations of 2-MIB (Li *et al.* 2007a). However, whether actinomycetes in this lake contribute to its taste and odour problems as well is unknown. Therefore, to provide some evidence on actinomycetes' contribution to odour occurrence, the present study was designed to isolate and identify the potential odour-producing actinomycetes from the sediments in Lake Lotus.

MATERIALS AND METHODS

Isolation of odour-producing actinomycetes from sediments in Lake Lotus

Ten grams of sediment collected from Lake Lotus in March 2006 was mixed with 90 ml sterile water containing 0.05 g phenol to reduce bacteria growth, and appropriate serial dilutions of suspensions were spread onto agar plates of Gause medium in triplicate (Zhao & He 2002). Inoculated plates were incubated at 28°C for 7–10 days until the colonies were visible, and actinomycete colonies were identified on the basis of their particular morphological characteristics. Colonies of actinomycetes were picked up and further purified on Gause plates several times. All isolates were prepared and grown in Gause, TSB (Trypticase Soy Broth) (Klausen *et al.* 2005) and M (Aoyama 1990) media for the accumulation of geosmin and 2-MIB and analysed by HSPME-GC-MS as follows.

Determination of geosmin and 2-MIB

Geosmin and 2-MIB from the actinomycetic culture medium were extracted by headspace solid phase micro-extraction (HSPME). Fibre assemblies (65 mm polydimethylsiloxane-divinylbenzene, Supelco 57310-U), a manual holder (Supelco 57330-U) and screw-capped vials (125 ml, with a PTFE-faced silicone rubber septum) for HSPME were used. Then, the fibre was retracted, placed in the injector of a gas chromatograph (Hewlett-Packard 6890 plus) equipped with a mass selective detector (Hewlett-Packard Model 5973) and desorbed in splitless mode for 2 min at 250°C. The capillary column (HP-5 MS, 30 m × 0.25 mm i.d. × 0.25 mm film thickness) was held at 60°C for 2 min, then programmed at 5°C/min to 200°C with 2 min hold, and finally at 20°C/min to 250°C with 5 min hold. The helium carrier gas was operated at a constant pressure of 120 kPa. The details of the method were described by Li *et al.* (2005, 2007b).

Production of geosmin and 2-MIB by actinomycete isolates

Based on the survey of actinomycete strains isolated from Lake Lotus, several isolates with detectable odours were selected for further investigation. The relationship between

the production of geosmin/2-MIB and biomass was investigated and compared on Gause, TSA (TSB with agar), M slant and liquid media, respectively. Seed cultures were incubated in Gause liquid medium for 18–24 h at 28°C and 200 rpm. Three kinds of slant agar medium were prepared in test tubes, which were inoculated with actinomycete seed cultures and incubated at 28°C. After 7 days, spores and mycelium on the slants were harvested and scraped into 9 ml sterile water as actinomycete culture suspensions for the determination of the odorous compounds. Appropriate dilutions of culture suspensions were maintained on the Gause plates to determine actinomycete density, expressed as cfu ml⁻¹. At the same time, 30 ml of three kinds of liquid medium were placed in 50-ml conical flasks and inoculated with seed cultures of actinomycete isolates. After cultivation for 7 days at 28°C and 200 rpm, geosmin and 2-MIB were determined by HSPME-GC-MS. In addition, 10–20 ml of liquid cultures, which were taken for dry cell weight measurement, were filtered through a dried membrane filter and washed with 10 ml of ultrapure water. The filter was dried to constant weight at ~110°C and weighed at room temperature. The dry biomass weight was obtained as the difference between the before and after dry weight measurements.

