

# Ribosomal Ribonucleic Acid Cistron Similarities and Deoxyribonucleic Acid Homologies of *Neisseria*, *Kingella*, *Eikenella*, *Simonsiella*, *Alysiella*, and Centers for Disease Control Groups EF-4 and M-5 in the Emended Family *Neisseriaceae*

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We detected distinct taxonomic relationships among the true *Neisseria* species, *Kingella kingae*, *Kingella denitrificans*, *Eikenella corrodens*, all *Simonsiella* species, the type strain of *Alysiella filiformis*, and members of Centers for Disease Control groups EF-4 and M-5. All these taxa constitute one large separate cluster having high levels of ribosomal ribonucleic acid cistron similarity (thermal denaturation temperature range, 74 to 81°C) in ribosomal ribonucleic acid superfamily III. There are at least four subbranches. We found high deoxyribonucleic acid (DNA)-DNA homology values between *Neisseria gonorrhoeae* and some other true *Neisseria* species and within the following species: *Simonsiella muelleri*, *Simonsiella crassa*, *Simonsiella steedae*, *Kingella denitrificans*, and *Eikenella corrodens*. All of the members of this large cluster have genome base compositions in the range from 42.8 to 57.7 mol% guanine plus cytosine. The molecular complexities of the genomic DNAs are  $2.2 \times 10^9$  to  $2.7 \times 10^9$  for *Simonsiella* and *Alysiella* species and  $1.4 \times 10^9$  to  $1.8 \times 10^9$  for the other members of this large cluster. We formally propose that this large cluster represents the emended family *Neisseriaceae* containing the following genera and groups: *Neisseria*, *Kingella* (not the generically misnamed *Kingella indologenes*), *Eikenella*, *Simonsiella*, *Alysiella* (not some misnamed strains), and Centers for Disease Control groups EF-4 and M-5. The genera and subgenera *Acinetobacter*, *Moraxella*, *Branhamella*, *Psychrobacter*, the false neisseriae, and *Kingella indologenes* should be removed from the *Neisseriaceae*, as they belong to superfamilies I and II.

In previous reports (48, 53) some of us showed that the bacterial family *Neisseriaceae*, as described in *Bergey's Manual of Systematic Bacteriology* (2), cannot maintain its present set of taxa and should be redefined. The genera *Acinetobacter* and *Moraxella*, including *Branhamella catarhalis*, the false neisseriae (*[Neisseria] ovis*, *[Neisseria] caviae*, and *[Neisseria] cuniculi*), and the species *[Kingella] indologenes* should be removed from the *Neisseriaceae*, leaving only the true *Neisseria* species and two *Kingella* species (*Kingella kingae* and *Kingella denitrificans*) as unequivocal members of this family. Throughout this paper we use brackets to indicate generic misnomers (according to our findings) for organisms whose correct generic names are not yet known. *Psychrobacter immobilis*, which was recently included in the *Neisseriaceae* (36), is also not a member of this family (R. Rossau, M. Gillis, et al., manuscript in preparation).

Screening of a great variety of bacterial taxa of uncertain affiliation by using deoxyribonucleic acid (DNA)-ribosomal ribonucleic acid (rRNA) hybridization revealed that *Eikenella corrodens*, *Simonsiella* species, *Alysiella* species, and members of Centers for Disease Control (CDC) groups M-5, EF-4a, and EF-4b are close genetic relatives of the true *Neisseria* species. In this study we examined the phylogenetic interrelationships among these taxa and several other gram-negative bacteria.

## MATERIALS AND METHODS

**Bacterial strains and media.** Most of the strains which we used are listed in Table 1; a few more strains are listed in Table 2. These organisms were grown as described by Rossau et al. (48). The CDC group M-5 strains and the *Alysiella filiformis* type strain were grown on heart infusion (Difco Laboratories, Detroit, Mich.) agar in Roux flasks at 33°C. The organisms mentioned below were grown in 500-ml batches in 2-liter Fernbach flasks which were moderately aerated on a gyratory shaker at 36°C. *Eikenella corrodens* was propagated in Mueller-Hinton broth (Oxoid Ltd., London, United Kingdom) supplemented with 25 µg of hemin per ml (hemin chloride [Fluka Ag., Buchs, Switzerland]; 22× concentrated stock solution in 4% [vol/vol] triethanolamine [E. Merck AG, Darmstadt, Federal Republic of Germany], sterilized by filtration) and with Vitox (Oxoid). CDC group EF-4 strains were grown in tryptic soy broth (Difco) supplemented with 0.5% (wt/vol) yeast extract (Oxoid) that was sterilized by filtration, and *Simonsiella* and *Alysiella* strains were grown in tryptic soy broth supplemented with 2% (vol/vol) horse serum (Oxoid) and Vitox (Oxoid). Mass cultures of *Kingella kingae* and *Kingella denitrificans* were grown in Schaedler broth (Bio-Mérieux, Charbonnières les Bains, France) or brain heart infusion broth (Oxoid) supplemented with 0.5% (wt/vol) yeast extract and 2% (vol/vol) horse serum as described above.

**Isolation of DNA.** DNA was isolated and purified by using the method of Marmur (40), with some modifications (48). Additional purification was achieved by preparative CsCl gradient centrifugation.

**Fixation of single-stranded DNA on nitrocellulose mem-**

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TABLE 1. List of most of the strains used, their DNA base compositions, and hybridization parameters of DNA-rRNA hybrids with various <sup>3</sup>H-labeled rRNAs

Sequence no. <sup>a</sup>	DNA from <sup>b</sup> :	Mol% G+C	rRNA from:							
			<i>Neisseria flavescens</i> ATCC 13120 <sup>T</sup>		<i>Simonsiella muelleri</i> ATCC 29453 <sup>T</sup>		<i>Eikenella corrodens</i> NCTC 10596 <sup>T</sup>		CDC group EF-4 strain ATCC 29858	
			<i>T<sub>m(e)</sub></i> (°C)	% RNA binding	<i>T<sub>m(e)</sub></i> (°C)	% RNA binding	<i>T<sub>m(e)</sub></i> (°C)	% RNA binding	<i>T<sub>m(e)</sub></i> (°C)	% RNA binding
1	<i>Neisseria gonorrhoeae</i> NCTC 8375 <sup>Tc</sup>	53.2	77.6	0.25	73.7	0.13	75.3	0.07	73.9	0.10
2	<i>Neisseria meningitidis</i> NCTC 10025 <sup>T</sup>	52.8	78.5	0.29	75.9	0.11				
3	<i>Neisseria lactamica</i> NCTC 10617 <sup>T</sup>	53.0	76.7	0.25	75.0	0.10	76.2	0.11	74.2	0.11
4	<i>Neisseria lactamica</i> NCTC 10616	53.8	77.5	0.24						
5	<i>Neisseria mucosa</i> CIP 59.51 <sup>T</sup>	51.6	78.9	0.20	75.6	0.09	75.1	0.09	75.3	0.09
6	<i>Neisseria subflava</i> ATCC 10555	49.6	79.3	0.21						
7	<i>Neisseria flavescens</i> ATCC 13120 <sup>T</sup>	49.6	80.6	0.24	75.4				74.3	
8	<i>Neisseria sicca</i> NRL 30016 <sup>T</sup>	51.2	78.7	0.31			76.4			
9	<i>Neisseria animalis</i> NCTC 10212 <sup>T</sup>	51.3	76.2	0.24	75.3	0.09	75.5	0.09	75.5	0.11
10	<i>Neisseria denitrificans</i> ATCC 14686 <sup>T</sup>	56.2	76.5	0.19	75.6	0.10	75.6	0.09	74.8	0.11
11	<i>Neisseria canis</i> ATCC 14687 <sup>T</sup>	49.6	76.3	0.34	73.6					
12	<i>Neisseria elongata</i> subsp. <i>elongata</i> NCTC 10660 <sup>T</sup>	53.1	75.9	0.20	75.8	0.09	76.1	0.10	75.5	0.10
13	<i>Neisseria elongata</i> subsp. <i>glycolytica</i> NCTC 11050	54.8	76.7	0.18	75.3	0.10	76.4	0.08	74.6	0.10
14	<i>Kingella kingae</i> NCTC 10529 <sup>T</sup>	48.2	75.1	0.23	76.3	0.11	75.3	0.08	74.6	0.10
15	<i>Kingella kingae</i> NCTC 10746	47.4	75.8	0.22	76.1	0.12	75.4	0.08	74.6	0.12
16	<i>Kingella denitrificans</i> NCTC 10995 <sup>T</sup>	53.5	76.0	0.28			76.5	0.13		
17	<i>Kingella denitrificans</i> NTCC 10997	55.9	76.4	0.26	74.9	0.16			75.7	0.12
18	[ <i>Kingella</i> ] <i>indologenes</i> NCTC 10717 <sup>T</sup>	48.2	63.3	0.17	62.5					
19	<i>Eikenella corrodens</i> NCTC 10596 <sup>T</sup>	56.4	75.1	0.36	74.2	0.11	80.0	0.13	75.4	0.11
20	<i>Eikenella corrodens</i> HIM 967-5	57.3	75.1	0.34	73.9	0.17	80.8	0.15	75.4	0.16
21	<i>Eikenella corrodens</i> HIM 815-5	54.6	74.6	0.26	73.2	0.11	80.2	0.11		
22	<i>Eikenella corrodens</i> HIM 801-1	57.0	76.0	0.29			80.3	0.12		
23	<i>Eikenella corrodens</i> HIM 930-8	53.6	75.5	0.26			78.9	0.11		
24	<i>Simonsiella crassa</i> ATCC 15533 <sup>T</sup>	47.0	75.8	0.42	75.9	0.26	75.4	0.20	75.0	0.27
25	<i>Simonsiella crassa</i> ATCC 29447	45.1	75.2	0.28	75.1	0.24	75.5	0.16	74.9	0.18
26	<i>Simonsiella steedae</i> ATCC 27409 <sup>T</sup>	52.2	74.4	0.19	74.4	0.13	75.4	0.09	74.3	0.08
27	<i>Simonsiella steedae</i> ATCC 27410	51.5	75.5	0.20	75.3	0.14	76.0	0.08	75.0	0.11
28	<i>Simonsiella steedae</i> ATCC 27398	53.8	75.3	0.28	74.1	0.10				
29	<i>Simonsiella muelleri</i> ATCC 29453 <sup>T</sup>	43.0	75.5	0.22	78.3	0.21	75.1	0.11	73.6	0.13
30	<i>Simonsiella muelleri</i> ATCC 29452	42.8	75.3	0.29	77.4	0.22	75.6	0.09	75.1	0.14
31	<i>Simonsiella muelleri</i> HIM 942-7	43.2	74.5	0.34	76.3	0.21	74.3	0.12	74.0	0.08
32	<i>Simonsiella</i> sp. strain ATCC 27381	57.7	75.5	0.29	74.5	0.13	75.1	0.09	74.1	0.14
33	<i>Alysiella filiformis</i> CCUG 3710 <sup>Td</sup>	48.7	75.0	0.29	75.2	0.12	75.2	0.10	74.8	0.11
34	[ <i>Alysiella</i> ] sp. strain ATCC 29468	40.5	63.1	0.18						
35	[ <i>Alysiella</i> ] sp. strain HIM 1018-2	45.0	65.2	0.11						
36	CDC group EF-4a strain ATCC 29858	49.3					75.5	0.09	78.0	0.05
37	CDC group EF-4a strain CDC T-191/78	50.9	74.9	0.19	75.1	0.08			78.8	0.12
38	CDC group EF-4b strain CDC T-194/78	50.5	76.3	0.22	75.4	0.09	75.2	0.11	77.5	0.11
39	CDC group EF-4a strain HIM 892-1	50.2	74.9	0.16	75.0	0.10			77.3	0.12
40	CDC group EF-4a strain CCUG 17349	50.4	74.2	0.15	75.0	0.10			77.7	0.14
41	CDC group M-5 strain CDC B5522	51.3	76.3	0.26	75.4	0.10	76.4	0.11	76.9	0.11
42	CDC group M-5 strain CCUG 4007	52.0	76.3	0.26					76.4	
43	<i>Chromobacterium violaceum</i> NCTC 9757 <sup>T</sup>	67.2	70.9	0.31						
44	<i>Aquaspirillum dispar</i> ATCC 27650	63	70.2	0.17						
45	<i>Janthinobacterium lividum</i> NCTC 9796 <sup>T</sup>	65.5	67.9	0.16						
46	<i>Bordetella bronchiseptica</i> NCTC 8761	69.5	67.4	0.08						
47	<i>Taylorella equigenitalis</i> NCTC 11184 <sup>T</sup>	37.6	67.6	0.22						
48	<i>Oligella urethralis</i> ATCC 17960 <sup>T</sup>	47.7	66.1	0.14						
49	<i>Oligella urethralis</i> WM 6	47.2	66.2	0.16						
50	<i>Oligella urethralis</i> WM 20	47.2	65.3	0.08						
51	[ <i>Pseudomonas</i> ] <i>woodsii</i> NCPPB 2441		68.2	0.08						
52	[ <i>Pseudomonas</i> ] <i>acidovorans</i> ATCC 15668 <sup>T</sup>	66.6	66.0	0.07						
53	<i>Xylophilus ampelinus</i> CNBP 2098	68.2	66.7	0.09						
54	<i>Moraxella bovis</i> CCUG 2133 <sup>T</sup>	42.5	64.6	0.15						
55	<i>Moraxella (Branhamella) ovis</i> ATCC 11227 <sup>T</sup>	45.9	65.1	0.16						
56	<i>Moraxella (Branhamella) cuniculi</i> ATCC 14688 <sup>T</sup>	46.1	61.7	0.12						

