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Pathovar-Specific Antigens of *Xanthomonas campestris* pv. *begoniae* and *X. campestris* pv. *pelargonii* Detected with Monoclonal Antibodies

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Two monoclonal antibodies specific for lipopolysaccharide antigens of *Xanthomonas campestris* pv. *begoniae* and pv. *pelargonii* reacted with all of their respective pathovar strains and not with 130 strains of other xanthomonads or 89 nonxanthomonads tested. These results, as well as previous results, indicate that pathovar-specific monoclonal antibodies were readily generated to strains of *X. campestris* pathovars that generally infect single hosts.

The phytopathogenic bacterial species *Xanthomonas campestris* is composed of numerous pathovars (4), that is, strains that are similar with respect to biochemical and bacteriological characteristics but different with respect to pathogenicity on one or more plant hosts. For example, *X. campestris* pv. *begoniae* causes a leaf spot disease of begonia, and *X. campestris* pv. *pelargonii* causes leaf spot, stem rot, and wilt of geranium, but neither of these pathogens infects the host species of the other. In attempts to distinguish between *X. campestris* pathovars by characteristics other than host reaction, several biochemical and genetic methods have been used, such as fatty acid analysis (13), DNA hybridization (14), phage typing (8, 12), and restriction fragment length polymorphism (6, 7, 11). As yet none of these methods has replaced testing of strains on suspected hosts for identifying *X. campestris* pathovars. Recently, monoclonal antibodies (MAbs) to several phytopathogenic bacteria were shown to be useful for identification, diagnosis, classification, and epidemiological purposes (1–3, 5, 17). Among the MAbs produced, one was specific for all 178 tested strains of *X. campestris* pv. *oryzae* (3), the etiological agent of bacterial leaf blight, and another was specific for all strains of *X. campestris* pv. *oryzicola* (3), the causal agent of bacterial leaf streak of rice. Neither of these MAbs reacted with numerous xanthomonads of other pathovars and species or with strains of other bacterial genera, and thus these MAbs were considered pathovar specific. However, after numerous attempts, no MAbs were generated that were specific for all strains of two other *X. campestris* pathovars, *campestris* (1) and *citri* (2; A. A. Benedict, A. M. Alvarez, C. Y. Mizumoto, and E. L. Civerolo, Phytopathology **75:** 1352, 1985). Therefore, MAbs were produced to other *X. campestris* pathovars to determine whether all strains of other pathovars could be identified by a single specific antigen, thus making available a rapid definitive method for identification of these pathovars. In this report, we present evidence that *X. campestris* pv. *begoniae* and pv. *pelargonii* can be identified with MAbs and that the pathovar-specific epitopes were associated with lipopolysaccharide (LPS).

The procedures used for production of MAbs have been reported previously (1). BALB/c mice were immunized twice, 14 days apart, by intraperitoneal injections of formalinized *X. campestris* pv. *begoniae* IPO-802 and IPO-803 or *X. campestris* pv. *pelargonii* X38. Selected antibody-producing cultures, as determined by the enzyme-linked immunosorbent assay (ELISA) described previously (3), were cloned twice by the limiting-dilution technique and grown as ascites in BALB/c mice. The resulting ascites were screened by reacting them with 26 strains of *X. campestris* pv. *begoniae* and 76 strains of *X. campestris* pv. *pelargonii*. For each pathovar, only a single attempt was necessary to produce pathovar-specific MAbs. Two MAbs were selected for further study, *X. campestris* pv. *begoniae*-specific MAb (Xbeg-1), clone 142-D7 (immunoglobulin G3*k*), and *X. campestris* pv. *pelargonii*-specific MAb (Xpel-1), clone 126-A75 (immunoglobulin G2a). Both MAbs reacted with all of their respective pathovar strains at 1:1,000 dilutions of ascitic fluid and not with any of 130 strains of other xanthomonads or 89 strains of other genera described previously (1, 3). The other *X. campestris* pathovars tested were *aberrans*, *alfalfae*, *amoraciae*, *begoniae*, *campestris*, *carotae*, *citri*, *dieffenbachiae*, *euphorbiae*, *hederae*, *holcicola*, *incanae*, *malvacearum*, *manihotis*, *pelargonii*, *phaseoli*, *oryzae*, *oryzicola*, *raphani*, *syngonii*, *translucens*, *urticae*, *vesicatoria*, and *vitians*. The ELISA activities of these MAbs are shown in Table 1.

