Distribution of *Pseudomonas syringae* Pathovars into Twenty-Three O Serogroups

MONIQUE SAUNIER, LAURENCE MALANDRIN, AND RÉGINE SAMSON*

Pathologie Végétale, Institut National de la Recherche Agronomique, 49071 Beaucouzé Cédex, France

Received 7 August 1995/Accepted 21 November 1995

Serological reactions of *Pseudomonas syringae* and *Pseudomonas viridiflava* were studied by Ouchterlony double diffusion. A total of 55 polyclonal antisera, containing anti-lipopolysaccharide (anti-LPS) precipitating antibodies, were cross-tested against antigenic suspensions of 51 strains. Twenty-three O serogroups were defined, primarily on the reaction of the type strains. Two families of O serogroups showed antigenic cross-reactivities (PHA, MOP1, MOP2, MOP3, HEL1, HEL2, and SYR1; PERSAVTOM1, PERSAVTOM2, DEL, POR, and SYR2). Ten O serogroups showed a clearcut specificity: APTPIS, TAB, VIR1, VIR2, VIR3, SYR3, SYR4, SYR5, HUS, and LAC. The last serogroup (RIB) contained strains with rough colony morphology and side chain-deficient LPSs, as evidenced by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The LPS basis of the O serogroups was demonstrated by immunoblotting. Serological reference strains were designated for all of the O serogroups, and correspondence was established between the O serogroups studied and seven previous serogroups (L. T. Pastushenko and I. D. Simonovich, *Mikrobiol. Zh.* 41:222–229 and 330–339, 1979). A total of 355 strains of *P. syringae* (*sensu lato*) belonging to 15 pathovars, not including pathovar *syringae*, were typed into the 23 described O serogroups. O serogroups were assigned after double-diffusion reactions, with each strain compared with serological references. The utility of O serogrouping to study *P. syringae* pathovar structure and diversity is discussed.

The *Pseudomonas syringae* complex can be defined as oxidase- and arginine dihydrolase-negative fluorescent pseudomonads which induce a hypersensitive reaction in tobacco plants (41). It is currently divided into 49 pathovars (62), each pathovar being specifically pathogenic to one or several plant species (3). However, the taxonomy of *P. syringae* is still unsettled. As an example, *P. syringae* pv. *savastanoi* was reinstated as a subspecies (21) and then elevated to a species on the basis of DNA-DNA hybridization studies (10). Whatever the taxonomic status of a given pathovar, there is still a need for improved identification methods for each pathogen. Most of the serological studies undertaken for identification of pseudomonads have been confined to a particular pathogen (7, 9, 20, 22, 32, 34, 36–38, 44, 47, 54, 55). One study attempted a global approach to the *P. syringae* group but with only local strains (42, 43).

A recent work showed that when rabbits were immunized with whole bacteria, they produced mainly anti-LPS (anti-cell wall lipopolysaccharide) antibodies (14). Phenol-water- and trichloroacetic acid-extracted antigens were responsible for the precipitation band observed in agarose when whole-cell diffusible antigens were used. LPS was shown to be spontaneously released from bacterial cells in suspension, diffusing in agar, thus allowing the use of such suspensions as reacting antigens for the Ouchterlony technique. Eight O serogroups, for which serological reference strains were assigned, were identified in a preliminary report: TAB, LAC, PHA, MOP1, MOP2, MOP3, APTPIS, and PERSAVTOM (47). A study of strains belonging to *P. syringae* pv. *aptata*, *P. syringae* pv. *tabaci*, *P. syringae* pv. *morsprunorum*, and *P. syringae* pv. *phaseolicola* revealed that each of the four pathovars formed homogeneous O serogroups distinct from the others (15).

The aim of this study was to define, by means of double-diffusion reactions of soluble LPS, O serogroups within *P. syringae* and *Pseudomonas viridiflava*, in order to form the basis of serotyping for the two species. A total of 368 strains representing 23 *P. syringae* pathovars (63, 64) and *P. viridiflava* strains were studied in order to monitor the serological diversity of the *P. syringae* complex and to check whether the serogroupings could be used for identification purposes.

MATERIALS AND METHODS

Bacteria. The 51 strains used to produce antisera, belonging to *P. viridiflava* and 18 pathovars of *P. syringae*, are listed in Table 1. The bacteria are named as described by Young et al. (63, 64). Although not validly named, strains designated *P. syringae* pv. *holci* (23), pv. *morihsuzi* (25), and pv. *porri* (46) were included in the study. The other strains classified in the serogroups are listed only in the result tables. LOPAT (levan, oxidase, potato, arginine, tobacco) tests (29) and some additional biochemical tests (18, 30), such as polypectate at pH 5.5 and 8.3, utilization of sorbitol, inositol, erythritol, DL-lactate, L-(+)-tartrate, and D-(-)-tartrate, were performed to detect strain mislabellings.

Antisera. Rabbits were immunized with bacteria grown in tubes of 5 ml of YP broth (0.3% yeast extract, 0.5% peptone) for 24 h at 23°C with shaking (220 strokes min⁻¹). Cells were harvested in exponential phase, washed with sterile phosphate-buffered saline (PBS) and resuspended in PBS (0.1 M phosphate; pH 7.2), with formalin (0.5%). Bacterial suspensions (10⁸ cells ml⁻¹) were administered to the rabbits (adults, Fauve de Bourgogne) in four intravenous injections within 2 weeks, and the animals were bled 6 days later. The injection protocol was sometimes repeated after 1 month's rest to increase antibody titer. Antisera were titrated by Ouchterlony double diffusion (39). A total of 55 antisera in 55 rabbits were prepared (Table 1).

Reacting antigens for double diffusion. Dense cell suspensions (10¹⁰ cells ml⁻¹), made in distilled water using bacteria collected from a King's medium B (24) plate after a 48-h incubation at 23°C in stationary phase, were used as reacting antigens. The stability of such suspensions during storage at 4 and -18°C was tested. The variability of the reactions was tested according to the growth media: LPGA (0.3% yeast extract, 0.5% peptone, 0.5% glucose, 1.5% agar), LPA (LPGA without glucose), NGA (0.3% beef extract, 0.5% peptone, 0.25% glucose, 1.5% agar) NSA (NGA with sucrose instead of glucose), and YDC (1% yeast extract, 2% glucose, 2% calcium carbonate, 1.5% agar).

Double-diffusion reaction. Ouchterlony double diffusion was performed with a 1% (wt/vol) agarose gel solubilized in borate-EDTA buffer (0.1 M sodium borate, 8 mM Na₂ EDTA · 2H₂O; pH 8.2) poured onto a glass plate (5.5 by 26.7 cm). Antibody and antigen wells (3-mm well diameter, 8-mm distance) were filled simultaneously (22 µl). Antisera were diluted 1:1 with glycerol for storage

* Corresponding author. Mailing address: Pathologie Végétale, Institut National de la Recherche Agronomique, BP 57, 42 rue Georges Morel, 49071 Beaucouzé Cédex, France. Phone: 33 41 22 57 27. Electronic mail address: samson@angers.inra.fr.

TABLE 1. Bacteria used to define the O serogroups

Species and pathovar ^a	CFBP no. ^b	Other designation ^c	Origin (host plant and country)	Homologous-antiserum code(s) ^d
<i>P. syringae</i> pathovars				
aptata	1617 ^T 2042	NCPPB 871 ^T	<i>Beta vulgaris</i> ; United States	168, 180
			<i>B. vulgaris</i> ; France	141
		IMV 45	<i>B. vulgaris</i> ; Ukraine	
		IMV 185	<i>B. vulgaris</i> ; Ukraine	
delphinii	2215 ^T	ICMP 52B ^T	<i>Delphinium</i> sp.; New Zealand	292
helianthi	1732	NCPPB 2639	<i>Helianthus annuus</i> ; France	223
	2149		<i>H. annuus</i> ; France	224, 320, 322, 332
holci		IMV 8299	<i>Sorghum</i> sp.; Ukraine	
lachrymans	1644	NCPPB 1096	<i>Cucumis sativus</i> ; Hungary	178, 190
		IMV 7591	<i>C. sativus</i> ; Ukraine	
		IMV 8535		
		IMV 458	<i>Brassica oleracea</i> cv. Botrytis; Ukraine	
lupini				
maculicola	2453		<i>Morus alba</i> ; France	330, 337
morihiuszi	1650	NCPPB 2422	<i>Prunus domestica</i> ; Switzerland	254
morsprunorum	2115		<i>Prunus cerasus</i> ; France	183, 192
	2351 ^T	NCPPB 2995 ^T	<i>P. cerasus</i> ; United States	286
persicae	1316	NCPPB 2324	<i>Prunus persica</i> ; France	IU5, 139
phaseolicola	1390 ^T	NCPPB 52 ^T	<i>Phaseolus vulgaris</i> ; Canada	150, 182, 284
	1882		<i>P. vulgaris</i> ; France	226
pisi	2105 ^T	ICMP 2452 ^T	<i>Pisum sativum</i> ; New Zealand	184, 285
	2258		<i>P. sativum</i> ; France	151
		IMV 7151	<i>P. sativum</i> ; Ukraine	
	3522		<i>P. sativum</i> ; France	
porri	1908 ^T		<i>Allium porrum</i> ; France	125, 135, 136
ribicola	2348 ^T	NCPPB 963 ^T	<i>Ribes aureum</i>	294
savastanoi	1670 ^T	NCPPB 639 ^T	<i>Olea europea</i> ; Yugoslavia	196, 255
	2090		<i>Nerium oleander</i> ; United States	289
syringae	602	NCPPB 2775	<i>Malus sylvestris</i> ; France	296
	1579	NCPPB 2769	<i>P. cerasus</i> ; France	338
	1669	NCPPB 294	<i>Populus</i> sp.; United Kingdom	299
	1679	NCPPB 1041	<i>Piper nigrum</i> ; Brazil	355
	1685	NCPPB 2264	<i>Zea mays</i> ; Yugoslavia	138
	1782	NCPPB 2810	<i>N. oleander</i> ; United States	297
	1894		<i>Z. mays</i> ; France	115
	1915		<i>Z. mays</i> ; France	327
	2009	ICMP 849	<i>Prunus avium</i> ; New Zealand	328, 336
	2336	Onion a	<i>Allium cepa</i> ; Japan	295
		IMV P-55	Fruit tree; United Kingdom	
		IMV 281	<i>Syringa vulgaris</i> ; United Kingdom	
		IMV 467	Fruit tree; Ukraine (<i>P. cerasi</i>)	
tabaci	1615	NCPPB 79	<i>Nicotiana tabacum</i> ; United States	189
	1699	NCPPB 1408	<i>N. tabacum</i> ; Hungary	149
	2106 ^T	NCPPB 1427 ^T	<i>N. tabacum</i> ; Hungary	167
		IMV 225	<i>N. tabacum</i> ; Ukraine	
tomato	1321		<i>Lycopersicon esculentum</i> ; Switzerland	246
	1326		<i>L. esculentum</i> ; France	227
	2212 ^T	NCPPB 1106 ^T	<i>L. esculentum</i> ; United Kingdom	191
	2545		<i>L. esculentum</i> ; France	140, 287
<i>P. viridiflava</i>	1141	NCPPB 1249	<i>Chrysanthemum morifolium</i> ; United Kingdom	302
	1468		<i>P. domestica</i> ; France	356
	2107 ^T	NCPPB 635 ^T	<i>Phaseolus</i> sp.; Switzerland	234

^a *P. syringae* (sensu lato) (41, 64).^b CFBP, French Collection of Phytopathogenic Bacteria, Angers, France.^c NCPPB, National Collection of Plant Pathogenic Bacteria, Harpenden, United Kingdom; IMV, Institute of Microbiology and Virology of the Academy of Sciences, Kiev, Ukraine; ICMP, International Collection of Micro-organisms from Plants, Auckland, New Zealand.^d One to four antisera were produced against each strain.

and were used at that concentration in order to obtain maximal reactions. The plates were kept in a humid chamber at 20°C and observed after 16, 24, and 72 h under tangential light. Antigens were positioned in the peripheral well. The reactions between strains and antisera were replicated two- to fivefold.

