Classification of *Pseudomonas syringae* with Monoclonal Antibodies Against the Core and O-side Chains of the Lipopolysaccharide

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ABSTRACT

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Six murine hybridomas producing monoclonal antibodies (MAbs) toward *Pseudomonas syringae* lipopolysaccharide (LPS) were established. Two of these, Ps-core-1 (IgG2a) and Ps-core-2 (IgG3), were found to be specific against an outer-core oligosaccharide epitope, and demonstrated reactivity only in immunoenzymatic assays. Core-specific antibodies recognized unsubstituted LPS bound to nitrocellulose paper, implying that the core-specific epitope is altered or masked by the attachment of the O-chain. The Ps-O:2-1, Ps-O:2-2, Ps-O:2-3 (all IgM), and Ps-O:3-1 (IgG3) MAbs were found to be reactive with O:2 and O:3 polysaccharides of LPS with known chemical structures. O-antigen-specific MAbs were positive for agglutination and precipitation reactions, enzyme-linked immunosorbent assay (ELISA), and immunoblotting analysis. The expression of MAb-specific epitopes by 223 strains belonging to 19 pathovars of *P. syringae,* 16 strains of 8 other pseudomonad spp. as well as 5 strains of *Agrobacterium* and *Xanthomonas* were studied in ELISA. Both core-specific MAbs recognized 99% of the strains from 17 pathovars of *P. syringae.* However, they did not react with any strains from *P. s. coriandricola* and *P. s. lachrymans* or strains of other species of bacteria. Bacteria exhibiting the O:2 phenotype of LPS were detected only within *P. s. atrofaciens* (28 strains), *P. s. glycinea,* (5 strains) and *P. s. syringae* (12 strains). The O:3 phenotype of LPS was detected among strains from *P. s. morsprunorum* (1 strain) and *P. s. syringae* (4 strains). The chemical bases of the O:2 and O:3 serogroup specificity were deduced and a new serological classification scheme for *P. syringae* proposed.

Additional keyword: serogrouping.

Immunological methods are widely used for rapid and accurate identification and classification of several human pathogenic bacteria. For example, *Pseudomonas aeruginosa* strains can be classified into various serogroups based on the immunological reactivity of their heat-stable somatic O-antigens (17,18). In contrast, these methods have rarely been applied for the detection and identification of phytopathogenic *Pseudomonas syringae,* the subject of the present study. An analysis of the Agricultural On-Line Access (Agricola) database citation to agricultural literature acquired by the U.S. National Agricultural Library and cooperating institutions revealed that since 1985 there have been 465 scientific papers that include information on *P. syringae.* Only five of these, however, are on serology (16).

None of the few early attempts to create a serological classification scheme for *P. syringae* has been completed (20,21,24). Lovrekovich et al (19), 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. In the work of Otta and English (21), approximately 450 isolates of bacteria classified as *P. syringae,* from more than 30 host plants, were divided into 10 distinct serotypes based on the reaction of their heat-stable antigens in gel-diffusion tests. According to the scheme proposed by Pastushenko and Simonovich (24) more than 300 strains of phytopathogenic bacteria tested using absorbed rabbit sera against O antigens have been ranked into I–IX(O:1–O:9 in this study) serogroups. More recently, Samson et al (5,28) distinguished six serogroups (PHA, MOP, LAC, TAB, APTPIS, and PER-SAV-TOM) of *P. syringae.* All of these serological studies investigated the reactivity of thermostable somatic antigens with absorbed antisera, posing problems with cross-reactivity and standardization of reagents (6,19,35). Furthermore, the sets of bacterial strains used by Otta and English (21), Pastushenko and Simonovich (24), and Samson et al (28) were not the same. Thus, the proposed classification schemes cannot be compared.

With *P. aeruginosa,* the diagnostic accuracy of immunological methods has been improved by the use of monoclonal antibodies (MAbs) instead of polyclonal antisera (13–15). Such reagents have also been generated against some *P. syringae* O-antigens, but information about their use in bacterial identification and classification is limited (2,30,33). MAbs should improve the serological classification of *P. syringae,* since both the chemical composition of the core oligosaccharides (4,37) and the chemical formulas of O-polysaccharides (OPS) of the lipopolysaccharide (LPS) (1,4,29,36,37) have recently been established for several strains of this species.

