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Effects of several metals on spore, biomass, and geosmin production by *Streptomyces tendae* and *Penicillium expansum*

CP Dionigi, TS Ahten and LH Wartelle

United States Department of Agriculture, New Orleans, LA 70179, USA

Cultures of *Streptomyces tendae* and *Penicillium expansum* grown on Actinomyces and Czapek's media, respectively, were exposed to 5 mg L^{-1} of manganese, magnesium, iron, cobalt, nickel, copper and zinc, supplied as sulfate salts. Only copper markedly increased geosmin (1α, 10β-dimethyl-9α-decalol), biomass, and spore production. Inductively coupled plasma-atomic emission spectrometric analysis of *S. tendae* and *P. expansum* cells did not indicate an accumulation of copper. Both 1 and 5 mg L^{-1} copper, as copper sulfate, increased total geosmin production in cultures of *S. tendae* on several media, but decreased production on others, suggesting that substrate composition affects responses to copper.

Keywords: off-flavor; heavy metals; geosmin production; *Streptomyces tendae*; *Penicillium expansum*

Introduction

Several taxa of cyanobacteria, bacteria, fungi, and other soil or aquatic organisms produce the earthy odorant geosmin (1α, 10β-dimethyl-9α-decalol, Registry No. GSM, 19700-21-1) [15,22] from sesquiterpene precursors [3], possibly by a pathway similar to that reported for the biosynthesis of dehydrogeosmin in higher plants [11]. The odor of geosmin and other microbial metabolites can prompt complaints concerning the safety and quality of indoor air, potable water, aquaculture-produced fish, and other resources [16,19,20,22]. In addition, volatile organic compounds may indicate populations that produce potential toxins and/or spores that may contribute to 'sick building syndrome' [10,23].

Copper is present in many architectural and agricultural substrates and may be added to resources as biocides, such as copper sulfate and copper chelate, to control problematic populations [1,4]. However, copper has been reported to increase spore and geosmin production by *Streptomyces tendae* and *Penicillium expansum* [8,9]. Although these microbes may reduce the quality of food and environmental resources [17,22], reports concerning the effects of metals on geosmin, biomass, and spore production by problematic microbes were not found. The purpose of this investigation was to determine the effects of additional metal cations on geosmin, biomass, and spore production by *S. tendae* and *P. expansum*.

Materials and methods

Inoculum and medium preparation

Cultures of *P. expansum* Link and *S. tendae* were obtained from JP Mattheis and RG Roberts of the USDA-ARS Tree Fruit Laboratory, Wenatchee, WA, USA and the American Type Culture Collection (ATCC 31160), respectively. *P. expansum* and *S. tendae* cultures were produced in culture plates containing Czapek medium [20] and Hickey–Tresner medium [12] at 20°C and 28°C, respectively as described by Dionigi and Champagne [8]. All media contained 1.5% (w/v) agar and were covered with a sterile 90-mm diameter 0.05-μm pore-size polycarbonate membrane. The media used for test cultures were: Czapek [20], Actinomyces [2], Nutrient (ATCC 24, Difco 0001), Hickey–Tresner [13], Yeast-malt extract (ATCC 196, Difco 0770), and Inorganic-salts-starch (ATCC 527, Difco 0772).

Metal solutions

$MnSO_4 \cdot H_2O$, $CuSO_4 \cdot 5H_2O$, $FeSO_4 \cdot 7H_2O$ and $ZnSO_4 \cdot 7H_2O$ were obtained from JT Baker Chemical Co, Phillipsberg, NJ, USA. $CoSO_4 \cdot 7H_2O$, $MgSO_4 \cdot 7H_2O$, and $NiSO_4 \cdot 6H_2O$ were from Mallinckrodt Specialty Chemical Co (Paris, KY, USA), Fisher Scientific (Fairlawn, NJ, USA) and EM Science (Cherryhill, NJ, USA) respectively. Each stock metal solution was prepared with deionized water and added prior to autoclaving the medium.

