Bacterial Seedling Blight of Tomato Caused by *Bacillus polymyxa*

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ABSTRACT

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Numerous necrotic flecks were observed on the stems, cotyledons, and occasionally, the roots of seedlings of 17 tomato cultivars germinated on water agar. A bacterium consistently isolated from lesions was identified as *Bacillus polymyxa*. This is the first known report of *B. polymyxa* causing seedling blight in tomato.

Reports of plant infections by *Bacillus* species are uncommon. *Bacillus subtilis* causes spotting of pepper fruits (23), partially decays peanut kernels (16,22), causes root deterioration, vascular dysfunction, and foliar veinal necrosis/marginal chlorosis in soybean (9,10), and rots potato tuber tissue (1). *B. mesentericus* (a synonym for *B. subtilis*) infects the lenticels and causes soft rot of potato tubers (4,7). White blotches and streaks on wheat are caused by *B. megaterium* pv. *cerealis* (12). Tobacco frenching symptoms are partially caused by toxin(s) produced by *B. cereus* (14).

In February 1981, the seed of 18 tomato (*Lycopersicon esculentum* L.) cultivars was tested for seedborne *Alternaria solani* and *Corynebacterium michiganense*. After germination on 2% water agar, seedlings of 17 cultivars developed necrotic flecks over the entire length of the hypocotyl; occasionally, general necrosis of whole seedlings occurred. *A. solani* was present on the seeds of several cultivars, but *C. michiganense* was never recovered. A white-pigmented, gram-variable bacterium was isolated consistently from lesions on seedlings from all cultivars. This report describes a new disease of tomato and its causal agent, *B. polymyxa* (Prazmowski) Mace. A preliminary report of this disease has already been published (6).

MATERIALS AND METHODS

Isolation of causal organism and pathogenicity studies. Excised lesions from diseased seedlings of several cultivars were streaked on plates of Difco nutrient agar. After incubation of the plates at 28 C for 96 hr, 10 bacterial colony types were observed. Representatives of the 10 types were streaked on nutrient agar plates to obtain pure cultures and later transferred to nutrient agar slants. The pathogenicity of each strain was determined using seeds of the cultivar Gardener's Delight (Johnny's Selected Seeds, Albion, ME), which had been free of symptoms in earlier screening tests. Tomato seeds were coated with a bacterial suspension of 10^6 colony-forming units (cfu) per milliliter prepared from 24-hr nutrient broth cultures, and seeds were plated directly on water agar plates. After 7 days at 28 C, necrotic flecks identical to those produced naturally appeared on seedlings inoculated with six bacterial strains that were identical in colony morphology. These six strains were studied further.

B. polymyxa strains 842 and 8523 were obtained from the American Type Culture Collection (ATCC). An additional strain, purchased from the North Central Culture Collection (NCCC), was obtained from Darryl Pratt, Department of Microbiology, University of Maine, Orono. These three type strains were used in all inoculations and biochemical tests.

Tomato cultivars Jetstar, Supersonic, Small Fry, Ramapo, Sunray, Roma, Rutgers, and New Yorker were obtained from Harris Seed Company, Rochester, NY. Seeds of these cultivars, which had been treated by the company with hot water/trisodium phosphate or thiram/captan, were symptomless when plated on water agar.

Four inoculation techniques using the nine strains were performed on the Harris cultivars: 1) seeds, coated with a bacterial suspension of 10^6 cfu/ml from 24-hr nutrient broth cultures, were incubated on water agar for 7 days at 28 C; 2) seeds, grown on water agar until the first leaves appeared, were sprayed with a fine mist of 10^6 cfu/ml (24-hr nutrient agar cultures suspended in sterile water and adjusted colorimetrically), transferred to new water agar plates, and allowed to incubate for 10 days at 28 C; 3) seeds, coated with a bacterial suspension of 10^6 cfu/ml prepared from 24-hr nutrient broth cultures, were grown in the greenhouse in sterile sand for 14 days; and 4) seeds, grown in sterile sand to the two-leaf stage, were sprayed to runoff with 10^6 cfu/ml, incubated in a humidity chamber for 24 hr, and kept on the greenhouse bench for 14 days. Seeds serving as controls were coated with nutrient broth and plated on water agar or planted in sand and sprayed with sterile water.

