Comparison of Tumorigenic Strains of *Erwinia herbicola* Isolated from Table Beet with *E. h. gypsophilae*

T. J. BURR, Associate Professor, B. H. KATZ, Former Research Technician, G. S. ABAWI, Professor, and D. C. CROSIER, Research Technician, Department of Plant Pathology, New York State Agricultural Experiment Station, Cornell University, Geneva 14456

ABSTRACT

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Tumorigenic strains of *Erwinia herbicola* were isolated from tumors on table beets (*Beta vulgaris*) collected from four commercial fields near Geneva, New York, in 1988. Strains were compared with *E. h. gypsophilae*, a saprophytic strain of *E. herbicola* (EH112Y), and strain C58 of *Agrobacterium tumefaciens*. Only strains of *E. herbicola* from beet were tumorigenic on beet slices and on roots of beet seedlings. These strains also infected stems of *Gypsophila paniculata*, causing spherical, compact tumors that contrasted with the elongate, necrotic tumors caused by *E. h. gypsophilae*. Strains of *E. herbicola* from beet and *E. h. gypsophilae* did not cause tumors on *Nicotiana glauca*. Strain C58 of *A. tumefaciens* was tumorigenic on roots of beet seedlings and stems of *N. glauca* but not on beet slices.

Bacterial pocket, a tumorous disease of sugar beet (*Beta vulgaris* L.) was reported in 1928 (1). The tumors that form on infected beets may be deeply indented with necrotic centers and be composed of many small nodules. The causal agent is a bacterium initially called *Bacterium beticola*, then changed to *Xanthomonas beticola* (Smith, Brown, and Townsend) Savulescu. The bacterium was described as yellow and aerobic with peritrichous flagella and has since been shown to be a facultative anaerobe and synonymous with *Erwinia herbicola* (Lohnis) Dye (13). Strains of *E. h. gypsophilae* (Brown) Miller, Quinn, and Graham cause tumors on *Gypsophila paniculata* L.(2,4).

Since 1972, we have observed in New York a tumorous disease of table beet that resembles crown gall and bacterial pocket disease. Although usually <0.1% of the beets have tumors, we observed an unusually high incidence of the disease in commercial fields and in our experiment station plots during 1988. In one of the commercial fields, the incidence of infected beets was as high as 10% in randomly collected samples. Tumors varied in size but were often 5 cm or greater in diameter and were present on the crowns of infected roots. Infected beets were usually culled because they could not be processed mechanically. We report *E. herbicola* as the cause of this disease and provide evidence that the host range of the strains from beet differs from that of *E. h. gypsophilae*.

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MATERIALS AND METHODS

Isolations from tumors. Beets with tumors were obtained from four commercial farms near Geneva, New York, in 1988. The beets were washed with tap water, soaked in sodium hypochlorite for about 10 min, and rinsed again in water. Pieces of the galls were then triturated in sterile distilled water, and triturates were streaked on medium 523 (8), yeast-mannitol agar, and Pseudomonas agar F (Difco). Schroth's medium (15) for isolation of biovar 1 of *Agrobacterium tumefaciens* (Smith and Townsend) Conn and New and Kerr medium (12) for isolation of biovar 2 were also used. Plates were incubated at 28 C for 3–7 days, and predominant colony types were subcultured for further testing. Later isolations from beet tumors were made on Miller-Schroth (MS) medium for isolation of *Erwinia* spp. (12).

Identification of strains. Strains were identified by the following tests (14): production of catalase and cytochrome oxidase, gelatin liquefaction, growth at 36 C on YDC agar, and soft rot of potato slices. Gram reaction according to Suslow et al (17), mucoid growth on 5% sucrose, colony type and color on MS medium, and anaerobic growth were also determined. Strains were tested for nitrate reduction (6), phenylalanine deaminase production (16), and production of relative levels of indoleacetic acid (IAA) and other indole-type compounds using the colorimetric assay of Gordon and Weber (7). Strains that were isolated from mature beet tumors were compared with known strains of *E. h. gypsophilae*, e.g., strain 0485-19, the original tumorigenic strain isolated from *G. paniculata* in 1932 (ATCC 13329) (2), and strains 1184-13 (ATCC 43348), 0585-3, and 1085-15 from D. Cooksey (University of California, Riverside). Strains EH3-1a, EH3-1b, and EH350-1 isolated from tumors on *G. paniculata* in Israel were received from S. Beer (Cornell University, Ithaca, NY), who also supplied strain EH112Y, a saprophytic strain of *E. herbicola*. Strain C58 of *A. tumefaciens* (tumorigenic biovar 1 strain isolated from a cherry gall by R. Dickey, Cornell University, Ithaca) was also included. All tests were repeated at least once.

