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Reaction of Airborne *Rhizobium meliloti* to Some Environmental Factors

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Survival of *Rhizobium meliloti* 102F5 in aerosols at 20 C was maximal at high relative humidity (RH) and minimal at low RH. Relatively high concentrations of NO_2, SO_2, or formaldehyde were needed to significantly reduce viability of *R. meliloti* in aerosols at 50% RH. Except for the reduction in activity of formaldehyde by SO_2, there was no additive or antagonistic effect of mixing pollutants. High environmental RH enhanced bactericidal activity of NO_2 and SO_2. High RH minimized and low RH accentuated the biological effect of ultraviolet light of 300 to 400 nm wavelength.

Organisms of the *Rhizobium* species are classical nonpathogenic nitrogen fixers which have been in use extensively for inoculating soil and legumes to increase nitrogen fixation (2). As far as we are aware, not a single incident of human infection, particularly among workers associated in the manufacturing and application of this inoculum, has been recorded. Accordingly, these organisms may be ideal for investigating microbial aerosol behavior in open atmospheres. This report is concerned with laboratory findings on behavior of airborne *R. meliloti* 102F5 in relation to relative humidity (RH), ultraviolet, light irradiation, and certain common gaseous atmospheric pollutants.

MATERIALS AND METHODS

Organisms. The test organism, *R. meliloti* 102F5, was kindly provided by J. C. Burton of the Nitragin Co., Milwaukee, Wis. Cultures were maintained on yeast extract-mannitol-agar. Cells used for aerosolization were 24-hr, second-passage cultures grown at 30 C in 100-ml volumes of modified mannitol yeast extract broth (5) of the following composition (in g/liter): mannitol, 10; K_2HPO_4, 0.5; $MgSO_4 \cdot 7H_2O$, 0.2; NaCl, 0.1; $CaCO_3$, 0.5; and yeast extract (Difco), 0.5. For use as a solid medium, 1.5% agar was added.

A frozen stock of spores of *Bacillus subtilis* var. *niger* was reconstituted by mixing 11.5 g with 100 ml of gelatin phosphate (2.0 g of gelatin and 4.0 g of Na_2HPO_4 per liter; *p*H 7.0). Suspensions for aerosolization consisted of measured volumes of *B. subtilis* var. *niger* spores and *R. meliloti* in gelatin phosphate that assayed at about 10^7 organisms per ml for each.

Aerosolization. The 500-liter stainless-steel rotating drum and the procedures used for aerosol generation, regulation of RH in the enclosed atmosphere, and methods for sampling and quantitation of aersols were similar to those described previously (9).

For assay of *R. meliloti* in mixed aerosols, which require 72 hr at 30 C for colony development, Brilliant Green at 150 μg/ml was added to the agar during preparation to suppress growth of *B. subtilis* var. *niger*. The latter, a faster grower, was readily distinguishable and countable after 24 hr at 30 C in the same medium without Brilliant Green.

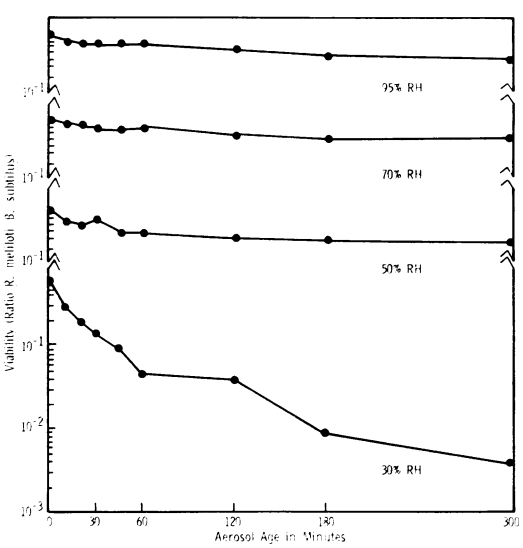

FIG. 1. *Viability of airborne Rhizobium meliloti 102F5 in relation to relative humidity at 20 C.*

Survival of test organisms was expressed as the ratio of test organism to tracer divided by the similar ratio of organisms as found in the spray suspension. The use of ratios rather than absolute numbers of cells gives data uninfluenced by common variables such as sampler efficiency and aerosol dilution.

