Physiological Studies of MIB- and Geosmin-Producing Cyanobacteria Isolated from the Phoenix Drinking Water Supply System

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Introduction

Laboratory experiments were designed to investigate factors associated with the production of 2-methlyisoborneol (MIB) and geosmin by two cyanobacteria, *Pseudanabaena sp.* and *Phormidium sp.* which were isolated from the Phoenix water supply system. These two volatile metabolites have been identified as the primary causes of musty and earthy tastes and odors (off-flavors) occurring in the Phoenix drinking water supply system during the summer and fall seasons. The effects of environmental conditions (i.e., temperature, light intensity, and lack of light) on cyanobacterial proliferation, cell autolysis, and the subsequent release of MIB and geosmin were investigated. Understanding the basic physiological responses of these MIB- and geosmin-producers to environmental factors is critical for development of effective field control measures to reduce or eliminate the off-flavors in the Phoenix drinking water supply system and also to be able to predict when off-flavor episodes are likely to occur.

Materials and Methods

Organisms: The cyanobacterium *Pseudanabaena* sp. was isolated at several sites along the Arizona Canal (e.g. Scottsdale Road, Central Avenue and Deer Valley). *Phormidium* sp. was isolated from the Verde River below Bartlett Lake and the Arizona Canal at Central Avenue. The two organisms were maintained in culture flasks containing BG-11 growth medium at 20 ^{0}C and 40 µmol m^{-2} s^{-1} of light.

Methods: All experiments were carried out in a batch mode. Temperature experiments were conducted in 100-ml flasks containing 40 ml algal culture. Flasks were placed onto a custom-designed thermo-gradient table varying in temperature from 10 to 35 ^{0}C (Fig. 1A). Light intensity experiments were conducted in 20-ml glass test tubes containing 10 ml of culture. Culture tubes were placed in a growth chamber providing light gradients ranging from 5 to 100 µmol m^{-2} s^{-1} (Fig. 1B). Algal growth was monitored by measuring cellular chlorophyll *a* and protein concentrations at designated time intervals. MIB and geosmin concentrations in algal cells and in growth medium were measured by gas chromatography and mass spectrometry (GC/MS) analysis.

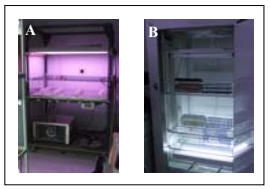

Fig. 1. A) The custom-designed thermogradiant table for conducting temperature experiments. B) A growth chamber showing light gradients for light intensity experiments.

Results

1. Identification of MIB- and Geosmin Producers

Isolated and purified *Pseudanabaena* sp. and *Phormidium* sp. were confirmed by GC/MS analysis to synthesize and release MIB and geosmin, respectively (Fig. 2).

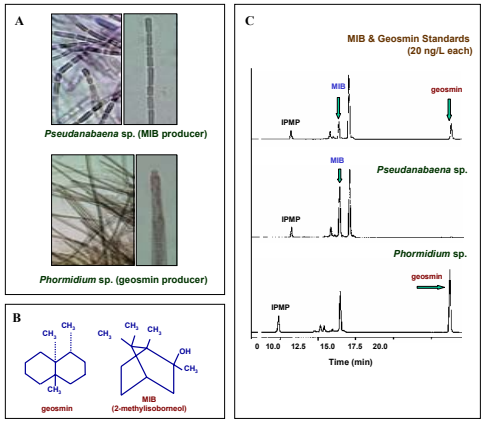

Fig. 2. A) Light micrographs of *Pseudanabaena* sp. and *Phormidium* sp.; B) Molecular structures of MIB and geosmin; and C) GC/MS spectra of MIB and geosmin standards and methanol-extracts of *Pseudanabaena* sp. and *Phormidium* sp.

2. Effect of Temperature

Both cyanobacteria exhibited temperature-dependent growth: the higher the temperature, the higher the growth rate at the range of temperatures tested (Fig. 3). Likewise, the higher temperatures also stimulated the biosynthesis and release of MIB and geosmin. Accordingly, both the highest cell-bound and the highest released MIB and geosmin into culture medium were detected in cultures at the higher temperatures tested (Fig. 4).

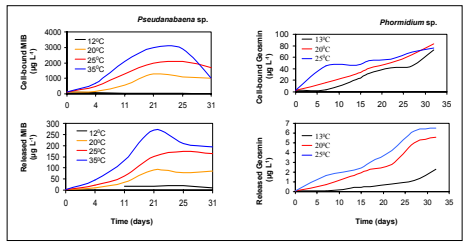

Fig. 4. Effect of temperature on cell-bound and released MIB and geosmin in cultures of *Pseudanabaena* and *Phormidium*.