Continued on following page

TABLE 1—Continued

Sequence no. <sup>a</sup>	DNA from <sup>b</sup> :	Mol% G+C	rRNA from:							
			<i>Neisseria flavescens</i> ATCC 13120 <sup>T</sup>		<i>Simonsiella muelleri</i> ATCC 29453 <sup>T</sup>		<i>Eikenella corrodens</i> NCTC 10596 <sup>T</sup>		CDC group EF-4 strain ATCC 29858	
			<i>T<sub>m(e)</sub></i> (°C)	% RNA binding	<i>T<sub>m(e)</sub></i> (°C)	% RNA binding	<i>T<sub>m(e)</sub></i> (°C)	% RNA binding	<i>T<sub>m(e)</sub></i> (°C)	% RNA binding
57	<i>Moraxella (Branhamella) caviae</i> ATCC 14659 <sup>T</sup>	46.9	62.6	0.10						
58	<i>Moraxella (Branhamella) catarrhalis</i> ATCC 25238 <sup>T</sup>	41.0	64.3	0.16						
59	<i>Acinetobacter calcoaceticus</i> Torry 7-40	40.7	62.3	0.17						
60	<i>Escherichia coli</i> B	52.2	62.7	0.13						
61	<i>Rhizobium meliloti</i> NZP 4009		58.0	0.05						

<sup>a</sup> Sequence numbers are not strain numbers.

<sup>b</sup> Abbreviations: ATCC, American Type Culture Collection, Rockville, Md.; CCUG, Culture Collection of the University of Göteborg, Department of Clinical Bacteriology, University of Göteborg, Göteborg, Sweden; CDC, Centers for Disease Control, Atlanta, Ga.; CIP, Collection de l'Institut Pasteur, Paris, France; CNBP, Collection Nationale de Bactéries Phytopathogènes, Beaucauzé, France; HIM, Hygiene-Institut, Marburg, Federal Republic of Germany; NCPPB, National Collection of Plant Pathogenic Bacteria, Plant Pathology Laboratory, Harpenden, United Kingdom; NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, United Kingdom; NRL, *Neisseria* Reference Laboratory, Harborview Medical Center, Seattle, Wash.; NZP, Applied Biochemistry Division, Department of Scientific and Industrial Research, Palmerston North, New Zealand.

<sup>c</sup> Most values were taken from reference 48; the other values were determined in our laboratories, except for the *Aquaspirillum dispar* value, which was taken from reference 29.

<sup>d</sup> This organism was also received separately as strain ATCC 15532<sup>T</sup> and as strain HIM 929-5<sup>T</sup>; the same results were obtained.

**brane filters and determination of the amount of DNA fixed.** High-molecular-weight denatured DNA was filter fixed as described by Gillis and De Ley (21). The amount of DNA fixed on a filter was determined by the method of Richards (44).

**DNA base composition.** The average moles percent guanine plus cytosine (G+C) of DNAs were determined spectropho-

tometrically by using the method of De Ley and Van Muylem (13) and the equation of De Ley (7).

**DNA-DNA hybridizations.** The degree of DNA binding (*D*), expressed as a percentage, was calculated from the initial renaturation rates by using the method of De Ley et al. (9); this value was a quantitative measure of the level of homology between the DNAs of a pair of strains. A model

TABLE 2. Hybridization parameters of the hybrids between DNAs from strains in the *Neisseriaceae* rRNA cluster and some misidentified strains and radioactively labeled rRNAs from various reference strains from different rRNA branches<sup>a</sup>

DNA from:	rRNA from:	<i>T<sub>m(e)</sub></i> (°C)	% rRNA binding
<i>Neisseria meningitidis</i> NCTC 10025 <sup>T</sup>	<i>Moraxella lacunata</i> ATCC 17952	63.0	0.10
<i>Neisseria mucosa</i> CIP 59.51 <sup>T</sup>	<i>Moraxella lacunata</i> ATCC 17952	64.6	0.13
<i>Neisseria mucosa</i> CIP 59.51 <sup>T</sup>	[ <i>Pseudomonas</i> ] <i>solanacearum</i> NCPPB 325 <sup>T</sup>	69.9	0.08
<i>Kingella denitrificans</i> NCTC 10995 <sup>T</sup>	<i>Moraxella lacunata</i> ATCC 17952	64.5	0.16
<i>Alysiella filiformis</i> CCUG 3710 <sup>T</sup>	<i>Moraxella lacunata</i> ATCC 17952	62.5	0.18
[ <i>Alysiella</i> ] sp. strain ATCC 29468	<i>Moraxella lacunata</i> ATCC 17952	0.15	
[ <i>Alysiella</i> ] sp. strain ATCC 29468	" <i>Moraxella paraphenylpyruvica</i> " CCUG 2651A <sup>b</sup>	73.2	0.06
[ <i>Alysiella</i> ] sp. strain ATCC 29468	<i>Brucella abortus</i> ATCC 23448 <sup>T</sup>	58.4	0.11
[ <i>Alysiella</i> ] sp. strain ATCC 29468	<i>Cardiobacterium hominis</i> ATCC 15826 <sup>T</sup>	63.5	0.12
[ <i>Alysiella</i> ] sp. strain HIM 1018-2	<i>Moraxella lacunata</i> ATCC 17952	76.9	0.11
[ <i>Alysiella</i> ] sp. strain HIM 1018-2	" <i>Moraxella paraphenylpyruvica</i> " CCUG 2651A	73.6	0.05
[ <i>Alysiella</i> ] sp. strain HIM 1018-2	<i>Oligella urethralis</i> WM 6	58.9	0.06
[ <i>Alysiella</i> ] sp. strain HIM 1018-2	<i>Cardiobacterium hominis</i> ATCC 15826 <sup>T</sup>	64.1	0.08
[ <i>Kingella</i> ] <i>indologenes</i> NCTC 10717 <sup>T</sup>	<i>Cardiobacterium hominis</i> ATCC 15826 <sup>T</sup>	73.6	0.15
[ <i>Kingella</i> ] <i>indologenes</i> NCTC 10883	<i>Cardiobacterium hominis</i> ATCC 15826 <sup>T</sup>	74.5	0.15
<i>Simonsiella crassa</i> ATCC 15533 <sup>T</sup>	<i>Chromobacterium violaceum</i> NCTC 9757 <sup>T</sup>	71.7	0.24
<i>Eikenella corrodens</i> NCTC 10596 <sup>T</sup>	<i>Alcaligenes faecalis</i> NCIB 8156 <sup>Tc</sup>	66.4	
<i>Eikenella corrodens</i> NCTC 10596 <sup>T</sup>	[ <i>Pseudomonas</i> ] <i>solanacearum</i> NCPPB 325 <sup>T</sup>	68.7	0.13
<i>Eikenella corrodens</i> NCTC 10596 <sup>T</sup>	<i>Escherichia coli</i> B	61.7	0.15
CDC group EF-4a strain CDC T-191/78	<i>Alcaligenes xylosoxidans</i> subsp. <i>denitrificans</i> ATCC 15173 <sup>T</sup>	66.6	0.12
CDC group EF-4a strain CDC T-191/78	[ <i>Alcaligenes</i> ] <i>paradoxus</i> ATCC 17713 <sup>T</sup>	66.2	0.15
CDC group EF-4a strain CDC T-191/78	<i>Acinetobacter calcoaceticus</i> ATCC 23055 <sup>T</sup>	60.6	0.16
CDC group EF-4a strain CDC T-191/78	<i>Actinobacillus lignieresii</i> NCTC 4189 <sup>T</sup>	64.1	0.16
CDC group EF-4b strain CDC T-194/78	<i>Alcaligenes xylosoxidans</i> subsp. <i>denitrificans</i> ATCC 15173 <sup>T</sup>	66.3	0.11
CDC group EF-4b strain CDC T-194/78	[ <i>Alcaligenes</i> ] <i>paradoxus</i> ATCC 17713	67.0	0.13
CDC group M-5 strain CDC B5522	<i>Chromobacterium violaceum</i> NCTC 9757 <sup>T</sup>	71.9	0.18

<sup>a</sup> Only results which have not yet been published by members of our research group are listed. For additional values see references 47, 48, and 53.

<sup>b</sup> Names in quotation marks have not been validated.

<sup>c</sup> NCIB, National Collection of Industrial Bacteria, Torry Research Station, Aberdeen, United Kingdom. For other abbreviations see Table 1, footnote b.

TABLE 3. DNA-DNA hybridization results expressed as the degree of binding between highly related neisseriae

Strain	D (%)						
	<i>Neisseria gonorrhoeae</i> NCTC 8375 <sup>T</sup>	<i>Neisseria meningitidis</i> NCTC 10025 <sup>T</sup>	<i>Neisseria lactamica</i> NCTC 10616	<i>Neisseria lactamica</i> NCTC 10617 <sup>T</sup>	<i>Neisseria mucosa</i> CIP 59.51 <sup>T</sup>	<i>Neisseria flavescens</i> ATCC 13120 <sup>T</sup>	<i>Neisseria subflava</i> ATCC 10555
<i>Neisseria gonorrhoeae</i> NCTC 8375 <sup>T</sup>	100						
<i>Neisseria meningitidis</i> NCTC 10025 <sup>T</sup>	74	100					
<i>Neisseria lactamica</i> NCTC 10616	58	56	100				
<i>Neisseria lactamica</i> NCTC 10617 <sup>T</sup>			96	100			
<i>Neisseria mucosa</i> CIP 59.51 <sup>T</sup>	33	34	37		100		
<i>Neisseria flavescens</i> ATCC 13120 <sup>T</sup>		31	34		36	100	
<i>Neisseria subflava</i> ATCC 10555			29		36	71	100

2600 spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio) equipped with a thermostatted cuvette chamber and a model 4225A graphic plotter (Hewlett-Packard Co., Palo Alto, Calif.) was used.

The DNA concentration in the cuvette was about 47 µg/ml, and the optimal renaturation temperature in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M citric acid, pH 7.0) was calculated from equation 21 of De Ley et al. (9). *D* values of about 30% or less were too high and should be disregarded.

**Molecular complexities of the genomes.** The molecular complexities (total molecular weights) were calculated from the initial renaturation rate constants by using the genome size of *Escherichia coli* B as a reference (20). The molecular complexities were calculated only for the DNAs used in the DNA-DNA hybridization experiments.

