# Proteins Unique to Phenotypically Distinct Groups of *Xanthomonas campestris* pv. *vesicatoria* Revealed by Silver Staining

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## ABSTRACT

Bouzar, H., Jones, J. B., Minsavage, G. V., Stall, R. E., and Scott, J. W. 1994. Proteins unique to phenotypically distinct groups of *Xanthomonas campestris* pv. *vesicatoria* revealed by silver staining. Phytopathology 84:39-44.

Silver staining of sodium dodecyl sulfate-lysed cells of *Xanthomonas campestris* pv. *vesicatoria* electrophoretically separated in polyacrylamide gel revealed broad, dark gray bands in the low molecular weight region. The molecular weight of these bands was characteristic for each of the two major phenotypic groups identified in our *X. c. vesicatoria* collection. A 32- to 35-kDa band, designated $\alpha$, was present in 192 of 197 tomato race 1 strains; whereas, a 25- to 27-kDa band, designated $\beta$, was present in all 55 strains of tomato race 2. Race 1 strains expressing an $\alpha$ band were unable to hydrolyze starch ($Amy^-$), and very few degraded pectate ($Pec^-$). In contrast, most race 2 strains were $Amy^+$ and $Pec^+$. The $\alpha$ and $\beta$ bands, which are unique to each of the *X. c. vesicatoria* subpopulations, were not revealed when the Coomassie blue or copper staining protocols were used and were characterized as heat-stable proteins. Silver staining of protein profiles and testing for amylolytic activity of the bacterium are relatively simple tests that can help assign uncharacterized strains to each *X. c. vesicatoria* phenotypic group.

*Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye, the causal agent of bacterial spot of tomato (*Lycopersicon esculentum* Mill.), was initially described by Doidge (7) and Gardner and Kendrick (9). This pathogen causes economically important losses on this host in the warm and humid Florida climate (20,21). In Florida, bacterial spot of tomato is caused by a group of strains (hereafter referred to as race 1 strains), which are pathogenically distinct from most strains received from South America (hereafter referred to as race 2 strains). Infection by the two organisms results in slightly different symptoms (26). Race 1 strains cause elevated spots with whitish margins, whereas race 2 strains induce flat or sunken leaf spots surrounded by water-soaked areas (1). In addition, the two groups differ in their abilities to infect the tomato genotype Hawaii 7998; race 2 strains are pathogenic, and race 1 strains trigger a hypersensitive reaction (29). The two groups of *X. c. vesicatoria* strains differ also in amylolytic and pectolytic activities (1,12), carbon substrate utilization (3), and lipopolysaccharide-associated antigens (12). DNA homology between the two groups was less than 50%, thus confirming the distinctiveness of the two groups at the genetic level (26).

Electrophoretic separation of cellular proteins in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) provides information on the similarity of strains within species and is widely used in bacterial classification (13). A preliminary study (2) comparing silver-stained protein profiles of *X. c. vesicatoria* strains indicated a relationship between protein bands and geographic origins of strains. In this paper, information is presented on the identification of unique protein bands for distinguishing the two groups of *X. c. vesicatoria* strains.

## MATERIALS AND METHODS

**Bacterial strains.** Two hundred fifty-two *X. c. vesicatoria* strains (Table 1) with different origins were analyzed. The strains were preserved at −70 C in 15% aqueous glycerol and cultured on nutrient agar (Difco Laboratories, Detroit, MI) prior to analysis.

**Race determination assays.** Growth of plants and pathogenicity tests on tomato accession Hawaii 7998 and cultivar Bonny Best were performed as reported (1,12). A bacterial suspension of $5 \times 10^8$ cfu/ml was infiltrated into the mesophyll of fully expanded leaflets. The plants were maintained at 24 C. Infiltrated areas were examined for necrosis daily for 3 days. Tomato race 1 strains are pathogenic on Bonny Best but induce a hypersensitive reaction (necrosis) on Hawaii 7998, whereas race 2 strains are pathogenic on both genotypes (29).

