Reclassification of *Eubacterium desmolans* as *Butyricicoccus desmolans* comb. nov., and description of *Butyricicoccus faecihominis* sp. nov., a butyrate-producing bacterium from human faeces

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A Gram-positive-staining, coccoid-shaped, non-motile, asporogenous, obligately anaerobic and butyrate-producing bacterium was recovered from a healthy human’s faeces. The organism was isolated by the enrichment culture technique using yeast extract-casein hydrolysate-fatty acids broth supplemented with 0.5 % mucin. Phylogenetic analysis of 16S rRNA gene sequences demonstrated that the novel strain should be classified as a member of the *Eubacterium desmolans*-related cluster in the family Ruminococcaceae. Furthermore, this analysis demonstrated that the type strains of *Butyricicoccus pullicaecorum* (95.6 %) and *Eubacterium desmolans* (94.7 %) were the closest phylogenetic neighbours to strain YIT 12789T. However, DNA–DNA reassociation values with these closest strains were less than 20 %. On the basis of the phenotypic, genotypic and chemotaxonomic features, the novel coccoid-shaped bacterium should be designated as a representative of a novel species of the genus *Butyricicoccus*, for which the name *Butyricicoccus faecihominis* sp. nov. is proposed. The type strain is YIT 12789T (=JCM 31056T=DSM 100989T). It is also proposed that *Eubacterium desmolans* be reclassified in the genus *Butyricicoccus* as *Butyricicoccus desmolans* comb. nov.

The diversity of bacterial microbiota in the human large intestine has been revealed through detailed analyses using molecular biological approaches (Harmsen et al., 2002; Eckburg et al., 2005; van den Bogert et al., 2011). In recent years, metagenomics-based methods have revealed the composition and function of the human gut microbial communities (Arumugam et al., 2011; Human Microbiome Project Consortium, 2012). In the large intestine of a healthy subject, the microbiota consists mainly of members of the *Bacteroides* group, the genus *Bifidobacterium*, the *Atopobium* cluster and the *Eubacterium cylindroides* group and members of the families *Lachnospiraceae* and *Ruminococcaceae*, accounting for approximately 90 % of intestinal bacteria (Harmsen et al., 2002). These bacteria constitute a balanced microbiota in the intestine and perform beneficial functions for the host, such as inhibition of the growth of pathogenic bacteria, decomposition of foods, synthesis of vitamins and immunomodulating activities (Gibson & Roberfroid, 1995; Prakash et al., 2011).

The gut microbiota produces short-chain fatty acids (acetate, propionate, butyrate, etc.), which are mainly utilized by the colonic epithelium in the gut and excreted in faeces. Butyrate is a preferable energy source in the colonic epithelium (Pryde et al., 2002) and induces IL-10-producing regulatory T-cells by epigenetic control (Furusawa et al., 2013). Eckhaut et al. (2013) showed that inflammatory bowel disease patients have lower counts of faecal butyrate-producing *Butyricicoccus* spp., and the oral administration of *Butyricicoccus pullicaecorum* attenuates symptoms of trinitrobenzenesulfonic acid-induced colitis in rats. For these reasons, butyrate or butyrate-producing bacteria have attracted increasing attention for clinical application. By utilizing *Clostridium leptum* group-specific PCR-DGGE (denaturing gradient gel electrophoresis) and clone library analysis, several unidentified butyrate-producing species were classified...
into the *Eubacterium desmolans*-related cluster (Shen et al., 2006). Therefore, we focused on isolating a novel butyrate-producing bacteria which belongs to the *E. desmolans*-related cluster from a human faecal sample.

