Serology and Pathology of Pseudomonas syringae

J. D. Otta and Harley English

Former Graduate Research Assistant and Professor, respectively, Department of Plant Pathology, University of California, Davis 95616. Present address of senior author: Department of Plant Science, South Dakota State University, Brookings 57006.

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ABSTRACT

Approximately 450 isolates of bacteria classified as *Pseudomonas syringae,* from more than 30 host plants, were received from around the world. Each isolate was studied for oxidase reaction, pathogenicity to peach seedlings, serological reaction, and antibiotic production as measured by inhibition of *Geotrichum candidum*. Sixty isolates were oxidase-positive, nonpathogenic on peach seedlings, and serologically distinct from pathogenic isolates (and hence considered misclassified). Most oxidase-negative isolates were confirmed as *P. syringae* by their pathogenicity to peach seedlings, and it is concluded that peach seedling pathogenicity can be used to detect *P. syringae* isolates originating from many hosts of this organism. Peach seedling pathogenicity was not correlated with the ability to inhibit *G. candidum*. The pathogenic isolates were divided into 10 distinct serotypes based on the reaction of their heat-stable antigens in gel-diffusion tests. Host of origin was generally not correlated with serotype. Composite antigens containing sonicated cells of seven of the major *P. syringae* serotypes were used to obtain composite antisera containing antibodies against the heat-stable antigens of each serotype. Rough and smooth isolates possessed serologically identical heat-stable antigens, were not consistently different pathologically, and did not agglutinate in up to 10% saline solutions. Isolates received as *P. aptata* and *P. morsprunorum* were similar enough to *P. syringae* in all tests to be considered synonymous with it. Phytopathology 61:443-452.

Additional key words: composite antisera, oxidase test.

The use of serological techniques for the identification and classification of phytopathogenic pseudomonads has been reported (10, 21, 23, 27, 28). One of the most promising of these studies is that by Lucas & Grogan (23) in which nearly 300 isolates of *Pseudomonas lachrymans* were divided into three serotypes using the species-specific heat-stable O antigen of this organism.

Perlasca (31), using agglutination tests, found a considerable variation in antigenic makeup among isolates of *P. syringae*. Mushin et al. (28) reported similar variation within *P. syringae* using antisera prepared against O antigens of this species.

Gel-diffusion methods have shown that the heat-stable antigens of *P. syringae* and *P. morsprunorum* differ from those of several other phytopathogenic pseudomonads (20, 21, 23, 29). Lovrekovich et al. (21) studied 14 isolates of *P. syringae,* two of *P. syringae* f. sp. *populea,* one of *P. syringae* var. *capsici,* and six of *P. morsprunorum,* using gel-diffusion methods with antisera prepared against whole cells. They divided these isolates into eight serological groups on the basis of their heat-stable antigens. The *P. syringae* and *P. morsprunorum* isolates could not be separated serologically into species-specific groups, leading to the conclusion that *P. morsprunorum* should be considered a synonym of *P. syringae*. The same investigators (20, 21) reported that antigen preparations must be heated to permit observation of the specific heat-stable reactions of *P. tabaci* and *P. syringae* in gel-diffusion plates. Grogan et al. (14) and Lucas & Grogan (23), however, found that untreated or heated antigen preparations of *P. lachrymans* gave the same specific reaction.

Lucas & Grogan (23, 25) found that rough isolates of *P. lachrymans* agglutinated in normal saline and differed serologically from, but were equal in pathogenicity to, smooth isolates. All rough isolates studied belonged to the same serotype regardless of the serotype of the smooth parent isolate. These findings, excepting pathogenicity, are in general agreement with observations made on organisms such as *Salmonella* spp. (8, 15, 34). However, morphologically rough isolates of *Salmonella* may still contain smooth O antigens (15). Gaby (12) found that smooth and rough isolates of *P. aeruginosa* could be separated by agglutination tests with antisera prepared against formalized cells. When "O" antisera prepared by injecting alcohol-treated cells were used, however, the smooth and rough isolates of this bacterium were identical serologically.

The oxidase test has been reported to be useful for separating saprophytic pseudomonads from most phytopathogens (19, 26). Phytopathogenic pseudomonads, including *P. syringae,* are generally reported to be oxidase-negative (13, 19, 26). DeVay et al. (6) found that all pathogenic *P. syringae* isolates tested were oxidase-negative. They also reported "no exception to the finding that pathogenic isolates of *P. syringae* produce a wide spectrum antibiotic". Loss of pathogenicity was correlated with loss of the ability to produce the antibiotic. Baigent (2), however, reported nonantibiotic producers to be pathogenic.

The purpose of this study was to determine the extent of the serological variation of the heat-stable antigens of *P. syringae*. A preliminary report of our investigations in which seven serotypes were identified has been published (30).

MATERIALS AND METHODS.—*Source and maintenance of isolates.*—The isolates of *Pseudomonas syringae* used, representative of a wide host range and geographical distribution, were obtained from the International Collection of Phytopathogenic Bacteria (ICPB), Davis, Calif., the National Collection of Plant Pathogenic Bac-

teria (NCPPB), England, and various individuals around the world (29). Isolates of several other *Pseudomonas* spp. were obtained for comparison with the *P. syringae* cultures. Nearly 450 isolates received as *P. syringae* and 70 isolates of various other *Pseudomonas* spp. were examined serologically by gel-diffusion methods. All isolates were maintained in sterile distilled water at room temperature (16) and on slants of King's Medium B agar (17). All cultures were dilution-plated, and single-colony isolates were selected for each colony type present. Single colonies were obtained from subcultures 2 additional times by dilution-plating before being placed in stock culture. Isolates used for the preparation of antisera were single-celled, by the methods of DeVay and Schnathorst.

