JOURNAL OF BACTERIOLOGY, Nov. 1996, p. 6459–6465 Vol. 178, No. 22
0021-9193/96/$04.00+0

Immunochemical Characterization of O Polysaccharides Composing the α-D-Rhamnose Backbone of Lipopolysaccharide of *Pseudomonas syringae* and Classification of Bacteria into Serogroups O1 and O2 with Monoclonal Antibodies

VLADIMIR OVOD,[1,2]* KLAUS RUDOLPH,[3] YURIY KNIREL,[4] AND KAI KROHN[1]

Institute of Medical Technology, University of Tampere, Tampere, Finland[1]; Department of Microbiology and Immunology, University of Kiev, Kiev, Ukraine[2]; Institut für Pflanzenpathologie und Pflanzenschutz der Georg-August-Universität, Göttingen, Germany[3]; and N. D. Zelinsky Institute of Organic Chemistry, Moscow, Russia[4]

Received 6 June 1996/Accepted 4 September 1996

Murine monoclonal antibodies (MAbs) reacting with *Pseudomonas syringae* lipopolysaccharide (LPS) O polysaccharides (OPS) composed of tetra- and tri-α-D-rhamnose repeats in the backbone [3)D-Rha(α1-3)D-Rha (α1-2)D-Rha(α1-2)D-Rha(α1] and [3)D-Rha(α1-3)D-Rha(α1-2)D-Rha(α1] were generated and used for immunochemical analysis and for serological classification of the bacteria. A total of 195 of 358 *P. syringae* strains tested representing 21 pathovars were shown to share a common epitope, 1a, and were classified into serogroup O1. All strains with pathovars aptata, glycinea, japonica, phaseolicola, and pisi, most of the strains with pathovars atrofaciens and striafaciens, and half of the strains with pathovar syringae were classified into serotypes O1a′, O1b, O1c, and O1d within serogroup O1. Serogroup-specific epitope 1a was inferred to be related to the (α1-2)D-Rha(α1-3) site of the OPS backbone. The serotype-specific epitopes 1b, 1c, 1d, and 1a′ were inferred as relating to the immunodominant lateral (α1-3)D-Rha, (β1-4)D-GlcNAc, and (α1-4)D-Fuc substituents and backbone-located site (α1-3)D-Rha(α1-2), respectively, of OPSs that share the common tetra-D-rhamnose repeats in the backbone. A total of 7.3% of the strains studied, all with pathovars morsprunorum and lapsa, were classified as serotypes O2a and O2d within serogroup O2. Serotype-specific epitope 2a was inferred as being related to the backbone-located site D-Rha(α1-3)D-Rha and epitope 2d to the immunodominant lateral (α1-4)D-Fuc residue of OPS consisting of tri-D-rhamnose repeats in the backbone. Epitope 2d alternated with 2a within the same LPS molecule and did not cross-react with epitope 1d. Serotypes O2a and O2d were observed in some strains correlating with the coexpression of the two chemotypes of OPS by the same strain. The serogroup O1-specific MAb Ps1a reacted weakly but definitely with all strains from serogroup O2. We propose serological formulas for serogroups O1 and O2 as well as for individual strains within these serogroups.

All oxidase-negative and arginine dihydrolase-negative fluorescent bacteria are currently classified as *Pseudomonas syringae* van Hall. This is the most prevalent species within the pseudomonads (36). More than 50 distinct pathovars have been identified to meet the needs of plant pathologists concerned with the differential pathogenicity of *P. syringae* strains (41, 50, 51). Although the bacterium was originally isolated from lilacs, the pathovars of *P. syringae* cause diseases on nearly every cultivated plant and on a hitherto-unknown number of wild plant species. The pathovars of *P. syringae* are becoming appreciated not only as phytopathogens but also as common epiphytes of healthy host and nonhost plant species, as well as ice nucleation agents capable of inciting frost damage in plants (11, 24).

The taxonomy (classification, nomenclature, and identification) of *P. syringae* in general and the trinominal nomenclature in particular are confusing, and it is difficult to identify each pathovar solely on the basis of biochemical and physiological features. Many *P. syringae* pathovars can infect only specific plant species, whereas others, especially *P. syringae* pv. syringae, possess a wide host range (3, 4, 9, 41). The ability of medical bacteriologists to provide rapid and accurate serological identification of gram-negative bacteria based on the lipopolysaccharide (LPS) diversity (25, 26, 30, 31) indicates a similar potential for the taxonomy of *P. syringae*. The serological features of *P. syringae* have not as yet been studied thoroughly (24). None of the earlier (33, 37, 40) or recent (10) attempts to create a serological classification scheme of *P. syringae* has been completed. A limited number of *P. syringae* strains have been studied with monoclonal antibodies (MAbs) (5, 35, 43, 48), known to be a powerful tool for taxonomic purposes (22, 23, 29, 45). The molecular structure of the O-polysaccharide (OPS) repeating units of LPS have recently been elucidated for many strains of *P. syringae* (1, 2, 8, 14, 32, 42). However, the discrepancies between the chemical structures of OPS (14) and the previously proposed serological classification scheme (37) could be clearly noticed. A detailed description of the molecular structure of the antigenic determinants of LPS is essential in order to study the biosynthesis of these macromolecules and the tremendous diversification in the OPS and to understand their role in plant pathogen interactions.

