Characterization of Epiphytic *Xanthomonas campestris* pv. *phaseoli* and Pectolytic Xanthomonads Recovered from Symptomless Weeds in the Dominican Republic

Rosendo Angeles-Ramos, Anne K. Vidaver, and Paula Flynn

First author: Department of Plant Protection, S.E.A., C.E.S.D.A., San Cristobal, Dominican Republic; second author: Department of Plant Pathology, University of Nebraska, Lincoln 68583-0722; third author: Department of Plant Pathology, Iowa State University, Ames 50011.

Proofs and reprints should be addressed to Anne K. Vidaver.

We thank Drs. Carol Ishimaru, Richard Dam, and Dermot Coyne for helpful advice; Dr. Myron Sasser for fatty-acid analysis; and Rosalie Focken and Dan Kuczmarski for technical assistance.

This research was supported in part by US-AID Title XII, Grant DAN-1310-G-SS-60008-00.

This manuscript has been assigned Journal Series 9031, Agricultural Research Division, University of Nebraska.

Accepted for publication 1 February 1991 (submitted for electronic processing).

ABSTRACT

Angeles-Ramos, R., Vidaver, A. K., and Flynn, P. 1991. Characterization of epiphytic *Xanthomonas campestris* pv. *phaseoli* and pectolytic xanthomonads recovered from symptomless weeds in the Dominican Republic. Phytopathology 81:677-681.

Xanthomonas campestris pv. *phaseoli* and pectolytic xanthomonads were isolated from symptomless weeds collected inside and adjacent to fields of dry edible beans (*Phaseolus vulgaris*) showing common blight symptoms in the Dominican Republic. Strains of *X. c. phaseoli* were identified on the basis of characteristic yellow colony pigmentation; starch hydrolysis on the semi-selective medium MXP; pathogenicity on dry bean leaves and pods; absence of extracellular melanin 'fuscans' pigment on modified nutrient broth yeast extract and King's medium B; and isolation of xanthomonadin pigment with a characteristic R_f value of 0.45 determined by thin-layer chromatography. Pectolytic xanthomonads also had most of the aforementioned properties, except that they failed to induce disease on bean leaves, caused an atypical incompatible response on bean pods, showed strong pectolytic activity on crystal violet pectate medium, and grew at 4 C on yeast extract-dextrose-calcium carbonate medium. Single-dimension polypeptide analysis of cellular proteins by polyacrylamide gel electrophoresis showed that the two groups of bacteria were distinctly different in their overall profiles. The percentage of pathogenic strains from 77 weed samples was 22% of the 132 strains collected. The majority of the pathogenic strains (91%) were recovered from inside infected bean fields. These results suggest that weeds may not be an important inoculum source for the common blight bacterium, but that they could serve as a reservoir of inoculum and harbor atypical xanthomonads of unknown function as well.

Many plant pathogenic microorganisms grow on leaf surfaces of healthy plants without causing visible symptoms (18). Several species of pathogenic bacteria have a resident stage either on their hosts or nonhosts in which they multiply and are the most likely source of inoculum for disease initiation on crops (11,26). Some bacterial pathogens can colonize bean (*Phaseolus vulgaris* L.) and multiply as foliar epiphytes before disease outbreaks (23). *Xanthomonas campestris* pv. *phaseoli*, which causes common blight of dry beans, can be present as an epiphyte on bean pods, seeds, and leaves (3,27,28).

Weeds play a role in the survival and dissemination of several

© 1991 The American Phytopathological Society

bacterial pathogens (6,7,13,16,17,21,22). None of the previous studies on *X. c. phaseoli* associated with weeds (2,8,25) deals with naturally occurring populations on symptomless plants. Heavy weed infestations are frequent problems in bean-growing areas around the world, particularly in the tropics. Cafati and Saettler (2) suggested that weeds, as well as associated crops such as maize, could function as important inoculum sources of common blight in tropical and semitropical bean-production regions.

Atypical xanthomonads have been found to be associated in nature with various plants. Such xanthomonads have been reported to be saprophytic in apple buds (20), while others have been isolated from bean debris (9) and soft rots of fruits and vegetables (19). Thus far, it appears that these xanthomonads, especially those that are pectolytic, can be opportunistic pathogens or survive epiphytically on cultivated plants and weeds. However, their taxonomic status still needs to be clarified (10).

