NOTES

Phylogenetic Analysis of *Ruminococcus flavefaciens*, the Type Species of the Genus *Ruminococcus*, Does Not Support the Reclassification of *Streptococcus hansenii* and *Peptostreptococcus productus* as *Ruminococci*

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The 16S rRNA gene sequence of the type strain of *Ruminococcus flavefaciens*, the type species of the genus *Ruminococcus*, was determined by PCR direct sequencing. A comparative sequence analysis showed that *R. flavefaciens* is phylogenetically related to a small cluster (cluster IV of Collins et al. [M. D. Collins, P. A. Lawson, A. Willems, J. J. Cordoba, J. Fernandez-Garayzabal, P. Garcia, J. Cai, H. Hippe, and J. A. E. Farrow, Int. J. Syst. Bacteriol. 44:812–826, 1994]) of organisms which includes several *Clostridium* and *Eubacterium* species. *R. flavefaciens* was found to be phylogenetically only remotely related to *Ruminococcus gnavus*, *Ruminococcus torques*, *Peptostreptococcus productus*, and *Streptococcus hansenii*. These findings demonstrate that the genus *Ruminococcus* is not a monophyletic group, and the proposed transfer of *P. productus* and *S. hansenii* to this genus (T. Ezaki, N. Li, Y. Hashimoto, H. Miura, and H. Yamamoto, Int. J. Syst. Bacteriol. 44:130–136, 1994) is not supported.

The taxonomic positions of *Peptostreptococcus productus* (Prévot 1941) Smith 1957 and *Streptococcus hansenii* (Holdeman and Moore 1974) have always been uncertain (5, 7, 12). *P. productus* is saccharolytic and differs markedly in this respect from other peptostreptococci, which are asaccharolytic and use peptones and amino acids as their energy sources. *P. productus* also differs from other peptostreptococci in containing a meso-diaminopimelic acid directly cross-linked murein (type A1 γ) and in having a significantly higher DNA G+C content (4, 8). *S. hansenii* closely resembles *P. productus* (e.g., in saccharolytic behavior) but differs from most authentic streptococcal species in being obligately anaerobic and in having a type A1 γ murein (12). Ezaki et al. (4, 11), using 16S rRNA gene sequencing, demonstrated that *P. productus* and *S. hansenii* are phylogenetically closely related to *Ruminococcus gnavus*, *Ruminococcus torques*, *Coprococcus eutactus*, and *Clostridium coccoides*. On the basis of these findings and phenotypic evidence, Ezaki et al. proposed that *P. productus* and *S. hansenii* should be reclassified in the genus *Ruminococcus* (4) as *Ruminococcus productus* and *Ruminococcus hansenii*, respectively. In a more comprehensive analysis of low-G+C-content gram-positive bacteria (2), *P. productus* and *S. hansenii* were found to be members of a large cluster (cluster XIVa) which included 14 clostridial species, "*Acetitomaculum ruminis*," *Coprococcus eutactus*, *Eubacterium cellulosolvens*, and *Roseburia cecicola*. Furthermore, the closest relative of *P. productus* and *S. hansenii* was clearly *Clostridium coccoides*, not *R. torques* (2). In this context it is pertinent to note the suggestion of several authors (1, 8) that the ovoid fermentative ruminococcal species (*Ruminococcus callidus*, *R. gnavus*, *Ruminococcus lactaris*, *Ruminococcus obeum*, and *R. torques*) may represent a separate

genus distinct from the spherical ruminococci *Ruminococcus albus* and *Ruminococcus bromii* and the type species of the genus *Ruminococcus*, *Ruminococcus flavefaciens*. Thus, the genus *Ruminococcus* may be heterogeneous, and a close phylogenetic relationship to *R. gnavus* and *R. torques* does not necessarily imply a close affinity with *R. flavefaciens*. To establish the validity of transferring *P. productus* and *S. hansenii* to the genus *Ruminococcus*, we determined the 16S rRNA gene sequence of *R. flavefaciens*, and in this paper we describe the results of a phylogenetic analysis.

The almost complete 16S rRNA gene sequence of the type strain of *R. flavefaciens* (NCFB 2213 [= ATCC 19208]) was determined by using PCR direct DNA sequencing. 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 DNA was extracted and purified as described previously (10). A large fragment of the 16S rRNA gene was amplified by PCR by using conserved primers (5' GAGAGTTTGATCCTGGCTCAGGA and 5' AAGGAGGTGATCCAGCCGCA) proximal to the 5' and 3' ends of the gene as described by Hutson et al. (9). The fragment was purified by using a Prep-A-Gene kit (Bio-Rad, Hercules, Calif.) according to the manufacturer's instructions and was sequenced by using a *Taq* DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, Calif.) and a model 373A automatic sequencer (Applied Biosystems).