Isolations from beet seedlings. A naturally occurring tumor was observed on one 6-wk-old beet seedling out of several hundred that were grown in the greenhouse to use for pathogenicity tests. Isolations were made from the tumor on MS medium as described above.

Because the tumor was observed on a noninoculated beet seedling growing in a greenhouse potting mixture, we investigated the potential seedborne nature of the pathogen by attempting to isolate tumorigenic bacteria from germinated beet seeds. About 500 seeds from two seed lots were germinated in moistened sterile sand. Five groups of 10 germlings about 2 wk old were randomly selected

from each seed lot, suspended in 9 ml of sterile distilled water, and stirred on a vortex stirrer for about 1 min. Then, 100-μl volumes of the seed wash were spread on MS medium and plates were incubated at 28 C for 1 wk, during which time colonies resembling *E. herbicola* were subcultured.

Pathogenicity tests. Whole mature table beets were soaked in sodium hypochlorite for 15 min and 95% ethanol for 5 min, then rinsed in running tap water for about 15 min. Beets were cut in cross-sectional slices about 0.5 cm thick and placed on the surface of moistened sterile perlite in petri dishes. Slices were inoculated with bacterial strains that were grown on 523 medium for 48 hr at 28 C; inoculum was collected on the flat end of a sterile toothpick and spread in a cross pattern on the surface of a beet slice. Slices were incubated at room temperature for 3 wk. At least two slices were inoculated for each strain, and inoculations were repeated at least once. Controls consisted of inoculations with sterile distilled water.

Beet seedlings (cv. Ruby Queen) were grown in sterile sand for 6 wk, then transplanted into a 1:1 mixture of sterile sand and soil. Bacterial inoculum was prepared from 48-hr cultures grown on 523 medium at 28 C. At the time of transplanting, seedlings were wounded at the crown with an insect-mounting pin, and bacterial growth that was collected on a sterile toothpick was smeared on the wound. Ten seedlings were inoculated per strain, and seedlings were potted individually in the greenhouse. Control plants were wounded as above and inoculated with sterile distilled water. The seedlings were grown for 6 wk, then evaluated for tumors. The experiment was repeated once.

All strains were also inoculated onto *G. paniculata* and *Nicotiana glauca* Graham. Young rooted plants of *G. paniculata* that had been propagated by

Fig. 1. Mature table beet infected by *Erwinia herbicola*.

Table 1. Four strains of *Erwinia herbicola* isolated from table beet (EH1188, EH2188, EH3188, EH4188) compared with strains of *E. h. gypsophilae* (Gyp.), a saprophytic strain of *E. herbicola* (EH112Y), and strain C58 (biovar 1) of *Agrobacterium tumefaciens*

	No. positive reactions/no. strains tested			
Test	**Beet**	**Gyp.**	**EH112Y**	**C58**
Gram	0/4	0/7	0/1	0/1
Growth				
Miller-Schroth medium	4/4	7/7	1/1	0/1
5% Sucrose	4/4	6/7	1/1	1/1
36 C	4/4	7/7	1/1	1/1
Anaerobic	4/4	7/7	1/1	0/1
Gelatin liquefaction	4/4	4/7	1/1	0/1
Oxidase	0/4	0/7	0/1	1/1
Catalase	4/4	7/7	1/1	1/1
Nitrate reduction	4/4	7/7	1/1	0/1
Phenylalanine deaminase	4/4	7/7	1/1	0/1
Potato soft rot	0/4	0/7	0/1	0/1
Indoleacetic acid production[a]	0.90	0.62	0.80	0.19

[a] Average colorimetric reading from groups of strains or single strains at A_{540nm} using Gordon and Weber procedure (7).

