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Phylogeny of *Bacteroides*, *Prevotella*, and *Porphyromonas* spp. and Related Bacteria

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The phylogenetic structure of the bacteroides subgroup of the cytophaga-flavobacter-bacteroides (CFB) phylum was examined by 16S rRNA sequence comparative analysis. Approximately 95% of the 16S rRNA sequence was determined for 36 representative strains of species of *Prevotella*, *Bacteroides*, and *Porphyromonas* and related species by a modified Sanger sequencing method. A phylogenetic tree was constructed from a corrected distance matrix by the neighbor-joining method, and the reliability of tree branching was established by bootstrap analysis. The bacteroides subgroup was divided primarily into three major phylogenetic clusters which contained most of the species examined. The first cluster, termed the prevotella cluster, was composed of 16 species of *Prevotella*, including *P. melaninogenica*, *P. intermedia*, *P. nigrescens*, and the ruminal species *P. ruminicola*. Two oral species, *P. zoogleoformans* and *P. heparinolytica*, which had been recently placed in the genus *Prevotella*, did not fall within the prevotella cluster. These two species and six species of *Bacteroides*, including the type species *B. fragilis*, formed the second cluster, termed the bacteroides cluster. The third cluster, termed the porphyromonas cluster, was divided into two subclusters. The first contained *Porphyromonas gingivalis*, *P. endodontalis*, *P. asaccharolytica*, *P. circumdentaria*, *P. salivosa*, [*Bacteroides*] *levii* (the brackets around genus are used to indicate that the species does not belong to the genus by the sensu stricto definition), and [*Bacteroides*] *macacae*, and the second subcluster contained [*Bacteroides*] *forsythus* and [*Bacteroides*] *distasonis*. [*Bacteroides*] *splanchnicus* fell just outside the three major clusters but still belonged within the bacteroides subgroup. On the basis of bootstrap analysis, the remaining species, [*Bacteroides*] *putredinis*, *Rikenella microfusus*, and two misclassified, free-living species of *Cytophaga*, appeared to form an additional subgroup separate from the bacteroides and other subgroups of the CFB phylum. However, on classification by single-base signatures, these species belonged within the bacteroides subgroup. Additional single-base signatures defined and differentiated clads within the bacteroides subgroup. With few exceptions, the 16S rRNA data were in overall agreement with previously proposed reclassifications of species of *Bacteroides*, *Prevotella*, and *Porphyromonas*. Suggestions are made to accommodate those species which do not fit previous reclassification schemes.

The taxonomy of the "bacteroides" has undergone significant changes in the past few years. Species of this bacterial group were once loosely defined as obligately anaerobic, gram-negative, nonsporulating, pleomorphic rods. In addition, guanine-plus-cytosine contents of the DNA of the bacteroides were shown to range broadly from 28 to 61 mol% (10). Attempts to better classify this bacterial group have been made on the bases of physiologic characteristics (10), electrophoretic patterns of dehydrogenases (34), cellular fatty acid and sugar composition (3, 4, 18), lipid analysis (19, 26), serology (15), and bacteriophage typing (2). The diversity of this bacterial group was further demonstrated in studies using 16S rRNA oligonucleotide cataloging (25), rRNA-DNA hybridization (12), DNA homology (11, 36), and 5S rRNA comparative analysis (35). On the basis of this heterogeneity, Shah and Collins (30–32) proposed that the genus *Bacteroides* be divided into the following three genera: (i) *Bacteroides* sensu stricto, consisting of saccharolytic, nonpigmenting species such as the type species, *B. fragilis*, and its relatives; (ii) *Prevotella*, consisting of moderately saccharolytic, bile-sensitive, predominantly oral species, such as *P. melaninogenica* and *P. intermedia*; and (iii) *Porphyromonas*, generally consisting of asaccharolytic, black-pigmenting species, such as *P. gingivalis* and *P. asaccharolytica*. Detailed descriptions and extensive literature reviews of each of these genera are reported by Shah (28, 29). For those species that did not fit the description of these three genera, additional new genera were proposed (28).

The cytophaga-flavobacter-bacteroides (CFB) group represents 1 of the 10 phyla that encompass all bacteria (21, 37). As defined by Gherna and Woese (9), the CFB phylum is composed of five major phylogenetic subgroups: cytophaga, flavobacter, sphingobacter, saprospira, and bacteroides. In the present study, 16S rRNA sequences from 39 strains of species of *Bacteriodes*, *Prevotella*, *Porphyromonas*, and related bacteria were obtained to expand our understanding of the phylogeny of the bacteroides subgroup and to determine the validity of previously proposed schemes for reclassification of these bacteria.

MATERIALS AND METHODS

Bacterial strains and culture conditions. Strains of *Bacteroides*, *Prevotella*, and *Porphyromonas* and related species and reference strains used in this study are listed in Table 1. Brackets around the generic epithet are used to designate species that do not belong by the sensu stricto definition to the genus indicated. Included in Table 1 are sites of initial isolation and GenBank accession numbers for 16S rRNA sequences. Strains of *Bacteroides*, *Prevotella*, *Porphyromonas*, and related

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TABLE 1. Bacterial strains and GenBank accession numbers of 16S rRNA sequences

