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Effects of carbon source, phosphorus concentration, and several micronutrients on biomass and geosmin production by *Streptomyces halstedii*

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The effects of various carbon sources, phosphorus concentration, and different concentrations of the micronutrients calcium, cobalt, copper, iron, manganese, potassium, and zinc were determined on biomass dry weight production, geosmin production, and geosmin/biomass (G/B) values for *Streptomyces halstedii*, a geosmin-producing actinomycete isolated from the sediment of an aquaculture pond. Of the substrates tested, maltose as a sole carbon source promoted maximal growth by *S. halstedii* while mannitol promoted maximal geosmin production, and galactose yielded the highest G/B values. Fish-food pellets and galactose were poor substrates for growth. Increasing phosphorus concentrations enhanced geosmin production and G/B values. Of the seven micronutrients tested, zinc, iron, and copper had the most profound effects on biomass and geosmin production. Increasing zinc concentrations promoted biomass production while inhibiting geosmin production and G/B values; increasing concentrations of copper and iron inhibited biomass and geosmin production. Increased copper concentrations had the greatest effect in preventing growth and geosmin production by *S. halstedii*. *Journal of Industrial Microbiology & Biotechnology* (2001) **26,** 241–247.

Keywords: actinomycete; biomass production; geosmin; micronutrients; off-flavor; *Streptomyces halstedii*

Introduction

Off-flavor episodes in channel catfish production ponds are most commonly due to the presence of the earthy-odor compound geosmin [22] and/or the musty-odor compound 2-methylisoborneol (MIB) [28,29]. The unpalatable flavor imparted to the flesh of catfish by these compounds causes economic losses to the catfish industry due to the reduced supply of marketable "on-flavor" fish, the inability to sell market-size fish, and the costs incurred during off-flavor removal [36]. Geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol) [17] and 2-methylisoborneol (1,2,7,7-tetramethyl-*exo*-bicyclo-heptan-2-ol) [18] are produced by actinomycetes [2,19,34,41] and cyanobacteria [21,27,30,34,38]. Although cyanobacteria are attributed with being major contributors to off-flavor episodes in aquaculture ponds in the southeastern United States, actinomycetes have been implicated as the cause of earthy and musty off-flavor episodes in other aquatic ecosystems such as municipal water supply reservoirs [16,41]. Actinomycetes may contribute to off-flavor episodes in aquaculture ponds and lakes following cyanobacterial bloom die-offs [35].

The effects of several environmental and chemical factors on geosmin synthesis by actinomycetes have been studied in attempts to better define the role of actinomycetes in off-flavor episodes and to determine conditions favorable for production of off-flavor compounds [35]. Most geosmin-producing actinomycetes isolated thus far have been identified as belonging to the genus *Streptomyces*. Studies on environmental and chemical factors influencing geosmin production by *Streptomyces* spp. have included effects of different temperatures [3,11,14,41], pH [3,14,41], and carbon sources [14,37,41]. Dionigi *et al* [12] studied the effects of several metals on biomass and geosmin production by *Streptomyces tendae*.

The micronutrients tested in this study include calcium, cobalt, copper, iron, manganese, potassium, and zinc. These micronutrients have been identified as having considerable influence on the yield of several actinomycete secondary metabolites [42].

Differences in micronutrient concentrations can greatly influence secondary metabolite production by actinomycetes, and geosmin is a secondary metabolite of actinomycetes. The presence, or particular concentration, of a specific micronutrient may induce (or repress) the geosmin biosynthetic pathway and stimulate (or diminish) its production. This study determines the effects of carbon source, phosphorus concentration, and several micronutrients (trace elements) on biomass and geosmin production by *Streptomyces halstedii*.

Materials and methods

Culture conditions

S. halstedii, a geosmin-producing actinomycete isolated from the sediment of an Alabama aquaculture pond [34], was used. For each study, 250 ml of liquid medium in 1-l Erlenmeyer flasks (in triplicate) were inoculated with a spore/propagule preparation of *S. halstedii* and incubated 5 days at 200 rpm at 30°C.

