

Reclassification of *Oribaculum catoniae* (Moore and Moore 1994) as *Porphyromonas catoniae* comb. nov. and Emendation of the Genus *Porphyromonas*

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A partial 16S rRNA gene sequence of the type strain of *Oribaculum catoniae* was determined by using PCR direct sequencing. A comparative sequence analysis demonstrated that this species, although saccharolytic, is phylogenetically a member of the genus *Porphyromonas*. On the basis of the phylogenetic and phenotypic distinctiveness of *O. catoniae*, we formally propose that this species should be reclassified in the genus *Porphyromonas*, as *Porphyromonas catoniae* comb. nov. An emended description of the genus *Porphyromonas* is presented.

Oribaculum catoniae was described by Moore and Moore (10) to accommodate a group of organisms which previously were referred to as “*Bacteroides* D26” and originated from the gingival crevices of humans with gingivitis or periodontitis and from humans with healthy gingivae (10–12). *O. catoniae* comprises obligately anaerobic, saccharolytic, non-spore-forming, rod-shaped bacteria that do not grow well in the presence of 20% bile and phenotypically most closely resemble species of the genus *Prevotella* (10). *O. catoniae*, unlike *Prevotella* species, usually produces major amounts of propionate and moderate levels of succinate as metabolic end products (10). This feature and the highly characteristic cellular fatty acid composition of *O. catoniae* strains were considered sufficient to merit the creation of the new genus *Oribaculum* within the *Bacteroidaceae* (10). In order to clarify the precise taxonomic position of *O. catoniae*, we determined the almost complete 16S rRNA gene sequence of the type strain of this species, and in this paper we report the results of a comparative sequence analysis.

A freeze-dried culture of the type strain of *O. catoniae* (ATCC 51270) was obtained from the American Type Culture Collection, Rockville, Md. Lyophilized cells were resuspended in 500 μ l 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 the suspension by the method of Lawson et al. (6). The 16S rRNA gene was amplified by using universal primers proximal to the 5' and 3' ends of the gene as described previously (5). PCR products were purified by using a Prep-A-Gene kit (Bio-Rad, Hercules, Calif.) according to the manufacturer's instructions and were sequenced by using a *Taq* DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, Calif.) and model 373A automated sequencer (Applied Biosystems, Inc.). The sequence was confirmed by analyzing a duplicate freeze-dried culture that was obtained separately from the American Type Culture Collection. The sequence determined was compared with the sequences of other bacteria that were obtained from the EMBL and GenBank data libraries to establish the nearest neighbors of *O. catoniae*. The new sequence was subsequently aligned with the sequences of phylogenetic relatives by using the PILEUP program (2), and the alignments were corrected manually. In the

comparative phylogenetic analyses approximately 100 bases at the 5' end of the rRNA sequence were not used because of alignment uncertainties. The phylogenetic analysis was performed by using the PHYLIP package (3). 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 by using the programs SEQBOOT, DNADIST, NEIGHBOR, and CONSENSE.

The partial 16S rRNA sequence of *O. catoniae* which we determined consisted of 1,470 nucleotides, representing approximately 96% of the total primary sequence. This sequence was compared with the sequences of other reference species in the EMBL and GenBank libraries and was found to be most closely related to the sequences of members of the genus *Bacteroides* and related genera. The highest levels of sequence similarity (approximately 85.2 to 89.5%) were found with species of the genus *Porphyromonas*. Similar, albeit slightly lower, levels of sequence relatedness were found with *Bacteroides* and *Prevotella* species (levels of relatedness, approximately 82.7 to 84.5 and 79.7 to 82.5%, respectively). A significantly lower level of similarity was found with members of the genus *Rikenella* (level of relatedness, 83.9%). A matrix of the sequence similarity values obtained for *O. catoniae* and its close relatives is shown in Table 1. A tree showing the phylogenetic relationships of *O. catoniae* and members of the genera *Bacteroides*, *Prevotella*, and *Porphyromonas* is shown in Fig. 1. From the treeing program it was evident that *O. catoniae* forms a distinct subline within the *Porphyromonas* group.

