Association of *Pseudomonas syringae* pv. *syringae* With a Leaf Spot Disease of Tomato Transplants in Southern Georgia

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Supported by USDA, Hatch, state, and grower-supplied funds allocated to the Georgia Agricultural Experiment Stations.
We thank J. Fowler and J. Hamm for technical assistance.
Accepted for publication 2 April 1981.

ABSTRACT

Jones, J. B., McCarter, S. M., and Gitaitis, R. D. 1981. Association of *Pseudomonas syringae* pv. *syringae* with a leaf spot disease of tomato transplants in southern Georgia. Phytopathology 71:1281-1285.

An outbreak of a foliar disease on tomato transplants in southern Georgia during 1980 was originally diagnosed as bacterial speck caused by *Pseudomonas tomato* (*P. syringae* pv. *tomato*). Isolates from diseased tissue yielded a green fluorescent pseudomonad that was oxidase- and arginine dihydrolase-negative and induced a hypersensitive reaction in tobacco but, in pathogenicity tests, did not cause typical bacterial speck symptoms. Physiological, biochemical, and pathogenicity tests demonstrated that the 1980 Georgia isolates were more similar to *P. syringae* (*P. syringae* pv. *syringae*) than to *P. tomato*. Most isolates of the new bacterium were similar to *P. syringae* in producing syringomycin, in using erythritol and DL-lactate as substrates, and in forming ice nuclei. The 1980 isolates typically caused necrotic leaf spots with halos on California Blackeye 3 cowpea and necrotic reactions on Lovell peach, both known hosts of *P. syringae,* whereas *P. tomato* isolates usually caused no necrotic symptoms. The new bacterium also caused brown necrotic lesions without halos on leaves of Chico III tomato plants after infiltration with the bacteria or when wounds were made. These results show a fluorescent pseudomonad other than *P. tomato* may be responsible for necrotic lesions on tomato transplants and indicate the need for additional testing to differentiate the bacteria during certification procedures.

Additional key words: Lycopersicon esculentum, bacterial diseases.

In the spring of 1980, a high incidence of a bacterial leaf spot disease occurred on tomato (*Lycopersicon esculentum* Mill.) transplants grown in southern Georgia for shipment to fruit-producing areas of the northern United States and Canada. Numerous isolations from tissue made on medium B of King et al (9) consistently yielded a green fluorescent pseudomonad. Further testing showed that the organism was both oxidase-negative (11) and arginine dihydrolase-negative (27) and induced a hypersensitive response in tomato (10), suggesting that the isolates were pathogenic (7,13,16,21). Bacterial speck has been a serious problem on tomato transplants in southern Georgia since 1978 (*unpublished*); therefore, *Pseudomonas tomato* (Okabe) Alstatt (*P. syringae* pv. *tomato*) was the suspected pathogen, although field symptoms sometimes differed from those observed in previous outbreaks of bacterial speck. Foliar spots that developed in 1980 frequently were larger than those usually observed for bacterial speck, and the halos common around lesions (2,5,19) often were absent. When greenhouse pathogenicity tests were conducted, only one of 20 isolates collected in 1980 produced typical bacterial speck symptoms on susceptible Chico III tomato plants. Inoculations of greenhouse-grown plants with four isolates collected in 1979 also failed to produce speck symptoms. We concluded that *P. tomato* was not responsible for much of the bacterial foliar disease observed in 1979 and 1980.

Other than reports on *P. tomato* (2,5,19), little has been reported on fluorescent pseudomonads pathogenic to tomato. Wilkie and Dye (29) reported that a pseudomonad, identified as *P. syringae,* from tomato produced light brown lesions without halos after artificial inoculation of healthy plants. Volcani (28) also isolated an organism from tomato that was identified as *P. syringae,* but the organism was not characterized completely and was described as a facultative anaerobe. *Pseudomonas viridiflava* also has been associated with lesions on tomato leaves (1,3), but its role as a pathogen is not clear (1). The limited information on the plant pathogenic fluorescent pseudomonads associated with tomato and the need for positive identification by regulatory personnel suggested a need to characterize the isolates differing from *P. tomato* that could be isolated from diseased tomato transplants.