The present study is part of a series of investigations in which MAbs against the LPS of different *P. syringae* pathovars with established chemical structures of the OPS (36, 37) were produced and utilized for serological classification, epitope mapping, and OPS structure prediction. Here we describe antibodies specific for the surface-exposed common outer-core epitope as well as for the polysaccharides O:2 and O:3 of *P. syringae.* The distribution of the specific epitopes among bacterial strains belonging to different pathovars of *P. syringae* was studied. Based on our results and earlier findings on the OPS structures of these bacteria, we discuss the chemical bases of O:2 and O:3 serogroup-

specificities and propose a new serological classification scheme for *P. syringae*.

MATERIALS AND METHODS

Bacterial strains and culture conditions. Strains are given in Table 1. The strains Ro (rough, piliated), HB (smooth, piliated), Ro+1 (rough, nonpiliated), SP-10 (smooth) and SP-35 (smooth) of *P. s. phaseolicola* were obtained from M. Romantchuck (University of Helsinki, Finland). Bacteria were grown on potato-dextrose agar (Difco Laboratories, Detroit, MI) at 28 C for 48 h.

LPS preparation. Bacterial cells grown on solid medium and scraped into phosphate buffered saline (PBS) (20 mM NaH_2PO_4/Na_2HPO_4, 14 mM NaCl, pH 7.4) were sedimented by centrifugation at room temperature at 2,000 *g* for 20 min and washed twice with PBS. Two grams of wet bacterial biomass were resuspended in 1 ml of Tris-HCl (20 mM)/NaCl (150 mM)/EDTA (10 mM), pH 7.4 (TBS-EDTA) and sonicated (Sonorex Super RK 510H, Bandelin Electronic, Berlin, Germany) at 60 C for 25 min. The preparations were then heated at 100 C for 1 h, centrifuged as above and the supernatants (soluble LPS preparations) stored at −20 C for subsequent analysis.

Production of MAbs. The hybridoma cell lines producing MAbs were generated according to standard hybridoma technology (3). Briefly, 10-wk-old female Balb/c mice were immunized with an intraperitoneal 0.4 ml injection containing 5×10^7 heat-killed

TABLE 1. Reactivity of murine monoclonal antibodies in enzyme-linked immunosorbent assay with different strains of bacteria

Species, pathovar, strain	Reactivity with monoclonal antibodies[a]	
	Ps-core-1, Ps-core-2	Ps-O:2-1, PsO:2-2, Ps-O:2-3
Pseudomonas syringae		
apii		
GSPB[b] 2153	P	N
aptata		
GSPB 61, 62, 1084, 1090, 1284, 1287, 1289	P	N
IMV 8544, 8545	P	N
atrofaciens		
ICMP[c] Y1852, Y4516, Y5011, Y5016	P	Y1852, Y5011
IIPGR[d] V1, V3, V4, V5, V7, V12, V14, V18, V23, V28, V33, V40, V46, V58	P	V1, V3, V4, V5, V7, V12, V18, V23, V33, V40
IMV[e] 234a, 870x[f], 948, K-1025[f], 1052[f], 1392, 2399, 2846, 7194, 7836, 7932, 8099, 8116, 8122[f], 8241, 8247, 8281, 8408, 8410a, 8413, 8540[f], 8548, 8904, 8999, 9005[f], 9006[f], 9010	P	870x, K-1025, 1052, 8122, 8540, 9005, 9006
IPPG[g] Pa1, Pa7, Pa9, Pa13, Pa14, Pa16, Pa17, Pa19, Pa22, Pa24, Pa26, Pa39	P	Pa7, Pa9, Pa13, Pa17, Pa22, Pa24, Pa26, Pa39
NCPPB[h] 117, 2612, 2712S, 2818	P	2712S
capsici		
GSPB 109	P	N
coriandricola		
GSPB 1798, 1816, 1914, 1963, 1964, 2150, 2151	N	N
NCPPB 3115, 3780, 3781	N	N
coronafaciens		
NCPPB 600	P	N
glycinea		
IMV L-25, 8541	P	N
GSPB 1834, 1835, 1836, 1837, 1838, 1969, 1993, 1997, 2003	P	1834, 1836, 1969, 1997, 2003
japonica		
ICMP Y6305	P	N
lachrymans		
GSPB 69, 77, 82, 229	N	N
IMV 0-3, 1-3, 5a, 10A, 13, r-14, 40, 40a, 182, 249b, 250b, 435a 617, 7591, 7593, 7594, 7595	N	N
maculicola		
IMV 381	P	N
GSPB 2145	P	N
morsprunorum		
IMV CF-4[f], 175-2[f], 186[f]	P	N
GSPB 836, 854, 877, 883, 887, 1013, 1207, 1481	P	N
papulans		
NCPPB 2848	P	N
persicae		
GSPB 1025	P	N

