Pathogenicity and Characterization of *Erwinia ananas* Causing a Postharvest Disease of Cantaloup Fruit

B. D. BRUTON, Agricultural Research Service, U.S. Department of Agriculture, Lane, OK 74555; J. M. WELLS, Agricultural Research Service, U.S. Department of Agriculture, 600 E. Mermaid Lane, Philadelphia, PA 19118; G. E. LESTER, Agricultural Research Service, U.S. Department of Agriculture, Weslaco, TX 78596; and C. L. PATTERSON, Oklahoma State University, Department of Plant Pathology, Lane 74555

ABSTRACT

Bruton, B. D., Wells, J. M., Lester, G. E., and Patterson, C. L. 1991. Pathogenicity and characterization of *Erwinia ananas* causing a postharvest disease of cantaloup fruit. Plant Dis. 75:180-183.

Cantaloups (*Cucumis melo* var. *reticulatus*) grown in the Rio Grande Valley of Texas developed small, water-soaked lesions after harvest. Lesions were shallow, firm, and, in many cases, not easily detected until the peel was removed. A yellow bacterium was isolated on nutrient agar and used to fulfill Koch's postulates. The bacterium was nonfluorescent, gram-negative, rod-shaped (0.5–1.1 × 0.9–3.5 μm), motile, and capable of anaerobic growth. Mucoid colonies were produced on high sugar media. The bacteria grew at 36 C and on Miller-Schroth medium. The pathological and physiological attributes of the bacterium fit the description of *Erwinia ananas*. Strains from cantaloup reacted positively in ELISA tests with antibodies against reference cultures of *E. ananas*, and cellular fatty acid profiles were identical to those of the *E. ananas-E. herbicola* group. This disease of cantaloup extends the known host range of *E. ananas* and may become an economically important postharvest decay of melons grown in the Rio Grande Valley of Texas.

Additional keyword: serology

The Rio Grande Valley of Texas is a major supplier of cantaloup (*Cucumis melo* L. var. *reticulatus* Naudin) fruit to the continental United States and Canada during April, May, and June. Because of the shipping distance, the melons must maintain a shelf life of 10–14 days. During the spring of 1985 and periodically since then, melons produced in the Rio Grande Valley and stored for 14 days at 4 C and 85% relative humidity (RH) developed conspicuous water-soaked lesions that detracted from the market value of the fruit. Affected tissues were firm and appeared clear and translucent or had a slight yellow-brown discoloration. The lesions extended 1–2 mm below the epidermis (Fig. 1). On close examination, the epidermis also appeared slightly water-soaked. Lesion diameters seldom extended beyond 10 mm. In some instances, as many as 87% of the melons were affected. A yellow bacterium was isolated from symptomatic tissue on nutrient agar. A similar disease of honeydew melons (*Cucumis melo* L. var. *inodorus* Naudin), brown spot, is caused by *Erwinia ananas* (3,14). The only other recent report of a postharvest blemish on cucurbits caused by a yellow bacterium is that of bacterial brown spot of cucumber, caused by *Xanthomonas campestris* pv. *cucurbitae* (Bryan) Dye (13).

The purpose of this study was to identify and demonstrate pathogenicity of the yellow bacterium isolated from cantaloup and to compare biochemical and serological characteristics of strains of the pathogen with *E. ananas* and other yellow bacteria associated with postharvest diseases.

MATERIALS AND METHODS

Isolation of strains and Koch's postulates. Bacteria were isolated from cantaloup fruits grown in the Rio Grande Valley that had water-soaked lesions. Melons were soaked in 95% ethanol for 3 min. Next, the epidermis was excised to expose the affected tissues. Portions of the lesions were transferred directly to nutrient agar (Difco) and incubated for 2 days at 27 C. Colonies were purified by streaking on nutrient agar plates and single colonies were selected. Four strains (TX-540, TX-541, TX-542, and TX-543), isolated from different melons, were used in the study. Reference strains of *E. ananas* and other genera of bacteria were obtained from the American Type Culture Collection (ATCC) or from colleagues (Table 1).

