Pepper (*Capsicum annuum*) Soft Rot Caused by *Erwinia carotovora* subsp. *atroseptica*

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ABSTRACT

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Erwinia carotovora subsp. *atroseptica* caused soft rot of pepper (*Capsicum annuum*) fruit in field plots at Beltsville, MD, in 1994. The pathogen was identified as *E. carotovora* subsp. *atroseptica* based on carbohydrate utilization and fatty acid profiles. This is the first report of a bacterial fruit rot of pepper caused by *E. carotovora* subsp. *atroseptica*. Inoculation techniques were evaluated for reliable induction of pepper soft rot. Inoculation of puncture wounds provided more consistent lesion development than inoculation of Carborundum-induced abrasion wounds or nonwounded tissue. Significant differences in susceptibility of puncture-inoculated pepper genotypes to *E. carotovora* subsp. *atroseptica*–induced soft rots were noted. Virulence of *E. carotovora* subsp. *atroseptica*, *E. carotovora* subsp. *carotovora*, and *E. chrysanthemi* were compared at warm (23°C) and cool (10°C) temperatures. *E. carotovora* subsp. *carotovora* and *E. chrysanthemi* caused the greatest soft rot decay of pepper fruit at 23°C. At 10°C, fruits inoculated with *E. carotovora* subsp. *atroseptica* were the most severely affected, suggesting that *E. carotovora* subsp. *atroseptica* has the potential to cause significant postharvest decay losses during cool storage conditions.

Additional keywords: fruit decay, inoculation technique

Soft rot of peppers (*Capsicum annuum* L.) affects both mature and immature fruit and is a common postharvest problem in most production areas in the U.S. Bacterial soft rot caused by *Erwinia* species is the most destructive postharvest market disease of bell pepper fruit (3,13). Soft rot erwinias are common on the aerial surfaces of pepper plants, in the root zone, and in the soil (4). These bacteria invade pepper fruits through wounds in the fruit walls or stems, typically during harvest and handling. Bacterial soft rot is characterized by water-soaking and rapid softening of the tissues. Infections that originate at wounds in the fruit wall typically spread lengthwise throughout the fruit whereas infection at the stem end proceeds quickly through the stem and calyx lobe tissues. Underlying tissue is macerated, leaving a sunken, wrinkled, fragile skin that is easily ruptured. Under humid conditions and optimum temperatures, the entire fruit can be reduced to a watery mass within 3 to 6 days. Wash water on packing lines is a common source of inoculum. Addition of chlorine to wash water may eliminate bacteria from the fruit surface, but does not disinfest internal tissues nor offset the adverse effects of washing (4). Although hot water treatments may significantly reduce bacterial soft rot, subsequent hydro-cooling results in greater decay of infected fruit (10,14).

Bacterial soft rot was observed on pepper fruits in field plots at Beltsville, MD, in September 1994 following cool, wet weather. This study was undertaken to identify the putative causal organism, develop a reliable method to evaluate the reactions of diverse *C. annuum* genotypes to infection by soft rot erwinias, and evaluate potential differences among *Erwinia* species for inducing pepper soft rot under warm and cool temperatures typical of production and postharvest environments.

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MATERIALS AND METHODS

Pathogens. *Erwinia carotovora* subsp. *atroseptica* strains were isolated from water-soaked lesions on field-grown sweet bell pepper fruits harvested from a field plot at Beltsville, MD, in September 1994. Strains of all the *Erwinia* species utilized in this study were evaluated for pectolytic activity on crystal violet pectate medium (5), florescence on King's medium B (11), growth at 37°C, and sensitivity to erythromycin (8). Pepper fruits were inoculated with strains 1-4 and 1-26, and the strains reisolated from the resultant lesions that were similar to those observed in the field, and evaluated as described above. All strains used in this study were further characterized with the Biolog Microstation System and the Biolog GN Database (release 3.50; Biolog Inc., Hayward, CA) to determine carbohydrate utilization. These strains were further characterized via gas chromatographic analysis of fatty acid methyl esters (FAME) by Microbial ID, Inc. (Newark, DE). *E. carotovora* subsp. *carotovora* strains 285 and 394 and *E. chrysanthemi* strains 219 and 229 were obtained from C. Ishimaru, Colorado State University, Fort Collins, CO.