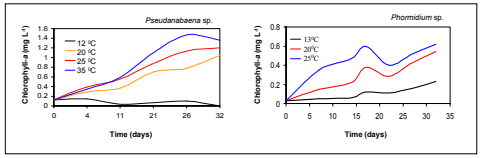

Fig. 3. Effect of temperature on the growth of *Pseudanabaena* sp. and *Phormidium* sp.

3. Effect of Light

While chlorophyll-*a* concentration was highest at lower light levels, total protein content was greatest at the highest light levels for both species (Fig. 5). This discrepancy is likely due to the reduced need for chlorophyll-*a* at higher light levels. Odorous compound production was maximized at mid-range light levels (between 20 and 50 µmol m^{-2} s^{-1} of light). Both organisms retained a greater concentration of cell-bound MIB and geosmin than they released into the environment (Fig. 6).

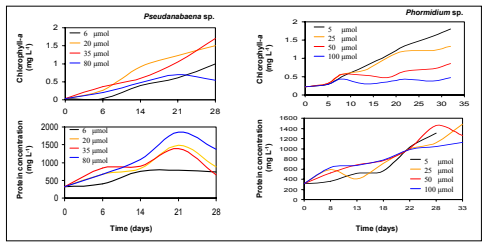

Fig. 5. Effects of light intensity on chlorophyll-*a* and protein content of *Pseudanabaena* sp. and *Phormidium* sp.

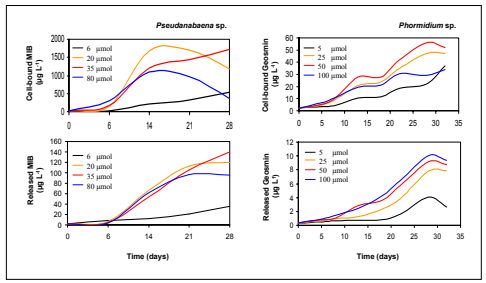

Fig. 6. Effects of light intensity on cell-bound and released MIB and geosmin concentrations in cultures of *Pseudanabaena* sp. and *Phormidium* sp.

4. Effect of Darkness (Decomposition)

Cultures were grown under optimal growth conditions (26 ºC and 30 µmol m^{-2} s^{-1} of light) for one or two weeks. Thereafter, culture tubes for both species were placed into a dark chamber. Measurements were taken daily for biomass (chlorophyll-*a* content) and cell-bound and released MIB/Geosmin concentrations. These experiments showed increased cellular releases of odorous compounds 4 days after dark incubation and continued for two weeks. Odorous compound levels in the medium doubled over this time span for both species, while total cellular content dropped to almost zero (Fig. 7). This suggests that cell death and subsequent lysis contributes to rapid increases in MIB/Geosmin levels in the environment.

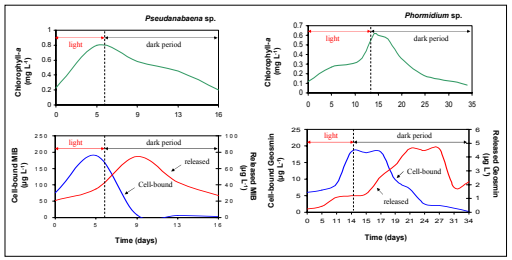

Fig. 7. Effect of darkness on cell lysis and release of MIB/geosmin in cultures of *Pseudanabaena* sp. and *Phormidium* sp.

Summary

- Two cyanobacteria, *Pseudanabaena* Sp. and *Phormidium* Sp., isolated from the Arizona Canal and Verde River are confirmed producers of 2-methyisoborneol (MIB) and geosmin, respectively.
- Over the temperature range encountered in nature, both *Pseudanabaena* and *Phormidium* accumulated more biomass and produced greater quantities of MIB/geosmin at higher temperatures.
- Over a range of light levels, both *Pseudanabaena* and *Phormidium* accumulated more biomass (protein) at the highest light levels, whereas MIB and geosmin production were the highest at mid-range light levels.
- Intracellular MIB and geosmin concentrations generally increased with an increase in biomass. Concomitant with intracellular accumulation, a corresponding increase in release of MIB and geosmin occurred.
- Exponential growth phase cultures of *Pseudanabaena* and *Phormidium* placed in the dark exhibited a rapid decline in intracellular MIB and geosmin and a rapid increase in released MIB/geosmin. Cell lysis and decomposition of MIB/geosmin producers may produce large spikes of these compounds in water supplies.
- Greater knowledge of the physiological responses of cyanobacterial producers of MIB and geosmin (off-flavor compounds) is critical to developing effective measures to predict when off-flavor episodes may occur, and to reduce or eliminate these off-flavors in the Phoenix water supply system.

Acknowledgements

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