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Outer Membrane Proteins and Lipopolysaccharides in Pathovars of *Xanthomonas campestris*

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Variations in the outer membrane proteins (OMPs) and lipopolysaccharides (LPSs) of 54 isolates belonging to 16 different pathovars of *Xanthomonas campestris* were characterized. OMP samples prepared by sarcosyl extraction of cell walls and LPS samples prepared by proteinase K treatment of sonicated cells were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in the presence of 4 M urea. In general, the OMP and LPS profiles within each pathovar were very similar but different from the profiles of other pathovars. Heterogeneity in OMP and LPS profiles was observed within *X. campestris* pv. campestris, *X. campestris* pv. translucens, and *X. campestris* pv. vesicatoria. LPSs were isolated from six *X. campestris* pathovars, which fell into two major groups on the basis of O antigenicity. The O antigens of *X. campestris* pv. begoniae, *X. campestris* pv. graminis, and *X. campestris* pv. translucens cross-reacted with each other; the other group consisted of *X. campestris* pv. campestris, *X. campestris* pv. pelargonii, and *X. campestris* pv. vesicatoria. A chemical analysis revealed a significant difference between the compositions of the neutral sugars of the LPSs of those two groups; the LPSs of the first group contained xylose and a 6-deoxy-3-*O*-methyl hexose, whereas the LPSs of the other group lacked both sugars.

Xanthomonas campestris is a plant pathogen that causes bacterioses in a variety of plant species world-wide. This gram-negative bacterial species has been divided into more than 140 pathovars on the basis of the host plants of *X. campestris* isolates (44). Some pathovars exhibit a high degree of host specificity, whereas others infect a wider range of plant species that usually belong to one plant family. Electrophoretic analyses of cellular proteins and cellular fatty acid analyses, as well as DNA-DNA hybridization studies, have revealed structural differences between isolates of different pathovars and, on the other hand, conservation of certain phenotypic or genetic characteristics in strains of many, but not all, pathovars (20, 43–46).

The surface structures of bacteria are important in forming the physical and functional barriers between bacterial cells and their environment. In gram-negative bacteria, surface antigens influence bacterium-plant interactions. Lipopolysaccharide (LPS) induces resistance to phytopathogenic pseudomonads and inhibits hypersensitivity reactions in plants (16, 31). LPS is also important in the symbiosis between rhizobia and their leguminous host plants because it serves as a recognition target for plant lectins and triggers metabolic events leading to primary host infection (8, 37). Outer membrane proteins (OMPs) involved in iron acquisition are important for the virulence of *Erwinia chrysanthemi* (14).

Relatively little is known about the structure and variability of surface antigens in *X. campestris* pathovars. The exopolysaccharide has been characterized chemically (7). *X. campestris* mutants lacking exopolysaccharide exhibit reduced virulence, suggesting that exopolysaccharide plays a role in the pathogenetic process (7). The electrophoretic patterns of the OMPs of only a few *Xanthomonas* pathovars have been studied (32, 35), and the LPSs of a number of *Xanthomonas* isolates have been characterized chemically (19, 34, 47, 48) but not serologically. A serological grouping based on antisera against whole cells has been described for the genus *Xanthomonas* (13), and more recent studies performed with monoclonal antibodies have focused on identification of individual pathovars (2–4). As a first step in clarifying the role of cell surface components in pathogenicity and host tropism of *X. campestris*, we characterized the variation in the OMPs and LPSs of a wide array of *X. campestris* pathovars. Our main interest was to assess whether the OMP patterns and LPS characteristics are related to the division of *X. campestris* pathovars.

MATERIALS AND METHODS

Bacteria. A total of 54 *X. campestris* isolates representing 16 pathovars were examined (Table 1). The strains were obtained from National Collection of Plant Pathogenic Bacteria, Harpenden, United Kingdom (52 strains), and from Statens Plantevern, Ås, Norway (*X. campestris* pv. pelargonii 85-37 and 86-5). For the OMP analysis, the bacteria were grown with shaking at 150 rpm in Luria broth at 28°C for 16 h (39). For isolation and electrophoretic analyses of LPSs and for immunization, the bacteria were grown on NYG agar at 28°C for 48 h (42). For agglutination assays, the bacteria were grown with shaking at 150 rpm in NYG broth for 16 h. *Salmonella enterica* serovar typhimurium SL696 and SL1181 were used as LPS reference strains and were grown on Luria agar at 37°C for 24 h (50).

OMP profiles. Samples for the OMP analysis were prepared basically as described by Achtman et al. (1). Briefly, bacterial cells suspended in 10 mM Tris-HCl (pH 8.0) were sonicated in an ice bath twice for 5 s, the cells were removed, and the membranes were collected by centrifugation at 15,000 × *g* for 60 min. The membranes were sus-

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TABLE 1. Bacterial strains and OMP pattern types

Taxon	Strain(s)	OMP type[a]
X. campestris pv. begoniae	2226, 3003, 3021	4
X. campestris pv. campestris	45, 279, 528, 1043, 1929, 3207, 3290, 3291	5
	1648	16
X. campestris pv. cerealis	3212	10
X. campestris pv. citri	3135, 3136, 3232, 3233, 3234[b]	1
X. campestris pv. cucurbitae	2597, 3168	8
X. campestris pv. daturi	2932, 2935	15
X. campestris pv. graminis	2700, 3020, 3040, 3041, 3066	10
X. campestris pv. mangiferaeindicae	2885, 3124	3
X. campestris pv. oryzae	3002, 3022, 3177, 3301	9
X. campestris pv. pelargonii	2985, 3119, 85-37, 86-5[c]	7
X. campestris pv. phaseoli	3035	2
X. campestris pv. phlei	3231	10
X. campestris pv. poae	3230	10
X. campestris pv. pruni	3155, 3156	6
X. campestris pv. translucens	973, 2389, 2904, 2920, 3170, 3176	10
	3215	14
X. campestris pv. vesicatoria	3206	11
	422	12
	3240	13
	2968, 3178	15
S. enterica serovar typhimurium	SL696, SL1181[d]	

