DNA Base Composition and Taxonomy of Phytopathogenic and Other Enterobacteria

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SUMMARY

Using lysates and purified DNA, base ratios were determined by the buoyant density ultracentrifugation and the absorbance-temperature transition techniques; the correspondence was very high between the two methods. The DNAs from a large number of *Erwinia* species, mostly phytopathogenic and nonphytopathogenic, but including some 'Erwinia-like' bacteria from animal sources and from other genera of the family Enterobacteriaceae were studied. The DNA base ratios of these bacteria fell mainly in the range 50–58 % GC, consistent with their taxonomic placement in the family Enterobacteriaceae. Certain assemblages of *erwinias* could be grouped on the basis of GC content into clearly differentiated clusters, which, accidentally or otherwise, correlated neatly with certain existing nomenclatural groupings. It was not possible, from the GC values alone, to decide whether the present multiplicity of *Erwinia* species can be justified as separate species, or whether and how they might be combined into fewer species, or whether and how they might be demoted to some infraspecific level. Indeed, it is not clear whether the genus *Erwinia*, defined as it is solely on the basis of plant habitat, should be maintained or whether its species might not, just as rationally, be distributed throughout other genera of the family Enterobacteriaceae. Classifications of the genus *Erwinia* on over-all phenotypic similarity result in a small number of specific taxa which, on the basis of the present study, are seen to contain organisms of different GC contents. In the present state of the art, one can only be agnostic about the amount of such differences in GC content which corresponds to separate taxa or to a particular categorical level (generic, specific, infraspecific) in the present hierarchical taxonomic scheme.

INTRODUCTION

The genus *Erwinia* was proposed originally by a committee (Winslow *et al.* 1917) with the candid admission that there was insufficient information for a more rational nomenclature: '...the plant pathogens...seem to us perhaps to deserve distinct generic rank. They resemble the proteus group in their Gram-negative reaction, general growth characters, tendency toward slime formation, and generally negative indole reaction, but their fermentative powers are less vigorous and the exact biochemical changes they produce in carbohydrates are unknown. We suggest that all

the peritrichate plant pathogens be for the present grouped under a new genus to be called *Erwinia* . . . '.

Erwin Frink Smith, the pioneer phyto bacteriologist who was commemorated by this act, stated, quite resolutely (cf. Jones, 1939, p. 43): 'I have no sympathy with those who would . . . lump everything indiscriminately . . . the species I have mentioned ought not be put into one genus (*Erwinia*) simply because they are plant pathogens.' His advice went unheeded, and the causal agent of fire blight of pear—the first disease of plants proven to be caused by bacteria (Burrill, 1882)—is today the type species (*Erwinia amylovora*) of the genus *Erwinia*. This impractical nomenclatural concept—in which taxa of phytopathogenic bacteria are segregated from other bacteria solely upon the basis of plant pathogenicity—has persisted to the present time. In *Bergey's Manual* (1957) the genus *Erwinia* is placed in the family Enterobacteriaceae, fenced off from its probable affinities not only by the nomenclatural barrier, but also by the insularity ensuing from the completely separate developments of plant and animal bacteriology.

Early in the twentieth century several bacterial soft-rot diseases of plants were studied and ascribed causally to several different bacterial 'species' which usually were named in accordance with the host plant from which they had been isolated. The genus *Erwinia* was sufficiently vague in concept to accommodate these soft-rot bacteria. Despite the early comparative studies (Jones, 1901; Harding & Morse, 1909; Jennison, 1923) which showed the essential similarity of most soft-rot bacteria to the one first described—now called *Erwinia carotovora*—a multiplicity of species names was used, with various definitions, but most frequently based on the curious 'new host—new species' notion (Starr, 1959; Stolp, Starr & Baigent, 1965). However, it is clear that the usual soft-rot bacteria are as different from *E. amylovora* as, for example, *Enterobacter cloacae* is from *Shigella dysenteriae*. Non-phytopathogenic yellow-pigmented bacteria are common on plants and some of these have been referred to the genus *Erwinia* (Billing & Baker, 1963; Graham & Hodgkiss, 1967). Finally, a group of yellow-pigmented bacteria from animals and humans is thought to be related to *Erwinia* (Muraschi, Friend & Bolles, 1965; von Graevenitz & Strouse, 1966; Whitcomb, Shapiro & Richardson, 1966; Graham & Hodgkiss, 1967; W. H. Ewing, personal communication; I. J. Slotnick, personal communication).

