Deoxyribonucleic Acid Relatedness Among *Erwiniae* and Other Enterobacteria

II. Corn Stalk Rot Bacterium and *Pectobacterium chrysanthemi*

DON J. BRENNER,¹ GEORGE R. FANNING, AND ARNOLD G. STEIGERWALT¹

Division of Biochemistry, Walter Reed Army Institute of Research, Washington, D.C. 20012

Relatedness of *pectobacteria* pathogenic to corn, grass, and sugar cane, and of isolates of *Pectobacterium chrysanthemi* pathogenic for chrysanthemum, dahlia, and dieffenbachia was determined by deoxyribonucleic acid (DNA) reassociation. Heterologous DNA preparations were allowed to reassociate. Thermal elution chromatography was used to separate reassociated nucleotide sequences from unreacted sequences and to determine the thermal stability of related sequences. DNA relatedness data indicate that corn stalk rot bacteria and strains pathogenic on grass are highly interrelated. Furthermore, strains of both types are distinct from *Pectobacterium carotovorum* and are most closely related to *P. chrysanthemi*. *P. chrysanthemi* strains fall into two relatedness groups, one of which contains isolates from chrysanthemum and guayule and the other isolates from dahlia or dieffenbachia. A sugar cane pathogen is 65% related to both the corn stalk rot bacteria and to strains of *P. chrysanthemi*. It is suggested that all of these organisms be considered as *P. chrysanthemi* and that the corn stalk rot and grass isolates be designated *P. chrysanthemi* pathovar *zeae*.

Deoxyribonucleic acid (DNA) from *Pectobacterium carotovorum* and *Pectobacterium chrysanthemi* were recently shown to reassociate only to about 40% (4). The purpose of this study was to assess the taxonomic position of corn stalk rot bacteria within *Enterobacteriaceae* by interspecific DNA reassociation. Reactions were carried out to determine relatedness of corn stalk rot bacteria to *pectobacteria* in general and, more specifically, to assess relatedness of corn stalk rot bacteria to *P. carotovorum* and *P. chrysanthemi*. The taxonomic position of the corn stalk rot pathogen has been a subject of controversy for a number of years (20). For reasons stated previously (4), the generic name *Pectobacterium* for soft-rot bacteria is used in this report. This is in accord with Waldee's recommendation that soft-rot-producing *erwiniae* should be placed in the genus *Pectobacterium* (24). The new combinations of *Pectobacterium* species have been proposed previously (4). The organisms referred to as *Erwinia* species are those species considered to be *Erwinia sensu stricto*. These are the species that cause wilt, blight, and necrosis diseases as opposed to soft-rot diseases. In Table 1 the organisms are listed as we received them; however, for the purpose of this study, *P. chrysanthemi*

strains that are pathogenic to corn are designated as corn stalk rot bacteria. For clarity, *Erwinia*, in parentheses, is used in addition to *Pectobacterium* when we refer to work of authors who do not subscribe to the use of *Pectobacterium*.

A bacterial disease of corn, characterized by top and stalk soft rot, causes substantial crop loss in widely separated geographic areas (1, 9-11, 14, 15, 19). Corn stalk rot isolates from these areas were similar in key cultural characteristics, performance in physiological tests (10, 11, 19), and in pathogenesis after inoculations of the stalks of corn plants (10, 16). With regard to physiological characteristics these isolates were more like *P. chrysanthemi* and related species than *P. (Erwinia) carotovorum*. Neither *P. carotovorum* nor *P. chrysanthemi*, however, were pathogenic to corn (10).

Starr and Mandel (22) completed a study of the DNA base composition of a large number of isolates of *Erwinia* and *Pectobacterium (Erwinia)* species. In this group were a number of isolates of the corn stalk rot bacterium including isolates from the United States (ICPB EM 107, 108, 109, 110, 111, 112) and Egypt (ICPB EC 209, 210). In addition to these isolates, Mandel subsequently determined guanine plus cytosine (G+C) contents for nine isolates from corn isolated in India and sent to A. Kelman by M. Payak. The G+C contents of DNAs from these

¹ Present address: Enteric Section, Center for Disease Control, Atlanta, GA 30333.

