Comparative Base Sequence Homologies of the Deoxyribonucleic Acids of *Erwinia* Species and Other *Enterobacteriaceae*

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Twenty-six strains representing ten species of *Erwinia* were examined by deoxyribonucleic acid (DNA)-DNA hybridization techniques for relatedness to each other as well as to other members of the family *Enterobacteriaceae*, including the genera *Escherichia*, *Salmonella*, *Klebsiella*, *Shigella*, and *Proteus*. DNA homologies among the different *Erwinia* species were in most cases below 50%, even under nonstringent annealing conditions. In most instances DNA homologies between *Erwinia* species and *Escherichia*, *Salmonella*, *Klebsiella*, and *Shigella* species showed about the same amount of relatedness as in *Erwinia*-to-*Erwinia* combinations. The molecular hybridization data indicate that the genus *Erwinia* is a loosely composed group of bacteria that often have no greater affinities to each other than to other enteric bacteria. Furthermore, the data do not support some of the previously proposed taxonomic divisions within the genus *Erwinia*.

The genus *Erwinia*, established in 1917 in honor of the phytopathologist Erwin F. Smith by a nomenclature committee headed by Winslow et al. (25), was initially based heavily on plant pathogenicity with little emphasis on other phenotypic characters. Thereafter many new species were placed in this genus almost solely on the basis of host parasitism. Taxonomic subdivisions within the genus *Erwinia* are currently based on morphological, biochemical, and physiological tests, but the emphasis on plant parasitism has led to the creation of numerous "new" species. Martinec and Kocur (21) suggested that many *Erwinia* spp. be lumped into two groups, with *E. amylovora* and *E. carotovora* being the typical species of the groups. Dye (1st Int. Congr. Plant Pathol., London, July 1968, Abstr. 51) recommended consolidating the numerous *Erwinia* species into five species (*E. amylovora*, *E. carotovora*, *E. herbicola*, *E. uredovorus*, and *E. stewartii*). Ewing and Fife (11) placed *E. herbicola*, *E. lathyri*, and other erwinias of this kind into the genus *Enterobacter* under a single species, *E. agglomerans*. Physiological-biochemical tests are useful pragmatically but are not always successful in establishing systematic relationships. We believe that deoxyribonucleic acid (DNA) re-association measurements are more accurate and realistic from a strictly taxonomic point of

view. The suggestion has been made previously that the genus *Erwinia* be dissolved and the members distributed among the enterobacteria (23). On the basis of heterogeneous DNA base compositions, Starr and Mandel (24) suggested that one can only be agnostic about the status of the genus. We have, therefore, attempted to study the validity of the genus *Erwinia*, its relationship to the other genera in the family *Enterobacteriaceae*, and the proposed subdivision of the genus on the basis of DNA sequence homologies. Brenner et al. (5) recently reported DNA-homology data for 5 strains of *Erwinia* and 11 strains of other organisms belonging to the family *Enterobacteriaceae*. Their data indicate a low but significant homology between the erwinias and between the erwinias and other members of the family *Enterobacteriaceae*. Our data, obtained by a hybridization technique different from that used by Brenner et al., confirm this same trend with a wider range of species and strains.

MATERIALS AND METHODS

Bacterial cultures. The source of cultures is listed in Table 1. Bacteria were grown with shaking in medium 523 broth (14) and harvested by centrifugation during the late log phase, washed in BE buffer (0.05 M

sodium borate, Na₂ ethylenediaminetetraacetic acid, pH 9.0), and stored in a frozen state (-20 C).

Labeling of DNA. For ³²PO₄-labeled DNA, cells were grown in low-phosphate medium (12). Carrier-free ³²PO₄-phosphoric acid (Schwartz-Mann, Orangeburg, N.J.) was added to the medium (1 µCi/ml of medium) and the cells were then harvested in the late log phase. The same procedure applied for ¹⁴C-DNA except that cells were grown in medium 925 salts (17) plus 0.7% glucose, 1 mM deoxyguanosine, nicotinic acid, (50 µg/ml), and 0.05% Casamino Acids. ¹⁴C-uridine (50 mCi/mole) plus ¹⁴C-adenine (52 mCi/mole) (Schwartz-Mann) were added to give a final concentration of 0.5 µCi/ml of medium. Specific activities were from 10,000 to 15,000 counts per min per µg for ³²PO₄-labeled DNA and 6,500 counts per min per µg for ¹⁴C-labeled DNA.