[a] See Fig. 1 for OMP types.
[b] Strain 3233 belongs to pathogenicity group C; the other *X. campestris* pv. citri strains belong to pathogenicity group A (National Collection of Plant Pathogenic Bacteria) (17).
[c] Strains 85-37 and 86-5 were obtained from Statens Plantevern, Ås, Norway. The other *X. campestris* strains were obtained from the National Collection of Plant Pathogenic Bacteria, Harpenden, England.
[d] Strain SL696 is an LPS chemotype S strain (50), and strain SL1181 is an LPS chemotype Rd_1 strain (50).

pended in 10 mM Tris-HCl (pH 8.0), and sarcosyl was added to a final concentration of 1.5% (vol/vol). After incubation at room temperature for 20 min, the membranes were collected by centrifugation at 15,000 × *g* for 90 min and suspended in electrophoresis sample buffer. The samples were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) by the system of Laemmli (24) in 0.75-mm-thick slab gels (gel concentration, 12%) containing 4 M urea. A low-molecular-weight calibration kit (Pharmacia, Uppsala, Sweden) was used as a molecular weight standard. We have purified fimbriae and flagella from one of the test strains, *X. campestris* pv. vesicatoria 3240 (33a). The polypeptides of these structures do not comigrate with the major OMPs, indicating that the OMP preparation from this strain was not significantly contaminated by fimbriae or flagella.

LPS analysis. LPSs were isolated from acetone-dried bacterial cells by using the phenol-water method (49); LPSs were collected from the aqueous phase. After dialysis against water, each extract was treated with RNase (Boehringer, Mannheim, Germany) in 50 mM Tris-HCl (pH 7.5) at 37°C overnight and washed three times with water by centrifugation for 4 h at 100,000 × *g* and 4°C.

The purified LPSs were subjected to the following chemical analyses. The thiobarbituric assay (23) for the estimation of 3-deoxy-D-mannooctulosonic acid was performed after the LPSs were hydrolyzed in 1 M HCl at 100°C for 2 h or in 0.1 M sodium acetate (pH 4.4) at 100°C for 1 h (6). The phosphate content was determined by the method of Lowry et al. (29), and the hexosamine content after hydrolysis (4 M HCl, 100°C, 16 h) was determined by a modified Morgan-Elson method (38). Fatty acids were liberated and derivatized as described previously (18). Qualitative and quantitative analyses of carbohydrate components were performed by a modification of the procedure of Hollingsworth et al. (22). Briefly, the LPSs were methanolyzed (methanolic 0.5 M HCl, 86°C, 45 min) and carboxyl reduced with $NaBD_4$. The reduced methyl glycosides were hydrolyzed with 1 M trifluoroacetic acid (100°C, 2 h), carbonyl reduced with $NaBH_4$, and peracetylated. The resulting alditol acetates were analyzed by gas-liquid chromatography and gas-liquid chromatography–mass spectrometry. During gas-liquid chromatography–mass spectrometry, hexitol acetates derived from hexuronic acids were monitored on the basis of the mass fragment at *m/z* 219. Neutral sugars were liberated by hydrolysis (0.1 M HCl, 48 h, 100°C) and analyzed as alditol acetates (36) by gas-liquid chromatography. Gas-liquid chromatography of the alditol acetates was performed with a model 5890A gas chromatograph (Hewlett-Packard, Avondale, Pa.) equipped with type NB9C fused silica capillary column (25 m by 0.32 mm [inside diameter]; Nordion Instruments, Helsinki, Finland). The temperatures of the injection port and the flame ionization detector were 260 and 300°C, respectively; the temperature was kept at 170°C for 3 min and then increased 3°C/min up to 260°C. Fatty acid methyl esters were analyzed in a type HP-5 column (25 m by 0.32 mm) by using the same temperature program. Electron impact mass spectrometry was performed with a model 5890 gas chromatograph combined with a model 5970 mass selective detector (Hewlett-Packard). Chemical ionization mass spectrometry was performed with an instrument obtained from VG Trio, Manchester, United Kingdom, using ammonia as the reactant gas. The ion source temperature was 170°C.

To analyze LPS profiles by SDS-PAGE, cells were suspended in water (1.5 ml) to give an A_{420} of 0.4. The suspension was sonicated three times for 30 s and centrifuged at 15,000 × *g* for 60 min, and the supernatant was discarded. A total of 50 μl of lysing buffer (21) was added to the deposited material, and the tube was kept at 100°C for 10 min. After 25 μg of proteinase K (Boehringer) was added,

