Taxonomy of Some Recently Described Species in the Family *Enterobacteriaceae*

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The taxonomic positions of several recently described species, *Levinea malonatica*, *Levinea amalonatica*, *Citrobacter diversus*, and *Enterobacter agglomerans*, were investigated by numerical analysis. A set of 141 strains, for which a total of 240 characters was recorded, was analyzed and also compared with representatives of a set of 384 strains of bacteria, examined in an earlier study, representing genera within the family *Enterobacteriaceae*. Three clusters of *Citrobacter* spp. were observed, *Citrobacter freundii*, *Citrobacter* spp., and *Levinea amalonatica*, with strains received as *Citrobacter diversus* and *Levinea malonatica* clustering with the *Citrobacter* spp. *Citrobacter intermedius* was concluded to be synonymous with *C. freundii*. *L. malonatica*, from the results of this study, was included in the species *C. diversus*. Hydrogen sulfide-positive strains of *Escherichia coli* were not judged to warrant separate species status. *Klebsiella aerogenes*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, and *Klebsiella edwardsii* were found to be highly related (similarity values > 90%). It is proposed that these species be merged into a single species, *Klebsiella pneumoniae*.

In a recently published study, 384 strains of bacteria representing genera within the family *Enterobacteriaceae* and the genus *Yersinia* were subjected to numerical analysis (38). Thirty-three phenetic clusters were distinguished, and these, for the most part, corresponded to the established species within the family *Enterobacteriaceae*. Useful information concerning taxonomic relationships among the *Enterobacteriaceae* was obtained. However, some members of the *Enterobacteriaceae*, including *Erwinia*, and the more recently described species *Levinea malonatica*, *Levinea amalonatica*, *Citrobacter diversus*, *Enterobacter agglomerans*, and *Erwinia*, were not represented. Therefore, an additional 141 strains of *Enterobacteriaceae* were examined, and the results were compared with those of the previous study.

MATERIALS AND METHODS

Bacterial strains. A list of the 141 strains included in the analysis is given in Table 1. The name *Citrobacter intermedius* has been kept through this paper, although Sedláč (58) in the 8th edition of *Bergey's Manual of Determinative Bacteriology* has applied the name *Citrobacter intermedius* to *C. diversus* (*L. malonatica*) and *L. amalonatica*.

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's stain (11) and by electron microscopy. Gram reaction was determined using the Hucker modification of the Gram staining procedure (11). 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. Pigmentation was recorded from observation of growth on the media of King et al. (41), yeast extract-mannitol agar (27), 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 44.5 °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)amino-methane and citrate phosphate buffer. Hemolysis was detected on heart infusion agar (Difco) containing 2% washed sheep red blood cells. Antibiotic sensitivities were tested on heart infusion agar (Difco) containing one of the following antibiotics: penicillin (2.5 U and 10 U), dihydrostreptomycin (2.5 µg, 10 µg, and 30 µg), chloramphenicol (2.5 µg, 10 µg, and 30 µg), erythromycin (15 µg), kanamycin (30 µg), aureomycin (30 µg), novobiocin (10 µg and 30 µg),

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TABLE 1. Strains assigned to clusters in Fig. 1 and 2

Cluster identification	Strain computer no.	Strain received as:	Source ^a
<i>Proteus mirabilis</i>	3	<i>P. mirabilis</i> 71	R. Sakazaki
	4	<i>P. mirabilis</i> 72	R. Sakazaki
	5	<i>P. mirabilis</i> 79	R. Sakazaki
<i>Proteus morganii</i>	6	<i>P. morganii</i> 2191	R. Sakazaki
	7	<i>P. morganii</i> 2192	R. Sakazaki
	8	<i>P. morganii</i> 2193	R. Sakazaki
<i>Edwardsiella tarda</i>	9	<i>E. tarda</i> 1223	R. Sakazaki
	10	<i>E. tarda</i> 2000	R. Sakazaki
	11	<i>E. tarda</i> 1343	R. Sakazaki
<i>Salmonella typhi</i>	12	<i>S. typhi</i> 113	R. Sakazaki
	13	<i>S. typhi</i> 111	R. Sakazaki
	14	<i>S. typhi</i> 112	R. Sakazaki
<i>Proteus rettgeri</i>	15	<i>P. rettgeri</i> 103	R. Sakazaki
	16	<i>P. rettgeri</i> 101	R. Sakazaki
	17	<i>P. rettgeri</i> 102	R. Sakazaki
<i>Escherichia coli</i> H ₂ S+	19	<i>E. coli</i> H ₂ S+ N114	R. Sakazaki
	20	<i>E. coli</i> H ₂ S+ N111	R. Sakazaki
	21	<i>E. coli</i> H ₂ S+ N110	R. Sakazaki
	22	<i>E. coli</i> H ₂ S+ N46	R. Sakazaki
	23	<i>E. coli</i> H ₂ S+ N76	R. Sakazaki
	24	<i>E. coli</i> H ₂ S+ N73	R. Sakazaki
	25	<i>E. coli</i> H ₂ S+ N103	R. Sakazaki
	26	<i>E. coli</i> H ₂ S+ N68	R. Sakazaki
	27	<i>E. coli</i> H ₂ S+ N99	R. Sakazaki
	28	<i>E. coli</i> H ₂ S+ N63	R. Sakazaki
	29	<i>E. coli</i> H ₂ S+ N21	R. Sakazaki
	30	<i>E. coli</i> H ₂ S+ N5	R. Sakazaki
	31	<i>E. coli</i> H ₂ S+ N2	R. Sakazaki
	32	<i>E. coli</i> H ₂ S+ N28	R. Sakazaki
	33	<i>E. coli</i> 1326-70	R. Sakazaki
<i>Escherichia coli</i>	34	<i>E. coli</i> 1441-70	R. Sakazaki
	35	<i>E. coli</i> 1324-70	R. Sakazaki
	36	<i>E. coli</i> 1120-70	R. Sakazaki
<i>Hafnia alvei</i>	37	<i>E. coli</i> 1233-70	R. Sakazaki
	38	<i>H. alvei</i> 13	R. Sakazaki
	39	<i>H. alvei</i> 7081	R. Sakazaki
<i>Citrobacter freundii</i> /intermedius	40	<i>H. alvei</i> 5621	R. Sakazaki
	41	<i>H. alvei</i> 887-61	R. Sakazaki
	42	<i>C. freundii</i> 577-67	R. Sakazaki
	43	<i>C. freundii</i> 423-71	R. Sakazaki
	44	<i>C. freundii</i> 511-71	R. Sakazaki
	45	<i>C. intermedius</i> 5	R. Sakazaki
	46	<i>C. intermedius</i> 1	R. Sakazaki
	47	<i>C. intermedius</i> 4	R. Sakazaki
	48	<i>C. intermedius</i> 2	R. Sakazaki
	49	<i>C. freundii</i> 624-71	R. Sakazaki
<i>Salmonella</i> spp.	50	<i>C. intermedius</i> 3	R. Sakazaki
	51	<i>C. freundii</i> 624-70	R. Sakazaki
	52	<i>S. enteritidis</i>	R. Sakazaki
	53	<i>S. meleagridis</i>	R. Sakazaki
	54	<i>S. derby</i>	R. Sakazaki
	55	<i>S. infantis</i>	R. Sakazaki
	56	<i>S. livingstone</i>	R. Sakazaki
	57	<i>S. arizonae</i> 888	R. Sakazaki
	58	Unknown	R. Sakazaki
	59	<i>S. arizonae</i> 114	R. Sakazaki
<i>Levinea amalonatica</i>	60	<i>L. amalonatica</i> 25406	ATCC
	61	<i>L. amalonatica</i> 25405	ATCC
	62	<i>L. amalonatica</i> 25407	ATCC
<i>Citrobacter diversus</i> /Levinea malonatica	63	<i>L. malonatica</i> 25409	ATCC
	64	<i>L. malonatica</i> 527-71	R. Sakazaki

TABLE 1—Continued

Cluster identification	Strain computer no.	Strain received as:	Source ^a
	65	<i>C. diversus</i> 1066-70	NCDC
	66	<i>C. diversus</i> 2572-70	NCDC
	67	<i>C. diversus</i> 2737-70	NCDC
	68	<i>C. diversus</i> 3613-63	NCDC
	69	<i>C. diversus</i> 2292-70	NCDC
	70	<i>C. diversus</i> 2393-70	NCDC
	71	<i>C. diversus</i> 2524-70	NCDC
	72	<i>L. malonatica</i> 25408	ATCC
	73	<i>L. malonatica</i> 25410	ATCC
	74	<i>L. malonatica</i> 528-71	R. Sakazaki
	75	<i>C. diversus</i> 1381-70	NCDC
	76	<i>C. diversus</i> 2469-70	NCDC
<i>Enterobacter cloacae</i>	78	<i>E. cloacae</i> 102-71	R. Sakazaki
	79	<i>E. cloacae</i> 116	R. Sakazaki
	80	<i>E. cloacae</i> 101-71	R. Sakazaki
	81	<i>E. cloacae</i> 1679	R. Sakazaki
	82	<i>E. cloacae</i> 2011	R. Sakazaki
	83	Unidentified 216	R. Sakazaki
	84	Unidentified 221	R. Sakazaki
	85	Unidentified 215	R. Sakazaki
<i>Enterobacter agglomerans</i>	86	<i>E. agglomerans</i> 184-71	NCDC
	87	<i>E. agglomerans</i> 6-71	NCDC
	88	<i>E. agglomerans</i> 1379-71	NCDC
	89	<i>E. agglomerans</i> 185-71	NCDC
	90	<i>E. agglomerans</i> 459-71	NCDC
	91	<i>E. agglomerans</i> 219-71	NCDC
	92	Unidentified 213	R. Sakazaki
<i>Klebsiella pneumoniae</i>	94	<i>K. pneumoniae</i> 72	R. Sakazaki
	95	<i>K. pneumoniae</i> 65	R. Sakazaki
	96	<i>K. oxytoca</i> 13183	ATCC
	97	<i>K. oxytoca</i> 833-64	R. Sakazaki
	98	<i>K. oxytoca</i> 13182	ATCC
	99	<i>K. oxytoca</i> 1296-63	R. Sakazaki
	100	<i>K. oxytoca</i> 1210-70	R. Sakazaki
	101	<i>K. edwardsii</i> 9496	NCTC
	102	<i>K. edwardsii</i> 5054	NCTC
<i>Klebsiella rhinoscleromatis</i>	111	<i>K. rhinoscleromatis</i> i-501-56	Ørskov
	112	<i>K. rhinoscleromatis</i> i-1-50	Ørskov
	113	<i>K. rhinoscleromatis</i> i-236-53	Ørskov
	114	<i>K. rhinoscleromatis</i> i-255-53	Ørskov
	115	<i>K. rhinoscleromatis</i> i-6-50	Ørskov
	116	<i>K. rhinoscleromatis</i> i-9-50	Ørskov
	117	<i>K. rhinoscleromatis</i> i-235-53	Ørskov
	118	<i>K. rhinoscleromatis</i> i-256-53	Ørskov
	119	<i>K. rhinoscleromatis</i> i-234-53	Ørskov
	120	<i>K. rhinoscleromatis</i> i-11-50	Ørskov
<i>Klebsiella ozaenae</i>	121	<i>K. ozaenae</i> 9-72	R. Sakazaki
	122	<i>K. ozaenae</i> 160	R. Sakazaki
	123	<i>K. ozaenae</i> 130	R. Sakazaki
	124	<i>K. ozaenae</i> 2015-62	R. Sakazaki
	125	<i>K. ozaenae</i> 15-72	R. Sakazaki
<i>Enterobacter liquefaciens</i>	129	<i>E. liquefaciens</i> 2214	R. Sakazaki
	130	<i>E. liquefaciens</i> 1689	R. Sakazaki
	131	<i>E. liquefaciens</i> 1493	R. Sakazaki
<i>Serratia marcescens</i>	132	<i>S. marcescens</i> 2121-68	R. Sakazaki
	133	<i>S. marcescens</i> 16	R. Sakazaki
	134	<i>S. marcescens</i> 2187-68	R. Sakazaki
	135	<i>S. marcescens</i> 2249-68	R. Sakazaki
	136	<i>S. marcescens</i> 14	R. Sakazaki
Strains not clustered	1	<i>E. liquefaciens</i> 222	R. Sakazaki
	2	Unknown coliform 224	R. Sakazaki

