Soft Rot of Crisphead Lettuce Incited by *Erwinia carotovora* subsp. *carotovora* in Hawaii

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ABSTRACT

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Erwinia spp. pathogenic in lettuce were isolated from 120 samples of diseased crisphead lettuce. Frequency of recovery ranged from 96.7 to 100%. Ten isolates were similar to *Erwinia carotovora* subsp. *carotovora* when compared in biochemical and pathological tests with known strains. A typical soft rot isolate from lettuce was compared with a known strain of *E. carotovora* subsp. *carotovora* (EC105) with respect to pathogenicity to a susceptible and resistant lettuce cultivar. In both cultivars, extensive maceration occurred 2 days after inoculation in the pith region of mature plants. Differences in the amount of rot were observed in relation to bacterial strain and cultivar when the midribs of wrapper leaves were inoculated. Multiplication of a lettuce soft rot strain was similar in the leaf midribs of a resistant as compared with a susceptible lettuce cultivar; however, maceration occurred earlier and at lower population levels in the latter.

Crisphead lettuce (*Lactuca sativa* L.) is the largest vegetable crop grown in Hawaii with 227 hectares in production yielding ~3.7 $\times 10^6$ kilograms (6). However, these figures reflect only 40% of the total consumption in the Hawaiian Islands. During the past 5 yr from 5 to 6.4 $\times$ 10^6 kg of lettuce were imported annually (6).

The ability of farmers in Hawaii to increase crisphead lettuce production is severely restricted by several diseases. Of these diseases, bacterial soft rot account for the major share of the losses (2,3). Severe reductions in yield occur annually during the winter and spring months when soft rot losses of up to 90% are not uncommon.

Soft rot of lettuce has been reported in Arizona (16), New York (1), and Florida (17). Those investigators attributed the rot to several bacterial pathogens including *Pseudomonas cichorii, P. marginalis, Xanthomonas campestris* subsp. *vitians, Erwinia carotovora, Erwinia* sp., and *Pseudomonas* sp. The disease symptoms described for *P. cichorii, P. marginalis,* and *X. campestris* subsp. *vitians* (1) are unlike those observed here. Symptoms are similar to those described for *E. carotovora* and *Erwinia* sp. (16,17). The results of preliminary investigations (2) indicated that *E. carotovora* subsp. *carotovora* was the major pathogen involved in soft rot of lettuce in Hawaii. This paper reports results of subsequent investigations of isolation from diseased materials, pathogenicity, and phenotypic comparisons of lettuce soft rot strains with *E. carotovora* subsp. *carotovora, E. carotovora* subsp. *atroseptica, E. chrysanthemi*, and strains of *Erwinia* spp. from sugar beet.

MATERIALS AND METHODS

Bacterial strains. Ten typical strains of *Erwinia* were isolated from crisphead lettuce exhibiting initial soft rot disease symptoms collected from Kula, HI. Lettuce strains were compared in morphological, biochemical, and physiological tests with eight strains of *E. carotovora* subsp. *carotovora*, two strains of *E. carotovora* subsp. *atroseptica*, two strains of *E. chrysanthemi*, and two strains of sugar beet *Erwinia* sp. Lettuce strains PV19 and PV19S (a streptomycin-resistant mutant of PV19 induced with ethane methyl sulfonate and *E. carotovora* subsp. *carotovora* ICPB EC105 were used in pathogenicity tests on resistant and susceptible lettuce cultivars. Known bacterial strains used for comparisons and their origins are listed in Table 1. All strains of *Erwinia* were maintained on nutrient agar (Difco, Detroit, MI 48232) slants.

Isolation of the lettuce pathogen. Lettuce plants exhibiting initial disease symptoms, a reddish to brown discoloration of the vascular tissues at the cut stem end, were collected from several commercial farms for pathogen isolation tests in the laboratory. The outer surface of the stem was removed with a sterile scalpel after surface sterilization and a small piece was taken from the discolored vascular bundles. This tissue was triturated in 1 ml of sterile saline (0.85% NaCl) with a mortar and pestle and streaked on Miller-Schroth (MS) (12) agar medium and yeast extract-dextrose-calcium carbonate (YDC) agar medium. The isolates were tentatively identified as *Erwinia* sp. based on their growth on MS agar, anaerobic fermentation of glucose (5), and ability to macerate potato slices.