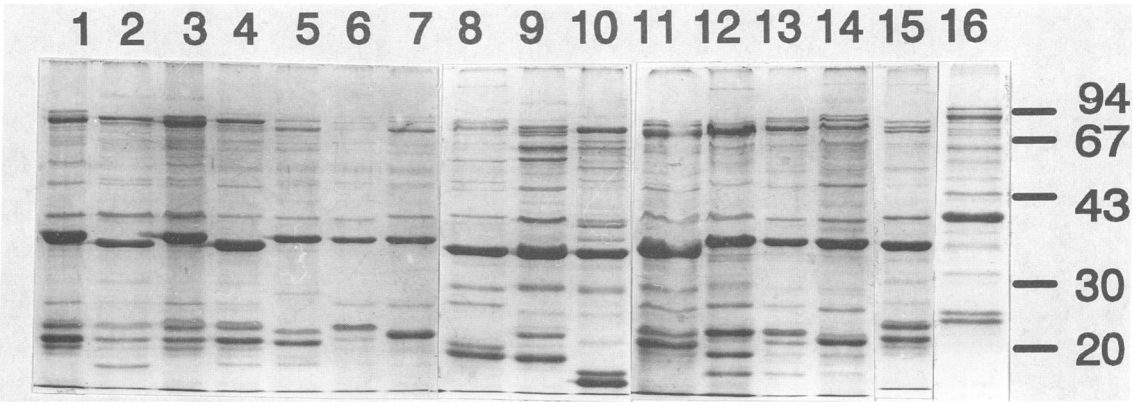

FIG. 1. OMP profiles (Coomassie blue-stained 12% SDS–PAGE gel) of *X. campestris* strains. Lane 1, *X. campestris* pv. citri 3232; lane 2, *X. campestris* pv. phaseoli 3035; lane 3, *X. campestris* pv. mangiferaeindicae 2885; lane 4, *X. campestris* pv. begoniae 3003; lane 5, *X. campestris* pv. campestris 528; lane 6, *X. campestris* pv. pruni 3156; lane 7, *X. campestris* pv. pelargonii 2985; lane 8, *X. campestris* pv. cucurbitae 2597; lane 9, *X. campestris* pv. oryzae 3002; lane 10, *X. campestris* pv. translucens 973; lane 11, *X. campestris* pv. vesicatoria 3206; lane 12, *X. campestris* pv. vesicatoria 422; lane 13, *X. campestris* pv. vesicatoria 3240; lane 14, *X. campestris* pv. translucens 3215; lane 15, *X. campestris* pv. daturi 2932; lane 16, *X. campestris* pv. campestris 1648. The migration distances of molecular mass standards (in kilodaltons) are indicated on the right.

the samples were incubated at 58°C for 2 h and then at 37°C for 14 h. The lysate (5 to 50 μl) was then used for SDS-PAGE. Purified LPSs were dissolved to a concentration of 0.1 mg/ml in lysing buffer, incubated at 100°C for 5 min, and applied to SDS-PAGE gels. The samples were electrophoresed in 12.5 or 15% polyacrylamide slab gels containing 3.2 M urea, and the gels were stained with silver (41).

Immunological methods. Anti-O antisera were raised by injecting rabbits with heat-treated (100°C, 1 h) and formalin-fixed bacterial cells (26). The agglutination titers of the antisera against heat-killed bacteria were determined in microtiter plates by using standard procedures (26). The bacterial suspensions were adjusted to an A_{600} of 1.5.

Immunoblotting of the purified LPSs with the antisera was performed by using the procedure of Towbin et al. (40). After transfer, the nitrocellulose membranes were incubated with gentle agitation in 5% (vol/vol) bovine serum albumin (BSA) in Tris-buffered saline (TBS) (10 mM Tris-HCl [pH 7.5], 0.9% [wt/vol] NaCl) for 30 min, rinsed in TBS, and incubated with 1/50 to 1/200 dilutions (in 1% BSA–TBS) of the antisera for 1.5 h. The membranes were then washed three times for 15 min in TBS containing 0.05% (vol/vol) Tween 20 (Sigma Chemical Co., St. Louis, Mo.) and incubated with biotinylated anti-rabbit immunoglobulin G (diluted 1/1,000 in 1% BSA–TBS; Sigma) for 1.5 h. The membranes were washed three times, incubated in an avidin-peroxidase (Sigma) solution (1/1,000 dilution in 1% BSA–TBS) for 1.5 h, washed, and incubated with 0.05% (wt/vol) diaminobenzidine (Sigma) in Tris-HCl buffer (pH 7.5) containing 0.04% (vol/vol) hydrogen peroxide, until color developed.

RESULTS

OMP profiles. A total of 16 different OMP profiles (Fig. 1) were identified among the 54 strains. The OMP profile type for each strain is shown in Table 1. In general, strains belonging to a given pathovar produced an OMP pattern that was unique for and conserved within the pathovar. Heterogeneity in the OMP profiles was observed within *X. campestris* pv. translucens (Fig. 2A and Table 1) and *X. campestris* pv. campestris (Fig. 2C and Table 1). The five *X. campestris* pv. vesicatoria strains produced four different OMP profiles (Fig. 2B). OMP pattern 10 (Fig. 1) was detected in *X. campestris* pv. cerealis, *X. campestris* pv. poae, *X. campestris* pv. phlei, *X. campestris* pv. translucens, and *X. campestris* pv. graminis (Table 1).

LPS profiles. LPSs from the following six *X. campestris* strains were purified and analyzed in detail: *X. campestris* pv. begoniae 3021, *X. campestris* pv. campestris 1929, *X. campestris* pv. graminis 3041, *X. campestris* pv. pelargonii 2985, *X. campestris* pv. translucens 2920, and *X. campestris* pv. vesicatoria 3178. On SDS-PAGE gels these LPSs produced bimodal ladder-like LPS patterns with only a few bands or no bands representing molecular species having intermediate O-chain lengths (Fig. 3A). Better resolution was achieved for the low-molecular-weight species by analyzing LPSs on a 15% polyacrylamide gel (Fig. 3B). The six strains produced different LPS profiles.

