Chemotaxonomy of bacteroides: A review

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The loose definition of *Bacteroides*, some species of which are important etiologic agents of oral diseases, has enabled isolates with only marginal similarities to be reposited in this genus. Many attempts have been made over the years to improve the taxonomy of this heterogeneous group of bacteria. The present article reviews major chemotaxonomic characters and techniques that have been used for this purpose: pigmentation, metabolites, whole-cell fatty acids, phospholipids, isoprenoid quinones, carbohydrates of lipopolysaccharide, whole-cell proteins, peptidoglycans, enzymes, pyrolysis mass spectrometry, DNA composition, restriction fragment length polymorphisms of DNA and ribosomal (r) RNA, homology of DNA and RNA, DNA-rRNA hybridization, and 16S and 5S rRNA oligonucleotide cataloging and sequencing. Despite improvements in their taxonomy, some bacteroides are still misclassified. Suggestions for further improvements in the taxonomy of bacteroides are made. □ *Bacteria;* Bacteroides; *chemistry; chemosystematics;* Porphyromonas; Prevotella

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Bacteroides was previously defined as obligately anaerobic, gram-negative, non-sporulating, pleomorphic rods with a range in the guanine (G) plus cytosine (C) contents varying broadly from 28 to 61 mol% (1). Unfortunately, this loose definition enabled isolates with only marginal similarities to be reposited in the genus. Many attempts have been made over the years to improve the taxonomy of this heterogeneous group of bacteria. Despite these efforts, some bacteroides are still misclassified. Chemotaxonomy, defined as the study of chemical variation in living organisms, and the use of chemical characters for classification and identification, have won wide popularity among taxonomists classifying oral pathogens, such as *Actinobacillus actinomycetemcomitans* and related organisms (for a review, see Olsen (2)). Since bacteroides are considered major etiologic agents in oral diseases such as periodontal and endodontic infections and in submucosal infections, the present article will give an overview of important contributions made in their classification and identification, using chemotaxonomic characters and techniques (Table 1). The review will end with suggestions for further improvements in the taxonomy of bacteroides. For additional information on the taxonomy of this group of bacteria, reviews by Holdeman et al. (1), Shah (3, 4), and Tanner et al. (5) should be consulted.

Genus *Bacteroides*, *Prevotella*, and *Porphyromonas*

To start with the end, Shah & Collins (6–8), prompted by the increasing taxonomic diversity of the bacteroides group, recently proposed that the genus *Bacteroides* should be divided into the following three genera: 1) *Bacteroides sensu stricto*, consisting of saccharolytic, non-pigmenting species such as the type species, *B. fragilis*, and its close relatives; 2) *Prevotella*, consisting of moderately saccharolytic, bile-sensitive, predominantly oral species, such as *Pr. melaninogenica* and *Pr. intermedia*; and 3) *Porphyromonas*, generally consisting of asaccharolytic, black-pigmenting species, such as *P. gingivalis* and *P. asaccharolytica*.

Table 1. Chemosystematic characters/techniques used to improve the taxonomy of bacteroides

Pigmentation of colonies
Metabolites
Whole-cell fatty acids
Phospholipids
Isoprenoid quinones
Carbohydrates of lipopolysaccharide
Whole-cell proteins
Peptidoglycans
Enzymes
Pyrolysis mass spectrometry
DNA composition
DNA restriction fragment length polymorphisms
rRNA restriction fragment length polymorphisms
DNA homology
RNA homology
DNA-rRNA hybridization
16S and 5S rRNA oligonucleotide cataloging and sequencing

Table 2. New genera/species derived from the genus *Bacteroides*

New designation	Old designation
Anaerorhabdus	*Bacteroides furcosus*
Fibrobacter	*Bacteroides succinogenes*
Megamonas	*Bacteroides hypermegas*
Mitsuokella	*Bacteroides multiacidus*
Rikenella	*Bacteroides microfusus*
Ruminobacter	*Bacteroides amylophilus*
Sebaldella	*Bacteroides termitidis*
Tissierella	*Bacteroides praeacutus*
Capnocytophaga	*Bacteroides ochraceus*
Eikenella	*Bacteroides corrodens*
Dichelobacter	*Bacteroides nodosus*
Oribacilum catoniae	*Bacteroides* VPI D26
Catonella morbi	*Bacteroides* VPI D42
Hallella seregens	*Bacteroides* VPI D12
Johnsonella ignava	*Bacteroides* VPI D19
Dialister	*Bacteroides pneumosintes*

Recently, *Pr. nigrescens* was established as a new species in the genus *Prevotella* (9), and *P. circumdentaria* and *P. salivosa* (from cats) in the genus *Porphyromonas* (10). Additional genera, most of which are monospecific, have been proposed for some of the most atypical bacteroides (Table 2) (3, 11, 12).