Bacterial genus and species	Source and strain[a]	Isolation location	Accession no.
Bacteroides			
B. eggerthii	NCTC 11185^{T}	Human feces	L16485
B. fragilis	ATCC 25285^{T}	Appendix abscess	M11656[b]
B. ovatus	NCTC 11153^{T}	Human feces	L16484
B. thetaiotaomicron	ATCC 29148^{T}	Human feces	L16489
B. uniformis	ATCC 8492^{T}	Human and swine feces	L16486
B. vulgatus	ATCC 8482^{T}	Human feces	M58762[b]
[*B.*] *distasonis*[c]	ATCC 8503^{T}	Human feces	M86695[b]
[*B.*] *forsythus*	FDC 338^{T}	Human periodontal pocket	L16495
[*B.*] *levii*	ATCC 29147^{T}	Bovine rumen	L16493
[*B.*] *macacae*	ATCC 33141^{T}	Macaque periodontal pocket	L16494
[*B.*] *putredinis*	ATCC 29800^{T}	Human feces	L16497
[*B.*] *splanchnicus*	NCTC 10825^{T}	Abdominal abscess, feces	L16496
Porphyromonas			
P. asaccharolytica	ATCC 25260^{T}	Empyema	L16490
P. circumdentaria	NCTC 12469^{T}	Feline gingiva	L26102
P. endodontalis	ATCC 35406^{T}	Infected root canal	L16491
P. gingivalis	ATCC 33277^{T}	Human gingival sulcus	L16492
P. salivosa	NCTC 11632^{T}	Feline oral cavity	L26103
Prevotella			
P. bivia	ATCC 29303^{T}	Urogenital tract	L16475
P. buccae	ATCC 33574^{T}	Gingival crevice	L16477
P. buccae	ATCC 33690	Human periodontal pocket	L16478
P. buccalis	ATCC 35310^{T}	Dental plaque	L16476
P. corporis	ATCC 33547^{T}	Cervical swab	L16465
P. denticola	ATCC 35308^{T}	Dental plaque	L16467
P. denticola	ATCC 33185	Maxillary atrium	L16466
P. disiens	ATCC 29426^{T}	Urogenital tract	L16483
[*P.*] *heparinolytica*	ATCC 35895^{T}	Subgingival plaque	L16487
P. intermedia (serotype I)	ATCC 25611^{T}	Empyema	L16468
P. loescheii	ATCC 15930^{T}	Gingival crevice	L16481
P. melaninogenica	ATCC 25845^{T}	Sputum	L16469
P. melaninogenica	ATCC 43982	Human, atypical	L16470
P. nigrescens (*intermedia* serotype II)	ATCC 33563^{T}	Dental plaque (gingivitis)	L16471
P. nigrescens (*intermedia* serotype II)	ATCC 25261	Laryngotomy wound	L16479
P. oralis	ATCC 33269^{T}	Human periodontal pocket	L16480
P. oris	ATCC 33573^{T}	Gingival crevice	L16474
P. oulera	ATCC 43324^{T}	Human gingival crevice	L16472
P. ruminicola subsp. *ruminicola*	ATCC 19189^{T}	Bovine rumen	L16482
P. veroralis	ATCC 33779^{T}	Human oral cavity	L16473
[*P.*] *zoogleoformans*	ATCC 33285^{T}	Human gingival sulcus	L16488
Rikenella microfusus	ATCC 29728^{T}	Quail cecum	L16498
Other			
[*C.*] *fermentans*	ATCC 19072^{T}	Marine mud	M58766[b]
[*C.*] *salmonicolor*	ATCC 19043^{T}	Marine mud	M62422[b]
Cytophaga heparina	ATCC 13125^{T}	Free-living	M61766[b]
Cytophaga lytica	ATCC 23178^{T}	Beach mud	M28058[b]
Flavobacterium aquatile	ATCC 11947^{T}	Fresh water	M28236[b]
Flavobacterium ferrugineum	ATCC 13524^{T}	Free-living	M28237[b]
Flavobacterium gleum	ATCC 35910^{T}	Vagina	M58772[b]
Flavobacterium meningosepticum	ATCC 13253^{T}	Spinal fluid	M58776[b]
Saprospira grandis	ATCC 23119^{T}	Marine water	M58795[b]
Sphingobacter mizutae	ATCC 33299^{T}	Fetal ventricular fluid	N58796[b]
Chlorobium vibrioforme	DSM 260^{T}	Marine water and mud	M62791, M27804[d]

[a] Strains were obtained from the following sources: ATCC; NCTC, London, United Kingdom; Forsyth Dental Center (FDC), Boston, Mass.; and DSM, Deutsche Sammlung von Mikroorganismen (DSM), Braunschweig, Germany.

[b] Source citation is from reference 9.

[c] Brackets around the genus name indicate that the species does not fulfill the sensu stricto definition of its given genus.

[d] Source citation is from reference 38.

TABLE 2. Sequencing primers for the bacteroides subgroup[a]

Primer no.	Position	Sequence (5′–3′)	Specificity
1	104–121	`GTTACTCACCCGTGCGCC`	Bacteroides[b]
2	288–307	`ACCTTCCTCTCAGAACCCCT`	Bacteroides[c]
3	344–358	`ACTGCTGCCTCCCGT`	Most bacteria
4	519–536	`GWATTACCGCGGCKGCTG`	Most bacteria
5	553–572	`TAAACCCAATAAATCCGGAT`	Most bacteria[d]
6	786–803	`CTACCAGGGTATCTAATC`	Most bacteria
7	907–926	`CCGTCAATTCMTTTRAGTTT`	Most bacteria[e]
8	1092–1114	`GGGTTGCGCTCGTTATGGCACTT`	Bacteroides[f]
9	1225–1242	`CCATTGTAACACGTGTGT`	Bacteroides
10	1392–1406	`ACGGGCGGTGTGTRC`	Most bacteria[e]
11	1487–1505	`CCTTGTTACGACTTAGCCC`	Bacteroides

[a] The sequences are complementary to 16S rRNA at the positions listed (*Escherichia coli* nomenclature [5]). Base codes are standard IUB codes for bases and ambiguity.

[b] Specific for most of the prevotella (except for *P. nigrescens* and *P. intermedia*) and bacteroides clusters. Excludes most of the porphyromonas cluster and remaining species.

[c] Specific for all of the prevotella and bacteroides clusters. Of the remaining species, specific for [*B.*] *forsythus*, [*B.*] *distasonis*, [*B.*] *splanchnicus*, and *R. microfusus* only.

[d] Except for *P. asaccharolytica* and *P. endodontalis*.

[e] Except for *P. asaccharolytica*.

[f] Except for [*B.*] *putredinis*, [*B.*] *splanchnicus*, and *R. microfusus*.

species were grown anaerobically at 37°C for 3 to 5 days on Trypticase soy (BBL) agar supplemented with sheep blood.

Isolation and purification of rRNA. RNA was isolated and partially purified by a modification (23) of the procedure of Pace et al. (22).

16S rRNA sequencing. RNA was sequenced directly by using a modification of the Sanger dideoxy-chain termination technique in which reverse transcriptase was used to elongate primers complementary to conserved regions of the 16S rRNA (16). The primers used in this study are shown in Table 2. Since some universally conserved primers (7) were not suitable for sequencing of many *Prevotella*, *Bacteroides*, *Porphyromonas*, and related species, additional specific primers were developed (Table 2; primers 1, 2, 5, 8, 9, and 11). Other details of the sequencing protocol have been described previously (6, 23).

16S rRNA sequence analysis. Programs for data entry, editing, sequence alignment, secondary structure comparison, similarity matrix generation, and phylogenetic tree construction were written in Microsoft QuickBASIC for use on IBM PC-AT and compatible computers. Our sequence data base contains approximately 500 sequences comprising those determined in our laboratory, published sequences, and those available from the Ribosomal Database Project (20). The Ribosomal Database Project data base presently contains approximately 2,000 sequences. Similarity matrices were constructed from the aligned sequences by using only those sequence positions for which data on 90% of strains was available. The similarity matrices were corrected for multiple base changes by the method of Jukes and Cantor (13). The neighbor-joining method of Saitou and Nei (27) was used for phylogenetic tree construction. The reliability of tree nodes was analyzed by using the bootstrapping program MEGA (14). Three hundred bootstrap trees were generated, and bootstrap confidence levels were determined.

Nucleotide sequence accession numbers. The sequences for the bacterial strains examined are available for electronic retrieval from the EMBL, GenBank, and DDBJ nucleotide sequence data bases under the accession numbers listed in Table 1.

RESULTS AND DISCUSSION

Approximately 1,475 bases (or 95%) of the 16S rRNA sequence were determined for each of the 39 strains of *Prevotella*, *Bacteroides*, *Porphyromonas*, and related microorganisms examined in this study. Sequences for *Prevotella denticola* ATCC 35308^{T} and *P. denticola* ATCC 33185 differed by 5 bases. Sequences for *Prevotella buccae* ATCC 33574^{T} and *P. buccae* ATCC 33690 were identical, and sequences for *Prevotella nigrescens* ATCC 33563^{T} and *P. nigrescens* ATCC 25261 differed by 3 bases. Only the sequence of the type strain for each of these three species was used for phylogenetic analysis.