Physiological tests.—The oxidase reaction of all isolates was determined by Eddy's modification of Kovacs' oxidase test (18) as reported by Steel (33). Growth from 18- to 24-hr-old cultures on Medium B was used in all cases.

Isolates of *P. viridiflava,* selected isolates of *P. syringae,* and many isolates of oxidase-negative pseudomonads from cankers of French prune (*Prunus domestica* L.) were compared by the techniques of Lelliot et al. (19) for their ability to induce potato soft rot.

The ability of each isolate to inhibit *Geotrichum candidum* was studied by methods of DeVay et al. (6) with the following modification: Difco casamino acids (0.2%) were added to the potato-dextrose agar to give better fungus growth (32).

Selected isolates of *P. syringae, P. aptata,* and *P. morsprunorum* were tested for ability to liquefy gelatin at 24 C and 30 C as described by Misaghi & Grogan (26), and for utilization of tartrate as a sole carbon source, using the methods of Baker (3). Readings for both tests were taken at 3-day intervals for 30 days. Thirty min before readings were taken, all gelatin tubes were placed in ice water to ascertain their remaining gelling ability.

Pathogenicity tests.—The pathogenicity of each isolate was determined by placing drops of standardized bacterial suspensions (ca. 10^8 cells/ml) on the upper three internodes of 30-day-old peach seedlings, and wounding with a sharp pointed scalpel. Sterile water and suspensions of *P. fluorescens* served as controls. Plants were placed in a controlled environmental chamber (13 C, 12-hr day), and after 1 week, symptoms were observed with pathogenicity being noted as necrosis around the site of inoculation.

The pathogenicity of morphologically smooth and rough isolates of *P. syringae* was compared by inoculating 4-month-old peach seedlings with six smooth and four rough isolates. Three of each of the smooth and rough isolates were *G. candidum* inhibitors, while three smooth and one rough isolate were noninhibitory. The rough isolates used in this study were obtained as sectors in colonies of the smooth isolates. One hundred seedlings were divided into 10 groups of 10 each. Each isolate was inoculated into the third internode from the top of one seedling selected at random from each group. Canker length was read to the nearest mm 7 days after inoculation.

Six-month-old sugarbeets were inoculated with *P. syringae* and *P. aptata* suspensions (ca. 10^4, 10^6, and 10^8 cells/ml) by rubbing the inoculum over leaf surfaces previously dusted with Carborundum, by wounding an area beneath a drop of inoculum on the petioles with a teasing needle, or by atomizing the inoculum onto plants, one-half of which had been covered with plastic bags for the previous 24 hr. Sterile water checks were used for all inoculations. Plants were left in the greenhouse and observed daily.

Serological techniques.—Selected pathogenic cultures of *P. syringae, P. aptata,* and *P. morsprunorum* used for antisera production were grown for 24-36 hr at 24 C in Roux flasks containing 100 ml of a solid medium consisting of 2 g Difco casamino acids, 15 ml glycerol, 1.5 g K_2HPO_4, 0.4 g $MgSO_4$, and 20 g Difco Bacto agar/1 liter of distilled water. The bacterial cells were harvested by adding to each Roux flask 10 ml of 0.05 M PO_4 buffer, pH 7.2, in 0.85% saline solution. The resulting cell suspensions were centrifuged at 10,000 *g* for 10 min at 4 C. The pellet was resuspended in the buffer and centrifuged as before. This pellet was resuspended (1 g bacteria/10 ml buffer solution), and 35- to 40-ml portions were sonicated at max intensity in a Bronwill Biosonik, Model U-20, for 15 min or until the suspension became opalescent. The protein concentration, determined by the Lowry method (22), was adjusted to 2.5 mg/ml with buffered saline solution. Four ml of this suspension were emulsified with 4 ml of Freund's incomplete adjuvant and injected subcutaneously into the backs of 4-5-lb. New Zealand White virgin doe rabbits. This injection was repeated 2 times, at weekly intervals. Rabbits were bled by cardiac puncture 1 week after the final injection.

Seven isolates of *P. syringae,* one representing each of seven serotypes of this species, were grown in Roux flasks, harvested, and sonicated as described previously. Each of the seven suspensions was divided into three equal volumes. One portion of each suspension received no treatment. A second portion of each was autoclaved for 15 min at 15 lb./inch^2 and 121 C, while the third portion was heated for 1 hr at 100 C. The resulting suspensions were combined for each treatment to form three composite antigens (15). Each of these suspensions was used to inject two rabbits. Each rabbit received subcutaneous injections containing 5 ml of one of the antigen preparations emulsified with 5 ml of Freund's incomplete adjuvant. The injections were repeated 5 times at weekly intervals. The rabbits were bled as previously described.

Antigens suspected of being the O or somatic antigen were isolated using trichloroacetic acid (TCA) as described by Lucas & Grogan (24). The isolated antigens were tested in gel-diffusion plates with antisera prepared by injecting sonicated cells of the parent isolates. Antisera were prepared against these "purified" antigens by injecting rabbits subcutaneously with an emulsion of 4 ml of Freund's incomplete adjuvant and 4 ml of buffered saline solution containing 1.5 mg/ml of the antigen. Five injections were given at weekly intervals. The resulting antisera were of low titer, but usable for gel-diffusion techniques. Peach seedlings were injected

with the isolated antigens using concentrations of 1, 5, 10, and 20 mg/ml.

The methods of Lucas & Grogan (23) were used for titer determinations, gel-diffusion tests, and antigen preparation for gel-diffusion tests with the following modifications: Both rough and smooth isolates of *P. syringae* used for agglutination tests were suspended in 0.85% NaCl, and cell suspensions of both were heated at 100 C for 1 hr in some tests before being placed in antigen wells. After initial studies, all gel-diffusion plates were placed at 3 C, resulting in sharper bands than at room temperature.