We have recently initiated a systematic investigation with the aim of producing MAbs to LPS of *P. syringae* with elucidated primary chemical structures of OPS and using these immu-

* Corresponding author. Mailing address: Institute of Medical Technology, University of Tampere, P.O. Box 607, Fin-33101, Tampere, Finland. Phone: 358-31-2157757. Fax: 358-31-2157332. Electronic mail address: Ltvlov@uta.fi.

TABLE 1. Partial characterization of murine MAbs against OPSs of *P. syringae* lipopolysaccharide

Bacterial strain used for immunization	Former serogroup[a]	MAb[b]	Isotype[c]	O serotype designation	Reactivity with homologous LPS by[d]:			
					AG	PR	ELISA	WB
P. syringae strains								
IMV 7836 pv. atrofaciens		Ps1a	IgG3	1a	±	−	+	+
		Ps1a′	IgG3	1a′	±	−	+	+
		Ps1x	IgG1	1x	+	+	+	+
IMV K-1025 pv. atrofaciens	II	Ps1b	IgM	1b	+	+	+	+
NCPPB 2612 pv. atrofaciens	IV	Ps1c	IgM	1c	+	+	+	+
IMV 120a pv. phaseolicola	VI	Ps1d	IgM	1d	+	+	+	+
GSPB 883 pv. morsprunorum	II	Ps2a	IgG1	2a	±	±	+	+
IMV P-55 pv. morsprunorum	III	Ps2d	IgG3	2d	+	+	+	+
B. cepacia IMV 3181		Bc1a	IgG3		+	+	+	+

[a] Former serogroup identified by Pastushenko and Simonovich (37).
[b] Ps1b and Ps2d were previously identified as Ps-O:2-1 and Ps-O:3-1, respectively (35).
[c] Ig, immunoglobulin.
[d] AG, agglutination; PR, precipitation; WB, Western blotting.

noreagents to classify the bacteria serologically and to define the specific epitopes. In our previous report (35), the core- and O-chain-specific MAbs Pscor1, Ps-O:2-1 (new name, Ps1b), and Ps-O:3-1 (new name, Ps2d) were partially characterized and evaluated for their potential use in serological testing.

Here we report the production and characterization of new MAbs to *P. syringae* OPSs composed of tetra- and tri-α-D-rhamnosyl repeats in the backbone. With these and the previously published (35) MAbs, 195 (54.5%) and 26 (7.3%) of the 358 strains of *P. syringae* were classified into serotypes O1a′, O1b, O1c, O1d, and O1x within serogroup O1 and into serotypes O2a and O2d within serogroup O2. The correlation described between the chemical structures of the OPSs and the specificities of MAbs enabled us to shed some light on the molecular basis of the immunological specificity of the corresponding epitopes.

MATERIALS AND METHODS

Bacterial strains and culture conditions. A total of 358 strains of *P. syringae* representing 21 pathovars (apii, aptata, atrofaciens, cannabina, capsici, coriandricola, coronafaciens, glycinea, japonica, lachrymans, lapsa, maculicola, morsprunorum, papulans, persicae, phaseolicola, pisi, striafaciens, syringae, tabaci, and tomato), 25 strains of *P. syringae* with unknown pathovars, 27 strains of other species of the genus *Pseudomonas* (*P. aeruginosa*, *P. avenae*, *P. cichorii*, *P. fluorescens*, *P. gladioli*, and *P. solanacearum*), 1 strain of *Burkholderia* (*Pseudomonas*) *cepacia*, 2 strains of *Agrobacterium tumefaciens*, and 4 strains of *Xanthomonas campestris* were used in this study. The strains were obtained from the Göttinger Strain Collection of Phytopathogenic Bacteria (GSPB), Göttingen, Germany; the International Collection of Microorganisms from Plants (ICMP), Auckland, New Zealand; the Institute of Introduction and Plant Genetic Resources (IIPGR), Sadovo-Plovdiv, Bulgaria; the Institute of Microbiology and Virology (IMV), Kiev, Ukraine; the Institute of Plant Protection in Göttingen (IPPG), Göttingen, Germany; and the National Collection of Plant Pathogenic Bacteria (NCPPB), Harpenden, United Kingdom. *P. syringae* (17 strains) and *P. fluorescens* (10 strains) isolated from tissue cultures of apples and the surfaces of apple buds and stems intended for propagation were a gift from M. Uosukainen and M.-L. Saleva (University of Helsinki, Finland). All bacteria were cultivated on solid potato dextrose agar (Difco Laboratories, Detroit, Mich.) or LB medium at 28°C for 48 h.