(*continued on next page*)

[a] Only four strains P55, 218, 839, and 8414 from pv. *syringae* and one strain 854 from pv. *morsprunorum* were positive with monoclonal antibody Ps-O:3-1. All strains tested were positive (P) or negative (N), respectively, with corresponding MAbs. When only some strains were positive these are listed; rare negative reactivity strains are indicated by a superscript N.
[b] Göttinger Sammlung Phytopathogener Bakterien, Göttingen, Germany.
[c] International Collection of Microorganisms from Plants, Auckland, New Zealand.
[d] Institute of Introduction and Plant Genetic Resources, Sadovo, Plovdiv, Bulgaria.
[e] Institute of Microbiology and Virology, Kiev, Ukraine.
[f] *P. syringae* strains grouped earlier (24) using rabbit polyclonal sera into O:2 serogroup.
[g] Institute of Plant Protection, Göttingen, Germany.
[h] National Collection of Plant Pathogenic Bacteria, Harpenden, UK.
[i] Gift from M. Romantchuck, University of Helsinki, Finland.
[j] *P. syringae* strains grouped earlier (24) using rabbit polyclonal sera into O:3 serogroup.

cells of *P. s. syringae* strain NCPPB 281 (two mice), *P. s. atrofaciens* IMV K-1025 (two mice), or *P. s. syringae* IMV P-55 (two mice) emulsified in Freund's incomplete adjuvant (Difco)/PBS (1:1). Intraperitoneal booster immunizations with the same immunogens were given on day 12. A third, 0.2 ml, booster containing 10^7 bacterial cells was given 2 wk later through a tail vein. The mice were killed 3 days later and their spleens removed. The primed splenocytes were fused with Sp2 myeloma cells in 50% polyethylene glycol (PEG 4000; Merck, Darmstadt, Germany). Fused cells were distributed into 10 flat-bottom 96-well plates and hybridomas were selected in culture medium containing hypoxanthine, aminopterin, and thymidine. Antibody production was monitored by enzyme-linked immunosorbent assay (ELISA). Selected cell lines were cloned and subcloned by limiting dilution, and established hybridomas were used to produce specific immunoglobulins in RPMI 1640 medium. Immunoglobulins from the cell culture supernatants were prepared as described previously (22) and their isotypes determinated by double immunodiffusion against antimouse immunoglobulin class- and subclass-specific rabbit antisera (Sigma, Corp. St. Louis, Missouri).