Inoculation of peppers. Pepper fruit at the mature green stage of development were harvested from field grown plants of 37 USDA breeding lines and commercially available cultivars of *C. annuum* and used to evaluate inoculation techniques and pepper soft rot resistance. Plants were grown in Keyport fine loam soils at Beltsville, MD. The plant materials represented a diverse collection of *C. annuum* germ plasm and included bell, banana, cherry, cheese, cayenne, chili, and ornamental peppers. Bacterial inoculum was prepared from 24-h-old nutrient broth cultures (Difco, Detroit, MI) that were continuously agitated at 25°C. Inoculum suspensions were adjusted with sterile nutrient broth to 1.4×10^9 CFU/ml based on previously determined correlations between optical density and bacterial concentrations.

Whole fruit of peppers were inoculated with *E. carotovora* subsp. *atroseptica* strain 1-4 by the following method: puncturing with a 1-mm-diameter glass needle to a depth approximately two-thirds the thickness of the fruit pericarp and covering the wound with an inoculum-soaked 1.25-cm sensi-disc (Schleicher and Schuell, Keene, NH) filter paper containing 100 µl of aqueous bacterial suspension (1.4×10^9 CFU/ml); abrasion of the pepper surface with 600 mesh Carborundum sufficient to remove surface wax followed by application of the inoculum-soaked sensi-disc; and application of the inoculum-soaked sensi-disc on a nonwounded surface. A sensi-disc containing 100 µl of sterile nutrient broth was applied to the fruit surface of control fruit for respective treatments.

Inoculated fruit were placed atop hardware cloth support screens (12.5 × 12.5 mm mesh) in 31 × 17 × 8 cm plastic boxes with tap water beneath the support screens.

Boxes were covered with snug fitting lids that permitted air exchange, yet allowed for maintenance of high relative humidity, and maintained at 23°C in the dark for 72 h. The experiment used three fruit per line or cultivar for each inoculation method tested. Individual fruit within each treatment were assigned to boxes in a completely randomized design. The experiment was conducted twice with a mid (August 9) and late (September 9) season harvest of fruit from the same field plot. The severity of soft rot decay was assessed by removing water-soaked tissue with a spatula and recording fresh weight of remaining healthy tissue (wt2) for comparison with initial fresh weight of fruit prior to inoculation (wt1) as described by Goth et al. (9). These data were transformed by the logit of the square root of proportional weight loss [(wt1 – wt2)/wt1] to conform to a normal distribution. Analysis of variance on the transformed data was obtained with the SAS (SAS Institute, Cary, NC) general linear models procedure with genotypes and inoculation techniques treated as fixed effects. Variances between the two harvest dates were homogeneous, indicating that the two data sets could be combined for analysis.

Strain virulence and temperature effect. Virulence of *E. carotovora* subsp. *atroseptica* (strains 1-4 and 1-26), *E. carotovora* subsp. *carotovora* (strains 285 and 394), and *E. chrysanthemi* (strains 219 and 229) on green bell pepper fruit obtained from a local vendor was evaluated at different temperatures (10 and 23°C) common in production and postharvest environments. Fruit were longitudinally halved and placed on wire support screens with the cut side adjacent to the screen. The external surface of the fruit was puncture inoculated as described above. The extent of soft rot decay in 10 fruit for each bacteria × temperature treatment was determined as described after 48 and 72 h and after 72, 96, and 120 h for 23 and 10°C treatments, respectively. Data were transformed by the logit of the square root of proportional weight loss to conform to a normal distribution. The data represent the results of two replicate experiments. The data were combined since variances between experiments were homogeneous. Statistical analysis was performed with the SAS general linear models procedure with species considered a fixed effect. Data are presented as proportional weight loss for clarity of interpretation since relative rankings of respective treatments for transformed and untransformed data did not differ.

