Numerical Taxonomy and Deoxyribonucleic Acid Reassociation in the Taxonomy of Some Gram-Negative Fermentative Bacteria

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One hundred and five strains of gram-negative, fermentative bacteria, representing members of the *Enterobacteriaceae* together with strains of *Vibrio* and *Aeromonas*, were subjected to numerical taxonomy and deoxyribonucleic acid (DNA)/DNA reassociation studies. The numerical taxonomy sorted the strains into major clusters corresponding to *Edwardsiella tarda*, *Escherichia coli*, *Citrobacter freundii*, the tribe *Klebsiellae*, and *Vibrio alginolyticus*. Smaller clusters could be identified as *Serratia marcescens*, *Hafnia alvei*, and *Shigella* species. Seven reference strains were used in the DNA/DNA reassociation studies. *Klebsiella pneumoniae* at 60 C shared reassociation values of 80 to 100% with other strains of *Klebsiella*, 37 to 64% with *Enterobacter* spp., and lower values with *Hafnia*, *Citrobacter*, *Serratia*, and *Escherichia coli*. Phenetically, *K. pneumoniae* and *Enterobacter aerogenes* shared high relatedness, although they are only 41 to 64% related in the reassociation studies. *Hafnia*, *Salmonella typhimurium*, and *Edwardsiella tarda* showed little relatedness to any of the other strains included in this study. Both reference strains of *Escherichia coli* shared genetic resemblances greater than 65% at 60 C with *Shigella* and at least 70% with other strains of *Escherichia coli*. Overall, the agreement between numerical taxonomy and the DNA/DNA reassociation studies was satisfactory, with correlation coefficients ranging from 0.5 to 0.9. The choice of reference strains may well affect the correlation between the two techniques, and it is suggested that hypothetical median organisms be determined from the phenetic groupings and that actual strains that approximate to the hypothetical median organisms be used as reference strains for DNA reassociation.

The taxonomy of the *Enterobacteriaceae* has been influenced strongly by clinical workers who require rapid and convenient identification methods and by tradition. Some of the inconsistencies in the taxonomy of the *Enterobacteriaceae* will be discussed in another paper (R. Johnson, R. Colwell, R. Sakazaki, and K. Tamura, submitted for publication), noteworthy among them being the nomenclature of the genus *Salmonella* and the taxonomy of *Shigella*. In addition, there is a direct contradiction between the molecular and traditional viewpoints of the species of *Proteus*, which are maintained by the latter in a single genus, based

on intuitive, or alpha, classifications (2), whereas evidence from numerical taxonomy indicates the possible existence of three genera, with suggestion that at least two genera are supported by deoxyribonucleic acid (DNA) base composition data.

Work has been underway in our laboratories on studies involving the application of numerical taxonomy and molecular genetic techniques to the classification of the *Enterobacteriaceae*. Our initial approach was to examine a wide range of strains representative of the *Enterobacteriaceae*. In this study, we attempted to correlate the results obtained from numerical taxonomy with those obtained from DNA reassociation experiments. This approach has been termed polyphasic (9), and it has so far been used to good effect in studies on pseudomonads, vibrios, and oxidase-negative moraxellas (1, 10, 17, 20).

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MATERIALS AND METHODS

Bacterial strains. Ninety-nine strains, representative of genera of the *Enterobacteriaceae* and the genus *Vibrio*, were received as freeze-dried cultures or on agar slants. All strains included in the study were subcultured on brain heart infusion agar and examined for purity. Several of the cultures exhibited colonial variation, and each variant was treated as a separate strain for the numerical taxonomy. Six strains were included as reference cultures (see Table 1), bringing the total number of strains tested to 105.

Tests and computation. Each strain was subjected to a series of morphological, physiological, biochemical, and nutritional tests, yielding 238 characters. The tests and methods have been previously described (11; Johnson et al., submitted for publication). A preliminary analysis of the characters indicated that 110 were of little use in discriminating between strains, because either all, or nearly all, the strains were positive or they were all negative. These characters were omitted from the data matrix before the computation. The final data matrix comprised 105 strains, each coded for 128 characters. The data were analyzed on an IBM 370/165 Systems computer using a numerical taxonomy program and procedure of analysis that will be described in a later paper (Johnson et al., submitted for publication). Similarity coefficients (negative matches excluded) were calculated between all strains, and groups were formed by a single-linkage clustering method.