Ultraviolet irradiation. Mixed aerosols were con-

tained in a 500-liter rotating drum with one face constructed of 0.01-inch (0.025 cm) cellulose acetate to permit entry of radiant energy. The source of ultraviolet light (300 to 400 nm) was a bank of six 4-ft (1.2 m) fluorescent lamps (General Electric no. F40BL) placed at a distance to give energy intensity equivalent to 0.1 that of sunlight at noon on a clear day in May in the Laboratory compound, as measured by an RCA 935 photocell fitted with a Corning 7-51 filter (1). The aerosols were irradiated continuously for 4.5 hr after an initial equilibrating period of 30 min in total darkness.

Atmospheric pollution. For this series of experiments, mixed aerosols were generated and stored in a 50-ft³ (12.24 m³) cylinder in which stirred settling conditions were created by a motor-driven fan mounted on the bottom (8). Pollutants used were NO_2, SO_2, and Formalin vapor. At 60 min after zero aerosolization time, the desired concentration of a pollutant was carefully metered or, as in the case of Formalin, atomized into the cylinder. Quantitative analyses were made according to methods recommended by the California State Department of Public Health (3).

RESULTS AND DISCUSSION

The effects of RH on survival of aerosolized *R. meliloti* at 20 C are shown in Fig. 1. A similar pattern of survival was found in experiments conducted at 2 C. As indicated in Fig. 1, RH levels of 95, 70, and 50% did not significantly affect viability over a 5-hr period, whereas 30% RH effected an accelerated loss of viability. This is in agreement with Webb (6), who observed maximal survival in aerosols of *Serratia marcescens*, *Escherichia coli* and *Staphylococcus albus* at high RH.

The effect of ultraviolet irradiation of aerosols at selected RH levels is shown in Fig. 2. The germicidal effect of the radiant energy was highest at

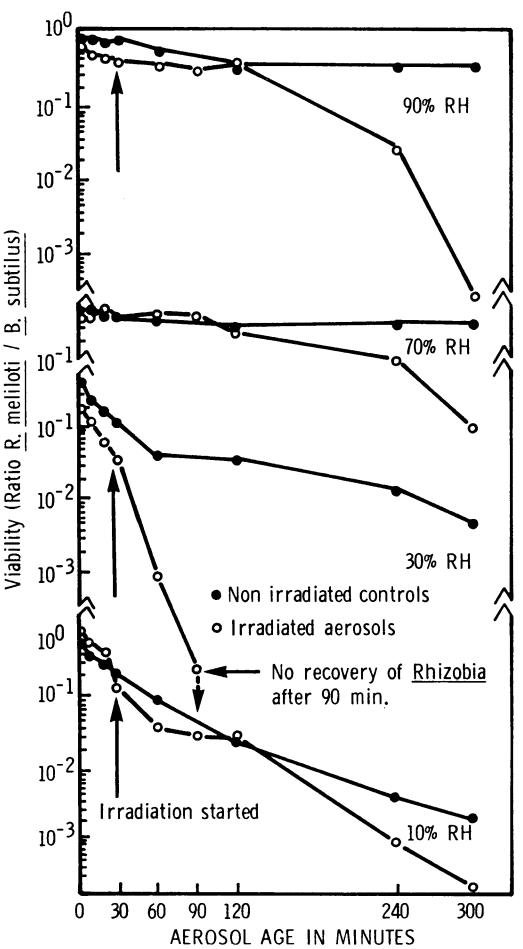

FIG. 2. *Influence of ultraviolet irradiation on a mixed aerosol of Rhizobium meliloti 102F5 and Bacillus subtilis var. niger at 20 C and indicated relative humidities. Irradiation began 30 min after aerosolization was completed.*

