

Chromobacterium, *Eikenella*, *Kingella*, *Neisseria*, *Simonsiella*, and *Vitreoscilla* Species Comprise a Major Branch of the Beta Group *Proteobacteria* by 16S Ribosomal Ribonucleic Acid Sequence Comparison: Transfer of *Eikenella* and *Simonsiella* to the Family *Neisseriaceae* (emend.)

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The 16S ribosomal ribonucleic acid sequences of the type strain and three other strains of *Eikenella corrodens*, the type strains of *Alcaligenes faecalis*, *Alcaligenes xylosoxydans* subsp. *denitrificans*, *Chromobacterium fluviatile*, *Chromobacterium violaceum*, *Kingella denitrificans*, *Kingella kingae*, and *Pseudomonas cepacia*, and a strain of *Vitreoscilla stercoraria* were determined by direct sequencing of bacterial ribosomal ribonucleic acid by a modified Sanger method. These sequences were compared with previously published sequences of *Neisseria gonorrhoeae* and *Pseudomonas testosteroni* and unpublished sequences of *Nitrosolobus* sp., *Nitrosomonas europaea*, *Rhodocyclus gelatinosus*, *Rhodocyclus purpura*, *Simonsiella muelleri*, and *Spirillum volutans*. All of the bacteria sequenced in this study were members of the beta group of the class *Proteobacteria*, formerly called "purple bacteria and their relatives." A phylogenetic tree was constructed based upon the sequence homologies. One of the findings of this study is that *Eikenella corrodens* is related, as indicated by percentage of sequence homology, to the following organisms: *Kingella denitrificans* (97.7%), *Simonsiella muelleri* (95.7%), *Kingella kingae* (96.2%), *Neisseria gonorrhoeae* (95.1%), *Vitreoscilla stercoraria* (94.4%), *Chromobacterium violaceum* (91.7%), and *Chromobacterium fluviatile* (89.8%). These bacteria constitute a newly recognized branch of the beta group *Proteobacteria*. The remaining species, including *Pseudomonas cepacia*, *Alcaligenes faecalis*, and *Alcaligenes xylosoxydans* subsp. *denitrificans*, are members of the major cluster of the beta group *Proteobacteria*. On the basis of our data we propose that the genera *Eikenella* and *Simonsiella* be placed in the family *Neisseriaceae*.

The initial goal of this research was to determine the phylogenetic position of *Eikenella corrodens*, a gram-negative, facultatively anaerobic, rod-shaped bacterium that has been implicated as a potential pathogen in periodontal disease (14, 59). The lack of knowledge regarding the relationship of *Eikenella corrodens* to species in other genera is reflected by its placement in the "other genera" group of facultatively anaerobic gram-negative rods in *Bergey's Manual of Systematic Bacteriology*, vol. 1 (18). Sequencing of ribosomal ribonucleic acid (rRNA) subunits has proven to be an extremely powerful tool for determining the phylogeny of both procaryotes and eucaryotes (64). Rapid sequencing methods now make it possible to determine the 16S rRNA sequence for a bacterial strain in less than 1 week (28). Using these methods, we obtained the sequence for the type strain of *Eikenella corrodens*. By comparing the *Eikenella corrodens* sequence with 125 eubacterial sequences in our data base, we found that this organism is a member of the beta group of the class *Proteobacteria*, formerly called "purple bacteria and their relatives" (52; Dewhirst and Paster, J. Dent. Res. 67[Special Issue]:395, abstract no. 2261, 1988). The beta group of the *Proteobacteria* corresponds to rRNA superfamily III of De Ley (8). Because few beta group *Proteobacteria* 16S rRNA sequences were available for comparison to determine the position of *Eikenella corrodens* within this group, sequences were obtained for *Alcaligenes faecalis*, *Alcaligenes xylosoxydans* subsp. *denitrificans*, *Chromobacterium fluviatile*, *Chromobacterium violaceum*, and *Pseudomonas cepacia*. Of these species, *Eikenella*

corrodens was most closely related to *Chromobacterium violaceum*, with a level of sequence homology of 92%. As we were completing the sequences for these organisms, the sequence for the 16S rRNA gene of *Neisseria gonorrhoeae* was published (45). A sequence comparison indicated that *Neisseria gonorrhoeae* and *Eikenella corrodens* had a level of sequence homology of 95%. This information prompted us to examine three additional organisms, *Kingella denitrificans*, *Kingella kingae*, and *Vitreoscilla stercoraria*, which we believed would be closely related based upon the results of 16S cataloging studies (66) and rRNA-deoxyribonucleic acid (DNA) hybridization studies (9-13, 46, 47). Thus, the expanded goal of this research was to examine the phylogeny of the beta group *Proteobacteria*.

