Special Topics

Isolation and Characterization of Phages Useful for Identifying *Pseudomonas syringae* pv. *tomato*

D. A. Cuppels

Assistant plant pathologist, Research Centre, Agriculture Canada, University Sub Post Office, London, Ontario N6A 5B7.
Appreciation is expressed to C. Olivier for excellent technical assistance.
Accepted for publication 6 April 1983.

ABSTRACT

Cuppels, D. A. 1983. Isolation and characterization of phages useful for identifying *Pseudomonas syringae* pv. *tomato*. Phytopathology 73:1376-1381.

Sixteen phages were isolated from tomato field soil and plant debris with six *Pseudomonas syringae* pv. *tomato* strains as the propagating hosts. Fifty-five strains of *P. syringae* pv. *tomato* and 51 strains from other pathovars of *P. syringae* were tested for lytic responses to these phages. Phage sensitivity patterns did not change with time or after passage through tomato plants. Four of the phages, PT1, PT18, PT20, and PT32 had a high degree of specificity for *P. syringae* pv. *tomato*. PT32, for example, lysed 90% of the virulent *P. syringae* pv. *tomato* strains tested, but less than 4% of the strains from other pathovars of *P. syringae*. None of the isolates of *P. syringae* pv. *syringae* from tomato and less than half of the avirulent strains of *P. syringae* pv. *tomato* tested were lysed by these phages. Phages PT1 and PT18, which have isometric heads and long, striated, noncontractile tails, were members of morphological group B1. Phages PT20 and PT32, which have isometric heads and short, noncontractile tails, were members of morphological group C1. When used in combination with selected physiological characters (D(−) tartrate, erythritol, and DL-lactate utilization and polypectate degradation), phage sensitivity patterns clearly distinguished virulent strains of *P. syringae* pv. *tomato* from the other pathovars of *P. syringae* that were tested.

Additional key words: bacterial speck of tomatoes, *Lycopersicon esculentum*.

Since the first major disease outbreak in 1978, bacterial speck has become an increasingly serious problem for the Ontario tomato grower. The disease, characterized by small brown-to-black lesions on tomato fruits and leaves, is caused by *Pseudomonas tomato* (Okabe) Alstatt (*P. syringae* pv. *tomato*). This pathovar is difficult to distinguish morphologically and physiologically from other pathovars of the species, particularly *P. syringae* pv. *syringae*, which also has been isolated from necrotic lesions on tomato (14). Furthermore, nonpathogenic, oxidase-negative, fluorescent pseudomonads resembling phytopathogens such as *P. syringae* pv. *tomato* commonly occur on plant surfaces (6). Therefore, it is essential that rapid, accurate diagnostic tests be developed for the bacterial speck pathogen. At present, the most useful laboratory tests for differentiation are erythritol and DL-lactate utilization (14). The ultimate diagnostic test for a plant pathogen is that of pathogenicity for the suspected host plant. Unfortunately, pathogenicity tests are time-consuming and not always possible.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

Phages also can be used to distinguish closely-related bacterial plant pathogens (7). Phage sensitivity patterns, for example, have shown that the cherry and plum pathotypes of *P. syringae* pv. *morsprunorum* are two distinct homogeneous groups (8). Although *P. syringae* pv. *tomato* has been tested for sensitivity to phages from *P. syringae* pv. *syringae* (6), no phages specific to *P. syringae* pv. *tomato* have been reported.

The present study is concerned with the isolation and characterization of phages specific to *P. syringae* pv. *tomato*. These phages may be used in combination with selected physiological tests to identify the bacterial speck pathogen.

MATERIALS AND METHODS

Bacterial strains. A total of 55 strains of *P. syringae* pv. *tomato*, 10 *P. syringae* pv. *papulans*, 34 *P. syringae* pv. *syringae*, one *P. syringae* pv. *lachrymans*, two *P. syringae* pv. *glycinea*, two *P. syringae* pv. *phaseolicola*, one *P. viridiflava*, one *P. syringae* pv. *antirrhini*, one *P. syringae* pv. *tabaci*, and two *P. marginalis* were used in this study. The strains of *P. syringae* pv. *tomato* and 12 strains of *P. syringae* pv. *syringae* were isolated from infected tomato plants from several different tomato-growing regions of the world, including Canada, the United States, Australia, New Zealand, and Europe.

Media and culture methods. Unless otherwise stated, all bacterial cultures were grown on nutrient broth-yeast extract medium (NBY) (24) at 25 C for 48 hr.

Phage isolation and maintenance. Thirty-seven phages were isolated from samples of soil and plant material from beneath tomato plants infected with *P. syringae* pv. *tomato*. The tomato plants were collected from seven different locations in southern Ontario: Tilbury, Picton, Simcoe, Ridgetown, Trenton, Talbotville, and Wellington. *P. syringae* pv. *tomato* AV80, SM78-1, PST6, DCT6D1, 167, and 804 were used as the propagating strains. A 70-g sample of soil and decaying tomato plant tissue was added to 50 ml of a log phase NBY culture of the propagating strain and the mixture was incubated for 48 hr at 25 C on a rotary shaker (175 rpm). The culture was filtered through a Whatman No. 1 filter paper and the filtrate was centrifuged at 8,000 rpm for 15 min. The supernatant was shaken with chloroform (5%, final concentration), allowed to settle 1–2 hr at 22 C, serially diluted, and spotted onto lawns of the same propagating strain. Single plaques were removed from the soft agar and the phage were purified by two more passages through the propagating strain. High-titer (10^9–10^{12} plaque forming units [pfu]/ml) phage stocks were prepared as previously described by Billing (5) and stored at 4 C for up to 8 wk. Phage stocks can also be stored at −75 C for at least 6 mo without a significant loss of titer.

