The Use of Pathovar-Indicative Bacteriophages for Rapidly Detecting *Pseudomonas syringae* pv. *tomato* in Tomato Leaf and Fruit Lesions

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ABSTRACT

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A suspension of macerated tomato leaf or fruit lesions was added to molten nutrient broth yeast extract (NBY) soft agar and the soft agar was poured over the surface of an NBY agar plate. Once the agar had hardened, the routine test dilutions (RTDs) of four *Pseudomonas syringae* pv. *tomato*-indicative (PT phages) were spotted onto the agar surface. Phage lysis zones indicating the presence of *P. syringae* pv. *tomato* appeared within 18–36 hr. A minimum of about 8×10^4 colony-forming units (cfu) of *P. syringae* pv. *tomato* per lesion was required for clear zones to appear. Lesions on leaves inoculated with strain DCT6D1 contained an average of 2.9×10^6 cfu 8 days after inoculation. *P. syringae* pv. *tomato* DCT6D1 was detected by the PT phage test 43 days after inoculation. Thirty-eight tomato fields and five fresh-produce markets were surveyed for the bacterial speck pathogen by the PT phage method of detection and by the isolation-physiological characterization (IPC) method. Identical results were obtained with both methods for all 34 leaf samples and 68 of 100 fruit samples. Thirty of the fruit samples were positive by the IPC method only. Thus, the PT phage test was a better detection method for leaf lesions than for fruit lesions. All 77 isolates of *P. syringae* pv. *tomato* obtained from the tomato fields were sensitive to the PT phages and pathogenic for tomato. The predominant bacteria in fruit lesions negative for *P. syringae* pv. *tomato* were identified as saprophytic fluorescent pseudomonads.

Additional key words: *Lycopersicon esculentum, Pseudomonas fluorescens, Pseudomonas marginalis, Pseudomonas putida, Pseudomonas viridiflava.*

Pseudomonas syringae pv. *tomato* (Okabe) Alstatt is the causal agent of bacterial speck, a leaf-spotting disease of tomatoes. Bacterial speck lesions, especially on fruit, vary in size, shape, and color and sometimes can be confused with lesions produced by other tomato pathogens. Identification of the pathogen as *P. syringae* pv. *tomato* is an essential, but time-consuming, process; the pathogen must first be isolated and purified and then be characterized and tested for pathogenicity. Although *P. syringae* pv. *tomato* has been the subject of several serological studies (4,9,11,12), no highly specific serological detection methods are presently available.

Phages specific for a particular species or subspecific group of bacteria have been used to help identify both animal and plant pathogens (3,5,7,10). Four phages highly specific for the bacterial speck pathogen, PT1, PT18, PT20, and PT32, recently were isolated from the plant debris and soil present in tomato fields (8). When used in combination with carefully selected physiological tests, PT phage sensitivity patterns offer a reliable means of identifying *P. syringae* pv. *tomato* without having to test for pathogenicity. Other bacterial pathogens of tomato, such as *Xanthomonas campestris* pv. *vesicatoria* and *Corynebacterium michiganense*, as well as several saprophytic and phytopathogenic fluorescent pseudomonad strains, are not sensitive to these phages. Phage tests are rapid; phage lysis zones usually become visible within 18–24 hr of inoculation. The objective of the present study was to develop a rapid presumptive test for *P. syringae* pv. *tomato* based on sensitivity to the PT phages. The microorganisms present in tomato leaf and fruit lesions were tested directly, without preliminary isolation and purification, for sensitivity to the phages. The identity of the microorganisms was then confirmed by means of physiological and pathogenicity tests.

MATERIALS AND METHODS

Bacteria and phage. Unless otherwise stated, the bacterial strains used in this study (Table 1) were grown on nutrient broth-yeast extract (NBY) agar (18) at 25 C for 48 hr. *P. syringae* pv. *tomato* phages PT1, PT18, PT20, and PT32 were propagated and maintained as previously described (8). Phage stocks were diluted to their routine test dilution (RTD) in 10 mM potassium phosphate buffer (pH 7.2) containing 1 mM magnesium sulfate (PM). RTD is the dilution that just gives confluent lysis of the propagating host strain (1). The RTDs were shaken with chloroform (5%, final concentration) and stored at 4 C for up to 2 wk.