The semi-selective medium, MXP (5), developed for isolation and recovery of *X. c. phaseoli*, enabled us to assess populations of naturally occurring common bacterial blight strains and atypical xanthomonads recovered from symptomless weeds in tropical dry bean fields in the Dominican Republic.

MATERIALS AND METHODS

Weed sampling and bacterial isolations. A total of 77 weed samples, representing 21 weed species, was collected at random from both inside and outside (2–10 m from the borders) of common blight diseased fields of dry beans in San Juan (southwestern region) and Higuey (eastern region), and a week after harvest in Constanza (northern region) in the Dominican Republic during the fall, winter, and summer of 1986–1987. These fields had a moderate incidence of common blight, ranging from 20 to 50% of plants with symptoms. From four to 11 different weed species were sampled, depending on the frequency of their occurrence, in selected quadrants of fields. In general, three to five bulked leaves from each sample of weed species were individually collected in plastic freezer bags and placed in a cooler with ice packets for shipment to Lincoln, NE, where the samples were processed upon receipt (approximately 48–96 h after collection). Weeds were identified in the laboratory before shipment.

Leaves were detached from the stem; protective gloves were used and changed with each sample. Bacteria were isolated from each sample of plant material by adding 10 ml of buffer (12.5 mM K_2HPO_4 with 10 mM $MgSO_4$, pH 7.1) to leaves in a plastic freezer bag and shaking the contents for 2 h at 180 rpm. Tenfold serial dilutions were prepared from each sample and plated onto duplicate MXP plates (5). Characteristic starch-hydrolyzing, yellow colonies were counted after incubation at 26 C for 2 or 3 days. Bacteria were purified by transferring two single colonies three times onto yeast extract-dextrose-calcium carbonate (YDC) medium (24); 132 strains were selected for further analysis. Strains were stored on YDC or nutrient broth yeast extract agar (NBY) plates (24) at 4 C and also in 60% sterile glycerol in buffer at −20 C. Representative cultures were lyophilized for long-term storage.

Bacteria used for comparative purposes were: *X. c. phaseoli* strains DRL-827, A6LB1, DR-200, and A9Xcp 67 (avirulent); *X. c. phaseoli* 'fuscans' strain A10; *X. c. campestris* strain A12; *X. c. vesicatoria* strain A11; *Erwinia herbicola* strain C9-1B; *E. carotovora* subsp. *carotovora* strain SR-204; and *Pseudomonas fluorescens* strain A4. All strains, except *E. carotovora* strain SR-204 obtained from A. Kelman (University of Wisconsin) and *E. herbicola* strain C9-1B obtained from C. Ishimaru (Colorado State University), were from our collection.

Pathogenicity tests. Dry bean plants of Dark Red Kidney cultivar Charlevoix were grown under greenhouse conditions at 28 ± 3 C in 10 pots, each containing two plants. Trifoliate leaves of 20-day-old plants were inoculated individually with one of 89 bacterial strains recovered from weeds collected in the San Juan and Higuey locations. Trifoliate leaves of about the same age on each plant were inoculated by using the micropipette-tip inoculation method (1), which consisted of touching a bacterial colony with a 200-μL micropipette tip and lightly pressing it against the abaxial surface of a leaflet at five different inoculation points parallel to the midrib. After continued growth in the greenhouse, plants were evaluated at 3, 7, and 11 days after inoculation for common blight symptoms in comparison with control strains. A second experiment was done with 13 strains from Constanza. Appropriate buffer controls were used.

A third experiment was done on immature bean pods. The surface of a 2- to 3-cm long pod was inoculated by using a mild circular motion with the micropipette tip at five different points. Seventy-four strains were inoculated to the pods of two 79-day-old bean plants of Dark Red Kidney cultivar Charlevoix in the greenhouse. Evaluation of symptoms was done as described. At 21 days after inoculation and continued incubation in the greenhouse, 24 pods at different developmental stages (green to fully mature) were individually harvested and evaluated for the presence of bacteria on the seed. Three to five seeds were removed aseptically from each pod, placed on NBY plates, incubated at 26 C, and evaluated for bacterial growth for up to 6 days.