Identification of isolated actinomycetes

Several actinomycete colonies selected on the basis of their odour production ability in liquid cultures were cultivated in 20 ml Gause liquid medium at 200 rpm and 28°C for 7 days. Spores and mycelium were then collected by centrifugation at 5,000 rpm for 20 min and stored in a freezer until used. By adding ultrasonic waves to disrupt cells, DNA was extracted from cells according to *Microbiology Experiment* (Zhao & He 2002). Identification of selected strains was performed by 16S rRNA sequence analysis with the degenerated primers 8-27F (5'-AGAGTTT-GATCCTGGCTCAG) and 1523-1504R (5'-AAGGAGGT-GATCCAGCCGCA-3') with a polymerase chain reaction (PCR). The volume of the reaction mixtures was 50 µl, containing 2 µl of DNA, 0.2 µM of each primer, 2 µM of dNTP, 2.5 U of Taq polymerase, 25 mM MgCl₂ and 10 × reaction buffer. PCR amplification was carried out under the following conditions: 10 min at 94°C followed by

35 cycles of 30 s at 94°C, 30 s at 56°C and 45 s at 72°C, with a final step at 72°C for 5 min. The PCR products were electrophoretically separated and visualized in 1% agarose gels stained with ethidium bromide using a 250 bp ladder. PCR products were then pooled from two reactions and purified with the PCR products reclamation kit (Generay Biotech Shanghai Co., Ltd) according to the manufacturer's introductions. Pooled amplification products were then sequenced. Similar sequence searches were conducted using the National Center for Biotechnology Information BLAST network service (blastn) at www.ncbi.nlm.nih.gov. The closest related sequences from previous cultured bacteria were downloaded from Genbank. A multiple sequence alignment of those sequences together with sequences of the six actinomycete isolates was executed using the program CLUSTALW, where all positions with gaps were removed. The alignment data were then used for neighbour-joining analysis to obtain phylogenetic trees with 1,000 replicates (MEGA version 3.1).

RESULTS

Isolation and investigation of odorous compounds produced by actinomycetes from sediments in Lake Lotus

Fifty-five actinomycete isolates were obtained from sediments in Lake Lotus and cultivated in Gause, M and TSB liquid media for the accumulation of odorous compounds and analysed by HSPME-GC-MS. Thirty-four (62%) of the isolated strains produced geosmin or 2-MIB or both in M liquid cultures, while, for the Gause and TSB liquid cultures, only 24 (44%) and 23 (42%) of the isolated strains produced odorous compounds, respectively (Table 1). Of the odour-producing isolates, 11 (20%) and 5 (9%) isolates produced 2-MIB and 21 (38%) and 23 (42%) isolates produced geosmin in Gause and TSB media, respectively (Table 1). The above results indicate that 2-MIB production was much higher in Gause medium than in TSB. Compared with those two media, 14 (25%) strains produced 2-MIB and 33 (60%) strains produced geosmin in M medium. The data suggest that M medium was the most favourable for the production of both geosmin and 2-MIB.

Table 1 | Production of odorous compounds by isolated actinomycetes from sediments in Lake Lotus in liquid media

Odour production characteristics	Number of isolates (percentage of total isolates)		
	Gause	TSB	M
MIB only	3 (5)	0 (0)	1 (2)
Geosmin only	13 (24)	18 (33)	20 (36)
Both MIB and geosmin	8 (15)	5 (9)	13 (24)
MIB	11 (20)	5 (9)	14 (25)
Geosmin	21 (38)	23 (42)	33 (60)
Odorous compounds	24 (44)	23 (42)	34 (62)
No odorous compounds	31 (56)	32 (58)	21 (38)

Note: Detection limit of geosmin was 1.0 ng l^{-1} and detection limit of 2-MIB was 1.7 ng l^{-1} .

When actinomycetes were cultivated in M liquid medium, 20 (36%) strains produced geosmin only, 13 (24%) strains produced both geosmin and 2-MIB, and 1 (2%) produced 2-MIB only. Therefore, among the odour-producing strains, most generated geosmin only and some produced both geosmin and 2-MIB, but few of the isolated strains produced 2-MIB only. Actinomycetes cultivated in TSB and Gause media showed similar performance. The above results indicate that plenty of actinomycetes in the sediments could produce geosmin and 2-MIB, and thus they may be one of the contributors to the earthy/musty odour occurring in water columns.

