Xanthomonas campestris pv. *asclepiadis*, pv. nov., Causative Agent of Bacterial Blight of Milkweed (*Asclepias* spp.)

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ABSTRACT

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Milkweed (*Asclepias* spp.) is currently grown in Nebraska as an alternative crop, principally for its floss. Plants with symptoms of leaf blight were observed in commercial milkweed fields and field plots from 1987 to 1993. A yellow-pigmented, gram-negative bacterium was consistently isolated from leaves and also from infected or infested seed, stems, roots, and flowers. The pathogen was identified using polypeptide and substrate-utilization profiles, fatty acid and pigment analysis, and growth on a semiselective medium. Host range tests on members of the milkweed family and on plants representing various commercial crops grown in Nebraska showed the pathogenic specificity of the bacterium for five of six *Asclepias* species tested. The pathogen appears to be a new pathovar of *Xanthomonas campestris*. The name proposed for this bacterium is *Xanthomonas campestris* pv. *asclepiadis*.

Additional keywords: *Asclepias speciosa* Torr., *Asclepias syriaca* L.

Properties of milkweed (*Asclepias* spp.) floss have spurred interest in development of the plant as an alternative crop (19). The floss is currently used as insulation for comforters and clothing products. The floss was a strategic war material and substituted for Kapok in life jackets (4,10). Milkweed has medicinal value and can be a source of natural rubber and chemical derivatives (1,10). Several milkweed species are also serious perennial weeds in some crop production areas (9,19).

A disease of milkweed was observed in cultivated fields in Nebraska starting in 1987 and has appeared each subsequent year. Characteristic symptoms consisted of leaf blight and wilt of young plants, often progressing to death of the plant. Symptoms included early leaf chlorosis, frequently beginning on one side of the leaf and restricted by the midrib, followed by necrosis. As necrosis became severe, defoliation was common. Symptoms were observed on leaves of all ages. Severity increased during the growing season. The incidence of disease increased from 10 to 90% as the season progressed. The effect on floss yield was confounded by other organisms that infect the plant and by insect damage. However, the disease reduced stands and affected the general health of the crop.

Bacteria were consistently isolated from diseased milkweed tissue, and these bacteria were characterized as xanthomonads. No other bacterial diseases of milkweed have been reported previously (5).

The purpose of this study was to determine the nature of the causal agent, investigate its host range, and further characterize the disease. Preliminary reports have been published (11,12).

MATERIALS AND METHODS

Isolation of the pathogen. Isolations were made from plants with blight symptoms collected from fields in Minden, Paxton, and Scottsbluff, Nebraska; these locations are separated by about 94 km. Later collections were made from other locations in Nebraska and Garden City, Kansas. Affected leaves from five to seven plants per sampling site were washed under running tap water for approximately 15 min. Pieces of tissue (1 to 3 cm^2) adjacent to necrotic areas were ground in 12.5 mM PO_4 buffer, pH 7.1, using a mortar and pestle. Serial dilutions were plated onto a nutrient broth–yeast extract medium, NBY, (17) and incubated at 25°C for 3 to 5 days. Single colonies of a yellow bacterium consistently isolated from symptomatic tissue were transferred three times onto fresh NBY agar to assure purity.

From plant material at locations listed above, similar isolations were made from stems, roots, seed, and flowers of diseased and asymptomatic plants. From three different seed lots, 5 g of seed were independently macerated in 20 ml of PO_4 buffer in a Waring blender with a small cup attachment. Aliquots of several 10-fold dilutions were plated on NBY agar. Seed were obtained from diseased milkweed plants in Minden, from milkweed grown in Garden City, and from Natural Fibers Corporation, Ogallala, Nebraska. Stem, root, and flower assays were similar to those described for leaves.