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0031-949X/81/12128105/$03.00/0

This paper reports the results of a comparative study of the morphology, physiology, and pathogenicity of unidentified fluorescent isolates from tomato and known isolates of *P. tomato* from tomato and *P. syringae* from other hosts.

MATERIALS AND METHODS

Bacterial isolates. Forty-five isolates were used in the various tests. Fifteen were known or suspected isolates of *P. tomato,* four obtained from D. L. Coplin (OARDC, Wooster, OH), two from M. Sasser (University of Delaware, Newark), one from R. E. Stall (University of Florida, Gainesville), and eight were from transplants grown in Georgia in 1978. Four of the 45 were known isolates of *P. syringae* (*P. syringae* pv. *syringae*), one each from W. M. Dowler (USDA, Beltsville, MD), J. D. Otta (South Dakota State University, Brookings), R. D. Wilcoxson (University of Minnesota, St. Paul), and E. I. Zehr (Clemson University, Clemson, SC). Two of these were from peach and two were from wheat. Twenty-two were unidentified green-fluorescent isolates obtained from tomato transplants in Georgia during 1979 and 1980. Four isolates (three from soil and one from decaying fish) of the fluorescent saprophyte *P. fluorescens* were included for comparison. All isolates were maintained either in tubes containing distilled water at room temperature or on slants of nutrient yeast glucose agar (nutrient agar, 23 g; yeast extract, 5 g; glucose, 10 g; and water, 1 L, pH 6.8) at 6 C. The isolates were grown on medium B of King et al (9) (KMB) for 48 hr at 25 C for inoculum production.

Morphological characters. All isolates were stained by the Hucker modification of the Gram-staining procedure (15), and the morphology and Gram reaction were determined. Flagellation of 12 representative isolates (two of *P. tomato,* two of *P. syringae,* seven of the 1979–1980 isolates, and one of *P. fluorescens*) was determined after staining by a silver-plating method (20). Culture and colony comparisons were made on KMB in plates and on autoclaved potato slants in tubes.

Physiological and biochemical reactions. Selected tests used to characterize *Pseudomonas* spp. (7,13,16,21,25), as well as routine bacteriological tests (15), were employed. Standard methods (15) were used to compare isolates for their relationship to free oxygen, motility in stabs of semisolid agar, action on litmus milk, citrate utilization in Koser's citrate medium, nitrate reduction, starch hydrolysis (plate tests), liquefaction of 15% gelatin medium, production of acetylmethylcarbinol, methyl red reaction, and the presence of catalase. Casein hydrolysis was determined by patching isolates on plates of milk agar (22). Arginine dihydrolase activity was determined by Thornley's method with her medium 2A (27). Lipase production was determined by streaking isolates on Tween-80 agar (23). Presence of β-glucosidase was determined using Hugh and Leifson's (8) peptone basal medium with 0.5% (w/v) arbutin added (6). Levan production was determined by streaking isolates on plates of nutrient sucrose agar (13). Tests for 2-ketogluconate were conducted as described by Misaghi and Grogan (16) except that potassium gluconate replaced sodium gluconate in the test medium. Determination of NaCl tolerance was made in broth (Difco nutrient broth supplemented with 5% w/v NaCl). Ammonia production from amino acids was determined by inoculating tubes of peptone water broth (10 g Bacto peptone, 5 g NaCl, 1 L distilled water, pH 7.2) and testing after 4 days with Nessler's reagent. The development of a dense yellow-orange precipitate was considered positive for ammonia production. Urease activity was determined with urea broth medium (0.1 g yeast extract, 9.1 g KH_2PO_4, 9.5 g Na_2HPO_4, 20 g urea, 25 ml of 0.04% aqueous solution of phenol red, and 1 L distilled water, pH 7.0). A change in color of the medium to a bright red after 2–4 days indicated hydrolysis of urea with liberation of ammonia. Hydrolysis of aesculin was determined by Sneath's method (24) in a liquid medium. Capacity of isolates to use certain organic substrates (trehalose, erythritol, mannitol, sorbitol, DL-alanine, DL-lactate, L(+)-tartrate, and D(−)-tartrate) was determined as described by Misaghi and Grogan (16) except that Seakem agarose (Marine Colloids Div., FMC Corp., Rockland, ME 04841) was used to solidify the media. Glucose, sucrose, and lactose as carbohydrate sources were tested by adding 1% (w/v) of each compound separately to Hugh and Leifson's semisolid basal medium (8). Stabbed tubes were either covered with melted Vaseline or left uncovered to determine whether the compounds were used fermentatively or oxidatively, respectively (8). The oxidase reaction of each isolate was determined by smearing cells from cultures grown on KMB for 48 hr onto moistened Taxo differentiation disks (BBL, Bioquest, Division of Becton, Dickson, and Co., Cockeysville, MD 21030). Potato soft rot capacity (13) was determined for three randomly selected isolates of *P. tomato,* two of *P. syringae,* and eight of the 1979–1980 transplant isolates.

