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Phylogenetic Placement of *Dialister pneumosintes* (formerly *Bacteroides pneumosintes*) within the *Sporomusa* Subbranch of the *Clostridium* Subphylum of the Gram-Positive Bacteria

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The nucleotide sequence of the 16S rRNA gene of the type strain of *Dialister pneumosintes* was determined. Phylogenetic analysis revealed that this species belongs to the *Sporomusa* branch of the *Clostridium* subphylum of the gram-positive bacteria and should therefore be excluded from the family *Bacteroidaceae*. Within this branch, which encompasses several other gram-negative taxa, such as *Acidaminococcus*, *Pectinatus*, *Phascolarcobacterium*, *Quinella*, *Selenomonas*, and *Zymophilus*, *Dialister* showed a specific, albeit distant, affinity with the genera *Megasphaera* and *Veillonella*.

The genus *Dialister* was recently revived to accommodate the gram-negative, obligately anaerobic, nonfermentative, rod-shaped species *Bacteroides pneumosintes* (11). *B. pneumosintes* was originally described by Olitsky and Gates as *Bacterium pneumosintes* in 1921 (12) and was first isolated from nasopharyngeal secretions of patients with influenza during the epidemics of 1918 through 1921 (12). The species was later placed in the genus *Dialister* and subsequently transferred to the genus *Bacteroides* in 1970 (8). During the 1980s the genus *Bacteroides* has undergone major taxonomic revisions, with many of the species being transferred to new genera (viz., *Anaerorhabdus*, *Fibrobacter*, *Megamonas*, *Mitsuokella*, *Porphyromonas*, *Prevotella*, *Rikenella*, *Ruminobacter*, *Sebaldella*, and *Tissierella*) (see reference 13 for a review). Shah and Collins in 1989 (14) proposed that the genus *Bacteroides* be restricted to the saccharolytic, nonpigmented species of the *Bacteroides fragilis* group. *B. pneumosintes* differs markedly from this genus as defined by Shah and Collins (14), and its true taxonomic affiliations remained uncertain. On the basis of phenotypic criteria, Moore and Moore (11) have recently resurrected the genus *Dialister*, to accommodate *B. pneumosintes*. In this study, we have sequenced the 16S rRNA gene of the type strain of *Dialister pneumosintes* to determine its phylogenetic proximity to the genus *Bacteroides* and other members of the family *Bacteroidaceae*.

A freeze-dried culture of the type strain of *D. pneumosintes* (ATCC 33048^T) was obtained from the American Type Culture Collection, Rockville, Md. Because of the phenotypic similarities of *Veillonella* and *Dialister* species (e.g., small cell size and non-spore-forming, nonmotile anaerobes), the 16S rRNA genes of three *Veillonella* species from the human oral cavity were also sequenced. *Veillonella atypica* (DSM 20739^T), *Veillonella dispar* (DSM 20735^T), and *Veillonella parvula* (DSM 2008^T) were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany. Lyophilized cells were resuspended in 500 μl of TES buffer (0.05 M Tris-HCl, 0.005 M EDTA, 0.05 M NaCl, pH 8.0), and genomic DNA was extracted directly from this cell suspension by the method of Lawson et al. (10). The 16S rRNA genes were amplified as described previously (9) and purified by using a Prep-A-Gene kit (Bio-Rad, Hercules, Calif.) according to the manufacturer's instructions. The 16S rDNA fragments were sequenced by using a Taq DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems Inc., Foster City, Calif.) and an automated sequencer (Applied Biosystems Inc.; model 373A). The new sequences were compared with sequences of reference organisms, which were obtained from the EMBL and GenBank data libraries. Once the closest known phylogenetic relatives had been established, sequences were aligned by using the PILEUP program (3), and the alignments were corrected manually. For comparative phylogenetic analyses approximately 100 bases at the 5′ end of the rRNA sequence were excluded because of alignment uncertainties. Phylogenetic analyses were performed with the PHYLIP package (4). A distance matrix was calculated by using the program DNADIST, and a phylogenetic tree was calculated by the neighbor-joining method with the program NEIGHBOR. The stability of the relationships was assessed by the bootstrap method with the programs SEQBOOT, DNADIST, NEIGHBOR, and CONSENSE.