We have undertaken a comprehensive study of the genus *Erwinia* and related bacteria, including investigations on such aspects as nutrition (Starr & Mandel, 1950); end-products of glucose dissimilation in *E. amylovora* (Sutton & Starr, 1959), in *E. carotovora* (Kraght & Starr, 1952), and in other erwinias (in preparation); uronic acid catabolism (Kilgore & Starr, 1959), pectolytic enzymes (Kraght & Starr, 1953; Starr & Moran, 1962; Nasuno & Starr, 1966; Moran, Nasuno & Starr, 1968*a, b*); pigment formation (Starr, Cosens & Knackmuss, 1966); bacteriophage and bacteriocin relationships (in preparation); pathogenicity (Starr, 1947; Starr, Cardona & Folsom, 1951; in preparation). In this report, we present the DNA base ratios of representative cultures of *Erwinia* species and other enterobacteria. These data, together with the other information becoming available about these organisms, should eventually provide the basis for clarification of the taxonomic relationships of these phytopathogenic and other enterobacteria.

METHODS

Organisms. The majority of the phytopathogenic bacteria were obtained from the International Collection of Phytopathogenic Bacteria (ICPB) maintained at the Department of Bacteriology, University of California, Davis. Many of these cultures are also accessioned in the National Collection of Plant Pathogenic Bacteria, Harpenden, Herts., England. The strains were chosen to be representative of the broadest possible range of geographical sources, host plants, and date of isolation. 'Erwinia-like' bacteria isolated mainly from plant materials were provided by E. Billing (Billing & Baker, 1963) and D. C. Graham (Graham & Hodgkiss, 1967); those isolated from American white-tail deer, humans and leafhoppers were provided by T. F. Muraschi (Muraschi *et al.* 1965), I. J. Slotnick, R. F. Whitcomb (Whitcomb *et al.* 1966), W. H. Ewing, and A. von Graevenitz (von Graevenitz & Strouse, 1966). Other enterobacteria came from the American Type Culture Collection (ATCC); these were selected to represent designated type or neotype cultures as far as these are available. Further details about the cultures are given in Tables 1-3.

For clarity in this presentation, we will use the genus and species designations under which the culture was received. Several trivial names are used here in accordance with present practices of workers in this field: 'erwinia', meaning any organism referred to the genus *Erwinia* with no connotation regarding origin (plant, animal) or phytopathogenicity; 'Erwinia-like' bacteria, meaning definitely non-phytopathogenic erwinias from plants or animals; 'pectobacteria', meaning pectolytic (i.e. soft-rot) members of the genus *Erwinia*; 'true-Erwinia', meaning the non-pectolytic phytopathogens (*E. amylovora* and other non-pectolytic species) of the genus *Erwinia*; 'enterobacteria', meaning any member of the family Enterobacteriaceae, including all of the foregoing categories.

Deoxyribonucleic acid isolation and analysis. Lysates of bacteria for CsCl buoyant density ultracentrifugation were prepared from slant cultures, as previously described (Mandel, 1966). Purified samples of DNA were prepared by the method of Marmur (1961) from organisms from 2 l. cultures grown in Penassay broth (Difco) at 30° with rotary agitation. These samples also were analysed in the ultracentrifuge for CsCl buoyant density, and their melting temperatures in 0.15 M-NaCl containing 0.015 M-sodium citrate at pH 7.0 were determined by changes in extinction at 260 m μ (nm.) (Mandel & Marmur, 1968). The DNA base ratios were estimated from mean buoyant densities by means of the equation of Schildkraut, Marmur & Doty (1962), and from the T_m by means of the equation of Marmur & Doty (1962). Each DNA sample was analysed at least twice by CsCl buoyant density equilibrium centrifugation, and many were additionally examined by the extinction/temperature transition-technique. Following the usual convention, the DNA base ratios are expressed as molar percentages of guanine+cytosine ('GC content') in terms of the total base content.