Production of geosmin and 2-MIB by actinomycete isolates

Six actinomycete isolates, L2, L18, L32, L33, L46 and L50, which produced detectable levels of geosmin and 2-MIB, were selected for study of their odour-producing abilities in Gause, M and TSB liquid media as well as on agar slants. Biomass and odour production by these actinomycete isolates varied dramatically among different media (Figure 1). It is indicated in Figure 1 that TSB was quite suitable for the yield of biomass. Isolate L50 was an exception, with a maximal biomass of 2.48 mg ml^{-1} (in dry weight) in Gause liquid medium. Compared with Gause medium, the yielded biomass was further decreased in M medium. However, for strain L2 in Gause medium, the yielded biomass in dry weight was only 0.06 mg ml^{-1} . Other than L2, five other isolates produced relatively higher biomass in Gause than in

M liquid cultures. However, higher biomass production did not result in higher production of odorous compounds; therefore, using a medium promoting growth of actinomycetes did not seem to stimulate the production of geosmin and 2-MIB. Of the three complex and defined media, M liquid medium was found to support the highest yields of geosmin and 2-MIB (over 10^2 ng mg^{-1} dry weight), whereas the production of odour compounds was much lower in Gause medium (Figure 1). In comparison with Gause medium, the ability of TSB to promote the odour-producing abilities for those isolates that could produce both geosmin and 2-MIB is very limited. Overall, M medium was the most suitable for the production of both geosmin and 2-MIB, while TSB was the most favourable for actinomycete growth.

The geosmin concentrations in six actinomycetes ranged from 4.93 to 142.85, 0.49 to 170.30, and 75.85 to $666.87 \text{ ng mg}^{-1}$ in biomass of dry weight when grown in Gause liquid medium, TSB and M liquid medium, respectively (Figure 1). The 2-MIB concentrations produced by the three isolates L-32, L-33 and L-46 ranged from 4.69 to 22.22 ng mg^{-1} dry weight in Gause medium, from 0 to 1.14 ng mg^{-1} in TSB and from 126.13 to $288.25 \text{ ng mg}^{-1}$ in M medium, respectively. Consequently, the geosmin and 2-MIB producing abilities of the six isolates differed, even when they were grown in the same medium. In addition, one of the isolates showed different tendencies to produce geosmin and 2-MIB in different media. In M medium, geosmin concentrations from isolates L-2, L-18 and L-50 were 4.7–6.5 times higher than in TSB and Gause media. However, for isolates L-32, L-33 and L46, which produced both geosmin and 2-MIB, geosmin concentrations in M medium were approximately 210.9, 154.6 and 39.6 times higher than in TSB medium. However, 2-MIB from L-46 was not detected when it was incubated in TSB. 2-MIB concentrations from L-32 and L-33 in M medium were about 383.8 and 110.6 times higher than in TSB. Consequently, strains that produced both geosmin and 2-MIB were more easily affected by liquid media than those strains that produced geosmin only.

Plate cultures were also used to relate production of odorous compounds to production of spores because solid slants more closely approximate the environments in which actinomycetes grow in sediments. Actinomycetes grown on Gause, TSA and M slant agar media showed different