We analyzed the LPS profiles of proteinase K-treated cells for 33 individual isolates belonging to the six pathovars. The *X. campestris* pv. graminis (Fig. 4D), *X. campestris* pv. begoniae, and *X. campestris* pv. pelargonii (data not shown) strains produced LPS patterns that were homogeneous within each pathovar and were indistinguishable from the patterns shown for the pathovar representatives in Fig. 3. In contrast, *X. campestris* pv. campestris, *X. campestris* pv. translucens, and *X. campestris* pv. vesicatoria (Fig. 4) were heterogeneous. The LPSs of *X. campestris* pv. campestris 45, 279 and 3291 differed in the high-molecular-weight region from the LPSs of the other *X. campestris* pv. campestris strains, whereas the low-molecular-weight species of LPS were very similar in all *X. campestris* pv. campestris isolates and distinguishable from those of other pathovars (Fig. 4A). *X. campestris* pv. campestris 45 and 279 contained up to seven detectable classes of molecules with high mobilities and one diffuse brown-staining molecular species with a low electrophoretic mobility. The seven *X. campestris* pv. translucens strains were divided into four clearly different electrophoretic types (Fig. 4B). On the basis of high-molecular-weight LPS molecules, strains 2389, 2920, and 3170 were similar, and strain 973 differed slightly. Strains 2904, 3176, and 3215 were unique. Two LPS types were detected among *X. campestris* pv. vesicatoria LPSs (Fig. 4C). Strains 422,

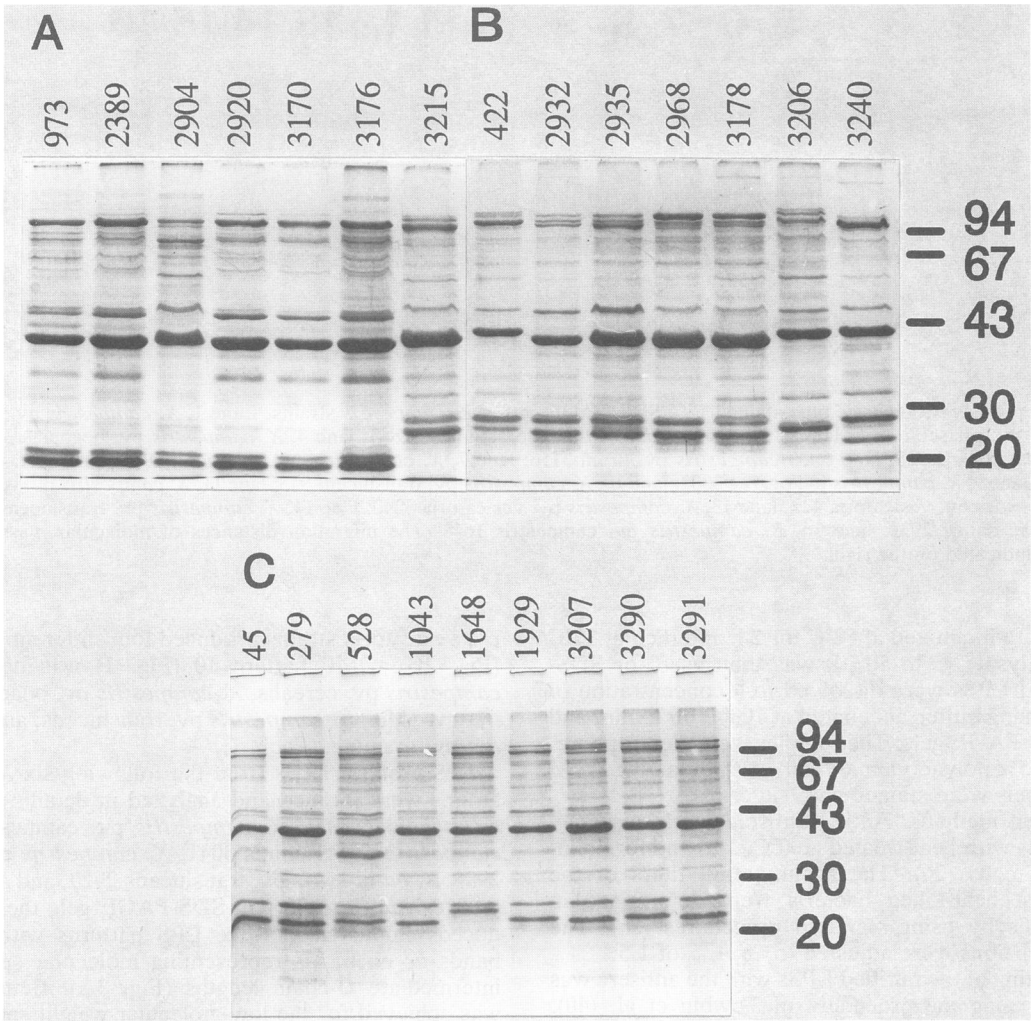

FIG. 2. OMP profiles of *X. campestris* pv. translucens 973, 2389, 2904, 2920, 3170, 3176, and 3215 (A), *X. campestris* pv. vesicatoria 422, 2968, 3178, 3206, and 3240 and *X. campestris* pv. daturi 2932 and 2935 (B), and *X. campestris* pv. campestris 45, 279, 528, 1043, 1648, 1929, 3207, 3290, and 3291 (C). For the gel technique used see the legend to Fig. 1.

3206, and 3240 formed one type, and the strains 2968 and 3178 formed another.

The LPS profiles of proteinase K-treated cells of the remaining 21 isolates representing 10 pathovars were also analyzed. Only two strains, *X. campestris* pv. daturi 2932 (Fig. 4C) and *X. campestris* pv. cucurbitae 3168 (data not shown), were devoid of bands exhibiting low mobility, indicating that the LPSs of these strains lack polymeric O side chains. The smooth *X. campestris* pv. daturi strain, strain 2935, produced an LPS profile similar to that of *X. campestris* pv. vesicatoria 2968. The LPS profiles of *X. campestris* pv. citri (five strains), *X. campestris* pv. mangiferaeindicae (two strains), *X. campestris* pv. oryzae (four strains), *X. campestris* pv. phaseoli (one strain), and *X. campestris* pv. pruni (two strains) were similar within each pathovar but clearly different from the profiles produced by other pathovars (data not shown). An exception was *X. campestris* pv. citri 3233 belonging to pathogenicity group C. The LPS profiles of the smooth strains *X. campestris* pv. cucurbitae 2597 and *X. campestris* pv. vesicatoria 422 exhibited a similar ladder-like structure (data not shown). The LPS patterns of *X. campestris* pv. poae and *X. campestris* pv. cerealis were similar to the *X. campestris* pv. translucens 973 LPS pattern shown in Fig. 4B.