**Preparation of <sup>3</sup>H-labeled rRNAs.** Labeled rRNAs from *Neisseria flavescens* ATCC 13120<sup>T</sup> (T = type strain), *Simonsiella muelleri* ATCC 29453<sup>T</sup>, *Eikenella corrodens* NCTC 10596<sup>T</sup>, and CDC group EF-4 strain ATCC 29858 were prepared as described previously (47). The *Neisseria flavescens* type strain is a genotypic representative of the genus *Neisseria* (Tables 1 and 3).

**DNA-rRNA hybridizations.** The DNA-rRNA hybridizations were performed as described by De Ley and De Smedt (10), except that the volumes were reduced by a factor of 0.6 and the ribonuclease concentration was standardized as described by Van Landschoot et al. (53). The temperature at which one-half of a DNA-rRNA duplex is denatured [ $T_{m(e)}$ ] is a reliable measure of the genotypic relationships between organisms. The higher the  $T_{m(e)}$ , the higher the level of relatedness. The percentage of rRNA binding is the amount of rRNA (in micrograms) bound to 100 µg of filter-fixed DNA after ribonuclease treatment. The latter parameter is not a measure of genetic relatedness but can often be used to differentiate between taxa with similar  $T_{m(e)}$  values on rRNA cistron similarity maps.

## RESULTS

**DNA-rRNA hybridizations.** Table 1 shows the parameters of the DNA-rRNA hybrids when we used <sup>3</sup>H-labeled rRNAs

from *Neisseria flavescens* ATCC 13120<sup>T</sup>, *Simonsiella muelleri* ATCC 29453<sup>T</sup>, *Eikenella corrodens* NCTC 10596<sup>T</sup>, and CDC group EF-4 strain ATCC 29858. Various rRNA cistron relationships are shown in Fig. 1 through 6. In the upper part of Fig. 1, a cluster is delineated; this cluster includes strains of several *Neisseria* species (sequence no. 1 through 13 in Table 1), two *Kingella* species (sequence no. 14 through 17), three *Simonsiella* species (sequence no. 24 through 32), one *Alysiella* sp. strain (sequence no. 33), *Eikenella corrodens* (sequence no. 19 through 23), CDC group EF-4 (sequence no. 36 through 40), and CDC group M-5 (sequence no. 41 and 42). Other members of rRNA superfamily III (sequence no. 43 through 53) are located at  $T_{m(e)}$  values between 66.0 and 70.9°C (Table 1 and Fig. 1 and 6). [*Kingella*] *indologenes* (sequence no. 18) and members of rRNA superfamily I (sequence no. 60), rRNA superfamily II (sequence no. 34, 35, and 54 through 59), and rRNA superfamily IV (sequence no. 61) are all located in the area below a  $T_{m(e)}$  value of 65.2°C on the rRNA similarity map (Fig. 1) and the  $T_{m(e)}$  similarity dendrogram (Fig. 6).

Table 2 shows the hybridization parameters of the hybrids between DNAs from some members of the *Neisseriaceae* cluster, [*Kingella*] *indologenes*, and two strains misidentified as *Alysiella* sp. and rRNAs from reference strains belonging to other rRNA branches.

**DNA-DNA hybridizations.** The DNA-DNA hybridization results are shown in Tables 3 through 5 and Fig. 6. The mean standard deviation for the separate hybridization results was 4% *D*. Individual results are discussed below.

**Molecular complexities of the genomes and mean moles percent G+C values.** The molecular complexities of the genomes of the strains belonging to *Neisseria* species, *Kingella kingae*, *Kingella denitrificans*, *Eikenella corrodens*, and CDC groups EF-4 and M-5 were fairly low ( $1.4 \times 10^9$  to  $1.8 \times 10^9$ ); the *Simonsiella* species genomes had molecular complexities of  $2.2 \times 10^9$  to  $2.4 \times 10^9$ , and the *Alysiella filiformis* genome had a molecular complexity of  $2.7 \times 10^9$  (Table 6). The mean moles percent G+C range in the *Neisseriaceae* cluster (sequence no. 1 through 42 except

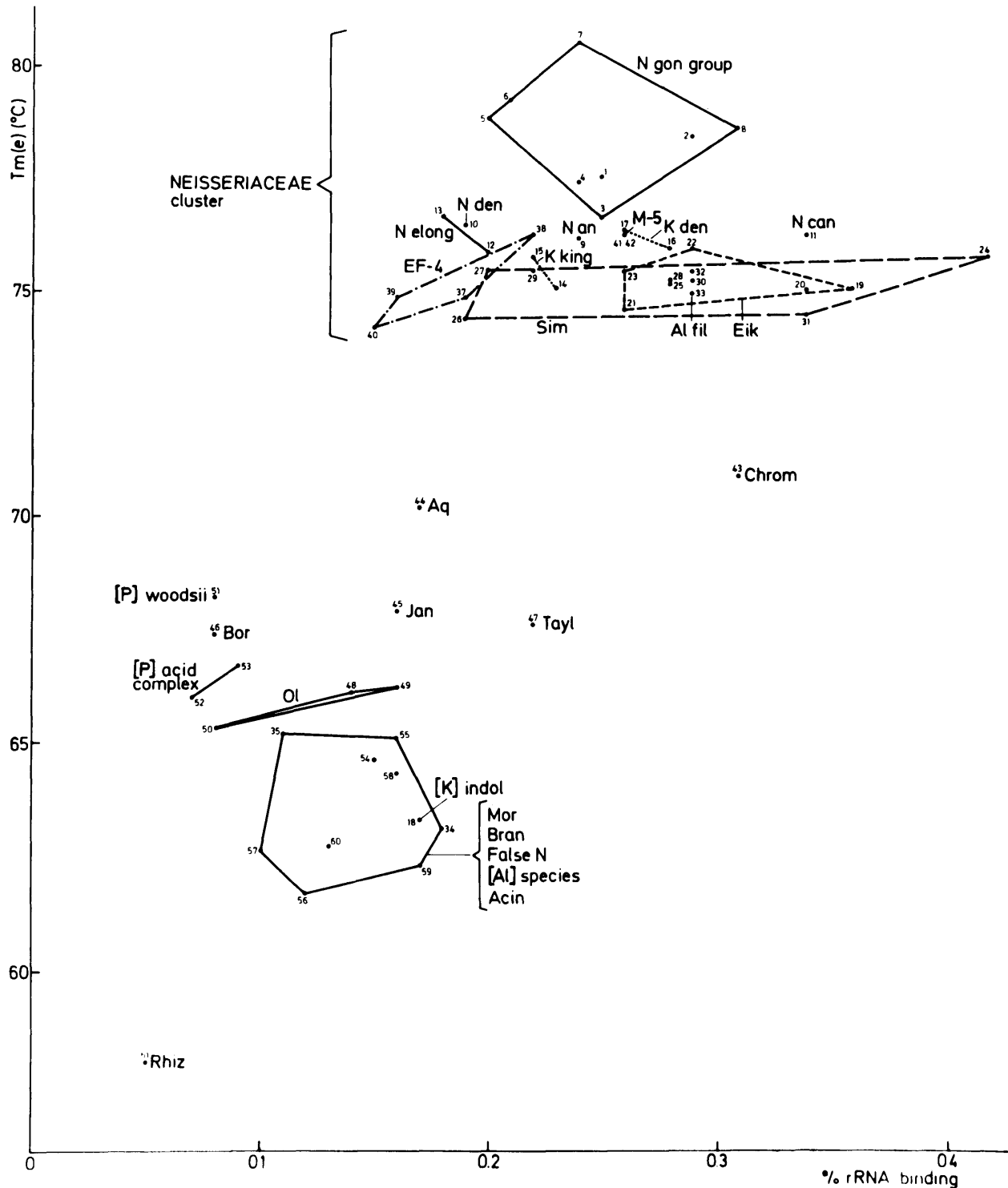


FIG. 1. rRNA similarity map of hybrids between <sup>3</sup>H-labeled rRNA from *Neisseria flavescens* ATCC 13120<sup>T</sup> and DNAs from a variety of bacteria. Each strain is represented by its sequence number (see Table 1). Abbreviations: Acin, *Acinetobacter*; Al, *Alysiella*; [AI], misnamed *Alysiella*; Al fil, *Alysiella filiformis*; Aq, *Aquaspirillum dispar*; Bor, *Bordetella*; Bran, *Branhamella*; Chrom, *Chromobacterium*; E, *Escherichia*; EF-4, CDC group EF-4; Eik, *Eikenella corrodens*; Jan, *Janthinobacterium*; K, *Kingella*; K den, *Kingella denitrificans*; [K] indol, [*Kingella*] *indologenes*; K king, *Kingella kingae*; N, *Neisseria*; N an, *Neisseria animalis*; N can, *Neisseria canis*; N den, *Neisseria denitrificans*; N elong, *Neisseria elongata*; N gon, *Neisseria gonorrhoeae*; Mor, *Moraxella*; M-5, CDC group M-5; Ol, *Oligella*; [P], misnamed *Pseudomonas*; [P] acid, [*Pseudomonas*] *acidovorans*; Rhiz, *Rhizobium*; Sim, *Simonsiella*; S cras, *Simonsiella crassa*; S muel, *Simonsiella muelleri*; S sp, *Simonsiella* species; S st, *Simonsiella steedae*; Tayl, *Taylorella*.

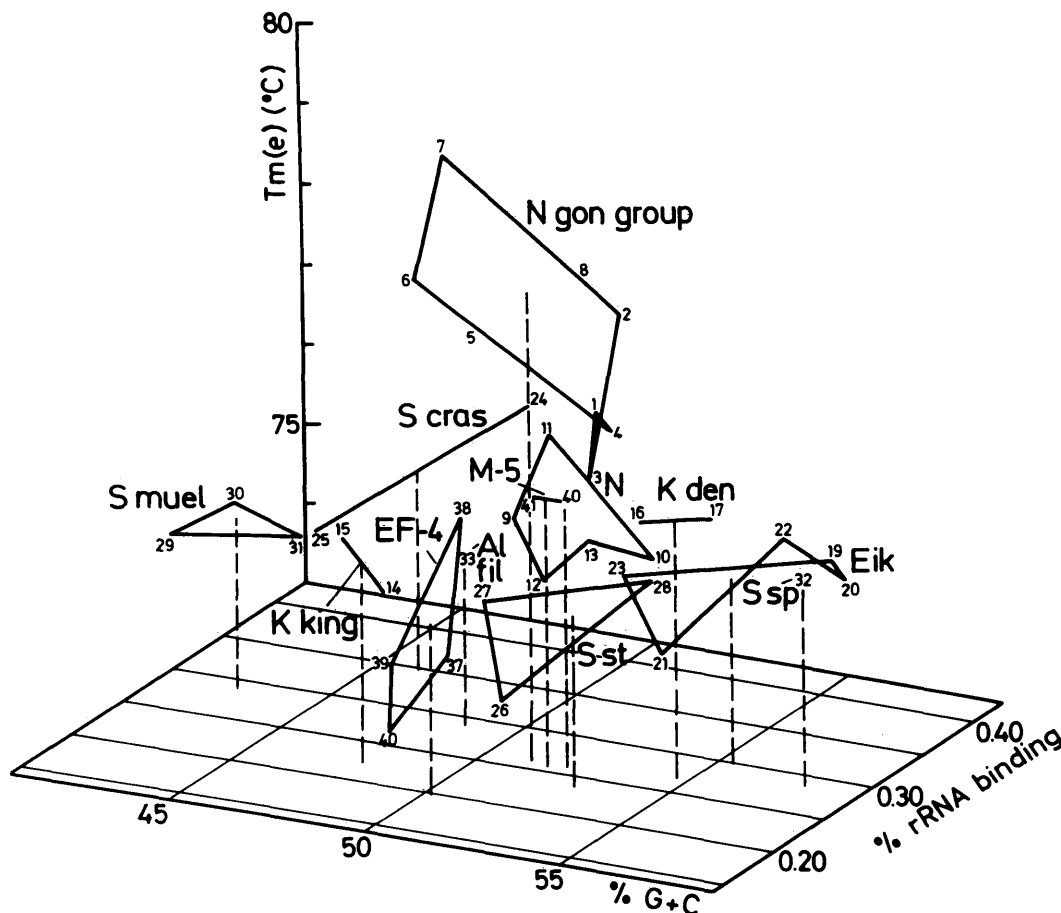


FIG. 2. Positions of the strains belonging to the *Neisseriaceae* cluster (Fig. 1) in a genotypic space defined by (i) the  $T_{m(e)}$  values of the DNA-rRNA hybrids with *Neisseria flavescens* ATCC 13120<sup>T</sup> rRNA, (ii) the percentages of rRNA binding, and (iii) the mean moles percent G+C values of the total genomes. For sequence numbers see Table 1. Sequences no. 9 through 13 indicate the positions of the named neisseriae other than those of the *Neisseria gonorrhoeae* group. For abbreviations see the legend to Fig. 1.

sequence no. 18, 34, and 35) was rather broad (42.8 to 57.7 mol%) (Table 1).

