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Development and application of a molecular assay to detect and monitor geosmin-producing cyanobacteria and actinomycetes in the Great Lakes

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ABSTRACT

Geosmin is a potent earthy-smelling sesquiterpene responsible for the majority of biologically induced taste-and-odor (T/O) episodes in drinking and recreational water, and major economic losses to commercial, farmed and sports fisheries. Geosmin is produced by a range of microorganisms, notably by cyanobacteria and actinomycetes. However, the effective management of geosmin T/O episodes has been hindered by our inability to identify the major biological sources, and absence of sensitive methods for early detection of T/O events. The main goal of this study was to develop taxon-specific PCR and RT-PCR assays that facilitate early detection of potential geosmin releases and identification of the likely biological sources. We developed a molecular assay for the detection and expression of geosmin synthase (*geoA*), a gene involved in geosmin biosynthesis in cyanobacteria and actinomycetes. Using the molecular probes, we detected *geoA* in a wide range of geosmin-producing taxa in both laboratory cultures and environmental samples which provided an important new database of *geoA* sequences from a diversity of GSM producers, allowing an evaluation of their phylogenetic relationships based on this gene. This study indicated that potential geosmin producers in the Laurentian Great Lakes water and sediment samples are predominantly represented by members of the Nostocaceae, and in particular *Anabaena* spp. No correlations were apparent between gene expression and ambient temperature, nutrient concentrations and total phytoplankton biomass. Rather, evaluation of the *geoA* expression pattern in the environmental samples tested from various watersheds and seasons suggested constitutive gene expression.

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Introduction

Geosmin (trans-1, 10-dimethyl-trans-9-decalol) and 2-methylisoborneol (2-MIB) are terpenoid secondary metabolites belonging to the sesquiterpenes and monoterpenes, respectively. These compounds have been studied extensively since their identification in the 1960s as two of the most common sources of biologically induced taste-and-odor (T/O) (Gerber and Lechevalier, 1965) and the confirmation of cyanobacteria and actinomycetes as their major producers in source- and drinking-water and aquaculture systems worldwide (Jüttner, 1995; Jüttner and Watson, 2007; Medsker et al., 1968; Persson, 1996; Zaitlin and Watson, 2006). Cyanobacteria are considered to be the dominant source of geosmin in aquatic environments, particularly in eutrophic systems (Jüttner et al., 1986; Jüttner and Watson, 2007; Matsumoto and Tsuchiya, 1988; Persson, 1988), while actinomycetes make a significant contribution to geosmin and 2-MIB related T/O during runoff events or where there is a significant water–sediment interaction (Jensen et al., 1994; Zaitlin et al., 2003). Cyanobacteria and actinomycete species of both planktonic and benthic origin produce these compounds (Jüttner, 1984; Medsker et al., 1968; Schrader and Dennis, 2005) and while many studies have focused on planktonic producers, benthic taxa that cause sometimes significant T/O episodes are often overlooked (Izaguirre and Taylor, 2004; Jüttner and Watson, 2007). This study focused on geosmin-producing cyanobacteria and actinomycetes from both planktonic and benthic habitats.

Geosmin is relatively stable to chemical and biological degradation and resists conventional water treatment. It is one of the most common causes of off-flavor in drinking water and farmed fish, and incurs substantial costs to treatment and lost revenue (Cook et al., 2001; Hanson, 2003; Howgate, 2004; Smith et al., 2002). Successful prediction and control of geosmin events require knowledge of the producers and the factors affecting geosmin biosynthesis. Environmental factors such as light, temperature, nutrients, chlorophyll concentration and bacterial interaction are suggested to correlate to geosmin production (Aoyama et al., 1995; Bowmer et al., 1992; Naes and Post, 1988; Rosen et al., 1992; Saadoun et al., 2001; Watson and Ridal, 2004). Despite this knowledge, taste and odor outbreaks are still unpredictable and continue to occur in freshwater systems worldwide, including the lower Great

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Lakes (Lakes Erie, Michigan and Ontario) (Taylor et al., 2006; Watson et al., 2008).

