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Taxonomy and Emended Description of Strains of *Erwinia* Isolated from *Musa paradisiaca* Linnaeus

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The phytopathogenic bacterium which causes soft rot of the pseudostem of *Musa paradisiaca* Linnaeus has been designated previously as *Erwinia carotovora* subsp. *paradisiaca*, *E. paradisiaca*, *E. carotovora* subsp. *chrysanthemi*, and *E. chrysanthemi*. Thirty strains were examined and compared with strains of closely related *Erwinia* species and their subspecies. The strains from *M. paradisiaca* were found to be phenotypically similar to *E. chrysanthemi* strains on the basis of the following characteristics: pectate degradation; production of phosphatase, indole, acetoin, and acid from ethanol; growth at 36 to 37°C; susceptibility to erythromycin; gas from glucose; utilization of malonate; no growth in 5% NaCl; and no production of acid from α-methyl-*d*-glucoside, trehalose, maltose, lactose, or palatinose. They could be distinguished from 322 strains of *E. chrysanthemi* isolated from 22 other plant hosts by the following phenotypic properties: production of acid from D-arabinose and raffinose; utilization of sodium tartrate; no production of lecithinase or of acid from inulin, mannitol, or sorbitol; and inability to liquefy gelatin.

Soft rot of the pseudostem of plantain (*Musa paradisiaca* Linnaeus) was initially observed in Puerto Tejada, Colombia, a small town near Cali (13). The disease later spread into the Cauca Valley and eventually into other areas of Colombia. The pathogen may cause an 80 to 90% reduction of production within 5 years after initial infection in a plantation. Thus far, the pathogen and disease have been identified and reported only in Colombia.

Llanos (13) used pathogenicity tests to demonstrate a bacterium as the causal agent. After completion of some physiological tests, Fernández-Borrero tentatively concluded that the bacterium should be included in the genus *Erwinia* (9). Victoria and Barros (22) studied the pathogen more thoroughly and reported that the bacterium was similar to *E. carotovora*, but they also noted that there were some striking differences. They proposed that the pathogen be designated *E. carotovora* subsp. *paradisiaca*. Fernández-Borrero and López-Duque (10) verified the differences between the plantain strains and *E. carotovora* and elevated the pathogen to specific rank as *E. paradisiaca*. Barzic et al. (3) included a strain from *M. paradisiaca*, supplied by Dickey, in a comparative study with other erwiniae and placed the strain in a group designated *E. carotovora* subsp. *chrysanthemi*. Samson and Nassan-Agha (17) have identified three plantain strains (from Dickey) as *E. chrysanthemi*.

Several of our original strains of the plantain pathogen and several from culture collections were examined, and the phenotypic characters were determined by the procedures used for the separation of *Erwinia* species in *Bergey's Manual of Determinative Bacteriology* (12). Strains of the "*E. carotovora* group" were included in the comparative study reported here.

MATERIALS AND METHODS

Bacterial strains. The 30 strains of the pathogen isolated from soft-rotted pseudostems of *M. paradisiaca* collected in Colombia are listed in Table 1. The strains were compared with 322 strains of *E. chrysanthemi*, 77 strains of *E. carotovora* subsp. *carotovora*, 16 strains of *E. carotovora* subsp. *atroseptica*, 2 strains of *E. cypripedii*, and 1 strain of *E. rhapontici*; the details concerning these strains are listed elsewhere (7). All strains were maintained on Difco nutrient agar at 4°C.

Morphological and cultural properties. The Hucker modification of Gram stain (19) was used to observe cell morphology, and flagella were observed by the method of Blenden and Goldberg (4). Cultural characteristics and pigment production were determined on nutrient agar and yeast extract-dextrose-$CaCO_3$ agar (2).

Physiological and biochemical properties. The methods used for determining the physiological and biochemical characters of the strains have been described previously (7).

Base composition of DNA. The deoxyribonucleic acid (DNA) of selected strains was extracted and

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TABLE 1. *Strains of Erwinia isolated from M. paradisiaca in Colombia, South America, and included in this study*

