Numerical Taxonomy Study of the *Enterobacteriaceae*

R. JOHNSON, R. R. COLWELL, R. SAKAZAKI, AND K. TAMURA

Department of Microbiology, University of Maryland, College Park, Maryland 20742, and National Institute of Health of Japan, 284, Kamiosaki-Chojamaru, Shinagawa-Ku Tokyo, Japan

Three hundred and eighty-four strains of bacteria representing the genera of the *Enterobacteriaceae* were examined over a wide range of biochemical, physiological, and morphological characters. The data were subjected to numerical analysis, and the resulting 33 clusters were equated as far as possible with established taxa within the *Enterobacteriaceae*. The clusters formed three groups. Group A corresponded to the tribe *Klebsiellae* and consisted of the genera *Enterobacter*, *Klebsiella*, and *Serratia*. The data suggest that *Enterobacter* and *Klebsiella* could be combined into a single genus, whereas *Hafnia alvei* should be retained in a genus separate from *Enterobacter*. Group B comprised the tribes *Edwardsiellae*, *Salmonelleae*, and *Escherichieae*. Strains of *Edwardsiella* species fell into two clusters, suggestive of possible biotype differences or perhaps two separate species. Many of Kauffmann's biochemical subgroup 1 salmonellae could be combined into a single species, which on grounds of priority should be designated *Salmonella enteritidis* (Gaertner) Castellani and Chalmers. However, there are other subgroup 1 serotypes which clearly do not belong in this species. Further study is suggested. Three species of *Shigella*, i.e., *S. boydii*, *S. flexneri*, and *S. dysenteriae*, were not separated by the analyses performed in this study. Either the three species cannot be identified on the basis of the biochemical characters employed or their identification requires considerable modification. Also included in group B were members of the genus *Yersinia*. Group C, representing the tribe *Proteae*, requires considerable revision, if a classification reflecting both molecular genetic and phenetic taxonomic relationships is to be attained.

With the application of computers to the analysis of data recorded in taxonomic studies, microbial classification has, in the last 15 years, received increased attention and interest. Papers published on the application of numerical techniques to bacterial taxonomy now run into many hundreds (11), and the majority of these have enabled considerable progress to be made in this field.

One area in which the application of numerical taxonomic techniques has been lacking is in examining relationships among members of the *Enterobacteriaceae*. Several papers have been published in which the authors examined a wide range of bacteria, among which were included some of the enteric organisms (22, 26, 65). In addition, individual groups of this family have been selected for study, notably *Serratia* (12, 27), *Erwinia* (49), *Salmonella* (48), the tribe *Klebsiellae* (4), and, more recently, *Obesumbacterium* (56). Yet, only a single study exists in which a representative selection of the whole family has been subjected to numerical analysis (44). Unfortunately, the results from this relatively limited analysis were inconclusive. That

is, the *Enterobacteriaceae* appeared to be made up of a continuous spectrum of bacteria showing little evidence of generic or specific subgroupings.

A larger study (4) including mainly members of the tribe *Klebsiellae* and reference strains of other enteric genera did show evidence of generic subgroupings. However, this study remains open to criticism because of the small number of characters recorded for each strain.

One of us (R. S.) accumulated a large amount of data on the *Enterobacteriaceae* and, in view of the noticeable lack of preceding studies, the data were subjected to numerical analysis. The results obtained, for the most part, bear out the currently accepted classification of this group and point out not only areas of taxonomic inconsistency, but also those areas where more research is required.

MATERIALS AND METHODS

Bacterial strains. Data were accumulated on a total of 384 strains representing the majority of the genera of the *Enterobacteriaceae*, together with representatives of the genus *Yersinia*. (A complete list of the strains used can be found in Tables 1 and 3.)

Morphology. Strains were subcultured onto Trypticase soy agar (BBL) and incubated at 30 C for 19 to 24 h. Cell form was examined by staining with Loeffler methylene blue. An India ink wet-film method was used to detect capsules. Flagella were observed using Leifson stain (13) and by electron microscopy. Gram reaction was determined using the Hucker modification of the Gram staining procedure (13). Colonial morphology was described from cultures grown on Trypticase soy agar (BBL) for 18 to 24 h at 30 C. Growth in nutrient broth was determined using heart infusion broth (Difco) followed by incubation at 30 C for up to 5 days. Pigmentation was recorded from observation of growth on the media of King, Ward, and Raney (39), yeast extract-mannitol agar (23), and heart infusion agar (Difco) containing either 0.2% (wt/vol) DL-phenylalanine or 0.5% (wt/vol) tyrosine.

Physiology and resistance. Tolerance to sodium chloride was detected in nutrient broth (Difco) containing 0, 0.5, 3.0, 5.0, 7.0, or 10% (wt/vol) NaCl. Temperature growth ranges (0 to 44 C) for cultures were determined in nutrient broth (Difco). Range of pH (4.0 to 10.0) for growth was tested on nutrient agar (Difco) in which the pH was adjusted after autoclaving, with tris(hydroxymethyl)aminomethane (Tris) and citrate phosphate buffer. Hemolysis was detected on heart infusion agar (Difco) containing 2% washed sheep red cells. Antibiotic sensitivities were tested on heart infusion agar (Difco) containing one of the following antibiotics: penicillin (10 U), dihydrostreptomycin (2.5 μ g and 10 μ g), chloromycetin (2.5 μ g, 10 μ g, and 30 μ g), erythromycin (15 μ g), kanamycin (30 μ g), aureomycin (30 μ g), novobiocin (30 μ g), terramycin (2.5 μ g and 30 μ g), or tetracycline (30 μ g). Sensitivity to the vibriostatic agent 0/129 was detected by placing several crystals on freshly inoculated heart infusion agar (Difco).

Biochemical reactions. The mode of glucose metabolism was tested using the oxidation-fermentation medium of Hugh and Leifson (BBL). Production of acid and gas from other carbohydrates or carbohydrate derivatives was detected in peptone water (Difco) containing Andrade's indicator and 1% (wt/vol) filter-sterilized carbohydrate. Other reactions included in the analyses were as follows: production of dextran or levan on nutrient agar (Difco) containing 5% (wt/vol) sucrose; methyl red and Voges-Proskauer in MRVP broth (BBL); oxidase by the methods of Kovacs (43) and Gaby and Free (25); catalase activity was detected in heart infusion broth (Difco) after incubation for 24 h, followed by the addition of several drops of 20% hydrogen peroxide; phosphatase following the method of Baird-Parker (2); reduction of nitrate and nitrite determined in peptone water (Difco) containing 0.1% potassium nitrate or potassium nitrite; gelatin liquefaction on Kohn charcoal-gelatin (41) on agar slant cultures incubated for 10 days at 30 C; agar digestion by the method of Colwell and Wiebe (13); action in litmus milk; growth and hydrolysis on skim milk agar made from heart infusion agar (Difco) containing 1% skim milk; ammonia production from Trypticase peptone water (BBL); hydrogen sulfide production from semisolid (ss) me-

dium containing 2% thiotone (BBL), 0.5% NaCl, and 0.02% ferric ammonium citrate; hydrogen sulfide from cystine detected in a semisolid medium containing 1% Trypticase, 0.02% L-cystine, 0.5% NaCl, and 0.02% ferric ammonium citrate; Christensen urease (BBL); indole production determined by the method of Macfarlane, Oakley, and Anderson (50); hydrolysis of Tweens 20, 40, 60, and 80 on Sierra medium (61); citrate utilization on Koser (42), Simmons (62), and Christensen media (10); phenylalanine deaminase detected on phenylalanine agar of Ewing, Davis and Reavis (19); amino acid decarboxylases in Møller medium (Difco); starch hydrolysis on heart infusion agar (Difco) containing 0.2% starch; aesculin hydrolysis by the method of Vaughn and Levine (70); serum digestion on Loeffler inspissated serum incubated for 10 days at 30 C; egg white digestion on Dorset egg slants (BBL) incubated for 10 days at 30 C; beta-galactosidase detected on the medium of LeMinor and Ben Hamida (46); deoxyribonuclease on deoxyribonuclease medium (BBL); and utilization of organic acids as sole carbon sources determined in Simmons agar base (62) (using the following sodium salts as carbon sources—malonate, formate, acetate, lactate, pyruvate, malate, fumarate, oxalate, alginate, and benzoate). Amino acid requirements were determined by the method of Colwell and Wiebe (13) using the following amino acids: L-phenylalanine, L-arginine, L-lysine, ornithine, β -alanine, L-proline, and L-tryptophane. Growth, slime production, and reduction of gluconate were recorded on Haynes medium (28). Growth on SS agar (Difco) and on brilliant green agar (BBL) were also recorded. Utilization of the organic acids D-tartrate, mucate, and citrate was detected on the medium of Kauffmann and Petersen (38).

Computation of data. A total of 216 characters were investigated, although not all characters were recorded for every strain. The average number of characters per strain was 176, of which 131 were common to all strains. Fifty-one characters were either positive or negative for all strains.

The data were coded into binary form using 1 for positive and 0 for negative. Noncomparable or missing characters were coded as 3. All computations were carried out on an IBM 370/165 electronic computer using a numerical taxonomy program developed by one of us (R.R.C.). Similarity coefficients using the coefficient of Jaccard, S_J , in which negative matches are excluded from the calculations, and simple matching coefficients, S_M , which takes negative matches into consideration, were computed between every pair of strains. Subsequent clustering was derived by single linkage analysis using the S_J coefficients (66).

RESULTS

The results of the initial computations are shown in Fig. 1 to 5. Thirty-three clusters were formed and are represented in Fig. 1 by shaded triangles. Examination of Fig. 1 reveals the existence of three large groups, A, B, and C, at a similarity level of 75 to 76%. These groups bear

FIG. 1. Dendrogram showing the arrangement of all strains of Enterobacteriaceae after single linkage clustering. Similarities determined using Jaccard's coefficient, S_J .

FIG. 2. Similarity matrix showing relationships between clusters 1 to 10 (cluster numbers given in Fig. 1).

some resemblance to the tribes outlined by Edwards and Ewing (14). Group A is equivalent to Edwards and Ewing's tribe IV, *Klebsiellae*; group B includes tribes I to III, i.e.,

Escherichieae, *Edwardsiellae*, and *Salmonelleae*, respectively; whereas group C is equivalent to tribe V, *Proteae*.

Of the 384 strains, 344 fell into one or another

FIG. 3. Similarity matrix showing relationships between clusters 11 to 22 (cluster numbers given in Fig. 1).

of the clusters. The remaining 40 strains, representing just over 10% of the total, were either associated with particular clusters, but were

atypical in some characters, or could not be placed accurately into any cluster.

In view of the large number of clusters, it is

FIG. 4. Similarity matrix showing relationships between clusters 23 to 28 (cluster numbers given in Fig. 1).

difficult, except in a monograph, to discuss the internal homogeneity of each in detail. Relevant information, such as actual strains, the number of strains, and the phenon levels at which each of the clusters form, can be readily extracted from Fig. 1 to 5 and Table 1.

Eight clusters contained strains which had been received under more than one specific epithet. Cluster 3 (see Table 1) contained 11

strains received under the names *Klebsiella aerogenes* or *Klebsiella* species. Taken in conjunction with cluster 2 and some intermediate strains, it forms part of a larger cluster which can be designated the *Enterobacter aerogenes*/*Klebsiella aerogenes* complex. This complex forms at the 90% phenon level, and within it two clusters, *E. aerogenes* and *K. aerogenes*, form at the 94 and 93% phenon levels, respec-

FIG. 5. Similarity matrix showing relationships between clusters 29 to 33 (cluster numbers given in Fig. 1).

tively (see Fig. 1 and 2).

Strains received under the name *Arizona arizonae* fell into two clusters, 20 and 21. In cluster 20, three strains of *Arizona* formed a tight group at the 93% phenon level; the group also included a single strain of *Salmonella seminoe*. A further six strains of *Arizona* were found in cluster 21, together with 31 strains of various *Salmonella* serotypes belonging to the Kauffmann biochemical subgroups I, II, and IV.

