

Bacterial Wilt of Perilla Caused by *Pseudomonas solanacearum* and its Transmission

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ABSTRACT

Hsu, S.-T., Hong, W.-F., Tzeng, K.-C., and Chen, C.-C. 1993. Bacterial wilt of perilla caused by *Pseudomonas solanacearum* and its transmission. *Plant Dis.* 77:674-677.

In Taiwan, a wilt disease of perilla (*Perilla crispera*) was caused by *Pseudomonas solanacearum* race 1, biovar 3. Strains from perilla were pathogenic to solanaceous and other hosts, but strains from solanaceous and other hosts were not pathogenic to perilla. Stem inoculations of perilla plants with *P. solanacearum* resulted in 90–100% wilting, but perilla plants rarely wilted when planted in infested soil. However, various population levels of *P. solanacearum* were detected in roots of 88% and in stems of 76% of asymptomatic plants grown in infested soil. The pathogen was mechanically transmitted from a wilted plant to healthy plants. The symptomless carriers also served as an inoculum source for the spread of the pathogen to healthy perilla plants. Leaves of perilla are harvested periodically during the growing season by mechanical means, resulting in a high incidence of the disease.

distilled water (SDW) in screw-capped test tubes at room temperature. Before they were used for each test, each strain was streaked on TZC medium (22) and incubated at 30 C for 48–72 hr. Single fluidal colonies were then transferred to agar plates of medium 523 (20) or TZC medium without tetrazolium salt and incubated again at 30 C for 48 hr.

Phenotypic characterization. Strains from perilla were compared with strain PS95 of *P. solanacearum* from tomato in various tests. Bacterial cultures were incubated at 30 C for all tests unless otherwise stated. Colony characteristics were observed on TZC medium after incubation for 48 hr. Production of acids from the oxidation of carbohydrates used for biovar identification was determined by the method of Hayward (11). Accumulation of poly- β -hydroxybutyrate was determined by growing bacteria on nutrient glucose agar containing 0.5% DL- β -hydroxybutyrate and observing bacterial cells stained with sudan black B solution (14). Formation of brown diffusible pigment was tested on Hayward's medium containing 0.1% L-tyrosine (11); formation of fluorescent pigments was tested on King's B medium (23). Oxidase was determined by Kovacs's method (24). Arginine dihydrolase was tested according to Thornley (31). Urease test was performed by the method of Christensen (5). Phosphatase and sulfatase were tested on plates of nutrient agar containing 0.01% phenolphthalein phosphate and 0.01% phenolphthalein sulfate, respectively, according to the methods described by Collins (6). Esterase activity (Tween 80 hydrolysis), phenylalanine deaminase, and production of H₂S and indole were determined by methods described by Fahy and Hay-

Perilla (*Perilla crispera* Tanaka) is an important spice crop grown in central Taiwan. Processed leaves are used mostly as food additives for flavoring and coloring. The crop usually is sown during January and February, and is harvested beginning in late April or early May. About 10 harvests can be made during a single growing season. Leaves are harvested by clipping off the plant top by machine. In 1987, a destructive wilt disease occurred in several fields in major perilla-growing regions. The disease was first noticed after harvesting shoot tops. It spread rapidly throughout the field along the clipping direction. Symptoms of the disease were characterized by black discoloration on the cut end of the stem and epinasty of leaves which progressed down the stem. Infected plants subsequently wilted and died. Preliminary results from isolations of suspect pathogens on Kelman's tetrazolium chloride agar (TZC) medium (22) and stem inoculations of perilla with the suspect pathogen suggested that the disease was caused by *Pseudomonas solanacearum* (Smith) Smith. This wilt is the most destructive disease on perilla.

Bacterial wilt caused by *P. solanacearum* affects a wide range of host plants (21). In Taiwan, bacterial wilt occurs on tomato, tobacco, pepper, eggplant, potato, peanut, strawberry, bird-of-paradise, sesame, castor bean, radish, comfrey, and sugar apple (17). Strains of *P. solanacearum* from solanaceous hosts and bird-of-paradise have been grouped into race 1 (4,19,34); however, several subgroups which differ in virulence to

certain hosts are found among these strains (4,19). Most of these strains are biovar 3; some are biovar 4; and only a few from tobacco are biovar 2 (4,18,34) according to the classification scheme of Hayward (11). Because bacterial wilt has not been reported on perilla, we characterized strains of *P. solanacearum* from perilla, compared their pathogenicity on several hosts with strains from other hosts, determined the importance of stem infection in wilt induction, and determined how readily the pathogen could be spread in plants by clipping. A preliminary report of this study has been published (15).