The partial 16S rRNA gene sequence of *D. pneumosintes* ATCC 33048^T consisted of 1,504 nucleotides (corresponding to positions 29 to 1504 of the *Escherichia coli* 16S rRNA). Sequence similarity calculations revealed that the closest known phylogenetic relatives of *D. pneumosintes* are the gram-negative organisms of the *Sporomusa* subbranch of the gram-positive bacteria (approximately 81 to 88% sequence similarity). Members of the *B. fragilis* group of species were phylogenetically far removed from *D. pneumosintes*, exhibiting approximately 70 to 72% sequence similarity (data not shown). Table 1 shows the percent sequence similarities of *D. pneumosintes* with members of the *Sporomusa* subbranch. A distance matrix tree based on calculated evolutionary distances is shown in Fig. 1 and places *D. pneumosintes* within the *Sporomusa* subbranch of the *Clostridium* subphylum. It is evident from both distance calculations and the treeing program that within the *Sporomusa* subbranch, the closest known relatives of *D. pneumosintes* are *Megasphaera elsdenii* and the human oral *Veillonella* species. The significance of this grouping was measured by bootstrapping (Fig. 1). The sequence divergences observed between *D. pneumosintes* and *M. elsdenii* (approximately 11.5%) and between *D. pneumosintes* and *Veillonella* species (12.2 to 12.8%) are clearly indicative of separate genera.

The results of the present study support the assignment of *B. pneumosintes* to a new genus, *Dialister* (11). Moore and Moore (11) placed *Dialister* in the family *Bacteroidaceae*, inasmuch as

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TABLE 1. Similarity values for the 16S rRNA genes of *D. pneumosintes* and other members of the *Sporomusa* subbranch of the *Clostridium* subphylum of the gram-positive bacteria

Taxon	% Similarity with[a]: *Dialister pneumosintes*	*Megasphaera elsdenii*	*Veillonella parvula*	*Sporomusa paucivorans*	*Sporomusa termitida*	*Clostridium quercicolum*	*Acidaminococcus fermentans*	*Phascolarcobacterium faecium*	*Pectinatus cerevisiiphilus*	*Pectinatus frisingensis*	*Quinella ovalis*	*Selenomonas sputigena*	*Selenomonas ruminantium* subsp. *ruminantium*	*Selenomonas lactifex*
Megasphaera elsdenii	88.5													
Veillonella parvula	87.7	89.6												
Sporomusa paucivorans	84.3	84.6	85.9											
Sporomusa termitida	81.4	83.4	84.2	93.2										
Clostridium quercicolum	83.8	86.4	86.9	90.0	88.0									
Acidaminococcus fermentans	84.9	84.9	86.3	87.6	85.5	86.2								
Phascolarcobacterium faecium	83.9	85.7	86.1	89.0	86.9	88.2	90.6							
Pectinatus cerevisiiphilus	85.2	86.4	87.0	88.1	86.0	88.1	85.4	86.2						
Pectinatus frisingensis	84.4	86.4	85.6	86.3	85.2	86.8	84.1	84.8	95.0					
Quinella ovalis	81.8	81.9	81.9	82.8	81.4	84.0	82.8	83.7	83.9	83.4				
Selenomonas sputigena	84.8	85.4	85.6	86.2	84.6	88.3	86.8	86.5	88.7	88.9	85.5			
Selenomonas ruminantium subsp. *ruminantium*	85.9	85.8	86.6	87.1	85.5	88.6	87.3	87.1	89.4	88.4	85.7	90.9		
Selenomonas lactifex	85.0	86.2	87.4	88.6	86.9	90.3	87.7	87.4	89.0	88.4	84.9	90.0	93.9	
Zymophilus paucivorans	84.0	85.9	86.9	88.2	88.6	89.0	86.3	86.4	88.2	88.4	82.4	88.5	89.8	91.9

[a] A continuous stretch of 1,259 nucleotides was used for the calculations.