Physiological and biochemical tests. The majority of tests were performed twice with the API 50 research system (Catalogue number 5010; Analytab Products, Inc., Plainview, NY 11803). The basal medium used in these tests was Hutner's mineral salt (15) at pH 6.8, plus 0.1% yeast extract (Difco).

Other tests performed as described by Dye (5) included: nitrate reduction, production of reducing substances from sucrose, growth in 5% NaCl, production of lecithinase, protopectinase activity, and fermentation of glucose.

Results of tests for nitrate reduction, phenylalanine deaminase, urease, indole, H_2S production, lysine decarboxylase, the Voges-Proskauer reaction, and aesculin hydrolysis were confirmed by using Patho Tec's Rapid I-D System (General Diagnostics Division, Warner-Lambert Company, Morris Plains, NJ 07950).

Lettuce isolates were compared with seven strains of *E. carotovora* subsp. *carotovora* (ICPB EC105, ICPB EC248, ICPB EC249, UCBPP82, UCBPP196, UCBPP202, and UCBPP232) and two strains of *E. carotovora* subsp. *atroseptica* (UCBPP143 and UCBPP149) (see Table 1). Because of the difficulty in determining reducing substances from sucrose tests, strains were also tested for the utilization of, and production of acid from, palatinose. Filter-sterilized palatinose was added to Hutner's mineral salts agar and to Hugh-Leifson basal medium (7) at 0.2% (w/v), and cultures were examined daily for 7 days for growth or acid production. All tests were carried out at 30 C.

Cellular morphology. Gram reaction was determined using the method of Skerman (14) and flagellation by the method of Mayfield and Innis (11).

Pathogenicity tests. Pathogenicity of lettuce isolates and *E.*

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carotovora subsp. *carotovora* ICPB EC105 was determined by inserting a small amount of a 2-day-old bacterial culture grown on nutrient agar into the cut stem end of mature crisphead lettuce. Plants were incubated in a moist environment at 24 C and observed for rotting 2, 3, and 5 days after inoculation.

Severities of infections caused by lettuce strain PV19, and *E. carotovora* subsp. *carotovora* ICPB EC105 in a field resistant (Salinas) and a susceptible (Calmar) crisphead lettuce cultivar were compared. Five heads of each cultivar were inoculated with each strain in two separate experiments. Mature lettuce heads of each cultivar were harvested and surface sterilized in 0.5% sodium hypochlorite (10% Clorox) for 10 min just prior to inoculation. Inoculum from 24-hr-old cultures was suspended in sterile distilled water at about 10^8 cfu/ml. A dissecting needle was dipped into individual bacterial suspensions and stabbed into either the detached midribs of the outermost wrapper leaf or the cut stem end. Inoculated plant materials were incubated in a moist environment at 25–27 C. The leaves were observed daily for rotting for 3 days after inoculation.

Multiplication of lettuce strain PV19S within detached midribs of the outermost wrapper leaves of a resistant (cultivar Salinas) and susceptible (cultivar Calmar) cultivar was determined. Detached lettuce midribs were infiltrated under vacuum with a cell suspension of 3×10^2 cfu/ml and incubated at 24 C in a polyethylene bag. Population densities of the bacterium within the midribs were estimated at 0, 5, 24, 30, and 48 hr after infiltration for each cultivar. Three 5-mm-diameter cylinders (~0.025 gm dry weight) were removed from each of three midrib sections with a cork borer. Disks were immediately triturated in 2 ml of sterile saline (0.85% NaCl) with a mortar and pestle, and a 10-fold endpoint dilution of the suspension was made in sterile saline. The last three dilutions were plated on Luria agar supplemented with 400 μg/ml of streptomycin sulfate (Nutritional Biochemicals Corp., Cleveland, OH 44128). Each dilution was replicated three times. Plates were incubated at 37 C and the populations estimated after 24 hr.

