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Effects of Selected Environmental Conditions on Biomass and Geosmin Production by *Streptomyces halstedii*

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The effects of bicarbonate concentration, atmospheric carbon dioxide level, and reduced atmospheric oxygen on biomass and geosmin production and geosmin/biomass (G/B) values for *Streptomyces halstedii*, a producer of the off-flavor compound geosmin, were determined. In addition, a study was performed to determine possible synergistic relationships between a cyanobacterium, *Oscillatoria tenuis* UTEX #1566, and *S. halstedii* in the enhancement of actinomycete growth and/or geosmin production. These studies took into consideration those conditions that can occur during cyanobacterial bloom die-offs. Increasing bicarbonate concentration caused slight decreases in geosmin production and G/B for *S. halstedii*. Increasing atmospheric oxygen promoted geosmin production and G/B while lower oxygen levels resulted in a decrease in geosmin production and G/B by *S. halstedii*. Biomass production by *S. halstedii* was adversely affected by reduced oxygen levels while changes in bicarbonate concentration and atmospheric carbon dioxide levels had little effect on biomass production. Sonicated cells of *O. tenuis* UTEX #1566 promoted biomass production by *S. halstedii*, and *O. tenuis* culture (cells and extracellular metabolites) and culture supernatant (extracellular metabolites) each promoted geosmin and G/B yields for *S. halstedii*. In certain aquatic systems, environmental conditions resulting from cyanobacterial blooms and subsequent bloom die-offs could favor actinomycete growth and off-flavor compound production by certain actinomycetes.

Key words: Actinomycetes, biomass, cyanobacteria, geosmin, *Streptomyces*

Most off-flavor episodes occurring in aquatic environments (e.g, aquaculture ponds, municipal water reservoirs, and lakes) in the southeastern United States are attributed to the presence of the earthy-odor compound geosmin and/or the musty-odor compound 2-methylisoborneol (MIB). Geosmin and MIB are produced by actinomycetes (3, 10, 11, 24) and cyanobacteria (14, 16, 17, 22, 23, 27). Cyanobacteria are attributed with being the main cause of such off-flavor episodes in aquaculture ponds; however, actinomycetes may play a role in off-flavor episodes in lakes and drinking-water reservoirs (32).

Carbon dioxide levels can be expected to be higher in lakes and ponds if respiration is occurring at a faster rate than photosynthesis. Such periods of higher carbon dioxide levels can exist in these waters during the early morning and during and after phytoplankton bloom die-offs (5). Research on the effects of elevated carbon dioxide levels and reduced oxygen levels on biomass and geosmin production by a geosmin-producing actinomycete would help to determine if actinomycetous propagules located in the mud/water interface of lakes and ponds might contribute to off-flavor episodes under such conditions.

The effect of bicarbonate levels on biomass and geosmin production by an actinomycete was also studied. Concentrations of carbon dioxide increase in aquaculture ponds following phytoplankton bloom die-offs, and corresponding increases in bicarbonate levels will occur [carbon dioxide in natural waters will react with bases in rocks and soils to form bicarbonate (4)]. In addition, as carbon dioxide removal from pond water increases during intense periods of photosynthesis by phytoplankton, bicarbonate levels will decrease as bicarbonate ions dissociate in an attempt to maintain equilibrium of the system and replace removed carbon dioxide. Bicarbonate buffers water against sudden pH changes, and water pH determines the contribution of bicarbonate to the total measurable alkalinity in water. Highest bicarbonate concentrations occur in aquaculture ponds when the water

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pH is 8.2~8.4 (4). Boyd (4) states that total alkalinity levels for natural waters can range from less than 5 mg/L to more than 500 mg/L. The effect of bicarbonate concentration on biomass and geosmin production by actinomycetes may help to determine the importance of this aquatic ecosystem variable in relation to conditions in lakes and aquaculture ponds that promote or inhibit off-flavor compound production by actinomycetes.

The effects of sudden die-offs of phytoplankton blooms in lakes and ponds on the physiology and metabolism of actinomycetes in relation to biomass and geosmin production have not been investigated. Enhancement of geosmin production by actinomycetes after phytoplankton bloom die-offs would suggest that a synergistic relationship may exist between geosmin-producing actinomycetes and cyanobacterial species which may constitute significant blooms but can not be shown to produce the off-flavor compounds geosmin or MIB in laboratory culture. Cyanobacterial cell biomass and metabolites released by the lysis of senescent cyanobacterial cells may provide compounds and nutrients which promote primary and/or secondary metabolism by actinomycetes. Precursor compounds used in the production of geosmin may also be provided during phytoplankton bloom die-offs. Also, enhancement of biomass production by actinomycetes would provide greater capacity for geosmin production when a shift to secondary metabolite production occurs.