Preparation of LPS. Several LPS preparations were produced and used in this study. (i) LPS was isolated from bacterial cells of the strains of *P. syringae* (Table 1) with known primary structures of OPS (14, 42) by phenol-water extraction (47) followed by ultracentrifugation at 100,000 × *g* for 1.5 h, ethanol precipitation, and dialysis against distilled water. These preparations were used in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western immunoblotting. (ii) LPS from the same strains were also prepared according to the proteinase K digestion method described by Hitchcock and Brown (12) and used in the same experiments. (iii) The fractions enriched with LPS were produced from all bacterial strains used in this study as described previously (34) and studied by enzyme-linked immunosorbent assay (ELISA) with MAbs. Briefly, the bacterial cells grown on potato dextrose agar were harvested and washed twice with phosphate-buffered saline (pH 7.4) by centrifugation at room temperature at 2,000 × *g* for 20 min. Two grams of wet bacterial biomass was resuspended in 2 ml of Tris-HCl (20 mM)-NaCl (150 mM)-EDTA (10 mM) (pH 7.4), heated at 100°C for 1 h, and then sonicated (Sonorex Super RK 510H; Bandelin Electronic, Berlin, Germany) at 70°C for 10 min. The cells were separated by centrifugation, and the supernatants were collected and stored at −20°C.

Production and selection of MAbs. Bacterial cells of *P. syringae* IMV K-1025 pv. atrofaciens, NCPPB 2612 pv. atrofaciens, IMV 120a pv. phaseolicola, GSPB 883 (= C28) pv. morsprunorum, IMV P-55 pv. morsprunorum and *B. cepacia* IMV 3181 with elucidated primary chemical structures of OPSs (14–19, 42) and *P. syringae* IMV 7836 pv. atrofaciens with an unknown primary structure of OPS of LPS were used as an immunogens in mice (Table 1). The antibodies Ps1b and Ps2d (designated previously as Ps-O:2-1 and Ps-O:3-1, respectively) had been produced and partially characterized by us previously (35). The murine hybridoma cell lines producing MAbs were generated according to the standard technology. Details concerning the antigen preparation, dose and schedule of immunization, recloning of hybridomas by limiting dilution, and MAb isotype determination have been previously published (34, 35). In order to select the appropriate MAbs, all hybridoma supernatants were first screened by ELISA with the LPS from the homologous strain. Positive clones were subsequently rescreened with LPS preparations from the reference *P. syringae* strains with known structures of the O repeats (including the LPSs with O repeats consisting of L-rhamnose and mixed L,D-rhamnose in the backbone) (14–19, 42). In order to prove the OPS specificity, those MAbs reacting by ELISA with only the homologous reference LPS were then analyzed by Western blotting with the same antigen. MAbs to all *P. syringae* OPSs with elucidated primary structures of the O repeats (14–19, 42) were finally produced (data not shown). In the preliminary screening of 358 strains of *P. syringae*, one smooth strain (IMV 7836) of pathovar atrofaciens with an unknown OPS structure did not react with any of the produced antibodies. A new set of MAbs to this strain was then produced, and the unique ones, Ps1a, Ps1a′, and Ps1x, were selected for this study (Tables 1 and 2).

The MAbs from hybridoma cell lines were designated as Ps and Bc indicating *P. syringae* and *B. cepacia*, respectively; the first digit refers to the O-chain serogroup; the small letter indicates the OPS serotype (epitope) (Table 1).

Agglutination, Ouchterlony immunodiffusion, ELISA, SDS-PAGE, and Western immunoblotting. All assays (agglutination, Ouchterlony immunodiffusion, ELISA, SDS-PAGE, and Western immunoblotting) were performed basically as described previously (34, 35). The serotyping potential of the MAbs was examined by ELISA with LPS preparations from all bacterial strains used in this study. The LPS prepared by water-phenol extraction (47) or by the proteinase K digestion method (12) was run in SDS-PAGE (21) with 5% stacking and 12.5% separating gels. Biopolymers were electrotransferred from gel onto nitrocellulose membranes (44), and the blots were treated and immunoenzymatically developed as described previously (35). In order to verify the carbohydrate specificity of the produced MAbs, mild periodate oxidation of homologous antigens (5 mM periodic acid [pH 4.5]) that destroy the carbohydrate vicinal hydroxyl groups on sugars without altering the protein or lipid epitopes was carried out by the method described by Woodward et al. (49).