DNA preparation. DNA was prepared by a modification of Marmur's technique (19). *Erwinia* spp. were lysed by a combination of freeze-thawing, lysozyme, and sodium dodecyl sulfate treatments, in that order. The lysed cells were extracted twice with phenol and twice with chloroform-octanol (24:1, v/v), and the DNA was then precipitated with 0.6 vol of freezing isopropanol. Treatments with pancreatic ribonuclease (preheated at 80 C for 10 min; 50 µg/ml, 37 C, 2 hr) and Pronase (predigested for 2 hr at 37 C; 1 mg/ml; 37 C, 6 to 12 hr) were followed by additional chloroform-octanol extractions and repeated precipitations with chilled isopropanol. In a few cases where persistent polysaccharide contamination was a problem, Kirby's methoxyethanol extraction procedure (15) was followed. The final preparation of DNA was dissolved in 0.1 × SSC (1 × SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0). The purity of each DNA preparation was checked by determination of absorbancy ratios, 260/280 nm (1.85 to 1.90) and 260/230 nm (2.2 to 2.3), by sharp melting (*T_m*) curves which included hyperchromicity values of 35 to 40%, and in some cases by appropriate *T_m* values.

For purifying sheared labeled DNA, the methods of Berns and Thomas (1) as modified by Brenner and Cowie (2) were used. DNA was sheared by repeated passage of the solution through syringes with 27 and 32 gauge needles. For competitive hybridization, competitor DNA (1 mg/ml) was sheared at 20,000 psi in a French pressure cell. Sheared DNA fragments in 0.1 M sodium phosphate buffer (pH 6.8) were collected on hydroxyapatite, eluted with 0.5 M of the same buffer, and dialyzed extensively against 0.1 × SSC. The ³²P-DNA solutions were passed through metrical filter discs (Gelman Instrument Co., Ann Arbor, Mich.) to remove any possible ³²PO₄ (as polyphosphates) contamination.

Melting profiles and calculation of percent GC. The procedures of Marmur and Doty (20) were used to calculate the mole percent guanine plus cytosine (GC) in the DNA from *T_m* values. DNA was diluted in 0.1 × SSC to approximately 50 µg/ml, and a deoxyadenosine or deoxyguanosine solution in 0.1 × SSC of equal absorbancy at 260 nm was used for a reference. The sample was melted in a Beckman Acta III dual-beam recording spectrophotometer equipped with matching thermal cuvette holders with inductive