Table 1. Response of pepper cultivars and breeding lines to *Erwinia carotovora* subsp. *atroseptica*

Genotype	Mean proportional weight loss	Logit of (proportional weight loss)$^{1/2}$	Market type
Cherry Sweet	0.95[y]	3.65 a[z]	Cherry
92C22	0.91	3.31 a	Long wax
90C44	0.92	2.98 ab	Tabasco
Fiery Festival	0.71	2.82 ab	Tabasco
Vallalat	0.69	2.09 ac	Pimiento
Little Dickens	0.54	2.05 ad	Tabasco
Thai Hot	0.61	1.50 be	Tabasco
90C40	0.64	1.31 bf	Tabasco
Sunnybrook	0.58	1.28 bg	Bell
90C53	0.56	1.24 bg	Tabasco
Petite Sarah	0.55	0.77 ch	Short wax
Purple Beauty	0.46	0.71 ch	Bell
92C27C	0.43	0.63 ci	Cheese
Jalapa	0.41	0.58 ci	Jalapeno
Sweet Chocolate	0.40	0.54 ci	Bell
Pimento	0.46	0.53 ci	Pimiento
Cubanelle	0.40	0.52 ci	Cuban
92C27D	0.45	0.36 ci	Cheese
92C24	0.34	0.27 ci	Bell
92C26	0.34	0.23 ci	Cuban
TC-M	0.31	0.17 di	Cheese
92C23	0.28	0.07 ei	Cuban
Long Red Cayenne	0.37	0.06 ei	Cayenne
Sweetheart 901	0.35	–0.06 ei	Pimiento
92C27B	0.28	–0.12 ei	Cheese
Garafarm	0.28	–0.14 ei	Pimiento
92C25	0.22	–0.30 ei	Cuban
92C28	0.16	–0.44 fi	Bell
92C29	0.19	–0.52 fi	Bell
Ariane	0.17	–0.54 fi	Bell
92C27A	0.22	–0.62 fi	Bell
Corona	0.15	–0.64 gi	Bell
Lady Bell	0.16	–0.65 gi	Bell
North Star	0.15	–0.75 hi	Bell
Anaheim M	0.04	–0.89 hi	Chili
Midway	0.11	–1.00 hi	Bell
Labamba	0.06	–1.26 i	Cuban

[y] Values are based on assays of six fruits following puncture inoculation with *E. carotovora* subsp. *atroseptica* strain 1-4. Severity was indicated by proportional weight loss by comparing weight of tissue before (wt1) and after (wt2) incubation of inoculated fruit [(wt1 – wt2)/wt1]. Mean proportional weight loss for controls = 0.018.

[z] Logit of the square root of proportional weight loss. Letters denote mean separations by Duncan's multiple range test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Field reaction. Soft rot lesions were apparent on 5 to 10% of harvested fruit with infection more prevalent in fruit harvested from low-lying areas of the field. *E. carotovora* subsp. *atroseptica* was repeatedly isolated from the margins of the lesions. Harvest was preceded by a 2-week period of wet, cool weather (maximum temperature range 19 to 31°C; minimum temperature range 13 to 22°C; fog and measurable precipitation reported on 9 and 8 days, respectively, during this period) (17).

