Deoxyribonucleic Acid Relatedness Among *Erwiniae* and Other *Enterobacteriaceae*: the Gall, Wilt, and Dry-Necrosis Organisms (Genus *Erwinia* Winslow et al., *sensu stricto*)

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Species representing the genus *Erwinia* (*sensu stricto*, the gall-, wilt-, and dry-necrosis-producing phytopathogens) were examined for relatedness to each other, to pectobacteria, and to other members of the family *Enterobacteriaceae*. The technique employed to show relatedness was deoxyribonucleic acid (DNA) hybridization and hydroxyapatite chromatography. The results indicate that all *erwiniae* are significantly related to *Pectobacterium* and to other enterobacteria. DNA relatedness reveals six species of *Erwinia*: *E. amylovora*, *E. nigrifluens*, *E. quercina*, *E. rubrifaciens*, *E. salicis*, and *E. tracheiphila*. *E. nigrifluens*, *E. rubrifaciens*, and *E. salicis* show close intrageneric relatedness, whereas the other species exhibit as much relatedness to members of other genera as to the other *Erwinia* species. The data support the two-genus concept of *Erwinia* and *Pectobacterium*.

As presently constituted in *Bergey's Manual* (1), the genus *Erwinia* Winslow et al. contains an extremely diverse assortment of organisms, all of which are supposed phytopathogens. Organisms that cause wilts, galls, or dry necrosis, but not soft rot, comprise the genus *Erwinia sensu stricto*. It has been suggested that organisms causing soft-rot diseases be placed in the genus *Pectobacterium* Waldee (23). We have followed this suggestion in earlier publications (5; D. J. Brenner, A. G. Steigerwalt, G. R. Fanning, and A. Kelman, unpublished data) and shall continue to do so in this report.

Since the latest edition of *Bergey's Manual*, which was published in 1957, several investigators have studied taxonomic parameters in *erwiniae*. Although often disagreeing on specific taxonomic and nomenclatural designations, the investigators usually agree that the tribe *Erwinieae* contains four kinds of organisms. In addition to phytopathogenic *erwiniae* and pectobacteria, there are yellow-pigmented organisms which in many cases are of doubtful phytopathogenicity. These organisms are often isolated from animals and man. Ewing and Fife (12) placed them in *Enterobacter* as *E. agglomerans*. Finally, existing strains of *Erwinia dissolvens* and *Erwinia nimipressuralis* are thought of as either grossly atypical *erwiniae* or

members of *Klebsiella* or *Enterobacter*. Unpublished studies from our laboratory indicate that one of our *E. dissolvens* strains belongs to *Klebsiella*. The other strains of *E. dissolvens* and *E. nimipressuralis* are most closely related to *Enterobacter cloacae*.

Two of our previous studies dealt with members of *Pectobacterium* (5; Brenner, Steigerwalt, Fanning, and Kelman, unpublished data). This report treats the phytopathogenic species of *Erwinia sensu stricto*, as typified by *Erwinia amylovora*. Interspecific deoxyribonucleic acid reassociation reactions were carried out to assess molecular relatedness among *Erwinia* species and between these organisms and other enterobacteria.

MATERIALS AND METHODS

Bacterial strains. The strains used in this study are listed in Table 1.

Methods. The general protocol for the experiments reported here is to choose a series of organisms as reference strains, label their deoxyribonucleic acid (DNA) with $^{32}\text{PO}_4$, shear and denature the labeled DNA, and allow it to reassociate with a 1500-fold excess of similarly prepared, unlabeled DNAs from the homologous organism and other organisms of interest (4). The concentration of labeled DNA (0.1 $\mu\text{g}/\text{ml}$) is small enough to prevent significant reassociation of