RESULTS AND DISCUSSION

The GC contents of cultures ascribed to the genus *Erwinia* and to other genera of the Enterobacteriaceae are presented in Tables 1-3. Table 1 contains the buoyant densities and calculated GC contents of the DNAs from the cultures of phytopathogenic erwinias, obtained by analysis of lysates. Table 2 presents the buoyant densities and

Table 1. *Buoyant density in CsCl and GC content of DNA in lysates of cultures of Erwinia species and other phytopathogenic Enterobacteriaceae*

	Strain (ICPB)	Mean density (g./cm. ³)	GC (mole %)
<i>E. amylovora</i>	EA 11	1.7125	53.6
	EA 131	1.713	54.1
	EA 137	1.7127	53.8
	EA 146	1.713	54.1
	EA 162	1.713	54.1
	EA 167	1.7125	53.6
	EA 169	1.713	54.1
<i>E. ananas</i>	EA 101	1.7125	53.6
	EA 133	1.712	53.1
	EA 180	1.713	54.1
	EA 181	1.7125	53.6
<i>E. aroideae</i>	EA 13	1.711	52.0
	EA 14	1.712	53.1
	EA 144	1.710	51.0
<i>E. atroseptica</i>	EA 112	1.7103	51.3
	EA 153	1.712	53.1
<i>E. carnegieana</i>	EC 186	1.710	51.0
	EC 187	1.7098	50.8
	EC 188	1.7107	51.7
	EC 189	1.7103	51.3
	EC 189	1.710	51.0
	EC 191	1.710	51.0
	EC 221	1.710	51.0
	EC 222	1.7105	51.5
<i>E. carotovora</i>	EC 223	1.710	51.0
	EC 105	1.710	51.0
	EC 131	1.7115	52.6
	EC 138	1.711	52.0
	EC 150	1.7115	52.6
	EC 153	1.7107	51.7
	EC 208	1.7113	52.3
<i>f.sp. parthenii</i>	EC 115	1.7115	52.6
<i>E. cassavae</i>	EC 11	1.714	55.1
<i>E. chrysanthemi</i>	EC 16	1.714	55.1
	EC 175	1.714	55.1
	EC 183	1.715	56.1
	EC 205	1.716	57.1
Corn stalk-rot pathogen	EM 107	1.7125	53.6
	EM 108	1.713	54.1
	EM 109	1.712	53.1
	EM 110	1.7125	53.6
	EM 111	1.7125	53.6
	EM 111 mut	1.7125	53.6
	EM 112	1.713	54.1
	EC 209	1.7125	53.6
	EC 210	1.7125	53.6
	EC 210 mut	1.7125	53.6
<i>E. cypripedii</i>	EC 155	1.713	54.1
	EC 160	1.7135	54.6
<i>E. cytolytica</i>	EC 207	1.715	56.1
<i>E. dieffenbachiae</i>	ED 102	1.714	55.1
	ED 103	1.715	56.1

Table 1 (cont.)

	Strain (ICPB)	Mean density (g./cm. ³)	GC (mole %)
<i>E. dissolvens</i>	ED 105	1.7125	53.6
	ED 106	1.716	57.1
	ED 109	1.7165	57.6
	ED 110	1.714	55.1
	ED 111	1.7147	55.8
	ED 112	1.716	57.1
	ED 113	1.7155	56.6
	ED 114	1.716	57.1
<i>E. mangiferae</i>	EM 101	1.7135	54.6
<i>E. milletiae</i>	EM 102	1.714	55.1
	EM 113	1.713	54.1
	EM 114	1.714	55.1
<i>E. nigrifluens</i>	EN 104	1.715	56.1
<i>E. nimipressuralis</i>	EN 1	1.714	55.1
<i>E. oleraceae</i>	EO 1	1.7095	50.5
<i>E. quercina</i>	EQ 101	1.714	55.1
	EQ 102	1.7135	54.6
<i>E. rhapontici</i>	ER 1	1.710	51.0
	ER 102	1.712	53.1
	ER 106	1.712	53.1
<i>E. rubrifaciens</i>	ER 103	1.711	52.0
	ER 104	1.7115	52.6
	ER 105	1.7115	52.6
<i>E. salicis</i>	ES 4	1.7103	51.3
	ES 102	1.7105	51.5
<i>E. solanisapra</i>	ES 101	1.710	51.0
<i>E. tracheiphila</i>	ET 5	1.711	52.0
	ET 102	1.7095	50.5
	ET 106	1.709	50.0
' <i>Aerobacter aerogenes</i> ' (banana strain)	3151	1.715	56.1
' <i>Bacterium stewartii</i> '	SS 11	1.714	55.1
	SS 102	1.7135	54.6
' <i>Xanthomonas uredovorus</i> '	XU 103	1.712	53.1
	XU 104	1.712	53.1