The 16S rRNA sequences for the type strain of *Prevotella oulera* obtained from the American Type Culture Collection (ATCC) (ATCC 43324) and from the National Collection of Type Cultures (NCTC) (NCTC 11871) were shown to be identical to that of *Rikenella microfusus* ATCC 29728^{T}. Phenotypic characteristics determined by ATCC for the type strain of *P. oulera* did not match those traits previously described. Accordingly, a replacement strain of *P. oulera* (strain WPH 179^{T}, equivalent to ATCC 43324^{T}) was obtained from the Scottish Anaerobic Reference Laboratory, University of Edinburgh Medical School, Edinburgh, Scotland. The phenotypic characteristics of the replacement strain matched those of the originally described type strain. NCTC and all individuals who received the previously deposited ATCC type strain of *P. oulera* were informed of this error by ATCC. The sequence for the replacement strain was used for phylogenetic analysis and was deposited with GenBank.

Table 3 shows a similarity matrix that was constructed on the basis of 1,430 base position comparisons for 16S rRNA sequences of 36 strains of species of *Prevotella*, *Bacteroides*, *Porphyromonas*, and related microorganisms, including two representative species of each of the four other subgroups of the CFB phylum (9). *Chlorobium vibrioforme*, a member of the green sulfur bacterial phylum, was used as the outgroup, since this species represents the phylum that branches closest to the CFB phylum (9). Sequences for [*Cytophaga*] *fermentans* and [*Cytophaga*] *salmonicolor* were also included in these analyses, since it was previously suggested that these species were members of the bacteroides subgroup of the CFB phylum (9). A phylogenetic tree (Fig. 1) was derived from the corrected distance matrix by the neighbor-joining method. As shown in Fig. 1, the bacteroides subgroup of the CFB phylum was divided into three major phylogenetic clusters that contained most of the species of *Prevotella*, *Bacteroides*, *Porphyromonas*, and related microorganisms listed in Table 1. [*Bacteroides*] *splanchnicus* fell just outside the three clusters but still was clearly a member of the bacteroides subgroup. Four of the species tested, [*Bacteroides*] *putredinis*, *R. microfusus*, [*C.*] *fermentans*, and [*C.*] *salmonicolor*, formed a deeper cluster that fell outside of [*B.*] *splanchnicus*.

The first major cluster, termed the prevotella cluster, of the bacteroides subgroup was composed of most of the species placed in the genus *Prevotella*, with an average interspecies similarity of approximately 91%. The stability of this cluster was verified by bootstrap analysis with a confidence level of 100%. Species of this cluster are isolated primarily from human oral or urogenital sources, with the notable exception of *Prevotella ruminicola*, which is among the more numerous bacteria isolated from the rumen of most ruminants (10). *P. ruminicola* is the deepest branch of this cluster. It was recently proposed that *P. intermedia* serotype II be reclassified as a new pigmented species of *Prevotella*, *P. nigrescens*, on the basis of physiologic characteristics, DNA-DNA homology, and multilo-