Biochemical characterization. To confirm and enhance the starch hydrolysis reaction observed on dilution plates of MXP, selected strains were grown on the same medium modified with twice the amount of the dyes, methyl violet 2B (60 μl/L; 1% solution in 20% ethanol) and methyl green (120 μl/L; 1% aqueous solution). Starch hydrolysis was observed directly and by flooding the plates with 10% Lugol's iodine solution after 3 days.

Selected strains were evaluated for 'fuscans' extracellular brown pigment (melanin) production on King's medium B (15) and in 7.5-$\times$0.9-cm test tubes containing NBY medium devoid of glucose and $MgSO_4$ (C. Ishimaru, *personal communication*). Pectolytic activity was determined by streaking the bacterial strains onto crystal violet pectate medium (CVP) (24). *E. c. carotovora* and *X. c. campestris* were used as positive controls.

Thin-layer chromatography of xanthomonadin pigment. The procedure used was a modification of the method of Irey and Stall (12). Seventeen selected strains were grown at 26 C on King's medium B. After 2 days, bacterial cells were scraped from the surface and suspended in 1.5 ml of methanol in 2-ml plastic screw-cap vials. The suspension was boiled at 90 C for 10 min in a Temp-blok Module Heater (Curtin Matheson Scientific Inc., Houston, TX) and then cooled to room temperature. After centrifugation for 10 min at 15,000 rpm (Eppendorf model 5413 centrifuge; Brinkmann Instruments, Co., Westbury, NY) to pellet the cell debris, the supernatant containing the methanol suspension was concentrated to a final volume of 1 ml by evaporation at 75 C for 40–50 min. After cooling, 5 μl of the yellow concentrated extracts were spotted with a 100-μl precision micropipette onto channeled precoated thin-layer chromatography glass plates of silica gel 60, 250 μm thick with preabsorbent spotting areas (Whatman International Std., Maidstone, England). Spots were placed on each channel at 5 mm below the silica gel preabsorbent interface and thoroughly dried before development with methanol. The chamber was saturated previously with the developing solvent. Separations were performed at ambient temperature and the R_f values were calculated from measurements made from the silica gel preabsorbent area interface. Xanthomonadin extracted from *X. c. phaseoli* strain A6LB1 and the yellow pigment of *E. herbicola* strain C9-1B were spotted as controls.

Polyacrylamide gel electrophoresis of total cellular proteins. The method of Carlson and Vidaver (4) with minor modifications, principally the substitution of a linear (12%) resolving gel (P. Flynn and A. Vidaver, *unpublished data*) was used. Representative pathogenic and nonpathogenic strains were examined.

RESULTS

Bacterial isolations and populations. Of the 132 weed samples collected in three locations, only four did not yield bacterial colonies on MXP. Some bacteria that grew on MXP did not hydrolyze starch, and some others were unable to grow when

transferred to YDC. Yellow, mucoid colonies that hydrolyzed starch were selected for further analysis. When strains were transferred after 2 days of incubation at 26 C to storage at 4 C, colonies of strains that were later identified continued to grow on YDC plates, whereas pathogenic strains grew slowly, if at all, over a period of 4–6 wk. Populations of *X. c. phaseoli* and atypical xanthomonads obtained from weed leaves ranged from undectable to greater than 10^6 colony forming units (cfu) per sample (Table 1). The great majority of samples yielded 10^2–10^4 cfu/sample. In only six cases were both pathogenic and nonpathogenic xanthomonads recovered from the same weed sample. However, high numbers of nonpathogenic strains may have masked recovery of low numbers of *X. c. phaseoli*.

Pathogenicity tests. Strains from weeds that were pathogenic to bean leaves induced a small water-soaked zone around the inoculation point after 3–4 four days. After 7 days, a light yellow, irregular zone was observed around a light brown center. The chlorotic zone intensified in brightness by 10–11 days. Later, tissues around the site of inoculation became necrotic and collapsed. By 2 wk, the yellow zone was expanded and covered an ample area around the necrotic brown center. Plants inoculated with known *X. c. phaseoli* strains gave the same response. Pectolytic strains induced a weak, incompatible response on bean leaves. Small, light tan spots were observed after 2–3 days of incubation.