RESULTS.—*Physiological and pathological tests for identifying P. syringae.*—Sixty of 448 isolates received as *P. syringae* were oxidase-positive; the remainder were oxidase-negative. The oxidase-positive reactions were our first indication that some of the supposed *P. syringae* isolates had been misidentified.

Pathogenicity tests with the same isolates on peach seedlings supported the results of the oxidase tests. None of the 60 oxidase-positive isolates were pathogenic. Of the oxidase-negative isolates, 351 were pathogenic, including 41 rough isolates, and 37 isolates were nonpathogenic. Pathogenic isolates produced necrotic cankers 5 to 100 mm long, while no necrosis was observed with water or *P. fluorescens*-inoculated checks. Ten of the nonpathogenic oxidase-negative isolates were later found to be serologically identical to the pathogenic *P. syringae* isolates. The remaining 27 nonpathogenic oxidase-negative isolates, along with the 60 oxidase-positive isolates, were shown to differ serologically from the pathogenic *P. syringae* isolates. Six of the oxidase-negative nonpathogenic isolates were tentatively identified as *P. viridiflava* because of their ability to cause a soft rot of potato slices identical to that produced by *P. viridiflava* isolates. All six of these isolates and the known *P. viridiflava* isolates produced a dark bluish-green water-soluble pigment on yeast dextrose calcium carbonate agar.

All eight isolates of *P. aptata*, 18 of 19 isolates of *P. morsprunorum*, and one of three isolates of *P. pisi* studied were pathogenic to peach seedlings and produced symptoms identical to those produced by *P. syringae*. Forty isolates representing 18 other nomenspecies of phytopathogenic pseudomonads were all nonpathogenic on peach seedlings (29).

Of 351 *P. syringae* and eight *P. aptata* isolates pathogenic on peach seedlings, 271 inhibited the growth of *G. candidum* and 88 did not (Table 1). Host of origin, but not serotype, appears correlated somewhat with ability to produce the antibiotic, as shown by the high percentage of inhibitory isolates originating from peach and sugarbeets and the low percentage originating from cherry and plum.

Rough isolates of *P. syringae* were obtained from smooth parent isolates (25) (Fig. 1), were received as the rough form, or, in two cases, were isolated by us from cankers on French prune trees. Two types of rough colonies were observed in addition to the smooth (S) colony types (Fig. 1). Three cultures consisting of

TABLE 1. Inhibition of *Goetrichum candidum* by isolates of *Pseudomonas syringae*

	No. inhibitory (+) and noninhibitory (−) isolates in serotype															
	Ia		Ib		II		IIIa,b,c		IVa&b		V		VI		Totals	
Host of origin	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
Apple	1						1								2	
Almond					24	5			1	1					25	6
Apricot	1				15	7			1						17	7
Bean	1				3		3	1	2				1	1	10	2
Cherry	1	3	13	10	13	5	6	1					1		34	19
Citrus							3	1							3	1
Crataegus spp.								2								2
Lilac							9	3							9	3
Pea							1	1							1	1
Peach			1	1	20		4		2				3		30	1
Pear	3	3	6	2	3	1	2	1							14	7
Pepper							1	1			1	1			2	2
Plum	4	1		3	2	2	2		1						9	6
Poplar							1	1							1	1
Prune	4	2			78	20	3								85	22
Raspberry					2										2	
Sugarbeet													8[a]		8	
Tomato			2												2	
Misc. hosts, one isolate each		1		1			4		2		1		2		9	2
Unknown			1	6			6						1		8	6
Totals	15	10	23	23	160	40	46	12	9	1	2	1	16	1	271	88

[a] All eight isolates received as *P. aptata*.

dry, wrinkled, rough colonies (R2) were observed, and in each case the host of origin was lilac. The morphology of this R2 type agrees with the description of the colony type in the first reported case of isolation of *P. syringae* from lilac in the United States (4). A second dull, rough colony type (R1) was commonly found mixed with smooth, glistening colony types in culture or was obtained as a sector of smooth colonies when smooth cultures were dilution-plated onto Medium B agar. All 41 rough isolates observed possessed heat-stable antigens serologically identical to those produced by one of the smooth isolates. Rough isolates obtained as sectors of smooth colonies possessed the same heat-stable antigen as did their smooth parent culture (Fig. 2). These rough isolates did not agglutinate in 0.2 to 10% saline solutions, and all but three were pathogenic on peach seedlings.

The relative pathogenicity of rough and smooth antibiotic and nonantibiotic-producing isolates was determined (Table 2). This experiment demonstrated that nonantibiotic-producing isolates, as indicated by *G. candidum* inhibition, not only are pathogenic, but may