TABLE 1. *Culture sources*

Organism	Source
<i>Erwinia amylovora</i> FB1	M. Schroth, UC, Berkeley
<i>E. amylovora</i> ICPB EA 167 ^a	M. P. Starr, UC, Davis
<i>E. amylovora</i> ICPB EA 178	M. P. Starr, UC, Davis
<i>E. carotovora</i> 3D31	R. H. Segall, USDA, Orlando, Fla.
<i>E. carotovora</i> EC201	M. Schroth, UC, Berkeley
<i>E. carotovora</i> 3D36	C. I. Kado, UC, Davis
<i>E. nigrifluens</i> 130-C	E. E. Wilson, UC, Davis
<i>E. nigrifluens</i> 131-B	E. E. Wilson, UC, Davis
<i>E. nigrifluens</i> ICPB EN104	M. P. Starr, UC, Davis
<i>E. nigrifluens</i> 5D313	J. M. Gardner, UC, Davis
<i>E. rubrifaciens</i> 6D34	E. E. Wilson, UC, Davis
<i>E. rubrifaciens</i> 6D321	E. E. Wilson, UC, Davis
<i>E. rubrifaciens</i> 6D325	J. M. Gardner, UC, Davis
<i>E. rubrifaciens</i> 6D326	J. M. Gardner, UC, Davis
<i>E. rubrifaciens</i> 6D327	J. M. Gardner, UC, Davis
<i>E. rhapontici</i> 8D31	E. E. Wilson, UC, Davis
<i>E. aroideae</i> ICPB EA14	M. P. Starr, UC, Davis
<i>E. nimipressuralis</i> 12D3	E. E. Wilson, UC, Davis
<i>E. dissolvens</i> 14D3	E. E. Wilson, UC, Davis
<i>E. uredovora</i> ICPB XU 102	M. P. Starr, UC, Davis
<i>E. herbicola</i> ICPB 3161	M. P. Starr, UC, Davis
<i>E. herbicola</i> Y46	A. K. Chatterjee, UC, Davis
<i>E. herbicola</i> 25D35	J. M. Gardner, UC, Davis
<i>E. herbicola</i> 25D36	C. I. Kado, UC, Davis
<i>E. herbicola</i> ICPB 2950	M. P. Starr, UC, Davis
<i>E. herbicola</i> ICPB 2858A	M. P. Starr, UC, Davis
<i>Escherichia coli</i> AB 295	A. J. Clark, UC, Berkeley
<i>E. coli</i> Q13	A. J. Wahba, U. Sherbrooke Quebec
<i>Klebsiella aerogenes</i> 19D3	D. G. Gilchrist, UC, Davis
<i>K. pneumoniae</i> M5A1	R. C. Valentine, UC, Berkeley
<i>Salmonella typhimurium</i> LT-2	J. L. Ingraham, UC, Davis
<i>Shigella dysenteriae</i> NCPB 32	National Center for Primate Research, U. C. Davis
<i>S. flexneri</i> NCPB 27	National Center for Primate Research, U. C. Davis
<i>Proteus mirabilis</i> 27D3	E. L. Biberstein, UC, Davis
<i>Agrobacterium tumefaciens</i> 1D135	C. I. Kado, UC, Davis
<i>Pseudomonas viridiflava</i> PV-4	P. C. Pecknold, UC, Davis

^a ICPB, International Collection of Phytopathogenic Bacteria (ICPB), Department of Bacteriology, University of California (UC), Davis.

stirrers and with a linear temperature programmer connected to an accurately calibrated platinum thermocouple probe ($\pm 0.001^\circ\text{C}$) positioned directly in the DNA solution during melting. (The programmer, matching cuvettes, and thermocouple were redesigned for improved accuracy through the cooperation of Beckman Instruments, Fullerton, Calif.). The temperature was increased at a rate of 0.5°C per min through the range of DNA melting. Melting curves were recorded on a Honeywell x-y recorder.

Determination of homology. Denatured DNA ($600\ \mu\text{g}$ in $100\ \text{ml}$ of $5 \times \text{SSC}$) was immobilized on 47-mm diameter nitrocellulose filters (Schleicher and Schuell, B-6; reference 13). The amount of DNA retained on 6-mm filter discs (cut from 47-mm filters) was checked by diphenylamine (6) and found to be approximately $15\ \mu\text{g}/\text{disc}$ in all cases. Filters were placed in glass vials (15 by $45\ \text{mm}$) and pretreated with the preincubation mixture of Denhardt (8) for about $6\ \text{hr}$. The liquid was removed with a vacuum pipette. DNA duplex formation in formamide-SSC was done by the method described by McConaughy et al. (22). A 0.1-ml amount of $20 \times \text{SSC}$, $0.2\ \text{ml}$ of 99% formamide (Mallinckrodt reagent), and $0.1\ \text{ml}$ of 0.5 to $1.0\ \mu\text{g}$ of sheared, heat-denatured, labeled DNA were added to the vials (i.e., $15:1$ filter DNA to radioactive DNA). Sheared, labeled DNA was heat-denatured by heating at 100°C for $10\ \text{min}$ and immersing in an ice bath. The vials were shaken in a temperature-controlled water bath for approximately $48\ \text{hr}$. Hybridization was performed at temperatures ranging from 30 to 48°C (about 18°C to 36°C below T_m of labeled DNA), depending on the experiment. Each sample was run in triplicate. The filters were washed twice with $0.5\ \text{ml}$ of 48% formamide- $5 \times \text{SSC}$ and once in $4\ \text{ml}$ of $3 \times \text{SSC}$ at the hybridization temperature, transferred to scintillation vials, dried, and counted in a nonaqueous scintillation fluid ($4\ \text{g}$ of diphenyloxazole and $0.1\ \text{g}$ of 1,4-bis(2-[5-phenyloxazolyl])-benzene/1 toluene). From 40 to 50% of the homologous radioactive DNA was bound by filter DNA. The optimal time for maximum reassociation was about $50\ \text{hr}$ (Fig. 1, inset). Filters lost no detectable DNA during this period as tested by the diphenylamine reaction. In competition experiments a mixture containing $0.1\ \text{ml}$ of $30 \times \text{SSC}$, $0.3\ \text{ml}$ of 98% formamide, $0.15\ \text{ml}$ of sheared, heat-denatured, competitor DNA (total $150\ \mu\text{g}$), and $0.05\ \text{ml}$ of radioactive DNA ($1\ \mu\text{g}$) was incubated with the filters. Percent competition was calculated as follows:

