Deoxyribonucleic Acid Relatedness of *Proteus* and *Providencia* Species

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Deoxyribonucleic acid reassociation was used to determine relatedness among protei and providenciae and between these organisms and other members of the family *Enterobacteriaceae*. The results indicate the following. (i) *Proteus mirabilis*, *Prot. morganii*, and *Providencia stuartii* are homogeneous species. (ii) *Prot. vulgaris*, *Prot. rettgeri*, and *Prov. alcalifaciens* each contain more than one deoxyribonucleic acid relatedness group. (iii) A group of urea-positive strains previously called *Prot. rettgeri* biogroup 5 are, in fact, members of *Prov. stuartii*. (iv) *Prot. myxofaciens* is a valid species. (v) Protei are only distantly related to all other *Enterobacteriaceae*. Taxonomic revisions consistent with these observations are discussed. These include a proposal to place the currently recognized species of *Proteus* into one of three genera: the genus *Proteus*, containing *Prot. mirabilis* Hauser, *Prot. vulgaris* Hauser, and *Prot. myxofaciens* Cosenza and Podgwaite; a separate genus *Morganella* Fulton, for *Prot. morganii* (Winslow et al.) Yale; and the genus *Providencia* containing *Prov. alcalifaciens* (De Salles Gomes) Ewing, *Prov. stuartii* (Buttiaux et al.) Ewing, and *Prov. rettgeri* comb. nov.

In *Bergey's Manual of Determinative Bacteriology*, 8th ed., Lautrop (29) recognized five species of *Proteus* Hauser: *Prot. vulgaris* Hauser, *Prot. mirabilis* Hauser, *Prot. morganii* (Winslow, Kligler, and Rothberg) Yale, *Prot. rettgeri* (Hadley, Elkins, and Caldwell) Rustigian and Stuart, and *Prot. inconstans* (Ornstein) Shaw and Clarke. Ewing (13-16) placed *Prot. inconstans* in the genus *Providencia* Ewing and recognized two species: *Prov. alcalifaciens* (De Salles Gomes) Ewing and *Prov. stuartii* (Buttiaux et al.) Ewing.

Ewing described four biogroups in *Prov. alcalifaciens* and two biogroups in *Prov. stuartii*. These are based on the production of gas from glucose and the fermentation of adonitol and inositol (17; see Table 12).

Several other taxonomic proposals for *Proteus* and *Providencia* have been reviewed by Rauss (38). The best known of these is Kauffmann's recommendation of four genera: *Proteus*, *Morganella*, *Rettgerella*, and *Providencia* (26). Kauffmann included *Prot. mirabilis* and *Prot. vulgaris* in the species *Prot. hauseri*. The four-genus concept with *Prot. hauseri* was endorsed by Rauss (38), and Coetzee (7) endorsed the four-genus system while retaining *P. mirabilis* and *P. vulgaris*.

Johnson et al. (24) studied 50 strains of *Pro-*

teus and *Providencia* by numerical taxonomy. They objected to separating *Proteus* from *Providencia* mainly on the basis of urease production. They also noted a large proportion of biochemically atypical strains of protei. This observation and observations of multiple antibiotic resistance in protei are well documented (8, 9, 12, 13, 16, 17, 21, 22, 30, 32, 33). Expanding upon the earlier work of Namioka and Sakazaki (31), Penner et al. (34) divided *Prot. rettgeri* into five biogroups based on acid production from salicin, L-rhamnose, D-mannitol, adonitol, and D-arabitol. Biogroup (BG) 5 did not produce acid from the three polyhydric alcohols and resembled some strains of *Prot. stuartii*. These authors suggested that their BG 5 may be intermediate between *Prot. rettgeri* and *Providencia*.

In a review (7), Coetzee documented serological differences in enzymes among protei. Therefore, there are antigenic, structural, and biochemical data that suggest genetic divergence among protei. With the exception of *Prot. morganii* (50 mol% guanine plus cytosine [G+C] in the deoxyribonucleic acid [DNA]), *Proteus* and *Providencia* species have substantially lower G+C ratios than other *Enterobacteriaceae*, whose DNA contains 50 to 58 mol% G+C. *Prot. mirabilis* and *Prot. vulgaris* contain 38 to 40 mol% G+C (19, 23, 29), *Prot. rettgeri* has 39 to 41.5 mol% G+C, and providenciae are reported to have 40 to 42 mol% G+C.

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A few laboratories have reported DNA relatedness studies on protei (1, 2, 7, 11, 25), and the data from these studies are summarized by Coetzee (7). These preliminary data indicate that *Prot. mirabilis* and *Prot. vulgaris* contain similar base sequences throughout a substantial portion of their genomes (1, 7), whereas neither of these species is closely related to other protei or to *Escherichia coli* (1, 2, 7). *Prot. morganii* and *Prot. rettgeri* do not exhibit substantial relatedness to each other, to *Providencia*, or to *E. coli* (7, 11).

In this study, we determined the degree of DNA relatedness within and among species of *Proteus* and *Providencia* and also the relatedness of these organisms to other *Enterobacteriaceae*. A revised classification of the tribe *Proteeae* is proposed based on nucleic acid relatedness data. There are also some nomenclatural problems and a need for neotype strains to be designated. These problems will be considered in a subsequent paper.

MATERIALS AND METHODS

Bacterial strains and media. The organisms used in this study are listed in Table 1. *Proteus* and *Providencia* strains are listed by the name under which they were received. Organisms were maintained on brain heart infusion agar or nutrient agar. Routine cultivation was carried out in brain heart infusion broth on a dry-air rotary shaker at 37°C. The medium used for labeling cells with $^{32}\text{PO}_4$ was described previously (2).

Preparation of DNA. A modification of the method of Berns and Thomas (2) was used in preparing both unlabeled and $^{32}\text{PO}_4$ -labeled DNA. DNA was sheared by sonic oscillation to an average double-stranded molecular weight of approximately 2.5×10^5 . Labeled DNA fragments were denatured by boiling for 4 min, rapidly cooled to 60°C, purified further by passage through a hydroxyapatite (HA) column equilibrated with 0.14 M phosphate buffer (PB; equimolar mixture of NaH_2PO_4 and Na_2HPO_4 , pH 6.8) plus 0.4% sodium dodecyl sulfate (SDS), and held at 60°C. Any labeled material bound to the column under these conditions was discarded. This procedure removes cross-linked DNA and other contaminating material that retains the ability to bind to HA after boiling and quick cooling. After this procedure, the zero-time binding (label bound to HA immediately after DNA is denatured) is usually less than 3%. In these experiments, the specific activity of labeled DNA was 2.5×10^4 to 1×10^5 cpm/ μg .

DNA reassociation. Labeled DNA fragments at a concentration of 0.1 $\mu\text{g}/\text{ml}$ (2,500 to 10,000 cpm/ml) plus 150 μg of unlabeled DNA fragments per ml contained in 0.28 M PB were thermally denatured, quickly cooled, and then incubated at either 60 or 75°C for 16 h to reach an approximate C_{0t} of 100 (DNA concentration $\times$ time units; reference 4) for the unlabeled DNA. After incubation, the PB was diluted to a concentration of 0.14 M, and the mixture was applied to

HA to separate single-stranded DNA from reassociated DNA. HA was equilibrated with 0.14 M PB plus 0.4% SDS and held at the temperature at which the DNA reassociation reaction had been carried out (60 or 75°C).