TABLE 1—Continued

Cluster identification	Strain computer no.	Strain received as:	Source ^a
	18	<i>E. coli</i> H ₂ S+ N59	R. Sakazaki
	77	<i>C. diversus</i> 2555-70	NCDC
	93	<i>Erwinia carotovora</i> 979	CCH
	103	Unknown coliform 222	R. Sakazaki
	104	<i>Erwinia carotovora</i> 630	CCM
	105	<i>Enterobacter agglomerans</i> 258-71	NCDC
	106	<i>Erwinia carotovora</i> 1016	CCM
	107	<i>Erwinia carotovora</i> 1012	CCM
	108	<i>Erwinia herbicola</i> Aj 2189	Tokyo Univ.
	109	<i>Erwinia herbicola</i> Aj 2190	Tokyo Univ.
	110	<i>Enterobacter agglomerans</i> 5379-71	NCDC
	126	<i>Erwinia herbicola</i> Aj 2671	Tokyo Univ.
	127	<i>Enterobacter liquefaciens</i> 225	R. Sakazaki
	128	<i>Hafnia alvei</i> 114	R. Sakazaki
	137	<i>Enterobacter agglomerans</i> 3737-71	NCDC
	138	<i>Enterobacter agglomerans</i> 1741-71	NCDC
	139	<i>Erwinia amylovora</i> 1020	CCM
	140	<i>Erwinia amylovora</i> 1017	CCM
	141	<i>Enterobacter agglomerans</i> 1743-71	NCDC

^a Key to sources given in footnote a, Table 3.

tetracycline (2.5 µg and 30 µg), tetracycline (2.5 µg, 10 µg, and 30 µg), or polymyxin B (50 U). Sensitivity to Vibriostat 0/129 was detected by placing several crystals of the reagent 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 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 (44) and Gaby and Free (29); 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 by 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 (42) on agar slant cultures incubated for 10 days at 30 C; agar digestion by the method of Colwell and Wiebe (11); action on 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 medium 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); Rustigan urease (56); indole production determined by the method of Macfarlane et al. (49); hydrolysis of Tweens 20, 40,

60, and 80 on Sierra medium (59); corn oil lipase detected on the corn oil medium of Hugo and Beveridge (36); hydrolysis of tributyrin on heart infusion agar containing tributyrin; citrate utilization on Koser (43), Simmons (60), and Christensen media (9); phenylalanine deaminase detected on phenylalanine agar of Ewing et al. (23); amino acid decarboxylases in Møller medium (Difco) containing arginine, lysine, ornithine, or glutamic acid; starch hydrolysis on heart infusion agar (Difco) containing 0.2% starch; esculin hydrolysis by the method of Vaughn and Levine (65); 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; cooked meat digestion tested in cooked meat medium (Difco) incubated for 10 days at 30 C; beta-galactosidase detected by the method of Le Minor and Ben Hamida (48); deoxyribonuclease on deoxyribonuclease medium (BBL); and utilization of the following organic compounds as sole carbon sources determined in Simmons agar base (60) (organic esters were in the form of the sodium salt)—acetate, citrate, malonate, lactate, fumarate, succinate, pyruvate, D-malate, L-malate, formate, tartrate, oxalate, glutarate, DL-glycerate, D-glucuronate, α-ketoglutarate, aconitate, enanthate, propionate, butyrate, valerate, isobutyrate, isovalerate, caproate, caprylate, caprate, benzoate, DL-β-hydroxybutyrate, para-hydroxybenzoate, para-aminobenzoate, gamma-aminobutyrate, aminobutyrate, phenylacetate, quinate, hippurate, alginate, glutamate, aspartate, N-acetylglucosamine, ethanol, propanol, β-alanine, spermine, betain, sarcosine, L-α-alanine, D-α-alanine, threonine, glycine, arginine, ornithine, lysine, leucine, serine, histidine, phenylalanine, tryptophan, tyrosine, citrulline, valine, isoleucine, and proline. Other reactions included in the analyses were as follows: growth, slime production, and oxidation of gluconate were recorded on Haynes

medium (34); chitin hydrolysis was determined by the method of Benton (4); ammonia from arginine was detected in arginine broth of Niven et al. (52); acid from cystine using Hinshaw medium (35); hydrolysis of tyrosine on the medium of Gordon and Smith (32); xanthine hydrolysis on xanthine agar of Gordon and Mihm (31); tryptophan deaminase tested by the method of Singer and Volcani (61); utilization of malonate in Ewing medium (23); hydrolysis of alginate on Davis and Ewing medium (15); pectate hydrolysis on Starr medium (63); decomposition of phenylpropionic acid by the method of d'Alessandro and Comes (1); utilization of 1% (vol/vol) putrescine as sole carbon source detected in ammonium salt medium; growth on nutrient agar (Difco) containing one of the following inhibitory substances: 0.1% (wt/vol) Tergitol 7, triphenyltetrazolium chloride (0.001% and 0.01% [wt/vol]), cetrimide (0.1%), potassium tellurite (0.3% [wt/vol]), or sodium azide (0.05% [wt/vol]). Growth on SS agar (Difco), brilliant green agar (BBL), deoxycholate citrate agar (Difco), TCBS (Difco), and in tetrathionate (Difco) and selenite (Difco) broths was also recorded. Utilization of the organic acids D-tartrate, mucate, and citrate was detected on the medium of Kauffmann and Petersen (40).

Computation of data. A total of 240 characters was investigated. The data were coded in binary notation, using 1 for positive and 0 for negative. Noncomparable or missing characters were coded as 3 and were not included in subsequent calculations. All characters were assigned equal weight, and subsequent computations were carried out on an IBM 370/165 electronic computer using a numerical taxonomy program developed by one of us (R.R.C.). Similarities between every pair of strains were calculated using both the coefficient of Jaccard, S_J , in which negative matches are excluded from the calculations, and by the simple matching coefficient, S_M , which includes negative matches in the similarity computation. Subsequent clustering was derived by single linkage analysis using the S_J coefficient (62).

RESULTS

The results of the initial computations are shown in Fig. 1 and 2. The 141 strains formed a large group at the 59% phenon level. Within this large group, 19 clusters were distinguished at varying levels of similarity. Twenty-one strains did not fall into any of the 19 clusters, although some were atypical in only a few characters. At least seven strains showed little similarity to any of the other strains, indicating wide divergence from the main group.

Thirteen of the 19 clusters were readily identified as belonging to the following species: *Serratia marcescens*, *Serratia liquefaciens*, *Klebsiella ozaenae*, *Klebsiella rhinoscleromatis*, *Enterobacter cloacae*, *Salmonella* species, *Hafnia alvei*, *Escherichia coli*, *Proteus rettgeri*, *Salmonella typhi*, *Edwardsiella tarda*, *Proteus*

morganii, and *Proteus mirabilis*. The remaining six clusters provided interesting taxonomic information and were analyzed further.

Fourteen strains of *E. coli* capable of producing hydrogen sulfide when grown on peptone iron agar joined with a non- H_2S producer at 81% similarity. Thirteen of the strains, all capable of producing hydrogen sulfide, joined at 85%. Members of this cluster shared highest similarities with other strains of *E. coli* which did not produce hydrogen sulfide. Characters useful for differentiating the two clusters are shown in Table 2.

Eight strains received as *Citrobacter freundii* or *C. intermedius* formed a separate cluster at the 86% similarity level and were joined by a single strain of *C. freundii* (strain 42 in Fig. 1) at 84%. Four of the nine strains were labeled *C. freundii*, and the remaining five strains were labeled *C. intermedius*. The strains did not separate into two subgroups on the basis of their specific epithets, although one character, production of hydrogen sulfide on peptone iron agar, was positive for the *C. freundii* strains and negative for *C. intermedius* strains.

Fourteen strains received as *Levinea malonatica* and *Citrobacter diversus* formed a cluster at 89% similarity. Here again there was no evidence of subgroups corresponding to the nomenclatural designation, although strains labeled *L. malonatica*, unlike those of *C. diversus*, were able to hydrolyze esculin and were unable to utilize citrate, when grown in the medium of Kauffmann and Petersen, or produce acid from cystine. All 14 strains formed a cluster which joined at the 84% phenon level with three strains of *L. amalonatica*. These latter strains, which included the type strain (ATCC 25405, our strain 61), formed a highly homogenous group at 94% and differed from the *L. malonatica/C. diversus* cluster in eight characters, among which were included ability to produce acid from adonitol and to grow in Møller potassium cyanide medium and on propanol and *para*-hydroxybenzoate as sole carbon sources. Propionate was not utilized as a sole carbon source nor was malonate reduced in Ewing medium.