TABLE 1. *Bacterial isolates examined*

Designation of cultures as received	Sender ^a	Host	Country	Source, person(s)
<i>Arizona hinshawii</i> 1	CDC		U.S.A.	— ^b
<i>Citrobacter freundii</i> 5118-60	CDC		U.S.A.	—
<i>Edwardsiella tarda</i> 1126-64	CDC		U.S.A.	—
<i>Enterobacter aerogenes</i> 1627-66	CDC		U.S.A.	—
<i>E. aerogenes</i> banana strain 3151	ICPB		U.S.A.	Starr
<i>Enterobacter cloacae</i> 1347-71	CDC		U.S.A.	—
<i>Erwinia amylovora</i> 7400	ATCC	Photinia	U.S.A.	Ark
<i>E. amylovora</i> EA 178	ICPB		U.S.A.	Starr
<i>Erwinia ananas</i> EA 181	ICPB	Pineapple	U.S.A.	Starr/Graham, Spiegelberg (MF28)
<i>Erwinia aroideae</i> EA 3	ICPB	Tobacco	S. Carolina	Starr/Burkholder, Holdeman
<i>E. aroideae</i> 12286 (ICPB EA 189)	ATCC	Tobacco	S. Carolina	Burkholder (SR23)/Holdeman
<i>E. aroideae</i> 25207 (ICPB EA 191)	ATCC	Carrot	U.S.A.	Waldee
<i>Erwinia atroseptica</i> EA 112	ICPB		Canada	Starr/Garrard (15)
<i>E. atroseptica</i> 7404	ATCC	Squash	California	Ark
<i>Erwinia carnegieana</i> 13452 (ICPB EC 251) (nonpectolytic; <i>Klebsiella pneumoniae</i> ?)	ATCC	Giant cactus	U.S.A.	Burkholder/Brown
<i>E. carnegieana</i> EC 223 (pectolytic)	ICPB	Prickly pear	U.S.A.	Starr/Alcorn (62-47-2)
<i>E. carnegieana</i> EC 186 (pectolytic)	ICPB	Giant cactus	U.S.A.	Starr/Alcorn (1)
<i>Erwinia carotovora</i> 495	ATCC	Carrot	U.S.A.	Rogers/Jones
<i>E. carotovora</i> 15359 (ICPB EC 153)	ATCC	Pepper	New Zealand	Starr/Kraght/Dye/Stolp
<i>E. carotovora</i> f. sp. <i>parthenii</i> EC 115	ICPB	Guayule	U.S.A.	Starr/Campbell
<i>E. carotovora</i> SR 144 (74V)	DAFS	Corn	Israel	Quinn/Lelliott/Volcani
<i>Erwinia chrysanthemi</i> EC 16	ICPB	Chrysanthemum	U.S.A.	Starr/Burkholder
<i>E. chrysanthemi</i> SR 31 (ICPB EC 176), fluidal colony type	UW	Chrysanthemum	U.S.A.	Kelman/Starr/Burkholder
<i>E. chrysanthemi</i> SR 32	UW	Chrysanthemum		Kelman/Starr/Burkholder
<i>E. chrysanthemi</i> EC 205	ICPB	<i>P. oxycardium</i>	U.S.A.	Starr/McFadden (B111)
<i>E. chrysanthemi</i> 11662 (EC 16)	ATCC	Chrysanthemum	U.S.A.	Burkholder (EC 16)
<i>E. chrysanthemi</i> 11663	ATCC	Chrysanthemum	U.S.A.	Burkholder (EC 17)
<i>E. chrysanthemi</i> SR 56 (ICPB EC 209) (corn stalk rot bacterium)	UW	Corn	Egypt	Kelman/Starr/Sabet (EG 48)
<i>E. chrysanthemi</i> SR 80 (ICPB EC 252) (corn stalk rot bacterium)	UW	Corn	Wisconsin	Kelman
<i>E. chrysanthemi</i> SR 84 (corn stalk rot bacterium)	UW	Corn	India	Kelman/Payak (I-1-M-1)
<i>E. chrysanthemi</i> SR 98 (ICPB EC 253) (corn stalk rot bacterium)	UW	Corn	N. Carolina	Kelman
<i>E. chrysanthemi</i> EC 210 (corn stalk rot bacterium)	ICPB	Corn	Egypt	Starr/Sabet
<i>E. chrysanthemi</i> EM 107 (UW SR 94) (corn stalk rot bacterium)	ICPB	Corn	N. Carolina	Starr/Kelman (C ₁ B ₁)
<i>E. chrysanthemi</i> SR 140 (221 IPV-BO) (NCPBP 2347) (corn stalk rot bacterium)	IPV-BO	Corn	Italy	Mazzucchi
<i>E. chrysanthemi</i> SR 141 (IPV-BO 226) (NCPBP 2348) (corn stalk rot bacterium)	IPV-BO	Corn	Italy	Mazzucchi

TABLE 1—Continued

Designation of cultures as received	Sender ^a	Host	Country	Source, person(s)
<i>E. chrysanthemi</i> SR 142 (IPV-BO 228) (corn stalk rot bacterium)	IPV-BO	Corn	Italy	Mazzucchi
<i>E. chrysanthemi</i> SR 143 (IPV-BO 229) (corn stalk rot bacterium)	IPV-BO	Corn	Italy	Mazzucchi
<i>E. chrysanthemi</i> SR 145 (NCPBP 377)	DAFS	Corn	Rhodesia	Quinn/Lelliott/Sabet
<i>E. chrysanthemi</i> SR 146 (420 R)	DAFS	Grass	Australia	Quinn/Hayward
<i>E. chrysanthemi</i> SR 147 (420 S)	DAFS	Grass	Australia	Quinn/Hayward
<i>E. chrysanthemi</i> SR 148 (430)	DAFS	Grass	Australia	Quinn/Hayward
<i>E. chrysanthemi</i> SR 149 (15/184)	DAFS	Sugar cane	Australia	Quinn/Hayward
<i>Erwinia cypripedii</i> EC 155	ICPB	Cypripedium	USA	Starr/Ark (4773)
<i>E. cypripedii</i> EC 160	ICPB	Cypripedium	USA	Starr/Ark
<i>Erwinia dieffenbachiae</i> ED 102	ICPB	Dieffenbachia	USA	Starr/McFadden
<i>E. dieffenbachiae</i> ED 103	ICPB	Dieffenbachia	USA	Starr/McFadden
<i>Erwinia cytolytica</i> EC 207	ICPB	Dahlia	Roumania	Starr/Lazar
<i>Erwinia dissolvens</i> ED 105	ICPB	Corn	Arkansas	Starr/NRRL B-154/Wal-dee W-4/Rosen
<i>Erwinia herbicola</i> 3163	ICPB	Oat seed		Starr/Graham/Webley (D11)
<i>E. herbicola</i> 14589	ATCC		USA	
<i>E. herbicola</i> 486-71	CDC	Animal	USA	—
<i>E. herbicola</i> 1645-71	CDC	Animal	USA	—
<i>E. herbicola</i> 1744-71	CDC	Animal	USA	—
<i>Erwinia lathyri</i> EL 102	ICPB	Banana (<i>Musa</i> sp.)	Israel	Starr/Volcani
<i>Erwinia nigrifluens</i> EN 104	ICPB	Persian walnut	California	Starr/Wilson
<i>Erwinia nimipressuralis</i> EN 1	ICPB	Elm	U.S.A.	Starr/Carter
<i>Erwinia oleraceae</i> EO 1	ICPB	Cauliflower	Canada	Starr/Davey/Harrison
<i>Erwinia quercina</i> EQ 101	ICPB	Live Oak	U.S.A.	Starr/Hildebrand
<i>Erwinia rubrifaciens</i> ER 105	ICPB	Persian Walnut	U.S.A.	Starr/Wilson (540)
<i>Erwinia salicis</i> ES 102	ICPB	Cricket-bat-willow	England	Starr/Lelliott/Dowson
<i>Erwinia solanisapra</i> ES 101	ICPB	Potato	U.S.A.	Starr/Smith
<i>E. solanisapra</i> 8061	ATCC	Potato	U.S.A.	Ark/Stapp
<i>Erwinia tracheiphila</i> 27004	ATCC	Cucumber		Starr/Dowson/Jones
<i>Erwinia uredovora</i> 19321 (NCPBP 800) (ICPB XU 102)	ATCC	Cereal rusts	New Zealand	Hayward
<i>Escherichia coli</i> K-12	WASH		U.S.A.	
<i>Klebsiella pneumoniae</i> 2	CDC	Animal	U.S.A.	—
<i>Proteus mirabilis</i> 1	WRAIR		U.S.A.	Falkow
<i>Salmonella typhimurium</i> LT2	NIH		U.S.A.	Theodore
<i>Serratia marcescens</i>	USA	Corwin		
<i>Shigella flexneri</i> 2a 24570	WRAIR	Animal	U.S.A.	Falkow
<i>Xanthomonas uredovora</i> XU 104 (<i>E. uredovora</i>)	ICPB	Uredia of <i>P. graminis</i>	Hungary	Starr/Klement (0.049)