Detection of lysogeny in the propagating strains. The procedure for propagating high titer phage stocks (5) was followed exactly except that no virus was added to the propagating strains of *P. syringae* pv. *tomato* (DCT6D1, AV80, SM78-1, 167, 804, and PST6). The resulting preparations were diluted and assayed for plaques on five *P. syringae* pv. *syringae* strains, seven *P. syringae* pv. *papulans* strains, and 11 *P. syringae* pv. *tomato* strains. The assay was done in triplicate for each propagating strain.

Purification of phage. Phage were purified by cesium chloride (CsCl) block density gradients followed by CsCl equilibrium gradients (10). Purified phage stocks were stored in λdil (10 mM tris-HCl and 10 mM $MgSO_4 \cdot 7H_2O$, pH 7.5) plus 25% glycerol at −75 C.

Phage sensitivity tests. Phage stocks were diluted in 10 mM potassium phosphate buffer (pH 7.2) containing 1 mM magnesium sulfate (PM) to their routine test dilution (RTD). The RTD is the dilution which just gives confluent lysis of the propagating host strain (2). Bacteria from 24-hr-old NBY agar cultures were suspended in PM buffer. This suspension was used to prepare bacterial lawns with NBY soft agar (0.7% agar) (10^7 colony-forming units [cfu] per lawn). These lawns were spotted (15 μl per drop) with the phage RTDs and incubated at 25 C for 24 hr. Turbid confluent zones, clear confluent zones, and zones with individual plaques were recorded as positive reactions; no lysis and extremely faint zones of lysis were recorded as negative reactions.

Electron microscopy. A drop of purified phage ($\sim 10^{11}$ pfu/ml) was placed on a carbon-backed copper grid for 2 min and then removed. The grid was washed with 0.1 M ammonium acetate and then stained with 0.6% phosphotungstate plus 150-μg/ml bacitracin for phages PT20 and PT32 and with 0.6% ammonium molybdate plus 150-μg/ml bacitracin for phages PT1 and PT18. Fifty particles of each phage strain were measured for head diameter and tail length.

Characterization of purified phages. Nucleic acid was extracted from purified phage by a previously described procedure (10). A 1- to 2-μg sample of the nucleic acid was then treated with either 3 μg of deoxyribonuclease (DNAse) I (Sigma) in the presence of 10 mM $MgSO_4 \cdot 7H_2O$ or 3 μg of ribonuclease (RNAse) A (Sigma Chemical Co., St. Louis, MO 63178) for 25 min at 32 C. The nucleic acid was subjected to electrophoresis on a 0.7% agarose gel in a tris-borate buffer as described previously (9). As a control, the nucleic acid was subjected to electrophoresis without prior nuclease treatment.

Phages PT1, PT18, PT20, and PT32 were tested for stability at 50, 60, 70, and 80 C. Small tubes containing 0.5 ml of phage suspension ($\sim 2 \times 10^6$ pfu/ml in λdil) were placed in a water bath at the designated temperature for 10 min and then rapidly chilled on ice. The phage suspension subsequently was assayed for pfus on the appropriate host strain.

Physiological test methods. All bacterial strains used in this study were tested for oxidase and fluorescent pigment production (15). Procedures for determining their ability to degrade polypectate at pH 4.9 and 8.3 also have been described (13). Utilization of D(−) tartrate, DL-lactate, erythritol, and homoserine as carbon sources was determined with the minimal medium of Ayers et al (3,20). Ayers' medium, supplemented with the desired carbon source, was spot-inoculated with 15-μl drops of a bacterial suspension (5×10^7 cfu/ml in PM buffer, pH 7.2) and incubated 4 days at 25 C.

Pathogenicity tests. All bacterial strains were tested for pathogenicity on 4- to 6-wk-old tomato plants (*Lycopersicon esculentum* Mill. 'Bonny Best') approximately 15 cm tall that had been preincubated in plastic bags at 25 C for 24 hr immediately prior to inoculation. The second set of true leaves was sprayed with a water suspension of carborundum (45-μm[320-grit]; Fisher Scientific Co., Pittsburgh, PA 15219), and then washed with distilled water. Bacterial test strains were grown on NBY agar overnight at 25 C. A distilled water suspension of the bacteria ($\sim 2 \times 10^5$ cfu/ml) then was sprayed on the carborundum-treated leaves. When the leaves had dried, the plants were covered with plastic bags and incubated for 24 hr at 25 C. The bags were removed and the plants were incubated another 6 days. Lesions that developed on the second set of true leaves were counted, leaf areas were measured with a portable Lambda area meter (Lambda Instruments Corp., Lincoln, NE 68504), and the number of lesions per square centimeter were calculated.