The results of our analysis do not support the proposal that the genus *Oribaculum* should be recognized as a phylogenetically distinct genus. Both the levels of sequence similarity and the tree branching patterns clearly demonstrate that *O. catoniae* is a member of the genus *Porphyromonas*. The fatty acid pattern and the G+C content reported for *O. catoniae* (10) are not incompatible with this finding. For example, *O. catoniae* contains approximately equal amounts of iso- and anteiso-C_{15:0} as the predominant fatty acids (10). Although in other porphyromonads iso-C_{15:0} generally predominates, these differences are essentially quantitative. Furthermore, *Bacteroides levii*, which is also a member of the *Porphyromonas* cluster (13), contains approximately equal quantities of these fatty acids (14). Similarly, the G+C content of *O. catoniae* (49 mol%) is

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TABLE 1. Similarity values for the 16S rRNA genes of *Porphyromonas*, *Bacteroides*, and *Prevotella* species
% Similarity with:

Taxon	<i>Bacteroides eggerthii</i>	<i>Bacteroides fragilis</i>	<i>Bacteroides heparinolyticus</i>	<i>Bacteroides ovatus</i>	<i>Bacteroides thetaiotaomicron</i>	<i>Bacteroides uniformis</i>	<i>Bacteroides vulgatus</i>	<i>Bacteroides zoogloformans</i>	<i>Prevotella bivia</i>	<i>Prevotella buccae</i>	<i>Prevotella buccalis</i>	<i>Prevotella corporis</i>	<i>Prevotella denticola</i>	<i>Prevotella disiens</i>	<i>Prevotella intermedia</i>	<i>Prevotella loeschii</i>	<i>Prevotella melaninogenica</i>	<i>Prevotella nigrescens</i>	<i>Prevotella oralis</i>	<i>Prevotella oris</i>	<i>Prevotella ouloris</i>	<i>Prevotella ruminicola</i>	<i>Prevotella veroralis</i>	<i>Bacteroides forsythus</i>	<i>Porphyromonas asaccharolytica</i>	<i>Porphyromonas cangingivalis</i>	<i>Porphyromonas canoris</i>	<i>Porphyromonas cansulci</i>	<i>Porphyromonas circumdentaria</i>	<i>Porphyromonas endodontalis</i>	<i>Porphyromonas gingivalis</i>	<i>Porphyromonas macacae</i>	<i>Bacteroides levii</i>	<i>Porphyromonas catoniae</i>	
<i>Bacteroides fragilis</i>	93.5																																		
<i>Bacteroides heparinolyticus</i>	93.1	90.8																																	
<i>Bacteroides ovatus</i>	94.6	94.5	91.6																																
<i>Bacteroides thetaiotaomicron</i>	94.2	94.6	91.6	97.4																															
<i>Bacteroides uniformis</i>	95.8	93.4	94.2	94.8	94.3																														
<i>Bacteroides vulgatus</i>	91.6	91.3	91.3	91.1	91.0	92.4																													
<i>Bacteroides zoogloformans</i>	92.4	90.2	96.9	91.3	91.0	93.8	91.0																												
<i>Prevotella bivia</i>	86.1	84.6	85.0	86.2	85.1	86.5	83.9	84.8																											
<i>Prevotella buccae</i>	83.5	82.3	84.3	83.9	83.5	84.8	83.9	84.2	88.8																										
<i>Prevotella buccalis</i>	84.5	82.8	84.4	84.7	83.5	85.8	83.5	84.1	90.2	89.5																									
<i>Prevotella corporis</i>	84.9	83.5	84.7	85.1	84.8	85.7	83.5	84.0	91.2	89.6	90.4																								
<i>Prevotella denticola</i>	84.0	82.2	85.2	83.6	82.8	84.6	83.2	85.1	89.8	91.4	89.0	90.8																							
<i>Prevotella disiens</i>	85.0	84.1	83.8	85.1	84.4	85.1	82.4	82.9	92.0	87.6	89.7	91.9	89.7																						