Strains that induced ambiguous reactions on plants were screened for the avirulence gene *avrRxv* (31) present in race 1 strains; the *avrRxv* gene is associated with hypersensitive reaction in Hawaii 7998 (32). Screening was performed by polymerase chain reaction (PCR) amplification with a thermal cycler according to standard protocols (10). The PCR solution contained PCR-amplification buffer (supplied by the manufacturer with the enzyme), 100 $\mu$M of each deoxynucleoside triphosphate, 50 $\mu$M of each primer, 100 ng of genomic DNA, and 1.25 U of *Taq* DNA polymerase. Template DNA was initially denatured at 95 C for 1 min. A total of 30 PCR cycles were run under the following conditions: denaturation at 95 C for 0.5 min, annealing at 64 C for 0.5 min, and DNA extension at 72 C for 0.75 min. A 680-bp region of the sequenced *avrRxv* gene (32; GenBank accession number L20423) was amplified with 25-mer primers RST27 (5′-AGTCGCGCGGACATTTAGCCCCGCC) and RST28 (5′-CGTCGATGGTGCGCCTGGAATGCGC) chosen from the sequence. Oligonucleotide primers were synthesized with the 394 DNA synthesizer (ABI Applied Biosystems, Foster City, CA) by

the Interdisciplinary Center for Biotechnology Research (University of Florida, Gainesville). PCR-amplified DNA was detected with agarose gel electrophoresis according to standard procedures (23).

**Assay for amylolytic and pectolytic activity.** The ability of strains to degrade starch was tested on two different media. The first was brilliant cresyl blue-starch medium (19) devoid of ammonium sulfate, brilliant cresyl blue, and methylene green. The second consisted of nutrient agar supplemented with 1% starch. The strains unable to degrade starch did not grow on the modified brilliant cresyl blue-starch medium, whereas amylolytic ($Amy^+$) strains grew profusely. Both $Amy^+$ and $Amy^-$ strains grew on the nutrient agar supplemented with starch. Starch hydrolysis was revealed by the production of an opaque zone surrounding bacterial growth in the medium. To confirm that starch hydrolysis had occurred, plates were flooded with Lugol's iodine (1% iodine, 2% potassium iodide), and clear unstained zones were formed where starch had been hydrolyzed.

Pectolytic activity was assayed as described (1) on modified crystal violet-pectate medium (25) devoid of crystal violet and thallium nitrate. After several days at room temperature, a depression developed in the medium surrounding the growth of each pectolytic ($Pec^+$) strain.

**Electrophoresis of whole-cell proteins.** The strains were characterized by one-dimensional SDS-PAGE of whole-cell proteins according to the method of Laemmli (15) as described by Jackman (11). Each strain was grown in nutrient broth (Difco Laboratories) at 28 C on an orbital shaker (200 rpm) for 18 h. Cells from a 1.5-ml sample were harvested by centrifugation (16,000 *g* for 10 min) and washed twice in sterile deionized water. The cell pellet (about 20 mg wet weight) was resuspended in 180 μl of 10% sorbitol, and the suspension was mixed with an equal volume of double-strength lysis buffer (125 mM Tris-hydrochloride, pH 6.8; 4% SDS; 10% 2-mercaptoethanol; 20% glycerol; and 0.01% bromophenol blue). The suspension was heated at 95 C for 10 min before it was loaded; it was then separated through a discontinuous gel (10 mm of stacking gel and more than 100 mm of separation gel). The separation gel (10% total acrylamide; 375 mM Tris-hydrochloride, pH 8.8; and 0.1% SDS) and the stacking gel (5% total acrylamide; 125 mM Tris-hydrochloride, pH 6.8; and 0.1% SDS) solutions were deaerated for 15 min, and polymerization was initiated by adding 0.05% ammonium persulfate and 0.005% tetramethylethylenediamine. The wells were loaded with 10 μl of sample; controls included protein molecular weight standards and extracts from group A strain 75-3 and group B strain ICPB 167. Electrophoresis was carried out at 4 C in a Protean II double slab vertical electrophoresis cell (BioRad Laboratories, Richmond, CA) filled with electrophoresis buffer (250

