Ruminococcus champanellensis sp. nov., a cellulose-degrading bacterium from human gut microbiota

Christophe Chassard,† Eve Delmas,† Céline Robert,† Paul A. Lawson2 and Annick Bernalier-Donadille1

1Unité de Microbiologie, INRA, Centre de Recherches de Clermont Ferrand – Theix, 63 122 Saint Genès-Champangelle, France
2Department of Botany and Microbiology, University of Oklahoma, Norman, OK 73019, USA

A strictly anaerobic, cellulolytic strain, designated 18P13T, was isolated from a human faecal sample. Cells were Gram-positive non-motile cocci. Strain 18P13T was able to degrade microcrystalline cellulose but the utilization of soluble sugars was restricted to cellobiose. Acetate and succinate were the major end products of cellulose and cellobiose fermentation. 16S rRNA gene sequence analysis revealed that the isolate belonged to the genus Ruminococcus of the family Ruminococcaceae. The closest phylogenetic relative was the ruminal cellulolytic strain Ruminococcus flavefaciens ATCC 19208T (<95% 16S rRNA gene sequence similarity). The DNA G+C content of strain 18P13T was 53.05 ± 0.7 mol%. On the basis of phylogenetic analysis, and morphological and physiological data, strain 18P13T can be differentiated from other members of the genus Ruminococcus with validly published names. The name Ruminococcus champanellensis sp. nov. is proposed, with 18P13T (=DSM 18848T =JCM 17042T) as the type strain.

The human large intestine harbours a large diversity of bacterial communities that play a key role in health and disease through their involvement in nutrition, pathogenesis and immunology (Cummings & Macfarlane, 1991; Salminen et al., 1998). A proper understanding of the diversity and functionality of species in the human gut ecosystem is therefore of considerable importance. Over the past 20 years, the microbiota composition has been investigated using both culture- and molecular-based methods and results have revealed the extensive diversity of this ecosystem (Eckburg et al., 2005; Chassard et al., 2008b; Qin et al., 2010). The microbiota is mainly composed of bacteria belonging to three major phyla: ‘Bacteroidetes’, ‘Firmicutes’ and ‘Actinobacteria’. The genus Ruminococcus represents an important phylogenetic taxon, belonging to phylum ‘Firmicutes’, and corresponds to 5–15% of the total bacterial population in the colon (Chassard et al., 2008b; Ramirez-Farias et al., 2009).

Presently, the genus Ruminococcus is not monophyletic and is divided into two phylogenetically separate groups. Group I is located within rRNA cluster IV and includes Ruminococcus flavefaciens, the type species of the genus. In the latest edition of Bergey’s Manual of Systematic Bacteriology, members of group I were included in the family Ruminococcaceae and should be considered as Ruminococcus sensu stricto (Rainey, 2009a). Members of group II are located within rRNA cluster XIVa, which is now recognized as the family Lachnospiraceae, a large group of phenotypic and phylogenetic heterogeneous genera (Rainey, 2009b). Recently, a number of misclassified Ruminococcus species and a Clostridium species in group II were reclassified in the genus Blautia (Liu et al., 2008). The remaining ruminococci within group II most likely constitute the nuclei of novel genera and should not be considered true ruminococci.

The genus Ruminococcus comprises anaerobic Gram-positive cocci with a fermentative metabolism for which carbohydrates, but not amino acids, serve as substrates for growth (Hungate, 1966; Rainey & Janssen, 1995). R. flavefaciens and Ruminococcus albus, isolated from the rumen, are the only Ruminococcus species able to degrade cellulose (Flint et al., 2008). Ruminococcus bromii and Ruminococcus callidus, isolated from the human gut, are able to degrade other complex polysaccharides such as starch or xylan (Leitch et al., 2007). However, the presence of cellulolytic Ruminococcus-like strains in the human

Abbreviation: DMA, dimethyl acetal.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 18P13T is AJ515913.

Two supplementary figures and two supplementary tables are available with the online version of this paper.
gut was reported by Robert & Bernalier-Donadille (2003). More recently, a real-time PCR analysis was developed to quantify cellulose-degrading rumiococci in human faeces and used to confirm their presence in the human gut, especially in methane-excreting individuals (Chassard et al., 2010). The present study reports the isolation and characterization of a novel cellulose-degrading isolate from human faeces, strain 18P13\(^T\), which belongs to the genus *Ruminococcus*.