Cluster 25 contained 37 strains which were received under the names *Shigella boydii*, *Shigella flexneri*, or *Shigella dysenteriae* (see Table 1). This cluster formed at a level of 86%, and although Fig. 4 shows evidence for the existence of two subclusters within cluster 25,

these subclusters did not correspond with accepted *Shigella* species. Instead, representative strains of the above three species were found in each.

Cluster 28 contained 11 strains, nine strains of *Yersinia enterocolitica* which formed a group at the 89% level and were joined by another strain of *Y. enterocolitica* and an unidentified *Shigella* species at levels of 86 and 85%, respectively.

The remaining three clusters containing mixed strains were representatives of the genus *Proteus* (see Fig. 1 and 5). Cluster 31 contained 10 strains of *Proteus morganii* which grouped at 90% and were then joined by a single strain of *Proteus mirabilis* at 88% similarity. Cluster 32 contained eight strains of *Proteus vulgaris*,

TABLE 1. *Strains assigned to clusters in Fig. 1^a*

Cluster no. and identification	Strain serial no.	Strain received as	Source ^b
1. <i>Enterobacter cloacae</i>	3	<i>Enterobacter cloacae</i> 2015-53	R. Sakazaki
	4	<i>Enterobacter cloacae</i> 1302-54	R. Sakazaki
	5	<i>Enterobacter cloacae</i> 1609-53	R. Sakazaki
	6	<i>Enterobacter cloacae</i> 1366-53	R. Sakazaki
	7	<i>Enterobacter cloacae</i> 116-54	R. Sakazaki
	8	<i>Enterobacter cloacae</i> 0427-53	R. Sakazaki
	9	<i>Enterobacter cloacae</i> 558-53	R. Sakazaki
	10	<i>Enterobacter cloacae</i> SA153	R. Sakazaki
	13	<i>Enterobacter aerogenes</i> 1414-69	R. Sakazaki
	14	<i>Enterobacter aerogenes</i> 1412-69	R. Sakazaki
2. <i>Enterobacter aerogenes</i>	15	<i>Enterobacter aerogenes</i> 138-54	R. Sakazaki
	16	<i>Enterobacter aerogenes</i> 195-57	R. Sakazaki
	17	<i>Enterobacter aerogenes</i> 1405-69	R. Sakazaki
	18	<i>Klebsiella aerogenes</i> 293-61	R. Sakazaki
3. <i>Klebsiella aerogenes</i>	19	<i>Klebsiella aerogenes</i> 919	R. Sakazaki
	20	<i>Klebsiella aerogenes</i> 56	R. Sakazaki
	21	<i>Klebsiella aerogenes</i> 222-60	R. Sakazaki
	22	<i>Klebsiella aerogenes</i> 1470	R. Sakazaki
	23	<i>Klebsiella aerogenes</i> 281-51	R. Sakazaki
	24	<i>Klebsiella aerogenes</i> 1193	R. Sakazaki
	25	<i>Klebsiella aerogenes</i> 104-54	R. Sakazaki
	26	<i>Klebsiella aerogenes</i> 105-54	R. Sakazaki
	27	<i>Klebsiella</i> sp. 164-69	R. Sakazaki
	28	<i>Klebsiella aerogenes</i> 313	R. Sakazaki
4. <i>Klebsiella pneumoniae</i>	35	<i>Klebsiella pneumoniae</i> 271-54	R. Sakazaki
	36	<i>Klebsiella pneumoniae</i> 244-54	R. Sakazaki
	37	<i>Klebsiella pneumoniae</i> 13-53	R. Sakazaki
	38	<i>Klebsiella pneumoniae</i> 871-55	R. Sakazaki
	39	<i>Klebsiella pneumoniae</i> 1111-53	R. Sakazaki
	40	<i>Klebsiella</i> sp. 179-69	R. Sakazaki
	41	<i>Enterobacter liquefaciens</i> 1783-67	R. Sakazaki
5. <i>Enterobacter liquefaciens</i>	42	<i>Enterobacter liquefaciens</i> 2318-68	R. Sakazaki
	43	<i>Enterobacter liquefaciens</i> 1312-67	R. Sakazaki
	44	<i>Enterobacter liquefaciens</i> 1689-69	R. Sakazaki
	45	<i>Enterobacter liquefaciens</i> 2214-69	R. Sakazaki
	46	<i>Enterobacter liquefaciens</i> 1638-69	R. Sakazaki
	47	<i>Enterobacter liquefaciens</i> 2108-69	R. Sakazaki
	48	<i>Enterobacter liquefaciens</i> 1493-69	R. Sakazaki
	49	<i>Enterobacter liquefaciens</i> 1111-65	R. Sakazaki
	51	<i>Klebsiella rhinoscleromatis</i> 1218-53	R. Sakazaki
6. <i>Klebsiella rhinoscleromatis</i>	52	<i>Klebsiella rhinoscleromatis</i> 119-53	R. Sakazaki
	53	<i>Klebsiella rhinoscleromatis</i> 1230-34	R. Sakazaki
	54	<i>Klebsiella rhinoscleromatis</i> 136-55	R. Sakazaki
	58	<i>Klebsiella ozaenae</i> 19-69	R. Sakazaki
7. <i>Klebsiella ozaenae</i>	59	<i>Klebsiella</i> sp. 171-69	R. Sakazaki
	60	<i>Klebsiella</i> sp. 172-69	R. Sakazaki
	61	<i>Klebsiella ozaenae</i> 13-69	R. Sakazaki
	62	<i>Klebsiella ozaenae</i> 11-69	R. Sakazaki
	63	<i>Klebsiella ozaenae</i> 8-69	R. Sakazaki
	64	<i>Klebsiella ozaenae</i> 9-69	R. Sakazaki
	65	<i>Klebsiella ozaenae</i> 21-69	R. Sakazaki
	66	<i>Klebsiella ozaenae</i> 10-69	R. Sakazaki
	67	<i>Klebsiella ozaenae</i> 16-69	R. Sakazaki

^a For unassigned strains, see Table 3.

^b Key to sources: R. Sakazaki and N.I.H. Japan, National Institute of Health of Japan, 284, Kamiosaki-Chojamaru, Shinagawa-Ku, Tokyo; Eveland, W.C. Eveland, 406th Medical General Laboratory, U.S. Army, A.P.O. 500 c/o Postmaster, San Francisco; Costin, Romania, I.D. Costin, The State Inspection for Hygiene, Timisoara, Romania; F. Kauffman, Statens Seruminstitut, Artager Boulevard 80, Dk-2300 Copenhagen S, Denmark; WHO Int. Salmonella Center, World Health Organization, International Salmonella Center, Institut Pasteur, Paris; NCDC, National Communicable Disease Center, Atlanta, Ga.; NCTC National Collection of Type Cultures, Central Public Health Laboratory, Colindale Avenue, London, N.W. 9, England; Univ. of Tottori, University of Tottori, 1-6-chome, Tachikawa-cho, Tottori-shi, Japan; M.R.E. Porton, Microbiological Research Establishment, Porton Down, Wiltshire, England; Fort Collins, Colorado State University, Fort Collins, Colo.; Kitasato Inst. Microbiol. Res. Est., Kitasato Institute, Microbiological Research Establishment, Tokyo, Japan; Univ of Lund, Sweden, Lunds Universitet, Fack, 221 01 Lund 1, Sweden; ATCC, American Type Culture Collection, 12301 Parklawn Drive, Rockville, Md.

TABLE 1—Continued

Cluster no. and identification	Strain serial no.	Strain received as	Source ^b
8. <i>Klebsiella</i> sp.	68	<i>Klebsiella</i> sp.	170-69
	69	<i>Klebsiella</i> sp.	154-69
	70	<i>Klebsiella</i> sp.	159-69
	71	<i>Klebsiella</i> sp.	184-69
	72	<i>Klebsiella</i> sp.	163-69
	73	<i>Klebsiella</i> sp.	142-69
	74	<i>Klebsiella</i> sp.	153-69
	75	<i>Klebsiella</i> sp.	140-69
	76	<i>Klebsiella</i> sp.	156-69
	77	<i>Klebsiella</i> sp.	148-69
9. <i>Serratia marcescens</i>	78	<i>Klebsiella</i> sp.	144-69
	79	<i>Klebsiella</i> sp.	133-68
	80	<i>Klebsiella</i> sp.	177-69
	81	<i>Serratia marcescens</i>	3130-68
	82	<i>Serratia marcescens</i>	2121-68
	83	<i>Serratia marcescens</i>	2249-68
	84	<i>Serratia marcescens</i>	2187-68
	85	<i>Serratia marcescens</i>	2242-68
	86	<i>Serratia marcescens</i>	2963-68
	87	<i>Serratia marcescens</i>	2638-68
10. <i>Hafnia alvei</i>	88	<i>Serratia marcescens</i>	2241-68
	89	<i>Serratia marcescens</i>	2191-68
	90	<i>Serratia marcescens</i>	2630-68
	91	<i>Hafnia alvei</i>	56211
	92	<i>Hafnia alvei</i>	100-794
	93	<i>Hafnia alvei</i>	P344
	94	<i>Hafnia alvei</i>	887-61
	95	<i>Hafnia alvei</i>	441-61
	96	<i>Hafnia alvei</i>	P253
	97	<i>Hafnia alvei</i>	1190-61
11. <i>Edwardsiella tarda</i>	98	<i>Hafnia alvei</i>	732-61
	99	<i>Hafnia alvei</i>	70811
	100	<i>Hafnia alvei</i>	P228
	101	<i>Edwardsiella tarda</i>	449
	102	<i>Edwardsiella tarda</i>	122
	103	<i>Edwardsiella tarda</i>	121
	104	<i>Edwardsiella tarda</i>	55
	105	<i>Edwardsiella tarda</i>	51
	106	<i>Edwardsiella tarda</i>	39
	107	<i>Edwardsiella tarda</i>	9
12. <i>Edwardsiella</i> sp.	108	<i>Edwardsiella tarda</i>	41
	109	<i>Edwardsiella tarda</i>	123
	110	<i>Edwardsiella tarda</i>	130
	111	<i>Edwardsiella</i> sp.	1-1
	112	<i>Edwardsiella</i> sp.	1-4
	113	<i>Edwardsiella</i> sp.	3-1
	114	<i>Edwardsiella</i> sp.	11-3
	115	<i>Edwardsiella</i> sp.	8-5
	116	<i>Edwardsiella</i> sp.	8-1
	117	<i>Edwardsiella</i> sp.	6-2
13. <i>Salmonella typhi</i>	118	<i>Edwardsiella</i> sp.	8-3
	119	<i>Edwardsiella</i> sp.	8-2
	120	<i>Edwardsiella</i> sp.	10-3
	122	<i>Salmonella typhi</i>	T68-1
	123	<i>Salmonella typhi</i>	T68-6
	124	<i>Salmonella typhi</i>	T68-5
	125	<i>Salmonella typhi</i>	T68-12
	126	<i>Salmonella typhi</i>	T68-10
	127	<i>Salmonella typhi</i>	T68-9
	128	<i>Salmonella typhi</i>	T68-7
14. <i>Salmonella gallinarum</i>	129	<i>Salmonella typhi</i>	T68-8
	130	<i>Salmonella typhi</i>	T68-13
	131	<i>Salmonella typhi</i>	T68-11
	133	<i>Salmonella gallinarum</i>	412-D67
	134	<i>Salmonella gallinarum</i>	414-D67
			Costin, Romania
			Costin, Romania