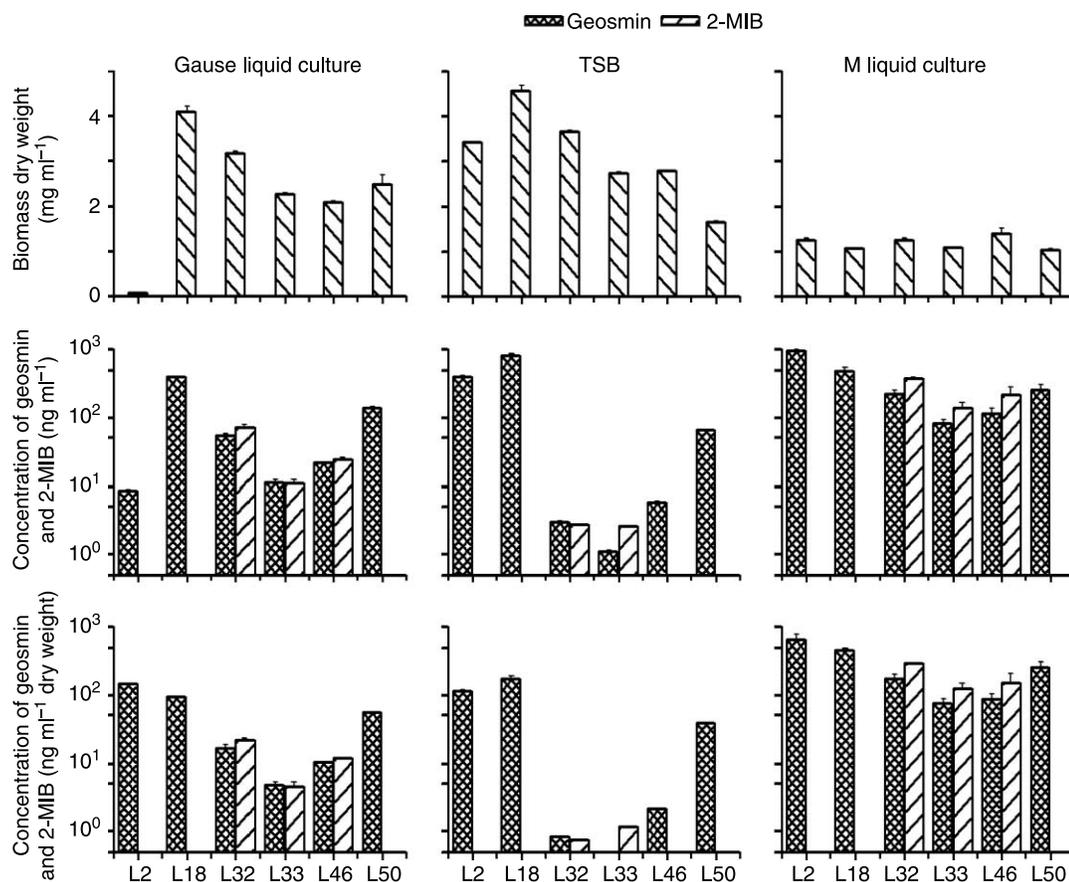


Figure 1 | Concentration of geosmin and 2-MIB produced by six actinomycetes in liquid media.

patterns from those grown in liquid cultures (Table 2). Maximal biomass production (cfu ml^{-1}) occurred on M or Gause slants; maximal odour production ($\text{ng}/10^7 \text{cfu}$) occurred in different media. Except for isolate L2, actinomycetes were covered with many spores on Gause and M agar media as evaluated by visual examination, while slight sporulation was observed in TSA by visual examination combined with DIC microscope (OLYMPUS BX2-DIC-F04). Isolate L2 produced large numbers of spores on the TSA and M slants and a small quantity on the Gause slant, perhaps because Gause and M slant agar media can promote sporulation. Most streptomycetes species will not sporulate in liquid cultures, and solid slants promote more spores (Jensen *et al.* 1994). However, attempts to determine the relationship between biomass on solid slants and odour production are subject to many sources of difficulties. First of all, the production of geosmin and 2-MIB in laboratory media may not be comparable to what would be observed in

sediments or other natural environments. Additionally, the concentration of odorous compounds produced on solid slants could vary markedly depending on their growth periods. For one isolate, quantification of biomass expressed as cfu ml^{-1} was not always consistent with mg ml^{-1} . Combined with the above results (Figure 1), it can be concluded that TSB promoted more mycelium formation and Gause and M agar medium supported sporulation for most of actinomycetes.

Although isolates producing both geosmin and 2-MIB showed considerable variations in levels of geosmin and 2-MIB production when they were cultivated in different media, including liquid and solid forms, there was a strong correlation between the concentration of geosmin and 2-MIB (ng ml^{-1}). The ratio of 2-MIB/geosmin was about 1.54/1.0 ($R = 0.838$) (Figure 2). Isolates that produced both geosmin and 2-MIB played an important role in 2-MIB production. Therefore, it was considered that the detectable

Table 2 | Concentration of geosmin and 2-MIB produced by six actinomycete strains cultivated on slants

