Bacterial Leaf Spot of *Hibiscus rosa-sinensis* Incited by *Pseudomonas syringae* pv. *hibisci*

J. B. JONES, GCREC, University of Florida, Bradenton; A. R. CHASE, AREC, University of Florida, Apopka; B. C. RAJU, Yoder Brothers, Alva, FL; and J. W. MILLER, Division of Plant Industry, Gainesville, FL

ABSTRACT

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Strains of *Pseudomonas syringae* that cause leaf spot of *Hibiscus rosa-sinensis* in Florida were found to be physiologically and pathogenically distinct from *P. syringae* pv. *syringae* (*P. s. syringae*). Strains isolated from hibiscus utilized L(+)tartrate but not DL-lactate and did not produce syringomycin or antimetabolites against *Escherichia coli*. Four of five strains of *P. s. syringae* were positive for the latter two tests, and all strains of *P. s. syringae* were negative for growth on L(+)tartrate and positive for growth on DL-lactate. On the basis of biochemical and pathogenicity tests, the bacterium was also distinct from two other *P. syringae* pathovars. We propose that the strains isolated from *H. rosa-sinensis* be designated *P. s. hibisci*. Cultivars of *H. rosa-sinensis* varied in susceptibility to this pathovar.

A leaf spot has been observed on cuttings of *Hibiscus rosa-sinensis* L. in Florida for many years (2). Isolations from the spots have consistently yielded a bacterium that produces a fluorescent pigment and is negative for oxidase and arginine dihydrolase. A bacterial blight of hibiscus was described previously in Japan (14). The bacterium originally was designated *Bacterium hibisci* and more recently was placed in *Pseudomonas syringae* (15) with questionable status because of the lack of an extensive study of its biochemical and physiological characteristics. In current nomenclatural convention (15), strains previously placed in *P. syringae* would now be identified as *P. syringae* pv. *syringae* (*P. s. syringae*). Because the description by Nakada and Takimoto (14) predated contemporary tests for bacterial identification, the bacteria isolated in Florida cannot be identified as being the same as those isolated from hibiscus in Japan. According to the scheme of Hildebrand and Schroth (6) for the identification of fluorescent pseudomonads, strains that have been isolated recently from hibiscus were not as close biochemically to *P. s. syringae* as they were to some other pathovars of *P. syringae*.

The purposes of this report are to describe the disease and to present data on the basis of which we designate the pathogen as a new pathovar of *P. syringae*. In addition, we report the relative susceptibility of several cultivars of hibiscus to this bacterium.

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MATERIALS AND METHODS

Lesions in leaves of *H. rosa-sinensis* were triturated in sterile deionized water, and the triturated suspension was plated on nutrient-yeast-dextrose agar (9) and medium B of King et al (KMB) (10). Selected isolates were tested for induction of a hypersensitive reaction (11) on tomato (*Lycopersicon esculentum* Mill.) and tobacco (*Nicotiana tabacum* L.), oxidase reaction (9), arginine dihydrolase (18), Gram stain reaction (17), presence of flagella (4), utilization of various carbon sources supplied at 0.2% in the minimal medium of Misaghi and Grogan (13) slightly modified (9), β-glucosidase (16), levan (16), oxygen requirement (16), soft rotting of potato (16), syringomycin (5), antimetabolite toxin (3), and ice-nucleating activity (12).

Selected isolates were tested for pathogenicity on *H. rosa-sinensis*, on F_2C tobacco (*Nicotiana tabacum* L.), and on Marketer cucumber (*Cucumis sativa* L.). Strains of *P. s. syringae* used in these tests were from wheat (one), cherry (two), and tomato (five). Single strains of *P. s. phaseolicola*, *P. s. tomato*, *P. s. coronofaciens*, *P. s. glycinea*, and *P. s. pisi* were also tested. Tobacco and cucumber plants were grown in a mixture of Florida peat, sand, vermiculite, and perlite (5:3:3:1, v/v). Calcium carbonate, dolomitic lime, hydrated lime, superphosphate, Micromax (Sierra Chemical Co., Milpitas, CA), and Osmocote (Sierra) were applied at 5.54, 5.54, 0.549, 1.39, 0.83, and 6.57 kg/m^3, respectively. The plants were placed in a mist chamber at 25–28 C for 16 hr before inoculation. For inoculum production, cultures were grown on KMB for 48 hr at 28 C. A suspension of 10^8 colony-forming units (cfu) per milliliter was obtained by turbidity adjustment and was gently misted on both surfaces of the foliage. Inoculated plants were placed in polyethylene bags for 36 hr. The plants were observed 7 days later for symptoms.