ELISA. Microtitre ELISA plates (Maxisorb, Nunc, Roskilde, Denmark) were coated with 50 μl of LPS solution (100 μl of soluble LPS preparation, undigested or digested with proteinase K in 1.9 ml of PBS) and incubated either at 4 C or at room temperature for 10–12 or 2 h, respectively. The oxidation of bound LPS

TABLE 1. (*continued from preceding page*)

Species, pathovar, strain	Reactivity with monoclonal antibodies[a]	
	Ps-core-1, Ps-core-2	Ps-O:2-1, PsO:2-2, Ps-O:2-3
phaseolicola		
GSPB 567, 665, 1547, 1549, 1550, 1551, 1678a	P	N
IMV 120a, 4012, 4013 (rough), 4270, 7842 (rough)	P	N
NCPPB 52	P	N
NPSW 3121	P	N
Romantchuk[i] HB, Ro, Ro+1, SP-10, SP-35	P	N
pisi		
GSPB 105, 231, 1206, 1447	P	N
IMV 7151, 7157	P	N
syringae		
GSPB 839, 1004, 1150, 2035, 2036	P	N
ICMP Y3023, Y3080, Y3406, Y4965	P	Y4965, 90a, 193, 197, 288, 467, 1279, 1297
IMV P-55[j], 90a[f], 181, 193[f], 197[f], 218[j], 288[f], 435[f], 467[f], 1055a 1279[f], 1297[f], 7921, 7923, 8299, 8300, 8302, 8414[j]	435[N], 8300[N], 8302[N]	
IPPG A106, B301D, DG100, DG101A, 1DG112, DG118, DG124, DG130, RDG136A, DG142, Ru1441, Ru1686	P	1DG112, RDG136A, Ru1686
IIPGR V14, V37, V38	P	N
NCPPB 281, 1023, 1053, 2509, 2842	P	1023
striafaciens		
ICMP Y4483	P	N
tabaci		
GSPB 114, 117, 1209, 1789	P	N
IIPGR GK35-1A2, T1A12, T ECHANDI, T RUFTY, T2-14, T-45, T1128A1, T1133A1, T1130A2, T1133A2	P	N
IMV 223, 225	P	N
tomato		
GSPB 477, 478, 483, 487, 492, 493, 505, 518, 1776, 1778	P	N
IIPGR 140, Ro	P	N
IMV 8290	P	N
P. aeruginosa		
PAO-1, 140 (clinic), 1960 (clc.), 1961 (clc.)	N	N
P. avenae		
ICMP Y3183	N	N
P. cepacia		
IMV 3181	N	N
P. cichorii		
ICMP 9048, 9416	N	N
P. fluorescens		
IMV B-25, 4125, 8937	N	N
P. gladioli pv. *alliicola*		
IMV 89, 8494, 8495	N	N
P. putida		
IMV B-39	N	N
P. solanacearum		
ICMP 9049	N	N
Agrobacterium tumefaciens		
NCPPB 2437	N	N
Xanthomonas campestris		
pv. *campestris*		
IMV 8003b, 8162	N	N
pv. *cucurbitae*		
IMV 8635, 8689	N	N

with sodium periodate was carried out by the method of Woodward et al (34). The antigen-coated plates were washed twice with PBS, pH 7.4, containing 0.1% (v/v) Tween 20 and blocked with PBS containing 1.5% (w/v) bovine serum albumin. Bound antibodies were immunoenzymatically visualized as described previously (22,34).

Whole cell agglutination and immunodiffusion. MAbs precipitated from culture supernatants with 50% saturated ammonium sulfate were dialyzed against PBS. Two hundred microliters of serial twofold antibody dilutions were added to single wells in U-bottom 96-well plates (Flow Laboratories, Irvine, UK). Ten microliters of a suspension containing 10^{10} heat-killed bacterial cells per milliter in PBS were added into each well, mixed, and allowed to settle for 10 h at room temperature. The agglutination was monitored visually. Double-diffusion assays were performed in 1% (w/v) agarose gel in PBS. The wells were filled with sonicated and heat-killed bacterial cells and MAb prepations were added to the antibody wells. Precipitin lines were observed visually after 24–48 h of incubation at room temperature.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis and immunoblotting analysis. Soluble LPS preparations or whole bacterial cells prepared by the proteinase K method of Hitchcock and Brown (7) were run in sodium dodecyl sulfate–polyacrylamide gel electrophoresis (12) with 5% stacking and 12.5% separation gels. Because purified LPS does not transfer onto nitrocellulose paper (NCP) (25) as efficiently as outer membrane proteins, we also used enzymatically untreated antigen preparations containing LPS closely associated with outer membrane proteins in the immunoblotting analysis. Biopolymers were electrotransferred from the gel onto NCP (31), and the blots were treated and immunoenzymatically developed as described previously (23). The oxidation of transferred antigens (LPS) with sodium periodate was carried out by the method of Woodward et al (34).