Many of the bacterial species examined in this study are not commonly encountered and may be as unfamiliar to readers as they were to us prior to these studies. Therefore, a brief description of these organisms is given below. These bacteria have been isolated from a variety of habitats and are quite diverse in their phenotypic traits. Most of the organisms, even those normally found in soil and water, are occasionally pathogenic for humans. *Alcaligenes faecalis* and *Alcaligenes xylosoxydans* subsp. *denitrificans* (previously *Alcaligenes denitrificans*) occur in water and soil and are saprophytic inhabitants of the intestinal tracts of vertebrates (21, 22, 49). Strains have been isolated from clinical samples of blood, urine, and purulent discharges (21, 23, 35). *Alcaligenes faecalis* has received recent attention as the etiological agent of turkey coryza (31). *Chromobacterium violaceum* and *Chromobacterium fluviatile* are violet-pigmented organisms whose normal habitat is soil and water

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TABLE 1. Sources and accession numbers of the strains sequenced

Organism	Strain ^a	GenBank accession no. ^b
<i>Alcaligenes faecalis</i>	ATCC 8750 ^T	M22508
<i>Alcaligenes xylooxidans</i> subsp. <i>denitrificans</i>	ATCC 15173 ^T	M22509
<i>Chromobacterium violaceum</i>	ATCC 12472 ^T	M22510
<i>Chromobacterium fluviatile</i>	ATCC 33051 ^T	M22511
<i>Eikenella corrodens</i>	ATCC 23834 ^T	M22512
	FDC 373	M22513
	FDC 558	M22514
	FDC 1073	M22515
<i>Kingella denitrificans</i>	ATCC 33394 ^T	M22516
<i>Kingella kingae</i>	ATCC 23330 ^T	M22517
<i>Pseudomonas cepacia</i>	ATCC 25416 ^T	M22518
<i>Vitreoscilla stercoraria</i>	VT1	M22519

^a ATCC, American Type Culture Collection; FDC, Forsyth Dental Center. Strain VT1 was obtained from W. R. Strohl.

^b The GenBank accession number for all 12 sequences is M22467.

(36, 37, 50). There are isolated reports of fatal septicemia caused by *Chromobacterium violaceum* (20). *Eikenella corrodens* is routinely isolated from human oral cavities. It has been recovered from Bartholin's gland, uterine, thyroid, head and neck, and other abscesses as well as from cases of endocarditis, meningitis, and osteomyelitis (3, 25, 41, 61; O. Riche, V. Vernet, and P. Megier, Letter, Lancet ii:1089, 1987). *Eikenella corrodens* has been detected in elevated numbers from sites of periodontal tissue destruction and may be involved in periodontal disease (14, 59). *Kingella denitrificans* and *Kingella kingae* are occasional members of the rhinopharyngeal flora in humans (51). *Kingella kingae* has been recovered from cases of osteoarthritis, osteomyelitis, spondylitis, endocarditis, meningitis, and septicemia (5, 6, 42). *Pseudomonas cepacia* (previously named *Pseudomonas multivorans* and "*Pseudomonas kingae*") is both a phytopathogen and, occasionally, a pathogen for humans (39). It is one of the major pulmonary pathogens in patients with cystic fibrosis (16, 57). *Simonsiella* spp. are morphologically unique multicellular filamentous bacteria (disk bacteria) that are found in the oral flora of vertebrates, including sheep, cattle, dogs, rabbits, chickens, and humans (26). *Simonsiella muelleri* has been encountered occasionally in oral clinical samples (63). *Vitreoscilla stercoraria* is a colorless, filamentous, gliding bacterium that was first isolated from cow dung and is found in freshwater, stream sediments, and soil (32, 34, 56). The cytochrome *o* of this nonpathogenic organism has been studied extensively (15).

MATERIALS AND METHODS

Bacterial strains and culture conditions. Bacterial strains were obtained from the American Type Culture Collection, Rockville, Md., Forsyth Dental Center, Boston, Mass., and W. R. Strohl, Ohio State University, Columbus. The strains which we examined are shown in Table 1. *Vitreoscilla stercoraria* VT1 rRNA was provided by David Lane, Gene-Trak Systems, Framingham, Mass. Strains of *Eikenella corrodens* were cultured at 37°C in commercially available Todd-Hewitt broth (BBL Microbiology Systems, Cockeysville, Md.) under an 80% N₂-10% CO₂-10% H₂ atmosphere. All other strains except the *Chromobacterium fluviatile* strain were cultured aerobically in commercially available Trypticase soy broth (Difco Laboratories, Detroit, Mich.) at 37°C; *Chromobacterium fluviatile* was grown at 25°C. The

bacteria were harvested during the late logarithmic growth phase.

Isolation and purification of rRNA. rRNAs were isolated and partially purified by a modification of the procedure of Pace et al. (38), as previously described (40).

16S rRNA sequencing. rRNA was sequenced by using a modified Sanger dideoxy chain termination technique in which primers complementary to conserved regions were elongated by using reverse transcriptase (28). The details of our protocol have been described previously (40). In these studies, four additional primers were used to obtain the complete 16S rRNA sequence except for about 50 bases at the 3'-terminal end. The sequences for the additional primers and the positions which they complement are as follows: 5'-ACTGCTGCCTCCCGT-3', positions 344 to 358; 5'-CTA CCAGGGTATCTAATC-3', positions 786 to 803; 5'-GG TTGCGCTCGTTGCGGG-3', positions 1,096 to 1,113; and 5'-TACGGYTACCTTGTTACGACT-3', positions 1,493 to 1,513 (Y indicates the presence of either cytosine or thymine at a position; numbering is relative to the *Escherichia coli* sequence). All primers used in this study were purchased from the Microchemistry Department, Harvard Biolabs, Harvard University, Cambridge, Mass.