$$\% \text{ competition} = \frac{[\text{cpm bound (homologous)}] - [\text{cpm bound in presence of heterologous DNA}]}{[\text{cpm bound (homologous)}] - [\text{cpm bound in presence of homologous DNA}]} \times 100.$$

It was shown by a competition curve that $150\ \mu\text{g}$ of homologous DNA competitor was near the saturation level of this system (Fig. 1).

Melting of filter hybrids. Washed radioactive filters in $1.0\ \text{ml}$ of $0.1 \times \text{SSC}$ or $5 \times \text{SSC}$ - 48% formamide were heated at various temperatures in an equilibrated linear thermal gradient bar (14) for $30\ \text{min}$. Duplicate tubes containing the filters were then rapidly cooled in

ice, and the filters were washed, dried, and counted as above.

RESULTS

Percent GC. The mole percent GC values of the DNA (Table 2) indicate that a fairly wide range of values (from 51 to 57%) exists within the genus *Erwinia* and that a similar range exists among the other organisms of the family *Enterobacteriaceae* tested. From these data alone, the *erwinias* cannot be distinguished from other members of the family. Furthermore, no correlation was observed when the differences in percent GC and DNA relatedness to test species were plotted.

DNA homologies. Radioactive DNA from four *Erwinia* species (*E. amylovora*, *E. herbicola*, *E. carotovora*, and *E. rubrifaciens*) was hybridized to filter-bound DNA from other *erwinias* as well as from other organisms in the

FIG. 1. Competition between *Erwinia rubrifaciens* 6D321 DNA and 6D321 ^{14}C -DNA for hybridization to homologous filter DNA. Hybridization without competitor was set at 100% . Hybridization with or without competitor DNA was done at 45°C in $0.6\ \text{ml}$ of $5 \times \text{SSC}$ in 48% formamide. Inset: Time course for direct hybridization of 6D321 ^{14}C -DNA to homologous filter DNA. Hybridization was done at 41°C in $0.4\ \text{ml}$ $5 \times \text{SSC}$ in 48% formamide. Hybridization at $50\ \text{hr}$ was set at 100% . Hybridization was extended to $160\ \text{hr}$ to insure that a single plateau was reached by $50\ \text{hr}$.

TABLE 2. Percent GC values for DNA preparations and DNA homologies between *Erwinia rubrifaciens* and other members of the family *Enterobacteriaceae*