Chemotaxonomic characters/ techniques

Pigmentation of colonies

Pigmentation of bacteroides colonies on blood agar may vary depending on the medium (13). Pigmentation of *Prevotella* and *Porphyromonas* species varies from shades of brown to green, changing to black on prolonged incubation. *Porphyromonas* species develop pigment because of their production of major amounts of protoheme rather than protoporphyrin (14). *Pr. intermedia*, *Pr. melaninogenica*, *Pr. loescheii*, *Pr. corporis*, and some strains of *Pr. denticola* produce protoheme and protoporphyrin. *Pr. denticola* is a slow producer of pigment: some strains do not form pigment even after 3 weeks of incubation (15). Pigment production is also variable among strains of *Pr. melaninogenica* (16). A non-pigmented *Pr. corporis* would be phenotypically virtually identical to *Pr. disiens*. If they do not produce pigment, *Pr. denticola* and *Pr. melaninogenica* can easily be confused with *Pr. buccalis*, *Pr. veroralis*, and *Pr. oralis*. Pigmentation on blood agar can no longer be considered a reliable characteristic in the taxonomy of bacteroides.

Metabolites

The genus *Bacteroides sensu stricto* contains species that produce major amounts of acetate and succinate as metabolic end products from peptone yeast glucose medium (17, 18). *Porphyromonas* species form *n*-acetic and butyric acids as major end products with lower levels of propionic, isobutyric, and isovaleric acids (4, 6). Metabolic acids were recently used to distinguish between oral non-pigmented *Prevotella* species (19). Most species produced succinic and acetic acid as major products and lactic acid as a minor product. Production of isovaleric, isobutyric, propionic, and phenylacetic acids was variable. Metabolic acids for classification and identification of *Bacteroides*, *Prevotella* and *Porphyromonas* species have also been reported elsewhere (20–25). Furthermore, the pattern of carbohydrate utilization, as

determined with high-pressure liquid chromatography in a defined medium, has been used to differentiate *Bacteroides* species (26). The pattern of metabolic acids is a useful adjunct in the taxonomy of bacteroides.

Whole-cell fatty acids

Authors have grouped bacteroides on the basis of similarity indices (27, 28) and multivariate analyses of cellular fatty acid data (29–32). Multivariate analyses discerned false bacteroides such as *B. gracilis* and *B. ureolyticus* from true bacteroides. Diagnostic keys based on ratios among the significant fatty acids in the cellular profile have also been used (33). Furthermore, grouping has been made on the basis of the patterns of C_{15} non-hydroxy acids detected (34). In summary, the *B. fragilis* group has a-15:0 as the predominant cell fatty acid, and the *Porphyromonas* group i-15:0 as the major acid (14). In the fermentative pigmented species of the *Prevotella* group a-15:0 dominates, and in non-pigmented saccharolytic *Prevotella* species, a-15:0, i-15:0, and 15:0 are predominant. Obviously, the amount and type of cellular fatty acids are useful criteria for grouping bacteroides.

Phospholipids

Sphingophospholipids in *Bacteroides* species constitute 40% to 70% of the total lipids (34) and are considered important features to substantiate the genus (7, 27, 35). Sphingolipids are absent from false *Bacteroides* species such as *B. amylophilus*, *B. furcosus*, *B. hypermegas*, *B. multiacidus*, and *B. succinogenes* (36, 37). Identified sphingophospholipids in bacteroides include ceramide-1-phosphoethanolamine (34), ceramide-1-phosphoglycerol and its phosphate ester (38, 39), and ceramide-1-phosphocholine (sphingomyelin) (40).

Isoprenoid quinones

The *B. fragilis* group of organisms is relatively homogeneous in its respiratory quinone content, usually containing major proportions of menaquinone (MK)-10 or MK-11, or both (7) (Table 3). In *Porphyromonas* the principal respiratory quinones are unsaturated menaquinones with 9 or 10 isoprene units (6). *P. gingivalis* strains have menaquinones with nine isoprene units. *P. asaccharolytica* possesses menaquinones with 10 isoprene units, whereas the polyprenyl side chain of *P. endodontalis* is not known (4). In *Prevotella*, unsaturated menaquinones with 10 to 13 isoprene units dominate (8). *B. nodosus*, *B. preacutus*, and other atypical *Bacteroides* species such as *B. furcosus*, *B. hypermegas*, and *B. multiacidus* lack both ubiquinones and menaquinones (18, 41, 42). *B. gracilis* contains MK-6, but the methyl-substituted naphthoquinone thermoplasmaquinone-6 is the major quinone (43). Isoprenoid quinones are helpful criteria to define genera among bacteroides.