RESULTS

Six strains of *P. syringae* pv. *tomato* (804, 167, DCT6D1, PST6, SM78-1, and AV80) were used as enriching-propagating strains in the isolation of 37 phages from Ontario tomato field soil and infected tomato plant debris. A preliminary survey of the sensitivity of 38 strains of *P. syringae* pv. *tomato*, 22 *P. syringae* pv. *syringae*, and three *P. syringae* pv. *papulans* to the RTDs of these phages indicated that only 16 of the 37 were distinct phage strains. The remaining 21 phage strains were discarded. The origins, propagating strains of *P. syringae* pv. *tomato* strains, RTDs, and plaque morphologies of these 16 strains are listed in Table 1.

Thirty-three strains of *P. syringae* were used as indicators to test the six propagating strains (Table 1) for the presence of temperate phage. All propagating strains, except PST6, released phage without induction. The highest temperate phage titers (pfu/ml) were obtained with indicator strain *P. syringae* pv. *syringae* NCPPB281: DCT6D1, $1.9 \pm 0.9 \times 10^5$; 804, $5.5 \pm 0.8 \times 10^2$; SM78-1, $4.3 \pm 2.5 \times 10^5$; 167, $8.8 \pm 3.7 \times 10^3$; and AV80, $3.5 \pm 2.1 \times$

10^5. Since PT phage stocks, particularly those prepared with DCT6D1, SM78-1, and AV80, were kept at high titers ($\geq 10^{10}$ pfu/ml), the presence of temperate phage did not interfere with the phage sensitivity tests (Fig. 1).

The RTDs of the 16 phages of *P. syringae* pv. *tomato* (PT) were spotted on lawns of the following: 55 strains of *P. syringae* pv. *tomato*, 22 strains of *P. syringae* pv. *syringae*, 12 strains of *P. syringae* pv. *syringae* isolated from tomato plants, and 10 strains of *P. syringae* pv. *papulans*. As shown in the histogram (Fig. 1), >70% of the strains of *P. syringae* pv. *tomato* were lysed by 13 of the PT phages. *P. syringae* pv. *papulans*, an apple pathogen, also was lysed by several PT phages. In addition to the three pathovars shown in Fig. 1, the PT phages were tested against *P. marginalis* (two strains), *P. viridiflava* (one strain), and five other pathovars of *P. syringae*: *P. syringae* pv. *tabaci* (one strain), *P. syringae* pv. *lachrymans* (one strain), *P. syringae* pv. *antirrhini* (one strain), *P. syringae* pv. *glycinea* (two strains), and *P. syringae* pv. *phaseolicola* (two strains). No zones of lysis or plaques were formed on lawns of any of these strains except *P. antirrhini*. PT10, PT11, PT15, PT19, PT23, and PT34 formed confluent zones of lysis on this strain.

TABLE 1. Source, RTD[a], and plaque morphology of *Pseudomonas syringae* pv. *tomato* phages

Phage	Source[b]	Enrichment host strain		RTD	Plaque morphology[c]
PT 1	Tilbury	SM78-1	(Georgia)	3.0×10^5	<1 mm, C, H
2	Picton	SM78-1	(Georgia)	1.3×10^5	<1 mm, C, H
8	Simcoe	167	(Ontario)	1.0×10^5	2–1 mm, C, H
10	Ridgetown	804	(Ohio)	1.6×10^4	5–4 mm, C, H
11	Tilbury	AV80	(Nebraska)	8.0×10^3	5–4 mm, C, H
14	Ridgetown	AV80	(Nebraska)	4.8×10^5	<1 mm, C
15	Trenton	AV80	(Nebraska)	2.6×10^4	5–4 mm, C, H
16	Picton	AV80	(Nebraska)	4.0×10^5	<1 mm, C
17	Simcoe	DCT6D1	(Ontario)	5.0×10^4	5–4 mm, C, H
18	Simcoe	DCT6D1	(Ontario)	4.3×10^5	2–1 mm, C, H
19	Ridgetown	DCT6D1	(Ontario)	4.0×10^4	2–1 mm, T
20	Talbotville	DCT6D1	(Ontario)	1.5×10^5	2–1 mm, T
23	Tilbury	AV80	(Nebraska)	2.5×10^4	5–4 mm, C, H
27	Wellington	PST6	(Ontario)	4.6×10^4	<1 mm, T
32	Tilbury	804	(Ohio)	7.5×10^4	2–1 mm, C, H
34	Tilbury	AV80	(Nebraska)	3.0×10^4	6–5 mm, C, H

[a] RTD = routine test dilution. The lowest concentration (plaque-forming units per milliliter) of phage required to produce confluent lysis on a lawn of the propagating strain.
[b] Phage were isolated from tomato field soil and plant debris collected at various locations throughout Ontario, Canada.
[c] C = clear, H = halo, and T = turbid.

