Identification and Nutritional Differentiation of the Erwinia Sugar Beet Pathogen from Members of *Erwinia carotovora* and *Erwinia chrysanthemi*

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ABSTRACT

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A disease of sugar beet denoted by soft rot and vascular necrosis is caused by a distinct group of *Erwinia carotovora* strains and is named *Erwinia carotovora* subsp. *betavasculorum* subsp. nov. A comparative study was made of the nutritional properties of 99 strains of identified and unidentified soft-rotting *Erwinia* species from several hosts, including sugar beet. Seventy-one strains were placed either in *E. carotovora* subsp. *carotovora*, *E. carotovora* subsp. *atroseptica*, or *E. carotovora* subsp. *betavasculorum* subsp. nov., or one unclassified group in this species depending on similarities of nutritional properties. Nutritional and physiological tests useful for distinguishing subspecies of *betavasculorum* from one or more of the other Erwiniae include: growth of α-methylglucoside, D-lactate, ethanol, L-lysine, maltose, palatinose, D-asparagine, and ethanol; either no, or very slow, growth on cellobiose, galacturonate, melibiose, malonate, and raffinose; no indole or phosphatase production; no gas from glucose; resistant to erythromycin; growth at 36 C; and the production of reducing substances from sucrose. Twenty-eight strains were placed into *E. chrysanthemi* and divided into six subdivisions. These six subdivisions corresponded to previously described species varieties or *formae speciales* and are generally equated with the host from which they were originally isolated.

A new soft rot disease of sugar beet was observed in the San Joaquin Valley, California, in 1972 and isolation from diseased plants consistently yielded a soft-rotting *Erwinia* sp. (23). Results of several early studies (19,21) suggest that the sugar beet pathogen might be a variant of *Erwinia carotovora* subsp. *atroseptica* or possibly *E. chrysanthemi* (10). However, our investigations (11,23) of its pathogenic and nutritional properties indicate that it is distinct from the presently described subspecies of *E. carotovora.*

Since the late 1800s, several bacterial pathogens causing rots of beets have been reported, but the descriptions of most were too vague to consider them as valid species (9). *Erwinia aroideae* and

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TABLE 1. Sources of strains of *Erwinia* species and subspecies used in this study to differentiate the sugar beet *Erwinia*