<i>Prevotella intermedia</i>	84.3	82.3	84.4	84.7	83.6	85.0	83.0	83.7	91.2	88.0	88.9	92.4	89.8	93.6																					
<i>Prevotella loeschii</i>	84.2	82.5	84.4	84.4	83.7	84.6	83.2	84.8	90.5	90.2	90.8	90.8	90.5	89.0	89.9																				
<i>Prevotella melaninogenica</i>	86.0	84.0	85.3	85.7	85.1	86.3	84.2	85.0	92.8	89.1	89.0	92.7	93.5	91.3	91.6	90.6																			
<i>Prevotella nigrescens</i>	84.6	83.5	85.3	84.4	83.7	85.6	84.0	85.1	90.9	88.9	90.0	93.0	91.3	91.8	94.1	90.3	91.7																		
<i>Prevotella oralis</i>	83.9	81.9	84.9	84.1	83.6	85.0	83.8	85.2	89.5	89.4	91.1	89.0	88.9	87.8	87.8	91.2	89.5	89.1																	
<i>Prevotella oris</i>	85.0	82.5	84.5	83.9	83.1	85.6	83.4	84.6	92.0	90.2	91.5	91.0	91.4	89.9	90.7	91.1	92.0	90.7	89.4																
<i>Prevotella ouloris</i>	87.0	85.1	85.6	86.2	86.1	86.6	85.1	85.4	92.1	89.3	90.1	92.5	91.5	93.2	91.7	90.4	93.6	91.1	89.3	93.1															
<i>Prevotella ruminicola</i>	85.3	83.7	84.0	85.1	84.7	85.9	84.8	84.0	90.2	87.6	89.0	88.5	88.3	88.4	88.4	88.5	89.8	88.0	88.2	88.8	89.0														
<i>Prevotella veroralis</i>	85.2	83.0	84.1	85.4	84.5	85.6	83.4	83.9	92.5	89.6	89.2	92.2	92.4	91.8	91.1	90.1	97.0	90.9	88.5	91.9	93.7	89.5													
<i>Bacteroides forsythus</i>	86.4	86.1	85.0	86.6	86.5	86.5	86.1	84.6	82.4	81.1	81.1	82.2	81.3	82.0	81.6	81.6	82.8	81.8	81.0	82.0	83.9	81.0	82.2												
<i>Porphyromonas asaccharolytica</i>	82.4	81.8	80.5	82.9	82.9	82.4	81.8	80.9	80.0	79.6	78.4	79.8	78.2	79.6	78.6	79.1	79.6	78.5	78.4	79.3	81.0	79.1	79.6	84.6											
<i>Porphyromonas cangingivalis</i>	84.2	83.1	82.9	84.4	83.7	84.1	83.5	82.6	80.4	79.5	78.4	79.6	79.4	79.8	78.9	80.3	80.8	78.8	79.8	79.6	81.4	79.5	80.1	88.4	86.4										
<i>Porphyromonas canoris</i>	83.0	81.2	80.9	83.6	83.6	81.8	82.1	80.6	78.4	77.5	77.7	77.9	76.8	77.3	77.4	79.8	78.8	77.3	78.2	77.9	79.6	78.1	77.7	87.9	85.0	91.1									
<i>Porphyromonas cansulci</i>	83.8	82.5	81.4	83.4	82.8	83.3	83.0	81.2	79.6	78.4	78.5	79.1	78.1	79.3	79.4	79.0	79.8	78.9	79.1	78.5	81.2	77.6	79.2	89.0	85.8	89.8	89.3								
<i>Porphyromonas circumdentaria</i>	83.3	82.8	82.5	84.3	84.2	84.1	82.4	82.3	80.2	79.9	78.9	79.8	79.2	80.2	80.2	80.7	78.9	78.8	79.2	81.2	80.5	80.1	86.5	87.7	87.5	85.7	86.9								
<i>Porphyromonas endodontalis</i>	83.8	82.9	81.9	84.3	83.6	83.9	82.5	81.2	80.4	79.8	78.9	80.4	79.6	80.6	79.8	80.0	81.0	79.9	78.8	79.4	81.8	79.6	80.7	87.2	88.7	88.8	86.0	87.8	93.7						
<i>Porphyromonas gingivalis</i>	84.5	83.9	83.1	84.8	84.2	84.7	83.9	82.4	81.0	80.2	80.7	80.4	79.9	81.2	78.0	80.6	80.6	80.5	79.7	80.7	81.7	79.6	79.8	88.5	85.9	88.7	88.2	89.8	86.1	87.2					
<i>Porphyromonas macacae</i>	84.0	83.2	82.7	86.0	84.9	84.0	83.6	82.8	80.0	80.6	79.4	80.4	79.2	80.5	79.1	80.6	80.6	79.8	79.4	79.8	81.7	79.6	80.2	88.6	86.6	89.0	88.5	89.1	87.9	87.8	88.8				
<i>Bacteroides levii</i>	84.9	84.1	82.8	84.8	85.2	84.4	83.2	81.9	79.4	78.0	78.8	79.8	78.7	80.3	78.9	79.4	81.1	78.8	78.6	79.4	80.9	79.0	79.2	87.5	85.3	91.5	89.1	87.6	86.3	87.0	86.4	88.3			
<i>Porphyromonas catoniae</i>	84.5	83.0	83.4	84.4	84.2	84.4	83.6	82.7	80.4	80.1	80.2	81.2	79.7	80.8	80.7	81.4	81.4	80.7	80.2	81.1	82.5	80.1	80.5	88.5	85.2	88.5	88.1	88.0	86.1	87.1	89.1	89.5	87.6		

