Relations between abundance of potential geosmin- and 2-MIB-producing organisms and concentrations of these compounds in water from three Australian reservoirs

Niels O. G. Jørgensen, Raju Podduturi and Michele A. Burford

ABSTRACT

Relationships between the abundance of potential geosmin- and 2-methylisoborneol-producing (2-MIB) cyanobacteria and bacteria, and concentrations of the two taste and odour compounds (T&Os) were examined in a 7 day incubation of natural water from the surface and bottom of three reservoirs in southeast Queensland, Australia. Only a single known T&O-producing cyanobacterium (*Geitlerinema* spp.) was detected by microscopy at low density, and only in one reservoir. Densities of potential T&O-producing *Streptomyces* (determined by quantitative polymerase chain reaction (qPCR) assay) were highest in the bottom water and varied from 0.7×10^3 to 775×10^3 cells L^{-1}. Geosmin ranged from 6 to 59 ng L^{-1} (with the highest concentrations in the bottom water), while 2-MIB varied from 6 to 47 ng L^{-1} (with the highest concentration in surface water). Concentrations of both compounds declined during the incubation under both light and dark conditions. Presence of the geosmin synthase gene, *geoA,* in cyanobacteria and *Streptomyces* was examined by different PCR approaches. Cloning of PCR products from amplification of *geoA* showed a high similarity to *geoA* in cyanobacteria, but not to streptomycetes. Our results demonstrate that more research on the ecology and molecular biology of T&O producers is required to better understand the dynamics of T&Os and to monitor emerging T&O episodes.

Key words | 2-MIB, cyanobacteria, geosmin, Queensland, *Streptomyces*, water reservoirs

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INTRODUCTION

Freshwater reservoirs provide drinking water in many urban regions, but taste and odour compounds (T&Os), produced by microorganisms in the reservoirs, may spoil the quality of the drinking water. Typical T&Os in the reservoirs are geosmin (which gives an earthy flavour) and 2-methylisoborneol (2-MIB; which gives a mildewed flavour) (Juttner & Watson 2007). Although non-toxic, tainting by geosmin and 2-MIB makes the water non-palatable to many consumers and introduces fear of bacterial contamination of the water (Antonopoulou *et al.* 2014).

T&Os are produced by certain species of cyanobacteria and various non-phototrophic bacteria, especially *Streptomyces* species, but recent research suggests that bacteria belonging to the *Myxococcales* order may also produce T&Os in freshwater (Auffret *et al.* 2013). Concentrations and seasonal fluctuations of T&Os, as well as potential T&O-producing organisms, have been studied in several reservoirs and natural waters, yet there is limited knowledge on processes controlling production of the T&Os (Srinivasan & Sorial 2011). For example, the influence of specific environmental conditions, such as light, inorganic and organic matter and temperature, on the abundance of potential T&O-producing organisms has only been studied in a few cases (Jüttner & Watson 2007; Matsushita *et al.* 2015).

Since removal or reduction of T&Os from drinking water requires expensive and time-consuming procedures,

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e.g., treatment by particulate activated charcoal or advanced oxidation procedures (Bruce *et al.* 2002; Antonopoulou *et al.* 2014), there is demand for alternative approaches to ensure lower levels of T&Os in drinking water. One such approach is the ability to predict upcoming T&O episodes by detection of the densities of geosmin- and/or 2-MIB-producing organisms in the water. Recently, molecular methods for detection of essential genes in the synthesis of geosmin and 2-MIB, i.e., geosmin synthase (*geoA*) or MIB synthase (*MIBS*) genes, have been published (Ludwig *et al.* 2007; Kakimoto *et al.* 2014; Kutovaya & Watson 2014). For monitoring of emerging T&O problems in drinking water reservoirs, e.g., caused by cyanobacteria, the density of geosmin-producing cyanobacteria can be estimated from the number of *geoA* genes (Tsao *et al.* 2014) at regular intervals during periods of expected high geosmin concentrations. This may allow water treatment operators to change to alternative sources of water.

In this study, we wished to test a practical application, e.g., by public water quality laboratories, of recent polymerase chain reaction (PCR) methods for detection and quantification of geosmin-producing phytoplankton and bacteria by amplification of the *geoA* gene. For detection of *geoA* in *Streptomyces* and cyanobacteria in water reservoirs in southeast Queensland, Australia, the general primers by Ludwig *et al.* (2007) were applied, while two *geoA*-specific primer sets for quantitative PCR, one set targeting *geoA* in selected *Streptomyces* (Auffret *et al.* 2011), the other targeting *geoA* in certain species of cyanobacteria and actinomycetes, e.g., *Streptomyces* (Kutovaya & Watson 2014), were applied. Water from the surface and bottom of three reservoirs with known T&O problems was incubated for a week at *in situ* temperatures to monitor changes in geosmin and 2-MIB relative to the abundance of potential T&O producers.