TABLE 1. List of *Xanthomonas campestris* pv. *vesicatoria* strains and their phenotypic characteristics

| Strain designation | Origin | Host[a] | Race[b] | Protein[c] | Amylolytic activity | Pectolytic activity | Source[d] |
|---|---|---|---|---|---|---|---|
| Group A[e] | | | | | | | |
| BA30-1 | Argentina | P | 1 | α | — | — | 1 |
| XV599 | Bahamas | P | 1 | α | — | — | 12 |
| XV317, XV322, XV326, XV363, XV368, XV371, XV374 | Barbados | P | 1 | α | — | — | 12 |
| IAPAR9696, IAPAR9699, IAPAR9700, IAPAR9701 | Brazil | P | 1 | α | — | — | 14 |
| XV461, XV492, XV494, XV496, XV497, XV516 | Costa Rica | P | 1 | α | — | — | 12 |
| XV235, XV274, XV276, XV290, XV293, CNBP 1604 | Guadeloupe | P | 1 | α | — | — | 12,16 |
| 90-27, 90-29, 90-30, 90-39 | Korea | P | 1 | α | — | — | 11 |
| XV880, XV883, XV888, XV950, XV990, XV996, XV999, B95 | Mexico | P | 1 | α | — | — | 12,22 |
| XV666, XV677, XV688, XV690, XV700, XV711, XV722, XV728, XV730, XV733, XV737, XV744, XV760 | Puerto Rico | P | 1 | α | — | — | 12 |
| LMG 913 | Senegal | P | 1 | α | — | — | 18 |
| 859-8, 985-A1, 985-B7, A1, A3 | Spain | P | 1 | α | — | — | 15 |
| 87-37, 87-40, 87-44, 87-47, 87-48, 87-56 | Taiwan | P | 1 | α | — | — | 21 |
| XV37, XV62, XV63, 69-1, 76-4, 80-5, 81-18, 82-4, 86-22, 86-46, 87-13 | Florida | P | 1 | α | — | — | 12,19 |
| EWCII, 71-1 | Hawaii | P | 1 | α | — | — | 2,7 |
| XV300, XV306, XV309, XV313, XV315, XV347, XV352, XV356, XV358, XV377, XV380, XV382, XV384 | Barbados | T | 1 | α | — | — | 12 |
| XV207, XV210, XV217, XV220, XV225, XV229, XV231, XV249, XV252, XV256, XV261, XV270, XV280, XV286, CNBP 2484 | Guadeloupe | T | 1 | α | — | — | 12,16 |
| XV535 | Guatemala | T | 1 | α | — | — | 12 |
| XC1-D | India | T | 1 | α | — | — | 6 |
| 91-66 to 91-80, XV955, XV957, XV960, XV966, | | | | | | | |

(*continued on next page*)

[a] Host of origin: P = pepper; T = tomato; U = unknown.
[b] Tomato race: 1 = hypersensitive reaction on Hawaii 7998; 2 = disease on Hawaii 7998.
[c] Molecular weight: α = 32–35 kDa; β = 25–27 kDa.
[d] Source of strains: 1 = A. Alippi, Catedra de Fitopatologia, Universidad Nacional de la Plata, La Plata, Buenos Aires, Argentina; 2 = A. Alvarez, Department of Plant Pathology, University of Hawaii 96822; 3 = American Type Culture Collection, 12301 Parklawn Dr., Rockville, MD 20852; 4 = C. Bender, Department of Plant Pathology, Oklahoma State University, Stillwater 74078; 5 = B. Canteros, Est. Exp. Agropecuaria, INTA, C. C. No. 5, Bella Vista, Corientes 3432, Argentina; 6 = R. Chand, Indian Institute of Horticultural Research, Sadashwanagar, Bangalore 560080, India; 7 = A. A. Cook, Plant Pathology Department, University of Florida, Gainesville 32611; 8 = B. Dhanvantari, Agriculture Canada Research Station, Harrow, N0R 1G0; 9 = R. Felix-Gastelum, Campbell Research and Technology, Apartado Postal 185, Guasave Sinaloa, 81000 Mexico; 10 = A. Hibberd, Department of Primary Industries, Redlands Research Center, Cleveland, Queensland, Australia; 11 = B. K. Hwang, Department of Agricultural Biology, Korea University, Anamdong, Sungbukku Seoul 136-701, Korea; 12 = J. B. Jones, G.C.R.E.C., University of Florida, Bradenton 34203; 13 = Z. Klement, Plant Protection Institute, Hungarian Academy of Sciences, 1525 Budapest, Hungary; 14 = R. M. V. B. C. Leite, Instituto Agronomico do Parana, C. P. 1331, Londrina, Parana, Brazil; 15 = M. M. Lopez, Instituto Valenciano de Investigaciones Agrarias, Apartado Oficial 46113, Moncado, Valencia, Spain; 16 = J. Luisetti, Collection National de Bactéries Phytopathogènes, INRA, Route de St. Clément, Beaucouzé, 49000 Angers, France; 17 = H. Nagai, Instituto Agronomico, 1481 Avenida Barao de Itapura, 13100 Campinas, Sao Paulo, Brazil; 18 = Y. Ping, LMG Culture Collection, Laboratorium voor Microbiologie, Universiteit Gent, K. L. Ledeganckstraat 35, B-9000 Gent, Belgium; 19 = R. E. Stall, Plant Pathology Department, University of Florida, Gainesville 32611; 20 = M. Starr, International Collection of Phytopathogenic Bacteria, University of California, Davis 95616; 21 = A. T. Tschanz, Asian Vegetable Research and Development Center, P. O. Box 42, Shanhua, Tainan, Taiwan; 22 = J. C. Watterson, Petoseed Research Center, 37437 State Hwy. 16, Woodland, CA 95695.
[e] Phenotypic groups according to Stall et al (26).

