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NOTE

Ruminococcus faecis sp. nov., Isolated from Human Faeces

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Bacterial strain Eg2^T, an anaerobic, Gram-positive, non-motile, and non-spore-forming coccus, was isolated from human faeces. The optimal temperature for its growth was 37°C. Oxidase activity was negative, but catalase activity was positive. The strain was able to hydrolyze esculin and to produce acids from the fermentation of several substrates, including glucose. Lactic and acetic acids were the main products of glucose fermentation. The major fatty acids present in this strain were $C_{16:0}$, $C_{14:0}$, and $C_{18:1}$ *cis*11 DMA. The G+C content was 43.4 mol%. Based on the 16S rRNA gene sequence, strain Eg2^T was closely related to species of the genus *Ruminococcus* (96.3% similarity to *R. torques* and 96.2% similarity to *R. lactaris*), and its taxonomic position was placed within the Clostridium cluster XIVa. Based on phenotypic, chemotaxonomic, genotypic, and phylogenetic evidence, we propose that this novel strain be assigned to the genus *Ruminococcus* and be named *Ruminococcus faecis* sp. nov. The type strain is Eg2^T (=KCTC 5757^T =JCM 15917^T).

***Keywords*:** gut bacteria, clostridium cluster XIVa, *Ruminococcus faecis* sp. nov., taxonomy

Since the completion of the Human Genome Project, the contribution of symbiotic human gastrointestinal tract (GI-tract) microbiota to normal physiology and predisposition to disease has been the focus of many studies (Turnbaugh *et al.*, 2007). The gastrointestinal tract microbiota in adults is dominated by two divisions of Bacteria, the phyla *Firmicutes* and *Bacteroidetes* (Backhed *et al.*, 2005; Eckburg *et al.*, 2005; Wang *et al.*, 2005), with *Firmicutes* comprising around 64-76% of the gut microbiota (Eckburg *et al.*, 2005; Frank *et al.*, 2007; Ley *et al.*, 2008 Hattori and Taylor, 2009). The Firmicutes, Gram-positive bacteria with a low G+C content are mainly composed of the class *Clostridia*, which is divided into three major Clostridium clusters, cluster IV, IX, and XIV, as determined by culture-independent approaches based on the 16S rRNA gene sequence (Collins *et al.*, 1994; Eckburg *et al.*, 2005; Wang *et al.*, 2005; Frank *et al.*, 2007; Ley *et al.*, 2008). Clostridium cluster XIV (XIVa and b) constitutes a majority of the class *Clostridia*, and encompasses the genera *Eubacterium*, *Roseburia*, *Ruminococcus*, *Dorea*, *Lachnospira*, *Butyrivibrio*, and *Coprococcus* (Collins *et al.*, 1994; Eckburg *et al.*, 2005). The genus *Ruminococcus*, composed of Gram-positive and non-spore-forming cocci, falls into Clostridium cluster IV and XIVa, with 16 species reported in this genus to date. *Ruminococcus* species are known to inhabit the rumen of animals and to digest dietary fibers (Devillard *et al.*, 2004; Rincon *et al.*, 2004). Two species of *Ruminococcus*, *R. gauvreauii*, and *R. luti*, were found in human faeces, not in the rumen of cattle (Simmering *et al.*, 2002; Domingo *et al.*, 2008). Here, we employed a polyphasic approach to describe a *Ruminococcus*-like strain, named *Ruminococcus faecis* sp. nov., isolated from human faeces.