Bacterial extracts for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Bacteria were washed in PBS and suspended in extraction Tris buffer (1 M; pH 6.8) containing SDS (2%), 2-mercaptoethanol (4%), and glycerol (10%). Suspensions were heated at 100°C for 10 min and treated with proteinase K (2.5 mg ml⁻¹) to remove proteins before electrophoresis (19).

SDS-PAGE and immunoblotting. SDS-PAGE was performed with 3.75% stacking and 14% separating acrylamide 0.75-mm-thick slab gels (28). After electrophoresis, the components were electrotransferred from gels to nitrocellulose membranes (59) with a Sartoblot system (Sartorius, Palaiseau, France) according to the manufacturer's instructions. The nitrocellulose membranes were then blocked overnight by incubation in skim milk (10%), rinsed in TBS-Tween (Tris-buffered saline [pH 7.2], 0.05% Tween), and then incubated for 1 h at 37°C with a 1:200 dilution of rabbit antiserum. The blots were washed three times with TBS-Tween and then incubated for 1 h at 37°C with a 1:1,000 dilution

TABLE 2. Double-diffusion reactions of 16 *P. syringae* pathovar strains with the corresponding antisera: definitions of the O-serogroups PHA, MOP1, MOP2, MOP3, HEL1, HEL2, SYR1, APTPIS, and TAB

CFBP strain no. (pathovar)	Reaction with the indicated antisera ^a									O serogroup
	150, 182, 226, 284	115, 254	183, 192	286	223	224, 320, 322, 332	296	141, 151, 168, 180, 184, 285	149, 167, 189	
1390 ^T (phaseolicola) 1882 (phaseolicola)	+++	+++	—	++s	—	+w	—	—	—	PHA
1650 (morsprunorum) 1894 (syringae)	+++	+++	++s	+++	—	—	—	—	—	MOP2
2115 (morsprunorum)	+w	++s	+++	+++	—	+w	—	—	—	MOP1
2351 ^T (morsprunorum)	+++	+++	+++	+++	—	+w	—	—	—	MOP3
1732 (helianthi)	+w	—	+w	++s	+++	+++	—	—	—	HEL1
2149 (helianthi)	+w	—	+w	++s	+w	+++	—	+w	—	HEL2
602 (syringae)	—	—	—	++s	—	++s	+++	—	—	SYR1
1617 ^T (aptata) 2042 (aptata) 2105 ^T (pisi) 2258 (pisi)	—	—	—	—	—	+w	—	+++	+w	APTPIS
1615 (tabaci) 1699 (tabaci) 2106 ^T (tabaci)	—	—	—	—	—	—	—	+w	+++	TAB

^a Homologous reactions are indicated by boldface type (reactions between antisera and immunizing bacteria). —, no reaction; +w, weak and inconstant spur; ++s, strong spur (remarkable partial identity); and +++, identity band.

of goat anti-rabbit immunoglobulin conjugated with alkaline phosphatase (Sigma, St. Louis, Mo.). The blots were washed three times with PBS-Tween, and the substrate mixture (33 μ l of NBT [*p*-nitrophenylphosphate] [75 mg ml⁻¹] and 25 μ l of BCIP [5-bromo-4-chloro-3-indolylphosphate] [50 mg ml⁻¹] in 7.5 ml of Tris, pH 9.6) was added. As soon as coloration appeared, the blots were rinsed in distilled water and kept in a cold humid chamber. Some of the polyacrylamide gels were silver stained directly to show LPS patterns (60).

RESULTS

Identification results. In the identification tests, all bacteria reacted as expected except two strains, CFBP 2104, the pathotype strain of *P. syringae* pv. lachrymans, and CFBP 2348, the pathotype strain of *P. syringae* pv. ribicola. Strain CFBP 2104^T was negative for utilization of polypectate (pH 8.3), inositol, L-(+)-tartrate, and erythritol and positive for D-(–)-tartrate. Pathogenicity tests performed with the pathovar lachrymans strains revealed that strain CFBP 2104^T was not pathogenic. The results indicate that CFBP 2104^T reacts more like *P. syringae* pv. syringae than like *P. syringae* pv. lachrymans. Strain CFBP 2348^T was fluorescent, oxidase negative, arginine-dihydrolase negative, levan variable, pectolytic at pH 8.3, and tobacco hypersensitivity negative. All these criteria are consistent with a *P. viridiflava* strain.

Ouchterlony serological reactions. The product of the homologous antigen-antibody reaction, i.e., the reaction between an antigen preparation and antiserum obtained by immunization with the same preparation (5), appeared as a single, thick, dense band with a slightly diffuse margin. The band appeared 10 to 18 h after the wells were filled. Spurs were carefully observed, and a distinction was made between stable spurs associated with a strong band (remarkable partial identity) and faint spurs associated with a temporary band.

A total of 55 antisera that contained precipitating antibodies

were obtained (Table 1). When a pathovar appeared to be serologically heterogeneous in preliminary tests (e.g., *P. syringae* pv. morsprunorum), additional strains of the pathovar were examined.

Definition of 23 O serogroups. The 51 strains used for the immunizations were tested against all antisera. A serogroup was defined as a group of strains giving a specific pattern by Ouchterlony reactions with a given antiserum. Twenty-three O serogroups were established (Tables 2 to 4).

The serological relationships between serogroups PHA, MOP, HEL, SYR1, APTPIS, and TAB are indicated in Table 2. MOP1 and MOP2 serogroup members presented both common and specific epitopes. The serogroups PHA and MOP2 differed only in a lack of common epitopes between PHA and MOP1. MOP3 was a serogroup of wide specificity, sharing epitopes with PHA, MOP1, and MOP2. MOP3 also might share epitopes with HEL1, HEL2, and SYR1 strains, although nonreciprocal reactions were found. In Table 3, relationships between PERSAVTOM1, PERSAVTOM2, DEL, POR, and SYR2 are indicated. PERSAVTOM2 strains possessed additional epitopes compared with PERSAVTOM1. The epitopes shared by the two PERSAVTOM serogroups were absent from the DEL serogroup. The remaining nine O serogroups were specific (Table 4).

Immunoblotting analysis. After digestion with proteinase K and enzyme-linked immunosorbent assay (ELISA) blot staining, bacterial components showed a ladder-like pattern (12) typical of LPS side chain antigens (Fig. 1). The serological specificity demonstrated by Ouchterlony tests was confirmed by the immunoblots. Thus, the APTPIS antiserum 285 stained only the extracts of *P. syringae* pv. pisi CFBP 2105^T and *P. syringae* pv. aptata CFBP 1617^T. The remaining extracts, be-

TABLE 3. Double-diffusion reactions of 11 *P. syringae* pathovar strains with the corresponding antisera: definitions of the O-serogroups PERSAVTOM1, PERSAVTOM2, DEL, POR, and SYR2

CFBP strain no. (pathovar)	Reaction with the indicated antisera ^a					O serogroup
	139, IU5, 191, 196, 227, 246, 255, 289	140, 287	292, 295	125, 135, 136	138	
1316 (persicae)						
1670 ^T (savastanoi)						
2090 (savastanoi)	+++	+w	+w	—	—	PERSAVTOM1
1321 (tomato)						
1326 (tomato)						
2212 ^T (tomato)						
2545 (tomato)	+++	+++	+++	+w	—	PERSAVTOM2
2215 ^T (delphinii)	+w	++s	+++	+w	—	DEL
2336 (syringae)						
1908 ^T (porri)	—	+w	++s	+++	++s	POR
1685 (syringae)	—	—	—	++s	+++	SYR2

^a Homologous reactions are indicated by boldface type. —, no reaction; +w, weak and inconstant spur; ++s, strong spur (remarkable partial identity); and +++, identity band.

longing to O serogroups VIR2, RIB, PERSAVTOM1, VIR1, and PHA, showed no reaction.

Stability of the serogroupings and RIB variation. The stability of antigen reactivity was tested for serogroups PERSAVTOM (strains CFBP 1316, 1670^T, and 2212^T), APTPIS (strains

CFBP 1617^T and 2105^T), TAB (strains CFBP 1615, 1699, and 2106^T), and MOP (strains CFBP 1650, 2115, and 2351^T). The results were not affected by the age of the culture (1 to 10 days), the conditions of storage of the bacterial suspension (1 to 6 days at 4°C, 1 to 6 months at –18°C), or the use of the

TABLE 4. Double-diffusion reactions of 13 *P. syringae* pathovar strains and *P. viridiflava* strains against the corresponding antisera: definitions of the O-serogroups VIR1, VIR2, VIR3, SYR3, SYR4, SYR5, HUS, LAC, and RIB

CFBP strain(s)	Reaction with the indicated antisera ^a											O serogroup
	Antisera of Table 2	Antisera of Table 3	234	297, 299, 302	327	328, 336, 338	330, 337	190	355	356	294	
1390 ^T , 1882, 1650, 2351 ^T , 2115, 1732, 2149, 602, 1894, 1617 ^T , 2042, 2105 ^T , 2258, 1615, 1699, 2106 ^T	See Table 2	—	—	—	—	—	—	—	—	—	— ^b	
1316, 1670 ^T , 2090, 1321, 1326, 2212 ^T , 2545, 2215 ^T , 1908 ^T , 1685, 2336	—	See Table 3	—	—	—	—	—	—	—	—	— ^c	
2107 ^T (<i>P. viridiflava</i>)	—	—	+++	—	—	—	—	—	—	—	+w	VIR1
1141 (<i>P. viridiflava</i>)	—	—	—	+++	—	—	—	—	—	—	+w	VIR2
1669, 1782 (<i>P. syringae</i> pv. <i>syringae</i>)												
1915 (<i>P. syringae</i> pv. <i>syringae</i>)	—	—	—	—	+++	—	—	—	—	—	—	SYR3
1579, 2009 (<i>P. syringae</i> pv. <i>syringae</i>)	—	—	—	—	—	+++	+w	—	—	—	—	SYR4
2453 (<i>P. syringae</i> pv. <i>morihiuszi</i>)	—	—	—	—	—	—	+++	—	—	—	—	HUS
1644 (<i>P. syringae</i> pv. <i>lachrymans</i>)	—	—	—	—	—	—	—	+++	—	—	—	LAC
1679 (<i>P. syringae</i> pv. <i>syringae</i>)	—	—	—	—	—	—	—	—	+++	—	—	SYR5
1468 (<i>P. viridiflava</i>)	—	—	—	—	—	—	—	—	—	+++	—	VIR3
2348 ^T (<i>P. syringae</i> pv. <i>ribicola</i>)	—	—	—	—	—	—	—	—	—	—	+++	RIB
3522 (<i>P. syringae</i> pv. <i>psi</i>)												

^a Homologous reactions are indicated by boldface type. —, no reaction; +w, weak and inconstant spur; and +++, identity band.