Bacteria. The taxa of bacteria used in this study are listed in Table 1. Besides the milkweed pathogen, other taxa and pathogens of host plants grown in Nebraska were included. Working cultures were maintained in a sterile glycerol medium by suspending several colonies in 1.5 ml of 50 mM PO_4 buffer; 1.2 ml of the suspension was added to 0.8 ml of glycerol, vortexed, and stored at –20°C. Cultures were also lyophilized in a skim milk medium (20% Bacto dehydrated skim milk [Difco Laboratories, Detroit, MI] in double-distilled water) for long-term storage at –20°C.

Pathogenicity tests. Pathogenicity of the bacterium was tested on 30-day-old milkweed seedlings (*Asclepias syriaca* L. and *A. speciosa* Torr.) established in 15-cm-diameter clay pots containing a potting medium of soil, peat moss, sand, and vermiculite (2:2:1:1). The medium was steamed for 1 h at 82°C. Seed was obtained from Natural Fibers Corporation. Plants were grown under greenhouse conditions, with temperature fluctuations between 24 and 35°C and a maximum natural light level of approximately 600 $\mu E \cdot s^{-1} \cdot m^{-2}$. Day length varied between 10 and 14 h. The plants were watered daily or as needed.

Either stems or leaves of plants were inoculated. For stem inoculations, approximately 0.1 ml of a cell preparation containing 10^6 CFU/ml was inserted into stems about 1 to 2 cm from the soil level with a 26 G gauge 1/2-in needle fitted onto a 1-cm^3 syringe. Inoculum was prepared from 3-day-old cultures grown at 25°C on NBY plates. Cells were suspended in 12.5 mM PO_4 buffer containing 10 mM $MgSO_4$. For leaf inoculations, a 100-µl pipette tip was touched to fresh colonies of

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bacteria grown for 24 to 48 h at 25°C and inoculated onto the adaxial surface of leaves with slight wounding by pipette (3). Symptom development was monitored up to 30 days after inoculation. The bacterium was reisolated from symptomatic tissue using the methods described above.

The effect of two temperatures on bacterial blight development in milkweed was tested in growth chambers (Environmental Growth Chamber, Chagrin Falls, OH) using one growth chamber at 25°C and the other at 32°C. Light and dark periods were set at 16 and 8 h, respectively. Low-pressure sodium and halide lights provided illumination. Test plants were grown from seed at each temperature. After 60 days, 10 to 12 plant stems were inoculated with a cell suspension containing approximately 10^7 CFU/ml. Control plants received buffer solution. The experiment was performed two times.

In another test, greenhouse-grown plants were stem inoculated with the bacterium 30, 60, or 90 days after planting by the method previously described. Six to 10 plants were inoculated on each date. Control plants were treated with buffer. The test was performed twice.

To assess systemic disease, milkweed rhizomes from eight diseased field plants collected from two different fields were planted in the greenhouse, and regrowth was observed for symptom development. In addition, root and rhizome tissue from 10 asymptomatic greenhouse-grown milkweed seedlings were inoculated in the greenhouse with a culture of strain 10007 containing 10^6 CFU/ml using the syringe inoculation procedure. Plants were removed from pots, inoculated, replanted, and observed for 60 days. Three seedlings received only sterile PO_4 buffer. The experiment was repeated twice.

Host range. The host range of the bacterium was determined from inoculation onto representative North American species of *Asclepias* (20; Table 2). Seeds were obtained from Jess R. Martineau, Native Plants, Inc., Salt Lake City, UT. In three independent tests, nine to 10 plants of each type were stem inoculated with a representative strain (10007) of the bacterium, as described above. Preliminary experiments with six independently isolated strains from the separate locations mentioned above showed no detectable differences in virulence to *A. syriaca* or in in vitro tests. Plants were evaluated for symptom development, and the bacterium was reisolated from symptomatic tissue by methods described previously.

