A Bacterial Leaf Spot Caused in Yam Bean by *Pseudomonas syringae* pv. *phaseolicola*

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This article reports the results of investigations on a bacterial leaf spot previously recognized by A. P. Martinez, A. C. Hayward, and I. W. Budjenhagen who kindly provided some of the original strains of the pathogen.

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**ABSTRACT**


A bacterium causing leaf spot of yam bean, *Pachyrhizus erosus* (L.) Urb., with symptoms consisting of small brown or water-soaked spots surrounded by a chlorotic or yellow area (Fig. 1), has been observed occasionally in Hawaii since 1969. However, no record of any bacterial disease of yam bean has been found in the literature. The observed symptoms are similar to those of halo blight of common bean (*Phaseolus vulgaris* L.) (8) caused by *P. syringae* pv. *phaseolicola* (ISPP List, 1980), syn. *Pseudomonas phaseolicola* (Burk.) Dowson.

The recorded host range of *P. phaseolicola* is restricted to *Phaseolus* spp. (1,3,17,23), *Macropodium altropurpureum* (siratro) (9), Glycine spp. (9,23), *Pueraria thumbergiana* (kudzu vine) (1,3), and *Dolichos* sp. (soybean weed, NCPPP 1647), and there is a doubtful record on Vigna *sinesis* (1). In Hawaii, *P. phaseolicola* has been recorded only on *P. vulgaris*. Other bacterial nematodes which may incite similar symptoms on some bean species are *P. syringae* pv. *glycinea* (23), pv. *tabaci* (1,3,21), pv. *syringae*, and *Xanthomonas phaseoli* (1,3).

The purpose of this study was to identify the yam bean (YB) pathogen by a combination of plant inoculations, biochemical tests, and toxin assays.

**MATERIALS AND METHODS**

**Strains.** Isolations of the yam bean pathogen were made from yam bean leaves showing symptoms of halo blight. Bacterial strains used in comparative experiments were *P. syringae* pv. *phaseolicola* HB-20, HB-33, HB-36 (supplied by M. N. Schroth, University of California, Berkeley), G50 and G50 tox (19); *P. glycinea* R-6 (supplied by E. G. Keen, University of California, Riverside); *P. syringae* ATCC 19310, pv. *tabaci* ICPB PT5, pv. *tomato* ICPB PT111, pv. *lachrymans*, and *P. fluorescens*. *Escherichia coli* K-12 (N100) was supplied by N. J. Panopoulos, University of California, Berkeley.

**Bacteriological characterization.** Nutritional and biochemical tests (15) were performed on 18 strains that showed similar colony appearance on triphenyltetrazolium chloride medium (10) when isolated from yam bean. Three to five replicates of each test were carried out with each bacterial strain. Tests for fluorescent pigment production, presence of oxidase and arginine dihydrolase,
production of levan, and hydrolysis of aesculin were conducted according to Lelliott et al (13). For testing utilization of substrates, we employed the solid mineral base medium of Palleroni and Doudoroff (16). Filter-sterilized carbon sources were added to a final concentration of 0.1% before the plates were poured. Utilization of KNO₃ as sole nitrogen source was tested on the solid mineral base medium containing 0.2% glucose but no NH₄Cl.

Bacterial cell dimensions were determined on safranin and Gram-stained smears from 48-hr cultures on King’s medium B (KMB) (11). Motility and presence of flagella were determined by the method of Mayfield and Inness (14).

**Pathogenicity.** Strains were first screened for pathogenicity on yam bean and Phaseolus vulgaris ‘Red Kidney’ in the greenhouse (20–30°C) and later in a controlled environment chamber (20–22°C). Plants were inoculated by lightly spraying cell suspensions on young trifoliate leaves, followed by a 4-hr period in a humid chamber. For comparative pathogenicity tests on yam bean, P. vulgaris cultivars Red Kidney and Red Mexican UI-3, inoculum was sprayed onto the undersides of recently unfolded primary leaves at 7–14 kPa to obtain uniform water-soaking, followed by 4 hr in a humid chamber at 24°C. Inocula consisted of cell suspensions (from 24-hr cultures) adjusted to approximately 10⁸ cells per milliliter. Since YB strains differed somewhat in the severity of symptoms induced on yam bean and Red Kidney bean, three strains (A192-1, A192-2, and A192-4), which caused the entire range of pathogenic responses, were selected for further studies.

**TABLE 1.** Comparison of bacteriological properties of bacterial strains from yam bean with three pathovars of Pseudomonas syringae

<table>
<thead>
<tr>
<th>Properties</th>
<th>Reactions of strains or pathovars:</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Yam bean strains</td>
<td>pv. phaseolicola</td>
<td>pv. phaseolicola</td>
<td>pv. tabaci syringae</td>
</tr>
<tr>
<td>Growth on</td>
<td></td>
<td>HB-33</td>
<td>HB-36</td>
<td></td>
</tr>
<tr>
<td>L(+)-tartrate</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D(+)-xylose</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D(-)-sorbitol</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>meso-erythritol</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>i-ribitol</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>n-mannitol</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Use of 0.1% KNO₃</td>
<td>as sole N source</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pitting of sodium polypectate pH 5.0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>pH 8.2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Action on litmus milk</td>
<td>Alk</td>
<td>Alk, R</td>
<td>Alk</td>
<td>D</td>
</tr>
</tbody>
</table>

4Alk = alkaline reaction, R = reduction, D = digestion (peptonization), + = positive reaction, and – = negative reaction.