Pathogenicity of the strains was tested on mature cantaloup, cucumber (*Cucumis sativas* L.), and honeydew purchased from a local vendor in Atoka, OK. The test fruit were surface-sterilized with 95% ethanol, and a syringe was used to inject 10 μl of a suspension of 10^6 colony-forming units per milliliter (cfu/ml) into potential infection courts. Three different fruit were each inoculated in eight to 10 sites approximately 3 mm below the epidermis for each test strain. Inoculated fruit were held at 15 C and approximately 95% RH for 14 days. Water-soaked tissues extending beyond the original inoculation site were considered evidence of disease. Bacteria were reisolated from diseased tissue by the methods previously described and identified based on colony characteristics, selected physiological tests, and fatty acid profiles. Data were analyzed by analysis of variance (ANOVA) and means were compared by Duncan's multiple range test.

Morphological examinations and cultural characterization. Colonies were examined at ×25 with a dissecting microscope, and unstained cells were examined at ×450 with a phase contrast microscope. Cells were stained by the Huckner method (6). Oxygen requirements were determined by culturing the bacteria in nutrient broth under a controlled atmosphere at 27 C. Culture flasks were fitted with gas inlets, inoculated with cells in log phase, and connected to lines through which flowed compressed air or nitrogen gas. Growth of the unknown strains after 48 hr was compared with that of an obligate aerobe, *X. c.* pv. *pruni* (Smith) Dye (strain XP1). Growth and mucoid colony formation were tested on the Miller-Schroth medium, a substrate specific for

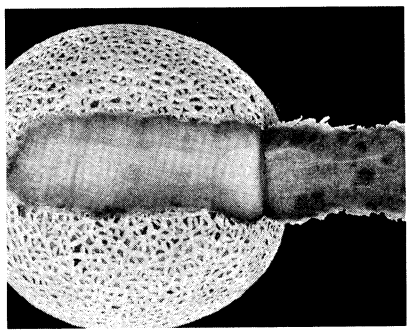

Fig. 1. Cantaloup fruit with small water-soaked lesions beneath the epidermal tissues caused by *Erwinia ananas*.

Accepted for publication 23 August 1990.

Table 1. Source of strains of *Erwinia ananas* and other phytobacteria

Bacterium	Strain designations	Source[a]	Host of origin or reference
Erwinia ananas	TX-540, TX-541, TX-542, TX-543	Original isolations	Cantaloup fruits, TX
E. ananas	X5	ATCC 35397	Honeydew melons, Guatemala
E. ananas	X6	ATCC 35398	Honeydew melons, Ecuador
E. ananas	X7	ATCC 35399	Honeydew melons, Ecuador
E. ananas	X9	ATCC 35400	Honeydew melons, CA
E. ananas	5R	Original isolation	Honeydew melons, Guatemala
E. ananas	9R	Original isolation	Honeydew melons, CA
E. ananas	T1	ATCC 11530	Pineapple fruitlets, HI
E. ananas	T2	ATCC 23822	Banana
E. ananas	T3	ATCC 33244	Pineapple fruitlets
E. herbicola	109, 112Y, 130 141, 171, 261	S. Beer, Cornell University, Ithaca, NY	...
E. amylovora	WV-55	T. Van der Zwet, USDA-ARS, Kearneysville, WV	(2)
E. carotovora subsp. *carotovora*	ATCC 15713	ATCC, Rockville, MD	Potato
	E21, E22, E31, E37, E40, E60	H. Moline, USDA-ARS, Beltsville, MD	Potato (4)
Xanthomonas campestris	XB1, XP1, XP19, XPh1, XCu-1, XM1,	E. Civerolo, USDA-ARS, Beltsville, MD	
Cytophaga johnsonii	PF-062	C. Liao, USDA-ARS, Philadelphia, PA	(9)
	ATCC 17061	ATCC, Rockville, MD	...

[a] ATCC = American Type Culture Collection, Rockville, MD.

Erwinia (12), and on nutrient agar supplemented with 5% glucose, respectively. Motility was determined on a stab-inoculated semisolid medium (11).

