# Effects of Temperature and Oxygen Concentration on Geosmin Production by *Streptomyces tendae* and *Penicillium expansum*

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*Streptomyces tendae* cultures incubated at 30 and 45 °C for 48 h produced more biomass and more of the earthy odorant geosmin ($1\alpha,10\beta$-dimethyl-$9\alpha$-decalol) than those incubated at 10 and 20 °C, indicating an association between biomass production and geosmin accumulation in these bacterial cultures. However, the fungus *Penicillium expansum* incubated at 20 °C produced ca. 85% more biomass than those incubated at 40 °C, and cells incubated at 40 °C contained an order of magnitude higher concentration of geosmin than those incubated at 20 °C. Exposure to 10% $O_2$ and 90% $N_2$ increased the accumulation of geosmin by *P. expansum* cells compared to ambient atmosphere-incubated controls. However, $O_2$ enrichment (30% $O_2$, 70% $N_2$) increased the geosmin accumulation by cultures of *S. tendae* compared to ambient atmosphere-incubated controls.

## INTRODUCTION

Geosmin ($1\alpha,10\beta$-dimethyl-$9\alpha$-decalol) (see Figure 1) is a widely occurring (Maga, 1987) and potent earthy odorant produced by several types of filamentous bacteria, cyanobacteria, and other organisms (Dionigi, 1993). Recently, the fungus *Penicillium expansum* Thom, which occurs on apples, pears, cherries, and other commodities, has been reported to produce geosmin (Mattheis and Roberts, 1992). Although geosmin is not mutagenic or highly toxic (Dionigi et al., 1993), it is one of the most potent odorants known (Buttery and Garibaldi, 1976). As little as 10–20 ng of geosmin $L^{-1}$ can impart an earthy odor to potable water (Hrudey and Low, 1992). Although geosmin negatively impacts the flavor quality of many food and water resources, geosmin is considered a positive flavor attribute in red table beets (Maga, 1987) and could be useful in perfumery (Finato et al., 1992).

Despite the impact of geosmin on food and water resources, there is a paucity of information concerning the effects of environmental parameters on geosmin biosynthesis. Geosmin production by filamentous bacteria has been reported to occur at 15–38 °C (Blevins, 1980) and may be fostered by increased aeration (Blevins, 1980; Wood et al., 1983). However, the optimal temperatures and $O_2$ concentrations for geosmin biosynthesis were not reported, and no information was found concerning the effects of these parameters on geosmin biosynthesis by fungi. The intent of this investigation was to compare the effects of increasing temperature and $O_2$ concentration on bacterial and fungal sources of geosmin.

## MATERIALS AND METHODS

**Culture Methods.** The isolates of *P. expansum* and of *Streptomyces tendae* Ettlinger used were obtained from J. P. Mattheis and R. G. Roberts (U.S. Department of Agriculture, Wenatchee, WA) [see Mattheis and Roberts (1992)] and the American Type Culture Collection (ATCC 31160), respectively. Cultures of *P. expansum* and *S. tendae* were grown in polystyrene Petri dishes (100-mm diameter) containing 20 mL of Czapek medium (Raper and Thom, 1949) or Hickey–Tresner medium (Hickey and Tresner, 1952), respectively. Each medium was solidified with 1.5% (w/v) bacteriological grade agar (Difco Laboratories, Detroit, MI). Molten medium was allowed to cool and covered with a sterile polycarbonate membrane with a mean pore diameter of 0.05 μm (Poretics Corp., Livermore, CA). The
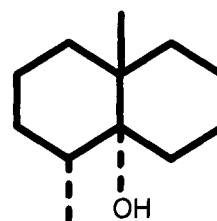


**Figure 1.** Geosmin ($1\alpha,10\beta$-dimethyl-$9\alpha$-decalol), an earthy odorant produced by microorganisms.

upper surface of the membrane was inoculated with a 100-μL aliquot of either *P. expansum* or *S. tendae* spores suspended in sterile water.

**Culture Conditions and Experimental Design.** A dual-chamber incubator (Queue Systems, Model 2720) fitted with gastight doors and capable of independently controlling temperature and flow of air, $O_2$, $N_2$, and $CO_2$ into each chamber was used to achieve specific temperature and atmospheric conditions. Temperature effects were investigated in a series of complete randomized block experiments in which cultures of *S. tendae* and *P. expansum* were incubated at either 10, 20, 40, or 45 °C for 48 h in an ambient atmosphere. The uniformity of conditions within controlled environmental chambers over time cannot be assumed (Lee and Rawlings, 1982). Therefore, to provide appropriate controls, cultures of both taxa were simultaneously incubated at 30 °C in the other chamber of the incubator and harvested with the temperature treatment cultures.

