Pseudomonas ficuserectae sp. nov., the Causal Agent of Bacterial Leaf Spot of *Ficus erecta* Thunb.

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Pseudomonas ficuserectae, a new nonfluorescent, phytopathogenic pseudomonad species, is described. This bacterium produces dark brown, water-soaked spots on the leaves and stems of *Ficus erecta* Thunb., resulting either in defoliation or shoot blight on severely infected plants. The colonies on nutrient agar plates are white, circular, and 3 to 4 mm in diameter after 6 days. *P. ficuserectae* is similar to *Pseudomonas amygdali* in many properties. The differences between these two species include size of colonies on agar plates, hydrolysis of Tween 80, production of H₂S, utilization of ribose, raffinose, mannitol, sorbitol, and malonate, and pathogenicity. The deoxyribonucleic acid base composition of the type strain of *P. ficuserectae*, strain L7, is 59 mol% guanine plus cytosine. The specific epithet of this new species reflects the pathogenicity of the bacterium on *F. erecta* Thunb.

Since 1973, a bacterial disease has been observed in Japan on the tree *Ficus erecta* Thunb. (Japanese common name, Inubiwa), which is often planted in gardens as an ornamental plant. This disease is characterized by small, water-soaked spots on the leaves and shoot blight, and a nonfluorescent pseudomonad has been consistently isolated from diseased tissues. Therefore, I performed a taxonomic study comparing this organism with three nonfluorescent pseudomonads, *Pseudomonas andropogonis* (Smith) Stapp 1928 (14), *Pseudomonas amygdali* Psallidas and Panagopoulos 1975 (8), and *Pseudomonas pseudoalcaligenes* subsp. *citrulli* Schaad et al. 1978 (10), which are similar in some respects to the bacterium isolated from diseased *F. erecta* tissues. *Pseudomonas syringae* pv. *phaseolicola* (Burkholder) Young et al. 1978 (16) was also included as a reference bacterium.

MATERIALS AND METHODS

Bacteria. The bacterial strains used in this study are listed in Table 1. Eleven cultures of the *Ficus* bacterium were isolated from new lesions formed on young leaves and twigs by the conventional plating method on yeast extract-peptone agar (0.5% yeast extract, 1.0% peptone, 1.5% agar, pH 6.8). Small white colonies grew on the plates in 2 days at 28°C. Single colonies were selected and grown on yeast extract-peptone agar slants. The cultures were maintained at 4°C for routine work.

Pathogenicity. Inoculation tests were made on *F. erecta* Thunb., *Ficus carica* L. (cultivar unknown), *Morus alba* L. (cultivar unknown), and *Prunus armeniaca* L. cv. Wasesuimitsu; 2-year-old plants grown from cuttings were inoculated either by spraying with bacterial suspensions (concentration, 10⁸ cells per ml) over young shoots or by puncturing mature leaves and

stems with a needle through drops of bacterial suspension placed on the plant surface. The inoculated plants were kept in a mist chamber for 24 h and then transferred to a greenhouse, where disease development was monitored.

Bacteriological properties. Cell size was measured under a light microscope after staining with Ziehl carbolfuchsin (12). Poly-β-hydroxy-butyrate accumulation was detected either by staining with sudan black (9) or by observing under a phase-contrast microscope a wet mount of cells grown on a synthetic medium (0.05% NH₄H₂PO₄, 0.02% MgSO₄ · 7H₂O, 0.02% KCl, 5% glucose, pH 6.8) for 3 to 5 days. The modified Yamanaka method was used for flagellar staining (11). Reactions for catalase, amino acid decarboxylase, amylase, urease, and Kovacs oxidase, reduction of KNO₃, and production of acetoin, 2-ketogluconate, and indole were tested by method 1, and production of H₂S was tested by method 3 described in Cowan and Steel's *Manual for the Identification of Medical Bacteria*, 2nd ed. (2). Phenylalanine deaminase was tested on 2-day-old phenylalanine agar slant cultures by method 2 of the same manual. Methods described in this manual were also used to determine the results for the litmus milk reaction, gelatin liquefaction, the methyl red test, growth inhibition by KCN, the Hugh-Leifson test, and esculin hydrolysis. The methods of Dye were used to test utilization of asparagine as a sole source of carbon and nitrogen and tolerance to NaCl (4). The production of reducing substances from sucrose, arginine dihydrolase, and levan formation were tested by methods described in the *Laboratory Guide for Identification of Plant Pathogenic Bacteria* (9). Utilization of carbon sources was tested by using the basal media developed by Ayers et al. (1) and by Starr (15). Denitrification was tested by the method of Stanier et al. (13). Pigmentation was tested on King B medium (5).