Data analysis. A program set for data entry, editing, sequence alignment, secondary structure comparison, homology matrix generation, and dendrogram construction for 16S rRNA data was written in Microsoft QuickBASIC for use with an IBM PC-AT computer and other compatible computers (40). Ribonucleic acid sequences were entered and aligned as previously described (40). Our sequence database contains approximately 125 sequences comprised of sequences determined in our laboratory, previously published sequences, and unpublished sequences provided to us by other scientists. In the beta group *Proteobacteria*, the sequences for *Neisseria gonorrhoeae* NCTC 8375^T (T = type strain) and *Pseudomonas testosteroni* ATCC 11996^T (67) were obtained from the literature. Unpublished sequences for *Rhodocyclus gelatinosus* TG-6, *Rhodocyclus purpura* 6770, *Nitrosomonas europaea* Nm35, *Nitrosolobus* sp. strain unknown, and *Spirillum volutans* neotype strain ATCC 19554 were kindly provided by Carl Woese, University of Illinois, Urbana. The unpublished sequence for *Simonsiella muelleri* ATCC 29453^T was provided jointly by Carl Woese and Gen-Probe, San Diego, Calif. In the construction of the phylogenetic tree, the following five members of the gamma group of the *Proteobacteria* were chosen for an outgroup: *Actinobacillus lignieresii* reference strain ATCC 19393 (Dewhirst and Paster, unpublished sequence), *Escherichia coli* (2), *Haemophilus influenzae* ATCC 33391^T (Dewhirst and Paster, unpublished sequence), *Proteus vulgaris* strain unknown (4), and *Ruminobacter amylophilus* DMS 1361^T (33). Phylogenetic trees were constructed by the modified unweighted pair group method of Li (30).

RESULTS

By using seven primers, we were able to determine about 95% (1,431 to 1,477 bases) of the total sequence for each of the bacterial 16S rRNAs examined. These sequences are shown in Fig. 1 aligned with, and numbered relative to, the *Escherichia coli* sequence (2). The sequences of *Eikenella corrodens* FDC 373, FDC 558, and FDC 1073 were identical and are represented by a consensus sequence in Fig. 1. The sequence for *Eikenella corrodens* ATCC 23834^T differed from the consensus sequence shown in Fig. 1 by the insertion of uracil between positions 94 and 95 and the replace-