The potential for cultivated crops to serve as hosts of the bacterium was tested (Table 2) with strains 10007, SB-K, and M-9. Pathovars of *X. campestris* known to be pathogenic on these respective plant species were used as positive controls (Table 2). For each host group, 30 plants were inoculated, and the experiment was performed twice. Negative control plants were tested with sterile buffer. The plants were grown in growth chambers at 22 or 28°C and also under greenhouse conditions in a minimum of three pots per treatment. Stems or leaves were inoculated 18 to 20 days after planting. Plants were evaluated for symptoms up to 30 days after inoculation.

To assess the possibility that the pathogen was a previously described pathovar of plants grown in the state, representatives of known *X. campestris* pathovars were tested for pathogenicity on milkweed (*A. syriaca* and *A. speciosa*). The type strain of *X. campestris* was included, along with pathovars of crops grown in Nebraska (Table 2). Known pathovars were inocu-

Table 1. Strains of bacteria tested for pathogenicity, characterization, and comparison with the milkweed pathogen

Bacterium	Strain	Source
Milkweed pathogen	10007, SB-K, M-9, P-f1	Nebraska
Xanthomonas campestris pv. *campestris* (Pammel) Dawson	528	NCPPB[a]
X. c. pv. *carotae* (Kendrick) Dye	5723	ICMP[a]
X. c. pv. *glycines* (Nakano) Dye	11766	ATCC[a], Nebraska
X. c. pv. *holcicola* (Elliott) Dye	3103	ICMP, Nebraska
X. c. pv. *phaseoli* (Smith) Dye	DRL-827, LB-1	Dominican Republic, Nebraska
X. c. pv. *translucens* (Jones, Johnson & Reddy) Dye	Js-2A, Js-1X	Nebraska
X. c. pv. *vesicatoria* (Doidge) Dye	XvL-2, 83-LT	Nebraska
X. albilineans (Ashby) Dowson	196	ICMP
X. axonopodis Starr & Garces	50-76	ICMP
X. fragariae Kennedy & King	10056	ICMP
X. populi (Ridé) Ridé & Ridé	5816-87	ICMP
Erwinia herbicola (Lohnis) Dye	112Y	C. Ishimaru, Colorado State Univ.
Clavibacter michiganensis subsp. *nebraskensis* (Vidaver & Mandel) Davis, Gillaspie, Vidaver & Harris	CN72-2	Nebraska
C. m. subsp. *insidiosus* (McCulloch) Davis, Gillaspie, Vidaver & Harris	P9	Pennsylvania

[a] Abbreviations: ATCC = American Type Culture Collection, Rockville, MD; NCPPB = National Collection of Plant Pathogenic Bacteria, Harpendon, England; ICMP = International Collection of Micro-Organisms from Plants, Auckland, New Zealand. Strains from international culture collections are pathotype strains.

Table 2. Susceptibility of milkweed species and various crop plants tested with pathogenic strains from milkweed and with known pathogens

		Susceptibility to pathogen	
Scientific name	Common name (cultivar)	Milkweed[a]	Known[b]
Asclepias syriaca L.	Common milkweed	+[c]	–[d]
A. speciosa Torr.	Showy milkweed	+	–[d]
A. tuberosa L.	Butterfly milkweed	+	–[d]
A. erosa Torr.		+	–[d]
A. syriaca × *A. speciosa*		+	–[d]
A. subulata Decne.		–	–[d]
Glycine max L.	Soybean (Harosoy)	–	+[e]
Lycopersicon esculentum Mill.	Tomato (Rutgers 10020-17248)	–	+[f]
Medicago sativa L.	Alfalfa (B-54)	–	+[g]
Phaeolus vulgaris L.	Dry bean (DRK Charlevoix)	–	+[h]
Sorghum bicolor L.	Sorghum (Bug Off)	–	+[i]
Triticum aestivum L.	Wheat (Michigan Amber)	–	+[j]
Zea mays L.	Maize (Golden Cross Bantam)	–	+[k]