Materials and Methods

Microorganisms

A geosmin-producing actinomycete isolated from the sediment of an Alabama aquaculture pond and identified as *Streptomyces halstedii* (24) was used. *Oscillatoria tenuis* UTEX #1566 obtained from the University of Texas Culture Collection of Algae, Austin, Texas, was used in the study on the determination of the effects of cyanobacterial cell biomass and metabolites on biomass and geosmin production by *S. halstedii*. During this study, *O. tenuis* UTEX #1566 did not produce any detectable amounts of geosmin or 2-methylisoborneol.

Culture conditions

To determine the effect of bicarbonate concentration on biomass and geosmin production by *S. halstedii*, 250 mL of a defined Romano-Safferman (RS) medium (21) with 2% (v/v) glycerol as a carbon source were placed in 1-L Erlenmeyer flasks (in triplicate). In order to maintain desired bicarbonate concentrations used in the study, the RS media was adjusted to pH 8.3 using sodium hydroxide pellets before the addition of potassium bicarbonate ($KHCO_3$) as a source of bicarbonate. The RS media was filter-sterilized using Nalgene 0.2 μm pore-size membrane filters (Nalge Company, Rochester, New York), inoculated with a spore preparation of *S. halstedii*, and incubated 5 days at 200 rpm at 30 °C in a New Brunswick Scientific model G25 incubator-shaker (New Brunswick Scientific Co., Inc., Edison, New Jersey). Control cultures contained no added bicarbonate.

For the study of the effect of carbon dioxide levels on biomass and geosmin production by *S. halstedii*, 250 mL of filter-sterilized RS broth (pH 9.0) with 0.05% (w/v) yeast extract, 2% (v/v) glycerol as the carbon source, and 0.2% (w/v) ammonium chloride (NH_4Cl) as a nitrogen source were placed in 1-L Erlenmeyer flasks (in quadruplicate) which were covered with Morton closures. These flasks were inoculated with a spore preparation of *S. halstedii* and incubated for 5 days at 200 rpm shaking at 30 °C in a New Brunswick Scientific model G25 top-loaded incubator-shaker. Vacuum grease was applied to small openings in the door of the incubator and to the rubber door gasket to help form a seal to maintain consistent internal atmospheric conditions within the incubator. To obtain a 5.0%±0.2% carbon dioxide atmosphere in the incubator chamber, a mixture of carbon dioxide (Airco, The BOC Group, Inc., Murray Hill, New Jersey) and air (5% and 95%, respectively) was continuously purged through the chamber at a measured flow rate of 80 mL/min using a flowmeter (Cole-Parmer Instrument Company, Niles, Illinois). To obtain a 10%±0.2% carbon dioxide atmosphere in the incubator chamber, a mixture of carbon dioxide and air (10% and 90%, respectively) was continuously purged through the chamber at a measured flow rate of 80 mL/min. The door of the incubator-shaker remained sealed during the 5-day incubation period. Control cultures were incubated simultaneously for 5 days in a New Brunswick Scientific Inova model 4000 incubator-shaker (New Brunswick Scientific Co., Inc., Edison, New Jersey) at 200 rpm shaking at 30 °C in an ambient atmosphere without any addition of carbon dioxide. Carbon dioxide levels in the incubator used for the control cultures were presumed to be approximately 0.03% to 0.05%, which is the carbon dioxide level in earth's atmosphere at sea level (31).

To determine the effect of reduced oxygen levels on biomass and geosmin production by *S. halstedii*, 250 mL of a 1% (w/v) yeast extract-1% (w/v) dextrose (YD) broth were placed in 1-L Erlenmeyer flasks which were covered with Morton closures. Flasks were divided into two sets (four flasks per set). One set of flasks contained YD broth adjusted to pH 9 by using sodium hydroxide (NaOH) pellets. The other set of

flasks contained YD broth adjusted to pH 7.5 by using a 1.0 N NaOH solution. All media were filter-sterilized using Nalgene 0.2 μm pore-size membrane filters. Media in each flask were inoculated with a spore preparation of *S. halstedii* and incubated 5 days at 200 rpm shaking at 30°C. Two flasks from each pH set were placed in the chamber of a New Brunswick Scientific model G25 incubator-shaker, and compressed nitrogen gas (Airco, The BOC Group, Inc., Murray Hill, New Jersey) was continuously purged through the chamber at a rate of 90 mL/min to reduce oxygen levels in the chamber. Vacuum grease was again used to seal all visible openings in the door of the incubator and to help form a better seal with the rubber door gasket. The remaining two flasks from each pH set were incubated simultaneously as controls for 5 days at 200 rpm shaking at 30°C in a New Brunswick Scientific Inova model 4000 incubator-shaker which contained an ambient atmosphere of approximately 21% oxygen (the oxygen content of the earth's atmosphere at sea level) (31).

