Brown Stem of Celery Caused by *Pseudomonas cichorii*

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ABSTRACT

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An unusually severe outbreak of a petiole necrosis of celery, known as brown stem, was observed in the winter of 1993 in southern Florida. *Pseudomonas cichorii* was consistently recovered from naturally infected tissue. Bacterial strains were gram-negative rods, fluorescent, oxidase-positive, and arginine dihydrolase-negative. Negative reactions were recorded for levan production, potato soft rot, and fermentation of glucose. Strains utilized mannitol, D-aspartate, and *m*-tartrate for growth. No growth was recorded for D-arabinose, cellobiose, sucrose, trehalose, benzoate, DL-lactate, D-tartrate, erythritol, or sorbitol. A hypersensitive response was recorded in leaf tissue of pepper cv. Early Calwonder. Brown stem and typical leaf spot symptoms were readily reproduced with test strains in greenhouse-grown celery.

Sporadic outbreaks of a petiole necrosis of celery (*Apium graveolens* L. var. *dulce* (Mill.) Pers.), known locally as "brown stem," have been observed in Florida for over 40 yr (5). Symptoms of this malady consist of a firm, brown discoloration throughout the petiole. Damage is especially evident in the heart region at the base of the stalk, but brown streaks may be seen along most of the length of the petiole. Browning is confined to the ground parenchyma; vascular bundles appear as islands of healthy green among diseased cortical and pith tissue. No specific cause for brown stem has been established, but the prevailing opinion has been that it is a physiological disorder (5).

During the 1992–1993 winter vegetable season, a particularly severe outbreak of brown stem occurred in celery production fields throughout the Everglades Agricultural Area (EAA). The problem was especially evident on celery approaching harvest. All nine fields surveyed had some brown stem, with average incidence about 5% infected plants per field. Losses to the industry were estimated at $5 million.

Isolations from diseased tissue on King's medium B (KMB) (9) consistently yielded a fluorescent pseudomonad. This study was undertaken to establish the identity and pathogenicity of pseudomonad strains associated with brown stem and to reproduce symptoms in healthy celery.

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MATERIALS AND METHODS

Pathogen isolation. Celery stalks with brown stem symptoms were cut at the base in the field. Samples were transferred to the laboratory, and isolations were made within 2 hr. Individual diseased petioles were stripped from the stalk, thoroughly washed in running tap water, and air-dried. Petioles were then dipped in 95% ethanol and flamed. A flamed and cooled inoculation needle with a right-angle bend was inserted through the margin of diseased tissue and, without removal, streaked onto duplicate plates of KMB (9) supplemented with 50 μg/ml of cycloheximide. Plates were incubated at 27 C for 48 hr. Fluorescent colonies characteristic of *Pseudomonas* spp. were purified by restreaking on KMB. Eight strains (B1–B8) were chosen for further study. Cultures were maintained in sterile 15% glycerol at −70 C. Working cultures were stored up to 1 mo on KMB slants at 4 C.

Physiological and biochemical tests. The following tests were used to characterize strains: Gram stain (13), oxidase (10), and arginine dihydrolase reactions (16); levan production (11); fermentation of glucose (6); nitrate reduction to nitrite and N_2 (11); potato soft rot (11); and hypersensitivity of leaf tissue in pepper cv. Early Calwonder (7).

Carbon source utilization was evaluated using the basal medium of Ayers et al (1) into which filter-sterilized substrates from 1% (w/v) solutions were added to a final concentration of 0.1% (w/v). Carbon sources tested were: D-arabinose, cellobiose, sucrose, trehalose, D-aspartate, benzoate, DL-lactate, D-tartrate, *m*-tartrate, erythritol, mannitol, and sorbitol. The medium was solidified with 1.7% agar, and duplicate plates for each carbon source were streaked with loopfuls of each strain grown for 24 hr at 27 C on KMB slants. Plates were compared at 3, 4, and 14 days for growth and acid or base reaction with control plates that had no added carbon source. Three strains of *Pseudomonas cichorii* (Swingle) Stapp, originally isolated from blighted leaf blades in celery seedbeds in September 1992 (15), were included for comparison.

Pathogenicity tests. All eight strains were tested for ability to produce brown stem symptoms in greenhouse-grown celery cv. June Belle 1622. Plants were grown from seed to near maturity in a Pahokee muck soil in 13-cm plastic pots. Sterile toothpicks were coated with bacterial cells taken from 48-hr growth at 27 C on KMB plates and inserted into celery at the first node above the petiolar base. Three to four petioles were inoculated on each of two plants per strain. A known strain of *P. cichorii* isolated from a celery leaf blade lesion was included in these tests. Petioles treated similarly with noninfested toothpicks served as controls. Plants were enclosed immediately in clear plastic bags. Bags were opened momentarily after 24 hr to remove toothpicks, resealed, and left undisturbed for 5 days. An extended period of high humidity was used to parallel the daily periods of heavy dew and fog common to the EAA in the winter season. Following removal of bags, plants were watered at the pot rim to prevent cross-contamination among treatments. The tests were conducted in the greenhouse where the maximum temperature was 27 C and minimum temperatures were between 13 and 19 C.

Plants were evaluated for disease development 11 days after inoculation. Ratings were made on a disease severity scale where 1 = no symptoms and 5 = extensive deep brown discoloration of pith and cortical tissue. The length from the inoculation site to the distal margin of the brown streak was also measured. *P. cichorii* was always reisolated from artificially infected celery petioles.