Specific name of strain as: Received	Identified[a]	UCPPB[b] strain designation	Source	Isolated from
Sugar beet *Erwinia*				
USB	Ecb	162	Moses Lake, WA	Sugar beet
WSB	Ecb	163	Moses Lake, WA	Sugar beet
Mad SB	Ecb	164	Madera County, CA	Sugar beet
Mont B1	Ecb	165	Monterey, CA	Sugar beet
Mont B2	Ecb	166	Monterey, CA	Sugar beet
Mont P1	Ecb	167	Monterey, CA	Sugar beet
Mont P2	Ecb	168	Monterey, CA	Sugar beet
B74-2	Ecb	169	Monterey, CA	Sugar beet
SB4	Ecb	170	Bakersfield, CA	Sugar beet
SB5	Ecc	171	Kern County, CA	Sugar beet
SB6	Ecb	172	Kern County, CA	Sugar beet
SB7	Ecb	173	Santa Maria County, CA	Sugar beet
SB10	Ecb	174	Sacramento County, CA	Sugar beet
SB11	Ecb	175	King County, CA	Sugar beet
SB13	Ecu	176	Lone Tree, CA	Soil
Sh1	Ecb	177	Belridge, CA	Sugar beet
Sh2	Ecb	178	Shandon, CA	Soil
Sh5	Ecb	181	Shandon, CA	Sugar beet
Sh6	Ecb	182	Shandon, CA	Sugar beet
Sh7	Ecb	183	Shandon, CA	Sugar beet
Sh8	Ecb	184	Shandon, CA	Sugar beet
Sh9	Ecb	185	Shandon, CA	Sugar beet
SAR1	Ecb	186	Sargent, CA	Sugar beet
SAR2	Ecb	187	Sargent, CA	Sugar beet
Beet 2	Ecb	189	Imperial County, CA	Sugar beet
Beet 3	Ecb	190	Imperial County, CA	Sugar beet
Beet 4	Ecb	191	Imperial County, CA	Sugar beet
UR7	Ecb	193	Kern County, CA	Sugar beet
UR8	Ecu	194	Kern County, CA	Sugar beet
Erwinia carotovora subsp. *atroseptica*				
EA143	Eca	140	A. Kelman	Potato
EA2	Eca	141	T. D. Miller	UK[c]
SR54	Eca	142	A. Kelman	Potato
E70	Ecc	143	S. Alcorn	UK
309	Eca	144	NCPPB[d]	Potato
334	Eca	145	NCPPB	UK
433	Eca	146	NCPPB	Potato
434	Eca	147	NCPPB	Potato
436	Eca	148	NCPPB	Potato
549	Eca	149	NCPPB	Potato
1042	Eca	150	NCPPB	Potato
1278	Eca	151	NCPPB	Soil
1449	Eca	152	NCPPB	Potato
3467-73	Eca	154	PDDCC[e]	Soil
1-119-66	Eca	155	PDDCC	Soil
1390-25	Eca	156	PDDCC	Soil
1745-66	Ecu	157	PDDCC	Potato
979	Ecu	158	NCPPB	Delphinium
1590	Eca	159	NCPPB	Soil
2043	Eca	160	NCPPB	Potato
Erwinia carotovora subsp. *carotovora*				
EC109	Ecc	78	ICPB[f]	UK
EC124	Eca	80	ICPB	UK
···	Ecc	81	D. C. Sands	Potato
···	Ecc	82	D. C. Sands	Potato
EC34	Ecc	84	T. D. Miller	UK
EC66	Ecc	86	T. D. Miller	UK
EC25	Echz	87	T. D. Miller	Corn
EC148	Ecc	88	T. D. Miller	UK
312	Ecc	90	NCPPB	Potato
Ec101	Ecu	91	S. Alcorn	UK
392	Ecc	93	NCPPB	Cucumber
395	Ecc	94	NCPPB	UK
547	Ecc	95	NCPPB	*Persea*
552	Ecc	96	NCPPB	Corn
929	Ecc	97	NCPPB	*Zantedeschia*
1276	Ecu	98	NCPPB	Potato
1281	Eca	99	NCPPB	Soil
1847	Ecc	100	NCPPB	Mexican pepper
2349	Ecc	101	NCPPB	Pumpkin
216	Ecc	216	Oregon	Potato
4a	Ecu	219	California	Potato
Ger #1	Ecc	243	California	Geranium
Erwinia chrysanthemi pv. *parthenii*				
EC121	Echpa	1016	ICPB	Guayule
EC196	Echpa	1017	ICPB	Guayule
Erwinia chrysanthemi pv. *dieffenbachiae*				
ED103	Echd	···	ICPB	Dieffenbachia
Erwinia chrysanthemi				
Ech2	Echdia	104	T. D. Miller	Poinsettia
402	Echch	108	NCPPB	Chrysanthemum
453	Echdia	109	NCPPB	Carnation
518	Echd	112	NCPPB	Carnation
1065	Echz	113	NCPPB	Corn
1385	Echdia	114	NCPPB	Dahlia
1490	Echd	115	NCPPB	Dieffenbachia
1514	Echd	116	NCPPB	Dieffenbachia
1955	Echdia	117	NCPPB	Dahlia
2308	Echd	118	NCPPB	Dieffenbachia
2309	Echch	119	NCPPB	Chrysanthemum
2348	Echz	120	NCPPB	Corn
3310-72	Echdia	121	PDDCC	Carnation
3349-73	Echdia	122	PDDCC	Dahlia
3724-74	Echdia	123	PDDCC	Carnation
4010-74	Echdia	124	PDDCC	Carnation
2357-68	Echz	125	PDDCC	Corn
1564-66	Echd	126	PDDCC	Dieffenbachia
427	Echch	127	NCPPB	Chrysanthemum
1956	Echdia	129	NCPPB	Dahlia
2339	Ecc	130	NCPPB	Chrysanthemum
EC174	Echdia	132	ICPB	Dianthus
EC177	Echdia	134	ICPB	Dianthus
EC179	Echd	135	ICPB	Dieffenbachia
EC242	Echz	137	ICPB	Corn
EC201	Echp	138	ICPB	Philodendron