^b Strain CFBP 2149, +w.

^c Strains CFBP 1316^T, 2212^T, and 2545, +w.

FIG. 1. Immunoblot of proteinase K-digested bacterial extracts. Nitrocellulose was stained with rabbit antiserum 285 (*P. syringae* pv. pisi 2105^T) diluted 1:200 and anti-rabbit alkaline phosphatase-conjugated immunoglobulin reacting with NBT plus BCIP substrate. Lanes: 1, no extract; 2, *P. viridiflava* CFBP 1141 (O-serogroup VIR2); 3, *P. syringae* pv. ribicola CFBP 2348^T (RIB); 4, *P. syringae* pv. tomato CFBP 2212^T (PERSAVTOM1); 5, *P. syringae* pv. pisi CFBP 2105^T (APTPIS); 6, *P. syringae* pv. savastanoi CFBP 1670^T (PERSAVTOM1); 7, *P. viridiflava* CFBP 2107^T (VIR1); 8, *P. syringae* pv. persicae CFBP 1316 (PERSAVTOM1); 9, *P. syringae* pv. aptata CFBP 1617^T (APTPIS); 10, *P. syringae* pv. phaseolicola CFBP 1390^T (PHA). Note the ladder-like pattern of the two reacting extracts revealing LPS side chains of O-serogroup APTPIS.

different culture media. Some inconsistency occurred when cells were grown on a carbohydrate-deficient medium (such as LPA), which resulted in the loss of the weak cross-reactions. More striking was the change of some strains from the initial serogroup to the RIB serogroup, for instance, when *P. syringae* pv. tomato CFBP 2212^T and *P. viridiflava* CFBP 2107^T were subcultured on medium containing calcium carbonate (YDC) or were stored as water suspensions. We termed this phenomenon a "serological jump." Notice, however, that strain *P. syringae* pv. pisi CFBP 3522 was typed as RIB immediately after isolation in our laboratory (13).

Side chain-deficient LPSs of the RIB strains. By comparing extracts from *P. syringae* pv. pisi CFBP 2105^T (APTPIS) and 3522 (RIB) and *P. syringae* pv. aptata CFBP 1617^T (APTPIS) on silver-stained acrylamide gels, it was observed that they all possessed a strong band corresponding to the fast-migrating LPS core (Fig. 2, band b). However, extracts from strain CFBP 3522 did not show the typical ladder-like pattern of LPS side chains (bands a). *P. syringae* pv. ribicola CFBP 2348^T gave a banding pattern similar to that of strain CFBP 3522 (not shown).

Comparison with the serogroups of Pastushenko and Simonovich. Nine of our O serogroups were checked for agreement with the serogroups of Pastushenko and Simonovich (42, 43) by comparison with strains from Kiev's Institute of Microbiology and Virology (Table 5). PHA corresponded to VI, MOP2 corresponded to III, SYR1 and HEL1 corresponded to II, APTPIS corresponded to IV, TAB corresponded to VIII, PERSAVTOM1 and PERSAVTOM2 corresponded to I, and LAC corresponded to IX.

Serological reactions of 15 pathovars of *P. syringae*. The 31 strains of *P. syringae* pv. aptata and the 21 strains of *P. syringae* pv. pisi isolated from *Beta vulgaris* and *Pisum sativum*, respectively, presented the same serological reactions (Table 6). The majority of them belonged to the O serogroup APTPIS. However, one *P. syringae* pv. aptata strain (Janse PD202) and one *P. syringae* pv. pisi strain (NCPPB 1653) belonged to HEL2, and one *P. syringae* pv. pisi strain (CFBP 3522) was RIB. Pathovars atrofaciens, coronafaciens, and striafaciens showed a high de-

gree of heterogeneity: four serogroups for pathovar atrofaciens (APTPIS, MOP2, SYR1, and SYR2), two serogroups for pathovar atropurpurea, three serogroups for pathovar coronafaciens (PERSAVTOM2, RIB, and SYR2), and three serogroups for pathovar striafaciens (DEL, RIB, and SYR1).

The 48 strains of *P. syringae* pv. glycinea, isolated from *Glycine max*, were equally distributed into serogroups APTPIS and HEL2. Upon initial isolation of strains from soybean plants and testing, it was noted that serogroup was linked to colony appearance on LPGA (SH163 and SH171 series of isolates). The circular, fluidal, semiopaque colonies gave an APTPIS reaction, whereas the smooth, star-shaped, translucent colonies were HEL2. The morphology of the colonies also was linked to the proteolytic activity of the strains on a gelatin medium (the APTPIS strains were protease negative; the HEL2 strains were protease positive). Variation among colonies isolated from the same host plant sample was not observed with the *P. syringae* pv. aptata isolates (SA33, SA84, and SB30 series of isolates).

Fourteen of fifteen strains of *P. syringae* pv. helianthi belonged to the serogroup HEL2. Three of these strains also cross-reacted with HEL1, whose serological reference strain is CFBP 1732. The *P. syringae* pv. helianthi type strain, CFBP 2067, was found to belong to the RIB serogroup. Forty-one *P. syringae* pv. phaseolicola strains were studied, originating from different hosts and representing the nine described races of Taylor et al. (57). All belonged to the serogroup PHA except for the five isolates from *Vigna radiata* (ex *Phaseolus aureus*), which reacted as APTPIS, and strain 1354A, which was RIB. The *P. syringae* pv. morsprunorum strains isolated from *Prunus avium*, *Prunus cerasus*, and *Prunus domestica* belonged mainly to the serogroups MOP1 (28 strains) and MOP3 (8 strains). Three strains belonged to MOP2. Five of six strains tested isolated from *Prunus armeniaca* belonged to MOP2.

The 11 strains designated *P. syringae* pv. lachrymans were assigned to three serogroups: LAC (nine strains), TAB (one strain), and PERSAVTOM1 (for type strain CFBP 2104 only). The eight strains of *P. syringae* pv. tabaci isolated from tobacco plants belonged to TAB, whereas the two strains isolated from

FIG. 2. Silver-stained-gel migrations of proteinase K-digested bacterial extracts. Lanes: 1, *P. syringae* pv. pisi 2105^T (O-serogroup APTPIS); 2, *P. syringae* pv. pisi CFBP 3522 (RIB); 3, *P. syringae* pv. aptata CFBP 1617^T (APTPIS). The ladder-like pattern of LPS side chains (a) and the fast-migrating LPS core (b) are indicated.

TABLE 5. Correspondence between some O serogroups of *P. syringae* from the present study and serogroups of Pastushenko and Simonovich (42, 43)

O serogroup	CFBP serological reference strain (pathovar)	IMV strain(s) belonging to the same serogroup (pathovar)	Pastushenko and Simonovich's serogroup
PHA	1390 ^T (phaseolicola)	8299 (holci)	VI
MOP2	1650 (morsprunorum)	P-55 (syringae)	III
SYR1	602 (syringae)	467 (cerasi)	II
HEL1	1732 (helianthi)	8535 (lupini)	II
APTPIS	1617 ^T (aptata) 2105 ^T (pisi)	45 and 185 (aptata) 7151 (pisi)	IV
TAB	1615 (tabaci)	225 (tabaci)	VIII
PERSAVTOM1	1316 ^T (persicae) 1670 ^T (savastanoi) 2212 ^T (tomato)	458 (maculicola)	I
PERSAVTOM2	2545 (tomato)	281 (syringae)	I
LAC	1644 (lachrymans)	7591 (lachrymans)	IX

soybean plants belonged to MOP2. All 21 strains of *P. syringae* pv. persicae and all 37 strains of *P. syringae* pv. savastanoi belonged to the same O serogroup, PERSAVTOM1. Pathovar tomato strains were distributed in three O serogroups, PERSAVTOM1 (24 strains), PERSAVTOM2 (17 strains), and RIB (three strains from tomato plants and one strain from pepper plants).

DISCUSSION

By using a panel of strains belonging to *P. syringae* and *P. viridiflava*, 23 O serogroups were defined on the basis of LPS reactions in Ouchterlony double-diffusion tests. The O serogroups described here take into account global reactions that reveal several epitopes reacting at the same time. Spur formation in double-diffusion reveals so-called remarkable distinct epitopes. Such epitopes could not be elucidated by agglutination reactions, immunofluorescent staining, or ELISA without absorption of the common antibodies of polyclonal antisera. The greater the number of strains tested, the more likely that new epitopes will be identified. This might explain why group II of Pastushenko and Simonovich (42, 43) corresponds to O serogroups SYR1 and HEL1 (Table 5), since strains that belong to SYR1 and HEL1 must share some epitopes (Table 2). The same rationale explains the partition of Pastushenko and Simonovich's serogroup I into PERSAVTOM1 and PERSAVTOM2. Recently, two serotypes, O:2 and O:3, tentatively have been proposed by using four monoclonal antibodies (MAbs) with specificity towards LPS side chains (40). MAbs Ps-O:2-1, Ps-O:2-2, and Ps-O:2-3 react with strain IMV 467 (*P. syringae* pv. syringae), which is SYR1 in our classification, and MAB Ps-O:3 reacts with strain IMV P-55, which is MOP2. Serotype O:2 recognizes strains belonging to several of our O serogroups (SYR1, IMV 467; APTPIS, CFBP 3180; and SYR2, CFBP 3182). Inversely, it does not recognize all APTPIS strains (CFBP 3181). It is possible that LPS antigens are composed of epitopes present in variable quantities, major epitopes that would altogether define O serogroups and minor epitopes that might be present in some strains in relatively minor proportions (31). Therefore, the classification proposed

by Ovod et al. (40) with serotype O:2 could be based on minor epitopes.