The epitopes were associated with surface antigens, since both MAbs gave strong confluent immunofluorescence and agglutinated the bacterial cells. By the immunoelectron microscopy technique of Robinson et al. (15) described previously (3), Xbeg-1 and Xpel-1 showed similar distributions on the cell surfaces (Fig. 1). LPS extracts of *X. campestris* pv. *begoniae* and *X. campestris* pv. *pelargonii* prepared from either living or Formalin-killed bacteria by the hot-phenol method (16) and purified by centrifugation for 6 h at 80,000 × *g* contained the specific antigens as determined by ELISA and Western blots (immunoblots). Western blots were performed by heating LPS diluted 1:1 with 2× sample buffer at 100°C for 90 s and fractionating them by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, using 5% stacking gels, 7.5% separating gels, and the buffer system of Laemmli (10). After electrophoresis, components were electrotransferred from gels to nitrocellulose. The nitrocellulose was blocked with 5% nonfat dry skim milk (9) in phosphate-buffered saline containing 0.02% azide (Blotto) overnight, rinsed in phosphate-buffered saline, and then reacted with 1:1,000 dilutions of MAbs in 1:3 Blotto. After

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TABLE 1. Titration by ELISA of MAbs Xbeg-1 and Xpel-1 specific for *X. campestris* pv. *begoniae* and *pelargonii*, respectively

Reciprocal dilution of MAb (10^{-3})	Titration by ELISA of[a]:	
	Xbeg-1[b]	Xpel-1[c]
1	1.14	1.60
2	0.72	1.35
4	0.39	1.11
8	0.16	0.85
16	0.07	0.45
32	0.06	0.26
64	0	0.20
128	0	0.12

[a] Averages of duplicate A_{450}s measured with a Titertek Multiskan plate reader.
[b] Reacted with Formalin-killed *X. campestris* pv. *begoniae* cells (A_{600}, 0.1).
[c] Reacted with Formalin-killed *X. campestris* pv. *pelargonii* cells (A_{600}, 0.1).

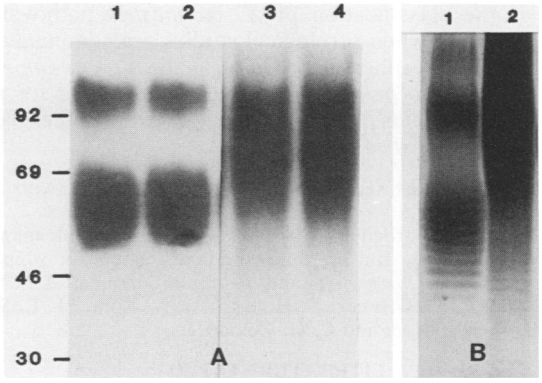

FIG. 2. (A) Western blots of purified LPS of *X. campestris* pv. *begoniae* (lanes 1 and 2) and *X. campestris* pv. *pelargonii* (lanes 3 and 4) reacted with MAbs Xbeg-1 and Xpel-1, respectively. Lanes 1 and 3, untreated LPS; lanes 2 and 4, LPS treated with proteinase K for 6 h at 37°C. (B) Silver-stained LPS in polyacrylamide electrophoresis gels of *X. campestris* pv. *begoniae* (lane 1) and *X. campestris* pv. *pelargonii* (lane 2).

washing, protein A labeled with ^{125}I was added for 1 h. Blots were autoradiographed on Kodak X-Omat AR5 film with an intensifying screen at −70°C. Each MAb was specifically bound by the respective purified LPS. The Xbeg-1 blots had two regions of binding at approximately 60 and 98 kildodaltons (Fig. 2). The ladder pattern characteristic of LPS was most striking with the lower-molecular-weight blot. The Xpel-1 blot showed a single dispersed blot without the distinctive ladder pattern at about 80 kilodaltons. Gels stained by the silver stain method for LPS also showed LPS in the same regions as the immunoblots. Each antigen treated with either RNase, proteinase K (Fig. 2), or pronase bound specific MAb. However, the ability of *X. campestris* pv. *pelargonii* LPS to bind MAb was greatly reduced when it was heated to 100°C for 30 min in phosphate-buffered saline. Thus, these two pathovar-specific antigens, as well as one *X. campestris* pv. *oryzae*-specific MAb (XCO-2) that reacts with most but not all *X. campestris* pv. *oryzae* strains (3), were associated with LPS.