Yam bean strains A192-1, A192-2, and A192-4 (which reacted similarly).

**RESULTS**

The 18 YB strains reacted similarly in nutritional and biochemical tests and showed all characteristics of Group Ia in the determination scheme of Lelliott et al (13) for phytopathogenic fluorescent pseudomonads. They resembled pv. phaseolicola in that they did not reduce nitrate, produce indole or H₂S, liquify gelatin, hydrolyze starch or aesculin. Acid was produced oxidatively within 4 days from fructose, galactose, glucose, and glycerol. No acid was produced in 20 days on lactose or maltose. Growth occurred in 2 and 3%, but not 4% NaCl medium. No lipolytic activity occurred on Tween-80 agar after 2 days. On litmus milk, all pv. phaseolicola and YB strains except two produced an alkaline reaction in 2 wk. Inhibition zones in antibiotic sensitivity tests were similar to known strains of pv. phaseolicola (19).

In further tests the three selected YB strains behaved uniformly, and differed from pv. tabaci and pv. syringae in substrate utilization, hydrolysis of aesculin, utilization, of KNO₃ as N source, ability to cause pitting of polypectate, and action on litmus.

**TABLE 2.** Results of plant inoculations with bacterial strains from yam bean and three pathovars of Pseudomonas syringae

<table>
<thead>
<tr>
<th>Plant</th>
<th>Yam bean strains</th>
<th>pv. phaseolicola</th>
<th>pv. tabaci syringae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phaseolus vulgaris</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Red Kidney’</td>
<td>A192-1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A192-2</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>A192-4</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Phaseolus vulgaris</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Red Mexican UI-3’</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Pachyphytus erosus (yam bean)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Detached pods</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. vulgaris</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Hawaiian Wonder’</td>
<td>w</td>
<td>wn</td>
<td>w</td>
</tr>
</tbody>
</table>

4Leaf reactions: + = water-soaking only, ++ = water-soaking and systemic chlorosis, and – = hypersensitive necrosis. Pod reactions: w = 50 to 90% sites water-soaked; wn = 90 to 100% sites water-soaked, some with a brownish necrotic surface layer; W = 100% sites water-soaked; and N = 90 to 100% sites necrotic.

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milk (Table 1). The organism was indistinguishable from the tested pv. phaseolicola strains except for its ability to utilize D-mannitol as sole carbon source for growth.

A comparative study of pathogenicity of the three YB strains and strains of pv. phaseolicola, pv. syringae, and pv. tabaci revealed that only the YB strain A192-4 and the pv. phaseolicola strains produced systemic symptoms in inoculated yam bean and Red Kidney bean plants (Table 2). Water-soaked and brownish lesions surrounded by chlorotic halos occurred at primary infection sites on young trifoliate leaves, with chlorosis and distortion of subsequently emerging trifoliate leaves (Fig. 1). YB strains A192-1 and A192-2 caused persistent water-soaking of inoculated leaves of yam bean and Red Kidney bean but did not cause chlorosis or systemic symptoms. In yam bean, as in Red Kidney bean, primary and trifoliate leaves became increasingly resistant as they became older. Plants recovered from chlorotic symptoms when maintained in the greenhouse at 25–30°C. At the cell concentration applied, pv. syringae and pv. tabaci caused hypersensitive necrosis in inoculated yam bean and Red Kidney bean plants. Pseudomonas syringae pv. phaseolicola strains HB-36, G50, and G50 tox caused water-soaking of inoculated primary leaves of bean cultivar Red Mexican U1-3, whereas strain HB-33, the three tested YB strains, and other phytopathogenic pseudomonads all caused hypersensitive necrosis.

Bean pods inoculated with pv. phaseolicola developed water-soaked lesions 2–5 mm in diameter after 3 days incubation. Necrotic, brownish lesions resulted at sites inoculated with pv. tabaci and pv. syringae. All YB isolates produced water-soaked lesions at most inoculation sites, but lesions often were less than 3 mm in diameter (Table 2).

In the E. coli microbial assay (Table 3), pv. phaseolicola strains HB-33, HB-36, and YB strain A192-4 produced large clear zones of inhibition of E. coli K-12 after preincubation at 18°C. In some replicates the inhibition zones were slightly turbid, but small, clear inner halos were apparent within the large zones around colonies of strains HB-33 and A192-4. With YB strains A192-1 and A192-2, some replicates produced only small halos while others caused no inhibition zones. Pseudomonas syringae ATCC 19310 produced turbid inhibition zones, and pv. tabaci PT5 produced a small clear zone (Fig. 2). After preincubation at 31°C, pv. phaseolicola and YB strains either produced no inhibition zones or only small halos (Fig. 3). Incubation at 31°C did not change results with pv. syringae whereas with pv. tabaci larger zones were produced at 31°C than at 18°C. In subsequent trials pv. phaseolicola strain G50 behaved similarly to other pv. phaseolicola strains and to YB strain A192-4, while G50 tox and HB-20 resembled A192-1 and A192-2. Pseudomonas fluorescens caused no inhibition zone, pv.