For the study of the effects of cyanobacterial cell biomass and metabolites on biomass and geosmin production by *S. halstedii*, an axenic cyanobacterial culture of *O. tenuis* UTEX #1566 was inoculated into four Kontes cytolift bioreactors (Kontes, Vineland, New Jersey) containing approximately 450 mL of BG11 medium (20) per bioreactor. The bioreactors were placed in an ambient light atmosphere of 45~50 $\mu Em^{-2}s^{-1}$ as provided by two white fluorescent lights held vertically on opposite sides of each bioreactor. Filtered air (0.2 μm pore-size acrodisc filter; Gelman Sciences, Ann Arbor, Michigan) was introduced into each bioreactor at a flow rate of approximately 84 mL/min as measured by a flowmeter (Cole-Parmer Instrument Company, Niles, Illinois). Incubation of *O. tenuis* UTEX #1566 was at room temperature (23~25°C), and was allowed to continue for 21 days to permit a large amount of cyanobacterial cell biomass to accumulate. Before harvesting the cells, a wet mount was performed to confirm that the cultures were still axenic.

Cyanobacterial cells were harvested by centrifugation of the culture for 20 min at 16,000 × g in a Sorvall refrigerated superspeed centrifuge model RC-5B (DuPont Instruments, Wilmington, Delaware). Sterilized centrifuge tubes were used to prevent contamination of the culture. Supernatant from each tube was retained in a sterile 125 mL Erlenmeyer flask for future use in the study. Four pellets of cyanobacterial cell biomass were obtained and rinsed with a sterile 0.85% saline solution before the pellets were recentrifuged. After recentrifugation, the supernatant was removed and the wet biomass weight for each pellet was determined. Two of the pellets (0.15 g and 0.258 g) were resuspended in 5 mL of RS salts medium (without a carbon source and yeast extract) and subjected to sonication (55 w at 50% pulse for 6 min in 2 min intervals with 2 min pauses between each interval) using a model W-375 sonicator (Heat Systems-Ultrasonics, Inc., Farmingdale, New York).

Chlorophyll *a* and carotenoids were obtained from the *O. tenuis* cultures. Cyanobacterial cells were harvested by centrifugation of approximately 400 mL of *O. tenuis* culture for 20 min at 8,000 × g in a Sorvall refrigerated superspeed centrifuge model RC-5B. The supernatant was removed and the pellet was resuspended in 80% aqueous acetone (extractant) overnight at 4°C in the dark. On the following day, the pellet and extractant were centrifuged for 20 min at 16,000 × g. The supernatant was then placed in a sterile glass petri dish, and any remaining acetone was evaporated in a filtered (0.2 μm pore-size acrodisc filter) stream of nitrogen gas (helps prevent oxidation of chlorophyll *a* and carotenoids). The remaining aqueous solution was resuspended in RS salts medium before addition to *S. halstedii* cultures. To determine the content of chlorophyll *a* in *O. tenuis* cells, 10-mL aliquots of *O. tenuis* culture were filtered through glass-fiber filters (25 mm, type A/E; Gelman Sciences, Ann Arbor, Michigan) under vacuum and extracted with 5 mL of a 4:1 solution of 90% acetone:methanol. Chlorophyll *a* was determined using a spectrophotometer (Spectronic 20; Bausch and Lomb Inc., Rochester, New York) set at

Table 1. Treatment of flask-cultures of *Streptomyces halstedii*

Flasks used	Treatment per flask
2 (Controls)	25 mL sterile, ultra-pure water added
3	25 mL of BG11 media
3	25 mL of RS media [pH 9 with 2% (v/v) glycerol and 0.05% (w/v) yeast extract]
2	Pellet* of *Oscillatoria tenuis* UTEX #1566 cells suspended in 25 mL RS salts media
3	25 mL of supernatant recovered from the centrifugation of *O. tenuis* UTEX #1566 culture grown in the bioreactor
3	25 mL of *O. tenuis* UTEX #1566 culture obtained directly from the bioreactor
2	25 mL of sonicated *O. tenuis* UTEX #1566 cells suspended in RS salts medium
2	25 mL of RS salts media containing chlorophyll *a* and carotenoids extracted from *O. tenuis* UTEX #1566 cells

* Pelleted wet biomass weight added to cultures was as follows: 428 mg to flask A and 227 mg to flask B.

665 nm (18).

During the time that the culture of *O. tenuis* UTEX #1566 was being grown, cultures of *S. halstedii* were being prepared. Initially, *S. halstedii* was grown in 1-L of RS medium [pH 9 with 2% (v/v) glycerol and 0.05% (w/v) yeast extract] in a 2-L Erlenmeyer flask at 200 rpm shaking at 30°C in a New Brunswick Scientific model G25 incubator-shaker for 5 days. Cells were harvested by centrifugation of the culture for 20 min at 12,000 × g in a Sorvall refrigerated superspeed centrifuge model RC-5B. Pellets were washed in a sterile 0.85% saline solution, centrifuged (20 min at 16,000 × g), and resuspended in 1-L of RS salts media (pH 9 without a carbon source and yeast extract). Twenty 500-mL Erlenmeyer flasks, each containing 100 mL of the resuspended *S. halstedii* cells in RS salts media, were utilized (Table 1). After treatment of the specified flask-cultures, the cultures were incubated at 200 rpm shaking at 30°C for 5 days.

