Ethylene Production by Soil Microorganisms

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Ethylene-producing strains of Penicillium cyclopium and P. crustosum were isolated from soil. These isolates produced ethylene on a variety of carbon growth substrates including phenolic acids. The quantities of ethylene produced on the various substrates varied, and the substrate-ethylene production pattern for P. cyclopium strains differed significantly from that of P. crustosum strains.

Ethylene has been identified as a component of soil atmospheres and, under certain conditions, has been shown to reach a concentration sufficiently high to influence plant growth and development (10–12, 14). The ethylene is apparently of microbial origin (10), but conflicting claims have been made to the relative contributions of various groups of microorganisms in the process of soil ethylene formation.

Several species of ethylene-producing soil bacteria (9), fungi (5, 8), and yeasts (8) have been isolated. It has been suggested (8, 9) that methionine is the precursor of ethylene in both fungal and bacterial isolates.

Previous work (4) has shown that phenolic acids, which are present in soil (13), promote ethylene production by the soil isolate Penicillium cyclopium. This note reports on attempts at isolating ethylene-producing microorganisms from soil by employing various types of isolation media.

Air-dried deciduous forest soil (10 g) was blended with sterile water (90 ml) for 1 min. Agar media in petri dishes were inoculated with 0.1-ml portions of 10-fold dilutions of the suspension and incubated at 22°C. After 7 days the petri dish lids were replaced with modified petri dish bases. The modification consisted of inserting and sealing in each base a rubber serum cap (Suba-Seal, Freeman & Co., Barnsley, Yorkshire, England), which facilitated sampling of the gas atmosphere above the cultures. Each petri dish was then sealed with adhesive tape. Gas samples were removed daily and analyzed for ethylene. Colonies on plates that contained >1.0 μl of ethylene per liter were subcultured on the same medium used for their isolation and were then tested for the ability to produce ethylene in pure culture. All media contained the following (grams per liter): NH₄NO₃, 2.0; KH₂PO₄, 0.5; K₂HPO₄, 0.5; MgSO₄·7H₂O, 0.4; KCl, 0.5; CaCO₃, 0.01; FeSO₄·7H₂O, 0.01; MnCl·4H₂O, 0.01; NaMoO₄·2H₂O, 0.01; ZnSO₄·7H₂O, 0.01; Oxoid agar no. 1, 15.0. All other medium components were dissolved in 0.1 M phosphate buffer, adjusted to pH 7, filter sterilized, and added to autoclaved (121°C, 15 min) mineral salts-agar. Three isolation media were used: (i) soil extract medium, which contained 20% (vol/vol) of an extract prepared by autoclaving (121°C, 15 min) equal volumes of soil and water, centrifuging (3,000 × g, 15 min), and filtering the supernatant with Whatman no. 1 filter paper; (ii) G-M medium, which contained glucose (13.3 g/liter) and methionine (5.0 g/liter); and (iii) V-Y medium, which contained vanillic acid (13.3 g) and yeast extract (3.3 g/liter). Other media contained single carbon sources added to give final concentrations of 10 g/liter.

Cultivation of isolates was carried out on solid slope media (10 ml) in Universal culture bottles plugged with cotton wool. Prior to gas analysis, the cotton wool plugs were replaced by alcohol-sterilized Suba-Seals. Gas samples were analyzed by ethylene using a Pye series 104 chromatogram fitted with a glass column (1.5 m by 4 mm), packed with Porapak N (80 to 100 mesh), and with a flame ionization detector. The oven temperature was maintained at 100°C. Ethylene levels of 0.05 μl/liter were detectable with this system.

Significant levels (>1 μl/liter) of ethylene were detected within 10 days of sealing the plates containing the organisms growing on V-Y or G-M medium, but not until after 25 days for organisms growing on soil extract medium. Although the three isolation media used supported growth of bacteria, yeasts, and fungi, all of the isolates that produced ethylene in pure culture were found to belong to the genus Penicillium. Nine ethylene-producing isolates were obtained: seven on V-Y medium, one on G-M medium, and one on soil extract medium. The isolates were identified by the Common-
wealth Mycological Institute (Kew, Surrey, England). Three of the ethylene producers isolated on vanillic acid-yeast extract medium were identified as strains of *P. crustosum*. The other isolates were identified as strains of *P. cyclopium*.

The nine isolates were grown on media containing various carbon sources. The quantities of ethylene produced by the various strains depended on the carbon source (Table 1). *P. cyclopium* strains produced significantly higher quantities of ethylene when grown on media containing acetate or tricarboxylic acid cycle intermediates than on media containing glucose even though growth on the latter substrate was substantially greater. A completely opposite pattern was observed for *P. crustosum* strains. Little or no ethylene was produced on media containing acetate or tricarboxylic acid cycle intermediates, although growth on these substrates was similar to that obtained with *P. cyclopium* strains. Glucose supported good ethylene production. This suggests that ethylene production occurs via different pathways in *P. cyclopium* and *P. crustosum*. Alternatively, different regulatory mechanisms exist in both organisms.

The results indicate that ethylene biosynthesis in *P. cyclopium* strains is similar to that for *P. digitatum* (3). The results also support previous findings (3, 4, 6, 7) that methionine, which is an immediate precursor of ethylene in plant tissue (1) and a postulated precursor in *Mucor hiemalis* (2) and bacteria (9), is not an immediate precursor of ethylene in *Penicillium* spp.

Significant increases in ethylene production were obtained with all isolates cultivated in media containing complex supplements such as yeast extract or mycological peptone (a component of Sabaroud dextrose medium). These large increases cannot be accounted for by the relatively smaller increases in mycelial mass.

It was found that all of the phenolic acids tested supported growth and ethylene production in strains of *P. cyclopium* and *P. crustosum*. This supports our previous suggestion (4) that phenolic acid-utilizing organisms may contribute to the total production of ethylene in soil, particularly in soils containing high levels of degradable phenolic polymers.

**LITERATURE CITED**


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