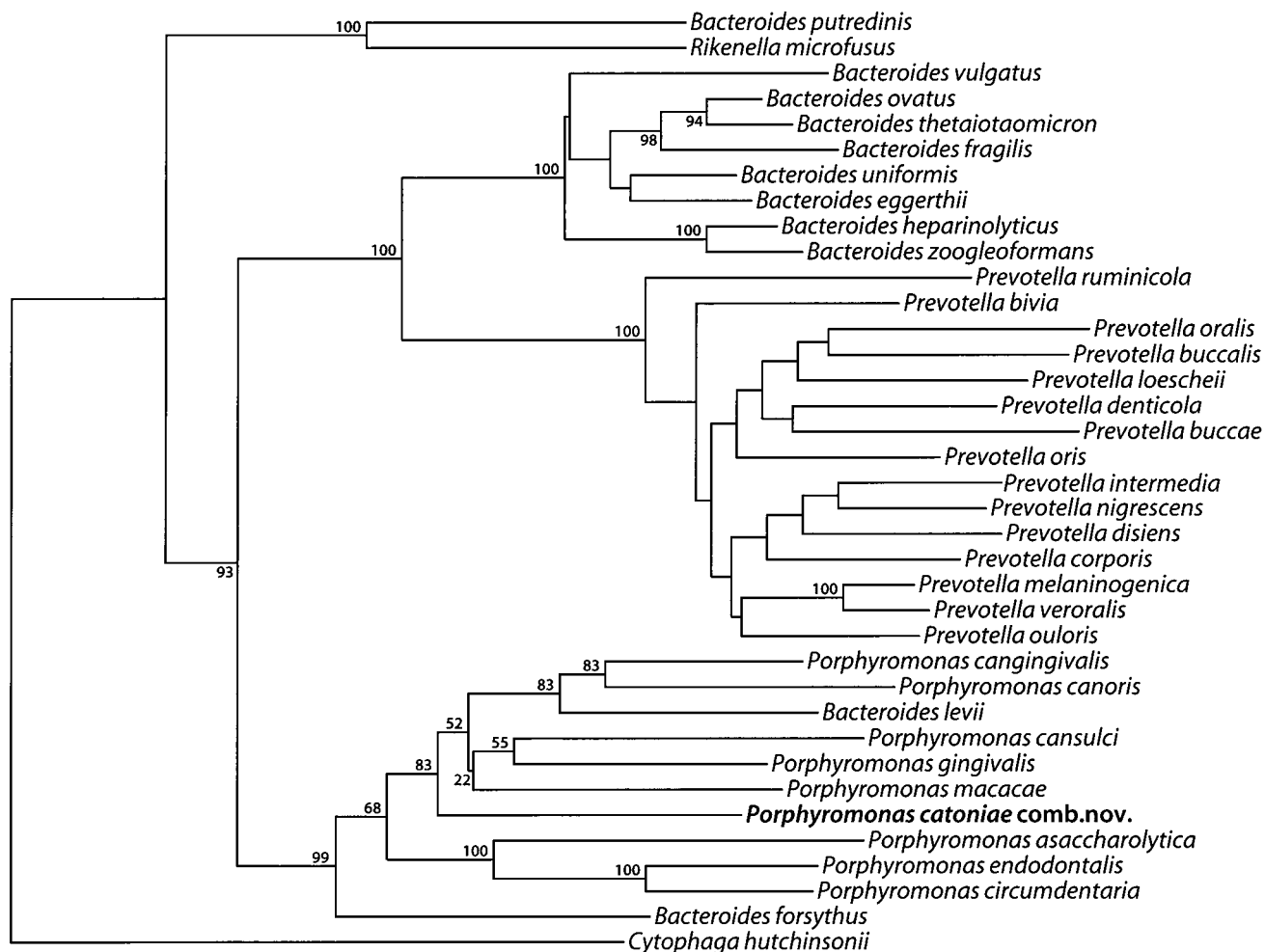


FIG. 1. Unrooted phylogenetic tree showing the position of *P. catoniae* within the *Bacteroidaceae*. Bootstrap values greater than 90% are given at the branching points, and all bootstrap values are shown for the *Porphyromonas* cluster.

within the accepted range (46 to 54 mol%) for the genus *Porphyromonas* (15). In contrast to most *Porphyromonas* species, *O. catoniae* is saccharolytic. However, recent phylogenetic observations indicate that several weakly saccharolytic "*Bacteroides*" species are members of the genus *Porphyromonas* (e.g., *Bacteroides macacae* [7, 13] and *Bacteroides levii* [13]) and should be reclassified as *Porphyromonas* species. *O. catoniae* exhibits approximately 10.5 to 14.8% sequence divergence with other recognized porphyromonads and exhibits no close affinity with any other species. Unlike the other *Porphyromonas* species, it does not produce a black pigment on blood agar. The degree of sequence divergence and the distinct phenotypic characteristics of *O. catoniae* justify reclassification of this taxon as a separate species within the genus *Porphyromonas* (15).