Optimal bacterial concentration for detection of PT phage lysis zones on NBY agar. Exponentially growing NBY broth cultures of *P. syringae* pv. *tomato* AV80 ($1.7 \pm 0.07 \times 10^8$ colony-forming units (cfu) per milliliter) and DCT6D1 ($1.6 \pm 0.7 \times 10^8$ cfu/ml) were diluted in PM buffer (10^{-1}, 2×10^{-2}, 10^{-2}, 5×10^{-3}, and 10^{-3}); 0.1 ml

TABLE 1. Origin of bacterial strains used in the development of a rapid detection method for *Pseudomonas syringae* pv. *tomato*

Strain	Host	Origin
P. syringae pv. *tomato*		
AV80	tomato	Vidaver, Nebraska
AV80 Sm^r	tomato	Cuppels, Nebraska
DCT6D1	tomato	Cuppels, Ontario
DCT6D1 Sm^r	tomato	Cuppels, Ontario
P. syringae pv. *syringae*		
NCPPB2748	tomato	Dye, New Zealand
NCPPB2749	tomato	Dye, Australia
P. syringae pv. *glycinea*		
B3	soybean	Gnanamanickam, Ontario
Xanthomonas campestris pv. *vesicatoria*		
3D4	tomato	Bonn, Ontario
T5-5	tomato	Bonn, Ontario
Corynebacterium michiganense		
C801	tomato	Bonn, Ontario

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of each dilution was added to 2.5 ml of molten NBY soft (0.7%) agar. The soft agar was poured over the surface of NBY agar plates. Once the agar had set, the RTDs of the four *P. syringae* pv. *tomato*-indicative phages were spotted onto the agar surface (15 μl/drop). After 18–48 hr of incubation at 25 C, the plates were examined for zones of lysis. The number of colony-forming units per milliliter for each culture was determined by the spread plate method (14).

Effect of PT phage-insensitive bacteria in *P. syringae* pv. *tomato* lawns on the formation of PT phage lysis zones. Exponentially growing cells of *P. syringae* pv. *tomato* AV80 were mixed with exponentially growing cells of PT phage-insensitive bacteria in the following ratios: 3×10^6 to 7.5×10^5 cfu (4:1); 3×10^6 to 1.5×10^6 cfu (2:1); 3×10^6 to 3×10^6 cfu (1:1); and 3×10^6 to 6×10^6 cfu (1:2). The mixture was added to 2.5 ml of molten NBY soft agar and the soft agar was poured over the surface of NBY agar plates. Drops of phage were placed on the agar and the plates were incubated as previously described.

PT phage test for plant tissue samples. All fruit and leaf specimens were washed thoroughly with tap water and blotted dry with paper towels. Tissue samples (~3 mm^2) were excised from the tomato fruit or leaf, macerated in 0.5 ml PM buffer with a sterile glass rod, and incubated at 25 C for 15–30 min. A 0.1-ml sample of this suspension was added to 2.5 ml of molten NBY soft agar and the soft agar was poured over the surface of an NBY agar plate. The RTDs of the four phages indicative for *P. syringae* pv. *tomato* were spotted (15 μl/drop) onto the agar surface and the plates were incubated at 25 C for 18–48 hr.

Determination of the number of bacteria in a bacterial speck lesion. Tomato plants (*Lycopersicon esculentum* Mill. 'Bonny Best') were inoculated with *P. syringae* pv. *tomato* DCT6D1 Sm^r and AV80 Sm^r (two plants per strain) as described previously (8). Eight days after inoculation, lesions were excised from the leaves and macerated in 0.5 ml of PM buffer. The suspension was incubated at 25 C for 15–30 min and then serially diluted in PM buffer. The number of colony-forming units per lesion was determined by the spread plate method (14) and using NBY-streptomycin (200 μg/ml) agar.

Effect of leaf lesion age on detection of *P. syringae* pv. *tomato*. Four to 6-wk-old tomato plants (*Lycopersicon esculentum* Mill. 'Bonny Best') ~15 cm high were inoculated with *P. syringae* pv. *tomato* AV80 and DCT6D1 (eight plants per strain) as previously described (8). At 8, 10, and 15 days after inoculation, two lesions (~3 mm^2) were excised from leaf tissue, macerated in 0.5 ml of PM buffer, and incubated at 25 C for 15 min. These suspensions were tested for the presence of PT phage-sensitive bacteria by the above procedure. At 19, 22, 25, 29, 32, 36, 39, 43, and 64 days after inoculation, two tissue samples, one containing two lesions and the other three (~5 mm^2), also were tested for the presence of PT phage-sensitive bacteria.