Preparation of DNA and DNA reassociation. The conditions used for DNA reassociation and separation of single-stranded from reassociated DNA on hydroxyapatite have been published (4-8, 12).

RESULTS

Numerical analysis. Of the 105 strains, 103 clustered at the 63% similarity (S) level (Fig. 1 and 2). Two reference strains, *Bacillus cereus* strain 99 and *Micrococcus* strain 98, joined the large phenon at lower similarity levels, 56 and 50% S, respectively. The 63% phenon was divisible into three groups. Twenty-two strains of *Edwardsiella tarda* formed a tight cluster at 90% S before joining the main group. Three strains of *Vibrio* and one *Pseudomonas* sp. (strains 92w, 92t, 96, and 94) also joined the main group at 63% S. The main group, consisting of 77 strains, formed at the 65% S level. The vibrio strains were positioned at one end of the largest cluster of strains and included eight strains of *Vibrio alginolyticus*, clustering at 85% S before being joined by a single strain of *Vibrio parahaemolyticus* at 79% S (Fig. 1). Two additional vibrio strains, 93s and 93r, and a single strain of *Aeromonas hydrophila*, strain 22, joined the remaining strains, which were all members of the *Enterobacteriaceae*, at 69 and 66% S (Fig. 2).

Within the *Enterobacteriaceae*, six clusters could be distinguished. Fifteen strains of *Escherichia coli* and *Alkalescens-Dispar* formed a cluster at 80% S, with three more strains joining at 79% (cluster 1, Fig. 2). Clusters 2, 3, and 4 joined at a level of 81% S. Cluster 2 contained eleven strains of *Citrobacter freundii*, which grouped at 87% S, before being joined by the single strain, 38, of *Salmonella typhimurium* at 84% S. Cluster 3, designated as the tribe *Klebsiellae*, contained 12 strains grouping at 82% S. Included in this group were representatives of the genera *Klebsiella*, *Enterobacter*, and *Erwinia*. The fourth cluster, consisting of two strains of *Serratia marcescens*, 13 and 37, plus a single strain of *Enterobacter liquefaciens*, 58, clustered at the 83% S level.

Cluster 5 contained three strains, two of which, 18 and 54, were received as *Enterobacter hafniae*, whereas the third strain, 40, was received as *Citrobacter freundii*. Strain 40 was reidentified as *E. hafniae* on the basis of being methyl red negative and Voges-Proskauer positive and by its ability to decarboxylate lysine and ornithine. It did, however, provide an unusual result in giving a positive reaction in Møllers arginine decarboxylation medium, although it is known that *E. hafniae* strains capable of carrying out this reaction do occur. Cluster 6 contained five strains of *Shigella* which fused at 77% S before joining with the other clusters at 73% S.

The identification of strains used in this study was checked against data given by Colwell (10) and Edwards and Ewing (15). With the exception of strain 40, discussed above, no serious discrepancies were apparent. No attempt was made to verify the identification of the *Erwinia* strains because of the unsatisfactory state of the taxonomy of these organisms and because they did not fall into a well-defined cluster.

DNA reassociation. A comparison of the results of the DNA/DNA reassociation experiments with those groupings obtained from the numerical analysis is shown in Table 2. The order of the strains is the same as that given in Fig. 2. The percentage of relative reassociation obtained at both 60 and 75 C was recorded, since it has been shown that for members of *Enterobacteriaceae* 60 C represents the optimal temperature for reassociation of single-stranded DNA into duplexes, whereas 75 C is a more stringent temperature, at which only closely related sequences can form stable duplexes (3). Thus, the denaturation temperature for a heterologous duplex formed at 75 C approximates more closely the denaturation