Biomass and geosmin accumulation

Each membrane with its surface biomass was placed in a 20-ml glass liquid scintillation vial, weighed, and covered with 12 ml of gas chromatography-grade hexane. One hundred microliters of a 25 mg kg^{-1} stock solution of 2-undecanone in ethanol and 100 μl of a 25 mg kg^{-1} stock solution of chlorododecane in ethanol were added to each vial as internal standards. Geosmin was detected and quantified as described by Dionigi and Champagne [8] using a gas chromatograph equipped with a flame ionization detector, autosampler, and computing integrator [7].

Determination of metal content

Cultures of *S. tendae* and *P. expansum* were established in 90-mm petri dishes on polycarbonate membrane-covered Actinomyces and Czapek agar media [21], respectively. Each medium contained either 5.0 mg L^{-1} of copper, manganese, magnesium, iron, cobalt, nickel, or zinc. After 48 h of incubation, 1 g of mycelium was removed from

Correspondence: CP Dionigi, United States Department of Agriculture, Agricultural Research Service, Southern Regional Research Center, 1100 Robert E Lee Blvd, PO Box 19687, New Orleans, LA 70179, USA
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Table 1 Response of *S. tendae* and *P. expansum* cultures to supplementation with several metals[a]

Metal	*S. tendae*			*P. expansum*		
	Fresh weight (g)	Geosmin (mg L^{-1})	Total Geosmin (mg $culture^{-1}$)	Fresh weight (g)	Geosmin (mg L^{-1})	Total Geosmin (mg $culture^{-1}$)
Untreated	0.50 ± 0.001	1.32 ± 0.24	0.66	2.19 ± 0.06	0.30 ± 0.01	0.66
Mg	0.53 ± 0.01	1.44 ± 0.09	0.76	2.24 ± 0.05	0.25 ± 0.01	0.57
Mn	0.53 ± 0.004	0.99 ± 0.11	0.53	2.16 ± 0.06	0.28 ± 0.01	0.61
Fe	0.55 ± 0.02	2.04 ± 0.13	1.12	2.15 ± 0.06	0.29 ± 0.01	0.62
Co	0.55 ± 0.01	1.22 ± 0.17	0.67	1.79 ± 0.04	0.28 ± 0.01	0.51
Ni	0.51 ± 0.001	0.64 ± 0.06	0.33	2.20 ± 0.05	0.38 ± 0.01	0.82
Cu	0.66 ± 0.01	5.27 ± 0.46	3.48	2.55 ± 0.12	0.70 ± 0.03	1.79
Zn	0.52 ± 0.001	0.68 ± 0.08	0.35	2.01 ± 0.03	0.22 ± 0.01	0.44

[a](5 mg L^{-1}) ± 2 s.e.m., $n = 4$.