Characterization of causal organism. The following tests (19,21,22,25) were used to identify the six natural strains and three type strains: Gram stain, flagellar stain by the silver plating method (17); spore stain (25), spreading or nonspreading growth in Difco motility medium; and growth on nutrient dextrose agar, 5% sheep's blood agar (incubated aerobically and anaerobically), Sabouraud's dextrose agar, MacConkey agar, Salmonella-Shigella (SS) agar, and in 5, 7, and 10% NaCl broth. Biochemical tests included nitrate reduction; esculin hydrolysis; gelatin liquefaction and hydrolysis; indole production; Voges-Proskauer; methyl red test; urease; oxidase; catalase; phenylalanine deaminase; citrate utilization; decomposition of casein and tyrosine; production of hydrogen sulfide, levan, potato and tomato soft rot; toleration of 0.2% triphenyl tetrazolium chloride; starch hydrolysis; ammonia evolution; ONPG (*O*-nitrophenyl-β-D-galactopyranoside) test for β-galactosidase; triple sugar iron test; and lysine decarboxylase. Acid and gas production were also monitored in a minimal medium containing 1% carbohydrate and phenol red indicator after 7 days (20). Compounds tested were glucose (dextrose), sucrose, maltose, lactose, xylose, arabinose, and glycerol. Acid production was tested in a minimal medium containing 10% glucose, mannitol, arabinose, and xylose solutions. Tobacco plants were also infiltrated with bacterial inocula to determine whether the hypersensitive reaction could be

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induced. In all tests, bacterial inoculum consisted of cells taken from 24-hr slant cultures, and media were incubated at 28 C unless indicated otherwise. All tests were repeated.

Host range and control. Seeds of jimsonweed (*Datura stramonium* L.), pepper (*Capsicum annuum* L.), egg plant (*Solanum melongena* L.), and petunia (*Petunia hybrida* L.) were inoculated with a bacterial suspension of 10^6 cfu/ml of each strain, plated on water agar, and incubated for 7 days at 28 C.

Seeds of seven naturally infested tomato cultivars were hot-water treated at 50 C for 0, 12, and 18 min and at 90 C for 0, 14, 30, 60, and 120 sec. Fifty seeds per treatment were plated on water agar or moistened sterile filter paper to test for disease symptoms. Experiments were repeated.

RESULTS

Pathogenicity studies. The disease was observed on the following cultivars of tomato: Bonny Best, Coldset, Crimson Trellis, Earlibright, Earlirouge, Marglobe, Moira, Napoli, Nova, Spectrum, Sub-Artic Early, Sub-Artic Maxi, Sub-Artic Midi, Sub-Artic Plenty, Tiny Tim, Trimson, and Whippersnapper. Symptoms included dark brown necrotic flecks either localized or along the entire length of the hypocotyl (Fig. 1A), distortion and necrosis of the emerging leaflets, poorly developed root systems that sometimes were necrotic, and sunken stem lesions that eventually killed the seedlings (Fig. 1B). In all excised lesions, large numbers of oozing bacterial cells were observed.

Six bacterial strains isolated from diseased seedlings induced symptoms comparable to those observed in natural infections either when seeds were coated with bacteria or when seedlings were sprayed with bacterial suspensions (Fig. 1C). These six isolates also induced necrosis on stems and leaflets of plants growing in the greenhouse (Fig. 1D). Plants inoculated with the three type strains of *B. polymyxa* developed symptoms similar to those described before.

Bacterial strains identical to the original strains were reisolated from all inoculated plants.

Characterization of causal organism. Colonies of the six pathogenic bacterial strains were translucent, white, and thin with amoeboid spreading on nutrient agar. Colony type on 5% sheep's blood agar (both aerobic and anaerobic) was serrate, flat, and translucent with zones of β-hemolysis. Mucoid, raised colonies were produced on potato-dextrose agar and nutrient-dextrose agar. No growth was observed on MacConkey agar or SS agar; abundant growth occurred on Sabouraud's dextrose agar.