calculated GC contents of the DNAs in the lysates of the cultures of non-phytopathogenic *Erwinia*-like bacteria. Table 3 presents comparative calculations of the GC contents determined both in CsCl and by thermal denaturation on purified samples of DNA extracted from representative *Erwinia* cultures and reference cultures of other enterobacteria. Table 4 summarizes tests of statistical significance of pairwise differences in densities between selected sets of *Erwinia* species; there is no necessity to equate such statistically significant differences with taxonomic significance.

The data have been applied to several purposes. Where the cultures have already been assigned to a specific taxon, the uniformity of the taxon has been tested with regard to the uniformity of GC contents. Various proposals for combining existing taxa have been examined in the light of the GC data. The wisdom of referring the non-phytopathogenic *Erwinia*-like bacteria to the family Enterobacteriaceae has been evaluated in terms of the GC values. The homogeneity of the family Enterobacteria-

ceae, with the various erwinias included, is scrutinized. The rationality of maintaining a genus *Erwinia* is considered. Since a rather large number of DNAs were examined by both buoyant density and transition temperature techniques, comparisons are made between the two methods (Table 3).

Table 2. *Buoyant density in CsCl and GC content of DNA in lysates of non-phytopathogenic 'Erwinia-like' bacteria*

Source or Name	Strain (ICPB)	Mean density (g./cm. ³)	GC (mole %)
Plant materials (Billing)	2650-651	1.714	55.1
	2650-653	1.713	54.1
	2650-656	1.7145	55.6
	2650-658	1.712	53.1
	2650-661	1.714	55.1
	2650-662	1.7135	54.6
<i>Erwinia lathyri</i>	EL 101 B	1.714	55.1
	EL 102	1.714	55.1
	EL 103	1.714	55.1
	EL 104	1.7115	52.6
	EL 105	1.712	53.1
	EL 106	1.7165	57.7
	EL 107	1.713	54.1
<i>Erwinia herbicola</i>	2553	1.714	55.1
	2554	1.713	54.1
	3161	1.714	55.1
	3162	1.712	53.1
	3163	1.714	55.1
' <i>Xanthomonas trifolii</i> '	XT 109	1.7145	55.6
	XT 110	1.7145	55.6
Deer (Muraschi)	2948	1.7149	56.0
	2949	1.714	55.1
	2950	1.715	56.1
Leafhopper (Whitcomb)	2973-L	1.7115	52.6
	2973-S	1.7125	53.6
Man (Slotnick)	2858-A	1.7145	55.6
	2858-B	1.714	55.1
Man (Muraschi)	2953	1.7155	56.6
	2956	1.713	54.1
Man (von Graevenitz)	2984	1.719	60.2
	2986	1.7185	59.7
	2987	1.715	56.1
Man (Ewing)	2992	1.714	55.1
	3080	1.7145	55.6
' <i>Bacterium typhi flavum</i> '	3164	1.714	55.1
	3165	1.714	55.1

The proposal of Waldee (1945) to separate the soft-rot bacteria (as a genus *Pectobacterium*) from the rest of the genus *Erwinia* is neither supported nor confirmed by the GC data. The assortments within these two groups, the pectobacteria and the true-*Erwinia*, as reviewed by Graham (1964), can now be assessed in terms of GC contents. *Erwinia amylovora* (53.9% GC) could accommodate *E. cassavae* and *E. mangiferae*. The DNAs of *E. salicis* and *E. tracheiphila*, on the other hand, are 4% to 5% lower in GC content than those of *E. amylovora* and its relatives. In addition,

the yellow-pigmented erwinias (Dye, 1963; Graham & Hodgkiss, 1967)—‘*Xanthomonas uredovorus*’, *E. ananas*, *E. lathyri*, and ‘*Bacterium stewartii*’—as well as many of the yellow *Erwinia*-like bacteria, have GC contents not unlike the *E. amylovora* group and lower than the *Pseudomonadaceae* genera in which some of them had earlier