TABLE 1. Summary of strains studied^a

Univ. Md. strain no.	Designation	Culture collection no.	Univ. Md. strain no.	Designation	Culture collection no.
1L	<i>Aeromonas hydrophila</i>	ATCC 9071	48	<i>Aerobacter cloacae</i>	ATCC 961-39-03
1S			49	<i>Citrobacter freundii</i>	
1R			50	<i>Escherichia coli</i>	
4	Alkaescens-Dispar	02 7040-59	51	Alkaescens-Dispar	01 111 69
5	<i>Escherichia coli</i>	09 Bi 316-42	52	<i>Klebsiella pneumoniae</i>	Type 1-35-A-1
5T			53	<i>Aerobacter aerogenes</i>	
6	<i>Citrobacter freundii</i>	4a, 4b: 5, 6 Na19	54	<i>Enterobacter hafniae</i>	4360-67
7	<i>Escherichia coli</i>	02A U9-41	55	<i>Klebsiella ozaenae</i>	Type 4
8	<i>Shigella flexneri</i>	(Newcastle) 6	56	Alkaescens-Dispar	04-C2
9	Alkaescens-Dispar	01-111-69	57	<i>Citrobacter freundii</i>	6, 4b, 5b: 72 Stu 260 B
10	Alkaescens-Dispar	03			
11	<i>Shigella dysenteriae</i>	3	58	<i>Enterobacter liquefaciens</i>	6136-66
12	<i>Enterobacter aerogenes</i>	865-65	59	<i>Klebsiella ozaenae</i>	Type 4
13	<i>Serratia marcescens</i> subsp. <i>kiliensis</i>		60S	<i>Edwardsiella tarda</i>	1126-64
14	<i>Shigella boydii</i>	(Etousa) 7	60R	<i>E. tarda</i>	1126-64
14T	<i>S. boydii</i>	(Etousa) 7	61	<i>E. tarda</i>	5497-61
15	<i>Citrobacter freundii</i>	3a, 3b, 1c: 4, 5 Na2C	62	<i>E. tarda</i>	2995-65
16	<i>Shigella dysenteriae</i>	1	63	<i>E. tarda</i>	4226-59
17	<i>Salmonella typhi</i>	643	64	<i>E. tarda</i>	3889-64
18	<i>Hafnia</i>	CDC 962-68	66	<i>E. tarda</i>	2334-67
19	<i>Citrobacter freundii</i>	8a, 8b: (21) 25, 26 Mich 10	67	<i>E. tarda</i>	3882-61
			68R	<i>E. tarda</i>	3592-64
			68S	<i>E. tarda</i>	3592-64
			69R	<i>E. tarda</i>	1795-62
			69S	<i>E. tarda</i>	1795-62
20	<i>Shigella boydii</i>	1	70	<i>E. tarda</i>	6243-61
21	<i>Klebsiella pneumoniae</i>	Type-2	71	<i>E. tarda</i>	5343-61
22	<i>Aeromonas hydrophila</i>	ATCC 11439	72	<i>E. tarda</i>	1483-59
23	Alkaescens-Dispar	04 774-56	73	<i>E. tarda</i>	3888-64
24	<i>Escherichia coli</i>	04 U4/41	74	<i>E. tarda</i>	5159-63
25	Alkaescens-Dispar	08 1143-51	75	<i>E. tarda</i>	4411-68
26	<i>Shigella sonnei</i> smooth		76	<i>E. tarda</i>	4862-60
27	<i>S. dysenteriae</i>	2	77	<i>E. tarda</i>	2634-69
29S	<i>Citrobacter freundii</i>	ETS #07	80	<i>E. tarda</i>	4751-66
29R	<i>C. freundii</i>	ETS #07	80T	<i>E. tarda</i>	4751-66
30	Alkaescens-Dispar	06 4878-54	81	<i>Erwinia carotovora</i>	7-69
31	Alkaescens-Dispar	07 1868-62	82	<i>Erwinia carnegiana</i>	7-69
32	<i>Escherichia coli</i>	01A U5/41	83	<i>Erwinia dissolvens</i>	7-69
33	<i>Citrobacter freundii</i>	2a, 1b: 6, 5 Na23	84	<i>Erwinia aroideae</i>	7-69
34	<i>Escherichia coli</i>	025 E 47a	85	<i>Vibrio alginolyticus</i>	159-70
35	<i>E. coli</i>	081 strain no. H5	86	<i>V. alginolyticus</i>	165-70
36	<i>E. coli</i>	K12-1	87	<i>V. alginolyticus</i>	157-70
37	<i>Serratia marcescens</i>	1201-65	88	<i>V. alginolyticus</i>	156-70
38	<i>Salmonella typhimurium</i>	LT2	89	<i>V. alginolyticus</i>	166-70
39	<i>Citrobacter freundii</i>	1a, 1b, 1c: 1, 2 Na1A	90	<i>V. alginolyticus</i>	163-70
40	<i>C. freundii</i>	5a, 5b, 4b: 63 6331-50	91R	<i>V. alginolyticus</i>	164-70
41S	<i>C. freundii</i>	29a, 29b, 74 Balm	91W	<i>V. alginolyticus</i>	164-70
41R	<i>C. freundii</i>	29a, 29b, 74 Balm	92W	<i>Vibrio</i> sp. (Khartoum)	M
42	Alkaescens-Dispar	05 3123-63	92T	<i>Vibrio</i> sp. (Khartoum)	M
44	<i>Proteus morganii</i>		93S	<i>Vibrio</i> sp. (Khartoum)	SF
45	<i>Enterobacter cloacae</i>	121-65	93R	<i>Vibrio</i> sp. (Khartoum)	SF
46	<i>Klebsiella pneumoniae</i>	Type 1	94	<i>Vibrio cholerae</i>	ATCC 14033
47	<i>K. rhinoscleromatis</i>	Type 3	95	<i>Escherichia coli</i>	ATCC 11303
			96	<i>Pseudomonas</i> sp. Ox- Sawyer	
			97	<i>Vibrio parahaemolyticus</i>	Sak-3
			98	<i>Micrococcus</i> sp.	CB 234
			99	<i>Bacillus cereus</i>	