mM Tris, 192 mM glycine, and 0.1% SDS). A constant current of 25 mA per gel was applied until the bromophenol blue had reached the separation gel (0.75 h). The current was then increased to 35 mA per gel until the tracking dye had migrated about 100 mm through the separation gel (approximately 2.75 h).

Different staining techniques were compared because not all proteins were stained with the same intensity by the different staining protocols (6,22). Polyacrylamide gels were stained at room temperature with the following techniques: 0.125% Coomassie brilliant blue R-250 (11), 0.3 M $CuCl_2$ (16), a silver staining kit (Sigma silver stain kit, Sigma Chemical Co., St. Louis, MO) (18), or a combined Coomassie blue-silver staining procedure (6). In the latter procedure, proteins were initially stained with Coomassie blue, destained with 50% methanol-10% acetic acid, and finally stained with the silver staining kit.

**Preliminary characterization of silver-stained group-specific bands.** Silver stains both proteins and lipopolysaccharides (LPS); therefore, a preliminary characterization of group-specific bands was performed by comparing SDS-PAGE of LPS, whole-cell extracts from lysed cells, heat-treated (45 min at 121 C) whole-cell extracts, and extracts treated with proteinase K (Boehringer Mannheim Biochemicals, Indianapolis, IN). The phenol-water procedure (30) was used to extract LPS from cells grown in nutrient broth at 28 C for 18 h. Lyophilized LPS was diluted in sterile deionized water to a final concentration of 1 mg/100 μl. A proteinase K treatment of protein extracts and LPS samples was performed overnight in a water bath at 60 C with 100 ng of enzyme per 1 μl of substrate. To improve silver staining of LPS, the polyacrylamide gel was immersed for 5 min in 0.7% periodic acid-40% ethanol-5% acetic acid, as described by Tsai and Frasch (27).

## RESULTS AND DISCUSSION

Comparisons were made of protein profiles in SDS-PAGE gels stained with Coomassie blue, copper, silver, and the Coomassie-silver combination. SDS-PAGE analysis of whole-cell extracts of 20 randomly selected *X. c. vesicatoria* strains resulted in reproducible profiles made up of a large number of discrete bands. Silver (Fig. 1) or Coomassie-silver stained a band that was easily distinguishable from other bands. This band was broader and more diffuse than most bands in the profile, and its dark gray color contrasted with the orange to brown color of most other bands in the profile. This band was not visible when stained with Coomassie blue (Fig. 1) or copper (not shown). Although selective detection of a band by only one of the stains is not unusual (22), staining of this peculiar band in *X. c. vesicatoria* strains by silver may be the result of the superior affinity of this stain