Observed with the light microscope, bacteria were gram-variable, rod-shaped ($2.0–4.0 \times 0.8–1.0$ μm), and motile by means of peritrichous flagella. Bacterial cells did not form chains. Older nutrient agar cultures contained high numbers of bacterial cells with subterminal to terminal spores producing a distinct swelling of vegetative cells (Fig. 2).

All nine strains produced catalase, β-galactosidase (ONPG-positive), reduced nitrate, hydrolyzed esculin and starch, tolerated 0.2% triphenyl tetrazolium chloride, rotted potato and tomato tissue, decomposed casein, produced levan, evolved ammonia, hydrolyzed and liquefied gelatin, and were positive for tobacco HR, methyl red, and Voges-Proskauer tests. Each isolate turned the slant and butt of triple sugar iron tubes acid.

None of the strains produced oxidase, urease, phenylalanine deaminase, indole, hydrogen sulfide, or lysine decarboxylase, utilized citrate, decomposed tyrosine, or grew in either 5, 7, or 10% NaCl.

Acid was produced by all strains from all carbon sources and gas was produced in most cases.

On the basis of these tests, the six isolates were identified as *B. polymyxa* according to the scheme of Bergey's

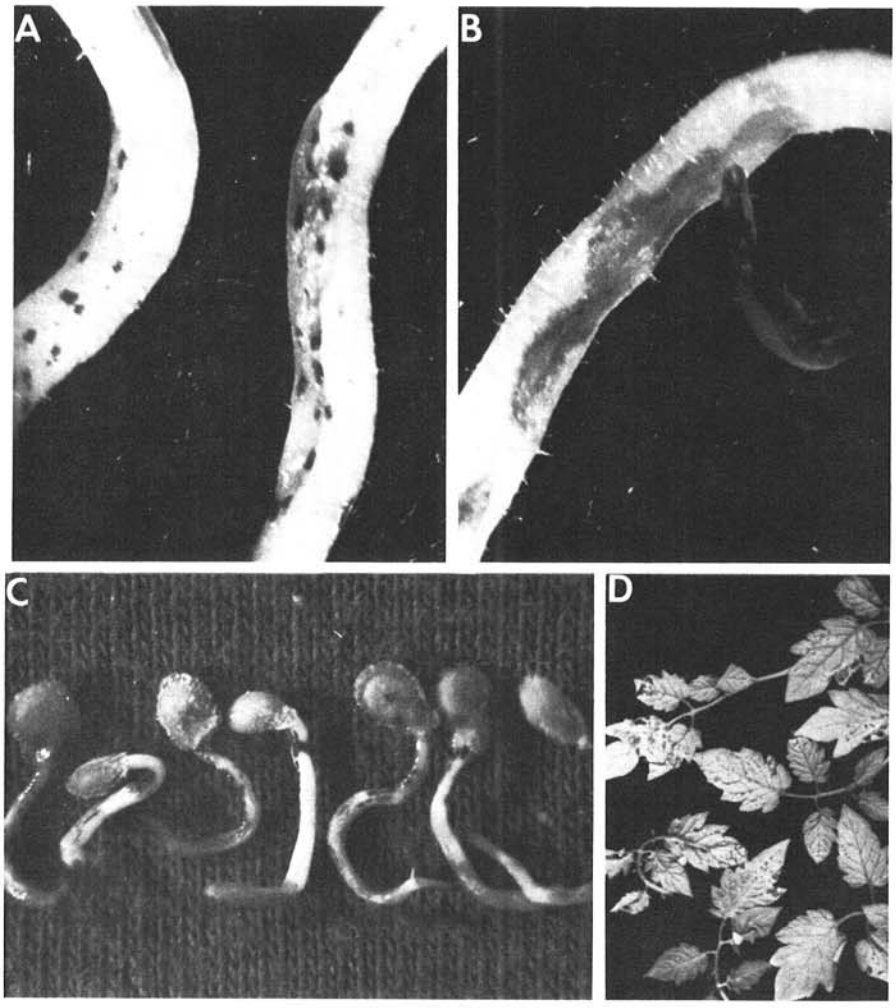

Fig. 1. Seedling blight of tomato caused by *Bacillus polymyxa*: **(A)** Flecking and **(B)** sunken lesions along the hypocotyl of naturally infected seedlings eventually girdled the plants. **(C)** Flecking on hypocotyls of artificially inoculated young seedlings. **(D)** Necrotic spots on leaflets of artificially inoculated, greenhouse-grown seedlings.