TABLE 1—Continued

Cluster no. and identification	Strain serial no.	Strain received as	Source ^b
15. <i>Salmonella abortusequi</i>	135	<i>Salmonella gallinarum</i> 416-D67	Costin, Romania
	136	<i>Salmonella gallinarum</i> 355	Costin, Romania
	137	<i>Salmonella gallinarum</i> 359	Costin, Romania
	138	<i>Salmonella gallinarum</i> 353	Costin, Romania
	139	<i>Salmonella gallinarum</i> 358	Costin, Romania
	140	<i>Salmonella gallinarum</i> 357	Costin, Romania
	141	<i>Salmonella gallinarum</i> 354	Costin, Romania
	142	<i>Salmonella gallinarum</i> 356	Costin, Romania
	143	<i>Salmonella abortusequi</i> Hik	R. Sakazaki
	144	<i>Salmonella abortusequi</i> Ura	R. Sakazaki
	145	<i>Salmonella abortusequi</i> Hid	R. Sakazaki
	146	<i>Salmonella abortusequi</i> 26	F. Kauffmann
	147	<i>Salmonella abortusequi</i> Wh2	R. Sakazaki
	148	<i>Salmonella pullorum</i> 1345-D67	Costin, Romania
16. <i>Salmonella pullorum</i>	149	<i>Salmonella pullorum</i> 1139-D67	Costin, Romania
	150	<i>Salmonella pullorum</i> 64-D67	Costin, Romania
	151	<i>Salmonella pullorum</i> 1343-D67	Costin, Romania
	152	<i>Salmonella pullorum</i> 1334-D67	Costin, Romania
	153	<i>Salmonella pullorum</i> 21F	R. Sakazaki
	154	<i>Salmonella pullorum</i> 23F	R. Sakazaki
	155	<i>Salmonella pullorum</i> 66-D67	Costin, Romania
	156	<i>Salmonella paratyphi</i> A T67-193	R. Sakazaki
17. <i>Salmonella paratyphi</i> A	157	<i>Salmonella paratyphi</i> A T67-180	R. Sakazaki
	158	<i>Salmonella paratyphi</i> A T67-131	R. Sakazaki
	159	<i>Salmonella paratyphi</i> A T67-474	R. Sakazaki
	160	<i>Salmonella paratyphi</i> A T67-504	R. Sakazaki
	161	<i>Salmonella paratyphi</i> A T67-191	R. Sakazaki
	162	<i>Salmonella paratyphi</i> A T67-238	R. Sakazaki
	163	<i>Salmonella paratyphi</i> A T67-505	R. Sakazaki
	164	<i>Salmonella paratyphi</i> A T67-475	R. Sakazaki
	165	<i>Salmonella paratyphi</i> A T67-263	R. Sakazaki
	166	<i>Salmonella paratyphi</i> A T67-263	R. Sakazaki
18. <i>Salmonella sendai</i>	167	<i>Salmonella sendai</i> 619-65	R. Sakazaki
	168	<i>Salmonella sendai</i> 620-65	R. Sakazaki
	169	<i>Salmonella sendai</i> 622-65	R. Sakazaki
	170	<i>Salmonella sendai</i> 621-65	R. Sakazaki
	171	<i>Salmonella sendai</i> 618-65	R. Sakazaki
	172	<i>Citrobacter freundii</i> 10	R. Sakazaki
	173	<i>Citrobacter freundii</i> 7	R. Sakazaki
19. <i>Citrobacter freundii</i>	174	<i>Citrobacter freundii</i> 3	R. Sakazaki
	175	<i>Citrobacter freundii</i> 5	R. Sakazaki
	176	<i>Citrobacter freundii</i> 2	R. Sakazaki
	177	<i>Citrobacter freundii</i> 4	R. Sakazaki
	178	<i>Citrobacter freundii</i> 9	R. Sakazaki
	179	<i>Citrobacter freundii</i> 12	R. Sakazaki
	180	<i>Citrobacter freundii</i> 12	R. Sakazaki
20. <i>Arizona arizonae</i>	181	<i>Arizona arizonae</i> A3	R. Sakazaki
	182	<i>Arizona arizonae</i> A1	R. Sakazaki
	183	<i>Arizona arizonae</i> A2	R. Sakazaki
	184	<i>Salmonella seminale</i> 1685	WHO Int. Salmonella Center
21. <i>Salmonella</i> sp.	185	<i>Salmonella chameleon</i> 1684	WHO Int. Salmonella Center
	186	<i>Salmonella ochsenzoll</i> 1550	WHO Int. Salmonella Center
	187	<i>Salmonella argentina</i> 1672	WHO Int. Salmonella Center
	188	<i>Salmonella bonaire</i> 1594	WHO Int. Salmonella Center
	189	<i>Salmonella tuindorp</i> 1386	WHO Int. Salmonella Center
	190	<i>Salmonella houten</i> 1375	WHO Int. Salmonella Center
	191	<i>Salmonella munsburg</i> 1636	WHO Int. Salmonella Center
	192	<i>Salmonella parera</i> 1499	WHO Int. Salmonella Center
	193	<i>Salmonella soesterberg</i> 1403	WHO Int. Salmonella Center
	194	<i>Arizona arizonae</i> 82	R. Sakazaki
	195	<i>Arizona arizonae</i> 81	R. Sakazaki
	196	<i>Arizona arizonae</i> 75	R. Sakazaki
	197	<i>Arizona arizonae</i> 77	R. Sakazaki
	198	<i>Arizona arizonae</i> 74	R. Sakazaki
	199	<i>Salmonella eilbek</i>	WHO Int. Salmonella Center
	200	<i>Salmonella beloha</i>	WHO Int. Salmonella Center
	201	<i>Salmonella bilthoven</i>	WHO Int. Salmonella Center
	202	<i>Salmonella arizonae</i> 76	R. Sakazaki

TABLE 1—Continued

Cluster no. and identification	Strain serial no.	Strain received as	Source ^a
22. <i>Salmonella choleraesuis</i>	203	<i>Salmonella nairobi</i>	WHO Int. Salmonella Center
	204	<i>Salmonella setubal</i>	WHO Int. Salmonella Center
	205	<i>Salmonella artis</i>	WHO Int. Salmonella Center
	206	<i>Salmonella basel</i>	WHO Int. Salmonella Center
	207	<i>Salmonella ngozi</i>	WHO Int. Salmonella Center
	208	<i>Salmonella haarlem</i>	WHO Int. Salmonella Center
	209	<i>Salmonella locarno</i>	WHO Int. Salmonella Center
	210	<i>Salmonella derby</i> 1195-67	R. Sakazaki
	211	<i>Salmonella montevideo</i> 1274-67	R. Sakazaki
	212	<i>Salmonella enteritidis</i> 577-67	R. Sakazaki
	213	<i>Salmonella typhimurium</i> 1630-67	R. Sakazaki
	214	<i>Salmonella livingstone</i> 1197-67	R. Sakazaki
	215	<i>Salmonella anatum</i> 1237-67	R. Sakazaki
	216	<i>Salmonella meleagridis</i> 1200-67	R. Sakazaki
	217	<i>Salmonella infantis</i> 1215-67	R. Sakazaki
	218	<i>Salmonella bredeney</i> 1248-67	R. Sakazaki
	219	<i>Salmonella heidelberg</i> 1263-67	R. Sakazaki
	220	<i>Salmonella kiambu</i> 1244-67	R. Sakazaki
	221	<i>Salmonella krefeld</i> 1271-67	R. Sakazaki
	222	<i>Salmonella choleraesuis</i> 1390	R. Sakazaki
	223	<i>Salmonella choleraesuis</i> 1481	R. Sakazaki
	224	<i>Salmonella choleraesuis</i> 1-68	R. Sakazaki
23. <i>Escherichia coli</i>	225	<i>Salmonella choleraesuis</i> 1348	R. Sakazaki
	226	<i>Salmonella choleraesuis</i> 1480	R. Sakazaki
	229	<i>Escherichia coli</i> 026	R. Sakazaki
	230	<i>Escherichia coli</i> 0125	R. Sakazaki
	231	<i>Escherichia coli</i> 0126	R. Sakazaki
	232	<i>Escherichia coli</i> 0128	R. Sakazaki
	233	<i>Escherichia coli</i> 0119	R. Sakazaki
	234	<i>Escherichia coli</i> 086	R. Sakazaki
	235	<i>Escherichia coli</i> 055	R. Sakazaki
	236	<i>Escherichia coli</i> 044	R. Sakazaki
	237	<i>Escherichia coli</i> 193-68	R. Sakazaki
	238	<i>Escherichia coli</i> 905-65	R. Sakazaki
	239	<i>Escherichia coli</i> 0146	R. Sakazaki
	240	<i>Escherichia coli</i> 897-65	R. Sakazaki
	241	<i>Escherichia coli</i> 916-65	R. Sakazaki
	242	<i>Escherichia coli</i> 632-65	R. Sakazaki
	243	<i>Escherichia coli</i> 911-65	R. Sakazaki
	244	<i>Escherichia coli</i> 1184-68	R. Sakazaki
	245	<i>Escherichia coli</i> AD12	R. Sakazaki
	246	<i>Escherichia coli</i>	R. Sakazaki
	247	<i>Escherichia coli</i> 1186-68	R. Sakazaki
	248	<i>Escherichia coli</i> AD4	R. Sakazaki
24. <i>Shigella sonnei</i>	249	<i>Escherichia coli</i> AD3	R. Sakazaki
	250	<i>Shigella sonnei</i> 70-101	R. Sakazaki
	251	<i>Shigella sonnei</i> 70-177	R. Sakazaki
	252	<i>Shigella sonnei</i> 70-176	R. Sakazaki
	253	<i>Shigella sonnei</i> 70-92	R. Sakazaki
	254	<i>Shigella sonnei</i> 70-117	R. Sakazaki
	255	<i>Shigella sonnei</i> 70-76	R. Sakazaki
	256	<i>Shigella sonnei</i> 70-75	R. Sakazaki
	257	<i>Shigella sonnei</i> 70-87	R. Sakazaki
	258	<i>Shigella sonnei</i> 70-56	R. Sakazaki
	259	<i>Shigella sonnei</i> 70-69	R. Sakazaki
25. <i>Shigella</i> sp.	260	<i>Shigella boydii</i> 7 5369-51	NCDC
	261	<i>Shigella flexneri</i> 2b 9768	NCTC
	262	<i>Shigella flexneri</i> 2a 4807-62	NCDC
	263	<i>Shigella flexneri</i> 4a 608-62	NCDC
	264	<i>Shigella flexneri</i> Y 9730	NCTC
	265	<i>Shigella flexneri</i> 5 9727	NCTC
	266	<i>Shigella flexneri</i> 6 9729	NCTC
	267	<i>Shigella boydii</i> 2 2234-60	NCDC
	268	<i>Shigella boydii</i> 4 9770	NCTC
	269	<i>Shigella dysenteriae</i> 8 9346	NCTC
	270	<i>Shigella flexneri</i> 3b 9724	NCTC