^aAbbreviations: Univ. Md., University of Maryland, College Park, Md.; ATCC, American Type Culture Collection, Rockville, Md. Species given with strain numbers without culture collection designation were obtained from Walter Reed Army Institute of Research, Washington, D. C.

FIG. 1. Dendrogram indicating the arrangement of 105 strains after clustering by single linkage.

temperature of a homologous duplex, since fewer unpaired bases occur. It has been found that highly related organisms usually share at least 70 to 75% DNA (8). Binding values less than this are interpreted as indicating that significant divergence has occurred and that the organisms are not members of the same species.

DNA from *Klebsiella pneumoniae* II gave high reassociation values only when compared with the DNA of strains found in cluster 3 and with strain 47, *Klebsiella rhinoscleromatis*, which fell outside cluster 3. Values for DNA relatedness ranging from 81 to 91% were obtained for those strains received as *Klebsiella*

FIG. 2. Similarity matrix corresponding to the dendrogram in Fig. 1.

species (strains 55, 59, 52, 46, and 47; Fig. 2), indicating high genetic similarity, as well as good agreement with the close phenetic similarity shown in Fig. 1 and 2. Four strains, *Enterobacter aerogenes* 12, *Enterobacter cloacae* 48 and 45, and *Erwinia dissolvens* 83 (Fig. 2), were included in cluster 3 by the numerical analysis but showed low homology values of 34 to 51% with the reference strain *K. pneumoniae* II. One strain, 53, received as *Aerobacter aerogenes*, grouped phenotypically with *K. pneumoniae*. On the basis of its nonmotility and positive lysine decarboxylase activity, it

appears to be a typical strain of *K. pneumoniae*. However, the DNA of this strain demonstrated only 56% relatedness with *K. pneumoniae* II DNA at 60 C and only 22% at 75 C. In contrast, high DNA relatedness (77 to 88%) for this strain and the two reference strains of *Enterobacter aerogenes* was noted (Table 2).

Strains of the other clusters showed only slight relatedness (12 to 32%) with *K. pneumoniae* II. The two reference strains of *Enterobacter aerogenes*, 1627/66 and 2164/70, shared large portions of their genomes with strains 53 and 12. Stringent (75 C) and nonstringent (60

C) binding were relatively similar, with little drop in reassociation, indicating few unpaired bases at 60 C.

Enterobacter hafniae 4360-67 appeared to have diverged significantly from all other members of *Enterobacteriaceae*. The DNA of this strain was 55% related when compared with the DNA of strain 18, also *E. hafniae*, whereas all other homologies were 30% or less.

Salmonella typhimurium LT2 DNA also yielded low reassociation values when reacted with the other DNA preparations. Two strains of *Citrobacter freundii*, 41R and 41S, both derived from a single parent strain, showed 58% homology at 60 C with LT2 but only 25% at 75 C.