Procedure for detection of *P. syringae* pv. *tomato* on field tomato plants. Two tissue suspensions, one containing two lesions and the other three, were prepared from each field sample and tested for the presence of PT phage-sensitive bacteria as described. The tissue suspensions then were streaked on King's B medium (13). Fluorescent bacteria appearing after 48 hr of incubation at 25 C were purified and tested for utilization of erythritol, DL-lactate, and D(−)tartrate as carbon sources, oxidase and pectolytic enzyme production, and phage sensitivity (8). All fluorescent strains were tested for pathogenicity on 4- to 6-wk-old tomato plants.

Additional characterization tests for fluorescent pseudomonads. All fluorescent bacteria that were isolated from, but not pathogenic on tomato, were tested for levan and arginine dihydrolase production, potato soft rot, and a hypersensitivity reaction on tobacco leaves (15). The hypersensitivity reaction was tested with 30-day-old tobacco plants (*Nicotiana tabacum* L. 'White Gold').

These bacteria were streaked on King's medium (13) and yeast extract-dextrose-calcium carbonate (YDC) (18) agar, incubated 48 hr at 25 C, and then examined for phenazine pigment production.

Gelatinase production was determined by flooding inoculated plates of nutrient agar plus 0.7% gelatin with 15% mercuric chloride in 20% (v/v) hydrochloric acid. The presence of gelatinase was indicated by zones of clearing around bacterial growth. The bacteria were tested for ability to use trehalose as a carbon source. The minimal medium and assay procedures have been described (2,8).

Ability to grow at 41 C was determined by inoculating 10 ml of prewarmed NBY broth with ~10^7 cfu of the test strain and incubating the culture at 41 C on a rotary shaker for 36 hr. A control culture was incubated at 25 C for 36 hr.

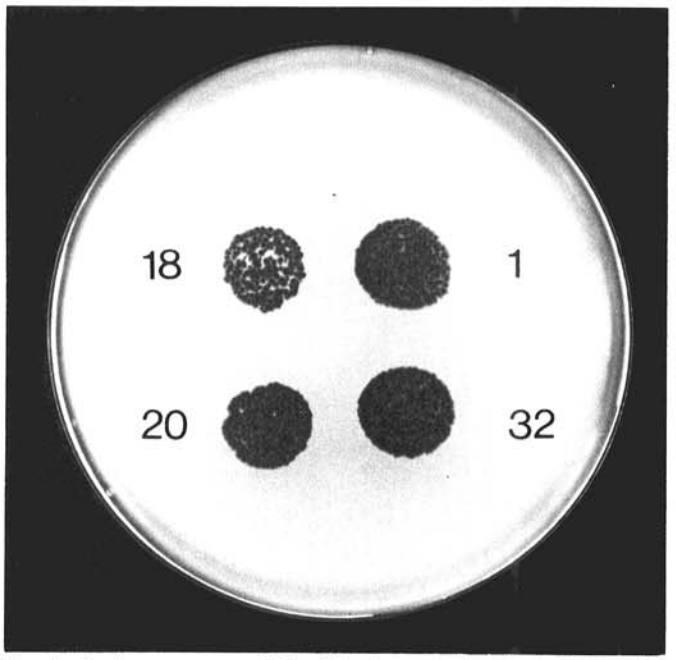

Fig. 1. Lysis zones caused by PT phage (which is indicative for *Pseudomonas syringae* pv. *tomato*) on NBY agar inoculated with a suspension of two tomato bacterial speck leaf lesions. Following inoculation with 0.1 ml of the 0.5-ml leaf suspension, the agar was spotted (15 μl/drop) with phages PT1, PT18, PT20, and PT32 at routine test dilutions (RTD) and then incubated at 25 C for 18 hr.