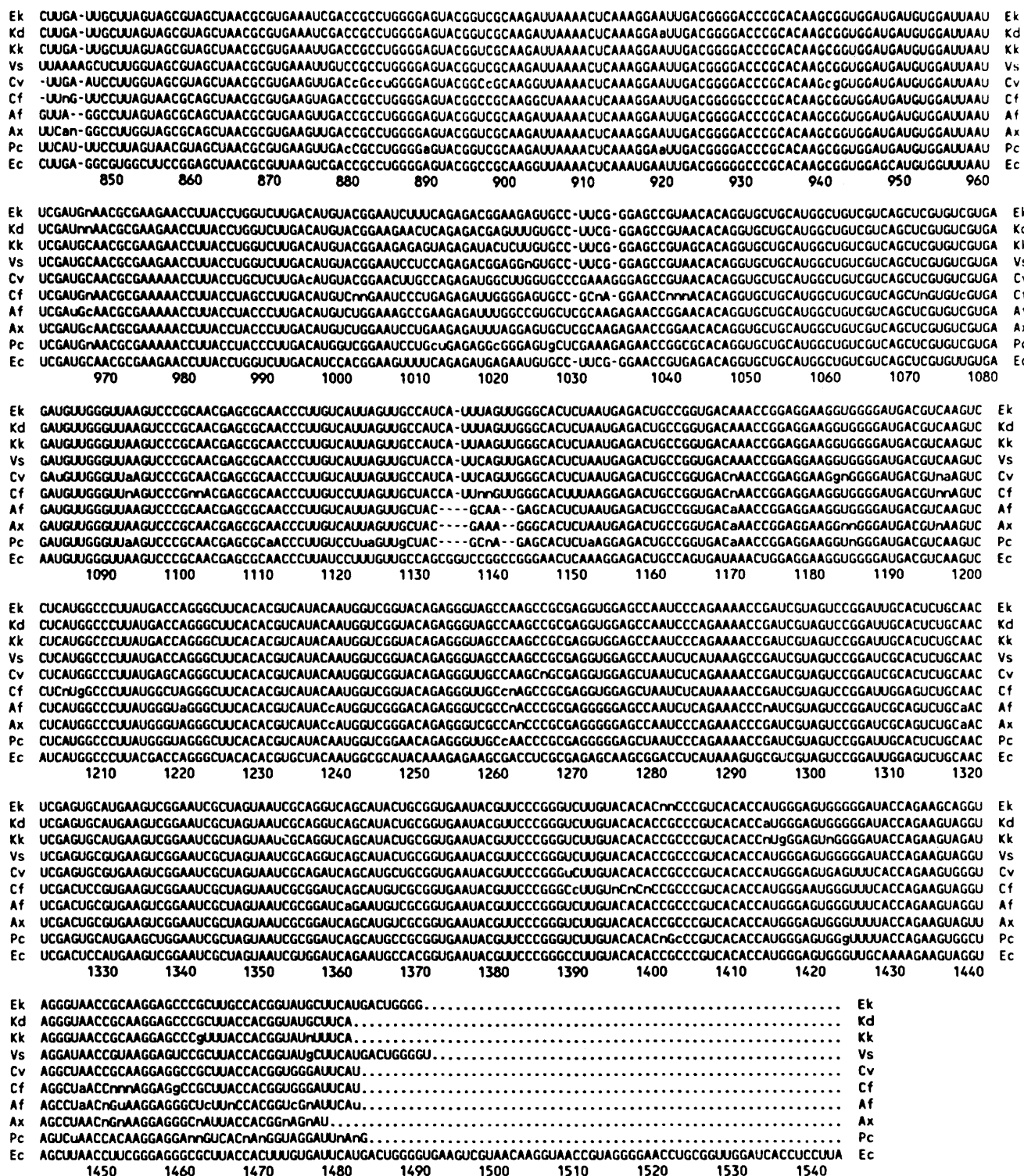


FIG. 1. Aligned sequences of the organisms studied. Aligned sequences of *Eikenella corrodens* (Ek), *Kingella denitrificans* (Kd), *Kingella kingae* (Kk), *Vitreoscilla stercoraria* (Vs), *Chromobacterium violaceum* (Cv), *Chromobacterium fluviatile* (Cf), *Alcaligenes faecalis* (Af), *Alcaligenes xylosoxydans* subsp. *denitrificans* (Ax), *Pseudomonas cepacia* (Pc), and *Escherichia coli* (Ec) are shown. A, adenine; C, cytosine; G, guanine; U, uracil. n, base position that could not be determined. Lower-case letters indicate some uncertainty in base identity. Dashes indicate gaps that were inserted for alignment of sequences, and dots indicate regions that were not sequenced.