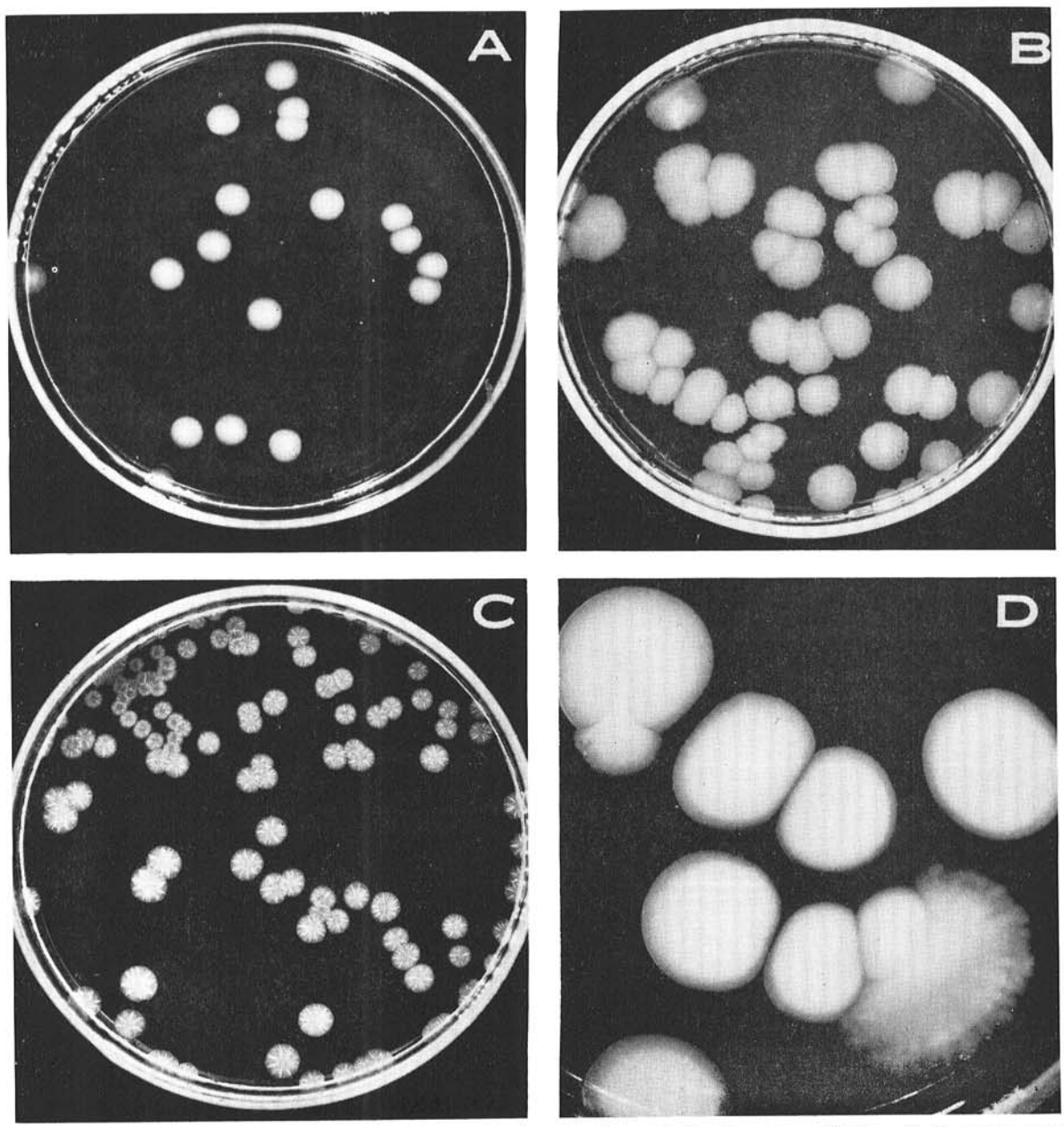

Fig. 1. Characteristic colony types of *Pseudomonas syringae* as observed after 1 week on Medium B. The three main colony types were **A)** a smooth glistening type; **B)** a dull, grainy rough-R1 type; and **C)** a dry, wrinkled, rough-R2 type. **D)** The R1 type was obtained readily from the smooth type.

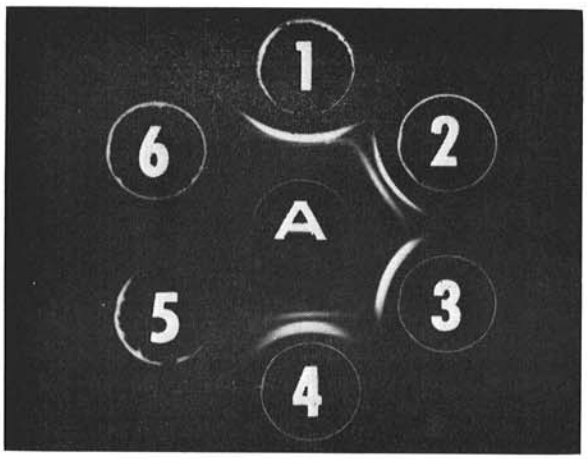

Fig. 2. Identity of the heat-stable antigens of rough and smooth isolates of *Pseudomonas syringae*. Center well (A) contains antisera to *P. syringae* serotype VI. Outer wells contain heavy bacterial cell suspensions of serotypes VI-S untreated (1), VI-S heated 1 hr at 100 C (2), VI-R untreated (3), VI-R heated 1 hr at 100 C (4), Ia-S untreated (5), and IIIa-S untreated (6).

be equally pathogenic to antibiotic-producing isolates under the conditions used. Also, rough isolates may be more, less, or equally pathogenic to smooth isolates.

Sugarbeets inoculated in petiole wounds with suspensions of either *P. syringae* or *P. aptata* (ca. 10^4, 10^6, and 10^8 cells/ml) developed general necrosis around the site of inoculation after 2 to 3 days, with necrotic streaks running up the petioles. There was no apparent difference between the symptoms produced by *P. syringae* and *P. aptata* (Fig. 3). Neither atomizing nor rubbing the cell suspensions onto leaves produced any visible symptoms.

Gelatin liquefication and tartrate utilization are two tests reported to be of value in separating *P. syringae* and *P. morsprunorum* (13). Table 3 gives results of studies of these two characters with selected isolates of these species.

TABLE 2. Pathogenicity on Lovell peach seedlings of rough, smooth, antibiotic-producing and nonantibiotic-producing isolates of *Pseudomonas syringae*

Isolate no.	*Geotrichum candidum* inhibition	Colony morphology[a]	Mean canker lengths (mm)[b]
SS-1S	—	S	8.0 a
857R	—	R	14.2 a
B-15+	+	S	22.1 ab
B-15—	—	S	25.2 ab
SS-1R	+	R	33.5 b
857S	—	S	42.4 bc
B-3-AS	+	S	47.2 bc
862aS	+	S	48.3 bc
862aR	+	R	53.2 c
B-3-AR	+	R	54.0 c

[a] S = smooth; R = rough.