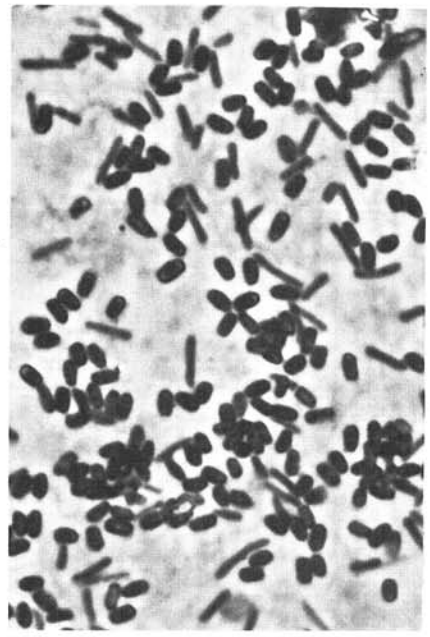

Fig. 2. Cells of *Bacillus polymyxa* stained with malachite green and safranin. Note typical rod-shaped cells and shorter stubby cells with distinctive swelling (×670).

Manual (3). Table 1 summarizes the most important characters of this species and compares the tomato strains and type strains with information supplied by Bergey's Manual and Gordon et al (11).

Host range and control. Symptoms comparable to those observed in tomato seedlings were observed on jimsonweed, pepper, and eggplant seedlings inoculated with the nine *B. polymyxa* strains. Petunia seedlings were not infected by the bacterium.

In most cases, hot-water seed treatment did not effectively control the disease (Table 2). Treatment of seeds at 90 C for 60 sec resulted in complete control of blight symptoms in the cultivars Marglobe, Moira, and Earlibright and partial control in Coldset. Treatment of seeds at 50 C for 18 min resulted in complete control only in the cultivar Marglobe. Exposure of seeds to 90 C for 120 sec killed all of the seeds.

DISCUSSION

This is not the first report of *B. polymyxa* as a plant pathogen; the bacterium is known to rot potato tuber tissue (2,7,8,13). Jackson and Henry (13) noted that temperatures required for the organism's pectolytic activity (30 C) normally would not be encountered in potato fields or storage areas. The bacterium can extensively rot carrot, onion, and cucumber slices and iris stems (8) and induce spots on pepper fruits (23). *B. polymyxa* is an important cause of bacterial pit and bacterial blotch of the cultivated mushroom (24).

Dowson (8) previously reported that *B. polymyxa* rotted green tomato fruits after inoculation. Madhok and Ud-Din (15) detected a subepidermal soft rot of mature tomato fruits. They named the organism *B. frutodestruans*. On the basis of the limited number of biochemical tests they performed in 1943 with this bacterium, it is difficult to be certain whether *B. polymyxa* actually caused the tomato rot. At present, *B. frutodestruans* is not recognized as a separate species and has not been formally characterized (5).

The bacterium, which was isolated from the diseased tomato seedlings and caused comparable symptoms in artificially inoculated seedlings is *B. polymyxa*. *B. polymyxa* is most often confused with the closely related species *B. macerans* and *B. circulans*. The tomato bacterium was adept at forming gas, producing gas in media containing arabinose, glucose, glycerol, lactose, mannitol, sucrose, and xylose. Growth on nutrient-dextrose agar was heaped and mucoid, and growth on nutrient agar was spreading in nature. Bacterial cells were swollen by the presence of spores. These characters separate the species from many other *Bacillus* species, but they are held in common with *B. macerans*. However, *B. macerans* does not decompose casein and is negative for Voges-Proskauer; both of these tests were positive for the tomato bacterium. *B. circulans* does not produce gas from arabinose, mannitol, or xylose and is also negative for Voges-Proskauer. On the basis of these characters and comparable results obtained with the three type strains, the pathogen was identified as *B. polymyxa*.