Growth measurement and culture extraction

Before biomass determinations were performed, wet mounts of each culture were prepared for microscopic examination to determine if contamination had occurred during the course of the study. A 50-mL aliquot of each 250-mL culture (for studies on bicarbonate concentration, carbon dioxide level, and reduced oxygen) was centrifuged in a Sorvall refrigerated superspeed centrifuge model RC-5B for 20 min at 16,000 × g and pellets were dried to constant weight in a drying oven set at 80 °C. The remaining 200 mL of culture in each flask were distilled, recovering 40 mL (20% of culture volume) of distillate. For the study on the effects of cyanobacterial cell biomass and metabolites on biomass and geosmin production by *S. halstedii*, a 25-mL aliquot of each 125-mL actinomycetous culture was centrifuged for 20 min at 16,000×g, and pellets were dried to constant weight. The remaining 100 mL of culture in each flask were distilled, recovering 20 mL (20% of culture volume) of distillate. Distillates were extracted successively with 20 and 10% (v/v) volumes of analytical grade methylene chloride (12). Combined extracts were concentrated in a stream of dry air to 0.5 mL. Approximately 300 mL of *O. tenuis* UTEX #1566 culture remaining in one of the bioreactors were distilled with recovery of 60 mL (20% of remaining culture volume) of distillate. This distillate was extracted successively with 20 and 10% (v/v) volumes of methylene chloride, and combined extracts were concentrated to 0.1 mL in a stream of dry air for gas chromatographic analysis to confirm that neither geosmin nor MIB were synthesized. Cultures of *S. halstedii* grown for 5 days produced maximal amounts of biomass and geosmin (final) for the medium and growth conditions we used in this study.

Gas chromatographic analysis

Quantitation of geosmin was achieved using a Perkin-Elmer model 8500 gas chromatograph (Perkin-Elmer Corp., Norwalk, Connecticut) equipped with a flame ionization detector (FID). A Stabilwax fused silica capillary column (length, 30 m; inner diameter, 0.25 mm; film thickness, 0.25 μm) (Supelco, Inc., Bellefonte, Pennsylvania) was used. The column temperature was set at 80°C for 2 min, then programmed successively to 200°C at 6°C/min and iso-time of 1 min, and finally, to 250°C at 10°C/min and iso-time of 7 min. The injector and detector were set at 300°C, the average linear gas velocity was set at 20 cm/sec with helium used as the carrier gas, and the split ratio was set at 50:1 with splitting and purging of the injected sample occurring after 1 min of run time in the splitless-injection mode. Borneol was added as an internal standard to concentrated extracts before gas chromatographic analysis. A borneol stock solution (10,000 mg/L in hexane) was added in 5 μL volumes to each 0.5 mL concentrated extract sample to yield a final concentration of 100 mg/L borneol in the extract samples. Borneol (100 mg/L) and different concentrations of a certified standard of geosmin (Wako Chemicals USA, Inc., Dallas, Texas) were used to make a cubic fit standard curve from PE Nelson Omega system hardware (Perkin-Elmer). This curve was used to identify and quantify geosmin in the concentrated extracts. The mini- mal geosmin concentration detectable was 20 ng/L using the gas chromatographic conditions previously described.

Data Analysis

Mean and standard deviations of the results of biomass and geosmin production and G/B values for *S. halstedii* from each study were determined. Regression analysis was used to determine the relationships of bicarbonate concentration and carbon dioxide level with biomass and geosmin production and G/B values for *S. halstedii*.

Results and Discussion

The co-occurrence of a particular cyanobacterial species with off-flavor episodes in water and fish has implicated cyanobacteria as the main source of the problem (2, 6, 14, 16, 22, 26, 29). Armstrong *et al.* (1) found a strong correlation between the occurrence of off-flavor episodes and cyanobacteria in Alabama catfish ponds. The association of geosmin presence in Alabama catfish ponds and the abundance of species of cyanobacteria such as *Anabaena*, *Aphanizomenon*,

and *Microcystis* was made by van der Ploeg (29). However, the contribution of off-flavor compound-producing actinomycetes to off-flavor episodes in lakes and aquaculture ponds cannot be completely discounted.

Actinomycetous propagules have been found to be present in greater numbers in lake sediments than in lake waters (32, 34). Although streptomycetes in lake muds have been shown to be relatively inactive, possibly due to anaerobic conditions (15), the activity of streptomycetous propagules at the mud/water interface in lakes and aquaculture ponds has not been extensively studied but may play a role in contributing to earthy and musty off-flavor episodes. Dmitrieva and Rodionova (9) observed an extension of hyphae without an increased rate of multiplication of nuclear bodies in submerged cultures of *Streptomyces streptomycini*. A study by Ram *et al.* (19) revealed a rapid increase in populations of aerobic bacteria in samples of suspended sediment taken from an intensely fed and aerated experimental fish aquarium over a period of 42 days. Increased aeration has been reported to enhance geosmin production by filamentous bacteria (3, 35), and Dionigi and Ingram (8) found that oxygen enrichment (30% O_2) of the ambient atmosphere used for incubation increased geosmin production in cultures of *Streptomyces tendae*. Oxygen levels are usually low in the benthic environments of lakes and ponds, and almost all sediments in lakes and ponds are anaerobic below a depth of a few centimeters (4). However, in most catfish production ponds, sediment is constantly being resuspended (and aerated) by the bottom-dwelling characteristics of the catfish.