TABLE 3. Similarity matrix

	% Similarity and % difference compared with[a]:																			
Bacterial species	*Pm*1	*Pm*2	*Pve*	*Pde*	*Pco*	*Pdi*	*Pin*	*Pni*	*Pou*	*Por*	*Pbl*	*Pbu*	*Pol*	*Plo*	*Pbi*	*Pru*	*Bov*	*Bth*	*Bfr*	*Beg*
Prevotella melaninogenica	—	99.0	97.2	93.8	92.9	92.2	91.5	91.2	93.6	91.4	88.6	89.0	89.0	90.0	92.4	89.2	85.3	84.9	84.2	85.9
Prevotella melaninogenica 43982	1.0	—	98.0	93.9	93.1	92.4	91.4	91.1	94.1	91.7	88.9	89.2	88.6	90.1	91.9	88.7	85.5	85.0	84.2	85.7
Prevotella veroralis	2.9	2.0	—	92.6	92.4	92.2	91.2	90.6	93.8	91.4	88.8	89.5	88.1	89.6	92.2	89.0	85.2	84.8	83.6	85.4
Prevotella denticola	6.5	6.4	7.7	—	91.1	89.8	89.7	91.2	91.3	91.3	88.4	91.2	88.4	89.9	89.4	87.8	83.4	83.1	82.4	84.5
Prevotella corporis	7.5	7.3	8.0	9.4	—	92.4	92.6	92.9	92.5	90.6	90.0	89.3	88.4	90.2	91.2	88.0	85.0	85.0	83.8	85.2
Prevotella disiens	8.3	8.0	8.3	10.9	8.1	—	93.7	91.9	93.2	89.7	89.3	87.4	87.5	88.5	91.9	87.9	85.1	85.0	84.4	85.4
Prevotella intermedia	9.1	9.2	9.4	11.0	7.7	6.6	—	94.0	91.3	89.9	88.1	87.7	87.0	89.2	90.8	87.6	84.3	83.9	82.7	84.4
Prevotella nigrescens	9.3	9.4	10.1	9.3	7.5	8.6	6.3	—	90.5	89.8	88.5	88.4	87.9	89.4	89.8	86.6	83.8	83.5	82.7	84.4
Prevotella oulera	6.7	6.2	6.5	9.2	7.9	7.1	9.3	10.2	—	92.3	89.3	89.0	88.9	89.4	91.9	88.8	85.5	85.7	85.2	86.8
Prevotella oris	9.1	8.8	9.2	9.3	10.0	11.1	10.9	10.9	8.1	—	91.4	90.4	89.0	90.6	91.7	88.6	83.6	83.0	82.9	84.9
Prevotella buccalis	12.4	12.0	12.1	12.6	10.7	11.5	13.0	12.5	11.5	9.2	—	89.2	90.6	89.9	89.9	88.7	84.3	83.7	83.2	84.8
Prevotella buccae	11.9	11.7	11.3	9.4	11.6	13.8	13.5	12.7	11.9	10.2	11.7	—	89.3	90.5	88.6	87.2	83.7	83.6	82.2	83.6
Prevotella oralis	11.9	12.3	12.9	12.6	12.6	13.7	14.3	13.2	12.0	11.9	10.0	11.5	—	91.0	89.4	87.9	83.8	83.2	81.8	83.7
Prevotella loescheii	10.8	10.6	11.2	10.8	10.6	12.5	11.7	11.4	11.5	10.1	10.9	10.2	9.6	—	90.0	88.1	84.2	83.6	82.7	84.3
Prevotella bivia	8.0	8.6	8.2	11.4	9.4	8.5	9.8	11.0	8.6	8.8	10.8	12.3	11.4	10.7	—	89.6	85.9	85.4	84.9	86.3
Prevotella ruminicola	11.7	12.3	11.9	13.3	13.1	13.1	13.5	14.7	12.1	12.4	12.2	14.1	13.2	13.0	11.1	—	84.6	84.3	84.0	85.0
Bacteroides ovatus	16.4	16.1	16.5	18.8	16.7	16.6	17.6	18.2	16.1	18.5	17.7	18.4	18.2	17.8	15.6	17.3	—	96.9	94.1	94.2
Bacteroides thetaiotaomicron	16.8	16.8	17.0	19.2	16.7	16.7	18.2	18.7	15.8	19.2	18.3	18.6	19.0	18.5	16.2	17.7	3.1	—	95.0	93.8
Bacteroides fragilis	17.7	17.8	18.5	20.0	18.2	17.5	19.7	19.7	16.4	19.4	19.0	20.3	20.9	19.6	16.8	18.0	6.2	5.1	—	93.4
Bacteroides eggerthii	15.6	15.9	16.3	17.4	16.5	16.3	17.5	17.5	14.5	16.7	17.0	18.5	18.3	17.6	15.1	16.8	6.0	6.5	7.0	—
Bacteroides uniformis	16.0	16.3	16.6	17.7	16.5	16.9	17.3	17.1	15.7	16.7	16.2	17.7	17.9	17.8	15.1	16.2	6.2	5.7	7.3	4.7
Bacteroides vulgatus	17.7	17.9	18.4	18.8	18.5	19.1	18.4	18.5	16.8	18.8	18.3	18.2	18.7	18.8	17.7	16.9	9.5	8.9	9.1	8.9
[*Prevotella*] *heparinolytica*	17.1	17.3	18.3	16.7	17.7	18.3	17.7	17.2	16.7	17.7	17.6	17.8	17.6	17.5	16.7	18.0	9.5	8.5	10.1	7.6
[*Prevotella*] *zoogleoformans*	17.6	17.6	18.7	17.0	18.5	19.5	18.8	17.4	17.1	17.9	18.3	18.2	17.1	17.3	17.0	18.3	10.1	9.5	10.9	8.7
Porphyromonas asaccharolytica	24.8	24.5	24.6	26.5	24.2	24.0	25.4	26.7	23.2	25.6	26.1	24.9	26.4	25.4	24.2	26.3	20.8	20.0	21.4	20.9
Porphyromonas endodontalis	22.5	22.1	22.6	24.4	23.0	22.4	23.6	24.2	21.7	24.3	25.0	23.2	25.5	23.1	22.9	24.7	18.1	18.6	19.4	17.9
Porphyromonas circumdentaria	23.2	23.3	23.4	25.4	24.1	23.4	23.8	25.2	22.8	24.9	25.7	23.6	26.0	23.4	23.4	23.9	17.8	17.6	19.9	17.9
Porphyromonas gingivalis	22.5	22.1	23.1	22.9	22.3	21.4	23.0	23.0	21.5	22.4	22.5	22.3	23.8	22.4	21.4	23.8	17.4	17.2	17.6	16.9
Porphyromonas salivosa	24.1	23.8	24.3	25.0	23.8	23.5	25.5	24.7	22.4	24.7	25.3	23.1	25.3	23.0	24.2	25.4	17.2	18.1	19.2	18.7
[*Bacteroides*] *macacae*	24.8	24.5	25.0	26.1	24.0	24.3	25.8	25.3	23.0	25.6	25.9	23.4	26.0	23.5	24.7	26.3	17.2	18.2	19.4	19.5
[*Bacteroides*] *levii*	21.8	22.3	23.8	24.8	24.1	22.3	24.3	24.3	22.4	23.9	25.1	25.6	24.7	23.3	23.4	24.9	17.2	16.7	17.6	16.4
[*Bacteroides*] *forsythus*	20.1	20.0	20.4	21.9	21.0	20.5	21.4	22.1	19.3	21.3	22.7	22.1	22.1	21.5	20.6	22.7	15.6	15.3	15.6	15.1
[*Bacteroides*] *distasonis*	22.2	22.4	23.5	23.2	23.6	24.4	24.8	24.3	21.1	23.1	23.4	23.4	22.9	21.4	22.7	22.1	15.3	15.9	16.1	16.2
[*Bacteroides*] *splanchnicus*	21.2	21.4	21.7	23.7	22.3	21.6	22.5	22.4	20.6	22.9	24.7	24.9	23.7	22.9	22.5	22.9	18.7	18.5	20.1	18.3
[*Bacteroides*] *putredinis*	22.9	23.2	23.9	24.9	23.1	22.8	23.2	23.4	22.3	24.3	24.6	24.8	24.3	23.1	22.7	24.6	21.6	21.3	22.0	20.0
Rikenella microfusus	23.1	23.6	24.2	24.0	23.2	22.1	24.2	23.3	20.9	22.8	23.1	22.5	24.1	22.1	23.8	22.9	21.2	20.6	20.2	19.2
[*Cytophaga*] *fermentans*	22.5	23.0	23.7	24.5	23.0	23.1	25.2	24.0	21.2	24.0	24.9	25.1	24.5	23.9	23.0	23.8	18.4	17.8	18.8	17.3
[*Cytophaga*] *salmonicolor*	21.5	22.0	22.6	23.6	21.9	22.6	23.6	23.7	20.6	23.6	23.3	24.6	23.6	24.3	22.3	23.7	17.5	17.1	18.3	16.2
Sphingobacter mizutae	24.9	25.0	25.2	26.4	26.2	26.4	26.8	26.9	23.6	25.2	25.6	25.5	27.3	25.0	25.9	26.7	22.3	21.9	24.7	21.8
Cytophaga heparina	25.5	26.1	26.3	27.1	25.5	24.5	25.9	27.6	24.2	26.8	26.6	26.1	27.6	26.3	25.1	27.6	21.4	21.8	22.6	22.6
Flavobacterium aquatile	26.1	26.3	26.8	27.0	25.6	27.3	28.8	27.5	25.2	27.4	28.5	28.1	27.2	27.2	26.2	26.6	23.9	23.5	24.8	24.3
Cytophaga lytica	25.7	25.9	26.0	27.9	25.4	25.8	27.3	27.5	24.9	26.4	26.1	26.6	27.7	26.8	24.1	24.4	21.3	22.0	22.6	21.4
Flavobacterium meningosepticum	23.5	23.5	23.3	25.0	24.1	26.1	27.7	26.9	24.4	25.1	26.7	24.5	26.2	25.2	25.5	25.7	21.9	21.3	22.8	22.2
Flavobacterium gleum	25.9	25.6	25.2	28.4	27.5	27.7	29.7	29.4	26.0	27.7	29.0	27.9	28.9	27.6	27.1	28.3	24.3	23.5	24.5	24.0
Saprospira grandis	29.8	30.0	29.5	31.3	30.7	29.1	31.2	31.6	29.4	31.2	30.8	31.0	31.5	29.1	29.5	30.7	29.2	29.4	30.0	29.3
Flavobacterium ferrugineum	30.2	30.3	30.9	30.7	31.4	30.2	32.3	32.2	29.7	31.7	31.4	32.5	32.1	31.7	29.7	29.2	25.7	26.2	26.2	26.5
Chlorobium vibrioforme	36.4	36.4	36.5	36.7	37.3	37.8	38.0	37.2	35.1	36.0	36.9	36.8	37.8	36.1	37.1	36.5	33.3	33.6	34.0	33.1

[a] Numbers above the diagonal are percentages of similarity and those below the diagonal are percentages of difference corrected for multiple base changes. Abbreviations above the columns represent the species listed vertically, in the same order.

cus enzyme electrophoresis profiles (33). By 16S rRNA sequence comparisons, *P. intermedia* (previously classified as *P. intermedia* serotype I) and *P. nigrescens* clearly represented separate species at only 94.7% similarity. These data agree with previous rRNA-DNA hybridization data that suggested the two serotypes warranted separate species designation (12). On the basis of phenotypic characteristics, Shah and Collins placed the oral species [*P.*] *heparinolytica* and [*P.*] *zoogleoformans* in the genus *Prevotella* (28, 31). However, on the basis of 16S rRNA sequence comparisons, they clearly fall with other members in the genus *Bacteroides*.