TABLE 1. Known *Erwinia* spp. included in comparisons with strains isolated from lettuce in Hawaii

Strain[a]	Host	Received from:
Erwinia carotovora subsp. *carotovora*		
ICPB EC105	Potato	M. P. Starr (originally from P. A. Ark)
ICPB EC248 (ATCC 25270)	...[b]	M. P. Starr (originally from D. J. Brenner)
ICPB EC249 (ATCC 25272)	...	M. P. Starr (originally from D. J. Brenner)
ICPB EC250 (ATCC 15359)	...	M. P. Starr (originally from D. J. Brenner)
UCBPP182	Potato	M. N. Schroth
UCBPP196	...	M. N. Schroth (originally from M. Harrison)
UCBPP202	Potato	M. N. Schroth (originally from M. E. Stanghellini)
UCBPP232	Potato	M. N. Schroth
Erwinia carotovora subsp. *atroseptica*		
UCBPP143	...	M. N. Schroth (originally from S. Alcorn)
UCBPP149 (NCPPB549 VI)	...	M. N. Schroth
Erwinia chrysanthemi		
ICPB EC16 (ATCC 11662)	...	M. P. Starr (originally from W. H. Burkholder)
ICPB EC205 (ATCC 29264)	*Philodendron oxycardium*	M. P. Starr (originally from L. A. McFadden)
Erwinia sp.		
UCBPP 176	Sugar beet	M. N. Schroth
UCBPP 193	Sugar beet	M. N. Schroth

[a] Acronyms: ICPB = International Collection of Phytopathogenic Bacteria. UCBPP = Plant Pathology Department of the University of California, Berkeley.
[b] ... = Host unknown.

RESULTS

Isolation from infected lettuce. *Erwinia* spp. were consistently isolated from a high percentage of the diseased materials sampled; recovery ranged from 96.7 to 100% (Table 2). A fluorescent pseudomonad soft rotter was isolated from only one lettuce sample.

Comparison of 10 lettuce isolates in morphological, biochemical, and pathological tests with strains of *E. carotovora* subsp. *carotovora*, and of *E. carotovora* subsp. *atroseptica* and *E. chrysanthemi* indicated that the pathogen was closely related to *E. carotovora* subsp. *carotovora*. The lettuce strains were Gram-

TABLE 2. Detection of soft-rot *Erwinia* spp. and *Pseudomonas* sp. from diseased crisphead lettuce

Farm	Diseased heads sampled (no.)	*Erwinia* sp. (%)	*Pseudomonas* sp. (%)
A	30	96.7	3.3
B	30	100.0	0.0
C	30	96.7	0.0
D	30	100.0	0.0

TABLE 3. Comparison of key physiological characters of lettuce rotting isolates from Hawaii, *Erwinia carotovora* subsp. *carotovora*, and *E. carotovora* subsp. *atroseptica*

Test or substrate	Lettuce *Erwinia* spp. isolates (10 strains)	*Erwinia carotovora* subsp. *carotovora* (7 strains)	*Erwinia carotovora* subsp. *atroseptica* (2 strains)
Pectate degradation	+[a]	+	+
Potato rot	+	+	+
Starch hydrolysis	−	−	−
Growth at 36 C	+(80)	+(43)	−
Growth at 39 C	+(60)	−(57)	−
Acetoin	+(90)	+	+
Indole	+(60)	−	+(50)
Growth in 7% NaCl	+	+	+
Lecithinase	−	−	−
Acid from:			
l-O-methyl-α-D-glucopyranoside	−(90)	−(87)	−
l-O-methyl-β-galactopyranoside	+(90)	+(87)	+
D(+)xylose	+	+	+
Sucrose	+	+	+
D(+)raffinose	+	+	+
Dulcitol	−	−	−
Adonitol	−	−	−
D(+)melizitose	−	−	−
Lactose	+	+	+
Sorbitol	−(90)	−	−
Maltose	−(90)	−	−
Amygdalin	+(80)	+	+
Inulin	−(80)	−	−
Utilization of citrate	+(90)	+	+

[a] Symbols: + = all strains positive; − = all strains negative; ( ) = percentage of strains + or −.

negative rods, oxidase negative, and fermented glucose within 24 hours. These strains grew well on MS agar with the growth turning orange, which is characteristic of the groups typified by *E. carotovora*. A comparison of key physiological and biochemical characters is summarized in Table 3.

