Association of *Enterobacter cloacae* with Rhizome Rot of Edible Ginger in Hawaii

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ABSTRACT

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Edible ginger is a popular spice crop that is grown in Hawaii primarily for the fresh market, and as such, rhizome quality is of paramount importance. In our studies, a Gram-negative, facultative anaerobic, rod-shaped bacterium was consistently isolated from decayed as well as symptomless ginger rhizomes. The bacterium was identified as *Enterobacter cloacae* by biochemical assays and 16S rDNA sequence analysis. Rot symptoms, which usually occurred in the central cylinder of the rhizome, were characterized by yellowish-brown to brown discolored tissue and firm to spongy texture. In inoculation experiments, ginger strains of *E. cloacae* produced basal stem and root rot, with foliar chlorosis and necrosis in tissue-cultured ginger plantlets, and discolored and spongy tissue in mature ginger rhizome slices and whole segments. In other hosts, ginger strains of *E. cloacae* caused internal yellowing of ripe papaya fruit and internal rot of onion bulbs. All strains that caused symptoms in inoculated plants were reisolated and identified as *E. cloacae*. Our studies suggest that *E. cloacae* can exist as an endophyte of ginger rhizomes, and under conditions that are favorable for bacterial growth, or host susceptibility, including maturity of tissues, rhizome rot may occur. Rhizome quality may be impacted by the presence of *E. cloacae* under conditions such as high temperature, high relative humidity, and low oxygen atmosphere that may affect the development of decay, and such conditions should be avoided during post-harvest handling and storage. The association of *E. cloacae* with a rhizome rot of ginger is a new finding.

Additional keywords: bacterial wilt, *Enterobacteriaceae*, extracellular polysaccharide (EPS), modified atmosphere packaging, *Ralstonia solanacearum*

Edible ginger (*Zingiber officinale* Roscoe) is a popular spice crop grown in Hawaii, primarily on the island of Hawaii, with annual production at approximately 6.35 million kg (14 million lbs) and value at about \$4.3 million (20). Bacterial wilt disease, caused by *Ralstonia solanacearum* (Smith) Yabuuchi, is the most devastating and destructive disease of ginger. It is characterized by wilt, eventual death of ginger plants, and rhizomes with reduced quality due to discoloration and/or rotted tissue (14,37). Beginning in 2001, we attempted to isolate and collect *R. solanacearum* strains from bacterial wilt-infected

field plants grown on the Hamakua coast of the island of Hawaii. However, a facultative anaerobic, gram negative, rod-shaped bacterium was repeatedly isolated along with the targeted bacteria. The bacterium was later identified as *Enterobacter cloacae* (Jordan) Hormaeche & Edwards. Strains of *E. cloacae* were also isolated from symptomless ginger rhizomes obtained from local supermarkets.

E. cloacae is the most frequently isolated *Enterobacter* species from man and animals (28) and is commonly found on or in plants, insects, and many sources in our environment (e.g., water, sewage, soil, freshly harvested vegetables) (5,15,18,19, 23,28,30,31). In addition to being a human pathogen (31), *E. cloacae* is a pathogen of plants. Examples of plant hosts include elm trees (7,25), coconut (10), orchid (36), corn (29), onion bulb (4,8,33), apple (30), papaya fruit (26,27), and mung bean sprouts (38).

We report on the identification and characterization of *E. cloacae* strains isolated from ginger rhizomes, their pathogenic responses on ginger and other hosts, and discuss some of the factors that may trigger disease development in ginger.

MATERIALS AND METHODS

Isolations from ginger rhizomes obtained from field and supermarket. Bacterial strains were isolated from 80 ginger plants with bacterial wilt symptoms collected from two ginger farms located on the Hamakua coast of the island of Hawaii in 2001 and 2002, and from 20 symptomless rhizomes purchased from a supermarket in Hilo in 2001. Ginger rhizomes from the field were rinsed in tap water to remove excessive debris, disinfected 5 min in 0.5% sodium hypochlorite, once in the field and a second time in the laboratory, then rinsed in sterile distilled water (SDW) for 1 min, drained, and air-dried before aseptically cross-sectioning the rhizomes and excising approximately 3 mm³ tissue sections from the endodermal ring or the central cylinder. The tissue sections were soaked in tubes of 3 to 5 ml of SDW for at least 1 h before streaking one to two loopsful of each suspension onto PT-M2 agar medium, a modified (no salt) agar version of peptone-yeast extract-medium (ATCC Medium 1366) (2) containing 1.8% bacto-agar, 1% peptone, 0.5% yeast extract, and 0.001% triphenyltetrazolium chloride (TTC; Sigma Chemical Co., St. Louis, MO; added to sterile, molten agar prior to pouring). Plates were incubated at room temperature (22°C) or 30°C, and single colonies were isolated and restreaked at least two consecutive times to purify the strains. Stock cultures were stored in SDW in test tubes at 20°C or at room temperature. Strains selected for long-term storage were stored in 15% glycerol at minus 80°C.

Separation of *E. cloacae* and *R. solanacearum* was initially difficult on Kelman's medium (17) with reduced TTC (0.001%) because of high amounts of bacterial extracellular polysaccharide (EPS) produced by *R. solanacearum*. The PT-M2 medium was used in isolation procedures to reduce EPS production of *R. solanacearum*. Aerobic strains, including putative *R. solanacearum*, were grown on PT-M2 at room temperature. *E. cloacae* and other facultative anaerobes were grown at 30°C on PT-M4 agar medium, (PT-M2 plus 0.25% sodium chloride), which was more favorable for growth.

Identification and characterization of bacterial strains. Purified cultures were initially characterized using Difco oxida-

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tive-fermentative (OF) basal medium with 1% glucose as a carbohydrate source (13). The inoculated medium was layered with sterile mineral oil to produce anaerobic conditions, and the tubes were incubated at 30°C for 24 h. Facultative anaerobes were identified as *Enterobacter* sp. or other members of the *Enterobacteriaceae* using API 20E strips (bioMérieux, Inc., USA office, Durham, NC) incubated at 30°C for 18 to 24 h. Selected strains were further characterized and compared with an *E. cloacae* strain from fruit fly (27) by various tests that included: (i) phenylalanine deaminase (9); (ii) acid production from filter-sterilized, 10% solutions of glucose, cellobiose, lactose, or α -methyl-glucoside incorporated into Difco OF basal medium (carbohydrate final concentration, 1%) under anaerobic conditions; (iii) growth on several media including yeast extract-dextrose-calcium carbonate medium (YDC) (35) and Miller-Schroth medium (MS) (22); and (iv) pectate degradation with modified crystal violet-pectate medium (CVP) (32). The tests were repeated one or more times to confirm strain identification. Cell measurements of selected bacterial strains were determined using a Leica Laborlux D light microscope on Gram-stained cells from 3-day-old cultures.

Identification of *Enterobacter* species was confirmed using PCR amplification from total DNA using the Y1 and Y2 primers that are complementary to highly conserved sequences within the 16S rDNA of the alpha proteobacteria (39). Following purification and sequencing, a NCBI BLASTN search (1) was conducted on an approximately 300-bp sequence. The type strain for *E. cloacae*, ATCC 13047, was included as a positive control.

Pathogenicity studies on bacterial strains. Pathogenicity studies were conducted using strains B193-3 and KN1-19 isolated from rotted ginger rhizomes from the island of Hawaii, and *E. cloacae* strain Dd-18 isolated from the oriental fruit fly *Bactrocera dorsalis* (Hendel) and characterized in earlier studies (15,27).

