

Occurrence of *Pseudomonas corrugata* on Tomato in California

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ABSTRACT

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Tomato pith necrosis was detected for the first time in the United States on the tomato cultivar Casino Royal in San Diego, CA. Symptoms of infection were stunting, stem lesions, and necrosis of the pith, which produced scattered hollow areas. Based on physiological, biochemical, and pathogenicity tests, the pathogen was identified as *Pseudomonas corrugata*.

An unusual disease causing pith necrosis of tomato was observed in the spring of 1976 and every year since then in tomato-growing areas near San Diego, CA. In March 1981, several San Diego County fields along the Mexican border had as many as 10% of the plants infected.

Plants appeared slightly stunted with varying degrees of gray or brown water-soaked lesions on the stem. The infected stems seemed to remain firm when externally examined, but when cut open, the pith was hollow with scattered dark brown necrotic areas (Fig. 1). In less severely affected plants, light to dark brown pith discoloration occurred with slight necrosis of the external stem. A bacterium was consistently isolated from the discolored tissues adjacent to the necrotic pith.

A similar disease incited by *Pseudomonas corrugata* Scarlett et al on glasshouse tomatoes was reported in 1978

from England (9). The early tomato crop in San Diego County is grown under plastic tents and is heavily fertilized, approaching conditions of glasshouse culture in England. Because the environmental conditions and disease symptoms were strikingly similar, a study was undertaken to identify the plant-pathogenic bacteria isolated from cultivar Casino Royal field tomatoes and to compare them with known strains of *P. corrugata* and several other plant-pathogenic pseudomonads.

MATERIALS AND METHODS

Isolation of the pathogen. Pith tissue at the margin of a necrotic area was surface-sterilized with 75% ethanol and macerated in 5 ml of sterile distilled water. This suspension was examined microscopically and streaked on plates of King's medium B (5) and nutrient agar. Plates were incubated for 48 hr at 25 C. All strains were maintained on nutrient agar during the study.

Bacterial cultures used for comparative studies. Three unknown strains designat-

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Table 1. Comparison of biochemical and physiological tests of *Pseudomonas syringae*, *P. lachrymans*, *P. gladioli*, *P. cichorii*, *P. cepacia*, *P. corrugata*, and bacterial strains isolated from tomato

Comparative tests	Strains											Tomato strains		
	<i>P. syringae</i>	<i>P. lachrymans</i>	<i>P. gladioli</i>	<i>P. cichorii</i>	<i>P. cepacia</i>		<i>P. corrugata</i>				A	B	C	
	5D4214	3D43	551	9D46	UCBPP 446	2D42	NCPBB 2445	NCPBB 2456	NCPBB 2447	NCPBB 2458				
Onion bulb rot	-	-	-	-	+	+	+	+	+	+	+	+	+	+
Carrot root rot	-	-	-	-	-	-	-	-	+	+	-	-	-	
Celery rot	-	-	-	-	+	+	+	+	+	+	+	+	+	
Tobacco hypersensitivity	+	+	+	+	+	+	+	+	+	-	+	+	+	
Oxidase	-	-	-	+	+	+	+	+	+	+	+	+	+	
Fluorescent pigment	+	+	+	+	-	-	-	-	-	-	-	-	-	
2-Ketogluconate	-	-	-	-	-	-	-	-	-	-	-	-	-	
Lipase	-	-	+	-	+	+	-	-	-	-	-	-	-	
Starch hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-	-	
Arginine dihydrolase	-	-	-	-	-	-	+	+	+	+	+	+	+	
Levan	+	+	-	-	-	-	-	-	-	-	-	-	-	
Nitrate reduction	-	-	-	-	-	-	-	-	-	-	-	-	-	
DL phenylalanine deaminase	-	-	-	-	-	-	-	-	-	-	-	-	-	
Lecithinase	-	-	-	-	-	-	-	-	-	-	-	-	-	
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	
Gelatin hydrolysis	+	-	+	-	-	-	-	+	+	+	+	+	+	
Pectinase	-	-	-	-	+	-	-	-	-	-	-	-	-	
Growth in 6% NaCl	-	-	-	-	+	+	+	+	+	+	+	+	+	
Growth in 41 C	-	-	-	-	+	-	+	+	+	+	+	+	+	
Poly-β-hydroxybutyrate	-	-	-	-	+	+	+	+	+	+	+	+	+	
Number of polar flagella	NT ^a	NT	NT	NT	NT	NT	1-3	1-3	1-3	1-3	1-3	1-3	1-3	

