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Identification of Geosmin as a Volatile Metabolite of *Penicillium expansum*

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Cultures of *Penicillium expansum* produce a musty, earthy odor. Geosmin [1,10-trans-dimethyl-trans(9)-decalol] was identified by gas chromatography-mass spectrometry from headspace samples of *P. expansum* cultures. Olfactory comparison of *P. expansum* cultures with a geosmin standard indicated geosmin is the primary component of the odor associated with *P. expansum*.

Members of the genus *Penicillium* Link are numerous and adapted to a wide range of habitats. *Penicillium expansum* Link is most widely known as a pathogen of stored pome fruits (11), but it has also been isolated from other stored fruit and oilseed crops (12, 13). *P. expansum* and other species have long been associated with distinctive odors. Raper and Thom (12) described the odor of various penicillia as being "strong moldy or earthy" (*P. expansum* and *P. crustosum* Thom), "moldy" (*P. claviforme* Bain. and *P. olivino-viride* Biourge ≡ *P. viridicatum* Westling fide Pitt [11]), "fragrant apple" or "walnut" smelling in old age (*P. purpurogenum* Stoll), like "spoiling citrus fruit" or "unpickled dill" [*P. digitatum* (Pers. ex Fr.) Sacc.], and "perfume-like" (*P. decumbens* Thom).

P. expansum is one of several fungi responsible for postharvest losses of apples, pears, and cherries. Fruit decayed by *P. expansum* has a penetrating, pungent, earthy odor. In addition to postharvest losses due to decay, the presence of *P. expansum* in stored apples is of concern because of the possible production of the mycotoxin patulin, which can contaminate apples (17) and apple products, when apples decayed by *P. expansum* are processed (16). Several European countries have established maximum limits for patulin contamination (15).

Apple and pear fruit are regularly stored for many months in sealed controlled-atmosphere rooms. Decay caused by *P. expansum* can spread from fruit to fruit during storage and handling; therefore, it would be advantageous to identify storage rooms with incipient decay problems to minimize subsequent losses. The objective of this study was to identify the volatile compound(s) responsible for the characteristic odor of *P. expansum* as an initial step in their evaluation as indicators of decay in stored products.

(A preliminary report of a portion of this data has been published previously [10].)

Two isolates of *P. expansum* were used throughout these studies, a spontaneous white cultural mutant (RR86-58) selected from an isolate obtained from 'Bing' sweet cherries and another isolate (RR89-30) obtained from flume water in an apple-packing plant. Wide-mouth 1-liter bottles for media (three replications) containing sterile Czapek agar (12) were inoculated with 1.0 ml of a conidial suspension (10^4 conidia ml^{-1}) and then capped using an inverted polyethylene beaker with two gas lines bored through the base. A polycarbonate filter (0.2-μm pore size, 25-mm diameter) was installed in one line to prevent contamination of the cultures; the other line was capped with a cork, and the vessels were incubated at 18°C. Growth and development of the cultures were examined microscopically daily.

Volatiles were collected at 24-h intervals by dynamic headspace sampling from inoculated vessels or blanks containing only Czapek agar. Compressed air, purified with a column containing potassium permanganate-coated molecular sieve (Purafil), activated charcoal, calcium hydroxide, molecular sieve, and Tenax, was passed through the jar, and volatiles were collected on a solid sorbent trap (Tenax TA, 50 mg) connected to the outlet port. The total air volume collected was 3 liters. Volatiles were introduced into a gas chromatograph-mass spectrometer by using thermal desorption and cryofocusing (6). Traps were desorbed with a hot-air gun at 200°C, and a 1-m section of fused silica capillary tubing was used as a cryoloop inserted into a dewar of liquid N_2. Following desorption and cryofocusing, volatiles were introduced to the gas chromatograph by exchanging the liquid N_2 dewar for a container of hot (100°C) water. A Hewlett-Packard 5890A-5971A gas chromatograph-mass selective detector system equipped with a DB-Wax column (60 m, 0.25-mm inner diameter, 0.25-μm film thickness; J & W Scientific) was used for analysis. The GC temperature program was an initial oven temperature of 35°C held for 5 min, increased to 50°C at 2°C min^{-1}, increased to 200°C at 5°C min^{-1}, and held for 5 min at 200°C. Linear gas velocity for the He carrier was 30 cm s^{-1}. Initial identification of compounds was made by using the Wiley-NBS library. Samples were analyzed in scan mode (20 to 200 atomic mass units) and data files were subsequently examined for base peaks with an atomic mass unit measurement of 112. Comparison of spectra and relative retention time with those of authentic standards were used to confirm identification. Geosmin quantitation was accomplished by using an external standard obtained from Chris Dionigi (U.S. Department of Agriculture, Agricultural Research Service, New Orleans, La.).

Geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol; Fig. 1) (7) was identified in the headspace samples collected from *P. expansum*. Comparison of samples and a geosmin standard indicated that retention times (Fig. 2) and fragmentation patterns (Fig. 3) were nearly identical. Olfactory comparison of the geosmin standard and *P. expansum* cultures by five

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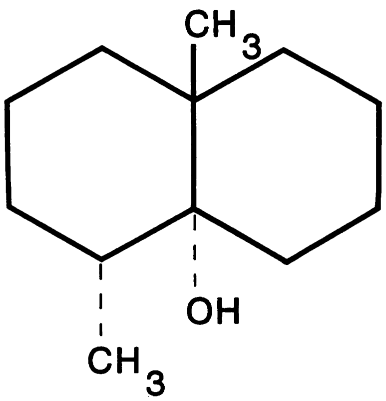

FIG. 1. Structure of geosmin.

laboratory personnel indicated geosmin was the main component of the odor associated with *P. expansum*. Geosmin is also produced by algae (14), bacteria (5), and the fungus *Chaetomium globosum* Kunze: Fr. (8). Geosmin concentrations as low as 10 ng/liter can impart a musty off-flavor and odor to drinking water supplies (4), and geosmin imparts a muddy off-flavor when taken up by fish (9, 18). Geosmin has also been identified as a component of the off-flavor of dry beans (3) and contributes to the natural flavor of red table beets (1).