The chemical structure of LPS O side chains of our serological reference strains (53) revealed that strains CFBP 1617^T (*P. syringae* pv. aptata) and CFBP 2105^T (*P. syringae* pv. pisi) both have O-antigen repeating units of a rhamnose backbone substituted with *N*-acetylglucosamine. This structure is identical to the structure of *Pseudomonas wieringae* LPS determined by Knirel et al. (26). Pastushenko and Simonovich (42) classified *P. wieringae* in their serogroup IV, which can be assimilated to our serogroup APTPIS as shown in Table 5. Strain CFBP 1650 of *P. syringae* pv. morsprunorum, the reference strain for O-serogroup MOP2, possesses an LPS side chain (53) identical to the side chain of the LPS of *P. syringae* pv. syringae IMV P-55 (27). Strain IMV P-55 belongs to serogroup III of Pastushenko and Simonovich and to our O-serogroup MOP2. Thus, it is well established in the case of *P. syringae* that the differential serological behaviors of the serogroups are closely linked to the chemical structures of the LPS side chains.

The RIB O serogroup deserves special mention. It was formed on the basis of serological reactivity of the pathotype strain CFBP 2348 of *P. syringae* pv. ribicola. It is not clear whether this bacterium is a true pathovar of *P. syringae*, since its physiological properties revealed that it might be a *P. viridiflava* strain. Nevertheless, its LPS defines a serogroup, RIB, that shows peculiar properties. Two strains, *P. syringae* pv. tomato CFBP 2212^T (PERSAVTOM1) and *P. viridiflava* CFBP 2107^T (VIR1), happened to change their serogroup suddenly to become RIB. This sudden serological jump was observed on rugose-dissociated cultures. The SDS-PAGE patterns of the RIB strains (*P. syringae* pv. pisi CFBP 3522 and *P. syringae* pv. ribicola CFBP 2348^T) after silver staining confirm that the RIB LPSs showed no side chain antigens.

The O serogroups of *P. syringae* are designated here by letters representing the pathovar names to avoid confusing number systems (47). Furthermore, to standardize the serological studies, it was proposed previously (47) that the same serological references be used at the international level. We propose 14 new serological reference strains for the 14 addi-

TABLE 6. O-serogroup determination of strains belonging to 15 pathovars of *P. syringae*

Pathovar, source, and CFBP strain	Alternate designation	Country of origin	Yr of isolation	Race	O serogroup
pv. aptata from <i>Beta vulgaris</i>					
1617 ^{Tsa}	NCPPB 871 ^T	United States	1959		APTPIS
1625	NCPPB 873	United States	1959		APTPIS
1906	Samson 1357-1	France	1979		APTPIS
2042	Samson SA33-5	France	1981		APTPIS
2134	Samson SA84-6	France	1981		APTPIS
2135	Samson SB30-3	France	1982		APTPIS
2279	Luisetti JA100-1	France	1981		APTPIS
2280	Luisetti JB47	France	1982		APTPIS
2471	Janse PD 565	The Netherlands	1985		APTPIS
2472	Janse PD 196	The Netherlands	1979		APTPIS
2473	Janse PD 197	The Netherlands	1979		APTPIS
3315	NCPPB 2712	Italy	1974		APTPIS
	SA33-1, ^b SA33-2, SA33-4, SA33-6, SA33-7	France	1981		APTPIS
	SA84-3, ^b SA84-4, SA84-5, SA84-8	France	1981		APTPIS
	SB30-3, ^b SB30-4, SB30-5, SB30-6, SB30-7, SB30-11, SB30-12	France	1982		APTPIS
	IMV 45	Ukraine			APTPIS
	IMV 185	Ukraine			APTPIS
2507	Janse PD 202	The Netherlands	1979		HEL2
pv. atrofaciens from <i>Triticum aestivum</i>					
1622	NCPPB 1868	Canada	1966		SYR1
1623	NCPPB 2613	New Zealand	1967		SYR1
1629	NCPPB 1870		1966		SYR1
2213 ^T	ICMP 4394 ^T	New Zealand	1968		APTPIS
2256	Alivizatos WGR1	Greece	1983		MOP2
2257	Alivizatos WGR5	Greece	1983		SYR1
	Rudolph Pa5	Germany			APTPIS
	Rudolph Pa7	Germany			SYR2
3179	Rudolph Pa8	Germany			APTPIS
3180	Rudolph Pa9	Germany			APTPIS
3181	Rudolph Pa14	Germany			APTPIS
	Rudolph Pa16	Germany			SYR1
3182	Rudolph Pa17	Germany			SYR2
3183	Rudolph Pa18	Germany			SYR2
3184	Rudolph Pa20	United States			SYR1
3185	Rudolph Pa21	Bulgaria			SYR1
pv. atropurpurea					
From <i>Agrostis</i> sp.					
1628	NCPPB 1768	United Kingdom	1965		PERSAVTOM1
From <i>Lolium</i> sp.					
1639	NCPPB 2396	Japan	1967		SYR2
2340 ^T	NCPPB 2397 ^T	Japan	1967		PERSAVTOM1
pv. coronafaciens from <i>Avena sativa</i>					
1618	NCPPB 874	Germany	1959		PERSAVTOM2
1626	NCPPB 1253	United Kingdom	1962		SYR2
1627	NCPPB 1356	Canada	1954		RIB
1640	NCPPB 2481	Kenya	1970		PERSAVTOM2
1725	NCPPB 2680	New Zealand	1969		PERSAVTOM2
1728	NCPPB 2816	Canada	1933		PERSAVTOM2
1791	NCPPB 2823	Canada	1958		PERSAVTOM2
2216 ^T	NCPPB 600 ^T	United Kingdom	1958		SYR2
3362	Cunfer C28	United States			SYR2
pv. glycinea from <i>Glycine max</i>					
1646	NCPPB 1271	United States	1962	4 ^c	APTPIS
2214 ^T	ICMP 2189 ^T	New Zealand	1968	4	APTPIS
3187	Samson SH163-1	France	1988		APTPIS
3189	Samson SH171-2	France	1988		APTPIS
3190	Rat SJ130-1	United States	1988		APTPIS
3192	Rat SJ130-5	United States	1990		APTPIS
3193	Chauveau SJ309-17	United States	1990		APTPIS

Continued on following page

TABLE 6—Continued

Pathovar, source, and CFBP strain	Alternate designation	Country of origin	Yr of isolation	Race	O serogroup
3194	Chauveau SJ309-22	Canada	1990		APTPIS
3212	ICMP 3482	New Zealand	1963	4	APTPIS
3357	Keen R1	United States		1	APTPIS
3359	Keen R5	United States		5	APTPIS
3360	Keen R6	United States		6	APTPIS
	SH163-2, ^b SH163-3, SH163-4, SH163-5, SH163-6, SH163- 18, SH163-19	France	1988		APTPIS
	SH171-3, ^b SH171-5, SH171-6, SH171-7, SH171-8, SH171-9	France	1988		APTPIS
1518	NCPPB 2070	United States	1962	4	HEL2
1560	NCPPB 2752	France	1974	4	HEL2
1561	Rat 118-7	France	1974		HEL2
1562	Rat 118-1	France	1974		HEL2
1563	NCPPB 2753	France	1974	4	HEL2
1638	NCPPB 1883	United States	1951	4	HEL2
3188	Samson SH163-8	France	1988		HEL2
3210	NCPPB 1245	Canada	1943	4	HEL2
3356	Keen R0	United States		0	HEL2
3358	Keen R4UI	United States		4	HEL2
3361	Fett A29-2	United States		4	HEL2
	SH163-7, ^b SH163-9, SH163- 10, SH163-11, SH163-12, SH163-13, SH163-14, SH163-15, SH163-16, SH163-17	France	1988		HEL2
	SH171-1, ^b SH171-4	France	1988		HEL2
pv. <i>helianthi</i> from <i>Helianthus annuus</i>					
1643	NCPPB 1055	Ethiopia	1961		HEL2 ^d
1732	NCPPB 2639	Canada	1974		HEL2 ^d
2043	Samson SA35-1	France	1981		HEL2
2149 ^s	Samson SA51-1	France	1981		HEL2 ^d
	SA35-2, SA35-3, SA35-4, SA35-5, SA35-6, SA35-7, SA35-10, SA35-11	France	1981		HEL2
	SA51-2, SA51-4	France	1981		HEL2
2067 ^T	NCPPB 2640 ^T	Mexico			RIB
pv. <i>lachrymans</i> from <i>Cucumis sativus</i>					
70	NCPPB 2754	France	1960		LAC
1644 ^s	NCPPB 1096	Hungary	1957		LAC
1729	NCPPB 277	Denmark	1950		LAC
2261	Samson SC57-5	France	1983		LAC
2263	Marinescu PLX6	Rumania	1981		LAC ^c
2275	Marinescu B72	Rumania	1981		LAC ^c
2276	Marinescu B9	Rumania	1981		LAC
2460	Chauveau 8339ID2	France	1983		LAC ^c
3592	IMV 7591	Ukraine			LAC
1633	NCPPB 541	Canada	1951		TAB
2104 ^{Tf}	NCPPB 537 ^T	United States	1935		PERSAVTOM1
pv. <i>morsprunorum</i> From <i>Prunus armeniaca</i>					
	Prunier T11-16	France	1986		MOP1
	Prunier S42-2	France	1985		MOP2
	Prunier T9-1	France	1986		MOP2
	Prunier T28-49	France	1986		MOP2
	Prunier V44-27	France	1988		MOP2
	Prunier V62-1	France	1988		MOP2
From <i>Prunus avium</i>					
1647	NCPPB 1781	Italy	1965		MOP1
1741	NCPPB 2787	Greece	1961		MOP1
2007	ICMP 3429	New Zealand	1973		MOP1
2627	Gardan E82	France	1973		MOP3
From <i>Prunus cerasus</i>					
4	Ridé 4	France	1958		MOP1
542	Prunier 130-4	France	1964		MOP1