![Fig. 1. Yam bean leaves showing chlorotic lesions at primary infection sites of A, strain A192-4 and B, systemic chlorosis and distortion following infection by strain A192-4.](image1)

![Fig. 2. Growth inhibition of Escherichia coli K-12 by Yam bean (YB) strains and Pseudomonas syringae pathogens, preincubated at 18°C.](image2)
lachrymans caused turbid zones at 18 and 31 C, pv. tomato caused inhibition zones like those of pv. phaseolicola or pv. syringae only at 18 C, and pv. glycinea produced similar zones at both 18 and 31 C (Table 3).

Extracts of yam bean and Red Kidney bean leaves inoculated with pv. phaseolicola strain G50 and YB strain A192-4 caused inhibition of bean OCT, whereas extracts of leaves inoculated with YB strains A192-2 and A192-4 yielded no inhibition of OCT activity. Sensitivity of the enzymatic assay was similar for phaseotoxin from bean leaves and culture filtrates, in contrast with the E. coli assay, which required approximately 100-fold higher concentrations of toxin from inoculated plants than from culture.

After passage through a Sephadex G15 column, extracted culture filtrates of YB strain A192-4 yielded a single peak of OCT inhibitory activity at a K_{m} (0.25) corresponding to that of phaseotoxin from cultures of pv. phaseolicola. Toxin production by strain A192-4 declined during prolonged maintenance in culture, but was restored when the strain was resolicited from inoculated bean leaves.

**DISCUSSION**

The YB strains resembled pv. phaseolicola and were distinct from pv. tabaci and pv. syringae in biochemical and nutritional tests that distinguished these pathovars (2,7,15,22,23). Although YB strains differed from pv. phaseolicola strains used in the present study in α-mannitol utilization, several other strains of pv. phaseolicola are capable of utilizing mannitol (22,23).

Phytoxins-producing and nonproducing strains of the YB pathogen occur, as they do in pv. phaseolicola. Toxigenic and nontoxigenic YB strains, and similar pv. phaseolicola strains, induce the same symptoms, including temperature dependence of chlorotic symptoms (8). The water-soaked lesions induced by YB strains in detached pods are typical of pv. phaseolicola (24). The smaller lesion size caused by YB strains relative to the pv. phaseolicola strains tested suggests that YB strains are less aggressive on the bean cultivar used.

The distinct phytoxins produced by pv. phaseolicola, pv. tabaci, and pv. syringae (18) might be useful as determinative criteria for these bacteria, if simple, rapid, and specific tests for the phytoxins were available. A rapid microbiological assay for toxin(s) produced by pv. phaseolicola has been described (25).

In our investigation, YB strain A192-4 caused inhibition of E. coli K-12 similar to that caused by known toxigenic strains of pv. phaseolicola. The small inhibition zones produced by nontoxigenic pv. phaseolicola strains, and by some toxigenic strains at 31 C, suggest that diffusible products other than phytoxins also inhibit E. coli in the assay. Moreover, inhibition zones caused by some other toxigenic pseudomonads resembled those of pv. phaseolicola. Gasson (4,5) also demonstrated that other toxin-producing phytopathogenic pseudomonads inhibit E. coli. Thus, caution is required in interpreting the results of the E. coli bioassay, especially as a determinative test. The mechanisms of inhibition of E. coli by dissimilar phytoxins apparently differ, as evidenced by reversal of inhibition zones by different amino acids (5,25). It may, therefore, be possible to devise variations of the technique specific for particular phytoxins, by altering the assay conditions.

Results of the microbial assay obtained by using overlaid cultures were consistent with those of the enzymatic bioassay on extracts from inoculated bean leaves, confirming phytoxins production by YB strain A192-4 and no phytoxins production by strains A192-1 and A192-2. The enzymatic bioassay demonstrated potent inhibition of OCT by the phytoxins from the YB pathogen, which is characteristic of pv. phaseolicola (20). The nontoxigenic YB strains may represent variants selected in culture, since toxin production by strain A192-4 declined during maintenance in culture. We have observed similar decline in toxin production by some pv. phaseolicola strains (unpublished). Restored levels of toxin production in such strains resolicited from bean leaves suggests that selection in the host plant favors toxin-producing cells.

On the basis of biochemical features, plant reactions, and phytoxins characteristics, we conclude that the pathogen causing leaf spot of yam bean is pv. phaseolicola. This is the first report of Pachyrrhizus erosus as a host of pv. phaseolicola. Yam bean may be significant as an alternative host for the bean halo-blight pathogen in tropical regions where this perennial vine is widely cultivated as a food crop.

**LITERATURE CITED**