Two major points for discussion emerge from this study. First, in regard to application, MAbs Xbeg-1 and Xpel-1 can be used for the identification of begonia leaf spot and geranium leaf spot and stem rot, respectively. These diseases result in serious losses of begonia and geranium plants, and rapid identification of the respective pathogen in propagative material is required for production of disease-free stock plants for the ornamental plant industry. Since these MAbs detected all tested strains of these pathovars, their use in an ELISA or immunoblot would be reliable and could be used to develop a more rapid method for identification than current bioassay methods.

A second point is that the ease of generating taxon-specific MAbs appears to be inversely related to the heterogeneity of the host range of the xanthomonad. Pathovar-specific MAbs were produced that reacted specifically with all strains of an *X. campestris* pathovar that generally infects a single host, as was observed with pathovars *oryzae* (3) and *oryzicola* (3), which infect only rice, pathovar *begoniae*, which infects only begonia plants, and pathovar *pelargonii*, which infects geranium plants. On the other hand, for certain *X. campestris* pathovars that infect several host genera, such as pathovars *campestris* (1), *dieffenbachiae* (R. L. Bonner, A. M. Alvarez, J. Berestecky, and A. A. Benedict, Phytopathology **77**:1725, 1987), *citri* (2; A. A. Benedict, A. M. Alvarez, C. Y. Mizumoto, and E. L. Civerolo, Phytopathology **75**: 1352, 1985), and *vesicatoria* (A. M. Alvarez and A. A. Benedict, unpublished data), no MAbs were found that reacted with all strains of the pathovars. In the latter cases, distinct serological subgroups were apparent, and with *X. campestris* pv. *campestris*, serologically different strains varied with respect to virulence on the respective host (A. M. Alvarez, A. A. Benedict, G. Or, and C. Y. Mizumoto, Phytopathology **77**:1725, 1987). We have pointed out the heterogeneity of *X. campestris* pv. *citri* strains on the basis of analysis with a battery of MAbs. Clearly a reevalu-

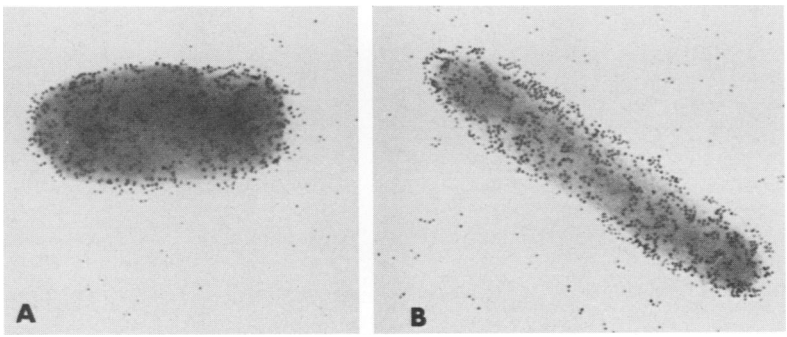

FIG. 1. Immunoelectron micrographs of *Xanthomonas campestris* pv. *begoniae* (A) and *Xanthomonas campestris* pv. *pelargonii* (B) reacted with 1:10 dilutions of MAbs Xbeg-1 and Xpel-1, respectively. Magnification, ×31,250.

ation of the classification of *X. campestris* pathovars is warranted, and when serological studies are complemented with genetic and other methods of analysis, *X. campestris* pv. *begoniae* and *pelargonii* might be considered for reinstatement to species, as has been suggested for other pathovars of *X. campestris* (7).

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