Carbohydrates of lipopolysaccharide

On the basis of negative results with the widely used thiobarbituric assay (TBA) it was claimed that *Bacteroides* has no 2-keto-3-deoxy-octonate (KDO) and heptose in its lipopolysaccharide (LPS) (44–46). KDO deficiency was proposed as a taxonomic criterion for this genus. Unfortunately, a negative TBA does not necessarily exclude the presence of KDO, which can be phosphorylated in the native LPS. Dephosphorylation of LPS from *B. fragilis* with 50% aqueous hydrofluoric acid before the TBA gave a positive response (47). Whereas previous investigations had reported absence of heptose and KDO in *B. gingivalis* LPS, Johne et al. (48) detected these substances in small amounts here. Later, Kumada et al. (49), Bramanti et al. (50), Bramanti & Holt (51), and Fujiwara et al. (52) observed that KDO occurs as a phosphorylated molecule and that strong hydrolysis is needed to detect it. KDO has also been found in *B. ovatus* (53), *Pr. intermedia* (48, 54), and *B. fragilis* (47) and seems to be a common component in LPS of gram-negative bacteria. Its usefulness for the taxonomy of bacteroides has been overestimated.

Table 3. Major menaquinones in *Bacteroides**

Major quinone	Organism
MK-10, MK-11	*B. fragilis, B. ovatus, B. thetaiotaomicron, B. vulgatus, Pr. oralis, B. eggerthii, Pr. melaninogenica, Pr. oulora, Pr. veroralis*
MK-11, MK-12	*Pr. ruminicola, Pr. denticola*
MK-12, MK-13	*Pr. buccalis, B. pentosaceus,*
MK-13	*Pr. oralis*
MK-9	*P. gingivalis, B. levii, B. splanchnicus*
MK-10	*P. asaccharolytica, B. distasonis*
MK-11	*Pr. intermedia*

* Adopted from Ref. 34.

Whole-cell proteins

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to compare cellular protein patterns from 91 periodontal isolates with those from reference strains of non-pigmented *Prevotella* species (55). A close correlation appeared between patterns of results from SDS-PAGE profiles and biochemical and gas–liquid chromatography (GLC) tests. For most isolates SDS-PAGE protein profiles enabled good differentiation and characterization. Variant SDS-PAGE profiles divided clinical isolates of *Pr. buccae* into two subgroups and those of *Pr. veroralis* in five subgroups. Similar distinction was achieved between *Pr. loescheii*, *Pr. oralis*, *Pr. melaninogenica*, and *Pr. denticola* (56). Despite the advantages of this system, comparative electrophoresis of proteins has not yet won wide popularity in bacteroides taxonomy.

Peptidoglycan

The cell wall peptidoglycan of *Bacteroides sensu stricto* and *Prevotella* contains *meso*-diaminopimelic acid (7, 8). The peptidoglycan of *Porphyromonas* does not contain diaminopimelic acid as the diamino acid, but lysine (6). Peptidoglycan composition is a useful chemical criterion to substantiate bacteroides taxa.

Enzymes

Dehydrogenase patterns. Cellular dehydrogenase patterns have been useful for defining the genus *Bacteroides* (57, 58). Whereas *Bacteroides sensu stricto* species contain glucose-6-phosphate dehydrogenase (G-6PDH), 6-phosphogluconate dehydrogenase (6-PDGH), malate dehydrogenase (MDH), and glutamate dehydrogenase (GDH), those of *Prevotella* and *Porphyromonas* have only MDH and GDH (6–8). There was considerable heterogeneity, however, among such enzyme patterns within *B. fragilis* strains isolated from a wide variety of clinical sites (59). Whereas 27 strains of *B. fragilis* produced single bands of both G-6PDH and MDH during enzyme electrophoresis, 25 strains produced multiple bands of one or both enzymes.

Multilocus enzyme electrophoresis. Strains of *Pr. intermedia* were recently subjected to multilocus enzyme electrophoresis, DNA-DNA reassociation, and physiologic tests (60). Two separate groups of strains were discernible, of which one (serogroup I) had electrophoretically fast-migrating MDH and GDH bands and showed homology with the type strain ATCC 25611 DNA. The other strain group (serogroup II and III) had slower-moving enzymes and hybridized with the DNA probe of reference strain ATCC 33563. *Pr. nigrescens* was named to contain the ATCC 33563 homology group.

API ZYM/RapID-ANA systems. The reactions of isolates in the 19 substrates of the API ZYM system did not enable differentiation between *Pr. melaninogenica*, *Pr. denticola*, *Pr. loescheii*, and *B. levii* (61).

With a set of 20 additional substrates it became possible to differentiate all the oral and non-oral black-pigmented bacteroides species tested. Similarly, the RapID-ANA System required additional tests to differentiate several phenotypically similar bile-inhibited *Bacteroides* species from humans (62) and *B. fragilis* and related organisms (63).