Seven strains, six of which were received as *Enterobacter agglomerans*, and the other, as an unidentified coliform, grouped together at the 79% similarity level. All strains were motile and five of the seven were aerogenic. All were capable of growth in Møller potassium cyanide medium; however, this group showed considerable variation in a great majority of characters recorded.

Finally, nine strains received as *Klebsiella*

FIG. 1. Similarity (S) value matrix prepared from the numerical taxonomy analysis of the 141 strains included in the study.

Similarity %

TABLE 2. Characters distinguishing hydrogen sulfide-positive strains of *Escherichia coli* from the more typical hydrogen sulfide-negative strains^a

Character	H ₂ S-positive strains (14) ^b	H ₂ S-negative strains (4) ^b	Strain 33 (1) ^b
Acid from raffinose	—	+	+
H ₂ S from peptone in agar	+	—	—
Gluconate reduction (Haynes)	+	—	—
Ornithine decarboxylase	3/14+	+	+
Growth at 44.5 C.	12/14+	—	—
Growth on 0.1% cetrinide	+	1/4+	+
Acid from 5% sucrose	—	+	+
β-Methyl glucosamine	12/14+	—	+

^a The intermediate strain (no. 33) is also included. Key: +, positive; —, negative.

^b Numbers in parentheses indicate numbers of strains.

pneumoniae, *Klebsiella oxytoca*, or *Klebsiella edwardsii* formed a cluster. These strains grouped at 85% similarity, and, within this cluster, seven strains of *K. pneumoniae* and *K. oxytoca* formed a group at 87%, whereas the two strains of *K. edwardsii* formed a separate group at 86% before joining the other strains. *K. oxytoca* strains were able to hydrolyze starch, produce indole, and produce acid from α-methyl glucosamine but not to produce hydrogen sulfide from cysteine. They were also able to utilize enanthate as a sole source of carbon. The strains received as *K. pneumoniae* did not share these characteristics. On the whole, strains of *K. pneumoniae* and *K. oxytoca* tended to be more biochemically active than those of *K. edwardsii*. *K. pneumoniae* gave positive results for the following characters, whereas negative results were obtained for *K. edwardsii*: acid from lactose and melibiose, growth on 4.5% sodium chloride, hydrogen sulfide from cysteine, growth on 0.1% cetrinide and glycerol, and growth on tartrate, benzoate, and hippurate as sole carbon sources.

Preliminary analysis of the data obtained for the 141 strains indicated that the strains received as *C. freundii* and *C. intermedius* should be placed in the same species. *C. diversus* and *L. malonatica* also appeared to be synonymous. *L. malonatica*, however, was observed to be distinct from this *C. diversus*/*L. malonatica* group. *K. pneumoniae*, *K. oxytoca*, and *K. ed-*

wardsii appeared to constitute another single species, whereas some strains of *E. agglomerans* shared sufficient characters that they grouped together, although other strains received as *E. agglomerans* or *Erwinia* species were distinctly different and constituted the majority of the strains which did not belong to any of the 19 clusters.

DISCUSSION

In general, the results obtained from the phenetic analysis were similar to those obtained previously (38). To determine the stability of the clusters formed by those strains not included in the previous study, all of the strains included in this study were combined with 196 strains whose characteristics have already been reported (38). A computer analysis was undertaken in which the new total of 337 strains (Table 3) was compared over 196 features. Similarities between every pair of strains were calculated using the Jacard coefficient, and subsequent clustering was derived by single-linkage analysis. A simplified dendrogram showing the arrangement of the strains is illustrated in Fig. 3. The general arrangement of the clusters was observed to be similar to that described previously, as was expected (38).

The main points of interest are as follows: the difference between the two clusters of *Citrobacter* (*C. freundii* and *Citrobacter* spp.); the relationships of the hydrogen sulfide-producing strains of *E. coli* to those strains incapable of producing hydrogen sulfide; the large cluster designated *Klebsiella aerogenes* and *Enterobacter aerogenes*; and, lastly, the wide dispersion of strains received as *Enterobacter agglomerans* or *Erwinia* species.

All strains received as *Citrobacter diversus* and *Levinea* species clustered with the *Citrobacter* species, although, within this cluster, three strains of *L. amalonatica* again formed a separate subgroup at a very high level of similarity. Strains labeled as *C. freundii* or *C. intermedius* were located in the *C. freundii* cluster.

Strains of *Escherichia coli* which did not produce hydrogen sulfide (strains 34, 35, 36, 37, 905–65, 916–65, 897–65, and 193–68) fell in close proximity to the H₂S-positive strains but were somewhat dispersed. However, they could be distinguished on the basis of selected characters, as shown in Table 2.

The cluster designated *Klebsiella aerogenes* and *Enterobacter aerogenes* contained strains

FIG. 2. Dendrogram showing separation of strain clusters.

TABLE 3. Strains assigned to clusters in Fig. 3

Cluster identification	Strain computer no.	Strain received as:	Source ^a
Strains not clustered	138	<i>Enterobacter agglomerans</i> 1741-71	NCDC
	141	<i>Enterobacter agglomerans</i> 1743-71	NCDC
	2	Unknown coliform 224	R. Sakazaki
	1	<i>Enterobacter liquefaciens</i> 222	R. Sakazaki
	140	<i>Erwinia amylovora</i> 1017	CCM
<i>Proteus rettgeri</i>	139	<i>Erwinia amylovora</i> 1021	CCM
	17	<i>P. rettgeri</i> 102	R. Sakazaki
	16	<i>P. rettgeri</i> 101	R. Sakazaki
<i>Proteus mirabilis</i>	15	<i>P. rettgeri</i> 103	R. Sakazaki
	5	<i>P. mirabilis</i> 79	R. Sakazaki
	4	<i>P. mirabilis</i> 72	R. Sakazaki
Strain not clustered	3	<i>P. mirabilis</i> 71	R. Sakazaki
	29	<i>E. coli</i> H ₂ S+ N21	R. Sakazaki
		<i>Y. pestis</i> A29	MRE, Porton
		<i>Y. pestis</i> 012	MRE, Porton
		<i>Y. pestis</i> F9581	MRE, Porton
<i>Yersinia pestis</i>		<i>Y. pestis</i> EV9-26-70	NIH, Japan
		<i>Y. pestis</i> A1122	Fort Collins
		<i>Y. pseudotuberculosis</i> no. 7	Univ. of Tottori, Japan
		<i>Y. pseudotuberculosis</i> 83	Univ. of Tottori, Japan
		<i>Y. pseudotuberculosis</i> no. 2	Univ. of Tottori, Japan
<i>Yersinia pseudotuberculosis</i>		<i>Y. pseudotuberculosis</i> 1779	Univ. of Tottori, Japan
		<i>Y. pseudotuberculosis</i> 24	Univ. of Tottori, Japan
		<i>Y. enterocolitica</i> MYO	Univ. of Lund, Sweden
		<i>Y. enterocolitica</i> MY079	Univ. of Lund, Sweden
		<i>Y. enterocolitica</i> ALBANY 5189	Univ. of Lund, Sweden
<i>Yersinia enterocolitica</i>		<i>Y. enterocolitica</i> LUCAS 110	Univ. of Lund, Sweden
		<i>Y. enterocolitica</i> 23715	ATCC
		<i>Enterobacter agglomerans</i> 258-71	NCDC
		<i>Salmonella gallinarum</i> 353	Costin, Romania
		<i>Edwardsiella</i> sp. 1-4	R. Sakazaki
<i>Edwardsiella</i> sp.		<i>Edwardsiella</i> sp. 1-1	R. Sakazaki
		<i>Edwardsiella</i> sp. 3-1	R. Sakazaki
		<i>Edwardsiella</i> sp. 3-1	R. Sakazaki
<i>Edwardsiella tarda</i>	11	<i>E. tarda</i> 1343	R. Sakazaki
	9	<i>E. tarda</i> 1223	R. Sakazaki
	10	<i>E. tarda</i> 2000	R. Sakazaki
		<i>E. tarda</i> 41	R. Sakazaki
		<i>E. tarda</i> 9	R. Sakazaki
<i>Proteus morganii</i>		<i>E. tarda</i> 39	R. Sakazaki
		<i>E. tarda</i> 51	R. Sakazaki
	6	<i>P. morganii</i> 2191	R. Sakazaki
	7	<i>P. morganii</i> 2192	R. Sakazaki
	8	<i>P. morganii</i> 2193	R. Sakazaki
Strain not clustered	128	<i>Hafnia alvei</i> 114	R. Sakazaki
		<i>S. boydii</i> 9770	NCTC
		<i>S. boydii</i> 2234-60	NCDC
		<i>S. dysenteriae</i> 9760	NCTC
		<i>S. dysenteriae</i> 9720	NCTC
<i>Shigella</i> spp.		<i>S. boydii</i> 1050-50	NCDC
		<i>S. boydii</i> 2064-59	NCDC
		<i>S. dysenteriae</i> 4379-60	NCDC
		<i>S. flexneri</i> 9989	NCTC
		<i>S. dysenteriae</i> 9719	NCTC
Strains not clustered		<i>Shigella flexneri</i> 1b 4558-60	NCDC
		<i>Shigella flexneri</i> 2b 9768	NCTC
		<i>Shigella flexneri</i> 2a 4807-62	NCDC
<i>Shigella sonnei</i>		<i>S. sonnei</i> 70-56	R. Sakazaki
		<i>S. sonnei</i> 70-69	R. Sakazaki