TABLE 1—Continued

Cluster no. and identification	Strain serial no.	Strain received as	Source ^a
	271	<i>Shigella flexneri</i> X	9769
	272	<i>Shigella boydii</i> 5	341-69
	273	<i>Shigella boydii</i> 11	1529-69
	274	<i>Shigella dysenteriae</i> 7	9763
	275	<i>Shigella dysenteriae</i> 10	9351
	276	<i>Shigella boydii</i> 1	2064-59
	277	<i>Shigella flexneri</i> 3a	9989
	278	<i>Shigella dysenteriae</i> 2	9719
	279	<i>Shigella boydii</i> 5	9733
	280	<i>Shigella dysenteriae</i> 1	4379-60
	281	<i>Shigella boydii</i> 13	9361
	282	<i>Shigella boydii</i> 8	9354
	283	<i>Shigella boydii</i> 10	9358
	284	<i>Shigella boydii</i> 3	1050-50
	285	<i>Shigella boydii</i> 6	9771
	286	<i>Shigella dysenteriae</i> 3	9720
	287	<i>Shigella dysenteriae</i> 6	9762
	288	<i>Shigella dysenteriae</i> 5	9761
	289	<i>Shigella dysenteriae</i> 9	9348
	290	<i>Shigella dysenteriae</i> 4	9760
	291	<i>Shigella boydii</i> 12	9772
	292	<i>Shigella boydii</i> 11	9360
	293	<i>Shigella boydii</i> 14	9766
	294	<i>Shigella boydii</i> 15	9365
	295	<i>Shigella boydii</i> 9	9356
	296	<i>Shigella flexneri</i> 1b	4558-60
26. <i>Yersinia pseudo-tuberculosis</i>	298	<i>Yersinia pseudotuberculosis</i>	7
	299	<i>Yersinia pseudotuberculosis</i>	24
	300	<i>Yersinia pseudotuberculosis</i>	83
	301	<i>Yersinia pseudotuberculosis</i>	1779
	302	<i>Yersinia pseudotuberculosis</i>	2
	303	<i>Yersinia pseudotuberculosis</i>	1
	304	<i>Yersinia pseudotuberculosis</i>	Nishizawa
	305	<i>Yersinia pseudotuberculosis</i>	R2
27. <i>Yersinia pestis</i>	306	<i>Yersinia pestis</i>	Bryans
	307	<i>Yersinia pestis</i>	MP6
	308	<i>Yersinia pestis</i>	Ev 9-26-70
	309	<i>Yersinia pestis</i>	A1122
	310	<i>Yersinia pestis</i>	A29
	311	<i>Yersinia pestis</i>	012
	312	<i>Yersinia pestis</i>	F9581
	313	<i>Yersinia pestis</i>	M11-40
	314	<i>Yersinia pestis</i>	027
28. <i>Yersinia enterocolitica</i>	320	<i>Yersinia enterocolitica</i>	70
	321	<i>Yersinia enterocolitica</i>	6613
	322	<i>Yersinia enterocolitica</i>	Albany 5819
	323	<i>Yersinia enterocolitica</i>	Lucas 110
	324	<i>Yersinia enterocolitica</i>	23715
	325	<i>Yersinia enterocolitica</i>	MY079
	326	<i>Yersinia enterocolitica</i>	MYO
	327	<i>Yersinia enterocolitica</i>	Daniels
	328	<i>Yersinia enterocolitica</i>	Becht 51
	329	<i>Yersinia enterocolitica</i>	Vache
	330	<i>Shigella</i> sp.	3341
29. <i>Proteus rettgeri</i>	340	<i>Proteus rettgeri</i>	R2
	341	<i>Proteus rettgeri</i>	R4
	342	<i>Proteus rettgeri</i>	R1
	343	<i>Proteus rettgeri</i>	R3
	344	<i>Proteus rettgeri</i>	R7
	345	<i>Proteus rettgeri</i>	R5
	346	<i>Proteus rettgeri</i>	R6
30. <i>Providencia</i>	350	<i>Providencia inconstans</i>	42-68
	351	<i>Providencia inconstans</i>	38-68
	352	<i>Providencia inconstans</i>	P3

TABLE 1—Continued

Cluster no. and identification	Strain serial no.	Strain received as	Source ^b
31. <i>Proteus morganii</i>	353	<i>Providencia inconstans</i> 37-68	R. Sakazaki
	354	<i>Providencia inconstans</i> P24	R. Sakazaki
	355	<i>Proteus morganii</i> M1095	R. Sakazaki
	356	<i>Proteus morganii</i> M1093	R. Sakazaki
	357	<i>Proteus morganii</i> M1090	R. Sakazaki
	358	<i>Proteus morganii</i> M1091	R. Sakazaki
	359	<i>Proteus morganii</i> M1096	R. Sakazaki
	360	<i>Proteus morganii</i> Ni14	R. Sakazaki
	361	<i>Proteus morganii</i> M1097	R. Sakazaki
	362	<i>Proteus morganii</i> Ni17	R. Sakazaki
32. <i>Proteus vulgaris</i>	363	<i>Proteus morganii</i> Ni15	R. Sakazaki
	364	<i>Proteus morganii</i> M1094	R. Sakazaki
	365	<i>Proteus mirabilis</i> 1443-68	R. Sakazaki
	366	<i>Proteus vulgaris</i> 1445-68	R. Sakazaki
	367	<i>Proteus vulgaris</i> 1480-68	R. Sakazaki
	368	<i>Proteus vulgaris</i> 1458-68	R. Sakazaki
	369	<i>Poteus vulgaris</i> 1445-68	R. Sakazaki
	370	<i>Proteus vulgaris</i> 1488-68	R. Sakazaki
	371	<i>Proteus vulgaris</i> 1477-68	R. Sakazaki
	372	<i>Proteus vulgaris</i> 1440-68	R. Sakazaki
33. <i>Proteus mirabilis</i>	373	<i>Proteus mirabilis</i> 1437-68	R. Sakazaki
	374	<i>Proteus vulgaris</i> Pr. 5	R. Sakazaki
	375	<i>Proteus vulgaris</i> Pr. 2	R. Sakazaki
	376	<i>Proteus mirabilis</i> P 11	R. Sakazaki
	377	<i>Proteus mirabilis</i> Pr. 14	R. Sakazaki
	378	<i>Proteus mirabilis</i> Pr. 12	R. Sakazaki
	379	<i>Proteus mirabilis</i> Pr. 7	R. Sakazaki
	380	<i>Proteus mirabilis</i> Pr. 8	R. Sakazaki
	381	<i>Proteus mirabilis</i> Pr. 9	R. Sakazaki
	382	<i>Proteus mirabilis</i> Pr. 11	R. Sakazaki

seven of which clustered at 91% before being joined by the remaining strain of *P. vulgaris* and a single strain of *P. mirabilis* at 90% similarity. The final cluster, 33, contained seven strains of *P. mirabilis* forming at 90% and joined by a single strain of *P. vulgaris* at 88% similarity.

Characters associated with clusters. All strains included in this study were gram-negative rods with rounded ends. They tended to be of medium length, stout, and arranged singly. Colonies on agar invariably had an entire edge, and except for six strains were non-pigmented. Growth occurred over the temperature range 25 to 37 C and pH range 5.5 to 7.0. No strain required the addition of NaCl to support growth in nutrient broth (Difco). All strains were fermentative in Hugh and Leifson oxidation-fermentation medium, were catalase positive, and reduced nitrate.

Characters which were uniformly negative included the ability to grow at 3 C; pH 4.0, 4.5, or 10.0; and in the presence of 10% sodium chloride; Kovac's and Gaby's oxidase; reduction of nitrite; digestion of egg white; sensitivity to 10 U of penicillin; and the ability to grow on L-phenylalanine or oxalate.

In determining characters which would differ-

entiate between the clusters, only those 131 characters which were common to all strains were considered. After eliminating characters which were uniformly positive or negative for all strains, it was decided that a further 24 characters had poor discriminating value because either the number of strains which gave a positive reaction was very small or the differentiation between character states presented some difficulty. Among the 24 characters deleted were morphological characters such as size of colony, turbidity in broth, pigmentation, production of acid from mannose, digestion of casein, serum digestion, growth in 7% sodium chloride, growth at pH 5.0, 8.0, and 9.0, sensitivity to 10 µg of dihydrostreptomycin or 30 µg of kanamycin, and growth on L-tryptophane.

Final differentiation between clusters was based on 56 characters. At this point it seemed possible that these characters may have been chosen subjectively by us and, in fact, may give rise to a completely different arrangement if the strains were clustered on only these characters. This possibility was investigated by examining each of the 33 clusters for the 56 characters. A hypothetical median strain was calculated for each cluster by recording each

character as positive if more than 85% of the strains in that cluster were positive or recording the character as negative if more than 85% of the strains were negative. Any character which fell in between the 15 to 85% limits was scored as not comparable. The final data matrix thus contained 33 hypothetical median strains scored for 56 characters. The data were computed using a matching coefficient (because the number of characters was small) and clustered by single linkage analysis. The resulting dendrogram is shown in Fig. 6. Comparison of Fig. 1 and 6 shows that the two dendrograms are virtually identical except for one or two minor changes. We thus concluded from this exercise that the characters retained are a representative section of the total characters and that they successfully differentiate the 33 clusters. The individual characters of the clusters are shown in Table 2.

Position of atypical strains. An analysis of the 40 atypical strains is outlined in Table 3. The nearest neighbor refers to the final species name given to a cluster or clusters in this

analysis with which the given strain is in juxtaposition and not to an individual strain in the analyses. The results in which the atypical strain differed from its nearest neighbor are then given as "aberrant" results. In many cases the differences between the atypical strains and their nearest neighbors are few enough to enable the atypical strains to be identified. Two examples are *Enterobacter cloacae* 2-55 and *Enterobacter aerogenes* 114-50, which were both anaerogenic. Gas production was recorded for each of these strains from nine carbohydrates, and there is every reason to believe that they are correlated. No strain was able to produce gas from other sugars if it was unable to do so from glucose. The metabolism of most carbohydrates or carbohydrate derivatives occurs in the *Enterobacteriaceae* via the Embden-Meyerhoff-Parnas or hexose monophosphate pathways, both resulting in the formation of pyruvate. Pyruvate then may further undergo a "phosphoroclastic" split, resulting in the formation of formate and acetyl coenzyme A. At this stage a nonspecific formic hydrogenlyase system would

FIG. 6. Dendrogram based on single linkage clustering of hypothetical median strains. Similarities were determined using S_M , simple matching co-efficient.

TABLE 2. Biochemical characters of the clusters^a

Character	Cluster no. and name	
	1	2
<i>Enterobacter cloacae</i>	+	+
<i>Enterobacter aerogenes</i>	+	+
<i>Klebsiella aerogenes</i>	+	+
<i>Klebsiella pneumoniae</i>	+	+
<i>Enterobacter liquefaciens</i>	+	+
<i>Klebsiella rhinoscleromatis</i>	+	+
<i>Klebsiella ozaenae</i>	+	+
<i>Klebsiella</i> sp.	+	+
<i>Serratia marcescens</i>	+	+
<i>Hafnia alvei</i>	+	+
<i>Edwardsiella tarda</i>	+	+
<i>Edwardsiella</i> sp.	+	+
<i>Salmonella typhi</i>	+	+
<i>Salmonella gallinarum</i>	+	+
<i>Salmonella abortusequi</i>	+	+
<i>Salmonella pullorum</i>	+	+
<i>Salmonella paratyphi</i> A	+	+
<i>Salmonella sendai</i>	+	+
<i>Citrobacter freundii</i>	+	+
<i>Arizona arizonae</i>	+	+
<i>Salmonella</i> sp.	+	+
<i>Salmonella choleraesuis</i>	+	+
<i>Escherichia coli</i>	9/21 ^a 16/21	+
<i>Shigella sonnei</i>	+	+
<i>Shigella</i> sp.	+	+
<i>Yersinia pseudotuberculosis</i>	+	+
<i>Yersinia pestis</i>	+	+
<i>Yersinia enterocolitica</i>	+	+
<i>Proteus rettgeri</i>	+	+
<i>Providencia alcalifaciens</i>	+	+
<i>Proteus morganii</i>	+	+
<i>Proteus vulgaris</i>	+	+
<i>Proteus mirabilis</i>	+	+
Motility 30 °C	+	+
Gas from glucose	+	+
Acid from	+	+
Malose	+	+
Lactose	+	+
Sucrose	+	+
Galactose	+	+
Adonitol	+	+
Arabinose	+	+
Cellobiose	+	+
Dulcitol	+	+
Glycerol	5/8	+
Inositol	5/8	+
Melbiose	+	+
Melzitose	+	+
Raffinose	+	+
Rhamnose	+	+
Salicin	3/8	+
Sorbitol	+	+
Trehalose	+	+
Xylose	+	+
Indole	+	+
Methyl red	4/11	3/5
Voges Proskauer	2/5	+
Christensen citrate	+	+
Gelatin	+	+
Urease	2/5	+
K ON	7/9	+
Arginine decarboxylase	+	+
Lysine decarboxylase	+	+
Ornithine decarboxylase	+	+
H ₂ S from peptone	+	+
H ₂ S from thiosulfate	6/10	+