RESULTS

Generation and partial characterization of MAbs. The murine MAbs selected for this study are listed in Table 1. All MAbs reacted strongly with antigens from serologically related *P. syringae* strains by ELISA (optical density at 492 nm of 1.5 to 2.5), whereas no significant background (optical density at

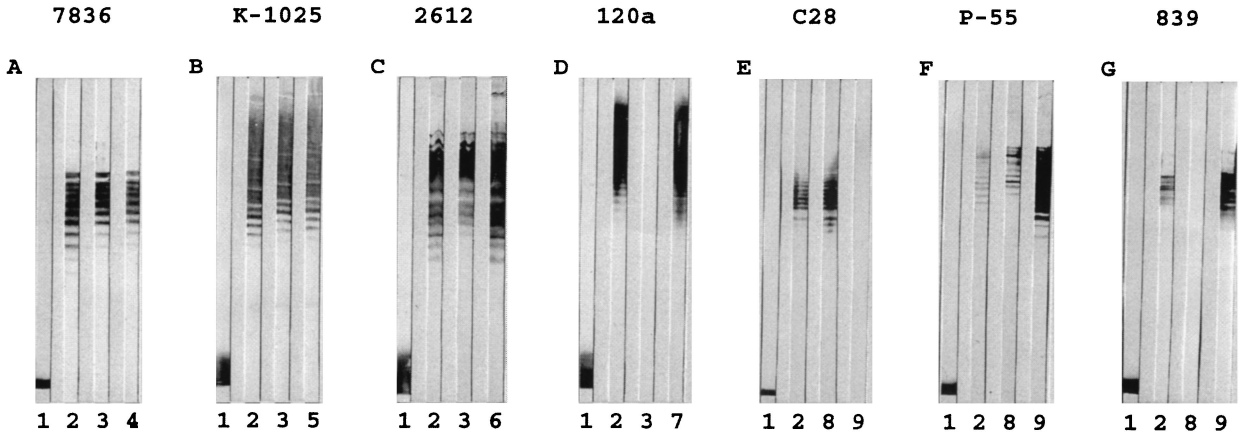

FIG. 1. Results of Western immunoblotting of LPS of *P. syringae* 7836 pv. atrofaciens (A), K-1025 pv. atrofaciens (B), 2612 pv. atrofaciens (C), 120a pv. phaseolicola (D), C28 pv. morsprunorum (E), P-55 (F), and 839 (G) after separation by SDS–12.5% PAGE and visualization with murine MAbs Pscor1, Ps1a, Ps1a′, Ps1x, Ps1b, Ps1c, Ps1d, Ps2a, and Ps2d (lanes 1 to 9, respectively).

492 nm of <0.2) was observed with the antigens from the non-serologically-related bacteria. Most MAbs (except Ps1a, Ps1a′, and Ps2a) strongly agglutinated homologous bacterial cells and precipitated soluble antigens, suggesting cell surface localization and multivalency of the specific antigens. In Western immunoblotting these antibodies reacted with LPSs extracted from homologous strains to give profiles with ladder-like bands (Fig. 1) typical for the smooth LPS with different O chain lengths (22, 39). Proteinase K digestion and high-temperature treatment of the LPS preparations used had no effect on antibody reactivity. Periodate oxidation of antigens, either coated on ELISA plates or electrotransferred onto nitrocellulose membranes (data not shown), resulted in the loss of reactivity with all antibodies, confirming the carbohydrate nature of the antigenic determinants. These results verify the specificity of the MAbs against the O chains of LPS.

Serotyping and serogrouping of *P. syringae* strains with MAbs. To assess the serotyping potential of the MAbs, we examined their reactivity by ELISA with soluble LPS preparations extracted from the bacteria used in this study. All *P. syringae* strains with pathovars aptata, glycinea, japonica, phaseolicola, and pisi as well as 72 (87.8%), 3 (75%), and 31 (50%) strains with pathovars atrofaciens, striafaciens, and syringae, respectively, and 20 (80%) *P. syringae* strains for which the pathovar(s) has not been identified cross-reacted strongly with the MAb Ps1a (Table 2). The strains within this group were subdivided into subgroups 1b, 1c, and 1d according to their reactivities with the corresponding MAbs Ps1b, Ps1c, and Ps1d. MAb Ps1x reacted with only the homologous strain IMV 7836 (data not shown). The antibody Ps1a′ cross-reacted with all strains from subgroups 1b, 1c, and 1x but not with strains from 1d. This observation indicates that epitope 1a′ is subgroup specific. We did not find it necessary, however, to subdivide the strains to classify this epitope into a distinct subgroup, as it would have merely complicated the classification scheme. Since all strains from subgroups 1a′, 1b, 1c, and 1d cross-reacted with MAb Ps1a, they were classified into serogroup O1 as serotypes O1a′, O1b, O1c, and O1d, respectively (Table 2). All strains with pathovars aptata and pisi were classified into a single serotype, O1c, and strains with pathovar phaseolicola were classified into a single serotype, O1d, whereas the strains with pathovar glycinea were divided between serotypes O1b and O1c. Different races of pathovars glycinea and phaseolicola were not differentiated by LPS serology (data not shown). Altogether, 87.8% of the strains with pathovar atrofaciens were classified into serogroup O1. This allows us to make a preliminary suggestion that only the strains with pathovar atrofaciens classified into this serogroup should be considered as a genuine atrofaciens pathovar. It is, however, essential that this suggestion be confirmed by cultural, biochemical, and epidemiological tests.