All inoculum for this study was prepared from cultures grown on PT-M4 agar medium at 30°C for 3 to 5 days. A bacterial suspension for each strain was prepared by scraping cells from individual cultures into 15 to 20 ml of SDW and adjusting to optical density (OD) approximately 0.5 A_{600} using a Turner SP-830 spectrophotometer, which was equivalent to 10^8 to 10^9 CFU/ml. SDW was used as a control.

Inoculation tests were performed on: (i) aseptic tissue-cultured ginger plantlets in test tubes; (ii) ginger rhizome slices in petri dishes; (iii) ginger rhizome segments stored under low oxygen (vacuum-sealed bags) or aerobic (without vacuum-sealed bags) conditions; (iv) young ginger plants grown in pots in the greenhouse; (v) papaya fruit; and (vi) yellow onion bulbs.

(i) Inoculation of ginger plantlets in test tubes. Tissue-cultured ginger plantlets, grown in test tubes (25 mm diameter $\times$ 150 mm long) on Murashige and Skoog agar medium (24), were obtained from the Pacific Basin Tropical Plant Genetic Resource Management Unit (USDA-ARS-PBARC, Hilo, HI). Each test tube contained a single plantlet (approximately 7 to 10 cm high) that was inoculated either with 0.5 ml of bacterial suspension (described earlier) or 0.5 ml of SDW (control). To determine the effect of wounding on disease development, half of the plantlets for each bacterial or control treatment were wounded by nicking the root area with a sterile scalpel; the other half of the plantlets were not wounded. Four plantlets for each bacterial or control treatment were placed randomly in test tube racks and incubated at 30°C in a continuously illuminated incubator (fluorescent cool, daylight, 15W, VWR 2020). Four days after inoculation, and every 2 to 4 days thereafter for approximately 14 days, individual plantlets were evaluated for foliar and rhizome/root symptoms (chlorosis or necrosis, and discoloration or rot, respectively) using a disease severity rating scale of 1 to 5 where 1 = no symptoms, 2 = slight (up to 25%) foliar and/or rhizome/root symptoms, 3 = moderate (up to 50%) foliar and/or rhizome/root symptoms, 4 = severe (up to 75%) foliar and/or rhizome/root symptoms, and 5 = dead plantlet. The experimental design was a randomized complete block, split plot with replicates consisting of two to four plantlets and where the main plot was inoculation method and the subplot was bacterial treatments. The experiment was conducted four times, twice with both wounded and nonwounded plantlets, and twice with only nonwounded plantlets. Disease severity ratings were performed on three experiments (experiments 2, 3, and 4).

(ii) Ginger rhizome slice inoculations. Ginger rhizomes ('Chinese' type, a commercial variety characterized by light yellow flesh and a mildly pungent flavor) of two maturities were used: (i) immature ginger rhizomes obtained from 5- to 7-month-old plants initiated from tissue culture, transplanted into Sunshine mix (90% peat moss) and volcanic cinder media (1:1), and grown in a greenhouse under ambient conditions, and (ii) mature, field-grown rhizomes purchased from a local produce packinghouse. The rhizomes were maintained in aerated fiberboard cartons at room temperature (22°C) until use.

The rhizomes were washed in tap water, air-dried, and then cross-sectioned into approximately 3-mm-thick slices (cross-section lengths of at least 2 cm) with a flame-sterilized knife. Each slice was briefly flamed on both surfaces and rehydrated by dipping in SDW. Four slices were placed single-layered onto sterile, SDW-moistened filter paper in a petri dish

(100 mm diameter). Rhizome slices of each maturity group were inoculated with bacterial suspension or with SDW (control).

Two inoculation methods were used in separate experiments. In the first method, sterilized toothpicks coated at the tip with bacterial culture or SDW were stab-inoculated into each ginger slice at the central cylinder or at the outer cortex (endodermal layer). In the second method, ginger slices were puncture-wounded, and 100 μ l of SDW or bacterial suspension at approximately 10^7 , 10^8 , and 10^9 CFU/ml (as determined by viable counts of serial dilutions of the initial suspension at OD = 0.5 A_{600}) was pipette-inoculated into the center of each slice. Four slices of each rhizome maturity group were inoculated at each of the inoculation positions in the first experiment. Four slices of each rhizome maturity group were inoculated with each of the bacterial concentrations or SDW (control) treatments in the second experiment. The inoculated slices in petri dishes were incubated in plastic Ziploc storage bags at 30°C until symptoms were observed, after approximately 12 to 14 days, and then were evaluated. Ginger slices were rated for severity of rot symptoms based on a scale of 1 to 5 where 1 = healthy tissue, 2 = slight (up to 25%) rot (slight discoloration), 3 = moderate (up to 50%) rot (discoloration and tissue breakdown), 4 = severe rot (50 to 75% of slice affected), and 5 = complete rot (up to entire slice affected).

The experimental design was a randomized complete block, split-split plot with replicates consisting of four slices and where the main plot was rhizome maturity, the subplot was inoculation site or bacterial concentration (first or second experiment, respectively), and the sub-subplot was bacterial treatments. The first experiment was conducted three times and the second experiment twice.

(iii) Inoculation of ginger rhizome segments. Immature to recently matured ginger rhizomes ('Chinese' type) were purchased from a farmers' market in Hilo. Rhizomes were washed in tap water, surface disinfected in 0.5% sodium hypochlorite for 3 min, rinsed in distilled water, then drained and air-dried before cutting into segments (at least 9 cm lengths, 82 g mean weight) with a sterilized knife. The cut surfaces were flamed to "seal" the wound tissue. Each rhizome segment was injected with approximately 0.3 ml of bacterial suspension (10^8 to 10^9 CFU/ml) at one to three selected sites, depending on the rhizome size, using a sterile 3 cc tuberculin syringe fitted with a 23-gauge needle. The inoculated sites were then covered with autoclavable tape to prevent cross-contamination. Controls were treated similarly with SDW. At least five sites were inoculated for each of two sets of segments for each bacterial or SDW treatment. One

set of inoculated rhizome segments of the same treatment was vacuum-sealed, one to three segments at a time, in sealing bags using a Mini Vacuum/Professional packaging machine (Minipack-America, same as Minipack-Torre – Italy), creating low oxygen storage conditions. The second set of inoculated rhizomes was stored under aerobic conditions (not in bags). All rhizomes were placed in fiberboard cartons, incubated at 30°C for 6 to 8 days, then cut and evaluated for discoloration and other rot symptoms. The experimental design was a randomized complete block, split plot with replicates consisting of at least five inoculations and where the main plot was storage conditions (low oxygen or aerobic) and the subplot was bacterial treatments. The experiment was conducted three times.

(iv) Inoculation of ginger plants in greenhouse. Tissue culture-initiated ginger plants were grown in 15-cm-diameter plastic pots containing Sunshine potting soil (Sun Gro Horticulture Canada, Ltd. of

Sun Gro Horticulture Inc., Bellevue, WA), vermiculite, and perlite (1:1:1), and a thin surface layer of sterilized coffee husks. Plants were fertilized with 13-13-13 Nutricote fertilizer granules with microelements, 180-day release formulation (Chisso-Asahi Fert. Co., Ltd., Nichimen Corp., Tokyo). The 4-month-old plants with five to seven leaves were approximately 34 cm tall at the time of inoculation. All roots were wounded before inoculation by cutting into the root zone with a clean paring knife at a distance approximately 3 to 5 cm from the base of the plant and to a depth of about 5 cm. About 18 ml of bacterial suspension (10^8 CFU/ml) or SDW was poured into the trough formed by the wounding procedure. Four plants were inoculated for each bacterial strain or SDW treatment.