For the study on the effects of various carbon sources on biomass and geosmin production, Romano–Safferman (RS) [33]

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medium with yeast extract (0.05%, w/v) and a carbon source (2.0%, w/v) was used. Media were adjusted to pH 9 using sodium hydroxide (NaOH) pellets and then filter sterilized using Nalgene 0.2-μm pore-size membrane filters (Nalge, Rochester, NY). Carbon sources tested included cellobiose, fructose, galactose, glucose, lactose, maltose, sucrose, glycerol, mannitol, acetate, citrate, lactate, succinate, aspartate, glutamate, and 32% crude protein fish-food pellets (manufactured for the Alabama Farmers Cooperative, Decatur, AL). Control cultures did not contain any added carbon source.

To study the effects of phosphorus concentration on growth and geosmin production, an RS broth with 2% (v/v) glycerol as a carbon source and monobasic sodium phosphate ($NaH_2PO_4 \cdot H_2O$) as a source of phosphorus was used. This medium was adjusted to pH 9 using NaOH pellets and filter sterilized using Nalgene 0.2-μm pore-size membrane filters.

Total phosphorus (P) concentrations in aquaculture pond waters seldom exceed 1000 μg/l with a reported average of 170 μg/l in fertilized fish ponds in Alabama [4]. The highest phosphorus concentration tested in this study was 1123 μg P/l (36.2 μM). Control cultures did not contain any added P source.

To determine the effects of different concentrations of several micronutrients on biomass and geosmin production, a defined RS broth (pH 9) with 2% (v/v) glycerol as a carbon source was used; yeast extract, which could possibly provide several trace elements, was not added. Demineralized water was used to prepare stock solutions and media. The micronutrients studied included calcium, cobalt, copper, iron, manganese, potassium, and zinc.

Fish-pond sediment samples obtained seasonally during a 2-year period were analyzed by the United States Department of Agriculture Soil Testing Laboratory, Auburn University, Alabama, to determine the concentrations of various micronutrients in the pond sediments and help identify levels for testing. The RS medium used for the potassium effects study contained monobasic sodium phosphate ($NaH_2PO_4 \cdot H_2O$) instead of dibasic potassium phosphate (K_2HPO_4) as listed in the original medium recipe [33]. A stock solution (100 mg/l) of copper sulfate ($CuSO_4 \cdot 5H_2O$) was prepared for the copper effects study. A 200 mg/l stock solution of cobaltous chloride ($CoCl_2 \cdot 6H_2O$), 200 mg/l stock solution of manganous chloride ($MnCl_2 \cdot 4H_2O$), and 10 mg/l stock solution of ferrous sulfate ($FeSO_4 \cdot 7H_2O$) were prepared for the cobalt effects, manganese effects, and iron effects studies, respectively. Dibasic potassium phosphate ($K_2HPO_4 \cdot 3H_2O$), calcium carbonate ($CaCO_3$), and zinc powder were weighed to obtain necessary concentrations of each for the potassium effects, calcium effects, and zinc effects studies, respectively. Concentrations above 3.3 and 430.7 μM of calcium and iron, respectively, were not tested due to incomplete solubilization of these elements in the media at these higher levels.

Biomass measurement and culture extraction

A 50-ml sample of each 250-ml culture was centrifuged for 20 min at 16,000$\times$g, the supernatant was removed, and pellets were dried to constant weight at 80°C. The remaining 200 ml of culture in each flask were distilled, recovering 40 ml (20% of culture volume) of distillate. Distillates were extracted successively with 20% and 10% (v/v) volumes of analytical grade methylene chloride [20]. Combined extracts were concentrated in a stream of dry air to 0.5 ml for gas chromatographic analysis. If geosmin was not detected in the extracts concentrated to 0.5 ml, these extracts were further concentrated to 0.1 ml before repeating gas chromatography.

Analytical tests

Quantitation of geosmin was achieved using a Perkin-Elmer model 8500 gas chromatograph (GC) (Perkin-Elmer, Norwalk, CT) equipped with a flame ionization detector (FID). A Stabilwax fused silica capillary column (30 m$\times$0.25 mm i.d.$\times$0.25 μm film thickness; Supelco, Bellefonte, PA) 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/s with helium used as the carrier gas, and splitting and purging of the injected sample (50:1) occurred 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 g/l in hexane) was added in 5.0-μl volumes to each 0.5 ml of concentrated extract sample immediately before gas chromatography 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, Dallas, TX) 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.