Bean pods inoculated with pathogenic strains developed symptoms earlier than leaves. About 3 days after inoculation, inoculation sites were visibly water-soaked. After 7 days, the spots became larger, and greasy water-soaked zones were observed. By 11 days, the greasy spot often spread on the pod surface, depending on the physiological stage of the pod (Fig. 1). Occasionally, a purple discoloration occurred around or mixed with the water-soaked area.

The pectolytic xanthomonads caused a very strong incompatible response on pod tissues, but the intensity of the reaction was less on older pods. At 24 h after inoculation, the tissues were noticeably brown. A few strains caused transient water-soaking. After 3 days, the area around the inoculation sites had a reddish brown color. Younger pods showed a dark brown rotlike zone after 11 days (Fig. 1).

Strains that were pathogenic on leaves also were pathogenic on pods. In some cases, bacterial slime emerged from inoculation sites on pods. Bacterial slime was produced by seven of 52 nonpathogenic strains and only one of 22 pathogenic strains. Seeds from two of the nine pods collected that had an incompatible reaction resulted in bacterial growth on NBY, while the seeds from all seven pods inoculated with pathogenic strains yielded typical growth on NBY. In general, the pods that were green and mature after inoculation had more externally contaminated seeds than those pods that became dry. Seeds from buffer controls and noninoculated pods did not yield any xanthomonads.

Twenty-two of the presumptive 102 xanthomonads were *X. c. phaseoli* (Table 2). These came from 14 samples making up eight weed species. The remaining strains nonpathogenic to beans were isolated from different samples composing 21 weed species. The ratio of pathogenic to nonpathogenic strains was 1:3.6. Most pathogenic strains (91%) were recovered from weeds collected inside diseased fields. None of the 13 strains from weeds collected after harvest caused common blight symptoms on bean plants.

Biochemical characteristics. The starch hydrolysis reaction on MXP sometimes was faint. Increasing the amount of methyl green and methyl violet twofold in MXP intensified the hydrolysis zones

TABLE 1. Epiphytic populations of *Xanthomonas campestris* pv. *phaseoli* and atypical xanthomonads isolated from weeds in the Dominican Republic

Location and weed species[b]	Family	Number of samples	Population ($\log_{10}$ cfu/ml)[a] *X. c. phaseoli*	Xanthomonads
San Juan				
Aeschynomene americana (IF)[c]	Leguminosae	4	4.56	4.38
Cassia tora (IF)	Leguminosae	3	ND[d]	5.07
Cleome viscosa (OF)	Capparidaceae	3	ND	6.51
Euphorbia heterophylla (IF)	Euphorbiaceae	2	ND	4.76
Euphorbia heterophylla (OF)	Euphorbiaceae	2	ND	4.91
Lagascea mollis (IF)	Compositae	2	ND	4.90
Lagascea mollis (OF)	Compositae	3	ND	5.47
Macroptilium lathyroides (OF)	Leguminosae	3	ND	4.58
Panicum maximum (OF)	Gramineae	3	ND	4.39
Parthenium hysterophorus (IF)	Compositae	2	ND	5.46
Rhynchosia minima (OF)	Leguminosae	3	4.80	5.78
Higuey				
Acanthospermum hispidum (IF)	Compositae	3	5.43	3.65
Barreria laevis (OF)	Rubiaceae	3	ND	3.83
Corchorus aestuans (OF)	Tiliaceae	1	ND	5.08
Echinochloa colona (IF)	Gramineae	3	4.45	ND
Eleusine indica (OF)	Gramineae	3	ND	3.71
Euphorbia heterophylla (IF)	Euphorbiaceae	3	3.22	ND
Leptochloa filiformis (IF)	Gramineae	3	5.20	NE[e]
Leptochloa filiformis (OF)	Gramineae	3	ND	5.00
Malachra alceaefolia (OF)	Malvaceae	3	3.51	4.76
Malvastrum sp. (OF)	Malvaceae	2	ND	4.40
Portulaca oleracea (IF)	Portulacaceae	3	3.77	5.10
Constanza				
Acanthospermum hispidum (AH)	Compositae	2	ND	4.14
Amaranthus sp. (AH)	Amaranthaceae	3	ND	4.32
Chenopodium album (AH)	Chenopodiaceae	3	ND	ND
Portulaca oleracea (AH)	Portulacaceae	3	ND	4.06
Setaria sp. (AH)	Gramineae	3	ND	2.52