[*P.*] *heparinolytica*, [*P.*] *zoogleoformans*, *Bacteroides eggerthii*, *B. fragilis*, *B. ovatus*, *B. thetaiotaomicron*, *B. uniformis*, and *B. vulgatus* formed the second major cluster, termed the bacteroides cluster, of the bacteroides subgroup with average interspecies similarities of approximately 93%. The stability of this cluster was also verified by bootstrap analysis with a confidence level of 100%. The average similarity between members of the bacteroides cluster and the prevotella cluster was approximately 85%. The close phylogenetic relationship between [*P.*] *heparinolytica* and [*P.*] *zoogleoformans* (96.6% similarity) was expected because of the high DNA homology (42%) between these two species (1). Unfortunately, DNA hybridizations between these two species and the six species of *Bacteroides* listed above were not performed. To accommodate [*P.*] *heparinolytica* and [*P.*] *zoogleoformans* within the genus *Bacteroides*, it will be necessary to modify the current descriptions of *Bacteroides* and *Prevotella* with respect to certain phenotypic criteria (28, 31). For example, *Bacteroides* species are presently described as being isolated from only mammalian and human fecal sources and thus would not include the oral species [*P.*] *heparinolytica* and [*P.*] *zoogleoformans*.

The third major cluster, termed the porphyromonas cluster, of the bacteroides subgroup was divided into two subclusters. The first subcluster contained *P. gingivalis*, *P. asaccharolytica*, *P. endodontalis*, *P. circumdentaria*, *P. salivosa*, [*Bacteroides*] *levii*, and [*Bacteroides*] *macacae*. The second subcluster contained only two members, [*Bacteroides*] *forsythus* and [*Bacteroides*] *distasonis*. The average interspecies similarity within the porphyromonas cluster was approximately 87%. The average similarity between members of the porphyromonas and prevotella clusters was approximately 80% and between members of the porphyromonas and bacteroides clusters was about 84%. As shown in Fig. 1, the stability of the porphyromonas cluster