(*continued*)

[a] Designation and abbreviations of received strains after reidentification: *Erwinia carotovora* subsp. *betavasculorum*, Ecb; *E. carotovora* subsp. *carotovora*, Ecc; *E. carotovora* subsp. *atroseptica*, Eca; unclassified strains, Ecu; *E. chrysanthemi* pv. *zeae*, Echz; pv. *chrysanthemi*, Echch; pv. *dieffenbachiae*, Echd; pv. *dianthicola*, Echdia; *philodendron* strains, Echp; and pv. *parthenii*, Echpa.
[b] UCPPB (University of California, Plant Pathology, Berkeley).
[c] UK, United Kingdom (unknown).
[d] NCPPB (National Collection of Plant Pathogenic Bacteria, P. Roberts, curator).
[e] PDDCC (Plant Diseases Division Culture Collection, D. W. Dye, curator).
[f] ICPB (International Collection of Phytopathogenic Bacteria, M. P. Starr, curator).

TABLE 2. Diagnostic tests and substrates useful in distinguishing *Erwinia carotovora* (including subspecies *betavasculorum*) from *Erwinia chrysanthemi*

Diagnostic tests	% Strains positive: *E. carotovora*	*E. chrysanthemi*
Production of		
gas from D-glucose	0	100[a]
indole	0	100[a]
phosphatase	0	100[a]
Sensitivity to		
erythromycin	0	99[a]
Utilization of		
cis-aconitate	0	21
γ-aminobutryate	0	21
citrate	76	100
galacturonate	63	100
gallate	0	25
D-glutamate	0	14
D-lactate	46	0
L-lactate	82	100
β-lactose	100	39
malonate	0	93[b]
maltose	66	4[c]
palatinose	70	4[d]
rhamnose	99[e]	36
salicin	100	89
L-serine	100	32
D-tartrate	0	39
L-tartrate	0	32
m-tartrate	1[f]	86
trehalose	94[g]	0

[a] According to Dickey (6).
[b] Strains 124, 128 negative (−).
[c] Strain 113 positive (+).
[d] Strain 129+.
[e] Strain 91−.
[f] Strain 98+.
[g] Strains 157−, 158−, 186−, 187−.

E. betivora were listed as causing soft-rot of beets and sugar beets, respectively; but because descriptive data were inadequate for species designation, both were listed as synonyms of *E. carotovora* in the eighth edition of Bergey's Manual (12). In addition, neither of the species was reported to cause vascular necrosis of sugar beet. To clarify the identification and characterization of the sugar beet pathogen, other soft-rot groups were studied for comparative purposes and those data are presented. We present evidence based on nutritional studies and physiological tests that the sugar beet pathogen differs from other Erwiniae soft-rotting species and subspecies and propose the name *Erwinia carotovora* subsp. *betavasculorum*. Reports of portions of this study have been published (11,23).

MATERIALS AND METHODS

Bacterial strains included in this study are listed in Table 1. Carbon source utilization was determined by incorporating 0.1% (w/v) of a carbon source into a simple mineral-base medium (1) and placing a 0.03-ml drop of turbid bacterial suspension on the medium. Growth or nongrowth was noted after 3, 7, 14, and 21 days of incubation at 28 C. Most carbon sources were sterilized by autoclaving, although a few required filtration (17). Acid production from various substrates was determined at 5 days by using the method of Dye (7,8). Production of reducing the substances from sucrose and growth at 37 C were determined by the methods of Dye (7). A colorimetric method also was used to measure quantitatively the production of reducing substances from sucrose (5,16). Each nutritional test was performed at least two or more times.

DNA base composition. DNA was isolated from three sugar beet strains harvested in the late logarithmic phase of growth (13). The temperature (*Tm*) corresponding to the midpoint of the hyperchromic shift was determined by the method of Marmur and Doty (14). Purified DNA was diluted to $A_{260} = 1.0$ in saline citrate (SC) buffer (0.15 M NaCl, 0.015 sodium citrate, pH = 7.0) and in 1:10 dilution of SC buffer. Samples were dialyzed against known concentrations of SC buffer to insure uniformity of their ionic strengths. Samples were placed in 0.3-ml Teflon-stoppered quartz cuvettes having a 1-cm light path. Samples were analyzed on a Gilford 250 spectrophotometer equipped with Model 2527 thermoprogrammer. The temperature within the cuvette chamber was raised from 70 to 100 C at the rate of 0.25 C/min. Two samples, one blank and one sample of reference calf thymus DNA, were analyzed simultaneously by using this equipment. Each sample was analyzed four times at each buffer concentration to confirm the reproducibility of *Tm* values.