TABLE 1. (*continued from preceding page*)

| Strain designation | Origin | Host[a] | Race[b] | Protein[c] | Amylolytic activity | Pectolytic activity | Source[d] |
|---|---|---|---|---|---|---|---|
| XV970, XV977, XV980, XV1002, XV1004, XV1007, XV1011, XV1013, XV1015, XV1035, XV1038, XV1040 | Mexico | T | 1 | α | — | — | 9,12 |
| XV438, XV439, XV453, XV481, XV504, XV526, XV529, XV531, XV540, XV541 | Nicaragua | T | 1 | α | — | — | 12 |
| LMG 914 | Senegal | T | 1 | α | — | — | 18 |
| 87-21, B111 | Taiwan | T | 1 | α | — | — | 21,22 |
| LMG 667 | Unknown | T | 1 | α | — | — | 18 |
| XV770, XV773, XV777, XV780, XV784, XV788, XV790, XV795, XV799, XV804 | U.S. Virgin Islands | T | 1 | α | — | — | 12 |
| XV18, XV39, XV58, XV67, XV85, XV116, XV118, XV121, XV122, XV127, XV128, XV820, XV821, XV915, XV929, XV936, 69-10, 75-3, 75-4, 83-4, 85-16, 86-2, B82 | Florida | T | 1 | α | — | — | 12,19,22 |
| PAP32, XV1596 | Hungary | U | 1 | α | — | — | 13 |
| Group B | | | | | | | |
| BA21-1, BA27-1 | Argentina | P | 2 | β | + | + | 1 |
| IAPAR8020 | Brazil | P | 2 | β | + | + | 14 |
| B122 | California | P | 2 | β | + | + | 22 |
| B80 | California | P | 2 | β | + | + | 22 |
| BA23-1, BA28-1, BA29-1, BV1-1, BV3-1, BV3-5, BV4-1, BV5-1, BV5-3a, BV5-4a, BV6-1, BV7-3a, BV8-1, 79-2 | Argentina | T | 2 | β | + | + | 1,5,19 |
| 0226 | Australia | T | 2 | β | + | + | 10 |
| IAPAR546, IAPAR8012, IAPAR8013, IAPAR8015, IAPAR8016, IAPAR8071, IAPAR9697, X525-85 | Brazil | T | 2 | β | + | + | 14,17 |
| XV522 | Costa Rica | T | 2 | β | + | + | 12 |
| CNBP 30, CNBP 1545-92 | France | T | 2 | β | + | + | 16 |
| XV456, XV457, XV469, XV550 | Guatemala | T | 2 | β | + | + | 12 |
| LMG 925 | Hungary | T | 2 | β | + | + | 18 |
| ATCC 35937, LMG 911, LMG 916, LMG 917 | New Zealand | T | 2 | β | + | + | 3 |
| 643 | Spain | T | 2 | β | + | + | 15 |
| ATCC 11551 | Indiana | T | 2 | β | + | + | 3 |
| XV10, XV15 | Oklahoma | T | 2 | β | + | + | 4 |
| XV8035 | Hungary | U | 2 | β | + | + | 13 |
| 81-6 | Indiana | U | 2 | β | + | + | 7 |
| Unusual phenotype | | | | | | | |
| 8, 21, 31, 35 | Canada | T | 1 | α | — | + | 8 |
| XV560 | Guatemala | T | 1 | α | — | + | 12 |
| XCV1-A, XCV1-C | India | T | 1 | α | — | + | 6 |
| XV655 | Puerto Rico | P | 1 | β | — | — | 12 |
| XV330, XV334, XV338, XV343 | Barbados | P | 1 | β | + | + | 12 |
| XV440, XV441 | Costa Rica | T | 2 | β | — | — | 12 |
| CNBP 2625 | Réunion Island | T | 2 | β | — | — | 16 |
| B61 | Florida | P | 2 | β | + | — | 22 |
| BV5-4b, BV20-3a | Argentina | T | 2 | β | + | — | 5 |
| 0350 | Australia | T | 2 | β | + | — | 10 |
| ICPB 167 | New Zealand | T | 2 | β | + | — | 20 |
| LAB2 | Hungary | U | 2 | β | + | — | 13 |

for this type of molecule (18). When samples of the same strain were run repeatedly, it was found that the electrophoretic mobility of the silver-specific band was constant, but the intensity and sharpness of the band varied. It became evident that this band was of diagnostic value when its position in the profile was correlated with the phenotypic grouping of the strains. The band present in group A (i.e., $Amy^-$, $Pec^-$) strains had an estimated molecular weight of 32–35 kDa and was designated $\alpha$; whereas in group B (i.e., $Amy^+$, $Pec^+$) strains, it had an estimated molecular weight of 25–27 kDa and was designated $\beta$ (Fig. 1).