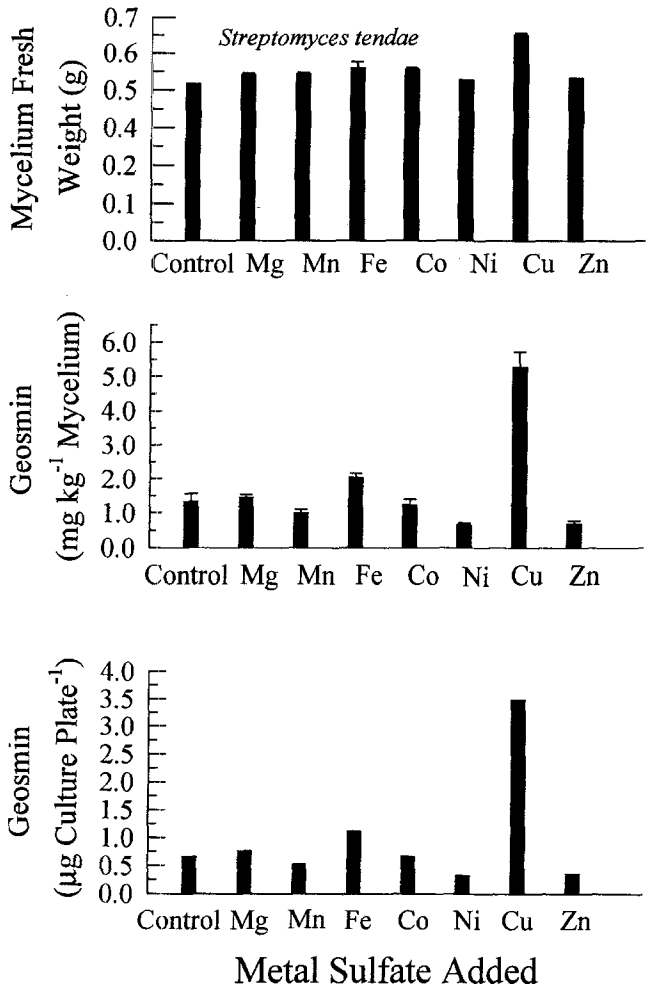

Figure 1 Effects of 1 and 5 mg L^{-1} each of sulfate salts of magnesium, manganese, iron, cobalt, nickel, and zinc with and without 1 and 5 mg L^{-1} copper on biomass, geosmin concentration, and the total geosmin content of *Streptomyces tendae* cultures. Narrow bars are ± 2 s.e.m., $n = 4$.

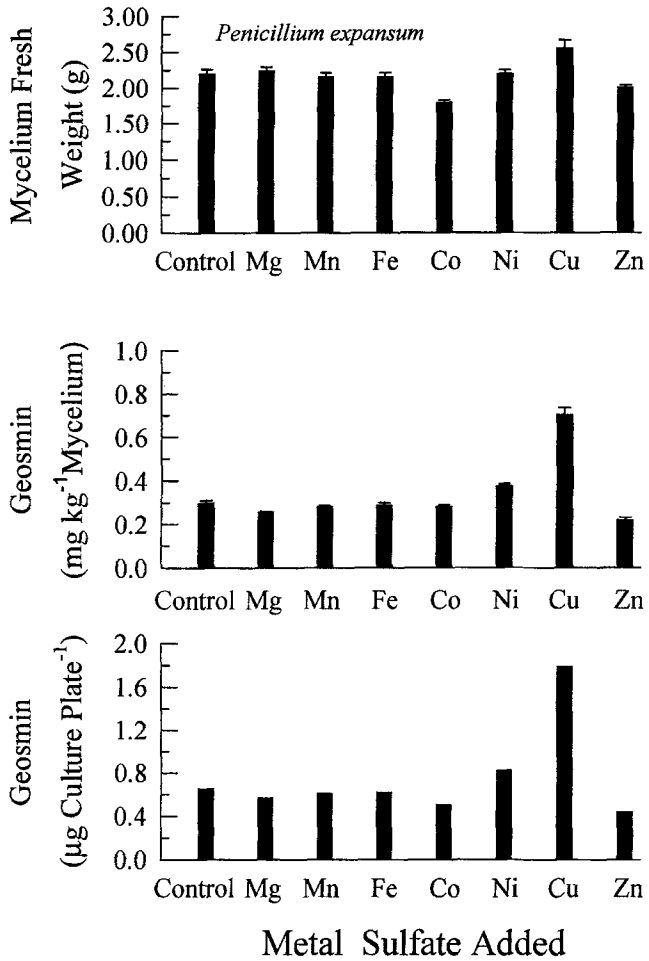

Figure 2 Effects of 1 and 5 mg L^{-1} each of sulfate salts of magnesium, manganese, iron, cobalt, nickel, and zinc with and without 1 and 5 mg L^{-1} copper on biomass, geosmin concentration, and the total geosmin content of *Penicillium expansum* cultures on Czapek medium. Narrow bars are ± 2 s.e.m., $n = 4$.

each membrane, rinsed in 10 ml of double-glass-distilled ultra-filtered sterile water and pelleted by centrifugation. The wash solution was discarded and this process was repeated before the mycelium was digested in 10 ml of 5%(v/v) trace metal-grade nitric acid (Fisher Scientific). The acid digest was analyzed by Inductively Coupled Atomic Plasma Spectrometry (Leeman Labs, Inc, Lowell, MA, USA) [8]. Spectral lines at 228.616, 324.754, 238.204, 285.213, 257.610, 232.003, and 213.856 nm were independently used for determining cobalt, copper, iron, magnes-