G+C content of DNA. Deoxyribonucleic acid (DNA) was isolated and purified by the method of Marmur

TABLE 1. *Pseudomonas* strains included in this study

Taxon	Strain(s)	History ^a	Habitat
<i>Pseudomonas ficuserectae</i>	L2, L3, L5, L6, L7, L8, L9, L10, L11, S1, S2 ^b	Goto	<i>F. erecta</i>
<i>Pseudomonas pseudoalcaligenes</i> subsp. <i>citrulli</i>	PDDCC 6521, PDDCC 6522	Schaad (PDDCC)	Watermelon
<i>Pseudomonas amygdali</i>	PDDCC 3918 ^T , PDDCC 3920	Panagopoulos (PDDCC)	Almond
<i>Pseudomonas andropogonis</i>	PA1 ^c	Goto	Sorghum
<i>Pseudomonas andropogonis</i>	PA21 ^c	Goto	Clover
<i>Pseudomonas andropogonis</i>	PA31 ^c	Goto	<i>Strelitzia</i>
<i>Pseudomonas syringae</i> pv. <i>phaseolicola</i>	PP1 ^c	Goto	Kidney bean

^a Person who isolated the strain(s). PDDCC, Plant Diseases Division Culture Collection, Auckland, New Zealand.

^b Strains L2, L3, L5, L6, L7, L8, L9, L10, and L11 were isolated from leaves, and strains S1 and S2 were isolated from stems.

^c Culture Collection of Phytopathogenic Bacteria, at the Laboratory of Plant Pathology, Shizuoka University, Shizuoka, Japan.

(6). The guanine-plus-cytosine (G+C) content of the DNA was calculated by using the equation of Marmur and Doty (7) and the thermal denaturation temperature, which was measured with a Gilford model 250 spectrophotometer. DNA of *Micrococcus luteus* (Schroeter) Cohn strain IAM 1056 was used as a reference; the base composition of this DNA was 72 mol% G+C.

RESULTS AND DISCUSSION

The 11 strains of the *Ficus* bacterium were identical in their morphological, physiological, and biochemical properties. In addition, as shown below, they were distinct from strains of three other phytopathogenic nonfluorescent species (*Pseudomonas amygdali*, *Pseudomonas pseudoalcaligenes* subsp. *citrulli*, *Pseudomonas andropogonis*) and *Pseudomonas syringae* pv. *phaseolicola*. The name *Pseudomonas ficuser-*

ectae sp. nov. is proposed for the new strains.

***Pseudomonas ficuserectae* sp. nov.** *Pseudomonas ficuserectae* (fi.cus.e.recta. *Ficus erecta* name of host species; L. n. *ficuserectae* of *Ficus erecta*) cells are gram-negative, non-encapsulated, nonsporeforming rods with an average size of 2.0 by 0.5 μ m and are motile by means of one to five polar flagella (Fig. 1). Poly- β -hydroxybutyrate granules accumulate. Colonies on yeast extract-peptone agar plates at 28°C are white, transparent, circular, convex, and 0.1 to 0.3 mm in diameter after 2 days and 2 to 3 mm in diameter after 6 days. Growth on yeast extract-peptone agar slants becomes very viscid after several days.

Pseudomonas ficuserectae was identical to the other four species studied in being positive for the following tests: motility, aerobic growth, catalase reaction, oxidative metabolism of fruc-

FIG. 1. *Pseudomonas ficuserectae* cells.