TABLE 1. *Bacterial strains studied*

Organism	Source ^a
<i>Citrobacter diversus</i> 1066-70	CDC
<i>C. freundii</i> 460-61	CDC
<i>Edwardsiella tarda</i> 1483-59	CDC
<i>E. tarda</i> 3888-64	CDC
<i>Enterobacter aerogenes</i> 1627-66	CDC
<i>E. agglomerans</i> (<i>Erwinia herbicola</i>) EH 103	ICPB
<i>E. agglomerans</i> (<i>E. herbicola</i>) 2553	ICPB
<i>E. agglomerans</i> (<i>E. herbicola</i>) 14589	ATCC
<i>E. agglomerans</i> (<i>E. lathyri</i>) EL 102	ICPB
<i>E. agglomerans</i> (<i>E. uredovora</i>) 19321	ATCC
<i>E. agglomerans</i> 486-51	CDC
<i>E. agglomerans</i> 1645-71	CDC
<i>E. cloacae</i> 256-4	NIH
<i>E. cloacae</i> 1347-71	CDC
<i>E. hafniae</i> 4360-67	CDC
<i>Erwinia amylovora</i> EA 169	ICPB
<i>E. amylovora</i> EA 178	ICPB
<i>E. amylovora</i> 7400	ATCC
<i>E. amylovora</i> 7401	ATCC
<i>E. amylovora</i> 15357	ATCC
<i>E. amylovora</i> 15580	ATCC
<i>E. amylovora</i> 19382	ATCC
<i>E. nigrifluens</i> EN 101	ICPB
<i>E. nigrifluens</i> EN 104	ICPB
<i>E. quercina</i> EQ 101	ICPB
<i>E. quercina</i> EQ 102	ICPB
<i>E. rubrifaciens</i> ER 103	ICPB
<i>E. rubrifaciens</i> ER 105	ICPB
<i>E. salicis</i> ES 102	ICPB
<i>E. salicis</i> 15712	ATCC
<i>E. tracheiphila</i> 27004	ATCC
<i>Escherichia coli</i> K-12	U. of Washington
<i>Klebsiella pneumoniae</i> 2	CDC
<i>Pectobacterium carnegiana</i> EC 186	ICPB
<i>P. carotovorum</i> 495	ATCC
<i>P. chrysanthemi</i> SR 32	U. of Wisconsin
<i>P. chrysanthemi</i> pathovar <i>zeae</i> SR 80	U. of Wisconsin
<i>P. cypripedii</i> EC 155	ICPB
<i>P. rhapontici</i> ER 106	ICPB
<i>Proteus mirabilis</i> 1	WRAIR
Red Mouth Bacterium	CDC
<i>Salmonella choleraesuis</i> 36	CDC
<i>S. typhimurium</i> LT2	NIH ¹
<i>Serratia marcescens</i> SM6	WRAIR

^a CDC, Center for Disease Control, Atlanta, Ga., from W. H. Ewing and G. Hermann; ICPB, International Collection of Phytopathogenic Bacteria, Univ. of California, Davis, from M. P. Starr; ATCC, American Type Culture Collection, Rockville, Md.; NIH, National Institutes of Health, Bethesda, Md., from V. J. Brenner; NIH¹ from T. Theodore; WRAIR, Walter Reed Army Institute of Research.

labeled DNA fragments with one another during the course of incubation. The concentration of unlabeled DNA is sufficiently high to insure complete reassociation of labeled DNA with unlabeled DNA (70 to 97% in practice). When the reaction is completed, reassociated (also termed hybridized, duplexed, or related) DNA is separated from unreacted DNA on hydroxyapatite (HA). At the phosphate concentration employed, double-stranded DNA binds to HA, but single-stranded DNA is eluted. The DNA bound to HA is eluted either by raising the phosphate concentration or by increasing the temperature until the DNA is denatured and elutes as single-stranded DNA (3). Two parameters of relatedness may be assessed. Percentage of relative relatedness is determined by normalizing the amount of DNA bound to HA in heterologous reactions to that bound in the homologous reaction where labeled and unlabeled DNA are from the same organism. Divergence in reassociated nucleotide sequences can be approximated by comparing the thermal stability of heterologous DNA duplexes with that of the homologous DNA duplex. Since each 1% of unpaired bases results in an approximate 1 C decrease in thermal stability (17), the decrease in heterologous DNA duplex stability may be expressed as percentage of divergence. These methods have been discussed in detail previously (4).