MATERIALS AND METHODS

Bacterial strains. Thirty-five strains of *P. solanacearum* were isolated from infected perilla plants collected from four locations in central Taiwan during 1987–1989. Strains of the bacterium from other hosts (Table 1) were obtained from the collection at the Department of Plant Pathology, National Chung Hsing University, Taichung, Taiwan. All strains were maintained as suspensions in sterile

Table 1. Pathogenicity of strains of *Pseudomonas solanacearum* from perilla and other host plants to perilla and tomato

Strain	Host origin	Pathogenicity ^a	
		Perilla	Tomato
Pr strains (35 strains)	Perilla	+	+
PS 21,70,75,95	Tomato	—	+
PS 27,31, TI 45	Tobacco	—	+
PS 86,91,96	Sweet pepper	—	+
PS 76,99,100	Eggplant	—	+
PS 60,92	Potato	—	+
PS 103,104,109	Peanut	—	+
BP 2,4,6	Bird-of-paradise	—	+
R 2,3,4,5,6,7,8,9	Radish	—	+

^a The pathogenicity test was repeated at least twice, and in each test 10 plants were used for each strain. + = pathogenic (induced wilting after stem inoculation), and — = not pathogenic.

Accepted for publication 9 March 1993.

ward (8). Gram stain, nitrate reduction, catalase, methyl red and Voges-Proskauer test, and hydrolysis of starch, gelatin, and esculin were performed by standard methods (30). Simmon's citrate medium was employed for the citrate utilization test (29). Pectate degradation was tested according to Hildebrand (13). Growth at 40 C was performed by adding one loopful of a dilute bacterial suspension to nutrient broth and incubating in a water bath regulated at 40 C. Nutrient broth containing 1 and 2% NaCl was used for the salt tolerance test.

Pathogenicity tests. The plants used for inoculations were perilla cv. Suo Mien Tzu Su, tomato (*Lycopersicon esculentum* Mill. 'Known-you 301'), tobacco (*Nicotiana tabacum* L. 'Wan Kuo Seh'), pepper (*Capsicum annuum* L. 'Blue Star'), potato (*Solanum tuberosum* L. 'Kennebec'), eggplant (*Solanum melongena* L. 'Pingtung Long'), and peanut (*Arachis hypogaea* L. 'Tainan No. 9'). Perilla, tomato, tobacco, pepper, and eggplant were seeded in vermiculite; and seedlings were transplanted into 18-cm pots containing natural field soil (sandy loam, pH 5.9, organic matter 0.8%), one plant per pot. Seeds of peanut and tuber seed pieces of potato were planted directly in pots, and one plant was allowed to develop in each pot. All plants except potato were grown in a greenhouse at 25–35 C. Potato plants were grown in the greenhouse at 16–28 C. When plants were 12–18 cm tall, they were inoculated by a stem-prick method. Bacterial cells from a 48-hr agar plate culture were picked with a sterile toothpick and inserted into the stem at the axil of the third expanded leaf below the stem apex. The toothpick was left in position. Control plants were similarly pricked with sterile toothpicks. Ten plants of each host were inoculated with each strain of the bacterium. The experiment was repeated at least twice. The inoculated plants were placed in the greenhouse at temperatures as described above and observed for disease development for 4 wk. Disease severity was rated weekly according to the following scale: 0 = no symptoms, 1 = one leaf partially wilted, 2 = one or two leaves wilted, 3 = three or more leaves wilted, 4 = all leaves wilted, and 5 = plant dead.

Wilt induction by stem inoculation and soil infestation. To determine disease development in perilla plants through stem and root infections, 2-mo-old perilla plants produced as described were inoculated with perilla strain Pr 52 by the stem-prick method as described for pathogenicity tests, or were transplanted into pots containing soil infested with strain Pr 52 at a concentration of 10^7 cfu/g dry soil. The infested soil was prepared by adding 1 L of a bacterial suspension adjusted to approximately 10^9 cfu/ml to 10 kg of soil, and mixing thoroughly by hand. Ten plants were used

for each inoculation method. Plants were observed for wilting after 4 wk. For comparison, tomato plants were inoculated similarly by both methods. All inoculations were repeated six times.