In 0.14 M PB, single-stranded DNA does not bind to HA, whereas double-stranded DNA is adsorbed to HA. Four 15-ml portions of 0.14 M PB plus 0.4% SDS at the incubation temperature removed unreacted DNA. The HA was then treated with either four 15-ml portions of 0.4 M PB (at which salt concentration double-stranded DNA is eluted from HA) or in a series of 15-ml portions of 0.14 M PB buffer at increasing 5°C increments up to 100°C (when thermal stability of reassociated DNA was of interest). As the incubation temperature exceeds the $T_{m(e)}$ (the temperature at which 50% of the DNA bound to HA is denatured) of various stability classes of DNA, the resulting single-stranded fragments are eluted from HA, and a thermal elution profile is obtained. The HA was finally washed with one 15-ml portion of 0.4 M PB to elute any material that remained bound to HA. All eluates were collected in counting vials and assayed directly by Cerenkov radiation in a scintillation counter (6). In control experiments, the reassociation of labeled DNA in 0.28 M PB was less than 1%. Relative binding values were not corrected for the background reassociation of labeled DNA.

DNA relatedness is the amount of labeled DNA reassociation in a reaction between labeled and unlabeled DNA (heterologous reaction) relative to the amount of labeled DNA reassociation in a homologous reaction where labeled and unlabeled DNA are from the same source. Relatedness is expressed in percent. Percent relatedness may be conveniently expressed as relative binding ratio (RBR). The equation used is: $\text{RBR} = \% \text{ relatedness} = [(\% \text{ heterologous DNA bound to HA}) / (\% \text{ homologous DNA bound to HA})] \times 100$. Relatedness is arbitrarily deemed to be close or high (RBR = 65% or more), moderate (RBR = 25 to 60%), or distant (RBR = less than 25%). Percent divergence (D) is calculated on the assumption that a 1°C decrease in thermal stability of a DNA duplex is caused by each 1% of unpaired bases within that duplex (28). For example, consider organisms A and B that are 50% related. An A-A duplex has a mean thermal stability of 91°C, whereas an A-B duplex has a mean thermal stability of 81°C. The D in related DNA is 10%.

Glossaries of DNA association terms helpful to the nonspecialist are given in references 3 and 42.

The amount of reassociation obtained in homologous control reactions, the thermal stability of these DNA duplexes, and the amount of reassociation of controls containing labeled DNA only are shown in Table 2.

RESULTS

Relatedness of protei to other *Enterobacteriaceae*. There has not been a thorough study of the relatedness of protei to other *Enterobacteriaceae*. These relationships were determined by labeling DNA from one or more strains of each species of *Proteus* and *Providencia* and reacting these preparations with DNA from

TABLE 1. *Bacterial strains used*

Strain	Atypical ^a biochemical reactions	Source
<i>Bacillus subtilis</i> 168		J. Marmur
<i>Citrobacter freundii</i> 80-68		CDC ^b
<i>Edwardsiella tarda</i> 1483-59		CDC
<i>Enterobacter aerogenes</i> 1627-66		CDC
<i>Enterobacter agglomerans</i> 1645-71		CDC
<i>Enterobacter agglomerans</i> 19321		ATCC ^c
<i>Enterobacter cloacae</i> 1347-71		CDC
<i>Enterobacter cloacae</i> 4562-70		CDC
<i>Erwinia amylovora</i> EA 178		ICPB ^d
<i>Erwinia quercina</i> EQ 102		ICPB
<i>Erwinia salicis</i> 15712		ATCC
<i>Escherichia coli</i> K-12		U. Washington
<i>Hafnia alvei</i> (<i>Enterobacter hafniae</i>) 962-68		CDC
<i>Klebsiella pneumoniae</i> 2		CDC
<i>Pectobacterium carotovorum</i> 495		ATCC
<i>Pectobacterium chrysanthemi</i> SR 32		A. Kelman
<i>Pectobacterium cypripedii</i> EC 155		ICPB
<i>Proteus mirabilis</i> (<i>americanus</i>) 4675		ATCC
<i>P. mirabilis</i> 1		S. Falkow
<i>P. mirabilis</i> PR 11		CDC
<i>P. mirabilis</i> PR 14		CDC
<i>P. mirabilis</i> PR 22		CDC
<i>P. mirabilis</i> PR 23K		CDC
<i>P. mirabilis</i> PR 25		CDC
<i>P. mirabilis</i> 82-73		CDC
<i>P. mirabilis</i> 412-66	Lac ⁺ Ind ⁺	CDC
<i>P. mirabilis</i> 1140-73		CDC
<i>P. mirabilis</i> 1788-72	Ind ⁺	CDC
<i>P. mirabilis</i> 1922-73		CDC
<i>P. mirabilis</i> 2098-73	PPA ⁻	CDC
<i>P. mirabilis</i> 2113-72	Ure ⁻	CDC
<i>P. mirabilis</i> 14253		ATCC
<i>Proteus mitajiri</i> 21136		ATCC
<i>Proteus morganii</i>		S. Falkow
<i>P. morganii</i> 102		CDC
<i>P. morganii</i> 105-52		CDC
<i>P. morganii</i> 141-49		CDC
<i>P. morganii</i> 1166-73		CDC
<i>P. morganii</i> 1167		CDC
<i>P. morganii</i> 1255-73		CDC
<i>P. morganii</i> 1422-73		CDC
<i>P. morganii</i> 1640-73	Orn ⁻	CDC
<i>P. morganii</i> 1881-73		CDC