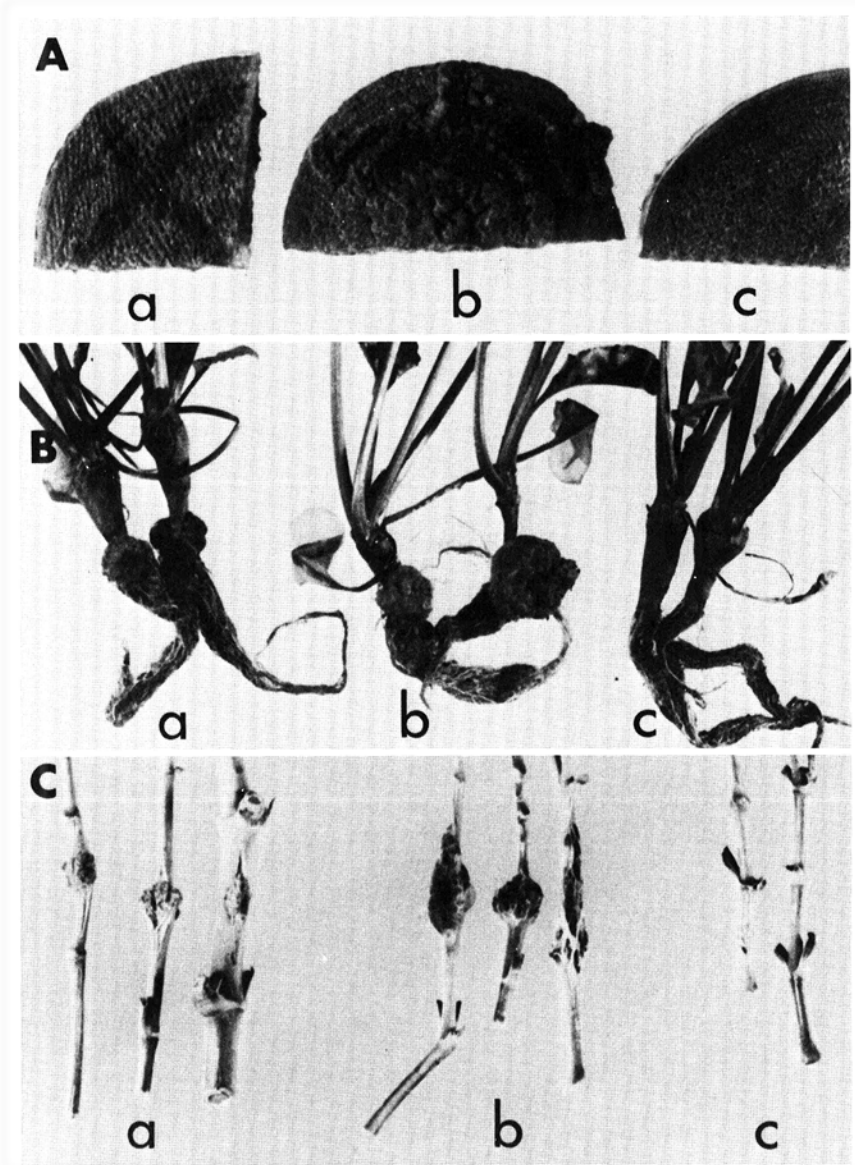

Fig. 2. (A) Tumor development on beet slices 2 wk after inoculation with (a) *Agrobacterium tumefaciens* strain C58, (b) *E. herbicola* strain 2188 (EH2188), and (c) sterile distilled water. **(B)** Tumor development on taproots of beet seedlings inoculated with (a) EH2188, (b) C58, and (c) sterile distilled water. **(C)** Differences in tumor morphology on stems of *Gypsophila paniculata* inoculated with (a) EH2188 and (b) *E. h. gypsophilae* strain 1184-13; (c) noninoculated control (inoculation with C58 caused same reaction).

tissue culture were potted and grown in the greenhouse for about 2 wk, at which time young stems were wounded with a sterile pin and inoculated as described above. Stems of about 1-mo-old *N. glauca* plants that had been grown from seed were inoculated with each strain as described above. In addition, a strain isolated from the naturally occurring beet seedling tumor and 14 strains isolated from the seedling washes from two beet seed lots were tested for tumorigenicity by inoculation on *G. paniculata* and *N. glauca*. Control plants were wounded as above and inoculated with sterile distilled water. Final observations of tumors were made 1 mo after inoculation. The tests were repeated at least once.