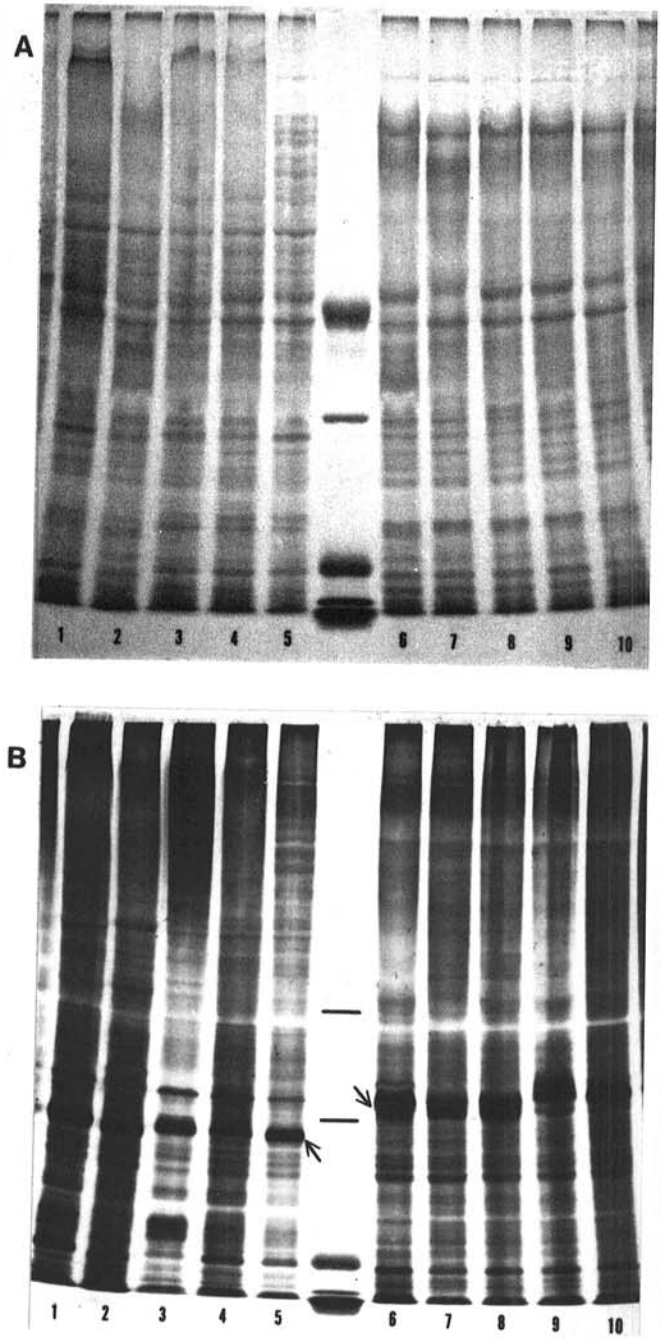


**Fig. 1.** Electrophoregrams of denatured proteins from sodium dodecyl sulfate-lysed cells of *Xanthomonas campestris* pv. *vesicatoria* stained with **A,** Coomassie blue and **B,** silver. Lanes 1–10: strains ATCC 35937, XV334, LAB2, X525-85, ICPB 167, 75-3, 87-13, XV300, XV990, and XV1040, respectively. Silver staining (**B**) revealed a broad, dark gray band of about 25–27 kDa (arrow) among group B (i.e., race 2, amylolytic [$Amy^+$] and pectolytic [$Pec^+$]) strains (lanes 1, 3, 4, and 5) and the race 1, $Amy^+$, $Pec^+$ Barbados strain (lane 2). A band similar in appearance but of about 32–35 kDa ($\alpha$ band; lanes 6–10) was present in group A strains (i.e., race 1, $Amy^-$, $Pec^-$) strains. These unique bands were not stained with Coomassie blue (**A**). Protein molecular weight markers in the center lane are from top to bottom: ovalbumin (43 kDa), carbonic anhydrase (29 kDa), $\beta$-lactoglobulin (18 kDa), and lysozyme (14 kDa).