[b] Data were analyzed statistically by Duncan's multiple range test. Statistical groupings ($P = .01$) for vertical comparison are shown by letters following the numbers. Values having a letter in common do not differ significantly.

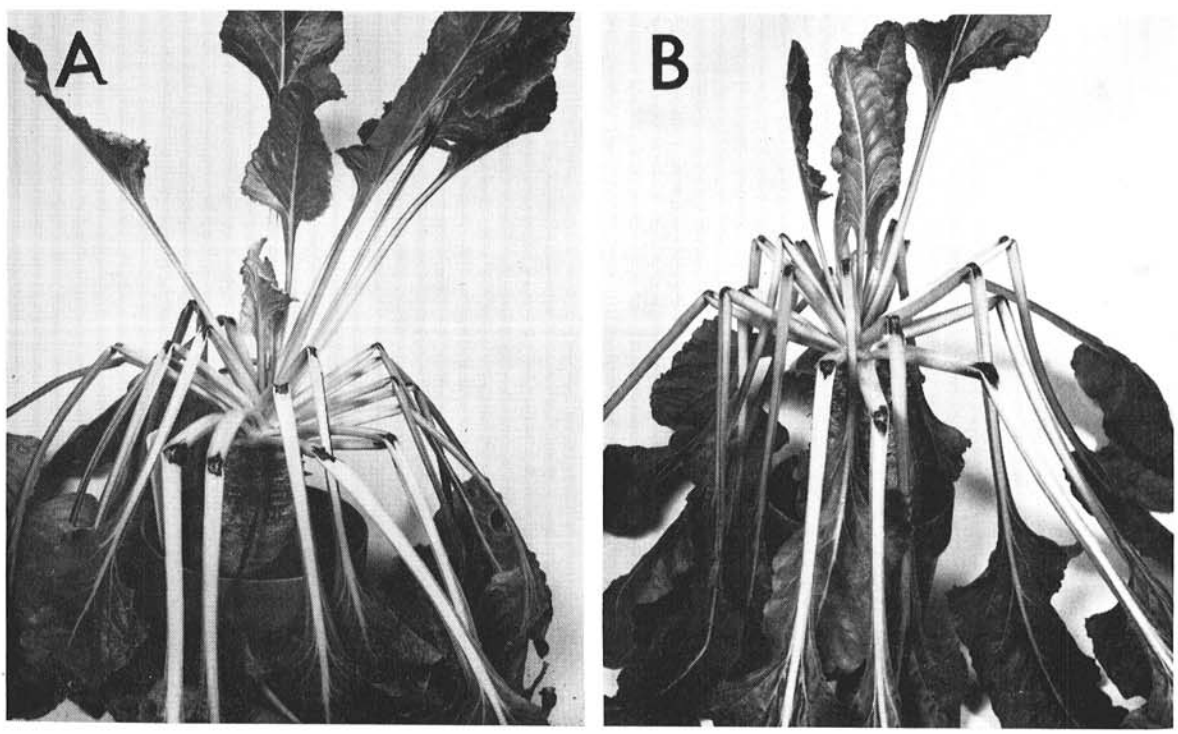

Fig. 3. Sugarbeets with similar symptoms produced by *Pseudomonas syringae* or *P. aptata*. Pictures taken 7 days after inoculation with **A)** *P. syringae;* or **B)** *P. aptata.*

TABLE 3. Comparison of selected isolates received as *Pseudomonas syringae* and *Pseudomonas morsprunorum* for serotype, gelatin liquefaction, and tartrate utilization

Serotype	Isolates received as	No. isolates	Gelatin liquefaction[a]		Tartrate utilization[a]	
			+	−	+	−
Ia	*P. syringae*	18	14	4	0	18
Ia	*P. morsprunorum*	1	1	0	0	1
Ib	*P. syringae*	12	12	0	1	11
Ib	*P. morsprunorum*	18	0	18	18	0
II	*P. syringae*	41	28	13	1[b]	40
III	*P. syringae*	32	27	5	0	32
IV	*P. syringae*	10	10	0	0	10
V	*P. syringae*	3	3	0	0	3
VI	*P. syringae*	9	9	0	0	9
Total		144	104	40	20	124

[a] Figures indicate number of isolates positive (+) or negative (−).
[b] The one tartrate (+) isolate was gelatin liquefaction (−).

Serology of P. syringae.—Ouchterlony gel-diffusion tests demonstrated that unheated antigen preparations of *P. syringae* isolates had several antigens in common, but one antigen that formed a line of precipitate close to the antigen well permitted a division of these isolates into subspecific serological groups (Fig. 4). The common bands tended to obscure the specific reaction with some of the antisera, but in most cases the specific band was easily discernible. Heating the antigens at 100 C for 1 hr practically eliminated the common antigens, but had a negligible effect on the heat-stable specific antigens. Ten distinct *P. syringae* serotypes were demonstrated by utilizing these specific heat-stable antigens, and were designated serotypes Ia, Ib, II, IIIa, IIIb, IIIc, IVa, IVb, V, and VI (29). There were no cross-reactions of the heat-stable antigens in reciprocal gel-diffusion tests with six of the serotypes (Fig. 4), but cross-reactions noted by spur formations were observed with the heat-stable antigens of some of the other serotypes (Fig. 5, Table 4).