it is "composed of anaerobic, gram-negative, nonsporing bacilli." However, this description also covers most of the taxa of the *Sporomusa* subbranch (1, 2). The family *Bacteroidaceae* is phylogenetically a member of the *Flavobacterium-Cytophaga* phylum of the gram-negative bacteria. It is clear, however, that several genera included in the *Bacteroidaceae* as currently defined (7) are phylogenetically far removed from the genus *Bacteroides* and in fact have a closer affinity to the *Clostridium* subphylum of the gram-positive bacteria (e.g., *Fusobacterium* and *Leptotrichia* [1], *Butyrivibrio* [5], and *Pectinatus* and *Selenomonas* [6]). Therefore, a more specific definition of the family *Bacteroidaceae*, restricted to those genera (e.g., *Porphyromonas* and *Prevotella*) that are phylogenetically closely related to the genus *Bacteroides*, is needed. Although much work is still required before the higher taxonomic groupings within the *Clostridium* subphylum can be resolved with confidence, it is evident from the phylogenetic data presented here that the genus *Dialister* should be accommodated in the suprageneric grouping currently termed the *Sporomusa* subbranch.

Nucleotide sequence accession numbers. The 16S rRNA

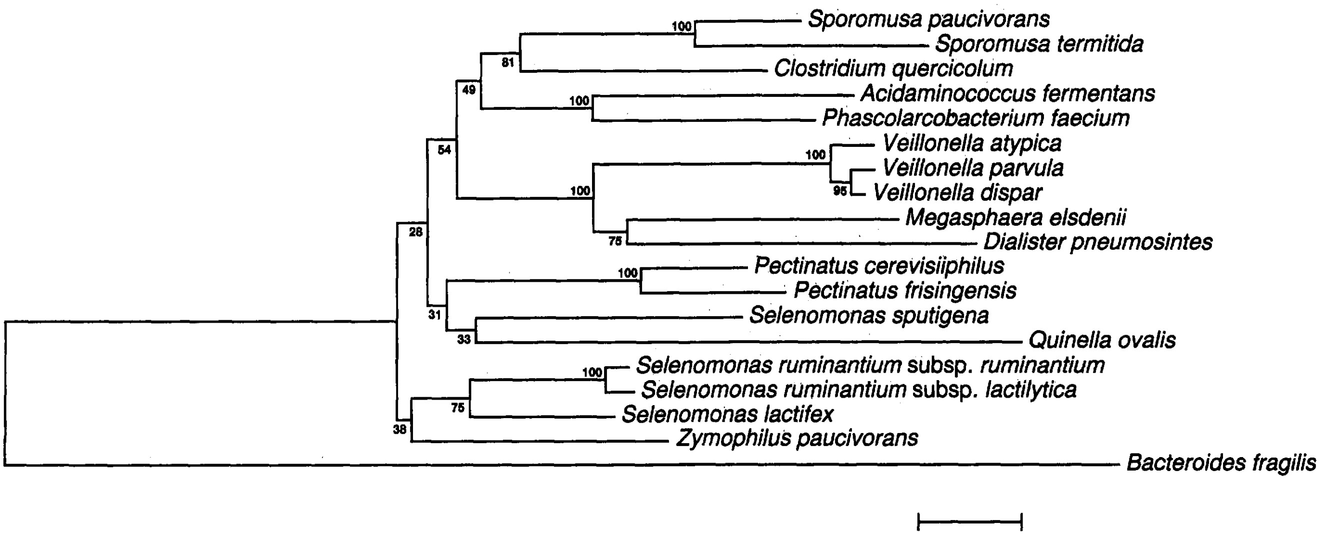

FIG. 1. Unrooted phylogenetic tree showing the position of *D. pneumosintes* within the *Sporomusa* subbranch of the *Clostridium* subphylum of the gram-positive bacteria. Bootstrap values are given at the branching points. Values of 90 or above are considered significant. The bar represents a 2% sequence difference.

gene sequences of *D. pneumosintes* ATCC 33048^T, *V. atypica* DSM 20739^T, *V. dispar* DSM 20735^T, and *V. parvula* DSM 2008^T have been deposited in the EMBL data base under accession numbers X82500, X84007, X84006, and X84005, respectively.

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