Physical and chemical changes in aquatic systems induced by the occurrences and die-offs of cyanobacterial blooms could play a role in stimulating off-flavor compound production by actinomycetes. This stimulation could lead to even higher concentrations of geosmin and/or MIB concentrations in the water during or after the occurrence of cyanobacterial blooms (including blooms of cyanobacterial species not producing off-flavor compounds). The complexity of ecological factors promoting off-flavor compound production by cyanobacteria and actinomycetes necessitates an understanding of the entire aquatic ecosystem including changes of physical and chemical factors occurring during periods of phytoplankton proliferation. The possible impact of such changes on off-flavor compound production by actinomycetes may help to predict and to control the occurrence of off-flavor episodes. The synergistic relationship between off-flavor producing actinomycetes and cyanobacteria as well as the ecological effects of phytoplankton bloom occurrences and die-offs on aquaculture pond water chemistry in relation to the promotion of geosmin production by actinomycetes needs further study. Phytoplankton bloom die-offs can occur during periods of high light intensity and calm, warm weather whereby light injury to phytoplankton cells at or on the water surface initiates the massive death of the phytoplankton (4). As dead phytoplankton decompose, dissolved oxygen is quickly depleted, concentrations of carbon dioxide and ammonia increase, and water pH decreases (5). An increase in the concentration of carbon dioxide causes a decrease in water pH and eventually leads to an increase in the concentration of bicarbonate in water as carbonic acid dissociates to yield bicarbonate (4). Conversely, carbon dioxide removal from lake or aquaculture pond water can occur during periods of high photosynthesis by phytoplankton. This

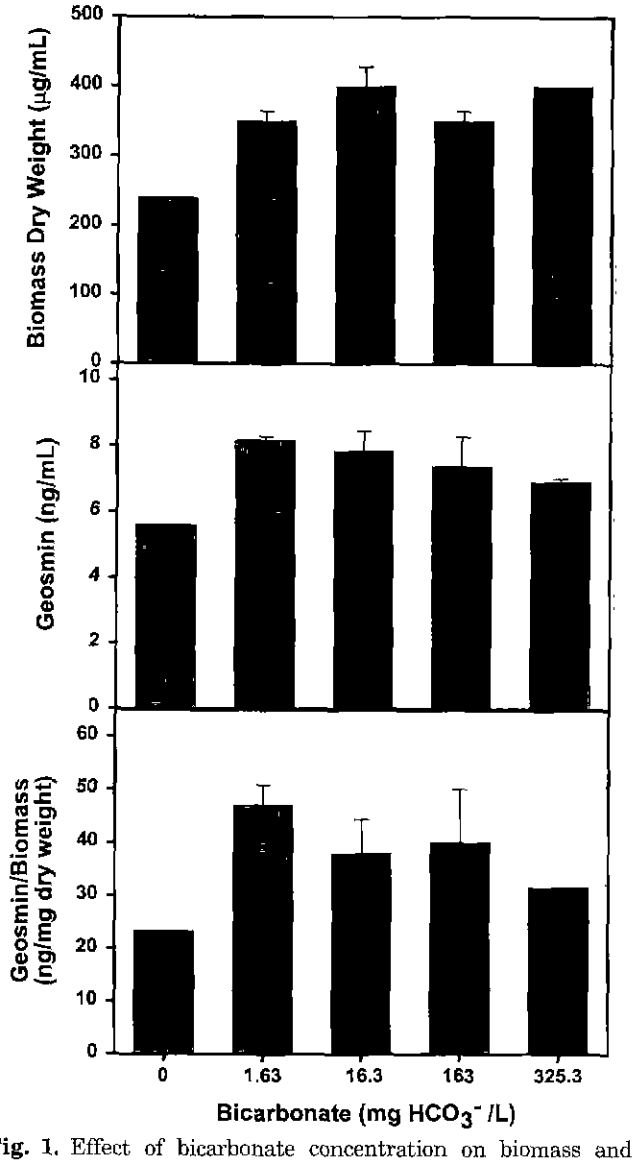

Fig. 1. Effect of bicarbonate concentration on biomass and geosmin production by *Streptomyces halstedii*. Bars represent standard errors.

removal of carbon dioxide from the water causes an increase in water pH and carbonate concentration and a subsequent decrease in bicarbonate concentration.