RESULTS

All strains of the soft-rotting Erwiniae tested (with the few exceptions noted) had many characteristics in common. They all

TABLE 3. Nutritional and physiological properties useful for the identification of *Erwinia carotovora* subsp. *betavasculorum* and other subspecies of *Erwinia carotovora*

	% Strains positive			
Tests	*E. carotovora* subsp. *carotovora* (Ecc) (19 strains)	*E. carotovora* subsp. *atroseptica* (Eca) (19 strains)	*E. carotovora* subsp. *betavasculorum* (Ecb) (26 strains)	Unclassified (Ecu) (7 strains)
Growth at 36 C	100%	0%	100%	86%[a]
Reducing substance from sucrose (Benedict's reagent)	11[b]	77[b]	100	100
Acid from α-methyl glucoside	0	100	100	43[c]
Growth on:				
allantoin	5(14)[de]	0	62(3–14)[e]	14(14)[e]
D-asparagine	84(14)[f]	21(3–14)[f]	100(3)	43(3–14)[f]
cellobiose	100(3)	100(3–7)	0	86(3–7)[g]
citrate	100(3)	100(3–7)	38(7–14)[h]	100(3)
ethanol	21(3–7)[i]	0	100(3)	29(3)[i]
galacturonate	100(3)	100(3)	0	100(3)
α-methyl-glucoside	0	63(7–14)[j]	96(3–7)[j]	43(3–21)[j]
D-lactate	0	26(7–14)[k]	100(3–7)	29(3)[k]
L-lactate	84(3–14)[l]	63(3–7)[l]	100(3–7)	71(3–7)[l]
L-lysine	5(7)[m]	0	100(3–14)	0
maltose	0	100(3–7)	100(7–14)	29(7)[n]
melibiose	100(3–7)	95(3–7)[o]	0	100(3)
mucate	100(3)	100(3–14)	100(7–14)	100(3–14)
palatinose	0	100(3–7)	100(3–7)	43(3–7)
raffinose	95(3–7)[p]	100(3)	15(7–14)[q]	100(3)
salicin	100(3–14)[q]	100(3–14)[q]	100(3)	100(3–14)[q]
L-serine	100(3–7)	100(7–14)	100(3–7)	100(3–14)
triacetin (mutants)	74(14–28)[r]	0	8(14–28)[r]	29(14–28)[4]

[a] Ecu strain 98 was negative (−).
[b] Ecc strains 84 and 90 were positive (+); Eca strains 146−, 152−, 154−, and 159−.
[c] Ecu strains 91+, 176+, 219+.
[d] Numbers in parentheses are number or range of days for growth to occur.
[e] Ecc strain 86+ and Ecu strain 219+; Ecb strains 164−, 165−, 166−, 169−, 170−, 173−, 174−, 178−, 184−, and 186−.
[f] Ecc strains 81−, 86−, and 100−; Eca strains 99+, 144+, 154+, and 155+; and Ecu strains 91+, 176+, and 219+.
[g] Ecu strain 157−.
[h] Growth for nearly all strains was poor and many mutants appeared; Ecb strains 165+, 166+, 168+, 172+, 175+, 178+, 186+, and 191+.
[i] Ecu strains 98+, 176+, and 219+; and Ecc strains 86+, 90+, and 94+.
[j] Eca strains 145−, 150−, 152−, 154−, 155−, 156−, and 160−; and Ecb strain 178−; and Ecu strains 91+, 176+, and 219+.
[k] Eca strains 80+, 99+, 145+, 150+, and 156+; and Ecu strains 98+, and 219+.
[l] Ecc strains 81−, 94−, 130−; Eca strains 99−, 141−, 142−, 145−, 147−, 149−, 151−, 152−, 154−; and Ecu strain 194−.
[m] Ecc strain 86+.
[n] Ecu strains 98+, 219+.
[o] Eca strain 80−.
[p] Ecc strain 93− and Ecb strains 165+, 166+, 169+, 175+.
[q] Growth very poor with few scattered mutant colonies appearing with most strains.
[r] Ecc strains 81−, 86−, 93−, 95−, 96−, 97−, and 143−; Ecb strains 165+, and 186+; and Ecu strains 157+, and 158+.

