

³⁵Sulfur Diffusion from Bean Leaves in Relation to Growth of *Pseudomonas phaseolicola*

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ABSTRACT

Growth phases of *Pseudomonas phaseolicola*, when infiltrated into susceptible bean leaves, consisted of an initial 10- to 12-hr lag phase followed by logarithmic growth with generation times of 2 to 2.5 hr. Greater diffusion of ³⁵Sulfur from infected leaves was first detected between 18 and 24 hr. The rate of the increased diffusion from diseased leaves, expressed as the differences in diffusion from healthy and diseased tissues, became logarithmic after 24 hr following an initial flush of ³⁵Sulfur between 18 and 24 hr. *Phytopathology* 62: 300-301.

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Altered permeability of host cells early in the disease process has been associated with several diseases caused by bacteria (1, 2, 9). Though the significance is still conjectural, one possibility is that there are not sufficient nutrients in the intercellular spaces to support bacterial multiplication without permeability changes. If true, then permeability changes should be detected when bacterial multiplication begins. This was examined by comparing the growth of *Pseudomonas phaseolicola* in bean leaves and cellular permeability changes by measuring the efflux of ³⁵Sulfur (³⁵S).

All experiments were conducted with *P. phaseolicola* (isolate HB 36) grown on King B medium (7). Leaves of 10-day-old bean plants (*Phaseolus vulgaris* L. 'Red Kidney') were infiltrated under vacuum with a cell suspension of 2.85×10^6 cells/ml. Control leaves for the ³⁵S experiment were infiltrated with water.

The population of the bacterium within bean leaves was measured at 3, 6, 9, 12, 18, 24, 48, and 72 hr by cutting five 13-mm discs from each of the primary leaves of two plants, grinding these in 20 ml of distilled water with a mortar and pestle, and plating a dilution series on King B medium. Colonies were counted after 48-hr incubation at 28 C. Eight replications were made at each time.

The growth curve of *P. phaseolicola* in bean leaves (Fig. 1) indicates that a lag phase of ca. 9 to 12 hr occurs before logarithmic growth begins. Generation

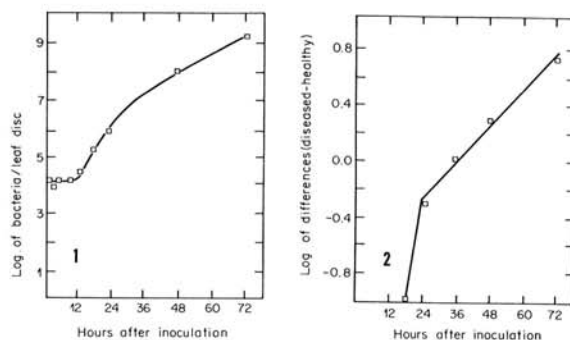


Fig. 1-2. 1) Growth curve of *Pseudomonas phaseolicola* in leaves of Red Kidney beans. Each point represents eight replications. 2) The log of the difference in the diffusion of ³⁵Sulfur from healthy Red Kidney bean leaves and leaves infected with *Pseudomonas phaseolicola*. Each point represents eight replications.

times during the early log phase were 2 to 2.5 hr (some variation from a logarithmic rate would be expected because of leaf expansion).

Roots of 10-day-old bean plants were washed free of soil, the leaves inoculated with the pathogen as previously described, and the root systems immediately placed in bottles containing 100 ml of 0.33 strength Hoagland's solution and 20 ml of 0.006 M Na₂³⁵SO₄ (activity of 10 μc/ml) for the remainder of the experiment. The inoculated plants were grown in a greenhouse cubicle at 21 C. At each measurement time, ten 13-mm discs were cut from the two primary leaves of a plant and shaken in a reciprocal shaker (100 strokes/min) for 1 hr at 27 C in 10 ml of water. After removal of a 0.5-ml aliquant to measure radioactivity in the diffusate, the samples were frozen for 24 hr, and again shaken for 1 hr. Another 0.5-ml aliquant was removed after shaking to measure the total cytoplasmic diffusate. One-half ml of Pirie's reagent was added to the 0.5-ml aliquants in scintillation vials for counting according to the method of Jeffay et al. (6). Radioactivity determinations were made with a Beckman liquid scintillation spectrometer.

The degree of ³⁵S diffusion from leaf discs was expressed as the percentage of the nonfrozen leaf-disc diffusate to the total cellular diffusate. The amount of ³⁵S diffusing from healthy leaves was subtracted from that of diseased leaves to obtain the net effect of the disease upon ³⁵S diffusion. A plot of the logarithms of these differences indicated that ³⁵S diffusion from diseased leaves increased disproportionately to healthy leaves after 18 hr (Fig. 2). The rate of diffusion difference became logarithmic after 24 hr, although the rate was much slower than between 18 and 24 hr.

Pseudomonas phaseolicola exhibited a clearly defined lag phase of growth in bean under the conditions of our tests. This is in contrast to the findings of Ercolani & Crosse (3) and Omer & Wood (8), using the same host-pathogen combination. The generation times which we obtained also were considerably shorter. Perhaps these differences

occurred because the bean cultivars used were different, as were the media used for growing the pathogen.

One may speculate on the significance of the ^{35}S diffusion difference plot. It appears that greater diffusion occurs several hours after initiation of logarithmic growth of the pathogen, thus indicating that diffusion changes are not required for at least initial bacterial multiplication. The sharp increase in diffusion followed by a much slower logarithmic rate suggests that a threshold is reached whereby some event is initiated which provides for the increased ^{35}S diffusion. The inoculation procedure used (infiltration of a large number of bacteria into a leaf) ensures that this event would begin simultaneously in a number of loci or plant cells, and would explain the sudden sharp rise in diffusion. As the bacteria continue to multiply logarithmically and to spread, the event would then be triggered in additional loci at a log rate.

This event which is initiated could be a breakdown of membrane permeability leading to the increased leakage of ^{35}S , or perhaps a new pathway is activated as suggested by Hancock (4). It is further tempting to speculate that an event similar to the hypersensitive response is involved. A suspension of 10^8 cells/ml of *P. phaseolicola* often will induce a hypersensitive reaction in plants (5). Collapse of the plant cells occurs 6 to 20 hr after infiltration of the bacteria. Conversion of the growth curve to fit the number of cells actually infiltrated into the leaves suggests that

the population reached a level corresponding to ca. 10^8 cells/ml prior to detection of increased diffusion.

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