^aNT = not tested.

ed A, B, and C were originally isolated from infected tomato plants taken from fields in San Diego County. Four cultures of *P. corrugata* (NCPPB 2445, 2456, 2447, and 2458) were obtained from the National Collection of Plant Pathogenic Bacteria, Harpenden, England. Two cultures of *P. cepacia* (UCBPP 446 and UCDPP 23D42) were obtained from M. N. Schroth, University of California, Berkeley, and C. I. Kado, University of California, Davis, respectively, one culture of *P. gladioli* (UCBPP 551) from M. N. Schroth, one culture of *P. lachrymans* (3D43 UCDPP), one culture of *P. syringae* (5D4214 UCDPP), and one culture of *P. cichorii* (9D46 UCDPP) from C. I. Kado.

Characterization of bacterial strains. All strains were characterized by the following biochemical tests: ability of the bacteria to rot onion bulbs, carrot roots, and celery stems (strains were tested for soft-rotting potential in moisture chambers at 25 C); Gram stain; hypersensitivity of inoculated tobacco (6); growth at 36 and 42 C in nutrient broth; production of levan (1); 2-ketogluconate reduction (8); nitrate reduction (8); presence of arginine dihydrolase (11); oxidase reduction (8); liquefaction of gelatin (8); production of fluorescent pigment (5); production of pectinase at pH 5.0, 7.0, and 8.5 (2); presence of lipase (10); starch hydrolysis (4); lecithinase test (3); phenylalanine deamination (10); tolerance to 3.0 and 6.0%, w/v, NaCl in nutrient broth; and catalase production (2). The following carbohydrates were tested as carbon sources in nutrient broth: arabinose, aesculin, cellobiose, citrate, dextrose, dextrin, galactose, glycerol, inulin,

inositol, lactose, levulose, mannose, maltose, mannitol, rhamnose, raffinose, salicin, sorbitol, saccharose, and xylose.

Bacteria were examined for size, shape, and flagella arrangement by transmission electron microscopy (TEM). Strains were grown for 16 hr in nutrient broth before TEM examination.

Pathogenicity tests. Pathogenicity of the three unknown strains was compared with four known strains of *P. corrugata*. Strains were grown for 5 days on nutrient agar at 24 C. Suspensions were prepared by flooding plates with 10 ml of sterile distilled water. Four-week-old tomato plants of Casino Royal, Bonny Best, Homestead, and Walter cultivars were inoculated by injecting one drop of the bacterial suspension through the stem nodes into the pith with a 1-ml syringe. Plants were drip-irrigated under plastic tents and grown at 20 C. After 6 wk, external and internal symptoms were observed and bacteria were reisolated from areas of discolored pith.

RESULTS

Isolation and characterization of the

pathogen. Nonfluorescent bacteria that produced slightly irregular, rounded, mucoid, buff yellow colonies when grown on King's medium B for 48 hr were consistently isolated from field-grown tomatoes. Bacteria were gram-negative, rod-shaped, about 1.5–3.0 × 0.5 μm, and motile by one to three polar flagella. After seven days, the bacteria produced acid in arabinose, aesculin, cellobiose, citrate, dextrose, dextrin, glycerol, inulin, lactose, mannose, maltose, mannitol, rhamnose, raffinose, sorbitol, saccharose, and xylose. Acid was not produced in galactose, inositol, levulose, and salicin. The *P. corrugata* strains used the same carbohydrates as the three San Diego tomato strains. The results of biochemical and physiological comparison tests completed on the three San Diego tomato strains were similar to the known strains of *P. corrugata* (Table 1). All of these tomato strains were biochemically and physiologically different from the other plant-pathogenic pseudomonads tested.