Continued on following page

TABLE 6—Continued

Pathovar, source, and CFBP strain	Alternate designation	Country of origin	Yr of isolation	Race	O serogroup
1564	NCPPB 2755	France	1974		MOP1
1566	Prunier 768-7	France	1968		MOP1
1567	Luisetti E12-16	France	1974		MOP1
1568	Luisetti E21-2	France	1974		MOP1
2115	Prunier G88-9	France	1975		MOP1
2116	Prunier E13-3	France	1974		MOP1
2119	Prunier E13-20	France	1974		MOP1
2331	Prunier N118-1	France	1980		MOP1
2332	Garrett EM78	United Kingdom	1976		MOP1
	Garrett EM-2-25	United Kingdom			MOP1
	Garrett EM4-18	United Kingdom			MOP1
	Garrett EM6	United Kingdom			MOP1
	Garrett EMC5	United Kingdom			MOP1
	Garrett EMC9rb	United Kingdom			MOP1
	Garrett EMC48	United Kingdom			MOP1
	Prunier 761-4	France			MOP1
	Prunier N118-28	France	1980		MOP1
	Prunier N126-2	France	1980		MOP1
	Prunier R12-1	France	1984		MOP1
	Prunier R36-1	France	1984		MOP1
	Prunier R39-1	France	1984		MOP1
	Prunier M101-1	France	1979		MOP2
1064	Prunier 560-2	France	1967		MOP3
1544	Gardan E8-2	France	1974		MOP3
	Garrett EM2-14	United Kingdom			MOP3
	Garrett EM4-15	United Kingdom			MOP3
	Garrett EMBC-2	United Kingdom			MOP3
	Prunier R19-2	France	1984		MOP3
From <i>Prunus domestica</i>					
1565	NCPPB 2756	France	1965		MOP1
1736	NCPPB 1095	United Kingdom	1960		MOP1
1650	NCPPB 2422	Switzerland	1965		MOP2
1658	NCPPB 2320	Rumania	1969		MOP2
2351 ^{TS}	NCPPB 2995 ^T	United States			MOP3
pv. <i>persicae</i> from <i>Prunus persica</i>					
1067	Vigouroux 509	France	1967		PERSAVTOM1
1316 ^S	NCPPB 2324	France	1970		PERSAVTOM1
1570	NCPPB 2758	France	1969		PERSAVTOM1
1571	NCPPB 2759	France	1969		PERSAVTOM1
1572	NCPPB 2760	France			PERSAVTOM1
1573 ^T	NCPPB 2761 ^T	France	1974		PERSAVTOM1
1574	NCPPB 2762	France	1974		PERSAVTOM1
1575	NCPPB 2763	France	1970		PERSAVTOM1
2023	NCPPB 2764	France	1968		PERSAVTOM1
2024	Gardan 827-4	France	1969		PERSAVTOM1
2025	Luisetti G114-3	France	1975		PERSAVTOM1
2026	Luisetti H55	France	1976		PERSAVTOM1
2027	Luisetti K53-3	France	1977		PERSAVTOM1
2028	Luisetti K55-3	France	1977		PERSAVTOM1
2029	Luisetti K55-4	France	1977		PERSAVTOM1
2030	Luisetti K71-11	France	1977		PERSAVTOM1
2031	Luisetti N69-2	France	1980		PERSAVTOM1
2032	Luisetti N119-1	France	1980		PERSAVTOM1
2033	Luisetti N125-2	France	1980		PERSAVTOM1
2034	Luisetti N219-5	France	1980		PERSAVTOM1
2820	Luisetti N125-1	France	1980		PERSAVTOM1
pv. <i>phaseolicola</i>					
From <i>Desmodium</i> sp.					
3645	Taylor 1516A	Tanzania	1986	5 ^g	PHA
From <i>Dolichos</i> sp.					
1667	NCPPB 1647	Tanzania	1964	8	PHA
From <i>Lablab purpureus</i>					
3644	Taylor 1375A	Kenya	1985	5	PHA
3651	Taylor 1449B	Ethiopia	1985	7	PHA
From <i>Macroptilium atropurpureum</i>					
3636	Taylor 1650	Australia	1972	2	PHA

Continued on following page

TABLE 6—Continued

Pathovar, source, and CFBP strain	Alternate designation	Country of origin	Yr of isolation	Race	O serogroup
From <i>Neonotonia wightii</i>					
3653	Taylor 1542A	Tanzania	1986	7	PHA
From <i>Phaseolus coccineus</i>					
1662	NCPPB 604	United Kingdom	1958		PHA
3632	Taylor 1281A	United Kingdom	1984	1	PHA
3633	Taylor 1474A	United Kingdom	1985	1	PHA
3634	Taylor 1996A	Italy	1988	1	PHA
From <i>Phaseolus vulgaris</i>					
1390 ^{TS}	NCPPB 52 ^T	Canada	1949		PHA
1429	IPO 38	The Netherlands			PHA
1508	Rudolph Ha1a	Germany	1972		PHA
1652	NCPPB 1103	Hungary	1956		PHA
1739	Rat HT	France	1975		PHA
1743	ICMP 718	New Zealand	1953		PHA
1882	Trigalet P	France	1978		PHA
3635	Taylor 882	United States	1987	2	PHA
3637	Taylor 1839A	North Yemen	1987	2	PHA
3638	Taylor 1301A	Tanzania	1984	3	PHA
3639	Taylor 1427B	Tanzania	1985	3	PHA
3640	Taylor 2475A	Colombia	1989	3	PHA
3641	Taylor 1302A	Rwanda	1984	4	PHA
3642	Taylor 1385A	Burundi	1985	4	PHA
3643	Taylor 1499A	Rwanda	1985	4	PHA
3646	Taylor 2708A	Malawi	1990	5	PHA
3647	Taylor 1299A	Tanzania	1984	6	PHA
3648	Taylor 2368A	South Africa	1989	6	PHA
3649	Taylor 2694A	Zimbabwe	1990	6	PHA
3657	Taylor 2656A	South Africa	1990	8	PHA
3658	Taylor 2659A	South Africa	1990	8	PHA
3660	Taylor 2709A	Malawi	1990	9	PHA
3661	Taylor 2732E	United Kingdom	1990	9	PHA
3650	Taylor 1354A	Kenya	1985	7	RIB
From <i>Vigna angularis</i>					
3655	Taylor 1674	New Zealand	1970	7	PHA
3659	Taylor 1675	New Zealand	1970	8	PHA
From <i>Vigna radiata</i> ^h					
1659	NCPPB 2437	United States	1971	7	APTPIS
1747	NCPPB 2798	United States			APTPIS
2277	NCPPB 2800	United States			APTPIS
3652	Taylor 1537C	Tanzania	1986	7	APTPIS
3662	Taylor 1646	Tanzania	1965		APTPIS
pv. <i>psi</i> from <i>Pisum sativum</i>					
1436	NCPPB 2766	France	1973	6 ⁱ	APTPIS
1541	NCPPB 1359	Canada	1946		APTPIS
1651	NCPPB 300	Canada	1951		APTPIS
1668	NCPPB 2222	Italy	1969	1	APTPIS
1676	NCPPB 2354	New Zealand	1969		APTPIS
1744	NCPPB 1434	Hungary	1956	6	APTPIS
2105 ^{TS}	NCPPB 2585 ^T	New Zealand	1969	2	APTPIS
2258	Samson SB19-1	France	1982		APTPIS
2452	Chauveau 229A11	France	1983		APTPIS
2703	Taylor 1576A	The Netherlands	1986	2	APTPIS
2704	Taylor 870A	United States	1975	3	APTPIS
2705	Taylor 870B	United States	1975	3	APTPIS
2706	Taylor 895A	United States	1975	4	APTPIS
2707	Taylor 974B	United States	1978	5	APTPIS
2708	Taylor 974C	United States	1978	5	APTPIS
2709	Taylor 1704B	France	1986	6	APTPIS
3195	Taylor 202, ICMP 815	United States	1944	2	APTPIS
3196	Taylor 299A, ICMP 2955	New Zealand	1970	1	APTPIS
	IMV 7151	Ukraine			APTPIS
1745	NCPPB 1653	South Africa	1961		HEL2
3522	Samson SG30-5	France	1987		RIB
pv. <i>savastanoi</i>					
From <i>Olea europea</i>					
71	Ridé 71	Tunisia	1960		PERSAVTOM1

Continued on following page

TABLE 6—Continued

Pathovar, source, and CFBP strain	Alternate designation	Country of origin	Yr of isolation	Race	O serogroup
1020	Eskandari LN1	France	1967		PERSAVTOM1
1670 ^{TS}	NCPPB 639 ^T	Yugoslavia			PERSAVTOM1
1746	NCPPB 2327	Italy	1969		PERSAVTOM1
2069	Yahiaoui K237	Algeria	1977		PERSAVTOM1
2070	Gardan KC2-1	Algeria	1977		PERSAVTOM1
2074	Yahiaoui 49	Algeria	1977		PERSAVTOM1
2075	Yahiaoui 50	Algeria	1977		PERSAVTOM1
From <i>Nerium oleander</i>					
1672	NCPPB 1343	United States	1961		PERSAVTOM1
1839	Ridé NO11	France	1970		PERSAVTOM1
2088	Yahiaoui L7-21	Algeria	1978		PERSAVTOM1
2090	Gardan L86-1	United States	1978		PERSAVTOM1
From <i>Jasminium</i> sp.					
1751	NCPPB 2788	Greece	1968		PERSAVTOM1
From <i>Fraxinus excelsius</i>					
1663	NCPPB 1006				PERSAVTOM1
1666	NCPPB 1464	United Kingdom	1959		PERSAVTOM1
1793	Ridé Fe60	France	1974		PERSAVTOM1
1794	Ridé Fe261	France	1974		PERSAVTOM1
1795	Ridé Fe311	France	1974		PERSAVTOM1
1838	Ridé Fe406	France	1974		PERSAVTOM1
2093	Gardan L39-4	France	1978		PERSAVTOM1
2158	Ridé Fe30	France	1974		PERSAVTOM1
2159	Ridé Fe241	France	1974		PERSAVTOM1
2160	Ridé Fe400	France	1975		PERSAVTOM1
2161	Ridé Fe51	France	1975		PERSAVTOM1
2162	Ridé Fe56	France	1975		PERSAVTOM1
2163	Ridé Fe61	France	1975		PERSAVTOM1
2164	Ridé Fe83	France	1976		PERSAVTOM1
2166	Ridé Fe92	France	1976		PERSAVTOM1
2167	Ridé Fe93	France	1976		PERSAVTOM1
2168	Ridé Fe502	France	1977		PERSAVTOM1
2169	Ridé Fe506	France	1977		PERSAVTOM1
2170	Ridé Fe331b	France	1974		PERSAVTOM1
2171	Ridé Fe500	France	1977		PERSAVTOM1
2172	Ridé Fe505	France	1977		PERSAVTOM1
2174	Ridé Fe58	France	1975		PERSAVTOM1
2175	Ridé Fe600	France	1975		PERSAVTOM1
2176	Ridé Fx2	Algeria	1977		PERSAVTOM1
pv. striafaciens					
From <i>Avena sativa</i>					
1674 ^T	ICMP 3961 ^T		1966		RIB
1686	NCPPB 2480	Zimbabwe	1971		DEL
From <i>Secale</i> sp. × <i>Triticum</i> sp.					
1750	NCPPB 2713	Mexico	1973		SYR1
pv. tabaci					
From <i>Nicotiana tabacum</i>					
1503					TAB
1615 ^S	NCPPB 79	United States	1935		TAB
1621	NCPPB 1866		1966		TAB
1699	NCPPB 1408	Hungary	1959		TAB
1721	NCPPB 1238	Malaysia	1962		TAB
1774	NCPPB 1234	Zambia	1962		TAB
2106 ^T	NCPPB 1427 ^T	Hungary	1959		TAB
	IMV 225	Ukraine			TAB
From <i>Glycine max</i>					
1775	PERSAVTOM1	Australia	1972		MOP2
1786	PERSAVTOM1	Australia	1972		MOP2
pv. tomato					
From <i>Lycopersicon esculentum</i>					
1318	Burki 167	Switzerland			PERSAVTOM1
1320	Burki 190	Switzerland	1969		PERSAVTOM1
1322	Burki 180	Switzerland			PERSAVTOM1
1323	Berniac 6	France	1971		PERSAVTOM1
1324	Berniac 2	France	1971		PERSAVTOM1