Physiological tests. Acid production from carbohydrates was tested in phenol red broth base (11). The carbohydrates adonitol, L-arabinose, D-cellobiose, dextrin, dulcitol, D-galactose, glycerol, maltose, and D-melibiose were filter-sterilized and added to stock solutions of phenol red broth base at a final concentration of 1% (11). Four milliliters of stock preparations of base plus carbohydrate were aseptically transferred to sterile screw-cap tubes and inoculated with 0.1 ml of a cell suspension in 0.01 M phosphate buffer, pH 7.0. Suspensions were prepared from cells grown for 24 hr at 27 C on nutrient agar and washed twice by centrifugation in phosphate buffer. Final concentrations were approximately 10^5–10^6 cfu/ml. Tubes were incubated without agitation at 27 C for 72 hr. Development of a yellow color within 48 hr was considered a positive reaction for acid production. Development of color between 48 and 72 hr was considered a weakly positive reaction. The production of catalase, oxidase, and the extracellular enzymes coagulase and lipase was determined by methods previously described (15). Hydrogen sulfide production was tested with lead acetate-impregnated strips (Difco) suspended over nutrient broth cultures; a dark color indicated a positive reaction. Indole production was determined with Broth 2 by the microtechnique method of Arnold and Weaver (1). Absence of a color change in the medium was considered a negative reaction. The nitrate reduction test was conducted as described by MacFaddin (11). No color development in the *d*-naphthylamine-sulfonic acid reaction (phase 1) and a pink to red color change in the zinc dust reaction (phase 2), indicating the presence of unreduced nitrate, were considered negative reactions.

Table 2. Pathogenicity of reference strains of *Erwinia ananas* and cantaloup strains on selected cucurbits[w]

Isolate	Lesion diameters on inoculated host (mm)[x]		
	Cantaloup	Cucumber	Honeydew
X9	6.9 a	4.0 a	2.4 a
5R	3.5 b	2.5 bc	2.4 a
TX-541	2.8 b	2.7 b	2.2 a
X7	2.6 b	2.4 bc	1.5 c
9R	2.6 b	2.3 bc	2.1 abc
TX-540	2.6 b	4.1 a	1.9 abc
T3	2.4 b	2.4 bc	0.6 d
T1	2.4 b	2.0 cd	2.0 abc
X5	2.2 b	1.4 d	1.5 c
X6	2.2 b	1.5 d	2.1 ac
TX-543	1.8 b	2.4 bc	1.5 bc
H_2O control[y]	0.8 c	0.4 e	0.1 d
Control[z]	0.0 c	0.0 e	0.0 d

[w] Host tissues inoculated by syringe with a 10-µl suspension of bacteria (1×10^6 cfu/ml) and incubated for 15 days at 15 C and 95% RH.

[x] Numbers represent average lesion diameters of 10 inoculated sites per fruit, replicated three times. Means followed by the same letter or letters within each column are not significantly different by Duncan's multiple range test ($P = 0.05$).

[y] Inoculated with sterile water.

[z] Sterile needle prick.

Serology. Enzyme-linked immunosorbent assay (ELISA) was performed with the avidin-biotin-peroxidase complex (ABC) technique described previously (7). Reactions, tested in duplicate wells, were measured by an EIA Gilford PR-50 automatic analyzer system (Gilford Instrument Labs, Oberlin, OH). Test antigens were prepared from membrane proteins of different strains of *E. ananas* by the method of Yakrus and Schaad (16). Antigen protein was measured with the Lowry method (10) and coated on microtiter plates at a final concentration of 2 µg of protein per well. Antibodies were prepared from ascites fluid of Swiss-Webster mice immunized with membrane proteins of strain X5 of *E. ananas*. The fluid was assayed at 0×, 10×, and 20× dilutions in phosphate-buffered saline (PBS).

Fatty acid analysis. Fatty acid composition was determined by gas-liquid chromatography (GLC) analysis of methyl esters with a Varian 3700 gas chromatograph (Varian Instruments, Palo Alto, CA) equipped with a flame ionization detector. Cells were grown on King's medium B agar for 1, 3, or 6 days at 27 C. The fatty acids were extracted, saponified, and methylated by a modification of the method of DeBoer and Sasser (5) described previously (14). Indi-

vidual fatty acids were identified by co-chromatography with known standards (Supelco, Supelco Park, Bellefonte, PA); confirmation of unsaturated, hydroxy-substituted, and cyclopropane acids was by methods also previously described (14). Data were expressed as percentages of total cellular fatty acids, which were averages from three determinations per culture at 1, 3, or 6 days. Major fatty acids were defined as those comprising at least 1% of the total.