Strain identity. Characteristics of soft rot organisms isolated from field-grown pepper fruit at Beltsville, MD, were consistent with profiles of *E. carotovora* subsp. *atroseptica*. *E. carotovora* subsp. *atroseptica* strains 1-4 and 1-26 produced deep pitting on crystal violet pectate medium and nonfluorescent colonies on King's medium B, produced reducing sugars from sucrose and acid from alpha-methyl glucoside, failed to grow at 37°C, and were sensitive to erythromycin. Growth of strains on 41 additional carbohydrate sources as part of the Biolog microbial analysis system and analysis of carbohydrate utilization via the Biolog GN Database also indicated these isolates were *E. carotovora* subsp. *atroseptica* (Biolog similarity indexes of 0.582 and 0.800 for strains 1-4 and 1-26, respectively). Fatty acid profiles obtained from strains 1-4 and 1-26 yielded Microbial Identification System (ver. 3.9; Microbial ID, Inc., Newark, DE) similarity indexes of 0.955 and 0.951, respectively, when matched with *E. carotovora* subsp. *atroseptica* library standards. The identity of *E. carotovora* subsp. *carotovora* strains 285 and 394 (Biolog similarity indexes of 0.805 and 0.621, respectively; FAME similarity indexes of 0.948 and 0.867, respectively;) and *E. chrysanthemi* strains 219 and 229 (Biolog similarity indexes of 0.776 and 0.797, respectively; FAME similarity indexes of 0.878 and 0.879, respectively) was also confirmed. Utilization of cellular fatty acid composition to delineate *E. carotovora* subspecies status has been reported (7).

To our knowledge, this is the first report of a bacterial fruit rot of pepper caused by *E. carotovora* subsp. *atroseptica*. *E. carotovora* subsp. *carotovora* has been reported to cause pepper soft rot (4,13). A number of reports that document pepper soft rot (2, 10,15,20) fail to delineate *E. carotovora*

subspecies status. In the Northern Hemisphere, *E. carotovora* subsp. *atroseptica* is commonly isolated from potato tubers with soft rot (18) and has also been isolated from tomato (*Lycopersicon esculentum* Mill.) and Chinese cabbage (*Brassica campestris* var. *pekinensis* (Lour.) Rupr.) (6). Based on the relatively few documented reports of *E. carotovora* subsp. *atroseptica* soft rot of different hosts, Smith and Bartz (19) suggest that typical low temperature strains of *E. carotovora* subsp. *atroseptica* may have a restricted host range.

Inoculation of pepper. Significant differences were evident among inoculation techniques for inducing soft rot by *E. carotovora* subsp. *atroseptica* ($F = 288$; $P \leq 0.01$). Puncture inoculation was more effective than other treatments in establishing soft rot decay (mean proportional tissue weight loss = 0.402). Significant differences were not noted between treatments in which inoculum was applied to Carborundum-abraded (mean proportional tissue weight loss = 0.037) or nonwounded fruit surfaces (mean proportional tissue weight loss = 0.045). Because of this, further analyses were limited to the puncture inoculation data subset. Little or no soft rot caused by resident bacteria was noted in control fruit for respective treatments (mean proportional weight loss = 0.018, 0.014, and 0.011 for punctured, abraded, and nonwounded inoculation sites, respectively).

Significant differences in susceptibility among *C. annuum* genotypes to soft rot established via puncture inoculation were observed ($F = 4.78$, $P \leq 0.01$; Table 1). Fruit pungency did not appear to be associated with soft rot among genotypes. Although the pungent variety Anaheim M was among the varieties exhibiting the least fruit weight loss, the pungent variety Thai Hot and pungent breeding lines 90C44 and 92C22 were the most susceptible to fruit decay. Likewise, a relationship between fruit shape and soft rot was not apparent. The sweet bell variety Sunnybrook for example, was among the most susceptible to fruit decay whereas the sweet bells Midway and North Star exhibited significantly less tissue loss. Similarly, Bartz and Stall (2) noted no correlation between pungency or fruit shape and pepper soft rot. The observed variation in genotypic response to soft rot infection suggests that soft rot resistance could be increased in pepper. Additional research is required to evaluate the feasibility of transferring appreciable soft rot resistance to susceptible pepper genotypes.

Differences in fruit soft rot between harvest dates were not noted ($F = 0.79$, $P > 0.05$). The uniform response across harvest dates suggests a stable response among diverse *C. annuum* genotypes to soft rot infection and broad applicability of the puncture inoculation technique. The puncture inoculation technique evaluated resistance in fruit flesh. Compared with the fruit stem or calyx, fruit walls are more susceptible to soft rot infection (2).