The effects of $O_2$ were investigated in a series of complete randomized block experiments in which cultures of *S. tendae* and *P. expansum* were incubated at 20 and 30 °C, respectively, in atmospheres containing various amounts of $O_2$. Specific atmospheric conditions were established by injecting either $N_2$ or $O_2$ into the incubator chamber as required to achieve either 5, 10, or 30% $O_2$. To provide appropriate controls, cultures of *S. tendae* and *P. expansum* were established in the other chamber of the incubator and incubated in air (ca. 20% $O_2$ and 80% $N_2$). Cultures were harvested 48 h after inoculation. The concentration of $CO_2$ was maintained at 0.1%. All gasses were filtered through a 0.2-μm pore size filter at a flow rate of 0.5 L min$^{-1}$ prior to injection into the chamber, and conditions in the chambers were allowed to stabilize for at least 48 h after new set points were entered.

Replications of the temperature and atmospheric conditions were achieved by repeating each experiment at least once. Randomization of incubator chambers was achieved by switching the condition imposed by each chamber between each repetition of the experiment. In addition, each culture plate was replicated at least once. Treatment data were compared to the controls
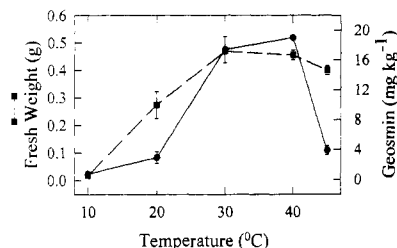
**Figure 2.** Effect of temperature on biomass accumulation and concentration of geosmin in *S. tendae* cultures 48 h after inoculation. Vertical bars are ±1 SE of the mean, *n* = 11, 12, 54, 16, and 10 for the 10, 20, 30, 40, and 45 °C data, respectively.

and subjected to ANOVA procedures for experiments replicated in time as described by McIntosh (1983).

**Biomass and Geosmin Analysis.** Cells were harvested by lifting the polycarbonate membrane off the surface of the medium and placing it in a tared 20-mL glass scintillation vial. The net weight of the cells was recorded prior to the membrane and cells being covered with 12 mL of gas chromatography grade hexane. Internal standards consisting of 100-$\mu$L aliquots of 25 ppm of 2-undecanone and chlorododecane were added to each vial. Each vial was sealed and stored at 0 °C until the solvent could be filtered through a bed (ca. 5 g) of anhydrous sodium sulfate held in individual glass preparatory funnels plugged with glass wool. Each scintillation vial and membrane was rinsed with two 3-mL aliquots of hexane, and the rinsate was also poured over the sodium sulfate bed. The filtrate was collected in glass evaporation tubes and concentrated under a stream of $N_2$ at 22 °C to ca. 500 $\mu$L with an automated evaporator (Turbovap evaporator, Zymark Corp., Hopkinton, MA). The filtrate was further concentrated by hand under a stream of $N_2$ to ca. 15–80 $\mu$L and transferred to a sealed glass autosampler vial for analysis by gas chromatography as described by Johnsen and Kuan (1987).

Gas chromatography was performed by a Hewlett-Packard Model 5890 gas chromatograph equipped with a flame ionization detector programmed as described by Dionigi et al. (1990). The retention times of 2-undecanone, chlorododecane (Sigma Chemical Co., St. Louis, MO), and geosmin (Wako Chemical Co., Richmond, VA) authentic standards were determined. Sample peaks exhibiting retention times corresponding to those of the authentic standards were verified by gas chromatography/mass spectrometry and recorded. The concentration of geosmin in each sample was calculated relative to the 2-undecanone peak area and sample weight. These data were verified by comparing them to those calculated from the chlorododecane peak areas.

## RESULTS AND DISCUSSION

**Temperature Effects.** *S. tendae* cultures incubated at 30 and 40 °C for 48 h produced more biomass than those incubated at 10 and 20 °C (Figure 2). These *S. tendae* cells also contained a higher concentration of geosmin than those incubated at 10 and 20 °C (Figure 2), indicating an association between relatively rapid biomass production and increased geosmin accumulation in these bacterial cultures. In contrast, the fungus *P. expansum* did not exhibit an association between rapid biomass production and increased geosmin accumulation. Cultures of *P. expansum* incubated at 20 °C exhibited more biomass than those incubated at 40 °C at 48 h after inoculation (Figure 3). However, cells incubated at 40 °C contained about an order of magnitude higher concentration of geosmin than those incubated at 20 °C (Figure 3).