During an investigation of the cellulose-degrading community of the human gut, we isolated five cellulolytic, Gram-positive, anaerobic cocci from two healthy human faecal samples (Robert & Bernalier-Donadille, 2003). The isolates were assigned to the genus *Ruminococcus* on the basis of 16S rRNA gene sequence analysis, their closest relatives being *R. flavefaciens* ATCC 19208\(^T\) and *R. callidus* ATCC 27760\(^T\). The isolates were further shown to represent a new phylogenetic lineage and could be quantified by PCR using specific primers (Chassard et al., 2010). Two isolates, strains 18P13\(^T\) and 25F8, shared >99% 16S rRNA gene sequence similarity. Strain 18P13\(^T\) was chosen for further analysis as it was the most efficient in degrading microcrystalline cellulose.

Strain 18P13\(^T\) was isolated from a fresh faecal sample of a 38-year-old methane-excreting healthy human female who had not received antibiotics in the last 3 months, had no diagnosed gastrointestinal disease, consumed a diverse diet and had a normal body mass index (18–25 kg m\(^{-2}\)). The isolation procedure has been described previously (Robert & Bernalier-Donadille, 2003). Briefly, faecal dilutions were inoculated into liquid basal cellulolytic (BC) medium containing 100 mg Whatman no. 1 filter paper as the sole energy source. Cellulolytic strains were isolated from the highest dilution showing filter paper degradation, using solid BC medium containing 0.7% (w/v) hydrated Sigmacell 50 cellulose as the sole energy source. Strain 18P13\(^T\) was isolated using the roll-tube technique (Hugate, 1969) from the 10\(^{-8}\) dilution. Strain 18P13\(^T\) was routinely cultivated in BC medium and its growth and phenotypic characteristics were determined in semi-synthetic BC medium (Robert & Bernalier-Donadille, 2003). Strain 18P13\(^T\) was stored at −80 °C on solid medium.

Cell morphology and motility were determined using 18-h-old cultures grown with cellobiose by phase-contrast microscopy and electron microscopy after negative staining of the whole cell with 2% uranyl acetate. Gram staining was performed with the conventional stain. Cell morphology and wall structure were further observed by transmission electron microscopy (Philips 400) of ultrathin sections after staining with uranyl acetate and lead citrate (Bernalier et al., 1996).

Cells of strain 18P13\(^T\) were cocci (about 1.3 μm in diameter). Cells stained Gram-positive and thin sections showed a Gram-positive cell-wall structure (Supplementary Fig. S1, available in IJSEM Online). Negatively stained cells revealed the absence of flagella. Viable cells were not recovered from cultures held at 100 °C for 10 min and no spores were detected either in cellulose- or cellobiose-grown cultures incubated for more than 15 days at 37 °C. Strain 18P13\(^T\) was unable to grow on agar plates and was thus cultivated on agar medium using the roll-tube technique with 100% CO\(_2\) in the gas phase. Colonies on BC medium containing cellobiose were white with a brown centre, translucent, circular with entire margins and 1.5–2.5 mm in diameter after 24 h.

DNA extraction, amplification and sequencing of the 16S rRNA gene of strain 18P13\(^T\) were performed as reported previously (Chassard et al., 2008a). Briefly, cells were grown for 24 h in 50 ml BC medium containing 2 g cellobiose l\(^{-1}\), harvested by centrifugation and subjected to DNA extraction (Easy DNA kit; Invitrogen). The 16S rRNA gene was amplified using the universal primers F8 and 1492R. Sequencing reactions were performed with the Dye Terminator Cycle Sequencing Ready Reaction kit (Perkin Elmer), according to the manufacturer’s instructions. The closest known relatives were determined by performing database searches using FASTA (Lipman & Pearson, 1985). These sequences and those of other related strains were retrieved from GenBank and aligned with the newly determined sequences using SEQtools and TreeView (Page, 1996).