The host of origin of the *P. syringae* isolates was generally not correlated with serotype whenever a fairly large sampling of isolates was studied from a given host (Table 5). Exceptions were lilac isolates, all of which belonged to serotype IIIa. Almond, apricot, and prune isolates were also somewhat uniform serologically, though this uniformity may be misleading because most of the isolates from these hosts were isolated by us from a limited geographical area in California. It is worth noting that each of the serotypes is composed of isolates from more than one host.

A comparison was made of our serological groupings and those of Lovrekovich et al. (21) with 12 isolates of *P. syringae* and *P. morsprunorum,* which were common to both investigations. The serological groupings of both investigations and results of our oxidase and peach pathogenicity test with these isolates are presented in Table 6. None of the five isolates that were nonpathogenic on peach belonged to any of the serotypes representative of 351 isolates which we considered valid *P. syringae.*

Composite antisera.—Inoculation of rabbits with composite antigens composed of isolates representative of seven major *P. syringae* serotypes resulted in individual antisera that reacted in a specific manner with all the *P. syringae* serotypes (Fig. 6). The best composite antisera were obtained when the composite antigens were heated at 100 C for 1 hr before injection.

Comparison of serology of P. syringae with other Pseudomonas nomenspecies.—Many of the 21 *Pseudomonas* nomenspecies studied in gel-diffusion tests did not produce the specific line of precipitate with any of the 10 *P. syringae* serotypes. However, all isolates of

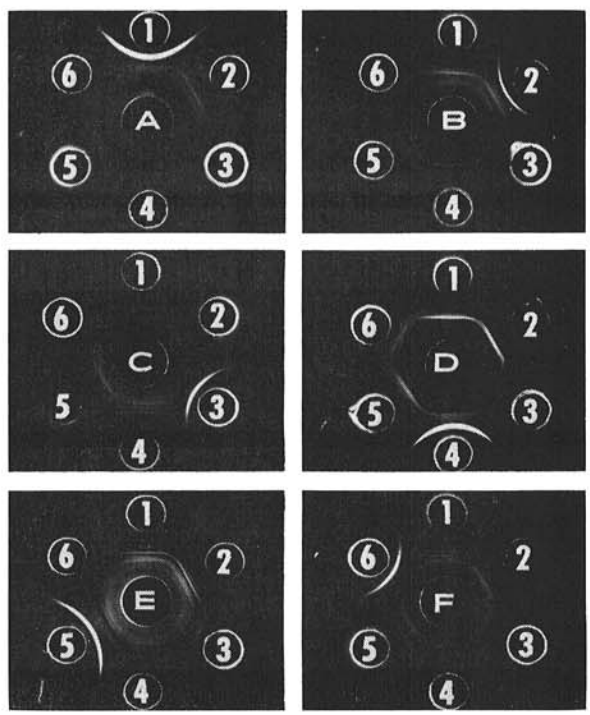

Fig. 4. Specific serological reactions of six independent serotypes of *Pseudomonas syringae.* Center wells contain antisera to *P. syringae* serotype Ia (A), II (B), IIIa (C), IVa (D), V (E), VI (F). Outer wells in each pattern contain untreated heavy bacterial cell suspensions of *P. syringae* serotype Ia (1), II (2), IIIa (3), IVa (4), V (5), and VI (6). Note that there are no apparent cross-reactions of the specific antigens of these six serotypes.

TABLE 4. Reaction of the heat-stable antigens of *Pseudomonas syringae* serotypes as observed in gel-diffusion tests[a]

Antisera of serotype	Antigens of serotype									
	Ia	Ib	II	IIIa	IIIb	IIIc	IVa	IVb	V	VI
Ia	++	+	−	−	−	−	−	−	−	−
Ib	−	++	−	−	−	−	+	+	−	−
II	−	−	++	−	−	−	−	−	−	−
IIIa	−	−	−	++	+	+	−	−	−	−
IIIb	−	−	−	+	++	+	−	−	−	−
IIIc	−	−	−	+	+	++	−	−	−	−
IVa	−	+	−	−	−	−	++	+	−	−
IVb	−	+	−	−	−	−	+	++	−	−
V	−	−	−	−	−	−	−	−	++	−
VI	−	−	−	−	−	−	−	−	−	++

[a] ++ = Homologous reaction of heat-stable antigens; + = partial cross-reaction of heat-stable antigens; − = negative reaction with heat-stable antigens.