TABLE 3—Continued

Cluster identification	Strain com- puter no.	Strain received as:	Source ^a
Strains not clustered		<i>S. sonnei</i> 70-75	R. Sakazaki
		<i>S. sonnei</i> 70-76	R. Sakazaki
		<i>Escherichia coli</i> 905-65	R. Sakazaki
		<i>Escherichia coli</i> 916-65	R. Sakazaki
		<i>Escherichia coli</i> 897-65	R. Sakazaki
		<i>Escherichia coli</i> 193-68	R. Sakazaki
<i>Escherichia coli</i> H ₂ S+	19	<i>E. coli</i> H ₂ S+ N114	R. Sakazaki
	32	<i>E. coli</i> H ₂ S+ N28	R. Sakazaki
	18	<i>E. coli</i> H ₂ S+ N59	R. Sakazaki
	26	<i>E. coli</i> H ₂ S+ N68	R. Sakazaki
	22	<i>E. coli</i> H ₂ S+ N46	R. Sakazaki
	31	<i>E. coli</i> H ₂ S+ N2	R. Sakazaki
	30	<i>E. coli</i> H ₂ S+ N5	R. Sakazaki
	28	<i>E. coli</i> H ₂ S+ N63	R. Sakazaki
	24	<i>E. coli</i> H ₂ S+ N73	R. Sakazaki
	23	<i>E. coli</i> H ₂ S+ N76	R. Sakazaki
	27	<i>E. coli</i> H ₂ S+ N99	R. Sakazaki
	25	<i>E. coli</i> H ₂ S+ N103	R. Sakazaki
	29	<i>E. coli</i> H ₂ S+ N21	R. Sakazaki
	20	<i>E. coli</i> H ₂ S+ N111	R. Sakazaki
	21	<i>E. coli</i> H ₂ S+ N110	R. Sakazaki
<i>Salmonella typhi</i>	14	<i>S. typhi</i> 112	R. Sakazaki
	13	<i>S. typhi</i> 111	R. Sakazaki
	12	<i>S. typhi</i> 113	R. Sakazaki
Strains not clustered	35	<i>E. coli</i> 1324-70	R. Sakazaki
	36	<i>E. coli</i> 1120-70	R. Sakazaki
	37	<i>E. coli</i> 1233-70	R. Sakazaki
		<i>S. choleraesuis</i> 1348	
<i>Salmonella</i> spp.	33	<i>E. coli</i> 1326-70	R. Sakazaki
	34	<i>E. coli</i> 1441-70	R. Sakazaki
		<i>S. seminare</i> 1685	WHO Collabo. Salmo- nella Center
		<i>S. arizonae</i> 1	R. Sakazaki
		<i>S. arizonae</i> 2	R. Sakazaki
		<i>S. arizonae</i> 3	R. Sakazaki
	57	<i>S. arizonae</i> 888	R. Sakazaki
	58	Unknown coliform	R. Sakazaki
	59	Unknown coliform 114	R. Sakazaki
		Unknown coliform 74	R. Sakazaki
		Unknown coliform 75	R. Sakazaki
		<i>S. chameleon</i>	WHO Collabo. Salmo- nella Center
		<i>S. ochsenzoll</i>	WHO Collabo. Salmo- nella Center
		<i>S. soesterberg</i>	WHO Collabo. Salmo- nella Center
		<i>S. parera</i>	WHO Collabo. Salmo- nella Center
		<i>S. bonaire</i>	WHO Collabo. Salmo- nella Center
		<i>S. houten</i>	WHO Collabo. Salmo- nella Center
		<i>S. munda</i> berg	WHO Collabo. Salmo- nella Center
		<i>S. tuindorp</i>	WHO Collabo. Salmo- nella Center
		<i>S. argentina</i>	WHO Collabo. Salmo- nella Center
		<i>S. beloha</i>	WHO Collabo. Salmo- nella Center

TABLE 3—Continued

Cluster identification	Strain com- puter no.	Strain received as:	Source ^a
		<i>S. eilbek</i>	WHO Collabo. Salmo- nella Center
		<i>S. nairobi</i>	WHO Collabo. Salmo- nella Center
		<i>S. bilthoven</i>	WHO Collabo. Salmo- nella Center
		<i>S. ngozi</i>	WHO Collabo. Salmo- nella Center
		<i>S. basel</i>	WHO Collabo. Salmo- nella Center
		<i>S. artis</i>	WHO Collabo. Salmo- nella Center
		<i>S. locarno</i>	WHO Collabo. Salmo- nella Center
		<i>S. setubal</i>	WHO Collabo. Salmo- nella Center
		<i>S. haarlem</i>	WHO Collabo. Salmo- nella Center
	55	<i>S. infantis</i>	R. Sakazaki
	54	<i>S. derby</i>	R. Sakazaki
		<i>S. krefeld</i>	R. Sakazaki
		<i>S. kiambu</i>	R. Sakazaki
		<i>S. meleagridis</i>	R. Sakazaki
		<i>S. montivideo</i>	R. Sakazaki
		<i>S. typhimurium</i>	R. Sakazaki
	53	<i>S. meleagridis</i>	R. Sakazaki
		<i>S. enteritidis</i>	R. Sakazaki
		<i>S. anatum</i>	R. Sakazaki
		<i>S. livingstone</i>	R. Sakazaki
		<i>S. derby</i>	R. Sakazaki
		<i>S. infantis</i>	R. Sakazaki
		<i>S. bredeney</i>	R. Sakazaki
		<i>S. heidelberg</i>	R. Sakazaki
	52	<i>S. enteritidis</i>	R. Sakazaki
	56	<i>S. livingstone</i>	R. Sakazaki
Strain not clustered		<i>Citrobacter freundii</i> 10	R. Sakazaki
<i>Citrobacter freundii</i>	50	<i>C. intermedius</i> 3	R. Sakazaki
		<i>C. freundii</i> 1	R. Sakazaki
	45	<i>C. intermedius</i> 5	R. Sakazaki
	43	<i>C. freundii</i> 423-71	R. Sakazaki
		<i>C. freundii</i> 6	R. Sakazaki
	49	<i>C. freundii</i> 624-71	R. Sakazaki
	44	<i>C. freundii</i> 511-71	R. Sakazaki
		<i>C. freundii</i> 5	R. Sakazaki
		<i>C. freundii</i> 3	R. Sakazaki
		<i>C. freundii</i> 2	R. Sakazaki
		<i>C. freundii</i> 4	R. Sakazaki
		<i>C. freundii</i> 9	R. Sakazaki
		<i>C. freundii</i> 12	R. Sakazaki
		<i>C. freundii</i> 7	R. Sakazaki
	42	<i>C. freundii</i> 577-67	R. Sakazaki
	47	<i>C. intermedius</i> 4	R. Sakazaki
	46	<i>C. intermedius</i> 1	R. Sakazaki
Strains not clustered	48	<i>C. intermedius</i> 2	R. Sakazaki
	51	<i>C. freundii</i> 624-70	R. Sakazaki
	93	<i>Erwinia carotovora</i> 979	CCM
<i>Citrobacter</i> spp.	76	<i>C. diversus</i> 2469-70	NCDC
	64	<i>Levinea malonatica</i> 527-71	R. Sakazaki
	63	<i>Levinea malonatica</i> 25409	ATCC

TABLE 3—Continued

Cluster identification	Strain com- puter no.	Strain received as:	Source ^a
<i>Citrobacter diversus</i>	74	<i>Levinea malonatica</i> 528-71	R. Sakazaki
	72	<i>Levinea malonatica</i> 25408	ATCC
	73	<i>Levenia malonatica</i> 25410	ATCC
	65	<i>C. diversus</i> 1066-70	NCDC
	68	<i>C. diversus</i> 3613-63	NCDC
	69	<i>C. diversus</i> 2292-70	NCDC
	67	<i>C. diversus</i> 2737-70	NCDC
	71	<i>C. diversus</i> 2524-70	NCDC
	66	<i>C. diversus</i> 2572-70	NCDC
	75	<i>C. diversus</i> 1381-70	NCDC
	70	<i>C. diversus</i> 2393-70	NCDC
	77	<i>C. diversus</i> 2555-70	NCDC
	62	<i>Levinea amalonatica</i> 25407	ATCC
	61	<i>Levinea amalonatica</i> 25405	ATCC
<i>Salmonella</i> spp.	60	<i>Levinea amalonatica</i> 25406	ATCC
		<i>S. sendai</i> 618-65	R. Sakazaki
		<i>S. pullorum</i> 1334-D67	Costin, Romania
		<i>S. paratyphi</i> A T67-131	R. Sakazaki
<i>Hafnia alvei</i>		<i>S. abortusequi</i> WH2	R. Sakazaki
		<i>H. alvei</i> 56211	Eveland
		<i>H. alvei</i> P344	Eveland
		<i>H. alvei</i> 100-794	Eveland
		<i>H. alvei</i> 887-61	R. Sakazaki
		<i>H. alvei</i> 70811	Eveland
	38	<i>H. alvei</i> 13	R. Sakazaki
	39	<i>H. alvei</i> 7081	R. Sakazaki
	40	<i>H. alvei</i> 5621	R. Sakazaki
		<i>H. alvei</i> 441-61	R. Sakazaki
	41	<i>H. alvei</i> 887-61	R. Sakazaki
		<i>H. alvei</i> 1190-61	R. Sakazaki
		<i>H. alvei</i> 732-61	R. Sakazaki
		<i>H. alvei</i> P253	Eveland
		<i>H. alvei</i> P228	Eveland
<i>Enterobacter cloacae</i>		<i>Enterobacter cloacae</i> 2-55	R. Sakazaki
	79	<i>Enterobacter cloacae</i> 116	R. Sakazaki
	80	<i>E. cloacae</i> 101-71	R. Sakazaki
		<i>E. cloacae</i> 717-53	R. Sakazaki
	81	<i>E. cloacae</i> 1679	R. Sakazaki
	82	<i>E. cloacae</i> 2011	R. Sakazaki
	78	<i>E. cloacae</i> 102-71	R. Sakazaki
		<i>E. cloacae</i> SA 153	R. Sakazaki
		<i>E. cloacae</i> 558-53	R. Sakazaki
		<i>E. cloacae</i> 116-54	R. Sakazaki
		<i>E. cloacae</i> 0427-53	R. Sakazaki
		<i>E. cloacae</i> 1366-53	R. Sakazaki
		<i>E. cloacae</i> 1609-53	R. Sakazaki
		<i>E. cloacae</i> 1302-54	R. Sakazaki
		<i>E. cloacae</i> 2015-53	R. Sakazaki
<i>Klebsiella</i> and <i>Enterobacter</i> <i>aerogenes</i>		<i>E. aerogenes</i> 114-54	R. Sakazaki
		<i>E. aerogenes</i> 2-56	R. Sakazaki
		<i>E. aerogenes</i> 1414-69	R. Sakazaki
		<i>E. aerogenes</i> 1412-69	R. Sakazaki
		<i>E. aerogenes</i> 138-54	R. Sakazaki
		<i>E. aerogenes</i> 195-57	R. Sakazaki
		<i>E. aerogenes</i> 1405-69	R. Sakazaki
	100	<i>K. edwardsii</i> 9496	NCTC
	96	<i>K. oxytoca</i> 13183	ATCC
	98	<i>K. oxytoca</i> 13182	ATCC
	97	<i>K. oxytoca</i> 833-64	R. Sakazaki