MAb Ps2a reacted with 20 of the 21 strains with pathovar morsprunorum (Table 2). Some of the Ps2a-positive strains with pathovar morsprunorum (GSPB 854, IMV P-55, 218, and 8414) also reacted with MAb Ps2d, while strain GSPB 839 as well as all strains with pathovar lapsa tested reacted only with MAb Ps2d. As can be seen in Table 2, all strains with pathovars morsprunorum and lapsa cross-reacted slightly but clearly with serogroup O1-specific MAb Ps1a by ELISA (optical density at 492 nm of 0.5 to 0.8) and Western immunoblotting (Fig. 1). However, since the strains with pathovar morsprunorum have been shown to possess the OPS backbones consisting of tri-α-D-rhamnose residues (Fig. 2F to H) and could be differentiated from other pathovars by culture and biochemical tests (4, 7),

TABLE 2. Serogrouping and serotyping of *P. syringae* strains by murine MAb reactivity

P. syringae pathovar[a]	No. of strains tested	No. of strains classified into serogroup (serotype) by reactivity to indicated MAb						
		O1 (O1a′, O1b, O1c, O1d)					O2 (O2a, O2d)	
		Ps1a	Ps1a′	Ps1b	Ps1c	Ps1d	Ps2a	Ps2d
aptata	11	11	11	—[b]	11	—	—	—
atrofaciens	82	72	62	36	26	10	—	—
glycinea	18	18	18	6	12	—	—	—
japonica	6	6	6	1	5	—	—	—
lapsa	5	5	—	—	—	—	—	5
morsprunorum	21	21	—	—	—	—	20	5
phaseolicola	25	25	—	—	—	25	—	—
pisi	9	9	9	—	9	—	—	—
striafaciens	4	3	3	3	—	—	—	—
syringae	62	31	29	16	13	2	—	—
Unknown	25	20	3	2	1	17	—	—

[a] Only the pathovars reacting with MAbs are shown. Unknown, the pathovars of these 25 strains tested are unknown.

[b] —, no detectable reactivity.