TABLE 1—Continued

Strain	Atypical ^a biochemical reactions	Source
<i>P. morganii</i> 2698-75	Orn ⁻	CDC
<i>P. morganii</i> 3205-70	Orn ⁻	CDC
<i>P. morganii</i> 3483-75	Orn ⁻	CDC
<i>P. morganii</i> 5832-72	Ind ⁻	CDC
<i>P. morganii</i> 5946-71	Ind ⁻ PPA ⁻	CDC
<i>P. morganii</i> 6166-50		CDC
<i>P. morganii</i> 9027-75		CDC
<i>P. morganii</i> 25830		ATCC
<i>P. morganii</i> 73-011043	Orn ⁻	CDC
<i>Proteus myxofaciens</i> 19692		ATCC
<i>Proteus rettgeri</i> BG 5b 285-75	Lac ⁺	CDC
<i>P. rettgeri</i> BG 5b 286-75	Lac ⁺	CDC
<i>P. rettgeri</i> BG 5b 416-72	Ind ⁻ Lac ⁺	CDC
<i>P. rettgeri</i> BG 1a 666-48	Rha ⁻	CDC
<i>P. rettgeri</i> BG 5b 697-75	Lac ⁺	CDC
<i>P. rettgeri</i> BG 2b 1163		CDC
<i>P. rettgeri</i> BG 3a 1165		CDC
<i>P. rettgeri</i> BG 1b 1226-73	Ino ⁻ Cit ⁻	CDC
<i>P. rettgeri</i> BG 2a 1266		CDC
<i>P. rettgeri</i> BG 1a 1387-73		CDC
<i>P. rettgeri</i> BG 3a 1519-73	Ure ⁻ Cit ⁻	CDC
<i>P. rettgeri</i> BG 5b 1586-76	Cit ⁻ Ino ⁻	CDC
<i>P. rettgeri</i> BG 5b 1631-75		CDC
<i>P. rettgeri</i> BG 3a 3737-74		CDC
<i>P. rettgeri</i> BG 5b 3899-72	Ind ⁻	CDC
<i>P. rettgeri</i> BG 5b 4100-74		CDC
<i>P. rettgeri</i> 4709-72	Ind ⁻ Ado ⁻ Ery ⁻ Rha ⁻ Tar ⁻	CDC
<i>P. rettgeri</i> 1a 4884-72	Mot ⁻ Gas ⁺ Lac ⁺ Suc ⁺ Raf ⁺ Tar ⁻	CDC
<i>P. rettgeri</i> BG 3a 4926-50		CDC
<i>P. rettgeri</i> BG 3a 5364-72		CDC
<i>P. rettgeri</i> BG 5b 5833-72	PPA ⁻	CDC
<i>P. rettgeri</i> BG 5b 6281-73	Lac ⁺	CDC
<i>P. rettgeri</i> BG 1a 9250		ATCC
<i>P. rettgeri</i> BG 5b 15267	Ind ⁻	A. Sonnenwirth
<i>Proteus sphingidis</i> 6911		ATCC
<i>Proteus vulgaris</i> PR1		CDC
<i>P. vulgaris</i> PR3		CDC
<i>P. vulgaris</i> PR 7		CDC
<i>P. vulgaris</i> PR 10		CDC
<i>P. vulgaris</i> PR 19		CDC
<i>P. vulgaris</i> 169		CDC
<i>P. vulgaris</i> 636-73	Ure ⁻	CDC
<i>P. vulgaris</i> 645-72	H ₂ S ⁻ Ind ⁻	CDC
<i>P. vulgaris</i> 931-72		CDC
<i>P. vulgaris</i> 1070-73	Nit ⁻ H ₂ S ⁻	CDC
<i>P. vulgaris</i> 1608-73		CDC
<i>P. vulgaris</i> 1808-73	Ind ⁻	CDC
<i>P. vulgaris (paraamericanus)</i> 6059		ATCC
<i>Providencia alcalifaciens</i> BG 1 671-66		CDC
<i>P. alcalifaciens</i> BG 1 1045-67		CDC
<i>P. alcalifaciens</i> BG 1 1805-66		CDC
<i>P. alcalifaciens</i> BG 1 1915-67		CDC
<i>P. alcalifaciens</i> BG 1 2424-66		CDC
<i>P. alcalifaciens</i> BG 1 3013-68		CDC
<i>P. alcalifaciens</i> BG 1 3055-67		CDC
<i>P. alcalifaciens</i> BG 1 4077-67		CDC
<i>P. alcalifaciens</i> BG 1 4673-67		CDC

TABLE 1—Continued

Strain	Atypical ^a biochemical reactions	Source
<i>P. alcalifaciens</i> BG 1 5209-66		CDC
<i>P. alcalifaciens</i> BG 1 5288-66		CDC
<i>P. alcalifaciens</i> BG 1 5574-66		CDC
<i>P. alcalifaciens</i> BG 2 325-69		CDC
<i>P. alcalifaciens</i> BG 2 355-69		CDC
<i>P. alcalifaciens</i> BG 2 3370-67		CDC
<i>P. alcalifaciens</i> BG 2 3397-67		CDC
<i>P. alcalifaciens</i> BG 2 3402-67		CDC
<i>P. alcalifaciens</i> BG 2 5097-67		CDC
<i>P. alcalifaciens</i> BG 2 9886		ATCC
<i>P. alcalifaciens</i> BG 3 Hart 034		CDC
<i>P. alcalifaciens</i> BG 3 132-68		CDC
<i>P. alcalifaciens</i> BG 3 156		CDC
<i>P. alcalifaciens</i> BG 3 1588-72		CDC
<i>P. alcalifaciens</i> BG 3 3574-67		CDC
<i>P. alcalifaciens</i> BG 3 12013		ATCC
<i>P. alcalifaciens</i> BG 3 26240		CDC
<i>P. alcalifaciens</i> BG 3 26250		CDC
<i>P. alcalifaciens</i> BG 3 26260		CDC
<i>P. alcalifaciens</i> BG 4 809-75	Ind ⁻	CDC
<i>P. alcalifaciens</i> BG 4 1831-75		CDC
<i>P. alcalifaciens</i> BG 4 5350-50		CDC
<i>Providencia stuartii</i> BG 5 430-70		CDC
<i>P. stuartii</i> BG 5 937-67		CDC
<i>P. stuartii</i> BG 5 2308-68		CDC
<i>P. stuartii</i> BG 5 2896-68		CDC
<i>P. stuartii</i> BG 5 4217-68		CDC
<i>P. stuartii</i> BG 5 4379-72		CDC
<i>P. stuartii</i> BG 5 5130-67		CDC
<i>P. stuartii</i> BG 6 508		CDC
<i>P. stuartii</i> BG 6 1153-67		CDC
<i>P. stuartii</i> BG 6 1160-67		CDC
<i>P. stuartii</i> BG 6 4105-66		CDC
<i>P. stuartii</i> BG 6 4579-50		CDC
<i>P. stuartii</i> BG 6 4596-50		CDC
<i>P. stuartii</i> BG 6 5956-66		CDC
<i>P. stuartii</i> BG 6 5966-66	PPA ⁻	CDC
<i>P. stuartii</i> 25825		CDC
<i>P. stuartii</i> 34185		CDC
<i>Salmonella typhimurium</i> LT2		T. Theodore
<i>Serratia liquefaciens</i> 446-68		CDC
<i>Serratia marcescens</i> SM6		WRAIR ^c
<i>Serratia rubidaea</i> 934-72		CDC
<i>Shigella flexneri</i> 24570		S. Falkow
<i>Yersinia enterocolitica</i> 497-70		CDC
<i>Yersinia pestis</i>		L. Corwin

^a Abbreviations for biochemical reactions: Lac, lactose; Ind, indole; Ure, urease; Orn, ornithine; Ino, inositol; Gas, gas from glucose; Nit, nitrate reduction; Cit, Simmons citrate; PPA, phenylalanine deaminase; Mot⁻, nonmotile; Tar, Jordan's tartrate; Suc, sucrose; Raf, raffinose; Rha, L-rhamnose; Ado, adonitol; Ery, erythritol.