All inoculated media were incubated at 25 C. Duplicate tubes or plates were run with each organism-medium combination, and tests were repeated when unexplained variation occurred.

Tobacco hypersensitivity. Interveinal areas of mature leaves of tobacco (*Nicotiana tabacum* L. 'Hicks') were infiltrated with a suspension (10^8 colony-forming units [cfu]/ml) of each isolate, and readings were made after 24 hr (10).

Syringomycin production. Syringomycin production was determined as described by Devay et al (4) except that Difco potato-dextrose agar was used instead of a naturally prepared formula. *Geotrichum candidum* Link ex Pers. was used as the bioassay organism (4).

Ice nucleation. Isolates were grown on glycerol agar and tested for their capacity to form ice nuclei as described by Lindow et al (14) except that 6-cm-diameter aluminum weighing pans were floated on an ethanol-ice water mixture adjusted to −5 to −10 C. Ten 10-μl droplets of the suspensions (10^8 cfu/ml) in 0.1 M phosphate buffer (pH 7.0) were placed on the surface of the aluminum pans. An isolate was considered to produce ice nuclei if more than one droplet froze within 30 sec.

Pathogenicity tests. All or representative isolates of each species or group were tested for pathogenicity on tomato (Chico III), cowpea (*Vigna unguiculata* (L.) Walp. subsp. *unguiculata* 'California Blackeye 3'), and peach (*Prunus persica* (L.) Batsch 'Lovell'). Cowpea and peach were used because they are suitable diagnostic hosts for *P. syringae* (12,17,18). Suspensions containing approximately 10^8 cfu/ml were used for all inoculations except where indicated otherwise. A suspension of each isolate was applied to runoff onto all surfaces of 6-wk-old (16–18 cm tall) Chico III tomato plants with a Burgess Model 862 paint sprayer (Vibrocrafters, Inc., Grayslake, IL 60030) held 30–35 cm from the plant surface to minimize leaf infiltration. If isolates failed to produce definite symptoms after spray inoculation of tomato, three other inoculation methods were tested: sand bags soaked in the suspensions were rubbed onto leaves to provide injury; leaves were partially infiltrated with inoculum by atomization close to the leaves (5 cm from the surface) with the paint sprayer; and leaves were infiltrated with a suspension (10^4 cfu/ml) by immersing the foliage and placing plants under a partial vacuum (64 to 76 cm of Hg) and releasing the vacuum abruptly. All plants except those inoculated by vacuum infiltration were enclosed in clear polyethylene bags for 36 hr to provide high humidity. Plants were placed in a growth chamber at 18–20 C except that in one study a 27 C chamber was also used. Symptoms were recorded 10–14 days after inoculation. Three-week-old cowpea plants were dusted with carborundum and rubbed with a cotton-tipped applicator soaked in a bacterial suspension (12). Inoculated plants were placed under continuous mist for 24 hr at 25 C and held for an additional 10 days to observe symptom development. Peach trees (25–30 cm tall) grown from cuttings were inoculated by injecting a suspension with a syringe and needle into the tips of rapidly growing shoots until water soaking occurred (17,18). Canker formation or the death of the shoot was considered a positive reaction after 5–6 days in a growth chamber at 25 C.