MATERIALS AND METHODS

Surface and bottom water were collected on February 5th, 2014, at three reservoirs close to Brisbane city in southern Queensland, Australia. The reservoirs were Leslie Harrison Dam (LHD; 13,206 ML full supply, 4.5 m mean depth), Little Nerang (9,280 ML full supply, 23.6 m mean depth) and Wyaralong (102,883 ML full supply, 8.4 m mean depth). These reservoirs were chosen as they represented the range of background concentrations of geosmin and 2-MIB. LHD had low concentrations and Little Nerang and Wyaralong had high concentrations throughout the 2013/2014 summer. Five litre water samples were collected from the surface and near the bottom of the reservoirs using a Van Dorn sampler and transported to the laboratory on the same day. Surface samples were placed in plastic bags incubated under a light intensity of 10 μmol photon (PAR) m^{-2} s^{-1} using a cool white fluorescent light on a 12:12 h day: night cycle, and bottom waters were incubated in the dark in a constant temperature room (28 °C) (Figure 1). Dissolved oxygen concentrations in the bags were not monitored, but considering the large headspace volume and the frequent opening due to sampling, we did not expect a low oxygen concentration in the dark-incubated bags.

Subsamples were collected from the plastic bags at the commencement of incubation and for 5 more days over a 7 day period for geosmin and 2-MIB analyses, enumeration of algae, cyanobacteria and bacteria, and PCR analysis for *Streptomyces* spp. and abundance of the *geoA* gene in microorganisms in the water. Glass vials were filled to the brim with water samples for geosmin and 2-MIB analyses, and chilled until analysed. Samples for counting of algae and cyanobacteria were fixed in Lugol's solution and counted using Sedgewick Rafter cells under a compound microscope. Densities of algae and cyanobacteria were converted to biovolumes according to Hillebrand *et al.* (1999) using

Figure 1 | Samples incubating on light table in transparent bags.

biovolumes previously determined for species in reservoirs in the region (Ann Chuang, unpublished data). Densities of bacteria were determined by epifluorescence microscopy at 100 × magnification after staining with SYBR Gold according to Shibata *et al.* (2006). Cell numbers in 10 randomly selected areas were counted on each filter.

For molecular studies, water samples of 500 mL were filtered onto 0.2 μm 47 mm diameter membrane filters and frozen until analysed. Each filter was cut into three equal pieces; the pieces were treated by three-fold 20 min freeze–thaw cycles (−70 °C–65 °C) to improve cell wall destruction and improve extraction of DNA. DNA was extracted using the PowerWater kit by Mobio Laboratories Inc. (USA). Unfortunately, the DNA samples from February 6th and 13th were destroyed during extraction. Instead, DNA was extracted from samples taken on February 7th and 11th. Densities of streptomycete bacteria were determined by quantitative PCR (qPCR), combining TaqMan probe and primer technology (www.appliedbiosystems.com), according to Rintala & Nevalainen (2006) and Lylloff *et al.* (2012). Filters from the start and end of the incubation period were selected for analysis.

qPCR assays for quantification of the *geoA* gene were adapted from Auffret *et al.* (2011) and Kutovaya & Watson (2014). The primer sets of AMgeo-F (GAGTACATCGAGATGCGCCGCAA) AMgeo-R (GAGAAGAGGTCGTTGCGCAGGTG) and geo_cya543F (ATCGAATACATYGARATGCG) geo_cya728R (ACTTCTCTYTGRTAGGA) were applied to target *geoA* of *Streptomyces* and cyanobacteria, respectively. The qPCR reactions were performed with a 20 μL reaction mixture containing the final concentration of 1 × RealQ PCR master mix with green dye (Ampliqon, Denmark), 200 nM of each primer (AMgeo-F&R for *Streptomyces* and geo_cya543F&728R for cyanobacteria), 0.2 mg BSA mL^{-1} and 2 μL DNA template. The cycling conditions were as follows: heat activation at 95 °C for 15 min, 40 cycles of denaturation at 95 °C for 30 sec, annealing at 61 °C (*Streptomyces*) and 55 °C (cyanobacteria) for 30 sec and extension at 72 °C for 60 sec, followed by melting curve analysis of the amplified product. Template DNA of *Streptomyces coelicolor* A3(2) and *Oscillatoria* spp. 327/2 (the strain mentioned in Suurnakki *et al.* (2015)) were used as positive controls for the PCR amplification. DNA extracted from conidia in two *Streptomyces* species was used for construction of the calibration curve (gene copy number in *Streptomyces* vs. C_t value) as in Lylloff *et al.* (2012).