Increasing concentrations of bicarbonate (0–325.3 mg HCO_3^-/L) were found to have little effect on mean biomass dry weight production by *S. halstedii*, while mean geosmin production and G/B values decreased with increasing bicarbonate concentrations (Fig. 1). Greatest geosmin production (8.18 ng/mL) and greatest G/B value (47.0 ng/mg dry weight) for *S. halstedii* occurred at the lowest bicarbonate concentration tested (1.63 mg HCO_3^-/L).

High levels of carbon dioxide were found to promote geosmin production and G/B values (Fig. 2). With an atmosphere containing 10% carbon dioxide, *S. halstedii* cultures produced a mean geosmin concentration of 106.42 ng/mL and a mean G/B value of 78.6 ng/mg dry weight. At carbon dioxide levels of 0.03 to 0.05%, the lowest mean production of geosmin (15.86 ng/mL) and lowest mean G/B value (11.0 ng/mg dry weight) occurred. Mean production of biomass dry weight by *S. halstedii* was not influenced greatly by carbon dioxide levels although a small reduction of mean biomass dry weight did occur at the 10% carbon dioxide level. Tetracyclinogenesis (tetracycline formation) by *Streptomyces aureofaciens* has been found to peak at approximately 2% CO_2 (30), demonstrating the carbon dioxide-effect on secondary metabolism. Carbon dioxide levels increase during die-offs of phytoplankton blooms and will also be higher in those areas of lake or pond waters where respiration is

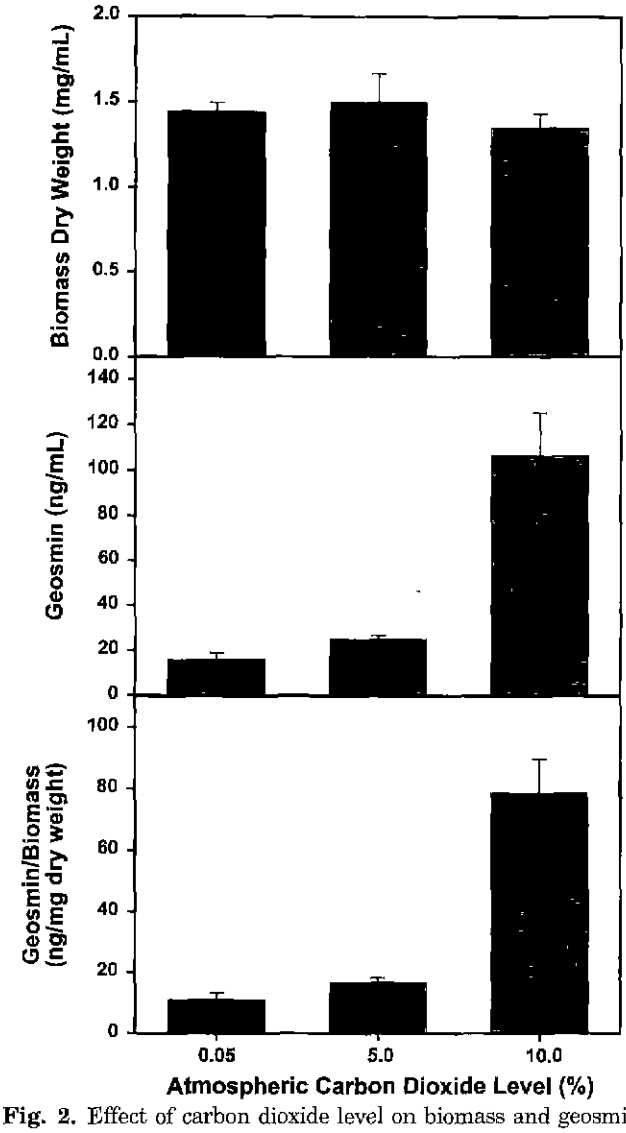

Fig. 2. Effect of carbon dioxide level on biomass and geosmin production by *Streptomyces halstedii*. Bars represent standard errors.