^a Abbreviations: CDC, Center for Disease Control, Atlanta, Ga.; ICPB, International Collection of Phytopathogenic Bacteria, University of California, Davis, Calif.; ATCC, American Type Culture Collection, Rockville, Md.; UW, University of Wisconsin (A. Kelman, Department of Plant Pathology), Madison, Wis.; WRAIR, Walter Reed Army Institute of Research, Washington, D.C.; WASH, University of Washington, Seattle, Wash.; IPV-BO, Istituto di Patologia Vegetale, Bologna, Italy; DAFS, Department of Agriculture and Fisheries for Scotland, East Craigs, Edinburgh, Scotland; SR numbers indicate the University of Wisconsin culture collection of A. Kelman.

^b —, CDC cultures are from Ewing, Hermann, or Martin.

cultures were very similar. The buoyant density of seven cultures was 1.7125 g/cm³ (53.6 mol% G+C) and of two cultures it was 1.7130 g/cm³ (54.1 mol% G+C). Thus, the G+C contents of Indian isolates do not differ significantly from those recorded for isolates from Egypt and the United States. These G+C determinations differed significantly from those of *P. (Erwinia) carotovorum* (52.1 mol%), *P. (Erwinia) chrysanthemi* (56 mol%), and *Erwinia dissolvens* (56.3 mol%).

On the basis of their findings with respect to *P. (Erwinia) chrysanthemi*, Starr and Mandel (22) questioned the wisdom of considering *P. (Erwinia) chrysanthemi* to be a variety of *P. (Erwinia) carotovorum* (5, 8) or of reducing all of the soft-rot producers and related species of *Pectobacterium (Erwinia)* to one species, *P. (Erwinia) carotovorum*, as Martinec and Kocur (13) suggested.

MATERIALS AND METHODS

Information regarding the strains used in this study is listed in Table 1. Organisms were maintained on meat extract agar or brain heart infusion agar. Routine cultivation of organisms was carried out in brain heart infusion broth or nutrient broth. The medium employed for labeling cells with ³²PO₄ has been described (2). Cells were cultivated on a dry-air rotary shaker held at 23 to 25°C, 30°C, or 37°C, depending on the growth characteristics of the various species (4). The preparation of both labeled and unlabeled DNA and the shearing of DNA has been described (2, 4).

In all cases, 0.1 μg of sheared, ³²PO₄-labeled DNA (specific activity = 7 × 10⁴ to 1.5 × 10⁵ cpm/μg) per ml was reacted with 150 μg of sheared, unlabeled DNA per ml of 0.28 M phosphate buffer (equimolar mixture of Na₂HPO₄ and NaH₂PO₄, pH 6.8) for 16 h at 60 or 75°C. At an incubation temperature of 60°C, optimal reassociation of related sequences occurred, whereas nonspecific duplex formation was precluded. At 75°C reassociation was restricted to highly similar related sequences as judged by duplex thermal stability.

After incubation, samples were diluted to 0.14 M phosphate buffer, and sodium dodecyl sulfate was added to 0.4%. Using a batch procedure (3), reassociated DNA was separated from unreacted DNA on hydroxyapatite. Unreacted DNA did not bind to hydroxyapatite. The reassociated DNA bound to hydroxyapatite was eluted in a series of washes with 0.4 M phosphate buffer.

A homologous control reaction plus nine heterologous samples were processed simultaneously. Observed homologous DNA reassociation was arbitrarily deemed 100%. The reassociation of heterologous DNA reactions was determined relative to that of homologous DNA. Heterologous reactions were carried out three to four times at 60°C and twice at 75°C.

RESULTS

SR80 is the corn stalk rot bacterium used as the reference strain in this study. Relatedness data obtained from heterologous DNA reassociation reactions with ³²PO₄-labeled DNA from SR80 are presented in Table 2. The six corn stalk rot bacteria tested included isolates from North Carolina and Wisconsin, as well as from Egypt and India. DNAs from these organisms showed an average of 89% relatedness to DNA from SR80 in reactions carried out at 60°C. The thermal stability of these DNA reassociation products was 3 to 4°C lower than the thermal stability of a homologous SR80 DNA duplex. We assumed that each 1°C decrease in thermal stability reflected the presence of approximately 1% unpaired (noncomplementary) bases within the DNA duplex (12). Therefore, some 3 to 4% of unpaired bases (base sequence divergence) were present in the DNA sequences shared by corn stalk rot bacteria. Relatedness of SR80 to other corn stalk rot bacteria remained high (85%) when reactions were carried out at 75°C, at which temperature only highly complementary duplexes are stable.

SR80 shows 68 to 83% relatedness to strains of *P. chrysanthemi*, *P. cytolytica*, *P. dieffenbachiae* and *P. carotovorum* f. sp. *parthenii*. As other authors have recognized (5), *P. (Erwinia) cytolytica*, *P. (Erwinia) dieffenbachiae*, and *P. (Erwinia) carotovorum* f. sp. *parthenii* are synonyms of *P. (Erwinia) chrysanthemi*. We have used the separate species designation throughout this paper, rather than assume, a priori, that they are all synonymous with *P. chrysanthemi*. The average extent of reaction was 74%, and about 10% unpaired bases were present in the related sequences. Most of these related sequences (54% average relative binding) were stable in reactions carried out at 75°C. The average relatedness of SR80 to all other pectobacteria was 37%. These related sequences had diverged to a point where they now contained 10 to 20% unpaired bases. Further evidence of extensive divergence was apparent from the fact that only one-fourth of the DNA duplexes formed at 60°C were stable in 75°C reactions.