Laboratory no.	Strain	Source[a]	Location
221	141	1	La Cumbre, Palmira
228	322	1	La Cumbre, Palmira
235	A-110	2	Palmira, Valle
236	B-131	2	Palmira, Valle
324	C-15	2	Collection of Plantain Varieties, Palmira
325	C-16	2	Collection of Plantain Varieties, Palmira
326	C-18	2	Collection of Plantain Varieties, Palmira
327	C-415	2	Collection of Plantain Varieties, Palmira
329	C-425	2	Collection of Plantain Varieties, Palmira
330	C-426	2	Collection of Plantain Varieties, Palmira
331	C-427	2	Collection of Plantain Varieties, Palmira
399	St L-13-1	2	Hacienda Santa Lucia
400	St L-13-3	2	Hacienda Santa Lucia
401	St L-14-1	2	Hacienda Santa Lucia
332	St L-25	2	Hacienda Santa Lucia
333	St L-35	2	Hacienda Santa Lucia
334	St L-36	2	Hacienda Santa Lucia
335	St L-37	2	Hacienda Santa Lucia
337	St L-39	2	Hacienda Santa Lucia
338	G-331	2	ICA, Centro Experimental, Palmira
409	17-22	2	Caicedonia
410	18-13	2	Caicedonia
414	20-23	2	Cerrito
416	22-3	2	Manizales
418	23-4	2	Manizales
419	24-2	2	Manizales
420	24-4	2	Manizales
443	NCPPB 2511	3	Cenicafé, Chinchiná
444	NCPPB 2512	3	Cenicafé, Chinchiná
445	NCPPB 2513	3	Cenicafé, Chinchiná

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purified by the method of Marmur (15), and the guanine plus cytosine (G+C) composition was determined by the thermal denaturation technique (14) and by the use of a Gilford 240 spectrophotometer. The relationship between melting point temperature (T_m) and G+C content of different DNA samples in standard saline citrate (14) was represented by the following equation: moles percent G+C = 2.44 (T_m − 69.4) (6).

Pathogenicity. Cells to be used as inocula were grown on slants of yeast extract-dextrose-$CaCO_3$ agar for 24 h at 27°C. Cells were removed from the medium by a dissecting needle and introduced into small pieces (4 by 8 cm) of plantain pseudostem (*M. paradisiaca*) from healthy mature plants, and each piece was placed in a closed petri dish with wetted filter paper. In addition, cells were collected on the tips of sterile toothpicks and were inserted near the base of 60- to 80-day-old plantain seedlings (*Musa balbisiana* Linnaeus). The controls were prepared by the insertion of a sterile needle or toothpick into a pseudostem piece or seedling, respectively. The occurrence of tissue degradation was recorded at 4, 6, 8, and 12 days after inoculation.

RESULTS

The bacterium isolated from *M. paradisiaca* was a gram-negative, non-sporeforming rod which occurred singly or in pairs. Cells from a 24-h-old nutrient agar culture had average dimensions of 0.6 by 1.7 μm. The cells were motile and peritrichous; four to six flagella were commonly discernible as previously reported (22). The colonies on nutrient agar after 24 h at 27°C were convex, slightly to moderately irregular and undulate, pale cream-colored, and butyrous and averaged 1.5 to 2.5 mm in diameter; on yeast extract-dextrose-$CaCO_3$ agar, the colonies were convex or somewhat umbonate, irregular, undulate, light tan, butyrous, and 2.5 to 3.5 mm in diameter.

The phenotypic characteristics for which all strains of *Erwinia* from *M. paradisiaca* were positive include the following: facultatively anaerobic; pectate degradation; potato soft rot; gas

from glucose; catalase production; phosphatase production; β-galactosidase production; indole production; nitrate reduction; growth at 36 and 39°C; susceptibility to penicillin G (2 U); susceptibility to erythromycin (15 μg); H_2S from sodium thiosulfate; KCN inhibition; utilization of sodium citrate, sodium malonate, sodium tartrate, and *meso*-tartrate; and acid from D-fructose, D-galactose, D-mannose, D-arabinose, L-arabinose, D-ribose, D-xylose, L-rhamnose, D-cellobiose, D-melibiose, D-raffinose, glycerol, ethanol, esculin, salicin, and α-D-galacturonic acid. The phenotypic characteristics for which all strains of *Erwinia* from *M. paradisiaca* were negative include the following: cytochrome oxidase production; casein hydrolysis; gelatin liquefaction; phenylalanine deamination; gluconate oxidation; blue pigment on yeast extract-dextrose-$CaCO_3$ agar; arginine decarboxylase; growth in 5% NaCl; deoxyribonuclease production; urease production; lecithinase production; and acid from D-maltose, D-lactose, D-trehalose, palatinose, D-melezitose, starch, inulin, dextrin, D-mannitol, adonitol, dulcitol, D-sorbitol, *i*-inositol, and α-methyl-*d*-glucoside.

All strains produced acetoin except strain 235. All strains grew in 1% peptone medium (12) without carbohydrate and produced a slight increase (0.36 to 0.90 unit) in the pH of the medium at 7 days after inoculation at 27°C. Slight to moderate growth also occurred in the basal medium of Ayers et al. (1) without carbohydrate, and a slight decrease in pH (0.05 to 0.51 unit) was recorded.