Inoculated plants were maintained in a greenhouse at the University of Hawaii-Hilo campus farm located approximately 6 mi south of Hilo, under a 30% shade screen, at ambient temperature (22 to

41°C) and relative humidity (34 to 100%). Plants were watered daily by an automated irrigation system, and Nutricote fertilizer (17 g) was applied as needed. Treatment replicates consisted of four plants that were arranged on a greenhouse bench in a completely randomized block design, and foliar symptoms were rated weekly using the 1 to 5 disease rating scale. The experiment was conducted three times.

(v) Papaya fruit inoculations. One-half- to three-quarter-ripe papaya fruit (*Carica papaya* L.) of three cultivars ('Kapoho Solo', 'Rainbow', and 'Sun Up') were obtained from commercial sources on the island of Hawaii. 'Rainbow' is the F1 hybrid resulting from a cross between 'Kapoho Solo' and the transgenic cultivar 'Sun Up'. Fruits were washed in tap water, air-dried, and surface disinfected by wiping with 70% ethanol. The fruits were inoculated at randomized sites along the longitudinal transect. Each site was injected with 0.5 ml of a bacterial suspension (10^8 to 10^9 CFU/ml) or SDW, using a sterile 3 cc tuberculin syringe fitted with a 23-gauge needle, then covered with autoclavable tape. Sixteen to 23 'Kapoho Solo', 20 'Rainbow', or 20 'Sun Up' fruits were inoculated each time in repeated, separate experiments for each cultivar. Fruit were incubated in cartons at 23 to 28°C until ripe (4 to 5 days), then evaluated for internal yellowing symptoms (yellow discolored flesh and rotting odor) (27). The experimental design for 'Kapoho Solo' and 'Rainbow' fruit was a randomized complete block design (two experiments) or a randomized incomplete block design (one experiment) where each fruit was inoculated with all bacterial treatments (complete block) or with at least two of the three *E. cloacae* strains (incomplete block). Strains B193-3 and Dd-18 were utilized in all three experiments, and data were pooled for analysis of each cultivar. The experimental design for 'Sun Up' fruit was a randomized complete block design where each fruit was inoculated with all bacterial treatments. The experiment was conducted two times. Replicates consisted of the number of fruit inoculated in each experiment for each cultivar, as described.

(vi) Onion bulb inoculations. Commercial, grade A, mature yellow onion (*Allium cepa* L.) bulbs (approximately 64 mm diameter) were obtained from a local supermarket. After removing the outer protective scale leaf and two layers of fleshy scale leaf, the bulbs were washed in tap water, air-dried, and then inoculated. Each onion bulb was injected at the stem-end with 0.5 ml of bacterial suspension (10^9 CFU/ml) or SDW, using a sterile 10 cc syringe fitted with a 22-gauge needle. Four to five bulbs were injected for each treatment, individually placed in plastic storage bags (Ziploc), and incubated at 30°C in a Fisher Isotemp Low-Temperature incubator (Fisher Scientific,

Table 1. Biochemical and physiological characteristics of *Enterobacter asburiae*, *E. cloacae* strain from oriental fruit fly (Dd-18), and strains B193-3 and KN1-19 from ginger rhizome

Characteristic	<i>E. asburiae</i> ^r	<i>E. cloacae</i> ^s	Strain Dd-18 <i>E. cloacae</i> ^t	Strain B193-3	Strain KN1-19
API 20E tests					
β-Galactosidase	+	+	+	+	+
Arginine dihydrolase	V ^u	+	V	+	+
Lysine decarboxylase	— ^u	—	—	—	—
Ornithine decarboxylase	+	+	+	+	+
Citrate utilization	+	+	+	+	+
Hydrogen sulfide	—	—	—	—	—
Urease	+	—	—	—	—
Tryptophan deaminase	—	—	—	—	—
Indole	—	—	—	—	—
Voges-Proskauer	—	+	+	+	+
Gelatin liquefaction	—	+	—	—	—
Acid from					
Glucose	+	+	+	+	+
Mannitol	+	+	+	+	+
Inositol	—	—	—	—	—
Sorbitol	+	+	+	+	+
Rhamnose	—	+	+	+	+
Sucrose	+	+	+	+	+
Melibiose	—	+	+	+	+
Amygdalin	+	+	+	+	+
Arabinose	+	+	+	+	+
Oxidase	—	—	—	—	—
Nitrate reduction	+	+	+	+	+
Catalase ^v	+	+	+	W ^u	+
Other tests					
Anaerobic growth ^w	+	+	+	+	+
Phenylalanine deaminase ^x	—	—	—	—	—
Pectate degradation ^y	—	—	—	—	—
Acid from ^z					
Cellobiose	+	+	+	+	+
Lactose	+	+	+	+	+
α-Methyl-D-glucoside	+	+	+	+	+

^r Data from Brenner et al., 1986 (6).

^s Data from Richard, 1984 (28).

^t Same strain characterized in a previous study (27).

^u + = positive reactions; — = negative reactions; W = weak reactions; V = variable results.

^v Results by using hydrogen peroxide, USP 3%.

^w Difco OF basal medium with 1% glucose (final concentration), inoculated and layered with sterile mineral oil.

^x Method of Ewing et al., 1957 (9).

^y Method using modified crystal violet-pectate (CVP) medium (32).

^z Difco OF basal medium with 1% carbohydrate source (final concentration).

Pittsburgh, PA). After 11 to 18 days, the bulbs were cut transversely through the center and evaluated for incidence and severity of rot symptoms using a 1 to 5 rating scale where 1 = healthy, 2 = slight (up to 25%) rot (slight discolored or flaccid tissue), 3 = moderate (up to 50%) rot (internal tissue generally discolored and flaccid), 4 = severe rot (50 to 75% of bulb affected), and 5 = complete rot (up to entire bulb affected) (4,33). The experimental design was a randomized complete block with replicates consisting of four to five bulbs. The experiment was conducted two times.

Reisolation of inoculated bacterial strains. Reisolation and identification using API 20E strips of the two ginger strains and the fruit fly strain from symptomatic tissue of inoculated plants were performed to fulfill Koch's postulates and conducted according to methods described earlier, with some modifications. Tissue-cultured ginger plantlets were rinsed in distilled water to remove agar from roots, and excised tissue pieces from all reiso-

lated plants (ginger, papaya, onion) were disinfected for 2 min in 0.5% sodium hypochlorite and drained on clean Kimwipes tissue. The excised sections were placed in tubes of SDW, macerated aseptically with a sterile glass rod, and allowed to soak 1 h before streaking the liquid suspension on PT-M2 agar medium. Isolations from selected plants inoculated with SDW were similarly performed.

Data analysis. Data were analyzed for main effects, or for main and interactive effects (factorial analysis), by the general linear models (GLM) procedure of SAS, version 8.0 (SAS Institute, Inc., Cary, NC). Analyses were performed on nontransformed data except for arcsine-square root transformations that were performed before analysis on proportion of infected onion bulbs according to protocols described in Snedecor & Cochran (34). Means separation (where appropriate) were performed by pairwise comparisons of means by the *t* test of the least squares means (LSMeans) option of GLM, or by Fisher's protected LSD test, at $P = 0.05$,

using version 8.0 of SAS statistical software.

RESULTS

Bacterial strains: isolation and symptoms. Rhizomes with rot symptoms attributed to *E. cloacae* or *R. solanacearum* were not easily distinguishable, and both species were repeatedly isolated from ginger plants with foliar symptoms (14,37) of bacterial wilt disease. PT-M2 medium facilitated the isolation and separation of both species by reducing EPS production by *R. solanacearum*.