The two reference strains of *Escherichia coli*, O2A and BB, both shared high homologies (>70%) with other strains of the *E. coli* cluster, including strains received as *E. coli* and *Alkalescens-Dispar*. Also noteworthy were the high reassociation values obtained for strains of the *Shigella* cluster, 65 to 81% at 60 C. Three strains, 26, 9, and 27 (Fig. 2), although not falling within the *E. coli* or *Shigella* clusters, demonstrated high relative binding with the two reference strains of *E. coli*. The single strain of *Salmonella typhimurium*, 38, reacted with the *E. coli* reference strains, sharing approximately 45% binding at 60 C and only 10% at 75 C, indicating few closely related DNA sequences.

Strains of *Edwardsiella tarda*, when reacted with the reference DNA, yielded reassociation values in the range 10 to 22%. This group appears to have diverged significantly from other members of *Enterobacteriaceae*, a conclusion in agreement with the position of these strains in the phenetic analysis (Fig. 1 and 2).

DISCUSSION

In general, the agreement between the clustering obtained from the numerical analysis and the DNA reassociation data was acceptable, although there were certain inconsistencies. The strains subjected to numerical analysis grouped into three rather loose, i.e., less tightly homogeneous, clusters. These included: (i) vibrio strains, for which no reassociation data were available; (ii) 22 strains of *Edwardsiella tarda*, which shared extremely high intragroup phenetic similarities (>90% S) yet were clearly separated from the majority of the remaining strains; and (iii) other members of *Entero-*

bacteriaceae. It will be reported that two biotypes or even two separate species of *Edwardsiella* may exist (Johnson et al., submitted for publication). However, all the strains used in this study appear to be typical of *Edwardsiella tarda*. The low phenetic similarity of this group, when compared with the other genera of the *Enterobacteriaceae*, is reflected in the low reassociation values noted for their DNA.

Figure 3 summarizes the degree of DNA reassociation observed for the clusters obtained from the numerical analysis. Cluster 3 was split into separate species, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, and *Enterobacter cloacae*. Strains of *Erwinia* were omitted because the data were insufficient.

By using the criterion for species relatedness as 75% DNA reassociation, it can be argued that *Shigella* and *Escherichia coli* are highly related and should be included in the same genus. *Alkalescens-Dispar* strains, as a general conclusion, are identical to strains of *Escherichia coli*. Although the nearest neighbors of the *Shigella* strains are members of the *E. coli* cluster, the phenetic similarities between the two groups were unexpectedly low, in the range 46 to 70%, with a mean of 59%. Thus the DNA relatedness was significantly higher than the observed phenetic similarities, whereas it would be expected that either they would be about equal or the phenetic similarities would be higher than the DNA homologies. Biochemically, the six strains of the *Shigella* cluster behaved typically, as judged by characters to be given (Johnson et al., submitted for publication) or following the scheme of Edwards and Ewing (15). This anomaly has been noted in an earlier study (7).

Klebsiella pneumoniae strains shared significant DNA relatedness between themselves and when compared with *Klebsiella rhinoscleromatis*, strain 47 (85%). The suggestion that *Enterobacter* and *Klebsiella* could be combined in the genus *Klebsiella* (Johnson et al., submitted for publication) needs to be evaluated in terms of what level of DNA relatedness constitutes a species, genus, etc. (3). The two genera are about 37 to 64% related at 60 C, with a drop to 24 to 42% for *Enterobacter aerogenes* DNA reacted with *Klebsiella pneumoniae* DNA at 75 C and even lower for *Enterobacter cloacae* DNA reacted with *K. pneumoniae* DNA at 75 C (10 to 15%). On the basis of commonly used tests, *Klebsiella* and *Enterobacter* may sometimes prove difficult to differentiate. Only motility and ornithine de-

TABLE 2. DNA homologies between selected reference strains and strains used in the numerical taxonomy analyses