Phage PT27, which represented 14 of the original 37 phage strains, was the most common PT phage, but it also had one of the more restricted host ranges. Only 33% of the strains of *P. syringae* pv. *tomato* were lysed by PT27. Four of the phages, PT1, PT18, PT20, and PT32, had a pronounced, although not absolute, specificity for the strains of *P. syringae* pv. *tomato* (Fig. 1; Table 2). Only two strains from the other pathovars of *P. syringae* were lysed by PT20 and PT32: *P. syringae* pv. *syringae* NCPPB281 (lilac) and GN2 (corn). Likewise, PT1 lysed only three strains from the other pathovars (one *P. syringae* pv. *syringae* from lilac and two *P. syringae* pv. *papulans*) and PT18 only five strains from other pathovars (*P. syringae* pv. *syringae* from lilac and soybean and three strains of *P. syringae* pv. *papulans*). PT1, PT18, PT20, and PT32 did not form plaques or zones of lysis on lawns of the strains of *P. syringae* pv. *syringae* from tomato.

The morphology of phages PT1, PT18, PT20, and PT32 that attack *P. syringae* pv. *tomato* was studied by electron microscopy of negatively-stained preparations. PT1 and PT18, which had isometric heads and long noncontractile tails, belonged to Ackermann's viral morphological group B1 (1) (Fig. 2). The head and tail of PT1 measured 60 ± 3.9 nm and 227 ± 7.7 nm, respectively; those of PT18 measured 60 ± 3.4 nm and 226 ± 9.2 nm, respectively. The tails of PT1 and PT18 are markedly striated with a basal plate and four fibers at the tip. PT1 and PT18 had almost identical patterns of thermal stability (Fig. 3). Complete

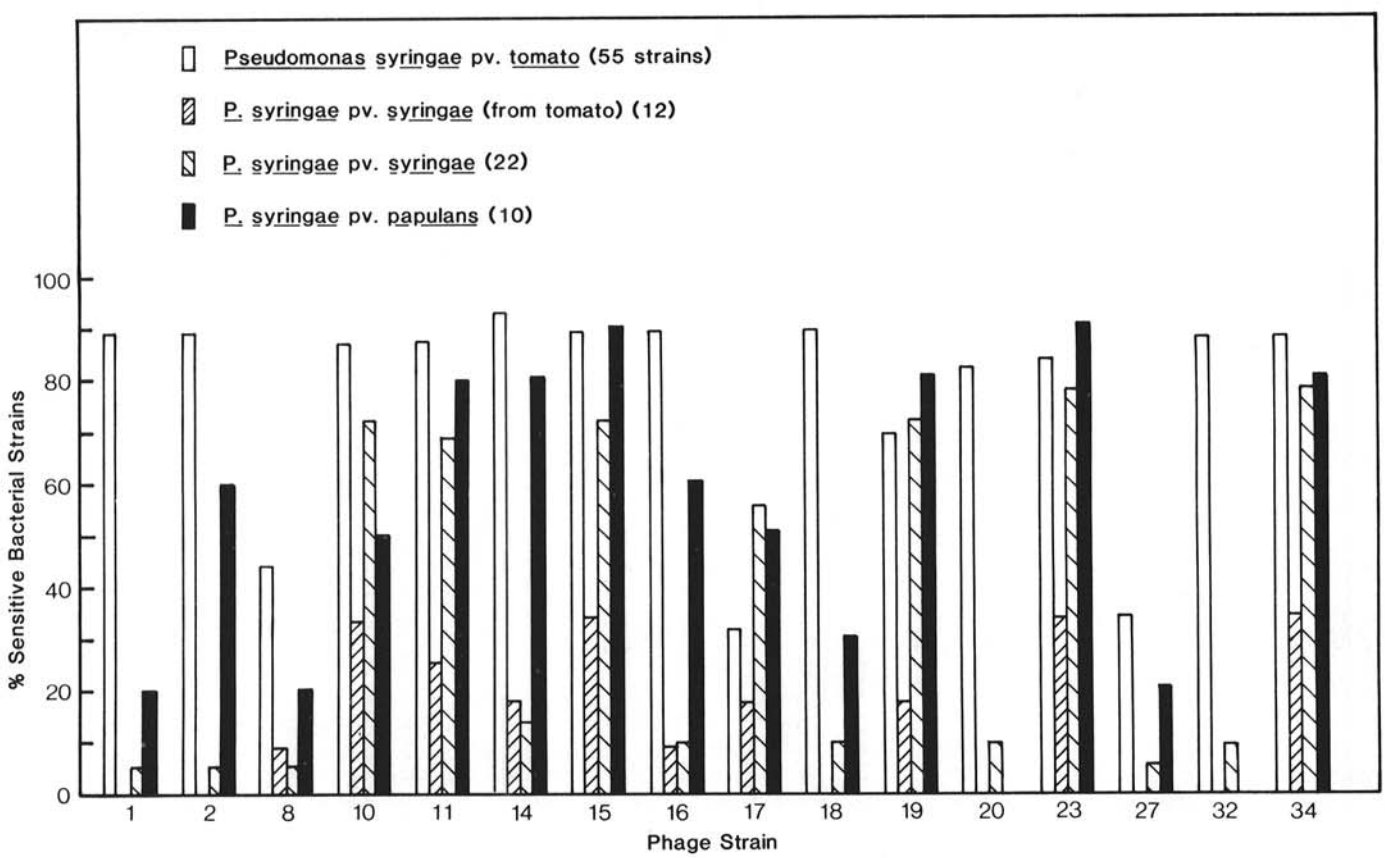

Fig. 1. Sensitivity of selected pathovars of *Pseudomonas syringae* to lysis by *Pseudomonas syringae* pv. *tomato* phages.

inactivation occurred at 60 C.