## DISCUSSION

Because of the high evolutionary conservation of rRNA cistron sequences, rRNA similarities are very useful for determining more remote relationships among organisms. The rRNA cistron similarities of a great variety of gram-negative bacteria have been and are being studied extensively by members of the Gent research group, who are using a DNA-rRNA hybridization method (8, 10-12, 14-16, 21, 47, 48, 53) in combination with other methods, such as moles percent G+C and (occasionally) genome size determinations, DNA-DNA hybridization, numerical analysis of numerous phenotypic features, and protein gel electrophoresis. Based on the thermal stabilities of the DNA-rRNA hybrids [expressed as  $T_{m(e)}$  in degrees Celsius], these organisms can be grouped together in at least six large groups, which we have called rRNA superfamilies I through VI (sensu De Ley [8]). Members of an rRNA superfamily usually group together in a  $\Delta T_{m(e)}$  range that is, at most, about 12 to 14°C below the values of the homologous DNA-rRNA duplexes. Each rRNA superfamily probably corresponds to a taxonomic order. Each rRNA superfamily

consists of quite a number of rRNA branches. An rRNA branch contains all of the organisms which are less than 12 to 14°C  $\Delta T_{m(e)}$  removed in DNA-rRNA hybridization experiments from the reference organism, which is a carefully selected, well-known, type or reference strain. A group of rRNA branches containing taxa which have phenotypic and genotypic similarities and which are at most 5 to 8°C  $\Delta T_{m(e)}$  removed from each other we call an rRNA cluster. Some rRNA clusters or individual rRNA branches (depending on the number of labeled reference rRNAs available) correspond to a taxonomic family; examples are the *Alcaligenes-Bordetella* cluster (family *Alcaligenaceae*), the *Rhizobium-Agrobacterium-Phyllobacterium* cluster (family *Rhizobiaceae*), the *Pasteurellaceae* cluster, the *Enterobacteriaceae* rRNA branch, the *Vibrionaceae* rRNA branch, etc. Not all rRNA branches contain a taxonomic family; some contain only a genus or sometimes only a single species.

Each genotypic or phenotypic method has its own range of validity, and in most cases the methods, which cover approximately the same validity range, support each other. Taxonomic conclusions are often drawn by comparison with the taxonomic status of well-established taxa. The rRNA hybridization method is valid from about the order level to

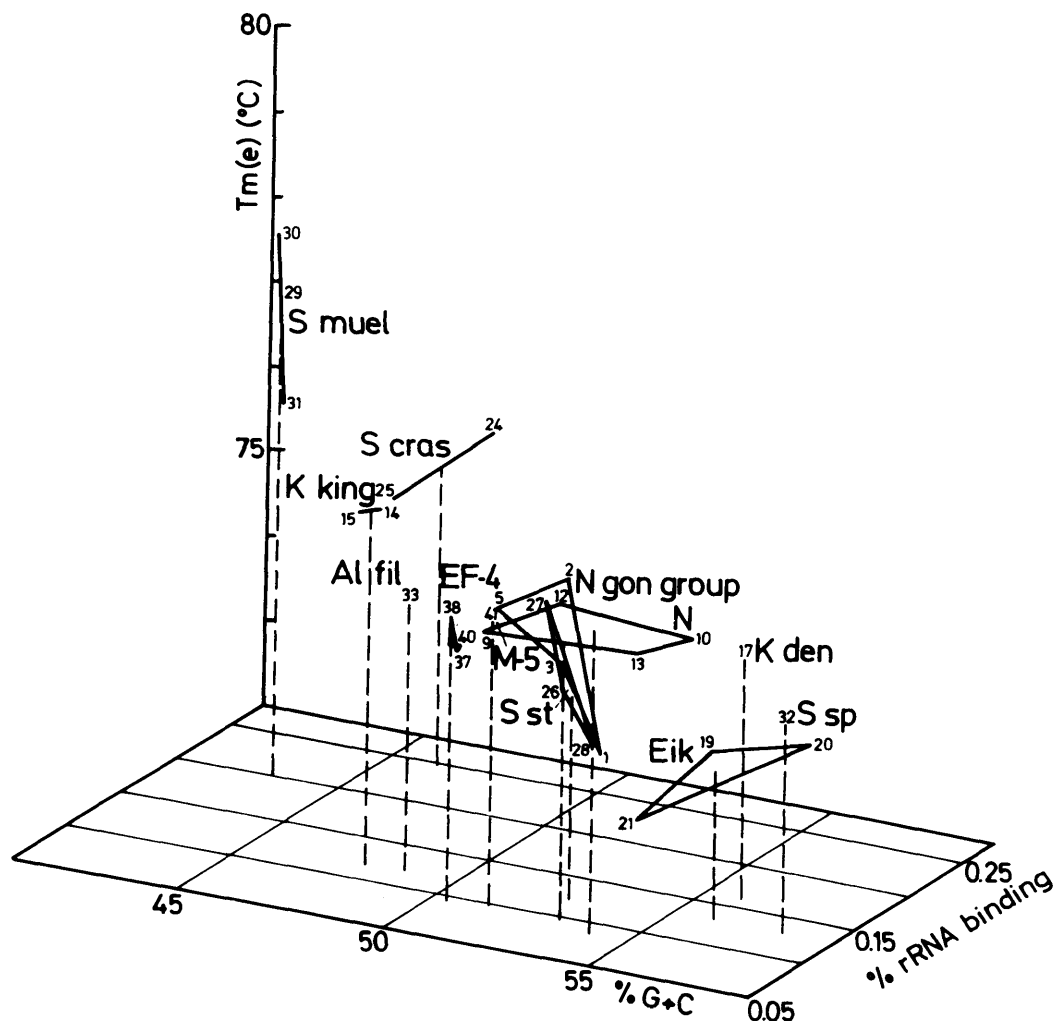


FIG. 3. Positions of the strains belonging to the *Neisseriaceae* cluster (Fig. 1) in a genotypic space defined by (i) the  $T_{m(e)}$  values of the DNA-rRNA hybrids with *Simonsiella muelleri* ATCC 29453<sup>T</sup>, (ii) the percentages of rRNA binding, and (iii) the mean moles percent G+C of the total genomes. For sequence numbers see Table 1. Sequences no. 9 through 13 indicate the positions of the neisseriae other than those of the *Neisseria gonorrhoeae* group. For abbreviations see the legend to Fig. 1.

about the subgenus or species level; rRNA cataloging, rRNA sequencing, and amino acid sequencing of cytochromes are valid from about the class level to about the genus or species level; DNA-DNA hybridization is valid between genera or within a genus depending on the heterogeneity of the taxon; numerical analysis of numerous phenotypic features (>100 features) is valid within or between genera; and numerical analysis of protein gel electropherograms is valid from the species level to the individual strain level. The taxonomic validity ranges of several other methods, such as fatty acid profiles, serology, zymograms, etc., are being examined.

***Neisseriaceae* cluster.** We found that the true neisseriae, *Kingella denitrificans*, *Kingella kingae*, various *Simonsiella* species, *Alysiella filiformis*, *Eikenella corrodens*, and CDC groups EF-4 and M-5 are all closely interrelated in a  $T_{m(e)}$  range from 73.2 to 80.8°C (Table 1 and Fig. 1 through 6). This cluster, which we refer to as the *Neisseriaceae* cluster, is definitely a member of rRNA superfamily III sensu De Ley (8), on which members of the Gent research group have already published extensively; the genera *Chromobacterium* (11) and *Janthinobacterium* (11), the generically misnamed [*Pseudomonas*] *acidovorans* and [*Pseudomonas*] *solan-*

*acearum* clusters (15, 16), the family *Alcaligenaceae* (12), and the genera *Oligella* (47), *Taylorella* (47), and *Dexia* (14) are other members of rRNA superfamily III. The closest neighbors of the *Neisseriaceae* rRNA cluster are the genus *Chromobacterium* and some members of the genus *Aquaspirillum* at about 8°C  $\Delta T_{m(e)}$ . All other members of rRNA superfamily III branch at an average  $\Delta T_{m(e)}$  of approximately 12°C. Organisms belonging to other rRNA superfamilies, such as the genera *Moraxella* and *Acinetobacter*, are at least 15°C  $\Delta T_{m(e)}$  away.

Each of the labeled reference rRNAs from *Neisseria flavescens* ATCC 13120<sup>T</sup>, *Simonsiella muelleri* ATCC 29453<sup>T</sup>, *Eikenella corrodens* NCTC 10596<sup>T</sup>, and CDC group EF-4 strain ATCC 29858 is a member of a separate rRNA branch. These branches diverge at a  $T_{m(e)}$  of 75.0°C (Fig. 6). There must be at least one additional rRNA branch in the *Neisseriaceae* cluster to accommodate *Simonsiella crassa*, *Simonsiella steedae*, *Simonsiella* sp., *Alysiella filiformis*, *Kingella kingae*, *Kingella denitrificans*, and some neisseriae.

***Neisseria*.** The use of genetic transformation (1) and DNA-DNA hybridization (Table 3 and Fig. 6) (18, 23, 27, 37, 45, 46, 55) within the true *Neisseria* revealed the existence of a