Figure 1 shows the distribution of relatedness values of SR80 to corn stalk rot bacteria, to *P. chrysanthemi* and related organisms, and to other pectobacteria. Each of the distribution-of-relatedness plots is symmetrically distributed around the mode relatedness value despite the relatively small number of strains tested. There is little overlap between the distribution of relatedness among corn stalk rot bacteria and that of SR80 with *P. chrysanthemi* and related strains. The distribution of relatedness

TABLE 2. Reassociation of DNA from corn stalk rot bacterium SR 80

Source of unlabeled DNA	% Relative binding at 60°C	ΔT_{me} ^a at 60°C	% Relative binding at 75°C
Corn stalk rot bacterium SR 80	100	—	100
Corn stalk rot bacterium SR 56	91	3.9	91
Corn stalk rot bacterium SR 84	91	4.1	93
Corn stalk rot bacterium SR 98	88	3.0	88
Corn stalk rot bacterium EC 210	86	3.9	86
Corn stalk rot bacterium EM 107	87	3.2	79
Avg	89	3.6	87
<i>P. chrysanthemi</i> fluidal SR 31	68	9.5	52
<i>P. chrysanthemi</i> EC 16	83	8.1	65
<i>P. chrysanthemi</i> SR 32	72	9.2	58
<i>P. chrysanthemi</i> EC 205	77	10.2	65
<i>P. chrysanthemi</i> 11662	76	9.1	57
<i>P. chrysanthemi</i> 11663	73	10.0	54
<i>P. cytolytica</i> EC 207	79	11.3	46
<i>P. dieffenbachiae</i> ED 102	62	9.0	42
<i>P. dieffenbachiae</i> ED 103	78	11.1	51
<i>P. carotovorum</i> f. sp. <i>parthenii</i> EC 115	73	9.6	48
Avg	74	9.7	54
<i>P. carotovorum</i> 495	39	18.0	11
<i>P. carotovorum</i> 15359	37	16.6	10
<i>P. aroideae</i> EA 13	39	18.0	10
<i>P. aroideae</i> 12286	37	18.1	10
<i>P. aroideae</i> 25207	42	18.7	10
<i>P. atroseptica</i> EA 112	38	18.2	10
<i>P. atroseptica</i> 7404	35	17.9	9
<i>P. oleraceae</i> EO 1	42	18.4	8
<i>P. solanisapra</i> ES 101	39	17.9	8
<i>P. solanisapra</i> 8061	33	17.0	7
<i>P. carnegieana</i> EC 223	33	19.5	6
<i>P. carnegieana</i> 13452	27	18.1	8
<i>P. cypripedii</i> EC 155	39	17.5	5
Avg	37	18.0	9
<i>E. amylovora</i> 7400	30	18.8	6
<i>E. nigrifluens</i> EN 104	39	16.9	11
<i>E. quercina</i> EQ 101	34	17.6	6
<i>E. rubrifaciens</i> ER 105	39	16.1	10
<i>E. salicis</i> ES 102	37	16.7	7
<i>E. tracheiphila</i> 27004	23	19.9	2
Avg	34	17.7	7
<i>E. ananas</i> EA 181	30	17.6	6
<i>E. herbicola</i> 3163	28	16.5	3
<i>E. lathyri</i> EL 102	29	19.0	4
<i>X. uredovorus</i> XU 104	27	19.0	3
Avg	29	18.0	4
<i>E. dissolvens</i> ED 105	31	18.5	5
<i>E. nimipressuralis</i> EN 1	31	18.6	4
Avg	31	18.6	5
<i>E. coli</i> K-12	27	18.5	5
<i>S. flexneri</i> 2a 24570	28	17.9	4
<i>S. typhimurium</i> LT2	26	16.9	3
<i>E. aerogenes</i> banana strain 3151	28	18.0	5
<i>E. aerogenes</i> 1627-66	27	17.1	4
<i>C. freundii</i> 5118-60	30	18.1	4

TABLE 2—Continued

Source of unlabeled DNA	% Relative binding at 60°C	$\Delta T_{m(e)}$ ^a at 60°C	% Relative binding at 75°C
<i>S. marcescens</i> SM6 W2	34	16.8	7
Avg	29	17.6	5
<i>E. tarda</i> 1126-64	19	18.9	
<i>P. mirabilis</i> 1	9	20.9	
Avg	14	19.8	

^a $T_{m(e)}$, Thermal elution midpoint; that temperature at which 50% of the DNA bound to hydroxyapatite (at the 60 or 75°C incubation temperature) is eluted. $\Delta T_{m(e)}$ is the decrease in $T_{m(e)}$ between heterologous DNA reactions and the homologous corn stalk rot bacterium SR80 DNA reaction. The average extent of reassociation of corn stalk rot bacterium SR80 DNA was 85% at both the 60 and 75°C incubation temperatures. Corn stalk rot bacterium DNA had an average $T_{m(e)}$ of 92.0°C.

FIG. 1. Distribution of relatedness values between corn stalk rot organism strains and other pectobacteria. Values shown were obtained from 60°C reactions in which the reference strain was corn stalk rot bacterium strain SR80.

of SR80 to other pectobacteria is substantially shifted towards lower levels of reaction.

A similar extent of relatedness, and divergence within related sequences of DNA, is present between SR80 and strains of the *Erwinia amylovora* group of species. Slightly decreased relatedness (29% average) is found between SR80 and presumed members of *Enterobacter agglomerans* (*Erwinia herbicola*, *E. lathyr*, *E. ananas*, and *Xanthomonas uredovorus*; D. J. Brenner, unpublished data, and see reference 7). SR80 also shares one-third of its genome with members of other genera of enterobacteria, including *Escherichia*, *Shigella*, *Salmonella*, *Enterobacter*, *Citrobacter*, and *Serratia*. The lowest level of relatedness to SR80 is exhibited by *Edwardsiella tarda* (19%) and *P. mirabilis* (9%).

Strains of *P. chrysanthemi* that are not pathogenic to corn (including *P. dieffenbachiae*, *P. carotovorum* f. sp. *parthenii*, and *P. cytolytica*; see reference 4) exhibit comparable levels of relatedness to *P. carotovorum* (40%, reference 4) and to corn stalk rot bacteria (Table 2). Labeled DNA from strain SR32 originally isolated by Burkholder from chrysanthemum was used

to determine the degree of heterogeneity among these strains of *P. chrysanthemi* (Table 3). *P. chrysanthemi* strains fall into two groups based on relatedness to SR32. One group is 80 to 98% related at 60°C with little or no evidence of unpaired bases within related sequences and little or no decrease in relative relatedness at 75°C. The second group exhibits 65% relatedness to SR32, with some 8% unpaired bases. At 75°C the level of relative reassociation remains at 75% of the level seen at 60°C.