[a]Average of one to four weed samples. Individual samples of three to five leaves yielded either *X. c. phaseoli*, other xanthomonads, or neither.
[b]Weeds identified by R. Hansen, Plant Protection Division, S.E.A., C.E.S.D.A., San Cristobal, Dominican Republic.
[c]IF = inside field; OF = outside field; AH = inside field after harvest.
[d]ND = not detected.
[e]NE = not estimated.

for both pathogenic and nonpathogenic xanthomonads after 3 days of incubation.

Strains of *X. c. phaseoli* from weeds either did not grow or grew poorly, without any pectolytic activity, on CVP medium. In contrast, the nonpathogenic xanthomonads generally grew well and showed positive pectolytic activity after about 48 h. The pectolytic xanthomonads formed characteristic pits in the agar similar to those formed by *E. c. carotovora* and *X. c. campestris*.

None of the selected strains produced 'fuscans' pigment either on King's B or in modified NBY media. After 6 days at 26 C, the pigment was detected only in media inoculated with *X. c. phaseoli* strain A10.

Xanthomonadin pigment production. All test strains and *X. c. phaseoli* strain A6-LB contained yellow pigments with R_f values of about 0.45, which is typical of xanthomonadin. The yellow pigment of *E. herbicola* strain C9-1B had an R_f value of 0.78. Strains from weeds tended to show a second spot of about 0.65 R_f. The xanthomonadin spots disappeared a few minutes after drying the silica gel plates, as reported also by Irey and Stall (12).

Polypeptide profiles. Polypeptide bands of the pathogenic xanthomonad strains could be clearly distinguished from the nonpathogenic xanthomonads (Fig. 2). The band pattern of known strains of *X. c. phaseoli* were indistinguishable from the isolated pathogenic strains (not shown). The relationship of the nonpathogenic strains to other xanthomonads remains to be determined.

DISCUSSION

The semi-selective medium MXP (5) enabled recovery of *X. c. phaseoli* and pectolytic xanthomonads from symptomless weeds. The latter differ in a number of properties from the common blight bacterium. Their presence, often in high populations, suggests a role in plant interactions, particularly because they were found more frequently on weeds than *X. c. phaseoli*.

Gitaitis et al (10) and Gilbertson et al (9) also have found pectolytic xanthomonads to be epiphytic on weeds. Whether their bacteria or ours produce pectolytic enzymes on plant surfaces is unknown. At least some pectolytic strains of *X. c. campestris* have the potential to cause postharvest rots of fruits and vegetables (19). The induction of a limited rotlike symptom on some inoculated young pods by high concentrations of our atypical xanthomonads could have been due to production of pectolytic enzymes in such succulent tissue. While the relationships among these pectolytic xanthomonads remain to be determined, the usefulness of polypeptide analysis for identification and differentiation of bacteria was confirmed (14).

Medium B of King et al (15) is routinely used for detection of fluorescent *Pseudomonas* species. We also noted that a brown pigment was produced in King's medium B by *X. c. phaseoli* 'fuscans' variant strain A10. Thus, the absence of brown pigment on this medium and modified NBY suggests that no 'fuscans'

TABLE 2. Selected characteristics among strains of *Xanthomonas campestris* pv. *phaseoli* and pectolytic xanthomonads from weeds in the Dominican Republic

Characteristic	Number of strains tested	*X. c. phaseoli*	Pectolytic xanthomonads
Yellow colonies on MXP and YDC[a]	102	+[b] (22)[c]	+ (80)
Starch hydrolysis on MXP	102	+ (22)	+ (80)
Pathogenic on bean leaves	102	+ (22)	−[d] (80)
Incompatible response on pods	74	− (22)	+ (52)
Pathogenic on bean pods	74	+ (22)	− (52)
Pectolytic activity on CVP[a]	38	− (16)	+ (22)
'Fuscans' pigment on modified NBY and KB[a]	44	− (22)	− (22)
Xanthomonadin pigment[e]	17	+ (11)	+ (6)

[a]Media are: MXP, a semi-selective medium for *X. c. phaseoli*; YDC = yeast extract-dextrose-calcium carbonate agar; CVP = crystal violet pectate; NBY = nutrient broth yeast extract, and KB = King's medium B (5,15,24).
[b]Positive reaction.
[c]Numbers in parentheses are the number of strains that tested either positive or negative.
[d]Negative reaction.
[e]R_f value of 0.45; average of two silica gel plates.