There is a growing need for a sensitive, reliable and rapid diagnostic method for detecting, monitoring and managing geosmin events. Current methods used to identify geosmin producers are often inconclusive. Microscopy is widely used to monitor source waters for potential algal producers but is not applicable to actinomycetes and at best, yields tentative diagnostics, particularly with new case studies. It requires knowledge of likely producers based on the source-water (if recorded) or other studies, yet few species have been tested and confirmed as geosmin producers (e.g. Watson, 2003). Taxonomic expertise has traditionally relied on morphological traits which can be highly variable; current systematics are now increasingly integrating this with molecular and biochemical characteristics (e.g. Komárek, 2002; Komárek and Zapomělová, 2007, 2008). Per capita geosmin yield often differs among species and strains of the same species (Jüttner and Watson, 2007), and is further influenced by factors such as nutrients, light, temperature, and bacterial interactions (e.g. Aoyama et al., 1995; Bowmer et al., 1992; Naes and Post, 1988; Naes et al., 1989; Rosen et al., 1992; Saadoun et al., 2001). More recent efforts to use fluorescence in situ hybridization (FISH) and immunofluorescence techniques are limited by the non-specificity of these methods, which detect both geosmin producers and non-producers (Klausen et al., 2005; Nielsen et al., 2006). Finally, even though advanced analytical techniques such as gas chromatography–mass spectrometry (GC–MS) is useful for quantifying geosmin production, it cannot identify the biological source(s). In this study, we describe a molecular approach that can address many the limitations of the current techniques and provide an affordable and more effective method to detect and manage potential odor outbreaks.

Geosmin is synthesized from the universal sesquiterpene precursor, farnesyl diphosphate (FPP), in a two-step process catalyzed by a bifunctional sesquiterpene cyclase (also known as terpene synthase or geosmin synthase *geoA*) in the presence of Mg^{2+} (Giglio et al., 2008; Jiang et al., 2007; Ludwig et al., 2007). In this study, the geosmin synthase gene (*geoA*) was employed as a proxy to detect potential geosmin producers (using PCR) and evaluate the expression pattern (using RT-PCR). Molecular studies using the geosmin synthase have been done previously to better comprehend the nature of geosmin-producers in both cyanobacteria and actinomycetes (Agger et al., 2008; Cane et al., 2006; Giglio et al., 2011; Jiang and Cane, 2008; Ludwig et al., 2007). In recent work, Giglio et al. (2008, 2011) isolated and characterized this gene and demonstrated its universality for both cyanobacteria and actinomycetes. However, there is still little known about geosmin synthase distribution and expression in environmental samples. We developed a molecular assay for an early detection and monitoring of geosmin producers, and evaluated the *geoA* expression pattern in both planktonic and benthic cyanobacteria and actinomycetes in the Laurentian Great Lakes, notably Lakes Ontario and Erie, the most severely affected by taste–odor events (Watson et al., 2007, 2008). Using cyanobacterial and actinomycete strains isolated from the Great Lakes and other waterbodies we first developed molecular probes to evaluate their phylogenetic relationships based on their potential for geosmin production. We then applied these probes to water samples and sediments collected from across Lakes Erie and Ontario to evaluate the genetic sources and potential for geosmin production in these lakes.