utilized: L-arabinose, D-aspartate, L-aspartate, D-fructose, fumarate, D-galactose, D-glucose, gluconate, α-ketoglutarate, glycerol, *meso*-inositol, inulin, L-malate, mannitol, D-mannose, mucate (except UCPPB 91), D-ribose, saccharate (except UCPPB 91), succinate, sucrose, and D-xylose. None of the soft-rotting Erwiniae utilized: adipate, adonitol, β-alanine, anthranilate, L-arginine, azelate, benzoate, *p*-hydroxybenzoate, betaine, butanol, butyrate, β-hydroxybutyrate, caprate, choline, citraconate, citronellal, DL-citrulline, L-cysteate, L-cysteine, dulcitol, erythritol, formate, glutarate, glycine, glycolate, heptanoate, DL-homocysteine, DL-homoserine, L-isoleucine, isostearate, isovalerate, isovanillin, itaconate, lauryl alcohol, D-leucine, linolenate, D-lyxose, mandelate, melezitose, mesaconate, D-methionine, nicotinate, D-norvaline, L-norvaline, octyl alcohol, oxalate, pelargonate, L-phenylalanine, phytate, picolinate, pimelate, propionate, putrescine, D-quinate, quinolinate, rutin, saligenin, sarcosine, sebacate, D-serine (except strain 91), shikimate, sorbate (except strain 113), sorbitol (except strain 91), L-sorbose, stearate, stearyl alcohol, suberate, L-threonine, tripropionin, trigonelline, L-tyrosine, undecylenate, valerate, δ-amino-valerate, D-valine, L-valine, vanillate, vanillin, and xylitol.

The results of tests with acetate, heptadecanoate, isoascorbate, laurate, oleate, and DL-ornithine as substrates were inconclusive since some strains were positive in one trial but negative in others.

Based on substrate utilization patterns, the strains were divided into two groups, *E. carotovora* and *E. chrysanthemi.* Although the utilization of no single substrate could unequivocally distinguish *E. carotovora* from *E. chrysanthemi* strains, the two species could be separated on the basis of a combination of tests and the use of several substrates (Table 2).

Tests with the substrates D-alanine, methylalanine, β-hydroxymethylglutarate, DL-glycerate, hippurate, L-xylose, D-malate, D-pantothenate, DL-pipecolate, and L-proline were inconclusive for distinguishing *E. carotovora* subspecies. Equivocal results for *E. chrysanthemi* were obtained with allantoin, glutathionine, L-lysine, and myristate as substrates.

Twenty-one tests were useful in separating *E. carotovora* into three subspecies: *atroseptica, carotovora,* and the sugarbeet pathogen for which we propose the name *betavasculorum* (Table 3). Strains of *E. carotovora* subsp. *betavasculorum* (162, 163, 177, 189, and 193) have been deposited in the National Collection of Plant Pathogenic Bacteria, Harpenden, as NCPPB 2794, 2793, 2792, 3075, and 2795, respectively.

Characteristics of *E. carotovora* subsp. *betavasculorum* NCPPB 2795 (designated as the type strain) that agree with those of *E. carotovora* are as follows: cells are predominantly single; Gram-negative; straight rods with cell sizes ranging from 0.5 to 1.0 by 1.0 to 3.0 μm; endospores are not produced; peritrichous flagella; facultative anaerobic; indole, phosphatase, and pigment not produced; and resistant to erythromycin.