Rosco Diagnostic tablets. When strains (reference and clinical) of oral non-pigmented *Prevotella* species were tested for preformed enzyme profiles with Rosco Diagnostic tablets (19), discriminatory profiles were obtained with tests for β-xylosidase, β-glucuronidase, β-glucosidase, α-fucosidase, β-*N*-acetylglucosaminidase, and α-mannosidase. The results gave good species discrimination and correlated well with conventional biochemical and morphologic tests and production of volatile and non-volatile fatty acids by GLC. It is beyond doubt that enzymatic tests are valuable in bacteroides taxonomy.

Pyrolysis mass spectrometry

Collection strains and non-pigmented clinical isolates provisionally identified as *Prevotella* species were recently classified numerically on the basis of pyrolysis mass spectrometry (PMS) data (64). Cluster membership in the PMS classification correlated well with SDS-PAGE results. PMS and SDS-PAGE divided two species into subgroups: two in *Pr. buccae* and five in *Pr. veroralis*. There was considerable compositional heterogeneity in strains representing *Pr. zoogleoformans* and *B. pentosaceus*. Reaction patterns of conventional tests and SDS-PAGE results suggested some heterogeneity in these species but not to the extent found with PMS. The possibility of heterogeneity within currently accepted species and of new centers of variation (subgroups) within the genus, as demonstrated with PMS, should encourage the use of DNA hybridization as supplementary tests.

PMS clusters corresponding to the species of the genus *Porphyromonas* were clearly distinct from those of the genus *Prevotella* (65). Strains identified as *Pr. corporis* and *P. asaccharolytica* showed higher levels of compositional heterogeneity than was found in *Pr. intermedia*. PMS deserves further attention as a taxonomic tool for bacteroides.

DNA composition

The DNA base composition of *Bacteroides* species was given by Shah & Collins (7, 17, 18). They suggested that the genus *Bacteroides* should be restricted to those species whose DNA base composition is within the range G + C 39–48 mol% and which are biochemically and chemically related to the type species *B. fragilis*. The DNA base compositions of the genus *Prevotella* are within the approximate range of 40 to 52 mol% G + C (8), and those of species in the genus *Porphyromonas* within the range 46 to 54 mol% G + C (6). The relationships between the DNA contents of *Bacteroides*, *Prevotella*, and *Porphyromonas* species and related bacteria are shown in Fig. 1. Although DNA base composition is useful for describing new taxa, it is clear from Fig. 1 that *Bacteroides sensu stricto*, *Prevotella*, *Porphyromonas*, and other bacteroides have overlapping G + C ratios.

DNA restriction fragment length polymorphisms

By comparison of their *Eco*RI restriction fragment patterns after agarose gel electrophoresis, several strains from *B. fragilis*, *B. ovatus*, *B. vulgatus*, and *B. uniformis* could be differentiated (66). To determine the significance of low levels of homology (<24%), chromosomal restriction endonuclease analysis was combined with Southern hybridizations. The method was particularly useful for genetically similar strains of the same species sharing >75% homology.

rRNA restriction fragment length polymorphisms

The *Escherichia coli* rRNA operon *rrnB* was recently used as a ^{32}P-labeled hybridization probe in Southern blots of genomic DNAs from representative strains of the sac-