The sequence which we determined consisted of 1,448 nucleotides. Identical sequences were obtained for the type strains of *R. flavefaciens* obtained from different culture collections (NCFB 2213 and ATCC 19208). Database searches indicated that *R. flavefaciens* was a member of the *Clostridium* subphylum of the gram-positive bacteria. The highest levels of sequence relatedness were observed with *Clostridium leptum* (level of similarity, approximately 88.3%) and other members of *Clostridium* cluster IV (range of levels of similarity, approx-

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TABLE 1. Similarity values for the 16S rRNA genes of ruminococcal species and representatives of *Clostridium* clusters IV and XIVa

Species	% Similarity to:																					
	Cluster IV organisms							Cluster XIVa organisms														
	<i>Ruminococcus flavefaciens</i>	<i>Clostridium cellulosi</i>	<i>Clostridium leptum</i>	<i>Clostridium sporosphaeroides</i>	<i>Eubacterium desmolans</i>	<i>Eubacterium plautii</i>	<i>Eubacterium siraeum</i>	<i>Ruminococcus gnavus</i>	<i>Ruminococcus (Streptococcus) hansenii</i>	<i>Ruminococcus (Peptostreptococcus) productus</i>	<i>Ruminococcus torques</i>	<i>Clostridium aminophilum</i>	<i>Clostridium clostridiiforme</i>	<i>Clostridium coccoides</i>	<i>Clostridium nexile</i>	<i>Clostridium oroticum</i>	<i>Clostridium sphenoides</i>	<i>Clostridium symbiosum</i>	<i>Eubacterium cellulosolvens</i>	<i>Eubacterium fissicatena</i>	<i>Eubacterium formicigenans</i>	
<i>Clostridium cellulosi</i>	87.5																					
<i>Clostridium leptum</i>	88.3	88.3																				
<i>Clostridium sporosphaeroides</i>	86.2	88.7	93.4																			
<i>Eubacterium desmolans</i>	87.0	97.2	87.4	86.4																		
<i>Eubacterium plautii</i>	85.8	85.4	87.7	86.7	88.9																	
<i>Eubacterium siraeum</i>	88.5	86.8	86.6	85.7	86.7	86.8																
<i>Ruminococcus gnavus</i>	79.0	76.5	78.5	77.3	79.8	79.0	80.0															
<i>Ruminococcus (Streptococcus) hansenii</i>	83.9	80.2	82.5	80.7	82.6	82.9	83.6	86.5														
<i>Ruminococcus (Peptostreptococcus) productus</i>	83.1	80.0	82.3	80.3	82.6	82.5	82.2	84.7	93.0													
<i>Ruminococcus torques</i>	82.6	80.0	81.8	80.8	82.2	82.8	82.8	87.6	91.1	90.3												
<i>Clostridium aminophilum</i>	83.0	81.0	83.6	82.0	82.9	83.1	82.8	84.4	90.3	88.8	89.2											
<i>Clostridium clostridiiforme</i>	82.3	79.9	83.1	81.2	82.5	83.0	82.3	87.1	92.9	90.9	92.0	91.0										
<i>Clostridium coccoides</i>	84.6	81.1	83.6	81.6	84.1	84.6	83.6	88.0	95.6	96.1	92.9	91.0	93.6									
<i>Clostridium nexile</i>	83.3	80.2	82.6	81.6	82.9	82.5	83.0	88.7	91.7	89.8	94.2	89.6	92.9	92.2								
<i>Clostridium oroticum</i>	84.3	80.6	83.2	81.9	83.0	84.0	83.2	88.4	91.7	90.8	95.0	89.9	93.8	93.5	93.0							
<i>Clostridium sphenoides</i>	83.3	80.7	83.7	82.8	83.4	84.1	84.0	86.6	91.8	90.2	92.2	91.4	93.4	93.3	92.3	93.0						
<i>Clostridium symbiosum</i>	83.3	80.9	82.7	80.9	82.3	82.9	83.6	85.2	91.6	89.7	91.8	92.6	93.5	91.9	91.9	93.4	92.9					
<i>Eubacterium cellulosolvens</i>	81.2	79.4	82.0	80.7	81.8	81.7	82.2	84.8	91.5	89.4	89.4	90.7	91.4	91.9	90.1	89.8	90.6	90.3				
<i>Eubacterium fissicatena</i>	84.0	80.5	82.8	81.5	82.6	83.6	82.9	88.9	91.7	90.9	95.4	90.1	93.1	93.8	90.4	97.9	93.4	93.6	90.4			
<i>Eubacterium formicigenans</i>	83.8	80.3	82.6	81.3	82.5	82.8	83.6	88.3	91.3	89.7	92.3	89.4	92.0	92.3	90.8	93.9	93.0	91.5	90.8	94.4		
<i>Roseburia cecicola</i>	83.5	80.4	83.5	82.0	83.4	83.2	83.5	87.8	92.2	90.4	92.4	90.8	94.0	92.8	93.5	92.9	93.1	93.1	90.0	93.4	91.6	