RESULTS

Morphology and cultural characteristics. All 45 isolates studied were Gram-negative rods, similar in size and morphology. Some isolates produced short chains of larger than normal cells, but this characteristic was not confined to any one species or grouping. The

representative isolates that were stained had three to six lophotrichous flagella. All isolates produced a green to slightly yellow water-soluble fluorescent pigment on KMB. Individual isolates of *P. tomato, P. syringae,* and the 1979–1980 isolates from tomato transplants differed slightly in color and slime production on KMB, but differences were as great within as among species. The *P. fluorescens* isolates generally were more yellow and slimy on KMB than the three plant pathogenic groups. Isolates differed slightly in appearance on potato slants, but no distinct cultural characteristic was associated with a given species or group.

Physiological and biochemical reactions. None of the 45 isolates hydrolyzed starch, produced acetylmethylcarbinol from glucose, or gave a positive methyl red reaction. All or a high percentage of isolates, including *P. fluorescens,* were aerobic, motile, NaCl-tolerant, catalase-positive, active on litmus milk, gelatin liquefiers, and utilized citrate, glucose, sorbitol, mannitol, and DL-alanine (Table 1). Some isolates of the three pathogenic groups and *P. fluorescens* produced levan, hydrolyzed casein, and utilized sucrose and lactose oxidatively, but results were variable among isolates of one or more groups. All isolates of *P. tomato, P. syringae,* and the unidentified isolates from tomato transplants were negative for oxidase, arginine dihydrolase, nitrate reduction, 2-ketogluconate and L(+)-tartrate, whereas 50 to 100% of the *P. fluorescens* isolates were positive for these reactions. All or most isolates of the pathogen groups were positive for tobacco hypersensitivity, β-glucosidase, and aesculin hydrolysis, but all *P. fluorescens* isolates were negative. Only one of 15 *P. tomato* isolates tested used erythritol and DL-lactate as substrates, whereas most isolates of *P. syringae,* the unidentified tomato isolates, and *P. fluorescens* used the compounds. The four known isolates of *P. syringae* did not use D(−)-tartrate, but most isolates of *P. tomato, P. fluorescens,* and the unidentified tomato isolates did. All the isolates tested gave a negative potato soft rot reaction. Three of four of the *P. syringae* isolates and all 22 of the unidentified tomato isolates produced syringomycin when grown on potato-dextrose agar. Syringomycin was not detected in cultures of *P. tomato* and *P. fluorescens.* Ice-nucleating activity occurred frequently among isolates of *P. syringae* and the unidentified isolates, but only rarely (one of 15 isolates) in *P. tomato* and not at all in *P. fluorescens.*

Pathogenicity tests. Only the known isolates of *P. tomato* and one of the 1979–1980 isolates from transplants produced typical bacterial speck symptoms on Chico III tomato plants, regardless of the method of inoculation used (Table 1). All 15 isolates of *P. tomato* produced lesions with halos that first appeared 5–7 days after standard (spray) inoculation. The unidentified tomato isolates and the isolates of *P. syringae* sometimes produced small brown lesions without halos after spray inoculation. However, symptoms produced by these isolates were more pronounced when inoculum was infiltrated into the leaf or leaf wounds were provided. Small necrotic lesions without halos appeared in 5–6 days after vacuum infiltration of 10^4 cfu/ml into leaf tissue. Close atomization with a paint sprayer to obtain partial infiltration

TABLE 1. Comparison of isolates of known *Pseudomonas* spp. with unidentified *Pseudomonas* isolates from tomato transplants in various physiological, biochemical, and pathogenicity tests