Recently, workers have described several other *Porphyromonas* species (1, 4, 8, 9), some of which do not conform to the strict circumscription of the genus given by Shah and Collins (15). In view of the atypical properties of *O. catoniae* (e.g., saccharolytic metabolism, absence of protoheme production) and these newly defined species (1, 9), below we propose an emended description of the genus *Porphyromonas* (Shah and Collins 1988).

Emended description of *Porphyromonas* Shah and Collins 1988. The description below is based on that of Shah and Collins (15) and data in references 1, 4, 7 through 10, and 14.

Gram-negative, obligately anaerobic, non-spore-forming, nonmotile rods to coccobacilli. Most cells in broth are small (0.5 to 0.8 by 1.0 to 3.5 μm), but occasionally longer cells (length, 4 to 6 μm) may be formed. Colonies on blood agar plates are smooth (rarely rough), shiny, convex, and 1 to 3 mm in diameter. The colonies of most species are pigmented because of protoheme production. While the growth of most species is not affected by carbohydrates, some species are saccharolytic. Nitrogenous substances such as Proteose Peptone, Trypticase, and yeast extract markedly enhance growth. The optimum temperature for growth is 37°C. The major fermentation products are usually *n*-butyric and acetic acids; lower levels of propionic, isobutyric, and isovaleric acids may also be produced. Some species produce major amounts of propionic acid.

Malate dehydrogenase and glutamate dehydrogenase are present; glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase are absent from most species. Proteolytic activity is variable. The strains have a limited ability to ferment amino acids such as aspartate and asparagine. Indole

is produced. Nitrate is not reduced to nitrite. Starch and esculin are not hydrolyzed.

The cell wall peptidoglycan contains lysine as the diamino acid; 2-keto-3-deoxyoctulosonic acid is absent. The principal respiratory quinones are unsaturated menaquinones with 9 or 10 isoprene units. Both nonhydroxylated and 3-hydroxylated long-chain fatty acids are present. The nonhydroxylated fatty acids are predominantly methyl-branched-chain fatty acids. iso-C_{15:0} predominates in most species, although a few species contain comparable amounts of iso-C_{15:0} and anteiso-C_{15:0}. The 3-hydroxylated fatty acids are generally straight-chain saturated fatty acids.

The DNA base compositions range from 40 to 55 mol%. The type species is *Porphyromonas asaccharolytica*.

Description of *Porphyromonas catoniae* (Moore and Moore 1994) comb. nov. The following description is based on that of Moore and Moore (10). Cells of the type strain grown in peptone-yeast extract-glucose (PYG) broth are 0.6 µm wide by 0.8 to 1.7 µm long and occur in pairs and short chains. Cells grown in media containing fermentable carbohydrates may be highly vacuolated. Colonies incubated on blood agar for 2 days are 0.5 to 2 mm in diameter, circular, entire, flat to low convex, and transparent. Most strains are not hemolytic on rabbit blood agar; occasionally strains may be beta-hemolytic. No colonies having a dark pigment are produced. Abundant growth occurs in peptone-yeast extract or PYG broth. Broth cultures are turbid with a smooth to fine granular sediment. The pH of PYG broth cultures is 5.0 to 5.5. A major amount of propionic acid and moderate to major amounts of acetic and succinic acids are produced as metabolic end products. Small amounts or traces of lactic, formic, and isovaleric acids are also produced. Saccharolytic (10). Gelatin is hydrolyzed by most strains. Some strains hydrolyze milk and meat. H₂S is produced by some strains. Weak or no growth occurs in PYG containing 20% bile. The major cellular fatty acids are iso-C_{15:0} and anteiso-C_{15:0}; substantial amounts of iso-C_{13:0} are also present. Isolated from the gingival crevices of humans with gingivitis or periodontitis and from humans with healthy gingivae. The type strain is ATCC 51270. The G+C content of the type strain is 49 mol%.

The 16S rRNA gene sequence of strain ATCC 51270^T (T = type strain) has been deposited in the EMBL database under accession number X82823.

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