TABLE 3—Continued

Cluster identification	Strain com- puter no.	Strain received as:	Source ^a
<i>Klebsiella ozaenae</i>	99	<i>K. oxytoca</i> 1296-63	R. Sakazaki
	100	<i>K. oxytoca</i> 1210-70	R. Sakazaki
		<i>K. aerogenes</i> 919	R. Sakazaki
		<i>K. aerogenes</i> 293-61	R. Sakazaki
		<i>Klebsiella</i> sp. 164-69	R. Sakazaki
		<i>K. aerogenes</i> 1193	R. Sakazaki
		<i>K. aerogenes</i> 104-54	R. Sakazaki
		<i>K. aerogenes</i> 281-51	R. Sakazaki
		<i>K. aerogenes</i> 1470	R. Sakazaki
		<i>K. aerogenes</i> 222-60	R. Sakazaki
		<i>K. aerogenes</i> 105-54	R. Sakazaki
		<i>E. aerogenes</i> 14460	ATCC
		<i>E. aerogenes</i> 90-55	R. Sakazaki
		<i>E. aerogenes</i> 36-55	R. Sakazaki
	94	<i>K. pneumoniae</i> 72	R. Sakazaki
	95	<i>K. pneumoniae</i> 65	R. Sakazaki
		<i>K. aerogenes</i> 56	R. Sakazaki
		<i>K. pneumoniae</i> 213-51	R. Sakazaki
		<i>K. aerogenes</i> 313	R. Sakazaki
		<i>K. pneumoniae</i> 1111-53	R. Sakazaki
		<i>K. pneumoniae</i> 871-55	R. Sakazaki
		<i>K. pneumoniae</i> 13-53	R. Sakazaki
		<i>K. pneumoniae</i> 244-54	R. Sakazaki
		<i>K. pneumoniae</i> 271-54	R. Sakazaki
		<i>Klebsiella</i> sp. 141-69	R. Sakazaki
		<i>Klebsiella</i> sp. 150-69	R. Sakazaki
		<i>Klebsiella</i> sp. 186-69	R. Sakazaki
		<i>Klebsiella</i> sp. 179-69	R. Sakazaki
	127	<i>E. liquefaciens</i> 225	R. Sakazaki
	83	Unknown coliform 216	R. Sakazaki
	84	Unknown coliform 221	R. Sakazaki
	102	<i>K. edwardsii</i> 5054	NCTC
	125	<i>K. ozaenae</i> 15-72	R. Sakazaki
	123	<i>K. ozaenae</i> 130	R. Sakazaki
	122	<i>K. ozaenae</i> 160	R. Sakazaki
	121	<i>K. ozaenae</i> 9-72	R. Sakazaki
	124	<i>K. ozaenae</i> 2015-62	R. Sakazaki
		<i>K. ozaenae</i> 22-69	R. Sakazaki
		<i>K. ozaenae</i> 12-69	R. Sakazaki
		<i>K. ozaenae</i> 19-69	R. Sakazaki
		<i>K. ozaenae</i> 16-69	R. Sakazaki
		<i>K. ozaenae</i> 13-69	R. Sakazaki
		<i>K. ozaenae</i> 11-69	R. Sakazaki
		<i>K. ozaenae</i> 8-69	R. Sakazaki
		<i>K. ozaenae</i> 9-69	R. Sakazaki
		<i>K. ozaenae</i> 21-69	R. Sakazaki
		<i>K. ozaenae</i> 10-69	R. Sakazaki
		<i>Klebsiella</i> sp. 171-69	R. Sakazaki
		172-69	R. Sakazaki
		170-69	R. Sakazaki
		159-69	R. Sakazaki
		184-69	R. Sakazaki
		163-69	R. Sakazaki
		142-69	R. Sakazaki
		153-69	R. Sakazaki
		140-69	R. Sakazaki
		156-69	R. Sakazaki
		148-69	R. Sakazaki
		154-69	R. Sakazaki

TABLE 3—Continued

Cluster identification	Strain com- puter no.	Strain received as:	Source ^a
		144-69	R. Sakazaki
		133-69	R. Sakazaki
		177-69	R. Sakazaki
<i>Klebsiella rhinoscleromatis</i>	119	<i>K. rhinoscleromatis</i> i-234-53	Ørskov
	112	<i>K. rhinoscleromatis</i> i-1-50	Ørskov
	111	<i>K. rhinoscleromatis</i> i-501-56	Ørskov
	116	<i>K. rhinoscleromatis</i> i-9-50	Ørskov
	115	<i>K. rhinoscleromatis</i> i-6-50	Ørskov
	114	<i>K. rhinoscleromatis</i> i-255-53	Ørskov
	113	<i>K. rhinoscleromatis</i> i-236-53	Ørskov
	118	<i>K. rhinoscleromatis</i> i-256-53	Ørskov
	117	<i>K. rhinoscleromatis</i> i-235-53	Ørskov
	120	<i>K. rhinoscleromatis</i> i-11-50	Ørskov
		<i>K. rhinoscleromatis</i> 1218-53	R. Sakazaki
		<i>K. rhinoscleromatis</i> 136-55	R. Sakazaki
		<i>K. rhinoscleromatis</i> 119-53	R. Sakazaki
		<i>K. rhinoscleromatis</i> 1230-54	R. Sakazaki
<i>Enterobacter liquefaciens</i>	130	<i>E. liquefaciens</i> 1689	R. Sakazaki
	129	<i>E. liquefaciens</i> 2214	R. Sakazaki
	131	<i>E. liquefaciens</i> 1493	R. Sakazaki
		<i>E. liquefaciens</i> 1111-65	R. Sakazaki
		<i>E. liquefaciens</i> 1689-69	R. Sakazaki
		<i>E. liquefaciens</i> 1638-69	R. Sakazaki
		<i>E. liquefaciens</i> 2214-69	R. Sakazaki
		<i>E. liquefaciens</i> 2108-69	R. Sakazaki
		<i>E. liquefaciens</i> 1493-69	R. Sakazaki
		<i>E. liquefaciens</i> 2318-68	R. Sakazaki
		<i>E. liquefaciens</i> 1312-67	R. Sakazaki
		<i>E. liquefaciens</i> 1783-67	R. Sakazaki
Strains not clustered	103	Unknown coliform 222	R. Sakazaki
	85	Unknown coliform 215	R. Sakazaki
	92	Unknown coliform 213	R. Sakazaki
		<i>Enterobacter liquefaciens</i> D974	R. Sakazaki
	90	<i>Enterobacter agglomerans</i> 459-71	NCDC
<i>Serratia marcescens</i>		<i>S. marcescens</i> 3130-68	R. Sakazaki
		<i>S. marcescens</i> 2187-68	R. Sakazaki
		<i>S. marcescens</i> 2249-68	R. Sakazaki
		<i>S. marcescens</i> 2630-68	R. Sakazaki
		<i>S. marcescens</i> 2191-68	R. Sakazaki
		<i>S. marcescens</i> 2241-68	R. Sakazaki
		<i>S. marcescens</i> 2638-68	R. Sakazaki
		<i>S. marcescens</i> 2242-68	R. Sakazaki
		<i>S. marcescens</i> 2963-68	R. Sakazaki
		<i>S. marcescens</i> 2121-68	R. Sakazaki
	135	<i>S. marcescens</i> 2249-68	R. Sakazaki
	136	<i>S. marcescens</i> 14	R. Sakazaki
	134	<i>S. marcescens</i> 2187-68	R. Sakazaki
	133	<i>S. marcescens</i> 16	R. Sakazaki
	132	<i>S. marcescens</i> 2121-68	R. Sakazaki
Strains not clustered	91	<i>Enterobacter agglomerans</i> 219-71	NCDC
	89	<i>Enterobacter agglomerans</i> 185-71	NCDC
	88	<i>Enterobacter agglomerans</i> 1379-71	NCDC
	86	<i>Enterobacter agglomerans</i> 184-71	NCDC
	126	<i>Erwinia herbicola</i> Aj 2671	Tokyo Univ.
	108	<i>Erwinia herbicola</i> Aj 2189	Tokyo Univ.
	109	<i>Erwinia herbicola</i> Aj 2190	Tokyo Univ.
	87	<i>Enterobacter agglomerans</i> 6-71	NCDC
	110	<i>Enterobacter agglomerans</i> 5379-	NCDC

TABLE 3—Continued

Cluster identification	Strain com- puter no.	Strain received as:	Source ^a
	71		
	107	<i>Erwinia carotovora</i> 1012	CCM
	106	<i>Erwinia carotovora</i> 1016	CCM
	104	<i>Erwinia carotovora</i> 630	CCM
	137	<i>Enterobacter agglomerans</i> 3737-	NCDC
	71		

^a Abbreviations: Ørskov, F. Ørskov, WHO International Escherichia Reference Center, Statens Serum-institut, Artager Bld. 80, 2300 Copenhagen 5, Denmark; NIH, Tokyo, 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, Calif.; Costin, Romania, I. D. Costin, The State Inspection for Hygiene, Timisoara, Romania; WHO Collabo. Salmonella Center, World Health Organization Collaborative Salmonella Center, Institut Pasteur, Paris; NCDC, National Center for Disease Control, Atlanta, Ga.; NCTC, National Collection of Type Cultures, Central Public Health Laboratory, Colindale Avenue, London NW 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.; Univ. of Lund, Lund Universitet, Fack, 221 01 Lund 1, Sweden; ATCC, American Type Culture Collection, 12301 Parklawn Drive, Rockville, Md.; CCM, Czechoslovak Collection of Microorganisms, J. E. Purkyne University, tř. Obránců míru 10, Brno, Czechoslovakia; Tokyo Univ., University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan.

received as *K. oxytoca*, *K. edwardsii*, *K. pneumoniae*, *K. aerogenes*, and *E. aerogenes*. This arrangement was consistent in the earlier study as well as in this analysis.

Classification of the genus *Citrobacter*. The history of the genus *Citrobacter* centers around one well-established species, *C. freundii* (Braak) Werkman and Gillen 1932, and involves the search for a suitable species to accommodate those strains which do not produce hydrogen sulfide. The nomenclature of the group has been reviewed by Frederiksen (28) and by Ewing and Davis (22), although differing conclusions were reached. Recently, Sedláč (58) reintroduced *C. intermedius* in the 8th edition of *Bergey's Manual of Determinative Bacteriology*, making an already confusing situation more difficult. Results of important contributions to the taxonomy of *Citrobacter* by various investigators are summarized in Table 4.