Strain virulence and temperature effect. To evaluate the potential significance of *E. carotovora* subsp. *atroseptica* relative to *E. carotovora* subsp. *carotovora* and *E. chrysanthemi* in pepper soft rot, decay of inoculated fruit was evaluated at two temperatures common in commercial production and packing operations. Storage of pepper fruit at 23°C subsequent to inoculation resulted in greater fruit decay (ca. 0.70 to 0.80 proportional tissue weight loss) with strains of *E. carotovora* subsp. *carotovora* and *E. chrysanthemi* after 72 h storage compared with *E. carotovora* subsp. *atroseptica* (ca. 0.35 to 0.40 proportional weight loss) (Fig. 1). Similar trends between strains were also observed after 48 h of storage at 23°C.

In contrast to pepper soft rot observed at 23°C, *E. carotovora* subsp. *atroseptica* generally caused greater decay of pepper pods in cool storage (10°C) relative to that caused by *E. carotovora* subsp. *carotovora* and *E. chrysanthemi* (Fig. 2). After 120 h at 10°C, decay caused by *E. carotovora*

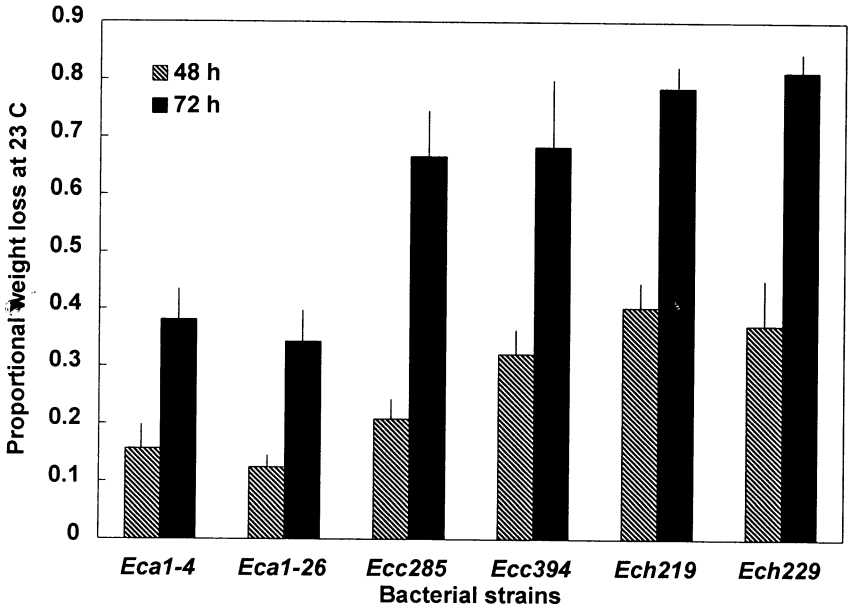

Fig. 1. Virulence of *Erwinia carotovora* subsp. *atroseptica* (*Eca*) strains 1-4 and 1-26, *E. carotovora* subsp. *carotovora* (*Ecc*) strains 285 and 394, and *E. chrysanthemi* (*Ech*) strains 219 and 229 after 48 and 72 h at 23°C. Bars indicate standard errors of the mean.