Source of unlabeled DNA	Percent GC ^b	Relative binding (%) ^a					
		Direct hybridization at (C)				Competitive hybridization at (C)	
		30	37	41	45	36	45
<i>Erwinia rubrifaciens</i> ER 103	51.7(51.5) ^c	100	100	100	100	100	100
<i>E. rubrifaciens</i> 6D327	51.7		103		100		100
<i>E. rubrifaciens</i> 6D325	51.3	104			100		
<i>E. rubrifaciens</i> 6D326	52.4	100		91	99		103
<i>E. rubrifaciens</i> 6D34	52.1	95		91	92	98	95
<i>E. nigrifluens</i> EN104	56.3	37		35	37	53	34
<i>E. nigrifluens</i> 130-C	56.7	44		27	32		
<i>E. nigrifluens</i> 131-B		48		36	41	67	
<i>E. nigrifluens</i> 5D313	56.3		42		37		33
<i>E. amylovora</i> EA167	54.1	36	24	17	15		
<i>E. amylovora</i> FB1	54.9	27		14	9		
<i>E. amylovora</i> EA178		36	23		13		12
<i>E. carotovora</i> 3D31	52.1	36		26			17
<i>E. carotovora</i> EC201	53.9	33		24	17	45	
<i>E. carotovora</i> 3D36		39			23		17
<i>E. herbicola</i> ICPB 3161	54.3	32		13	10		10
<i>E. herbicola</i> Y46	54.8	32		15	13		
<i>E. herbicola</i> 25D35	53.3		34		19		
<i>E. herbicola</i> 25D36	55.2	35		19	17		
<i>E. herbicola</i> 2950					19		9
<i>E. herbicola</i> 2858A					15		
<i>E. rhapontici</i> 8D31	53.2		28		19		
<i>E. aroideae</i> EA14	53.2	27		14	9		19
<i>E. nimipressuralis</i> 12D3	55.3	29		18	15		12
<i>E. dissolvens</i> 14D3	53.5				19		15
<i>E. uredovora</i> XU102	53.5		29		16		10
<i>Escherichia coli</i> AB295	51.9	38			16		
<i>E. coli</i> Q13		40			22		
<i>Klebsiella aerogenes</i> 19D3	58.2		31	15	20		14
<i>K. pneumoniae</i> M5A1	58.2	37			19		
<i>Salmonella typhimurium</i> LT-2	51.9	26		19	20		10
<i>Proteus mirabilis</i> 27D3	42.2				2		2
<i>Shigella flexneri</i> 27					6		10
<i>Shigella dysenteriae</i> 32					6		10
<i>Agrobacterium tumefaciens</i> ^d 1D135	60.5 ^e	13			0		1
<i>Pseudomonas viridiflava</i> ^d PV-4					0		
Calf thymus ^d					0		

^a For direct hybridization, 1.0 µg of ¹⁴C-labeled DNA in a total volume of 0.4 ml of 5 × SSC-48% formamide was incubated with 15 µg of unlabeled DNA fixed on 6-mm filters for 48 hr. For competitive hybridization, the reaction volume was increased to 0.6 ml, and 150 µg of sheared-unlabeled DNA was added to the reaction mixture.

^b Percent GC determined by optical thermal denaturation (20). Each value represents an average based on usually three or more determinations. GC variations from the mean were less than 0.6%.

^c Percent GC determined by melting radioactive filter hybrid (average of two determinations).

^d Not a member of the family *Enterobacteriaceae*.

^e From reference (14).

family. The hybridization data show that the degree of relative homologies was under 40% in most cases and clustered in this range (Tables 2-5). Incubation temperatures ranged from the stringent reannealing temperature of 48 C

(ca. 18 C below T_m) to the nonstringent reannealing temperature of 30 C (ca. 36 C below T_m). Above 37 C (ca. 29 C below T_m) there was no significant hybridization with DNA from *Agrobacterium tumefaciens* or *Pseudomonas*

viridiflava, which are not members of the family *Enterobacteriaceae*, or with calf thymus, indicating that hybridization conditions were specific. At the nonstringent temperature of 30 C, 8 to 13% homology was obtained with *A. tumefaciens*. Even at this temperature (36 C below T_m) most homologies among all other bacteria remained below 50%. Results of competitive hybridization experiments agreed with those of direct hybridization, and percent homologies, with one exception, fell in the same range as those of direct hybridization (10 to 50%). The only comparatively high hybridization values (ca. 40%) were between *E. rubrifaciens* and *E. nigrifluens* under stringent reannealing conditions (21 C below T_m). Competitive hybridization gave values as high as 67% for this combination at relatively nonstringent temperatures (36 C below T_m). Comparative DNA homologies of other erwinias and members of the family with *E. herbicola*, *E. amylovora*, *E. carotovora*, and *E. rubrifaciens* were clearly so low and tightly clustered that no meaningful differences were possible to define. *Proteus mirabilis* was the only exception within the family, since no homology was detected with *E. rubrifaciens*. This would be expected based on a 10% difference in GC.