Table 3. *Melting temperatures, buoyant densities in CsCl, and calculated GC contents of purified DNA samples from representative Erwinia and other Enterobacteriaceae*

Name	Strain	T_m (°C)	GC (mole %)	Buoyant density (g./cm. ³)	GC (mole %)
<i>Erwinia amylovora</i>	ICPB EA 11	91.0	53.0	1.7125	53.6
<i>E. aroideae</i>	ICPB EA 14	91.5	54.0	1.712	53.1
<i>E. carotovora</i>	ICPB EC 138	91.2	53.4	1.711	52.0
<i>E. chrysanthemi</i>	ICPB EC 16	92.1	55.6	1.714	55.1
<i>E. dissolvens</i>	ICPB ED 106	92.7	57.1	1.716	57.1
<i>E. lathyri</i>	ICPB EL 103	92.2	55.9	1.714	55.1
<i>E. milletiae</i>	ICPB EM 102	92.0	55.4	1.714	55.1
<i>E. nigrifluens</i>	ICPB EN 104	92.5	56.6	1.715	56.1
<i>E. nimipressuralis</i>	ICPB EN 1	92.0	55.4	1.714	55.1
<i>E. oleraceae</i>	ICPB EO 1	90.4	51.5	1.7095	50.5
<i>E. rhapontici</i>	ICPB ER 1	90.2	51.1	1.710	51.0
<i>E. salicis</i>	ICPB ES 4	90.8	52.5	1.7103	51.3
<i>E. tracheiphila</i>	ICPB ET 106	90.9	52.7	1.709	50.0
<i>Escherichia aurescens</i>	ATCC 12814	90.3	51.3	1.710	51.0
<i>E. coli</i>	K-12 Hfr G 6	90.4	51.5	1.710	51.0
<i>E. coli</i>	ATCC 11775	90.5	51.7	1.710	51.0
<i>E. coli</i>	ATCC 12814	90.3	51.3	1.710	51.0
<i>Enterobacter aerogenes</i>	ATCC 13048	91.8	54.9	1.712	53.1
<i>E. aerogenes</i>	ATCC 13882	92.1	55.6	1.715	56.1
<i>E. alvei</i>	ATCC 23280	89.2	48.7	1.707	48.0
<i>E. cloacae</i>	ATCC 13047	92.0	55.4	1.7135	54.6
<i>E. lipolyticus</i>	ATCC 14460	91.4	53.9	1.7115	52.6
<i>Klebsiella pneumoniae</i>	ATCC 13883	92.9	57.6	1.7123	53.4
<i>K. edwardsii</i> subsp. <i>edwardsii</i>	ATCC 13886	92.5	56.5	1.7155	56.6
<i>K. edwardsii</i> subsp. <i>atlantae</i>	ATCC 13887	92.6	56.9	1.715	56.1
<i>K. rhinoscleromatis</i>	ATCC 13884	92.7	57.1	1.7145	55.6
<i>Citrobacter freundii</i>	ATCC 8090	90.9	52.7	1.7115	52.6

Table 4. *Tests of statistical significance of pairwise differences in buoyant densities of the DNAs from selected sets of Erwinia species*

	$x \pm \sigma$	n	t	D.F.	ρ (sign ignored)
<i>E. carnegieana</i>	1.7100 $\pm$ 0.0008	22	3.78	37	0.001
<i>E. carotovora</i>	1.7111 $\pm$ 0.0009	17			
<i>E. amylovora</i>	1.7128 $\pm$ 0.0007	16	6.6	18	0.001
<i>E. salicis</i>	1.7105 $\pm$ 0.0006	4			
<i>E. chrysanthemi</i>	1.7148 $\pm$ 0.0009	8	8.45	29	> 0.001
‘Corn stalk rotters’	1.7126 $\pm$ 0.0005	23			
<i>E. amylovora</i>	1.7128 $\pm$ 0.0007	16	0.034	22	< 0.5
<i>E. ananas</i>	1.7125 $\pm$ 0.0005	8			
<i>E. carotovora</i>	1.7111 $\pm$ 0.0009	17	0.874	21	0.4
<i>E. aroideae</i>	1.7110 $\pm$ 0.0011	6			

