Influence of Growth Conditions on Production and Release of the Taste and Odor Compound Geosmin by A Cyanobacterium Isolated from the Phoenix Water Supply System

Qiang Hu[1], Milton Sommerfeld[1], Thomas Dempster [1], Paul Westerhoff[2], My-Linh Nguyen[2] and Larry Baker[3],

[1]Department of Plant Biology and [2]Department of Civil and Environmental Engineering, Arizona State University, Tempe, Arizona 85287

[3]Baker Environmental Consulting, 8001 Greenwood Drive, Moundview, MN 55112

Introduction

Frequent episodes of algae-related taste and odor problems in the Phoenix water supply have generated significant public concern about the perceived quality of the drinking water. A major compound associated with earthy and musty off-flavors in the water is called geosmin (trans-1, 10-dimethyl-trans-9-decalol). Geosmin is produced by a number of cyanobacteria. The human odor threshold concentration of geosmin in drinking water is approximately 5 ng L^{-1} (parts per trillion). Although taste and odor problems generally represent an aesthetic nuisance, recent studies have, in some cases, correlated the presence of algal toxins with the presence of taste and odor compounds. As consumers' awareness and expectation for drinking water quality rises, there is increased pressure on water utilities to prevent or remove off-flavors from drinking water sources.

Objectives of study:

- identify and isolate geosmin-producing cyanobacteria
- determine the effect of environmental and physiological parameters on production and release of geosmin

Site description

The water supply source for metropolitan Phoenix is primarily surface water from the Colorado, Salt and Verde Rivers and their reservoirs (Lake Pleasant, Bartlett and Saguaro Lakes). Water is then diverted into an extensive canal system, from which water is conveyed into water treatment plants (Figure 1).

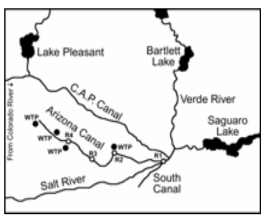

Figure 1. Schematic diagram of the metro Phoenix water supply system. Closed circles represent water treatment plants (WTPs) taking water from the Arizona Canal.

Production of geosmin in the Arizona Canal

Baseline monitoring conducted over the past three years has measured geosmin concentrations at 30 permanent sites throughout the metro Phoenix water supply system. Significant production of geosmin was found to occur in the Arizona Canal, which delivers water to four water treatment plants (WTPs). At the intake of the Deer Valley WTP, geosmin concentrations typically exceeded the human odor threshold level in late summer and fall (Figure 2).

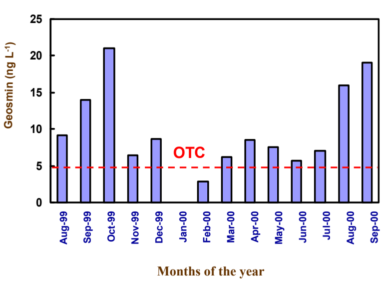

Figure 2. Concentrations of geosmin in the Arizona Canal near Deer Valley WTP throughout the year.

OTC: odor threshold concentration.

Isolation of geosmin-producing cyanobacterium

Species belonging to the genera *Oscillatoria, Phormidium, Pseudanabaena,* and *Spirulina* were the major cyanobacteria found in the Arizona Canal. One cyanobacterium identified as a geosmin producer was *Oscillatoria splendida* (Figure 3A). In the field, *O. splendida* was periphytic, forming localized and discontinuous patches on submerged canal walls and rocks, and under certain circumstances, dislodged mats in the water column. GC/MS analysis of supernatant from cultures of isolated *O. splendida* confirmed that it produces geosmin (Figure 3B).

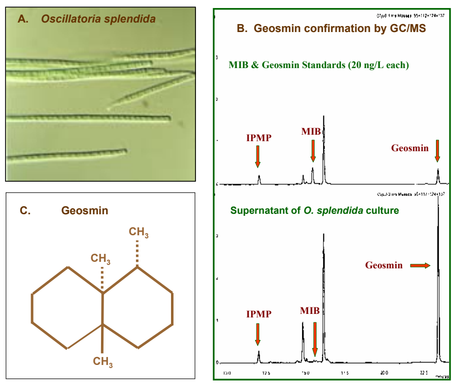

Figure 3. (A) Light photomicrograph of *O. splendida*, (B) GC/MS spectra of geosmin from a standard solution of 20 ng L^{-1} geosmin and MIB and the supernatant from cultures of *O. splendida*, and (C) molecular structure of geosmin.

Effect of growth phase

In batch culture, the amount of geosmin released from *O. splendida* cells was found to be biomass-dependent: the higher the biomass concentration (chlorophyll *a*), the higher the geosmin concentration in the medium (Figure 4A). Cellular content of geosmin followed essentially the same pattern as the increase in biomass. However, cell-bound geosmin per chlorophyll *a* was the highest when the cells were in the exponential growth phase (Figure 4B). It is concluded that the production of geosmin is constitutive in *O. splendida*, and the higher the specific growth rate the higher the cellular geosmin content.

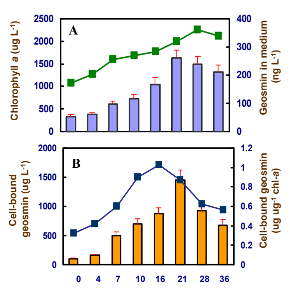

Figure 4.
(A) Changes in chlorophyll *a* concentration (bar graph) and geosmin concentration in medium (curved line).
(B) Changes in cell-bound geosmin (bar graph) and the ratio of cell-bound geosmin concentration to chlorophyll *a* (curved line) as a function of time.