Isolate	Slant	Odorous compounds (ng ml ⁻¹)		10 ⁷ cfu ml ⁻¹	Mean odorous compounds (ng/10 ⁷ cfu)	
		Geosmin	2-MIB		Geosmin	2-MIB
L2	Gause	ND	ND	0.168	–	–
	TSA	340.53 ± 0.9	ND	88.5	3.85	–
	M	200.92 ± 10.6	ND	144	1.40	–
L18	Gause	24.07 ± 3.0	ND	18.5	1.30	–
	TSA	78.05 ± 2.2	ND	10.3	7.58	–
	M	79.31 ± 8.0	ND	26.5	2.99	–
L32	Gause	179.54 ± 1.9	106.39 ± 4.1	45.5	3.95	2.34
	TSA	ND	ND	0.295	–	–
	M	59.31 ± 5.9	152.06 ± 21.5	24.2	2.45	6.28
L33	Gause	110.59 ± 2.4	335.51 ± 25.1	48.7	2.27	6.89
	TSA	ND	ND	0.45	–	–
	M	54.64 ± 0.4	152.06 ± 21.5	53	1.03	2.87
L46	Gause	63.90 ± 0.4	68.76 ± 3.5	46.3	1.38	1.49
	TSA	6.81 ± 0.2	ND	0.755	90.20	–
	M	82.63 ± 5.5	63.19 ± 3.1	20	4.13	3.16
L50	Gause	49.04 ± 0.9	ND	76	0.65	–
	TSA	29.26 ± 8.0	ND	0.34	86.06	–
	M	51.51 ± 5.4	ND	79.5	0.65	–

Notes: ND: not detected; –: not applicable; Detection limit of geosmin was 1.0 ng l⁻¹ and detection limit of 2-MIB was 1.7 ng l⁻¹.

concentration of 2-MIB in the water column in Lake Lotus may be dependent on the characteristics of musty odorous compounds produced by the actinomycetes in the sediments.

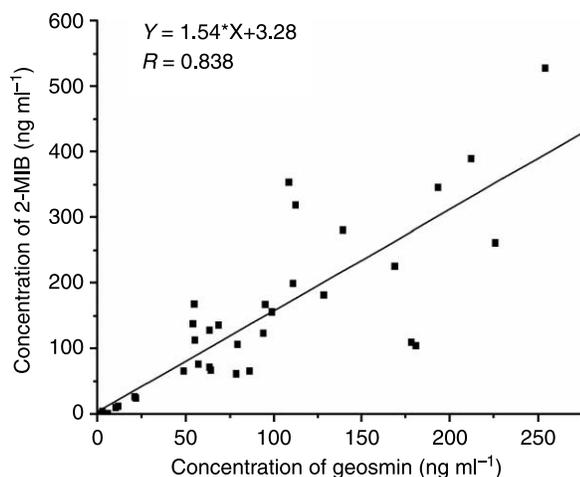


Figure 2 | Relationship between 2-MIB and geosmin in the actinomycetes that produced both the 2-MIB and geosmin under slant as well as liquid cultivation conditions.

Identification of isolated actinomycetes

The 16S rRNA sequences of the six actinomycetes were found to be a continuous stretch of 1,500 nucleotides (data not shown). Sequence analyses of 16S rRNA products were also conducted with the actinobacterial primers 8-27F and 1523-1504R. The relative 16S rRNA sequences of the selected actinomycetes were obtained from NCBI followed by phylogenetic BLAST analysis. The results revealed that the six actinomycetes were identified to six members of *Streptomyces*. A phylogenetic tree (Figure 3), using neighbour-joining analysis, indicated that strain L33 was identified as *Streptomyces tanashiensis*, while the other five strains could only be confirmed to the genus of *Streptomyces* according to 16s rRNA.

DISCUSSION

This study was conducted to investigate, isolate and identify actinomycetes that were able to produce geosmin and 2-MIB from lake sediments in Lake Lotus.