Cuttings of hibiscus cultivars for use in pathogenicity studies and cultivar susceptibility tests were obtained from commercial producers. The cuttings were rooted and grown in the following medium: Canadian peat, cypress shavings, and pine bark (2:1:1, v/v). The medium was amended with 4.4 kg of Osmocote, 4.2 kg of dolomitic lime, and 0.9 kg of Micromax per cubic meter. Inoculum for experiments with *H. rosa-sinensis* was produced on nutrient agar for 2 days at 32 C. The inoculum was adjusted to 10^8 cfu per milliliter. Plants were placed on a glasshouse bench, where they received intermittent mist (5 sec/30 min from 0800 to 2000 hours daily) for 24 hr before inoculation and during incubation.

RESULTS AND DISCUSSION

The leaf spot is generally characterized by a small irregular-shaped dark brown to black lesion that occasionally has a prominent chlorotic halo (Fig. 1). In no instance was any observable symptom associated with the flowers, petioles, or stems. The lesions were up to 0.5 mm on immature leaves and up to 3 mm on mature leaves. Lesion development on immature leaves was associated with a crinkled appearance of the leaf. Blighting as reported by Nakada and Takimoto (14) was not observed. In Florida, however, hibiscus plants are grown from cuttings, whereas in the original study, plants were grown from seed.

The leaf spot is most prevalent in Florida in January and February and is less prevalent in March. The leaf spot was only observed on plants grown under intermittent mist. We observed that the plants required a preinoculation mist period to maximize disease development. Disease development was optimum at 18 C and considerably less at 15 and 24 C (A. R. Chase, *unpublished*). The disease developed within 3 days when inoculated plants were incubated at 18 C and under high humidity. Generally, symptoms developed within 5–21 days under less than optimal environmental conditions. The low temperature optimum for disease development helps explain the

presence of this disease during the cool months.

A bacterium that produced a fluorescent pigment on KMB and was negative for oxidase and arginine dihydrolase was consistently isolated from leaf spots of hibiscus. The bacterium was a strict aerobe and a gram-negative rod with polar, lophotrichous flagella. It induced a hypersensitive reaction in tobacco.

The hibiscus strains were similar biochemically to *P. s. tabaci* and *P. s. lachrymans* (Table 1). According to Hildebrand and Schroth (6), utilization of L(+)tartrate, sucrose, and mannitol are tests useful for distinguishing those two bacteria from the other fluorescent phytopathogenic pseudomonads. The hibiscus strains were readily separated from *P. s. syringae* because of the inability of the former to produce syringomycin or an antimetabolite inhibitory to *Escherichia coli* and to utilize DL-lactate and also because of the inability of strains of the latter to utilize L(+)tartrate. In previous studies, *P. s. syringae* has been shown to be unable to utilize L(+)tartrate but generally did utilize DL-lactate (5,6,9,12). The hibiscus strains also utilized D(+)arabinose, *meso*-tartrate, DL-arginine, L-asparagine, D-saccharate, D(+)mannose, glutarate, and dextrose. The bacterial strains isolated from hibiscus did not utilize adonitol, D(−)arabinose, L(−)arabitol, maltose, betaine, glycollate, D(+)trehalose, rhamnose, dulcitol, cellobiose, and citraconnate.

In the first pathogenicity test, four hibiscus strains were compared with two strains each of *P. s. tabaci* and *P. s. lachrymans* for pathogenicity on cucumber and on tobacco. The hibiscus strains were negative for pathogenicity on both cucumber and tobacco, whereas *P. s. lachrymans* induced symptoms on cucumber only and *P. s. tabaci* was pathogenic on tobacco (Table 2). In a second experiment, four strains of *P. s. syringae*, three strains of hibiscus, two strains of *P. s. lachrymans*, one strain of *P. s. tabaci*, and one strain each of several other pathovars were compared for pathogenicity on *H. rosa-sinensis*. Two of three hibiscus strains produced a strong reaction on hibiscus, whereas none of the *P. s. syringae* strains or the other pathovars were pathogenic on hibiscus (Table 2). In other pathogenicity tests, all eight hibiscus strains tested induced pathogenic reactions on hibiscus.

Although the bacterium inducing the leaf spot of hibiscus had been identified previously as *P. syringae* (15), the hibiscus strains are distinct from *P. s. syringae* on the basis of the biochemical and pathogenicity data presented in this paper. Although they are more closely related to *P. s. tabaci* and *P. s. lachrymans*, they are pathologically distinct. The hibiscus strains were specific to *Hibiscus*, and the two other pathovars did not infect hibiscus.

In the varietal susceptibility study, one cultivar was considerably more susceptible than two other cultivars to the bacterium (Table 3). Brilliant Red had the highest number of lesions, whereas President had the lowest.