TABLE 3. Position of strains which fell outside the limits of the 33 clusters

Strain serial no.	Strain received as and source ^a	Nearest neighbor	Aberrant results
1	<i>Enterobacter cloacae</i> 2-55, R. Sakazaki	<i>Enterobacter cloacae</i>	Anaerogenic Mucate KP, negative Arginine dec., negative
2	<i>Enterobacter cloacae</i> 717-53, R. Sakazaki	<i>Enterobacter cloacae</i>	Sucrose, negative Cellobiose, negative Raffinose, negative Methyl red, positive Ornithine dec., negative Aesculin, negative Gluconate, negative Tetracycline sensitive Anaerogenic
11	<i>Enterobacter aerogenes</i> 114-54, R. Sakazaki	<i>Enterobacter aerogenes</i>	Inositol, negative Melezitose, negative Arginine dec., positive Growth on SS agar, negative
12	<i>Enterobacter aerogenes</i> 2-56, R. Sakazaki	<i>Enterobacter aerogenes</i>	Melezitose, negative Ornithine dec., negative Growth on SS agar, negative
29	<i>Enterobacter aerogenes</i> 36-55, R. Sakazaki	<i>Enterobacter aerogenes</i>	Melezitose, negative Ornithine dec., negative Growth on SS agar, negative
30	<i>Enterobacter aerogenes</i> 90-55, R. Sakazaki	<i>Enterobacter aerogenes</i>	Melezitose, negative Growth on SS agar, negative
31	<i>Enterobacter aerogenes</i> ATCC 14460	<i>Enterobacter aerogenes</i>	Melezitose, negative Gelatin, positive
32	<i>Klebsiella</i> sp. 150-69, R. Sakazaki	<i>Klebsiella pneumoniae</i>	Arabinose, negative Growth on pyruvate, positive Growth on brilliant green agar, negative
33	<i>Klebsiella</i> sp. 186-69, R. Sakazaki	<i>Klebsiella pneumoniae</i>	Arabinose, negative Glycerol, negative
34	<i>Klebsiella</i> sp. 141-69, R. Sakazaki	<i>Klebsiella pneumoniae</i>	Arabinose, negative
50	<i>Enterobacter liquefaciens</i> D974, R. Sakazaki	<i>Enterobacter liquefaciens</i>	Maltose, sucrose, raffinose, sorbitol, and xylose, all negative
55	<i>Klebsiella pneumoniae</i> 213-51, R. Sakazaki	<i>Klebsiella ozaenae</i>	Voges-Proskauer, positive Urease, positive Gluconate, positive Aesculin, positive
56	<i>Klebsiella ozaenae</i> 22-69, R. Sakazaki	<i>Klebsiella ozaenae</i>	Sucrose, inositol, and rhamnose, all positive
57	<i>Klebsiella ozaenae</i> 12-69, R. Sakazaki	<i>Klebsiella ozaenae</i>	Mucate KP, positive Glycerol, negative Indole, negative Christensen's citrate, negative Starch hydrolysis, negative Growth on 5% NaCl, negative D-tartrate KP, positive
121	<i>Escherichia coli</i> AD8, R. Sakazaki	<i>Escherichia coli</i>	Anaerogenic Trehalose, negative Trehalose, negative Mucate KP, positive Citrate, positive Sucrose, positive Indole, positive H ₂ S from thiosulfate, negative Lactate, negative Pyruvate, negative
132	<i>Salmonella pullorum</i> 1337-D67, Costin, Romania	<i>Salmonella pullorum</i>	Anaerogenic Trehalose, negative Trehalose, negative Mucate KP, positive Citrate, positive Sucrose, positive Indole, positive H ₂ S from thiosulfate, negative Lactate, negative Pyruvate, negative
166	<i>Salmonella pullorum</i> 1138-D67, Costin, Romania	<i>Salmonella pullorum</i>	Anaerogenic Trehalose, negative Trehalose, negative Mucate KP, positive Citrate, positive Sucrose, positive Indole, positive H ₂ S from thiosulfate, negative Lactate, negative Pyruvate, negative
180	<i>Arizona arizonae</i> 80, R. Sakazaki	<i>Salmonella</i> sp.	Anaerogenic Trehalose, negative Trehalose, negative Mucate KP, positive Citrate, positive Sucrose, positive Indole, positive H ₂ S from thiosulfate, negative Lactate, negative Pyruvate, negative
227	<i>Citrobacter freundii</i> 6, R. Sakazaki	<i>Citrobacter freundii</i>	Anaerogenic Urease, positive Lactose, negative Sucrose, positive
228	<i>Citrobacter freundii</i> 1, R. Sakazaki	<i>Citrobacter freundii</i>	Motility, salicin, both negative
297	<i>Yersinia pseudotuberculosis</i> 12, Univ. of Tottori, Japan	<i>Yersinia pseudotuberculosis</i>	Glycerol, negative Pyruvate, negative Lactate, negative Growth on 5% NaCl, negative Maltose, negative Glycerol, negative
315	<i>Escherichia coli</i> AD6, R. Sakazaki	<i>Escherichia coli</i>	Glycerol, negative Pyruvate, negative Lactate, negative Growth on 5% NaCl, negative Maltose, negative Glycerol, negative
316	<i>Escherichia coli</i> 633-65, R. Sakazaki	<i>Escherichia coli</i>	Glycerol, negative

^a For key to sources, see footnote *b* to Table 1.

TABLE 3—Continued

Strain serial no.	Strain received as and source ^a	Nearest neighbor	Aberrant results
317	<i>Escherichia coli</i> AD9, R. Sakazaki	<i>Escherichia coli</i>	Lactate, negative Pyruvate, negative H ₂ S from peptone, negative Glycerol, negative Growth on 5% NaCl, negative Lactate, negative Pyruvate, negative Glycerol, negative Sorbitol, negative Indole, negative Lactate, negative Pyruvate, negative Growth on 5% NaCl, negative Cellobiose, positive Citrate, negative Gluconate, positive Growth 44 °C, negative Growth 5% NaCl, negative
318	<i>Escherichia coli</i> 0127a, R. Sakazaki	<i>Escherichia coli</i>	Raffinose, positive Salicin, negative Dulcitol, positive Glycerol, positive Gluconate, positive Ornithine dec., positive Glycerol, positive Citrate, positive Gluconate, positive H ₂ S from peptone, positive Ornithine dec., positive
319	<i>Shigella</i> sp. 2710, Univ. of Lund, Sweden	<i>Escherichia coli</i>	
331	<i>Yersinia pseudotuberculosis</i> 51, Univ. of Tottori, Japan	<i>Yersinia pseudotuberculosis</i>	
332	<i>Shigella</i> sp. 1621, Univ. of Lund, Sweden	<i>Shigella</i> sp.	
333	<i>Shigella</i> sp. 3873, Univ. of Lund, Sweden	<i>Shigella</i> sp.	
334	<i>Escherichia coli</i> AD13, R. Sakazaki	No near relatives	
335	<i>Providencia inconstans</i> P9, R. Sakazaki	<i>Providencia</i>	Nonmotile; maltose, sucrose, arabinose, lactose, raffinose, and sorbitol, all positive Methyl red, negative Gluconate, positive Anaerogenic Adonitol, negative Melibiose, negative Trehalose, positive Urease, negative Adonitol, negative Inositol, positive Sorbitol, positive Aesculin, negative Maltose, negative Sucrose, positive Adonitol, negative Melibiose, negative Raffinose, positive Urease, negative Sucrose, positive Inositol, negative Urease, positive Inositol, rhamnose, Voges-Proskauer, urease, arginine dec., ornithine dec., all positive Starch, negative Inositol, rhamnose, urease, arginine dec., pyruvate, all positive Starch, negative Growth on 5% NaCl, positive Inositol, positive Rhamnose, positive Urease, positive Maltose, negative Sucrose, negative Melibiose, negative Voges-Proskauer, negative Voges-Proskauer, negative Gelatinase, negative
336	<i>Providencia inconstans</i> Pr. 1, R. Sakazaki	<i>Proteus rettgeri</i>	
337	<i>Providencia inconstans</i> P4, R. Sakazaki	<i>Providencia</i>	
338	<i>Providencia inconstans</i> P6, R. Sakazaki	<i>Proteus rettgeri</i>	
339	<i>Providencia inconstans</i> P17, R. Sakazaki	<i>Proteus rettgeri</i>	
347	<i>Proteus rettgeri</i> R8, R. Sakazaki	<i>Providencia</i>	
348	<i>Proteus rettgeri</i> R9, R. Sakazaki	<i>Providencia</i>	
349	<i>Proteus rettgeri</i> 1446-68, R. Sakazaki	<i>Providencia</i>	
383	<i>Proteus mirabilis</i> R12, R. Sakazaki	<i>Proteus mirabilis</i>	
384	<i>Proteus vulgaris</i> 1451-68, R. Sakazaki	<i>Proteus vulgaris</i>	

TABLE 4. Identification of the clusters

Cluster no.	Designation of majority of strains	Identification according to:	
		Bascomb et al. (4)	Edwards and Ewing (14)
1	<i>Enterobacter cloacae</i>	<i>Enterobacter cloacae</i>	<i>Enterobacter cloacae</i>
2	<i>Enterobacter aerogenes</i>	<i>Enterobacter aerogenes</i>	<i>Enterobacter aerogenes</i>
3	<i>Klebsiella aerogenes</i>	<i>Klebsiella aerogenes</i>	<i>Klebsiella pneumoniae</i>
4	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i>
5	<i>Enterobacter liquefaciens</i>	<i>Enterobacter liquefaciens</i>	<i>Enterobacter liquefaciens</i>
6	<i>Klebsiella rhinoscleromatis</i>	<i>Klebsiella rhinoscleromatis</i>	<i>Klebsiella rhinoscleromatis</i>
7	<i>Klebsiella ozaenae</i>	<i>Klebsiella ozaenae</i>	<i>Klebsiella ozaenae</i>
8	<i>Klebsiella</i> species	No equivalent	No equivalent
9	<i>Serratia marcescens</i>	<i>Serratia marcescens</i>	<i>Serratia marcescens</i>
10	<i>Hafnia alvei</i>	<i>Hafnia alvei</i>	<i>Enterobacter hafniae</i>
11	<i>Edwardsiella tarda</i>	<i>Edwardsiella tarda</i>	<i>Edwardsiella tarda</i>
12	<i>Edwardsiella</i> species	No equivalent	No equivalent
13	<i>Salmonella typhi</i>	<i>Salmonella typhi</i>	<i>Salmonella typhi</i>
14	<i>Salmonella gallinarum</i>	<i>Salmonella gallinarum</i>	<i>Salmonella enteritidis</i>
15	<i>Salmonella abortusequi</i>	No equivalent	<i>Salmonella enteritidis</i>
16	<i>Salmonella pullorum</i>	<i>Salmonella pullorum</i>	<i>Salmonella enteritidis</i>
17	<i>Salmonella paratyphi A</i>	<i>Salmonella paratyphi A</i>	<i>Salmonella enteritidis</i>
18	<i>Salmonella sendai</i>	No equivalent	<i>Salmonella enteritidis</i>
19	<i>Citrobacter freundii</i>	<i>Citrobacter freundii</i>	<i>Citrobacter freundii</i>
20	<i>Arizona arizonae</i>	<i>Salmonella</i> subgenus III	<i>Arizona hinshawii</i>
21	<i>Salmonella</i> species	No equivalent	<i>Salmonella enteritidis</i>
22	<i>Salmonella choleraesuis</i>	<i>Salmonella choleraesuis</i>	<i>Salmonella choleraesuis</i>
23	<i>Escherichia coli</i>	<i>Escherichia coli</i>	<i>Escherichia coli</i>
24	<i>Shigella sonnei</i>	<i>Shigella sonnei</i>	<i>Shigella sonnei</i>
25	<i>Shigella</i> species	<i>Shigella</i> species—other serotypes	No equivalent
26	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	No equivalent
27	<i>Yersinia pestis</i>	<i>Yersinia pestis</i>	No equivalent
28	<i>Yersinia enterocolitica</i>	<i>Yersinia enterocolitica</i>	No equivalent
29	<i>Proteus rettgeri</i>	<i>Proteus rettgeri</i>	<i>Proteus rettgeri</i>
30	<i>Providencia inconstans</i>	<i>Providencia alcalifaciens</i>	<i>Providencia alcalifaciens</i>
31	<i>Proteus morganii</i>	<i>Proteus morganii</i>	<i>Proteus morganii</i>
32	<i>Proteus vulgaris</i>	<i>Proteus vulgaris</i>	<i>Proteus vulgaris</i>
33	<i>Proteus mirabilis</i>	<i>Proteus mirabilis</i>	<i>Proteus mirabilis</i>

characters studied by these authors may be submitted as a possible criticism, our survey, which included far more characters, substantiates their general findings. Cluster 10, as judged by its position in Fig. 1 or 6, retains a position away from the *Enterobacter-Klebsiella-Serratia* complex and appears to justify its status as a separate genus, *Hafnia*, rather than to be included in *Enterobacter* as *Enterobacter alvei* or *Enterobacter hafniae* as has been suggested (20, 59). The molecular genetic dissimilarities, i.e., overall deoxyribonucleic acid (DNA) base composition between *Hafnia alvei* ATCC 23280, NCTC 8105, and 9540, have been reported at 48.0 to 48.7 mol% (56, 67). These values lie significantly below the general range of 52 to 58 mol% obtained with other *Enterobacter* species (29, 58). Furthermore, unpublished data of D. Brenner (personal communication) indicates that, in DNA/DNA hybridization studies, *Hafnia alvei* showed only 11 to 26% association with either *Enterobacter* or *Kleb-*

siella species, whereas reassociations between the DNAs of *Enterobacter* and *Klebsiella* were in the range of 40 to 100%.