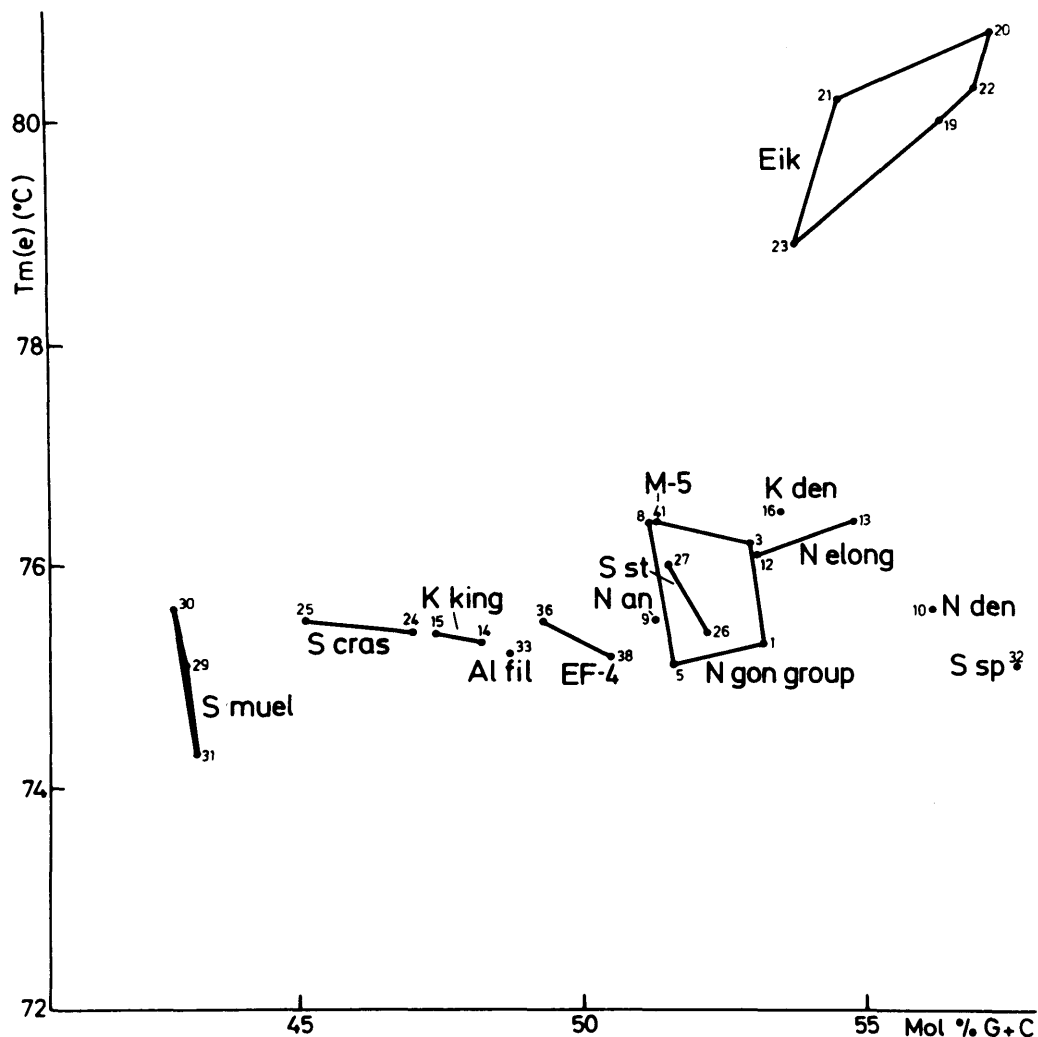


FIG. 4. Correlation between the mean moles percent G+C values of the total genomes and the  $T_{m(e)}$  values of hybrids between  $^3\text{H}$ -labeled rRNA from *Eikenella corrodens* NCTC 10596<sup>T</sup> and DNAs from strains of the *Neisseriaceae* cluster. For sequence numbers see Table 1. For abbreviations see the legend to Fig. 1.

large group (which we call the *Neisseria gonorrhoeae* group) with more than 30% *D*. The same group was also detected by using DNA-rRNA hybridization (Fig. 1 and 2). This group is subdivided into at least three subgroups. One subgroup contains *Neisseria flavescens*, *Neisseria subflava* (including *Neisseria perflava* and *Neisseria flava*), and *Neisseria sicca* strains. A second subgroup contains *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Neisseria lactamica*, *Neisseria polysaccharea*, and *Neisseria cinerea* strains. *Neisseria macacae* also belongs in this subgroup (23), but its exact taxonomic position is not yet clear; therefore, it was not included in Fig. 6. The third subgroup contains *Neisseria mucosa*.

Within the genus *Neisseria* our DNA-DNA hybridization results (Tables 3 and 4) are in excellent agreement with those of Riou and his collaborators (23, 45). Although Hoke and Vedros (27) apparently used the same hybridization method that we used in our study, their results deviated considerably from ours. Perhaps they used DNA concentrations that were too high for such small genomes, yielding very high renaturation rates and the possibility of misinterpretation.

The levels of DNA homology between the members of the

*Neisseria gonorrhoeae* group and other members of the genus *Neisseria* are at or below the reliability limit of about 30% *D* (Table 4) (46, 55); the latter organisms are in the  $T_{m(e)}$  range from 75.9 to 76.7°C versus rRNA from *Neisseria flavescens* (Table 1). The degree of binding (*D*) between *Neisseria elongata* subsp. *elongata* NCTC 10660<sup>T</sup> and *Neisseria elongata* subsp. *glycolytica* NCTC 11050 was 59%. Genetic transformation data demonstrated that *Neisseria elongata* is most probably a true *Neisseria* species. As far as we know, there is no genotypic evidence to support the inclusion of *Neisseria denitrificans*, *Neisseria animalis*, and *Neisseria canis* in the genus *Neisseria*. CDC group M-6 strains were not included in our genotypic studies. Phenotypically (49) and chemotaxonomically (41) these strains are very similar to *Neisseria elongata*. We cannot exclude the possibility that CDC group M-6 strains are nitrate-negative variants of *Neisseria elongata*, as suggested by Falsen (19).

Between *Neisseria* species and other representatives of the *Neisseriaceae* cluster no significant levels of DNA homology or higher levels of rRNA similarity were found (Tables 1, 4, and 5 and Fig. 1 through 6).

*Kingella*. The marginal relationship detected between *Kin-*



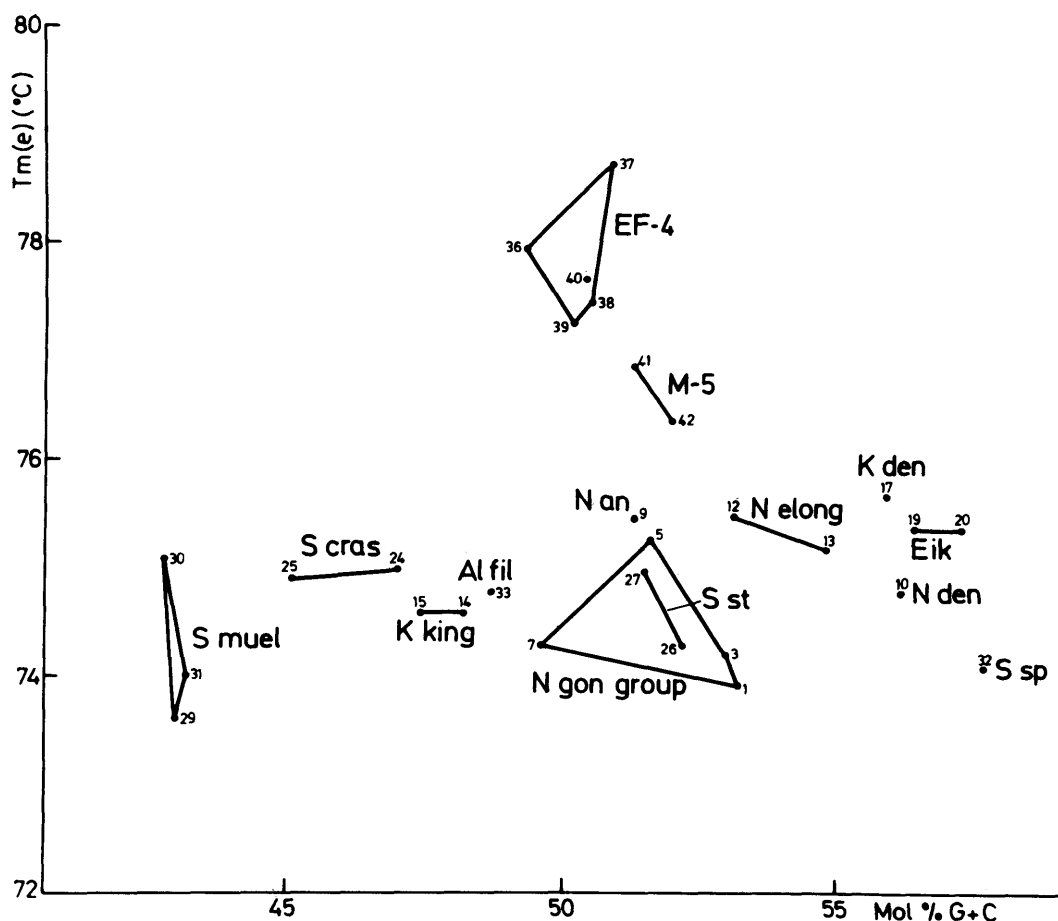


FIG. 5. Correlation between the mean moles percent G+C values of the total genomes and the  $T_{m(e)}$  values of hybrids between  $^3\text{H}$ -labeled rRNA from CDC group EF-4 strain ATCC 29858 and DNAs from strains of the *Neisseriaceae* cluster. For sequence numbers see Table 1. For abbreviations see the legend to Fig. 1.

*gella kingae* and *Neisseria* species by transformation experiments (3) and fatty acid analysis (32) was strengthened by our DNA-rRNA hybridization results (Table 1 and Fig. 1, 2, and 6). *Kingella kingae* and *Kingella denitrificans* are definitely part of the *Neisseriaceae* cluster. No DNA homology was found between *Kingella kingae* and *Kingella denitrificans* (Table 5). In all of our DNA-rRNA hybridizations both species were always separate (Fig. 2 through 5). Strains of both species showed generally negligibly low levels of DNA homology with all other strains belonging to the *Neisseriaceae* cluster. However, when *Simonsiella muelleri* rRNA was used, slightly higher  $T_{m(e)}$  values were observed with DNAs from *Kingella kingae* strains than with DNAs from other organisms, suggesting a possible relationship between the two taxa. Some other criteria for relationship are the rather low moles percent G+C values (Table 1), some similar phenotypic traits (39, 50), and similar fatty acid profiles with tetradecanoic acid as a prominent compound (32, 35). In other *Simonsiella* species tetradecanoic acid is also prominent (35). However, the genome complexity of *Kingella kingae* is only 60% that of *Simonsiella muelleri* (Table 6). Further clarification of the exact relationship between these taxa is needed. In view of their low moles percent G+C values, *Simonsiella crassa* and *Alysiella filiformis* might also be considered possible relatives of *Kingella kingae*.

Rossau et al. showed previously that [*Kingella*] indolo-

*genes* is not at all related to the other two *Kingella* species (Table 1) (48). A fairly high level of rRNA cistron similarity [ $T_{m(e)}$ , 74.1°C] was found between [*Kingella*] *indologenes* and *Cardiobacterium hominis* (Table 2) (U. Rothenpieler, R. Mutters, W. Frederiksen, R. Rossau, P. Segers, J. De Ley, and W. Mannheim, Abstr. XIV Int. Congr. Microbiol., P. B4-8, p. 49, 1986). Both of these taxa are part of a separate rRNA branch which is remotely related to rRNA superfamilies I and II [ $T_{m(e)}$ , 64.8°C] (48; U. Rothenpieler et al., manuscript in preparation).

***Simonsiella* and the “*Simonsiellaceae*.”** The family “*Simonsiellaceae*” is composed of two genera of gliding bacteria, *Simonsiella* and *Alysiella* (38). These strictly aerobic, gram-negative bacteria are found in the oral cavities of animals and human beings and are considered to be nonpathogenic. They are easily recognized by their typical multicellular micro-morphology. In the Reichenbach subdivision of gliding bacteria (43) the “*Simonsiellaceae*” were allocated to the order *Nostocales* within the cyanobacteria. However, our DNA-rRNA hybridization results revealed considerable rRNA cistron similarities among representative strains of this family and *Neisseria flavescens*, *Eikenella corrodens*, and CDC group EF-4 [ $T_{m(e)}$ , 73.6 to 76.0°C] (Table 1 and Fig. 1 through 6). Thus, genotypically, the “*Simonsiellaceae*” are completely unrelated to the cyanobacteria and most other gliding bacteria.

