Oxidation of ethylene by soil bacteria

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The course of the biological oxidation of ethylene by soil was dependent on the type of soil used as well as on other factors. As evidenced from an increase in oxidation rate, the ethylene-consuming microorganisms in soil could grow at the expense of ethylene, even when the gas was present at concentrations of 50 ppm or less. Five strains of bacteria strongly resembling each other were isolated from different soils. These pleomorphic, gram-positive, acid-fast, obligate aerobic, ethylene-oxidizing bacteria grew also on saturated alkanes and on ordinary carbon sources. An apparent $K_m$ for ethylene of approximately 40 ppm was estimated for whole-cell suspensions of strain E20 by following the disappearance of the gas from the atmosphere.

INTRODUCTION

Ethylene is an important naturally occurring gas which is functioning as hormonal regulator of growth and development of plants. Dose-response relationships are rather similar for most processes; a concentration of 0.01 ppm is normally required for a threshold effect, 0.1 ppm for a half-maximum effect and 10 ppm for a saturating dose. Plants also produce ethylene in quantities varying from organ to organ and with period of development (Abeles, 1973). In soil, ethylene may also be produced by fungi. Twenty-five percent of 228 species tested by Ilag and Curtis (1968) were able to evolve the gas. Methionine was the substrate for ethylene formation by a pure culture of *Mucor hiemalis* isolated from soil, but glucose was also required for maximum ethylene production (Lynch and Harper, 1974). The fungus was seen as the major producer of ethylene in soil (Lynch, 1975). Considine and Patching (1975) isolated a *Penicillium* sp. from soil which produced ethylene from phenolic acids. Other workers (Smith and Cook, 1974) stated that anaerobic spore-forming bacteria are the main ethylene-producing microorganisms. There is general agreement on anaer-
robiosis favouring the microbial formation of ethylene (Smith and Restall, 1971; Smith and Cook, 1974; Lynch, 1975; Considine and Patching, 1975). The concentration at which the gas sometimes occurs in soil under waterlogged conditions can be a significant factor in causing injury to crop plants (Smith and Restall, 1971).

The removal of ethylene from the soil atmosphere has received considerably less attention. From the work of Abeles et al. (1971) it has become evident that soil may remove ethylene in the presence of oxygen; sterilization eliminated this effect, indicating the involvement of microorganisms in the process. Experiments with steam-sterilized soils by Smith, Bremner and Tabatabai (1973) also showed that soil microorganisms were responsible for the uptake of ethylene. Formation as well as degradation of ethylene in rice soils were investigated by Yoshida and Suzuki (1975). Ethylene formation was markedly enhanced when organic matter was added to soil. The degradation activity associated with rhizosphere microorganisms was mainly found in continually submerged lowland soils.

The purpose of the work reported here was to study the microorganisms in soil which are responsible for the breakdown of ethylene. A preliminary report has appeared at an earlier date (de Bont, 1975).

MATERIALS AND METHODS

Media. The mineral salts solution (MS medium), used throughout the investigation, contained the following salts in 1 litre of deionized water: NaNO₃, 2.0 g; K₂HPO₄, 0.5 g; KH₂PO₄, 0.5 g; MgSO₄·7H₂O, 0.2 g; CaCl₂, 0.015 g; FeSO₄·7H₂O, 0.001 g; CuSO₄·5H₂O, 5 μg; H₃BO₃, 10 μg; ZnSO₄·7H₂O, 70 μg; MnSO₄·5H₂O, 10 μg; Na₂MoO₄·2H₂O, 100 μg; final pH, 6.8. The yeast extract-glucose medium (YEG medium) contained in one litre of tap water 10 g glucose and 7 g yeast extract. For solid media 1.5% agar was added.

Chemicals. Ethylene (99.0%) and other gaseous substrates (commercial purity) were obtained from the Matheson Co., East Rutherford, N.J.

Culture conditions. Weekly subcultures on slants of MS medium were kept in desiccators with approximately 5% ethylene in air. In growth experiments, ethylene and other gaseous substrates were also supplied at this concentration. Solid or liquid substrates were given at a concentration of 2 g per litre while ethylene oxide was injected as gas (5 ml) in sealed Erlenmeyer flasks (5 litre) containing five or more slants. The pure cultures were grown and kept at 30°C.

Soil samples. Samples from top layers of moist soil were divided into appropriate portions and put into Erlenmeyer flasks which were sealed with a suba-seal. Ethylene was injected and after 3 hours the first gas sample (0.1 ml) was withdrawn for gas-chromatographic analysis. Water was kept on top of the suba-seals. The soil samples were incubated at 25°C. Oxygen remained present