been included (see De Ley, 1968, for an independent study on this point). The *Erwinia*-like organisms isolated from American white-tail deer (Muraschi), from man (Ewing, Slotnick, Muraschi, and one of the three strains sent by von Graevenitz), from leafhoppers (Whitcomb), and the non-phytopathogenic plant *Erwinia*-like organisms received from Billing and from Graham have GC contents which are also similar to the *E. amylovora* range, and these are all similar to the data for *Klebsiella* and certain *Enterobacter* cultures. Two of the three *erwinia* strains isolated from humans by von Graevenitz & Strouse (1966) have higher GC contents (60.2 %, 59.7 % GC) than any other *Enterobacteriaceae* cultures examined in this study.

Fig. 1. The GC contents of the DNAs of representative *Erwinia* species. The values plotted are means $\pm$ one standard deviation.

Turning to the soft-rot *erwinias*, the *pectobacteria*, *Erwinia carotovora* (52.1 % GC) does not display any tendency to discrete GC contents for its relatives *E. aroideae*, *E. solanisapra*, *E. oleraceae*, and *E. atroseptica*, thus justifying the current practice of lumping these into one species. *Erwinia chrysanthemi* is characterized by 56 % GC and, on this basis, cannot be considered to be identical with *E. carotovora* (52.1 % GC). The several organisms rotting the stalks of corn plants (A. Kelman, unpublished) are significantly different (i.e. statistically so; Table 4) in their GC contents (53.7 % GC) from the *E. carotovora* (52.1 % GC), *E. chrysanthemi* (56 % GC), and *E. dissolvens* (56.3 % GC) cultures; they might form a taxon separate from these three, but whether at the specific or infraspecific level remains to be decided on other grounds. Similarly, the representatives of *E. carnegieana* (51.1 % GC) are distinct in their GC contents, as compared to the other soft-rot *erwinias*, and might be viewed as a separate taxon; here again, the hierarchical level cannot be decided from these data alone.

The other named strains which have been examined in this study fall into the range of 50–58 % GC values, typical of the other members of the family *Enterobacteriaceae* (Marmur, Falkow & Mandel, 1963; Hill, 1966; Rosypal & Rosypalová, 1966). The

extremes are reached with *Enterobacter alvei* ATCC 23280 (48.0% GC) and the aforementioned human *Erwinia* strain from von Graevenitz, ICPB 2984 (60.2% GC).

Based on a study of over-all phenotypic similarity, Martinec & Kocur (1963) have suggested that all the species presently in the genus *Erwinia* might be reduced to two species: *E. carotovora* and *E. amylovora* (and var. *salicis*). In a similar study with over 200 cultures and using some 80 traits, Dye (1968) has recommended reduction to five species: *E. amylovora* (and var. *salicis*, var. *tracheiphila*, var. *nigrifluens*, var. *quercina*), *E. herbicola* (and var. *ananas*), *E. uredovorus*, *E. stewartii*, *E. carotovora* (and var. *atropetetica*, var. *chrysanthemi*, var. *cypripedii*, var. *rhapontici*). Since following these recommendations would lead to taxa (specific and infraspecific) which the present DNA data show would be composed of organisms with statistically different GC contents (Table 4), we are at a loss to decide on the wisdom of these suggestions. In support of this view, the computer sorting of Lockhart & Koenig (1965) and Krieg & Lockhart (1966) shows no intensification of sorting when the S-values which they report for *Erwinia* species are plotted versus our data on their GC contents. Indeed, the range of link-sorting is less than the range of GC values throughout the family Enterobacteriaceae; hence, the traits used in the generation of these computer analyses are probably of little value in discriminating taxa at the generic level and below. The data may, of course, also mean that a species which is based on high over-all phenotypic similarity can include individual specimens with genetic make-ups varying over a range corresponding to several per cent in GC content.

We are not now prepared to equate discrete differences in GC contents with discrete taxa (see Rosypal & Rosypalová, 1966). In the present state of the art, who is to say what particular amount of difference in DNA base ratios or in DNA homologies justifies separating one taxon from another? We can not now be other than agnostic about the amount of such differences which would be required to place taxa in any particular position in the hierarchical taxonomic categories. All we can now say with assurance is that certain assemblages of *erwinias* can be grouped on the basis of GC contents into clearly differentiated clusters, and that these clusters—accidentally or otherwise—correlate neatly with certain existing nomenclatural groupings.