The comparison of relative relatedness values obtained at an optimal and stringent reassociation criterion provides a third index of relatedness. The optimal temperature for reassociation is 25 to 30 C below the temperature at which a given DNA is denatured, which in these experiments is 60 C. The 75 C temperature is the stringent incubation condition, at which only closely related sequences can reassociate. At 60 C, up to 20% unpaired bases may be present in DNA duplexes; at 75 C, less than 7% unpaired bases are tolerated (2).

The above-mentioned methods have been discussed in detail previously (3-5).

RESULTS

In Table 2 are shown the hybridization data from *Erwinia amylovora* EA 178 and *E. quercina* EQ 102 reactions carried out at 60 C. The seven strains of *E. amylovora* are extremely closely related. Relatedness of *E. amylovora* to other erwiniae is only 13 to 23%. A similar low level of relatedness is seen between *E. amylovora* and species of *Pectobacterium*, *Enterobacter*, *Klebsiella*, *Serratia*, *Escherichia*, *Salmonella*, *Citrobacter*, *Edwardsiella*, and *Proteus*. The two strains of *E. quercina* are highly interrelated (91%). *E. quercina* is also not highly related to any other species of enterobacteria, its relatedness being 16 to 32% with other species of *Erwinia*, 20 to 33% with pectobacteria, and 19 to 25% with other enterobacteria (except *Proteus mirabilis*: 7%).

A somewhat different pattern of relatedness

TABLE 2. Relatedness of *Erwinia amylovora* EA 178 and *Erwinia quercina* EQ 102 to various enterobacteria

Source of unlabeled DNA	% relative binding at 60 C of DNA from	
	<i>E. amylovora</i> EA 178	<i>E. quercina</i> EQ 102
<i>Erwinia amylovora</i> EA 178	100	18
<i>E. amylovora</i> ATCC 15357	105	23
<i>E. amylovora</i> EA 169	104	26
<i>E. amylovora</i> ATCC 19382	101	21
<i>E. amylovora</i> ATCC 7400	100	22
<i>E. amylovora</i> ATCC 15580	93	20
<i>E. amylovora</i> ATCC 7401	85	16
<i>E. quercina</i> EQ 101	13	100
<i>E. quercina</i> EQ 102	14	91
<i>E. rubrifaciens</i> ER 103	15	27
<i>E. rubrifaciens</i> ER 105	15	27
<i>E. salicis</i> ES 102	21	31
<i>E. salicis</i> ATCC 15712	21	30
<i>E. tracheiphila</i> ATCC 27004	23	15
<i>E. nigrifluens</i> EN 101	17	32
<i>E. nigrifluens</i> EN 104	16	31
<i>Pectobacterium carnegieana</i> EC 186	14	26
<i>P. carotovorum</i> ATCC 495	16	27
<i>P. chrysanthemi</i> pathovar <i>zeae</i> SR 80	16	33
<i>P. cypripedii</i> EC 155	22	20
<i>Enterobacter agglomerans</i> EL 102	23	21
<i>E. agglomerans</i> 2553	24	20
<i>E. agglomerans</i> EH 103	25	23
<i>Serratia marcescens</i> SM6	25	25
<i>Klebsiella pneumoniae</i> 2	19	21
<i>Enterobacter aerogenes</i> 1627-66	19	22
<i>Escherichia coli</i> K-12	17	23
<i>Citrobacter freundii</i> 460-61	17	19
<i>Enterobacter hafniae</i> 4360-67	15	19
<i>Salmonella typhimurium</i> LT2	14	21
<i>Edwardsiella tarda</i> 3888-64	17	
<i>Proteus mirabilis</i> 1	7	7

is seen with *E. salicis* ATCC 15712 (Table 3). *E. salicis* ES 102 and ATCC 15712 are indistinguishable. *E. salicis* is most closely related to *E. rubrifaciens*. These organisms exhibit 55% average relatedness at 60 C, and one-half of the related sequences remain stable at 75 C. Ten percent of the unpaired bases (divergence) are present in the duplexes formed at 60 C. *E. nigrifluens* is somewhat less related to *E. salicis* (45% binding, 12% divergence at 60 C; 18% binding at 75 C). The relatedness of *E. salicis* to other *Erwinia* species is 20 to 33% at 60 C, falling to about 5% at 75 C. These duplexes exhibit 15 to 17% divergence.