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TABLE 2. *Homologous DNA reactions*^a

Source of DNA ^b	60°C RBR	<i>T</i> _{m(e)}	75°C RBR
<i>Proteus mirabilis</i> PR 14	80	88	74
<i>P. mirabilis</i> PR 14 label only	3.7		1.7
<i>Proteus morganii</i> 25830	90	92	89
<i>P. morganii</i> 25830 label only	1.4		1.8
<i>Proteus rettgeri</i> 1163	87	89	80
<i>P. rettgeri</i> 1163 label only	2.3		2.0
<i>Proteus rettgeri</i> 3899-72	83	89	78
<i>P. rettgeri</i> 3899-72 label only	3.2		2.6
<i>Proteus rettgeri</i> 9250	87	89	80
<i>P. rettgeri</i> 9250 label only	3.6		2.9
<i>Proteus vulgaris</i> PR1	83	88	75
<i>P. vulgaris</i> PR1 label only	1.4		1.4
<i>Proteus vulgaris</i> 13315	67	88	65
<i>P. vulgaris</i> 13315 label only	1.4		3.8
<i>Providencia alcalifaciens</i> 3370-67	85	89	80
<i>P. alcalifaciens</i> 3370-67 label only	2.3		2.0
<i>Providencia alcalifaciens</i> 132-68	53	88	39
<i>P. alcalifaciens</i> 132-68 label only	3.0		
<i>Providencia alcalifaciens</i> 2617-71	87	89	78
<i>P. alcalifaciens</i> 2617-71 label only	2.8		2.5
<i>Providencia stuartii</i> 2896-68	80	89	72
<i>P. stuartii</i> 2896-68 label only	3.4		2.4
<i>Providencia stuartii</i> 3233-68	80		
<i>P. stuartii</i> 3233-68 label only	2.6		

^a Abbreviations: RBR, relative binding ratio; *T*_{m(e)}, thermal elution midpoint.

^b For each pair, the upper reaction contained 0.1 µg of labeled DNA plus 150 µg of unlabeled DNA, and the lower reaction contained 0.1 µg of labeled DNA in the absence of unlabeled DNA. The numbers shown are the arithmetic means of 10 or more reactions.

most species of *Enterobacteriaceae*. *Prot. morganii* was about 20% related to species other than protei (Table 3). All other species of *Proteus* and *Providencia* had an average of 6 to 13% relatedness to species other than protei (Table 3). In 75°C reactions, the relatedness of *Prot. morganii* to other *Enterobacteriaceae* fell to 5% or less, and that of other protei to other *Enterobacteriaceae* fell to between 0 and 2%.

***Prot. mirabilis* and *Prot. vulgaris*.** All of the DNA relatedness data for the protei in this

study are shown in Table 4. For convenience, summary tables have also been included for specific groups (Tables 5 to 11). *Prot. mirabilis* strains were 90% related with very little divergence within the reassociated sequences (Table 5). *Prot. mirabilis* was 55% related to *Prot. vulgaris*. The related sequences showed 12% *D*, and relatedness in 75°C reactions was 20%. *Prot. mirabilis* was less than 25% related to other *Proteus* and *Providencia* species.

Strains of *Prot. vulgaris* formed more than one DNA relatedness group (Table 6). Six strains were extremely closely related (94%) to reference strain PR1. Five other strains (931-72 group) were 70% related to PR1 with an average 7% *D*. The average reaction at 75°C of PR1 with these strains was 34%. *Prot. myxofaciens* was about 45% related to both *Prot. mirabilis* and *Prot. vulgaris* PR1.

The relatedness of *Prot. vulgaris* PR1 and *Prot. vulgaris* 13315 to other *Proteus* and *Providencia* species was 15 to 20% (Table 6).

***Prot. morganii*.** All tested strains of *Prot. morganii* were in one DNA relatedness group. The average relatedness was 90% in 60°C reactions and 85% in 75°C reactions (Table 7). The related sequences contained less than 1% unpaired bases. *Prot. morganii* was less than 20% related to all other protei in 60°C reactions and less than 5% related to them in 75°C reactions.

***Prot. rettgeri*, *Prov. alcalifaciens*, and *Prov. stuartii*.** *Prot. rettgeri* strains form three DNA relatedness groups. The *Prot. rettgeri* 1163 group is composed of strains that are mainly biochemically typical and belong to Penner's BG 1, 2, and 3 (34). These strains were 79% related and showed an average *D* of 3% (Tables 4 and 8). The average relatedness at 75°C was 63% (Table 8).

The 1163 group was about 20% related to other *Proteus* species and 35 to 40% related to *Providencia* species. Almost identical results were obtained (not shown) when we used labeled DNA from another member of the 1163 group (strain 9250).

All members of a second group of *Prot. rettgeri* strains, exemplified by strain 3899-72, are in the Penner BG 5b (34). This group was inseparable from *Prot. stuartii* on the basis of DNA reassociation (Tables 4 and 8). The 3899-72 group was 30 to 35% related to typical *Prot. rettgeri* and to *Prov. alcalifaciens* (Table 8) and 16 to 25% related to other *Proteus* species (Tables 4 to 8).

The third *Prot. rettgeri* relatedness group (666-48 group) is composed of five strains that are biochemically atypical. They were most closely related to *Prot. rettgeri* and *Providencia* species (30 to 45%, Tables 4 to 11). These strains did not appear to form a single group on the

TABLE 3. DNA relatedness of members of the tribe *Proteeae* to other *Enterobacteriaceae*

Source of unlabeled DNA	RBR ^a , 60°C								
	<i>Prot.</i> <i>mirabi-</i> <i>lis</i> PR 14	<i>Prot.</i> <i>rettgeri</i> 1163	<i>Prot.</i> <i>rettgeri</i> 9250	<i>Prot.</i> <i>rettgeri</i> 3899-72	<i>Prot.</i> <i>vul-</i> <i>garis</i> PR 1	<i>Prov.</i> <i>alcali-</i> <i>faciens</i> BG 2 3370-67	<i>Prov.</i> <i>stuartii</i> BG 5 2896-68	<i>Prov.</i> <i>stuartii</i> BG 6 3233-68	<i>Prot.</i> <i>mor-</i> <i>ganii</i> 25830
<i>Citrobacter freundii</i> 80-68	11	10			8	12			20
<i>Edwardsiella tarda</i> 1483-59	10	10	6	4	7	11	6	5	22
<i>Enterobacter aerogenes</i> 1627-66	12	10			7				21
<i>Enterobacter agglomerans</i> 1645-71	10	10				12			19
<i>Enterobacter agglomerans</i> 19321	10	9			7				19
<i>Enterobacter cloacae</i> 1347-71	10	11			7	13			21
<i>Enterobacter cloacae</i> 4562-70	11	11				12			23
<i>Enterobacter hafniae</i>	13	15	9	9	11	15	9	9	20
<i>Erwinia amylovora</i> EA 178	10	10			7	11			18
<i>Erwinia quercina</i> EQ 102	10	10							19
<i>Erwinia salicis</i> 15712	11	10				12			20
<i>Escherichia coli</i> K-12	12	12	8	9	8	13	5	8	24
<i>Klebsiella pneumoniae</i> 2	11	8	5	5		12	5	6	23
<i>Pectobacterium carotovorum</i> 495	12	11			9	16			21
<i>Pectobacterium chrysanthemi</i> SR 32	14	7							18
<i>Pectobacterium cypripedii</i> EC 155	13	9							22
<i>Salmonella typhimurium</i> LT2	12	11	8	8	9	13	6	6	22
<i>Serratia liquefaciens</i> 446-68	11	11							22
<i>Serratia marcescens</i> SM6	11	10	5	6	6	11		6	24
<i>Serratia rubidaea</i> 934-72	10	10							20
<i>Shigella flexneri</i> 24570	14	10							23
<i>Yersinia enterocolitica</i> 497-70	16	14			11	16			18
<i>Yersinia pestis</i>	13								15
Average	12	10	7	7	8	13	6	7	21
<i>Bacillus subtilis</i> 168	0	0.5			0.5				2

^a RBR, Relative binding ratio. See Materials and Methods for definition.

basis of biochemical profiles (Table 1). No strains from this group were labeled for use as reference strains in hybridization studies. It is therefore possible that they belong to two or more hybridization groups.