Among the clusters of group A, *Enterobacter cloacae*, *Enterobacter liquefaciens*, *Klebsiella rhinoscleromatis*, and *Serratia marcescens* are all recognized as valid species by the two reference systems. The relationships observed in this study between clusters 2, 3, and 4 are, however, open to different interpretations. Cluster 3 contained ten strains received as *Klebsiella aerogenes* and one strain received as *Klebsiella* species. Three strains of *Enterobacter aerogenes* (36-55, 90-55, and ATCC 14460) shared high similarities with both cluster 3 and cluster 2, which contained five strains of *Enterobacter aerogenes*. The five strains of *Enterobacter aerogenes* in cluster 2 could be distinguished from cluster 3 by their motility, production of acid from melezitose, negative urease, and positive ornithine decarboxylase.

Comparison of clusters 3 and 4 reveals that

they are differentiated only by growth on lactate, SS agar, and brilliant green bile agar, all positive in cluster 3. These characters hardly seem reasonable grounds for differentiating the two clusters into separate species. Bascomb et al. (4) listed growth in Møller KCN broth as the only definitive character separating *Klebsiella pneumoniae* from *Klebsiella aerogenes*/*K. oxytoca*. In addition, their five strains of *K. pneumoniae* were not clearly separated from their *K. aerogenes*/*oxytoca*/*K. edwardsii* clusters in their dendrogram. In our hands, two out of five strains of *K. pneumoniae* were capable of growth in KCN broth. We, therefore, agree with the classification of Edwards and Ewing in designating both clusters 3 and 4 as *K. pneumoniae*.

Clusters 7 and 8, *Klebsiella ozaenae* and *Klebsiella* species, respectively, showed differences in arabinose, methyl red, lysine decarboxylase, and utilization of mucate, although only lysine decarboxylase was absolutely definitive. It is possible that two biotypes of *K. ozaenae* exist, and on the basis of the results obtained in this study there are insufficient characters to warrant giving both biotypes species status. We propose, therefore, to recognize clusters 7 and 8 as comprising the same species, *K. ozaenae*, with biotypes A and B, respectively.

We separated the nine clusters of group A into seven species: *Enterobacter cloacae*, *Enterobacter aerogenes*, *Enterobacter liquefaciens*, *Klebsiella pneumoniae*, *Klebsiella ozaenae*, *Klebsiella rhinoscleromatis*, and *Serratia marcescens*. We feel, however, that the generic composition of these species merits some discussion. Bascomb et al. (4) have proposed the transfer of *Enterobacter liquefaciens* to the genus *Serratia*. The two species differ in the production of acid from adonitol, galactose, arabinose, raffinose, and xylose, and in aesculin hydrolysis, urease, and growth on SS agar. Studies involving larger numbers of strains, however, indicate that only arabinose and raffinose are definitive or nearly so (14, 18). Pigmentation is of little value because many fresh isolates of *Serratia marcescens* are nonpigmented. Their G+C base composition (*Serratia* 53 to 60%; *Enterobacter liquefaciens* 52 to 58%) would not preclude them from being placed in a single genus. Difficulties have been reported (18, 73) in distinguishing the two species and, consequently, we see no reason why *Enterobacter liquefaciens* should not be transferred to *Serratia* as *Serratia liquefaciens*.

Bascomb et al. further proposed that *Enterobacter aerogenes* be included in *Klebsiella* as *Klebsiella mobilis*. On the basis of the close

similarity between these species in this study, we find their argument not entirely without basis. Only five characters served to differentiate the two clusters, and three strains of *Enterobacter aerogenes* shared high similarities with both clusters. With the exception of motility, the original description of *Enterobacter* by Hormaeche and Edwards (31) would also be applicable to the genus *Klebsiella*. The presence of ornithine decarboxylase has also been cited as a differentiating character (14). These two characters can be considered insufficient for differentiating genera. Furthermore, there is no logical reason why motile and nonmotile species cannot be accommodated within a single genus (63).

The genus *Enterobacter* was proposed by Hormaeche and Edwards for motile strains of *Aerobacter aerogenes*, together with *Aerobacter cloacae*, nonmotile strains of *Aerobacter aerogenes* being placed in the genus *Klebsiella* as *Klebsiella pneumoniae*. Relevant papers concerning these taxonomic changes have been published by Hormaeche and Edwards (30-32), Hormaeche and Munilla (33), Edwards and Fife (15), Skinner (63), Carpenter et al. (9), and the Judicial Commission of the ICSB (Opinion 28 [54] and Opinion 46 [55]). In summary, the genera *Aerobacter*, *Cloaca*, and *Enterobacter* are synonymous. Both *Aerobacter* and *Cloaca* were placed on the list of rejected names by the Judicial Commission of the ICSB, whereas *Enterobacter* was conserved. The above authors agreed that nonmotile strains of *Aerobacter aerogenes* (not genuine nonmotile mutants) were identical to *Klebsiella pneumoniae*, and studies were undertaken to find adequate methods to differentiate the motile *Aerobacter* strains from the nonmotile *Klebsiella* strains. At this time only three species were involved, *Klebsiella pneumoniae*, *Aerobacter aerogenes*, and *Aerobacter cloacae*. Hormaeche and Munilla (33) differentiated *Klebsiella* and *Aerobacter* (*Cloaca*) on the basis of motility, gas production from inositol, glycerol, and insoluble starch, urease production, and arginine dihydrolase activity. On the basis of these results, however, Hormaeche and Munilla (33) were unable to identify strains of rhinoscleroma and ozaena bacilli. These authors formed the opinion that these particular strains were intermediates between the motile and nonmotile groups, but were more closely related to the motile *Cloaca*. The bacilli of rhinoscleroma and ozaena have now attained species status in the genus *Klebsiella*, and their biochemical properties serve only to obscure the generic differences between *Klebsiella* and *Enterobacter*.

Three more species have been proposed for inclusion in *Enterobacter*. The exclusion of two of these, *E. liquefaciens* and *E. hafniae*, has already been discussed. The third species, *Enterobacter agglomerans*, was proposed by Ewing and Fife (21) as the niche for the *herbicola/lathyr*i group of bacteria previously classified in the genus *Erwinia*. Although it can be accepted that several of the species of *Erwinia* will undoubtedly find more permanent homes in other genera of the *Enterobacteriaceae*, the strains of the species *E. agglomerans* are so biochemically diverse that the species is poorly defined, at best. Its position in *Enterobacter* has yet to be accepted, and we would hesitate to include it until more evidence is available. Of the species that remain in the genera *Klebsiella* and *Enterobacter*, only motility and ornithine decarboxylase effectively separate them into two genera. We have shown that, when tested over a large number of characters, they share a high overall similarity. Their DNA base ratios fall within the same range of 52 to 58 mol%. Evidence from DNA/DNA hybridization (8) indicates that they share significant amounts of common DNA sequences. Thus, it seems possible that all five species of *Klebsiella* and *Enterobacter* could be combined in a single genus which, by priority, would be *Klebsiella* Trevisan 1887. Although *Klebsiella* has been traditionally thought of as nonmotile, based on the nonmotility of the type species *K. pneumoniae*, there are many other genera, even within the *Enterobacteriaceae*, that contain motile and nonmotile species, so tradition need not be cited in this instance.

Further studies currently underway in our laboratories have been initiated so that certain questions such as the relationship of *Enterobacter agglomerans* to the *Klebsiellae* can be clarified.

Group B: the tribes Edwardsiellae, Salmonelleae, and Escherichiae. Group B, containing clusters 11 to 28, corresponds to the three tribes designated by Edwards and Ewing as *Edwardsiellae*, *Salmonelleae*, and *Escherichiae*. Strains received as *Edwardsiella tarda* or *Edwardsiella* species fell into two clusters, 11 and 12. Cluster 11 corresponded to typical *E. tarda* strains, whereas those in cluster 12 differed in being salicin positive, sucrose positive, and citrate positive. The two clusters joined at 92% similarity, indicating a possible biotype difference rather than the existence of a new species. On the basis of three characters it is premature to propose a new species for cluster 12, but attention should be drawn to the possi-

ble existence of two biotypes in *E. tarda*.

Clusters 13 to 22 represent an area of considerable taxonomic controversy in the *Enterobacteriaceae*. On the basis of studying 97 strains of *Salmonella* and *Arizona*, it would be somewhat presumptuous to propose specific changes in their taxonomy. Nevertheless, several points of interest deserve mention. Various suggestions have been made for the taxonomy of *Salmonella*, ranging from giving each serotype species status (34, 37), dividing the genus into four biochemical subgroups (35, 36, 47), limiting the number of species to *Salmonella typhi*, *S. choleraesuis*, and *S. enteritidis* (16), or giving species status to the so-called "classical" species (whatever they may be). In this study the strains of *Salmonella* and *Arizona* fell into nine clusters which may or may not prove to be species foci within the genus. However, it seemed possible that the inclusion of more strains of some serotypes than of others could have resulted in the formation of artificial clusters. This possibility was investigated by reducing the 97 strains to 41 so that only one strain of each serotype was represented. One strain of *Citrobacter freundii* was also included. This data matrix, comprising 42 strains and 180 characters, was computed as before and the resulting dendrogram is shown in Fig. 7. The name of each serotype and its Kauffmann biochemical subgroups have been included in Fig. 7 for comparative purposes.

The high phenetic similarities between the strains reflect the close biochemical similarities within the salmonellae. Nevertheless, there appear to be areas which may correspond to possible species foci. Many of the Kauffmann subgroups I, II, and IV serotypes can be placed in a single species which, as Ewing (16) suggested, could be named *Salmonella enteritidis*. *Arizona* strains are closely related to this group, and there is no substantive reason why they should be regarded as a separate genus (14). A number of serotypes of subgroups I did not fall into the *S. enteritidis* "species." Neither could they be regarded as *S. typhi* or *S. choleraesuis*. Other species would be required for serotypes now referred to as *S. gallinarum*, *S. abortusequi*, *S. paratyphi* A, *S. pullorum*, *S. sendai* and presumably others not included in this study. Ewing (16) has suggested that *S. typhi* and *S. choleraesuis* should be given species status. However, it would not be totally justifiable at the present time to place all other serotypes in the species *S. enteritidis*. Inevitably the species would portray a variable biochemical capability, resulting in strains being

Fig. 7. Single linkage clustering of representative strains of *Salmonella*.

included in *S. enteritidis* only because they could not be identified as *S. typhi* or *S. choleraesuis*. The net effect would be to reduce *S. enteritidis* to a repository species, a practice which has never proved to be sound taxonomic practice. A promising method of tackling the problems of *Salmonella* taxonomy would be to carry out a numerical analysis of all the serotypes, necessitating a collaborative approach among the *Salmonella* reference laboratories. Much of the data required are almost certainly already available in these laboratories, and the effort required to correlate the survey would surely be amply rewarded by the end result. Antigenic structure has not been found to correlate with biochemical patterns within the salmonellae, but this is not a significant problem in the formation of species within the genus.