[a] Strains 10007, SB-K, and M-9.
[b] All pathogens of crop plant species were tested on milkweed; positive controls produced expected disease symptoms in homologous hosts.
[c] + = positive, disease response; – = no disease or response.
[d] Delayed hypersensitivelike response in leaves of milkweed inoculated with *Xanthomonas campestris* pv. *holcicola*, *X. c.* pv. *campestris* 528, and *X. c.* pv. *glycines*, and in dry beans inoculated with the milkweed pathogen.
[e] *X. c.* pv. *glycines* 11766.
[f] *X. c.* pv. *vesicatoria* 83-LT.
[g] *Clavibacter michiganensis* subsp. *insidiosus* P9.
[h] *X. c.* pv. *phaseoli* LB-1.
[i] *X. c.* pv. *holcicola* 3103.
[j] *X. c.* pv. *translucens* Js-1x.
[k] *X. c.* pv. *holcicola* 3103 or *C. m.* subsp. *nebraskensis* CN72-2.

lated at about 10^7 CFU/ml, either by stab or micropipette inoculation, into their respective host plants. All these strains and strain 10007 of the milkweed pathogen were compared for pathogenicity on 60-day-old milkweed plants using the micropipette tip technique to inoculate leaves. Plants were observed for symptom development for 30 days after inoculation, and the experiment was performed twice.

Plants were set up in randomized complete block design whenever multiple strains or pathogens were tested. Responses were recorded as positive or negative.

Characterization of the pathogen. Characterized strains were from Nebraska unless otherwise indicated. Each strain was tested for gram reaction (15), growth on the semiselective medium MXP (8), xanthomonadin pigment (13), substrate utilization, fatty acid profile, and one-dimensional polypeptide profile (6).

Pigment analysis was performed using a modification of the procedure of Irey and Stall (2,13) with *X. c.* pv. *phaseoli* LB-1 as a positive control.

Substrate utilization profiles were obtained by using the GN MicroPlate System (Biolog, Inc., Hayward, CA) according to the manufacturer's recommendation. *X. campestris* pathovars (*X. c.* pv. *holcicola* Xhl, *X. c.* pv. *campestris* 528 NCPPB, *X. c.* pv. *glycines* 11766 ATCC) and *Erwinia herbicola* 112 Y were tested along with milkweed strains 10007, SB-K, and M-9.

Fatty acid compositions of strains 10007, SB-K, and M-9 of the milkweed pathogen were analyzed separately by M. Sasser (Microbial ID, Inc., Newark, DE). Some comparative studies have shown the usefulness of this technique in differentiating xanthomonads (7,18,21).