While exploring novel butyrate-producing bacteria, strain YIT 12789\感兴趣 was isolated from a healthy human adult who had not undergone antibiotic therapy in the previous 6 months. For isolation, fresh faecal sample was collected and immediately taken to the laboratory under anaerobic conditions. The faecal sample was homogenized and subsequently diluted with pre-reduced PBS solution without a metal salt [PBS (\(-\)); Nissui Pharmaceutical]. The faecal dilution was inoculated into yeast extract-casein hydrolysate-fatty acids (YCFA) broth (Lopez-Siles et al., 2012) supplemented with 0.5% carbohydrate [one of mucin (from porcine stomach; Wako Pure Chemical Industries), inulin (from chicory), arabinogalactan, xylan, lignin, pectin, cellu-lose, galactose, amylopectin, L-fucose, N-acetyl-d-galacto-samine or N-acetyl-d-glucosamine] and incubated at 37°C for 8–16 h in an anaerobic chamber (Coy Laboratory Products; atmosphere comprising N\(_2\)/CO\(_2\)/H\(_2\); 88 : 5 : 7). The result showed that the best carbohydrate for growth of *E. desmolans*-related cluster strains was mucin (see Table S1, available in the online Supplementary Material). Therefore, to increase the probability of increasing the numbers of the novel organism for improved recovery by cultivation, a combination of mucin and the enrichment culture method (González et al., 1996) were used. Cells of *E. desmolans*-related cluster strains are small and their density in bacterial culture broth is lower than that of other bacteria. In order to verify the optimal experimental conditions for accumulating the bacterial cells of *E. desmolans*-related cluster strains, three centrifugation conditions were employed, each for 5 min. From these results, the best centrifugation condition was centrifugation at 4000 g for 5 min. By repetitive subcultivation for seven times, the ratio of *E. desmolans*-related cluster strains grew by approximately 30 times from the initial ratio (see Fig. S1). After repetitive subcultivation for seven times, the enrichment sample was spread onto a yeast extract-casein hydrolysate-fatty acids supplemented with 1.0% glucose (YCFA) agar plate and incubated at 37°C for 3 days in a anaerobic chamber. Colonies in the *E. desmolans*-related cluster strains were screened by a fluorescence *in situ* hybridization (FISH) technique (Harmsen et al., 2002) using the *E. desmolans*-related cluster-specific probe pUNG260 (5’-CCATCGLTCAA-GACCAGCG-3’), which was designed in this study. This probe was designed from the oligonucleotide target sites, selected from the 16S rRNA gene sequences of the family *Ruminococcaceae* obtained from the GenBank/EMBL/DDBJ database, and its specificity was confirmed by using the Probe Match analysis function of the Ribosomal Database Project (Maidak et al., 1997). Among the 16 isolates which were identified as belonging to the *E. desmolans*-related cluster on the basis of the specific FISH probe and randomly amplified polymorphic DNA (RAPD)-PCR profiling, isolate KS-2 was designated as strain YIT 12789\感兴趣 (Fig. S2).

Chromosomal DNA used as a template for 16S rRNA gene sequence amplification was prepared from the novel strain according to the method of Matsuki et al. (2004). The 16S rRNA gene of the strain was amplified with 8F (5’-AGAGTTGTATCTGGGGCTAG-3’) and 15R (5’-AAGGAGGTGTA TCCARCGCGA-3’) bacterial universal primers (Irissawa & Okada, 2009). PCR was performed with an *Taq* DNA polymerase kit (Takara Bio). Each 30 µl reaction mixture contained 0.2 mM dNTPs, 1.5 mM MgCl\(_2\), 1.5 U of *Taq* polymerase, primers 8F and 15R (1 µM each) and 1 ng of template DNA. The PCR amplification programme consisted of initial heating at 95°C for 5 min; 35 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 1 min; and a final extension at 72°C for 5 min. Approximately 1500 bp of the PCR products were excised from the 1.5% agarose gel and purified using a High Pure PCR Product Purification Kit (Roche Diagnostics). The 16S rRNA gene sequences were determined by cycle sequencing using the BigDye Terminator v3.1 cycle sequencing kit (Thermo Fisher Scientific) according to the manufacturer’s instructions. The following primers were used: 8F, 15R, 520F (5’-CAGGAGTGCCAGACGCACGC-3’), 520R (5’-ACCGGGCCTGCTGGCC-3’), 800R (5’-CAGACTACT-CAGGTATATCA-3’), 930F (5’-GCACAGGCGTTGGAGCATGTTG-3’), 1100F (5’-CAGGAGCAACGAGCGAACC-3’) and 1100R (5’-AAGGTTGCGCTGTCG-3’). Sequences were obtained with an Applied Biosystems 3130 x1 Genetic Analyzer (Thermo Fisher Scientific). Closely related sequences were retrieved from the DDBJ (http://www.ddbj.nig.ac.jp) using the BLAST program (Altschul et al., 1990). Sequences were aligned and used to produce a phylogenetic tree by the neighbour-joining method (Saitou & Nei, 1987) using CLUSTAL W (Thompson et al., 1994). The statistical reliability of the tree was evaluated by bootstrap analysis of 1000 replicates (Felsenstein, 1985).