	Number of isolates with positive reactions			
Test for[a]	*P. tomato*	*P. syringae*	Unidentified tomato isolates	*P. fluorescens*
Number isolates[b] tested	15	4	22	4
Oxidase	0	0	0	4
Arginine dihydrolase	0	0	0	4
Levan	15	4	12	1
Nitrate reduction	0	0	0	2
NaCl tolerance (5%)	13	4	22	3
Ammonia	0	0	3	4
2-ketogluconate	0	0	0	3
β-glucosidase	15	4	22	0
Litmus milk				
Peptonization	12	1	20	3
Reduction	15	4	21	4
Gelatin liquefaction	14	3	20	2
Lipase	0	0	2	3
Urease	3	0	2	1
Casein hydrolysis	6	3	16	2
Aesculin hydrolysis	14	4	21	0
Citrate utilization	15	4	21	4
Other substrate utilization				
Sucrose (oxidatively)[c]	15	4	22	1
Lactose (oxidatively)[c]	15	1	6	3
Trehalose	1	0	1	3
Sorbitol	15	4	22	3
Erythritol	1	4	22	3
DL-lactate	1	3	22	4
L(+)-tartrate	0	0	0	3
D(−)-tartrate	15	0	17	3
Syringomycin	0	3	22	0
Tobacco hypersensitivity	15	4	22	0
Ice nucleation	1	3	18	0
Tomato pathogenicity[d]	15	0	1	0
Cowpea pathogenicity	0[e]	3	19	0

[a] All 45 isolates were aerobic, motile, catalase positive, and utilized glucose, mannitol and DL-alanine, but none hydrolyzed starch, produced acetylmethylcarbinol, or gave a positive methyl red reaction.

[b] The *P. tomato* isolates were obtained from D. L. Coplin, M. Sasser, R. E. Stall, and from our culture collection from Georgia plants. The *P. syringae* isolates were from wheat (two isolates) or peach (two isolates) and came originally from W. M. Dowler, J. D. Otta, R. D. Wilcoxson, and E. I. Zehr. The 22 unidentified isolates came from tomato transplants in Georgia. The *P. fluorescens* isolates were from soil (three isolates) or decaying fish (one isolate).

[c] None of the organisms used sucrose or lactose fermentatively as determined by Hugh and Leifson's method (8).

[d] Refers to production of typical bacterial speck symptoms with standard (spray) inoculation. The unidentified *Pseudomonas* isolates produced brown necrotic symptoms after wound inoculation and vacuum infiltration of inoculum.

[e] Twelve instead of 15 isolates of *P. tomato* were tested on cowpea.

produced irregular-shaped brown lesions in 5–7 days. Water-soaked lesions that later became dry and brown occurred along wounds resulting from sand bag inoculation. Isolates of *P. fluorescens* did not produce symptoms regardless of the method of inoculation.

All of the 12 unidentified tomato isolates and three of four isolates of *P. syringae* tested produced a necrotic reaction or tip death on shoots of young Lovell peach trees. Two of 12 isolates of *P. tomato* produced a mild necrotic reaction. Most isolates of *P. tomato* produced extensive reddening surrounding the point of inoculation, but this response was not considered a disease reaction.

Most of the unidentified tomato isolates (18 of 22 positive) and isolates of *P. syringae* (three of four positive) produced necrotic lesions surrounded by halos on California Blackeye 3 cowpea 10 days after inoculation (Table 1). All isolates of *P. tomato* and *P. fluorescens* failed to produce a typical disease reaction, although three isolates of *P. tomato* produced white, papery necrotic areas.

DISCUSSION

Tomato transplant producers in southern Georgia and tomato growers in the northern United States and Canada who receive Georgia transplants have been justifiably concerned about bacterial speck since a major outbreak first occurred in Georgia plant beds in 1978. Since that time, isolates of fluorescent pseudomonads from tomato transplants that were oxidase (11) and arginine dihydrolase (27) negative and tobacco hypersensitive positive (10) were usually assumed to be *P. tomato* as no similar bacterium had previously been associated with foliar lesions. Our results show that another green fluorescent pathogen very similar to *P. syringae* is also associated with foliar lesions on tomato and is capable of producing lesions after artificial inoculation. This new organism appears to be a weak pathogen compared with *P. tomato;* leaf infiltration with inoculum or wounds are required for symptom expression. In 1980, the disease occurred early in the season when the weather was cool to mild and humid, conditions that are also conducive to the development of bacterial speck (5,19).