TABLE 2. Phenotypic characteristics of *Pseudomonas ficuserectae*, *Pseudomonas amygdali*, *Pseudomonas pseudoalcaligenes* subsp. *citrulli*, *Pseudomonas andropogonis*, and *Pseudomonas syringae* pv. *phaseolicola*

Character	<i>Pseudo- monas ficuserectae</i>	<i>Pseudo- monas amygdali</i>	<i>Pseudomonas pseudoalcaligenes subsp. citrulli</i>	<i>Pseudomonas andropogonis</i>	<i>Pseudomonas syringae</i> pv. <i>phaseolicola</i>
Flagella	1-5 (polar)	1-6 (polar)	1 (polar)	1-3 (polar)	1-3 (polar)
Poly- β -hydroxybutyrate accumulation	+	+	-	+	-
Oxidase	-	-	+	-	-
Growth at 41°C	-	-	+	-	-
Growth at 4°C	+	+	-	+	+
Utilization of asparagine as C and N sources	+	+	-	+	+
Nitrate reduction	-	-	+	-	-
Levan formation	+	+	-	-	+
Gelatin liquefaction	-	-	$\pm$	-	-
Tween 80 hydrolysis	-	+	+	-	+
H ₂ S production	-	+	+	-	-
Oxidation of gluconate	-	-	+	-	-
Reducing substances from sucrose	+	+	+	-	+
Urease	-	-	+	+	-
Arginine decarboxylase	-	-	+	-	-
Growth in 4% NaCl	-	-	+	+	-
G+C content	59 mol%	61 mol%	66 mol% ^b	ND ^c	ND
Utilization of:					
L-Arabinose	+	+	-	+	+
D-Arabinose	-	-	-	-	-
Xylose	-	-	-	+	+
Ribose	-	+	+	+	+
Glucose	+	+	-	+	+
Mannose	+	+	-	+	+
Rhamnose	-	-	-	+	+
Cellobiose	-	-	-	-	+
Lactose	-	-	-	+	-
Saccharose	+	+	-	-	+
Melibiose	-	-	-	+	+
Raffinose	+	-	-	-	+
Melezitose	-	-	-	+	-
Ethanol	-	-	+	-	-
Glycerol	+	-	+	+	+
Inositol	-	-	-	+	-
Mannitol	-	+	-	+	+
Adonitol	-	-	-	+	-
Sorbitol	- ^d	+	-	+	-
Formate	-	-	-	-	+
Acetate	-	-	+	+	-
Lactate	-	-	+	+	-
Maleate	-	-	+	-	+
Malonate	+	+	+	+	+
Propionate	-	-	+	-	-
Caprate	-	-	+	-	-
Tartrate	-	-	+	-	-
β -Alanine	-	-	+	-	-
Serine	-	-	+	+	+
Leucine	-	-	+	-	-
γ -Aminobutyric acid	-	-	+	+	+
Valine	-	-	+	-	-
Ethylene glycol	-	-	+	-	-

^a +, Positive reaction; -, negative reaction; $\pm$, weak reaction; (d), delayed reaction.^b From reference 10.^c ND, Not determined.^d Strain S2 was positive.

TABLE 3. Main differences between *Pseudomonas ficuserectae* and *Pseudomonas amygdali*

Species	Size of colonies on nutrient agar plates after 7 days (mm)	Hydrolysis of Tween 80	Production of H ₂ S	Utilization of:					G+C content (mol%)
				Ribose	Raffinose	Glycerol	Mannitol	Sorbitol	
<i>Pseudomonas ficuserectae</i>	3-4	—	—	—	+	+	—	— ^a	59
<i>Pseudomonas amygdali</i>	0.3-1.0	+	+	+	—	—	+	+	61

^a Strain S2 was positive.

tose, alkaline reaction of litmus milk, growth in 3% NaCl, utilization of L-galactose, fructose, malate, fumarate, succinate, and citrate, and tobacco hypersensitive reaction. Negative reactions were obtained in the following tests: Gram stain, growth factor requirement, denitrification, utilization of arginine and betaine as carbon sources, arginine dihydrolase, starch hydrolysis, decarboxylase reaction with lysine and ornithine, phenylalanine deaminase, growth in 5% NaCl and 0.0075% KCN, indole production, methyl red test, acetoin production, potato rot, and utilization of D-galactose, maltose, trehalose, erythritol, dulcitol, xylan, inulin, glycogen, starch, carboxymethyl cellulose, salicin, α -methyl-D-glucoside, esculin, oxalate, glycolate, butyrate, sebacate, hippurate, arginine, betaine, valine, and ethylene glycol.