Strain	Strain ^a																		
	<i>Klebsiella pneumoniae</i> II (21)			<i>Enterobacter hafniae</i> 4360-67 (54)			<i>Salmonella typhimurium</i> LT2 (38)			<i>Escherichia coli</i> O2A (7)			<i>Escherichia coli</i> BB		<i>Enterobacter aerogenes</i> 1627-66		<i>Enterobacter aerogenes</i> 2164-70		
	H _{6,0}	H _{7,5}	S	H _{6,0}	H _{7,5}	S	H _{6,0}	H _{7,5}	S	H _{6,0}	H _{7,5}	S	H _{6,0}	H _{7,5}	H _{6,0}	H _{7,5}	H _{6,0}	H _{7,5}	
<i>Shigella</i> sp. 16 14T 14 20 11 8													71	66	48	67	68		
													70	65	48	81	79		
													70	65	58	81	79		
													67	53	62	73	59		
4 81 84	30	7.5	44				38	13	58				73	64	57	76	70		
													65	40	63				
	17	5.1	61	30	12.3	68													
<i>Hafnia alvei</i> 40 54 18	16	3.3	70	100	100	100	20	2	68										
	14	3.3	67	55.3	18.8	77													
<i>Serratia</i> 13 37 58	27	9.1	75	23.6	5.3	71													
	25	5.6	68	12.3	3.1	73													
<i>Klebsiellae</i> 55 59 52 46 53 21 12 82 48 1e 45 83	80	74	73	18.2	2.0	66													
	80	74	72	18.2	2.0	65													
	91	76	77	19.8	2.2	65													
	46	76	79	19.8	2.2	69													
	53	22	80	19.3	3.5	70													
	100	100	100	16.8	3.3	70													
	51	16	83	11.0	2.8	73													
<i>Citrobacter</i> 39 49 6	37	15	68	17.3	2.9	72													
	38	9.6	68	18.0	4.5	74													
	34	13	72	14.0	2.5	75													

[illegible]

TABLE 2.—Continued

Strain	Strain ^a											
	<i>Klebsiella pneumoniae</i> II (21)			<i>Enterobacter hafniae</i> 4360-67 (54)			<i>Salmonella typhimurium</i> LT2 (38)			<i>Escherichia coli</i> O2A (7)		
	<i>H</i> ₆₀		<i>H</i> ₇₅	<i>H</i> ₆₀	<i>H</i> ₇₅	<i>S</i>	<i>H</i> ₆₀	<i>H</i> ₇₅	<i>S</i>	<i>H</i> ₆₀	<i>H</i> ₇₅	<i>S</i>
	<i>H</i> ₆₀	<i>H</i> ₇₅	<i>S</i>	<i>H</i> ₆₀	<i>H</i> ₇₅	<i>S</i>	<i>H</i> ₆₀	<i>H</i> ₇₅	<i>S</i>	<i>H</i> ₆₀	<i>H</i> ₇₅	<i>S</i>
76												
70												
74												
67												
72												
69R	16	4.5	48	19.0	1.7	59	18	5	53			
69S	16	4.5	48	19.0	1.7	59	18	5	53			
77												
75												
62												
68R							10	1	52			
64							10	1	51			
68S												
Correlation coefficient (<i>H</i> ₆₀ / <i>S</i>)		0.65			0.76			0.9		0.5		

^aHomology (H) and similarity (S) are expressed as percentages. *H*₆₀ indicates homology at 60°C; *H*₇₅ indicates homology at 75°C.

<i>Shigella</i>									
<i>Hafnia</i>	55-100								
<i>Serratia</i>	12-24								
Klebsiellae	(<i>Klebsiella</i>)	30	16-20	25-27	80-100				
	(<i>Enterobacter aerogenes</i>)		11	15-32	41-64	78-94			
	(<i>Enterobacter cloacae</i>)		17-18		37-38	29-44			
<i>Citrobacter freundii</i>		18-22		28-32	22-36				
<i>Escherichia coli</i>	65-81	18-21		27-29	20-38		44-45	70-100	
<i>Edwardsiella</i>		19		16	16-22				

<i>Shigella</i>	<i>Hafnia</i>	<i>Serratia</i>	<i>Klebsiella</i>	<i>Enterobacter aerogenes</i>	<i>Enterobacter cloacae</i>	<i>Citrobacter freundii</i>	<i>Escherichia coli</i>	<i>Edwardsiella</i>
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FIG. 3. Inter- and intragroup DNA homology ranges based on groups formed in the numerical analysis.

carboxylase activity appear to be definitive, and yet these species are not closely related genetically. If this is the case, attention should be focused on a search for improved tests and testing methods for identification and classification of these bacteria. Also, it is necessary to point out that selection of strains as reference strains in the DNA experiment is critical. The preferred choice in such studies would be the strain most similar to the hypothetical median organism (20).