Although *B. polymyxa* was isolated consistently from all 17 tomato cultivars listed in Results, the cultivars Coldset, Sub-Arctic Midi, Sub-Arctic Plenty, Crimson Trellis, and Nova had a particularly high percentage of diseased seedlings. These cultivars may be more susceptible to seed infection. Seedling inoculations, however, indicated that all cultivars were equally susceptible to each of the bacterial strains under our test conditions. In contrast, inoculated seedlings of cultivars obtained from Harris expressed differences in susceptibility to the bacterium. Infection percentage was lowest for Rutgers (32.9), followed by Ramapo (56.3), New Yorker (57.9), Sunray (65.5), Jetstar (70.9), Supersonic (74.1), Roma (80.7), and Small Fry (81.3). Symptoms appearing on Rutgers, Ramapo, and New Yorker seedlings were also markedly less severe than those on seedlings of the other cultivars.

Cultures of *B. polymyxa* over 4 days old contained abundant spores (Fig. 2). The bacterium may exist in the endospore stage within tomato seed. Because endospores are typically resistant to heat and may survive several hours of boiling (18), unsuccessful attempts to inactivate the bacterium by heat-treating can easily be explained. Antibiotic treatments were not tested in this work, but these compounds may prove effective for control of the pathogen.

The source of the pathogen in infested

Table 1. Characterization and comparison of *Bacillus* strains with published descriptions

Test	Six strains[a]	Reference strains[b] 1	2	3	Bergey's Manual[c]	Gordon et al[c]
Gram reaction	V[d]	V	V	V	V	V
Motility	+	+	+	+	+	−
Catalase	+	+	+	+	+	+
Anaerobic growth	+	+	+	+	+	+
Growth at pH 5.7	+	+	+	+	+	+
Voges-Proskauer	+	+	+	+	+	+
Growth in:						
5% NaCl	−	−	−	−	−	−
7% NaCl	−	−	−	−	−	−
10% NaCl	−	−	−	−	−	−
Acid from:						
Glucose	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+
Xylose	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+
Starch hydrolysis	+	+	+	+	+	+
Use of citrate	−	−	−	−	−	−
Nitrate reduction	+	+	+	+	+	+
Decomposition of:						
Casein	+	+	+	+	+	+
Tyrosine	−	−	−	−	−	−

[a] Strains of *Bacillus* isolated from blighted tomato seedlings.
[b] American Type Culture Collection: 1 = *B. polymyxa* 842, 2 = *B. polymyxa* 8523, and 3 = North Central Culture Collection.
[c] Bergey's Manual (3) and Gordon et al (11).
[d] + = More than 85% of strains positive, − = more than 85% of strains negative, and V = variable character.

Table 2. Effect of hot-water seed treatment on the occurrence of tomato seedling blight

Cultivar	Percentage of seedlings with disease: 50 C[a] Untreated	12 Min	18 Min	90 C[b] Untreated	15 Sec	30 Sec	60 Sec
Nova	83	42	56	55	67	68	42
Sub-Artic Early	11	9	9	8	11	17	11
Marglobe	22	10	0	26	11	15	0
Sub-Artic Midi	79	80	90	78	50	59	88
Moira	21	11	22	15	25	10	0
Earlibright	28	18	25	19	14	10	0
Coldset	100	97	100	90	76	71	19

[a] One hundred seeds were placed in 50 C water for 12 or 18 min or left untreated. Seeds were then plated on water agar and rated for disease development after 6 days of incubation at 28 C.
[b] One hundred seeds were placed in 90 C water for 15, 30, or 60 sec or left untreated. Seeds were then plated on water agar and rated for disease development after 6 days of incubation at 28 C.

seed is unknown, although rotting tomato fruits may have been the source. *B. polymyxa* is widely distributed in soil, water, feces, and decaying vegetable matter (3). Therefore, it is likely that sufficient numbers of the bacterium were present in the field to infect fruits. Further work on this host-pathogen system is warranted to determine the susceptibility of other solanaceous hosts to *B. polymyxa* and to explain the prevalence of seed infestation observed.

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