For detection of the *geoA* gene in the water samples, the primer set CycFW (TGGTAYGTITGGGTITTYTTYTTY-GAYGAYCAYTT) and CycRW (CATRTGCCAYTCRTGIC CICCISWYTGCCARTCYTG), targeting both cyanobacteria and *Streptomyces* and designed by Ludwig *et al.* (2007), was applied. In brief, the PCR protocol was: 50 μL reaction mixture with 1.5 mM $MgCl_2$, 400 μM dNTPs, 600 nM of each primer, 20 μg BSA, 1 μL of 100% DMSO, 2.5 U of Taq DNA polymerase (vwr.com) and 2 μL of DNA template. The PCR cycling conditions included heat activation at 95 °C for 2 min, followed by 35 cycles of denaturation at 95 °C for 30 sec, annealing at 55 °C for 45 sec and extension at 72 °C for 60 sec, and finished with extension for 5 min at 72 °C.

The amplified PCR products were purified using a Qiagen gel extraction kit (qiagen.com), cloned into pGEM®-T Easy Vector (promega.com) and transformed into *Escherichia coli* (JM109 High Efficiency Competent Cells; Promega) in accordance with the manufacturers' guidelines. Positive clones were sequenced by GATC-Biotech (Germany). The sequences were submitted to NCBI and given accession numbers KX250357 to KX250366.

Concentrations of geosmin and 2-MIB were determined using a Teledyne Tekmar Stratum Purge and Trap Concentrator (Teledyne Tekmar, USA) connected to an Agilent 7890B GC System with an Agilent 5977A MS detector (Agilent Technologies, Australia) with a detection limit of 1 ng/L for both compounds. Nutrient concentrations were measured following standard colorimetric methods (APHA 2005). Total nutrients were initially digested in persulfate prior to the colorimetric analysis (Hosomi & Sudo 1986). The detection limit for dissolved inorganic nutrients was 0.002 mg L^{-1} and for total nutrients was 0.005 mg L^{-1}.

RESULTS

Geosmin and 2-MIB

In the Wyaralong reservoir, concentrations of 2-MIB (47.1 and 14.5 ng L^{-1} at surface and bottom, respectively) were higher than the corresponding concentrations of geosmin

(1.4 and 5.8 ng L^{-1}) at the start of the incubation (Figure 2). During the 7 day incubation, 2-MIB and geosmin concentrations both declined. Concentrations of 2-MIB were reduced to 6.9 (surface) and 2.7 ng L^{-1} (bottom), while geosmin concentrations were <1 ng L^{-1} after 7 days. In contrast to Wyaralong, concentrations of geosmin were higher than concentrations of 2-MIB in Little Nerang reservoir. Geosmin concentrations at the start of the experiment in the surface and bottom were 19.8 and 58.6 ng L^{-1}, respectively, while the corresponding concentrations of 2-MIB were 5.9 and 7.9 ng L^{-1}, respectively. As measured in Wyaralong samples, geosmin and 2-MIB concentrations declined during the incubation, with final concentrations of about 4 ng L^{-1} (geosmin) and 1 ng L^{-1} (2-MIB) measured at the end of the incubation. In the LHD reservoir, concentrations of geosmin and 2-MIB varied between 1 and 2 ng L^{-1} with a tendency to decline over time (data not shown).

Phytoplankton and bacteria

The abundance of phytoplankton cells varied significantly in the three reservoirs but the populations were all dominated by cyanobacteria, making up from 82.9 to 99.6% of the phytoplankton biovolume (Figure 3). At the start of the incubation, the total phytoplankton biovolume ranged from 6.13×10^9 to 18.16×10^9 μm^3 L^{-1} in the surface water of the LHD and Little Nerang reservoirs, respectively, to 1.28×10^9 to 5.81×10^9 μm^3 L^{-1} in the bottom water of the Little Nerang and LHD reservoirs, respectively. After 1 week of incubation, the phytoplankton biovolumes in the surface samples were unchanged in the Wyaralong and LHD reservoirs, but were reduced by 52% in the Little Nerang reservoir. In the bottom water, after a week, the biovolume in the LHD water was unchanged while the biovolume was two- and four-fold higher in the Wyaralong and Little Nerang reservoirs, respectively. The Wyaralong and Little Nerang bottom samples had the highest measured concentrations of total P, total N and ammonium (Table S1, available with the online version of this paper).