RESULTS

Formation of PT phage lysis zones on NBY agar inoculated with the bacterial speck pathogen or bacterial speck lesions. After 18 hr of incubation, PT phages PT1, PT18, PT20, and PT32 formed distinct lysis zones on NBY agar inoculated with $\geq1.6\times10^5$ cfu of *P. syringae* pv. *tomato* DCT6D1 and on NBY agar inoculated with $\geq1.65\times10^5$ cfu of *P. syringae* pv. *tomato* AV80. After 36 hr of incubation, clear zones appeared on NBY agar inoculated with $\geq3.0\times10^4$ cfu of strain AV80 and on NBY agar inoculated with $\geq3.2\times10^4$ cfu of strain DCT6D1. At lower concentrations the lawns were too sparse to give clearly defined zones, even with an extended incubation time of 48 hr. Therefore, when two bacterial speck leaf lesions are macerated in 0.5 ml of PM buffer and 0.1 ml of this suspension is used to form a lawn, the minimum number of bacteria that must be present in one lesion for phage lysis zones to appear is 75,000 to 80,000. Eight-day-old lesions on greenhouse-grown tomato plants inoculated with AV80 Sm^r had an average $6.0\pm0.2\times10^6$ cfu/lesion; lesions on plants inoculated with DCT6D1 Sm^r had an average $2.9\pm1.6\times10^6$ cfu/lesion. Thus, young bacterial speck lesions contain adequate numbers of bacteria for visualization of phage lysis zones (Fig. 1).

Visible phage lysis zones did not appear on plates prepared from AV80-induced lesions over 25 days old; the number of viable cells in these lesions had dropped below the detectable limit, 75,000. Visible phage lysis zones appeared on lawns prepared from 43-day-old, but not 64-day-old DCT6D1-induced lesions.

Plant material from sources other than bacterial speck lesions also was tested for the presence of PT phage-sensitive bacteria. Lawns prepared from tomato leaf tissue that had not been sprayed with *P. syringae* pv. *tomato* or from tissue that had been sprayed

but that was not part of a lesion did not develop phage lysis zones. *X. campestris* pv. *vesicatoria* T5-5 lesions, tomato leaf tissue sprayed with *Corynebacterium michiganense* C801, or leaf tissue exhibiting the necrotic flecks characteristic of infection caused by *P. syringae* pv. *syringae* likewise gave negative responses to the PT phage test. Lesions formed by other pathovars of *P. syringae* also were tested. Lysis zones did not form on lawns prepared from soybean lesions (*P. syringae* pv. *glycinae* B3); phage PT18 alone formed a zone on lawns prepared from apple lesions (*P. syringae* pv. *papulans*) but only at $\geq 10 \times$ RTD.

Effect of PT phage-insensitive bacteria in *P. syringae* pv. *tomato* lawns on the formation of PT phage lysis zones. *Xanthomonas campestris* pv. *vesicatoria* 3D4, causal agent of bacterial spot of tomato, and *P. syringae* pv. *syringae* NCPPB2748 and NCPPB-2749 are examples of PT phage-insensitive microorganisms that might be present on or in bacterial speck lesions isolated from field tomato plants. The presence of *P. syringae* pv. *syringae* NCPPB2748 or 2749 in a *P. syringae* pv. *tomato* AV80 lawn did not affect the appearance of phage lysis zones when *P. syringae* pv. *tomato* AV80 outnumbered *P. syringae* pv. *syringae* 4:1. However, when the ratio of AV80 to *P. syringae* pv. *syringae* was 2:1 or 1:1, the zones became turbid. When strains 2748 and 2749 outnumbered strain AV80 by 2:1, the zones disappeared or were barely visible. *X. campestris* pv. *vesicatoria* 3D4 has a much slower growth rate (doubling time = 2.1 hr) than strain AV80 (doubling time = 1.4 hr) and consequently had no effect on the appearance of phage lysis zones, even when it outnumbered strain AV80 by 2:1.

Detection of *P. syringae* pv. *tomato* in leaf and fruit spots on field tomato plants. In July 1983, tomato plant leaves with lesions resembling bacterial speck were collected from 22 southern Ontario tomato fields. Three Georgia transplant seedlings also were included in the survey. Lesion preparations from 18 of the 25 leaf sample sources gave positive reactions to the PT phage test. The predominant fluorescent bacteria isolated from these lesions were strains of *P. syringae* pv. *tomato*. All strains were oxidase positive, able to use D(−)tartrate but not erythritol and DL-lactate as carbon sources, and able to degrade pectate at pH 4.9, but not pH 8.3. All strains also produced typical bacterial speck symptoms on tomato plants. Lesion preparations from the remaining seven tomato plant samples gave a negative response to the PT phage test and did not contain fluorescent bacteria with the physiological and pathological characteristics of *P. syringae* pv. *tomato*. Thus, the PT phage test agreed 100% with physiological and pathological tests in the identification of fluorescent bacteria isolated from tomato leaf lesions.