Chemical characterization of the LPSs. LPSs purified from six strains were characterized chemically. These LPSs contained carbohydrates and phosphate in the proportions shown in Table 2. Glucose, rhamnose, and mannose were present in different amounts in each LPS. An analysis for neutral sugars revealed that galactose was absent in all LPSs. However, analysis of the LPS by the procedure involving methanolysis and carboxyl reduction by $NaBD_4$ showed that galactitol hexaacetate was present in substantial amounts in each LPS. As determined by gas-liquid chromatography–mass spectrometry, this substance produced, inter alia, a prominent mass fragment at *m/z* 219, indicating that the galactitol peak was exclusively derived from galacturonic acid. The fragment at *m/z* 219 and other fragments that indicated carboxyl reduction at C-6 were absent from the mass spectra of glucitol and mannitol hexaacetates. Therefore, the uronic acid component of *X. campestris* LPS was exclusively galacturonic acid. Additional sugar components were fucose (present in one LPS) and xylose (present in three LPSs). In each of the xylose-containing LPSs, a

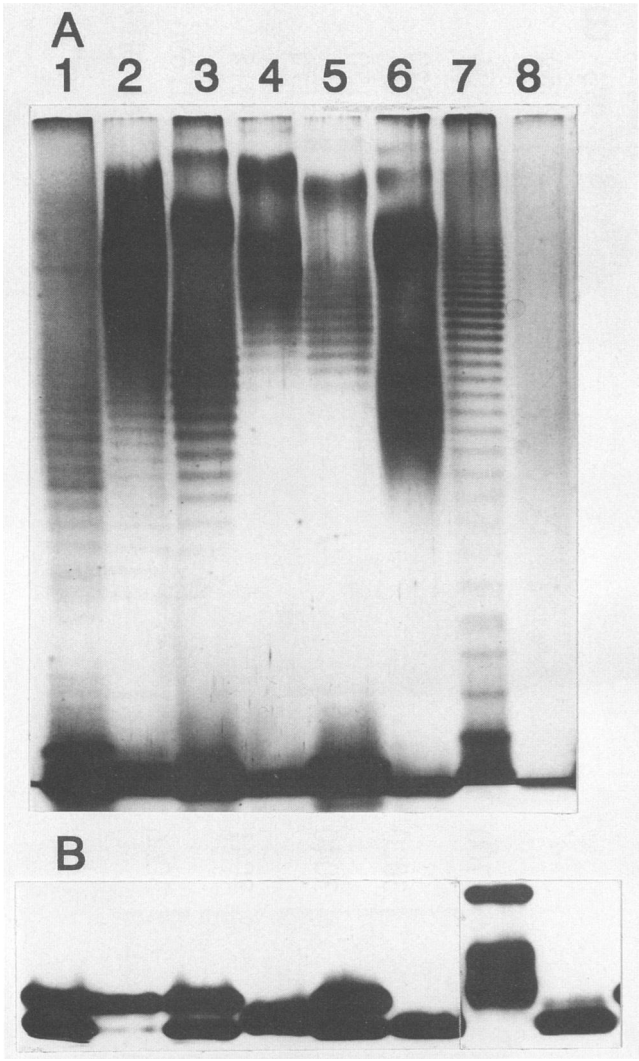

FIG. 3. Silver-stained SDS-PAGE of LPSs purified from six *X. campestris* pathovars and *S. enterica* serovar typhimurium control strains. (A) Profiles of 8 μg of each LPS in a 12.5% polyacrylamide gel. (B) Profiles of 4 μg of each LPS in a 15% polyacrylamide gel (only the low-molecular-weight region). Lane 1, *X. campestris* pv. campestris 1929; lane 2, *X. campestris* pv. pelargonii 2985; lane 3, *X. campestris* pv. vesicatoria 3178; lane 4, *X. campestris* pv. translucens 2920; lane 5, *X. campestris* pv. begoniae 3021; lane 6, *X. campestris* pv. graminis 3041; lanes 7 and 8, *S. enterica* serovar typhimurium SL696 and SL1181 proteinase K-treated cell envelopes, respectively.

6-deoxy-3-*O*-methyl hexose was identified, whereas in the LPSs of *X. campestris* pv. campestris and *X. campestris* pv. vesicatoria a 3-amino-3,6-dideoxyhexose was found. The electron impact mass spectrum of the 3-amino-3,6-dideoxyhexitol contained the same main peaks as those reported previously for acetylated 3-acetamido-3,6-dideoxymannitol (*m/z* 96, 110, 128, 156, 170, 195, 216, and 230) (30). Notably, we did not find heptoses in any of the *X. campestris* LPSs. An analysis of fatty acids (data not shown) revealed a pattern similar to that reported previously for *Xanthomonas* LPS (34). No qualitative differences in the LPS fatty acids were observed.

Immunological analysis. Antisera were prepared against the six *X. campestris* strains used for chemical characterization of LPS. The purified LPSs were used as antigens in immunoblotting experiments to determine the cross-reactivities of the LPSs. In addition, cultures of all *X. campestris* strains were used in agglutination tests with the six antisera.