RESULTS

Isolation of LPS-specific hybridomas. Hybridomas were identified in more than 30% of the tissue culture wells from every fusion experiment performed. After primary screening, 31 clones were shown to produce antibodies reacting with antigens of homologous strains in ELISA. Corresponding supernatants were then tested for ELISA reactivity against a limited number of *P. syringae* strains from serogroups O:1–O:9 (24), other pseudomonad spp., as well as bacteria belonging to *Xanthomonas* or *Agrobacterium* spp. Six hybridoma cultures were selected and subcloned by limiting dilution to yield monoclonal cell lines. Two of these cell lines, designated clones #1 and #2, (immunogen strain NCPPB 281) reacted with all initially tested strains of *P. syringae*. Three additional clones, designated #3, #4, and #5 (immunogen strain IMV K-1025), were specific for strains of the O:2 serogroup; one hybridoma, clone #6, produced MAb only against the homologous strain IMV P-55 (O:3 serogroup).

Characterization of the monoclonal antibodies. To further characterize the six MAbs they were used for immunostaining of homologous LPS preparations resolved by 12.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and electrically transferred onto NCP (Fig. 1). MAbs #1 and #2 detected fast-moving, low molecular weight components, presumably containing rough lipopolysaccharide (R-LPS). In contrast, MAbs #3, #4, #5, and #6 demonstrated reactivity with high molecular weight components in a ladderlike profile, previously shown to represent smooth lipopolysaccharide (S-LPS) molecules with different lengths of OPS (23, 25-27). MAbs #1 and #2 probably react with an epitope on the core oligosaccharide of LPS, whereas MAbs #3–6 detect epitopes within O-polysaccharide (OPS) of LPS of *P. syringae*.

Immunodiffusion against immunoglobulin isotype-specific antibodies indicated that one MAb (#1) was an IgG2a, three MAbs (#3-5) were IgM, and two (#2, #6) were IgG3 (Table 2). The four S-LPS-specific MAbs agglutinated heat-killed *P. syringae* cells and precipitated soluble LPS preparations, whereas the two R-LPS-specific antibodies reacted only in immunoenzymatic assays (ELISA and immunoblotting). Treatment of LPS-antigens, either coated on ELISA plates or electrotransferred onto NCP, with sodium periodate resulted in loss of reactivity with all six antibodies. Proteinase K digestion of LPS had no effect on antibody reactivity. Based on these results, the six hybridoma cell lines and the corresponding antibodies were designated Ps-core-1, Ps-core-2, Ps-O:2-1, Ps-O:2-2, Ps-O:2-3 and Ps-O:3-1. Ps indicates *P. syringae*; core and O:2- or O:3- refer to the LPS core designation and O:2 or O:3 O-antigen polysaccharide-specificity, respectively; the last digit indicates clone identification.

Serogrouping of bacterial strains with MAbs. To assess the serogrouping potential of the MAbs we tested their reactivity in ELISA with soluble LPS preparations of 272 bacterial strains (Table 1). Ps-core-1 and Ps-core-2 antibodies reacted with 99% of *P. syringae* strains (199/202) representing 17 pathovars but with none of the other species tested. All tested strains of *P. s. coriandricola* (10 strains) and *P. s. lachrymans* (21 strains) as well as three *P. s. syringae* strains (8300, 8302, and 435) did not react with these antibodies. Fourteen out of the 18 *P. syringae* strains previously classified as belonging to the O:2 serogroup (24) reacted with O:2 serogroup-specific MAbs.