In a chemical control test under greenhouse conditions with streptomycin (300 μg/g) and cupric hydroxide (53% a.i., 2.3 g/L) or cupric hydroxide mixed

Fig. 1. Leaf spot lesions on *Hibiscus rosa-sinensis* showing irregular shapes and prominent chlorotic halos.

Table 1. Comparison of *Pseudomonas syringae* (P. s.) strains isolated from *Hibiscus rosa-sinensis* with other phytopathogenic pseudomonads in physiological tests

Diagnostic tests	Hibiscus isolates (4)[a]	*P. s. tabaci* (2)	*P. s. lachrymans* (1)	*P. s. coronofaciens* (2)	*P. s. syringae* (5)	*P. s. tomato* (2)	*P. s. phaseolicola* (2)	*P. chicorii* (1)	*P. viridiflava* (1)
Utilization of									
β-Alanine	0[b]	0	0	0	0	0	0	0	0
Mannitol	4	2	1	2	5	2	0	1	1
Sorbitol	4	2	1	2	5	2	0	0	0
Erythritol	4	1	1	2	5	0	0	0	1
L(+)Tartrate	4	1	1	0	0	0	0	1	0
D(−)Tartrate	0	0	0	0	1	2	0	0	1
DL-Lactate	0	0	1	0	5	0	0	1	1
Sucrose	4	2	1	2	5	2	1	0	0
Trehalose	0	0	0	0	0	0	0	0	0
Production of									
β-Glucosidase	4	2	1	2	5	2	0	1	0
Ice-nucleating activity	4	2	1	2	5	0	0	0	1
Syringomycin	0	NT[c]	NT	NT	4	NT	NT	NT	NT
Antimetabolite against *Escherichia coli*	0	NT	NT	NT	4	NT	NT	NT	NT

[a] Number in parentheses represents number of strains tested.
[b] Number represents number of strains positive for a reaction.
[c] NT = not tested.

Table 2. Pathogenicity of various strains of *Pseudomonas syringae* (*P. s.*) on three plant hosts

Test organism	Test 1		Test 2
	Cucumber	Tobacco	Hibiscus
P. s. syringae (8)[a]	NT[b]	NT	0[c]
P. s. phaseolicola (1)	NT	NT	0
P. s. lachrymans (2)	2	0	0
P. s. tomato (1)	NT	NT	0
P. s. pisi (1)	NT	NT	0
P. s. coronofaciens (1)	NT	NT	0
P. s. tabaci (2)	0	2	0[d]
P. s. glycinea (1)	NT	NT	0
Hibiscus strains (4)	0	0	2[e]

[a]Number in parentheses is number of strains tested.
[b]NT = not tested.
[c]Number of strains positive for pathogenic reaction on the host.
[d]Only one strain tested on *Hibiscus rosa-sinensis*.
[e]Only three strains tested on *H. rosa-sinensis*.

Table 3. Susceptibility of seven cultivars of *Hibiscus rosa-sinensis* to *Pseudomonas syringae* pv. *hibisci*

Cultivar	No. of lesions/plant
Euterpe	16.3 ab[z]
White-Red Eye	10.7 a
Senorita	36.7 ab
President	6.2 a
Brilliant Red	56.1 b
Pink Versicolor	17.3 ab
Butterfly	21.9 ab

[z]Numbers followed by same letter are not significant at $P = 0.01$ according to Duncan's multiple range test.

with maneb (1.8 g/L), the bactericides were applied to hibiscus foliage and the plants were placed in mist for 24 hr before inoculation. Plants were inoculated by gently misting a suspension of 10^8 cfu/ml on the plants. The inoculated plants were placed back in the mist and rated for disease development after 7 days. Disease was not controlled on inoculated plants with any of the chemical treatments. Chemical control under this type of misting system does not appear feasible. Reduction of leaf moisture by eliminating overhead irrigation or minimizing it has suppressed disease in commercial operations (B. C. Raju, *personal communication*). Preinoculation moisture has been observed previously to enhance disease development (7,8).

The biochemical responses of *P. s. syringae* and the hibiscus strains are considerably different and are important enough to differentiate the hibiscus strains from *P. s. syringae*. Although the bacterium originally isolated from hibiscus in 1923 was placed in *P. syringae*, the correct contemporary taxonomic placement of this bacterium is not known. It was placed in *P. syringae* because of the limited bacteriological tests that were conducted (1,14). It is not possible to state conclusively that the bacterium originally identified by Nakada and Takimoto (14) is the same bacterium that was isolated from hibiscus in Florida.

We propose that the bacterium that causes leaf spot of *H. rosa-sinensis* be named *P. s. hibisci* to distinguish it from *P. s. syringae*. The type strain, designated P53, is deposited at the Agricultural Research and Education Center, Apopka, and at the Gulf Coast Research and Education Center, Bradenton.

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