Additional properties of *E. carotovora* subsp. *betavasculorum* NCPPB 2795 are as follows: growth occurs at 36 C, reducing substances are formed from sucrose, and the DNA base composition is 54.4 mol % G ± C. Organic substrates utilized as sole sources of carbon and energy include: L-arabinose, D-asparagine, D-aspartate, L-aspartate, cellobiose, ethanol, D-fructose, fumarate, D-galactose, galacturonate, D-glucose, α-methylglucoside, gluconate, α-ketoglutarate, DL-glycerate, glycerol, inosine,

TABLE 4. Characters useful for the identification of pathovars of *Erwinia chrysanthemi*

	Erwinia chrysanthemi pathovars (% strains positive)					
Tests	*zeae* (Echz) (5 strains)	*dieffenbachiae* (Echd) (5 strains)	*philodendroni*[a] (Echp) (1 strain)	*parthenii* (Echpa) (2 strains)	*chrysanthemi* (Echch) (4 strains)	*dianthicola* (Echdia) (11 strains)
Growth on:						
cis-aconitate	100%(3)[b]	0%	0%	0%	25%(7)[b]	0%
D-arabinose	100(3)	100(3–7)	100(3)	0	0	0
D-asparagine	100(3–7)	0	0	100(21)	100(14–21)	0
γ-aminobutyrate	100(3–7)	0	0	0	25(7–14)[c]	0
cellobiose	100(14)	100(7–14)	100(14)	100(14)	100(14)	0
ethanol	100(3–7)	0	0	100(3)	75(3–7)[d]	0
gallate	100(3)	0	0	50(7)[e]	0	0
glucuronate	100(3)	100(3–14)	100(3)	100(3)	100(3–14)	100(14)
D-glutamate	80(3–14)[f]	0	0	0	75(7–14)[g]	0
glycerate	100(3–7)	0	0	100(14)	0	100(3–14)
β-lactose	100(7–14)	0	100(14)	100(7–14)	100(3)	0
D-malate	100(3)	100(3)	100(3)	100(3)	0	100(3)
melibiose	100(3)	0	100(3)	100(3)	100(3)	64(3)[h]
L-proline	100(14)	0	0	0	0	0
raffinose	100(3)	0	100(3)	100(3)	100(3)	64(3)[i]
rhamnose	0	0	0	0	0	73(3)[j]
ribose	100(3)	100(3)	100(3)	100(14)	100(3)	100(3–14)
salicin	100(3)	100(3)	100(3)	100(3)	100(3)	73(3)[k]
L-serine	100(3)	60(307)	100(3)	100(3)	100(3)	55(7–14)[l]
D-tartrate	0	0	0	0	0	100(3)
L-tartrate	40(14)[m]	100(14)	0	100(14)	0	0
m-tartrate	100(3)	100(3)	100(3)	100(3–7)	0	100(7)
triacetin (mutants)	100(14–28)	100(14–21)	100(14)	100(14–21)	0	0

[a] The philodendron strain is not yet recognized as a pathovar.
[b] Number in parentheses is number of days for growth to occur. Echch strain 119 positive (+).
[c] Echch strain 108+.
[d] Echch strain 104 negative (−).
[e] Echpa strain 1017+.
[f] Echz strain 137−.
[g] Echch strain 104−.
[h] Echdia strain 114−, 121−, 123−, and 124−.
[i] Echdia strains 114−, 121−, 123−, and 124−.
[j] Echdia strains 114−, 112−, and 122−.
[k] Echdia strains 121−, 123−, and 124−.
[l] Echd strains 103− and 116−; Echdia strains 112+, 114+, 117+, 122+, 124+, and 128+.
[m] Echz strains 125− and 137−; and Echd strain 138−.

inositol, D-lactate, L-lactate, β-lactose, L-malate, mannitol, D-mannose, D-maltose, mucate, palatinose, rhamnose, D-ribose, saccharate, salicin, L-serine, succinate, sucrose, trehalose and D-xylose, but not *cis*-aconitate, adipate, adonitol, β-alanine, anthranilate, D-arabinose, D-arabitol, L-arabitol, L-arginine, azelate, benzoate, betaine, butyrate, γ-amino butyrate, caprate, caproate, caprylate, cellobiose, choline, citraconate, citrate, citronellal, DL-citrulline, creatine, L-cysteine, dextrin, dulcitol, erythritol, esculin, D-fucose, L-fucose, gallate, geraniol, D-glutamate, glutarate, glycine, glycolate, heptanoate, DL-homocysteine, DL-homoserine, L-isoleucine, isostearate, itaconate, kynurenate, kynurenine, laurate, lauryl alcohol, L-leucine, D-lysine, D-lyxose, malonate, melezitose, melibiose, mesaconate, D-methionine, myristate, nicotinate, D-norvaline, L-norvaline, octanol, oleate, orcinol, oxalate, pelargonate, phenylacetate, L-phenylalanine, phytate, phytol picolinate, pimelate, DL-pipecolate, propionate, propylene glycol, D-quinate, quinolinate, rutin, saligenin, sarcosine, sebacate, D-serine, shikimate, sorbate, sorbitol, L-sorbose, suberate, D-tartrate, *meso*-tartrate, L-threonine, trigonelline, valerate, D-valine, L-valine, L-xylose, or xylitol.