Comparative analysis of 16S rRNA gene sequences demonstrated that the novel strain should be classified as belonging to the members of the *E. desmolans*-related cluster in the family *Ruminococcaceae* (Rainey, 2009; Rainey & Lawson, 2016). On the basis of this analysis, the type strains of *Butyrivibrio fibrisolvens* YIT 12785\感兴趣 (95.6%) and *E. desmolans* YIT 10068\感兴趣 (94.7%) were the closest phylogenetic neighbours to strain YIT 12789\感兴趣 (Fig. 1). The database searches revealed that uncultivated bacterial clones from human faeces (Suau et al., 1999; Mai et al., 2006; Li et al., 2007, 2012) were closely related (>98%) to strain YIT 12789\感兴趣 (Fig. 1), indicating that the novel organism is present in human faeces and intestinal mucosa as a common member of the human indigenous microbiota.

For determining DNA G+C content and DNA–DNA reassociation values, the strains were cultured until the log phase in anaerobic YCFAG broth or GAM broth (modified *Nissui*; Nissui Pharmaceutical) with 1% inositol (for *E. desmolans*). Paraformaldehyde (20%) in PBS solution was added to the bacterial culture at a final concentration of 1%, and the culture was incubated overnight at 4°C. After washing with TE buffer (10 mM Tris and 1 mM EDTA, pH 8.0) three times, 1–2 g (wet weight) of bacterial cells was
resuspended in 6 ml TE buffer, and 2 ml EDTA solution (0.25 M EDTA and 50 mM Tris, pH 8.0) and 1 ml of cell-wall digesting enzyme solution [600 U acromopeptidase ml⁻¹ (Sigma) and 50 mg lysozyme ml⁻¹ (Wako Chemical)] were then added and incubated at 37 °C for 2h. Six hundred microlitres of SDS solution (20% SDS, 50 mM Tris and 20 mM EDTA, pH 8.0) and 150 µl 10 mg proteinase K solution ml⁻¹ were added to the cell lysate, and incubated at 65 °C for 6h. Genomic DNA molecules were extracted from the cell lysate according to a previously reported procedure (Mesbah et al., 1989).

DNA-DNA reassociation analyses were performed according to the microdilution-well technique, using photobiotin for DNA labelling (Ezaki et al., 1989). Reciprocal hybridization experiments were performed for every pair of strains at 46.4 °C for 2h in the presence of 50% formamide. For each hybridization, the highest and lowest values from eight replicate wells were excluded before the means of the six remaining values were calculated and recorded as a relatedness value. DNA–DNA relatedness values between strain YIT 12789T and Butyricicoccus pullicaeorum YIT 12789T and closely related species in the family Ruminococcaceae. Tree reconstructed by the neighbour-joining method on the basis of a comparision of approximately 1343 nt. Blautia cocoides DSM 935T (Clostridium cluster XIVa) was used as an outgroup. Bootstrap values (percentages) based on 1000 replications are given at nodes. Bar, 2% sequence divergence.

**Fig 1.** Phylogenetic tree based on 16S rRNA gene sequences showing the relationship of strain YIT 12789T with closely related species in the family Ruminococcaceae. Tree reconstructed by the neighbour-joining method on the basis of a comparison of approximately 1343 nt. Blautia cocoides DSM 935T (Clostridium cluster XIVa) was used as an outgroup. Bootstrap values (percentages) based on 1000 replications are given at nodes. Bar, 2% sequence divergence. 

Butyricicoccus faecihominis sp. nov.
Table 1. DNA G+C content and levels of DNA–DNA relatedness among strain YIT 12789T and representatives of closely related species

<table>
<thead>
<tr>
<th>DNA G+C content (mol%)</th>
<th>Percentage DNA relatedness with labelled DNA from:</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>YIT 12789T</td>
</tr>
<tr>
<td>Butyricicoccus faecihominis sp. nov.</td>
<td>YIT 12789T</td>
</tr>
<tr>
<td>Butyricicoccus pullicaecorum</td>
<td>YIT 12785T</td>
</tr>
<tr>
<td>E. desmolans</td>
<td>YIT 10068T</td>
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</tbody>
</table>

protocols. Next-generation sequencing of all libraries prepared was performed by the MiSeq system (Illumina). Paired-end 250 bp reads were used for contig construction. The DNA G+C contents calculated on the basis of the draft genome sequences of strain YIT 12789T, Butyricicoccus pullicaecorum LMG 24109T (NZ_AQOB01000000) and E. desmolans YIT 10068T were almost identical (54.3–55.6 mol%).