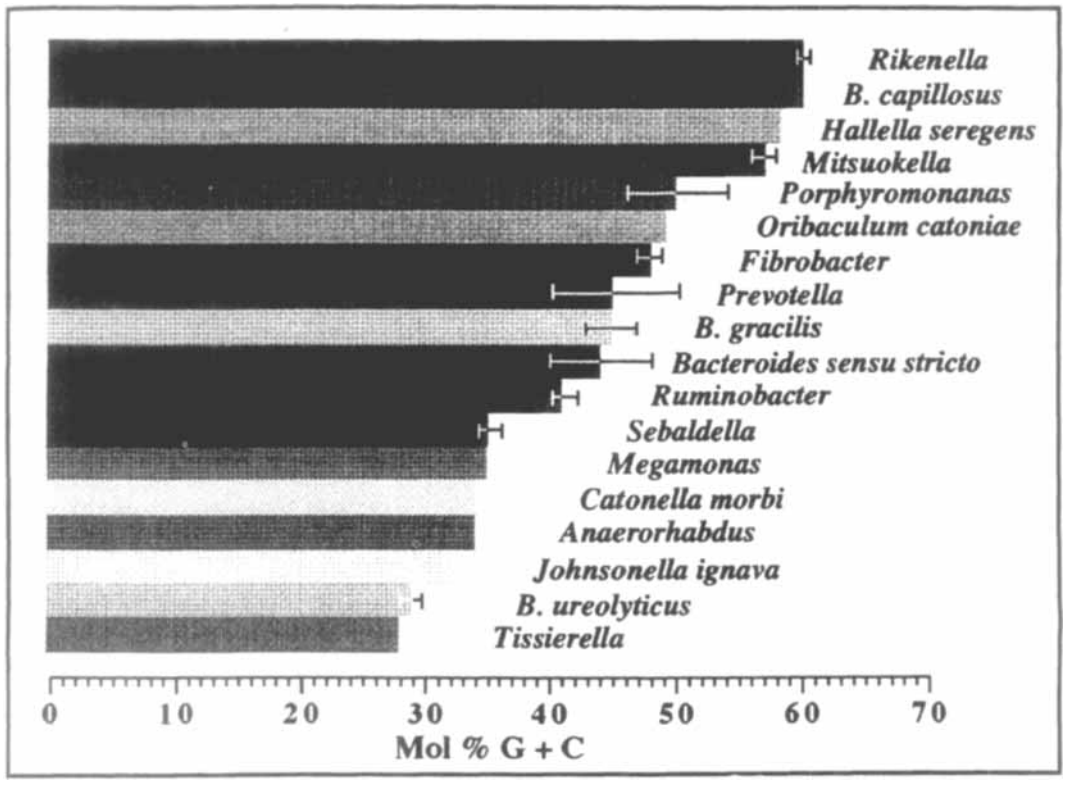

Fig. 1. Guananine (G) and cytosine (C) composition of DNA from typical and atypical bacteroides. G + C variation is indicated by horizontal bars.

charolytic, gram-negative obligate anaerobes of the genus *Bacteroides* (67). Nearly identical rRNA fragment patterns were produced when either the *E. coli rrnB* gene probe or homologous rRNA isolated from *B. fragilis* ATCC 25285 was used as a probe. In addition, a specific 16S or 23 *rrnB* gene probe was used to produce fragment patterns suitable for analysis. Specific fragment patterns were seen on autoradiograms for all but one (*B. ovatus*) of 8 of 10 recognized *Bacteroides* species tested. Restriction fragment length polymorphisms (RFLPs) were noticed for many of the strains, but these differences did not prevent species classification. The rRNA fragment patterns of the five *B. ovatus* strains displayed marked heterogeneity. Both rRNA RFLP and DNA RFLP seem to be useful taxonomic systems for bacteroides.

DNA homology

B. fragilis *and related organisms*. Major changes in the taxonomy of the genus *Bacteroides* involving DNA homology analysis have been reviewed by Barnes (68). *B. fragilis* was originally regarded as a single species with several subspecies: *B. fragilis* subspecies *fragilis*, *B. fragilis* subspecies *distasonis*, *B. fragilis* subspecies *eggerthii*, *B. fragilis* subspecies *ovatus*, *B. fragilis* subspecies *thetaiotaomicron*, *B. fragilis* subspecies *uniformis*, and *B. fragilis* subspecies *vulgatus* (69). DNA-DNA hybridization found all these subspecies to be genetically distinct (70). They were therefore reinstated to species rank (71). DNA homology studies with 340 strains of *B. fragilis* and other saccharolytic intestinal bacteroides demonstrated further subgroups within some of the species (72). DNA homology groupings were

also correlated with phenotypic characteristics in *B. fragilis* and other saccharolytic *Bacteroides* species (73). There was no general pattern between levels of intergroup homologies and phenotypic properties. At one time *B. uniformis* was considered identical with *B. thetaiotaomicron*, but homology studies showed them to be separate species (72).

Pr. melaninogenica *and related organisms.* Strains that produce black-pigmented colonies on blood agar used to be classified as *B. melaninogenicus* (*Pr. melaninogenica*) but were later differentiated into three subspecies on the basis of phenotypic features: *B. melaninogenicus* subspecies *asaccharolyticus* (*P. asaccharolytica*), *B. melaninogenicus* subspecies *intermedius* (*Pr. intermedia*), and *B. melaninogenicus* subspecies *melaninogenicus* (*Pr. melaninogenica*). Finegold & Barnes (74) proposed transfer of asaccharolytic strains into the new species *B. asaccharolyticus* (*P. asaccharolytica*). DNA-DNA homology data showed that this species was heterogeneous, and oral strains were gathered in a new species, *B. (P.) gingivalis* (75). *B. macaccae* (from monkeys) was established simultaneously. Later, *B. (P.) endodontalis* was formally created (76). DNA homology studies demonstrated several distinct groups within *B. melaninogenicus* subspecies *intermedius* (*Pr. intermedia*) and *B. melaninogenicus* subspecies *melaninogenicus* (*Pr. melaninogenica*) (77, 78). *B. melaninogenicus* subspecies *intermedius* (*Pr. intermedia*) contained three DNA homology groups yielding *B. intermedius* (*Pr. intermedia*) (two homology groups) and *B. corporis* (*Pr. corporis*) (79). *B. levii* was also established (79). Organisms resembling *B. melaninogenicus* subspecies *melaninogenicus* (*Pr. melaninogenica*) contained three DNA homology groups: *B. melaninogenicus* (*Pr. melaninogenica*), *B. (Pr.) loescheii* (80), and *B. (Pr.) denticola* (81). Holdeman & Johnson (80) amended the description of *B. (Pr.) denticola* as given by Shah & Collins (81) so as to include non-pigmenting organisms. A new species, *Pr. nigrescens* was recently created for the genetically distinct group of strains that hybridized with strain ATCC 33563 of *Pr. intermedia*, whereas the group of strains containing the type strain, ATCC 25611, was retained as *Pr. intermedia* (9, 60). Similar results were obtained through quantitative DNA hybridization by Fukushima et al. (82), who found that almost all strains of *Pr. intermedia* isolated from diseased sites (gingival pockets, periapical lesions) of adults belonged to the ATCC 25611 group, whereas strains isolated from the saliva of children belonged to the ATCC 33563 group.