Continued on following page

TABLE 6—Continued

Pathovar, source, and CFBP strain	Alternate designation	Country of origin	Yr of isolation	Race	O serogroup
1325	Berniac 3	France	1971		PERSAVTOM1
1326	Berniac 5	France	1971		PERSAVTOM1
1636	NCPBP 1367	Canada	1944		PERSAVTOM1
1785	NCPBP 2683	New Zealand	1972		PERSAVTOM1
1918	Bonn P803S	Canada	1978		PERSAVTOM1
1920	Bonn P808S	Canada			PERSAVTOM1
2212 ^{TS}	NCPBP 1106 ^T	United Kingdom	1960		PERSAVTOM1
	Rat 81.157.3	France	1981		PERSAVTOM1
	Rat 81.200.121	France	1981		PERSAVTOM1
	Rat 82.29.122	France	1982		PERSAVTOM1
	Rat 84.54.51	France	1984		PERSAVTOM1
	Rat 84.63.61	France	1984		PERSAVTOM1
	Rat 84.74.6	France	1984		PERSAVTOM1
	Rat 84.84.9	France	1984		PERSAVTOM1
	Rat 84.93.7	France	1984		PERSAVTOM1
	Rat 87.16.1	France	1987		PERSAVTOM1
	Rat 87.34.4	France	1987		PERSAVTOM1
	Rat 87.58.2	France	1987		PERSAVTOM1
	Rat 88.37.21	France	1988		PERSAVTOM1
1426	Paulin T3	France	1972		PERSAVTOM2
2545 ^S	Rat R4	France	1979		PERSAVTOM2
2546	Chauveau B117-1	France	1980		PERSAVTOM2
	83.47.2	France	1983		PERSAVTOM2
	83.49.3	France	1983		PERSAVTOM2
	83.85.3	France	1983		PERSAVTOM2
	83.96.41	France	1983		PERSAVTOM2
	84.74.1	France	1984		PERSAVTOM2
	84.84.10	France	1984		PERSAVTOM2
	84.93.3	France	1984		PERSAVTOM2
	85.10.1	France	1985		PERSAVTOM2
	85.12.1	France	1985		PERSAVTOM2
	87.33.12	France	1987		PERSAVTOM2
	87.34.3	France	1987		PERSAVTOM2
	87.49.3	France	1987		PERSAVTOM2
	88.14.4	France	1988		PERSAVTOM2
	88.48.1	France	1988		PERSAVTOM2
1696	NCPBP 269	Denmark	1949		RIB
	Rat 83.63.91	France	1983		RIB
	Rat 84.84.3	France	1984		RIB
From <i>Capsicum annuum</i>					
	Rat 83.135.2	France	1983		RIB

^a T, pathotype strain; S, serological reference.^b Authors' laboratory group of strains isolated from different lesions on different plants of the same diseased crop.^c Races of pv. glycinea according to Fett and Sequeira (9) and Barton-Willis et al. (2).^d Cross-reactions with HEL1 serogroup.^e Cross-reactions with RIB serogroup.^f Strain probably not pv. lachrymans.^g Races of pv. phaseolicola according to Taylor et al. (57).^h Ex-*Phaseolus aureus*.ⁱ Races of pv. pisi according to Taylor et al. (56).

tional O serogroups presented here. The serological reference can be the type strain of the pathovar, although this is not the case when the type strain is considered to be not representative of the pathovar or when there are several serogroups for a given pathovar. The new serological references proposed are as follows: *P. syringae* pv. *helianthi* CFBP 1732 for HEL1, *P. syringae* pv. *helianthi* CFBP 2149 for HEL2, *P. syringae* pv. *syringae* CFBP 602 for SYR1, *P. syringae* pv. *syringae* CFBP 1685 for SYR2, *P. syringae* pv. *syringae* CFBP 1915 for SYR3, *P. syringae* pv. *syringae* CFBP 2009 for SYR4, *P. syringae* pv. *syringae* CFBP 1679 for SYR5, *P. syringae* pv. *delphinii* CFBP 2215^T for DEL, *P. syringae* pv. *porri* CFBP 1908^T for POR, *P. viridiflava* CFBP 2107^T for VIR1, *P. viridiflava* CFBP 1141 for VIR2, *P. viridiflava* CFBP 1468 for VIR3, *P. syringae* pv. *mori-huszi* CFBP 2453 for HUS, and *P. syringae* pv. *ribicola* CFBP

2348^T for RIB. Although no cultural variants upon subculturing were observed for the serological reference strains after their introduction in our collection, note that the reactions of presumed isogenic strains in other collections would have to be checked.

The relationships of serogroups to pathovars showed several patterns. Some pathovars were homogeneous, either defining a unique serogroup (*P. syringae* pv. *porri* and POR) or being contained in a shared serogroup (e.g., APTIS for pathovars aptata and pisi and PERSAVTOM1 for pathovars persicae, tomato, and savastanoi). Some pathovars were heterogeneous, with either a low percentage of variants (e.g., pathovars aptata, helianthi, pisi, and lachrymans), an essentially bimodal distribution of serogroups (pathovar glycinea), or variation linked to the host of origin (TAB for pathovar tabaci isolates from

tobacco plants and MOP2 from soybean plants and PHA for pathovar *phaseolicola* isolates from different beans except for mung bean isolates, which are APTPIS). Finally, some variations were attributed to the existence of misidentified strains (pathotype strain of pathovar *lachrymans*).

Serology of pathovars *aptata*, *glycinea*, *helianthi*, *phaseolicola*, and *pisi*. Previous studies, performed with small numbers of strains, found serological homogeneity for pathovars *aptata* (35), *pisi* (54, 55), and *phaseolicola* (6, 16). Serological cross-reactions between pathovars *aptata* and *pisi* were previously noticed (35). These two pathovars as well as pathovar *glycinea* were classified in the same serogroup (serogroup IV) by Pastushenko and Simonovich (43).

The facts that the serogroup HEL2 within pathovars *aptata* and *pisi* is apparently rare (3.4 and 5.4%, respectively, of the strains tested in the present study) and that the previous studies included very few strains probably explain why serogroup HEL2 was not encountered before. In an extensive study performed in our laboratory with 2,673 isolates of *P. syringae* pv. *pisi* serotyped directly out of infected plant tissues (13), it was found that 11.4% of the isolates belonged to HEL2 and 88.5% of the isolates belonged to APTPIS. We also reported that faint cross-reactions, varying according to the antisera, between APTPIS and HEL2 strains were often noticed. These reactions suggest that the O antigens may share a few epitopes or that different LPS molecules could exist on a given bacterial outer membrane (52). The observation that one *P. syringae* pv. *pisi* isolate appeared to belong alternatively to serogroup APTPIS or HEL2 (13) supports the notion that bacteria have flexible LPS biosynthetic pathways. It would be interesting to test the possible role of lysogenic phages in such a serotype conversion and to study particularly the strain which presented a reversion from HEL2 to APTPIS.

The serological relation between the APTPIS and HEL2 serogroups is supported by the fact that *P. syringae* pv. *glycinea* strains (isolated from *G. max*, soybean) were distributed half in serogroup APTPIS and half in serogroup HEL2. Even from the same lesion, pathovar *glycinea* colonies seemed to have the same probability of being APTPIS or HEL2 (isolates SH163 and SH171). Heterogeneity of LPS side chains of pathovar *glycinea* was reported by Barton-Willis et al. (2), who observed a different composition of the LPS side chain of a race 4 strain (CFBP 3358) compared with that of single strains of races 1 (CFBP 3357), 5 (CFBP 3359), and 6 (CFBP 3360), whose side chains appeared identical. Their findings correlate with our results showing that CFBP 3358 belongs to HEL2 and that CFBP 3357, CFBP 3359, and CFBP 3360 belong to APTPIS. The two LPS types of *P. syringae* pv. *glycinea* were confirmed by Wingate et al. (61), who reported differential reactions of the same set of strains with two MAbs, probably directed towards LPSs of the two O serogroups since the epitopes were periodate sensitive. It is now clear that the LPS antigens are not linked to physiological races. We identified three race 4 strains in the APTPIS serogroup and seven race 4 strains in HEL2.

Although most *P. syringae* pv. *phaseolicola* strains belonged to the PHA serogroup, one group of strains acted differently: the five strains isolated from *V. radiata* that were APTPIS. CFBP 2277 reacted with probe pGi63, specific to *P. syringae* pv. *phaseolicola*, in dot blot assays (58), attesting to a phaseolotoxin gene. Why is there a serological clustering of the *V. radiata* strain group, which is distinct from the serogroup PHA otherwise shared by numerous isolates from various hosts?

Serology of pathovar *morsprunorum*. Although pathovar *morsprunorum* was found to be serologically homogeneous by Pastushenko and Simonovich (43) on the basis of a study of

seven strains, three other reports distinguished two distinct serological subgroups: 5 strains in a serotype A and one strain in a serotype B (33), 80 strains in a serotype D and 1 strain in a serotype C (36), and 1 strain in a serotype Ia and 18 strains in a serotype Ib (38). In the present study, 45 strains of *P. syringae* pv. *morsprunorum* were examined and three serogroups were identified: MOP1 (29 strains), MOP2 (8 strains), and MOP3 (8 strains). Serogroup MOP3, the antisera to which were obtained against the type strain CFBP 2351, acts as a superserogroup sharing some epitopes with MOP1 and some other epitopes with MOP2. Serogroup MOP2 itself shares a part but not all of its epitopes with serogroup PHA, the assignment of a serogroup to a given strain being based on a total-identity reaction with the serological reference by Ouchterlony double diffusion. Therefore, the LPS structures must have several common epitopes among the three MOP serogroups.

Serogroup reactions did not correspond to the host source of the strains. However, MOP1 and MOP3 were the most frequent serogroups among *P. syringae* pv. *morsprunorum* isolates from *P. avium*, *P. cerasus*, and *P. domestica*, whereas MOP2 was predominant for strains from *P. armeniaca* (apricot). The strains from apricot trees were isolated in only one area in south France, and it would be useful to check the serology of more strains of pathovar *morsprunorum* isolated from apricot trees in other areas before extrapolating to a serological cluster linked to apricot-derived strains.