Prov. alcalifaciens BG 1 and 2 strains were in one hybridization group with 89% average relatedness and 3% *D* at 60°C, and 74% average relatedness at 75°C (Table 9). Seven BG 1 strains and seven BG 2 strains were 90 to 100% related at both incubation temperatures and exhibited less than 1% *D*. Five additional BG 1 strains were 75 to 80% related to the reference B 2 strain (3370-67). In these reactions, *D* was 5 to 10%, and relatedness fell to between 41 and 60% in 75°C reactions. This level of relatedness implies divergence within strains of a single species that is not yet sufficient for the formation of two species. *Prov. alcalifaciens* BG 3 strains are 55% related to BG 1 and 2. *Prov. stuartii* and the three *Prot. rettgeri* groups are 35 to 48% related to BG 1 and 2. Relatedness of other *Proteus* species to BG 1 and 2 is 20%.

Prov. alcalifaciens BG 3 strains were all

closely related (Table 10). They were 45% related to *Prov. alcalifaciens* BG 1 and 2, 25 to 30% related to *Prov. stuartii* and the three *Prot. rettgeri* groups, and 10 to 15% related to other *Proteus* species.

All *Prov. stuartii* strains and the BG 5 strains of *Prot. rettgeri* (the 3899-72 group) were 90% related (Table 11). The other *Prot. rettgeri* groups and *Prov. alcalifaciens* groups were 35 to 40% related to *Prov. stuartii*. *Proteus* species were 14 to 29% related to *Prov. stuartii*.

There is a BG 4 in *Prov. alcalifaciens*. Several strains presumed to belong to BG 4 were indistinguishable from *Prov. stuartii* in DNA hybridization reactions. These strains were reexamined and shown to be inositol-positive, typical strains of *Prov. stuartii* (Table 12). DNA from three strains with the biochemical characteristics of *Prov. alcalifaciens* BG 4 were subsequently tested for relatedness to [³H]DNA of *Prov. stuartii* at the stringent 75°C incubation temperature. The RBR values obtained were 89, 88, and 75%. RBR values at 75°C with these strains and *Prov. alcalifaciens* BG 1, 2, 3, and *Proteus*

Source of unlabeled DNA	Source of labeled DNA															
	<i>P. mirabilis</i> PR 14		<i>P. P. morganii</i> 25830		<i>P. vulgaris</i> PR 1		<i>P. rettgeri</i> BG 2b 1163		<i>P. rettgeri</i> BG 5b 3899-72		<i>P. stuartii</i> BG 5 2896-68		<i>P. alcalifaciens</i> BG3 132-68		<i>P. alcalifaciens</i> BG2 3370-67	
	60 C RBR	75 C D RBR	60 C RBR	75 C D RBR	60 C RBR	75 C D RBR	60 C RBR	75 C D RBR	60 C RBR	75 C D RBR	60 C RBR	75 C D RBR	60 C RBR	D	60 C RBR	75 C D RBR
<i>P. mirabilis</i> t	87		15	18	1		51	12	17	15	2	13			8	22
<i>P. mirabilis</i> PR 11	88						52	10	17							
<i>P. mirabilis</i> PR 14	100	0 100	15				54	13	23	14		18			24	25 17 3
<i>P. mirabilis</i> PR 22	88	1 89					47									
<i>P. mirabilis</i> PR 23 K	90	1 91					50	11								
<i>P. mirabilis</i> PR 25	89	1 88	16				48									
<i>P. mirabilis</i> 82-73	88						46	12								
<i>P. mirabilis</i> 412-66	91	1					55									
<i>P. mirabilis</i> 1140-73	89	1					52	13								
<i>P. mirabilis</i> 1788-72	92	1	14				52									
<i>P. mirabilis</i> 1922-73	93						51	12								
<i>P. mirabilis</i> 2098-73	90						51	12	18							
<i>P. mirabilis</i> 2113-72	93	1 89	16				52	12	18	15					20	16 2
<i>P. mirabilis</i> (americanus) 4675	97	1 97					47	11							22	
<i>P. mirabilis</i> 14273	92		17	15	4		55	18	22							
<i>P. morganii</i>	18	8														
<i>P. morganii</i> 105-52			92													
<i>P. morganii</i> 141-49			86													
<i>P. morganii</i> 1166-73	19		92	1 88							29		10		22	
<i>P. morganii</i> 1167			89	1												
<i>P. morganii</i> 1255-73			89	2 82												
<i>P. morganii</i> 1422-73			91				17	14	1	12						
<i>P. morganii</i> 1640-73	19	12	87	1 83			14	2							11	
<i>P. morganii</i> 1881-73			86				13	3			16				22	16 4
<i>P. morganii</i> 5832-72			90	1 86											17	16 1
<i>P. morganii</i> 5946-71	16		94				13	12								
<i>P. morganii</i> 6166-50			95													
<i>P. morganii</i> 25830	17	13 4	100	0 100			15	15	6	25	9 8				21	
<i>P. vulgaris</i> PR 1	56	11 19	16	21 3			100	0 100	22	12 2		14		13	21	17 3
<i>P. vulgaris</i> PR 3	53						90	0 95				16				
<i>P. vulgaris</i> PR 7	54	12 17					97	1 100								
<i>P. vulgaris</i> PR 10	55	16					92		23							

TABLE 5. DNA relatedness of *Proteae* to *Proteus mirabilis* PR14

Source of unlabeled DNA	No. of strains ^a	60°C RBR ^b	D	75°C RBR
<i>Prot. mirabilis</i>	14	91	<1	91
<i>Prot. vulgaris</i>	13	56	12	20
<i>Prot. rettgeri</i> 1163 group	5	24	14	3
<i>Prot. rettgeri</i> 666-48 group	4	22	14	8
<i>Prot. rettgeri</i> 3899-72 group	5	25	14	
<i>Prot. morganii</i>	5	18	12	6
<i>Prov. alcalifaciens</i> BG 1, 2	6	23	12	3
<i>Prov. alcalifaciens</i> BG 3	1	20		
<i>Prov. stuartii</i>	5	20	13	4
<i>Prot. myxofaciens</i>	1	46	12	8

^a Number of strains tested in 60°C reactions. Fewer strains were tested for D (percent divergence) and for relatedness at 75°C (see Table 4). The homologous control reaction (100% by definition) is not included in the number of strains tested or in the calculations of RBR and D.

^b Relative binding ratio (RBR) values are arithmetic means obtained from the total number of strains tested. Relatedness values for individual strains are given in Table 4.