One *P. s. syringae* strain (435) and three (CF-4, 175-2, 186) from *P. s. morsprunorum* previously classified into the O:2 serogroup (24) did not react with these MAbs. The 38 additional strains among *P. s. atrofaciens, P. s. glycinea,* and *P. s. syringae,* which were not tested previously with rabbit serogroup-specific antisera, also were grouped into the O:2 serogroup.The O:3 serogroup-specific MAb Ps-O:3-1 reacted with a limited number

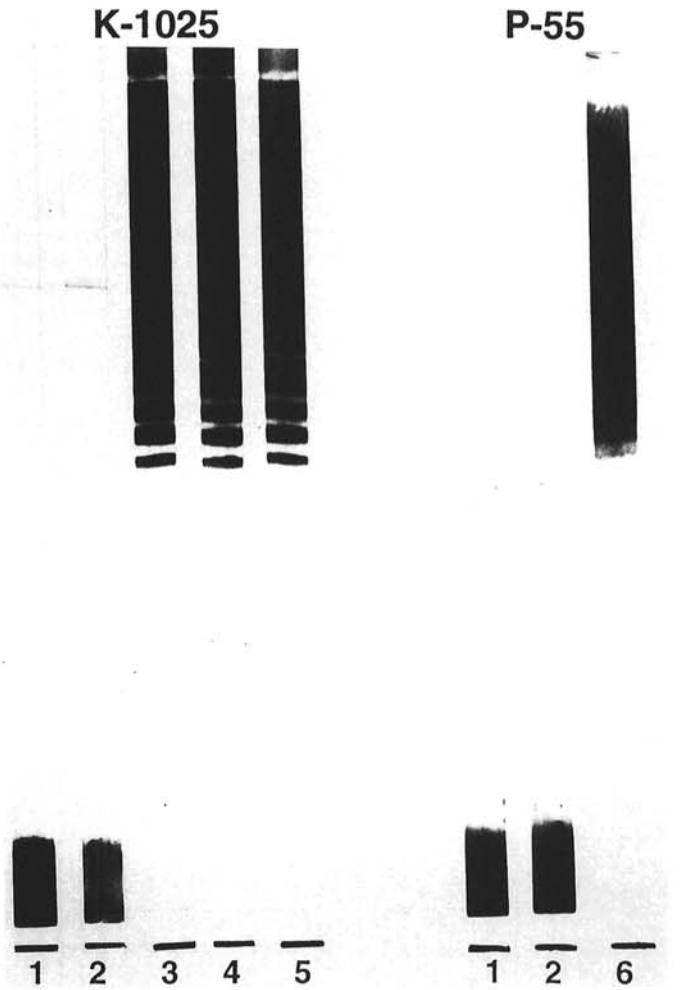

Fig. 1. Immunoblots of *Pseudomonas syringae* pv. *atrofaciens* K-1025 (A) and *P. s. syringae* P-55 (B) LPS after separation on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (12.5% gel) and visualization with murine monoclonal antibodies Ps-core-1 (#1), Ps-core-2 (#2), Ps-O:2-1 (#3), Ps-O:2-2 (#4), Ps-O:2-3 (#5) and Ps-O:3-1 (#6).

(5/233) of strains of *P. syringae* in ELISA. Only four *P. s. syringae* strains (P-55, 218, 839, and 8414) and one *P. s. morsprunorum* strain (854) were classified into the O:3 serogroup. None of the O:2 or O:3 serogroup-specific MAbs reacted with bacterial strains from other pseudomonad species or more distantly related phytopathogenic bacteria (Table 1).

DISCUSSION

We established six murine myeloma lines secreting MAbs specific for the surface-exposed, heat- and protease-resistant but periodate-sensitive molecules of *P. syringae* (Table 2). Four of the MAbs (Ps-O:2-1, Ps-O:2-2, Ps-O:2-3, and Ps-O:3-1) agglutinated whole bacterial cells and precipitated the antigens in agarose gels, suggesting multivalency of the antigenic epitopes (26). In immunoblots these antibodies reacted with the antigen to give ladderlike profile. Both features support our suggestion that the specific antigens are the O chains of S-LPS. The two other antibodies (Ps-core-1 and Ps-core-2) were unable to agglutinate or to precipitate the specific antigens. Moreover, in immunoblots the antibodies only reacted with fast-moving molecules characteristic of R-LPS (Fig. 1) (7, 23, 26-27). These two antibodies did not react with the S-LPS molecules, suggesting either that the core-specific epitope is sterically masked by the O-chain or that the O-chain is covalently attached to the core at the site defining the specific epitope.