Determination of populations of *P. solanacearum* in symptomless perilla plants. To determine whether symptomless perilla plants from infested soil became infected with *P. solanacearum*, the presence of the bacterium in stems and roots was assayed. Perilla plants (2 mo old) were transplanted individually into 18-cm pots containing soil infested with strain Pr 52 at a density of 5.6×10^7 cfu/g dry soil and grown in the greenhouse at 25–35 C. Populations of *P. solanacearum* in stems and roots were determined at 4, 7, 14, 21, and 28 days after transplanting. Five symptomless plants were selected randomly at each sample date and assayed separately. For the root-sample assay, root systems of plants were excised, washed with SDW, surface disinfected with 1% sodium hypochlorite for 5 min, and rinsed three times with SDW. Roots were blotted dry, weighed, and macerated in a mortar containing 10 ml of SDW. Aliquots (0.1 ml) of 10-fold dilutions of the suspension were spread onto a modified SM-1 medium (9,32). Colonies of *P. solanacearum* were counted after incubation at 30 C for 48–72 hr, and the number of colony forming units per gram fresh weight roots was calculated. For stem sample assays, a 3-cm stem segment from above the soil line was removed, weighed, and ground with 10 ml SDW in a mortar. Populations of *P. solanacearum* in the stem were determined similarly as for root samples.

Spread of *P. solanacearum* in plants by clipping. Wilted perilla plants, which were produced by stem inoculation with strain Pr 52 seven days previously, were used as inoculum-source plants. The stem apex of a wilted plant was removed at the second internode with sterile scissors. The contaminated scissors were then used to cut serially the stem apex of 40 healthy perilla plants. Control plants were similarly clipped with scissors that had been used to cut a healthy plant. Clipped plants were placed in a greenhouse and observed for wilt development. The number of *P. solanacearum* cells on the scissors after cutting a wilted plant was estimated by immersing the scissors into 10 ml of SDW, shaking them vigorously for 3 min, and plating the diluted washings onto TZC medium. The average number of cells of *P. solanacearum* on the scissors was calculated from five replications. Plants that did not show wilt symptoms 21 days after planting in the soil infested with strain Pr 52 (approximately 10^7 cfu/g dry soil) were also tested as a possible source of inoculum. Ten symptomless plants were tested. Each was clipped with the scissors, and the scissors were used to cut five healthy

plants as described above. The number of *P. solanacearum* cells in the 1-cm stem segment just below the cut end of the symptomless plant was also assayed. The clipped plants were kept in the greenhouse to observe subsequent symptom development.

RESULTS

Phenotypic characteristics. All strains from perilla were gram-negative and produced irregularly round or elliptical, smooth, fluidal, white colonies with light red centers on TZC medium. They produced a brown diffusible pigment on tyrosine medium, but none of them produced fluorescent pigments on King's B medium. They were positive for catalase, oxidase, phosphatase, urease, nitrate reduction, pectate degradation, citrate utilization, and poly- β -hydroxybutyrate accumulation; and all were negative for arginine dihydrolase, phenylalanine deaminase, sulfatase, H_2S and indole production, methyl red test, Voges-Proskauer test, and gelatin, esculin, Tween 80, and starch hydrolysis. All strains tolerated 1% but not 2% NaCl. They did not grow at 40 C. All strains produced acids from lactose, maltose, cellobiose, mannitol, sorbitol, and dulcitol and were therefore classified as biovar 3 of *P. solanacearum* according to Hayward's classification (11). The characteristics of strains from perilla were the same as those of strain PS95 from tomato, which was used for comparison.

Pathogenicity. With stem inoculation, all strains from perilla were pathogenic to perilla, inducing wilting of plants within 7–10 days after inoculation. However, the 29 strains from tomato, tobacco, pepper, eggplant, peanut, bird-of-paradise, and radish were not pathogenic to perilla (Table 1). These strains induced only browning in the inoculated site on some plants; wilting was not observed even after 2 mo. All of the strains from perilla and other hosts caused wilting of tomato within 7 days (Table 1).

All strains from perilla were highly virulent to tomato, eggplant, potato, and pepper, but varied in virulence to tobacco and peanut (Table 2).