Light intensity: 20 µmol m^{-2} s^{-1}
Temperature: 26 ^{0}C
Bars represent standard deviation of 3 replicates.

Effect of temperature

The specific growth rate of *O. splendida* increased with increasing temperature (Figure 5A). As temperature increased, the concentration of cell-bound geosmin increased. However, cell-bound geosmin per amount of chlorophyll *a* was significantly higher at lower temperatures (Figure 5B). When released geosmin was normalized on a per-chlorophyll *a* basis, more geosmin was released from the cells at lower temperatures (Figure 5C).

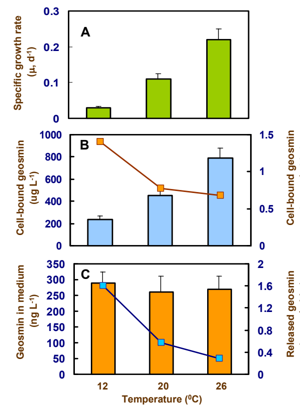

Figure 5. Effect of temperature on (A) the specific growth rate, (B) cellular content (bar graph), and cell-bound geosmin per chlorophyll a (curved line), and (C) release of geosmin into the culture medium (bar graph) and released geosmin per chlorophyll *a* (curved line) by *O. splendida*.

Samples taken on day 5 and 7 were used for calculation of the specific growth rate and on day 7 for analysis of geosmin concentration. Error bars indicate standard deviation of 3 replicates.

Effect of light intensity

An inverse relationship occurred between light intensity and the specific growth rate of *O. splendida* cultures (Figure 6A). Higher concentrations of geosmin in the medium occurred in cultures grown at lower light intensities (Figure 6B). On the other hand, a positive relationship between light intensity and cell-bound geosmin was evident (Figure 6C).

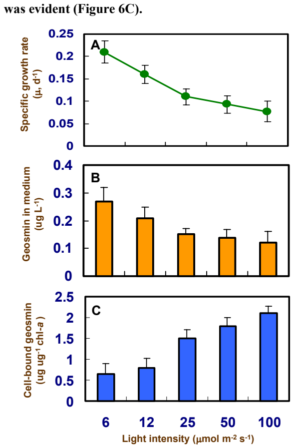

Figure 6. Effect of light intensity on (A) the specific growth rate, (B) geosmin in culture medium and (C) ratio of cell-bound geosmin to chlorophyll *a*.

Samples taken on day 6 were used for analysis of geosmin and on day 4 and 6 were used for calculation of the specific growth rate.
Error bars indicate standard deviation of 3 replicates.

Effect of dark incubation

Cultures of *O. splendida* were illuminated at 20 µmol m^{-2} s^{-1} and 20 ^{0}C for one week to reach the exponential growth phase and then transferred to dark at the same temperature for 25 days. The chlorophyll *a* concentration of the cultures remained stable for the first 14 days in dark, and thereafter declined. By day 25, chlorophyll *a* in the cultures was almost undetectable due to cellular decomposition (Figure 7A). Cell-bound geosmin increased by 50% over the first 7 days in dark, and then decreased. However, geosmin in the medium was low for the first 14 days, and then increased over the following 5 days. Thereafter, geosmin in the medium decreased rapidly (Figure 7B).

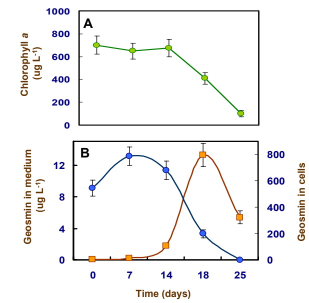

Figure 7. Changes in (A) chlorophyll *a* content and (B, ●) cell-bound and (B, ■) released geosmin into the medium as a function of time during dark incubation.

Culture conditions: *O. splendida* was maintained in BG-11 growth medium at 20 µ mol m^{-2} s^{-1} and 20 ^{0}C for one week, then transferred to a dark chamber. Samples were taken at selected time intervals for chlorophyll *a* and geosmin analysis. Error bars indicate the standard deviation of four replicates.

Effect of nitrate and phosphate

The growth of *O. splendida* and the production and release of geosmin were influenced by nitrate concentration in the Arizona Canal water (Figure 8).

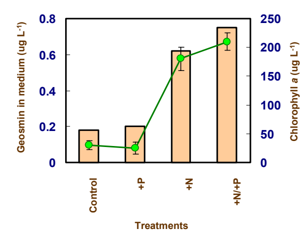

Figure 8. Effect of nitrate and phosphate concentrations on chlorophyll *a* concentration (solid line) and release of geosmin to the medium (bar graph).

Control = filtered raw water from the Arizona Canal;
+P = filtered raw water enriched with 1 mg L^{-1} of PO_4^{-3} (as P);
+N = filtered raw water enriched with 5 mg L^{-1} of NO_3^- (as N);
+P/+N = filtered raw water enriched with 1 mg L^{-1} of PO_4^{-3} and 20 mg L^{-1} of NO_3^- (as N).

Summary

We have identified and isolated a geosmin-producing cyanobacterium from the Phoenix water supply system that exhibits high intracellular concentrations and comparatively low extracellular release of geosmin, except upon cell lysis. Our results also illustrate that geosmin production is constitutive, but variable and influenced by growth conditions. This may explain the seasonal variability associated with cyanobacteria-related taste and odor problems. Further work is necessary to understand the physiological and metabolic basis for geosmin production, storage and release.

Acknowledgements

This research was supported by a grant from the City of Phoenix (Arizona) to Arizona State University