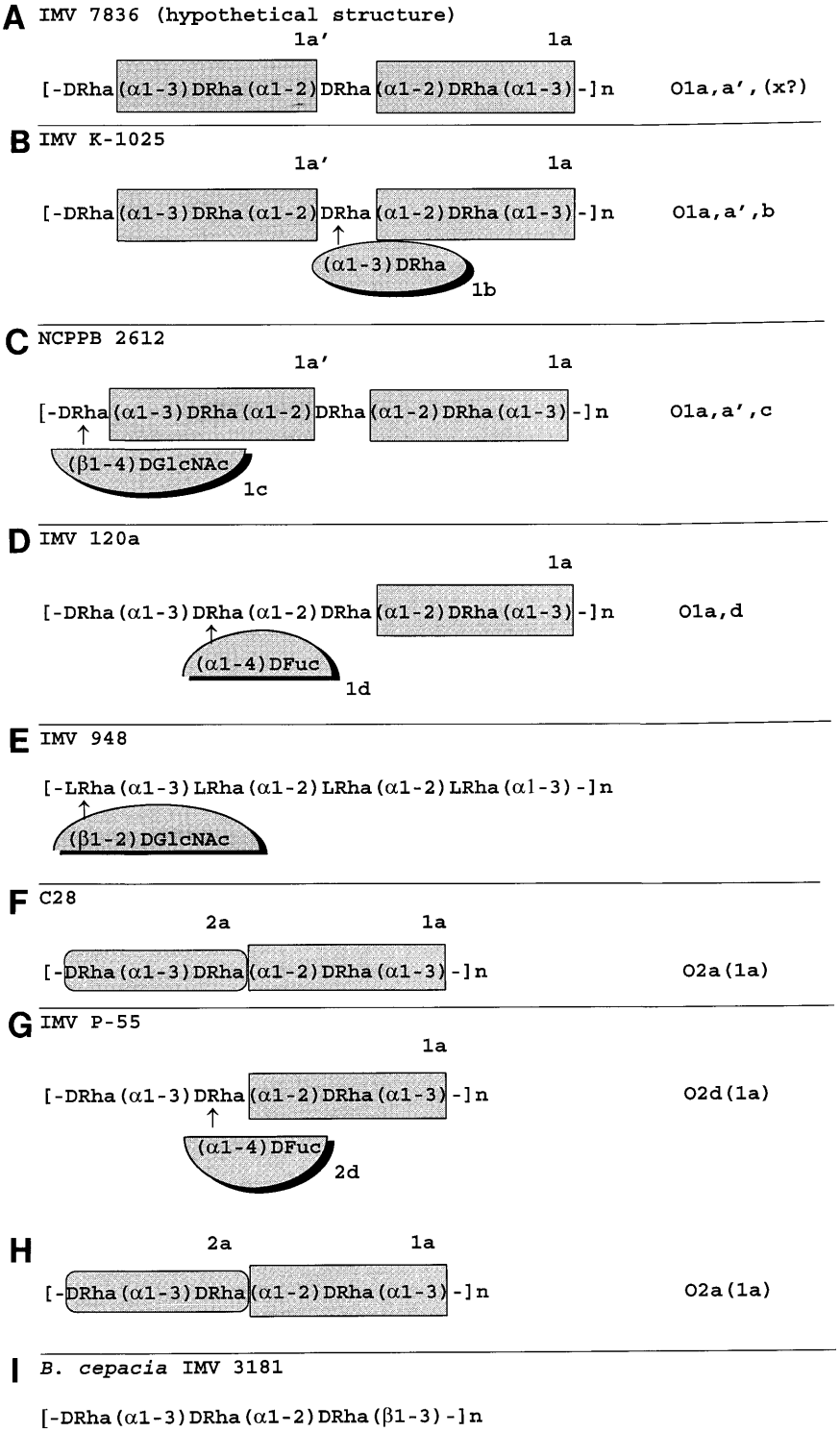

FIG. 2. Primary chemical structures and deduced epitopes of OPS repeats of lipopolysaccharide of *P. syringae* (A to H) and *B. cepacia* (I). The structures of most OPSs are given according to Knirel et al. (14–19), and that of strain C28 is given according to Smith et al. (42). To show the deduced epitopes better, we have slightly changed the presentation from the official recommendations of presentation of the chemical structures of OPS repeats in condensed form in two lines (20). Only the immunodominant residues of the inferred epitopes are shown. In panel E, a tentative structure of the main OPS repeating unit (14) is shown. On the right, the proposed serological formulas are given. DRha, D-rhamnose; LRha, L-rhamnose; DGlcNAc, 2-acetamido-3-deoxy-D-glucose; DFuc, D-fucose.

we propose that they be classified into a distinct O2 serogroup as serotypes O2a and O2d.

All *P. syringae* strains, which were classified into serogroups O1 and O2, reacted by ELISA with core-specific MAb Pscor1 (35) (data not shown). None of the MAbs specific to *P. syringae* strains reacted with *B. cepacia* IMV 3181. MAb Bc1a, produced against OPS of *B. cepacia*, did not cross-react with any of the *P. syringae* strains. Finally, none of the MAbs reacted

with LPS preparations from different strains of other *Pseudomonas* species and other bacterial genera (data not shown).

In summary, the common serological formula O1a,a′,b,c,d,x for designation of serogroup O1 and the formulas O1a,a′,x, O1a,a′,b, O1a,a′,c, and O1a,d for designation of the individual *P. syringae* strains (serological passport of the strain) within serogroup O1 as well as the formulas O2a(1a), O2a,d(1a), and O2d(1a) for the individual strains from serogroup O2 [O2a,d (1a)] that correlate with the chemical structures of the corresponding OPSs (see Discussion) can be proposed (Fig. 2).

Western immunoblotting. The representative strains from serogroups O1 and O2 (Table 1) were shown by immunoblotting with core-specific MAb Pscor1 (35) to possess rough LPS molecules which share a common epitope, cor1 (Fig. 1A to G, lanes 1). Western immunoblots further revealed that all MAbs to *P. syringae* reacted with homologous LPSs, indicating typical ladder-like banding profiles given by smooth LPS molecules with O chains of different lengths (Fig. 1A to F, lanes 2, 5, 6, 7, 8, and 9, respectively). Characteristically, all MAbs reacted only with the higher-molecular-weight bands of homologous and heterologous LPSs, indicating that a certain minimum number of O repeats is required for binding by MAbs. Mild periodate oxidation of the LPS molecules electrotransferred onto nitrocellulose membranes appears to remove the binding sites for all O-chain-specific MAbs (data not shown), confirming the carbohydrate nature of the specific epitopes.