Morphological and cultural characteristics and many of the routine physiological tests were of little value in distinquishing *P. tomato* from *P. syringae* or in assigning the 1979–1980 tomato isolates to one of the nomenspecies. However, several key tests were useful in showing that the new tomato isolates were very similar to the four known isolates of *P. syringae* from either peach or wheat. The unknown isolates, like the *P. syringae* isolates, utilized erythritol and DL-lactate, whereas the *P. tomato* isolates generally did not. However, unlike other workers (7,21), we were unable to use similarity in D(−)-tartrate utilization in assigning our isolates to *P. syringae*. The unknown isolates were also similar to *P. syringae* in producing syringomycin (4) and in the capacity to form ice nuclei (14). Pathogenicity tests on peach, and especially cowpea, also suggested similarity to *P. syringae*. Both are diagnostic hosts of *P. syringae,* and symptoms produced by the unknown tomato isolates were similar to those described by other workers (17,18). Slight differences among isolates of the new bacterium in pathogenicity and physiological reactions suggest heterogeneity in the field population.

It is difficult to determine whether the foliar disease that we observed was the same as that reported by Volcani (28) and Wilkie and Dye (29) because of the brevity of their reports and incomplete descriptions of the causal organism. The brown lesions without halos that we observed in the field and on artifically inoculated plants are at least similar to those described by Wilkie and Dye (29) after their greenhouse inoculations.

Our finding that more than one green fluorescent pseudomonad may cause foliar lesions on tomato transplants appears similar to the report of Taylor and Dye (26) on organisms associated with bacterial blight of pea in New Zealand. *P. syringae (P. syringae* pv. *syringae*) and *P. viridiflava,* as well as the usual *P. pisi* (*P. syringae* pv. *pisi*), were frequently isolated. *P. syringae* caused a disease indistinguishable from that caused by *P. pisi,* whereas *P. viridiflava* was considered to be a secondary invader. *P. viridiflava* has also been associated with lesions on tomato foliage (3), but unlike this organism, our tomato isolates did not rot potato and often produced levan (1,13).

The presence of a bacterial foliar disease on southern-grown tomato transplants is a threat to later production of fruit and is grounds for rejection of transplants by state regulatory authorities. Accurate disease diagnosis is essential before certification decisions can be made. Our results show that isolates of green fluorescent pseudomonads from transplants with characteristics (oxidase- and arginine-negative and tobacco hypersensitive positive) common to pathogenic types (7,13,16,21) must be further tested to determine their true identity. Our results and reports of others (7,16) show that the capacity of isolates to use erythritol and DL-lactate are acceptable tests for distinguishing *P. tomato* from *P. syringae*. Tests for syringomycin production and ice-nucleating capacity appear to be other laboratory tests of value in separating these organisms. Pathogenicity tests on tomato could be a final confirmation because symptoms caused by the two organisms are distinctly different.