All strains were positive in the following phenotypic characters: nitrate and aesculin reduction, production of β-galactosidase (ONPG), resistance to 50 μg erythromycin per milliliter, production of acid from: L−(+) arabinose, ribose, D−(+) xylose galactose, D−(+) glucose, fructose, D−(+) mannose, rhamnose, meso-inositol, mannitol, *N*-acetyl-glucosamine, arbutin, salicin, and D−(+) cellobiose; and were all negative for the production of arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, H_2S, urease, tryptophane deamination, and the production of acid from erythritol, D−(−) arabinose, methyl-xyloside, L(−) sorbose, methyl-D-mannoside, dextrin, amylose, starch, and glycogen.

The phenotypic characters, growth at 36 C, and production of reducing compounds from sucrose have been useful for differentiation between *E. carotovora* subsp. *carotovora* and *E. carotovora* subsp. *atroseptica*. Eight of 10 lettuce isolates, three of seven strains of *E. carotovora* subsp. *carotovora*, and neither of the strains of *E. carotovora* subsp. *atroseptica* grew at 36 C. Detection of reducing sugars produced from sucrose according to Dye's method was quite variable. This test was repeated on from three to six occasions with different results recorded for 10 of the 15 strains evaluated.

Results comparing several strains in respect to utilization and acid production from palatinose and production of sucrose reducing substances showed one of 10 lettuce isolates, one of eight strains of *E. carotovora* subsp. *carotovora*, one of two strains of *E. carotovora* subsp. *atroseptica*, none of two strains of *E. chrysanthemi*, and both of the strains of *Erwinia* from sugar beets utilized and fermented palatinose. In contrast, three of the 10 lettuce isolates, two of eight strains of *E. carotovora* subsp. *carotovora*, two of two strains of *E. carotovora* subsp. *atroseptica*, one of two *E. chrysanthemi* strains, and two of two *Erwinia* sp. strains from sugar beets produced reducing compounds from sucrose.

Pathogenicity test. All strains in this study rotted potato slices 24 hr after inoculation. All lettuce strains and *E. carotovora* subsp. *carotovora* ICPB EC105 macerated lettuce tissues 2 days after inoculation in the pith region of detached mature lettuce heads. No difference in severity of rot from pith inoculations could be detected between the bacterial strains or lettuce cultivars. However, invasion and tissue maceration from leaf midrib inoculations were more extensive in the susceptible cultivar (Calmar) than in the resistant cultivar (Salinas), and disease severity caused by EC105 was greater than that caused by PV19 (Table 4).

When detached petioles were infiltrated with a bacterial suspension of strain PV19S containing 3×10^2 cfu/ml, multiplication in the resistant cultivar (Salinas) tissues was similar to that in the susceptible cultivar (Calmar) tissues (Fig. 1). However, extensive maceration occurred earlier and at lower population levels in Calmar (after 24 hr) than in Salinas (after 48 hr).

TABLE 4. Reaction of a resistant and a susceptible crisphead lettuce cultivar to two strains of *Erwinia carotovora* subsp. *carotovora*

	Invasion of midrib tissue from inoculation point (mm after 1, 2, and 3 days)[y]								
	Isolate (PV19) (from lettuce)			*E. carotovora* subsp. *carotovora* ICPB EC105			Check (sterile H_2O)		
Cultivar	1 day	2 days	3 days	1 day	2 days	3 days	1 day	2 days	3 days
Calmar (susceptible)	5.5	5.4	6.4	19.3	25.8	NR[z]	0	0	0
Salinas (resistant)	1.0	1.0	1.0	4.7	15.3	NR	0	0	0

[y] Means of invasion distance were significantly different (susceptible versus resistant) ($P = 0.05$). Means for PV19 and EC105 were significantly different from the check ($P = 0.01$).
[z] NR = Not recorded.