An almost-complete sequence of the 16S rRNA gene (1368 nt) of strain 18P13\(^T\) was obtained. Phylogenetic analysis (Fig. 1) revealed that the isolate was most closely related to members of ruminococcal group I, the genus *Ruminococcus*, and this cluster was supported by a high bootstrap value (93%). All of the major nodes were confirmed by maximum-parsimony analysis (data not shown). Within the cluster, strain 18P13\(^T\) was most closely related to one of the cellulolytic strains from the rumen in the family *Ruminococcaceae*, *R. flavefaciens* ATCC 19208\(^T\), with which it shared <95% 16S rRNA gene sequence similarity. The other cellulolytic strain from the rumen, *R. albus* ATCC 27210\(^T\), exhibited only 86% 16S rRNA gene sequence similarity with strain 18P13\(^T\). Of the strains from the human gut, the closest relative to strain 18P13\(^T\) was *R. callidus* ATCC 27760\(^T\) (88% 16S rRNA gene sequence similarity). Although there is no precise correlation between 16S rRNA gene sequence divergence and species delineation, it is generally recognized that a divergence value >3% is significant (Stackebrandt & Goebel, 1994) and more recent data have demonstrated that this value can be decreased to 1.3% without loss of resolution (Stackebrandt & Ebers, 2006). Therefore, the tree topology and the 16S rRNA gene sequence divergence (5%) showed that the isolate represents a novel species in the genus *Ruminococcus*. 
The G+C content of the genomic DNA was determined using HPLC by the DSMZ (Braunschweig, Germany). The cellular fatty acids were also determined by the DSMZ, according to the Microbial Identification System (Microbial ID). The DNA G+C content of strain 18P13T was 53.05 ± 0.7 mol% (n=4), which was much higher than values reported for the genus Ruminococcus (e.g. 39–44 mol% for R. flavefaciens ATCC 19208T, 42.6–45.8 mol% for R. albus ATCC 27210T and 43 mol% for R. callidus ATCC 27760T; Buchanan & Gibbons, 1974; De Vos et al., 2009) and constituted a distinguishing characteristic. The predominant fatty acids of strain 18P13T were iso-C15:0 (26.57 %), anteiso-C15:0 (19.56 %), iso-C16:0 dimethyl acetal (DMA; 11.94 %) and iso-C16:0 (8.84 %) (Supplementary Table S1, available in IJSEM Online). This finding was consistent with the finding of Allison et al. (1962), who found that R. albus and R. flavefaciens produced 15- and 16-carbon fatty acids.

The presence of catalase and cytochrome oxidase was examined by standard methods. Growth at 25–45 °C (at intervals of 1 °C; pH 6.8) and at pH 5.5–7.5 (at intervals of 0.1 pH units; 37 °C) was determined in semi-synthetic BC medium containing 2 g cellobiose l−2, with the pH adjusted using NaHCO₃. Other physiological, biochemical and enzyme activity tests were performed using API 20 A, API 50 CH and API ZYM kits (bioMe®rieux), according to the manufacturer’s instructions, with incubation at 37 °C in an anaerobic chamber.

The results of the phenotypic analysis are given in Table 1 and the species description. Strain 18P13T was strictly anaerobic and required an O₂-free medium (100 % CO₂ headspace) at a redox potential sufficient to decolourize resazurin (E₉₀ = −50 mV). Cells of strain 18P13T did not possess catalase or cytochrome oxidase, as observed in R.

Fig. 1. Unrooted neighbour-joining tree based on 16S rRNA gene sequences (approx. 1320 nt), showing the phylogenetic relationships between strain 18P13T and some members of the families Ruminococcaceae and Lachnospiraceae. Bootstrap values (>90 %) based on 1000 replications are shown at branch nodes. Bar, 1 % sequence divergence.
flavefaciens, R. albus and R. callidus (Hungate, 1966; Buchanan & Gibbons, 1974; De Vos et al., 2009). With cellulbiose as the substrate, the isolate grew at 33–39 °C (optimum 39 °C) and at pH 6.5–6.8 (optimum pH 6.8). The optimal growth conditions corresponded with those found in the human colon. At 37 °C on cellulbiose BC medium, the doubling time was 1.9 h and the maximum optical density (OD600) was 1.1–1.3. Strain 18P13T did not require rumen fluid; growth was similar in cellulbiose BC medium (20 % rumen fluid) and in cellulbiose semi-synthetic BC medium (no rumen fluid).

The phenotypic characteristics of strain 18P13T are compared with those of its closest phylogenetic neighbours in Table 1. In addition to cellulose, strain 18P13T could utilize xylan and cellulbiose but no growth was observed with many other carbohydrates, including starch and glucose. The nutritional capacity of other members of the genus Ruminococcus, in particular R. flavefaciens, is reportedly restricted to a limited range of substrates (Hungate, 1966; De Vos et al., 2009) but in the current study the human gut strain R. callidus ATCC 27760T was able to use most of the substrates, but notably not cellulose. Strain 18P13T and R. flavefaciens ATCC 19208T were the most similar, but the isolate could be differentiated by the absence of lactose fermentation and no yellow pigment production. Strain 18P13T could be differentiated from R. callidus ATCC 27760T by its ability to use cellulose, but not starch, glucose, lactose, maltose, raffinose or sucrose.