TABLE 3—*Continued*

% Similarity and % difference compared with[a]:																											
Bun	*Bvu*	*Phe*	*Pzo*	*Pas*	*Pen*	*Pci*	*Pgi*	*Psa*	*Bma*	*Ble*	*Bfo*	*Bdi*	*Bsp*	*Bpu*	*Rmi*	*Cfe*	*Csa*	*Smi*	*Che*	*Faq*	*Cly*	*Fme*	*Fgl*	Sgr	Ffe	Cvi
85.6	84.2	84.7	84.3	78.9	80.6	80.0	80.5	79.4	78.9	81.1	82.4	80.8	81.5	80.3	80.1	80.6	81.3	78.8	78.4	77.9	78.2	79.9	78.1	75.4	75.1	71.2
85.3	84.1	84.4	84.3	79.1	80.9	80.0	80.9	79.6	79.1	80.7	82.5	80.7	81.4	80.0	79.8	80.2	81.0	78.7	78.0	77.8	78.1	79.9	78.3	75.3	75.1	71.2
85.1	83.7	83.7	83.5	79.1	80.5	79.9	80.1	79.2	78.7	79.6	82.1	79.9	81.1	79.6	79.3	79.7	80.5	78.6	77.8	77.4	78.0	80.0	78.6	75.6	74.6	71.1
84.3	83.4	85.0	84.8	77.7	79.1	78.5	80.3	78.7	78.0	78.9	81.0	80.0	79.7	78.8	79.4	79.1	79.8	77.8	77.2	77.3	76.7	78.7	76.3	74.4	74.8	71.0
85.2	83.6	84.2	83.6	79.3	80.2	79.4	80.7	79.6	79.5	79.4	81.7	89.8	80.7	80.1	80.0	80.2	81.0	77.9	78.4	78.3	78.4	79.4	77.0	74.8	74.3	70.6
84.9	83.1	83.7	82.8	79.5	80.7	79.9	81.4	79.8	79.3	80.7	82.1	79.2	81.3	80.3	80.9	80.1	80.5	77.7	79.1	77.1	78.2	78.0	76.8	75.9	75.1	70.3
84.6	83.7	84.2	83.4	78.4	79.8	79.6	80.2	78.4	78.2	79.3	81.4	78.9	80.5	80.0	79.3	78.6	79.7	77.5	78.1	76.1	77.1	76.8	75.5	74.5	73.8	70.2
84.7	83.6	84.7	84.4	77.5	79.3	78.6	80.2	79.0	78.5	79.2	80.9	79.2	80.6	79.9	79.9	79.5	79.7	77.4	76.9	77.0	77.0	77.4	75.7	74.2	73.8	70.7
85.9	85.0	85.0	84.7	80.1	81.1	80.3	81.3	80.6	80.2	80.7	83.0	81.6	82.0	80.7	81.8	81.5	82.0	79.8	79.3	78.6	78.8	79.2	78.0	75.7	75.5	72.0
85.0	83.4	84.3	84.1	78.3	79.2	78.8	80.7	78.9	78.3	79.5	81.5	80.1	80.2	79.2	80.3	79.5	79.8	78.6	77.5	77.1	77.7	78.7	76.8	74.4	74.2	71.4
85.5	83.7	84.3	83.8	77.9	78.7	78.2	80.6	78.5	78.1	78.7	80.4	79.9	79.0	79.0	80.1	78.8	80.0	78.3	77.6	76.3	78.0	77.5	75.9	74.7	74.4	70.9
84.2	83.8	84.2	83.8	78.8	80.0	79.7	80.7	80.1	79.9	78.3	80.8	79.9	78.8	78.9	80.5	78.7	79.0	78.4	77.9	76.6	77.6	79.1	76.7	74.6	73.6	70.9
84.1	83.5	84.3	84.7	77.7	78.4	78.0	79.6	78.5	78.0	79.0	80.9	80.2	79.7	79.3	79.4	79.1	79.8	77.1	76.9	77.2	76.9	77.9	76.0	74.2	73.9	70.3
84.2	83.4	84.4	84.6	78.4	80.1	79.9	80.6	80.2	79.8	80.0	81.3	81.4	80.3	80.1	80.9	79.5	79.2	78.7	77.8	77.2	77.5	78.6	76.9	75.9	74.2	71.3
86.3	84.3	85.0	84.8	79.3	80.3	79.9	81.4	79.3	79.0	79.9	82.0	80.4	80.5	80.4	79.6	80.2	80.7	78.1	78.7	77.9	79.4	78.4	77.3	75.6	75.5	70.7
85.5	84.9	84.0	83.7	77.8	79.0	79.6	79.6	78.4	77.8	78.8	80.4	80.9	80.3	79.0	80.3	79.6	79.7	77.6	77.0	77.6	79.1	78.3	76.4	74.8	75.8	71.1
94.0	91.1	91.1	90.5	81.8	83.9	84.2	84.4	84.6	84.6	84.7	85.9	86.2	83.5	81.3	81.5	83.7	84.4	80.7	81.4	79.5	81.5	81.0	79.3	75.8	78.2	73.1
94.5	91.6	91.9	91.0	82.5	83.5	84.3	84.6	83.9	83.9	85.0	86.2	85.7	83.6	81.4	82.0	84.2	84.7	81.0	81.1	79.9	81.0	81.4	79.8	75.7	77.9	72.9
93.1	91.4	90.6	89.9	81.4	82.9	82.5	84.3	83.0	82.9	84.3	85.9	85.5	82.3	80.9	82.3	83.3	83.8	79.0	80.5	78.9	80.5	80.4	79.1	75.2	77.9	72.7
95.4	91.6	92.8	91.8	81.8	84.1	84.0	84.9	83.4	82.8	85.3	86.3	85.4	83.8	82.5	83.1	84.6	85.5	81.1	80.5	79.2	81.4	80.8	79.4	75.7	77.7	73.2
---	92.7	94.1	93.6	81.4	83.9	84.1	85.0	83.2	82.9	84.1	85.7	86.3	83.1	81.5	82.6	84.1	84.9	81.3	80.6	78.5	81.2	80.3	79.7	75.0	77.7	72.6
7.7	---	91.7	91.0	81.5	83.3	83.2	84.7	83.5	83.0	83.3	85.8	84.2	82.4	80.1	80.3	82.4	83.7	79.4	79.2	78.2	79.4	79.3	78.6	75.1	76.4	72.6
6.1	8.8	---	96.7	80.0	82.1	82.7	83.2	82.3	81.9	82.9	84.5	84.5	82.7	80.2	80.8	81.7	83.2	79.8	79.4	77.5	78.7	78.8	78.0	74.3	76.3	73.0
6.7	9.6	3.4	---	80.2	81.5	82.7	82.9	82.2	82.0	81.8	83.9	84.3	81.9	79.8	80.5	81.2	82.6	79.3	78.6	77.7	78.4	78.3	77.8	73.7	76.7	72.6
21.4	21.2	23.3	23.0	---	87.8	86.8	85.1	84.9	84.7	84.8	84.5	81.7	79.9	81.4	79.8	80.4	80.6	77.3	78.6	77.8	77.9	79.2	76.4	75.7	76.7	72.3
18.1	19.0	20.4	21.2	13.7	---	93.7	87.7	87.8	87.6	86.9	86.0	83.2	80.8	82.1	82.6	83.5	83.6	81.2	80.2	79.3	81.0	80.3	78.4	76.4	75.1	73.3
17.9	19.1	19.7	19.6	14.5	6.6	---	86.5	87.5	87.5	86.2	85.4	83.0	80.2	82.6	83.0	83.2	83.3	80.8	79.3	78.5	81.1	80.5	78.7	76.1	75.5	73.9
16.8	17.1	19.0	19.4	16.6	13.3	14.8	---	88.8	88.3	86.5	87.5	83.8	81.6	82.2	82.2	82.5	83.1	80.7	80.7	78.9	80.2	80.9	78.7	76.8	75.8	72.4
19.1	18.6	20.2	20.3	16.9	13.3	13.7	12.2	---	99.6	87.8	87.1	84.9	80.1	82.0	82.8	84.0	82.8	79.7	80.1	78.4	79.7	79.5	77.2	75.4	75.9	73.2
19.4	19.3	20.7	20.5	17.1	13.6	13.6	12.8	0.4	---	87.3	86.6	84.5	80.0	81.5	82.7	83.7	82.5	79.3	79.7	78.0	79.7	79.0	76.8	74.9	75.5	72.9
17.9	18.9	19.4	20.9	17.0	14.4	15.3	14.9	13.4	13.9	---	87.1	84.9	82.8	82.8	82.9	84.2	85.0	80.7	80.5	78.2	80.1	81.3	77.8	75.0	75.7	71.8
15.8	15.7	17.4	18.2	17.3	15.5	16.2	13.6	14.2	14.7	14.2	---	88.7	84.8	84.0	83.2	84.2	84.1	81.0	80.6	78.8	80.3	81.9	79.1	75.7	77.2	74.1
15.1	17.8	17.3	17.6	20.9	19.0	19.3	18.2	16.8	17.4	16.9	12.3	---	83.5	82.1	82.3	83.0	83.0	80.3	80.2	78.3	79.0	79.9	77.5	74.8	77.3	73.4
19.2	20.0	19.7	20.7	23.4	22.1	22.9	21.1	23.1	23.3	19.6	17.0	18.6	---	83.2	82.1	84.2	85.8	80.0	79.5	80.9	80.4	82.7	79.9	77.5	76.9	71.7
21.2	23.1	22.9	23.5	21.4	20.4	19.9	20.3	20.6	21.2	19.5	18.0	20.5	19.1	---	88.4	84.5	84.8	81.6	81.8	81.2	80.8	83.5	81.8	79.3	77.5	73.7
19.8	22.8	22.2	22.6	23.5	19.8	19.3	20.3	19.5	19.7	19.5	19.0	20.2	20.5	12.5	---	86.9	86.2	83.6	82.9	80.7	82.4	83.7	81.8	77.0	75.9	73.3
17.9	20.0	21.0	21.7	22.7	18.6	19.1	20.0	18.0	18.4	17.7	17.7	19.3	17.7	17.4	14.4	---	90.3	83.3	83.1	85.5	85.6	85.3	82.4	78.2	78.3	74.1
16.9	18.3	19.0	19.8	22.4	18.5	18.9	19.2	19.6	19.9	16.7	17.8	19.2	15.8	17.0	15.2	10.4	---	83.3	83.0	82.7	83.5	85.3	83.0	78.5	78.2	73.6
21.6	24.0	23.5	24.2	27.1	21.6	22.1	22.3	23.7	24.2	22.3	22.0	22.9	23.3	21.1	18.5	18.8	18.9	---	89.1	79.1	81.1	83.4	81.2	78.5	75.7	74.2
22.4	24.4	24.1	25.2	25.2	23.0	24.3	22.3	23.1	23.7	22.5	22.4	22.9	23.9	20.8	19.5	19.2	19.3	11.8	---	80.5	81.8	82.7	79.1	78.9	78.0	73.5
25.3	25.8	26.7	26.4	26.3	24.2	25.4	24.8	25.5	26.0	25.7	24.9	25.6	22.1	21.7	22.3	16.1	19.7	24.5	22.5	---	89.6	86.1	83.4	79.5	79.4	73.7
21.7	24.1	25.1	25.5	26.2	21.9	21.8	23.0	23.6	23.7	23.1	22.9	24.6	22.8	22.1	20.1	16.0	18.6	21.7	20.8	11.2	---	85.5	82.0	78.4	77.7	72.8
22.9	24.2	24.8	25.7	24.4	22.8	22.5	22.1	23.9	24.6	21.5	20.7	23.4	19.6	18.6	18.4	16.3	16.4	18.8	19.6	15.4	16.2	---	94.1	82.0	79.1	76.0
23.6	25.2	26.0	26.3	28.3	25.5	25.1	25.1	27.2	27.7	26.3	24.5	26.8	23.4	20.8	20.9	20.1	19.2	21.7	24.5	18.7	20.5	6.1	---	80.5	78.4	74.3
30.5	30.2	31.4	32.3	29.4	28.4	28.7	27.8	29.8	30.5	30.4	29.4	30.8	26.8	24.3	27.5	25.7	25.3	25.3	24.8	24.0	25.5	20.6	22.5	---	79.1	73.3
26.5	28.4	28.5	28.0	27.9	30.2	29.6	29.2	29.1	29.6	29.3	27.1	27.0	27.6	26.8	29.0	25.6	25.7	29.3	26.0	24.0	26.5	24.5	25.5	24.5	---	72.3
34.2	34.1	33.3	34.1	34.5	33.0	32.1	34.4	33.2	33.8	35.4	31.7	32.9	35.2	32.3	33.1	31.8	32.5	31.6	32.7	32.4	33.8	29.0	31.5	33.1	34.6	---