In Wyaralong reservoir, the cyanobacteria were dominated by *Planktolyngbya minor* and *Gloeothece* spp. (making up 55 and 20% of all cells), while *Planktolyngbya limnetica* and *Gloeothece* spp. co-dominated in Little Nerang reservoir (Table S2, available with the online version of this paper). In the LHD reservoir, *Woronichinia* spp. made up about half of the cells. Among known T&O-producing

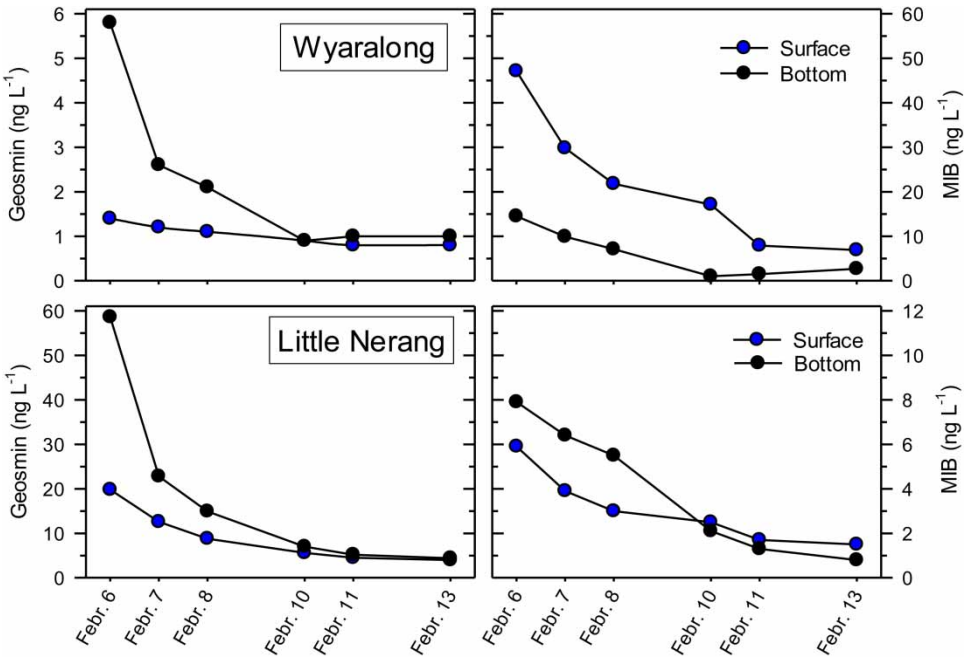

Figure 2 | Concentrations of geosmin and 2-MIB (ng L^{-1}) in surface and bottom water at Wyaralong and Little Nerang reservoirs. Single concentrations shown.

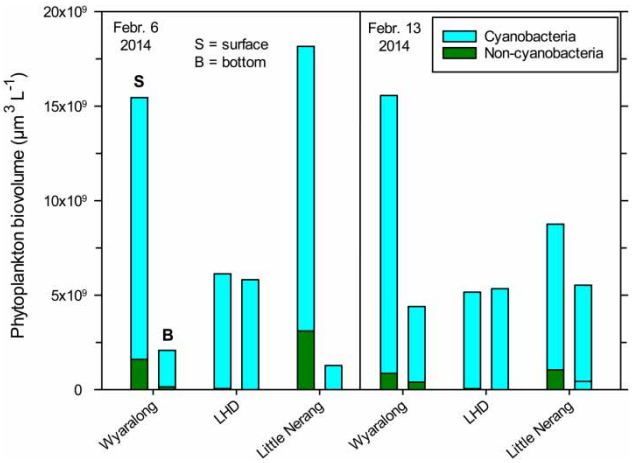

Figure 3 | Biovolume of cyanobacteria and non-cyanobacterial phytoplankton in the three reservoirs.

organisms, only *Geitlerinema* spp. was detected in Wyaralong surface water (1.2×10^3 cells L^{-1}), while the common T&O producers, such as *Dolichospermum* (formerly *Anabaena)* and *Oscillatoria*, were not present in measurable densities.

