Germination of Penicillium digitatum Spores as Affected by Solutions of Volatile Components of Citrus Fruits

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ABSTRACT

Volatile compounds known to occur in mature citrus fruits were evaluated for their effects on spore germination of *Penicillium digitatum*. The aldehydes tested inhibited germination, whereas esters, terpenes, and alcohols did not. The germination of spores was inhibited 50% or more by 1.0, 0.2, 0.1, and 0.06 mmole/liter of C_5, C_6, C_7, and C_8 aliphatic aldehydes, respectively. None of the compounds induced germination of the spores in distilled water.

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Because of the effects of some chemical pesticides on our environment, it is appropriate to search for naturally occurring components of fruits and vegetables which could aid in the prevention of their decay by microorganisms. A recent reference work on the dormancy and germination of spores (10) mentions a few instances of self-inhibition and the occurrence of inhibitors in soil, sea water, and higher plants. Some monoterpenes are reported to inhibit germination of radish seeds (1), and the flavone nobiletin has been indicated to be the main fungistat in tangerines which are resistant to "mal secco" caused by *Deuterophoma tracheiphila* (2). The phenolic compounds, protocatechuic acid and catechol, which occur in pigmented onions, prevent spore germination of two fungi pathogenic to onions (11). In addition, the pungent (volatile) components of onions have also been associated with disease resistance (9).

We tested volatile constituents of citrus fruits, including aldehydes, alcohols, esters, and terpenes, as inhibitors of germination of spores of *Penicillium digitatum* Sacc., one of the primary organisms responsible for decay of citrus fruits.

Spores were harvested from 2-week-old cultures of a "wild" strain of *P. digitatum* which had been isolated from, and maintained on, orange fruits. The spores were suspended in 200 ml of distilled water containing 1 drop of Tween 20 (polyoxyethylene sorbitan monolaurate) and washed by three successive centrifugations and resuspensions in distilled water.

All compounds tested except butyraldehyde are known components of citrus volatiles (8). Saturated aqueous solutions were prepared by agitating the slightly soluble compounds with distilled water for 1 hr. Test solutions were prepared by adding 2 drops of the saturated solution, or dilutions thereof, to 2 drops of a synthetic culture medium (4) and 1 drop of a washed spore suspension in Syracuse watch glasses. The pH of each test solution was near that of the slightly buffered synthetic medium, which was 4.32. Germination in the synthetic medium occurs over the pH range of 3 to 6, with a maximum at about pH 4 (4).

After incubation for 16 to 18 hr at 25 C, germination was stopped by adding a drop of Formalin to each dish. Germination was estimated as follows: trace (0 to 1%); poor (1 to 25%); fair (25 to 75%), and good (75 to 100%). Actual counts were made in quantitative tests with the C_5-C_9 aldehydes, valeraldehyde, hexanal, haptanal, octanal, and nonanal. All materials were evaluated by at least two tests with three replications.

Of all compounds tested, only aldehydes inhibited germination of *P. digitatum* spores. Alcohols, esters, and terpenes did not inhibit germination. The aldehydes tested and the resulting germinations were: butyraldehyde, poor; heptanal, trace; octanal, trace; nonanal, trace; decanal, poor; citral, fair; citronellal, fair; and lauric aldehyde, good. Alcohols were nerol, 2-hexanol, octanol, nonanol, decanol, geraniol, and citronellol. Esters were ethyl butyrate, citronellyl acetate, methyl isovalerate, and terpineyl acetate. Terpenes tested were terpinolene, *beta* pinene, myrcene, *para*-cymene, and limonene. None of the compounds induced germination of the spores in distilled water.

The solubilities of the C_5-C_9 aldehydes were 0.255 M, 0.0563 M, 0.0135 M, 0.0028 M, and 0.00077 M, respectively (3). Quantitative tests were conducted on dilutions of saturated solutions of these compounds, and, in general, the inhibitory effect increased with chain length (Fig. 1). The concentrations of the C_5-C_9 aldehydes giving 50% or more inhibition were 1.0, 0.2, 0.1, 0.06, and 0.2 mmoles/liter, respectively. Nonanal was less effective than octanal, and approximately equivalent to hexanal.

The potential of aldehydes as inhibitors has been noted in other instances. Major et al. (7) isolated

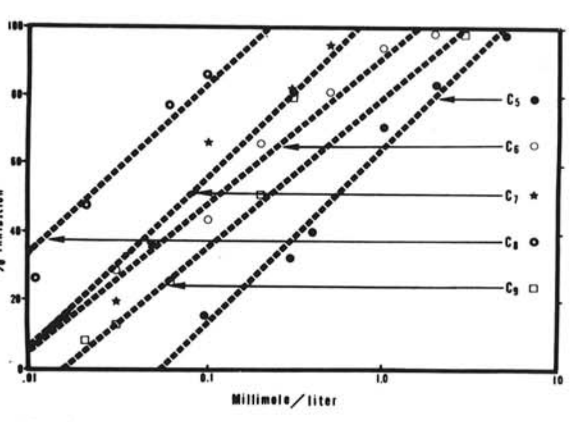

Fig. 1. Inhibition of germination of *Penicillium digitatum* spores by C_5-C_9 aldehydes. Each point is an average of at least three tests, with three replicates/test. Per cent inhibition

$$= \frac{\%\text{ germination in control} - \%\text{ germination in treated}}{\%\text{ germination in control}} \times 100.$$

a-hexenal from leaves of the tree *Ginkgo biloba*, and proposed that its presence may account for part of the resistance of the leaves to fungi. DeGreef & Van Sumere (5), in a study of compounds affecting the growth of *Saccharomyces cerevisiae*, concluded that aromatic aldehydes were probably more inhibitory than their corresponding acids and alcohols. In a related work (6) concerning the respiration of *S. cerevisiae*, the same authors proposed that aldehydes such as salicylaldehyde may act as potent uncouplers of oxidative phosphorylation similar in effect to 2,4-dinitrophenol. Naturally occurring aldehydes apparently can function as inhibitors of organisms causing diseases of plants and decay of fruits and vegetables.

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