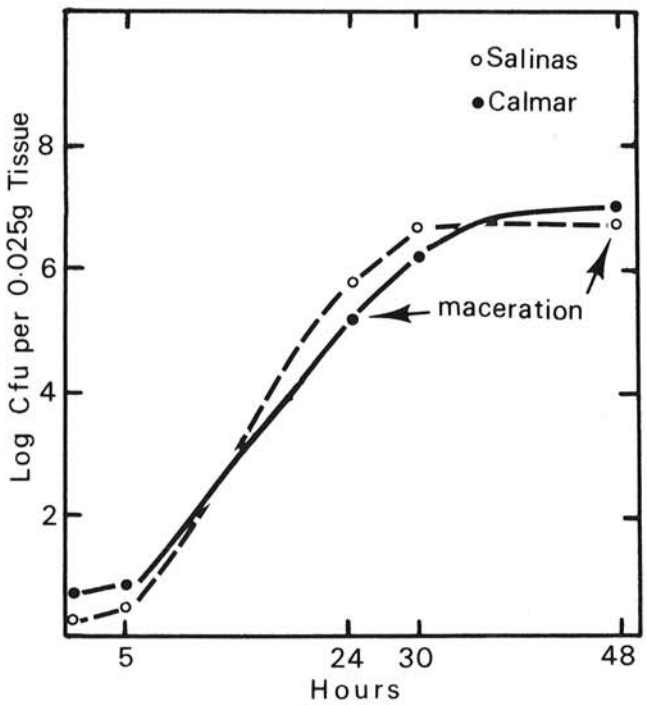

Fig. 1. Multiplication of *Erwinia carotovora* PV19S within the wrapper leaf midrib sections of a susceptible (cultivar Calmar) and a resistant (cultivar Salinas) crisphead lettuce within 48 hr after infiltration.

DISCUSSION

Several investigators (5,8) have identified biochemical and physiological tests useful for identifying members of the *E. carotovora* group. Of these, only three or four phenotypic characters are useful in differentiating *E. carotovora* subsp. *carotovora* from *E. carotovora* subsp. *atroseptica*. These tests include acid production from α-methyl glucoside and maltose, growth at 36 C, and the production of reducing compounds from sucrose. On the basis of Lelliott's description in Bergey's Manual (8), the lettuce strains were more similar to *E. carotovora* subsp. *carotovora*. The test for production of reducing substances from sucrose appeared to be unreliable for the separation of strains, because of inconsistent results with some strains. Lund (9) demonstrated that the major reducing compounds produced from sucrose by *E. carotovora* subsp. *atroseptica* are the disaccharides, palatinose, and 1-O-α-glucosylfructose (GF). Furthermore, Lund and Wyatt (10) and Sands and Dickey (13) demonstrated that these reducing sugars could be metabolized by strains of *E. carotovora* subsp. *atroseptica* but not by their strains of *E. carotovora* subsp. *carotovora*. Complete metabolism of these compounds could be affected by several factors such as environmental conditions, initial inoculum level, and physiological age of the bacterium. This could explain the different results recorded for *E. carotovora* subsp. *atroseptica* in my tests. The results for utilization of, or acid production from, palatinose were identical for all strains and should be more reliable for differentiation of *Erwinia* spp. into groups. These results are comparable to those reported by others (4,13). Some of the known strains used in these comparisons did not check out according to Lelliott's description and may have been mislabeled.

Several crisphead lettuce cultivars have been identified as being resistant to bacterial soft rot in the field (2,3). This study indicates that the presence and nature of this resistance may be detected in the midribs of the leaves, but not in the stem tissues. It may be possible to use mature detached wrapper leaf midribs to screen lettuce cultivars for resistance to Erwinia soft rot.

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