Serology of pathovars *lachrymans* and *tabaci*. In the present study, 9 of 11 pathovar *lachrymans* strains belonged to LAC, 1 belonged to TAB (CFBP 1633), and the type strain belonged to PERSAVTOM2. Lucas and Grogan (34) described a similar situation. They distributed 256 pathovar *lachrymans* isolates in three serogroups: 235 smooth strains in their serogroup I, 17 smooth strains in their serogroup II, and four rough strains in their serogroup III. Pastushenko and Simonovich (43) placed 16 strains of pathovar *lachrymans* in just one serogroup, IX. The lack of pathogenicity for cucumber plants of CFBP 2104 (the pathotype strain) and its biochemical characters support the opinion of Young et al. (63) that the present pathotype strain does not represent the pathovar. Thus, strain CFBP 1644 was assigned as the serological reference of the LAC serogroup instead of the pathotype strain.

Pastushenko and Simonovich (42) reported two serogroups for pathovar *tabaci*: VII and VIII. Their serogroup VIII, containing six strains, corresponds to our TAB serogroup. The seven TAB strains that we studied were tobacco isolates. Their serogroup VII contained two strains, one isolated from tobacco and the other from an unidentified host (43). Our second serogroup for pathovar *tabaci* (MOP2) contained two soybean isolates. Soybean wildfire disease was described in the United States (1). The causal bacteria appeared different from previously known pathovar *glycinea* isolates. They appeared more similar to pathovar *tabaci* because of the natural symptoms and because of their serology. Cross-inoculations of tobacco and soybean plants confirmed the hypothesis that pathovar *tabaci* strains, coming from diseased tobacco fields, would also infect soybean plants. However, it is not known if strains CFBP 1775 and 1786, isolated in Australia, could have originated from an equivalent situation. The only difference between CFBP 1775 and 1786 and the other pathovar *tabaci* strains was the fact that they belonged to a distinct serogroup (MOP2).

Recent studies of the LPS structure of LAC and TAB serological reference strains (53) revealed that LPS side chains of CFBP 1644 and 1615 presented the same backbone, consisting of α -D-, β -L-, and α -L-rhamnopyranoses, but differed by a Gl-

cNAc residue present as a monosaccharide branch for CFBP 1615.

Serology of pathovars persicae, savastanoi, and tomato. All strains of pathovar persicae (21 strains) and pathovar savastanoi (38 strains) showed a tight homogeneity (serogroup PERSAVTOM1), independent from the host of origin and geographical origin for pathovar savastanoi. The strains of *P. syringae* pv. persicae examined here were all from infected peach trees in France (45). Our results for pathovar savastanoi are consistent with those of the study of Janse (20) using 18 strains.

The 41 non-RIB pathovar tomato strains were almost equally shared between serogroups PERSAVTOM1 and PERSAVTOM2. The CFBP strains came from six countries, and the isolates from France were collected between 1981 and 1988. The two serogroups had common epitopes but also possessed specific epitopes justifying their separation. One serogroup was found by Fackrell and Sinha (8) for 16 strains of pathovar tomato. Jones et al. (22), studying 26 strains of pathovar tomato, placed 20 strains in a serovar I, placed 5 additional strains in an undefined serovar II, and left the last strain non-classified.

The similarity of the LPS antigens of pathovars tomato and savastanoi, combined with the fact that LPS is the dominant antigen of gram-negative bacteria, might explain why, even when MAb techniques were used, the two pathovars reacted in the same way with the clones obtained by Casano et al. (4).

Serology of pathovars atrofaciens and coronafaciens. A total of 127 *P. syringae* pv. atrofaciens strains from wheat were classified by Pastushenko and Simonovich (43) into four serogroups (II, IV, V, and VI). We found homology between their serogroups II, IV, and VI, and our serogroups SYR1, APTPIS, and PHA, respectively. A recent work on 100 barley isolates (17) reported an additional serogroup (I) that was identified with PERSAVTOM1. Thus, *P. syringae* pv. atrofaciens shows heterogeneity comparable to that of pathovar syringae, the serological study of which is being undertaken in our laboratory.

The three pathovars atropurpurea, coronafaciens, and striafaciens were regarded as synonyms (50) on the basis of physiological and pathological tests on a range of cereal and grass hosts as well as on the basis of serological agglutination and immunofluorescent cross-reactions (19 strains). Our present results do not support this observation of serological homogeneity, as the 15 strains studied here belonged to five O serogroups (if strains 1674^T and 1627, RIB, are disregarded because of their probable lack of LPS side chains). A reason for this discrepancy may relate to the differences of the strain samplings, since they were all different except for three (*P. syringae* pv. atropurpurea 1628, pv. striafaciens 1674, and pv. coronafaciens 3362).

The use of serology for identification purposes. The *P. syringae* group (sensu lato) does not present as much serological heterogeneity as has been suggested previously (48, 49). We showed, with a collection of diversified strains, that a pathovar generally belongs to one dominant serogroup (e.g., pathovar aptata, pathovar pisi, pathovar helianthi, pathovar phaseolicola from *Phaseolus vulgaris*, pathovar lachrymans, pathovar tabaci from *Nicotiana tabacum*, pathovar persicae, and pathovar savastanoi from all of its hosts). When the strains of some pathovars were split between two serogroups, the serogroups were related by common epitopes (MOP for pathovar morsprunorum from all hosts and PERSAVTOM for pathovar tomato). For these pathovars, a serological method such as double diffusion using pure cultures as antigens is an adequate tool to provide a characterization complementary to the few

discriminating biochemical features that are available. In several cases, such as for pathovars pisi and porri, O antigens alone were determinant criteria for routine identification purposes (3, 13).

There was no correspondence between O serogroupings and host specificity. The pathovar aptata, pisi, and glycinea strains may present the same O serogroup. Reciprocally, RIB strains and HEL2 strains of pathovar pisi were as pathogenic to pea plants as APTPIS strains. The only report which refers to LPS and virulence is the study of *P. syringae* pv. morsprunorum in relation to phage A7 (66). Virulent cherry strain C28 is sensitive to phage A7. A mutant resistant to phage A7 contained only rough LPS and displayed a reduced virulence. Another strain, a plum isolate, was avirulent and resistant to phage A7 and had a smooth LPS but with side chains containing a high glucose content, unlike the wild type.

There was no correspondence between O serogroupings and taxonomic relationships, although there were sometimes close associations. The cases in which serogroups overlap pathovars could indicate a close relationship between the pathovars. However, when the O serogroups are compared with the genomic species recently described by quantitative DNA-DNA hybridization (11, 51), it is clear that genomic relationships cannot account for all instances of serological relatedness. Pathovars aptata and pisi belong to the same genomic species, but pathovars persicae, savastanoi and tomato, although homogeneous in their O serogroups, are allocated in two genomic species.

The most probable reason for the O-serogroup homogeneity must be attributed to a clonal origin of the strains of most pathovars studied in this work. The ancestor of the pathovars would present diversified O types, from which pathogenic clones have emerged. Serological studies of *P. syringae* pv. syringae, which is a ubiquitous and opportunistic pathogen, are under way and should provide valuable information. This hypothesis of clonal structure of some pathovars deserves further investigation by molecular biology methods.

ACKNOWLEDGMENTS

We acknowledge John Taylor, Josef Nemeth, and Rostislav I. Gvozdyak for kindly providing strains and Pierre Guerin for having taken care of the rabbits. We are indebted to John Young for helpful comments on the manuscript.

REFERENCES

1. Allington, W. B. 1945. Wildfire disease of soybeans. *Phytopathology* **35**:857-869.
2. Barton-Willis, P. A., M. C. Wang, B. Staskawicz, and N. T. Keen. 1987. Structural studies of the O-chain polysaccharides of lipopolysaccharide from *Pseudomonas syringae* pv. *glycinea*. *Physiol. Plant Pathol.* **30**:187-197.
3. Bradbury, J. F. 1986. *Pseudomonas*, p. 110-185. In *Guide to plant pathogenic bacteria*. CAB International Mycological Institute, Kew, England.
4. Casano, J. F., S. Y. Hung, and J. M. Wells. 1987. Differentiation of some pathovars of *Pseudomonas syringae* with monoclonal antibodies. *Bull. OEPP/EPPO Bull.* **17**:173-176.
5. Chaparas, S. D., A. Lind, O. Ouchterlony, and M. Ridell. 1983. Terminology guidelines for serotaxonomic studies using immunodiffusion and immunoelectrophoresis. *Int. J. Syst. Bacteriol.* **33**:414-416.
6. Coléno, A. 1968. Utilisation de la technique d'immunofluorescence pour le dépistage de *Pseudomonas phaseolicola* (Burkh.) Dowson dans les lots de semences de haricot contaminés. *C. R. Acad. Sci. Agric. France* **18**:1016-1020.
7. Coléno, A. 1968. Intérêt des motifs antigéniques communs entre *Pseudomonas phaseolicola* (Burkh.) Dowson et *P. syringae* Van Hall pour le typage de cette dernière espèce. *C. R. Acad. Sci. Paris* **266**:2516-2519.
8. Fackrell, H. B., and R. C. Sinha. 1973. Serological analysis of *Pseudomonas syringae* pv. *tomato*. *Phytopathology* **73**:178-181.
9. Fett, W. F., and L. Sequeira. 1981. Further characterization of the physiological races of *Pseudomonas glycinea*. *Can. J. Bot.* **59**:283-287.
10. Gardan, L., C. Bollet, M. Abu-Ghorrah, F. Grimont, and P. A. D. Grimont. 1992. DNA relatedness among pathovar strains of *Pseudomonas syringae*