Having shown the specificity of the MAbs against OPS or outer-core epitopes of the LPS of *P. syringae,* we utilized these immunoreagents to analyze the presence of the corresponding epitopes in different pathovars of *P. syringae* as well as in other bacterial species. The results in Table 1 indicate that the core-associated epitope is highly conserved among strains belonging to different *P. syringae* pathovars and is expressed in most of the strains examined. Three strains of *P. s. syringae* (435, 8300, and 8302) were not recognized by core-specific MAbs, presumably due to chemical variation within the core oligosaccharide or masking effect of the O-chain.

Thus, *P. syringae* strains representing different pathovars share a serologically related LPS core. This observation is in agreement with the data showing that core oligosaccharides of *P. syringae* strains from different pathovars typically consist of rhamnose, glucose, galactosamine, glucosamine, KDO, alanine, and phosphorus (4,37). These results may be of taxonomic and diagnostic importance.

The O:2 serogroup-specific MAbs recognized, with some exceptions (Table 1), all bacterial strains that were previously classified into this serogroup using rabbit antisera (24). The great majority of *P. syringae* strains expressing the O:2 phenotype of LPS were from *P. s. atrofaciens* and *P. s. syringae*. In contrast, only five strains from *P. s. morsprunorum* and *P. s. syringae* expressed the O:3 phenotype in this study. The latter phenotype may rarely occur within *P. syringae* spp. in general.

The chemical structure of OPS has previously been established for some of the strains studied here (8–11). According to Knirel et al (11) the O units of LPS of *P. s. atrofaciens* strain K-1025 and of *P. s. syringae* strain 90a are identical branched pentasaccharide homopolymers of α-D-rhamnose (Table 3, key [A]). These two strains, as well as *P. s. syringae* strain 467, have identical serological reactivity with serogroup O:2 polyclonal sera (24) and also reacted with our O:2-specific MAbs (Table 1). However, strain 467 was reported (10) to contain linear trisaccharide O repeating units of α-D-rhamnose (Table 3, key [E]). An identical chemical structure was reported for strain C28 (= GSPB 883 in this study) of *P. s. morsprunorum* (29). Moreover, strains 218 and P-55 (10), which reacted with the O:3-specific MAb, and strain 435 (32), which reacted neither with the O:2- nor with the O:3-specific MAbs (Table 1), were shown to have OPS with similar backbones of trisaccharide α-D-rhamnose. In strains 218 and P-55, 35 or 85% of the O-units were substituted by the lateral (α→4)D-fucofuranosyl, respectively (Table 3, key [G]) and the OPS of the strain 435 has a lateral (α→3)D-rhamnosyl substituent (Table 3, key [F]). Taken together, these results suggest that serogroup O:2 and O:3 specificities cannot be based only on the chemical structure of the OPS backbones, because strains with the same backbone have different reactivities both with the polyvalent rabbit antisera and with our monoclonal antibodies. Nor can serogroup specificity be based solely on the lateral substituent, since similar (α→3)D-rhamnosyl structures are found in strains K-1025 and 90a, which react with the O:2 MAbs and in the strain 435, which was not reactive. Likewise, strains P-55 and 218, showing reactivity only with the O:3 MAb, have a lateral substituent on their OPS backbones similar to that on strains belonging to the O:6 serogroup (Table 3, key [B]).

We can, therefore, conclude that the serological reactivity of the LPS in *P. syringae* is determined by several features of the chemical structure of OPS including 1) the type of the sugar residues composing the backbone, 2) the number of the sugars in the repeating unit of the backbone, and 3) the type of the lateral substituents. It appears that the chemical structure responsible for the serogroup O:2 specificity consists of a pentasaccharide with an α-D-rhamnose backbone and with an immunodominant (α→3)D-rhamnosyl lateral substituent, and that serogroup O:3 specificity consists of a tetrasaccharide with an α-D-rhamnose backbone and immunodominant (α→4)D-fucosyl lateral substituent. This conclusion implies that the chemical structure of *P. s. syringae* strain 467 has been described incorrectly.