The presence and activity of *E. cloacae* in ginger rhizomes was associated with a range of symptoms from mild water-soaking, to dark yellow, or tan-brown discoloration in the central cylinder of rhizomes that appeared externally healthy. Severe symptoms included dark brown discoloration of the central cylinder, collapsed tissue, and a foul or putrid odor occasionally emitted from rhizomes that were rotted or spongy in texture and possibly produced in association with other bacteria.

Fig. 1. Symptoms produced by *Enterobacter cloacae* on ginger plantlets, rhizome slices and whole rhizome segments. **A and B,** Tissue-cultured ginger plantlets in test tubes at 30°C, 14 days after inoculation. **A,** Healthy plantlets that were inoculated with sterile distilled water (SDW). **B,** Foliar symptoms (dark yellow chlorosis, 'burning' necrosis) and basal root and stem rot on plantlets inoculated with B193-3. **C and D,** Mature ginger rhizome slices incubated in petri dishes after 12 to 14 days at 30°C. **C,** Healthy ginger slices that were inoculated with SDW. **D,** Mild rot symptoms (discoloration, water-soaking) on ginger slices inoculated with B193-3. **E to G,** Whole ginger rhizome segments after 6 to 8 days at 30°C in vacuum-sealed bags (**E, F**) or not in bags (**G**). **E,** Spongy and collapsed tissue of rhizome segment inoculated with KN1-19. **F,** Healthy tissue of whole ginger rhizome segment treated with SDW (bottom row, far left), and discolored or rotted tissue of rhizome segments inoculated with B193-3 (bottom row, center and right), KN1-19 (top row, left), and Dd-18 (top row, right). **G,** Healthy tissue of whole rhizome segment inoculated with SDW (center row, left) and discolored tissue of rhizome segments inoculated with B193-3 (top row, left and right), KN1-19 (bottom row, right), and Dd-18 (bottom row, left).

Foliar symptoms associated with *E. cloacae* based on laboratory and field observations were characterized by distinct, dark yellow chlorosis with amber-colored necrosis at margins or tips, and dieback symptoms. Wilt symptoms in the field occurred in association with *R. solanacearum*.

Rhizomes collected from farms and supermarket. Fourteen facultatively anaerobic strains with colony characteristics resembling *Enterobacter* sp. were isolated from 80 wilted ginger plants collected from two farms on the Hamakua coast. Eight similar strains were isolated from 20 symptomless ginger rhizomes purchased from a Hilo supermarket. The strains were presumptively identified to the genus *Enterobacter* sp. Four representative strains were identified as *E. cloacae* using API 20E strips (bioMerieux) after 18 to 24 h incubation at 30°C.

Characterization of strains. Biochemical and physiological characteristics of two representative strains from diseased ginger rhizomes (B193-3 and KN1-19) were similar to those of the fruit fly strain (Dd-18) (Table 1). The strains were a 95.2% identification match (based on biochemical profile) for *E. cloacae* on API 20E strips. All three strains were gram negative, catalase-positive, oxidase-negative, facultatively anaerobic, negative for pectate degradation and phenylalanine deaminase, and matched the biochemical and physiological characters described for *E. cloacae* (28), except for a negative reaction for liquefaction of gelatin (Table 1). The three strains differed from *E. asburiae* in tests for urease, Voges-Proskauer reaction, and acid production from rhamnose and melibiose (Table 1). The referenced characterization of *E. asburiae* (6) was positive for urease and negative for the other biochemical tests. *E. asburiae* and fruit fly strain Dd-18 were variable for the arginine dihydrolase reaction.

All three strains produced creamy-tan and mucoid colonies on YDC, orange colonies on MS, and colonies with pink to dark-pink centers and clear to translucent margins on PT-M2 and PT-M4. Two colony forms were observed on Kelman's medium with reduced TTC. Colonies of strains KN1-19 and Dd-18 were identical and had pink to dark-pink centers that sharply transitioned to a translucent margin (nonmucoid colony form), while B193-3 had colonies with similarly colored centers that diffused gradually toward a mucoid margin (mucoid colony form).

Bacterial cells of the three strains were single, straight rods that measured (mean $\pm$ standard error of the mean) as follows: 0.34 (± 0.012) $\mu\text{m} \times 1.52$ (± 0.041) μm for B193-3; 0.31 (± 0.006) $\mu\text{m} \times 1.37$ (± 0.029) μm for KN1-19; and 0.30 (± 0.010) $\mu\text{m} \times 1.35$ (± 0.033) μm for Dd-18.

The two ginger strains, B193-3 and KN1-19, fruit fly strain Dd-18, and type strain ATCC 13047 were all confirmed as *E. cloacae* with at least 99.7% similarity using 16S rDNA sequence analysis of an approximately 300-nucleotide sequence and a BLASTN search (BLASTN 2.2.6) (1). (GenBank accession number for B193-3 is AY780489.)

Pathogenicity tests on tissue-cultured ginger plantlets in test tubes. Ginger strains B193-3 and KN1-19 inoculated on tissue-cultured ginger plantlets in test tubes produced characteristic burning foliar symptoms consisting of chlorosis, tip burn, and marginal necrosis. Basal root or basal stem tissue developed dark-brown to black discoloration and rot (Fig. 1B; Table 2). Analysis of variance indicated no significant difference ($P > 0.05$) between plantlets that were wounded or not wounded and slight or no inoculation $\times$ bacterial treatment interaction (Table 3). Data were subsequently pooled for incidence of disease, disease severity rating,

and incidence of dead plants. Results for the plantlets inoculated with ginger strains of *E. cloacae* were significantly different ($P < 0.05$) from SDW control plantlets. When inoculated with B193-3 or KN1-19 strains, 96 to 100% of the plantlets developed foliar and/or root symptoms with a mean disease severity rating of 4.1 to 4.3, and 38 to 63% of the plantlets were dead after 14 days (Table 3). Tissue-cultured plantlets inoculated with fruit fly strain Dd-18 did not result in any plantlet deaths, although 33% of the plantlets developed foliar and/or root symptoms with a mean disease severity rating of 1.44 that was not significantly different ($P > 0.05$) from the SDW control. The SDW controls developed slight foliar tip chlorosis (mean severity rating of 1.31) that may have been caused by physiological stress (Fig. 1A; Table 3).

Pathogenicity tests on ginger rhizome slices. Toothpick inoculations with bacterial cells of fruit fly strain Dd-18 did not produce any disease symptoms on either mature or young ginger slices (*data not shown*). Mild symptoms (Fig. 1D; Table 2) were observed on mature, but not on young, ginger slices inoculated with ginger strains B193-3 and KN1-19 (13 to 19% incidence). Infection incidences at the central cylinder or endodermal layer inoculation sites were not statistically different ($P > 0.05$).

In a separate experiment in which wounded ginger slices from young or mature rhizomes were inoculated at the central cylinder with liquid inoculum (10^6 , 10^7 , or 10^8 CFU per site), all concentrations were equally effective in producing rot symptoms but were not statistically different from SDW controls (Table 4). There were no significant differences ($P > 0.05$) in incidences or severity ratings of rot symptoms among the three bacterial strains. The only main effect with means that were significantly different ($P < 0.001$) was rhizome age. Incidences of infection and disease severity ratings were significantly higher in mature ginger slices than in young ginger slices (Table 4). Analysis of variance indicated no interaction among the main effects (bacterial concentration, rhizome age, and bacterial treatment).