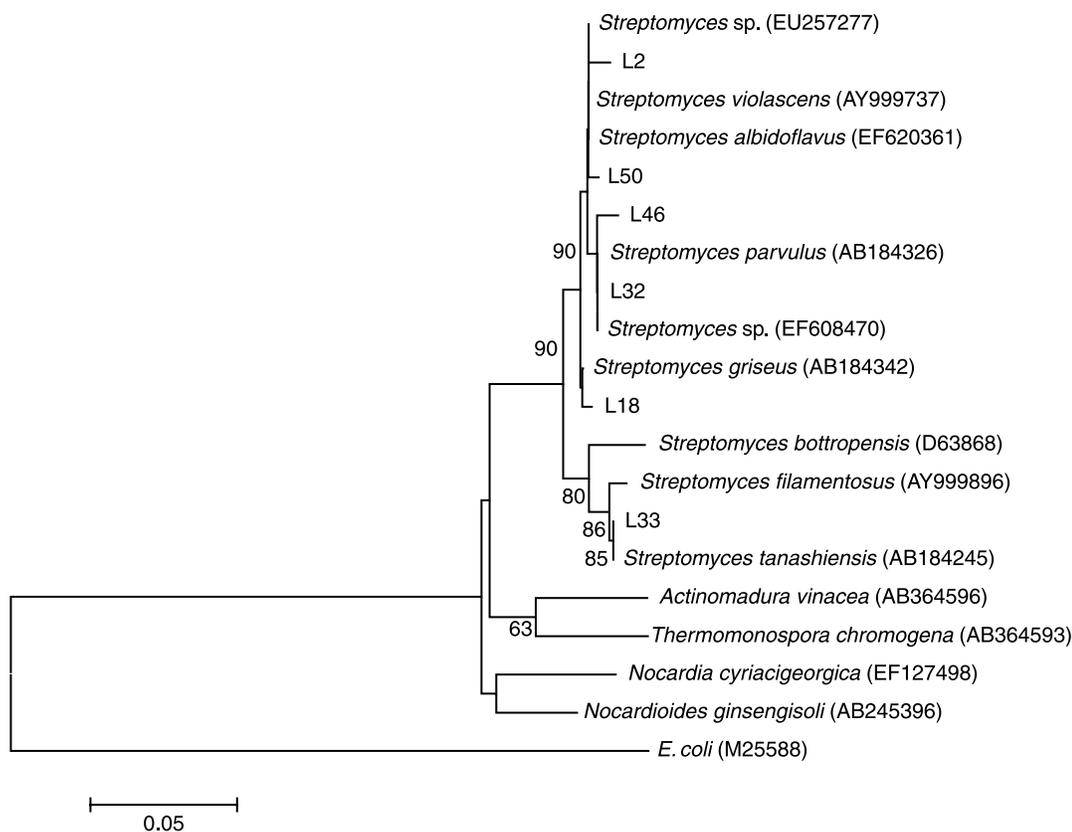


Figure 3 | Phylogenetic relationship between six actinomycete isolates and related sequences based on 16S rRNA gene sequences (neighbour-joining method).

Sugiura & Nakano (2000) isolated 40 actinomycete strains from sediments in Lake Kasumigaura, and all could produce both geosmin and 2-MIB. In our study, 55 actinomycete strains were isolated from sediments of Lake Lotus, but not all actinomycetes could produce the odorous compounds geosmin and 2-MIB. One strain showed different odour production performance when cultivated in different media. When the isolated actinomycetes were grown in M liquid medium, over half (62%) of them produced odorous compounds. However, only 44% and 42% of them produced odorous compounds in Gause and TSB media, respectively. Among the odour-producing isolates, the majority produced geosmin only, some produced both geosmin and 2-MIB, and the minority produced 2-MIB only. Similar results were reported by Jensen *et al.* (1994), who suggested that odour production by actinomycetes may vary with cultivation nutrient conditions. Odour-producing streptomycetes could also be isolated from the mud of the reservoir (Tung *et al.* 2006). Tung *et al.*'s report

showed that *Streptomyces malaysiensis* in M liquid cultures produced geosmin up to 4.5 ng mg^{-1} , which was much lower than geosmin (75.85 to $666.87 \text{ ng mg}^{-1}$ dry weight) produced by the six *Streptomyces* strains isolated from Lake Lotus. In addition, our three strains of *Streptomyces* sp. produced higher 2-MIB concentrations (126.13 to $288.25 \text{ ng mg}^{-1}$) than *S. malaysiensis* (2.4 ng mg^{-1}) and *Streptomyces caelestis* (less than 2.0 ng mg^{-1}). M medium has been reported to support good production of geosmin and 2-MIB by actinomycetes (Aoyama 1990; Jensen *et al.* 1994). Our study also verified this result. However, media in the laboratory are different from those in the natural environment, and no media exist that closely represent the conditions in the field. Therefore, it is necessary to know the detailed nutrient conditions that could affect the production of geosmin and 2-MIB in the field.