Degradation and fermentation of cellulose by strain 18P13T were studied in BC medium (10 ml per tube) containing 100 mg Avicel pH 101, Simgacell 101 or Whatman no. 1 filter paper cellulose as the sole energy source. The inocula were 0.4 ml of a 5-day-old culture on BC medium containing Avicel pH 101. Each incubation time and substrate was tested in triplicate. The kinetics of cellulose degradation were determined by measuring the dry weight of remaining cellulose, as described previously (Robert & Bernalier-Donadille, 2003). End products of cellulose fermentation were determined using 10-day-old cultures. Gases in the headspace and short-chain fatty acids in the supernatants were analysed by gas-phase chromatography (Robert et al., 2001). The production of formate, succinate, ethanol and lactate was measured using enzymic methods (Roche Boeringher Mannheim). End products of cellulbiose fermentation were also determined using 24-h-old cultures on semi-synthetic BC medium containing 2 g cellulbiose 1−1. Cellulolytic and xylanolytic activities were determined using CM-cellulose, Avicel pH 101 or xylan oat spelt as substrates and measuring the released reducing sugars colorimetrically (Forano et al., 1994; Devillard et al., 1999); glucose and xylose were used as standards and each assay was performed in triplicate. Protein was determined by the method of Bradford (1976) using bovine serum albumin as the standard.

Strain 18P13T could degrade cellulose from different sources (Avicel pH 101, Simgacell 101 and Whatman no. 1 filter paper; Supplementary Fig. S2, available in IJSEM Online); however, filter paper was degraded most during the 12-day incubation period. CM-cellulose activity [1.4 ± 0.2 μg glucose (mg protein)−1 h−1] and avicelase activity [0.9 ± 0.2 μg glucose (mg protein)−1 h−1] was measured in the supernatant fluid of 6-day-old cultures grown with Avicel pH 101. In addition, xylanase activity [3.3 ± 0.4 μg xylose (mg protein)−1 h−1] was detected in this culture. Similarly, R. flavefaciens has been shown to express both cellulase and xylanase activities (Flint et al., 2008). These enzymic processes may confer an ecological advantage on cellulolytic bacteria by enabling them to access the cellulose fibrils in plant cell walls and the matrix polysaccharides composed of xylan, pectin and mannans that are available within this complex ecosystem. The end products of cellulose fermentation by strain 18P13T were mainly acetate and succinate, with smaller quantities of ethanol, formate and lactate (Supplementary Table S2, available in IJSEM Online). The ratio of acetate and succinate varied according to the type of cellulose provided. In addition, strain 18P13T produced large amounts of H2 during cellulose degradation. Similarly, the cellulolytic species R. flavefaciens and R. albus ferment cellulose with great production of H2 (Buchanan & Gibbons, 1974); by contrast, these two species do not produce succinate from cellulose fermentation (Table 1).
On the basis of phenotypic, genotypic and phylogenetic differences, strain 18P13T could be clearly distinguished from all previously described species of the ruminococci. The isolate’s closest phylogenetic neighbours belonged to the genus Ruminococcus in the family Ruminococcaceae (ruminococcal group I) (De Vos et al., 2009; Rainey, 2009a). Therefore, it is proposed to classify the isolate as a representative of a novel species, Ruminococcus champanellensis sp. nov.

Description of Ruminococcus champanellensis sp. nov.

Ruminococcus champanellensis (cham.pae.nel.len’ sis. N.L. masc. adj. champanellensis of or belonging to Saint-Genes-Champanelle, where the type strain was first isolated).

Cells are non-motile cocci (up to 1.3 μm in diameter) and Gram-stain-positive, with a Gram-positive cell-wall ultrastructure. No heat-resistant endospores are formed. Strictly anaerobic. Cytochrome oxidase- and catalase-negative. Fermentable carbohydrates are required for growth. Ferments only cellulose, cellobiose and xylose; it does not ferment other carbohydrates including starch and pectin. Cellulose and cellobiose are metabolized to acetate and succinate. Grows at 33–39°C (optimum 39°C) and at pH 6.5–6.8 (optimum pH 6.8). Rumen fluid is not required for growth. Indole-negative, but able to hydrolyse aesculin.

The type strain, 18P13T (DSM 18848T=JCM 17042T), was isolated from human faeces of a methane-excreting healthy individual at the INRA centre of Clermont-Ferrand-Theix, Auvergne, France. The DNA G+C content of the type strain is 53.05 ± 0.7 mol%.

Acknowledgements

C.R. and C.C. were supported by fellowships from the French Ministère de la Recherche et de l’Enseignement Supérieur. We express many thanks to B. Lassalas for her help with the gas chromatography analyses of fermentation end products and to B. Gaillard-Martine for assistance with electron microscopy.

References