Relatedness of *P. chrysanthemi* SR32 to corn stalk rot bacteria at 60°C is 55 to 60% with about 9% unpaired bases. This level of relatedness falls to 35 to 40% in reactions carried out at 75°C. Other pectobacteria, erwiniae, and members of other genera in *Enterobacteriaceae* exhibit 20 to 25% relatedness to SR32. The related sequences show 13.5 to 17.5% divergence, and at 75°C relatedness falls to less than 10%.

Reference DNA from *P. dieffenbachiae* (ED 103) was used to assess relatedness in the *P. chrysanthemi* group that exhibits the lower level of relatedness to *P. chrysanthemi* SR32. These data (Table 4) confirm the existence of two levels of relatedness among *P. chrysanthemi*.

TABLE 3. Reassociation of DNA from *Pectobacterium chrysanthemi* SR 32

Source of unlabeled DNA	% Relative binding at 60°C	$\Delta T_{m(e)}$ ^a at 60°C	% Relative binding at 75°C
<i>Pectobacterium chrysanthemi</i> SR 32	100		100
<i>P. chrysanthemi</i> 11662	98	+1.0	98
<i>P. chrysanthemi</i> 11663	97	+0.5	
<i>P. chrysanthemi</i> EC 16	97	+0.5	94
<i>Pectobacterium carotovorum</i> f. sp. <i>parthenii</i> EC 115	82	1.3	75
<i>Pectobacterium dieffenbachiae</i> ED 103	65	8.3	47
<i>Pectobacterium cytolytica</i> EC 207	64	8.6	46
<i>P. chrysanthemi</i> EC 205	64	8.7	48
Corn stalk rot bacterium EM 107	59	9.2	
Corn stalk rot bacterium SR 56	58	9.7	41
Corn stalk rot bacterium EC 210	57	9.4	35
Corn stalk rot bacterium SR 80	56	8.7	34
Corn stalk rot bacterium SR 84	55	8.7	40
Corn stalk rot bacterium SR 98	54	9.1	35
<i>P. carotovorum</i> 495	26	15.0	8
<i>Pectobacterium carnegiana</i> EC 186	24	16.1	9
<i>Pectobacterium cypripedii</i> EC 155	19	17.5	
<i>P. cypripedii</i> EC 160	17	17.5	
<i>Erwinia salicis</i> ES 102	26	15.1	5
<i>Erwinia amylovora</i> EA 178	19	16.5	
<i>Erwinia tracheiphila</i> 27004	15	17.4	
<i>Erwinia herbicola</i> 14589	21	15.6	
<i>E. herbicola</i> 486-51	20	15.7	
<i>E. herbicola</i> 1645-71	20	16.6	
<i>E. herbicola</i> 1744-71	19	15.5	
<i>Erwinia uredovora</i> 19321	19	13.6	
<i>Escherichia coli</i> K-12	19	16.2	
<i>Arizona hinshawii</i> 1	18	16.2	

^a See Table 2 for definition of $T_{m(e)}$. The average extent of reassociation of *P. chrysanthemi* SR 32 DNA was 74% at 60°C and 79% at 75°C. The average $T_{m(e)}$ was 91.6°C.

themi strains. Relatedness of corn stalk rot bacteria and other enterobacteria to ED 103 is similar to the relatedness of these organisms to SR32.

An additional set of isolates from corn (Italy, Israel, Rhodesia), grasses (Australia), and sugar cane (Australia) was examined in some detail to assess further the taxonomic position of corn stalk rot bacteria and determine whether strains isolated from other monocotyledons could be differentiated on the basis of DNA relatedness. Reactions completed with DNA from two corn isolates from Italy, *P. chrysanthemi* SR143 and SR140 (Table 5), revealed that 98% of SR143 DNA is related to 88% of SR140 DNA, implying that the genome size of SR140 is some 10% larger than that of SR143. These organisms generally exhibit comparable levels of relatedness to all test organisms and thus will be considered together.

The additional corn isolates of *P. chrysanthemi*

are also highly interrelated (84 to 103%). These isolates are similarly highly related to the corn stalk rot bacteria, EM 107 from North Carolina, SR56 from Egypt, and SR80 from Wisconsin (87 to 93%), and to the *P. chrysanthemi* strains isolated from grasses (83 to 86%). In all of these reactions the thermal stability of DNA heteroduplexes is close to that of the homologous SR143 DNA duplex. The sugar cane isolate, *P. chrysanthemi* SR149, exhibited 65% relatedness to the corn isolates. In this case the $\Delta T_{m(e)}$ is 9°C. *P. carotovorum* SR144, which was isolated from corn, exhibited the same level of relatedness to SR143 as shown by *P. carotovorum* 495. Physiological tests by A. Kelman (personal communication) indicate that SR144 should not be considered to be in the *P. chrysanthemi* group. Relatedness of SR140 and SR143 to typical *P. chrysanthemi* strains isolated from dahlia, dieffenbachia, or chrysanthemum is similar to that observed previously

TABLE 4. Reassociation of DNA from *Pectobacterium dieffenbachiae* ED 103

Source of unlabeled DNA	% Relative binding at 60°C	$\Delta T_{m(e)}$ at 60°C ^a
<i>Pectobacterium dieffenbachiae</i> ED 103	100	—
<i>P. dieffenbachiae</i> ED 102	80	0.1
<i>Pectobacterium chrysanthemi</i> EC 205	79	3.0
<i>Pectobacterium cytolytica</i> EC 207	73	5.9
<i>P. chrysanthemi</i> SR 32	59	10.5
<i>P. chrysanthemi</i> EC 16	59	9.8
<i>P. chrysanthemi</i> 11662	59	10.3
<i>Pectobacterium carotovorum</i> f. sp. <i>parthenii</i> EC 115	56	10.4
<i>P. chrysanthemi</i> 11663	55	10.9
Corn stalk rot bacterium EM 107	60	10.1
Corn stalk rot bacterium SR 84	59	10.3
Corn stalk rot bacterium SR 56	59	10.7
Corn stalk rot bacterium SR 80	58	9.4
Corn stalk rot bacterium SR 98	56	9.8
Corn stalk rot bacterium EC 210	54	10.5
<i>P. carotovorum</i> 495	27	15.5
<i>Pectobacterium carnegieana</i> EC 186	24	16.6
<i>Pectobacterium cypripedii</i> EC 155	22	16.2
<i>P. cypripedii</i> EC 160	22	16.2
<i>Erwinia salicis</i> ES 102	27	15.8
<i>Erwinia amylovora</i> EA 178	18	17.0
<i>Erwinia tracheiphila</i> 27004	15	16.6
<i>Erwinia herbicola</i> 1744-71	22	16.6
<i>E. herbicola</i> 486-51	21	16.0
<i>E. herbicola</i> 1645-71	21	16.5
<i>E. herbicola</i> 14589	21	16.0
<i>Erwinia uredovora</i> 19321	20	16.2
<i>Escherichia coli</i> K-12	19	15.9
<i>Arizona hinshawii</i> 1	18	16.4