TABLE 2. Homology matrix^a

	Ek	Kd	Kk	Sm	Ng	Vs	Cv	Cf	Af	Ax	Pc	Pt	Rg	Ns	Ne	Rp	Sv	Ec	Pv	Ra	Hi	Al
Ek	—	97.7	95.7	96.2	95.1	94.4	91.7	89.8	90.0	89.2	89.6	86.4	87.8	87.0	88.4	88.1	88.3	83.0	83.3	82.5	82.6	82.7
Kd	2.4	—	95.8	95.9	95.0	93.1	91.7	89.5	89.8	88.6	89.1	85.3	87.1	86.0	87.8	87.9	88.4	83.3	83.3	82.8	83.6	82.8
Kk	4.5	4.3	—	95.0	94.3	93.2	90.9	89.1	89.1	89.1	89.6	85.7	86.7	86.3	87.4	87.8	88.4	82.5	83.2	82.3	81.8	81.8
Sm	3.9	4.2	5.1	—	93.8	94.0	90.7	89.3	90.0	89.1	88.2	86.0	86.9	85.8	87.5	87.8	88.3	82.9	83.2	82.3	82.6	82.8
Ng	5.0	5.1	6.0	6.5	—	93.9	90.5	88.7	88.6	89.1	89.0	85.3	86.7	86.2	87.8	87.5	87.4	82.6	83.2	82.4	81.5	80.9
Vs	5.8	7.2	7.2	6.3	6.4	—	92.3	89.9	89.2	89.2	89.1	87.4	88.7	86.9	88.0	88.2	89.2	83.3	84.6	83.1	83.5	82.6
Cv	8.8	8.8	9.8	9.9	10.2	8.1	—	91.5	89.1	89.5	90.5	87.7	89.7	87.4	89.1	89.8	88.3	83.1	83.2	83.8	82.9	83.0
Cf	10.9	11.3	11.8	11.5	12.3	10.8	9.1	—	89.6	89.0	90.0	88.1	88.8	87.1	89.0	89.4	88.1	83.4	83.5	83.1	82.8	83.2
Af	10.7	10.9	11.8	10.7	12.4	11.6	11.8	11.2	—	95.4	90.5	88.4	88.8	88.2	90.1	89.6	89.2	83.0	82.8	82.7	82.2	82.3
Ax	11.6	12.4	11.8	11.8	11.8	11.6	11.3	11.9	4.7	—	90.7	88.8	90.8	88.2	90.8	89.7	89.8	82.6	83.2	83.2	81.3	81.3
Pc	11.2	11.8	11.2	12.8	11.9	11.8	10.2	10.7	10.1	9.9	—	89.1	90.9	88.5	89.8	90.8	90.5	82.6	82.8	81.7	81.8	81.8
Pt	15.1	16.3	15.9	15.5	16.4	13.8	13.5	12.9	12.6	12.1	11.7	—	92.1	87.8	88.7	88.2	87.2	82.0	82.1	80.9	81.5	81.3
Rg	13.3	14.2	14.6	14.4	14.6	12.3	11.1	12.2	12.2	9.8	9.8	8.4	—	87.5	89.3	90.7	88.8	82.1	82.7	81.9	82.8	82.3
Ne	14.3	15.4	15.2	15.7	15.2	14.4	13.7	14.1	12.8	12.8	12.5	13.3	13.7	—	93.6	89.2	87.8	81.9	82.1	81.7	81.3	82.5
Ns	12.5	13.3	13.7	13.7	13.4	13.1	11.8	11.9	10.6	9.8	10.9	12.3	11.5	6.6	—	90.5	89.5	83.6	83.5	83.0	82.6	82.8
Rp	12.9	13.2	13.4	13.4	13.7	12.8	11.0	11.5	11.2	11.1	9.8	12.8	9.9	11.6	10.2	—	90.5	82.7	83.2	82.8	82.8	82.6
Sv	12.7	12.5	12.5	12.7	13.7	11.6	12.7	13.0	11.6	11.0	10.1	14.0	12.1	13.3	11.4	10.1	—	83.2	84.2	83.2	83.5	82.8
Ec	19.2	18.9	20.0	19.4	19.8	18.9	19.1	18.7	19.2	19.8	19.9	20.6	20.5	20.7	18.5	19.7	19.0	—	94.3	88.4	89.3	89.3
Pv	18.9	18.9	19.1	19.1	19.0	17.2	19.0	18.7	19.5	19.0	19.6	20.5	19.7	20.4	18.6	19.0	17.8	6.0	—	87.4	88.5	89.2
Ra	20.0	19.5	20.2	20.2	20.1	19.1	18.3	19.1	19.7	19.0	21.0	22.1	20.7	21.0	19.2	19.5	19.0	12.5	13.8	—	87.4	86.8
Hi	19.8	18.6	20.9	19.8	21.3	18.7	19.4	19.6	20.3	21.5	20.9	21.3	19.6	21.5	19.8	19.6	18.7	11.6	12.5	13.8	—	94.3
Al	19.7	19.5	20.8	19.6	22.0	19.8	19.3	19.1	20.1	21.5	20.8	21.5	20.1	19.9	19.6	19.8	19.5	11.5	11.7	14.6	5.9	—

^a Abbreviations: Ek, *Eikenella corrodens*; Kd, *Kingella denitrificans*; Kk, *Kingella kingae*; Sm, *Simonsiella muelleri*; Ng, *Neisseria gonorrhoeae*; Vs, *Vitreoscilla stercoraria*; Cv, *Chromobacterium violaceum*; Cf, *Chromobacterium fluviatile*; Af, *Alcaligenes faecalis*; Ax, *Alcaligenes xylosoxydans*; Pc, *Pseudomonas cepacia*; Pt, *Pseudomonas testosteroni*; Rg, *Rhodocyclus gelatinosus*; Ne, *Nitrosomonas europaea*; Ns, *Nitrosolobus* sp.; Rp, *Rhodocyclus purpurea*; Sv, *Spirillum volutans*; Ec, *Escherichia coli*; Pv, *Proteus vulgaris*; Ra, *Ruminobacter amylophilus*; Hi, *Haemophilus influenzae*; Al, *Actinobacillus lignieresii*. The numbers above the diagonal are uncorrected percentages of similarity. The numbers below the diagonal are percentages of difference corrected for multiple base changes by the method of Jukes and Cantor (19).

ment of the bases listed at positions 189, 1,006, and 1,010 by cytosine. The sequences in Fig. 1 are available for electronic retrieval from GenBank under the accession numbers given in Table 1.

A sequence homology matrix for 17 beta group and 5 gamma group *Proteobacteria* is presented in Table 2. The numbers above the diagonal are uncorrected percentages of homology calculated by using only those positions at which every one of the 22 sequences had determined bases (1,290 bases). Exclusion from the homology calculations of positions at which one or more of the sequences have missing data is essential for accurate phylogenetic tree construction by the modified method of Li (30). The numbers below the diagonal are difference values (100% minus the percentage of homology) corrected for multiple base changes by the method of Jukes and Cantor (19). Homology values calculated by not excluding any position (about 1,450 bases per comparison) were about 1% lower.