Isolations were made from all tumors that developed on the different indicator plants. Pieces of tumor tissue from beet slices, beet roots, and *G. paniculata* and *N. glauca* stems were triturated in sterile distilled water and streaked on MS medium or Schroth's medium. Colonies were characterized as *E. herbicola* as described above or as *A. tumefaciens* by typical growth on Schroth's medium.

RESULTS

Isolation of bacteria. Tumors on naturally infected table beets developed on the side or top of the roots and were frequently composed of many small nodules (Fig. 1). Numerous colonies with a uniform morphological type grew within 48 hr on 523, yeast-mannitol, and Pseudomonas agar F media and within 3–4 days on MS medium. Colonies were smooth, mucoid, and cream to yellow on all media except MS, on which they were orange. A few colonies that grew slowly on Schroth's medium for *A. tumefaciens* biovar 1 were isolated from some of the tumors but were not typical of those of *Agrobacterium*.

Identification of strains. Four strains (EH1188, EH2188, EH3188, and EH4188) that were isolated from beet tumors sampled from the different farms were identified as *E. herbicola* (Table 1). These strains were indistinguishable from other strains of *E. herbicola* except that EH3-1a, EH3-1b, and EH350-1 did not liquefy gelatin. Production of IAA-type compounds using the Gordon and Weber method (7) was not correlated with the tumorigenic ability of strains, since the nontumorigenic strains produced amounts that were equal to or greater than those produced by some of the tumorigenic strains. None of the strains rotted potato slices.

Pathogenicity tests. Only the strains of *E. herbicola* that were isolated from tumors on beets (EH1188, EH2188, EH3188, and EH4188) caused tumors on both beet slices and seedlings. Small tumors were visible on beet slices in about 10 days (Fig. 2A). None of the other bacteria tested induced tumor formation on beet slices. The beet strains of *E. herbicola* and *A. tumefaciens* caused tumors on the taproots of beet seedlings. All these tumors were morphologically indistinguishable from each other (Fig. 2B).

Strains of *E. herbicola* from beet and strains of *E. h. gypsophilae*, but not strain C58 of *A. tumefaciens*, caused tumors on *G. paniculata*. The tumors caused by *E. h. gypsophilae* were elongated and necrotic in the centers, whereas those incited by beet strains were more compact, spherical, and nonnecrotic (Fig. 2C). Strains 0485-19 and 0585-3 of *E. h. gypsophilae* and the saprophytic strain EH112Y of *E. herbicola* did not cause tumors when inoculated on any of the plants. Only *A. tumefaciens* strain C58 produced tumors on *N. glauca*. Strains were readily reisolated from the tumors that developed on the indicator plants. Bacteria isolated from tumors on plants inoculated with *E. herbicola* produced colonies characteristic of *E. herbicola* on MS medium but not on Schroth's medium. Strains from tumors of plants inoculated with *A. tumefaciens* produced colonies characteristic of *Agrobacterium* on Schroth's medium. Strains recovered from inoculated indicator plants were characterized using the methods described above.

Isolations from beet seedlings. The strain of *E. herbicola* isolated from the naturally occurring tumor that developed on a beet seedling caused tumors on beet slices and on *G. paniculata* that resembled tumors caused by the other tumorigenic strains of *E. herbicola* from beet. Isolations from the individual washes of groups of germinated beet seeds always yielded numerous colonies on MS medium that resembled *E. herbicola*. However, none of the 14 strains selected for tests on beet seedlings or on *Gypsophila* were tumorigenic.