The relatedness of *E. salicis* to pectobacteria is 21 to 42%. The highest pectobacterium reaction is with *Pectobacterium carnegieana*. *P. rhapontici* ER 106 is 95% related to *E. salicis* at 60 C and 100% related at 75 C. These duplexes show no evidence of divergence. We are convinced that this culture of strain ER 106 belongs, in fact, to *E. salicis* rather than to *P.*

rhapontici. The relatedness of *E. salicis* to other enterobacteria is 16 to 34%.

Relatedness patterns similar to that of *E. salicis* were obtained using reference DNA from *E. nigrifluens* EN 104 and *E. rubrifaciens* ER 105 (Table 4). The two *E. nigrifluens* strains used in this study are indistinguishable, as are the two *E. rubrifaciens* strains. *E. rubrifaciens* and *E. salicis* are again shown to be the most closely related species of *Erwinia* at both the 60 C and 75 C criteria. The relatedness of *Erwinia* species to ER 105 and EN 104 is 19 to 54%. Pectobacteria exhibit 20 to 34% relatedness to ER 105 and EN 104, and the relatedness of other enterobacteria (excluding *P. mirabilis*) to ER 105 and EN 104 is 16 to 25%.

No strain of *Enterobacter agglomerans* studied was highly related to *Erwinia* species (Tables 2-4). These strains included organisms previously designated *Erwinia herbicola*, *E. lathyri*, or *E. uredovora* (Table 1). Studies in which DNA from several other *Enterobacter*

TABLE 3. Relatedness of *Erwinia salicis* ATCC 15712 to enterobacteria

Source of unlabeled DNA	60 C		% relative binding at 75 C
	% relative binding	% divergence ^a	
<i>Erwinia salicis</i> ATCC 15712	100		100
<i>E. salicis</i> ES 102	98		100
<i>E. rubrifaciens</i> ER 105	60	11.7	34
<i>E. rubrifaciens</i> ER 103	52	9.2	28
<i>E. nigrifluens</i> EN 104	47	13.1	19
<i>E. nigrifluens</i> EN 101	45	11.3	17
<i>E. quercina</i> EQ 102	33	15.2	7
<i>E. amylovora</i> EA 178	22	15.9	6
<i>E. tracheiphila</i> ATCC 27004	20	17.0	4
<i>Pectobacterium carnegieana</i> EC 186	42	12.7	9
<i>P. chrysanthemi</i> SR 32	32	15.7	6
<i>P. cypripedii</i> EC 155	21	16.3	5
<i>P. rhapontici</i> ER 106	95	0.0	100
<i>Enterobacter agglomerans</i> 1645-71	33		
<i>E. agglomerans</i> 486-51	31		
<i>E. agglomerans</i> ATCC 19321	31		
<i>Citrobacter diversus</i> 1066-70	25		
<i>C. freundii</i> 460-61	27		
<i>Salmonella choleraesuis</i> 36	27	15.1	5
<i>Escherichia coli</i> K-12	22	16.2	7
<i>Edwardsiella tarda</i> 1483-59	21	15.8	4
<i>Enterobacter cloacae</i> 256-4	20		
<i>E. cloacae</i> 1347-71	20		
<i>Serratia marcescens</i> SM6	19		
Red Mouth Bacterium	17		
<i>Enterobacter aerogenes</i> 1627-66	16		
<i>E. hafniae</i> 4360-67	16		

^a Percent divergence is calculated on the assumption that each 1 C decrease in thermal stability of a heterologous DNA duplex compared to that of the homologous DNA duplex is caused by 1% unpaired bases within the duplex (17).

agglomerans was tested (data not shown) confirm the lack of a high level of relatedness between *E. agglomerans* and *Erwinia*. Dye (8) and Ewing and Fife (12) consider *Erwinia milletiae* and *E. cassavae* to be synonyms of *E. herbicola* (*Enterobacter agglomerans*). It must be noted, however, that our preliminary studies indicate the existence of several widely different hybridization groups in *Enterobacter agglomerans*.