Mispairing in the DNA hybrids was estimated by melting the filter-bound radioactive hybrids. Homologous hybrids produced sharp helix-to-coil transitions with T_m values similar to those obtained by the optical melting technique. On the other hand, heterologous hybrids melted over a relatively broad temperature range (Fig. 2 and 3). The thermal instability of the heterologous hybrids on filters indicates that even under moderately stringent hybridization conditions (22 C below T_m) mispaired bases were present in the hybrids. According to Laird et al. (16), a 1% alteration in base pairing will decrease the T_m by 0.7 C, although this value can vary by a factor of 2 (5). By this estimate (Fig. 2 and 3), about 8% and 14% base mispairing is present in the *E. nigrifluens*-*E. rubrifaciens* and *E. herbicola*-*E. rubrifaciens* DNA hybrids, respectively.

DISCUSSION

Our studies show that no distinct relationships are evident between the *Erwinia* species and between erwinias and other members of the family *Enterobacteriaceae* based on GC values

TABLE 3. DNA homologies between *Erwinia herbicola* and other members of the family *Enterobacteriaceae*

Source of unlabeled DNA	Relative binding (%) ^a				
	Direct hybridization at (C)			Competitive hybridization at (C)	
	39	40	48	39	46
<i>Erwinia herbicola</i> ICPB 3161	100	100	100	100	100
<i>E. herbicola</i> 25D31	96	101	96	96	
<i>E. herbicola</i> 25D36	120	110	114	99	
<i>E. rubrifaciens</i> 6D34	25	24	7	19	
<i>E. rubrifaciens</i> 6Γ ⁻ 21	16	16		15	5
<i>E. rubrifaciens</i> 6D326	19	11	7		
<i>E. amylovora</i> FB1	24	12	6		6
<i>E. amylovora</i> EA167	36	35	16		13
<i>E. carotovora</i> 3D31		16	5		
<i>E. carotovora</i> EC201	31	29	19		17
<i>E. nigrifluens</i> 130-C		14			5
<i>E. nigrifluens</i> 131-B	16	16	12	16	21
<i>E. nigrifluens</i> FN104	26	29	6		16
<i>E. nimipressuralis</i> 12D3	29	35	17	16	
<i>E. dissolvens</i> 14D3	33	35	8	15	21
<i>Escherichia coli</i> AB295	36		11	14	14
<i>Salmonella typhimurium</i> LT-2	39	29	11		
<i>Klebsiella aerogenes</i> 19D3	29	28	10	29	
<i>Agrobacterium tumefaciens</i> ^b 1D135	2		0	0	
Calf thymus ^b		0			

^aMethods were as described in Table 2 except that ³²P-₄-DNA was used.

^b Not a member of the family *Enterobacteriaceae*.

TABLE 4. DNA homologies between *Erwinia amylovora* and other members of the family *Enterobacteriaceae*

Source of unlabeled DNA	Relative binding (%) ^a				
	Direct hybridization at (C)			Competitive hybridization at (C)	
	30	39	44	35	46
<i>Erwinia amylovora</i> FB1	100	100	100	100	100
<i>E. amylovora</i> EA167	106	100	101	100	
<i>E. carotovora</i> 3D31		9	13	30	
<i>E. carotovora</i> EC201	49		8	44	28
<i>E. rubrifaciens</i> 6D34	35	24	14	20	22
<i>E. rubrifaciens</i> 6D321		24	15		
<i>E. rubrifaciens</i> 6D326	30	17	13		
<i>E. rubrifaciens</i> 6D325	40				
<i>E. herbicola</i> ICPB 3161	37		27	34	
<i>E. herbicola</i> Y46	45	35	22	36	22
<i>E. herbicola</i> 25D36	45	32	32	43	
<i>E. nigrifluens</i> 130-C	28	17		34	18
<i>E. nigrifluens</i> 131-B		27	13	34	10
<i>E. nigrifluens</i> EN104	34	19	15	41	10
<i>E. nimipressuralis</i> 12D3	34	23		36	
<i>E. dissolvens</i> 14D3		18	19	32	15
<i>Escherichia coli</i> 9D3	34	22	12	23	14
<i>Salmonella typhimurium</i> LT-2	57	26	19		18
<i>Klebsiella aerogenes</i> 19D3		18	19	37	
<i>K. pneumoniae</i> M5A1	42				
<i>Agrobacterium tumefaciens</i> ^b 1D135	8	1	0		
Calf thymus ^b		0			