^a See Table 2 for definition of $T_{m(e)}$. The average extent of reassociation of *P. dieffenbachiae* ED 103 DNA was 74%, and the average $T_{m(e)}$ was 91.8°C.

between corn stalk rot bacteria and *P. chrysanthemi* (63 to 73%, Tables 2 to 4).

Reactions using labeled DNA from the grass isolate, SR147, and the sugar cane isolate, SR149, are shown in Table 6. The grass isolates are essentially identical. They show 78 to 87% relatedness to the corn stalk rot bacteria and the *P. chrysanthemi* strains isolated from corn. DNA from the grass isolate SR147 is 60 to 68% related to typical strains of *P. chrysanthemi*. The sugar cane isolate exhibits a uniform level of relatedness (57 to 67%, 9 to 11.4°C $\Delta T_{m(e)}$) to corn and grass isolates and to typical *P. chrysanthemi*.

DISCUSSION

Investigators in many parts of the world (1, 9-11, 14-16, 19, 23, 25) have clearly established the causal relationship of a *Pectobacterium Erwinia*) to stalk and top rot disease of corn (*Zea*

mays L.). Sabet (19) was the first to describe the pathogen in detail. He found it to be physiologically similar to *P. (Erwinia) carotovorum*, and concluded that it should be classified as *Erwinia carotovora* f. sp. *zeae*.

Rosen (17, 18) previously described a stalk rot disease of corn caused by a bacterium subsequently classified as *Erwinia dissolvens* (Rosen) Burkholder. Since *E. dissolvens* was non-motile, hydrolyzed starch, produced indole and did not degrade pectin or liquefy gelatin, Waldee (24) concluded that this organism was not a species of *Erwinia*, and he placed it in the genus *Enterobacter* (*Aerobacter*). Rosen's original cultures (17, 18) are no longer available. The available cultures of *E. dissolvens* are not phytopathogenic and are considered to be closely related to *Enterobacter cloacae* (6, 8). DNA from *E. dissolvens* was shown to be most closely related to DNA from *Enterobacter cloacae* (Brenner, unpublished observation). Corn stalk rot bacterium SR80 is not closely related to *E. dissolvens* (31%).

Kelman et al. (11) showed that corn stalk rot pathogens differed significantly from other soft rot-producing species, but were reluctant to make a species designation. In contrast Sabet et al. (20) concluded that there was no justification for establishing a new species for the corn stalk rot bacterium and that *E. carotovora* f. sp. *zeae* would be the most appropriate designation.

Graham (8), after reviewing the complex taxonomic problems within the soft rot bacteria, supported the proposal of Waldee (24) to establish a separate genus, *Pectobacterium*, for these organisms. He also questioned the validity of the term *forma specialis* as a means of designating specific taxa within pectobacteria. This was due to the difficulty of determining the complete host range of a given soft rot pathogen and the implication of host restriction inherent in the term. Dye (5) supported the continued inclusion of soft rot species in *Erwinia*. He concluded that corn stalk rot pathogens were too similar to *P. (Erwinia) chrysanthemi* to merit their separation. In Dye's taxonomic scheme, *E. carotovora* var *chrysanthemi* was synonymous with Sabet's designation of *E. carotovora* f. sp. *zeae* (19).

Rangarajan and Chakravarti (16) compared isolates from Egypt, the United States, and India. They agreed with Graham (8) that the designation "*forma specialis zeae*" should be abandoned, and proposed that corn stalk rot bacteria be referred to as a strain of *E. carotovora*.

The six corn stalk rot bacteria initially tested, which include isolates from the United

TABLE 5. Reassociation of DNA from *Pectobacterium chrysanthemi* strains SR 143 and SR 140

Source of unlabeled DNA	Plant host	<i>P. chrysanthemi</i> SR 143 ^a		<i>P. chrysanthemi</i> SR 140 ^c : % Relative binding at 60°C
		% Relative binding at 60°C	$\Delta T_{m(e)}$ ^b at 60°C	
<i>Pectobacterium chrysanthemi</i> SR 143	Corn	100		88
<i>P. chrysanthemi</i> SR 140	Corn	98	1.0	100
<i>P. chrysanthemi</i> SR 142	Corn	103	+0.7	92
<i>P. chrysanthemi</i> SR 145	Corn	89	3.1	85
<i>P. chrysanthemi</i> SR 141	Corn	84	2.8	86
<i>P. chrysanthemi</i> SR 146	Grass	86	3.4	84
<i>P. chrysanthemi</i> SR 147	Grass	85	3.5	85
<i>P. chrysanthemi</i> SR 148	Grass	84	3.3	83
<i>P. chrysanthemi</i> SR 149	Sugar cane	66	9.0	64
<i>Pectobacterium carotovorum</i> SR 144	Corn	30	15.6	
Corn stalk rot bacterium EM 107	Corn	93	0.2	98
Corn stalk rot bacterium SR 80	Corn	89	1.9	90
Corn stalk rot bacterium SR 56	Corn	87	2.9	89
<i>P. chrysanthemi</i> SR 32	Chrysanthemum	68		73
<i>P. carotovorum</i> f. sp. <i>parthenii</i> EC 115		67	11.1	70
<i>P. chrysanthemi</i> EC 205	Chrysanthemum	66	10.7	65
<i>Pectobacterium dieffenbachiae</i> ED 103	Dieffenbachia	66	11.0	67
<i>P. chrysanthemi</i> 11662	Chrysanthemum	65	9.9	65
<i>Pectobacterium cytolytica</i> EC 207	Dahlia	63	10.9	64
<i>P. carotovorum</i> 495		31		
<i>Pectobacterium cypripedii</i> EC 155		24		
<i>Erwinia amylovora</i> EA 178		21		
<i>Klebsiella pneumoniae</i> 2		24		
<i>Enterobacter aerogenes</i> 1627-66		22		
<i>Enterobacter cloacae</i> 1347-71		22		
<i>Arizona hinshawii</i> 1		21		
<i>Serratia marcescens</i> SM6		20		
<i>Escherichia coli</i> K-12		18		

^a Source of ³²PO₄-labeled DNA.