Data analysis

Mean and standard deviations of biomass and geosmin production caused by differences in carbon source, phosphorus concentration, and micronutrient concentration were determined. Regression analyses were used to determine the relationships of geosmin, biomass, and geosmin/biomass (G/B) values with phosphorus concentration and micronutrient concentration.

Results and discussion

Carbon source effects

In actinomycetes, carbon sources that can readily serve as growth substrates, such as glucose, often repress secondary metabolism [10]. An example of catabolite repression of secondary metabolism in actinomycetes is that of actinomycin synthesis by *Streptomyces antibioticus* after glucose is added to the medium [15]. In some cases, repression of antibiotogenesis (antibiotic synthesis) has been linked to the repression of idiolite synthetases by certain carbon sources. For example, glucose, mannose, fructose, maltose, and lactose have been reported to repress *N*-acetylkanamycin amidohydrolase, a synthetase for kanamycin production by *Streptomyces kanamyceticus* [10]. For most actinomycetes, glucose is an excellent carbon source for growth compared to glycerol [40]. Exceptions include *Streptomyces clavuligerus*, a cephamycin producer, which does not utilize glucose but favors glycerol for growth while antibiotogenesis is inhibited [1]. Catabolite inhibition of secondary metabolism (idiolite production) by a specific carbon source can occur due to inactivation of those enzymes necessary for idiolite production. Cortés *et al* [9] have reported that glucose-6-phosphate inhibits deacetoxycephalosporin C synthetase in the actinomycete *Nocardia lactamdurans*.

In our study, maltose used as a sole carbon source yielded the greatest biomass production (810 μg dry weight/ml) by *S. halstedii* (Figure 1). Succinate, lactose, and mannitol also favored high biomass production (650, 580, and 580 μg dry weight/ml, respectively). Lowest biomass production (50 μg dry weight/ml) occurred with fish-food pellets used as the sole carbon source, with galactose and aspartate also yielding low biomass production (62.5 and 230 μg dry weight/ml, respectively). Lowest geosmin production (0.1 ng/ml) and G/B values (1.9 ng/mg dry weight) by *S. halstedii* occurred with fish-food pellets used as the sole carbon source. Citrate also yielded low amounts of geosmin (2.2 ng/ml) and G/B values (5.3 ng/mg dry weight). Highest geosmin production (145.4 ng/ml) occurred with mannitol, and the highest G/B value (635.9 ng/mg dry weight) occurred with galactose as the sole carbon source.