The presence of geosmin was initially detected from headspace samples collected 3 days after inoculation (Fig. 4) and was present thereafter. The appearance of geosmin in headspace samples coincided with the onset of sporulation under the stated culture conditions. Mature conidiophores, phialides, and very short conidial chains (1 to 3 conidia per phialide) were microscopically observed in the 3-day-old cultures. The appearance of geosmin also coincided with the

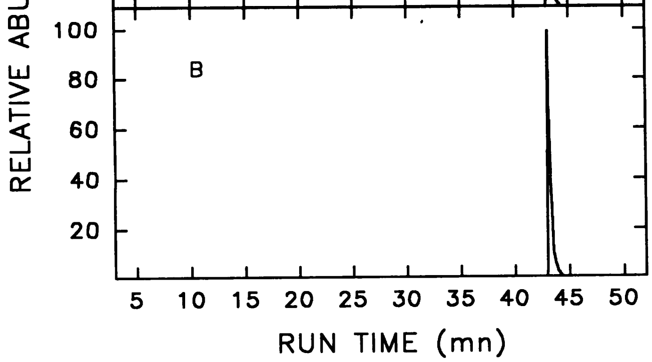

FIG. 2. Extracted ion chromatograms of headspace samples collected from *P. expansum* (RR86-58) (A) and sample of authentic geosmin (B).

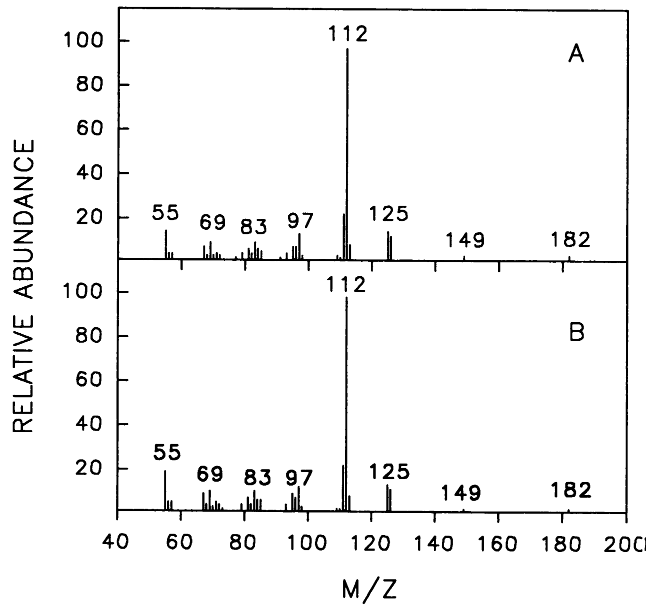

FIG. 3. Fragmentation pattern of geosmin in headspace samples collected from *P. expansum* (RR86058) (A) and geosmin standard (B).

development of the blue-gray pigmentation typically observed in cultures of *P. expansum* on Czapek agar; however, geosmin was also detected in headspace samples of sporulating cultures of the spontaneous white cultural mutant of *P. expansum* (RR86-58) (Fig. 2). Geosmin synthesis has previously been associated with sporulation in the actinomycete *Streptomyces odorifer* (2).

To our knowledge, this is the first report of geosmin produced by a *Penicillium* species. Many different fungi are

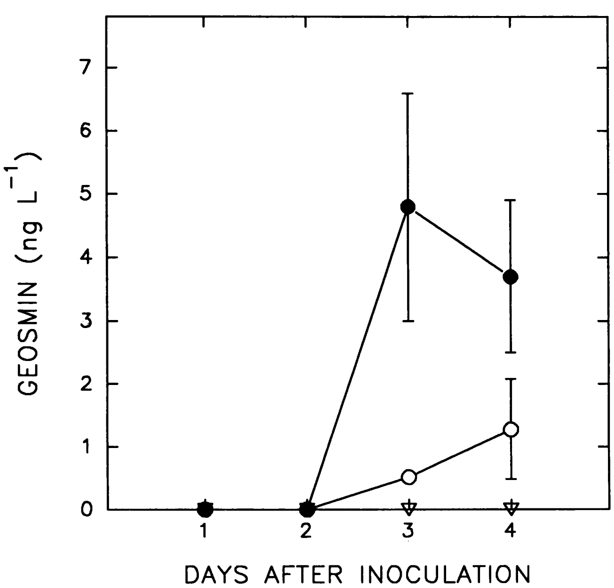

FIG. 4. Geosmin concentration in the headspace of 1-liter jars containing *P. expansum* (RR89-30) cultured on Czapek agar. Values are means ± standard deviations of replicate samples. ▽, controls; ○, experiment 1; ●, experiment 2.

associated with harvested fruit, including *Botrytis cinerea*, *Mucor piriformis* Fischer, and a number of species of *Penicillium*. To develop a practical means of using geosmin as an indicator of incipient losses due to *P. expansum*, it is necessary to determine whether other fungi produce geosmin in the postharvest environment.

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ADDENDUM IN PROOF

We acknowledge a previous report of geosmin synthesis by *Penicillium citrinum* and *P. farenosum* (A. Pisarnitskii and I. Egorov, Prikl. Biokhim. Mikrobiol. **24:**760–764, 1988).

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