Table 2 Metal concentration in *S. tendae* and *P. expansum* cells supplementation with several metals[a]

Metal	*S. tendae*		*P. expansum*	
	Untreated cells (mg kg^{-1})	Treated–Untreated cells (mg kg^{-1})	Untreated cells (mg kg^{-1})	Treated–Untreated cells (mg kg^{-1})
Mg	9.16 ± 0.70	−0.09 ± 0.15	2.92 ± 0.84	−0.40 ± 0.33
Mn	0.01 ± 0.003	0.50 ± 0.12	0.01 ± 0.003	0.21 ± 0.04
Fe	1.17 ± 0.18	0.26 ± 0.19	0.54 ± 0.06	0.65 ± 0.25
Co	0.02 ± 0.01	0.19 ± 0.02	0.01 ± 0.01	1.56 ± 0.36
Ni	0.05 ± 0.01	0.31 ± 0.02	0.07 ± 0.01	0.03 ± 0.02
Cu	0.00 ± 0.00	0.30 ± 0.02	0.00 ± 0.00	0.66 ± 0.54
Zn	0.88 ± 0.11	0.41 ± 0.15	0.17 ± 0.04	0.54 ± 0.09

[a](5 mg L^{-1}) ± 2 s.e.m., $n = 4$.

Table 3 Responses of *S. tendae* cultures to copper sulfate on several media[a]

Medium	0 mg L^{-1} Cu	1 mg L^{-1} Cu	5 mg L^{-1} Cu
		Fresh weight (g)	
Actinomyces	0.53 ± 0.02	0.70 ± 0.03	0.67 ± 0.02
Nutrient	0.51 ± 0.01	0.38 ± 0.05	0.40 ± 0.05
Hickey–Tresner	0.51 ± 0.03	0.54 ± 0.01	0.48 ± 0.01
Yeast-maltose	0.63 ± 0.03	0.59 ± 0.02	0.58 ± 0.02
Inorg-salts-starch	0.10 ± 0.002	0.11 ± 0.001	0.001 ± 0.002
		Geosmin (mg kg^{-1})	
Actinomyces	6.2 ± 0.2	20.9 ± 0.02	18.4 ± 6.5
Nutrient	17.5 ± 0.5	6.9 ± 0.02	15.3 ± 2.1
Hickey–Tresner	25.8 ± 3.2	25.1 ± 0.01	48.1 ± 1.9
Yeast-maltose	31.8 ± 1.4	21.4 ± 0.01	17.0 ± 2.7
Inorg-salts-starch	162.3 ± 16.3	142.6 ± 0.01	0.00 ± 0.0
		Total Geosmin (mg $culture^{-1}$)	
Actinomyces	3.3	14.5	12.3
Nutrient	9.0	25.8	6.1
Hickey–Tresner	13.2	13.5	23.0
Yeast-maltose	20.0	10.7	112.5
Inorg-salts-starch	16.9	16.0	0.0

[a]± 2 s.e.m., $n = 4$.

ium, manganese, nickel, and zinc concentrations in each sample respectively.

Statistical analysis

The effects of each metal on geosmin and biomass production in each microbe were determined in a series of randomized complete block experiments. Replication was achieved by establishing complete blocks within each experiment and by repeating each experiment. Data were combined and subjected to factorial analysis and orthogonal *a priori* contrasts [18].

Results

Cultures of *S. tendae* grown with copper exhibited a greater accumulation of biomass ($P \leq 0.0001$) and geosmin ($P \leq 0.0001$) than cultures treated with the other metals. These increases resulted in a total geosmin production that was about six-fold greater than that observed in untreated controls (Table 1). Supplementation with metals other than copper did not increase biomass or geosmin accumulation by *S. tendae* cultures compared to controls (Table 1). Hickey–Tresner medium contains cobalt [12], and *S. tendae* cultures grown on this medium exhibited consistent spore and geosmin production [5]. However, *S. tendae* cultures grown on Actinomyces medium supplemented with cobalt did not exhibit an increased geosmin production (Table 1).