Werkman and Gillen (67) proposed the genus *Citrobacter* based on studies of 15 strains. They subdivided the genus into seven species: *C. freundii*, *C. album*, *C. glycoligenes*, *C. intermedius*, *C. decolorans*, *C. diversum*, and *C. anindolicum*. However, only three species have been widely accepted.

Werkman and Gillen's description of *C. freundii* was based on two strains. Nevertheless, it has now become recognized as a legitimate species, although description of the species has been broadened on the basis of studies on larger numbers of strains (20). Their description of *Citrobacter intermedius* was based on four strains, and the species description differed

from *C. freundii* in several characteristics (see Table 5). Tittsler and Sandholzer (64) examined two of the strains (M8BK and 24R) which Werkman and Gillen assigned to *C. intermedius* and noted that both produced acid from salicin and hydrogen sulfide on lead acetate agar (the medium used by Werkman and Gillen).

It has since been confirmed by other authors that strain M8BK does, in fact, produce hydrogen sulfide and is capable of acidifying salicin. Detailed characterization of this strain at the American Type Culture Collection (ATCC 6750) indicated that it is a typical strain of *C. freundii*, as this species is presently defined (15). Table 5 shows characteristics employed by Werkman and Gillen for differentiation of *C. freundii* from *C. intermedius*. Also included in the table are results for the same characteristics given for *C. freundii*, as defined by Ewing. The single difference between *C. intermedius* and Ewing's definition of *C. freundii* is the production of hydrogen sulfide, the characteristic that Werkman and Gillen had recorded incorrectly. The differences between *C. freundii* of Werkman and Gillen and *C. intermedius* were more apparent than real, and they recede when larger numbers of strains are examined. In addition, the only authentic strain of *C. intermedius* now extant (ATCC 6750) has been shown to be a typical *C. freundii*. Thus, we must conclude that the two species are synonymous names, and we propose that *C. freundii* be retained on the grounds of priority.

Macierewicz (50) studied 27 strains which

FIG. 3. Taxonomic relationships among species of Enterobacteriaceae, *Yersinia*, and related organisms included in a combined analysis of a total of 337 strains.

failed to produce hydrogen sulfide. These were assigned to a new genus, *Padlewskia*. However, Macierewicz failed to propose any species for this genus. Frederiksen (28) proposed a new species, *Citrobacter koseri*, for 30 strains similar to those of Macierewicz, although they differed in the production of acid from adonitol, gelatin hydrolysis, and growth in Møller po-

tassium cyanide medium (see Table 4). In a study of 108 strains, Young et al. (68) proposed a new genus, *Levinea*, with two species, *L. malonatica* and *L. amalonatica*. Examination of Table 4 indicates that *L. malonatica* is identical to *C. koseri*, whereas *L. amalonatica* is identical to *Padlewskia*, a conclusion shared by Sedláč (58). *C. koseri* has priority over *L. ma-*

TABLE 4. Comparative study of relevant literature pertaining to the taxonomy of *Citrobacter diversus*, *C. intermedius*, *C. koseri*, and *Levinea species*^a

AUTHOR :	BURKEY 1928 (7)		MERKMAN AND GILLEN 1932 (63)		VAUGHN AND LEVINE 1942 (61)		FREDERIKSEN 1970 (27)		YOUNG ET AL., 1971 (64)		RICHARD ET AL. 1972 (51)		EHING AND DAVIS 1972 (21)		SEDLAK 1974 (54)	
	1	2	4		1942 (61)		1970 (27)		50	58	8	49	1972 (21)		A	B
DESIGNATION :	<i>C. diversus</i>	<i>C. diversus</i>	<i>C. intermedius</i>	<i>C. intermedius</i>	<i>C. intermedius</i>	<i>C. intermedius</i>	<i>C. koseri</i>	<i>C. koseri</i>	<i>L. avalonatica</i>	<i>L. avalonatica</i>	<i>L. avalonatica</i>	<i>L. avalonatica</i>	<i>C. diversus</i>	<i>C. diversus</i>	<i>C. intermedius</i>	<i>C. intermedius</i>
CHARACTERISTIC																
INDOLE	+	+	-	-	V	+	+	+	100	100	100	100	100	100	+	+
METHYL RED	-	IND.	+	+	+	+	/	/	100	100	100	100	100	100	+	+
VOGES PROSKAUER	-	-	-	-	-	-	-	-	0	0	0	0	0	0	-	-
CITRATE	/	+	+	+	+	+	+	+	100	100	100	100	100	100	+	+
HYDROGEN SULPHIDE PRODUCTION	/	+	-	-	-	-	W	W	0	0	0	0	0	0	-	-
UREASE	/	/	/	/	/	/	W	W	90	77.6	0	0	89	89	-	-
PHENYLALANINE DEAMINASE	/	/	/	/	/	/	-	-	0	0	0	0	0	0	-	-
MALONATE UTILIZATION	/	/	/	/	V	/	+	+	0	100	0	100	88.5	88.5	-	+
ARGININE DEC.	/	/	/	/	/	/	+	+	100	100	12	0	98	98	+	+
LYSINE DEC.	/	/	/	/	/	/	-	-	0	0	0	0	0	0	-	-
ORNITHINE DEC.	/	/	/	/	/	/	+	+	100	100	100	98	100	100	+	+
ACID FROM MANNITOL	+	+	+	+	+	/	+	+	100	98.3	100	100	100	100	+	+
LACTOSE	+	+	+	+	+	/	+	+	92	84.5	65	69	84	84	/	/
SUCROSE	+	+	-	-	V	-	V	V	0	8.6	0	23	17	17	-	-
SALICIN	+	+	-	-	V	+	+	+	98	98.3	100	100	91	91	-	-
ADONITOL	+	+	/	/	V	-	+	+	0	100	0	100	100	100	-	-
INOSITOL	+	+	-	-	V	-	L OR W	L OR W	0	0	0	12	0	0	/	/
DULCITOL	-	-	+	+	V	-	V	V	0	44.8	0	45	53	53	/	/
SORBITOL	+	+	+	+	V	+	+	+	100	100	100	100	99	99	+	+
MALTOSE	+	+	+	+	+	/	+	+	/	/	100	100	100	100	+	+
XYLOSE	+	+	+	+	+	+	+	+	/	/	100	100	100	100	+	+
GLUCONATE OXID.	/	/	/	/	/	/	/	/	/	/	/	/	/	/	-	-
GELATIN HYDROLYSIS	-	-	-	-	-	+	-	-	8	0	62	0	2	2	-	-
NON GROWTH	/	/	/	/	/	+	-	-	100	75.9	100	0	0	0	+	+
MOTILITY	-	-	+	+	+	/	+	+	/	/	/	/	93	93	+	+

KEY: + = POSITIVE, - = NEGATIVE, / = NO INFORMATION, V = VARIABLE, W = WEAK, IND = INDEFINITE, NUMERICAL VALUES ARE PERCENTAGES.

TABLE 5. Comparison of three species of *Citrobacter*, as described by Werkman and Gillen, with the evolved description of *C. freundii*^a

Character	Ewing 1971 (20)	Werkman and Gillen 1932 (67)			
	<i>C. freundii</i>	<i>C. freundii</i>	<i>C. intermedium</i>	<i>C. diversum</i>	
Indole	—	Weak	—	+	
Methyl red	+	+	+	Indefinite	
H ₂ S	(+)	+	—	+	
Motility	+	+	+	—	
Esculin	—	+	—	+	
Sucrose	V	+	—	+	
Salicin	V	+	—	+	
Galactose	/	+	+	—	
Dulcitol	V	—	+	—	
Inositol	—	+	—	+	
Raffinose	V	+	—	+	
Glycogen	/	—	/	+	
Melezitose	/	—	—	+	

^a Key: +, positive; —, negative; /, no information; V, variable; (+), delayed positive.

lonatica, whereas *L. amalonatica* should be retained over *Padlewskia*, since a type species of the latter was not proposed.

Ewing and Davis (22) also reported on strains similar to those described by Young et al. (68) and Frederiksen (28). Examination of the literature led them to conclude that the name *C. diversus* (Burkey) Werkman and Gillen was the appropriate species for many of the isolates, although they commented that there were some differences between the description provided by Werkman and Gillen and their own. Table 4 shows that *C. diversus*, as described by Ewing and Davis, is significantly similar in its characteristics to *C. koseri* and *L. malonatica*. Therefore, the logical conclusion is to consider these synonymous. It can be argued that the differences between the description by Werkman and Gillen and that by Ewing and Davis are sufficiently great to preclude consideration of the strains as belonging to the same species. Werkman and Gillen, for example, recorded *C. diversum* as nonmotile, giving an indefinite methyl red reaction, producing hydrogen sulfide, and not producing acid from inositol. The difference in hydrogen sulfide production can be explained by the test methods employed, since the lead acetate agar used by Werkman and Gillen is more sensitive than the triple sugar iron medium used by Ewing and Davis. On the basis of data for the two strains included in this study, we do not feel that the other characters are sufficient in number to determine that the strains are different species. Comparison of *C. diversus*, *L. malonatica*, and *C. koseri* gives a strong indication that all of those strains belong to the same species and that *C. diversus* has priority. The argument that the original description of *C. diversum*

may be inadequate appears dubious, even though some characteristics such as amino acid decarboxylase reactions were not recorded.

Ewing and Davis (22) also reported that strains of *L. amalonatica* and *Padlewskia* appeared to be identical to strains of *C. freundii* that were hydrogen sulfide negative and indole positive. Even though they retained these strains as variants of *C. freundii*, evidence appears to be accumulating that suggests either that there is a closer relationship to *C. diversus* or that these strains should be considered a separate species, intermediate between *C. freundii* and *C. diversus*. The phenetic analysis used in this study resulted in separation of *L. amalonatica* from the *C. diversus* group. Gross and Rowe (33) reported that strains of *L. amalonatica* possessed common O antigens not shared with *L. malonatica*, *C. koseri*, or *C. diversus*, although strains received under the latter names did share common O antigens. Perhaps the strongest evidence is provided by Crosa et al. (13) in deoxyribonucleic acid (DNA)-DNA homology studies of these organisms. Table 6 summarizes the ranges of homologies obtained at the less stringent temperature, i.e., 60 C. The results clearly show a division of the genus *Citrobacter* into three groups: *C. freundii* and some biochemically and serologically atypical strains; *L. amalonatica*; and *C. diversus*. The degree of relatedness appears to be similar among all of the groups. Results of this study are in agreement with those of Crosa et al., namely that the genus *Citrobacter* should consist of three taxa: *C. freundii*, *C. diversus*, and *L. amalonatica*. The authors postpone the naming of *L. amalonatica* as a species of *Citrobacter* until the legitimacy of the specific epithet "*amalonatica*" has been studied.