The results for the T_m and G+C determinations of the three strains isolated from *M. paradisiaca*, two strains of *E. chrysanthemi*, and a strain of *Escherichia coli* are given in Table 2. There were no striking differences in the T_m and G+C determinations of the plantain strains and the *E. chrysanthemi* strains.

TABLE 2. *DNA base composition of selected strains of Erwinia and a strain of E. coli*

Strain	T_m (°C)	G+C content of DNA (mol%)
221[a]	91.5	54.0
235[a]	91.8	54.7
327[a]	91.7	54.4
B-46[b]	92.0	55.1
NCPPB 2538[c]	91.9	54.7
E. coli[d]	90.6	51.7

[a] Isolated from *M. paradisiaca*.
[b] *E. chrysanthemi* isolated from philodendron (*Philodendron selloum*).
[c] *E. chrysanthemi* isolated from corn (*Zea mays*).
[d] Strain obtained from Steve Klevickis (Department of Bacteriology, University of Wisconsin, Madison).

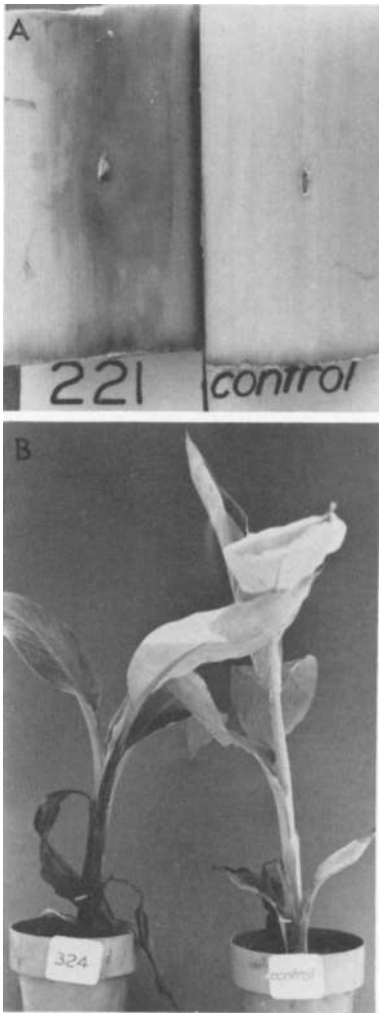

FIG. 1. *Typical symptoms produced by strains of Erwinia isolated from M. paradisiaca. (A) Development of pink to light-brown color and soft rot of pseudostem tissue (M. paradisiaca) around point of insertion of cells of strain 221 at 6 days after inoculation (left). Peudostem tissue punctured with sterile needle (right). (B) Dark-brown discoloration and soft rot of stem and leaf tissues of 80-day-old plantain seedlings (M. balbisiana) at 12 days after inoculation with cells of strain 324 (left). Toothpick infested with cells of strain 324 (left) and sterile toothpick (right) near base of stem denote the points of inoculation.*

All strains from *M. paradisiaca* caused tissue degradation of plantain pseudostem pieces and plantain seedlings. Typical results are shown in Fig. 1.

DISCUSSION

The problems inherent in the taxonomy of the soft-rot group of *Erwinia* species have been reviewed elsewhere (5, 7, 8, 11, 12, 20). The 30 strains from *M. paradisiaca* are very similar to *E. chrysanthemi* strains (Table 3) when compared with the results of comparative studies previously reported for phenotypically similar *Erwinia* species and their subspecies (5, 7, 8, 11, 18). Barzic et al. (3) designated one strain (their no. 1451) from plantain as *E. carotovora* subsp. *chrysanthemi*, and Samson and Nassan-Agha (17) designated three strains as *E. chrysanthemi* based primarily on the following characteristics: production of indole and phosphatase; growth at 37°C; utilization of malonate; no production of acid from α-methylglucoside or lactose; and no production of reducing substances from sucrose. These characteristics agree with our results (Table 3).

The DNA base compositions of the three plantain strains and two strains of *E. chrysanthemi* (Table 2) are in close agreement with the results obtained with strains of *E. chrysanthemi* and *Escherichia coli* (16, 21) and support the similarity of the five strains.