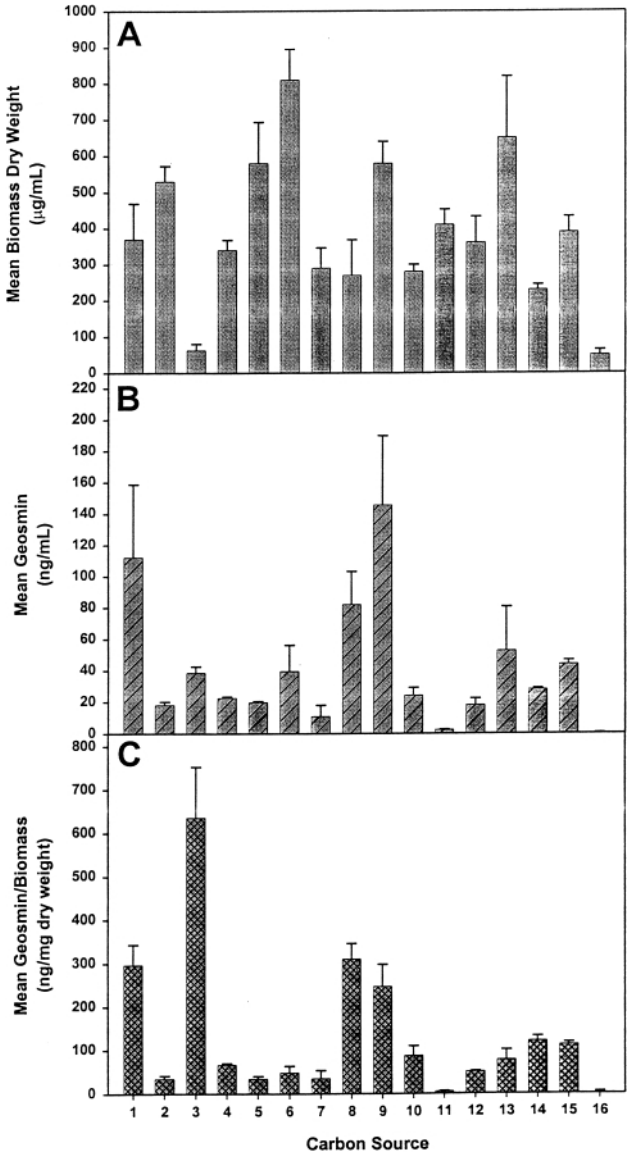

Figure 1 Effects of different carbon sources on yield of biomass (A), geosmin (B), and G/B (C) by *S. halstedii*. On *x*-axis, 1 = cellobiose, 2 = fructose, 3 = galactose, 4 = glucose, 5 = lactose, 6 = maltose, 7 = sucrose, 8 = glycerol, 9 = mannitol, 10 = acetate, 11 = citrate, 12 = lactate, 13 = succinate, 14 = aspartate, 15 = glutamate, and 16 = fish food. Values are obtained after subtracting control results. Narrow bars represent the standard deviation, $n = 3$.

Sivonen [37] reported that glucose, fructose, and mannitol are the most widely used carbon sources for growth by 18 actinomycete strains isolated from natural waters in Finland. In the same study, *Streptomyces flavogriseus* and *Streptomyces coelicolor* produced an earthy odor when grown in medium containing either glucose or mannitol. Also, *Streptomyces galbus* produced an earthy odor when grown in medium containing either glucose, fructose, or rhamnose. Weete *et al* [41] reported that cellobiose, glucose, and glycerol as sole carbon sources yielded greatest biomass dry weight production by a *Streptomyces* species (33L) isolated from the sediment of a lake. This *Streptomyces* species (33L) also produced the greatest amounts of geosmin and G/B when grown in medium using succinate as a sole carbon source.

Glycerol was substituted for glucose as the carbon source in the RS medium used in the other studies on the effects of chemical factors on biomass and geosmin production by *S. halstedii*. Glycerol favored growth as well as high G/B values (310.2 ng/mg dry weight) compared to the other carbon sources tested (Figure 1). The type of catfish food (32% crude protein) tested in this study appears to be a poor carbon source for promoting biomass and geosmin production by *S. halstedii*.

Phosphorus concentration effects

Phosphate can directly regulate secondary metabolism in actinomycetes. Martín and Demain [25] reported that biosynthesis of the antibiotic candicidin by *Streptomyces griseus* is controlled by phosphate concentration, with phosphate concentrations at 5–10 mM greatly inhibiting candicidin formation due to a decreased rate of precursor molecule incorporation into the antibiotic. In another study, production of the antibiotic tylosin by a *Streptomyces* species was inhibited by high phosphate concentrations (30 mM) while biomass production was unaffected [23]. Streptomycin biosynthesis is also inhibited by excessive phosphate concentrations ($\geq$10 mM) [32]. Once phosphate becomes depleted in growth media, biosynthesis of candicidin occurs [26]. One mechanism of phosphate control of secondary metabolism is by repression of phosphatases that dephosphorylate intermediates during aminoglycoside (a classification of antibiotics) biosynthesis [24].

In our study, maximal geosmin production (13.5 ng/ml) and the maximal G/B value (116.1 ng/mg dry weight) for *S. halstedii* occurred at the highest phosphorus concentration tested (36.2 μM) (Figure 2). Geosmin production did not occur at the lowest phosphorus concentrations tested (0.7 and 3.6 μM), while biomass production was generally higher at the higher phosphorus concentrations tested (18.1–36.2 μM) than at the lower phosphorus concentrations tested (0.7–7.3 μM). Regression analyses revealed that no strong linear relationship exists between phosphorus concentration (0.7–36.2 μM) and biomass and geosmin production by *S. halstedii*; however, an increase in phosphorus concentration strongly effected a linear increase in G/B values. Higher concentrations of phosphate ($\geq$5 mM) than used in our study have been found to inhibit idiolite biosynthesis [25].