The SDS-PAGE profiles of 252 *X. c. vesicatoria* strains from our worldwide collection (Table 1) were stained with silver for the presence of the $\alpha$ and $\beta$ bands. There was a high correlation between the presence of the $\alpha$ or $\beta$ band and other tested phenotypes (i.e., pathogenicity, starch hydrolysis, and pectate degradation). All race 2 strains expressed the $\beta$ band, and 97% of the 197 race 1 strains had the $\alpha$ band (Table 1). Amylolytic and pectolytic activities were generally associated with the presence of the $\beta$ band (Table 1). All $Amy^+$ strains expressed a $\beta$ band; in contrast, 192 of the 196 $Amy^-$ strains expressed the $\alpha$ band. Polypectate was degraded by 89% of the 56 $Amy^+$ strains (Table 1). Some of the $Pec^+$ strains strongly liquefied the pectate medium and multiplied actively, while others produced a limited depression at the surface of the gel and grew poorly on this medium. Of the 192 $Amy^-$ strains expressing an $\alpha$ band, seven had limited ability to degrade pectate. These seven $Pec^+$, race 1 strains belonged to a heterogenous group of 21 strains with unusual phenotype (Table 1). The remainder included five race 1 strains that expressed the $\beta$ band and nine race 2 strains that were unable to degrade starch and/or pectate. The race 1 affiliation of the unusual strains was confirmed by PCR-amplification signals of the 680-bp sequence within *avrRxv* (Fig. 2).

There was no correlation between the phenotypic groups and either geographic source or host of origin. Such an association between phenotype and origin could not be examined for the 21 strains with unusual phenotype because of their limited occurrence.

Preliminary characterization of silver-stained group-specific bands suggests that the $\alpha$ and $\beta$ bands are heat-stable proteins (Fig. 3). Subjecting the SDS-treated cell extract to 121 C resulted in the clearing of the LPS and of most of the protein bands but not of the group-specific band. Samples treated with proteinase K lost most of the bands present in the profiles of the group A strain 75-3 and the group B strain ICPB 167, including their group-specific $\alpha$ and $\beta$ bands, respectively. Proteinase K digestion of the SDS-treated cell extract revealed the LPS banding patterns. LPS extracts from strain 75-3 did not show the group-

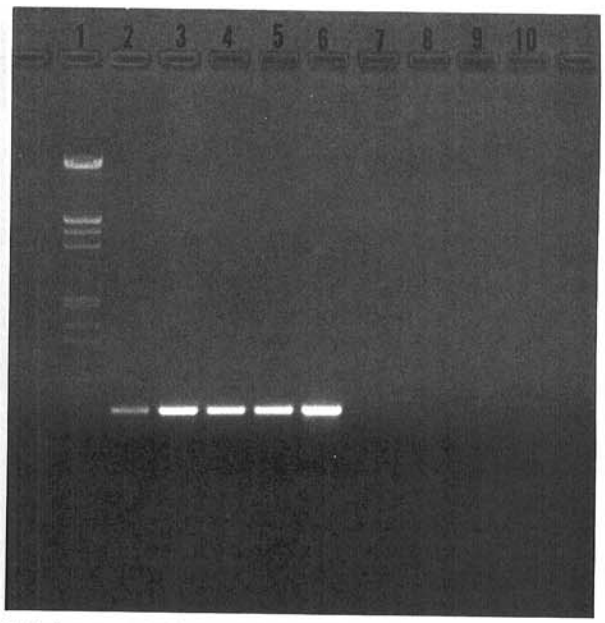


**Fig. 2.** Polymerase chain reaction (PCR) products of *Xanthomonas campestris* pv. *vesicatoria* strains obtained with primers from the *avrRxv* sequence. Lane 1, molecular weight markers from cleavage of lambda-DNA with *Eco*RI and *Hin*dIII; lanes 2–6, race 1 strains 87-13, XV207, XV334, 91-66, and XV1038, respectively, showing the 680-bp amplified fragment; lanes 7–10, race 2 strains CNBP 30, XV440, ATCC 11551, and ICPB 167, respectively, did not contain a PCR-amplified band.