TABLE 6. DNA relatedness of *Proteae* to *Proteus vulgaris* PR1^a

Source of unlabeled DNA	No. of strains	60°C RBR	D	75°C RBR
<i>Prot. vulgaris</i> PR1 group	6	94	<1	98
<i>Prot. vulgaris</i> 931-72 group	5	69	7	34
<i>Prot. mirabilis</i>	15	51	12	13
<i>Prot. rettgeri</i> 1163 group	4	17		
<i>Prot. rettgeri</i> 666-48 group	4	18		2
<i>Prot. rettgeri</i> 3899-72 group	4	19	17	7
<i>Prot. morganii</i>	4	14	14	4
<i>Prov. alcalifaciens</i> BG 1, 2	3	15	14	1
<i>Prov. alcalifaciens</i> BG 3	1	21	14	2
<i>Prov. stuartii</i>	5	15	16	2
<i>Prot. myxofaciens</i>	1	44	13	11

^a See footnotes to Table 5.

species yielded RBR values of below 10%.

Additional organisms. Several strains listed as *Proteus* species separate from the accepted species of *Proteus*, as well as varieties of accepted species, were included in this study (Table 4). *Prot. mirabilis* (*Prot. americanus*) is a typical *Prot. mirabilis* strain, *Prot. vulgaris pa-*

raamericanus is a typical *Prot. vulgaris* strain, and *Prot. mitajiri* is a typical *Prot. rettgeri* (1163 group) strain. *Prot. sphingidis* is, as stated by the ATCC, a member of *Serratia marcescens*. *Prot. myxofaciens* does appear to represent a new species of *Proteus* (see section above on *Prot. mirabilis* and *Prot. vulgaris*).

DISCUSSION

Proteus and *Providencia* were known to be only distantly related to *E. coli* (2, 7). We now know that protei are distantly related to all species of *Enterobacteriaceae* (Table 3; D. J. Brenner, unpublished data). Species of *Proteus* and *Providencia* are biochemically and enzymatically different in many aspects and show substantial heterogeneity in DNA relatedness (7, 11; Tables 4-11).

In agreement with Kauffmann (26) and Coetzee (7), we believe that the phenotypical and genotypical divergence present in protei necessitates the establishment of additional genera. There is also a need for additional speciation in some cases and for consolidation of species in other cases. The current classification of protei and recommendations for changes in the classification and nomenclature of these organisms are summarized in Table 13. Biochemical reactions for these organisms are given in Table 14.

All *Prot. mirabilis* strains, including atypical Lac⁺, Ind⁺, and Ure⁻ strains, are one genetic species. Seven strains of *Prot. vulgaris* (PR1 group) were 90% or more related. Five additional strains (931-72 group) were 65 to 75% related to the PR1 group. Where tested, relatedness fell to about 35% in 75°C reactions. Each of these

TABLE 7. DNA relatedness of *Proteae* to *Proteus morganii* 25830^a

Source of unlabeled DNA	No. of strains	60°C RBR	D	75°C RBR
<i>Prot. morganii</i>	12	90	<1	85
<i>Prot. mirabilis</i>	6	16	17	3
<i>Prot. vulgaris</i> PR1 group	3	14		
<i>Prot. vulgaris</i> 931-72 group	3	19		
<i>Prot. rettgeri</i> 1163 group	4	17	17	2
<i>Prot. rettgeri</i> 666-48 group	3	19	15	5
<i>Prot. rettgeri</i> 3899-72 group	3	16		
<i>Prov. alcalifaciens</i> BG 1, 2	6	18		
<i>Prov. stuartii</i>	5	17	17	2
<i>Prot. myxofaciens</i>	1	12		

^a See footnotes to Table 5.

TABLE 8. DNA relatedness of *Proteaceae* to *Proteus rettgeri* strains 1163 and 3899-72^a

Source of unlabeled DNA	<i>Prot. rettgeri</i> BG 2b 1163 ^b			<i>Prot. rettgeri</i> BG 5b 3899-72 ^b		
	No. of strains	60°C RBR	75°C RBR	No. of strains	60°C RBR	75°C RBR
<i>Prot. rettgeri</i> 1163 group	7	79	63	7	32	9
<i>Prot. rettgeri</i> 3899-72 group	5	33	5	4	88	86
<i>Prot. rettgeri</i> 666-48 group	5	45	12	5	37	16
<i>Prot. mirabilis</i>	5	20	2	2	16	
<i>Prot. vulgaris</i> PR1 group	3	23				
<i>Prot. vulgaris</i> 931-72 group	3	22	4			
<i>Prot. morganii</i>	4	22	4			
<i>Prov. alcalifaciens</i> BG 1, 2	6	39	5	4	32	
<i>Prov. alcalifaciens</i> BG 3	1	38		1	29	
<i>Prov. stuartii</i>	5	34	5	6	83	77
<i>Prot. myxofaciens</i>	1	26	6			

^a For additional information, see footnotes to Table 5.^b Source of labeled DNA.TABLE 9. DNA relatedness of *Proteaceae* to *Providencia alcalifaciens* BG 2 3370-67^a

Source of unlabeled DNA	No. of strains	60°C RBR	<i>D</i>	75°C RBR
<i>Prov. alcalifaciens</i> BG 1, 2	18	89	3	74
<i>Prov. alcalifaciens</i> BG 3	2	55	12	18
<i>Prov. stuartii</i>	9	38	13	10
<i>Prot. rettgeri</i> 1163 group	4	48	14	10
<i>Prot. rettgeri</i> 3899-72 group	1	35	16	5
<i>Prot. rettgeri</i> 666-48 group	2	40		6
<i>Prot. mirabilis</i>	4	22	17	3
<i>Prot. vulgaris</i> PR1 group	2	21	17	3
<i>Prot. vulgaris</i> 931-72 group	2	21	16	2
<i>Prot. morganii</i>	4	21	16	3
<i>Prot. myxofaciens</i>	1	24	17	2

^a See footnotes to Table 5.TABLE 10. DNA relatedness of *Proteaceae* to *Providencia alcalifaciens* BG 3 132-68^a

Source of unlabeled DNA	No. of strains	60°C RBR
<i>Prov. alcalifaciens</i> BG 3	8	88
<i>Prov. alcalifaciens</i> BG 1, 2	4	46
<i>Prov. stuartii</i>	4	30
<i>Prot. rettgeri</i> 1163 group	3	33
<i>Prot. rettgeri</i> 3899-72 group	4	23
<i>Prot. rettgeri</i> 666-48 group	5	28
<i>Prot. mirabilis</i>	1	16
<i>Prot. vulgaris</i> PR1 group	1	13
<i>Prot. vulgaris</i> 931-72 group	2	10
<i>Prot. morganii</i>	2	11

^a See footnotes to Table 5.

strains exhibited higher relatedness to *Prot. vulgaris* than to *Prot. mirabilis*. Thus far, there is no known biochemical correlation with the low relatedness at 75°C, and it is not known whether these strains belong to one or more DNA relatedness groups. Pending further study, they should remain in *Prot. vulgaris*.