Pathogenicity tests on ginger rhizome segments under low oxygen or aerobic conditions. Despite severe rotting symptoms and abundant gas (CO_2) that were produced under low oxygen storage conditions (Fig. 1E and F; Table 2), analysis of variance indicated no significant difference ($P = 0.2772$) in incidences of rot between inoculated ginger rhizome segments that were stored in vacuum-sealed bags or stored without bags (Fig. 1G; Table 5). There was no storage $\times$ bacterial treatment interaction. All three bacterial strains had significantly greater incidences ($P < 0.05$) of rot than the SDW control in the inocu-

Table 2. Description of symptoms resulting from artificial inoculation of various hosts with *Enterobacter cloacae* strains B193-3 and KN1-19 (ginger) and Dd-18 (fruit fly)

Host	Symptoms
Ginger	
Tissue-cultured plantlets in test tubes, 30°C	Rhizome: Brown discoloration; rot (ginger strains) Foliage: Dark yellow chlorosis; amber ("burning") necrosis (ginger strains); Weak or no symptoms (fruit fly strain)
Tissue-cultured plants in 15 cm pots, greenhouse	No rhizome or foliar symptoms (all strains)
Rhizome slices in petri dishes, 30°C	Water-soaked, tan-yellow central cylinder; spongy to collapsed tissue (ginger strains)
Rhizome segments in vacuum-sealed bags, 30°C	Water-soaked, tan-brown to gray central cylinder, or completely dark to "fluorescent" yellow; spongy to collapsed tissue; gas production; foul odor (all strains)
not in bags, 30°C	Less severe symptoms; no gas; no foul odor (all strains)
Papaya fruit	
'Kapoho Solo', 23 to 28°C	Internal yellowing (bright to "fluorescent" yellow; rot; foul odor) (all strains)
Onion bulb	
Yellow onion, 30°C	Internal rot (tan to dark brown; flaccid internal scales) (all strains)

lated rhizome segments that were combined for storage treatment (Table 5). Rhizome segments inoculated with SDW and stored under low oxygen conditions developed discoloration or abnormal texture due to unknown causes, while the SDW controls stored under aerobic conditions did not develop symptoms (*data not shown*). *E. cloacae* was not isolated from the SDW controls that were stored under either low oxygen or aerobic conditions (three and four isolation attempts, respectively).

Pathogenicity tests on potted ginger plants in a greenhouse. No disease symptoms were produced on greenhouse-grown ginger plants inoculated at the root zone with bacteria or SDW (*data not shown*).

Pathogenicity tests on other hosts: papaya fruit. The three cultivars of papaya exhibited different degrees of susceptibility when inoculated with B193-3, KN1-19, and Dd-18. All three bacterial strains produced internal yellowing symptoms (Table 2) in 75 to 80% of 'Kapoho Solo' fruit (Fig. 2A, second, third, and fourth from left) and in 17 to 25% of 'Rainbow' fruit; no symptoms were produced in 'Sun Up' fruit (Table 6). None of the SDW controls produced disease symptoms (Fig. 2A, far left).

Pathogenicity tests on onion bulb. Internal rot symptoms were produced in 33 to 100% of the onion bulbs inoculated with B193-3, KN1-19, or Dd-18 (Fig. 2B, middle and right; Table 7). There were no rot symptoms in the SDW-inoculated controls (Fig. 2B, left). Overall, the rot symptoms in onion bulbs were mild, with the exception of KN1-19, which produced moderate

symptoms (mean severity rating, 2.9) and incidence of rot (100%) that were significantly higher ($P < 0.05$) than the other strains and/or the SDW control (Table 7).

Reisolation of bacterial strains from inoculated plant hosts. Strains B193-3, KN1-19, and Dd-18 were reisolated and identified as *E. cloacae* on API 20E strips at least once and often several times from plant hosts that developed disease symptoms after inoculation with the respective bacterial strains, thereby fulfilling Koch's postulates and demonstrating pathogenicity. *E. cloacae* was not isolated from asymptomatic or symptomatic SDW controls. Rhizome quality of ginger slices and segments with symptomatic SDW controls was likely affected by other naturally occurring microorganisms (37).

DISCUSSION

Strains B193-3 and KN1-19 that were isolated from decayed ginger rhizomes were identified as *E. cloacae* (Jordan) Hormaeche & Edwards based on bacteriological tests and molecular analysis. This is the first report of *E. cloacae* associated with a ginger rot, although the bacterium is associated with other plant diseases (4,7,8,10,25–27,29,33,36,38).

The mucoid colony form of B193-3 differed from the nonmucoid colony form of KN1-19 and Dd-18, especially on media containing sugar carbohydrates (e.g., glucose in Kelman's medium). The importance of mucoid forms of *E. cloacae* is unknown, although EPS production may provide the bacterium with some protection in harsh environments (21), or may be

a factor in causing wilt symptoms in other hosts (10). In our studies, there was no statistical difference in incidence or severity of symptoms on ginger between the mucoid and nonmucoid ginger strains (B193-3 and KN1-19, respectively), although on onion, symptoms were slightly more severe with KN1-19 than with B193-3.

Ginger strains B193-3 and KN1-19 produced severe rhizome and foliar symptoms on inoculated ginger plantlets in test tubes. The foliar "burning" and basal stem or rhizome rot symptoms of ginger plantlets were similar to symptoms of coconut root (wilt) disease, which is characterized by yellowing and marginal necrosis of leaves and a "defective root system" (10). *E. cloacae* was isolated from diseased coconut roots; however, its pathogenicity on coconut plants was not confirmed, although an aqueous extract of a polysaccharide substance of the bacterium produced wilt symptoms on tomato seedlings (10).

The in vitro pathogenicity tests with tissue-cultured ginger plantlets afforded a convenient bioassay using pathogen-free plants, and although not the same as using plants grown under soil conditions, the tests showed differences among the ginger and fruit fly strains. The symptoms exhibited by the ginger plantlets when inoculated with the ginger strains were severe compared with the significantly fewer and milder disease symptoms when inoculated with the fruit fly strain.

Strains B193-3, KN1-19, and Dd-18 differed in pathogenic responses to ginger and onion, but there were no strain differences on three papaya cultivars. The vary-

Table 3. Effect of inoculation^a of wounded or nonwounded tissue-cultured ginger plantlets in test tubes with *Enterobacter cloacae* strains from ginger (B193-3, KN1-19) or fruit fly (Dd-18), or sterile distilled water (SDW) after about 14 days at 30°C

Effect	% disease incidence ^y		Disease severity rating ^{y,w}		% dead plants ^{y,x}	
	Rep.	Mean	Rep.	Mean	Rep.	Mean
Inoculation method						
Wounding	8	62.5	4	2.69	8	18.8
Nonwounding	16	54.7	12	2.83	16	28.1
Bacterial treatment						
<i>Enterobacter cloacae</i> B193-3	6	95.8 a ^y	4	4.34 a	6	62.5 a
<i>E. cloacae</i> KN1-19	6	100.0 a	4	4.09 a	6	37.5 a
<i>E. cloacae</i> Dd-18	6	33.3 b	4	1.44 b	6	0.0 b
SDW control	6	0.0 c	4	1.31 b	6	0.0 b
Analysis of variance						
Source	df	F value	df	F value	df	F value
Inoculation method (I)	1	0.00	1	2.81	1	0.50
Bacterial treatment (T)	3	17.33*** ^z	3	104.11***	3	12.94***
Experiment	3	1.64	2	1.43	3	0.67
I × T	3	0.26	3	2.26	3	4.50*

^a Two to four plantlets were inoculated with 0.5 ml of bacterial suspension (10^9 CFU/ml) or SDW, then were incubated in a continuously illuminated (fluorescent cool, daylight, 15W) incubator.