These data may be summarized as follows. (i) Neither *Erwinia amylovora* nor *E. quercina* exhibit significantly more than the core level of relatedness to any other species of *Enterobacteriaceae*. (ii) *Erwinia rubrifaciens*, *E. salicis*, and *E. nigrifluens* are about 50% interrelated. The range of relatedness of these organisms to other erwiniae overlaps with their relatedness range to *pectobacteria*. (iii) *Erwinia tracheiphila* shows its highest relatedness (38%) to *E. rubrifaciens*. (iv) All six species of *Erwinia* studied here fall into clearly different hybridization groups.

DISCUSSION

Gardner and Kado (13) obtained DNA hybridization data from several *Erwinia* species, particularly *Erwinia rubrifaciens* and *E. amylovora*. They used the DNA filter technique in the presence of formamide and assayed both direct DNA binding and DNA competition at several temperatures between 18 and 36 C below the thermal elution midpoint of their labeled DNA. Despite the use of a different method, largely different strains, and different temperatures of incubation, their data and those reported here are in good general agreement.

Results from the more recent studies attest to the existence of four groups of organisms within the genus *Erwinia* as presently described in *Bergey's Manual* (1). There is general agreement that one of these groups, the so-called "atypical erwiniae," is composed of organisms that belong, in fact, to *Enterobacter* or to *Klebsiella pneumoniae* (9, 10). Many, but

TABLE 4. Relatedness of *Erwinia nigrifluens* EN 104 and *Erwinia rubrifaciens* ER 105 to various enterobacteria

Source of unlabeled DNA	% relative binding		
	<i>E. nigrifluens</i> EN 104 ^a	<i>E. rubrifaciens</i> ER 105 ^a	
	60 C	60 C	75 C
<i>Erwinia nigrifluens</i> EN 104	100	42	21
<i>E. nigrifluens</i> EN 101	103	46	23
<i>E. rubrifaciens</i> ER 105	44	100	100
<i>E. rubrifaciens</i> ER 103	45	101	101
<i>E. salicis</i> ATCC 15712	47	53	31
<i>E. salicis</i> ES 102	45	54	30
<i>E. quercina</i> EQ 102	30	26	8
<i>E. quercina</i> EQ 101	29	25	9
<i>E. tracheiphila</i> ATCC 27004		38	7
<i>E. amylovora</i> ATCC 19382	24	23	
<i>E. amylovora</i> ATCC 15580	23	21	
<i>E. amylovora</i> ATCC 15357	23	23	
<i>E. amylovora</i> EA 178	22	22	7
<i>E. amylovora</i> ATCC 7400	22	20	7
<i>E. amylovora</i> ATCC 7401	19	20	7
<i>Pectobacterium carnegieana</i> EC 186	34	32	9
<i>P. carotovorum</i> ATCC 495	32	34	11
<i>P. chrysanthemi</i> pathovar <i>zeae</i> SR 80	28	28	8
<i>P. cypripedii</i> EC 155	21	20	7
<i>Enterobacter agglomerans</i> EH 103	23	22	7
<i>E. agglomerans</i> EL 102	21	20	7
<i>E. aerogenes</i> 1627-66	25	20	8
<i>Klebsiella pneumoniae</i> 2	25	19	
<i>Serratia marcescens</i> SM6	25	23	8
<i>Salmonella typhimurium</i> LT2	23	20	9
<i>Escherichia coli</i> K-12	21	20	8
<i>Citrobacter freundii</i> 460-61	19	17	8
<i>Enterobacter hafniae</i> 4360-67	18	19	6
<i>Edwardsiella tarda</i> 3888-64		16	
<i>Proteus mirabilis</i> 1	8	6	

^a Source of unlabeled DNA.

not all, strains of *P. carnegieana* do in fact belong to *K. pneumoniae* (5). Available cultures labeled *Erwinia dissolvens* or *Erwinia nimipressuralis* are either strains of *Enterobacter cloacae* or are quite closely related to *E. cloacae* (5, 9, 10). Further study is needed before these organisms can be placed in the appropriate species.