There is no compelling evidence from the GC data that the *Erwinia* group, either whole or in parts, comprises a taxon or taxa separable at the generic level from the other genera of the Enterobacteriaceae. Indeed, from Fig. 1 it will be seen that a case could be made for distributing the *erwinias* throughout the other genera of the family Enterobacteriaceae. Broadly based comparative studies now in progress are designed to clarify this matter; hence, no nomenclatural recommendations will be made here.

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REFERENCES

- BILLING, E. & BAKER, L. A. E. (1963). Characteristics of *Erwinia*-like organisms found in plant material. *J. appl. Bact.* **26**, 58.
Bergey's Manual of Determinative Bacteriology. (1957). 7th ed. Ed. by R. S. Breed, E. G. D. Murray and N. R. Smith. Baltimore: Williams and Wilkins Co.

- BURRILL, T. J. (1882). The bacteria: an account of their nature and effects, together with a systematic description of the species. *Illinois Ind. Univ., Report of the Board of Trustees* **11**, 93.
- DE LEY, J. (1968). DNA base composition of yellow *Erwinia* strains. *Antonie van Leeuwenhoek* **34**, 257.
- DYE, D. W. (1963). The taxonomic position of *Xanthomonas uredovorus* Pon *et al.* 1954. *N.Z. Jl Sci.* **6**, 146.
- DYE, D. W. (1968). A taxonomic study of the genus *Erwinia*. *1st. Int. Congr. Pl. Path.* (London, July 1968), Abstracts of Papers, 51.
- GRAEVENITZ, A. VON, & STROUSE, A. (1966). Isolation of *Erwinia* spp. from human sources. *Antonie van Leeuwenhoek* **32**, 429.
- GRAHAM, D. C. (1964). Taxonomy of the soft-rot coliform bacteria. *A. Rev. Phytopath.* **2**, 13.
- GRAHAM, D. C. & HODGKISS, W. (1967). Identity of Gram negative, yellow pigmented, fermentative bacteria isolated from plants and animals. *J. appl. Bact.* **30**, 175.
- HARDING, H. A. & MORSE, W. J. (1909). The bacterial soft rots of certain vegetables. *Tech. Bull. N. Y. St. agric. Exp. Stn.* **11**, 251.
- HILL, L. R. (1966). An index to deoxyribonucleic acid base compositions of bacterial species. *J. gen. Microbiol.* **44**, 419.
- JENNISON, H. M. (1923). Potato blackleg with special reference to the etiological agent. *Ann. Mo. bot. Gdn.* **10**, 1.
- JONES, L. R. (1901). A soft rot of carrot and other vegetables caused by *Bacillus carotovorus*. *Rep. Vermont. agric. Exp. Stn* **13**, 299.
- JONES, L. R. (1939). Biographical memoir of Erwin Frink Smith 1854–1927. *Biogr. Mem. natn. Acad. Sci.* **21**, 1.
- KILGORE, W. W. & STARR, M. P. (1959). Catabolism of galacturonic and glucuronic acids by *Erwinia carotovora*. *J. biol. Chem.* **234**, 2227.
- KRAGHT, A. J. & STARR, M. P. (1952). Fermentation of galacturonic acid and glucose by a strain of *Erwinia carotovora*. *J. Bact.* **64**, 259.
- KRAGHT, A. J. & STARR, M. P. (1953). Pectic enzymes of *Erwinia carotovora*. *Archs. Biochem. Biophys.* **42**, 271.
- KRIEG, R. E. & LOCKHART, W. R. (1966). Classification of enterobacteria based on overall similarity. *J. Bact.* **42**, 1275.
- LOCKHART, W. R. & KOENIG, K. (1965). Use of secondary data in numerical taxonomy of the genus *Erwinia*. *J. Bact.* **90**, 1638.
- MANDEL, M. (1966). Deoxyribonucleic acid base composition in the genus *Pseudomonas*. *J. gen. Microbiol.* **43**, 273.
- MANDEL, M. & MARMUR, J. (1968). Use of ultraviolet absorbance-temperature profile for determining the guanine plus cytosine content of DNA. *Meth. Enzym.* **12**, 195.
- MARMUR, J. (1961). A procedure for the isolation of deoxyribonucleic acid from micro-organisms. *J. molec. Biol.* **3**, 208.
- MARMUR, J. & DOTY, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J. molec. Biol.* **5**, 109.
- MARMUR, J., FALKOW, S. & MANDEL, M. (1963). New approaches to bacterial taxonomy. *A. Rev. Microbiol.* **17**, 329.
- MARTINEC, T. & KOCUR, M. (1963). A taxonomic study of the genus *Erwinia*. *Folia. Publ. Fac. Nat. Sci. J. E. Purkynye Univ. (Biologia)*, **4**, 1.
- MORAN, F., NASUNO, S. & STARR, M. P. (1968*a*). Extracellular and intracellular polygalacturonic acid *trans*-eliminases of *Erwinia carotovora*. *Archs Biochem. Biophys.* **123**, 298.
- MORAN, F., NASUNO, S. & STARR, M. P. (1968*b*). Oligogalacturonide *trans*-eliminase of *Erwinia carotovora*. *Archs Biochem. Biophys.* **125**, 734.
- MURASCHI, T. F., FRIEND, M. & BOLLES, D. (1965). *Erwinia*-like microorganisms isolated from animal and human hosts. *Appl. Microbiol.* **13**, 128.
- NASUNO, S. & STARR, M. P. (1966). Polygalacturonase of *Erwinia carotovora*. *J. biol. Chem.* **241**, 5298.
- ROSYPAL, S. & ROSYPALOVÁ A. (1966). Genetic, phylogenetic and taxonomic relationships among bacteria as determined by their deoxyribonucleic acid base composition. *Folia. Publ. Fac. Nat. Sci. J. E. Purkynye Univ. (Biologia)* **7**, 1.