Densities of bacteria in the water ranged from 0.7×10^9 cells L^{-1} in the surface water of the Little Nerang reservoir to 5.9×10^9 cells L^{-1} in the bottom water of the Wyaralong reservoir (Figure 4(a)). The highest densities of bacteria occurred in the bottom water (*t*-test; $p < 0.05$), except for the LHD reservoir, in which a 2.5-fold increase in bacterial density was observed in the surface water after a week. *Streptomyces* spp. bacteria were detected in all samples and the abundance was highest in the bottom samples, except for LHD, at the start of the incubation (Figure 4(b)). The abundance varied significantly and ranged from 0.7×10^3 cells L^{-1} in Little Nerang surface water to 775×10^3 cells L^{-1} in Wyaralong bottom water; the average density of all samples was $10^9 \times 10^3$ cells L^{-1}. The density declined during the incubation (*t*-test; $p < 0.08$) or was unchanged (LHD bottom water). Relative to the total bacterial density, on average 32 (range 1 to 131), among 1 million bacterial cells were streptomycetes.

A summation of changes in phyto- and bacterioplankton abundance and of changes in the concentration of geosmin and 2-MIB in the water during the 7 day incubation is shown in Figure 5. Overall, there was little change in cyanobacterial or algal biovolumes in the surface water irrespective of

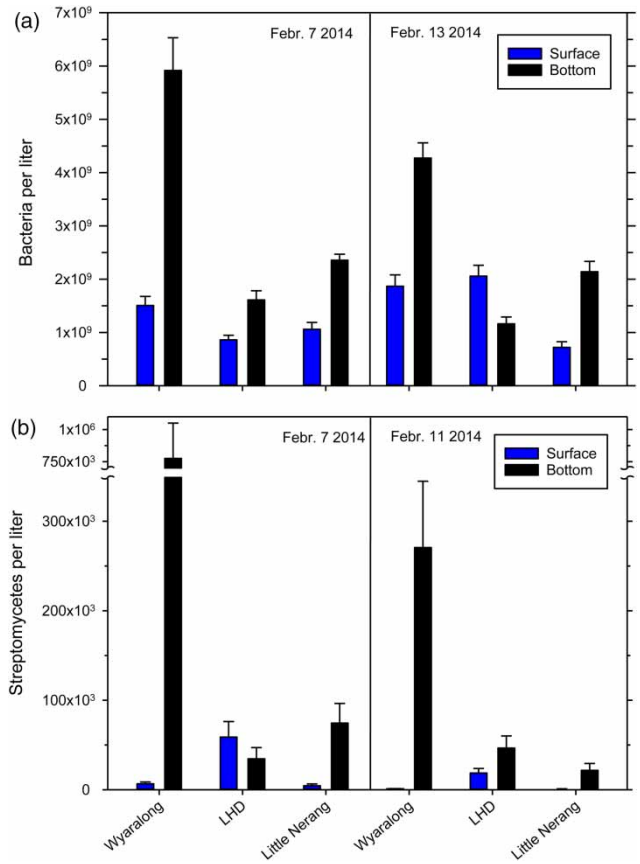

Figure 4 | (a) Mean (+SD) abundance of bacteria and (b) streptomycete bacteria in water (cells L^{-1}) from the three reservoirs. Due to loss of samples, bacteria were quantified on February 7th instead of 6th, and streptomycetes were quantified on February 7th and 11th due to destruction of DNA extracted from samples of February 6th and 13th. Means ± 1 SE (n = 10) shown for bacteria and means ± 1 STD (n = 3) shown for streptomycetes.

the reservoir, while there was an increase in the bottom waters from the Wyaralong and Little Nerang reservoirs. This contrasted with a decrease in geosmin and 2-MIB concentrations in Little Nerang and Wyaralong, and no change in LHD. Coincidentally, the densities of *Streptomyces* also decreased in all treatments, with the exception of LHD bottom water, while the most noticeable change in abundance of all bacteria was a doubling in the LHD surface water.