The close biochemical similarity between *Escherichia* and *Shigella* is illustrated in clusters 23 to 25. Clusters 23 and 24 present no problems in identification, corresponding to *Escherichia coli* and *Shigella sonnei*, respectively. Cluster 25 contains 37 strains of assorted *Shigella* species (other than *S. sonnei*) and bears out the views of Edwards and Ewing (14) that the species of *Shigella* (other than *S. sonnei*) cannot be classified using selected biochemical characters alone. The strains in cluster 25 were received as *S. boydii*, *S. flexneri*, and *S. dysenteriae*. No evidence of each species forming a subcluster within cluster 25 was obtained in this study.

Yersinia pseudotuberculosis, *Y. pestis*, and *Y. enterocolitica* showed close association with the other Group B clusters. The possible inclusion of *Yersinia* in the *Enterobacteriaceae* has al-

ready been suggested, based on biochemical studies (52, 68, 69), serology (71), DNA base composition (29, 58), and DNA/DNA hybridization data (57). From this study, it appears that, of the *Enterobacteriaceae*, the genera *Escherichia* and *Shigella* are biochemically the most similar to *Yersinia*. Further substantiation is provided by serological cross-reactions between a rough somatic antigen (serofactor 1) of *Y. pseudotuberculosis* and *E. coli*, *S. flexneri*, and *S. sonnei* (71). It should be mentioned, however, that there have also been reports of serological cross-reactions between *Y. pseudotuberculosis* and *Salmonella* (40), between *Y. enterocolitica* and *Brucella abortus* (1), and between *Yersinia* and *Pasteurella multocida* (72). The evidence from G+C base ratios would not preclude a close similarity, i.e., *Yersinia* falling within the range 46 to 48 mol% G+C, whereas *Escherichia-Shigella* and *Salmonella* are in the range 49 to 54 mol% G+C. Genetic transfer has been recorded between *E. coli* and both *Y. pseudotuberculosis* and *Y. pestis* (45, 51), and it has also been reported (64) that these species are sensitive to the same pesticides and colicins. It should be pointed out that most of this evidence is circumstantial; there is still a requirement for a detailed comparative study of *Yersinia*, *Pasteurella*, and the *Enterobacteriaceae*, a need which is being reviewed by the International Subcommittee on the Taxonomy of *Pasteurella*, *Yersinia*, and *Francisella* (53).

Group C: the tribe Proteae. Group C, corresponding to the Edwards and Ewing tribe *Proteae*, consists of 50 strains of *Proteus* and *Providencia* which fall into five clusters, 29 to 33. Examination of the characters shown in Table 2 indicates that many of the *Proteus-Providencia* strains were atypical. Characters listed by both Edwards and Ewing (14) and Bascomb et al. (3) as useful differentiating criteria for the species in these genera include indole (*Proteus mirabilis*, negative), ornithine decarboxylase (*Proteus mirabilis* and *Proteus morganii*, positive), acid from maltose (*Proteus vulgaris*, positive), urease (*Proteus*, positive, *Providencia*, negative), acid from adonitol (*Proteus rettgeri*, usually positive, *Providencia alcalifaciens*, positive), and acid from inositol (*Proteus rettgeri*, positive, *Providencia stuartii*, positive). Within the clusters 29 to 33, unusual results were obtained from the indole, ornithine decarboxylase, and maltose reactions.

The position of *Proteus* and *Providencia* in the *Enterobacteriaceae* is somewhat anomalous when the evidence from biochemical tests and molecular taxonomy is compared. Biochemical

characterization tests would logically place these genera within the *Enterobacteriaceae* as they are fermentative, often producing visible gas, motile (peritrichous), and reduce nitrate. Comparison of the DNA base ratio data for these genera with the rest of the family indicates that, except for *Proteus morganii*, there is probably little if any genetic relatedness. The overall DNA base composition for the *Enterobacteriaceae* lies within the range 49 to 60 mol%; *P. morganii*, at 50 to 53 mol%, falls within this range (29), but the remaining species of *Proteus* and *Providencia* have a considerably lower range of 38 to 42 mol%. Evidence from DNA/DNA hybridization studies (6, 7) also indicates extremely low genetic relatedness.

Kauffmann has suggested that the group should be divided into four genera (37). The genus *Proteus* would be retained for *P. vulgaris* and *P. mirabilis*, *Morganella* was proposed for *P. morganii*, *Rettgerella* was proposed for *P. rettgeri*, and *Providencia* would be retained. Breed, Murray, and Smith (5) placed all the species in a single genus *Proteus*, with *Providencia* being labeled as *Proteus inconstans*, as had been previously suggested (60). Edwards and Ewing (14), expressing dissent at including *Providencia* and *P. rettgeri* in the same genus as *P. vulgaris* and *P. mirabilis*, did not agree with Breed, Murray, and Smith (5), and suggested, instead, that one genus, *Proteus*, be retained for *P. vulgaris* and *P. mirabilis*, and a second genus be used for *P. rettgeri*, *P. morganii*, and *Providencia*. In light of the DNA base composition data, *P. morganii* would have to be removed from the latter group, presumably to a third genus. Kauffmann's proposal would appear to have certain merits. The arrangement of the clusters in this study reflects the close similarities between *P. vulgaris* and *P. mirabilis*. Molecular hybridizations indicate greater than 90% binding of their respective DNA (7), providing further strong evidence of their close similarity. *P. morganii* is phenetically similar to *P. vulgaris* and *P. mirabilis*, but the large difference in DNA base composition indicates little if any genetic similarity. Evidence for DNA hybridization between these species is lacking. *Proteus rettgeri* and *Providencia* have similar DNA base compositions, although this does not constitute high genetic similarity. Unfortunately, DNA/DNA hybridization data are also lacking in reference to this point of discussion. DNA from *P. mirabilis* showed only slight association (6 to 16%) (7) with these species. The observed DNA/DNA reassociation values were in the same range as between *P. mirabilis* and *E. coli*, *Salmonella*

typhimurium, *S. typhi*, and *Aerobacter aerogenes*, indicative of relatively insignificant relationships between *Proteus* and the other genera of the *Enterobacteriaceae*.

In the instance where available evidence indicates that substantial changes should be made in the taxonomy of the tribe *Proteae*, the dilemma arises as to which is the more important—a taxonomic scheme which, to the best of currently available knowledge, reflects the phenetic and genetic similarities and which, therefore, approaches the “natural” order, or a practical scheme, in which identification rather than phylogeny is of prime importance. In retaining *Proteus*, with its four species and *Providencia* as a separate genus, Edwards and Ewing appear to accept identification as the prime objective, although the separation of *Proteus* and *Providencia* solely on the basis of urease production is unacceptable to the numerical and molecular genetic taxonomist. It would appear that somewhere a “natural” order and the needs of identification should coalesce. On the basis of studying only 50 strains, many of which might be regarded as atypical, changes in the taxonomy of *Proteus* and *Providencia* are not, therefore, proposed. Nevertheless, the evidence for this is accumulating. Classification inevitably changes to accommodate the data derived from the sophisticated and more objective approaches to taxonomy. The changes suggested by data accumulated to date should be accepted, providing of course that there is consensus that the results are accurate, reproducible, and information rich. A practical necessity for identification is, nevertheless, paramount, particularly when dealing with a group of bacteria such as the *Enterobacteriaceae*, and those changes brought about as a result of the application of sophisticated laboratory techniques must eventually lead to the development of tests for identification which can be carried out quickly and inexpensively in the routine diagnostic laboratory.

In summary, omissions in the array of strains employed in this study should be noted, two of which already have been indicated. A numerical analysis of all the extant *Salmonella* serotypes should be pursued, perhaps a task best undertaken as a collaborative effort among the *Salmonella* reference laboratories around the world. The relationship of *Enterobacter agglomerans* to the *Klebsiellae* is under study in our laboratory, and the results of this work are being prepared for publication. Finally, confusion has recently arisen over a second species to be included in the genus *Citrobacter*. Various names have been proposed, including *Levine*,

(74) *Citrobacter koseri* (24) and *Citrobacter diversus* (17). None of these strains was included in the present study, but we are in the process of rectifying this omission in a further study involving them.

REPRINT REQUESTS

Address reprint requests to: Dr. R. R. Colwell, Department of Microbiology, University of Maryland, College Park, Md. 20742.

LITERATURE CITED

- Ahvonon, P., and K. Siever. 1969. *Yersinia enterocolitica* infection associated with *Brucella* agglutinins. *Acta Med. Scand.* 185:121-125.
- Baird-Parker, A. C. 1963. A classification of micrococci and staphylococci based on physiological and biochemical tests. *J. Gen. Microbiol.* 30:409-427.
- Bascomb, S., S. P. Lapage, M. A. Curtis, and W. R. Willcox. 1973. Identification of bacteria by computer: identification of reference strains. *J. Gen. Microbiol.* 77:291-315.
- Bascomb, S., S. P. Lapage, W. R. Willcox, and M. A. Curtis. 1971. Numerical classification of the tribe *Klebsiellae*. *J. Gen. Microbiol.* 66:279-295.
- Breed, R. S., E. G. D. Murray, and N. R. Smith. 1957. *Bergey's manual of determinative bacteriology*, 7th ed. Bailliere, Tindall and Cox Ltd., London.
- Brenner, D. J. 1970. Deoxyribonucleic acid divergence in *Enterobacteriaceae*. *Develop. Ind. Microbiol.* 11:139-153.
- Brenner, D. J., G. R. Fanning, K. E. Johnson, R. V. Citarella, and S. Falkow. 1969. Polynucleotide sequence relationships among members of *Enterobacteriaceae*. *J. Bacteriol.* 98:637-650.
- Brenner, D. J., A. G. Steigerwalt, and G. R. Fanning. 1972. Differentiation of *Enterobacter aerogenes* from *klebsiellae* by deoxyribonucleic acid reassociation. *Int. J. Syst. Bacteriol.* 22:193-200.
- Carpenter, K. P. et al. 1970. Request to the Judicial Commission that *Aerobacter* Beijerinck 1900 and *Aerobacter* Hormaeche and Edwards 1958 be declared rejected generic names. *Int. J. Syst. Bacteriol.* 20:221-224.
- Christensen, W. B. 1949. Hydrogen sulfide production and citrate utilization in the differentiation of the enteric pathogens and the coliform bacteria. *Res. Bull. Weld County Health Dept.* 1:3.
- Colwell, R. R. 1973. Genetic and phenetic classification of bacteria. *Advan. Appl. Microbiol.* 16:137-175.
- Colwell, R. R., and M. Mandel. 1965. Adansonian analysis and deoxyribonucleic acid base composition of *Serratia marcescens*. *J. Bacteriol.* 89:454-461.
- Colwell, R. R., and W. J. Wiebe. 1970. “Core” characteristics for use in classifying aerobic, heterotrophic bacteria by numerical taxonomy. *Bull. Ga. Acad. Sci.* 18:165-185.
- Edwards, P. R., and W. H. Ewing. 1972. Identification of enterobacteriaceae, 3rd ed. Burgess Publishing Company, Minneapolis.
- Edwards, P. R., and M. A. Fife. 1955. Studies on *Klebsiella-Aerobacter* group. *J. Bacteriol.* 70:382-390.
- Ewing, W. H. 1972. The nomenclature of *Salmonella*, its usage, and definitions for the three species. *Can. J. Microbiol.* 18:1629-1637.
- Ewing, W. H., and B. R. Davis. 1972. Biochemical characterization of *Citrobacter diversus* (Burkey) Werkman and Gillen and designation of the neotype strain. *Int. J. Syst. Bacteriol.* 22:12-18.
- Ewing, W. H., B. R. Davis, M. A. Fife, and E. F. Lessel.