^a Methods were as described in Table 2 except that ³²PO₄-DNA was used.^b Not a member of the family *Enterobacteriaceae*.

in combination with DNA-homology data. Attempts to group the *erwinias* into a few species by using GC contents alone is obviously misleading. For example, the GC values of 51% for *E. rubrifaciens* and 56% for *E. nigrifluens* are distant, and yet the DNA homology is closer between these two organisms than any other combination and was the highest encountered (32 to 41%). Starr and Mandel (24) noted that percent GC clusters among certain *erwinias*, notably the soft-rot-*E. carotovora* group, correlate well with groupings based on physiological data. However, they admit that such a correlation may be purely fortuitous. Instead, the range of GC values may indicate significant disparity among the *erwinias* and other organisms in the family. Irrespective of the GC data, our DNA reassociation experiments show very little homology between *Erwinia* species and the other organisms of the family tested. Even if some small differences in homologies were shown to be statistically different (e.g., 1 to 10% differences), the taxonomic significance of such small differ-

TABLE 5. DNA homologies between *Erwinia carotovora* 3D32 and other members of the family *Enterobacteriaceae*

Source of unlabeled DNA	Relative binding at 35 C (%) ^a
<i>Erwinia carotovora</i> EC201	100
<i>E. amylovora</i> FB1	12
<i>E. amylovora</i> EA167	24
<i>E. herbicola</i> ICPB 3161	14
<i>E. herbicola</i> Y46	29
<i>E. rubrifaciens</i> 6D321	25
<i>E. rubricaciens</i> 6D326	27
<i>E. nigrifluens</i> 131-B	45
<i>E. nigrifluens</i> EN104	34
<i>E. nimipressuralis</i> 12D3	27
<i>E. dissolvens</i> 14D3	25
<i>Escherichia coli</i> AB295	26
<i>Klebsiella aerogenes</i> 19D3	36
<i>Salmonella typhimurium</i> LT-2	33
<i>Agrobacterium tumefaciens</i> ^b 1D135	5

^a Direct hybridization done as described in Table 2 except that ³²PO₄-DNA was used.^b Not a member of the family *Enterobacteriaceae*.

FIG. 2. Thermal denaturation of homologous and heterologous DNA duplexes by *Erwinia rubrifaciens* 6D321 DNA:6D321 DNA (○). *E. rubrifaciens* 6D321:*E. nigrifluens* 5D313 DNA (●). Melting was done in $5 \times \text{SSC}$ -48% formamide. Sheared, denatured 6D321 ^3H -DNA (0.8 μg) in a total volume of 0.4 ml of $5 \times \text{SSC}$ -48% formamide was reacted with homologous 6D321 DNA and heterologous 5D313 DNA filters at 45°C. Filters were then washed and incubated in 1.0 ml of $5 \times \text{SSC}$ -48% formamide at various temperatures for 30 min.

ences would be doubtful. More intensive re-association techniques are necessary to study the family *Enterobacteriaceae*. For example, dissecting and enriching the genomes into related and unrelated sequences (e.g., using multiple competitors) may amplify genetic differences. Nevertheless, the *erwinias* appear to have certain genetic relatedness to other members of the family. Chatterjee and Starr (7) recently showed that drug-resistance factors and sex factors can be passed from *Shigella dysenteriae* and *Escherichia coli*, respectively, to *erwinias*. Our hybridization data suggest that regions of the *Erwinia* genome lend to such interchanges because of the small but significant sequence homologies. Kado et al. (*in press*) demonstrated that polyacrylamide-gel electropherograms of 70S ribosomal proteins from *E. rubrifaciens*, *E. nigrifluens*, *E. carotovora*, and *E. amylovora* are strikingly similar to those of *E. coli* and *Salmonella typhimurium*, whereas *A. tumefaciens* ribosomal protein patterns are quite different. These similarities in ribosomal structural proteins also imply a significant degree of relatedness and conservation of ribosomal genes. Our data suggest that a small but significant common core of relatedness is present throughout the genomes of the mem-