Prot. myxofaciens was isolated from living and dead gypsy moth larvae in New York and

TABLE 11. DNA relatedness of *Proteaceae* to *Providencia stuartii* BG 5 2896-68^a

Source of unlabeled DNA	No. of strains	60°C RBR	<i>D</i>	75°C RBR
<i>Prov. stuartii</i>	17	91	<1	
<i>Prov. rettgeri</i> 3899-72 group	5	91	<1	92
<i>Prov. alcalifaciens</i> BG 1, 2	6	34	12	4
<i>Prov. alcalifaciens</i> BG 3	2	34	13	7
<i>Prot. rettgeri</i> 1163 group	4	36		
<i>Prot. rettgeri</i> 666-48 group	5	40	11	14
<i>Prot. mirabilis</i>	2	14		
<i>Prot. vulgaris</i> PR1 group	2	15		
<i>Prot. vulgaris</i> 931-72 group	3	16		
<i>Prot. morganii</i>	1	29		

^a See footnotes to Table 5.TABLE 12. Biogroups of *Providencia* as defined by Ewing et al.^a

Species and biogroup	Gas	Adonitol	Inositol
<i>Prov. alcalifaciens</i>			
BG 1	+	+	-
BG 2	-	+	-
BG 3	+	-	-
BG 4	-	-	-
<i>Prov. stuartii</i>			
BG 5	-	-	+
BG 6	-	+	+

^a Reference 17.

Connecticut (10). The type strain (ATCC 19692) differed biochemically from all other protei (10). In the present study, this strain was about 50% related to *Prot. mirabilis* and *Prot. vul-*

TABLE 13. *Classification and nomenclature of Proteace*

<i>Bergey's Manual</i> (29)	DNA hybridization group	Taxonomic proposals and recommendations
<i>Proteus mirabilis</i>	<i>P. mirabilis</i>	<i>P. mirabilis</i>
<i>Proteus vulgaris</i>	<i>P. vulgaris</i> PR1 group <i>P. vulgaris</i> 931-72 group	<i>P. vulgaris</i> Retain as <i>P. vulgaris</i> pending further study
	<i>P. myxofaciens</i>	<i>P. myxofaciens</i> —a valid species
<i>Proteus morganii</i>	<i>P. morganii</i>	Change genus name to <i>Morganella</i>
<i>Proteus rettgeri</i>	<i>P. rettgeri</i> 1163 group <i>P. rettgeri</i> 3899-72 group BG 5 <i>P. rettgeri</i> 666-48 group	Transfer to <i>Providencia</i> Transfer to <i>Providencia stuartii</i> , urea ⁺ Include in <i>Providencia rettgeri</i> pending further study
<i>Proteus inconstans</i> A (<i>Providencia alcalifaciens</i>)	<i>P. alcalifaciens</i> BG 1, 2 <i>P. alcalifaciens</i> BG 3 <i>P. alcalifaciens</i> BG 4	<i>Providencia alcalifaciens</i> Make new species in <i>Providencia</i> when definitive biochemical data are available Transfer to <i>Providencia stuartii</i>
<i>Proteus inconstans</i> B (<i>Providencia stuartii</i>)	<i>P. stuartii</i> BG 5, 6	<i>P. stuartii</i>

garis. In *Bergey's Manual* (29), *Prot. myxofaciens* is reported to be identical with *Erwinia herbicola* (*Enterobacter agglomerans*). J. Leete (unpublished data) has shown that labeled *Prot. myxofaciens* DNA is 40 to 50% related to DNA from *Prot. mirabilis* and *Prot. vulgaris* but only about 10% related to DNA from a strain of *E. herbicola*. We conclude that *Prot. myxofaciens* is not a synonym of *E. herbicola* or of *Prot. mirabilis* (as stated in the *American Type Culture Collection Catalogue*, 12th ed.) but is a valid species both biochemically and genetically. The name *Prot. myxofaciens* has been validly published (10), and strain ATCC 19692 is the type strain for this species.

All *Prot. morganii* strains are highly related, including Orn⁻ and Ind⁻ strains. *Prot. morganii* DNA contains 50 mol% G+C, which is similar to the G+C content in *E. coli* and *Salmonella* rather than that of protei. It is related to most enteric bacteria at a 20% level and is not more than 20% related to other protei. For these reasons, *Prot. morganii* should be placed in a separate genus. Since *Prot. vulgaris* and not *Prot. morganii* is the type species of the genus *Proteus*, the rules of nomenclature allow the establishment of a new genus for *Prot. morganii*. The recognition of the genus *Morganella* (20) and the species *Morganella morganii* is appropriate. The neotype strain of *M. morganii* remains ATCC 25830.

At least three hybridization groups exist in *Prot. rettgeri*. The 1163 group includes strains from the Penner BG 1a, 1b, 2a, 2b, and 3a (34). This group consists of biochemically typical *Prot. rettgeri* strains. The 666-48 group contains five strains that are 35 to 50% related to the typical strains. All of these strains are biochemically atypical. It is quite possible that they do not constitute a single DNA relatedness group. Pending further biochemical and DNA work, they should remain in the species *Prot. rettgeri*. Both of these *Prot. rettgeri* groups are more closely related to *Providencia* species than to *Proteus* species. We propose that they be placed in the genus *Providencia* as *Providencia rettgeri* comb. nov.

The third DNA relatedness group observed in *Prot. rettgeri* is made up of BG 5b strains. By DNA relatedness, these strains are indistinguishable from *Prov. stuartii*. They must be viewed as urease-positive strains of *Prov. stuartii*.

All species of *Proteus* and *Providencia* are phenylalanine deaminase positive. The urea reaction is relied upon to separate *Proteus* (Ure⁺) from *Providencia*. Urease-negative *Proteus* strains are well documented. In fact, 5% of *Prot. vulgaris*, 10% of *Prot. mirabilis*, and 2% of *M. morganii* strains are urease negative (13). On the other hand, *Prot. rettgeri* is listed as 100% positive (13). However, there are several reports

TABLE 14. Biochemical reactions at $36 \pm 1^\circ\text{C}$ of the seven species in the tribe *Proteeae* as recognized in this paper