The isolate, designated as Eg2^T, was isolated from faeces obtained from a healthy 26-year-old male during a study on the diversity of cultivable intestinal microbiota of Koreans. The strain was isolated on Eggerth-Gagnon (EG) medium supplemented with 5% horse blood after incubation at 37°C for a two-day period under anaerobic conditions (in a Bactron II SHEL LAB Anaerobic Chamber, which contained a mixture of gases, N_2:H_2:CO_2=95:5:5). Then, the isolate was transferred and subcultured onto EG medium supplemented with 5% horse blood. The isolate was suspended in 10% skim milk (BBL) containing 10% glycerol and stored at -80°C. Two strains, *Ruminococcus torques* ATCC 27756^T and *Ruminococcus lactaris* ATCC 29176^T, obtained from ATCC (American Type Culture Collection), were used as references. All experiments were performed with the cells cultured in EG medium at 37°C and at a pH of 7.6-7.8 for three or four days, unless specified otherwise. Gram staining was performed using a Gram Staining kit (bioMérieux, France). Spore staining was with malachite green dye. Phase-contrast microscopy (ECLIPSE 50*i*, Nikon, Japan) was used to observe the morphology of cells, Gram and spore staining. Catalase and oxidase activities were tested with a solution of 3% (v/v) hydrogen peroxide and 1% (w/v) *p*-tetramethyl phenylenediamine (bioMérieux), respectively. The motility of the isolate was determined by stabbing the center of the column of PY-glucose medium containing 0.4% agar. For the description of other phenotypic characteristics, API ZYM, 20A and rapid ID 32A were applied according to the manufacturer's instructions (bioMérieux). The end products of glucose fermentation were analyzed using an HPLC system (940-LC High Performance Liquid Chromatograph (Varian, USA) with Alltech Prevail™ Organic Acid Columns (Grace Davison Discovery Sciences, USA). The isolate was cultured

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in PY-glucose medium. Lactic and acetic acid were produced from glucose fermentation. Data on fermentation products are used for the differentiation of species within the genus *Ruminococcus* as described by Moore *et al.* (1976). The fatty acid composition of the isolate was analyzed by gas chromatography (Hewlett Packard 6890, USA) and identified using the Microbial Identification software package (Sasser, 1990) after saponification, methylation and extraction, as described by Sherlock Microbial Identification Systems (MIDI, 1999). The isolate and two reference species were grown together on EG medium at 37°C, pH 7.6-7.8, for five days. The cellular fatty acids of the isolate were included in the species