PT20 and PT32 had isometric heads with short, thin, noncontractile tails and thus were members of morphological group C1 (Fig. 2). The heads were 57 ± 3.6 nm and 61 ± 2.8 nm, respectively; the tails, partially obscured by negative staining, were difficult to measure, but were ~27–29 nm long. PT20 was more heat-stable than PT32 at 50 C, but both were totally inactivated at 60 C (Fig. 3).

Nucleic acid extracted from purified particles of PT1, PT18, PT20, and PT32 was treated with either DNAse I or RNAse A. The products of the reactions, along with untreated controls, were subjected to electrophoresis on a 0.7% agarose gel. Only the

TABLE 2. Differentiation of *Pseudomonas syringae* pv. *tomato* from other closely-related *P. syringae* pathovars

	Number of positive strains from *P. syringae*:									
Diagnostic test	pv. *tomato* (55)[a]		pv. *antirrhini* (1)[a]		pv. *syringae* (from tomato) (12)[a]		pv. *syringae* (22)[a]		pv. *papulans* (10)[a]	
Utilization of:										
Homoserine	0		1[b]	(100)[c]	0		0		0	
Erythritol	0		0		9	(75)	19	(86)	10	(100)
DL-lactate	0		0		12	(100)	18	(82)	10	(100)
D(−) tartrate	55	(100)	1	(100)	9	(75)	5	(23)	1	(10)
Pectate degradation at:										
pH 4.9	54	(98)	1	(100)	0		2	(9)	8	(80)
pH 8.3	0		0		0		0		0	
Sensitivity to phage:										
PT1	49	(89)	0		0		1	(5)	2	(20)
PT18	49	(89)	0		0		2	(9)	3	(30)
PT20	45	(82)	0		0		2	(9)	0	
PT32	48	(87)	0		0		2	(9)	0	
Bacterial speck symptoms on tomato	51	(93)	0		0		0		0	

[a] Number of strains tested.
[b] s = slightly positive.
[c] Percent positive.

