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

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A strictly anaerobic, cellulolytic strain, designated 18P13^T, was isolated from a human faecal sample. Cells were Gram-positive non-motile cocci. Strain 18P13^T 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 19208^T (<95 % 16S rRNA gene sequence similarity). The DNA G+C content of strain 18P13^T was 53.05 ± 0.7 mol%. On the basis of phylogenetic analysis, and morphological and physiological data, strain 18P13^T can be differentiated from other members of the genus *Ruminococcus* with validly published names. The name *Ruminococcus champanellensis* sp. nov. is proposed, with 18P13^T (=DSM 18848^T=JCM 17042^T) 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.

Abbreviation: DMA, dimethyl acetal.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 18P13^T is AJ515913.

Two supplementary figures and two supplementary tables are available with the online version of this paper.

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

gut was reported by Robert & Bernalier-Donadille (2003). More recently, a real-time PCR analysis was developed to quantify cellulose-degrading ruminococci 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 Western diet that included at least 15 g dietary fibre per day 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 (Hungate, 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% CO₂ 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 (Rasmussen, 2002). The resulting alignment was corrected manually using GeneDoc (Nicholas *et al.*, 1997). A phylogenetic tree was constructed according to the neighbour-joining method (Saitou & Nei, 1987) and a bootstrap analysis was performed with 1000 replications 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*.

Fig. 1. Unrooted neighbour-joining tree based on 16S rRNA gene sequences (approx. 1320 nt), showing the phylogenetic relationships between strain 18P13^T 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.

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 18P13^T 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 19208^T, 42.6–45.8 mol% for *R. albus* ATCC 27210^T and 43 mol% for *R. callidus* ATCC 27760^T; Buchanan & Gibbons, 1974; De Vos *et al.*, 2009) and constituted a distinguishing characteristic. The predominant fatty acids of strain 18P13^T were iso-C_{15:0} (26.57%), anteiso-C_{15:0} (19.56%), iso-C_{15:0} dimethyl acetal (DMA; 11.94%) and iso-C_{16:0} (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⁻¹, 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 (bioMérieux), 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 18P13^T was strictly anaerobic and required an O₂-free medium (100% CO₂ headspace) at a redox potential sufficient to decolorize resazurin ($E'_0 = -50$ mV). Cells of strain 18P13^T did not possess catalase or cytochrome oxidase, as observed in *R.*

Table 1. Characteristics useful in the differentiation of strain 18P13^T from its closest phylogenetic neighbours

Strains: 1, *Ruminococcus champanellensis* sp. nov. 18P13^T; 2, *R. flavefaciens* ATCC 19208^T; 3, *R. albus* ATCC 27210^T; 4, *R. callidus* ATCC 27760^T. Data for columns 2 and 3 were taken from Buchanan & Gibbons (1974) and Rainey (2009a) and for column 4 from this study and Rainey (2009a). All strains are negative for catalase and fermentation of arabinose and mannitol. +, Positive; w/–, weakly positive or negative; –, negative; ND, no data available.

Characteristic	1	2	3	4
Cell size (µm)	0.9–1.3	0.8–0.9	0.8–2	0.7–0.9
Gram stain	+	+	+	+
Fermentation of:				
Cellulose	+	+	+	–
Xylan	+	+	+	+
Starch	–	–	–	+
Glucose	–	–	+	+
Cellobiose	+	+	+	+
Lactose	–	+	+	+
Mannose	–	–	+	–
Maltose	–	–	–	+
Raffinose	–	–	–	+
Sucrose	–	–	+	+
Xylose	–	–	–	w/–
Major fermentation products*	A,S	A,F,E	A,E	ND
DNA G + C content (mol%)	53.05	39–44	42.6–45.8	43

*A, acetate; E, ethanol; F, formate; S, succinate.

flavefaciens, *R. albus* and *R. callidus* (Hungate, 1966; Buchanan & Gibbons, 1974; De Vos *et al.*, 2009). With cellobiose 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 cellobiose BC medium, the doubling time was 1.9 h and the maximum optical density (OD₆₀₀) was 1.1–1.3. Strain 18P13^T did not require rumen fluid; growth was similar in cellobiose BC medium (20 % rumen fluid) and in cellobiose semi-synthetic BC medium (no rumen fluid).

The phenotypic characteristics of strain 18P13^T are compared with those of its closest phylogenetic neighbours in Table 1. In addition to cellulose, strain 18P13^T could utilize xylan and cellobiose 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 27760^T was able to use most of the substrates, but notably not cellulose. Strain 18P13^T and *R. flavefaciens* ATCC 19208^T were the most similar, but the isolate could be differentiated by the absence of lactose

fermentation and no yellow pigment production. Strain 18P13^T could be differentiated from *R. callidus* ATCC 27760^T by its ability to use cellulose, but not starch, glucose, lactose, maltose, raffinose or sucrose.

Degradation and fermentation of cellulose by strain 18P13^T were studied in BC medium (10 ml per tube) containing 100 mg Avicel pH 101, Sigmacell 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 Boehringer Mannheim). End products of cellobiose fermentation were also determined using 24-h-old cultures on semi-synthetic BC medium containing 2 g cellobiose l^{–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 18P13^T could degrade cellulose from different sources (Avicel pH 101, Sigmacell 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-cellulase 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 18P13^T 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 18P13^T 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 18P13^T 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.pa.nel.len'sis. N.L. masc. adj. *champanellensis* of or belonging to Saint-Genès-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 ultra-structure. No heat-resistant endospores are formed. Strictly anaerobic. Cytochrome oxidase- and catalase-negative. Fermentable carbohydrates are required for growth. Ferments only cellulose, cellobiose and xylan; 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. Urease is not detected and gelatin is not hydrolysed. Does not grow with glucose, sucrose, fructose, maltose, xylose, galactose, ribose, melibiose, mannose, lactulose, galacturonic acids, lactose, raffinose, arabinose, salicin, trehalose, mannitol, inositol, sorbitol or fucose. Does not produce acid from L-rhamnose, L-sorbose, dulcitol, arabitol, D-adonitol, erythritol, inulin, melibiose, xylitol, gentiobiose, turanose, D-lyxose, D-tagatose, fucose, glycerol, N-acetylglucosamine or amygdalin. With API ZYM, produces alkaline phosphatase, esterase, esterase lipase, naphthol-AS-BI-phosphohydrolase, α- and β-galactosidases, and β-glucosidase, but does not produce lipase, leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, β-glucuronidase, α-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase or α-fucosidase. The major fatty acids are iso-C_{15:0}, anteiso-C_{15:0}, iso-C_{15:0} DMA and iso-C_{16:0}.

The type strain, 18P13^T (=DSM 18848^T=JCM 17042^T), 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-Martinie for assistance with electron microscopy.

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comb. nov., *Blautia hansenii* comb. nov., *Blautia hydrogenotrophica* comb. nov., *Blautia luti* comb. nov., *Blautia producta* comb. nov., *Blautia schinkii* comb. nov. and description of *Blautia wexlerae* sp. nov., isolated from human faeces. *Int J Syst Evol Microbiol* **58**, 1896–1902.

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