DNA-rRNA hybridizations showed that the genus *Simon-*

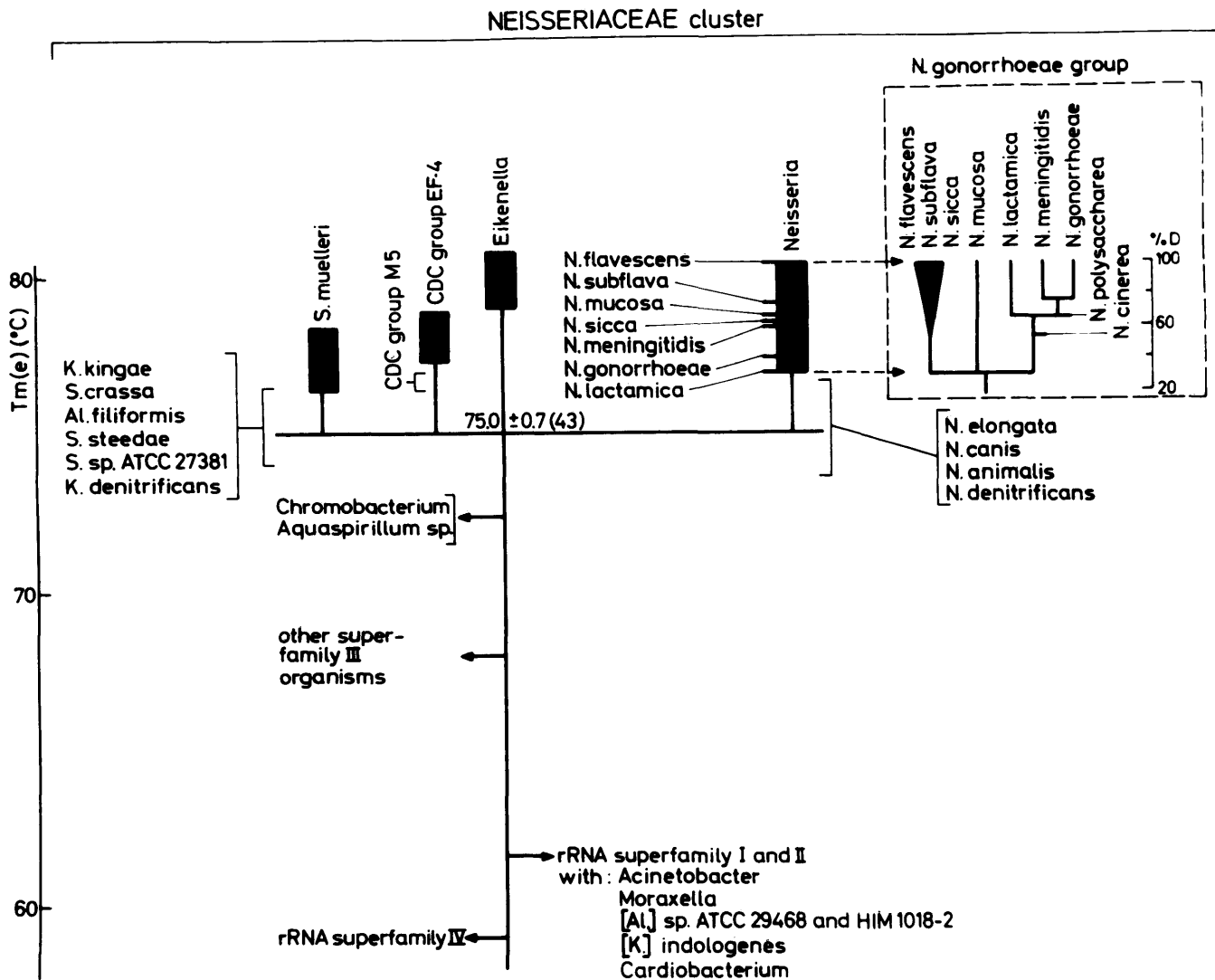


FIG. 6. Composite dendrogram showing the genotypic relationships of the taxa belonging to the *Neisseriaceae* cluster and other gram-negative bacteria. The rRNA cistron similarities are expressed as  $T_{m(e)}$  values (in degrees Celsius) versus *Simonsiella muelleri*, CDC group EF-4, *Eikenella corrodens*, and *Neisseria flavescens* rRNAs. The solid bars indicate the ranges of  $T_{m(e)}$  values for the clusters (see Fig. 1). The solid arrows indicate the average  $T_{m(e)}$  values at the branching points; these values were taken from previous work by members of the Gent research group. The position of CDC group M-5 is not yet completely certain. The upper few degrees Celsius of a  $T_{m(e)}$  dendrogram are not precise enough to represent the exact relationships between closely related taxa. More reliable relationships in the upper 3.5°C of the *Neisseria gonorrhoeae* group are shown in the insert DNA-DNA hybridization dendrogram (data from this study and references 23 and 45). In this case the upper 3.5°C of  $T_{m(e)}$  corresponds to a range from about 30 to 100% *D*.

*siella* is genotypically heterogeneous and that its species are distributed all through the *Neisseriaceae* cluster (Fig. 2 through 5). The three strains of *Simonsiella muelleri* stand out as a small separate rRNA branch; both strains of *Simonsiella crassa*, three strains of *Simonsiella steedae*, and one unspecified *Simonsiella* strain constitute individual separate groups. The degrees of DNA interrelatedness among the different *Simonsiella* species were low. The levels of DNA relatedness of the different *Simonsiella* species with *Kingella kingae*, *Kingella denitrificans*, *Neisseria gonorrhoeae*, *Eikenella corrodens*, and CDC group EF-4 were equally low (Table 5).

***Alysiella*.** Only one authentic member of the genus *Alysiella*, the type strain of *Alysiella filiformis*, is available from public culture collections. We received this organism from three different sources, as strain ATCC 15532<sup>T</sup>, CCUG

3710<sup>T</sup>, or HIM 929-5<sup>T</sup>. The results with the three strains were the same. This species, which was isolated from sheep, is also a member of the *Neisseriaceae* cluster (Table 1 and Fig. 1 through 6). It is separate from each of the four rRNA branches, and no DNA-DNA homologies could be demonstrated (Table 5).

Other isolates that were tentatively designated *Alysiella* sp. (e.g., strains ATCC 29468 and HIM 1018-2) were not significantly related to the type strain of *Alysiella filiformis* in terms of total DNA or DNA-rRNA hybridization (Tables 1 and 5 and Fig. 1 and 6). We established that strains ATCC 29468 and HIM 1018-2 have high levels of rRNA similarity [ $T_{m(e)}$ , 76.4 and 76.9°C, respectively] with *Moraxella lacunata* (Table 2), which clusters together with the genus *Acinetobacter* in rRNA superfamily II (48). It seems to be difficult, if not impossible, to discriminate false and true

TABLE 4. DNA-DNA hybridization results expressed as the degree of binding between strains in the *Neisseriaceae* rRNA cluster but not belonging to the group formed by *Neisseria gonorrhoeae* and highly related neisseriae<sup>a</sup>

Strain	D (%):							
	<i>Neisseria meningitidis</i> NCTC 10025 <sup>T</sup>	CDC group EF-4a strain CDC T-191/78	CDC group EF-4b strain CDC T-194/78	<i>Neisseria elongata</i> subsp. <i>elongata</i> NCTC 10660 <sup>T</sup>	<i>Neisseria animalis</i> NCTC 10212 <sup>T</sup>	<i>Neisseria denitrificans</i> ATCC 14686 <sup>T</sup>	CDC group M-5 strain CDC B5522	<i>Kingella denitrificans</i> NCTC 10995 <sup>T</sup>
<i>Neisseria meningitidis</i> NCTC 10025 <sup>Ta</sup>	100							
CDC group EF-4a strain CDC T-191/78	24	100						
CDC group EF-4b strain CDC T-194/78	25	31	100					
<i>Neisseria elongata</i> subsp. <i>elongata</i> NCTC 10660 <sup>T</sup>	23	20	14	100				
<i>Neisseria animalis</i> NCTC 10212 <sup>T</sup>	16	24	21	16	100			
<i>Neisseria denitrificans</i> ATCC 14686 <sup>T</sup>	15	19	20	18	32	100		
CDC group M-5 strain CDC B5522	11	19	24	13	13	12	100	
<i>Kingella denitrificans</i> NCTC 10995 <sup>T</sup>	19	11	19	19	21	15	14	100

<sup>a</sup> See Table 3. *Neisseria meningitidis* NCTC 10025<sup>T</sup> was used as a representative of the group formed by *Neisseria gonorrhoeae* and highly related neisseriae.

*Alysiella* sp. strains by means of conventional phenotypic criteria. The statement (52) that the genera *Simonsiella* and *Alysiella* belong to different RNA subdivisions and that the latter is closest to the genus *Acinetobacter* was probably not based on an analysis of the type strain of *Alysiella filiformis* and should be revised.

***Eikenella corrodens*.** *Eikenella corrodens* contains gram-negative, fastidious, facultatively anaerobic, nonmotile or twitching rods; these organisms pit agar surfaces and are biochemically rather inert. They grow well on blood agar in 5 to 10% CO<sub>2</sub> and their moles percent G+C range from 56 to 58. These organisms are normal human oral and intestinal inhabitants; many serious infections have been described, such as through human bites, abscesses, periodontal disease, osteomyelitis, etc.

Intraspecific DNA binding values obtained with a collection of strains that are phenotypically heterogeneous (with respect to hemolysis, nitrate reduction, hydrolases, oxidation of carbohydrates, colonial morphology, clinical significance) (Göthe et al., manuscript in preparation) essentially confirm the findings of Coykendall and Kaczmarek (5). These authors regard *Eikenella corrodens* as a molecularly homogeneous species with internal *D* values of 70 to 100%. The results of DNA-rRNA hybridization support this. Because of a positive oxidase reaction and a negative catalase reaction a possible relationship between *Eikenella corrodens* and *Kingella kingae* has been suggested (51). Serological cross-reactions between these two species have been observed (24). However, these taxa differ significantly in the moles percent G+C of their DNAs (8 mol%) (Table 1) and in their cellular fatty acid compositions (32, 42).

Some authors (22, 25, 26, 30) have suggested that the genus *Eikenella* belongs in the *Brucellaceae*. From our DNA-rRNA hybridization data (Table 1 and Fig. 1 through 6) we concluded that *Eikenella corrodens* is a member of the

*Neisseriaceae* cluster. From hybridizations with *Eikenella corrodens* rRNA (Table 1 and Fig. 4) and from DNA-DNA hybridization data (Table 5) we proved that *Eikenella* is a quite distinct genus in the family *Neisseriaceae*. Generally, *Eikenella corrodens* strains have higher octadecanoic acid contents (34 to 46%) (42) than other members of the cluster.

**CDC group EF-4.** CDC group EF-4 consists of arginine dihydrolase-positive subgroup EF-4a and arginine dihydrolase-negative subgroup EF-4b (4, 28). These bacteria, which originally were thought to be related to the genus *Pasteurella*, are part of the oral flora of dogs and cats and are often isolated from infections resulting from dog and cat bites. The rRNA cistron similarities (Table 1 and Fig. 1 through 6) show that both CDC group EF-4 subgroups are part of the *Neisseriaceae* rRNA cluster as a separate rRNA branch; they appear to be most closely related to CDC group M-5 (Fig. 5). The recently detected phenotypic heterogeneity within CDC group EF-4 (28, 56) is strengthened by our DNA-DNA hybridization results; subgroup EF-4a and EF-4b strains exhibit only a low degree of DNA binding (31% *D*) (Table 4) and thus can be considered to be separate taxa. No significant DNA-DNA homology was detected between CDC group EF-4 members and other members of the cluster (Tables 4 and 5). The rRNA similarity with *Neisseria* species is supported by similarities in fatty acid patterns (6).