A single strain of *Erwinia dissolvens*, 83, included in this study was found to be closely related phenetically with two strains of *Enterobacter cloacae* (45, 48) and a strain received as *Aeromonas hydrophila*, 1L. In our hands, strain 1L was oxidase negative, indole and methyl red negative, and Voges-Proskauer and Simmons citrate positive, and also decarboxylated arginine and ornithine. It appeared to be a typical strain of *Enterobacter cloacae*. The close phenetic relationship of *Erwinia dissolvens* to *Enterobacter cloacae* is substantiated by the 69 to 73% DNA/DNA reassociation found between these two species elsewhere (3). It seems likely that *Erwinia dissolvens* is not an *Erwinia* and should be reclassified at *Enterobacter cloacae* or as a new species of *Enterobacter*.

The proposal to include *Hafnia* in *Enterobacter* as *Enterobacter hafniae* (16) is debatable. *E. hafniae* appears to be a rather diffuse species, sharing little genetic relatedness with other species of *Enterobacter* or, for that

matter, with members of any of the other genera examined in this study. It thus appears to merit separate genus status. Further study should provide clarification of this point.

It has been reported that, on theoretical grounds, there should be a high correlation between phenetic similarities and DNA homologies, since both reflect to a greater or lesser extent the similarity of base sequences (14, 18, 21). The relationship between the two is not linear. Below approximately 60%, the homology values drop faster than phenetic similarities (13, 22). Explanations proposed for this phenomenon are several. It is possible that the limited number of features used in numerical taxonomy, reflecting only 10 to 20% of the genome (14), the choice of features, and the fact that in different genera the coding sequences for enzymes may differ, even though the resulting enzymes carry out the same biochemical reaction, can skew the numerical taxonomy results. In addition, incongruence will result from experimental error involved in the two techniques, i.e., experimental "noise" inherent in the methodologies. Also to be considered is the rather sharp drop in relatedness as measured by DNA reassociation when strains other than members of the same species are compared. It is significant that the range of 60 to 80% is less frequently observed, with reassociation of 40% noted for many interspecies and almost all intergeneric comparisons. It may well be necessary to explain this phenome-

non in order to understand the discrepancy between numerical taxonomy and DNA reassociation results.

Studies using numerical taxonomy and DNA reassociation methods are scarce in the literature. Colwell (10) reported correlation coefficients of 0.92 and 0.81 for reference strains of *Vibrio cholerae* and *V. parahaemolyticus* matched against other strains of these species. These are remarkably good values. Data extracted from a study on pseudomonads (10) revealed correlation coefficients ranging from 0.64 to 0.99 (only matches where the phenetic similarity between strains was greater than 60% were considered). This undoubtedly reflects a much greater divergence in *Pseudomonas* than has occurred in the two species of *Vibrio* considered.

Four of the seven reference strains used in the DNA homology studies were also included in the numerical taxonomy (Table 2). The homology values and their complementary phenetic similarities are recorded, and again, using only matches where the phenetic similarity values were greater than 60%, correlation coefficients ranging from 0.5 to 0.9 were calculated. These coefficients were obtained over the whole range of the organisms studied and not just within clusters. Again we see this as evidence of significant divergence with the *Enterobacteriaceae*. However, experimental error and the number and choice of phenetic characters used in the numerical taxonomy may contribute to lower correlation coefficients.

As in the pseudomonad study, the small number of comparisons that can be made from the data obtained in this study means that a detailed statistical analysis is not possible. Yet it is also impracticable to obtain homology values between all strains used in a taxonomic study. A better approach would be to carry out a detailed numerical taxonomic study, including sufficient strains of each species so that sizable clusters are obtained. From the clusters obtained in the numerical taxonomy analyses, hypothetical median organisms (20) can be calculated. These and other strains found to be highly related phenetically can be used as reference strains for other experimental approaches, such as DNA base composition determination and DNA reassociation and isozyme studies, in order that conclusions can be drawn from a truly polyphasic taxonomy.

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