Micronutrient effects

Micronutrients (trace elements) are known to affect the production of some secondary metabolites produced by actinomycetes. The concentration range of a trace element is much narrower for permitting secondary metabolism than the concentration range that permits superb vegetative growth [42]. Calcium and copper usually do not affect the secondary metabolism of actinomycetes [42]; these

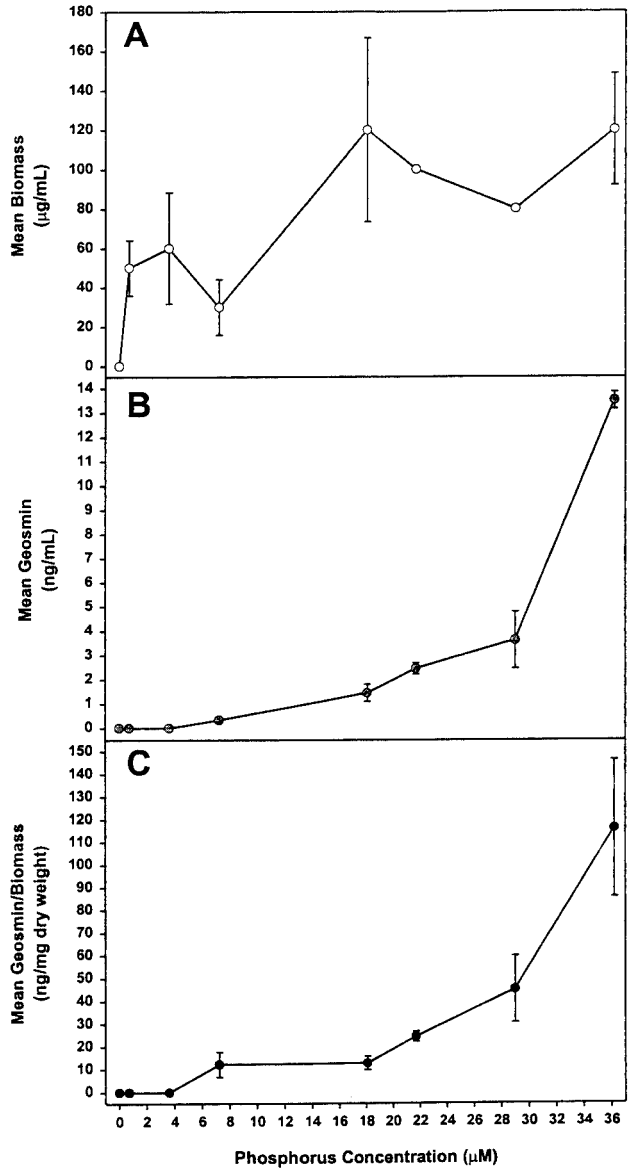

Figure 2 Effect of different concentrations of phosphorus on yield of biomass (A), geosmin (B), and G/B (C) by *S. halstedii*. Values are obtained after subtracting control results. Error bars represent the standard deviation, $n = 3$.

trace elements were included in this study since changes in the concentration of each element can have a direct impact on pond water chemistry. Calcium hardness of water is the concentration of calcium expressed as equivalent calcium carbonate and varies for fish ponds depending upon the amount of limestone present in the pond bottom or the amount of lime applied to the pond [7].