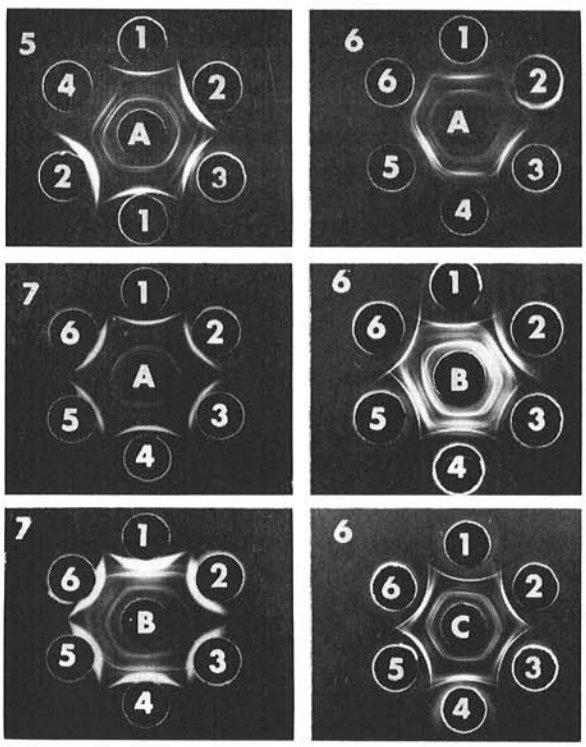

Fig. 5-7. **5)** Example of spur formation observed with *P. syringae* serotypes I, III, and IV. Center well contains antiserum to serotype IVb. Outer wells contain untreated heavy bacterial cell suspensions of serotypes Ib (1), IVb (2), IVa (3), and Ia (4). **6)** Serological reactions produced with antisera prepared against a composite antigen of *P. syringae* serotypes Ia, II, IIIa, V, and VI. Center wells contain antisera prepared against untreated antigens (A), antigens autoclaved 15 min at 15 lb./inch^2 at 121 C (B), or antigens heated 1 hr at 100 C (C). Outer wells contain untreated heavy bacterial cell suspensions of *P. syringae* serotype Ia (1), II (2), IIIa (3), IVa (4), V (5), or VI (6). **7)** Serological identity of the heat-stable antigens of *P. syringae* and *P. aptata*. Center wells contain antisera to *P. syringae* serotype VI (A) and *P. aptata* (B). Outer wells contain untreated heavy bacterial cell suspensions of *P. syringae* serotype VI (1 and 4) or isolates of *P. aptata* (2, 3, 5, and 6). Note the complete reciprocal identity of the heat-stable antigen bands nearest the antigen wells.

P. aptata, P. morsprunorum, and *P. pisi* that were pathogenic on peach seedlings were identical serologically with one of the *P. syringae* serotypes (Table 5). Reciprocal gel-diffusion tests with antigens and antisera of *P. aptata* and *P. syringae* serotype VI demonstrated the complete serological identity of the heat-stable antigens of these organisms (Fig. 7). Similar reactions f identity were observed with antigens and antisera of *P. morsprunorum* and *P. syringae* serotypes Ia and Ib. One of the 18 *P. morsprunorum* isolates belonged to serotype Ia, whereas the remaining 17 isolates belonged to serotype Ib. The *P. pisi* isolate was identical to *P. syringae* IIIa.

Isolates of some of the *Pseudomonas* nomenspecies that were not pathogenic on peach seedlings possessed heat-stable antigens that were serologically related to or identical with those possessed by one of the *P. syringae* serotypes (Fig. 8). Included in this group were isolates of *P. antirrhini, P. maculicola, P. mori, P. pisi, P. savastanoi, P. tomato,* and *P. viridiflava.*

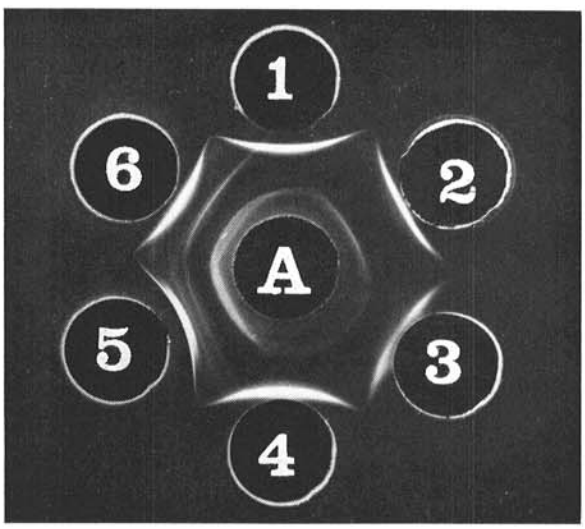

Fig. 8. Serological relationship of *Pseudomonas syringae* and isolates of various other *Pseudomonas* nomenspecies. Center well contains antisera to *P. syringae* serotype IIIb (A). Outer wells contain heavy bacterial cell suspensions of *P. syringae* IIIb (1 and 4), *P. antirrhini* (2), *P. savastanoi* (3), *P. tomato* (5), and *P. mori* (6).

TABLE 5. Relationship of host of origin and serotype of *Pseudomonas syringae* pathogenic to Lovell peach seedlings

Host of origin	No. isolates in serotype									
	Ia	Ib	II	IIIa	IIIb	IIIc	IVa	IVb	V	VI
Apple	1			1						
Almond			35				2			
Apricot	1[a]		22				1			
Bean	1		3	3		1		1		2
Cherry	4[a]	23[a]	18	4	3					1
Citrus				2	2					
Crataegus spp.				1	1					
Lilac				12						
Pea				2[b]						
Peach		2	24	3	1		2			1
Pear	6	8	4	3			1			
Pepper					2				2	
Plum	5	3[a]	4	2				1		
Poplar				2						
Prune	6		114	3						
Raspberry			2							
Sugarbeet										8[c]
Tomato		2								
Miscellaneous hosts, one isolate each[e]	1	1		2	1[d]	1	2		1	2
Unknown		7[a]		5	1					1
Total	25	46	226	45	11	2	8	2	3	15

[a] Includes some isolates received as *P. morsprunorum*.
[b] Includes one isolate received as *P. pisi*.
[c] All isolates received as *P. aptata*.
[d] Received as *P. passiflorae*.
[e] One isolate from each of the following hosts: Avocado, *Cyamopsis tetragonoloba, Forsythia suspensa,* millet, mock orange, nectarine, passion fruit, *Panicum milaceum,* sorghum, sudangrass, and walnut.