In August 1983, 100 tomato fruit samples were collected from 16 tomato fields in southern Ontario and five fresh-produce markets in London, Ontario. The bacterial speck pathogen was detected on fruit from 12 of the tomato fields, but on none of the market tomatoes. The PT phage method of detection agreed with the isolation-physiological characterization (IPC) method for 68 of the 100 samples taken. *P. syringae* pv. *tomato* was detected by the IPC method but not by the PT phage method in 30 of the remaining samples. Conversely, the pathogen was detected by the PT phage test but not by the IPC method in just two of the samples. Thus, in contrast to the results obtained with tomato leaf lesions, the IPC method provided a better means of detecting *P. syringae* pv. *tomato* in fruit lesions than did the PT phage test. All 43 strains of *P. syringae* pv. *tomato* isolated from tomato fruit were pathogenic to tomato and sensitive to the four PT phages. Two leaf-lesion samples were collected from one of the fields that had been surveyed for the presence of *P. syringae* pv. *tomato* on fruit. Although the pathogen had not been detected on the fruit, it was present in leaf lesions, as determined by both detection methods.

Characterization of fluorescent pseudomonads isolated from tomato fruit lesions. When the PT phage test was negative, fruit-lesion suspensions often contained large numbers of fluorescent pseudomonads and a fast-growing microorganism with large, fluidal, translucent, light orange colonies. Because of their prevalence in tomato fruit spots and similarity to the bacterial speck pathogen on King's B medium, 33 strains of these fluorescent pseudomonads were characterized further. They were insensitive to the PT phages and nonpathogenic for tomato plants. Based on the dichotomous key of Stolp and Gadkari (17), the determinative scheme of Lelliott et al (15), and the nutritional and biochemical study of Misaghi and Grogan (16), they were tentatively identified as *P. fluorescens* (13 strains), *P. putida* (six strains), *P. viridiflava* (five strains), *P. syringae* pv. *syringae* (one strain), and *P. marginalis* (one strain). Although closely related to *P. fluorescens* or *P. putida*, the remaining seven strains could not be classified.

DISCUSSION

In an earlier study, the majority (82–89%, depending upon the phage strain) of strains of *P. syringae* pv. *tomato* tested were sensitive to the four PT phages PT1, PT18, PT20, and PT32 (8). These phages infected not only Canadian strains of *P. syringae* pv. *tomato* but also U.S., Australian, New Zealand, Danish, Yugoslavian, and British strains. In the present study, all leaf lesions from which the pathogen was isolated gave a positive reaction to the PT phage test. None of the other fluorescent pseudomonads or bacterial plant pathogens isolated from Ontario tomato fields was sensitive to these phages. Demonstration of PT phage sensitivity for the bacteria present in tomato leaf lesions is a good presumptive test for *P. syringae* pv. *tomato*. However, since this method requires the presence of large numbers of bacteria, it would not be useful in screening symptomless seeds or seedlings for infestation by the pathogen.

Under greenhouse conditions, the number of cells of *P. syringae* pv. *tomato* in leaf lesions decreased with lesion age. In older lesions, numbers were less than optimum for a positive response to the PT phage test. The rate of decrease appeared to be strain-dependent. Presumably bacterial numbers also decline as lesions age in field material and therefore, if possible, only the younger leaves of field tomato plants should be used in the PT phage test. Positive test results have been obtained, however, with older field tomato leaves collected late in the season (mid-August).

The presence of PT phage-insensitive bacteria in lawns of *P. syringae* pv. *tomato* had a pronounced effect on the appearance of phage lysis zones. This effect was dependent upon the relative growth rates and cell numbers of *P. syringae* pv. *tomato* and the phage-insensitive bacteria. PT phage-insensitive bacteria also may contribute to false-negative reactions in the PT phage test with field tomato samples. Pathogen resistance to the phage was not a factor in false-negative reactions since all strains of *P. syringae* pv. *tomato* detected by the IPC method were phage-sensitive in pure culture. Perhaps *P. syringae* pv. *tomato* is better able to compete with PT phage-insensitive bacteria in leaf lesions than in fruit lesions. Such a hypothesis might help explain why the PT phage test was more effective for leaf lesions than for fruit lesions.