bers of the family *Enterobacteriaceae* including the *Erwinia* spp. tested here.

Many artificial groupings of *Erwinia* spp. were devised almost purely from phenotypic differences (e.g., pigment production, pathogenicity, sugar utilization, etc.). Some previous proposals to lump species of *Erwinia* are not supported by our data. For example, the proposal by Dye (9) that *E. rubrifaciens* and *E. nigrifluens* be considered as subspecies of *E. amylovora* is clearly artificial because of the low (13 to 15%) DNA homologies with *E. amylovora* obtained in our experiments under stringent conditions. DNA-homology data of Brenner et al. (5) also demonstrate that the soft-rot organisms, *E. aroideae* (considered identical to *E. carotovora* by Dye [10]) and *E. carnegiana*, were only 46% and 5% related, respectively, to *E. carotovora*. Ewing and Fife (11) proposed that *E. herbicola* be transferred to *Enterobacter* as *E. agglomerans* on the basis of numerous physiological tests. This change in nomenclature is not supported by the 10% relative homology we found between *E. herbicola* and *Klebsiella aerogenes* (*Enterobacter* is a member of the tribe *Klebsiellae*), regardless of the fact that homologies between *E. herbicola* and other *erwinias* were not much higher. These authors also recommended that *E. amylovora* be removed from the family *Enterobacteriaceae*; our homology data indicate on the other hand that *E. amylovora* has 12 to 19% relative genetic homologies with other members of the

FIG. 3. Thermal denaturation of homologous and heterologous DNA duplexes by *Erwinia rubrifaciens* 6D321 DNA: *E. rubrifaciens* 6D321 DNA (●). *E. rubrifaciens* 6D321 DNA:*E. herbicola* ICPB 3161 DNA (○). Conditions for hybrid formation and melting are the same as those described in Fig. 2.

family under relatively stringent annealing conditions. From these data, it appears that the taxonomic proposals cited above and made on the basis of physiological tests have been misleading.

The heterogeneity of DNA-DNA and DNA-ribonucleic acid homologies among *Shigella*, *Salmonella*, *Escherichia*, and *Proteus* groups was evident from the studies of Brenner et al. (2-4). Most of the inter- and intragenus relationships were below 50% on the basis of DNA homology as confirmed here. Under stringent hybridization conditions, as much as 25% homology divergence was observed among strains of *E. coli*; a 23% divergence in genome size was also found among these strains. This suggests that much evolutionary (i.e., polynucleotide) divergence has taken place among organisms in the family *Enterobacteriaceae*. The present data showing a small but significant homology between all erwinias tested also suggest evolutionary divergence among *Erwinia* spp. *E. rubrifaciens* and *E. nigrifluens* are distinct and specific pathogens of Persian walnut and show the highest degree of DNA homology in our studies. This may suggest that parallel evolution is reflected by common polynucleotide sequences of these two organisms. Whether the small segments of homologous base sequences found between two given species are common to many or all of the erwinias or of the other organisms in the family must be tested by other DNA-reassociation techniques.

It is not clear at what point on the homology scale the lines for intraspecies and intragenus relatedness should be drawn. How far, for instance, can a genus or species diverge in evolution and still retain its nomenclature? Obviously, such rules must be made with both arbitrary and pragmatic considerations. Whether the genus *Erwinia* should be dissolved and its members reshuffled among the other genera of the family *Enterobacteriaceae* will be an academic as well as a pragmatic exercise. Although genetic homologies provide the most valid basis for classification, it may be that, in quoting Mandel (18), "Like good cigars, a good species and a good classification is one which satisfies."

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