^b See Table 2 for definition of $T_{m(e)}$. Reassociation of DNA from strains SR 143 and SR 140 was 80 and 74%, respectively. The average $T_{m(e)}$ of DNA from strain SR 143 was 91.8°C.

States, Egypt, and India, exhibited DNA relatedness typical of a group of strains representing one bacterial species. This observation can be extended to the second series of corn isolates from Italy and Rhodesia (SR140-SR143, SR145). The grass isolates from Australia are virtually identical, and are not distinguishable from corn stalk rot bacteria based on DNA relatedness.

Depending upon the reference strain employed, corn stalk rot bacteria and isolates from grass exhibited 60 to 80% relatedness to *P. chrysanthemi* isolates from dahlia, dieffenbachia, or chrysanthemum (Tables 2, 5, 6). In reciprocal reactions where *P. chrysanthemi* (includes *P. dieffenbachiae*) was the source of reference DNA, relatedness to corn stalk rot bacteria was 54 to 60% (Tables 3, 4). These data imply genome size differences; for example,

72% of SR80 DNA is related to 56% of *P. chrysanthemi* SR32 DNA, and 78% of SR80 DNA is related to 58% of *P. dieffenbachiae* ED 103 DNA. Thus, the SR80 genome is apparently some 30 to 35% smaller than those of SR32 and ED 103. These genome size differences probably also account for the higher binding of SR80 to other *pectobacteria* and *erwiniae* (23 to 42%) than that seen with SR32 (15 to 26%) or ED 103 (15 to 30%).

P. carotovorum is 40% related to corn stalk rot bacteria (Table 2, and see reference 2). Furthermore, corn stalk rot bacterium-*P. carotovorum* DNA duplexes contain almost twice as many unpaired bases as corn stalk rot bacterium-*P. chrysanthemi* DNA duplexes. These DNA relatedness data, together with cultural, physiological, and pathogenic differences (10), indicate that the corn stalk rot bacterium is

TABLE 6. Reassociation of DNA from *Pectobacterium chrysanthemi* strains SR 147 and SR 149 at 60°C

Source of unlabeled DNA	Plant host	<i>P. chrysanthemi</i> SR 147 ^a		<i>P. chrysanthemi</i> SR 149 ^a	
		% Relative binding	$\Delta T_{m(e)}$ ^b	% Relative binding	$\Delta T_{m(e)}$
<i>Pectobacterium chrysanthemi</i> SR 147	Grass	100		67	11.1
<i>P. chrysanthemi</i> SR 148	Grass	99	+1.2	65	11.4
<i>P. chrysanthemi</i> SR 146	Grass	99	+0.6	65	10.3
<i>P. chrysanthemi</i> SR 149	Sugar cane	63	9.5	100	
<i>P. chrysanthemi</i> SR 141	Corn	87	2.3	67	10.4
<i>P. chrysanthemi</i> SR 140	Corn	86	4.4	65	11.4
<i>P. chrysanthemi</i> SR 145	Corn	85	1.7	68	10.5
<i>P. chrysanthemi</i> SR 142	Corn	83	2.4	64	11.0
<i>P. chrysanthemi</i> 143	Corn	78	3.6	57	10.5
<i>Pectobacterium carotovorum</i> SR 144	Corn	33	15.8		
Corn stalk rot bacterium SR 56	Corn	80	2.1	65	9.8
Corn stalk rot bacterium SR 80	Corn	79	3.6	66	9.8
Corn stalk rot bacterium EM 107	Corn	78	3.6	66	10.0
<i>P. chrysanthemi</i> SR 32	Chrysanthemum	68		63	10.5
<i>P. chrysanthemi</i> 11662	Chrysanthemum	65	9.7	60	9.7
<i>P. chrysanthemi</i> EC 205	Chrysanthemum	64	10.4	63	9.3
<i>P. carotovorum</i> f. sp. <i>parthenii</i> EC 115	Guayule	63	10.8	64	10.9
<i>Pectobacterium dieffenbachiae</i> ED 103	Dieffenbachia	61	11.1	66	9.0
<i>Pectobacterium cytolytica</i> EC 207	Dahlia	60	10.5	64	9.4
<i>P. carotovorum</i> 495		26			
<i>Pectobacterium cypripedii</i> EC 155		20			
<i>Erwinia amylovora</i> EA 178		19			
<i>Enterobacter cloacae</i> 1347-71		22			
<i>Enterobacter aerogenes</i> 1627-66		21			
<i>Klebsiella pneumoniae</i> 2		21			
<i>Arizona hinshawii</i> 1		19			
<i>Serratia marcescens</i> SM6		18			
<i>Escherichia coli</i> K-12		15			

^a Source of ³²P₀₄-labeled DNA.^b See Table 2 for definition of $T_{m(e)}$. Reassociation of DNA from strains SR 147 and SR 149 was 79 and 82%, respectively. The average $T_{m(e)}$ of these DNA duplexes was 92.3°C (SR 147) and 91.9°C (SR 149).

neither a forma specialis of *P. (Erwinia) carotovorum*, as suggested by Sabet (19, 20), Hingorani et al. (9), and Pauer (15), nor a strain of *P. (Erwinia) carotovorum* (16, 23, 25).

The sugar cane isolate, SR149, was about 65% related to corn stalk rot bacteria, as well as to isolates from grass, and strains of *P. chrysanthemi*. *P. chrysanthemi*, therefore, appears to contain four distinguishable hybridization groups.

Group 1 contains *P. chrysanthemi* SR32 and highly related strains 11662, 11663, EC 16, and EC 115. These organisms were all isolated from chrysanthemums, except EC 115, which was isolated from guayule. Guayule (*Parthenium*) is, like chrysanthemum, a member of the *Compositae*.