Seven diverse strains were identified as *E. carotovora*, but because they could not be placed into any of the subspecies they were listed in an unclassified group (Table 3). On the basis of utilization patterns with 25 different substrates, the strains of *E. chrysanthemi* included in our test could be divided into six clusters (Table 4), which correspond with pathovars *chrysanthemi, dianthicola, dieffenbachiae, parthenii, zeae,* and strains from *Philodendron* (25).

Measurement of reducing compound formation from sucrose. The method recommended in Bergey's Manual (12) for detecting reducing compounds formed by *E. carotovora* subsp. *atroseptica* from sucrose utilizes Benedict's reagent. However, results obtained with this method can be difficult to interpret and it was decided, therefore, to use the colorimetric method of Nelson (16) for comparison. Five strains each of *E. carotovora* subsp. *atroseptica*, subsp. *carotovora*, and subsp. *betavasculorum* and one unclassified strain were tested.

Fair agreement was generally obtained with both techniques (Table 5). Although the quantitative colorimetric estimation was more time consuming, it appeared more sensitive for measuring differences among organisms. Two to five times more reducing substances were accumulated by subsp. *betavasculorum* than by subsp. *atroseptica* strains.

DNA base composition. Melting temperatures for subsp. *betavasculorum* strains under both ionic conditions are listed in Table 6. The DNA's of the three strains do not differ significantly in their respective base compositions as the G + C content ranged from 54.1 to 54.6% with an average value of 54.4%. These values are intermediate between the values obtained for *E. carotovora* subsp. *carotovora* (50.5–53.1%), *E. carotovora* subsp. *atroseptica* (51.3–53.1%) and *E. chrysanthemi* (55.1–57.1%) (12).

DISCUSSION

The sugar beet pathogen is readily distinguished from other subspecies of *E. carotovora* and pathovars of *E. chrysanthemi* by using nutritional and physiological tests. The host range and symptom expression in sugar beet also aid in distinguishing this pathogen (23). Furthermore, Dickey (6) in a comprehensive study of erwiniae suggested that the sugar beet isolates possibly should be designated to a specific rank. This supports our study and we propose that the sugar beet pathogen be given the designation of *E. carotovora* subsp. *betavasculorum*. Strain number of NCPPB 2795 is designated as the type strain.

The decision to make the sugar beet pathogen a subspecies of *E. carotovora* rather than giving it species ranking was made after considerable debate and equivocation. Although it exhibits many similarities to *E. carotovora* subsp. *carotovora* and *E. carotovora* subsp. *atroseptica*, it also shows similarities to *E. chrysanthemi*. Inversely, it also differs from those pathogens in various characters. In the future, some of the *Erwinia* subspecies such as subsp. *betavasculorum* and pathovars of *E. chrysanthemi* may be elevated to the species rank. For example, our data show that some strains of *E. chrysanthemi* are as closely related to some strains of *E. carotovora* as are strains of *E. carotovora* subsp. *carotovora* to *E. carotovora* subsp. *atroseptica* on the basis of overall similarity. Generally, however, most research workers agree that *E. carotovora* subsp. *carotovora* and subsp. *atroseptica* are closely related and distinct from *E. chrysanthemi* on the basis of serology (20) and DNA-DNA homology (4,15) and other studies (3,18). Because of the variation among *E. carotovora* and *E. chrysanthemi* strains, the differences between these two groups were compared. Thus, some or most (but not all) of the *E. carotovora* strains (including subsp. *betavasculorum*) utilized α-methylglucoside, D-lactate or maltose; in contrast, none of the *E. chrysanthemi* strains utilized these compounds as carbon sources. Most of the *E. chrysanthemi* strains utilized *cis*-aconitate, D-arabinose, α-amino-butyrate, gallate, D-glutamate, D-tartrate, and L-tartrate, whereas none of the *E. carotovora* strains utilized these substrates.