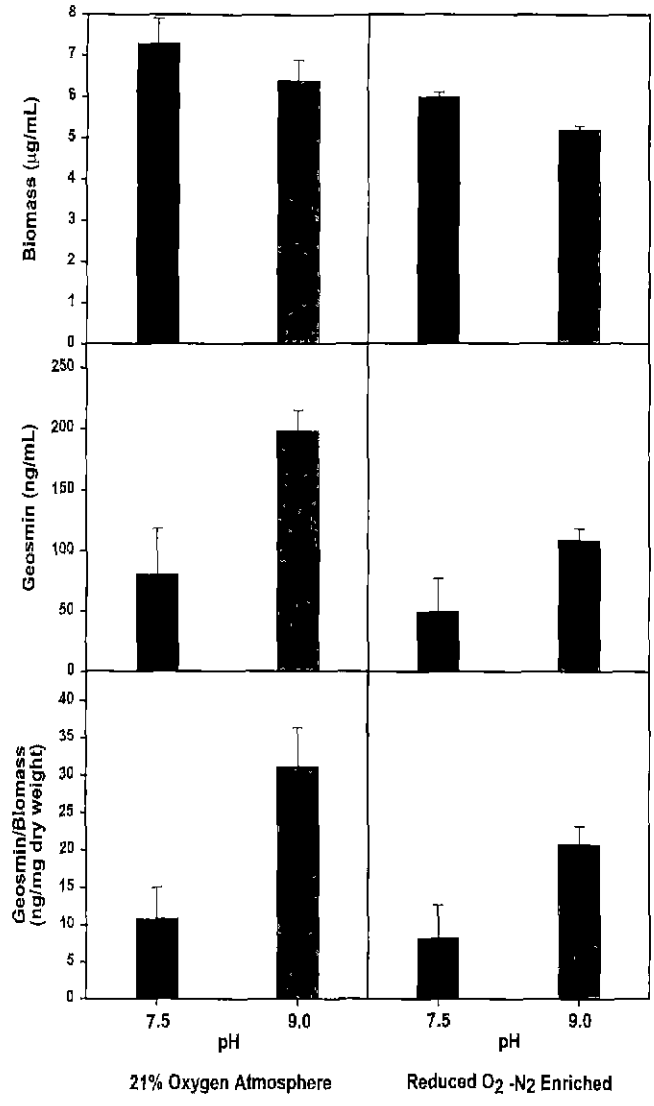

Fig. 3. Effect of reduced oxygen level on biomass and geosmin production by *Streptomyces halstedii*. Bars represent standard errors. Controls were incubated without a reduction of oxygen levels.

occurring more rapidly than photosynthesis.

Regression analyses comparing bicarbonate concentration (0~325.3 mg HCO_3^-/L) with biomass and geosmin production and G/B values for *S. halstedii* reveals no strong, linear relationships (data not shown). Regression analyses of increasing carbon dioxide level with biomass and geosmin production and G/B values for *S. halstedii* indicates strong, linear increases for geosmin and G/B values with an increase in carbon dioxide levels (0.05% to 10.0%) (data not shown). An increase in the carbon dioxide level in the ambient atmosphere evidently enhances geosmin production and G/B values for *S. halstedii*.

Reduced oxygen levels inhibited biomass and geosmin production as well as G/B values for *S. halstedii* grown at pH 7.5 and 9.0 (Fig. 3). Cultures of *S. halstedii* were grown at two different pH values to determine if growth and geosmin production at different pH conditions were affected in a similar manner by reduced oxygen level. The results in Fig. 3 indicate that reduced oxygen level has the same effect on *S. halstedii* grown at pH 7.5 and 9.0 by causing a decrease in biomass and geosmin production as well as for G/B values.

Lower dissolved oxygen levels occur in lakes and aquaculture ponds as phytoplankton decompose during and after phytoplankton bloom die-offs. In addition, lower dissolved oxygen levels can occur below the euphotic zone (defined as the strata in a lake or pond receiving at least 1% of the radiation striking the water surface) found in stratified lakes and deep aquaculture ponds (4). Many aerated aquaculture ponds usually do not undergo thermal stratification because of their shallow depth. However, the bottoms of these shallow ponds can become supersaturated with oxygen if the ponds are clear and an infestation of benthic algae and macrophytic plants are present (4).

Fig. 4 reveals that sonicated *O. tenuis* UTEX #1566 cells and RS salts media containing chlorophyll *a* and carotenoids from *O. tenuis* cells promoted biomass production by *S. halstedii*. Culture of *O. tenuis* (whole cells) and the supernatant from the centrifugation of *O. tenuis* culture yielded lowest biomass production (14 μg/mL and 8 μg/mL, respectively) by *S. halstedii*, while yielding maximal geosmin production (1,232.8 ng/L and 587.9 ng/L, respectively) and maximal G/B values (90.4 ng/mg dry weight and 90.1 ng/mg dry weight, respectively) for *S. halstedii*. Sonicated *O. tenuis* cells were a poor substrate for geosmin production (167.8 ng/L) by *S. halstedii* and also yielded a low G/B value (2.0 ng/mg dry weight) for *S. halstedii*.

The promotion of geosmin production by *S. halstedii* from the presence of *O. tenuis* culture (whole cells and metabolites) and the supernatant from the centrifugation of *O. tenuis* culture suggests that some