LITERATURE CITED

1. Billing, E. 1970. *Pseudomonas viridiflava* (Burkholder, 1930; Clara 1934). J. Appl. Bacteriol. 33:492-500.
2. Bryan, M. K. 1933. Bacterial speck of tomatoes. Phytopathology 23:897-904.
3. Burki, T. 1973. *Pseudomonas viridiflava* (Burkholder) Dowson and other fluorescent plant pathogenic *Pseudomonas* associated with lesions on tomato leaves. Int. Soc. Plant Pathol.: Phytobacteriology section, working group on *Pseudomonas syringae*, 2–6 April 1973. Angers, France. 118 pp. (mimeographed).
4. Devay, J. E., Lukezic, F. L., Sinden, S. L., English, H., and Coplin, D. L. 1968. A biocide produced by pathogenic isolates of *Pseudomonas syringae* and its possible role in the bacterial canker disease of peach trees. Phytopathology 58:95-101.
5. Goode, M. J., and Sasser, M. 1980. Prevention—the key to controlling bacterial spot and bacterial speck of tomato. Plant Dis. 64:831-834.
6. Hildebrand, D. C., and Schroth, M. N. 1964. β-glucosidase activity in phytopathogenic bacteria. Appl. Microbiol. 12:487-491.
7. Hildebrand, D. C., and Schroth, M. N. 1971. Identification of the fluorescent pseudomonads. Pages 281–287 in: H. P. M. Geesteranus, ed. Proc. Third Int. Conf. on Plant Pathogenic Bacteria. University of Toronto Press, Canada. 365 pp.
8. Hugh, R., and Leifson, E. 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various Gram-negative bacteria. J. Bacteriol. 66:24-26.
9. King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. 44:301-307.
10. Klement, Z., Farkas, G. L., and Lovrekovich, L. 1964. Hypersensitive reaction induced by phytopathogenic bacteria in the tobacco leaf. Phytopathology 54:474-477.
11. Kovacs, N. 1956. Identification of *Pseudomonas pyocyanea* by the oxidase reaction. Nature 178:703.
12. Lai, M., and Hass, B. 1973. Reaction of cowpea seedlings to phytopathogenic bacteria. Phytopathology 63:1099-1103.
13. Lelliott, R. A., Billing, E., and Hayward, A. C. 1966. A determinative scheme for the fluorescent plant pathogenic pseudomonads. J. Appl. Bacteriol. 29:470-489.
14. Lindow, S. E., Arny, D. C., and Upper, C. D. 1978. *Erwinia herbicola:* A bacterial ice nucleus active in increasing frost injury to corn. Phytopathology 68:523-527.
15. Manual of Microbiological Methods. 1957. Committee on Bacteriological Technic, Society of American Bacteriologists, eds. McGraw-Hill, New York. 315 pp.
16. Misaghi, I., and Grogan, R. G. 1969. Nutritional and biochemical comparisons of plant-pathogenic and saprophytic fluorescent pseudomonads. Phytopathology 59:1436-1450.
17. Otta, J. D., and English, H. 1971. Serology and pathology of *Pseudomonas syringae*. Phytopathology 61:443-452.
18. Perlasca, G. 1960. Relationships among isolates of *Pseudomonas syringae* pathogenic on stone fruit trees. Phytopathology 50:889-899.
19. Pohronezny, K., Volin, R. B., and Stall, R. E. 1979. An outbreak of bacterial speck on fresh-market tomatoes in south Florida. Plant Dis. Rep. 63:13-17.
20. Rhodes, M. E. 1958. The cytology of *Pseudomonas* spp. as revealed by the silver-plating staining method. J. Gen. Microbiol. 18:639-648.

21. Sands, D. C., Schroth, M. N., and Hildebrand, D. C. 1970. Taxonomy of phytopathogenic pseudomonads. J. Bacteriol. 101:9-23.
22. Shaffer, W. H., Jr. 1975. Procedures for the identification of bacterial plant pathogens. Pages 68–73 in: R. N. Goodman, ed. Proc. First Workshop on Phytobacteriology. Univ. of Missouri, Columbia. 73 pp.
23. Sierra, G. 1957. A simple method for the detection of lipolytic activity of micro-organisms and some observations on the influence of the contact between cells and fatty substrates. Antonie van Leeuwenhoek J. Microbiol. Serol. 23:15-22.
24. Sneath, P. H. A. 1956. Cultural and biochemical characteristics of the genus *Chromobacterium*. J. Gen. Microbiol. 15:70–98.
25. Stanier, R. Y., Palleroni, N. J., and Doudoroff, M. 1966. The aerobic pseudomonads: A taxonomic study. J. Gen. Microbiol. 43:159-271.
26. Taylor, J. D., and Dye, D. W. 1972. A survey of the organisms associated with bacterial blight of peas. N. Z. J. Agric. Res. 15:432-440.
27. Thornley, M. J. 1960. The differentiation of *Pseudomonas* from other Gram-negative bacteria on the basis of arginine metabolism. J. Appl. Bacteriol. 23:37-52.
28. Volcani, Z. 1954. An onion and tomato disease caused by a variety of *Pseudomonas syringae*. Bull. Res. Coun. Israel 4:171-175.
29. Wilkie, J. P., and Dye, D. W. 1974. *Pseudomonas tomato* in New Zealand. N. Z. J. Agric. Res. 17:131-135.