Polypeptide profiles of *Xanthomonas* species type strains and selected *X. campestris* pathovars (Table 1) were examined using modified procedures of Carlson and Vidaver (6). Cells were grown in NBY broth (without Mg or glucose) to an OD_{640} of 0.4. The cells were collected by centrifugation and washed three times by vortexing in 1 ml of 0.01 M Tris buffer, pH 8.0. The final pellet was resuspended in 200 µl of lysozyme (Sigma Chemical Company, St. Louis, MO) solution (4 mg/ml in 10 mM Tris, pH 8.0) and vortexed 1 min. This suspension was incubated 1 h at 37°C, 200 µl of 10% sodium dodecyl sulfate (SDS) (Sigma) was added, and the sample was vortexed again. The tube containing the sample was cooled at –20°C until frozen and subsequently thawed at 37°C. The freeze–thaw sequence was repeated twice. A buffer of 200 µl of 100 mM Tris, 10% glycerol, 2% SDS, 5% ß-mercaptoethanol, 4 mM EDTA, and enough bromphenol blue to give a pale color was added, and samples were either loaded immediately onto 3-mm-thick gels or maintained at –20°C until used. A 12% linear resolving gel (25 ml of distilled H_2O; 20 ml of 1.5 M Tris, pH 8.8; 32 ml of acrylamide solution [29.2 g of acrylamide + 0.8 g of N,N′-bismethyleneacrylamide brought to 100 ml in distilled H_20]; 0.8 ml of 10% SDS; 0.75 ml of 10% ammonium persulfate; 0.0375 ml of TEMED) was overlaid with a 4% stacking gel (9.15 ml of distilled H_20; 3.75 ml of 0.5 M Tris, pH 6.8; 1.95 ml of acrylamide solution, as described for resolving gel; 0.15 ml of 10% SDS; 0.15 ml of 10% ammonium persulfate, 7.5 µl of TEMED). Upper and lower buffer reservoirs were attached and filled with an electrode well buffer (57 g of glycine, 12.1 g of Tris, 10 ml of 10% SDS, and 0.8 ml of 3-mercaptopropionic acid per liter). With the apparatus placed in an ice bath, 60- to 80-µl samples were electrophoresed at constant 65 mA until just before the bromphenol blue in the sample began to elute from the gel (about 2 to 3 h). Prestained SDS molecular weight markers (Sigma) were used for comparison. Gels were held overnight in Coomassie stain (0.125% Coomassie Brilliant Blue R250, 50% methanol, 10% acetic acid). Gels were destained sequentially in solutions of 50% methanol with 10% acetic acid followed by 5% methanol with 7% acetic acid. The destained gels were photographed (Kodak Technical Pan Film TP 135-36, 58 green filter) and stored at room temperature in destaining solution for reference.

Fig. 1. Characteristic field symptoms of bacterial blight of milkweed.

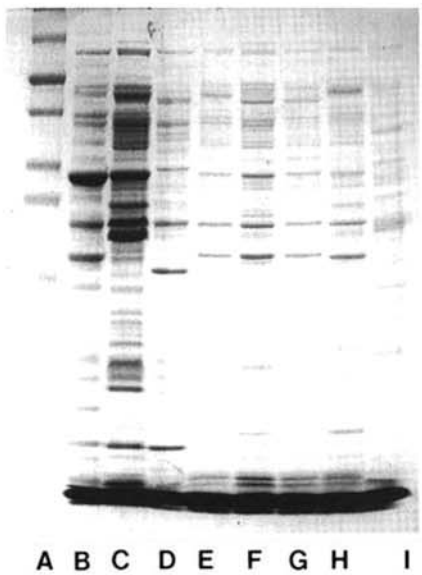

Fig. 2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of total cellular proteins from xanthomonads and *Erwinia herbicola*. Lane A, molecular size markers (approx. mol. wt. = 180,000, 116,000, 84,000, 58,000, 48,500, 36,500, 26,600); lane B, *Xanthomonas populi* 5816-87; lane C, *X. axonopodis* 50-76; lane D, *X. albilineans* 196; lane E, *X. fragariae* 10056; lane F, *X. campestris* pv. *phaseoli* DRL-827; lane G, *X. c.* pv. *glycines* C; lane H, milkweed pathogen 10007; lane I, *Erwinia herbicola* 112 Y.

RESULTS

Isolations and symptomatology. A mucoid, yellow, gram-negative, rod-shaped bacterium was consistently recovered from milkweed showing symptoms of leaf blight (Fig. 1). The organism also was isolated from stems, roots, flowers, and seed. Over a 3-year period, 32 strains were collected from various locations and plant organs. The 32 strains did not differ appreciably in virulence on *A. syriaca* and *A. speciosa*.

Typical symptoms of leaf chlorosis followed by necrosis developed on inoculated milkweed seedlings about 7 days after stem or leaf inoculation. No water-soaking accompanied disease development. Characteristically, symptoms developed on one side of a leaf vein and spread throughout the entire leaf, followed by symptoms appearing on other leaves. Affected leaves withered and died, and plants wilted and died as early as 20 days after inoculation. Stem tissue remained intact at the point of inoculation. Symptom development was delayed up to 18 days in mature plants. Lower leaves typically showed symptoms before upper leaves. The bacterium was reisolated from symptomatic organs but not from controls, which remained asymptomatic. Disease developed on plants inoculated at different ages and on plants grown at two different temperatures. However, plants grown at 32°C developed symptoms approximately 1 week earlier than plants grown at 25°C.