Test	<i>Proteus mirabilis</i>	<i>Proteus vulgaris</i>	<i>Proteus myxofaciens</i>	<i>Providencia alcalifaciens</i>	<i>Providencia stuartii</i>	<i>Providencia rettgeri</i>	<i>Morganella morganii</i>
Indole production	- ^{a2} ^b	+98	-	+99	+99	+100	+99.5
Methyl red test	+99	+93	+	+99.9	+100	+93	+97
Voges-Proskauer	v16	-0	+	-0	-0	-0	-0
Citrate (Simmons) utilization	v59	v11	+	+98	+93	+96	-0
H ₂ S (TSI) ^c production	+94	+95	-	-0	-0	-0	-0
Urease production	v88	+95	+	-0	v15 ^d	+99	+98
Phenylalanine deaminase	+99	+100	+	+97	+95	+98	+95
Lysine decarboxylase	-0	-0	-	-0	-0	-0	-0
Arginine dihydrolase	-0	-0	-	-0	-0	-0	-0
Ornithine decarboxylase	+99	-0	-	-1	-0	-0	+97
Motility, 36°C	+95	+95	-	+96	v86	+94	v88
Gelatin liquefaction, 22°C	+92	+91	+	-0	-0	-0	-0
KCN growth	+99	+100	+	+99	+99	+97	+99
Malonate utilization	-2	-0	-	-0	-0	-1	-5
D-Glucose-acid production	+100	+100	+	+100	+100	+100	+100
D-Glucose-gas production	+96	v86	+	v85	-0	v12	v86
Acid from:							
Lactose	-2	-0	-	-1	-4	-5	-0
Sucrose	v19	+95	+	v13	v31	v13	-1
D-Mannitol	-0	-0	-	-2	v13	+99	-0
Dulcitol	-0	-0	-	-0	-0	-0	-0
Salicin	-1	v58	-	-1	-2	v50	-0
Adonitol	-0	-0	-	+94	-4	+99	-0
Inositol	-0	-0	-	-1	+97	+93	-0
D-Sorbitol	-0	-0	-	-1	-3	-0	-0
L-Arabinose	-0	-0	-	-1	-4	-0	-0
Raffinose	-1	-0	-	-1	-5	-9	-0
L-Rhamnose	-2	-9	-	-0	-0	v75	-0
Maltose	-1	+96	+	-1	-3	-2	-0
D-Xylose	+96	v89	-	-1	-6	v15	-0
Trehalose	+98	v30	+	-4	+99	-1	v14
Cellobiose	-2	-0	-	-1	-10	-4	-0
α -CH ₃ -glucoside	-0	v80	+	-0	-0	-2	-0
Erythritol	-0	-3	-	-0	-0	v78	-0
Mannose	-	-	-	+	+	+	+
Esculin	-1	v59	-	-0	-0	v30	-0
Melibiose	-	-	-	-	-	-	-
D-Arabitol	-	-	-	-	-0	+99	-
Glycerol	+90	v70	+	v12	v12	v66	-5
Mucate	-0	-0	-	-0	-0	-0	-0
Jordan tartrate utilization	v88	+93	+	+100	+96	+96	+93
Sodium acetate utilization	v13	v23	-	v30	v81	v59	-0
Lipase production (corn oil)	+92	v87	+	-0	-0	-0	-0
Deoxyribonuclease production, 25°C	v	v	-	-	-	-	-
NO ₃ ⁻ → NO ₂ ⁻	+94	+100	+	+100	+100	+99	v89
Oxidase production	-0	-0	-	-0	-0	-0	-0
ONPG ^e production	-	-	-	-	-	-	-
Tyrosine clearing	+	+	-	+	+	+	+
Pectate liquefaction	-	-	-	-	-	-	-

^a Symbols: +, 90% or greater positive; -, 10% or less positive; v, 10.1 to 89.9% positive (all reactions are for 48 h).

^b The number gives the percentage of strains positive at 48 h (24 h for phenylalanine, NO₃⁻ → NO₂⁻, and oxidase). The data were tabulated by W. H. Ewing, B. R. Davis, F. W. Hickman, and J. J. Farmer III from over 1,000 cultures admitted to the Center for Disease Control for identification. Some of the data for *Prov. rettgeri* are taken from the paper by Penner et al. (34). The data for *Prot. myxofaciens* are based on only one strain, the neotype, ATCC 19692. When limited data are available, no percentage is given.

^c TSI, Triple sugar iron.

^d An estimate based on strains sent to Penner for serotyping.

^e ONPG, *o*-Nitrophenyl- β -D-galactopyranoside.

in the literature describing urease-positive strains of *Providencia* (*Prot. inconstans*) (5, 18, 27, 34, 35, 39–41, 43, 44). The overall biochemical reactions of urease-positive *Prov. stuartii* strains (or *Prot. rettgeri* BG 5b) are much closer to *Prov. stuartii* than to *Prot. rettgeri* (34, 44). The biochemical reactions most helpful in grouping the BG 5b strains with *Prov. stuartii* and separating them from *Prot. rettgeri* are trehalose (+), D-mannitol (–), adonitol (–), D-arabitol (–), meso-erythritol (–), L-rhamnose (–), and salicin (–) fermentation. The *Prot. rettgeri* 5b strains are also serologically related to *Providencia*. Thus it seems clear that the 3899-72 group (BG 5b) strains are, in fact, urease-positive strains of *Prov. stuartii*. We thus propose the transfer of *Prot. rettgeri* BG 5 to *Prov. stuartii*.

Of 674 *Prot. rettgeri* strains, Penner described only three BG 4 strains and four BG 5a strains (34). These rare members of 4 and 5a BG have not been tested for DNA relatedness.

The BG of *Providencia* are shown in Table 12. Adonitol-positive strains of *Prov. alcalifaciens* BG 1 and 2 are one hybridization group. Together, BG 1 and 2 account for 600 of 633 characterized strains of *Prov. alcalifaciens* (13). BG 3 strains represent less than 3% of the total isolates of *Prov. alcalifaciens*. The eight BG 3 strains studied belong to a hybridization group separate from the other hybridization groups of *Prov. alcalifaciens*. These strains apparently belong to a new species of *Providencia*. This recommendation will be made after all available BG 3 strains have been characterized biochemically.

BG 4 of *Prov. alcalifaciens* also represents less than 3% of the characterized isolates of *Prov. alcalifaciens*. They were placed arbitrarily in *Prov. alcalifaciens* by Ewing et al. (17), who realized that they were not clearly differentiable from *Prov. stuartii*. The three strains of BG 4 tested were indistinguishable from strains of *Prov. stuartii*, and we propose that they be transferred to *Prov. stuartii*. DNAs from all strains of *Prov. stuartii* BG 5 and 6 were very highly related.

In an evolutionary sense, the protei are at the fringe of the family *Enterobacteriaceae*. Relatedness between protei (except for *M. morganii*) and other *Enterobacteriaceae* is between 5 and 15%. This very low level of relatedness to other members of the family, coupled with the large degree of diversity between species of *Proteus*, can be taken as a basis for arguing that protei be placed in a separate family. Such a decision is unjustified because protei share the morphological and biochemical properties of *Enterobacteriaceae* and because conserved DNA sequences, such as those that specify ribosomal ribonucleic

acid, are highly related between protei and other *Enterobacteriaceae* (2) and substantially less related between *Enterobacteriaceae* and members of other families (25, 33).

Taxonomy is a science—the science of classification. As such, it is not static, but dynamic, and must respond to changes dictated by new knowledge. A genus should contain a group of related species. All too often, however, genera have been created on the basis of a single common characteristic. The best example is in the family *Bacillaceae* in which endospore formation is the single criterion for inclusion. The genetic diversity within members of the genera *Bacillus* and *Clostridium* within *Bacillaceae* is as great or greater than that found in the entire family of *Enterobacteriaceae*.

After reviewing the biochemical, serological, and morphological data on *Proteus* and *Providencia* as well as the DNA hybridization data, we proposed three generic changes. In our opinion, these changes are warranted scientifically. As proposed, there are three related species in *Proteus*, three related species in *Providencia*, and a single species in *Morganella*. Additional species will no doubt be added to each of these genera as additional knowledge is accumulated. For example, BG 3 strains of *Prov. alcalifaciens*, the 666-48 group of *Prot. rettgeri*, and the 931-72 group of *Prot. vulgaris* are likely candidates for future designation as distinct and separate species.

It should be emphasized that these proposals are not new concepts. Biochemical (7, 14, 20, 26, 36–38), serological (7, 26, 34), and morphological differences among species of *Proteus* and *Providencia* have been known to exist for 30 years. Investigators recognized that the tribe *Proteeae* should eventually contain more than one genus. Some made specific recommendations (20, 26, 36–38), whereas others preferred to maintain the single genus *Proteus* until additional data were available. Those data are now available and are consistent with the three genera we recognize herein.

A paper concerning the type or neotype strains of the species discussed herein is in preparation.

REPRINT REQUESTS

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