Cultures of *P. expansum* treated with copper sulfate exhibited increases in biomass and geosmin accumulation compared to untreated cultures (Table 1). These increases were similar to those observed in *S. tendae* cultures. Supplementation of these fungal cultures with sulfates of manganese, magnesium, iron, cobalt, nickel, or zinc did not increase biomass or geosmin accumulation over controls (Table 1).

Inspection of *S. tendae* and *P. expansum* cultures supplemented with copper indicated abundant spore production following 72 h of incubation, whereas spores were not observed in similar cultures treated with sulfates of manganese, magnesium, iron, cobalt, nickel, or zinc following 72 h or 7 days of incubation.

Cultures of *S. tendae* (Figure 1) and *P. expansum* (Figure 2) exposed to 1 or 5 mg L^{-1} copper alone and together with each of the other metals exhibited greater biomass and approximately three-fold increase in geosmin production compared to controls that were not supplemented with copper (Figures 1 and 2).

Metal analyses of *S. tendae* and *P. expansum* cells grown on medium supplemented with manganese, magnesium, iron, cobalt, nickel, copper, and zinc indicated that accumulation of these metals was not closely associated with changes in biomass and geosmin accumulation (Tables 1 and 2). For example, the concentration of copper observed in *S. tendae* and *P. expansum* (Table 2) cells was not different from the mean concentration of the other metals investigated. However, the concentration of geosmin in copper-treated *S. tendae* and *P. expansum* cells was different from the mean geosmin concentration in cells treated with each of the other metals investigated (Table 1). In addition, *P. expansum* cells grown on media supplemented with 5 mg L^{-1} cobalt indicated a greater concentration of this metal compared to copper-treated cells (Table 2). However, *P. expansum* cultures treated with cobalt exhibited a lower concentration of geosmin than those treated with copper (Table 1).

Exposure to either 1 or 5 mg L^{-1} copper increased total geosmin production in cultures of *S. tendae* grown on Actinomyces, Nutrient, and Hickey–Tresner media (Table 3). However, similar cultures grown on yeast-maltose-extract and inorganic-salts-starch media containing either 1 or 5 mg L^{-1} copper sulfate exhibited less geosmin than untreated controls (Table 3). Growth on inorganic-salts-starch medium supplemented with 5 mg L^{-1} copper sulfate was inhibited (Table 3).

Discussion

Cells of *S. tendae* grown on a cobalt-containing medium [13] exhibited more consistent geosmin and spore production than cells grown on Actinomyces medium [5]. However, cobalt did not induce geosmin biosynthesis in cultures of *S. tendae*, suggesting that component(s) other than cobalt may induce spore and geosmin production. Of the metals tested, only copper, applied alone and in combination with other metals, stimulated biomass and geosmin accumulation. The lack of stimulation by other metal-sulfates supports previous reports that increases in biomass and geosmin accumulation were not due to addition of sulfate moieties [8]. Metal bioavailability and accumulation by microorganisms can vary [14]. However, increases in geosmin biosynthesis were not correlated with a specific or markedly differential accumulation of copper compared with the other metals tested, suggesting that the observed effects were not due to a differential bioavailability or uptake of copper alone. Responses to copper varied among media, suggesting that substrate composition may alter the affects of copper in the field.

Geosmin is not highly cytotoxic or mutagenic [6]. However, microbial spores and odors are highly problematic in certain environments, and their occurrence may signal the presence of toxigenic populations [23]. *P. expansum* and *S. tendae* are eukaryotic and prokaryotic microbes, respectively, that exhibit differing environmental optima for biomass and geosmin accumulation [7]. If populations growing on architectural substrates [eg 10,20] exhibit similar responses, the bioavailability of copper may affect the occurrence and severity of problems associated with environmental odorants, and conditions such as 'sick building syndrome'. Achieving abundant and consistent growth, reproduction, and secondary metabolite production are important considerations in commercial use of microbes. For example, lowcost 'earthy' fragrances are of interest to the perfume industry [12]. Copper-supplementation may augment microbial growth, reproduction, and the biosynthesis of certain compounds of interest.

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