Cultures of *S. tendae* incubated at 45 °C contained a lower concentration of geosmin than those incubated at 30 and 40 °C (Figure 2). Geosmin is volatile (Pirbazari et al., 1992). Therefore, the reduced concentration of geosmin in cells incubated at 45 °C (Figure 2) may have been due in part to an increased volatilization of geosmin from the cells. Cultures of *P. expansum* exposed to 45 °C exhibited little biomass and geosmin, indicating that this
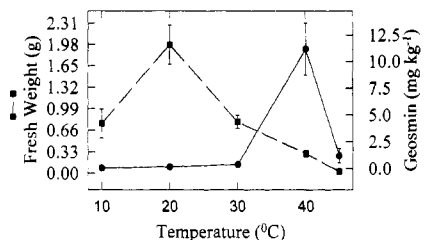


**Figure 3.** Effect of temperature on biomass accumulation and concentration of geosmin in *P. expansum* cultures 48 h after inoculation. Vertical bars are ±1 SE of the mean, *n* = 12 for each mean except for 30 °C mean, where *n* = 46.
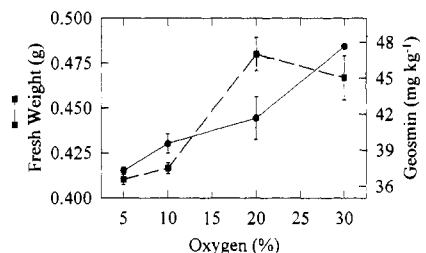


**Figure 4.** Effect of the proportion of $O_2$ in the atmosphere on biomass accumulation and concentration of geosmin in *S. tendae* cultures 48 h after inoculation. Vertical bars are ±1 SE of the mean, *n* = 5, 10, 26, and 12 for the 5, 10, 20, and 30% $O_2$ data, respectively.
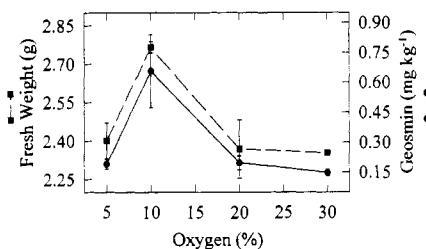


**Figure 5.** Effect of the proportion of $O_2$ in the atmosphere on the biomass accumulation and concentration of geosmin in *P. expansum* cultures 48 h after inoculation. Vertical bars are ±1 SE of the mean, *n* = 10, 10, 32, and 12 for the 5, 10, 20, and 30% $O_2$ data, respectively.

temperature may be near the maximum for this isolate (Figure 3), whereas cultures of *S. tendae* exposed to 45 °C accumulated biomass (Figure 2), indicating that *S. tendae* may be less sensitive to heat than *P. expansum*. The increased accumulation of geosmin in *P. expansum* cells exposed to 40 °C (Figure 3) indicates that geosmin biosynthesis may be associated with exposure to superoptimal temperatures in this taxon. The physiological function (if any) of geosmin biosynthesis is not fully known. Geosmin is lipophilic (Pirbazari et al., 1992), suggesting that the accumulation of geosmin in cells may alter the properties of lipophilic temperature-sensitive structures, such as membranes.

**Oxygen Effects.** Oxygen enrichment of the atmosphere increased geosmin accumulation by cultures of *S. tendae* (Figure 4). Dionigi et al. (1990) reported that mixed-function oxidase activity may be involved in the biosynthesis of geosmin by *S. tendae*. Since these enzymes require $O_2$ (Hodgson and Tate, 1976), geosmin biosynthesis in cells incubated in 30% $O_2$ and 70% $N_2$ may be enhanced by increased substrate availability. However, cultures of *P. expansum* incubated in 10% $O_2$ and 90% $N_2$ exhibited a higher concentration of geosmin than ambient controls (Figure 5). The isolate of *P. expansum* used was obtained from populations occurring on fruit stored in low $O_2$ and high $CO_2$ controlled atmosphere storage facilities [see Mattheis and Roberts (1992)]. This isolate may be more

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acclimated to reduced $O_2$ concentrations than *S. tendae*. The diversity of taxa reported to produce geosmin [see Dionigi (1993)] and the varied response of geosmin-producing taxa to temperature and atmospheric conditions indicate the complexity associated with reducing geosmin-producing populations and geosmin biosynthesis in food and water resources.

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