FIG. 1. Unrooted phylogenetic tree showing the position of *R. flavefaciens* within the *Clostridium* subphylum of the gram-positive bacteria.

imately 85.8 to 88.5%) (Table 1) (groups of Collins et al. [2]). *R. flavefaciens* exhibited significantly lower levels of sequence similarity with *R. gnavus* (79.0%), *R. torques* (82.6%), *R. (P.) productus* (83.1%), and *R. (S.) hansenii* (83.9%). We also performed a detailed phylogenetic analysis of *R. flavefaciens* within the *Clostridium* subphylum. A 1,310-nucleotide region (positions 101 to 1376; *Escherichia coli* numbering) was aligned by using the program PILEUP (3), and the alignment was corrected manually. A distance matrix was produced by using the program DNADIST of the PHYLIP package (6), and a tree was constructed by the neighbor-joining method with the program NEIGHBOR of the same package. Figure 1 is a tree which shows the position of *R. flavefaciens* within the *Clostridium* subphylum. Our treeing analysis confirmed that *R. flavefaciens* is closely related to members of *Clostridium* cluster IV, which includes three clostridial species (*Clostridium cellulosi*, *Clostridium leptum*, and *Clostridium sporosphaeroides*) and three eubacterial species (*Eubacterium desmolans*, *Eubacterium plautii*, and *Eubacterium siraeum*). In contrast, *R. (P.) productus*, *R. (S.) hansenii*, and other ruminococcal species were distantly related and were recovered in *Clostridium* cluster XIVa (Fig. 1).

It is evident from the comparative sequence analysis that the genus *Ruminococcus* is not a monophyletic group. *R. flavefaciens*, the type species of the genus, exhibits a distinct relationship with a phenotypically diverse (e.g., sporulating and non-sporulating) group of organisms referred to as *Clostridium* cluster IV (Fig. 1) (2). In contrast, the other ruminococcal

species which we examined are phylogenetically distantly related to *R. flavefaciens* and exhibit a far closer affinity with *Clostridium* cluster XIVa (2). The latter group contains organisms which exhibit a wide variety of phenotypes and has a level of intracluster sequence divergence of approximately 16.5%, which is more consistent with a suprageneric (i.e., family) association than a single genus. All of cluster XIVa requires extensive taxonomic revision since many of the species are currently classified in the genera *Clostridium*, *Eubacterium*, and *Ruminococcus* (Fig. 1), although they are in fact phylogenetically only remotely related to the type species of their respective genera. Since cluster XIVa contains a great variety of organisms not previously considered to be related, additional comparative phenotypic studies and sequence analyses (e.g., analyses that include other members of the genus *Ruminococcus*) will be necessary before a taxonomically acceptable subdivision of this cluster can be proposed. However, our data demonstrate that, irrespective of whether *R. (P.) productus*, *R. (S.) hansenii*, *R. gnavus*, and *R. torques* belong to a single genus, it is taxonomically incorrect to preserve the name *Ruminococcus* for these organisms. Therefore, we suggest that the names *Streptococcus hansenii* and *Peptostreptococcus productus* should be retained until the taxonomy and nomenclature of *Clostridium* cluster XIVa are resolved. As noted above, the species belonging to this cluster are currently assigned to a variety of genera (e.g., the genera *Acetivomaculum*, *Coprococcus*, and *Roseburia*), a fact which will have to be taken into account in

any future taxonomic revision involving *P. productus* and *S. hansenii*.

Nucleotide sequence accession number. The 1,448-nucleotide sequence which we determined has been deposited in the EMBL data library under accession number X83430.

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