Serogroup-specific MAb Ps1a strongly cross-reacted by immunoblotting (Fig. 1B to D, lanes 2) with LPSs possessing O chains that consist of tetra-α-D-rhamnose repeats in the backbone (Fig. 2B to D). The reactivity, however, was weak (Fig. 1E to G, lanes 2) for OPSs with tri-α-D-rhamnose repeats in the backbone (Fig. 2F to H). The similarities of the profiles of LPS bands reacted with the MAbs revealed with serogroup-specific MAb Ps1a and serotype-specific MAbs Ps1b, Ps1c, and Ps1d suggest the localization of corresponding epitopes within the same molecule of LPS (Fig. 1B to D). MAb Ps1a′ reacted with LPS of homologous strain IMV 7836 (Fig. 1A, lane 3) and LPS from serotypes O1b and O1c (Fig. 1B and C, lanes 3) but not from O1d (Fig. 1D, lane 3), whereas the MAb Ps1x was positive only with LPS from the homologous strain (Fig. 1A, lane 4). MAbs Ps1a, Ps1a′, and Ps1x, which had different serotyping potentials by ELISA (Table 2), revealed similar reaction profiles with homologous LPS bands (Fig. 1A, lanes 2 to 4), suggesting that corresponding epitopes 1a, 1a′, and 1x are located within the same LPS molecule.

The LPS of *P. syringae* C28 pv. morsprunorum (Fig. 2F) reacted with MAbs Ps1a and Ps2a (Fig. 1E, lanes 2 and 8), indicating that there are two different epitopes, 1a and 2a, within linear poly-D-rhamnan. Different reaction profiles of the bands were observed in Western immunoblots of LPS from strain IMV P-55 with MAbs Ps1a, Ps2a, and Ps2d (Fig. 1F, lanes 2, 8, and 9). The LPS from strain GSPB 839 reacted only with MAbs Ps1a and Ps2d (Fig. 1G, lanes 2 and 9), demonstrating that there are no molecules with linear O chains (see Discussion). The Western immunoblot data (Fig. 1) correlated well with the ELISA results (Table 2) and the structural chemistry of the corresponding LPS O repeats (Fig. 2).

DISCUSSION

We describe murine MAbs Ps1a, Ps1a′, Ps1b, Ps1c, Ps1d, Ps1x, Ps2a, and Ps2d (Table 1) specific to the linear and branched OPSs consisting of tetra- and tri-α-D-rhamnose repeats in the backbone with different lateral substituents (Fig. 2). The serotyping potential of these antibodies was evaluated by studying the distribution of the corresponding epitopes among the 358 strains of *P. syringae* representing 21 pathovars. Among the *P. syringae* strains studied, those with pathovars aptata, glycinea, japonica, phaseolicola, and pisi were shown to be serologically homogeneous and were classified into serotypes O1a′, O1b, O1c, O1d, and O1x within serogroup O1 (Table 2). No serological difference was observed for the races within pathovars glycinea and phaseolicola. The serological homogeneity of some of the above-mentioned pathovars had also been shown previously with polyclonal antisera (2, 10, 33, 37, 40). The serological findings with serotype-specific MAbs Ps2a and Ps2d (Table 1), the data on the OPS primary structures (Fig. 2F to H) and the cultural, biochemical and epidemiological characteristics (4, 7) of the strains with pathovar morsprunorum enabled us to classify these bacteria into serotypes O2a and O2d within serogroup O2. Thus, most *P. syringae* pathovars seem to be serologically homogeneous and could be identified with MAbs, a finding which may have important taxonomic (classification, nomenclature, and identification) implications.

Since the primary chemical structures of the OPSs of some of *P. syringae* strains classified now into serogroups O1 and O2 had been elucidated by Knirel et al. (14–19) and Smith et al. (42) (Fig. 2), we could draw preliminary conclusions as to the chemical compositions of the serogroup- and serotype-specific epitopes. The serogroup O1-specific epitope 1a was coexposed by all OPS molecules possessing linear or branched O units with tetra- or tri-α-D-rhamnose repeats in the backbone but with different lateral substituents in different positions. On the basis of the structural (Fig. 2A to H) and serological (Table 2; Fig. 1) data, we can infer that the epitope 1a is related to the Rha(α1-2)D-Rha(α1-3)D-Rha sequence of the OPS backbone with the immunodominant site (α1-2)D-rhamnose(α1-3). The epitope 1a′ was exposed by homologous strain 7836 and by strains from serotypes O1b and O1c (Fig. 1A to C). The fact that antibody Ps1a′ does not react with strains from serotype O1d could be due to the direct hindrance of epitope 1a′ by the lateral (α1-4)D-fucose residue (Fig. 1D). Thus, the most consistent interpretation is that epitope 1a′ is related to the D-Rha(α1-3)D-Rha(α1-2)D-Rha site of the OPS backbone consisting of tetra-D-rhamnose repeats. The residue (α1-3)D-Rha(α1-2) is immunodominant for epitope 1a′.