^y Data were analyzed by the least squares means option of the generalized linear models procedure at $P = 0.05$. The experiment was conducted four times, twice with both wounded and nonwounded plantlets, and twice with only nonwounded plantlets. Replicates consisted of two to four plantlets. Results are presented as means instead of LSMeans.

^w Data represent disease severity rating based on a scale where 1 = healthy (no symptoms), 2 = slight (up to 25%) foliar and/or rhizome/root symptoms, 3 = moderate (up to 50%) foliar and/or rhizome/root symptoms, 4 = severe (up to 75%) foliar and/or rhizome/root rot symptoms, 5 = dead plantlet. Disease severity ratings were performed on three experiments.

^x Data represent percent dead plantlets (mean disease severity rating of 4.5 to 5 on a scale of 1 = healthy to 5 = dead).

^y Means in columns, by effect category, followed by the same letter are not significantly different according to the *t* test of least squares means of percent disease incidence, disease severity rating, or percent dead plants at $P = 0.05$.

^z *, *** F value for effect significant at $P = 0.05$ or $P = 0.001$, respectively.

ing responses of Dd-18 on ginger (plantlets, slices, or segments) suggest either that Dd-18 is a weak pathogen of ginger or that ginger is a poor host for this strain. Other investigators reported no differences in pathogenicity among *E. cloacae* strains for onion (4) or papaya (27).

The failure to produce disease symptoms on ginger plants grown in pots under greenhouse conditions when inoculated with *E. cloacae* strains was unexpected because of our positive results in *in vitro* tests, and may be due to one or more of the following factors: (i) the young plants were not at a susceptible maturity (i.e., rhizome age was too young); (ii) temperature and relative humidity conditions were not optimal for infection; or (iii) aeration and low soil moisture were not conducive to infection (e.g., stagnant and waterlogged soil conditions may be needed for *E. cloacae* infection to occur). The first and last factors seem the most likely scenarios in the failure to obtain pathogenic responses on inoculated ginger plants in pots.

Mature tissues appear to be more susceptible to *E. cloacae* infection than young tissues. Infection rates in mature ginger

slices were significantly higher ($P < 0.05$) than in young slices in inoculations with the three bacterial strains in the second ginger slice inoculation experiment (i.e., pipette inoculation). Variation in tissue maturity of young rhizomes may have accounted for the absence of symptoms in inoculated ginger slices in the first experiment (i.e., toothpick inoculation) (5-month-old plants) and mild symptoms in the second experiment (7-month-old plants). Host or tissue maturity affecting susceptibility to *E. cloacae* infection was also reported by others. *E. cloacae* did not cause disease in young, growing onions, or in colorbreak and one-fourth-ripe papaya fruit, but it caused internal rot in mature onion bulbs (4) and internal yellowing infection in ripe papaya fruit (26,27).

In our investigations, *E. cloacae* strains were isolated from both healthy and diseased ginger rhizomes. In diseased ginger, *E. cloacae* was isolated along with other bacteria, including *R. solanacearum*. We showed that when inoculated alone, *E. cloacae* produced disease symptoms on ginger, papaya fruit, and onion bulbs, indicating that *E. cloacae* may act independ-

ently under certain conditions (e.g., host susceptibility), but at the same time, may also depend on physiological and biological factors in the host-pathogen (or host-microflora) complex for disease expression, demonstrating the opportunistic nature of this bacterium.

The occurrence of *E. cloacae* as an opportunistic pathogen has been observed in other hosts. *E. cloacae* or *Enterobacter* spp. were isolated alone, or with other bacterial strains from diseased onion bulbs with internal rot (4,8), from healthy and diseased watermelons with watermelon rind necrosis (12), and from bacterial leaf rot of *Odontioda* orchids (36). These studies concluded that *E. cloacae* and other bacteria are part of the natural microflora of both the host and the environment, and may become opportunistic pathogens under certain environmental and/or physiological conditions that favor the bacteria or cause the host to become susceptible to infection. Some of the reported environmental conditions included high temperatures (35 to 37°C) and high humidity. Similar observations, including isolation of several bacterial strains, difficulty in reproducing symptoms, and symptom development requiring moisture, were encountered by Kaneshiro et al. (16) when they studied the relationship between *E. clo-*

Table 4. Effect of bacterial concentration and ginger rhizome age on percent incidence and severity rating of rot symptoms of wounded ginger slices inoculated^v at the center of the central cylinder with strains of *Enterobacter cloacae* (B193-3, KN1-19, Dd-18) or sterile distilled water (SDW) after 12 to 14 days at 30°C

Effect	Rep.	% incidence ^w	Severity rating ^{w,x}
		Mean	Mean
Bacterial concentration			
0 CFU	12	25.00	1.75
10 ⁶ CFU	12	50.00	2.03
10 ⁷ CFU	12	35.42	1.92
10 ⁸ CFU	12	39.58	2.06
Rhizome age			
Mature	24	59.38 a ^y	2.65 a
Young	24	15.63 b	1.23 b
Bacterial treatment			
<i>E. cloacae</i> B193-3	16	48.44	2.12
<i>E. cloacae</i> KN 1-19	16	31.25	1.96
<i>E. cloacae</i> Dd-18	16	32.81	1.74
Experiment			
Test 1	24	46.88 a	2.36 a
Test 2	24	28.13 b	1.52 b
Analysis of variance			
Source	df	F value	F value
Bacterial concentration (C)	3	1.30	0.33
Rhizome age (A)	1	23.32*** ^z	33.23***
Bacterial treatment (T)	2	1.47	0.79
Experiment	1	4.28*	11.75**
C × A	3	0.93	0.53
T × C	6	0.50	0.28
T × A	2	0.36	0.09
T × C × A	6	0.13	0.27

^v Four ginger slices of mature or young rhizome maturity per concentration were inoculated at the center with 100 µl of cell suspension or SDW (0 CFU).

^w Data were analyzed by GLM procedure. The experiment was repeated once. Replicates consisted of four ginger slices. Results are presented as means instead of LSMeans.

^x Data represent severity ratings of rot symptoms of ginger slices based on 1 = healthy tissue, 2 = slight (up to 25%) rot (slight discoloration), 3 = moderate (up to 50%) rot (discoloration and tissue breakdown), 4 = severe rot (50 to 75% of slice affected), and 5 = complete rot (up to entire slice affected).

^y Means (same as LSMeans) in columns, by effect category, followed by the same letter are not significantly different according to the *t* test of least squares means of percent incidence or disease rating at $P = 0.05$.

^z *, **, *** F value for effect significant at $P = 0.05$, $P = 0.01$, or $P = 0.001$, respectively.

Table 5. Effect of storage in vacuum-sealed bags on percent incidence of rot symptoms of ginger rhizome segments inoculated^w with strains of *Enterobacter cloacae* (B193-3, KN1-19, Dd-18) or sterile distilled water (SDW) control after 6 to 8 days at 30°C

Effect	% incidence ^x	
	Rep.	Mean
Storage treatment		
Vacuum-sealed	12	42.29
Not vacuum-sealed	12	34.26
Bacterial treatment		
<i>E. cloacae</i> B193-3	6	53.05 a ^y
<i>E. cloacae</i> KN1-19	6	48.45 a
<i>E. cloacae</i> Dd-18	6	46.83 a
SDW control	6	4.77 b
Analysis of variance		
Source	df	F value
Storage treatment (S)	1	1.28
Bacterial treatment (T)	3	10.02*** ^z
Experiment	2	1.40
S × T	3	1.02

^w Young to recently matured ginger rhizome segments were inoculated by injecting approximately 0.3 ml at one to three sites with the same bacterial strain (cell suspension at 10⁹ CFU/ml) or SDW, then covered with tape.