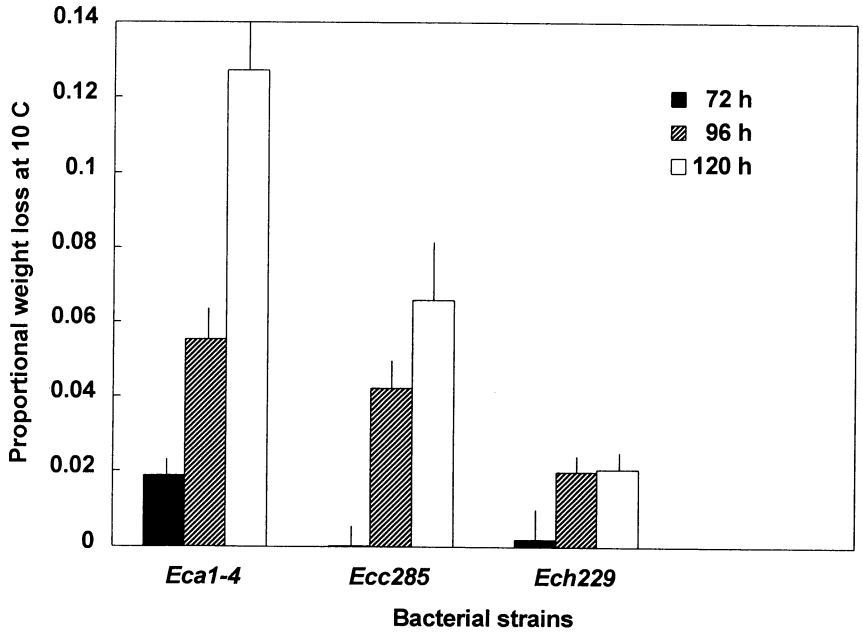

Fig. 2. Virulence of *Erwinia carotovora* subsp. *atroseptica* (*Eca*) strain 1-4, *E. carotovora* subsp. *carotovora* (*Ecc*) strain 285, and *E. chrysanthemi* (*Ech*) strain 229 after 72, 96, or 120 h at 10°C. Bars indicate standard errors of the mean.

subsp. *atroseptica* was approximately twice that observed for *E. carotovora* subsp. *carotovora* and approximately 6.5 times greater than that caused by *E. chrysanthemi*. While all strains produced appreciable decay at 23°C, *E. chrysanthemi* strains exhibited the least adaptability to induce soft rot at cool temperatures. Our results suggest that *E. carotovora* subsp. *carotovora* and *E. chrysanthemi* may cause greater losses at ambient temperatures in the field and during packing, but that *E. carotovora* subsp. *atroseptica* has the potential to cause greater decay under cool storage conditions in packing houses and wholesale and retail markets. In potato, *E. carotovora* subsp. *atroseptica* caused greater soft rot of potato tubers at 15°C (12) and produced black leg at lower soil temperatures than is commonly encountered when *E. carotovora* subsp. *carotovora* is the causative organism (16).

Inoculated pepper fruit stored at 10°C incurred considerably less soft rot than was observed in fruit stored at 23°C. Since cool temperatures merely slow the rate of decay in infected fruit, genetic resistance to soft rot would be of considerable value in reducing losses. Even small lesions that develop at cool temperatures render fruit unmarketable and serve as inoculum reservoirs for other fruit. Storage of wound-inoculated tomato fruit at temperatures less than 15°C delayed soft rot onset but resulted in an increased incidence of decay when fruit were returned to ambient conditions during ripening and marketing (1). In peppers, storage at nonfreezing temperatures below 10°C may further reduce the incidence of decay, but cause chilling injury and increased susceptibility to bacterial and fungal fruit decay upon transfer to higher temperatures (14).

In summary, we provide the first report of a bacterial soft rot of pepper caused by *E. carotovora* subsp. *atroseptica*. A reliable puncture inoculation technique was identified for inducing soft rot decay by *E. carotovora* subsp. *atroseptica* in pepper fruit. The inoculation technique was sufficient to evaluate differences in susceptibility of different *C. annuum* genotypes to *E. carotovora* subsp. *atroseptica*–induced soft rot. Variation in virulence of *E. carotovora* subsp. *atroseptica*, *E. carotovora* subsp. *carotovora,* and *E. chrysanthemi* under warm and cool conditions suggests that *E. carotovora* subsp. *atroseptica* has the potential to cause significant postharvest decay losses at cool storage conditions common in commercial markets.

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