Hydrogen sulfide variants of *Escherichia coli*. Judging by overall phenetic similarity and the individual biochemical test results, there is little doubt but that the hydrogen sulfide-producing strains of *Escherichia coli* belong with the more typical strains within the same species. The possibility exists, however, that they may be confused with *Citrobacter*. Some of the more useful differentiating characteristics are shown in Table 7. Lautrop et al. (46) and Darland and Davis (14) reported the occurrence of H₂S-positive strains. Thus, it appears that these strains represent a very small proportion of *E. coli* strains isolated from clinical specimens. They also exhibit biochemical and serological variability and do not represent a restricted biotype of the species. The property may sometimes be lost and can also be transferred to H₂S-negative strains; it has been

TABLE 6. DNA-DNA homologies determined among *Citrobacter* and *Levinea* spp. at 60 C^a

Species	DNA homology			
	<i>C. freundii</i>	<i>C. freundii</i> (atypical)	<i>L. amalonatica</i>	<i>L. malonatica</i>
<i>C. freundii</i>	90-100	69-77	53-56	51-58
<i>C. freundii</i> (atypical)	76-97	68-100	53-55	52-58
<i>L. amalonatica</i>	62-66	56-57	94-100	56-66
<i>L. malonatica</i>	63-68	55-59	57-63	86-100
<i>C. diversus</i>	65-70	59-61	57-58	83-85

^a Data summarized from Crosa et al. (13).TABLE 7. Selected characteristics differentiating hydrogen sulfide variants of *Escherichia coli* from citrobacters^a

Character	<i>E. coli</i>		<i>C. freundii</i>	<i>C. diversus</i>	<i>C. amalonatica</i>
	H ₂ S positive	H ₂ S negative			
Indole	+	+	-	+	+
Methyl red	+	+	+	+	+
Voges-Proskauer	-	-	-	-	-
Lysine dec.	+	+	-	-	-
Ornithine dec.	⁵ / ₁₄	+	-	+	+
KCN	-	-	+	-	+
Malonate			V	+	-
H ₂ S from thiosulfate	+	-	+	-	-
Gluconate	+	-	⁷ / ₁₇	-	-
Starch hydrolysis	+	-	-	-	-
Acid from raffinose	-	+	-	-	-
Acid from 5% sucrose	+	-	/	/	/
Acid from adonitol	-	-	-	-	+

^a For key to symbols, see footnote a, Table 5.

shown that the ability to produce hydrogen sulfide is associated with a plasmid (54).

The enigma of the clustering of *Klebsiella aerogenes* and *Enterobacter aerogenes* remains to be solved. In our previous study, we argued in favor of retaining *K. pneumoniae* for a group of strains received as *K. pneumoniae*, *K. aerogenes*, and some unidentified *Klebsiella* species (38). In this study, a similar cluster was formed, but in addition to the above-named strains, *K. oxytoca* and *K. edwardsii* also fell into this group. Kaluzewski (39) favored granting specific status to *K. oxytoca* for the indole-positive strains. Ørskov (53) retained the name *K. oxytoca* for those strains which are indole positive and liquefy gelatin. On the other hand, Cowan et al. (12) followed the earlier dictum of Lautrop (45) and excluded *K. oxytoca* from the genus altogether. They subdivided 176 strains of *Klebsiella* into six groups, differentiated on the basis of 13 characteristics. Four groups were assigned specific rank: *K. aerogenes*, *K. pneumoniae* (*sensu stricto*), *K. rhinoscleromatis*, and *K. ozaenae*, whereas the other two groups were given varietal status in a new species, *K. edwardsii*. The results of a phenetic analysis

(3) showed unequivocally that strains of *K. aerogenes*, *K. pneumoniae* (*sensu stricto*), *K. oxytoca*, and *K. edwardsii* were highly related, with similarities of >90%. It was conservatively argued that *K. pneumoniae* be retained separately from the *K. aerogenes/oxytoca/edwardsii* group.

The results obtained in our studies are almost identical to those of Bascomb et al. (3). However, we cannot accept that *K. pneumoniae* should be a separate species. The evidence to support such a choice is limited. The names of all four species (*K. pneumoniae*, *K. aerogenes*, *K. oxytoca*, and *K. edwardsii*) should be considered synonymous, and the designation *K. pneumoniae* (Schroeter) Trevisan 1887 should be used. However, Jain et al. (37) showed by DNA-DNA hybridization that the *Oxytocum* strains represent a distinct DNA homology from that of *K. pneumoniae* and they proposed a new genus of *Enterobacteriaceae* with one species for this group. Further study of *K. oxytoca* is required.

The high phenetic resemblance between *Klebsiella pneumoniae* and *Enterobacter aerogenes* is apparent from this analysis as well as

from the earlier study. Only two characteristics, motility and the presence of ornithine decarboxylase, definitely separate the two species, although the production of urease may also be helpful. Strains may occur which may be atypical in one or both of these characteristics. From the results of DNA relatedness studies, Brenner *et al.* (6) suggested that the genus *Klebsiella* could be expanded to include *E. aerogenes* strains. Matsumoto (51) demonstrated a close relationship between *K. pneumoniae* and *E. aerogenes* by recombination of chromosomal genes. Bascomb *et al.* (3) proposed the name *Klebsiella mobilis* for the organisms known as *E. aerogenes*.

Genus *Erwinia*. The present studies have not provided any help towards settling the taxonomic conundrum presented by the genus *Erwinia*. It would appear from the literature that three groups exist, corresponding to those described by Dye (16-19) and Lelliot (47). A fourth group of atypical *erwiniae* (19) has disappeared through reallocation of the strains to other already existing genera (5, 10). The three groups correspond to what are sometimes called the "true" *Erwinia* or "amylovora" group, i.e., those species which cause wilts, galls, or dry necrosis of plants; the "carotovora" group, corresponding to Waldee's *Pectobacterium* (66), or soft-rot organisms; and the herbicola-lathyr group, or *Enterobacter agglomerans* (24-26).

Evidence from phenotypic analyses and DNA-DNA homology studies (5, 7, 30) divides the amylovora group into six species and the carotovora group into five species. The agreement between the two approaches is extremely good, and the consensus of opinion is for retaining the two groups within the family *Enterobacteriaceae*, but probably as two separate genera, *Erwinia* and *Pectobacterium*, respectively.

The third group consists of yellow-pigmented strains, which are more and more frequently being isolated in clinical situations. These strains are of doubtful phytopathogenicity and have, in the past, been assigned a variety of names. Dye (18) and Lelliot (47) subdivided the group into three species, *Erwinia herbicola* (with two subspecies, subsp. *herbicola* and subsp. *ananas*), *Erwinia uredovora*, and *Erwinia stuartii*. However, Ewing and Fife (24-26) grouped all three species into a single species which they placed in the genus *Enterobacter* as *E. agglomerans*. Nevertheless, they found it necessary to subdivide *E. agglomerans* into 11 biogroups on the basis of reduction of nitrate, Voges-Proskauer, and indole reactions. Ewing and Fife (24) argued that many of the characteristics of these strains are uniform, and thus they can be included in a single spe-

cies. However, examination of their tabulated results presents a somewhat different picture. There is significant biochemical variability within this group, with no justification for creation of a species "ragbag" for purposes of assigning nomenclature to an otherwise unidentified isolate. It also appears that Ewing and Fife are not entirely convinced that the species *E. agglomerans* will not have to be divided at a later date or conceivably that a new genus will not have to be created for these strains. In citing some unpublished data, Brenner *et al.* (5) indicated that, on the basis of DNA-DNA homology studies, at least 10 different hybridization groups exist within the herbicola group. In view of the coincidence between his hybridization divisions and the phenetic divisions of Dye in the amylovora and carotovora groups, one wonders whether there will also be a striking similarity in the herbicola group.

Strains received as *Erwinia* species or *Enterobacter agglomerans* in this study exhibited as much biochemical diversity as reported by other workers. Figures 1 and 2 indicate that seven strains did form a cluster (designated *E. agglomerans*) at about 78% similarity. However, when the number of strains in the analysis was increased (Fig. 3), this cluster was not robust, and the strains were observed to be more loosely aggregated. On the basis of data for 20 strains of *Erwinia*, taxonomic recommendations may not be warranted except that very little justification is provided for *Enterobacter agglomerans* to be a species group as it is presently defined.

In conclusion, some useful observations concerning the taxonomy of several species and genera of the *Enterobacteriaceae* have been provided by this study. It is hoped that continuing investigations will further clarify those taxonomic issues which are as yet unresolved.

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The raw data have been submitted to the World Data Bank, Brisbane, Australia, and also are available from the authors upon request.