Five out of the six *Asclepias* species were susceptible to the pathogen (Table 2). Symptoms of leaf blight and wilt of plants were similar on all susceptible species. No other plant species was susceptible (Table 2); however, kidney bean leaves showed a hypersensitivelike response of localized chlorosis followed by necrosis approxi-

mately 7 days after inoculation. Known pathogens of crop plant species did not produce disease symptoms on milkweed (Table 2); however, *X. c.* pv. *holcicola, X. c.* pv. *campestris,* and *X. c.* pv. *glycines* did produce a delayed localized necrosis around inoculation points.

Asymptomatic rhizomes collected from diseased plants in the field and replanted in the greenhouse gave rise to leaves that developed symptoms of blight. In addition, all 10 healthy seedlings inoculated in rhizome or root tissue developed characteristic symptoms, whereas controls remained free of disease.

Strain classification and identification. All strains of the bacterium grew on and hydrolyzed starch in MXP. Fatty acid analysis indicated a similarity index of 0.4 to 0.5 to *X. campestris*. Pigment analysis showed xanthomonadin with characteristic R_f values ranging from 0.43 to 0.44. Control R_f values from a known xanthomonad ranged from 0.43 to 0.44, which is in the reported range (13) for xanthomonads.

Polypeptide profiles of five currently recognized species of *Xanthomonas* (*X. axonopodis*, *X. albilineans*, *X. fragariae*, *X. campestris,* and *X. populi*) were clearly distinguishable (Fig. 2). The profiles also showed a close similarity between *X. campestris* pathovars and *X. fragariae*. The yellow-pigmented *Erwinia herbicola* was clearly different from the xanthomonads. Although the milkweed pathogen was similar to *X. c.* pv. *holcicola*, *X. c.* pv. *phaseoli*, *X. c.* pv. *campestris*, and *X. c.* pv. *glycines* (Figs. 2 and 3), it could be separated from them. Polypeptide profiles from representative pathogenic milkweed strains from various locations and years were indistinguishable (Fig. 4).

Table 3. Carbon utilization testing with the Biolog GN MicroPlate system[a,b,c,d]

	Bacteria[e]						
	M-91	SB-K	10007	Xcg	Xcc	Xch	Eh
Glycogen	+	+	+	+	+	−	+
N-acetyl-D-glucosamine	+	+	+	+	+	−	+
L-fucose	+	+	+	+	+	−	+
D-galactose	+	+	+	+	+	−	+
Lactulose	+	+	+	+	−	−	+
D-melibiose	−	−	−	+	+	−	−
Psicose	+	+	+	+	+	−	+
cis-Aconitic acid	−	−	−	+	−	+	+
Malonic acid	+	+	+	+	+	−	+
Hydroxy L-proline	+	+	+	+	+	−	−
L-proline	+	+	+	+	+	−	+
L-serine	+	+	+	+	+	−	+
Glycerol	+	+	+	+	+	−	+
Alaninamide	+	+	+	+	+	+	−
α-Keto glutaric acid	+	+	+	+	+	+	−
Propionic acid	−	−	v	+	+	−	−
D-saccharic acid	−	−	−	v	+	−	+
Maltose	+	+	+	+	+	v	+
ß-Methyl-D-glucoside	−	−	−	−	−	v	+
Sucrose	+	+	+	+	+	v	+
Xylitol	−	−	−	−	−	v	−
Mono-methyl succinate	v	v	v	+	v	v	+
Acetic acid	v	v	v	−	v	−	−
α-Hydroxybutyric acid	−	−	−	v	−	−	−
Citric acid	−	−	v	+	+	−	+
α-Keto butyric acid	−	−	v	v	v	−	−
D,L-lactic acid	+	+	+	+	+	v	+
Succinamic acid	+	+	+	v	+	+	v
L-aspartic acid	v	−	v	−	−	−	+
Glycl-L-aspartic acid	v	−	v	−	−	−	+
Glycl-L-glutamic acid	+	v	v	+	+	+	+
L-threonine	−	−	v	v	v	−	−