- subsp. *savastanoi* Janse (1982) and proposal of *Pseudomonas savastanoi* sp. nov. Int. J. Syst. Bacteriol. **42**:606–612.
11. Gardan, L., H. Shafik, and C. Bollet. 1994. Taxonomy of some pathovars of *Pseudomonas syringae*, p. 419–420. In Proceedings of the 8th International Conference on Plant Pathogenic Bacteria 1992. Institut National de la Recherche Agronomique, Paris.
 12. Goldman, R. C., and G. Leive. 1980. Heterogeneity of antigenic-side-chain length in lipopolysaccharide from *Escherichia coli* O111 and *Salmonella typhimurium* LT2. Eur. J. Biochem. **107**:145–153.
 13. Grondeau, C., M. Saunier, F. Poutier, and R. Samson. 1992. Evaluation of physiological and serological profiles of *Pseudomonas syringae* pv. *pisi* for pea blight identification. Plant Pathol. **41**:495–505.
 14. Guillerit, C., and R. Samson. 1993. Serological specificity of the lipopolysaccharides, the major antigens of *Pseudomonas syringae*. J. Phytopathol. **137**:157–171.
 15. Guillerit, C., and R. Samson. 1993. Serological study of four pathovars of *Pseudomonas syringae*: *Ps. sy. aptata*, *Ps. sy. tabaci*, *Ps. sy. mors-prunorum* and *Ps. sy. phaseolicola*. J. Appl. Bacteriol. **74**:683–687.
 16. Guthrie, J. W. 1968. The serological relationship of races of *Pseudomonas phaseolicola*. Phytopathology **58**:716–717.
 17. Gvozdyak, R. I., and L. A. Pasichnik. 1991. The peculiarities of strains serogrouping of *Pseudomonas syringae* pv. *atropfaciens*, p. 412–418. In Proceedings of the 4th International Working Group on *Pseudomonas syringae* Pathovars 1992. University of Florence, Florence, Italy.
 18. Hildebrand, D. C., M. N. Schroth, and D. C. Sands. 1988. *Pseudomonas*, p. 60–80. In N. W. Schaad (ed.), Laboratory guide for identification of plant pathogenic bacteria. American Phytopathological Society Press, St. Paul, Minn.
 19. Hitchcock, P. J., and T. M. Brown. 1983. Morphological heterogeneity among *Salmonella* lipopolysaccharide chemotypes in silver-stained polyacrylamide gels. J. Bacteriol. **154**:269–277.
 20. Janse, J. D. 1981. The bacterial disease of ash (*Fraxinus excelsior*) caused by *Pseudomonas syringae* subsp. *savastanoi* pv. *fraxini*. II. Etiology and taxonomic considerations. Eur. J. Forest Pathol. **11**:425–438.
 21. Janse, J. D. 1982. *Pseudomonas syringae* subsp. *savastanoi* (ex Smith) subsp. nov., nom. rev., the bacterium causing excrescences on *Oleaceae* and *Nerium oleander*. Int. J. Syst. Bacteriol. **32**:166–169.
 22. Jones, J. B., D. L. Dawe, and S. M. McCarter. 1983. Separation of strains of *Pseudomonas syringae* pv. *tomato* into serovars by three serological methods. Phytopathology **73**:573–576.
 23. Kendrick, J. B. 1926. Holcus bacterial spot of *Zea mays* and *Holcus* species. Iowa Agric. Exp. St. Res. Bull. **100**:303–333.
 24. King, E. O., M. K. Ward, and D. E. Raney. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. **44**:301–307.
 25. Klement, Z., L. Lovrekovich, and M. Hevesi. 1960. Studies on the biochemistry, phage sensitivity and serological properties of *Pseudomonas* pathogenic to mulberry. Phytopathol. Z. **38**:18–32.
 26. Knirel, Y. A., G. M. Zdorovenko, V. M. Dashunin, L. M. Yakovleva, A. S. Shashkov, I. Y. Zakharova, R. I. Gvozdyak, and N. K. Kochetkov. 1986. Antigenic polysaccharides of bacteria. 15. Structure of the repeating unit of O-specific polysaccharide chain of *Pseudomonas wiesingae* lipopolysaccharide. Bioorg. Khim. **12**:1253–1262.
 27. Knirel, Y. A., G. M. Zdorovenko, A. S. Shashkov, S. S. Mamyan, L. M. Yakovleva, L. P. Solyanic, and I. Y. Zakharova. 1988. Antigenic polysaccharides of bacteria. 26. Structure of O-specific polysaccharide chains of LPS from *Pseudomonas cerasi* 467 and *Pseudomonas syringae* pv. *syringae* strains 218 and P-55, belonging to serogroups II and III. Bioorg. Khim. **14**:82–91.
 28. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
 29. Lelliott, R. A., E. Billing, and A. C. Hayward. 1966. A determinative scheme for the fluorescent plant pathogenic pseudomonads. J. Appl. Bacteriol. **29**:470–489.
 30. Lelliott, R. A., and D. E. Stead. 1987. Diagnostic procedures, p. 50. In T. F. Preece (ed.), Methods for the diagnosis of bacterial diseases of plants. Blackwell Scientific Publications, Oxford.
 31. Liu, P. V., H. Matsumoto, H. Kusama, and T. Bergan. 1983. Survey of heat-stable, major somatic antigens of *Pseudomonas aeruginosa*. Int. J. Syst. Bacteriol. **33**:256–264.
 32. Lovrekovich, L., and Z. Klement. 1961. Species-specific antigens of *Pseudomonas tabaci*. Acta Microbiol. Acad. Sci. Hung. **8**:303–310.
 33. Lovrekovich, L., Z. Klement, and W. J. Dowson. 1963. Serological investigation of *Pseudomonas syringae* and *Pseudomonas morsprunorum* strains. Phytopathol. Z. **47**:20–24.
 34. Lucas, L. T., and R. G. Grogan. 1969. Serological variation and identification of *Pseudomonas lachrymans* and other phytopathogenic nomenclatures. Phytopathology **59**:1908–1912.
 35. Mazzucchi, U. 1975. Vascular blackening in sugar beet taproots caused by *Pseudomonas syringae*. Phytopathol. Z. **84**:289–299.
 36. Oprea, F. 1971. Serological investigations on bacteria *Pseudomonas morsprunorum* and *syringae*. Rev. Roum. Biol. Ser. Bot. **16**:213–220.
 37. Otta, J. D. 1977. Occurrence and characteristics of isolates of *Pseudomonas syringae* on winter wheat. Phytopathology **67**:22–26.
 38. Otta, J. D., and H. English. 1971. Serology of *Pseudomonas syringae*. Phytopathology **61**:443–452.
 39. Ouchterlony, O. 1958. Diffusion-in-gel methods for immunological analysis. Prog. Allergy **5**:1–78.
 40. Ovod, V., P. Ashorn, L. Yakovleva, and K. Krohn. 1995. Classification of *Pseudomonas syringae* with monoclonal antibodies against the core and O-side chains of the lipopolysaccharide. Phytopathology **85**:226–232.
 41. Palleroni, N. J. 1984. Family I *Pseudomonadaceae*. Genus I *Pseudomonas*. 6 *Pseudomonas syringae*, p. 168–172. In N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. Williams & Wilkins, Baltimore.
 42. Pastushenko, L. T., and I. D. Simonovich. 1979. Groupes sérologiques des bactéries phytopathogènes du genre *Pseudomonas*. I. Parenté antigénique intraspécifique. Mikrobiol. Zh. **41**:222–229. (Translated by T. Babilion, Institut National de la Recherche Agronomique, Versailles, France.)
 43. Pastushenko, L. T., and I. D. Simonovich. 1979. Groupes sérologiques des bactéries phytopathogènes du genre *Pseudomonas*. II. Parentés antigéniques de diverses espèces. Mikrobiol. Zh. **41**:330–339. (Translated by T. Babilion, Institut National de la Recherche Agronomique, Versailles, France.)
 44. Perlasca, G. 1960. Relationships among isolates of *Pseudomonas syringae* pathogenic on stone fruit trees. Phytopathology **50**:889–899.
 45. Prunier, J. P., J. Luisetti, and L. Gardan. 1970. Etudes sur les bactérioses des arbres fruitiers. II. Caractérisation d'un *Pseudomonas* non fluorescent agent d'une nouvelle bactériose du pêcher. Ann. Phytopathol. **2**:181–197.
 46. Samson, R., and A. Benjama. 1984. Physiological and serological identification of *Pseudomonas syringae* pv. *porri* pathogenic to *Allium porrum*, p. 52–53. Proceedings of the 2nd Working Group on *Pseudomonas syringae* pathovars, 1984. The Hellenic Phytopathological Society, Athens, Greece.
 47. Samson, R., and M. Saunier. 1987. Désignation de références sérologiques pour six sérogroupes de pathovars de *Pseudomonas syringae* sur la base de leur lipopolysaccharide. Bull. OEPP/EPPO Bull. **17**:165–171.
 48. Schaad, N. W. 1979. Serological identification of plant pathogenic bacteria. Annu. Rev. Phytopathol. **17**:123–147.
 49. Schaad, N. W. 1988. Initial identification of common genera, p. 1–15. In N. W. Schaad (ed.), Laboratory guide for identification of plant pathogenic bacteria. American Phytopathology Society Press, St. Paul, Minn.
 50. Schaad, N. W., and B. M. Cunfer. 1979. Synonymy of *Pseudomonas coronafaciens*, *Pseudomonas coronafaciens* pathovar *zeae*, *Pseudomonas coronafaciens* subsp. *atropurpurea*, and *Pseudomonas striafaciens*. Int. J. Syst. Bacteriol. **29**:213–221.
 51. Shafik, H. 1994. Taxonomie des *Pseudomonas* phytopathogènes du groupe de *Pseudomonas syringae*: étude phénotypique et génotypique. Ph.D. thesis. University of Angers, Angers, France.
 52. Smith, A. R. W., S. M. Munro, R. Wait, and R. C. Hignett. 1995. Effect on lipopolysaccharide structure of aeration during growth of a plum isolate of *Pseudomonas syringae* pv. *morsprunorum*. Microbiology **140**:1585–1593.
 53. Spitali, M. 1993. Ph.D. thesis. University of Greenwich, London.
 54. Taylor, J. D. 1970. Bacteriophage and serological methods for the identification of *Pseudomonas phaseolicola* (Burkh.) Dowson. Ann. Appl. Biol. **66**:387–395.
 55. Taylor, J. D. 1972. Specificity of bacteriophages and antiserum for *Pseudomonas pisi*. N. Z. J. Agric. Res. **15**:432–440.
 56. Taylor, J. D., J. R. Bevan, I. R. Crute, and S. L. Reader. 1989. Genetic relationship between races of *Pseudomonas syringae* pv. *pisi* and cultivars of *Pisum sativum*. Plant Pathol. **38**:364–375.
 57. Taylor, J. D., D. M. Teverson, D. J. Allen, and M. A. Pastor-Corrales. 1996. Identification and origin of races of *Pseudomonas syringae* pv. *phaseolicola* from Africa and other bean growing areas. Plant Pathol. **45**:469–478.
 58. Tourte, C., and C. Manceau. 1994. Evaluation of a digoxigenin-labelled phaseolotoxin gene fragment as a DNA probe for the detection of *Pseudomonas syringae* pv. *phaseolicola* from bean seeds. Seed Sci. Technol. **22**:449–459.
 59. Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.
 60. Tsai, C. M., and C. E. Frasch. 1982. A sensitive silver stain for detecting lipopolysaccharides in polyacrylamide gels. Anal. Biochem. **119**:115–119.
 61. Wingate, V. P. M., P. M. Norma, and C. J. Lamb. 1990. Analysis of the cell surface of *Pseudomonas syringae* pv. *glycinea* with monoclonal antibodies. Mol. Plant-Microbe Interact. **3**:408–416.
 62. Young, J. M., J. F. Bradbury, R. E. Davis, R. S. Dickey, G. L. Ercolani, A. C. Hayward, and A. K. Vidaver. 1991. Nomenclatural revisions of plant pathogenic bacteria and list of names 1980–1988. Rev. Plant Pathol. **70**:211–221.
 63. Young, J. M., D. W. Dye, J. F. Bradbury, C. G. Panagopoulos, and C. F. Robbs. 1978. A proposed nomenclature and classification for plant pathogenic bacteria. N. Z. J. Agric. Res. **21**:153–177.
 64. Young, J. M., Y. Takikawa, L. Gardan, and D. E. Stead. 1992. Changing concepts in the taxonomy of plant pathogenic bacteria. Annu. Rev. Phytopathol. **30**:67–105.