Wilt induction by stem inoculation and soil infestation. Inoculation of perilla plants by stem puncture with strain Pr 52 resulted in 90–100% wilted plants, but plants transplanted in the infested soil rarely became diseased. In six separate soil-infestation tests (with 10 plants in each test), plants did not wilt in three tests; and 1, 2, and 3 plants wilted in each of the other three tests. When tomato was similarly inoculated, all plants wilted either by stem inoculation or by soil infestation. Similar results were obtained when the experiment was repeated with two other strains from perilla.

Population of *P. solanacearum* in symptomless plants grown in infested soil. Of 40 perilla plants transplanted into

infested soil, only four plants showed wilting after 28 days. Among the 25 symptomless plants tested from five sample dates (4, 7, 14, 21, and 28 days after transplanting), *P. solanacearum* was detected in the roots of 22 plants (88%) and in the stems of 19 (76%). Populations of *P. solanacearum* in the roots and the stems of individual plants varied considerably (Table 3).

Spread of the pathogen by clipping.

A pair of scissors carried an average of 7.8×10^5 cfu of *P. solanacearum* after cutting the stem of a wilted perilla plant. All 40 healthy perilla plants that had their stem apexes removed with the contaminated scissors wilted 14 days after clipping. Control plants similarly clipped with scissors used to cut a healthy plant remained healthy. *P. solanacearum* was detected in the stem apex of eight of 10

plants that did not show wilt symptoms 21 days after planting in the infested soil (Table 4). When these symptomless carriers were used as inoculum sources, *P. solanacearum* was transmitted by clipping from those containing a high number of bacteria (causing some healthy plants to wilt), but not usually from the plants containing a low number of bacteria (Table 4).

DISCUSSION

A wilt disease of perilla was shown to be caused by *P. solanacearum*. The cultural and physiological characteristics of the strains of bacteria from perilla were similar to the characteristics of the strains of *P. solanacearum* isolated from solanaceous and other hosts in Taiwan (18). Strains of *P. solanacearum* have been divided into different biovars on the

basis of the ability to oxidize three disaccharides and three sugar alcohols (11). All strains from perilla were grouped in biovar 3, which is the predominant biovar in Taiwan (4,18,34). Strains of *P. solanacearum* have also been divided into different races on the basis of the hosts primarily affected (1). Race 1 is a broad host-range group and affects many solanaceous and other plants; race 2 affects bananas and Heliconias; and races 3, 4, and 5 primarily affect potato, ginger, and mulberry, respectively (1,2,12). The strains from perilla caused disease not only on perilla but also on tomato, tobacco, potato, pepper, eggplant, and peanut, and could therefore be classified as race 1. Although strains from solanaceous and other hosts from Taiwan have previously been reported to belong to race 1 (4,19,34), none of those tested were pathogenic to perilla. Apparently, the occurrence of bacterial wilt on perilla is associated with a particular group of strains of *P. solanacearum* different from those strains from other hosts. It is not known where the perilla strain originated. Strains within race 1 that vary in host range exist in Taiwan (4,19). The recent wide cultivation of perilla may have selected a particular group of strains from previously undetected populations.

The soilborne nature of bacterial wilt on a wide range of hosts is well documented (7,21). However, on perilla, plants rarely became diseased when planted in the infested soil but wilted easily when their stems were inoculated. A cultivar of a host species may react differently to *P. solanacearum* with different inoculation methods (25,33). Thus, some tomato and tobacco cultivars were susceptible to stem inoculation but resistant to root inoculation (25,33). Apparently, the inability of *P. solanacearum* from soil to cause wilt in perilla plants did not result from the failure of the bacterium to infect roots, because high populations of *P. solanacearum* were detected in the roots and stems of most of the symptomless plants. Although *P. solanacearum* can infect the roots of resistant cultivars of host species such as pepper, castor bean, and tomato, and of presumed nonhosts such as bean and corn, without symptom expression (10), the lack of disease development on a susceptible plant like perilla, when infected systemically with the bacterium via soil, was unexpected.