Strains of *E. chrysanthemi* have been isolated and identified from many host plants (7). The plantain strains can be distinguished from strains of *E. chrysanthemi* from other hosts by the following physiological properties: production of acid from D-arabinose and raffinose; utilization of sodium tartrate; inability to liquefy gelatin; no production of lecithinase; and no production of acid from mannitol, sorbitol, or inulin. Thus, the plantain strains constitute a new phenotypic subdivision, VI (Table 4). Subdivisions I through V maintain their original identity and incorporate the same strains as previously described (7), although the scheme for separation of the subdivisions has been modified in Table 4. Samson and Nassan-Agha (17) have divided 129 strains of *E. chrysanthemi* from 17 hosts into eight groups, three of which are listed as biovars. They used six properties to separate the three plantain strains from the strains included in the three biovars and other groups. Four of the properties (acid production from D-arabinose, acid production from raffinose, no acid production from mannitol, and utilization of tartrate) are included in Table 4, and two properties (acid production from melibiose and lack of arginine degradation) agree with our results (see above).

A recent proposal has been made (23) that the term pathovar be used for phytopathogenic bacteria at the infrasubspecific level. Strains which possess a limited number of phenotypic differences and a unique host range are considered

TABLE 3. *Characteristics selected to indicate the relationship between Erwinia strains from M. paradisiaca and phenotypically similar Erwinia species and their subspecies*[a]

Characteristic	*Erwinia* strains from *M. paradisiaca*	*E. chrysanthemi*	*E. carotovora* subsp. *carotovora*	*E. carotovora* subsp. *atroseptica*	*E. cypripedii*	*E. rhapontici*
Pectate degradation	+[b]	+	+	+	−	−
Phosphatase production	+	+	−	−	v	v
Growth at 36 to 37°C	+	+	+	−	+	v
Indole production	+	+	−	−	−	−
Acetoin production	+	+	+	+	−	+
Susceptibility to erythromycin	+	+	−	−	+	+
Gas from glucose	+	+	v	v	+	−
Growth in 5% NaCl	−	v	+	+	+	+
Utilization of malonate	+	+	−	−	v	+
Acid from:						
α-Methyl-*d*-glucoside	−	−	−	+	−	v
Trehalose	−	−	+	+	+	+
Maltose	−	−	v	+	+	+
Lactose	−	v	+	+	−	+
Palatinose	−	−	−	+	−	+
Ethanol	+	+	−	−	+	−

[a] The phenotypic results for the *Erwinia* species and their subspecies are derived from previously published data (5, 7, 8, 11, 18). Two or more authors report the following for all species: facultatively anaerobic; reduction of nitrate; production of catalase and H_2S and of acid from fructose, galactose, mannose, arabinose, ribose, glucose, cellobiose, glycerol, mannitol, esculin, and salicin; utilization of citrate; and no production of oxidase, urease, arginine decarboxylase, or acid from adonitol. β-Galactosidase production and no production of deoxyribonuclease are reported by one or more authors.

[b] Symbols: +, 80% or more strains positive; −, 80% or more strains negative; v, 21 to 79% strains positive.

TABLE 4. *Comparison of some phenotypic characteristics of Erwinia strains from M. paradisiaca with those of strains of E. chrysanthemi isolated from other hosts*

Characteristic	Subdivision[a] (no. of strains)					
	I (22)	II (3)	III (25)	IV (199)	V (73)	VI (30)
Acid from:						
D-(−)-mannitol	+[b]	+	+	+	+	−
D-(−)-sorbitol	+	+	+	+	+	−
D-(−)-arabinose	+	−	−	+	−	+
D-(+)-raffinose	−	+	+	+	+(51)	+
Inulin	−	−	+	−	+	−
Utilization of sodium tartrate	+	+	−	+	+	+
Lecithinase	+	+	+	+	+(93)	−
Gelatin liquefaction	+	+	+	+	+(90)	−

[a] See Dickey (7) for original hosts of strains in infrasubspecific subdivisions I through V; subdivision VI includes only strains isolated from *M. paradisiaca*.

[b] Symbols: +, all strains positive; −, all strains negative; ( ), percentage of strains.

eligible for designation as pathovars. The following five pathovars of *E. chrysanthemi* have been proposed: *diffenbachiae, parthenii, zeae, chrysanthemi* (23), and *dianthicola* (7). Thus far, pathogenic bacterial strains which possess the phenotypic characters reported here (see above and Table 4) are unusual because they have been isolated only from *M. paradisiaca* plants collected in Colombia. If it is eventually determined that the plantain strains have a unique host range, it is proposed that the strains isolated from *M. paradisiaca* be designated *E. chrysanthemi* pathovar *paradisiaca*. It is suggested that strain B-131 (strain PDDCC 2349, Plant Disease Division Culture Collection, Aukland, New Zealand) be designated the reference strain.

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REPRINT REQUESTS

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