^x Percent incidence of inoculated sites with rot symptoms were analyzed by generalized linear models procedure. The experiment was conducted three times. Replicates consisted of at least five inoculated sites for each bacterial treatment per vacuum-seal storage treatment.

^y Means (same as LSMeans), by effect category, followed by the same letter are not significantly different according to the *t* test of least squares means of percent incidence at $P = 0.05$.

^z *** F value for effect significant at $P = 0.001$.

*aca*e and gray kernel disease of macadamia nut.

The presence of *E. cloacae* in the internal tissue of apparently healthy ginger rhizomes may impact rhizome quality if storage conditions enhance bacterial growth and/or favor the development of decay. This was demonstrated in our studies with noninoculated and inoculated ginger rhizomes stored in vacuum-sealed bags.

In a preliminary study (not published), mature ginger rhizomes produced from tissue-cultured starter plantlets that were free of *R. solanacearum* were stored under

either low oxygen or aerobic environments at 22 or 4°C. After 63 days of storage, vacuum-sealed bags stored at 22°C were inflated from gas production (85% CO₂, 0% O₂) and ginger rhizomes were flaccid, collapsed, stringy, and foul smelling, whereas the rhizomes stored at 4°C were in good (marketable) condition. *E. cloacae* was isolated from two out of three juice or tissue samples collected from the decayed rhizomes. Our current study corroborated these results by inoculating commercial ginger segments with B193-3, KN1-19, and Dd-18, and reproducing similar rot

symptoms under low oxygen storage conditions (i.e., vacuum-sealed bags) at 30°C for 6 to 8 days. The results illustrate the potential damaging effects of *E. cloacae* on ginger rhizome quality under high temperature and low oxygen storage conditions, and serve as a caution in the use of modified atmosphere packaging that may enhance the growth of microorganisms or favor certain groups of microorganisms when CO₂ levels are elevated (or O₂ levels are reduced) (5).

We conclude that *E. cloacae* is an opportunistic pathogen of edible ginger that

Fig. 2. Internal yellowing of papaya or internal rot of onion caused by *Enterobacter cloacae*. **A**, left to right, ‘Kapoho Solo’ papaya fruit treated with sterile distilled water (SDW) (far left), B193-3 (second from left), KN 1-19 (third from left), and Dd-18 (far right), after 4 to 5 days at 23 to 28°C. **B**, left to right, Yellow onion bulbs inoculated with SDW (left), B193-3 (middle), and Dd-18 (right), after 11 to 18 days at 30°C.

Table 6. Percent incidence of internal yellowing symptoms in papaya fruit (‘Kapoho Solo’, ‘Rainbow’, and ‘Sun Up’) inoculated^w with strains of *Enterobacter cloacae* (B193-3, KN1-19, Dd-18) or sterile distilled water (SDW) and incubated at 23 to 28°C for 3 to 5 days

Effect	% incidence ^x					
	‘Kapoho Solo’		‘Rainbow’		‘Sun-Up’	
	Exp.	Mean	Exp.	Mean	Exp.	Mean
Bacterial treatment						
<i>E. cloacae</i> B193-3	3	76.9 a ^y	3	20.1	2	0
<i>E. cloacae</i> KN1-19	2	80.0 a	2	25.0	2	0
<i>E. cloacae</i> Dd-18	3	75.2 a	3	16.8	2	0
SDW control	3	0.0 b	3	0.0	2	0
Analysis of variance						
Source	df	F value	df	F value	df	F value
Bacterial treatment	3	190.07*** ^z	3	3.39	3	...
Experiment	2	1.90	2	6.09*	1	...

^w Fruit of one-half to three-quarters ripeness stage were inoculated with 0.5 ml of 10⁸ to 10⁹ CFU/ml of bacterial suspension of each strain or SDW at separate sites on the same fruit. Inoculated fruit (16 to 23 per experiment for ‘Kapoho Solo’ and 20 per experiment for ‘Rainbow’ or ‘Sun Up’) were incubated until completely ripe.

^x Data for each cultivar were analyzed by the generalized linear models (GLM) procedure at *P* = 0.05. Experiments were conducted at least twice. Results are presented as means instead of LSMeans.

^y Means in columns followed by the same letter are not significantly different according to the *t* test of the least squares means of percent incidence at *P* = 0.05.

^z *, *** *F* value for effect significant at *P* = 0.05 or *P* = 0.001, respectively.

Table 7. Percent incidence and severity rating of internal rot of yellow onion bulbs inoculated^w with strains of *Enterobacter cloacae* (B193-3, KN1-19, and Dd-18) or sterile distilled water (SDW) after incubation at 30°C for 18 days

Treatment	% incidence ^x		Disease severity rating ^y	
	Exp.	Mean	Mean	
<i>E. cloacae</i>				
B193-3	2	50.0 ab ^z	1.70	b
KN1-19	2	100.0 a	2.85	a
Dd-18	2	32.5 b	1.65	b
SDW control	2	0.0 b	1.00	b

^w Mature onion bulbs were inoculated at the stem-end with approximately 0.5 ml of 10⁹ CFU/ml bacterial suspension or SDW. Inoculated bulbs (four to five per treatment) were stored individually in Ziploc type plastic storage bags.

^x Data were transformed (arcsine-square root) for analysis by GLM procedure then retransformed for presentation (34). The experiment was conducted twice.

^y Severity of rot symptoms was based on a ratings scale where 1 = healthy, 2 = slight (up to 25% discolored or flaccid tissue), 3 = moderate (up to 50% internal tissue discolored and flaccid), 4 = severe (50 to 75% of bulb affected), and 5 = complete (up to entire bulb affected).

^z Means in columns followed by the same letter(s) are not significantly different according to the LSD test at *P* = 0.05.

can cause rot symptoms in rhizomes and enhanced foliar burning symptoms in ginger plants under conditions that favor bacterial growth or host susceptibility. We believe *E. cloacae* can exist as an endophyte in healthy ginger rhizomes, similar to its occurrence in other monocots such as corn (3,11), rice (18), and sugarcane (23). However, in mature ginger, when certain environmental conditions such as high temperature, high relative humidity, and low oxygen atmospheres are present, the bacterium may attain high populations and produce disease symptoms.

Within the limited number of hosts we tested for pathogenicity, ginger appears to be less susceptible to rot than papaya fruit. Interestingly, different papaya cultivars showed varied levels of susceptibility to the three *E. cloacae* strains, ranging from susceptible by ‘Kapoho Solo’ to immune by ‘Sun Up’. ‘Rainbow’, an F1 hybrid between ‘Kapoho’ and ‘Sun Up’, exhibited an intermediate level of resistance compared with the two parental lines. Therefore, genetic factors also may determine host susceptibility to *E. cloacae* infection. This is an area of investigation that deserves further study. The interactions of *E. cloacae* with other bacteria (e.g., *R. solanacearum*) in the microflora of ginger also merit further investigation.

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LITERATURE CITED

- Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J. 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res.* 25:3389-3402.