In all SDS-PAGE and immunoblotting experiments, the antisera reacted with the low-molecular-weight region which probably represents molecular species containing a common core oligosaccharide linked to lipid A. On the basis of the cross-reactions of the LPS molecules having a ladder structure, the six pathovars could be divided into two main groups. The O antigens of *X. campestris* pv. campestris 1929 reacted with both *X. campestris* pv. pelargonii 2985 and *X. campestris* pv. vesicatoria 3178 antisera, and the O antigens of *X. campestris* pv. pelargonii 2985 and *X. campestris* vesicatoria 3178 reacted with *X. campestris* pv. campestris 1929 antiserum. However, the latter two O antigens did not cross-react. The other cross-reacting group contained *X. campestris* pv. begoniae 3021, *X. campestris* pv. graminis 3041, and *X. campestris* pv. translucens 2920 (Table 3). The results of the agglutination tests (Table 4) agreed well with the results of the immunoblotting experiments and also showed the presence of the two major groups. Of the other strains tested (data not shown), the *X. campestris* pv. cerealis, *X. campestris* pv. phlei, and *X. campestris* pv. poae strains had higher agglutination titers with antisera to members of the *X. campestris* pv. graminis-*X. campestris* pv. begoniae-*X. campestris* pv. translucens group. *X. campestris* pv. phaseoli and *X. campestris* pv. mangiferaeindicae strains were similar to *X. campestris* pv. pelargonii strains in their reactivity with the antisera. The smooth type of *X. campestris* pv. cucurbitae 2597 reacted with the *X. campestris* pv. campestris and *X. campestris* pv. vesicatoria antisera. The smooth *X. campestris* pv. daturi strain, strain 2935, had titers comparable to those shown by *X. campestris* pv. vesicatoria isolates; the only difference was that strain 2935 also cross-reacted significantly with the *X. campestris* pv. pelargonii antiserum.

DISCUSSION

The SDS-PAGE analyses of the OMP and LPS patterns showed that the strains belonging to an *X. campestris* pathovar are in most cases very similar and differ from isolates that belong to other pathovars. Our findings agree with the results obtained from restriction fragment length polymorphism and DNA-DNA homology analyses of *X. campestris* and from analyses of cellular proteins and fatty acids (15, 25, 43, 46) which have revealed conserved properties in *X. campestris* isolates and pathovars that infect members of one plant family (45). However, in some cases the pathovar division does not correspond to the observed DNA homology groups. In this study, similar LPS and protein profiles were obtained only for pathovars that attack related plants (i.e. members of the same plant family).

It appears that OMP and LPS patterns vary less in pathovars of *X. campestris* than in nonpathogenic plant-associated bacteria. OMP and LPS profiles exhibit great diversity and can be used for strain identification among plant root-colonizing *Pseudomonas* and *Rhizobium leguminosarum* biovars (9–11). OMP profiles varied only slightly within the *Xanthomonas* pathovars which we studied. Most pathovars were identifiable on the basis of their OMP profiles; the exceptions were *X. campestris* pv. translucens, *X. campestris* pv. graminis, *X. campestris* pv. cerealis, *X. campestris* pv. poae, and *X. campestris* pv. phlei, all of