survived bootstrap analysis with a confidence level of 96%. Although the generic positions of [*B.*] *levii* and [*B.*] *macacae* were previously uncertain, the 16S rRNA data are consistent with the previous suggestion that they are related to other species of *Porphyromonas* on the basis of physiologic characteristics (28). These data also agree with previous findings from rRNA-DNA hybridization studies which showed that [*B.*] *levii* and [*B.*] *macacae* clustered with species of *Porphyromonas* (12). Surprisingly, *P. salivosa* was found to be closely related to [*B.*] *macacae* at 99.5% sequence similarity. *P. salivosa* and [*B.*] *macacae* possess different phenotypic traits and are isolated from different hosts (17). More detailed experiments are needed to clarify the phylogenetic relatedness of these two species. In the second subcluster, [*B.*] *forsythus* and [*B.*] *distasonis* were related to each other at about 89% similarity and to members of the first subcluster of the porphyromonas cluster, with an average similarity of about 86%. Because of this phylogenetic depth, it is uncertain whether [*B.*] *forsythus* and [*B.*] *distasonis* should be considered species of *Porphyromonas* or whether they constitute one or more separate genera. Other criteria will have to be used to determine the taxonomic status of these two species. The phylogenetic position of [*Bacteroides*] *furcosus* remains to be determined; however, this species should fall within the porphyromonas cluster on the basis of rRNA-DNA hybridization data (12).

The phylogenetic relationships of species of the bacteroides subgroup determined in this study corresponded well with those obtained by Johnson and Harich from rRNA-DNA hybridization data (12). One discrepancy was that [*B.*] *distasonis* was placed with species of *Bacteroides*. However, in a review of their data, [*B.*] *distasonis* was equally distant from species of *Bacteroides* and species of *Porphyromonas*. Consequently, the 16S rRNA data presented here and elsewhere (9, 25) clearly place [*B.*] *distasonis* in the *Porphyromonas* cluster and do not support the recent proposal that it be included within the genus *Bacteroides* (28, 31).

[*B.*] *splanchnicus* branched off separately from the three major clusters described above. However, on the basis of a bootstrap analysis confidence level of 93% (Fig. 1), [*B.*] *splanchnicus* clearly belongs within the bacteroides subgroup of the CFB phylum. However, its exact position remains in question, with only a 68% bootstrap confidence level. On the basis of phenotypic data, the taxonomic position of [*B.*] *splanchnicus* has been uncertain except for the suggestion that it was not a species of *Bacteroides* (28). Since [*B.*] *splanchnicus*

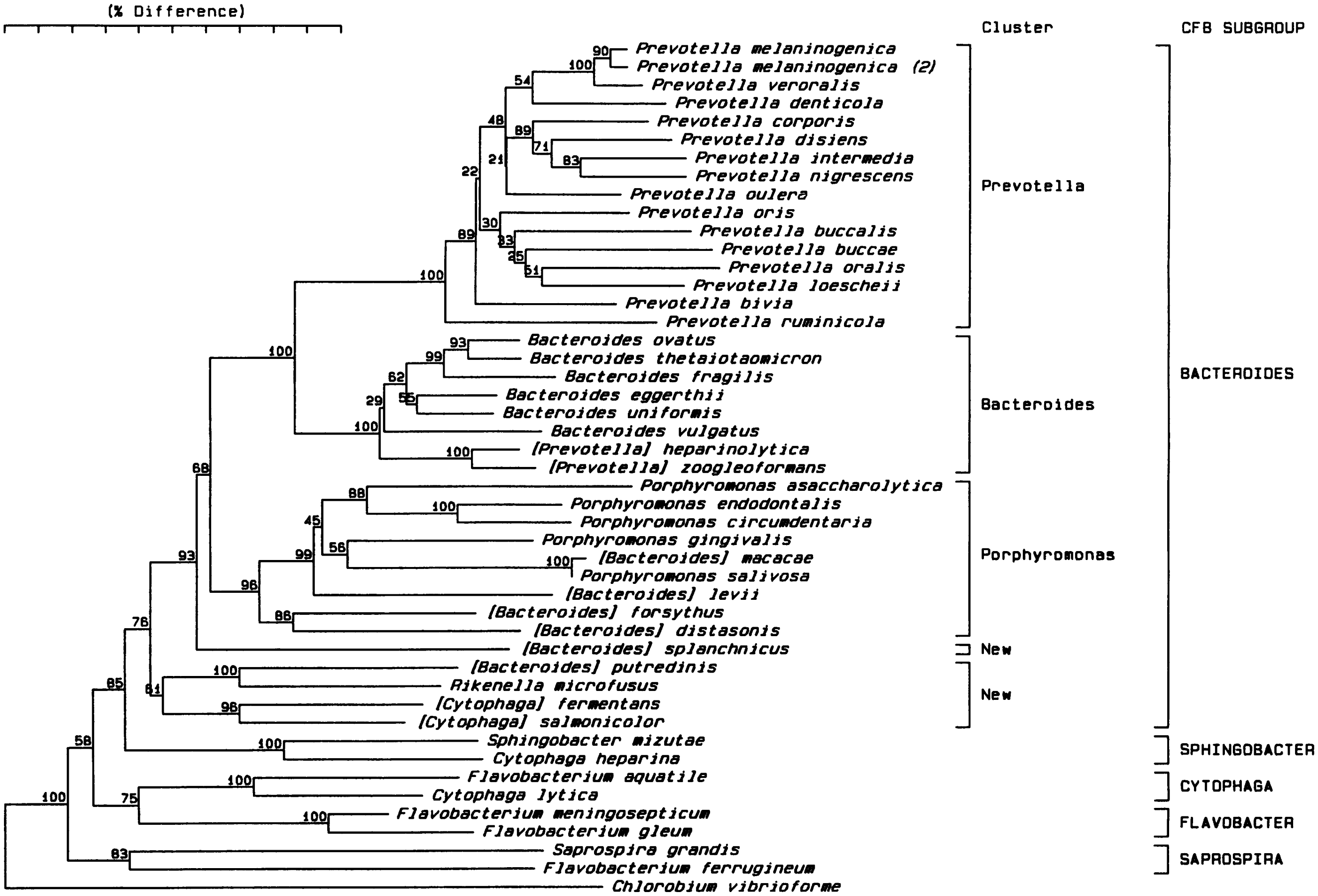

FIG. 1. Phylogenetic tree for the bacteroides subgroup of the CFB phylum. The five subgroups of the CFB phylum and the phylogenetic clusters within the bacteroides subgroup are indicated by brackets. Numbers above each node are confidence levels (%) generated from 300 bootstrap trees (14). The scale bar represents a 10% difference in nucleotide sequence determined by taking the sum of all of the horizontal lines connecting two species.

is not closely related to any other species of the subgroup, this species most likely represents a new genus.