The pathovar system (25) was not used in naming the sugar beet pathogen since the host range was not as distinct as the physiological properties that separated it from other *Erwinia* species. For example, *E. carotovora* subsp. *betavasculorum* in greenhouse tests infected potato, tomato and chrysanthemum (23). Furthermore, some *Erwinia* strains such as UCPPB 176 (NCPPB 3074) and UCPPB 193 (NCPPB 2795), which were isolated from soil and plant material, infected several hosts including sugar beet, but were distinct according to nutritional tests. Thus, identification

TABLE 5. Comparison of two techniques for determination of the amounts of reducing substances produced from sucrose by *Erwinia carotovora* subsp. *carotovora*, subsp. *atroseptica*, and subsp. *betavasculorum*

Strain	Test results	
	Benedict's reagent (reaction strength)	Nelson's procedure[a] (μg/ml)
E. carotovora subsp. *carotovora*		
81	+[b]	240[c]
82	−	210
88	−	230
94	++	460
100	−	400
E. carotovora subsp. *atroseptica*		
80	+	750
144	+++	760
149	+++	1,080
150	−	520
156	+++	840
E. carotovora subsp. *betavasculorum*		
162	+++	2,620
164	+++	1,920
167	+++	2,020
173	+++	2,580
189	+++	2,840
Unclassified		
158	+++	520

[a]See references 5 and 16.
[b]Estimated strength of reaction; + = slight brown color; ++ = brown color; +++ = dark brown color within 3 min.
[c]Glucose equivalents present in medium after 3 days of incubation at 28 C.

TABLE 6. DNA base composition of three strains of *Erwinia carotovora* subsp. *betavasculorum* as determined in two concentrations of saline citrate buffer

Sources	Mol % G + C		
	1.0 × SC[a]	0.1 × SC[a]	Average
Strains			
165	54.7 ± 0.7	54.4 ± 1.2	54.6
189	53.4 ± 1.2	54.7 ± 1.1	54.1
193	54.4 ± 1.0	54.4 ± 1.2	54.4
Calf thymus	42.0 ± 0.2	42.0 ± 1.2	42.0

[a]Concentration of saline citrate buffer used for dilution of DNA samples.

at this time cannot be made by simple host range study.

The resolution of the taxonomy of soft-rotting Erwiniae also cannot be done on the basis of a few determinative tests because of the diversity of strains, intermediate types (21), and the relationships that exist throughout the group. There likely are a number of *Erwinia* strains associated with sugar beets and other crop plants with a range of characters varying from those of the presently described species and subspecies. Accordingly, the taxonomic status of the Arizona Wilcox strains (21), associated with sugar beet, must await a comprehensive comparison with other *Erwinia* strains.

Although the main purpose of this investigation was to describe a means of separating *E. carotovora* subsp. *betavasculorum* from other subsp. of *E. carotovora*, there are some observations with the *E. chrysanthemi* strains which should be noted. In Bergey's Manual, 8th ed. (12), the following were listed as synonyms of *E. chrysanthemi*: *Pectobacterium parthenii* var. *dianthicola*, *E. carotovora* f. sp. *parthenii*, *E. carotovora* var. *zeae*, *E. dieffenbachiae*, and *P. carotovora* var. *graminearum*. Brenner et al (4) also placed *E. cytolytica* in this group. The lumping of these organisms into *E. chrysanthemi* was based on the lack of differential characteristics other than pathogenicity and host range data. However, our data (based on a limited number of strains in some cases) supported Dickey's findings (6) that *E. chrysanthemi* as currently constituted can be divided into distinct groups generally corresponding with the host from which they were originally isolated. Sufficient differences appear to exist that may justify species status for pathovars and strains of *E. chrysanthemi*: pv. *zeae*, pv. *dieffenbachiae*, pv. *parthenii*, pv. *dianthicola*, and *E. chrysanthemi* strains from *Philodendron*. Base composition studies (15,22), serology (20), and some determinative tests (2,6,24) also indicate that distinct differences exist among this group of organisms.

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