Pathogenicity tests. Lesions similar to those observed in the field developed on the four tomato cultivars tested: Walter,

Table 2. Comparison of disease response when known strains of *Pseudomonas corrugata* and bacteria strains isolated from tomatoes with symptoms of pith necrosis were inoculated to the tomato cultivars Walter, Homestead, Bonny Best, and Casino Royal

Tomato cultivar	Strain	Disease ratings	
		External symptoms ^a	Internal symptoms ^b
Walter	NCPPB 2445	2.4	2.2
	NCPPB 2456	1.2	1.4
	NCPPB 2447	2.8	3.0
	NCPPB 2458	0.2	1.0
	A	1.0	1.4
	B	1.4	1.6
	C	0.8	1.4
Homestead	NCPPB 2445	2.8	2.8
	NCPPB 2456	2.8	2.8
	NCPPB 2447	2.6	3.0
	NCPPB 2458	0.8	1.2
	A	2.0	2.4
	B	2.8	3.0
	C	1.2	2.9
Bonny Best	NCPPB 2445	2.0	1.0
	NCPPB 2456	2.4	1.0
	NCPPB 2447	2.6	2.9
	NCPPB 2458	0.4	1.0
	A	1.8	2.4
	B	3.0	2.6
	C	2.0	1.0
Casino Royal	NCPPB 2445	2.2	2.9
	NCPPB 2456	2.0	2.6
	NCPPB 2447	2.2	2.6
	NCPPB 2458	1.6	1.8
	A	2.0	2.9
	B	2.6	3.0
	C	2.0	2.9

^aExternal symptoms were rated from 0 to 3, depending on the amount of external discoloration of the stem. A wound site from the inoculation needle was rated as 0; discoloration of up to 0.2 cm as 1; discoloration from 0.2 to 1 cm as 2; and discoloration greater than 1 cm around the wound site as 3. Ratings are the mean of five plants; checks of each cultivar were rated as 0.

^bInternal symptoms were rated from 0 to 3, depending on the amount of internal discoloration of the pith. Normal pith with no discoloration was rated as 0; discoloration of up to 0.5 cm as 1; discoloration from 0.5 to 1 cm as 2; and discoloration greater than 1 cm from the inoculation site with associated hollow areas of pith as 3. Ratings are the mean of five plants; checks of each cultivar were rated as 0.

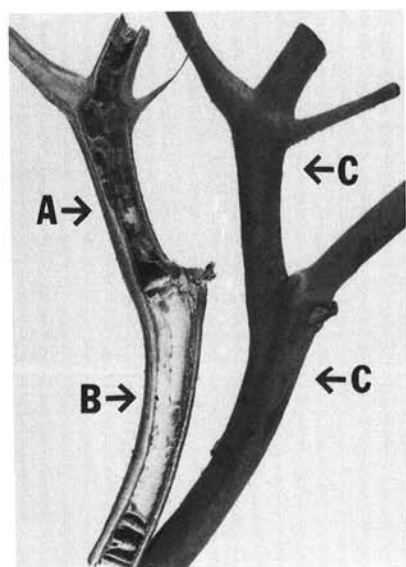


Fig. 1. Symptoms of tomato pith necrosis on naturally infected tomato cultivar Casino Royal, including (A) brown necrotic areas where the pith has been hollowed out, (B) whitish healthy areas of solid pith, and (C) gray or brown discoloration on the external stem surface.

Homestead, Bonny Best, and Casino Royal. Tomato cultivars demonstrated varying degrees of susceptibility both to the San Diego tomato strains and the known *P. corrugata* strains (Table 2). Strain "B" and *P. corrugata* NCPBB 2447 were the most virulent pathogens on all four tomato cultivars tested.

DISCUSSION

Although there are some minor differences (in ability to rot carrot roots and in gelatin hydrolysis) in physiological and biochemical characters (Table 1) among the tomato strains from California and those from England, we conclude that the California isolates are strains of *P. corrugata*. Although Lukezic (7) reported isolation of *P. corrugata* from

symptomless alfalfa roots in Pennsylvania, this is the first report of tomato pith necrosis caused by *P. corrugata* in California and in the United States.

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