It is most likely that the serotype-specific epitopes 1b, 1c, and 1d are related to the lateral immunodominant substituents of (α1-3)D-Rha, (β1-4)D-GlcNAc, and (α1-4)D-Fuc, respectively, which are all bound to the backbone-located D-rhamnose (Fig. 2B to D, respectively). MAb Ps1c does not react with the OPS that compose tetra-α-L-rhamnose in the backbone and the lateral substituent DGlcNAc attached by (β1-2) glycosidic linkage (*P. syringae* IMV 948 pv. atrofaciens) (Fig. 2E). The absence of the cross-reactivity could be explained by the different glycosidic linkages attaching the lateral D-GlcNAc to the main chain and presumably different D and L isomers of the neighboring rhamnose (Fig. 2C and E).

The observation that MAbs Ps2a and Ps1a react with rhamnan consisting of tri-α-D-rhamnose repeats suggested that there are two different epitopes, 1a and 2a, related to the D-Rha-(α1-2)D-Rha(α1-3)-D-Rha [immunodominant site, (α1-2)D-Rha(α1-3)] and D-Rha(α1-3)D-Rha sequences of the linear OPS, respectively (Fig. 2F). On the basis of the results of the immunological studies (Table 2) and the data on the structural chemistry (Fig. 2H and G), it could be inferred that epitope 2d is related to the lateral immunodominant (α1-4)D-fucose residue which, in fact, masks or directly blocks epitope 2a. Epitopes 2a and 2d are thus alternative epitopes within the same OPS molecule (Fig. 2G). We have, however, shown immunologically that some of the strains from serogroup O2, including

strain P-55, exhibit both these epitopes at the same time (Table 2; Fig. 2). This is consistent with molecular structural data demonstrating that strain P-55 possesses two distinct chemotypes of OPS, linear (15%) (Fig. 2H) and branched (85%) (Fig. 2G) (18). We have also identified one strain (GSPB 839) with pathovar morsprunorum and all strains with pathovar lapsa that expose only epitopes 1a and 2d, definitely indicating the existence of only one, branched type of OPS moiety of LPS. Therefore, we do not propose that the epitope 2a be treated as serogroup O2 specific.

As can be seen in Fig. 2D and G, we deduced that epitopes 1d and 2d, respectively, are related to the same lateral (α1-4) D-Fuc substituent. The specific antibodies Ps1d and Ps2d do not, however, cross-react. Thus, it seems that the number of D-rhamnose residues within the O repeats in the OPS backbone basically affects the immunological specificity of the lateral D-fucose residue.

None of the O2a and O2d phenotypes of OPS reacted by ELISA or immunoblotting (data not shown) with MAb Bc1a, produced against the OPS of *B. cepacia* (Fig. 2K). As can be seen in Fig. 2H and K, the difference between the chemical structures is seen only in the presence of (α1-3) or (β1-3) glycosidic linkages, which, thus, drastically change the entire conformation and immunological reactivity of the OPS.

The primary chemical structure of the OPS of strain 7836 (O1a,a′,x) seems to be unique and remains to be elucidated. On the basis of the serological and structural results, we predict that the OPS backbone of this strain consists of tetra-α-D-rhamnose repeats (Fig. 1A). It is likely that antigenic determinant 1x is related to the unknown lateral substituent within structure A. It would be also interesting to know why this structure is so rare in nature (only one strain with the serological formula O1a,a′,x among 358 strains of *P. syringae* was found) and what role it has in the evolution of OPS diversity.

The data described above indicate that the immunological behavior of oligosaccharide-specific epitopes is very complex and depends on the entire conformation of the LPS molecule, being strongly affected by the nature of glycosidic linkages (α or β anomeric configuration and position of substitution), D or L isomers, by neighboring residue(s), number of residues in O-unit repeats, and the total number of repeats within the O chain. These observations are consistent with suggestions made previously (6, 13, 22, 27, 28, 31, 38, 46) based on the results of the immunochemical investigation of polysaccharides from other species of bacteria. It is, however, obvious that to precisely determine the epitopes recognized by our MAbs, more-detailed analyses, especially competition ELISAs with monosaccharides and synthetic oligosaccharides with different compositions and lengths as competing antigens have to be carried out.

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