The second group contains the species *Erwinia cassavae* and *E. milletiae* (and possibly *E. vitivora*) as well as strains designated *E. herbicola*, *E. lathyri*, *E. uredovora*, *E. maydis*, *Xanthomonas trifolii*, *E. ananas*, etc. (9, 10). About two-thirds of these organisms produce a yellow pigment (10). Their validity as phytopathogens is, in many cases, questionable. These organisms are being isolated from human infection with increasing frequency (9, 22), a fact which has stimulated a good deal of interest in them on the part of medical bacteriologists. Dye has studied these organisms

from a taxonomic point of view (8); von Graevenitz (22) and Ewing and Fife (12) have studied clinical isolates. These organisms are usually treated as a single species. Ewing and Fife (12) proposed the name *Enterobacter agglomerans* for these organisms and described seven anaerogenic and four aerogenic biogroups. DNA-relatedness studies in progress indicate that these organisms are significantly, although distantly (usually 35% or less), related to *Erwinia*, *Pectobacterium*, and to most other *Enterobacteriaceae* (Brenner, unpublished results). These DNA-relatedness studies indicate the existence of at least four markedly different hybridization groups among these organisms. A more detailed discussion before more data are available is both academic at this time and beyond the scope of this paper.

A great many taxonomic schemes have been proposed for the remaining two groups in *Erwiniae*: the "true" *erwiniae*, including *E.*

amylovora, the type species of *Erwinia*, and the pectolytic erwiniae, including *Pectobacterium carotovorum*, the type species of *Pectobacterium*. Most of these proposals are of four types. (i) Remove the "true" erwiniae from *Enterobacteriaceae* (10, 23). (ii) Retain the genus *Erwinia* as presently constituted in *Bergey's Manual*. (iii) Split the genus into two genera or two groups, one containing the pectolytic and related organisms and the other containing "true" erwiniae (5, 6, 7, 23). (iv) Distribute these organisms to other genera of *Enterobacteriaceae* based on biochemical similarity and without regard for phytopathogenicity (16, 19).

There are compelling reasons to retain both the "true" and the pectolytic erwiniae in *Enterobacteriaceae*. These include biochemical and genetic evidence (see reference 20), as well as DNA-hybridization data obtained previously (5, 13) and in this study. The data relating to the question of whether these organisms should be retained in the genus *Erwinia*, split into *Erwinia* and *Pectobacterium*, or largely distributed throughout other genera of *Enterobacteriaceae* are excellently treated in the review by Starr and Chatterjee (20). We have supported the two-genus concept because of the different metabolic patterns exhibited by *Erwinia sensu stricto* and *Pectobacterium*, differences in type of disease production, and generally higher intragroup DNA relatedness. It is true that the guanine + cytosine (G + C)

composition in the DNA of these organisms does not correlate with the two-genus concept, as each genus contains organisms with 51% to about 57% G + C (21; and Table 5). The single-genus concept does not alleviate the heterogeneous distribution of G + C and does not take into account the biochemical and pathological differences between these organisms.

There are also many schemes for speciating erwiniae. Many of the earlier schemes, unfortunately, were based on the host from which the bacterium was isolated without information about host range of the newly isolated species or previously isolated species. This practice led to a variety of nomenclatural synonyms for the same species. Graham (15) and Dye (6-8) have carried out extensive studies to purge these synonyms from the literature. Objections to classification based on site of isolation have been firmly stated by both plant and medical bacteriologists (7, 11-14).

Some of the more recent proposals for speciation of *Erwinia* are shown in Table 5. The present *Bergey's Manual* (we are unaware of the makeup of *Erwinia* in the edition of *Bergey's Manual* that is in preparation) contains six species of *Erwinia sensu stricto* (1). Three of these, *E. cassavae*, *E. milletiae*, and *E. vitivora*, are from the *E. herbicola*, or *Enterobacter agglomerans*, group (8, 12). Three additional species, *E. nigrifluens*, *E. quercina*, and *E. rubrifaciens*, are not included in the current