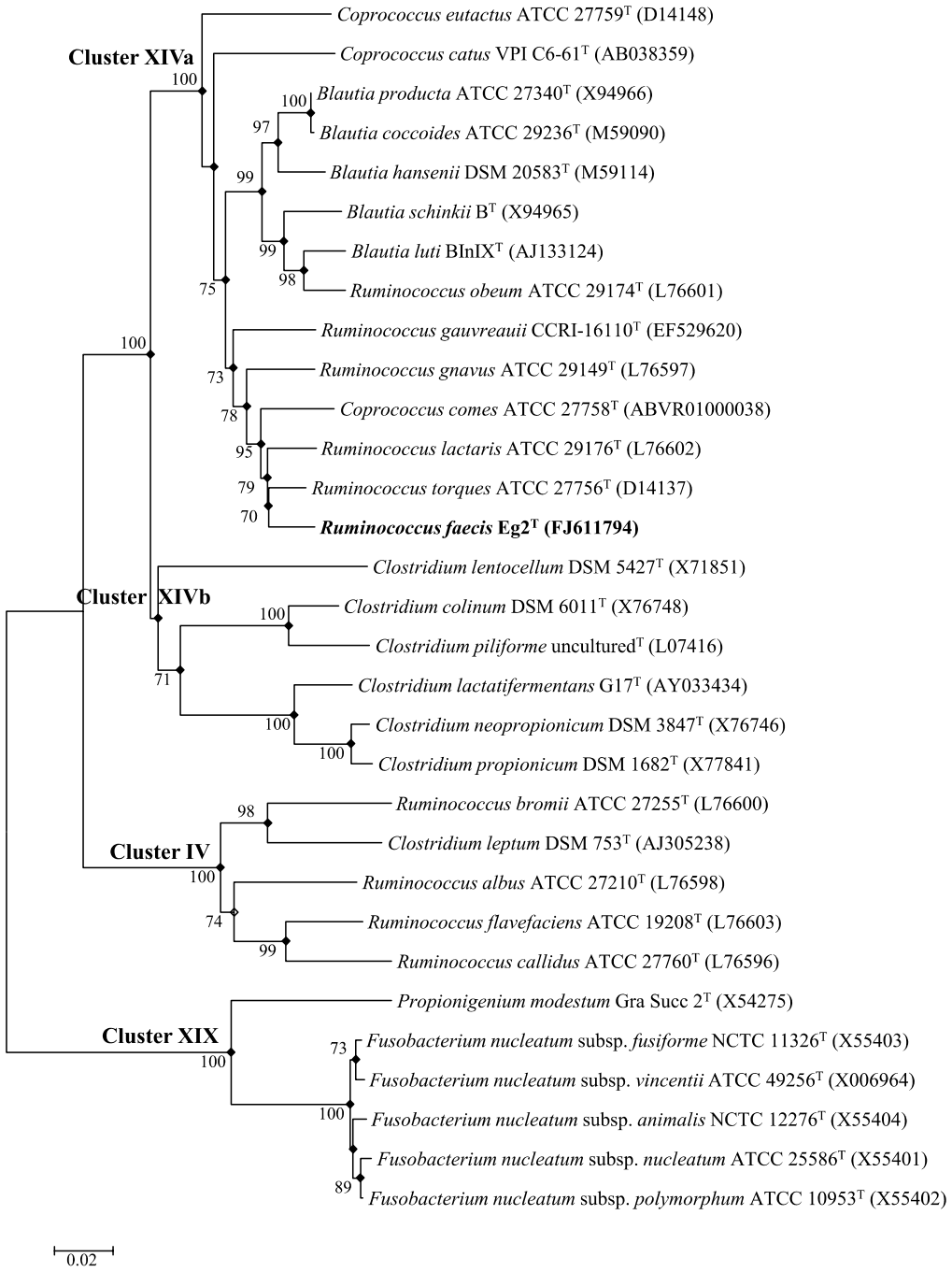

Fig. 1. Phylogenetic consensus tree based on the 16S rRNA gene sequences. Filled diamonds indicate generic branches that were present in phylogenetic trees generated by neighbor-joining, maximum-parsimony and maximum-likelihood algorithms and empty diamonds indicate generic branches that were present in both phylogenetic trees generated by neighbor-joining and maximum-parsimony algorithms. The numbers at the nodes indicate bootstrap values of the neighbor-joining algorithm as percentages of 1,000 replicates. Values below 70% are not indicated at the branch points. Bar, 0.02 substitutions per site.

description.

For a comparative study based on the 16S rRNA gene sequence, the 16S rRNA gene sequence of the isolate was amplified by Colony PCR with four bacteria-specific primers (8F, 968F, 518R, and 1492R) (Baker *et al.*, 2003). After purification (QIAquick® PCR Purification kit), the PCR product was sequenced using the BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, USA), according to the manufacturer's instructions. The reaction mixtures were analyzed by an automated DNA analyzer system (PRISM 3730XL DNA analyzer, Applied Biosystems). The partial fragments obtained in the sequencing were subsequently assembled by SeqMan software (DNASTAR). The 1,404-bp 16S rRNA gene sequence (GenBank accession no., FJ611794) of the isolate was assembled and compared with other sequences in EzTaxon (Chun *et al.*, 2007). The results indicated that the isolate was closely related to the type species belonging to the Clostridium cluster XIVa, such as the genera *Ruminococcus*, *Eubacterium*, *Coprococcus*, and *Clostridium*, in the phylum *Firmicutes*. The 16S rRNA gene sequence of the isolate was less than 97.0% similar to the corresponding sequences of type species of Clostridium cluster XIVa (96.3% to *Ruminococcus torques*, 96.2% to *Ruminococcus lactaris*, 95.8% to *Eubacterium contortum*, 95.4% to *Clostridium glycyrrhizinilyticum*, 95.2% to *Clostridium nexile*, and 95.0% to *Coprococcus comes*). A total of 30 16S rRNA gene sequences of Clostridium cluster XIV (XIVa and b) and IV (outgroup) collected from the GenBank database were aligned with the isolate using the multiple sequence alignment program CLUSTAL X (1.83) (Thompson *et al.*, 1997). The trimmed alignment was converted to MEGA format, and phylogenetic trees were generated by MEGA 5 (Kumar *et al.*, 2008). The phylogenetic trees were tested by randomly selecting 1,000 bootstrap replicates for neighbor-joining and maximum-parsimony algorithms (Kluge and Farris, 1969; Saitou and Nei, 1987). Maximum-likelihood analysis was also performed by randomly selecting 100 bootstrap replicates (Felsenstein, 1981). The phylogenetic analyses showed that the taxonomic position of the isolate was close to those of the members of Clostridium cluster XIVa, and also that the isolate formed a distinct taxonomic lineage within the cluster XIVa (Fig. 1). Moreover, a low level of 16S rRNA gene sequence similarity (lower than 97.0%) indicated that the isolate was phylogenetically distinct from its close relatives, *Ruminococcus torques* and *Ruminococcus lactaris* (Wayne *et al.*, 1987; Stackebrandt and Goebel, 1994). The genomic DNAs of the isolate and two references were extracted using the G-spin™ Genomic DNA Extraction kit (iNtRON Biotechnology, Korea). The G+C content of the isolates was determined using a fluorimetric method employing SYBR Green I and real-time PCR (Gonzalez and Saiz-Jimenez, 2002). The genomic DNAs of *Escherichia coli* K12 and the reference species were used as the calibration references (Gonzalez and Saiz-Jimenez, 2002). The G+C content of the isolate was estimated as being 43.4 mol%. Thus, the value is within the range of 39 to 46 mol% (Simmering *et al.*, 2002).