RESULTS

Pathogenicity tests. In general, the lesions induced by all bacterial strains on inoculated cantaloups were similar to those observed on the stored fruit in 1985 (Table 2). Strain X9, originally isolated from honeydew melons from California, appeared to be more aggressive in cantaloup and cucumber than the other strains. However, there was no consistent relationship between host of origin and aggressiveness. Average lesion diameters were larger on cantaloups (2.9 mm) and cucumbers (2.5 mm) than on honeydew melons (1.8 mm). *E. herbicola* (Lohnis) Dye was not included in the present experiment because it was determined to be nonpathogenic in preliminary studies.

Morphology and physiology of strains. Acid production from cellobiose was positive for known strains of *E. ananas* but negative for strains from cantaloup. However, the other physiological characteristics were the same for all strains of *E. ananas*, including those from cantaloup (Table 3).

Serology. Polyclonal mouse antibodies against strain X5 of *E. ananas* reacted with all other strains of *E. ananas*, as well as with the cantaloup strains (Table 4). The antibodies also reacted with *E. herbicola* at all dilutions and cross-reacted with *E. amylovora* at 1×. Optical density readings were less than 0.1 and considered negative for other species of bacteria. At 10× and 20× dilutions, the antibodies to *E. ananas* cross-reacted with only *E. herbicola*.

Fatty acid analysis. The major class of fatty acids consisted of saturated even-carbon straight chains (45–47%), unsaturated acids (32–38%), hydroxy-substituted acids (4–5%), and cyclopropane acids (8–13%) (Table 5). The distribution and quantities of cellular fatty acids were similar for all strains of the yellow bacteria from cantaloups and for all strains of *E. ananas* tested. Pathogenic strains of the cantaloup bacteria and of *E. ananas* reisolated from artificially inoculated hosts contained the same composition of fatty acids as the original strains, thereby fulfilling Koch's postulates.

Table 3. Characteristics of the cantaloup strains of *Erwinia ananas* and of ATCC strains isolated from honeydew melon

Characteristic	*Erwinia ananas*[a]	
	ATCC honeydew strains[b]	Cantaloup strains[c]
Cell dimensions	0.5–1.0 × 1.0–3.5 μm	0.5–1.1 × 0.9–3.5 μm
Motility	+	+
Colony characteristics	Entire, domed	Entire, domed
Pigmentation	Yellow	Yellow
Growth at 36 C	+	+
Growth on MS agar	+	+
Mucoid growth[d]	+	+
Anaerobicity	+	+
Pathogenic to pineapple	+	ND
Nitrogen reductase	−	−
Indole production	±	−
H_2S production	+	+
Acid production from:		
D-cellobiose	+	−
D-galactose	+	+
glycerol	±	±
maltose	+	+
D-melibiose	+	+
L-arabinose	+	+
adonitol	−	−
dextrin	−	−
dulcitol	−	−
Catalase	+	+
Oxidase	−	−
Coagulase	−	−
Lipase	−	−

[a] + = Positive reaction, − = negative reaction, ± = variable reaction, ND = not determined.
[b] ATCC 35397, 35399, and 35400.
[c] Original isolates TX-540, -542, and -543.
[d] On nutrient agar + 5% glucose.

Table 4. Optical density measurements by indirect ELISA of antigenic reactions of cantaloup strains, selected *Erwinia* spp., and other yellow bacteria with antiserum against strain X5 of *Erwinia ananas*

Antigen[a]		Antibody dilution[b]		
Bacterium	Strain	1×	10×	20×
Cantaloup strain	TX-540	0.373[c]	0.149	0.174
Cantaloup strain	TX-541	0.394	0.201	0.209
Cantaloup strain	TX-542	0.391	0.199	0.151
Cantaloup strain	TX-543	0.407	0.199	0.122
E. ananas[d]	X5	0.326	0.124	0.118
E. ananas	X7	0.563	0.475	0.392
E. ananas	X9	0.409	0.241	0.190
E. herbicola	109	0.434	0.160	0.149
E. herbicola	125	0.341	0.169	0.147
E. amylovora	WV-55	0.122	0.066	0.049
E. carotovora subsp. *carotovora*	ATCC 15713	0.098	0.071	0.009
Xanthomonas campestris pv. *campestris*	XCu-1	0.072	0.051	0.049
Cytophaga johnsonii	PF-062	0.019	0.010	0.011