DISCUSSION

Tumorigenic strains of *E. herbicola* from table beets differed from strains of *E. h. gypsophilae* in host range and in morphology of the tumors they caused on *G. paniculata*. Brown (1) first reported that strains of bacteria isolated from sugar beets with bacterial pocket disease had a limited host range, because they caused tumors on table beet but not on seven other species of plants susceptible to *A. tumefaciens*. In a later report (2), Brown showed that strains isolated from tumors on sugar beets infected *Gypsophila*, whereas tumorigenic strains from *Gypsophila* did not cause tumors on beet or 12 other genera of plants. Another more recent report (3) also showed that *E. h. gypsophilae* is host-specific. We also found that *E. h. gypsophilae* did not infect beet, but tumorigenic strains of *E. herbicola* from beet produced tumors on *Gypsophila* that were morphologically distinct from those produced by *E. h. gypsophilae*. Therefore, *E. herbicola* from beet is not host-specific, although it may have a very limited host range. The beet slice tumorigenicity assay appears to be a quick and specific method for measuring the tumorigenic nature of the beet strains of *E. herbicola* and should be useful for future research on this pathogen.

It is interesting that *E. herbicola*, a bacterium that is usually considered a saprophyte, causes important diseases of at least two plant species. It may be tumorigenic on other plants as well, and if a tumorous disease develops and tumorigenic strains of *Agrobacterium* cannot be readily isolated, the possibility of *E. herbicola* as the pathogen should be investigated. Crown gall has been reported on both table beet and sugar beet. Because tumors caused by *E. herbicola* closely resemble those caused by *A. tumefaciens*, visually distinguishing bacterial pocket disease from crown gall may at times be impossible. The relative importance of the these two diseases on table and sugar beet should be evaluated.

All tumorigenic strains from beet were characterized by biochemical and physiological tests as *E. herbicola* and were indistinguishable from saprophytic strain EH112Y and most strains of *E. h. gypsophilae*. Strain 0485-19 (ATCC 43348), which was nontumorigenic in our tests, was also previously found to be nontumorigenic (4,5). Three strains of *E. h. gypsophilae* from Israel (EH3-1a, EH3-1b, and EH350-1) did not liquefy gelatin in our tests. Brown (2) reported that strains from beet liquefied gelatin in 7–14 days, whereas strains from *Gypsophila* began to liquefy gelatin in 30 days and did not complete the reaction for 4 mo. We did not incubate our tests for longer than 3 wk and therefore may have failed to detect gelatin liquefaction by these strains. We also were unable to correlate the relative levels of indole-type compounds, as measured by the Gordon and Weber test, with tumorigenicity of strains. Our results may have been affected by the fact that this test does not measure IAA specifically but detects other indole-type compounds as well. Recently, however, Manulis et al (10) used high-pressure liquid chromatography to measure differences in IAA production by *E. h. gypsophilae* and *E. herbicola* and detected no correlation between IAA production and tumorigenicity.

Plasmid DNA from *E. h. gypsophilae* encodes for IAA production, which is potentially important in tumor formation on *Gypsophila*, and plasmid DNA sequences from *E. h. gypsophilae* have been shown to share homology with the IAA biosynthetic genes from *Pseudomonas savastanoi* (9). It has also been demonstrated that, in contrast to transformed tumor tissue of *A. tumefaciens*, growth of axenic tumor tissue from *Gypsophila* infected with *E. h. gypsophilae* requires media supplemented with

exogenous hormones (3). Tumorigenicity of *E. herbicola* from beet has not been investigated at the molecular level, and further research is needed to determine the role of plasmids in the tumorigenic response and the relatedness to tumorigenicity of *E. h. gypsophilae*.

Tumors on table beets result in an economic loss because infected beets cannot be mechanically processed. The incidence of the disease differs greatly from year to year. The incidence was high (greater than 2% in some fields) in 1988, a relatively dry growing season (12.2 cm of rainfall during June and July at Geneva, New York). The beet fields were not irrigated and plants were exposed to severe water stress that year, resulting in growth cracks at root crowns that possibly influenced disease development. In 1989, such dry conditions did not occur (18.8 cm rainfall during the same time period), and disease was present but at the low level observed in most years. Three samples of more than 100 beets each collected in 1989 from one commercial field had tumors on 0–0.7% of the taproots, with an overall average of 0.2%. Processors have indicated that this low incidence is typical.