PCR amplification of the *geoA* gene

Initial tests of the qPCR assay for the *geoA* gene in DNA extracted from cultures of *Streptomyces* and *Oscillatoria* demonstrated that the approach produced the expected amplification curves and C_t values, indicating that the PCR

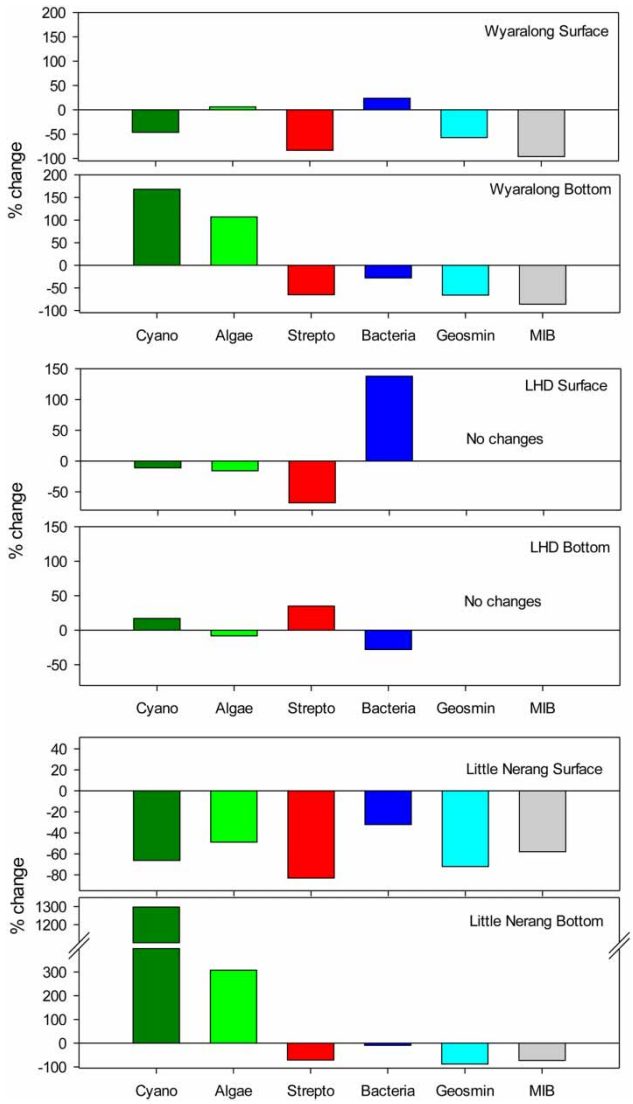

Figure 5 | Summary of changes in phyto- and bacterioplankton and geosmin and 2-MIB from the start (February 6th) to end of the incubation (February 13th), except for *Streptomyces* (February 7th to 11th) and bacteria (February 7th to 13th).

assay amplified *geoA* in the two groups of organisms. However, the qPCR assay did not form a successful product for *geoA* for DNA extracted from surface and bottom water samples of the three reservoirs. Incorrect melting point curves were found, implying a non-target amplification in the reaction.

In order to examine for the presence of the *geoA* gene in organisms in the reservoirs, a non-QPCR approach (using primer set CycFW and CycRW) was applied to DNA extracted from the bottom water of the Little Nerang reservoir, which had the highest geosmin content. As controls, DNA from cultures of *Streptomyces* and *Oscillatoria* were used. The PCR reaction generated positive products with the expected size from all samples. Taxonomic affiliation of the amplicons was examined by cloning into a vector, sequencing of 10 positive clones (sequences given in Table S3, available with the online version of this paper), and creation of a neighbour-joining phylogenetic tree of the cloned sequences. All sequences were identical to *geoA* in cyanobacterial strains and formed three different clusters. NCBI BLAST suggests that the sequences from one cluster were 88–89% identical to *Phormidium* sp. P2r sesquiterpene synthase (GenBank Identity (GI) 156707951), 85–86% identical to *Oscillatoria* sp. 327/2 geosmin synthase (GI: 730042929) and 85–86% identical to *Aphanizomenon gracile* WH-1 putative geosmin synthase (GI: 783305622). The sequences from the second cluster were 90–93% identical to *Phormidium* sp. P2r sesquiterpene synthase (GI: 156707951). The sequence in the third cluster was 71% identical to *Nostoc* sp. UK1 geosmin synthase (730042927). Sesquiterpene synthases include a large number of enzymes that form sesquiterpenes from farnesyl diphosphate. The amino acid sequence of geosmin synthase resembles that in some sesquiterpene synthases (Jiang *et al.* 2007), and the sesquiterpene synthase found in *Phormidium* sp. and other cyanobacteria might actually be geosmin synthase.