Based on the published descriptions of the chemical structure of the OPS of *P. syringae* having O-units with four α-D-rhamnose residues in its backbone (36,37) and the serological results obtained with polyclonal sera (24) and our MAbs, we propose that these strains should be arranged into a new serogroup O1 (Table 3). The strains within this serogroup should be classified into serotypes 1b (equals former serogroup O:2), 1c (= O:6), and 1d (= O:4). To be classified into a single serogroup O1, each strain, however, must possess one common epitope 1a in its OPS backbone (Table 3). Similarly, the strains having three a-D-rhamnose residues in the backbone should be arranged into a new serogroup O2 with serotypes 2a, 2b, and 2c (= O:3) (Table 3). In this paper, we demonstrated the presence of the 1b and 2c serotypes. In addition, analysis of published data obtained with polyclonal antisera demonstrated the presence of at least 1c (= O:6) and 1d (= O:4) serotypes (36,37). We have now been able to develop MAbs reacting with the 1c, 1d, and 2a serotypes as well as antibodies recognizing the 1a epitope specific for the O1 serogroup, and these will be described in future papers.

TABLE 2. Characterization of the murine monoclonal antibodies specific against *Pseudomonas syringae* lipopolysaccharide

Clone Number	MAb	Isotype	Reactivity in immunological assays				Antigen specificity
			Agglutination	Precipitation	ELISA[a]	Immunoblotting	
1	Ps-core-1	IgG2a	−	−	+	+	Core
2	Ps-core-2	IgG3	−	−	+	+	Core
3	Ps-O:2-1	IgM	+	+	+	+	OPS[b]
4	Ps-O:2-2	IgM	+	+	+	+	OPS
5	Ps-O:2-3	IgM	+	+	+	+	OPS
6	Ps-O:3-1	IgG3	+	+	+	+	OPS

[a]Enzyme-linked immunosorbent assay.
[b]O polysaccharide.

TABLE 3. Structure of repeating units and suggested serological formulas of O polysaccharides of *Pseudomonas syringae* strains containing D-rhamnose backbones

Pathovar, strain, chemical structure	(Key)	Reference	Serogroup[a]	Suggested serological formula
atrofaciens K-1025 *syringae* 90a [→3) DRha (α1→3) DRha (α1→2) DRha (α1→2) DRha (α1→] n ↑ (α3←1) DRha	A	11	O:2	O1a,b
atrofaciens 2399 *phaseolicola* 120a *syringae* 8299 [→3) DRha (α1→3) DRha (α1→2) DRha (α1→2) DRha (α1→] n ↑ (α4←1) DFuc	B	9	O:6	O1a,c
atrofaciens 2846 *glycinea* L-25 *syringae* 1055a and 7923 [→3) DRha (α1→3) DRha (α1→2) DRha (α1→2) DRha (α1→] n ↑ (β4←1) DGlcNAc	C	8	O:4	O1a,d
Hypothetical OPS structure [→3) DRha (α1→3) DRha (α1→2) DRha (α1→2) DRha (α1→] n	D			O1a,e,...
syringae (*cerasi*) 467 *morsprunorum* C28 (= GSPB 883) [→3) DRha (α1→3) DRha (α1→2) DRha (α1→] n	E	10, 29	O:2	O2a
syringae (*cerasi*) 435 [→3) DRha (α1→3) DRha (α1→2) DRha (α1→] n (α3←1) DRha	F	32	O:2	O2b
syringae P-55 and 218 [→3) DRha (α1→3) DRha (α1→2) DRha (α1→] n				O2a
and [→3) DRha (α1→3) DRha (α1→2) DRha (α1→] n ↑ (α4←1) DFuc	G	10	O:3	O2c

[a]Serogrouping based on results of Pastushenko and Simonovich (24) using rabbit-absorbed sera.

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