**CDC group M-5.** Members of CDC group M-5 also seem to be associated with infected dog bites (4, 49). They phenotypically resemble *Moraxella* species but are also similar in some respects to *Neisseria elongata* (49); therefore, they have sometimes been named "*Neisseria parelongata*" (19). The results of DNA-rRNA hybridizations with DNAs of CDC group M-5 strains indicate a relationship with CDC group EF-4 (Fig. 5). No significant DNA homology was found with any taxon tested (Table 4). The fatty acid profiles of CDC group M-5 strains essentially do not differ from those

TABLE 5. Results of DNA-DNA hybridizations expressed as the degree of binding between type or reference strains of the genera *Simonsiella*, *Alysiella*, *Kingella*, *Eikenella*, and *Neisseria* and CDC group EF-4

Strain	D (%):																			
	<i>Simonsiella muelleri</i> ATCC 29453 <sup>T</sup>	<i>Simonsiella muelleri</i> ATCC 29452	<i>Simonsiella muelleri</i> HIM 942-7	<i>Simonsiella crassa</i> ATCC 15533 <sup>T</sup>	<i>Simonsiella crassa</i> ATCC 29447	<i>Simonsiella steedae</i> ATCC 27409 <sup>T</sup>	<i>Simonsiella steedae</i> ATCC 27410	<i>Simonsiella steedae</i> ATCC 27398	<i>Simonsiella</i> sp. strain ATCC 27381	<i>Simonsiella</i> sp. strain ATCC 29466	<i>Alysiella filiformis</i> ATCC 15532 <sup>T</sup>	[ <i>Alysiella</i> ] sp. strain ATCC 29468	[ <i>Alysiella</i> ] sp. strain HIM 1018-2	<i>Kingella kingae</i> NCTC 10529 <sup>T</sup>	<i>Kingella denitrificans</i> NCTC 10995 <sup>T</sup>	<i>Kingella denitrificans</i> NCTC 10997	<i>Neisseria gonorrhoeae</i> NCTC 19424 <sup>T</sup>	<i>Eikenella corrodens</i> NCTC 10596 <sup>T</sup>	CDC group EF-4 strain CCUG 17349	
<i>Simonsiella muelleri</i> ATCC 29453 <sup>T</sup>	100																			
<i>Simonsiella muelleri</i> ATCC 29452	91	100																		
<i>Simonsiella muelleri</i> HIM 942-7	70	84	100																	
<i>Simonsiella crassa</i> ATCC 15533 <sup>T</sup>				100																
<i>Simonsiella crassa</i> ATCC 29447				80	100															
<i>Simonsiella steedae</i> ATCC 27409 <sup>T</sup>	30			27		100														
<i>Simonsiella steedae</i> ATCC 27410						80	100													
<i>Simonsiella steedae</i> ATCC 27398						94	88	100												
<i>Simonsiella</i> sp. strain ATCC 27381	30			23		40			100											
<i>Simonsiella</i> sp. strain ATCC 29466									97	100										
<i>Alysiella filiformis</i> ATCC 15532 <sup>T</sup>	14			35		13			29		100									
[ <i>Alysiella</i> ] sp. strain ATCC 29468	24					27			30		31	100								
[ <i>Alysiella</i> ] sp. strain HIM 1018-2													100							
<i>Kingella kingae</i> NCTC 10529 <sup>T</sup>	28			33		32			26		19			100 <sup>a</sup>						
<i>Kingella denitrificans</i> NCTC 10995 <sup>T</sup>	32			20		21			31		28			8 <sup>a</sup>	100					
<i>Kingella denitrificans</i> NCTC 10997															74	100				
<i>Neisseria gonorrhoeae</i> ATCC 19424 <sup>T</sup>	28					34			32		26						100			
<i>Eikenella corrodens</i> NCTC 10596 <sup>T</sup>	23			26		33			30		34			12	25		28	100		
CDC group EF-4 strain CCUG 17349	15			25		27			24		0							34	100	

<sup>a</sup> Data from Rothenpieler et al. (manuscript in preparation).

of CDC group EF-4 strains (6, 34, 41) and strains in the genus *Neisseria* (32). In contrast to the CDC group EF-4 strains, CDC group M-5 strains are asaccharolytic and do not reduce nitrate (49, 56).

**Taxonomic implications.** The most recent officially proposed taxonomic composition of the family *Neisseriaceae* is that of Bøvre (2). In addition to the genus *Neisseria*, this family includes the genera *Moraxella* (with two subgenera, *Moraxella* and *Branhamella*), *Acinetobacter*, and *Kingella*. The genus *Psychrobacter* was added in 1986 (36). Our results from this study and from previous studies (47, 48, 53) prove that the present family *Neisseriaceae* as such is not justified. It has to be emended by exclusion of the genera *Acinetobacter*, *Moraxella* (with its two subgenera, *Moraxella* and *Branhamella*), and *Psychrobacter* (Rossau et al., in preparation), which are members of rRNA superfamily II. The emended family *Neisseriaceae* has to correspond to our *Neisseriaceae* rRNA cluster, and it has to include the following genera and groups: true *Neisseria*, *Kingella* (not [*Kingella*] *indologenes*), *Simonsiella*, *Alysiella*, *Eikenella*, and CDC groups EF-4 and M-5. There is no justification for accepting the nonvalidated family "*Simonsiellaceae*," as it is a more recent proposal; both genera in this family should be included in the *Neisseriaceae*. The degree of genotypic homogeneity in the *Neisseriaceae* rRNA cluster [ $\Delta T_{m(e)}$  range, 6.4°C (Table 1)] is comparable to the degrees of homogeneity within some other genotypically well-defined

bacterial families, such as the *Alcaligenaceae* (12) [ $\Delta T_{m(e)}$  range, 6°C], the *Acetobacteriaceae* (21) [ $\Delta T_{m(e)}$  range, 5°C], and the *Enterobacteriaceae* [ $\Delta T_{m(e)}$  range, 9°C] (J. De Ley and R. Tytgat, manuscript in preparation).

All of the data presented in this paper justify the proposal for the emended family *Neisseriaceae*. The description below was compiled from references 2, 6, 17, 31, 33, 35, 38, 41, 42, 49, 50, 54, 56.

**Description of the *Neisseriaceae* (Prévot 1933, 119<sup>AL</sup>) emend.** *Neisseriaceae* (Neis.se.ri.a'ce.ae. M.L. fem. n. *Neisseria*, type genus of the family; -aceae, ending to denote family; M.L. fem. pl. n. *Neisseriaceae*, the *Neisseria* family). Organisms are coccal, coccoid, or rod shaped, or they exhibit a multicellular micromorphology (*Simonsiella* and *Alysiella*). Gram-negative, with a tendency to resist decolorization. Endospores are not formed. Flagella are absent. Swimming motility is not observed, but there may be twitching or gliding motility. Capsules and fimbriae may be present. Aerobic, but some species also grow anaerobically (*Kingella* sp. and *Eikenella corrodens*). Chemoorganotrophic and mesophilic. Oxidase active; no indole production. Biochemically rather inert. Some species are nutritionally fastidious; some are saccharolytic. The main habitat is the mucosal surfaces of human beings and other mammals. Most species are opportunistic, but some are primary pathogens of humans. The fatty acid profiles of the taxa examined show the presence of saturated or monounsaturated non-

TABLE 6. Molecular complexities of the genomes of strains (mostly type strains) belonging to the *Neisseriaceae* cluster

Strain	Genome mol wt ( $\times 10^9$ )
<i>Neisseria gonorrhoeae</i> NCTC 8375 <sup>T</sup>	1.5
<i>Neisseria meningitidis</i> NCTC 10025 <sup>T</sup>	1.4
<i>Neisseria lactamica</i> NCTC 10617 <sup>T</sup>	1.5
<i>Neisseria lactamica</i> NCTC 10616	1.5
<i>Neisseria mucosa</i> CIP 59.51 <sup>T</sup>	1.5
<i>Neisseria subflava</i> ATCC 10555	1.8
<i>Neisseria flavescens</i> ATCC 13120 <sup>T</sup>	1.4
<i>Neisseria animalis</i> NCTC 10212 <sup>T</sup>	1.4
<i>Neisseria denitrificans</i> ATCC 14686 <sup>T</sup>	1.7
<i>Neisseria elongata</i> subsp. <i>elongata</i> NCTC 10660 <sup>T</sup>	1.7
<i>Neisseria elongata</i> subsp. <i>glycolytica</i> NCTC 11050	1.5
<i>Kingella kingae</i> NCTC 10529 <sup>T</sup>	1.4 <sup>a</sup>
<i>Kingella kingae</i> NCTC 10746	1.8 <sup>a</sup>
<i>Kingella denitrificans</i> NCTC 10995 <sup>T</sup>	1.5
<i>Kingella denitrificans</i> NCTC 10997	1.8
<i>Eikenella corrodens</i> NCTC 10596 <sup>T</sup>	1.7
<i>Simonsiella crassa</i> ATCC 15533 <sup>T</sup>	2.4
<i>Simonsiella steedae</i> ATCC 27409 <sup>T</sup>	2.2
<i>Simonsiella muellerii</i> ATCC 29453 <sup>T</sup>	2.3
<i>Alysiella filiformis</i> ATCC 15532 <sup>T</sup>	2.7
CDC group EF-4a strain CDC T-191/78	1.6
CDC group EF-4b strain CDC T-194/78	1.8
CDC group M-5 strain CDC B5522	1.6

<sup>a</sup> U. Rothenpieler et al., personal communication.

branched fatty acids, predominantly with 14, 16, or 18 carbon atoms. Carbonic anhydrase is found in all of the taxa studied. Nearly all family members use ubiquinones as sole respiratory quinones.

G+C range, 41 to 58 mol% (determined mainly by the thermal denaturation method). Genome molecular complexity,  $1.4 \times 10^9$  to  $2.7 \times 10^9$ . All taxa are found within a  $\Delta T_{m(e)}$  range of 7.6°C (DNA-rRNA hybridization). The type genus is *Neisseria* Trevisan 1885, 105.

The following species are included at this time in the type genus (Fig. 6): *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Neisseria lactamica*, *Neisseria sicca*, *Neisseria subflava* (including *Neisseria perflava* and *Neisseria flava*), *Neisseria flavescens*, *Neisseria mucosa*, *Neisseria macacae*, *Neisseria cinerea*, *Neisseria polysaccharea*, *Neisseria elongata*, *Neisseria canis*, *Neisseria animalis*, and *Neisseria denitrificans*. The allocation of the last three species in this genus is not yet certain.

The following genera and other taxa are also included in this family (Fig. 6): *Kingella* Henriksen and Bøvre 1976, 449<sup>AL</sup> (excluding [*Kingella*] *indologenes*); *Eikenella* Jackson and Goodman 1972, 72<sup>AL</sup>; *Simonsiella* Schmid 1922, 504<sup>AL</sup>; *Alysiella* Langeron 1923, 116<sup>AL</sup>; and CDC groups EF-4 and M-5. The possible inclusion of the genus *Chromobacterium* and a few aquaspirilla (B. Pot, M. Gillis, C. Aerts, and J. De Ley, FEMS Symp. Evolution Prokaryotes, abstr. no. C8, 1984) in the *Neisseriaceae* [average  $\Delta T_{m(e)}$ , 8 to 9°C] is uncertain. The interrelatedness of some of these taxa within the emended family requires further investigation.

#### ACKNOWLEDGMENTS

J.D.L. is indebted to the Fonds voor Geneeskundig Wetenschappelijk Onderzoek (Belgium) for research and personnel grants. W.M. and co-workers gratefully acknowledge a grant from the Deutsche Forschungsgemeinschaft (Federal Republic of Germany). R.R. is indebted to the Instituut tot Aanmoediging van het Wetenschappelijk Onderzoek in Nijverheid en Landbouw (Belgium) for a scholarship.

We are indebted to E. Falsen (Göteborg, Sweden) for the gift of strains.