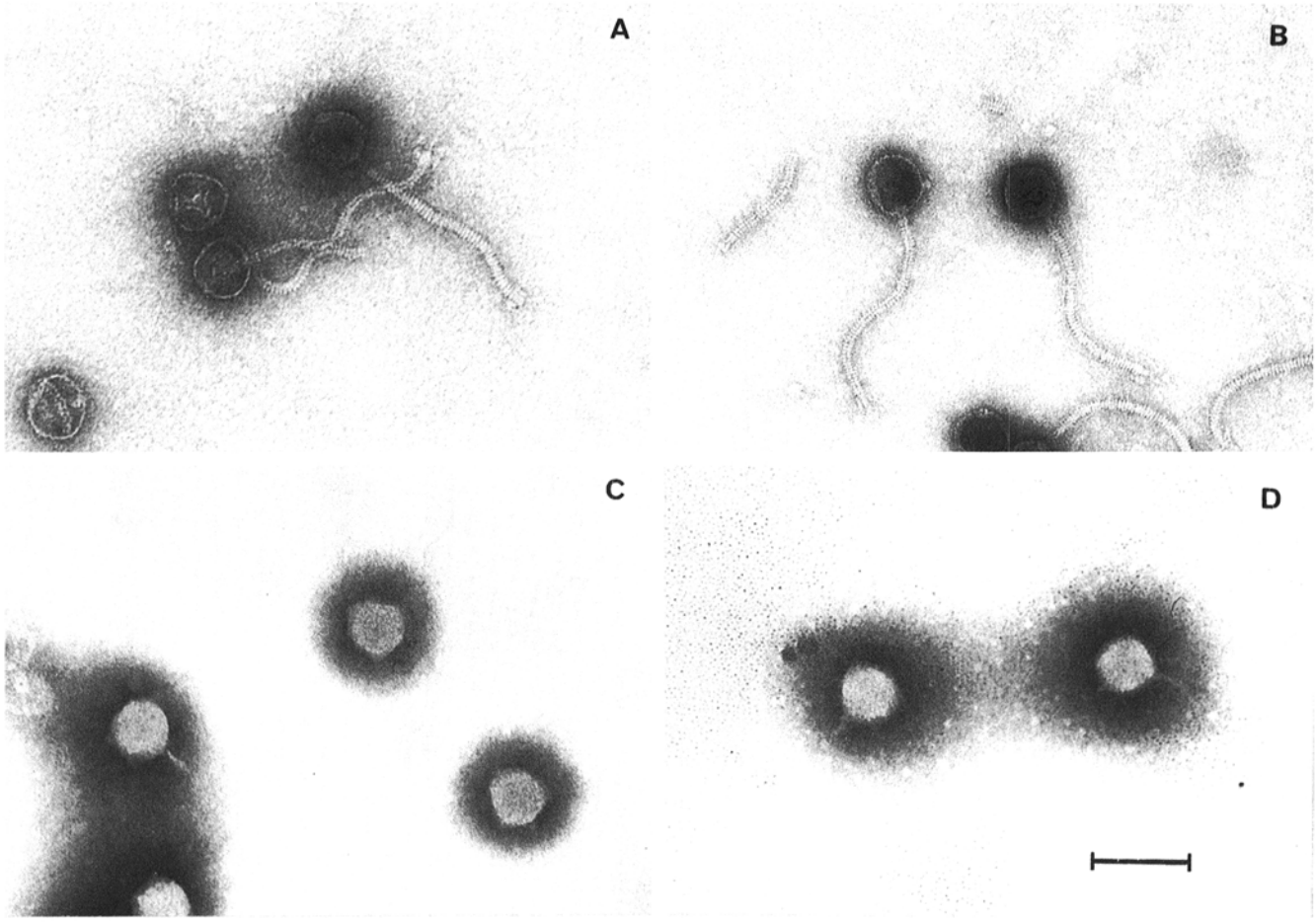

Fig. 2. Electron micrographs of negatively-stained *Pseudomonas syringae* pv. *tomato* phages **A,** PT1, **B,** PT18, **C,** PT20, and **D,** PT32. Bar = 100 nm.

controls and the RNAse-treated samples gave ethidium bromide-stained bands. Thus, the nucleic acid of all four phage strains was deoxyribonucleic acid (DNA). Since all four DNAs could be removed from 70% ethanol by being wound onto a glass rod, they were assumed to be of high molecular weight and double-stranded.

PT1 and PT18 were very similar, differing mainly in host range.

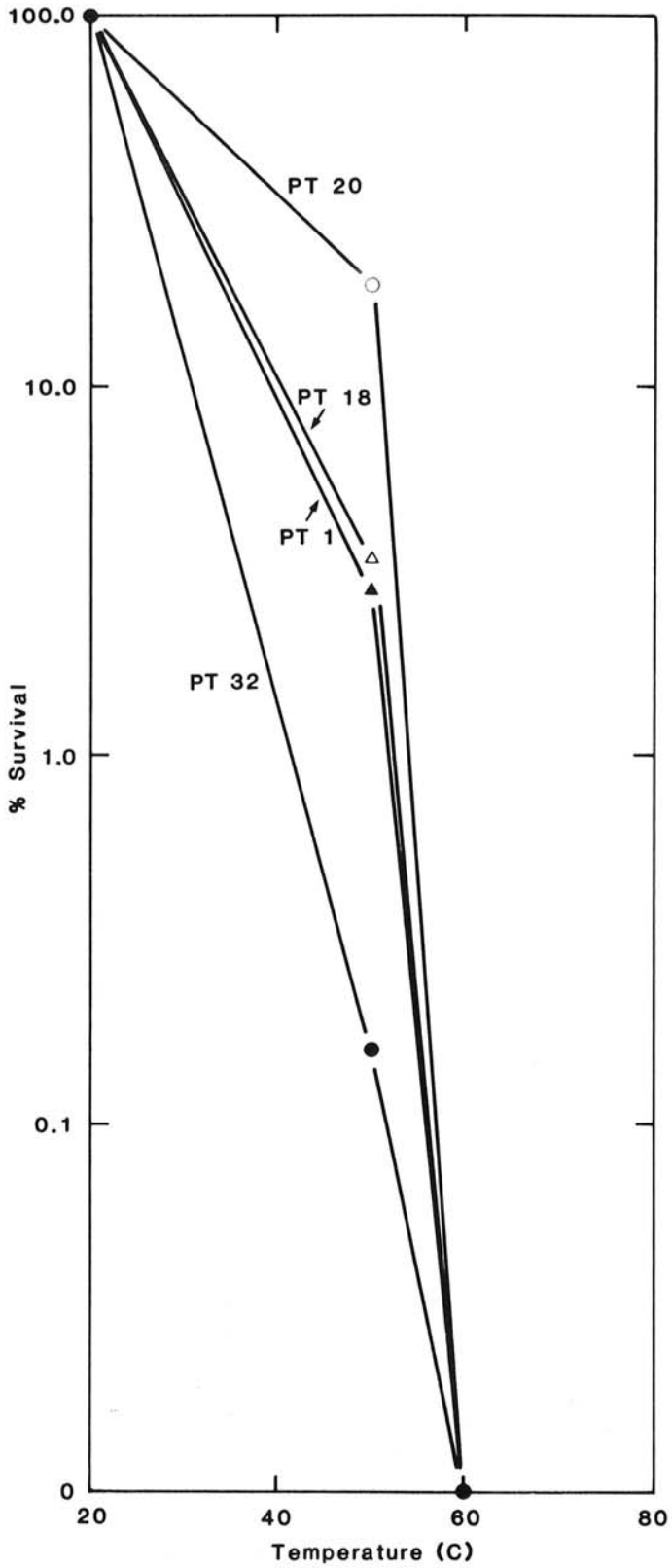

Fig. 3. Thermal stability of *Pseudomonas syringae* pv. *tomato* phages. Phage suspensions were incubated at the selected temperature for 10 min and then rapidly chilled on ice.