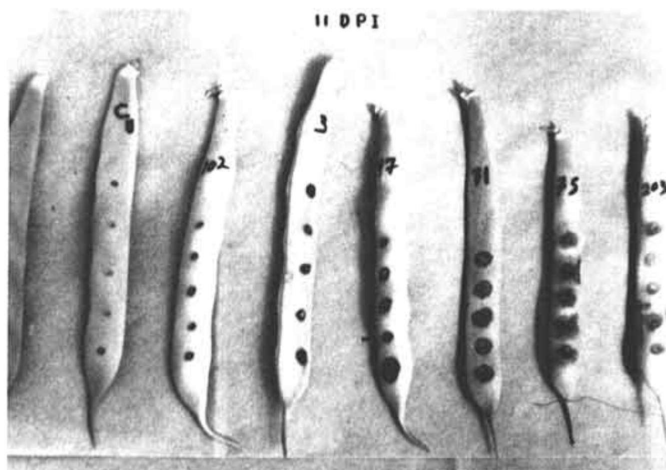

Fig. 1. Reactions produced by epiphytic xanthomonads isolated from weeds on dry bean pods 11 days after inoculation. Notice the rotlike spots and bacterial slime emerge from the lesions of fresh spots (no. 3). Strains 71, 75, and 203 were also pathogenic on leaves; strains 3, 97, and 102 were nonpathogenic to leaves. C = buffer control. First pod is a noninoculated control.

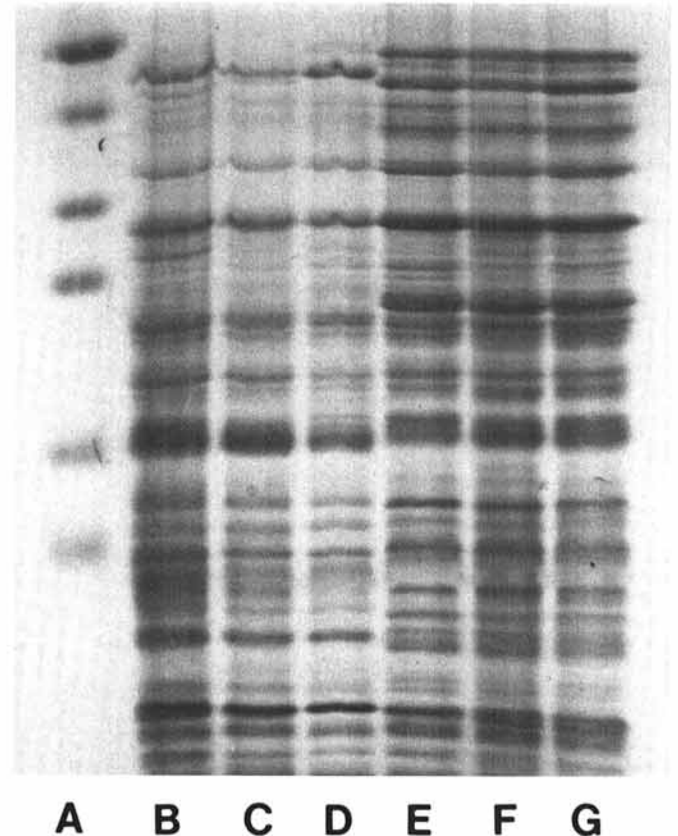

Fig. 2. Polyacrylamide gel electrophoresis of total cellular proteins of xanthomonads from weeds. Nonpathogenic strains 59, 67, and 102 are in lanes B, C, and D, respectively; pathogenic strains 15, 73, and 91 are in lanes E, F, and G, respectively. Markers are in lane A and range from 116 (top) to 26.6 kDa (bottom).

strains were isolated from symptomless weeds. Perhaps such variants are rarely found as epiphytes, at least on common tropical weeds.