REPRINT REQUESTS

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LITERATURE CITED

1. d'Alessandro, G., and R. Comes. 1956. Sul meccanismo della reazione dell'acido β -fenil-propionico negli enterobatteri. Boll. Ist. Sieroter. Milan. 35:202-213.
2. Baird-Parker, A. C. 1963. A classification of micrococci and staphylococci based on physiological and biochemical tests. J. Gen. Microbiol. 30:409-427.
3. 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.
4. Benton, A. G. 1935. Chitinovorous bacteria. A preliminary survey. *J. Bacteriol.* 29:449-465.
 5. Brenner, D. J., G. R. Fanning, and A. G. Steigerwalt. 1974. Deoxyribonucleic acid relatedness among erwiniae and other *Enterobacteriaceae*: the gall, wilt, and dry-necrosis organisms (genus *Erwinia* Winslow et al., sensu stricto). *Int. J. Syst. Bacteriol.* 24:197-204.
 6. 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.
 7. Brenner, D. J., A. G. Steigerwalt, G. V. Miklos, and G. R. Fanning. 1973. Deoxyribonucleic acid relatedness among erwiniae and other *Enterobacteriaceae*. I. The soft-rot organisms (genus *Pectobacterium* Waldee). *Int. J. Syst. Bacteriol.* 23:205-216.
 8. Burkey, L. A. 1928. The fermentation of cornstalks and their constituents. I. Studies on the pectin-fermenting bacteria. *Iowa State Coll. J. Sci.* 3:57-100.
 9. Christensen, W. B. 1949. Hydrogen sulphide production and citrate utilization in the differentiation of the enteric pathogens and the coliform bacteria. *Res. Bull. Weld County Health Dept.* 1:3.
 10. Colwell, R. R., R. Johnson, L. Wan, T. E. Lovelace, and D. J. Brenner. 1974. Numerical taxonomy and deoxyribonucleic acid reassociation in the taxonomy of some gram-negative fermentative bacteria. *Int. J. Syst. Bacteriol.* 24:422-433.
 11. 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.
 12. Cowan, S. T., K. J. Steel, C. Shaw, and J. P. Duguid. 1960. A classification of the *Klebsiella* group. *J. Gen. Microbiol.* 23:601-612.
 13. Crosa, J. H., A. G. Steigerwalt, G. R. Fanning, and D. J. Brenner. 1974. Polynucleotide sequence divergence in the genus *Citrobacter*. *J. Gen. Microbiol.* 83:271-282.
 14. Darland, G., and B. R. Davis. 1973. Biochemical and serological characterization of hydrogen sulfide positive variants of *Escherichia coli*. Center for Disease Control, Atlanta, Ga.
 15. Davis, B. R., and W. H. Ewing. 1966. The biochemical reactions of *Citrobacter freundii*. Center for Disease Control, Atlanta, Ga.
 16. Dye, D. 1968. A taxonomic study of the genus *Erwinia*. I. The "Amylovora" group. *N. Z. J. Sci.* 11:590-607.
 17. Dye, D. 1969. A taxonomic study of the genus *Erwinia*. II. The "Carotovora" group. *N. Z. J. Sci.* 12:81-97.
 18. Dye, D. 1969. A taxonomic study of the genus *Erwinia*. III. The "Herbicola" group. *N. Z. J. Sci.* 12:223-236.
 19. Dye, D. 1969. A taxonomic study of the genus *Erwinia*. IV. "Atypical" erwiniae. *N. Z. J. Sci.* 12:833-838.
 20. Edwards, P. R., and W. H. Ewing. 1972. Identification of *Enterobacteriaceae*, p. 1-362. Burgess Publishing Co., Minneapolis.
 21. Ewing, W. H. 1971. Biochemical characterization of *Citrobacter freundii* and *Citrobacter diversus*. Center for Disease Control, Atlanta, Ga.
 22. 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.
 23. Ewing, W. H., B. R. Davis, and R. W. Reavis. 1957. Phenylalanine and malonate media and their use in enteric bacteriology. *Publ. Health Lab.* 15:153-167.
 24. Ewing, W. H., and M. A. Fife. 1972. *Enterobacter agglomerans* (Beijerinck) comb. nov. (the *Herbicola*-*Lathyr*i bacteria). *Int. J. Syst. Bacteriol.* 22:4-11.
 25. Ewing, W. H., and M. A. Fife. 1972. *Enterobacter agglomerans*. The herbicola-lathyr*i* bacteria. Center for Disease Control, Atlanta, Ga.
 26. Ewing, W. H., and M. A. Fife. 1972. Biochemical characterization of *Enterobacter agglomerans*. Center for Disease Control, Atlanta, Ga.
 27. Fred, E. B., I. L. Baldwin, and E. McCoy. 1932. Root nodule bacteria and leguminous plants. *Univ. Wis. Stud. Sci.* 5:1-343.
 28. Frederiksen, 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). *Publ. Fac. Sci. Univ. J. E. Purkyne Brno.* 47:89-94.
 29. Gaby, W. L., and E. Free. 1958. Differential diagnosis of *Pseudomonas*-like microorganisms in the clinical laboratory. *J. Bacteriol.* 76:442-444.
 30. Gardner, J. M., and C. I. Kado. 1972. Comparative base sequence homologies of the deoxyribonucleic acids of *Erwinia* species and other *Enterobacteriaceae*. *Int. J. Syst. Bacteriol.* 22:201-209.
 31. Gordon, R. E., and J. M. Mihm. 1957. A comparative study of some strains received as nocardiae. *J. Bacteriol.* 73:15-27.
 32. Gordon, R. E., and M. M. Smith. 1955. Rapidly growing, acid fast bacteria. II. Species description of *Mycobacterium fortuitum* Cruz. *J. Bacteriol.* 69:502-507.
 33. Gross, R. J., and B. Rowe. 1974. The serology of *Citrobacter koseri*, *Levinea malonatica* and *Levinea amalonatica*. *J. Med. Microbiol.* 7:155-161.
 34. Haynes, W. C. 1951. *Pseudomonas aeruginosa*—its characterization and identification. *J. Gen. Microbiol.* 5:939-950.
 35. Hinshaw, W. R. 1941. Cysteine and related compounds for differentiating members of the genus *Salmonella*. *Hilgardia* 13:583-621.
 36. Hugo, W. B., and E. G. Beveridge. 1962. A quantitative and qualitative study of the lipolytic activity of single strains of seven bacterial species. *J. Appl. Bacteriol.* 25:72-82.
 37. Jain, K., K. Radsak, and W. Mannheim. 1974. Differentiation of the *Oxytocom* group from *Klebsiella* by deoxyribonucleic acid-deoxyribonucleic acid hybridization. *Int. J. Syst. Bacteriol.* 24:402-407.
 38. Johnson, R., R. R. Colwell, R. Sakazaki, and K. Tamura. 1975. Numerical taxonomy study of the *Enterobacteriaceae*. *Int. J. Syst. Bacteriol.* 25:12-37.
 39. Kaluzewski, S. 1967. Taxonomic position of indole-positive strains of *Klebsiella*. *Exp. Med. Microbiol.* 19:350-359.
 40. Kauffmann, F., and A. Petersen. 1956. The biochemical group and type differentiation of *Enterobacteriaceae* by organic acids. *Acta Pathol. Microbiol. Scand.* 38:481-491.
 41. 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.
 42. Kohn, J. 1955. A preliminary report of a new gelatin liquefaction method. *J. Clin. Pathol.* 6:249.
 43. Koser, S. A. 1923. Utilization of the salts of organic acids by the colon-aerogenes group. *J. Bacteriol.* 8:493-520.
 44. Kovacs, N. 1956. Identification of *Pseudomonas pyocyanea* by the oxidase reaction. *Nature (London)* 178:703.
 45. Lautrop, H. 1956. Gelatin liquefying *Klebsiella* strains (*Bacterium oxytocom* (Flügge)). *Acta Pathol. Microbiol. Scand.* 39:375-384.
 46. Lautrop, H., I. Orskov, and K. Gaarslev. 1971. Hydrogen sulphide producing variants of *Escherichia coli*. *Acta Pathol. Microbiol. Scand. Sect. B* 79:641-650.
 47. Lelliot, R. A. 1974. The genus *Erwinia*, p. 1-1246. In R. E. Buchanan and N. E. Gibbons (ed.), *Bergey's man-*

- ual of determinative bacteriology, 8th ed. Williams and Wilkins Co., Baltimore.
48. Le Minor, L., and F. Ben Hamida. 1962. Avantages de la recherche de la β -galactosidase sur celle de la fermentation du lactose en milieu complexe dans le diagnostic bactériologique en particulier des Enterobacteriaceae. Ann. Inst. Pasteur Paris 102:267-277.
 49. 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.
 50. Macierewicz, M. 1966. A proposal of a new group (genus) of Enterobacteriaceae. Med. Dosw. I. Mikrobiol. 18:333-339.
 51. Matsumoto, H. 1973. Genetic recombination between *Klebsiella pneumoniae* and *Enterobacter aerogenes*. Genet. Res. 21:47-55.
 52. Niven, C. F., K. L. Smiley, and J. M. Sherman. 1942. The hydrolysis of arginine by streptococci. J. Bacteriol. 43:651-660.
 53. Ørskov, I. 1957. Biochemical types in the *Klebsiella* group. Acta Pathol. Microbiol. Scand. 40:155-162.
 54. Ørskov, I., and F. Ørskov. 1973. Plasmid-determined H_2S character in *Escherichia coli* and its relation to plasmid-carried raffinose fermentation and tetracycline resistance characters. J. Gen. Microbiol. 77:487-499.
 55. Richard, C., B. Brisou, and J. Lioult. 1972. Étude taxonomique de "*Levinea*" nouveau genre de la famille des Entérobactéries. Ann. Inst. Pasteur Paris. 122:1137-1146.
 56. Rustigan, R., and C. A. Stuart. 1941. Decomposition of urea by *Proteus*. Proc. Soc. Exp. Biol. Med. 47:108-112.
 57. Sedláč, J. 1973. Present knowledge and aspects of *Citrobacter*. Curr. Top. Microbiol. Immunol. 62:41-59.
 58. Sedláč, J. 1974. The genus *Citrobacter*, p. 1-1246. In R. E. Buchanan and N. E. Gibbons (ed.), *Bergey's manual of determinative bacteriology*, 8th ed. Williams and Wilkins Co., Baltimore.
 59. Sierra, G. 1957. A simple method for the detection of lipolytic activity of microorganisms and some observations on the influence of the contact between cells and fatty substrates. Antonie van Leeuwenhoek J. Microbiol. Serol. 23:15-22.
 60. Simmons, J. S. 1926. A culture medium for differentiating organisms of the typhoid-colon aerogenes groups and for isolation of certain fungi. J. Infect. Dis. 39:209-214.
 61. Singer, J., and B. E. Volcani. 1955. An improved ferric chloride test for differentiating *Proteus-Providence* group from other *Enterobacteriaceae*. J. Bacteriol. 69:303-306.
 62. 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.
 63. Starr, M. P. 1947. Causal agent of bacterial root and stem disease of guayule. Phytopathology 37:291.
 64. Tittsler, R. P., and L. A. Sandholzer. 1935. Studies on the *Escherichia-Aerobacter* intermediates. J. Bacteriol. 29:349-361.
 65. Vaughn, R. H., and M. Levine. 1942. Differentiation of the "intermediate" coli-like bacteria. J. Bacteriol. 44:487-505.
 66. Waldee, E. L. 1945. Comparative studies of some peritrichous phytopathogenic bacteria. Iowa State Coll. J. Sci. 19:435-484.
 67. Werkman, C. H., and G. F. Gillen. 1932. Bacteria producing trimethylene glycol. J. Bacteriol. 23:167-182.
 68. 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.