A dendrogram representing the phylogenetic relationships for the organisms in Table 2 is shown in Fig. 2. The tree was calculated by using a modified unweighted pair group method in which differing branch lengths reflected differing numbers of base changes relative to other species in the tree. For example, *Pseudomonas testosteroni* had consistently lower levels of homology (higher difference values) with other species than *Rhodocyclus gelatinosus* did, which is reflected in the long branch length of *Pseudomonas testosteroni*. Among the more interesting relationships shown in Fig. 2 is that species of the genera *Chromobacterium*, *Eikenella*, *Kingella*, *Neisseria*, *Simonsiella*, and *Vitreoscilla* constitute a separate branch of the beta group *Proteobacteria*; we refer to this branch as the "Chromobacterium branch." *Eikenella corrodens*, *Kingella denitrificans*, *Kingella kingae*, *Neisseria gonorrhoeae*, and *Simonsiella muelleri* form a tight cluster within the *Chromobacterium* branch,

with interspecies levels of homology of more than 95%; we refer to this cluster as the "Neisseriaceae cluster." *Vitreoscilla stercoraria* is closely related to the *Neisseriaceae* cluster, with an average level of homology of 93%, while *Chromobacterium violaceum* and *Chromobacterium fluviatile* are more distant, with average levels of homology of 91 and 90%, respectively. We refer to the remaining beta group organisms as members of the "main beta group cluster."

DISCUSSION

The overall tree presented in Fig. 2 is relatively stable; however, the exact branching order within the areas enclosed in circles was somewhat sensitive to the choice of outgroup organisms or to the exclusion of highly variable regions from the homology calculations.

The deep branching of *Chromobacterium* species from other members of the beta group *Proteobacteria*, shown in Fig. 2, agrees with the phylogenetic relationships deduced from rRNA-DNA hybridization experiments (9). The close phylogenetic relationship among *Neisseria gonorrhoeae*, *Kingella denitrificans*, *Kingella kingae*, and *Chromobacterium violaceum* was expected based upon the rRNA-DNA hybridization studies of Rossau and co-workers (47). *Vitreoscilla stercoraria* has been shown by 5S rRNA sequencing to form a deep branch of the beta group *Proteobacteria* (53), but its relationship to other organisms of the *Chromobacterium* branch has not been examined.

The beta group of the *Proteobacteria* has previously been subdivided into three groups based upon 16S rRNA oligonucleotide cataloging results (64, 66). Each of the organisms sequenced in this study falls into the beta-2 group. The grouping of organisms into branches in this work differs from the grouping in the cataloging studies. This is not surprising since the cataloging methods preserve only about 40% of the

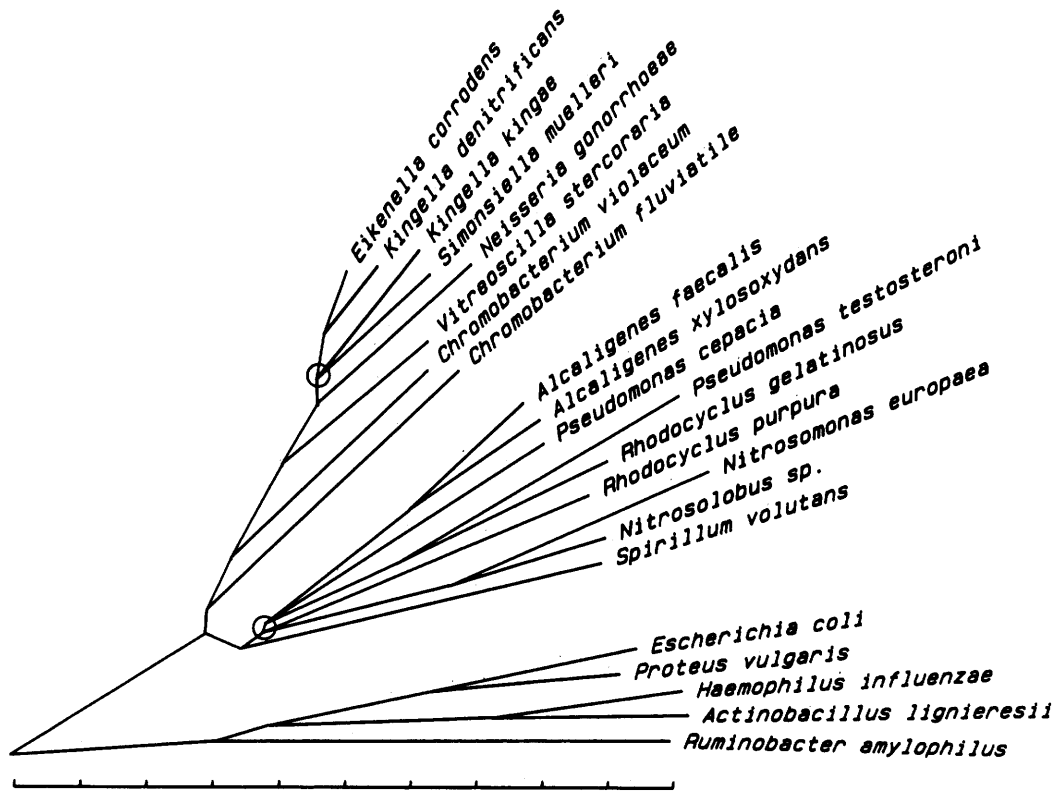


FIG. 2. Phylogenetic tree for the organisms examined in this study and those listed in Materials and Methods. The scale represents a 10% difference in nucleotide sequence as determined by taking the sum of all branch lengths connecting two species.