DISCUSSION

Analysis of results from the reservoirs showed three key results: (1) major difference in which T&O compound was most abundant in the reservoirs (prevalence of geosmin in Little Nerang and 2-MIB in Wyaralong) with the absence of the 2-MIB producing cyanobacteria; (2) unsuccessful quantification and detection of geosmin-producers by application of recent molecular approaches; (3) reduction of both geosmin and 2-MIB over the incubation period although both compounds are assumed to be relatively recalcitrant.

T&O concentrations vs. densities of cyanobacteria and *Streptomyces*

The difference in the dominance of either geosmin or 2-MIB as the major T&O compound between Little Nerang and

Wyaralong suggests that different organisms produced T&Os in the two reservoirs. Cyanobacteria can produce both geosmin and 2-MIB, or just one of the compounds, while all non-photosynthetic microorganisms that produce 2-MIB are also reported to produce geosmin (Jüttner & Watson 2007). In the light-exposed surface water of Wyaralong (but not in the 35 m deep bottom water of the reservoir) the high 2-MIB concentration of 47 ng L^{-1} might originate from cyanobacteria. However, only a single potential T&O-producing cyanobacterium, *Geitlerinema* spp., was identified in the surface water, and at low abundance. Further, in the *Geitlerinema* genus, only one species, *G. splendidum*, is known to produce T&O, and only geosmin, not 2-MIB (Jüttner & Watson 2007). Thus, cyanobacteria most likely did not produce the measured 2-MIB. Similarly, in the Little Nerang reservoir, where no known geosmin-producing cyanobacteria were identified in the water, the relatively high geosmin concentrations in the surface (19.8 ng L^{-1}) and bottom water (58.6 ng L^{-1}) most likely did not originate from cyanobacteria.

Other potential T&O producers in the water were streptomycete bacteria. The abundance of *Streptomyces* in the reservoirs (0.7×10^3 to 775×10^3 cells L^{-1}) fall within the range previously found for freshwater in southeast Queensland (Lylloff *et al.* 2012); but were streptomycete bacteria sufficiently abundant to produce the measured concentrations of geosmin and 2-MIB? Production of geosmin and 2-MIB by *Streptomyces* spp. in the water can be estimated, assuming that production rates from laboratory experiments with selected species of aquatic *Streptomyces* by Klausen *et al.* (2005) reflect conditions in the present reservoirs. Applying the highest cell-specific production rates by Klausen *et al.* (2005) (the observed range was 0.1–35 and 0.2–15 ag bacterium^{-1} h^{-1} for geosmin and 2-MIB, respectively) to the Wyaralong bottom water (water with the maximum density of *Streptomyces*), the time needed to produce the observed concentrations was 23 days for geosmin (15 ng L^{-1}) and 54 days for 2-MIB (50 ng L^{-1}). Thus, species of *Streptomyces* might have produced the measured geosmin and 2-MIB in the bottom water in Wyaralong. At other locations in the three reservoirs, the density of *Streptomyces* was significantly lower, and these bacteria were probably not the dominant producers of geosmin and 2-MIB. Also, only about 70% of known streptomycete species produce T&Os (geosmin and/or 2-MIB) (Jüttner & Watson 2007). This indicates that other T&O-producing organisms were present in the reservoirs, speculatively bacteria belonging to order *Myxococcales* as suggested by Auffret *et al.* (2013), but more studies are needed to confirm this.

In the dark incubation of water from the Wyaralong and Little Nerang reservoirs, a significant growth of especially the cyanobacteria *Planktolyngbya limnetica* and *Gloeothece* spp. occurred. Mixotrophic growth may explain the cell growth, at least for *Gloeothece*, since species within this genus are reported to use organic compounds for biosynthesis (Bandyopadhyay *et al.* 2010). The relatively high concentrations of total P and N and ammonium in the bottom water might, together with the DOC, have promoted the growth.

Detection of the *geoA* gene

The unsuccessful qPCR amplification of the geosmin synthase gene in cyanobacteria and *Streptomyces* with the applied quantitative assays could be caused by too few target cells in the water, or non-matching primers. The present qPCR assay for *geoA* was adopted from Kutovaya & Watson (2014), who designed functional PCR primers targeting *geoA* in isolated strains of cyanobacteria and actinomycetes (i.e., filamentous *Actinobacteria* such as *Streptomyces*), using separate primer sets. Since only one T&O-producing cyanobacterium (within the *Geitlerinema* genus) was identified by microscopy in the reservoirs, a low number of cyanobacterial *geoA* genes was expected. However, the lack of detection of *geoA* in *Streptomyces* by the qPCR assay was surprising, since these bacteria were abundant in the reservoirs. Kutovaya & Watson (2014) designed their primer sequences for actinomycetes on information from Auffret *et al.* (2011), who successfully detected *geoA* in isolated *Streptomyces* species and in environmental samples (biofilters from aquaculture systems). Although the qPCR assay by Kutovaya & Watson efficiently targeted *geoA* in their laboratory strains of actinomycetes, the authors did not detect the gene in the water or sediment of Canadian lakes. The failure to detect *geoA* in the environmental samples, as also observed in our study of Australian reservoirs, may reflect an undocumented genetic variability of the *geoA* gene in different geosmin-producing organisms (Ludwig *et al.* 2007).