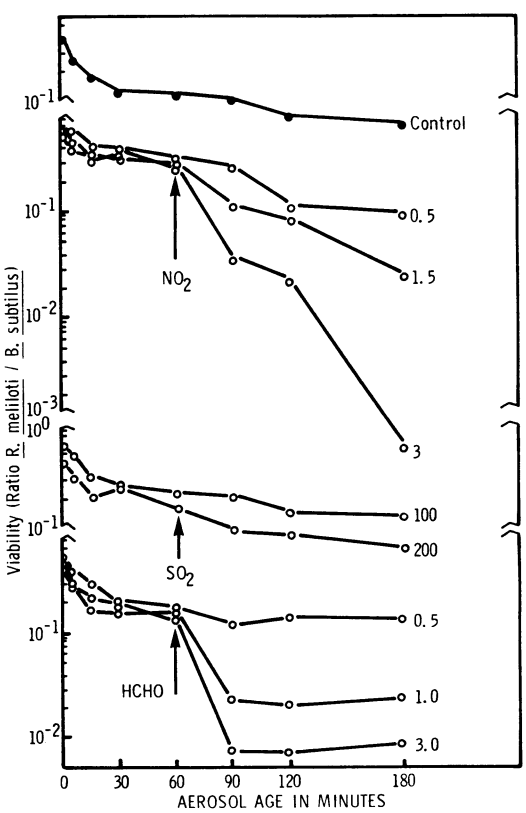

FIG. 3. *Bactericidal effect of gas pollutants (microliters per liter) on mixed aerosols of Rhizobium meliloti 102F5 and Bacillus subtilis var. niger held at 20 C ana 50% RH. Pollutant was introduced 60 min after generation of the aerosols.*

30% RH. At high RH, the effect of the light energy was delayed for several hours, but the ultimate decay rate was accelerated. This correlates with the observation of Webb (7) that RH modifies the extent of radiation damage. His data indicated that airborne *S. marcescens* was more sensitive to ultraviolet light at 65 to 55% RH range than at a higher RH level. This suggested to him that at this critical RH region bound water was removed from cell nucleoprotein, rendering the cells more susceptible to irradiation damage. On the other hand, since the organisms were affected to a lesser degree at a lower RH (10%), the removal of water can hardly be responsible for increased sensitivity to ultraviolet irradiation, as suggested by Webb.

The effect of introducing Formalin, NO_2, or SO_2 into mixed aerosols of *R. meliloti* and *B. subtilis* var. *niger* is shown in Fig. 3. *R. meliloti* was more sensitive to NO_2 than Formalin, but relatively insensitive to SO_2, requiring 200 μg/ml of the latter to affect viability significantly. The effect of formaldehyde was only transient, whereas the effect of the other pollutants was continued for some time after the introduction of the material. Combinations of the gases did not produce measurable synergistic or antagonistic effects, except that SO_2 reduced the efficacy of formaldehyde, since the typical bactericidal pattern characteristically associated with HCHO was not manifested. Instead, aerosol death rate was moderate, progressively increasing in a manner seen in SO_2 reaction. A possible explanation for this observation may be that, in the presence of moisture, SO_2 formed a bisulfite and combined with the highly reactive aldehyde group forming so-called addition products that may have altered the bactericidal activity of HCHO.

In experiments performed in high environmental RH (95%), which normally favors maximal aerosol stability as noted in Fig. 1, bactericidal activity of SO_2 and NO_2 appeared to be greater than at 50% RH. As little as 0.15 μliters/liter for NO_2 and 20 μliters/liter for SO_2 exhibited some degree of bactericidal activity at 95% RH. However, high RH did not increase the activity of HCHO.

Increased water content may favor or accelerate the formation of H_2SO_3 from SO_2 and HNO_3 and HNO_2 from NO_2 in situ (4). These acids are perhaps more toxic than the pollutants themselves. Also, the moisture may increase membrane permeability, resulting in an increased uptake of NO_2 and SO_2 by the cells.

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