- Atlas, R. M. 1993. Peptone Yeast Extract Medium. Page 704 in: *Handbook of Microbiological Media*. L. C. Parks, ed. CRC Press, Boca Raton, FL.
- Bacon, C. W., and Hinton, D. M. 1993. Endophytic nature of *Enterobacter cloacae* in roots of corn seedlings. (Abstr.) *Phytopathology* 83:1406.
- Bishop, A. L., and Davis, R. M. 1990. Internal decay of onions caused by *Enterobacter cloacae*. *Plant Dis.* 74:692-694.
- Brackett, R. E., and Splittstoesser, D. F. 2001. Fruits and Vegetables. Chapter 50. Pages 515-520 in: *Compendium of Methods for the Microbiological Examination of Foods*, 4th ed. F. P. Downes and K. Ito, eds. American Public Health Association, Washington, DC.
- Brenner, D. J., McWhorter, A. C., Kai, Akemi, Steigerwalt, A. G., and Farmer, J. J., III. 1986. *Enterobacter asburiae* sp. nov., a new species found in clinical specimens, and reassignment of *Erwinia dissolvens* and *Erwinia nimipressuralis* to the genus *Enterobacter* as *Enterobacter dissolvens* comb. nov. and *Enterobacter nimipressuralis* comb. nov. *J. Clin. Microbiol.* 23:1114-1120.
- Carter, J. C. 1945. Wetwood of elms. III. *Nat. Hist. Surv. Bull.* 23:407-448.
- Cother, E. J., and Dowling, V. 1986. Bacteria associated with internal breakdown of onion bulbs and their possible role in disease expression. *Plant Pathol.* 35:329-336.
- Ewing, W. H., Davis, B. R., and Reavis, R. W. 1957. Phenylalanine and malonate media and their use in enteric bacteriology. *Public Health Lab.* 15:153-167.
- George, M., Potty, V. P., and Jayasankar, N. P. 1976. Association of *Enterobacter* with coconut root (wilt) disease. *Curr. Sci.* 45(18):677-678.
- Hinton, D. M., and Bacon, C. W. 1995. *Enterobacter cloacae* is an endophytic symbiont of corn. *Mycopathologia* 129:117-125.
- Hopkins, D. L., and Elmstrom, G. W. 1977. Etiology of watermelon rind necrosis. *Phytopathology* 67:961-964.
- Hugh, R., and Leifson, E. 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various Gram negative bacteria. *J. Bacteriol.* 66:24-26.
- Ishii, M., and Aragaki, M. 1963. Ginger wilt caused by *Pseudomonas solanacearum* E. F. Smith. *Plant Dis. Rep.* 47:710-713.
- Jang, E. B., and Nishijima, K. A. 1990. Identification and attractancy of bacteria associated with *Dacus dorsalis* (Diptera: Tephritidae). *Environ. Entomol.* 19(6):1726-1731.
- Kaneshiro, W. S., Cavaletto, C. G., Tang, C. S.,

and Alvarez, A. M. 2003. Gray kernel disease of macadamia nut: Are bacteria involved? Online publication. *Plant Health Progress* doi:10.1094/PHP-2003-0825-01-HN.

- Kelman, A. 1954. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. *Phytopathology* 44:693-695.
- Ladha, J. K., Barraquio, W. L., and Watanabe, I. 1983. Isolation and identification of nitrogen-fixing *Enterobacter cloacae* and *Klebsiella planticola* associated with rice plants. *Can. J. Microbiol.* 29:1301-1308.
- Marchini, D., Rosetto, M., Dallai, R., and Marri, L. 2002. Bacteria associated with the oesophageal bulb of the medfly *Ceratitis capitata* (Diptera:Tephritidae). *Curr. Microbiol.* 44:120-124.
- Martin, D. A. 2002. Hawaii ginger root. *Hawaii Agric. Stat. Serv.* August 8, 2002. Hawaii Dept. Agriculture, Honolulu, HI.
- Mead, M. J., Nakas, J. P., and Tanenbaum S. W. 1993. Highly viscous polysaccharide produced by an *Enterobacter* isolate on a hemicellulose hydrolysate. *Biotechnol. Lett.* 15(4):389-392.
- 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.
- Mirza, M. S., Ahmad, W., Latif, F., Haurat, J., Bally, R., Normand, P., and Malik, K. A. 2001. Isolation, partial characterization, and the effect of plant growth-promoting bacteria (PGPB) on micro-propagated sugarcane *in vitro*. *Plant Soil* 237(1):47-54.
- Murashige, T., and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco culture. *Physiol. Plant* 15:473-497.
- Murdoch, C. W., and Campana, R. J. 1983. Bacteria species associated with wetwood of elm. *Phytopathology* 73:1270-1273.
- Nishijima, K. A. 1994. Papaya Diseases Caused by Bacteria. Internal Yellowing. Page 65 in: *Compendium of Tropical Fruit Diseases*. R. C. Ploetz, G. A. Zentmyer, W. T. Nishijima, K. G. Rohrbach, and H. D. Ohr, eds. American Phytopathological Society, St. Paul, MN.
- Nishijima, K. A., Couey, H. M., and Alvarez, A. M. 1987. Internal yellowing, a bacterial disease of papaya fruits caused by *Enterobacter cloacae*. *Plant Dis.* 71:1029-1034.
- Richard, C. 1984. Genus VI. *Enterobacter* Hormaeche and Edwards 1960, 72; Nom. Cons. Opin. 28, Jud. Comm. 1963, 38. Pages 465-469 in: *Bergey's Manual of Systemic Bacteriology*. Vol. 1. N. R. Krieg and J. G. Holt, eds. Williams & Wilkins, Baltimore, MD.
- Rosen, H. R. 1922. The bacterial pathogen of corn stalk rot. *Phytopathology* 12:497-499.
- Rossiter, M. C., Howard, D. J., and Bush, G. L. 1983. Symbiotic bacteria of *Rhagoletis pomonella*. Pages 77-84 in: *Fruit Flies of Economic Importance*. R. Cavalloro, ed. A. A. Balkema, Rotterdam.
- Sanders, W. E., and Sanders, C. C. 1997. *Enterobacter* spp.: Pathogens poised to flourish at the turn of the century. *Clin. Microbiol. Rev.* 10:220-241.
- Schaad, N. W. 1988. I. Initial Identification of Common Genera. Pages 1-15 in: *Laboratory Guide for the Identification of Plant Pathogenic Bacteria*, 2nd ed. N. W. Schaad, ed. American Phytopathological Society, St. Paul, MN.
- Schwartz, H. F., and Otto, K. 2000. First report of a bulb decay of onion by *Enterobacter cloacae* in Colorado. *Plant Dis.* 84:808.
- Snedecor, G. W., and Cochran, W. G. 1989. *Statistical Methods*, 8th ed. Iowa State University, Ames.
- Sutton, D. D., Ark, P. A., and Starr, M. P. 1960. The causal agent of bacterial brown rot of Cy-

- pripedium orchids. *Phytopathology* 50:182-186.
36. Takahashi, Y., Takahashi, K., Sato, M., Watanabe, K., and Kawano, T. 1997. Bacterial leaf rot of *Odontioda* orchids caused by *Enterobacter cloacae*. *Ann. Phytopathol. Soc. Jpn.* 63:164-169.
 37. Trujillo, E. E. 1964. Diseases of Ginger (*Zingiber officinale*) in Hawaii. Circ. 62. Hawaii Agricultural Experiment Station, University of Hawaii at Manoa.
 38. Wick, R. L., Rane, K. K., and Sutton, D. K. 1987. Mung bean sprout disease caused by *Enterobacter cloacae*. (Abstr.) *Phytopathology* 77:123.
 39. Young, J. P. W., Downer, H. L., and Eardly, B. D. 1991. Phylogeny of the phototrophic *Rhizobium* strain BTAi1 by polymerase chain reaction-based sequencing of a 16S rRNA gene segment. *J. Bacteriol.* 173:2271-2277.