

Isolation of *Pseudomonas phaseolicola* from Bean Leaves Exhibiting Systemic Symptoms

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ABSTRACT

Pseudomonas phaseolicola was present in bean leaves exhibiting chlorotic symptoms. Isolation of the pathogen from these leaves depended upon the technique employed. *Pseudomonas phaseolicola* was isolated from 39 of 40 leaves with an infiltration-centrifugation technique, whereas it was detected in only 16 of 40 leaves using a comminution technique, and then only in small numbers. Phytopathology 61:580-581.

Additional key words: *Pseudomonas glycinea*, soybean.

A common symptom of halo blight infection in the common bean (*Phaseolus vulgaris* L.) is chlorosis of leaves above a leaf exhibiting lesions. Reports have indicated that few bacteria exist in these leaves, and that they rarely can be isolated (1, 4, 5, 7). During development of a technique to remove bacteria from leaves by infiltration-centrifugation (2), however, we consistently isolated *Pseudomonas phaseolicola* from the chlorotic leaves. We therefore investigated whether or not the difficulty in detecting the pathogen was caused by the isolation technique.

Trifoliate leaves exhibiting chlorotic symptoms were selected from *P. vulgaris* 'Pencil Pod Wax', 'Red Kid-

ney Red Kote', 'Pinto UI-111', and 'Rogers Tender Crop', all of which had been inoculated on the primary leaves with *P. phaseolicola* strains HB-36, HB-38, or HB-43 2 weeks before. Half the leaves were surface-sterilized using a 0.5%-sodium hypochlorite solution. One leaflet from each leaf was infiltrated with water, centrifuged at 900 g for 5 min, and the recovered fluid containing the bacteria then streaked on King's B medium (3). Another chlorotic leaflet from the same leaf was comminuted in a mortar and pestle in 2 ml water and similarly streaked. Bacterial colonies that developed on the medium were identified as *P. phaseolicola* on the basis of fluorescence, oxidase test, colony morphology, and the conducting of sufficient pathogenicity tests to verify identification.

Results indicated that the isolation method greatly affects detection of *P. phaseolicola* in leaves exhibiting chlorotic symptoms. *Pseudomonas phaseolicola* was isolated from chlorotic leaves in 39 of 40 trials with the infiltration-centrifugation technique. Most streaked plates exhibited 40-200 colonies of the pathogen with very little contamination present, regardless of whether or not the leaf surface had been surface sterilized (Fig. 1). In contrast, *P. phaseolicola* was isolated in only 16 of 40 trials when the leaves were comminuted, a ratio similar to that obtained by Waitz & Schwartz (7). Furthermore, only a few colonies developed when the organism was isolated by the latter technique, and considerable contamination often was present in the nonsurface-sterilized leaf samples; this did not, however, appear to have prevented isolation of the pathogen. A related pathogen, *P. glycinea*, cannot be isolated from chlorotic leaves of soybean (6). Therefore, similar tests were conducted with *Glycine max* (L.) Merr. 'Lindarin' and 'Hawkeye' infected with strains R-2 and R-4 of the pathogen. Bacteria were not isolated from chlorotic leaves with either isolation technique. But considerable difficulty was experienced in removing fluid from leaves by centrifugation without severe injury to the leaves, a factor which could have influenced results of the test.

These tests emphasize the hazard of using one iso-

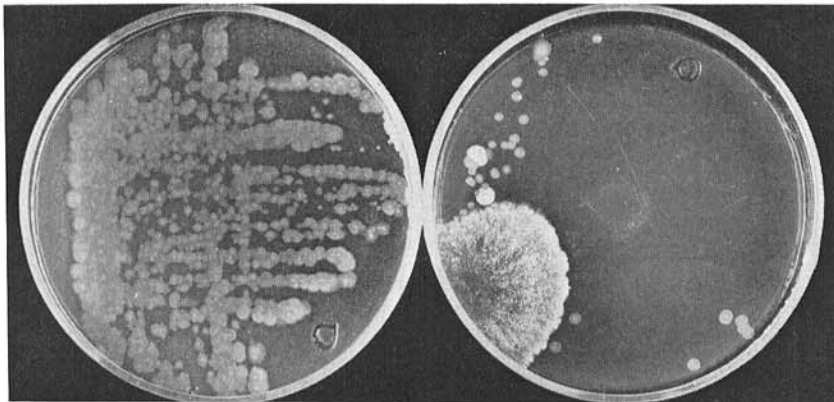


Fig. 1. Isolation of *Pseudomonas phaseolicola* from chlorotic leaflets of the same trifoliate leaf using a comminution technique (right) and an infiltration-centrifugation technique (left). None of the colonies on the comminution plate was *P. phaseolicola*, whereas nearly all colonies on the infiltration plate were those of the pathogen.

lation procedure to determine whether a pathogen is present or associated with particular symptoms and then attaching undue significance to negative results. Many phytopathogenic bacteria have been considered difficult to isolate. Two well-known examples of this are bacterial canker of poplar and bacterial blight of strawberry. In both cases, it was years after the recognition of the diseases before the true causal agents were isolated. Other bacteria are easily isolated at certain times in the disease cycle, but can only be isolated with difficulty at other times. An example of this is bacterial canker of stone fruit, where *P. syringae* is difficult to isolate at certain times of the year.

Reasons for difficulty in isolation of *P. phaseolicola* from ground chlorotic tissues are not apparent. Since bacteria may be present in substantial numbers, it is possible that a toxin is released when the tissues are damaged. But the question arises as to why this toxin would not be present in lesions where the bacteria are easily isolated.

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