When they were propagated in two different bacterial strains, their host sensitivity patterns differed by three bacterial strains. However, when they were propagated in the same bacterial strain, PST6, their sensitivity patterns differed by only one bacterial strain. In contrast, PT20 and PT32, when propagated in the same bacterial strain, 804, continued to have a host range difference of four bacterial strains. Whether PT18 and PT1 are different isolates of the same virus or two distinct viruses will require further characterization.

Strains of *P. syringae* pv. *tomato* have been included in several taxonomic studies of the phytopathogenic pseudomonads (6,13,14,18,19,22,25). Based on these studies, the physiological tests listed in Table 2 appeared to be among the best for differentiating *P. syringae* pv. *tomato* from related pathovars of *P. syringae*. Every strain of *P. syringae* tested (Table 2) was oxidase-negative and fluorescent. The majority of strains of *P. syringae* pv. *syringae*, all strains of *P. syringae* pv. *papulans*, but no strains of *P. syringae* pv. *tomato* were able to use DL-lactate and erythritol as carbon sources.

All strains of *P. syringae* pv. *tomato*, with one exception, were able to use D(−) tartrate and degrade polypectate at pH 4.9. Pectate degradation at pH 4.9 was a good test for differentiating *P. syringae* pv. *tomato* from *P. syringae* pv. *syringae*, but not from *P. syringae* pv. *papulans*. Conversely, utilization of D(−) tartrate separated strains of *P. syringae* pv. *tomato* from *P. syringae* pv. *papulans*, but not from *P. syringae* pv. *syringae*. Several representative strains of *P. syringae* pv. *tomato*, *P. syringae* pv. *syringae*, and *P. syringae* pv. *papulans* also were tested for production of glycoside hydrolases (12). No enzymes unique to *P. syringae* pv. *tomato* were found (*unpublished*).

Phage sensitivity tests showed as high a specificity for *P. syringae* pv. *tomato* as did the DL-lactate and erythritol utilization tests (Table 2). Three of the four avirulent strains of *P. syringae* pv. *tomato* tested were not lysed by PT1, PT18, and PT20 and two of the four were not lysed by PT32. If these avirulent strains were excluded from the survey, even higher percentages of the strains of *P. syringae* pv. *tomato* were sensitive to PT1, PT18, PT20, and PT32: 96, 96, 88, and 94%, respectively. Only one pathogenic strain of *P. syringae* pv. *tomato* was resistant to all four phages.

If phage sensitivity tests are to be used in bacterial identification or typing schemes, it is essential that the sensitivity patterns be reproducible. Thirty bacterial strains were retested for sensitivity to the 16 PT strains 1–2 mo after the initial survey had been performed. New RTDs were prepared from the phage stocks stored at −75 C. Changes occurred in only 3% of the 480 phage-bacteria combinations that were examined. The stability of the phage sensitivity patterns of two pathogenic strains of *P. syringae* pv. *tomato*, 804 Str^r and PST6 Str^r, after passage through the host plant also was determined. No changes occurred in the phage pattern for either strain.

DISCUSSION

PT1, PT18, PT20, and PT32 were the first phage strains to be isolated with a high degree of specificity for *P. syringae* pv. *tomato*. The most probable source of phages highly specific for a particular plant pathogen is the soil beneath infected host plants (7). Thus, it was not unexpected that other *P. syringae* phages isolated from soil, meat, and manure had no differential value for *P. syringae* pv. *tomato* (6). PT1, PT18, PT20, and PT32, although isolated from Ontario tomato fields, were able to infect *P. syringae* pv. *tomato* strains from all over the world, including Australia, New Zealand, Europe, and the U.S. Conversely, strains of *P. syringae* pv. *syringae* having the same habitat (tomato plant surfaces) as *P. syringae* pv. *tomato*, were not lysed by these phages.

Phage sensitivity tests can offer greater specificity in identifying bacteria than physiological or even serological tests (17,21,23). However, they rarely show an absolute specificity and thus cannot serve as the sole basis for identification or differentiation. Billing and Garrett (7) recommend that, for bacterial identification, more than one phage be used and that physiological tests be employed in conjunction with phage sensitivity patterns. Although several

taxonomic studies (6,13,14,18,19,22,25) have included *P. syringae* pv. *tomato*, relatively few strains of this pathogen have been characterized. Based on these studies as well as our own, DL-lactate, D(−) tartrate, and erythritol utilization combined with polypectate degradation at pH 4.9 were good diagnostic characters for differentiating *P. syringae* pv. *tomato* from *P. syringae* pv. *papulans* and *P. syringae* pv. *syringae*, which are two closely-related pathogens. When used in combination with phage (PT1, PT18, PT20, and PT32) sensitivity tests, they presented a rapid, reliable means of identifying the bacterial speck pathogen. Although more avirulent strains must be tested, phage sensitivity also might be a means of separating avirulent and virulent strains of *P. syringae* pv. *tomato*. All avirulent strains tested had reduced phage sensitivity; however, not all phage-resistant strains were avirulent (*unpublished*).