[a] The following carbon sources were utilized by all strains tested: dextrin, Tween 40, Tween 80, cellobiose, D-fructose, gentiobiose, α-D-glucose, D-mannose, D-trehalose, methyl pyruvate, succinic acid, bromo succinic acid, D-alanine, L-alanine, L-alanyl-glycine, L-glutamic acid.

[b] The following carbon sources were not utilized by the strains tested: adonitol, D-arabitol, *i*-erythritol, ß-hydroxybutyric acid, γ-amino butyric acid, *p*-hydroxy phenylacetic acid, itaconic acid, α-keto valeric acid, sebacic acid, L-leucine, L-phenylalanine, L-pyroglutamic acid, D-serine, D,L-carnitine, urocanic acid, phenyl ethylamine, putrescine, 2-amino ethanol, 2,3-butanediol, D-glucosaminic acid.

[c] The following carbon sources were utilized only by *Erwinia herbicola* 112Y: L-arabinose, *m*-inositol, α-lactose, D-mannitol, L-rhamnose, D-sorbitol, turanose, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, thymidine, D-glucuronic acid, quinic acid, glucuronamide, L-asparagine, L-histidine, L-ornithine, α-amino butyric acid, inosine, uridine, D,L-α-glycerol phosphate, glucose-1-phosphate, glucose-6-phosphate, α-cyclodextrin, and D-raffinose.

[d] + = carbon source utilized, − = carbon source not utilized, v = variable result.

[e] M9, SB-K, and 10007 are the milkweed pathogen; Xcg 11766 = *Xanthomonas campestris* pv. *glycines*, Xcc 528 = *X. c.* pv. *campestris*, Xch 3103 = *X. c.* pv. *holcicola,* and Eh 112Y = *Erwinia herbicola*.

Fig. 3. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of total cellular proteins from *Xanthomonas campestris* pathovars. Lane A, milkweed pathogen 10007; lane B, *X. c.* pv. *holcicola* Xhl; lane C, *X. c.* pv. *vesicatoria* XvL-2; lane D, *X. c.* pv. *glycines* C; lane E, *X. c.* pv. *campestris* 528; lane F, *X. c.* pv. *translucens* Js-2A; lane G, *X. c.* pv. *carotae* 5723; lane H, *X. c.* pv. *phaseoli* DRL-827; lane I, molecular size markers (same as Fig. 2).

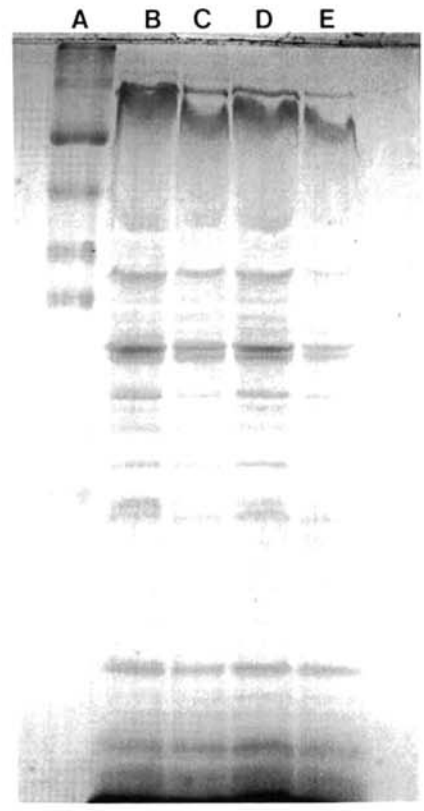

Fig. 4. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of total cellular proteins from pathogenic milkweed strains collected at various locations and times. Lane A, molecular size markers (same as Fig. 2); lane B, P-fl (collected in 1988 at Paxton, Nebraska); lane C, M-9 (collected in 1988 at Minden, Nebraska); lane D, SB-K (collected in 1988 at Scottsbluff, Nebraska); lane E, 10007 (collected in 1987 at Paxton).