The remaining species, i.e., *R. microfusus*, [*B.*] *putredinis*, and the two *Cytophaga* species, formed a cluster that branched outside of [*B.*] *splanchnicus*. [*B.*] *putredinis* and *R. microfusus* clustered together at about 89% similarity. It remains to be determined whether [*B.*] *putredinis* should be included within the genus *Rikenella* or forms a separate genus. The free-living species *C. fermentans* and *C. salmonicolor* clustered together at about 91% similarity. These species are clearly misclassified as species of *Cytophaga*, since they are not related to other *Cytophaga* species on the basis of 16S rRNA sequence comparisons (9). Consequently, a new genus should be created to accommodate them.

Because of a bootstrap confidence level of only 76%, it is not clear whether these remaining species fall within the bacteroides subcluster or form at least one additional subgroup of the CFB phylum. However, analysis of base signatures (Table 4) indicates that these species be included within the bacteroides subgroup. As shown in Table 4, two key base signatures—an A:U pair at base positions 947:1234 and a G at base position 1202—were found in members of the three major clusters, [*B.*] *splanchnicus*, *R. microfusus*/[*B.*] *putredinis*, and the two [*Cytophaga*] species but not in members of the other CFB subgroups or in most other bacterial phyla. Other base signatures, such as those at positions 42:400 and 897:902 indicate that the two [*Cytophaga*] species be included in the bacteroides subgroup while *R. microfusus*/[*B.*] *putredinis* be excluded. Signatures at positions 943:1340 excluded only [*C.*] *fermentans* but included members of the *Sphingobacter* subgroup of the CFB phylum.

The bacteroides cluster, the prevotella cluster, and the porphyromonas cluster were also defined according to single-base signatures, as shown in Table 4. Signatures were chosen on the basis of invariance of base composition within each of the clusters. Base signatures of special note are the U:A pair at positions 41:401 for members of the prevotella and bacteroides clusters. The ancestral composition, defined as the dominant base composition in sequences of most other bacteria (9), is R:Y at these positions. At position 547, a G occurs in sequences of all members of the prevotella cluster, whereas an A is the ancestral composition (with the exception of some *Corynebacterium* species). In addition, the base pair C:G at positions 1089:1096 is unique for members of the three major phylogenetic clusters of the bacteroides subgroup. All other bacteria possess a G:C at these positions.

Exact 16S rRNA similarity limits have not been established for defining specific taxa such as genus and species. Previously, we suggested that phylogenetic clustering of bacterial groups, rather than a specific similarity value, be used as a guide for

TABLE 4. Signature analysis of the bacteroides subgroup of the CFB phylum

Position of base or pair[a]	Base composition in:[b]							
	Prevotella cluster	Bacteroides cluster	Porphyromonas cluster	[*B.*] *splanchnicus*	*R. microfusus*, [*B.*] *putredinis*	[*C.*] *salmonicola*, [*C.*] *fermentans*	Other CFB subgroups	Ancestral composition[c]
41:401	**U:A**	**U:A**	G:C	G:C	G:C	G:C	G:C	G:C
42:400	**A:U**	**A:U**	**A:U**	**A:U**	G:C	**A:U**	G:C	G:C
122	G	**A**	G (A, *Pas*)	G	G	G	R	R
128:233	**C:G**	**C:G**	G:Y	G:C	G:C	G:C	R:Y	G:C
503:542	**C:G**	U:A	U:A	U:A	U:A	U:A	Y:R	—
511:540	**U:A**	C:G	C:G	C:G	C:G	C:G	C:G	C:G
547	**G**	A	A	A	A	A	A	A*
578:763	**A:U**	**A:U**	U:A	U:A	U:A	U:A	U:A (a:u)	Y:R
599:639	**G:C**	C:G	C:G	U:A	U:R	C:G	C:G	Y:R
600:638	**U:G**	A:U	R (C, *Bfo*):U	A:U	A:U	A:U	A:U (u:g)	—
770:809	**U:A** (C:G, *Plo*)	U:A	C:G	C:G	C:G	C:G	C:G (u:a)	C:G
772:807	**C:G** (U:A, *Plo*; U:G, *Pou*)	U:A	U:A	U:A	U:A	U:A	U:A	U:A
897:902	**G:C**	**G:C**	**G:C**	**G:C**	C:G	**G:C**	C:G (g:c)	C:G
943:1340	**A:U**	**A:U**	**A:U**	**A:U**	**A:U**	**A:U** and U:A	U:A (**A:U**[d])	Y:R*
947:1234	**A:U**	**A:U**	**A:U**	**A:U**	**A:U**	**A:U**	K:M	K:M*
996:1045	Y:R	U:A	A:U (C:G, *Bdi*)	U:R	A:U	U:A	U:A and A:U	A:C*
1089:1096	**C:G**	**C:G**	**C:G**	G:C	G:C	G:C	G:C	G:C*
1156	**Y**	G	G	G	G	G	R	R
1202	**G**	**G**	**G**	**G**	**G**	**G**	U	U
1224	Y	U	**G**	U	K	U	Y (r)	—
1247	Y	U	**G**	U	U	U	K	—

[a] Base position numbering of *E. coli* (5).

[b] Base signatures that define each clad or group of clads are underlined and in boldface type. Exceptions to signatures are noted in parentheses.

[c] Bacterial name abbreviations: *Pas*, *P. asaccharolytica*; *Bfo*, [*B.*] *forsythus*; *Plo*, *P. loescheii*; *Pou*, *P. oulera*. Nucleotide abbreviations: R, A or G; Y, C or U; K, G or U; M, A or C. Lowercase letters indicate a base or base pair that is present in 6% or less of the available sequences in the group listed.

[c] Defined as the dominant composition in the majority of the 10 bacterial phyla (9). *, >98% of the sequences have this base or base pair composition (excluding the clad or clads under signature analysis). —, ancestral composition is uncertain or not defined.

[d] Only the sphingobacter subgroup of the CFB phylum has the A:U pairing (9).

defining bacterial taxa (24). Depending upon the bacterial phylum under investigation, different limits have been used. For example, species of the genus *Treponema* cluster together with average interspecies similarities of 84% (24), whereas species of *Campylobacter* cluster together with average interspecies similarities of 94% (23). In general, species have been defined on the basis of sequence similarities of greater than 99%. However, the identification of strains of uncertain taxonomic status to species level can be verified by using other experimental methods, such as DNA-DNA hybridization (8).

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