In contrast to the qPCR assay, the non-QPCR assay using the degenerate primer set by Ludwig *et al.* (2007) indicated that *geoA* and related sesquiterpene genes actually did occur in microorganisms in the Little Nerang reservoir. As mentioned previously, the amino acid sequence of geosmin synthase and some sesquiterpene synthases are similar, and *geoA* might in fact be a sesquiterpene synthase (Ludwig *et al.* 2007). Surprisingly, the cloned sequences could only be related to known *geoA* genes (or sesquiterpenes genes) in cyanobacteria (in species belonging to the *Phormidium*, *Oscillatoria* and *Aphanizomenon* genera), and none of these cyanobacteria were identified in the Little Nerang reservoir. There were no matches to *Geitlerinema* due to lack of information about the *geoA* sequence for this genus. Within the *Phormidium* and *Aphanisomenon* genera, several species are known to produce geosmin, while species of *Oscillatoria* appear to produce 2-MIB rather than geosmin (Jüttner & Watson 2007). Among the three cyanobacteria identified in the clones, only *Aphanizomenon* sp. was found in Wyaralong surface water, but not in Little Nerang (Table S2). Sequences of the selected clones had no matches to known *geoA* sequences in streptomycetes, although *Streptomyces* occurred in the reservoirs.

The rather unsuccessful detection of *geoA*, using published primer sets for cyanobacteria and *Streptomyces*, suggests that existing information on molecular genetics of the *geoA* gene(s) is inadequate. Consequently, the design of suitable PCR primers, e.g., for monitoring of potential geosmin-producing organisms in water reservoirs, is correspondingly problematic. Recent studies on biosynthesis and molecular biology of geosmin and 2-MIB synthesis in selected species of cyanobacteria (Tsao *et al.* 2014; Suurnakki *et al.* 2015; Wang *et al.* 2015) may help to develop universal molecular approaches for targeting the *geoA* as well as the *MIBS* genes.

Degradation of T&O

Biological degradation of geosmin and 2-MIB is reported to be a slow process (Saito *et al.* 1999; Klausen *et al.* 2005), yet there was relatively fast reduction of both compounds during the week-long incubation in this study. In bioreactors with selected bacteria and in aquaculture waste treatment, stimulated biodegradation of geosmin and 2-MIB was measured (Ho *et al.* 2007; McDowall *et al.* 2009; Hsieh *et al.* 2010; Jiang *et al.* 2007), indicating that natural microbial populations include organisms with the capacity to degrade these compounds. Among specific bacterial species with a high catabolic capacity for geosmin and 2-MIB is *Bacillus subtilis* (Ma *et al.* 2015; Ma *et al.* 2016), and some species within the phylum *Proteobacteria* (Ho *et al.* 2007). The reduction in geosmin and 2-MIB in the reservoirs might indicate the presence of similar T&O-degrading organisms, but this was not studied. Loss of geosmin and 2-MIB by volatilization probably also occurred, but studies of the kinetics of volatilization of the two compounds at various temperatures and concentrations suggest that less than 10% was lost from the water as volatile compounds during the week-long incubation period (Li *et al.* 2012).

CONCLUSIONS

The purpose of this study was to test the application of available PCR-based methods for detection of geosmin-producing microorganisms in relation to actual abundance to geosmin in the water. Unfortunately, our results did not provide clues to specific geosmin producers in the reservoirs, and emphasize the need for more research on biology and molecular mechanisms that control production of T&O. Newly published information on the biosynthesis and molecular biology of geosmin and 2-MIB synthesis in selected cyanobacteria may help in designing universal PCR primers for both *geoA* and *MIBS* genes. If abundance of specific geosmin and 2-MIB producers can be detected by reliable PCR assays, a rapid prediction of emerging T&O episodes in water reservoirs can be achieved.

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