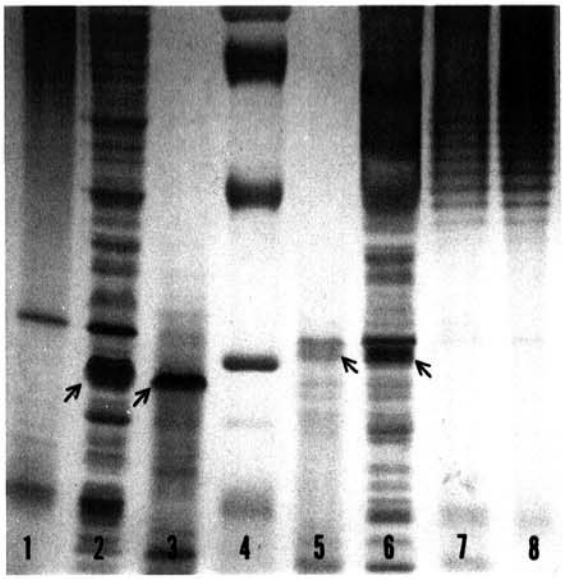


**Fig. 3.** Electrophoretic separation through a 10% acrylamide gel of protein and lipopolysaccharide (LPS) preparations treated with heat or chemicals to characterize the group-specific bands (indicated by an arrow) revealed with silver staining. Lane 1, group B strain ICPB 167 treated with sodium dodecyl sulfate (SDS) and proteinase K; lane 2, strain ICPB 167 treated with SDS; lane 3, sample in lane 2 autoclaved for 45 min; lane 4, protein molecular weight standards from top to bottom: bovine serum albumin (69 kDa), ovalbumin (46 kDa), and carbonic anhydrase (30 kDa); lane 5, group A strain 75-3 SDS lysed and autoclaved for 45 min; lane 6, protein profile of strain 75-3 treated with SDS; lane 7, sample in lane 6 treated with proteinase K (protein digestion reveals LPS ladder pattern identical to lane 8); lane 8, LPS of strain 75-3.

specific band $\alpha$.

This study demonstrated that the *X. c. vesicatoria* population is composed of at least two subpopulations that can be segregated by pathogenicity, amylolytic-pectolytic activities, and the presence of very distinct protein bands revealed by silver staining. The presence of $Amy^-$ and $Amy^+$ strains in *X. c. vesicatoria* is well known (4,5,8,14,17,24), but only with serology has a relationship been established with amylolytic activity of the strain (24). A recent numerical analysis performed on Coomassie blue-stained SDS-PAGE protein patterns of 17 strains also revealed two major clusters within *X. c. vesicatoria* (28). When we performed silver staining on profiles of strains from the Belgian LMG collection (Table 1), which are representative of these two clusters, strains assigned to clusters 3d and 7b of Vauterin et al (28) expressed the $\alpha$ and $\beta$ bands, respectively. Therefore, silver staining can be a simple, inexpensive alternative to scanning and cluster analysis of Coomassie blue-stained profiles, and it easily differentiates between the two major *X. c. vesicatoria* groups. A correlation was obtained between the presence of the silver-stained $\alpha$ or $\beta$ band and the genetic groupings obtained by pulsed-field gel electrophoresis of restricted genomic DNA and DNA homology studies performed on a limited number of strains (26). The latter technique indicated a low level ($\leq$46%) of genomic relatedness between the two *X. c. vesicatoria* groups, thus suggesting that they appear to represent different species. The finding of a correlation between *X. c. vesicatoria* subpopulations, defined by the size of the silver-specific band, and amylolytic activity suggests that silver staining of protein profiles and testing for starch hydrolysis are relatively simple assays that can be used to discriminate between the two major groups of *X. c. vesicatoria* strains.

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