#### LITERATURE CITED

- Bøvre, K. 1980. Progress in classification and identification of *Neisseriaceae* based on genetic affinity, p. 55–72. In M. Goodfellow and R. G. Board (ed.), *Microbiological classification and identification*. Academic Press, Inc. (London), Ltd., London.
- Bøvre, K. 1984. Family VIII. *Neisseriaceae* Prévot 1933, 119<sup>AL</sup>, p. 288–309. In N. R. Krieg and J. G. Holt (ed.), *Bergey's manual of systematic bacteriology*, vol. 1. The Williams & Wilkins Co., Baltimore.
- Bøvre, K., L. O. Frøholm, S. D. Henriksen, and E. Holten. 1977. Relationship of *Neisseria elongata* subsp. *glycolytica* to other members of the family *Neisseriaceae*. *Acta Pathol. Microbiol. Scand. Sect. B* 85:18–26.
- Clark, W. A., D. G. Hollis, R. E. Weaver, and P. Riley. 1984. Identification of unusual pathogenic Gram-negative aerobic and facultatively anaerobic bacteria, p. 144–147. Centers for Disease Control, Atlanta.
- Coykendall, A. L., and F. S. Kaczmarek. 1980. DNA homologies among *Eikenella corrodens* strains. *J. Periodontal Res.* 15: 615–620.
- Dees, S. B., J. Powell, C. W. Moss, D. G. Hollis, and R. E. Weaver. 1981. Cellular fatty acid composition of organisms frequently associated with human infections resulting from dog bites: *Pasteurella multocida* and groups EF-4, IJ, M-5, and DF-2. *J. Clin. Microbiol.* 14:612–616.
- De Ley, J. 1970. Reexamination of the association between melting point, buoyant density, and chemical base composition of deoxyribonucleic acid. *J. Bacteriol.* 101:738–754.
- De Ley, J. 1978. Modern methods in bacterial taxonomy: evaluation, application, prospects, p. 347–357. In *Proceedings of the 4th International Conference on Plant Pathogenic Bacteria*, Angers, vol. 1. Gibert-Clarey, Tours, France.
- De Ley, J., H. Cattoir, and A. Reynaerts. 1970. The quantitative measurement of DNA hybridization from renaturation rates. *Eur. J. Biochem.* 12:133–142.
- De Ley, J., and J. De Smedt. 1975. Improvements of the membrane filter method for DNA:rRNA hybridization. *Antonie van Leeuwenhoek J. Microbiol. Serol.* 41:287–308.
- De Ley, J., P. Segers, and M. Gillis. 1978. Intra- and intergeneric similarities of *Chromobacterium* and *Janthinobacterium* ribosomal ribonucleic acid cistrons. *Int. J. Syst. Bacteriol.* 28: 154–168.
- De Ley, J., P. Segers, K. Kersters, W. Mannheim, and A. Lievens. 1986. Intra- and intergeneric similarities of the *Bordetella* ribosomal ribonucleic acid cistrons: proposal for a new family, *Alcaligenaceae*. *Int. J. Syst. Bacteriol.* 36:405–414.
- De Ley, J., and J. Van Muylem. 1963. Some applications of deoxyribonucleic acid base composition in bacterial taxonomy. *Antonie van Leeuwenhoek J. Microbiol. Serol.* 29:344–358.
- De Smedt, J., M. Bauwens, R. Tytgat, and J. De Ley. 1980. Intra- and intergeneric similarities of ribosomal ribonucleic acid cistrons of free-living nitrogen-fixing bacteria. *Int. J. Syst. Bacteriol.* 30:106–122.
- De Vos, P., and J. De Ley. 1983. Intra- and intergeneric similarities of *Pseudomonas* and *Xanthomonas* ribosomal ribonucleic acid cistrons. *Int. J. Syst. Bacteriol.* 33:487–509.
- De Vos, P., M. Goor, M. Gillis, and J. De Ley. 1985. Ribosomal ribonucleic acid cistron similarities of phytopathogenic *Pseudomonas* species. *Int. J. Syst. Bacteriol.* 35:169–184.
- Edwards, R., and R. K. A. Feltham. 1983. Taxonomic implications of the cellular fatty acid content of the *Legionellaceae* and possibly related species. *FEMS Microbiol. Lett.* 17:251–255.
- Elwell, L. P., and S. Falkow. 1977. Plasmids of the genus *Neisseria*, p. 137–154. In R. B. Roberts (ed.), *The gonococcus*. John Wiley & Sons, Inc., New York.
- Falsen, E. 1988. Catalogue of strains CCUG, Culture Collection, 5th ed. University of Göteborg, Göteborg, Sweden.
- Gillis, M., and J. De Ley. 1975. Determination of the molecular complexity of double-stranded phage genome DNA from initial renaturation rates. The effect of DNA base composition. *J. Mol.*

- Biol. 98:447-464.
21. Gillis, M., and J. De Ley. 1980. Intra- and intergeneric similarities of the ribosomal ribonucleic acid cistrons of *Acetobacter* and *Gluconobacter*. Int. J. Syst. Bacteriol. 30:7-27.
  22. Goldstein, E. J. C., and M. E. Gombert. 1983. *Eikenella corrodens*: a new perspective, p. 1-43. In E. J. Bottone (ed.), Unusual microorganisms. Gram-negative fastidious species. Marcel Dekker, Inc., New York.
  23. Guibourdenche, M., M. Y. Popoff, and J. Y. Riou. 1986. Deoxyribonucleic acid relatedness among *Neisseria gonorrhoeae*, *N. meningitidis*, *N. lactamica*, *N. cinerea* and "*Neisseria polysaccharea*." Ann. Microbiol. (Paris) 137B:177-185.
  24. Haug, R. H., and S. D. Henriksen. 1969. The serology of *Moraxella kingii*. Acta Pathol. Microbiol. Scand. 75:641-647.
  25. Henriksen, S. D. 1969. Designation of the type strain of *Bacteroides corrodens* Eiken 1958. Int. J. Syst. Bacteriol. 19:375-376.
  26. Henriksen, S. D. 1969. Corroding bacteria from the respiratory tract. II. *Bacteroides corrodens*. Acta Pathol. Microbiol. Scand. 75:91-96.
  27. Hoke, C., and N. A. Vedros. 1982. Taxonomy of the neisseriae: deoxyribonucleic acid base composition, interspecific transformation, and deoxyribonucleic acid hybridization. Int. J. Syst. Bacteriol. 32:57-66.
  28. Holmes, B., and M. S. Ahmed. 1981. Group EF-4: a *Pasteurella*-like organism, p. 161-174. In M. Kilian, W. Frederiksen, and E. L. Biberstein (ed.), *Haemophilus*, *Pasteurella* and *Actinobacillus*. Academic Press, Inc. (London), Ltd., London.
  29. Hylemon, P. B., J. S. Wells, N. R. Krieg, and H. W. Jannasch. 1973. The genus *Spirillum*: a taxonomic study. Int. J. Syst. Bacteriol. 23:340-380.
  30. Jackson, F. L., and Y. E. Goodman. 1972. Transfer of the facultatively anaerobic organism *Bacteroides corrodens* Eiken to a new genus, *Eikenella*. Int. J. Syst. Bacteriol. 22:73-77.
  31. Jackson, F. L., and Y. Goodman. 1984. Genus *Eikenella* Jackson and Goodman 1972, 74<sup>AL</sup>, p. 591-597. In N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol 1. The Williams & Wilkins Co., Baltimore.
  32. Jantzen, E., K. Bryn, T. Bergan, and K. Bøvre. 1974. Gas chromatography of bacterial whole cell methanolysates. V. Fatty acid composition of *Neisseriae* and *Moraxellae*. Acta Pathol. Microbiol. Scand. Sect. B 82:767-779.
  33. Jantzen, E., K. Bryn, N. Hagen, T. Bergan, and K. Bøvre. 1978. Fatty acids and monosaccharides of *Neisseriaceae* in relation to established taxonomy. Nat. Inst. Public Health Ann. (Norway) 1:59-71.
  34. Jantzen, E., O. M. Kvalheim, T. A. Hauge, N. Hagen, and K. Bøvre. 1987. Grouping of bacteria by simca pattern recognition on gas chromatographic lipid data: patterns among *Moraxella* and rod-shaped *Neisseria*. Syst. Appl. Microbiol. 9:142-150.
  35. Jenkins, C. L., D. A. Kuhn, and K. R. Daly. 1977. Fatty acid composition of *Simonsiella* strains. Arch. Microbiol. 113:209-213.
  36. Juni, E., and G. A. Heym. 1986. *Psychrobacter immobilis* gen. nov., sp. nov.: genospecies composed of gram-negative, aerobic, oxidase-positive coccobacilli. Int. J. Syst. Bacteriol. 36:388-391.
  37. Kingsbury, D. T. 1967. Deoxyribonucleic acid homologies among species of the genus *Neisseria*. J. Bacteriol. 94:870-874.
  38. Kuhn, D. A. 1981. The genera *Simonsiella* and *Atysiella*, p. 390-399. In M. P. Starr, H. Stolp, H. G. Trüper, A. Balows, and H. G. Schlegel (ed.), The prokaryotes, vol. 1. Springer-Verlag, Berlin.
  39. Kuhn, D. A., D. A. Gregory, G. E. Buchanan, Jr., M. D. Nyby, and K. R. Daly. 1978. Isolation, characterization, and numerical taxonomy of *Simonsiella* strains from the oral cavities of cats, dogs, sheep, and humans. Arch. Microbiol. 118:235-241.
  40. Marmur, J. A. 1961. A procedure for the isolation of deoxyribonucleic acid from micro-organisms. J. Mol. Biol. 3:208-218.
  41. Moss, C. W., P. L. Wallace, D. G. Hollis, and R. E. Weaver. 1988. Cultural and chemical characterization of CDC groups EO-2, M-5, and M-6, *Moraxella (Moraxella)* species, *Oligella urethralis*, *Acinetobacter* species, and *Psychrobacter immobilis*. J. Clin. Microbiol. 26:484-492.
  42. Prefontaine, G., and F. L. Jackson. 1972. Cellular fatty acid profiles as an aid to the classification of "corroding bacilli" and certain other bacteria. Int. J. Syst. Bacteriol. 22:210-217.
  43. Reichenbach, H. 1981. Taxonomy of the gliding bacteria. Annu. Rev. Microbiol. 35:339-364.
  44. Richards, G. M. 1974. Modification of the diphenylamine reaction giving increased sensitivity and simplicity in the estimation of DNA. Anal. Biochem. 57:369-376.
  45. Riou, J. Y., and M. Guibourdenche. 1987. *Neisseria polysaccharea* sp. nov. Int. J. Syst. Bacteriol. 37:163-165.
  46. Riou, J. Y., M. Guibourdenche, and M. Y. Popoff. 1983. A new taxon in the genus *Neisseria*. Ann. Microbiol. (Paris) 134B:257-267.
  47. Rossau, R., K. Kersters, E. Falsen, E. Jantzen, P. Segers, A. Union, L. Nehls, and J. De Ley. 1987. *Oligella*, a new genus including *Oligella urethralis* comb. nov. (formerly *Moraxella urethralis*) and *Oligella ureolytica* sp. nov. (formerly CDC group IVe): relationship to *Taylorella equigenitalis* and related taxa. Int. J. Syst. Bacteriol. 37:198-210.
  48. Rossau, R., A. Van Landschoot, W. Mannheim, and J. De Ley. 1986. Inter- and intrageneric similarities of ribosomal ribonucleic acid cistrons of the *Neisseriaceae*. Int. J. Syst. Bacteriol. 36:323-332.
  49. Rubin, J. S., P. A. Granato, and B. L. Wasilauskas. 1985. Glucose-nonfermenting gram-negative bacteria, p. 330-349. In E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
  50. Snell, J. J. S. 1984. *Kingella* Henriksen and Bøvre 1976, 449<sup>AL</sup>, p. 307-309. In N. R. Krieg and J. G. Holt, Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
  51. Snell, J. J. S., and S. P. Lapage. 1976. Transfer of some saccharolytic *Moraxella* species to *Kingella* Henriksen and Bøvre 1976, with descriptions of *Kingella indologenes* sp. nov. and *Kingella denitrificans* sp. nov. Int. J. Syst. Bacteriol. 26:451-458.
  52. Stackebrandt, E. 1986. Das hierarchische System der Eubakterien: Problem und Lösungsansätze. Forum Mikrobiol. 9:255-260.
  53. Van Landschoot, A., R. Rossau, and J. De Ley. 1986. Intra- and intergeneric similarities of the ribosomal ribonucleic acid cistrons of *Acinetobacter*. Int. J. Syst. Bacteriol. 36:150-160.
  54. Vedros, N. A. 1984. *Neisseria* Trevisan 1885, 105<sup>AL</sup>, p. 290-296. In N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
  55. Vedros, N. A., C. Hoke, and P. Chun. 1983. *Neisseria macacae* sp. nov., a new *Neisseria* species isolated from the oropharynxes of rhesus monkeys (*Macaca mulatta*). Int. J. Syst. Bacteriol. 33:515-520.
  56. Weaver, R. E., D. G. Hollis, and E. J. Bottone. 1985. Gram-negative fermentative bacteria and *Francisella tularensis*, p. 309-329. In E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.