TABLE 6. Comparison of serological groupings of isolates of *Pseudomonas syringae* and *Pseudomonas monsprunorum* studied by the authors and by Lovrekovich et al. (21)

Isolate no.	*Pseudomonas* species	Received from	Oxidase reaction (authors')	Peach pathogenicity (authors')	Serological group no. (Lovrekovich et al.)	Serotype (authors')
C-86	*syringae*	Klement[a]	—	+	A	Ib
C-72	*syringae*	Klement	—	+	B	Ia
270	*morsprunorum*	Klement	—	+	B	Ia
524	*syringae*	Klement	—	+	C	IIIa
D281	*syringae*	Klement	—	+	C	IIIa
191	*syringae*	Klement	—	+	C	IIIa
365	*syringae*	NCPPB[b]	—	+	C	IIIa
296	*syringae* f. sp. *populea*	Klement	—	+	C	IIIa
Ps 140 = I 201	*syringae* var. *capsici*	ICPB[c]	—	—	D	—
C109	*syringae*	Klement	—	—	E	—
366	*syringae*	Klement	+	—	F	—
294	*syringae* f. sp. *populea*	ICPB	—	—	G	—
C162	*syringae*	Klement	+	—	H	—

[a] Klement = Zoltan Klement, Research Institute for Plant Protection, Budapest, Hungary.
[b] NCPPB = National Collection of Plant Pathogenic Bacteria, England.
[c] ICPB = International Collection of Phytopathogenic Bacteria, Davis, Calif.; — = negative oxidase, or pathogenicity test or did not give a serological reaction characteristic of any of the authors' 10 *P. syringae* serotypes.

Isolation of the heat-stable antigen of P. syringae.—The heat-stable antigen of *P. syringae* was isolated from both rough and smooth colony types by TCA extraction and ethanol precipitation. The isolated antigen was serologically identical to the heat-stable antigen of untreated whole cells. Antisera produced against these "purified" antigens were of low titer, but were usable for gel-diffusion reactions. These antisera had the same specificity for heat-stable antigens as antisera prepared with sonicated cells of the parent isolates. No symptoms were produced when peach seedlings were injected with the isolated antigen.

DISCUSSION.—A positive oxidase test was found to be an excellent criterion for eliminating from consideration as *P. syringae* many nonpathogenic pseudomonads found in association with diseased plant tissue or on healthy plant surfaces. Oxidase, peach pathogenicity, and gel-diffusion tests were used to confirm the identity of isolates received as *P. syringae*. From the study of nearly 475 isolates received as *P. syringae, P. morsprunorum,* and *P. aptata,* only 10 nonpathogenic isolates were found that gave a specific serological reaction indicative of *P. syringae*. In all other isolates of these species, the specific serological reaction was correlated with pathogenicity on peach seedlings. Thus, these isolates, originating from 30 different hosts, demonstrated that pathogenicity to peach seedlings can be used to detect *P. syringae* isolates originating from many hosts of this organism. The general susceptibility of peach seedlings to isolates originating from other hosts apparently does not apply to some of the other hosts of *P. syringae,* such as bean and tomato (31) or lilac (5). It can be concluded that Lovell peach is a suitable host for screening for pathogenic *P. syringae* isolates regardless of their host of origin.

The results of this study confirm the speculation of Lucas & Grogan (23) that the three *P. lachrymans* serotypes they studied might reflect a similar limited serological variation in other plant-pathogenic *Pseudomonas* nomenspecies. The occurrence of at least 10 serotypes of *P. syringae* is not surprising, considering its wide host range; and the existence of additional serotypes is quite probable. Although the occurrence of 10 or more serotypes appears to limit the usefulness of Ouchterlony gel-diffusion tests for identification, this problem is overcome by the ability to produce antisera against composite antigens as described for *Salmonella* spp. (15). Thus, one antiserum can detect all of the currently identified serotypes of *P. syringae*.

Spur formation as observed in serotypes I, III, and IV indicates that a given isolate may possess more than one heat-stable antigen or that the heat-stable antigen may possess one to several antigenic determinants, with these determinants overlapping to give serologically similar, though not identical, antigens. This parallels earlier results with *Salmonella* spp. (15), *P. medicaginis* var. *phaseolicola* (28), and *P. syringae* (21, 28).

The antigenic specificity and variability of *P. syringae* is probably associated with O-type antigens as in *P. lachrymans* (24). However, the failure of rough isolates to agglutinate in saline solutions and their serological identity to smooth isolates makes it apparent that the rough isolates of *P. syringae* studied do not correspond to rough isolates of most other gram-negative bacterial species (1, 8, 15, 24, 34). A report of the serological identity of the O antigens of smooth and rough isolates of *P. aeruginosa* (12) appears to describe a system identical to that observed with *P. syringae* here. Also, rough isolates of *Salmonella* spp. which contain the same O antigen as smooth isolates do occur, but such rough types are not frequently encountered (15).

Lovrekovich et al. (21), using heat stable antigens, established eight serological groups based on their study of 17 *P. syringae* and six *P. morsprunorum* isolates. The results of our oxidase, pathogenicity, and serological tests with 12 of these same isolates indicates that five of their eight serological groups are based on cultures which may not be valid *P. syringae*. However, the two studies do agree on the serological grouping of all isolates which we found to be pathogenic on peach seedlings.

Although tartrate utilization separates most isolates of *P. morsprunorum* from *P. syringae,* gelatin liquefaction by *P. syringae* was too variable for use in separating the two species. Also, the pathological, serological, and *G. candidum* inhibition tests pointed out similarities among *P. aptata, P. morsprunorum,* and *P. syringae*. Although *P. aptata* and especially *P. morsprunorum* may be pathologically or ecologically specialized forms, these two species probably are sufficiently similar to *P. syringae* to be considered synonymous with it, as suggested by previous investigators (5, 7, 9, 11, 13, 21).

The pathogenicity to peach seedlings of isolates of *P. syringae* and *P. morsprunorum* which were not inhibitory to *G. candidum* was demonstrated. This is contrary to results of DeVay et al. (6), but concurs with results of Baigent (2) and Perlasca (31).

The similarity or identity of the heat-stable antigens of *P. syringae* and some isolates of *P. antirrhini, P. maculicola, P. mori, P. pisi, P. savastanoi, P. tomato,* and *P. viridiflava* was noted and needs further study.

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