For morphological, cultural and biochemical testing according to standard techniques, strains were cultured in preduced, anaerobically sterilized GAM broth at 37 °C for 20 h. Cellulose morphology was observed using an optical microscope (BX-50; Olympus) and a scanning electron microscope (model S-3400N; Hitachi). After culturing, cells were washed with 0.05 M phosphate buffer (pH 7.0), cells were placed on Sempore (JEOL Datum), fixed with 2.5 % glutaraldehyde at 4 °C for 1 h, and post-fixed with 1 % osmium tetroxide at 4 °C for 1 h, dehydrated with a series of increasing ethanol concentrations (twice at 50, 70, 80, 90, 95 and 100 % for 20 min each) and soaked twice in 3-methyl butyl acetate for 20 min. The prepared cells were subsequently critical-point-dried in a critical point dryer (model HCP-2; Hitachi), sputter-coated with osmium plasma (model SC-701C-MC; SANYU Electron) and observed with a scanning electron microscope. A scanning electron micrograph of cells of strain YIT 12789T is shown in Fig. S3. The Gram reaction was determined using the Favor-G kit (Nissui Pharmaceutical). For spore formation, cells were cultured at 37 °C for 7 days using Duncan and Strong (DS) medium (Duncan & Strong, 1968). The metabolic end-products, after cultivation in the YCFAG broth at 37 °C for 3 days, were analysed by HPLC according to a previously reported procedure (Chonan et al., 1995). Hydrogen and carbon dioxide gases were determined with a gas chromatograph [Shimadzu GC-14A with thermal conductivity detection, packing material: Molecular Sieve 13X or WG-100 (GL Science), column temperature: 60 °C]. Biochemical characteristics were determined using the API 20 A, API Rapid ID 32A and API ZYM systems (bioMérieux) according to the manufacturer’s instructions. Prereduced peptone-yeast extract broth (Holdeman et al., 1977) was used as the basal medium for API 20 A. Sulfide production, indole formation and motility were tested using sulfide indole motility medium, according to the methods of Holdeman et al. (1977).

Cells for chemotaxonomic analyses were harvested from YCFAG broth or GAM broth with 1 % inositol (for E. desmolans) after anaerobic cultivation at 37 °C for 20 h. The cell-wall peptidoglycan was isolated after disruption of the cells by sonication with glass beads and subsequent total hydrolysis (4 M HCl, at 100 °C for 16 h) (Komaga & Suzuki, 1987). The composition of cellular fatty acids and the amino acid isomers in cell-wall hydrolysates were examined by TechnoSuruga Laboratory (Shizuoka, Japan). Diamino acids were identified from the total hydrolysate by using one-dimensional TLC in a methanol/pyridine/4 M HCl/water (80:10:4:26, by vol.) solvent system. Total amino acids were visualized using ninhydrin (contains acetamide) ethanol solution as a TLC stain (Tokyo Chemical Industry) followed by heating at 100 °C. These results showed that the major cellular fatty acids (≥10 %) of the novel isolate and the type strain of E. desmolans included predominately C18:1ω9c and C18:0 DMA, but Butyricicoccus pullicaecorum did not include C18:1ω9c: meso-diaminopimelic acid as the diamino acid was contained in the cell-wall peptidoglycan common to the three strains investigated (Table S2, Fig. S4).

Pure cultured Butyricicoccus faecihominis sp. nov. did not grow with mucin, indicating that this species cannot utilize mucin. Moreover, 16 carbohydrates were used, and it was determined that the species had almost no enzymes related to carbohydrate metabolism. L-Fucose was also not utilized. Nevertheless, the novel species was culturable by mucin enrichment culture. These results indicate that the novel isolate may have used the mucin degradation products produced by the mucin-degrading bacteria in faeces.

Various phenotypic characteristics of the novel strain differed from those of Butyricicoccus pullicaecorum and E. desmolans, which are the phylogenetically most closely related species (Table 2). According to the data obtained, the novel strain is genetically distinguishable from recognized species of the E. desmolans–related cluster in the family Ruminococcaceae and thus represents a novel species, for which the name Butyricicoccus faecihominis sp. nov. is proposed. We also propose that, on the basis of phylogenetic criteria and physiological, biochemical and chemotaxonomic characteristics, Eubacterium desmolans should be assigned to the genus Butyricicoccus as Butyricicoccus desmolans comb. nov.
Table 2. Differential characteristics of Butyricicoccus faecihominis sp. nov. YIT 12789T and the type strains of closely related members of the Eubacterium desmolans-related cluster in the family Ruminococcaceae