Pr. oralis *and related organisms.* Bacteria that resembled *B. ruminicola* subspecies *brevis* biotype 3 were isolated from human sources and were long considered to be identical with bovine strains. This strain group was later identified as *B. (Pr.) oris* (83). Loesche et al. (84) established the new species *B. (Pr.) oralis*. *B. (Pr.) oralis*-like strains contained three additional DNA homology groups, which were named *B. (Pr.) veroralis* (85), *B. (Pr.) buccalis*, and *B. (Pr.) denticola* (81). *B. (Pr.) oralis* was redefined to include a discrete species for which a type strain was deposited with the American Type Culture Collection. *B. (Pr.) buccae*, which had no DNA homology with *B. ruminicola* subspecies *ruminicola*, *B. ruminicola* subspecies *brevis*, or with *B. (Pr.) oralis*, was also created (83). *B. pentosaceus* was proposed as new species by Shah & Collins (81), *B. capillus* by Kornman & Holt (86), and *B. forsythus* by Tanner et al. (87). *B. capillus* and *B. pentosaceus* are later synonyms for *B. (Pr.) buccae* (88, 89), although doubt has been raised recently on their identity (55). Indole-positive bacteroides from humans and cats, phenotypically similar to *B. (Pr.) zoogleoformans*, were shown to have only 45% to 49% DNA homology with the type strain of *B. (Pr.) zoogleoformans* and were identified as *B. heparinolyticus* (*Pr.) heparinolytica*) (90). DNA-DNA hybridization has been the standard arbiter for the designation of *Bacteroides* species.

RNA homology

Levels of rRNA similarity were determined among 30 *Bacteroides* species using the membrane competition method and both

the 16S and 23S rRNA subunits (91). Three major clusters of species were established with intercluster homology values of 20% to 30%. One cluster consisted of *B. fragilis* and nine other saccharolytic species that were common fecal organisms. A second cluster contained *Pr. melaninogenica* and 11 other moderately saccharolytic species, many of which were commonly isolated from human oral cavities. The third cluster, which was heterogeneous, comprised *P. asaccharolytica* and four other mostly asaccharolytic species. There was a reasonably good correlation between rRNA homology and DNA homology values. Since the amount of RNA homology determined reflected the phylogenetic relatedness of the organisms, it was clear that the genus *Bacteroides* is a diverse phylogenetic group.

DNA-rRNA hybridization

In DNA-rRNA hybridizations the sequence homology between labeled 16S or 23S rRNA from a reference strain and the rRNA cistrons within the chromosomal DNA from a second organism are determined. Hybridization experiments were recently carried out between DNAs from more than 70 strains of *Campylobacter* species and related taxa and either ^{3}H-labeled 23S rRNAs from reference strains belonging to *C. fetus*, *C. concisus*, *C. sputorum*, *C. coli*, and *C. nitrofigilis* and a *Wolinella succinogenes* strain, or ^{3}H- or ^{14}C-labeled 23S rRNAs from 13 gram-negative reference strains (92). Three rRNA homology groups, related at or beyond the genus level, were found. *B. gracilis* and *B. ureolyticus* fell in the first rRNA homology group together with *C. fetus*, *C. hyointestinalis*, *C. concisus*, *C. mucosalis*, *C. sputorum*, *C. jejuni*, *C. coli*, *C. lari*, '*C. upsaliensis*', *W. curva* and *W. recta*. Later, transfer of the amended genus *Campylobacter* and *Arcobacter* to the new family Campylobacteraceae was proposed (93). *B. gracilis* and *B. ureolyticus* were also found to belong to this family (93) and, accordingly, are not true bacteroides.