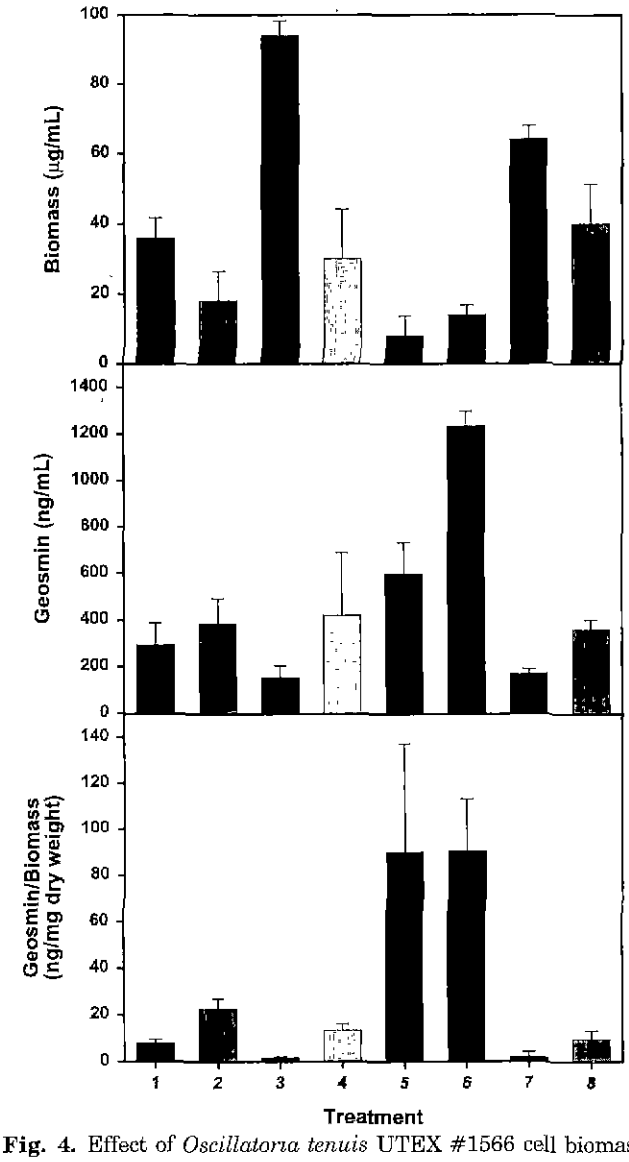

Fig. 4. Effect of *Oscillatoria tenuis* UTEX #1566 cell biomass and metabolites on biomass and geosmin production by *Streptomyces halstedii*. Treatment (per flask): 1=Control; 2=25 mL of BG11 media added; 3=25 mL of RS media with glycerol; 4=25 mL RS salts media with *O. tenuis* cell pellet; 5=25 mL of *O. tenuis* culture supernatant; 6=25 mL *O. tenuis* culture (435 ng/L chlorophyll *a*); 7=25 mL of sonicated *O. tenuis* cells (including cytoplasmic contents); 8=25 mL of RS salts media containing chlorophyll *a* and carotenoids.

cyanobacterial metabolite may be stimulatory to off-flavor production by actinomycetes. From the results of this study, a synergistic relationship between *O. tenuis* UTEX #1566 and *S. halstedii* in the promotion of actinomycete growth and geosmin production appears to exist. Silvey and Roach (25) reported that an observed increase in gram-negative heterotrophic bacteria and actinomycete populations in reservoir lakes of the southwestern United States after cyanobacterial bloom die-offs might be due to an increase in

the availability of metabolic byproducts from the lysis of senescent cyanobacterial cells. In addition, Silvey and Roach (25) suggested that, in aquatic environments, a dependency may exist by one group of microorganisms upon one or more other groups of microorganisms for at least a portion of its source of nutrition. Recently, van Hannen *et al.* (28) detected an increase in actinomycetes in lake water following lysis of cyanobacterial populations by viruses and suggested that actinomycetes may be able to rapidly assimilate dissolved compounds released due to the lysis. Metabolites from *O. tenuis* appear to be slightly more stimulatory than *O. tenuis* cell biomass for geosmin production by *S. halstedii* as evidenced by higher geosmin production in *S. halstedii* cultures containing *O. tenuis*-culture supernatant when compared to *S. halstedii* cultures containing *O. tenuis* cell pellets. The control and prevention of cyanobacterial blooms may help indirectly in reducing off-flavor episodes, even if the dominant species is a non-odor producer, and if additional synergistic relationships exist between cyanobacterial species and actinomycetes not tested in this study. In the absence of cyanobacterial blooms and off-flavor-producing species of cyanobacteria, actinomycetes may contribute to off-flavor episodes using cyanobacterial metabolites in the formation of off-flavor compounds. Additional field studies are needed to indicate a more direct link between cyanobacteria bloom formation/die-off and actinomycete development in ponds and lakes.

Management practices to control and prevent cyanobacterial blooms include the use of copper sulfate and chelated-copper compounds. Periodic applications of copper sulfate to aquaculture ponds have been useful in controlling populations of *Microcystis* (7), and van der Ploeg (29) found that applications of copper sulfate to shallow areas of monitored aquaculture ponds appeared to reduce an odor-producing *Oscillatoria* species. Although levels of *Oscillatoria* species were diminished, copper sulfate did not reduce the abundance of phytoplankton. In addition to the use of copper sulfate, the application of other phytotoxic compounds may be useful in controlling cyanobacteria. The discovery of compounds that are selectively toxic towards cyanobacteria, environmentally safe, and pose no threat to human health could be helpful in preventing off-flavor episodes in ponds, lakes, and reservoirs.

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