Colonies of pigmented *Xanthomonas* species are difficult to distinguish on many media from colonies of other yellow-pigmented bacteria. Irey and Stall (12) reported that xanthomonadin, a pigment characteristic of most *Xanthomonas* species and pathovars, had R_f values of 0.42–0.49 and that other yellow bacteria did not yield pigments of those R_f values. Likewise, Gitaitis et al (10) found R_f values of 0.36–0.49 for xanthomonadin extracted with methanol from yellow pectolytic xanthomonads. The average R_f value of 0.45 found for *X. c. phaseoli* and for pectolytic xanthomonad epiphytes on weeds in this study agrees with previous reports. The pectolytic strains also were identified by fatty-acid analysis as belonging to the genus *Xanthomonas* (M. Sasser, *unpublished data*).

Weed samples collected inside infected fields yielded more pathogenic strains of *X. c. phaseoli* than samples gathered outside those fields or after harvest. Thus, symptomless weeds with epiphytic common blight bacteria inside bean fields may contribute to secondary spread of the disease, whereas the role of weeds outside such fields may be less important as an inoculum source. The finding of eight weed species from six families that can act as symptomless hosts strongly indicates that many other weeds will undoubtedly be found harboring epiphytic *X. c. phaseoli*. However, because no pathogenic strains were detected on weeds 7 days after beans were harvested from a diseased field, weeds may play a limited role in bacterial survival for the long-term.

The role, if any, of the pectolytic xanthomonads remains to be determined. Such pectolytic xanthomonads isolated from dry bean leaves or debris have subsequently been found in Nebraska (C. Ishimaru and A. Vidaver, *unpublished data*) and Wisconsin (9), and thus are not peculiar to the tropics. Such strains may be progenitors of pathogenic xanthomonads, pathogens of some other crop or weeds, or simply saprophytic xanthomonads involved in normal degradation of plant material in the field.