16S and 5S rRNA oligonucleotide cataloging and sequencing

Bacteroides, Flavobacterium, *and* Cytophaga *cataloging*. Oligonucleotide cataloging studies have suggested that species from *Bacteroides*, *Flavobacterium*, and *Cytophaga* form a phylogenetically coherent and major cluster of eubacteria (94). Interestingly, distinctive lipid profiles (presence of menaquinones, sphingolipids, and branched-chain fatty acids) also link these bacteria (34), which have been characterized as a poorly understood pot pourri of phenotypes (95). Full sequencing of the 16S rRNA genes showed a natural relationship between the bacteroides (represented by the *B. fragilis* sequence) and a phylogenetic unit that comprised the flavobacteria, cytophagae, and flexibacteria and others (represented by the *Fl. heparinum* sequence) (96).

B. nodosus—*16S rRNA sequencing*. Comparison of partial sequences of the cloned 16S rRNA gene of *B. nodosus* with the sequences of *Kingella indologenes* and *Cardiobacterium hominis* indicated that *B. nodosus* belongs to a novel family in the gamma division of *Proteobacteria* (97). After direct 16S rRNA sequencing, *B. nodosus* was proposed transferred to the new genus *Dichelobacter* as *D. nodosus*. This genus was assigned then together with the genera *Cardiobacterium* and *Suttonella* to the new family Cardiobacteriaceae (11). Accordingly, *B. nodosus* is not a true bacteroides.

Bacteroides, Prevotella, *and* Porphyromonas—*16S rRNA sequencing*. Approximately 95% of the 16S rRNA sequences were recently determined for representative strains of species of *Prevotella*, *Bacteroides*, and *Porphyromonas* and related organisms (98–100). The species examined fell into three major phylogenetic clusters within the bacteroides subgroup. The first cluster, termed the *Prevotella* cluster, comprised 15 species of *Prevotella*, including *Pr. melaninogenica*, *Pr. intermedia*, *Pr. nigrescens*, and the non-oral *Pr. ruminicola*. Two oral species previously placed in the genus *Prevotella*, *Pr. zoogleoformans* and *Pr. heparinolytica*, did not fall within the *Prevotella* cluster. These two species and six species

of *Bacteroides*, including the type species of the genus, *B. fragilis*, formed the second cluster, termed the *Bacteroides* cluster. The *Porphyromonas* cluster contained *P. asaccharolytica*, *P. endodontalis*, *P. circumdentaria*, *P. gingivalis*, *B. macacae*, *P. salivosa*, and *B. levii*. *B. splanchnicus* constituted a distinct group. Furthermore, *B. forsythus* and *B. distasonis* formed another cluster outside the *Porphyromonas* group. *B. putredinis*, *Rikenella microfusus*, and two misclassified *Cytophaga* species, *Cy. fermentans* and *Cy. salmonicolor*, formed a sixth subgroup of the *Cytophaga–Flavobacterium–Bacteroides* phylum. Interestingly, a close relationship was found between *B. gracilis*, *B. ureolyticus*, *W. curva*, *W. recta*, and true campylobacters (101). The 16S rRNA data were thus in overall agreement with recently proposed reclassifications of species of *Bacteroides*, *Prevotella*, and *Porphyromonas* (6–8), with some exceptions.

5S rRNA sequencing. The 5S rRNA sequences were determined for *B. fragilis*, *B. thetaiotaomicron*, *B. capillosus*, *Pr. veroralis*, *P. gingivalis*, *Anaerorhabdus furcosus*, *Fusobacterium nucleatum*, *F. mortiferum*, and *F. varium* (102). In the 5S dendrogram, *Bacteroides* clustered together with *Cytophaga* and *Fusobacterium*. The intraphylum relationships deduced suggested that *Bacteroides* is related to *Cytophaga* rather than to *Fusobacterium*.

Future steps to improve the taxonomy of bacteroides

Genus Prevotella

Despite all the efforts made to improve the chemotaxonomy of bacteroides mentioned in the present review, some bacteroides remain misclassified. Doubt has been raised as to the validity of the proposed genus name *Prevotella* (8). This genus name was assigned in 1976 by Labroue to *Pr. bacteriogloeae* novum genus, nova species in the chlamydobacteria (103). Since this assignment was not validly published, it is not necessary to change the genus name assigned by Shah & Collins (8).

Pr. melaninogenica *and* Pr. loescheii

DNA homology studies showed that the VPI 9343 homology group might be considered distinct from VPI 2381, the type strain of *B. melaninogenicus* subspecies *melaninogenicus* (*Pr. melaninogenica*), and that the VPI D1C-20 homology group showed low homology with *B.* (*Pr.*) *loescheii*, although the respective groups were indistinguishable by phenotypic characteristics (80). Similar to *Pr. intermedia*, from which *Pr. nigrescens* was derived (9), it should be examined whether there are clinical or ecologic reasons for establishing new species from *Pr. melaninogenica* and *Pr. loescheii*.