information in the 16S rRNA molecules. The "signatures" which distinguish the *Chromobacterium* branch from other members of the beta group *Proteobacteria* are at the following positions shown in Fig. 2: guanine for cytosine at position 1,263, uracil or cytosine for guanine at position 1,272, guanine or adenine for cytosine or uracil position 1,443, and cytosine or uracil for guanine or adenine at position 1,459. These signatures fall in guanine-rich segments which are not recovered by T1 endonuclease digest cataloging. Additional positions at which the *Chromobacterium* branch organisms, except *Chromobacterium fluviatile*, differ from other beta group *Proteobacteria* include positions 987, 989, 1,040, 1,216, 1,218, 1,355, and 1,367 (Fig. 2).

The phylogenetic relationships presented in Fig. 2 have important taxonomic implications, especially for the family *Neisseriaceae*. Rossau and co-workers, who studied the inter- and intragenic similarities of rRNA cistrons of the *Neisseriaceae*, have recommended that the family should be limited to the genera *Neisseria* and *Kingella* and that the genera *Acinetobacter*, *Moraxella*, and *Branhamella* should be removed as they are members of the gamma group *Proteobacteria* (47). These authors also noted that *Kingella indologenes* is not related to the other *Kingella* species, but instead is a member of the gamma group *Proteobacteria*. The sequencing data support the close relationship previously observed between *Neisseria gonorrhoeae* and *Kingella denitrificans* and *Kingella kingae*. The equally close relationship of *Eikenella corrodens* and *Simonsiella muelleri* to these three species strongly argues for the inclusion of the genera *Eikenella* and *Simonsiella* in the family *Neisseriaceae*.

Evidence supporting a close relationship among *Neisseria gonorrhoeae*, *Kingella denitrificans*, and *Eikenella corro-*

dens includes the observation that strains of these species possess identical 25.2-megadalton conjugative plasmids that carry the TetM determinant for tetracycline resistance (24, 44). Strains of each of these species were able to donate the plasmid to several *Neisseria* species, but not to the more distantly related "false neisseria" *Branhamella catarrhalis*. A 9.4-kilobase plasmid encoding penicillin, streptomycin, and sulfonamide resistance isolated from *Eikenella corrodens* appears to be identical to a resistance plasmid commonly isolated from saprophytic *Neisseria* sp. strains (48). In contrast to the findings concerning the exchange of plasmids among these species, Tønnum et al. (60) could not show genetic transformation between *Eikenella corrodens* and *Neisseria* and *Kingella* species. We are somewhat surprised that these transformation studies were negative, but do not believe that negative results in one system detract or contradict positive results in other systems.

The four strains of *Eikenella corrodens* sequenced were selected for their diversity based upon previous serological studies (1) and typing by polyacrylamide gel electrophoresis protein profiles (58). As indicated above, the sequences of three of the strains were identical, and the fourth differed by only four bases. Our results indicate that *Eikenella corrodens* is a genetically homogeneous species, in agreement with the results of previous DNA-DNA hybridization studies (7) and genetic transformation studies (60). The genus *Eikenella* was proposed by Jackson because he realized that the species "*Bacteroides corrodens*" did not belong in the genus *Bacteroides* (17). From Figure 2, it is evident that *Eikenella corrodens* is more closely related to *Kingella denitrificans* than *Kingella kingae* is. This raises the question of whether *Eikenella corrodens*, *Kingella denitrificans*, and *Kingella kingae* belong in a single genus. Resolution of this

question should await a detailed examination of these species in which a uniform set of phenotypic traits is used.

The genera *Simonsiella* and *Alysiella* have previously been placed in the family *Simonsiellaceae* based upon their unusual morphology and gliding motility (54). In *Bergey's Manual of Systematic Bacteriology*, vol. 3, the family *Simonsiellaceae* is placed in the section including nonphotosynthetic, nonfruiting, gliding bacteria (29). While gliding motility has long been thought to be of phylogenetic significance, 16S rRNA oligonucleotide cataloging studies have shown that this is not true since gliding organisms occur in 8 of the 10 major divisions of the eubacterial kingdom as defined by Woese (43, 65). The phylogeny of the gliding bacteria is well described by Reichenbach et al. (43). This information suggests that future taxonomies will discard gliding bacteria as a higher taxon and place gliding genera with phylogenetically related nongliding genera. The family *Simonsiellaceae* is not phylogenetically coherent. Studies in which 16S rRNA oligonucleotide cataloging has been used have shown that the genus *Alysiella* belongs in the gamma group of the *Proteobacteria*, while the genus *Simonsiella* belongs in the beta group (43, 52). Phenotypic information linking the genus *Simonsiella* to the family *Neisseriaceae* includes the presence of carbonic anhydrase (D. A. Kuhn, D. Picken, and J. Mittelman, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, A39, p. 128).