Group 2 contains *P. chrysanthemi* strains

(including *P. cytolytica* and *P. dieffenbachiae*) ED 102, ED 103, EC 205, and EC 207. These strains were isolated from dahlia or dieffenbachia.

Group 3 contains all *P. chrysanthemi* isolates from corn or grass.

Group 4 contains SR149, the only isolate from sugar cane.

The host range of all of these organisms must be determined before a precise nomenclatural designation is proposed. If the host patterns shown above prove to be correct and mutually exclusive, a reasonably objective taxonomic scheme could be based upon the patho-variety concept.

Such a procedure is logical and practical for both taxonomists and plant pathologists. Data obtained thus far (Kelman, unpublished obser-

vations) indicate that this system of classification is valid for the corn pathogens. At this time, we feel justified in proposing *Pectobacterium chrysanthemi* pathovar *zeae* for strains pathogenic for corn and/or grasses.

Another pathovar of *P. chrysanthemi* was recently described (21). These organisms cause a severe wilt and root rot in the sweet potato.

ACKNOWLEDGMENT

We thank Arthur Kelman for suggesting this study and for supplying many of the strains. We are deeply indebted to him for continued help, insight, and a critical review of the manuscript. Dr. Kelman's investigation of the phytopathogenicity of corn stalk rot bacteria will be published separately.

REPRINT REQUESTS

Address reprint requests to: Dr. Don J. Brenner, Enteric Section, Center for Disease Control, Atlanta, GA 30333.

LITERATURE CITED

1. Anilkumar, T. B., and B. P. Chakravarti. 1960. The nutrition of two strains of *Erwinia carotovora*, casual organism of stalk rot of maize in India. *Abl. Bakteriologie* 11: 708-712.
2. Brenner, D. J., G. R. Fanning, K. E. Johnson, R. V. Citarella, and S. Falkow. 1969. Polynucleotide sequence relationships among members of the *Enterobacteriaceae*. *J. Bacteriol.* 98:637-650.
3. Brenner, D. J., G. R. Fanning, A. Rake, and K. E. Johnson. 1969. A batch procedure for thermal elution of DNA from hydroxyapatite. *Anal. Biochem.* 28:447-460.
4. Brenner, D. J., A. G. Steigerwalt, G. V. Miklos, and G. R. Fanning. 1973. Deoxyribonucleic acid relatedness among erwiniae and other enterobacteria. I. The soft-rot erwiniae or *Pectobacterium* group. *Int. J. Syst. Bacteriol.* 23:205-216.
5. Dye, D. 1969. A taxonomic study of the genus *Erwinia*. II. The *Carotovora* group. *N. Z. J. Sci.* 12:81-97.
6. Dye, D. 1969. A taxonomic study of the genus *Erwinia*. IV. Atypical erwiniae. *N. Z. J. Sci.* 12:833-839.
7. Ewing, W. H., and M. A. Fife. 1971. *Enterobacter agglomerans*. Center for Disease Control, Atlanta, Ga.
8. Graham, D. C. 1964. Taxonomy of the soft rot coliform bacteria. *Annu. Rev. Phytopathol.* 2:13-43.
9. Hingorani, M. K., V. J. Grant, and N. J. Singh. 1959. *Erwinia carotovora* f. sp. *zeae*, a destructive pathogen of maize in India. *Indian Phytopathol.* 12:151-157.
10. Hoppe, P. E., and A. Kelman. 1969. Bacteriol top and stalk rot disease of corn in Wisconsin. *Plant Dis. Reporter* 53:66-70.
11. Kelman, A., L. H. Person, and T. T. Herbert. 1957. A bacterial stalk rot of irrigated corn in North Carolina. *Plant Dis. Reporter* 41:798-802.
12. Laird, C. D., B. L. McConaughy, and B. J. McCarthy. 1969. On the rate of fixation of nucleotide substitutions in evolution. *Nature (London)* 224:149-154.
13. Martinec, T., and M. Kocur. 1963. Taxonomická studie rodu *Erwinia*. *Folia (Biol. 2) Fac. Sci. Nat. Univ. Purkynianae Brunensis* 4:1-163.
14. Mazzucchi, U. 1972. A bacterial stalk rot of maize (*Zea mays* L.) in Emilia. *Phytopathol. Mediterr.* 11:1-5.
15. Pauer, G. D. 1964. *Erwinia carotovora* f. sp. *zeae*, the bacterium causing stalk rot of maize in the Republic of South Africa. *S. Afr. J. Agric. Sci.* 7:581-582.
16. Rangarajan, M., and B. P. Chakravarti. 1971. *Erwinia carotovora* (Jones) Holland as the inciting agent of cornstalk rot in India. *Phytopathol. Mediterr.* 10:41-45.
17. Rosen, H. R. 1922. The bacterial pathogen of corn stalk rot. *Phytopathology* 12:497-499.
18. Rosen, H. R. 1926. Bacterial stalk rot of corn. *Phytopathology* 16:241-267.
19. Sabet, K. A. 1954. A new bacterial disease of maize in Egypt. *Emp. J. Exp. Agric.* 84:65-67.
20. Sabet, K. A., A. S. Samra, and H. A. El-Shafey. 1964. On the systemic position of the coliform bacteria responsible for stalk rot of maize. *Ann. Appl. Biol.* 53:267-274.
21. Schaad, N. W., and D. Brenner. 1977. A bacterial wilt and root rot of sweet potato caused by *Erwinia chrysanthemi*. *Phytopathology* 67:302-308.
22. Starr, M. P., and M. Mandel. 1969. DNA base composition and taxonomy of phytopathogenic and other enterobacteria. *J. Gen. Microbiol.* 56:113-123.
23. Volcani, Z. 1961. A maize stalk disease caused by a strain of *Erwinia carotovora* in Israel. *Israel J. Agric. Res.* 11:179-183.
24. Waldee, E. L. 1945. Comparative studies of some peritrichous phytopathogenic bacteria. *Iowa State Coll. J. Sci.* 19:435-484.
25. Zachos, D. R., G. G. Panagopoulos, and S. A. Makris. 1963. A disease of maize in Greece caused by *Erwinia carotovora* (Jones). *Holland Ann. Inst. Phytopathol. Benaki* 5:288-293.