Strains were also tested for production of typical leaf spot symptoms in blades of young celery plants. A cotton swab/Carborundum method was used to inoculate strains into leaf blades of June Belle 1622 plants as previously described

(15). Plants were evaluated for bacterial blight symptoms 5 days later. Symptoms produced by strains from brown stem lesions were compared with those produced by a known *P. cichorii* strain originally recovered from a blight lesion on a celery leaf blade. This test was repeated using a second known *P. cichorii* strain.

RESULTS

Physiological and biochemical tests. All bacterial strains were gram-negative rods, produced water-soluble, fluorescent pigment on KMB, and were oxidase-positive and arginine dihydrolase-negative (Table 1). Strains were negative for levan production, potato soft rot, and fermentation of glucose. Nitrate was not reduced to N_2, but four of eight brown stem strains reduced NO_3^- to NO_2^-. All strains utilized mannitol for growth, with production of acid. D-Aspartate and *m*-tartrate were also utilized, but alkaline metabolites were produced. No growth was supported by D-arabinose, cellobiose, sucrose, trehalose, benzoate, DL-lactate, D-tartrate, erythritol, or sorbitol. A typical hypersensitive response on leaves was recorded in 24 hr in Early Calwonder pepper.

Pathogenicity tests. All strains produced brown stem symptoms in celery within 6 days of inoculation. Typical brown streaks were evident in petioles, and some strains produced streaks of considerable length (Table 2). For example, strain B3 produced streaks averaging 30 cm long by 11 days after inoculation. In cross section it was evident that pith and cortex tissues were brown but vascular bundles remained green and healthy. The pathogen was easily recovered from inoculated, symptomatic petioles, both near the inoculation site and along extended brown streaks. No symptoms were observed in control plants, and *P. cichorii* was not recovered during isolation attempts. The eight brown stem strains and the known *P. cichorii* control strains produced characteristic leaf spots when swab-inoculated into blades.

DISCUSSION

Brown stem is another manifestation of the infection of celery by *P. cichorii*. Bacterial strains recovered from brown stem lesions in petioles are biochemically and physiologically indistinguishable from those causing typical bacterial blight lesions on celery leaf blades (2,15). Furthermore, brown stem strains incite typical symptoms on leaf blades of celery, and strains originally isolated from leaf blades incite brown stem when introduced into petioles. Other bacterial pathogens can cause different diseases in the same host. *Erwinia carotovora* (Jones) Bergey et al is a classic example of a bacterial pathogen of potato that can cause seed piece decay and poor stands, blackleg of mature plants in the field, and postharvest tuber rot (14). *P. cichorii* has been reported to cause lesions on celery petioles in New Zealand (17). However, there the lesions are of limited size and confined to the cortical tissue. In Florida, petioles of celery with brown stem are necrotic throughout the entire cortex and pith.

P. cichorii is a pathogen of increasing importance in Florida agriculture, attacking a range of crops (3,4,7,8,12). It has been found to cause a stem necrosis of chrysanthemum (7), with symptoms somewhat like those of celery brown stem.

Leaf spot symptoms caused by *P. cichorii* are a yearly occurrence in the EAA. Serious outbreaks of brown stem, on the other hand, are reported only about every 5 or 6 yr. The reasons for the sporadic nature of brown stem outbreaks are unknown. The weather in the winter of 1992–1993 was unusual, with frequent rainfall and strong winds. These conditions might be essential for brown stem expression. However, this hypothesis cannot be tested, as there are no reports of the specific weather conditions that coincided with previous brown stem epidemics (5). Identification of environmental conditions conducive to brown stem development is the subject of current research.

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Table 1. Results of physiological and biochemical tests for eight strains of *Pseudomonas* isolated from brown stem lesions on celery petioles in the 1993 winter season at Belle Glade, Florida

Test	Brown stem strains	*P. cichorii* strains from typical leaf spot symptoms
Gram reaction	−	−
Fermentation of glucose	−	−
Oxidase	−	−
Arginine dihydrolase	−	−
Levan	−	−
Potato soft rot	−	−
Nitrate reduction to N_2	−	−
Nitrate reduction to nitrite	±[a]	−
Hypersensitivity in pepper	+	+
Substrate utilization for growth		
D-Arabinose	−	−
Cellobiose	−	−
Sucrose	−	−
Trehalose	−	−
D-Aspartate	+	+
Benzoate	−	−
DL-Lactate	−	−
D-Tartrate	−	−
m-Tartrate	+	+
Erythritol	−	−
Mannitol	+	+
Sorbitol	−	−

[a] Four of eight strains were positive for reduction of nitrate to nitrite.

Table 2. Results of pathogenicity tests for strains of *Pseudomonas cichorii* isolated from brown stem lesions on celery petioles in the 1993 winter season at Belle Glade, Florida[a]

Strain	Average severity rating[b]	Average length of brown streaks[c] (cm)
B1	2.4	12.3
B2	3.9	26.0
B3	4.2	30.1
B4	2.9	16.6
B5	3.2	26.8
B6	3.9	25.1
B7	3.5	21.0
B8	3.6	18.8
Pc355[d]	2.8	13.5

[a] Based on toothpick inoculation of three or four petioles on each of two celery plants of cv. June Bell 1622 per strain. Ratings were made 11 days after inoculation.
[b] On a scale of 1–5, where 1 = no symptoms and 5 = extensive deep brown discoloration of pith and cortical tissue.
[c] Measured from inoculation site to distal margin of brown streaks.
[d] Typical leaf blight strain recovered from blade lesion of celery in 1992.

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