LITERATURE CITED

1. Arunakumari, A., and Vidaver, A. K. 1986. Identification of *Xanthomonas campestris* pv. *phaseoli* mutants with altered pathogenicity using a detached bean leaflet bioassay (Abstr.) Phytopathology 76:1136.
2. Cafati, C. R., and Saettler, A. W. 1980. Role of nonhost species as alternate inoculum sources of *Xanthomonas phaseoli*. Plant Dis. 64:194-196.
3. Cafati, C. R., and Saettler, A. W. 1980. Transmission of *Xanthomonas phaseoli* in seeds of resistant and susceptible *Phaseolus* genotypes. Phytopathology 70:638-640.
4. Carlson, R., and Vidaver, A. 1982. Taxonomy of *Corynebacterium* plant pathogens, including a new pathogen of wheat, based on polyacrylamide gel electrophoresis of cellular proteins. Int. J. Syst. Bact. 23:315-326.
5. Claflin, L. E., Vidaver, A. K., and Sasser, M. 1987. MXP, a semiselective medium for *Xanthomonas campestris* pv. *phaseoli*. Phytopathology 77:730-734.
6. Elango, F. N., and Lozano, J. C. 1981. Epiphytic survival of *Xanthomonas manihotis* on common weeds in Colombia. Pages 204-209 in: Proc. Int. Conf. Plant Pathog. Bact. J. C. Lozano, ed. CIAT. Cali, Colombia.
7. Ercolani, G. L., Hagedorn, D. J., Kelman, A., and Rand, R. E. 1974. Epiphytic survival of *Pseudomonas syringae* on hairy vetch in relation to epidemiology of bacterial brown spot of bean in Wisconsin. Phytopathology 64:1330-1339.
8. Gardner, M. W. 1924. A native weed host for bacterial blight of bean. Phytopathology 14:340.
9. Gilbertson, R. L., Rand, R. E., and Hagedorn, D. J. 1990. Survival of *Xanthomonas campestris* pv. *phaseoli* and pectolytic strains of *X. campestris* in bean debris. Plant Dis. 74:322-327.
10. Gitaitis, R. D., Sasser, M. J., Beaver, R. W., McInnes, T. B., and Stall, R. E. 1987. Pectolytic xanthomonads in mixed infection with *Pseudomonas syringae* pv. *syringae*, *P. syringae* pv. *tomato*, and *Xanthomonas campestris* pv. *vesicatoria* in tomato and pepper transplants. Phytopathology 77:611-615.
11. Hirano, S. S., and Upper, C. D. 1983. Ecology and epidemiology of foliar bacterial plant pathogens. Annu. Rev. Phytopathol. 21:243-269.
12. Irey, M. S., and Stall, R. E. 1981. Value of xanthomonadins for identification of pigmented *Xanthomonas campestris* pathovars. Pages 85-95 in: Proc. Int. Conf. Plant Pathog. Bact. J. C. Lozano, ed. CIAT. Cali, Colombia.
13. Jones, J. B., Pohronezny, K. L., Stall, R. E., and Jones, J. P. 1986. Survival of *Xanthomonas campestris* pv. *vesicatoria* in Florida on tomato crop residue, weeds, seeds, and volunteer tomato plants. Phytopathology 76:430-434.
14. Kersters, K., and DeLey, J. 1980. Classification and identification of bacteria by electrophoresis of their proteins. Pages 273-297 in: Microbiological Classification and Identification. M. Goodfellow and G. Boards, eds. Academic Press Inc., New York.
15. King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Clin. Med. 44:301-307.
16. Latorre, B. A., and Jones, A. L. 1978. Survival and pathogenicity to sour cherry of *Pseudomonas syringae* recovered from weeds and plant refuse. (Abstr.) Phytopathol. News 12:137.
17. Laub, C. A., and Stall, R. E. 1967. An evaluation of *Solanum nigrum* and *Physalis minima* as suscepts of *Xanthomonas vesicatoria*. Plant Dis. Rep. 51:659-661.
18. Leben, C. 1965. Epiphytic microorganisms in relation to plant disease. Annu. Rev. Phytopathol. 3:209-230.
19. Liao, C.-H., and Wells, J. M. 1987. Association of pectolytic strains of *Xanthomonas campestris* with soft rots of fruits and vegetables at retail markets. Phytopathology 77:418-422.
20. Mass, J. L., Finney, M. M., Civerolo, E. L., and Sasser, M. 1985. Association of an unusual strain of *Xanthomonas campestris* with apple. Phytopathology 75:438-445.
21. McCarter, S. M., Jones, J. B., Gitaitis, R. D., and Smitley, D. R., 1983. Survival of *Pseudomonas syringae* pv. *tomato* in association with tomato seed, soil, host tissue, and epiphytic weed hosts in Georgia. Phytopathology 73:1393-1398.
22. McCarter-Zorner, M. J., Harrison, M. D., Franc, G. D., Ovinn, C. E., Sells, I. A., and Graham, D. C. 1985. Soft rot *Erwinia* bacteria in the rhizosphere of weeds and crop plants in Colorado, United States and Scotland. J. Appl. Bacteriol. 59:357-368.
23. Morris, C. E., and Rouse, D. I. 1982. Diversity of epiphytic bacterial communities on bean (*Phaseolus vulgaris*) leaves and pods based on nutrient utilization. (Abstr.) Phytopathology 72:936.
24. Schaad, N. 1988. Laboratory Guide for Identification of Plant Pathogenic Bacteria. 2nd ed. The American Phytopathological Society, St. Paul, MN. 164 pp.
25. Schuster, M. L. 1970. Survival of bean bacterial pathogens in the field and greenhouse under different environmental conditions. (Abstr.) Phytopathology 57:830.
26. Schuster, M. L., and Coyne, D. P. 1974. Survival mechanisms of phytopathogenic bacteria. Annu. Rev. Phytopathol. 12:199-221.
27. Weller, D. M., and Saettler, A. W. 1980. Evaluation of seedborne *Xanthomonas phaseoli* and *Xanthomonas phaseoli* var. *fuscans* as primary inocula in bean blights. Phytopathology 70:148-152.
28. Weller, D. M., and Saettler, A. W. 1980. Colonization and distribution of *Xanthomonas phaseoli* and *X. phaseoli* var. *fuscans* in field-grown navy beans. Phytopathology 70:500-506.