Materials and methods

Cultures and cultivation

The actinomycetes and cyanobacteria strains used in the study are listed in Table 1 and include a mix of strains from different waterbodies. The cyanobacterial strains from the Laurentian Great Lakes, Lake Winnipeg and Lake of the Woods (S. Watson; EC strains) were initially isolated into sterile filtered lake water by micropipetting followed by repeated washings, and later transferred to growth media once established (Andersen, 2005). With the exception of the Maumee *Lyngbya wollei* mats, the cyanobacteria strains were monoalgal but not axenic. Stock cultures were maintained at 20 °C in Z8 media (Kotai, 1972) under a light regime of 16:8 h light:dark at 50 μmol photons m^{-2} s^{-1}, and transferred on a monthly basis using sterile technique. For the purposes of this study, inoculates were grown under the same conditions in 250 mL batch culture to late exponential phase (2–3 weeks). Attempts to isolate *L. wollei* BH-LE were not successful; however, we were able to maintain viable, non-axenic mats of this cyanobacterium under low light in algal medium for extended periods. The actinomycete strains were isolated from a Maumee River (Lake Erie, USA) *Lyngbya* mat onto BD Difco™ Actinomycete Isolation Agar (product # 212168, BD New Jersey USA) and cultured at 20 °C in TSB liquid media (T8907, Sigma Aldrich Canada) until stationary growth had been achieved.

Environmental samples

Information on water and sediment sampling station locations and dates can be found in Electronic Supplementary Material (ESM) Tables S1 and S2. At each site, water temperature was measured using a YSI profiler. Surface water was collected from a depth of 1 m using a Van Dorn (or equivalent) sampler and processed for the analysis of water chemistry, major nutrients (total phosphorus [TP], soluble reactive P [SRP], nitrate/nitrite [$NO_3^- + NO_2^-$], ammonia [NH_3]) and chlorophyll-a (chl*a*) by the National Laboratory for Environmental Testing in Burlington, Ontario using standard methods (Environment Canada, 1994). Total P was preserved with 1% (v/v) H_2SO_4 in 120 mL glass bottles and analyzed following persulfate digestion. Dissolved nutrients were analyzed from 0.45-μm membrane filtrates (Sartorius) prepared on site; biomass for chl*a* analysis was collected onto 45 mm GF/C filters (Whatman Corporation) and frozen until extraction. Subsamples were preserved in Lugol's iodine for later identification and enumeration of major algal and cyanobacterial taxa using the Utermöhl method (Lund et al., 1958). Water was filtered using a peristaltic pump onto 0.22-μm Sterivex cartridges (Millipore) and immediately frozen at −80 °C for nucleic acid extraction as described below. Surficial (top ~5 cm) sediment samples were collected using a Ponar sampler into 50 mL screw-capped polyethylene tubes and immediately frozen at −80 °C until analyzed.

Primers design and evaluation

All primers used in this study are shown in Table 2. Degenerate primers for cyanobacterial geosmin synthase, geo_cya543F and geo_cya728R, were developed based on the alignment of thirteen sequences (Fig S1). Nine of the sequences were obtained through testing the primers from Giglio et al. (2008, 2011) against genomic DNA extracted from cyanobacterial isolates and sequencing the positive samples. The other four geosmin synthase sequences were acquired from GenBank (*Anabaena ucrainica* CHAB1432 — HQ404996.1; *A. ucrainica* CHAB2155 — HQ404997.1; *Phormidium* sp. P2r — EF619621.1; *Nostoc punctiforme* PCC 73102 NPUNMOD — FJ010203.1). Degenerate primers for actinomycete geosmin synthase, geo_act532F and geo_act698R were designed taking into consideration an alignment from Auffret et al. (2011). The primers evaluation and validation was performed in two steps. First, the specificities of the new primers were tested using BLASTn and Primer-BLAST. Forward and reverse primers for both cyanobacterial *geoA* and actinomycete *geoA* assays indicated relatively high E-values with the geosmin synthase sequences in the GenBank and were the top hits with the intended targets (data not shown). Second, the primer efficacy was evaluated with template DNA from the isolates that have been previously confirmed as geosmin producing organisms by GC–MS (Watson, unpublished data). The isolates were the following: *Anabaena lemmermannii* GI CA799, *Aphanizomenon gracile* SAG 31–79, *Geitlerinema splendida* NIVA 244, *Phormidium* GI LM788 and *Actinomyces* sp. EC 012-1, 2, 3 (three isolates) (Table 1).

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