Additional characteristics of *Pseudomonas ficuserectae* that differentiate this species from *Pseudomonas amygdali*, *Pseudomonas pseudoalcaligenes* subsp. *citrulli*, *Pseudomonas andropogonis*, and *Pseudomonas syringae* pv. *phaseolicola* are shown in Table 2. *Pseudomonas ficuserectae* is clearly distinguished from *Pseudomonas andropogonis* and *Pseudomonas syringae* pv. *phaseolicola* by its limited ability to utilize sugars and sugar alcohols and from *Pseudomonas pseudoalcaligenes* subsp. *citrulli* in the limited number of organic acids utilized, as well as by its negative reactions in some physiological and biochemical tests. In contrast, *Pseudomonas ficuserectae* is similar to *Pseudomonas amygdali* in phenotypic characteristics (8). The differences between these two species are shown in Table 3. In addition to differences in seven biochemical properties and G+C content, the strains of *Pseudomonas amygdali* are quite distinctive in that their growth on yeast extract-peptone agar plates is retarded; colonies are only 0.3 to 1.0 mm in diameter after 7 days at 28°C in contrast to *P. ficuserectae* colonies, which are 3 to 4 mm in diameter. The delayed growth of *Pseudomonas amygdali* on agar plates is consistent even after repeated subculturing on the same medium.

The disease produced by *Pseudomonas ficuserectae* is characterized by leaf spots which develop beside the thick veins (Fig. 2A). These

spots are angular, 1 to 3 mm across, and dark green to dark brown, with a water-soaked appearance. The individual spots enlarge gradually and coalesce to form large lesions which invariably are surrounded by a yellow halo. Severely infected leaves often defoliate. In early spring,

FIG. 2. Plant infected with *Pseudomonas ficuserectae*. (A) Leaves showing spots which develop beside the veins. (B) Shoot blight.

the disease also develops on new shoots, causing shoot blight which results in dieback of the twigs (Fig. 2B).

In inoculation tests, *Pseudomonas ficuserectae* produced small, water-soaked dark green spots on the leaves of *F. erecta* 4 to 5 days after inoculation by the spraying method. These spots enlarged slowly to form angular lesions with or without halos, were 1 to 2 mm across after 10 days, and turned dark brown. The strains of *Pseudomonas amygdali*, *Pseudomonas pseudoalcaligenes* subsp. *citrulli*, *Pseudomonas andropogonis*, and *Pseudomonas syringae* pv. *phaseolicola* tested did not produce any symptoms on *F. erecta*. None of the bacteria studied, including *Pseudomonas ficuserectae*, was pathogenic on *F. carica* L., *M. alba* L., or *Prunus armeniaca* L. when either the pricking method or the spraying method of inoculation was used.

The base composition of *Pseudomonas ficuserectae* DNA is 59 mol% guanine plus cytosine.

The type strain is strain L7 (= ATCC 35104 = PDDCC 7848).

In 1980, *Pseudomonas syringae* pv. *fici* was reported from India as the pathogenic agent of bacterial leaf spot of *Ficus palmata* Forsk. (3). This bacterium shares with *Pseudomonas ficuserectae* many biochemical properties; however, these two organisms do differ in utilization of carbohydrates. The type strain of *Pseudomonas syringae* pv. *fici* has not been designated, and no culture has been available from any culture collection; thus, a direct comparison between *Pseudomonas ficuserectae* and *Pseudomonas syringae* pv. *fici* could not be undertaken.

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