In addition to the evidence presented above supporting the relationship of the genera *Eikenella* and *Simonsiella* to the genera *Neisseria* and *Kingella*, all four genera are oxidase positive and inhabit the mucous membranes of mammals. Perhaps the strongest evidence linking together the members of the *Neisseriaceae* cluster is a rare signature in their 16S rRNA, an adenine at position 585 paired with a uracil at position 756 (*Escherichia coli* numbering, as shown in Fig. 1). All other members of the alpha, beta, gamma, and delta groups of the *Proteobacteria* possess a guanine-cytosine pair at these positions.

The genus *Vitreoscilla* is also placed in the section which includes the nonphotosynthetic, nonfruiting, gliding bacteria in *Bergey's Manual of Systematic Bacteriology*, vol. 3 (55). In addition to our comments above regarding the lack of phylogenetic coherence of gliding bacteria, the genus *Vitreoscilla* is itself not phylogenetically coherent. Stahl and co-workers showed, by using 5S rRNA sequencing, that *Vitreoscilla stercoraria* forms a deep branch of the beta group *Proteobacteria* (the *Chromobacterium* branch in Fig. 2), while *Vitreoscilla filiformis* is related to *Rhodocyclus gelatinosus* in the main cluster of beta group *Proteobacteria*, and *Vitreoscilla beggiatoides* is a member of the gamma group *Proteobacteria* and is related to *Beggiatoa alba* (53). These results suggest that each of the three *Vitreoscilla* species should be placed in a separate genus. While *Vitreoscilla stercoraria* is closely related to the *Neisseriaceae* cluster, we do not propose its transfer to the family *Neisseriaceae*. As shown in Table 2, *Vitreoscilla stercoraria* has an average level of sequence homology of 93.7% with members of the *Neisseriaceae* cluster, while members of the cluster have an average level of sequence homology of 95.5% with one another. *Vitreoscilla stercoraria* does not possess the unique signature in its 16S rRNA characteristic of the *Neisseriaceae* cluster, is oxidase negative, and is a free-living organism.

Because it had been suggested previously that *Cardiobacterium hominis* may be related to the genera *Eikenella*, *Kingella*, and *Neisseria* (60), we sequenced the 16S rRNA from the type strain of *Cardiobacterium hominis*. Contrary to our expectations, sequence comparisons showed that

Cardiobacterium hominis is not a member of the beta group of the *Proteobacteria*, but rather is a deeply branching member of the gamma group of the *Proteobacteria* (unpublished data).

Based upon the results described in this paper and the results of other studies cited above, we propose that the genera *Eikenella* and *Simonsiella* be transferred to the family *Neisseriaceae*. The emended description below of the *Neisseriaceae* is based upon characteristics of the genera *Neisseria* (62), *Kingella* (51), *Eikenella* (18), and *Simonsiella* (27). The genera *Acinetobacter* and *Moraxella* are excluded based upon the findings of Rossau and co-workers (47) described above.

Emended description of the *Neisseriaceae* Prevot 1933, 119^{AL}. Coccobacillary organisms. Coccal forms occur singly, in pairs, or in masses, often with adjacent sides flattened. Rod-shaped forms occur singly, joined end to end to form pairs or chains, or joined along the long axis to form flattened filaments. Endospores are not formed. May be capsulated. Gram negative, but the cells often have a tendency to resist Gram decolorization. Flagella and swimming motility are absent. Strains of several species are fimbriated (piliated) and may show surface-bound twitching motility. Members of the genus *Simonsiella* possess gliding motility. Many species corrode agar. Aerobic, except members of the genus *Eikenella*, which require nitrate. Strains of some species may grow under anaerobic conditions. These organisms inhabit the mucous membranes of mammals and have an optimum growth temperature of 32 to 37°C. Most species are fastidious chemoorganotrophs that require a number of amino acids and vitamins for growth. Some species are saccharolytic, utilizing glucose and a limited number of other carbohydrates. Species in each of the genera can reduce nitrate to nitrite. Oxidase positive when tested with tetramethyl-*p*-phenylenediamine. Organisms do not contain branched-chain fatty acids in their cell membranes. Species possess a unique signature in their 16S rRNA that differentiates them from other members of the *Proteobacteria*, an adenine at position 585 and a uracil at position 756 (numbering relative to the *Escherichia coli* sequence).

The guanine-plus-cytosine content of the DNA ranges from 41 to 58 mol%.

The type genus is *Neisseria* Trevisan 1885, 105.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants DE-02847, DE-04881, and DE-08303 from the National Institutes of Health.

ADDENDUM IN PROOF

After this paper was submitted, Rossau et al. (R. Rossau, G. Vandebussche, S. Thielemans, P. Segers, H. Grosch, E. Göthe, W. Mannheim, and J. De Ley, Int. J. Syst. Bacteriol. 39:185-198, 1989) published phylogenetic data on members of the beta group *Proteobacteria*. Their results, based on rRNA-DNA and DNA-DNA hybridization experiments, are comparable to those obtained in this study.

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