[a] Membrane protein of indicated bacteria. Protein concentrations per well of microtiter plates standardized at 2 μg.
[b] Ascites fluid of Swiss-Webster mice immunized against membrane protein of X5 strain of *Erwinia ananas*. Antibody controls and normal mouse serum were negative.
[c] Average absorbance at 460 nm of duplicate tests per reaction. Readings of less than 0.1 were considered negative reactions.
[d] Homologous reaction.

DISCUSSION

E. ananas was recently established as a distinct species to accommodate the only member of the *E. herbicola* group that is pathogenic to plants (8). Therefore, by convention, *E. ananas* can be distinguished from *E. herbicola* by the property of phytopathogenicity and by some physiological properties. Among the distinguishing physiological characteristics are indole production and acid production from melibiose, inositol, cellobiose, and glycerol (positive for *E. ananas* and negative for *E. herbicola*). In addition, nitrate reduction and acid production from dulcitol is negative for *E. ananas* and positive for *E. herbicola* (8).

Physiological properties of the pathogenic bacterium isolated from lesions of cantaloup are consistent with those of *E. ananas* isolated from honeydew melons and pineapple fruitlets, except for indole production and acid from cello-

Table 5. Fatty acid analysis of a reference culture of *Erwinia ananas* and of bacterial strains isolated from cantaloup fruit

	Percentage of total fatty acids			
	E. ananas	Cantaloup isolates		
Fatty acid class[a]	ATCC 35400	TX-540	TX-543	Range[b]
Saturated straight chains, even carbon				
10:0	0.04	0.04	0.04	0.01–0.16
12:0	4.55	4.80	4.02	3.01–4.80
14:0	3.94	5.00	4.85	3.06–5.00
16:0	35.90	36.50	35.61	33.18–37.22
18:0	0.63	0.61	0.30	0.44–1.14
20:0	0.05	0.03	0.30	0–0.16
Total percentage	45.13	47.01	44.88	41.64–47.22
Saturated straight chains, odd carbons				
11:0	0.23	0.18	0.23	0–0.26
15:0	0.18	0.13	0.18	0–0.22
17:0	0.18	0.08	0.10	0–0.27
19:0	0.25	0.24	0.34	0.12–0.94
Total percentage	0.86	0.70	0.85	0.37–1.29
Unsaturated acids				
16:1	15.64	17.03	19.20	14.58–20.73
18:1	22.00	14.73	14.71	12.61–23.64
Total percentage	37.64	31.76	33.92	31.36–41.88
Hydroxy-substituted				
20H-14:0	0.10	0.00	0.00	0–0.77
30H-14:0	4.09	5.09	3.71	1.75–6.62
Total percentage	4.25	5.09	3.71	1.75–7.63
Branched chains				
i-17:1	0.05	0.20	0.05	0–0.46
a-17:0	0.20	0.27	0.57	0.01–0.57
Total percentage	0.27	0.75	0.62	0.04–2.15
Cyclopropane acids				
D-17:0	6.70	10.57	12.57	4.10–12.57
D-19:0	0.93	0.30	0.35	0.14–1.85
Total percentage	7.63	10.87	12.92	4.17–12.92
Unidentified				
Total percentage	3.85	3.73	3.14	3.11–7.91

[a] Data on fatty acids comprising less than 0.04% of the total not shown but are represented in the total percentages for each class.
[b] Percentage range of isolates TX-540, -541, -542, and -543.

biose, which were negative. Results of 14 other physiological tests were consistent with those of *E. ananas*. As expected, serological reactions and cellular fatty acid analyses could be used to distinguish strains of the *E. herbicola-E. ananas* group from the reference bacteria used here but not for making distinctions within the group. Phytopathogenicity was the primary criterion for making that distinction.