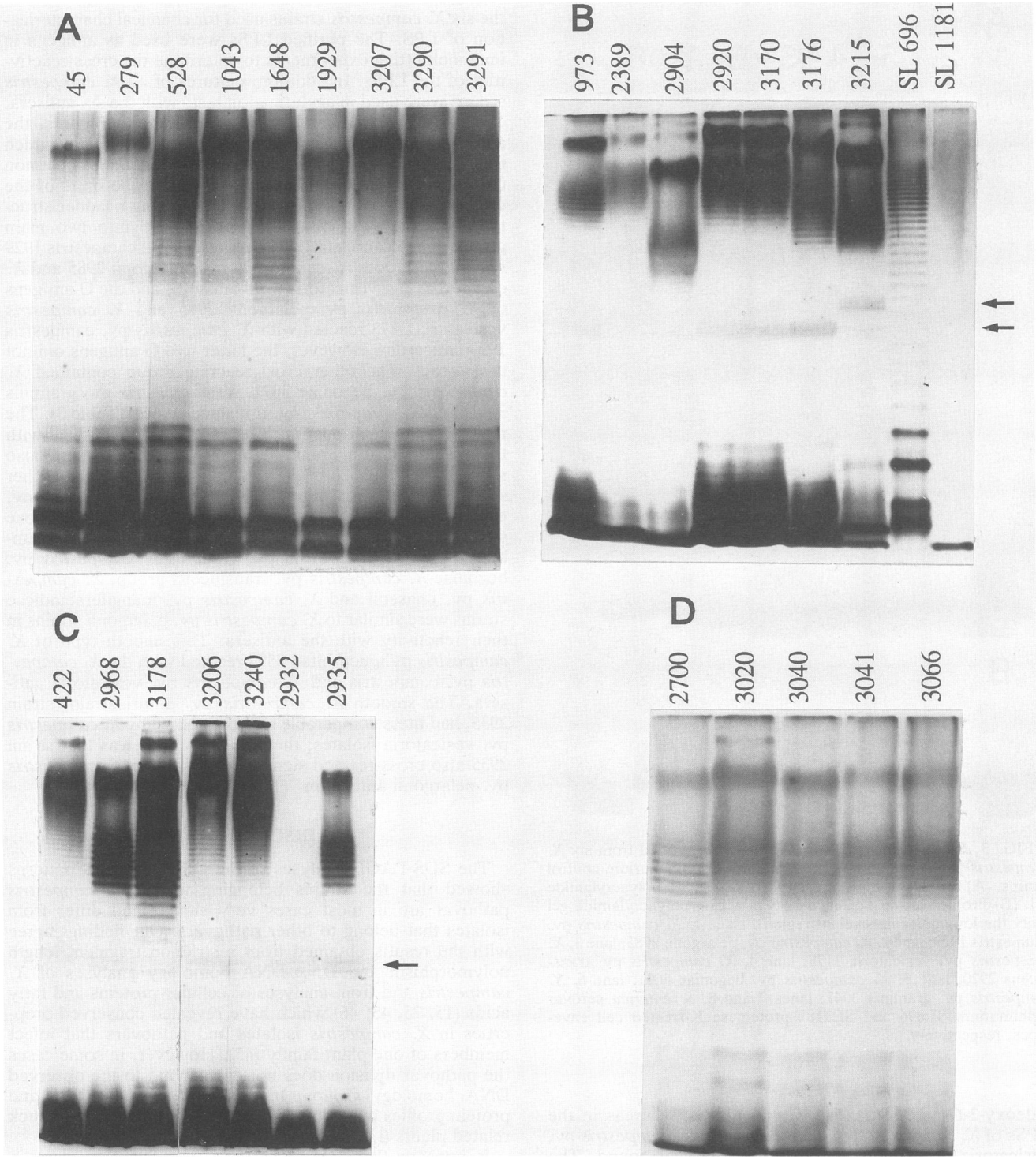

FIG. 4. Silver-stained SDS-PAGE of proteinase K-treated cells of *X. campestris* pv. campestris 45, 279, 528, 1043, 1648, 1929, 3207, 3290, and 3291 (A), *X. campestris* pv. translucens 973, 2389, 2904, 2920, 3170, 3176, and 3215 and *S. enterica* serovar typhimurium SL696 and SL1181 (B), *X. campestris* pv. vesicatoria 422, 2968, 3178, 3206, and 3240 and *X. campestris* pv. daturi 2932 and 2935 (C), and *X. campestris* pv. graminis 2700, 3020, 3040, 3041, and 3066 (D). Silver-stained proteinase K-resistant bands are indicated by arrows.

which all had OMP type 10 (Table 1). In general, the LPS profiles of the *X. campestris* isolates varied more than the OMP profiles. On the basis of LPS profiles, *X. campestris* pv. graminis was distinct from the other pathovars that produce the type 10 OMP pattern. In particular, the strains of *X. campestris* pv. campestris and *X. campestris* pv. translucens, both of which produced two types of OMP profiles, produced four (*X. campestris* pv. campestris) or

TABLE 2. Compositions of LPSs from *X. campestris* pathovars

Strain	Concn (μmol/mg) of:										
	Hexosamines	TBA^{+a}	Phosphate	Galacturonic acid	Rhamnose	Mannose	Glucose	Fucose	Xylose	3-*O*-methyl-6-deoxyhexose[b]	3-Amino-3,6-dideoxyhexose[b]
X. campestris pv. campestris 1929	0.55	0.10	1.2	0.21	0.31	0.24	0.28	0	0	0	0.03
X. campestris pv. vesicatoria 3178	0.46	0.10	0.95	0.31	1.3	0.23	0.26	0	0	0	0.23
X. campestris pv. pelargonii 2985	0.21	0.06	0.44	0.06	0.71	0.12	3.3	0.31	0	0	0
X. campestris pv. begoniae 3021	0.56	0.11	1.1	0.46	0.69	0.27	0.27	0	0.33	0.04	0
X. campestris pv. graminis 3041	0.54	0.07	0.81	0.21	1.8	0.25	0.49	0	0.33	0.16	0
X. campestris pv. translucens 2920	0.32	0.07	0.56	0.20	1.3	0.20	1.1	0	0.70	0.03	0

[a] TBA$^+$, thiobarbituric acid-positive material (estimated as 3-deoxy-D-mannooctulosonic acid). The LPS was hydrolyzed in 1 M HCl at 100°C for 2 h.
[b] The isomeric form was not determined, but it was the same in all LPSs.

five (*X. campestris* pv. translucens) types of LPS profiles (Fig. 4). All of the strains obtained from cereals or grasses except *X. campestris* pv. translucens 3215 differed from the other *Xanthomonas* strains by having only one major LPS molecule with a high mobility (Fig. 3B, lanes 4 and 6) instead of the two molecular species detected in the other strains (Fig. 3B, lanes 1 and 3). The extent of conservation of the OMP and LPS profiles within *X. campestris* pathovars is exemplified by the fact that we detected only one strain, *X. campestris* pv. translucens 3215, that differed completely from the other isolates of its pathovar in OMP and LPS patterns as well as in LPS antigenicity.

A chemical analysis of the LPSs from six pathovars showed that they fall into three major groups: those with a xylose- and 3-*O*-methyl-6-deoxyhexose-containing LPS (*X. campestris* pv. begoniae, *X. campestris* pv. graminis, *X. campestris* pv. translucens), those with a 3-amino-3,6-dideoxyhexose-containing LPS (*X. campestris* pv. campestris, *X. campestris* pv. vesicatoria), and those with a fucose-containing LPS (*X. campestris* pv. pelargonii) (Table 2). On the basis of LPS sugar composition, *Xanthomonas* pathovars have previously been roughly differentiated into three main groups containing either xylose or fucose or neither of these sugars (47, 48). Our analysis of LPSs revealed that small amounts of a 3-*O*-methyl-6-deoxyhexose not previously reported for *Xanthomonas* spp. were present in each of the xylose-containing LPSs. Although *O*-methyl sugars are found in LPSs of many bacterial groups (28), either as nonstoichiometric derivatives of their parent sugars (33) or as components of the terminal O-specific repeating unit (5), the general significance of these sugars is unclear. A 3-amino-3,6-dideoxyhexose, which was present in the LPSs of *X. campestris* pv. vesicatoria and *X. campestris* pv. campestris, has been detected previously as a major component in phenol-soluble LPS of *X. campestris* and LPS of another plant-associated bacterium, *Rhizobium leguminosarum* biovar trifolii (8, 19).