B. fragilis, B. ovatus, *and* B. thetaiotaomicron

Two DNA homology groups (possibly genosubspecies) were also detected in *B. fragilis* (73). *B. ovatus* and *B. thetaiotaomicron* had similar levels of heterogeneity in their DNA homology groups but were more complex, with a greater number of subgroups. The rationale for establishing new species in these groups should be investigated.

Pr. heparinolytica *and* Pr. zoogleoformans

Previous investigations based on phenotypic characteristics (8) suggested that the oral species *Pr. heparinolytica* and *Pr. zoogleoformans* should be transferred to the new genus *Prevotella*. This was not supported by 16S rRNA experiments (100), which clearly showed that they belong to the genus *Bacteroides*. By including *Pr. heparinolytica* and *Pr. zoogleoformans* in *Bacteroides*, this genus will contain oral bacteria in addition to fecal bacteria from mammalian and human sources. The existing descriptions of the genera *Bacteroides* and *Prevotella* (3, 8) should therefore be modified with regard to certain phenotypic criteria.

B. levii *and* B. macacae

16S rRNA studies showed that *B. levii* and *B. macacae* belong to the genus *Porphyromonas*, not to *Bacteroides* (100). *B.*

Table 4. Other *Bacteroides* of uncertain generic position*

B. capillosus
B. cellulosolvens
B. coagulans
B. galacturonicus
B. helcogenes
B. pectinophilus
B. polypragmatus
B. suis
B. tectum
B. xylanolyticus

* Adopted from Ref. 3.

levii and *B. macacae* resemble the *Porphyromonas* group also by producing mainly protoheme and having a similar dehydrogenase pattern (18). Like members of the genus *Porphyromonas*, *B. levii* produces acetic and butyric acid as metabolic end products and has a G + C content of 45 to 48 mol% (3). *B. levii* and *B. macacae* should probably be transferred to *Porphyromonas*.

B. forsythus *and* B. distasonis

B. forsythus and *B. distasonis* fell just outside the *Porphyromonas* 16S rRNA subcluster (100). Owing to the phylogenetic depth demonstrated for this cluster it is uncertain whether *B. forsythus* and *B. distasonis* are species of *Porphyromonas* or whether they constitute one or more separate genera. The whole-cell fatty acid profile of *B. forsythus* indicated that this organism should not be a member of the genus *Bacteroides* (104). DNAs from organisms fitting the phenotypic description of *B. distasonis* were very heterogeneous (72). The taxonomic status of these two species should be investigated further.

B. putredinis

After 16S rRNA sequencing *B. putredinis* and *R. microfusus* formed a cluster together with two misclassified *Cytophaga* species (100). Whether *B. putredinis* should be included in the genus *Rikenella* or form a separate genus remains to be determined.

B. splanchnicus

B. splanchnicus probably represents a separate genus, since it was not found related to any other species (100). *B. splanchnicus* differs from other species of the *B. fragilis* group in producing major amounts of *n*-butyric acid (20), in having isopentadecanoic acid as its major cellular carboxylic acid (27), and in possessing MK-9 as its major menaquinone isoprenalogue (18). In a numerical taxonomic study based on 111 phenotypic characters related to chemical composition and products, *B. splanchnicus* clustered with other *Bacteroides* species at the 0.88 similarity level (105).

B. gracilis *and* B. ureolyticus

B. gracilis and *B. ureolyticus* are closely related to *Campylobacter*, but more data are required before their appropriate taxonomic status can be determined (106).

Taxonomic position of other bacteroides

Several other species have been proposed as members of the genus *Bacteroides* (Table 4). Since many of them have little resemblance to classical bacteroides, their taxonomic position is uncertain. It is to be hoped that some of this uncertainty can be resolved by 16S rRNA sequence analysis, although this technique cannot measure all phylogenetic relationships equally well, especially not those between very recently diverged species (107). The integrated use of both phylogenetic and phenotypic characteristics, or so-called polyphasic taxonomy, has been recommended for the delineation of taxa at all levels from kingdom to genus (108, 109).

16S rRNA sequencing has shown, however, that *Mitsuokella multiacidus* and *Megamonas hypermegas*, previously belonging to the *Bacteroides*, are non-motile species of *Selenomonas* and *Pectinatus*, respectively (Paster, Dewhirst, Olsen, Fraser & Socransky, 1993; unpublished observations. Furthermore, *Sebaldella* (formerly *B.*) *termitidis* and *Leptotrichia buccalis* are related to the fusobacteria. However, the phylogenetic distance of these two species from the fusobac-

teria and each other warrants separate genus designation.

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