TABLE 5. Comparison of some taxonomic groupings for *Erwinia* species

Species	% G + C ^a	<i>Bergey's Manual</i> (1)	Martinez and Kocur (18)	Dye (6)	Present study
<i>Erwinia amylovora</i>	53.6-54.9	<i>E. amylovora</i>	<i>E. amylovora</i>	<i>E. amylovora</i>	<i>Erwinia amylovora</i>
<i>E. cassavae</i>	55.1	<i>E. cassavae</i>	<i>E. amylovora</i> subsp. <i>salicis</i>	<i>E. herbicola</i> subsp. <i>herbicola</i>	<i>Enterobacter agglomerans</i>
<i>E. milletiae</i>	54.1-55.1	<i>E. milletiae</i>	<i>E. amylovora</i>	<i>E. herbicola</i> subsp. <i>herbicola</i>	<i>Enterobacter agglomerans</i>
<i>E. nigrifluens</i>	56.1-56.7			<i>E. amylovora</i> subsp. <i>nigrifluens</i>	<i>Erwinia nigrifluens</i>
<i>E. quercina</i>	54.6-55.1			<i>E. amylovora</i> subsp. <i>quercina</i>	<i>E. quercina</i>
<i>E. rubrifaciens</i>	51.3-52.6			<i>E. amylovora</i> subsp. <i>rubrifaciens</i>	<i>E. rubrifaciens</i>
<i>E. salicis</i>	51.3-51.5	<i>E. salicis</i>	<i>E. amylovora</i> subsp. <i>salicis</i>	<i>E. amylovora</i> subsp. <i>salicis</i>	<i>E. salicis</i>
<i>E. tracheiphila</i>	50.0-52.0	<i>E. tracheiphila</i>	<i>E. amylovora</i>	<i>E. amylovora</i> subsp. <i>tracheiphila</i>	<i>E. tracheiphila</i>
<i>E. vitivora</i>		<i>E. vitivora</i>	<i>E. amylovora</i>	<i>Species incertae sedis</i>	?

^a All determinations of G + C are from Starr and Mandel (21). In addition, determinations of Gardner and Kado (13) are used for *E. amylovora*, *E. nigrifluens*, and *E. rubrifaciens*.

Bergey's Manual scheme. Martinec and Kocur (18) recognize only one species, *E. amylovora*, and one variety (subspecies), *E. amylovora* subsp. *salicis*. Dye also recognizes *E. amylovora* as the sole species but specifies five subspecies with the subspecific epithets *nigrifluens*, *quercina*, *rubrifaciens*, *salicis*, and *tracheiphila* (6).

Gardner and Kado (13) demonstrated that *E. amylovora*, *E. rubrifaciens*, and *E. nigrifluens* are separate species by virtue of their own DNA relatedness. Our data confirm and extend their findings, indicating that *E. amylovora*, *E. quercina*, *E. rubrifaciens*, *E. nigrifluens*, *E. salicis*, and *E. tracheiphila* are all distinct species. Of these, *E. salicis*, *E. nigrifluens*, and *E. rubrifaciens* are most closely related. The remaining species seem to share about the same level of core relatedness with *Erwinia* species, *Pectobacterium*, and members of other genera of *Enterobacteriaceae*.

Although the genera *Pectobacterium* and *Erwinia* both contain some organisms of differing G + C content, and some organisms that do not exhibit high levels of intragenus relatedness, they are not unique among *Enterobacteriaceae* in these respects. The genus *Enterobacter* and the genus *Proteus* contain species that exhibit 20% or less relatedness at the genus level (Brenner, unpublished observations). *Proteus* species differ in G + C content by as much as 13%.

We believe that the most logical present course of action is to retain *E. tracheiphila*, *E. quercina*, *E. amylovora*, *E. salicis*, *E. rubrifaciens*, and *E. nigrifluens* in the genus *Erwinia* on the basis of their similar biochemistry and pattern of disease production. The alternative would be four genera: one for *E. tracheiphila*, one for *E. quercina*, one for *E. amylovora*, and one for *E. salicis*, *E. rubrifaciens*, and *E. nigrifluens*. Such a scheme has no merit because it splits organisms without any purpose.

The strain received as *Pectobacterium rhapontici* ER 106 is indistinguishable from *E. salicis* (Table 3). This strain was included in our previous study (5) as the only representative of *P. rhapontici*. Therefore the conclusion that *P. rhapontici* is a valid species of *Pectobacterium* must be withheld until valid strains of *P. rhapontici* are examined.

REPRINT REQUESTS

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