In our study, increasing calcium concentrations (0.0–3.3 μM) caused a decrease in biomass and geosmin production by *S. halstedii* and in G/B values (Figure 3). Maximal biomass (830 μg dry weight/ml) and geosmin production (57.9 ng/ml), and the highest G/B value (68.9 ng/mg dry weight) occurred in the absence of calcium. Regression analysis revealed a linear relationship (r^2=0.81) between increasing calcium concentration and decreasing biomass production by *S. halstedii*. Liming can increase pond water and sediment pH [7], and a higher pH has been found to promote geosmin production in previous studies using *S. halstedii*

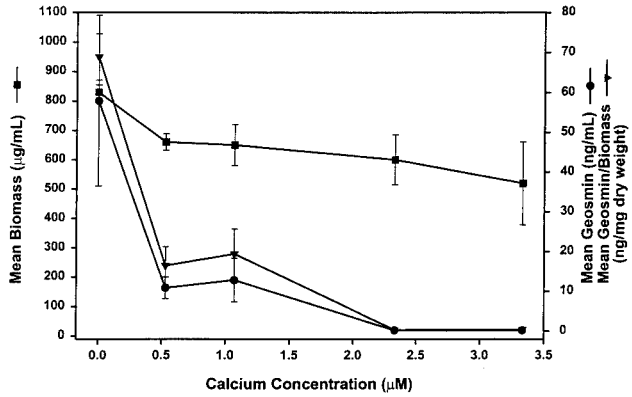

Figure 3 Effect of different concentrations of calcium on yield of biomass, geosmin, and G/B by *S. halstedii*. Values are obtained after subtracting control results. Error bars represent the standard deviation, $n = 3$.

and in another *Streptomyces* species [41]. However, increasing concentrations of calcium in pond water and sediments may help counteract the stimulatory effects of higher pH values that promote geosmin production by certain Streptomycetes [3].

Copper sulfate and/or chelated copper algicide application to fish ponds (and water reservoirs) to kill existing phytoplankton blooms would be expected to cause changes in pH, dissolved oxygen, ammonia, and carbon dioxide levels of pond waters [6]. Van der Ploeg [39] found that free copper concentrations in pond waters increased following copper sulfate treatments, but 50–70% of the applied copper disappeared from the pond water within 24 h after treatment. The free copper content of the pond water returned to pretreatment levels within 1–2 weeks. Free copper levels decline rapidly due to precipitation of copper as malachite [$Cu_2(OH)_2CO_3$] or tenorite (CuO), complexation with amino acids, carbonates, hydroxides, polypeptides, and humic substances, or chelation by organic matter such as during adsorption by pond sediments [5]. Following copper sulfate treatment of ponds, copper has been observed to accumulate in pond sediments due to precipitation and/or the adsorption of copper by these sediments [31].

Increasing concentrations of copper inhibited biomass and geosmin production by *S. halstedii* (Figure 4). Maximal biomass (620 μg/ml) and geosmin (20.0 ng/ml) production and maximal

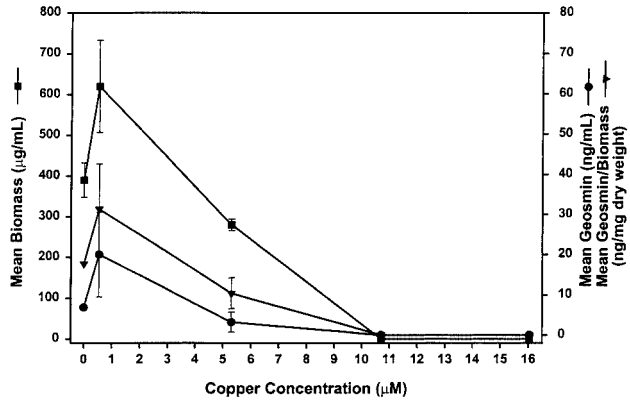

Figure 4 Effect of different concentrations of copper on yield of biomass, geosmin, and G/B by *S. halstedii*. Values are obtained after subtracting control results. Error bars represent the standard deviation, $n = 3$.

G/B value (31.3 ng/mg dry weight) occurred at 0.54 μM copper while complete inhibition of growth and geosmin production occurred at and above 10.7 μM copper. A very small amount of copper (0.54 μM) appears to stimulate geosmin production by *S. halstedii* (Figure 4). Regression analyses revealed linear relationships between an increase in copper concentration and a decrease in biomass production (r^2=0.82) and with a decrease in G/B values (r^2=0.76) for *S. halstedii*. Our results differ greatly from those of Dionigi *et al* [12] who reported growth and geosmin production by *S. tendae* on yeast–maltose medium containing 1 mg/l copper (15.74 μM) and 5 mg/l copper (78.7 μM). Although different species of Streptomycetes were used in each study, the large difference in results between the two studies is probably due to the difference in the substrate composition of the media used in each study [12].