1973. Biochemical characterization of *Serratia liquefaciens* (Grimes and Hennerty) Bascomb et al. (formerly *Enterobacter liquefaciens*) and *Serratia rubidaea* (Stapp) comb. nov. and designation of type and neotype strains. *Int. J. Syst. Bacteriol.* **23**:217-225.
19. Ewing, W. H., B. R. Davis, and R. W. Reavis. 1957. Phenylalanine and malonate media and their use in enteric bacteriology. *Pub. Health Lab.* **15**:153-167.
20. Ewing, W. H., and M. A. Fife. 1968. *Enterobacter hafniae* (the "Hafnia Group"). *Int. J. Syst. Bacteriol.* **18**:263-271.
21. Ewing, W. H., and M. A. Fife. 1972. *Enterobacter agglomerans* (Beijerinck) comb. nov. (the herbicolalathyrus bacteria). *Int. J. Syst. Bacteriol.* **22**:4-11.
22. Focht, D. D., and W. R. Lockhart. 1965. Numerical survey of some bacterial taxa. *J. Bacteriol.* **90**:1314-1319.
23. Fred, E. B., I. L. Baldwin, and E. McCoy. 1932. Root nodule bacteria and leguminous plants. *Univ. Wis. Studies Sci.* **5**: 343 pp.
24. Fredericksen, W. 1970. *Citrobacter koseri* (n. sp.) A new species within the genus *Citrobacter* with a comment on the taxonomic position of *Citrobacter intermedium* (Werkmann and Gillen). *Pub. Fac. Sci. Univ. J. E. Purkyne, Brno.* **47**:89-94.
25. Gaby, W. L., and E. Free. 1958. Differential diagnosis of *Pseudomonas*-like microorganisms in the clinical laboratory. *J. Bacteriol.* **76**:442-444.
26. Goodfellow, M. 1967. Numerical taxonomy of some named bacterial cultures. *Can. J. Microbiol.* **13**:1365-1374.
27. Grimont, P. A. D., and H. L. C. Dulong de Rosnay. 1972. Numerical study of 60 strains of *Serratia*. *J. Gen. Microbiol.* **72**:259-268.
28. Haynes, W. C. 1951. *Pseudomonas aeruginosa* — its characterization and identification. *J. Gen. Microbiol.* **5**:939-950.
29. Hill, L. R. 1966. An index to deoxyribonucleic acid base compositions of bacterial species. *J. Gen. Microbiol.* **44**:419-437.
30. Hormaeche, E., and P. R. Edwards. 1958. Observations on the genus *Aerobacter* with a description of two species. *Int. Bull. Bacteriol. Nomencl. Taxon.* **8**:111-115.
31. Hormaeche, E., and P. R. Edwards. 1960. A proposed genus *Enterobacter*. *Int. Bull. Bacteriol. Nomencl. Taxon.* **13**:71-74.
32. Hormaeche, E., and P. R. Edwards. 1960. Proposal for the rejection of the generic name *Cloaca* Castellani and Chalmers, and proposal of *Enterobacter* as a generic name with designation of type species and of its type culture. *Int. Bull. Bacteriol. Nomencl. Taxon.* **13**:75-76.
33. Hormaeche, E., and M. Munilla. 1957. Biochemical tests for the differentiation of *Klebsiella* and *Cloaca*. *Int. Bull. Bacteriol. Nomencl. Taxon.* **7**:1-20.
34. Kauffmann, F. 1959. On the principles of classification and nomenclature of Enterobacteriaceae. *Int. Bull. Bacteriol. Nomencl. Taxon.* **9**:1-6.
35. Kauffmann, F. 1963. Zur differentialdiagnose der *Salmonella* Sub-genera I, II and III. *Acta Pathol. Microbiol. Scand.* **58**:109-113.
36. Kauffmann, F. 1965. Weitere *Salmonella* species der Sub-genera II, III, and IV. *Acta Pathol. Microbiol. Scand.* **64**:367-372.
37. Kauffmann, F. 1966. The bacteriology of Enterobacteriaceae. E. Munksgaard, Copenhagen.
38. Kauffmann, F., and A. Petersen. 1956. The biochemical group and type differentiation of Enterobacteriaceae by organic acids. *Acta Pathol. Microbiol. Scand.* **38**:481-491.
39. King, E. O., M. K. Ward, and D. E. Raney. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. *J. Lab. Clin. Med.* **44**:301-307.
40. Knapp, W. 1955. Die diagnostische Bedeutung der antigenen Beziehungen zwischen *Past. pseudotuberculosis* und der *Salmonella* Gruppe. *Zentralbl. Bacteriol. Parasitenk. Infektionskr. Abt. 1 Orig.* **164**:57-59.
41. Kohn, J. 1953. A preliminary report of a new gelatin liquefaction method. *J. Clin. Pathol.* **6**:249.
42. Koser, S. A. 1923. Utilization of the salts of organic acids by the colon-aerogenes group. *J. Bacteriol.* **8**:493-520.
43. Kovacs, N. 1956. Identification of *Pseudomonas pyocyanea* by the oxidase reaction. *Nature (London)* **178**:703.
44. Krieg, R. E., and W. R. Lockhart. 1966. Classification of enterobacteria based on overall similarity. *J. Bacteriol.* **92**:1275-1280.
45. Lawton, W. D., B. C. Morris, and T. W. Burrows. 1968. Gene transfer in strains of *Pasteurella pseudotuberculosis*. *J. Gen. Microbiol.* **52**:25-34.
46. LeMinor, L., and F. Ben Hamida. 1962. Avantages de la recherche de la B-galactosidase sur celle de la fermentation du lactose en milieu complexe dans le diagnostic bacteriologique en particulier des Enterobacteriaceae. *Ann. Inst. Pasteur Paris* **102**:267-277.
47. LeMinor, L., R. Rhode, and J. Taylor. 1970. Nomenclature des *Salmonella*. *Ann. Inst. Pasteur Paris* **119**:206-210.
48. Lockhart, W. R., and J. G. Holt. 1964. Numerical classification of *Salmonella* serotypes. *J. Gen. Microbiol.* **35**:115-124.
49. Lockhart, W. R., and K. Koenig. 1965. Use of secondary data in numerical taxonomy of the genus *Erwinia*. *J. Bacteriol.* **90**:1638-1644.
50. Macfarlane, R. G., C. L. Oakley, and C. G. Anderson. 1941. Haemolysis and the production of opalescence in serum and lecithovitellin by the α -toxin of *Clostridium welchii*. *J. Pathol. Bacteriol.* **52**:99-103.
51. Martin, G., and F. Jacob. 1962. Transfert de L'episome sexuel d'*Escherichia coli* à *Pasteurella pestis*. *C. R. Acad. Sci.* **254**:3589-3590.
52. Martinevskij, I. L. 1968. Taxonomie du genre *Yersinia* et nomenclature de ses representants, p.361-368. In R. H. Regamey et al. (ed.), *International Symposium On Pseudotuberculosis*, Paris 1967. Symposia Series on Immunobiological Standardization, vol. 9, Karger, Basel.
53. Mollaret, H. H., and W. Knapp. 1971. Report (1966-1970) of the Subcommittee on *Pasteurella*, *Yersinia*, and *Francisella* to the International Committee on Nomenclature of Bacteria. *Int. J. Syst. Bacteriol.* **21**:157.
54. Opinion 28 of The Judicial Commission of the ICSB. 1963. Rejection of the bacterial generic name *Cloaca* Castellani and Chalmers and acceptance of *Enterobacter* Hormaeche and Edwards as a bacterial generic name with type species *Enterobacter cloacae* (Jordon) Hormaeche and Edwards. *Int. Bull. Bacteriol. Nomencl. Taxon.* **13**:38.
55. Opinion 46 of The Judicial Commission of the ICSB. 1971. Rejection of the generic name *Aerobacter* Beijerinck. *Int. J. Syst. Bacteriol.* **21**:110.
56. Priest, F. G., H. J. Somerville, J. A. Cole, and J. S. Hough. 1973. The taxonomic position of *Obesumbacterium proteus*, a common brewery contaminant. *J. Gen. Microbiol.* **75**:295-307.
57. Ritter, D. B., and R. K. Gerloff. 1966. Deoxyribonucleic acid hybridization among some species of the genus *Pasteurella*. *J. Bacteriol.* **92**:1838-1839.
58. Rosypal, S., and A. Rosypalova. 1966. Genetic, phylogenetic and taxonomic relationships among bacteria as determined by their deoxyribonucleic acid base composition. *Folia Prirodovedecke Fac. Univ. J. E. Purkyne Brno VII* **14**:31-91.
59. Sakazaki, R. 1961. Studies on the Hafnia group of

- Enterobacteriaceae. Jap. J. Med. Sci. Bio. 14:223-241.
60. Shaw, C., and P. H. Clarke. 1955. Biochemical classification of *Proteus* and *Providencia* cultures. J. Gen. Microbiol. 13:155-161.
61. Sierra, G. 1957. A simple method for the detection of lipolytic activity of micro-organisms and some observations on the influence of the contact between cells and fatty substrates. Antonie van Leeuwenhoek. J. Microbiol. Serol. 23:15-22.
62. Simmons, J. S. 1926. A culture medium for differentiating organisms of typhoid-colon aerogenes groups and for isolation of certain fungi. J. Infect. Dis. 39:209-214.
63. Skinner, C. E. 1957. Letters to the editor. Int. Bull. Bacteriol. Taxon. 7:139-140.
64. Smith, D. A., and T. W. Burrows. 1962. Phage and bacteriocin investigations with *Pasteurella pestis* and other bacteria. Nature (London) 193:397-398.
65. Sneath, P. H. A., and S. T. Cowan. 1958. An electro-taxonomic survey of bacteria. J. Gen. Microbiol. 19:551-565.
66. Sneath, P. H. A., and R. R. Sokal. 1973. Numerical taxonomy: the principles and practice of numerical classification, p. 1-573. W. H. Freeman, San Francisco.
67. Starr, M. P., and M. Mandel. 1969. DNA base composition and taxonomy of phytopathogenic and other enterobacteria. J. Gen. Microbiol. 56:113-123.
68. Stevens, M. 1969. Development and use of multi-inoculation test methods for a taxonomic study. J. Med. Lab. Technol. 26:253-263.
69. Thal, E. 1954. Untersuchungen uber *Pasteurella pseudotuberculosis*. Berlingska Boktryckeriet, Lund, Sweden.
70. Vaughn, R. H., and M. Levine. 1942. Differentiation of the "intermediate" coli-like bacteria. J. Bacteriol. 44:487-505.
71. Wetzler, T. F., H. E. Eitzen, J. A. Currie, and J. D. Marshall. 1968. Lipopolysaccharide-like antigens from *Pasteurella pseudotuberculosis* shared by various genera of the Enterobacteriaceae as demonstrated by hemagglutination tests, p.155-166. In: R. H. Regamey et al. (ed.), International symposium of *Pseudotuberculosis*, Paris 1967. Symposia Series on Immunobiological Standardization, vol. 9. Karger, Basel.
72. Wilson, G. S., and A. A. Miles. 1964. Topley and Wilson's principles of bacteriology and immunity, 5th ed. Edwards Arnold and Co., London.
73. Wolf, P. L., E. von der Nuehll, and M. Ludwick. 1972. A new test to differentiate *Serratia* from *Enterobacter*. Amer. J. Clin. Pathol. 57:241-243.
74. Young, V. M., D. M. Kenton, B. J. Hobbs, and M. R. Moody. 1971. *Levinea*, a new genus of the family Enterobacteriaceae. Int. J. Syst. Bacteriol. 21:58-63.