Because the bacterial disease of cantaloup caused by *E. ananas* described in this report detracts from the market quality of Texas-grown cantaloups, it can be classified as a postharvest disease. The disease, however, apparently originates in the form of field infections that remain quiescent until the fruit ripens. *E. ananas* is a relatively unusual phytopathogenic form of *E. herbicola*, a bacterium that appears to be ubiquitous. Presumably, expression of the disease in Texas requires specific, yet undetermined, environmental conditions or cultural practices. The brown spot disease of honeydew melons appeared in New York markets in 1983 and 1984 but has not been observed since. Obviously, the potential exists for future outbreaks and/or significant economic losses to occur. The epidemiology of the disease needs to be studied to determine the conditions for infection and disease development. We propose that this condition of cantaloups be called brown spot to relate it to the disease of similar etiology in honeydew melons.

LITERATURE CITED

1. Arnold, W. M., and Weaver, R. H. 1948. Quick microtechniques for the identification of cultures. J. Lab. Clin. Med. 33:1334.
2. Casano, F., Wells, J. M., and Van der Zwet, T. 1986. Fatty acid profiles of *Erwinia amylovora* as influenced by growth medium, physiological age and experimental conditions. J. Phytopathol. 121:267-274.
3. Ceponis, M. J., Wells, J. M., and Cappellini, R. A. 1985. Bacterial brown spot of honeydew melons. HortScience 20:302-303.
4. Civerolo, E. L., Sasser, M., Helkie, C., and Burbage, D. 1982. Selective medium for *Xanthomonas campestris* pv. *pruni*. Plant Dis. 66:39-43.
5. DeBoer, S. H., and Sasser, M. 1986. Differentiation of *Erwinia carotovora* spp. *carotovora* and *E. carotovora* ssp. *atroseptica* on the basis of cellular fatty acid composition. Can. J. Microbiol. 32:796-800.
6. Doetsch, R. N. 1981. Determinative methods of light microscopy. Pages 21-51 in: Manual of Methods for General Bacteriology. P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg, and G. B. Phillips, eds. American Society for Microbiology, Washington, DC.
7. Hsu, S. M., and Raine, L. 1981. Protein A, avidin and biotin in immunohistochemistry. J. Histochem. Cytochem. 29:1349-1353.
8. Lelloitt, R. A., and Dickey, R. S. 1984. Genus VII. *Erwinia* Winslow, Broadhurst, Buchanan, Krumweide, Rogers and Smith. 1920. Pages 469-476 in: Bergey's Manual of Systematic Bacteriology. Vol. I. N. R. Krieg and J. G. Holt, eds. Williams & Wilkins Co., Baltimore, MD.
9. Liao, C. H., and Wells, J. M. 1986. Properties of *Cytophaga johnsonii* strains causing spoiling of fresh produce at food markets. Appl. Environ. Microbiol. 52:1261-1265.
10. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. 1951. Protein measurements with the folin phenol reagent. J. Biol. Chem. 193:265-275.
11. MacFaddin, J. F. 1976. Biochemical Tests for Identification of Medical Bacteria. Williams & Wilkins Co., Baltimore, MD. 307 pp.
12. Schaad, N. W. 1988. Initial identification of common genera. Pages 1-12 in: Laboratory Guide for Identification of Plant Pathogenic Bacteria. N. W. Schaad, ed. American Phytopathological Society, St. Paul, MN.
13. Takikawa, Y., and Tsuyumu, S. 1988. Bacterial brown spot of cucumber by *Xanthomonas campestris* pv. *cucurbitae*. Proc. Kansai Plant Prot. Soc. 29:11-15.
14. Wells, J. M., and Raju, B. C. 1984. Cellular fatty acid composition of six fastidious, gram-negative, xylem-limited bacteria from plants. Curr. Microbiol. 10:231-236.
15. Wells, J. M., Sheng, W.-S., Ceponis, M. J., and Chen, T. A. 1987. Isolation and characterization of strains of *Erwinia ananas* from honeydew melons. Phytopathology 77:511-514.
16. Yakrus, M., and Schaad, N. W. 1979. Serological relationships among strains of *Erwinia chrysanthemi*. Phytopathology 69:517-522.