- SCHILDKRAUT, C. L., MARMUR, J. & DOTY, P. (1962). Determination of the base composition of deoxyribonucleic acid from its buoyant density in CsCl. *J. molec. Biol.* **4**, 430.
- STARR, M. P. (1947). The cause of bacterial root and stem disease of guayule. *Phytopathology* **37**, 291.
- STARR, M. P. (1959). Bacteria as plant pathogens. *A. Rev. Microbiol.* **13**, 211.
- STARR, M. P. & MANDEL, M. (1950). The nutrition of phytopathogenic bacteria. IV. Minimal nutritive requirements of the genus *Erwinia*. *J. Bact.* **60**, 669.
- STARR, M. P. & MORAN, F. (1962). Eliminative split of pectic substances by phytopathogenic soft-rot bacteria. *Science, N.Y.* **135**, 920.
- STARR, M. P., CARDONA, C. & FOLSOM, D. (1951). Bacterial fire blight of the raspberry. *Phytopathology* **41**, 915.
- STARR, M. P., COSENS, G. & KNACKMUSS, H.-J. (1966). Formation of the blue pigment indigoidine by phytopathogenic *Erwinia*. *Appl. Microbiol.* **14**, 870.
- STOLP, H., STARR, M. P. & BAIGENT, N. L. (1965). Problems in speciation of phytopathogenic pseudomonads and xanthomonads. *A. Rev. Phytopath.* **3**, 231.
- SUTTON, D. D. & STARR, M. P. (1959). Anaerobic dissimilation of glucose by *Erwinia amylovora*. *J. Bact.* **78**, 427.
- WALDEE, E. L. (1945). Comparative studies of some peritrichous phytopathogenic bacteria. *Iowa St. Coll. J. Sci.* **19**, 435.
- WHITCOMB, R. F., SHAPIRO, M. & RICHARDSON, J. (1966). An *Erwinia*-like bacterium pathogenic to leafhoppers. *J. Invertebr. Path.* **8**, 299.
- WINSLOW, C. E. A., BROADHURST, J., BUCHANAN, R. E., KRUMWIEDE, C. JUN., ROGERS, L. A. & SMITH, G. H. (1917). The families and genera of the bacteria. Preliminary report of the committee of the Society of American Bacteriologists on characterization and classification of bacterial types. *J. Bact.* **2**, 505.