Strain Eg2^T from human faeces represents a novel species belonging to the genus *Ruminococcus*. Based on the 16S rRNA gene sequence, it is closely related to *Ruminococcus* species and clearly falls into cluster XIVa (Fig. 1). The 16S rRNA gene sequence of strain Eg2^T (>3% dissimilarity with close relatives) presents a difference from other *Ruminococcus* species. Strain Eg2^T also has other characteristics of *Ruminococcus* species, such as being Gram-positive cocci that form chains, being non-spore-forming and lacking indole formation and nitrate reduction. Strain Eg2^T has similar phenotypic characters to *R. lactaris* and *R. torques*, but D-glucose, D-raffinose, and D-sorbitol reactions of strain Eg2^T were different from those of *R. lactaris* and *R. torques*. Moreover, strain Eg2^T yields mainly lactic and acetic acids from glucose fermen-

Table 1. Phenotypic characteristics of strain Eg2^T compared with close relatives. Strains: 1, strain Eg2^T; 2, *Ruminococcus torques* ATCC 27756^T; 3, *R. lactaris* ATCC 29176^T. Data were obtained from the present study. +, positive; -, negative.

Characteristics	1	2	3
API 20A			
D-Glucose	+	-	-
D-Lactose	+	+	-
D-Saccharose	-	+	-
D-Maltose	+	+	-
Salicin	-	+	-
D-Xylose	-	+	-
L-Arabinose	-	+	-
Glycerol	-	+	-
D-Mannose	-	+	-
D-Raffinose	+	-	-
D-Sorbitol	+	-	-
Indole formation	-	+	-
Gelatin hydrolysis	-	+	-
Esculin hydrolysis	+	-	-
API rapid ID 32A			
Reduction of nitrate	-	+	-
α-Galactosidase	+	-	-
α-Glucosidase	+	-	+
β-Glucosidase	+	-	-
N-Acetyl-β-glucosaminidase	-	-	+
Alkaline phosphatase	+	-	-
Arginine arylamidase	-	+	-
Proline arylamidase	-	+	-
Pyroglutamate arylamidase	-	+	-
Alanine arylamidase	-	+	-
Glycine arylamidase	-	+	-
Histidine arylamidase	-	+	-
Serine arylamidase	-	+	-
API ZYM			
Alkaline phosphatase	+	-	-
α-Galactosidase	+	-	-
β-Galactosidase	-	-	+
β-Glucuronidase	-	+	-
α-Glucosidase	+	-	-
β-Glucosidase	+	-	-
N-Acetyl-β-glucosaminidase	-	+	+
α-Mannosidase	-	+	-
End products of glucose fermentation[a]	L, A	L, a, E[b]	F, L, A, s[b]

[a] A/a, acetic acid; E, ethanol; L, lactic acid; s, succinic acid; F, formic acid. Capital letters indicate major end products.
[b] Data are from Moore *et al.* (1976).

tation. Strain Eg2^T is able to hydrolyze esculin, and its enzyme activities include α-galactosidase, α-glucosidase, β-glucosidase, and alkaline phosphate which are lacking in *R. lactaris* and *R. torques* (Table 1). On the basis of phenotypic, genotypic, and phylogenetic analyses, we propose that strain Eg2^T be assigned as a novel species of the genus *Ruminococcus* and that this species be named *Ruminococcus faecis* sp. nov.