The predominant PT phage-insensitive bacteria found in typical bacterial speck fruit lesions were of the same fluorescent pseudomonad species and pathovars of *P. syringae* pv. *syringae* as those that Burki (6) recovered from tomato leaf spots. Although Burki suggested that one of these bacteria, *P. viridiflava*, was the primary cause of atypical leaf spots, not one of the Ontario isolates was a tomato pathogen. The Ontario isolates probably were opportunistic saprophytes that appeared after bacterial speck lesions had been formed.

In summary, the phage sensitivity test is a simple and effective means of rapidly identifying a tomato leaf lesion as bacterial speck. Every bacterial speck leaf lesion collected in 1983 was sensitive to the PT phages. The test was not as effective with fruit lesion samples collected late in the season; such lesions often contained a preponderance of saprophytic fluorescent pseudomonads.

LITERATURE CITED

1. Adams, M. H. 1959. Bacteriophages. Interscience Publishers Inc., New York. 592 pp.
2. Ayers, S. H., Rupp, P., and Johnson, W. T. 1919. A study of the alkali-forming bacteria in milk. U.S. Dep. Agric. Bull. 782.
3. Billing, E., and Garrett, C. M. E. 1980. Phages in the identification of plant pathogenic bacteria. Pages 319-338 in: Microbial Classification

and Identification of Plant Pathogenic Bacteria. M. Goodfellow and R. G. Board, eds. Academic Press, Toronto. 408 pp.
4. Bosshard-Heer, E., and Vogelsanger, J. 1977. Überlebensfahigkeit von *Pseudomonas tomato* (Okabe) Alstatt in verschiedenen Boden. Phytopathol. Z. 90:193-202.
5. Brown, E. R., and Cherry, W. B. 1955. Specific identification of *Bacillus anthracis* by means of a variant bacteriophage. J. Infect. Dis. 96:34-39.
6. Burki, T. 1973. *Pseudomonas viridiflava* (Burkholder) Dowson and other fluorescent plant pathogenic pseudomonads associated with lesions on tomato leaves. Pages 26-34 in: ISPP Journées d'études sur les bacteries du groupe *Pseudomonas syringae*. 2–6 Avril 1973. INRA, Angers, France. 118 pp.
7. Cherry, W. B., Davis, B. R., Edwards, P. R., and Hogan, R. B. 1954. A simple procedure for the identification of the genus *Salmonella* by means of a specific bacteriophage. J. Lab. Clin. Med. 44:51-55.
8. Cuppels, D. A. 1983. Isolation and characterization of phages useful in the identification of *Pseudomonas syringae* pv. *tomato*. Phytopathology 73:1376-1381.
9. Fackrell, H. B., and Sinha, R. C. 1983. Serological analysis of *Pseudomonas syringae* pv. *tomato*. Phytopathology 73:178-181.
10. Hirsh, D., and Martin, L. D. 1983. Rapid detection of *Salmonella* spp. by using Felix-01 bacteriophage and high-performance liquid chromatography. Appl. Environ. Microbiol. 45:260-264.
11. Jones, J. B., Dawe, D. L., and McCarter, S. M. 1983. Separation of strains of *Pseudomonas syringae* pv. *tomato* into serovars by three serological methods. Phytopathology 73:573-576.
12. Jones, J. B., Gitaitis, R. D., and McCarter, S. M. 1983. Evaluation of indirect immunofluorescence and ice nucleation activity as rapid tests for identifying foliar diseases of tomato transplants incited by fluorescent pseudomonads. Plant Dis. 67:684-687.
13. King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. 44:301-307.
14. Koch, A. 1981. Growth measurement. Pages 179-207 in: Manual of Methods for General Bacteriology. P. Gerhardt, ed. American Society for Microbiology, Washington, DC. 524 pp.
15. 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.
16. Misaghi, I., and Grogan, R. G. 1969. Nutritional and biochemical comparisons of plant-pathogenic and saprophytic fluorescent pseudomonads. Phytopathology 59:1436-1450.
17. Stolp, H., and Gadkari, D. 1981. Nonpathogenic members of the genus *Pseudomonas*. Pages 719-741 in: The Prokaryotes. Vol. 1. M. P. Starr, H. Stolp, H. G. Truper, A. Balows, and H. G. Schlegel, eds. Springer-Verlag, New York. 1102 pp.
18. Vidaver, A. K. 1967. Synthetic and complex media for the rapid detection of fluorescence of phytopathogenic pseudomonads: Effect of the carbon source. Appl. Microbiol. 15:1523-1524.