P. solanacearum can be disseminated from plant to plant or from place to place by various means, such as root-to-root spread, infected planting materials, contaminated implements, and insects (3, 21,28). The pattern of disease spread observed in the perilla fields indicated that *P. solanacearum* was spread from infected to healthy plants primarily by mechanical means. During perilla's growing stages, young leaves are per-

Table 2. Virulence of 35 strains of *Pseudomonas solanacearum* from perilla on other host plants

Number of strains	Virulence rating ^a					
	Tomato	Eggplant	Potato	Tobacco	Pepper	Peanut
3	5.0	5.0	4.0-5.0	4.0-5.0	4.0-5.0	4.0-5.0
15	4.0-5.0	4.0-5.0	4.0-5.0	3.8-5.0	4.0-5.0	2.6-3.5
13	5.0	5.0	4.0-5.0	3.8-4.5	4.0-5.0	1.0-2.0
3	4.0-5.0	4.0-5.0	4.0-5.0	2.2-3.5	4.0-5.0	2.2-3.5
1	4.5	5.0	4.5	1.4	4.4	2.6

^a Based on disease ratings on a scale where 0 = no symptoms, 1 = one leaf partially wilted, 2 = one or two leaves wilted, 3 = three or more leaves wilted, 4 = all leaves wilted, and 5 = plant dead, and expressed as average disease indices of 10 plants 4 wk after stem inoculation.

Table 3. Populations of *Pseudomonas solanacearum* detected in roots and stems of asymptomatic perilla plants grown in soil infested with strain Pr 52 of the bacterium (10^7 cfu/g dry soil)

Days after planting	Root ^a		Stem ^b	
	A/B ^c	Population range	A/B	Population range
4	4/5	3.8×10^3 - 4.1×10^7	4/5	1.4×10^6 - 8.2×10^8
7	5/5	2.6×10^5 - 7.5×10^6	5/5	3.0×10^4 - 1.2×10^8
14	4/5	2.0×10^6 - 2.4×10^7	3/5	2.0×10^8 - 3.2×10^9
21	4/5	4.4×10^6 - 1.6×10^9	4/5	5.6×10^2 - 4.1×10^9
28	5/5	2.7×10^6 - 3.3×10^9	3/5	2.5×10^8 - 5.1×10^9

^a Population expressed as cfu/g fresh tissue of root.

^b Tissues taken from a 3-cm segment just above the soil line. Population expressed as cfu/g fresh weight.

^c A/B = number of plants containing *P. solanacearum*/number of plants assayed.

Table 4. Transmission of bacterial wilt in perilla by clipping the stem apex of symptomless plants infected with *Pseudomonas solanacearum* with a pair of scissors before cutting the stem apex of healthy plants

Symptomless plant ^a	<i>P. solanacearum</i> detected in the symptomless plant ^b	No. plants wilted/no. plants tested
1	0	0/5
2	2.1×10^3	0/5
3	3.4×10^7	3/5
4	4.0×10^4	1/5
5	0	0/5
6	7.8×10^3	0/5
7	8.7×10^6	2/5
8	9.9×10^4	0/5
9	2.0×10^4	0/5
10	2.1×10^4	1/5

^a Ten plants that did not show wilt symptoms 21 days after planting in soil infested with *P. solanacearum* strain Pr 52 (10^7 cfu/g dry soil). Each plant was clipped with scissors which were then used to cut five healthy plants.

^b Determined in 1-cm stem segment below the cut end. Population was expressed as cfu/g fresh weight.

idically harvested by the cutting machine. Because of this practice the pathogen spread rapidly, resulting in a high incidence of disease. Therefore, bacterial wilt on perilla is insignificant if infection occurs only through the roots, but it becomes a serious problem with the practice of mechanical harvesting. Thus, bacterial wilt of perilla may be considered a mechanically transmitted disease.

P. solanacearum from a single wilted plant clipped with scissors caused 40 healthy plants to wilt. The high efficiency of spread of *P. solanacearum* by clipping has been reported on tomato plants (26, 27). *P. solanacearum* was also spread by clipping from some systemically infected but symptomless plants to healthy plants. This indicates that although most perilla plants did not show wilt symptoms when planted in infested soil, some of these symptomless carriers may serve as important sources of inoculum in a field where mechanical harvest is practiced.

Because the bacterial wilt problem on perilla is a result of the clipping practice, preventing the spread of *P. solanacearum* during leaf harvest by the cutting machine should be the most important means for managing the disease. Our preliminary results from greenhouse tests indicated that treatment of contaminated scissors with disinfectants such as 0.5% sodium hypochlorite before clipping healthy plants prevented infection (16). However, a practical means of applying disinfectants to the cutting machine during operation remains to be studied.

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