Several other factors may influence seasonal differences in disease development. For example, beets planted in soils containing high nitrogen levels are more susceptible to infection (1). Also, the source of primary inoculum is unknown. Our isolation of the pathogen from a single gall on a noninoculated seedling growing in the greenhouse suggests that the pathogen may be seedborne. We feel that in this case the inoculum probably came from the seed, since the seedlings were grown in a pastuerized potting mixture. We failed to isolate the pathogen from other germinated seed, however, which may indicate that the levels of the pathogen in the seed were too low to be detected by the isolation procedure we used. Further investigations are needed to determine if the pathogen is seedborne and also to determine the possible predisposing effects of water stress and nutrition on disease development.

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LITERATURE CITED

1. Brown, N. A. 1928. Bacterial pocket disease of the sugar beet. J. Agric. Res. 37:155-168.
2. Brown, N. A. 1932. A gall similar to crown gall, produced on *Gypsophila* by a new bacterium. J. Agric. Res. 48:1099-1112.
3. Clark, E., Vigodsky-Haas, H., and Gafni, Y. 1989. Characteristics in tissue culture of hyperplasias induced by *Erwinia herbicola* pathovar *gypsophilae*. Physiol. Mol. Plant Pathol. 35:383-390.
4. Cooksey, D. A. 1986. Galls of *Gypsophila paniculata* caused by *Erwinia herbicola*. Plant Dis. 70:464-468.
5. De Cleen, M., and Deley, I. 1980. The presumed pathogenicity of *Agrobacterium gypsophilae*. Phytopathol. Z. 101:90-93.
6. Dye, D. W. 1968. A taxonomic study of the genus *Erwinia* I. The "amylovora" group. N.Z. J. Sci. 11:590-607.
7. Gordon, S. A., and Weber, R. P. 1951. Colorimetric estimation of indoleacetic acid. Plant Physiol. 26:192-195.
8. Kado, C. I., Heskett, M. G., and Langley, R. A. 1972. Studies on *Agrobacterium tumefaciens*: Characterization of strains 1D135 and B6, and analysis of the bacterial chromosome, transfer RNA and ribosomes for tumor inducing ability. Physiol. Plant Pathol. 2:47-57.
9. Manulis, S., Clark, E. M., Ophir, Y., Barash, I., and Gafni, Y. 1989. Molecular basis of IAA production in *Erwinia herbicola* pv. *gypsophilae*. (Abstr.) Phytopathology 79:1177-1178.
10. Manulis, S., Gafni, Y., Clark, E., Zutra, D., Ophir, Y., and Barash, I. 1991. Identification of a plasmid DNA probe for detection of strains of *Erwinia herbicola* pathogenic on *Gypsophila paniculata*. Phytopathology 81:54-57.
11. Miller, T. D., and Schroth, M. N. 1972. Monitoring the epiphytic population of *Erwinia amylovora* on pear with a selective medium. Phytopathology 62:1175-1182.
12. New, P. B., and Kerr, A. 1971. A selective medium for *Agrobacterium radiobacter* biotype 2. J. Appl. Bacteriol. 34:233-236.
13. Robbs, C. F., Kimura, O., and Ribeiro, R. D. D. 1981. Descricao de um novo patovar de *Xanthomonas campestris* em beterraba horticola e studo comparativo com *Xanthomonas beticola*. Fitopatol. Bras. 6:387-394.
14. Schaad, N. W., ed. 1980. Laboratory Guide for Identification of Plant Pathogenic Bacteria. American Phytopathological Society, St. Paul, MN. 72 pp.
15. Schroth, M. N., Thompson, J. P., and Hildebrand, D. C. 1965. Isolation of the *Agrobacterium tumefaciens–A. radiobacter* group from soil. Phytopathology 55:645-647.
16. Smibert, R. M., and Krieg, N. R. 1981. General characterization. Pages 409-443 in: Manual of Methods for General Bacteriology. P. Gerhardt, ed. American Society of Microbiology, Washington, DC. 524 pp.
17. Suslow, T. V., Schroth, M. N., and Isaka, M. 1982. Application of a rapid method for Gram differentiation of plant pathogenic and saprophytic bacteria without staining. Phytopathology 72:917-918.