Copper inhibits photosynthesis and respiration in algae [7] and may inhibit respiration in actinomycetes. Domek *et al* [13] found that copper impairs aerobic respiration in *Escherichia coli*. Copper sulfate application to fish ponds may actually inhibit geosmin production by actinomycetes in the water column for a brief period until copper concentrations in the pond return to preapplication levels. Inhibitory effects may be greater in pond (and lake) sediments where copper has accumulated following copper sulfate and chelated copper product treatments [31].

Maximal biomass production (1190 μg dry weight/ml) by *S. halstedii* occurred at a cobalt concentration of 11.2 μM while maximal geosmin production (12.9 ng/ml) and G/B value (14.4 ng/mg dry weight) occurred with 1.1 μM cobalt (Table 1). Statistical analyses did not reveal any strong, linear relationships between changes in cobalt concentration and biomass and geosmin production and G/B values. The substantial production of 8.1 ng geosmin/ml in the absence of cobalt indicates that this element is not essential for geosmin production by *S. halstedii*. Dionigi *et al* [12] found that cobalt did not induce geosmin biosynthesis in cultures of *S. tendae*.

Table 1 Effect of different concentrations of cobalt, iron, manganese, and potassium on biomass and geosmin production, and G/B values for *S. halstedii*

Micronutrient (μM)		Mean[a] biomass (μg/ml)	Mean[a] geosmin (ng/ml)	Mean[a] G/B (ng/mg dry wt.)
Co	0	960±113.1	8.1±2.0	8.4±1.1
	1.1	893±30.6	12.9±2.1	14.4±2.5
	5.6	900±0	6.5±1.2	7.2±1.3
	11.2	1,190±99.0	9.2±0.6	7.8±1.2
	33.6	1,160±28.3	11.7±2.4	10.1±1.8
Fe	0	670±14.1	16.8±1.3	25.1±2.5
	0.2	800±72.1	26.2±5.8	33.1±8.5
	0.4	730±70.7	2.8±1.1	3.8±1.1
	3.6	690±14.1	2.1±0.2	3.0±0.2
	47.9	480±141.4	1.3±1.0	3.2±3.0
	430.7	210±99.0	0.7±0.3	3.6±0.4
Mn	0	770±42.4	8.2±2.4	10.8±3.8
	15.2	760±141.4	11.2±2.0	15.3±5.4
	152.0	610±14.1	8.2±0.5	13.4±0.5
	1,820.0	680±84.9	3.5±3.2	5.0±4.0
K	0	300±28.3	3.2±1.9	10.3±5.3
	0.4	420±52.9	8.8±3.9	21.0±8.8
	1.8	573±41.6	13.3±2.3	23.2±2.9
	3.2	490±14.1	11.1±1.0	22.8±2.8

[a]Mean±SD (n=3).

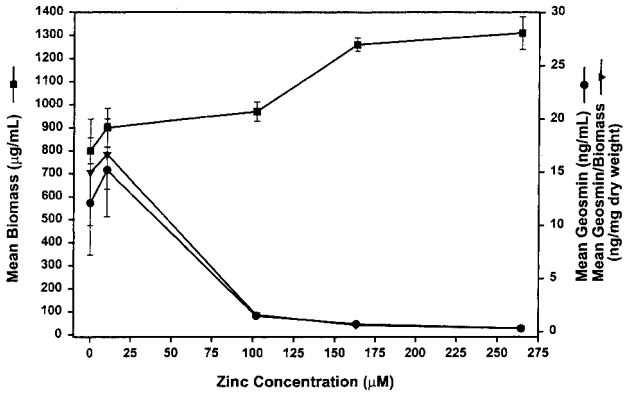

Figure 5 Effect of different concentrations of zinc on yield of biomass, geosmin, and G/B by *S. halstedii*. Values are obtained after subtracting control results. Error bars represent the standard deviation, n = 3.