Substrate utilization profiles showed the pathogenic milkweed strains were different from *X. c.* pv. *holcicola* and *E. herbicola*. However, there were no major differences from the other two pathovars tested (Table 3) in the 48 substrates utilized by them. The milkweed pathogen did not utilize D-melibiose and *cis*-aconitic acid, which distinguished it from *X. c.* pv. *glycines*. The pathogen did utilize lactulose but not D-melibiose, which differentiated it from *X. c.* pv. *campestris*. The milkweed pathogen was most similar to *X. c.* pv. *glycines*.

DISCUSSION

Bacterial blight was identified as a new disease of milkweed in Nebraska. The disease affected stand and yields of this alternative crop. The pathogen also was isolated from seed grown in Kansas. Characteristic symptoms included chlorotic and necrotic areas of leaves and subsequent wilt of young plants. Since the pathogen was isolated from seed, root tissue, and other plant parts, and growth from rhizomes of diseased plants developed symptoms of blight, the pathogen appears to be systemic.

All Nebraska fields were planted from the same seed source; thus the strains isolated in the different geographical regions may be of common origin. Consistent with this hypothesis is the inability to distinguish the strains by any of our criteria and the sensitivity of all the strains to two bacteriophages that were isolated from two different locations (P. Flynn, *unpublished*).

Isolation of a yellow-pigmented bacterium that could grow on MXP medium (8) suggested a relationship to *Xanthomonas*. Chemotaxonomic tests served to distinguish this bacterium from other yellow-pigmented bacteria and *X. campestris* species and pathovars. Biolog GN MicroPlates provided rapid, extensive, and cost-effective carbon oxidation profiles, and showed that the milkweed pathogen was most closely related to *X. c.* pv. *glycines* and *X. c.* pv. *campestris*. Extensive examination (537 strains, 28 pathovars) of xanthomonads with this assay system showed strong clustering of most *X. campestris* pathovars (14). In multiple characterization methods, the Biolog system was more useful than fatty acid profiles in differentiating xanthomonads from aroids (7). In other studies of a range of pathovars of *X. campestris*, fatty acid analyses enabled differentiation of some pathovars but not others (18,21). The similarity index of the milkweed pathogen was closest to *X. campestris*, but clearly different. In addition, we and others (2,16) have shown that polypeptide profiles were useful in distinguishing *Xanthomonas* species and pathovars. However, further work is needed to resolve the taxonomic relationships among *X. campestris* pathovars and the milkweed pathogen.

Host inoculations suggest relatedness among several *Xanthomonas* pathovars that produce a hypersensitivelike response in milkweed. These pathovars, *X. c.* pv. *campestris, X. c.* pv. *holcicola,* and *X. c.* pv. *glycines*, are highly related based on polypeptide and metabolic profiles.

Pathogen characterization and host range studies with the milkweed pathogen showed the bacterium to be distinct and differentiable from other *X. campestris* pathovars tested. Therefore, we suggest the bacterium constitutes a new pathovar. We propose the name *Xanthomonas campestris* pv. *asclepiadis*, based on the genus name *Asclepias*. A pathotype strain, *X. c.* pv. *asclepiadis* 10007 is proposed and has been deposited with the International Collection of Micro-Organisms from Plants, Auckland, New Zealand (formerly Plant Diseases Division Culture Collection).

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