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td>Isolation source</td>
<td>Human faeces</td>
<td>Cat faeces*</td>
<td>Chicken caecal content†</td>
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<tr>
<td>Acid from:</td>
<td></td>
<td></td>
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<tr>
<td>D-Glucose</td>
<td>+</td>
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<tr>
<td>Salicin</td>
<td>–</td>
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<tr>
<td>Sucrose</td>
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<tr>
<td>D-Xylose</td>
<td>–</td>
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<tr>
<td>Aesculin hydrolysis</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Indole production</td>
<td>+</td>
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<td></td>
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<tr>
<td>Enzyme activity:</td>
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<td></td>
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<tr>
<td>Alkaline phosphatase</td>
<td>+</td>
<td></td>
<td></td>
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<tr>
<td>Esterase (C4)</td>
<td>–</td>
<td>+</td>
<td></td>
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<tr>
<td>Leucine arylamidase</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>End products from glucose‡</td>
<td>B, f, H₂, CO₂</td>
<td>B, f, CO₂</td>
<td>B, f, H₂, CO₂</td>
</tr>
</tbody>
</table>

*Data from Morris et al. (1986).
†Data from Eeckhaut et al. (2008).
‡B, butyric acid; f, formic acid. Minor end-products are indicated by lower-case letters.

Description of Butyricicoccus faecihominis sp. nov.

Butyricicoccus faecihominis (fa.e.ci ho’mi.nis. L. n. faex faecis the dregs, faeces; L. gen. n. hominis of a human being; N.L. gen. n. faecihominis of human faeces).

Cells are coccoid-shaped (1.3×0.7 µm) and usually occur in pairs. Cells are Gram-stain-positive, non-motile, asporogenous and obligately anaerobic. After anaerobic growth at 37°C for 72 h, colonies on YCFAG agar plates are waxy, yellowish and circular, 1–2 mm in diameter. Aesculin and gelatin are not hydrolysed. Indole is produced. H₂S is not produced on sulhide-indole motility medium. Alkaline phosphatase, leucine arylamidase and acid phosphatase activities are detected, but no activities for esterase (C4), esterase lipase (C8), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-gluconidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase or urease. Acid is produced from D-glucose, but not from L-fucose, lactose, sucrose, maltose, salicin, D-xylene, D-arabinose, cellobiose, D-mannose, raffinose, sorbitol, rhamnose, trehalose, melezitose, D-mannitol or glyceral. Butyrate with a small amount of formate, and both H₂ and CO₂ are produced as the end products of glucose. Cells contain meso-diaminopimelic acid in their cell-wall peptidoglycan. Peptidoglycan structure is A1γ-type in the presence of Gln and Ala. The major cellular fatty acids (≥10%) are unsaturated C₁₈:₁ω⁹c and saturated C₁₈:₀ DMA and C₁₂:₀. Phylogenetic analysis of the 16S rRNA gene sequence places the species in the E. desmolans-related cluster in the family Ruminococcaceae.

The type strain is YIT 12789T (=JCM 31056T=DSM 100989T); it was isolated from human faeces. The DNA G+C content of Butyricicoccus faecihominis YIT 12789T is 55.6% based on the draft genome sequence.

Description of Butyricicoccus desmolans comb. nov.

Butyricicoccus desmolans (des mol.ans. Gr. n. desmos a bond; mod. chem. term desmolase an enzyme splitting a carbon-carbon bond; N.L. part. adj. desmolans making desmolase).


The description is based on the results of Morris et al. (1986). Forms non-haemolytic, circular to irregular, convex, shiny, entire and white to colourless colonies that are barely visible to the unaided eye. The cells are plump, short rods that are 0.8–1.1 µm wide by 1.7–2.3 µm long and occur singly or in short chains. Cells have capsules and are Gram-stain-positive and motile with four to six flagella. Inositol is the sole carbohydrate fermented. Amygdalin, arabinose, cellobiose, erythritol, ascelin, glycogen, fructose, glucose, lactose, maltose, mannitol, mannose, melezitose, melibiose, pectin, raffinose, rhamnose, ribose, salicin, sorbitol, sucrose, trehalose and xylose are not fermented. Indole is produced. H₂S is not produced on sulhide-indole motility medium. Acetate, butyrate and trace amounts of succinate

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and lactate are produced in inositol-enriched media. Hydrogen gas is not produced. Starch is not hydrolysed. Lipase, lecitinase, oxidase and catalase are not produced. Nitrate and resazurin are not reduced. The type strain is ATCC 43058T (=CCUG 27818=JCM 6566T). The DNA G+C content of strain ATCC 43058T is 35 mol% (as determined by the thermal denaturation method).

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References