All phage sensitivity tests were performed at the RTDs of the phages since false-positive reactions caused by lethal adsorption, bacteriocins, or temperate phages in the propagating strains are possible if undiluted phage preparations are used. None of the propagating strains produced bacteriocins active on other strains of *P. syringae* pv. *tomato* (*unpublished*). Temperate phages, however, were present in 12 of 25 strains of *P. syringae* pv. *tomato* that were tested (*unpublished*). Five of the six PT phage-propagating strains released temperate phages, but the concentration in the PT phage stocks was not high enough to interfere with phage sensitivity tests. Lysogeny is regarded as the norm, rather than the exception, for bacteria (11). Given the proper indicator strain and induction conditions, it is likely that most isolates of *P. syringae* pv. *tomato* would release temperate phage.

All phage strains, stored at −75 C in crude lysate or λdil plus 25% glycerol, maintained a high titer (10^{10}–10^{12} pfu/ml) with no evidence of plaque or host range mutation. Furthermore, the PT phage sensitivity patterns, like the plum and cherry phage types of *P. syringae* pv. *syringae* (8), were reproducible over time and after passage of the pathogen through the host plant. These results with phytopathogenic pseudomonads are in direct contrast to those obtained with the animal pathogen, *P. aeruginosa*, for which phage sensitivity patterns have been erratic (4).

LITERATURE CITED

1. Ackermann, H.-W. 1973. Tailed bacteriophages: Listing by morphological group. Pages 579–607 in: CRC Handbook of Microbiology. Vol. 1. A. I. Laskin and H. A. Lechevalier, eds. Chemical Rubber Company Press, Cleveland, OH. 924 pp.
2. Adams, M. H. 1969. Bacteriophages. Interscience Publishers, New York. 592 pp.
3. 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.
4. Bergan, T. 1978. Phage typing of *Pseudomonas aeruginosa*. Pages 169-199 in: Methods in Microbiology. Vol. 10. T. Bergan and J. Norris, eds. Academic Press, New York. 385 pp.
5. Billing, E. 1969. Isolation, growth, and preservation of bacteriophages. Pages 315-329 in: Methods in Microbiology. Vol. 3B. J. R. Norris and D. W. Ribbons, eds. Academic Press, New York. 369 pp.
6. Billing, E. 1970. Further studies on the phage sensitivity and the determination of phytopathogenic *Pseudomonas* spp. J. Appl. Bacteriol. 33:478-491.
7. Billing, E., and Garrett, C. M. 1980. Phages in the identification of plant pathogenic bacteria. Pages 319-338 in: Microbial Classification and Identification. M. Goodfellow and R. G. Board, eds. Academic Press, Toronto. 408 pp.
8. Crosse, J. E., and Garrett, C. M. 1963. Studies on the bacteriophagy of *Pseudomonas mors-prunorum*, *Ps. syringae* and related organisms. J. Appl. Bacteriol. 26:159-177.
9. Cuppels, D. A., Vidaver, A. K., and VanEtten, J. L. 1979. Resistance to bacteriophage $\phi 6$ by *Pseudomonas phaseolicola*. J. Gen. Virol. 44:493-504.
10. Davis, R. W., Botstein, D., and Roth, J. R. 1980. A manual for genetic engineering, advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 251 pp.
11. Hayes, W. 1968. The genetics of bacteria and their viruses. Blackwell Scientific Publications, London. 925 pp.
12. Hayward, A. C. 1977. Occurrence of glycoside hydrolases in plant pathogenic and related bacteria. J. Appl. Bacteriol. 43:407-411.
13. Hildebrand, D. C. 1971. Pectate and pectin gels for differentiation of *Pseudomonas* sp. and other bacterial plant pathogens. Phytopathology 61:1430-1436.
14. 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.
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. Lovrekovicn, L., Klement, Z., and Dowson, W. J. 1963. Serological investigation of *Pseudomonas syringae* and *Pseudomonas morsprunorum* strains. Phytopathol. Z. 47:19-24.
17. Misaghi, I., and Grogan, R. G. 1969. Nutritional and biochemical comparisons of plant-pathogenic and saprophytic fluorescent pseudomonads. Phytopathology 59:1436-1450.
18. Sands, D. C., Schroth, M. N., and Hildebrand, D. C. 1970. Taxonomy of phytopathogenic pseudomonads. J. Bacteriol. 101:9-23.
19. Sands, D. C., Schroth, M. N., and Hildebrand, D. C. 1980. *Pseudomonas*. Pages 36-44 in: Laboratory Guide for Identification of Plant Pathogenic Bacteria. N. W. Schaad, ed. The American Phytopathological Society, St. Paul, MN. 72 pp.
20. Sato, M., Takahashi, K., and Wakomoto, S. 1971. Properties of the causal bacterium of bacterial blight of Mulberry. *Pseudomonas mori* (Boyer et Lambert) Stevens, and its phages. Ann. Phytopathol. Soc. Jpn. 37:128-135.
21. Schroth, M., Hildebrand, D. C., and Starr, M. P. 1981. Phytopathogenic members of the genus *Pseudomonas*. Pages 701-718 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.
22. Taylor, J. D. 1972. Specificity of bacteriophages and antiserum for *Pseudomonas pisi*. N. Z. J. Agric. Res. 15:421-431.
23. 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.
24. Wilkie, J. P., and Dye, D. W. 1974. *Pseudomonas tomato* in New Zealand. N. Z. J. Agric. Res. 17:131-135.