Description of *Ruminococcus faecis* sp. nov.

Ruminococcus faecis (fa.e'cis. L. gen. n. *faecis*, of dregs, of faeces, referring to faecal origin).

Strain Eg2^T is an anaerobic, Gram-positive, non-motile, and non-spore-forming bacterium. The cells have a coccoid (1.0 μm in diameter) or oval (1.0 μm×1.5 μm) shape. The cells are generally observed in chains. After three days of culture at 37°C on EG medium, the colonies are 4.0-5.0 mm in diameter and have a white color, circular form with a toothed margin, dull surface, umbonate side view, and buttery texture. No zone of hemolysis is observed. The optimal temperature for growth is 37°C. The cells are oxidase-negative, but catalase-positive. The cells are negative for urease, indole formation and gelatin hydrolysis, but positive for esculin hydrolysis. In API 20A strips, acid production occurs from D-glucose, D-lactose, D-maltose, D-raffinose, and D-sorbitol, but not from D-saccharose, D-xylose, L-arabinose, D-cellobiose, D-mannose, L-rhamnose, D-mannitol, salicin, glycerol, D-melezitose, or D-trehalose. In API rapid ID 32A strips, it is positive for α-galactosidase, α-glucosidase, β-glucosidase, and alkaline phosphatase, but negative for β-galactosidase, N-acetyl-β-glucosaminidase, D-mannose fermentation, D-raffinose fermentation, glutamic acid decarboxylase, α-fucosidase, indole production, leucyl glycine arylamidase, alanine arylamidase, glutamyl glutamate arylamidase, urease, arginine dihydrolase, β-galactosidase-6-phosphate, α-arabinosidase, β-glucuronidase, reduction of nitrate, arginine arylamidase, proline arylamidase, phenylalanin arylamidase, leucine arylamidase, pyroglutamate arylamidase, tyrosine arylamidase, glycine arylamidase, histidine arylamidase, and serine arylamidase. In API ZYM strips, the cells are positive for alkaline phosphatase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, α-glucosidase, and β-glucosidase, but negative for leucine arylamidase, β-galactosidase, esterase (C4), esterase lipase (C8), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase. The fatty acids predominantly consist of $C_{16:0}$ (27.7%), $C_{14:0}$ (10.0%), $C_{18:1}$ *cis*11 DMA (9.0%), as well as several minor fatty acids: $C_{16:0}$ DMA (7.3%), $C_{18:1c11/t9/t6}$ (6.7%), $C_{16:1}$ *cis*9 DMA (5.7%), $C_{14:0}$ DMA (5.0%), $C_{18:2}$ *cis*9,12 (3.3%), $C_{18:1}$ *cis*9 (3.1%), $C_{18:0}$ (2.9%), $C_{17:1}$ *cis*9/$C_{17:2}$ (2.7%), $C_{16:1}$ *cis*9 (2.5%), $C_{12:0}$ (2.3%), $C_{18:1}$ *cis*9 DMA (2.0%), $C_{18:0}$ DMA (2.0%), $C_{15:2}$/$C_{15:1}$ *cis*7 (2.0%), $C_{13:1}$ *cis*12/$C_{11:1}$ 2-OH (1.9%), $C_{16:0}$ ALDE (1.5%), $C_{14:1}$ *cis*7 DMA (1.2%), and $C_{11:0}$ DMA (1.1%). The G+C content is 43.4 mol%.

The type strain, designated as Eg2^T (=KCTC 5757^T =JCM 15917^T), was isolated from human faeces.

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