According to previous reports, growth of actinomycetes under particular conditions is not a good predictor of odour production (Blevins *et al.* 1995; Schrader & Blevins 2001).

With continuous shaking cultivation in liquid media, TSB attained maximal mycelium growth, while maximal odour production occurred in M medium. The total number of actinomycetes in the natural environment does not elucidate the contribution of actinomycetes to taste and odour. However, when actinomycetes were cultivated on slants, M and Gause media supported the highest production of biomass, but odour production by different strains did not show consistency on one solid slant. It may be that odorous compounds produced on the solid slants were easily lost to the atmosphere. The production of geosmin and 2-MIB may also be related to the growth stage of the actinomycetes. Compared with liquid cultures, solid cultures supported more spore production. In some reports, actinomycetes that could not produce spores or aerial mycelium odours also lost or reduced the ability to produce geosmin (Bentley & Meganathan 1981; Dionigi *et al.* 1992). Some reports have shown a good correlation between concentration of geosmin and the number of spores (Schöller *et al.* 2002). Tung *et al.* (2006) also suggested that sporulation was linked to odorant generation. However, the present study did not replicate these results. Actinomycetes that produced more spores did not excrete more odorous compounds.

Concentrations of geosmin and 2-MIB produced by actinomycetes varied dramatically when they were grown in different media. Compared with isolates that produced geosmin only, concentrations of geosmin and 2-MIB from isolates that could produce both odours exhibited large variations. The reasons for this were unclear until now. However, for isolates that produced both odours, there was a significantly positive correlation between the changes in geosmin and 2-MIB (ng ml^{-1}). Earlier work showed that synthesis of both geosmin and 2-MIB was derived from mevalonate through the isoprenoid pathway (Bentley & Meganathan 1981). This indicates that geosmin and 2-MIB originate from one precursor, which may provide some explanation of our results. The trend of changes in concentrations of geosmin and 2-MIB was consistent in actinomycetes that could produce both odours. In this study, the ratio of 2-MIB/geosmin was about 1.54/1.0, which was different from previous reports, indicating that the ratio of geosmin to 2-MIB was 1.4 (Sugiura & Nakano 2000). It was demonstrated that actinomycetes play an important role in 2-MIB production, which may provide

some evidence that actinomycetes in sediments may contribute to odour occurrence in Lake Lotus. Numerous geosmin- and/or 2-MIB-producing actinomycetes have been isolated from sediments in the past decades (Johnston & Cross 1976; Sugiura & Nakano 2000; Zaitlin *et al.* 2003). Furthermore, the correlation between the number of actinomycetes and concentration of geosmin has also been investigated (Sugiura & Nakano 2000), suggesting that actinomycetes from sediments may potentially contribute to odour occurrence and must be taken into consideration. However, in order to evaluate the exact contribution of actinomycetes to odour occurrence in water bodies, it would be very critical to understand the detailed environmental factors and physicochemical conditions that stimulate the production of geosmin and 2-MIB in sediments.

CONCLUSIONS

Based on *in vitro* analyses, 55 actinomycete strains were isolated from sediments in Lake Lotus, of which 34 (62%), 24 (44%) and 23 (42%) strains produced odorous compounds in M, Gause and TSB liquid media, respectively. Of the odour-producing actinomycetes, most produced geosmin, some produced both odours and a few produced only 2-MIB. M liquid medium supported good production of geosmin and 2-MIB, TSB promoted good production of mycelium growth, and M and Gause slants stimulated more spore production than TSA for five of the six selected strains. Among them, one was *Streptomyces tanashiensis* and the other five were classified to genera *Streptomyces*. Odour production by isolates that produced both odours was more greatly influenced by liquid media than that of those that could produce geosmin only. The ratio of 2-MIB to geosmin from actinomycetes producing both odours under liquid and solid cultivation conditions was found to be 1.54 ($R = 0.838$), revealing that actinomycetes' role in odour events should be considered.

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