The division into three chemically different LPS groups (Table 2) was reflected in the reactivities of the anti-O antisera obtained with the six pathovars tested (Tables 3 and 4). In Western blots (immunoblots), O chains in the LPSs of *X. campestris* pv. begoniae, *X. campestris* pv. graminis, and *X. campestris* pv. translucens isolates cross-reacted with each other but not with LPSs from *X. campestris* pv. campestris, *X. campestris* pv. pelargonii, and *X. campestris* pv. vesicatoria isolates, which exhibited cross-reactivity (Table 3). Very similar patterns of reactivity were detected in Western blots prepared with the six purified LPS antigens and in agglutination assays performed with many isolates (Tables 3 and 4), which strongly suggests that O antigenicity in these six pathovars is conserved fairly well. The possibility that minor variability exists is suggested by the finding that the *X. campestris* pv. translucens and *X. campestris* pv. campestris isolates exhibited heterogeneity in their LPS profiles (Fig. 4) and also exhibited a wider range of reactivity in agglutination assays than the isolates of the other four pathovars did (Table 4). The immunological reactivity of *X. campestris* pathovars detected in this work is similar to the immunological reactivity obtained by Elrod and Braun (13) with agglutination assays.

The fact that variation in OMPs and LPSs is conserved within *X. campestris* pathovars raises the question of whether surface antigens have a function in determining pathovar host specificity. *X. campestris* pv. pelargonii, *X.*

TABLE 3. Immunoblot specificities and cross-reactivities of rabbit antisera to six *X. campestris* LPSs

Antiserum to:	Reactivity with O antigen in[a]:					
	X. campestris pv. campestris 1929	*X. campestris* pv. vesicatoria 3178	*X. campestris* pv. pelargonii 2985	*X. campestris* pv. begoniae 3021	*X. campestris* pv. graminis 3041	*X. campestris* pv. translucens 2920
X. campestris pv. campestris 1929	++	+	+	−	−	−
X. campestris pv. vesicatoria 3178	++	++	−	−	−	−
X. campestris pv. pelargonii 2985	+	−	++	−	−	−
X. campestris pv. begoniae 3021	−	−	−	++	++	+
X. campestris pv. graminis 3041	−/+	−	−	+	+	+
X. campestris pv. translucens 2920	−/+	−	−	++	++	++

[a] Reactivity scores were based on the intensity of immunostaining, as follows: ++, strong reaction; +, weak reaction; −, no reaction.

TABLE 4. Agglutination titers of *X. campestris* pathovars

Taxon	No. of strains tested	Titer of antiserum to[a]:					
		X. campestris pv. campestris 1929	*X. campestris* pv. vesicatoria 3178	*X. campestris* pv. pelargonii 2985	*X. campestris* pv. begoniae 3021	*X. campestris* pv. graminis 3041	*X. campestris* pv. translucens 2920
X. campestris pv. campestris	9	128–512	8–128	32–128	<8–32	<8–8	<8–16
X. campestris pv. vesicatoria	5	128–256	256–1,024	<8–64	<8–16	<8–16	<8–16
X. campestris pv. pelargonii	4	128–256	<8–16	256	<8–16	<8–16	<8–16
X. campestris pv. begoniae	3	32	8–16	16–32	1,024	128–256	128–256
X. campestris pv. graminis	5	16–32	8	8–32	256–512	128–256	128–256
X. campestris pv. translucens	7	8–16	<8–16	8–16	64–512	16–128	128–512

[a] The titer was the reciprocal of the highest dilution of antiserum that gave positive agglutination. The ranges of titers obtained with individual strains are shown.

campestris pv. begoniae, *X. campestris* pv. oryzae, *X. campestris* pv. mangiferaeindicae, *X. campestris* pv. citri, and *X. campestris* pv. pruni isolates characteristically infect members of only one plant genus or a few plant genera (27). Our study showed that these pathovars consist of isolates that have similar OMP and LPS types. Interestingly, the single *X. campestris* pv. citri strain that exhibited an LPS type distinct from that of the group A *X. campestris* pv. citri strains was a member of pathogenicity group C, which is known to have a geographical distribution and host specificity different from the geographical distribution and host specificity of the group A *X. campestris* pv. citri strains (17). *X. campestris* pv. pelargonii and *X. campestris* pv. begoniae have been shown previously to be uniform groups by DNA hybridization and electrophoretic analysis of cellular proteins (45). On the other hand, *X. campestris* pv. graminis isolates which share a common broad host specificity (12) are closely related according to the results of DNA hybridization analyses and electrophoresis of total proteins (46). This is in accordance with the similarity of their OMP and LPS profiles detected in this study. Strains assigned to *X. campestris* pv. vesicatoria infect various genera belonging to the family Solanaceae (27) and form at least two distinct subgroups on the basis of DNA homology data (44). Consistent with the latter finding is the fact that two major LPS types were detected for this pathovar (Fig. 4C); this pathovar also exhibited heterogeneity in its OMPs (Table 1). Notably, *X. campestris* pv. daturi, which also infects members of one genus belonging to the family Solanaceae, had OMP and LPS profiles identical to the profiles of the two *X. campestris* pv. vesicatoria isolates with the type 15 OMP profile. This may indicate that these strains have a common origin.

In this paper, we show that the variation in OMPs and LPSs is correlated with the pathovar groups of *X. campestris*; it remains to be established how surface antigens affect the pathogenic processes in *X. campestris* infections. It is also possible that the surface structures which we studied, OMPs and LPSs, are only indirectly involved in the infection processes but serve as useful surface markers for pathovars or clonal groups.

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