Maximal biomass (800 μg dry weight/ml) and geosmin (26.2 ng/ml) production by *S. halstedii* and maximal G/B value (33.1 ng/mg dry weight) occurred at 0.2 μM of iron (Table 1). Higher concentrations of iron tested in this study inhibited biomass and geosmin production. Lowest biomass (210 μg dry weight/ml) and geosmin production (0.7 ng/ml) occurred at 430.7 μM of iron while the lowest G/B value (3.0 ng/mg dry weight) occurred at 3.6 μM iron. Decreasing biomass production by *S. halstedii* was strongly related to increasing iron concentration above 0.2 μM; this inverse relationship was linear (r^2=0.83). A nonlinear relationship was found between an increase in iron concentration (0–430.7 μM) and geosmin production by *S. halstedii* even though low levels of iron (up to 0.2 μM) were stimulatory for geosmin production (Table 1). Although other studies have found a linear relationship between the amount of secondary metabolite produced and the amount of iron present, a decrease in secondary metabolite production eventually occurs as iron levels continue to increase [42]. For example, streptomycin production by *S. griseus* increases linearly up to 10 μM iron and then decreases as the iron concentration approaches 1000 μM [8]. Substantial geosmin production (16.8 ng/ml) occurred in the absence of iron indicating that iron is not essential for geosmin production by *S. halstedii*.

Manganese concentration did not have a strong effect on biomass production while increasing concentrations of manganese inhibited geosmin production (Table 1). Geosmin was produced in the absence of manganese indicating that manganese is not essential for geosmin production by *S. halstedii*. Increasing manganese concentration was slightly stimulatory for G/B values up to 15.2 μM. Decreasing geosmin production and G/B values correlated with increasing concentrations of manganese (r^2=0.77 and 0.83, respectively). Manganese concentrations control the quantity of spiramycin produced by *Streptomyces ambofaciens* without having an effect on vegetative growth [42].

Potassium concentration did not reveal a strong effect on biomass and geosmin production (Table 1). At 1.8 mM potassium, maximum biomass (573 μg dry weight/ml) and geosmin (13.3 ng/ml) production and G/B value (23.2 ng/mg dry weight) occurred. No strong, linear relationships were found for potassium concentration with biomass dry weight production, geosmin production, or G/B values. High amounts of potassium in fertilizers used for fish ponds would appear not to be a stimulating factor for geosmin production by actinomycetes based on the results of this study.

Maximal biomass production (1310 μg dry weight/ml) occurred at the highest zinc concentration tested (264.5 μM) (Figure 5). Geosmin production was inhibited and G/B values were less at high zinc concentrations, while greatest geosmin production (15.2 ng/ml) and G/B value (16.7 ng/mg dry weight) occurred at the lowest zinc concentration tested (10.2 μM). Biomass production increased with increasing zinc concentration while geosmin production and G/B values decreased. Regression analysis revealed strong relationships between an increase in zinc concentration effecting an increase in biomass production (r^2=0.90) while causing a decrease in geosmin production (r^2=0.75) and G/B values (r^2=0.76) for *S. halstedii*. Other studies [42] have found that lower zinc concentrations permit greater secondary metabolite production. Yields of streptothricin formed by *Streptomyces flavotricini* were enhanced by lowering zinc concentrations from 173 down to 0.6 μM [42]. Increases in zinc concentration promoted biomass production while inhibiting or repressing geosmin synthesis by *S. halstedii*.

Of the trace elements tested in this study, copper had the most profound effect on biomass and geosmin production and G/B values. Copper inhibited biomass and geosmin production completely at a concentration as low as 10.7 μM. Copper applied in the form of copper sulfate to the sediments of drained fish ponds might help prevent future off-flavor episodes.

The molecular events which occur to allow trace elements to control secondary metabolism in actinomycetes have not yet been determined. Elucidation of the complete biosynthetic pathway of geosmin production in actinomycetes would permit trace element effects studies on specific enzymatic steps in geosmin synthesis. Many physical and chemical factors appear to play a primary role in regulating geosmin production; however, until the complete geosmin biosynthetic pathway is known, the most imperative physical and chemical factors in directly regulating geosmin production will remain unknown.

Acknowledgements

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