Reclassification of *Eubacterium rectale* (Hauduroy et al. 1937) Prévot 1938 in a new genus *Agathobacter* gen. nov. as *Agathobacter rectalis* comb. nov., and description of *Agathobacter ruminis* sp. nov., isolated from the rumen contents of sheep and cows

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Three strains of a butyrate-producing bacterium were isolated from the rumen contents of grazing sheep and cows. The strains were anaerobic, with Gram-positive cell walls, straight-to-slightly-curved, rod-shaped, non-spore-forming and single flagellate. C14:1, C14:0, C16:0 and C16:1 were the predominant fatty acids. The cell-wall peptidoglycan type was A1c. The DNA G+C content varied from 41.4 to 42.2 mol%. 16S rRNA gene sequence similarities between the isolates and *Eubacterium rectale*, *Roseburia hominis* and *Roseburia intestinalis* were found to be 96, 95 and 95 %, respectively. The phylogenetic tree showed that the strains constituted a different taxon, separate from other taxa with validly published names and forming a cluster with strains of *Eubacterium rectale*. On the basis of phenotypic, chemotaxonomic and phylogenetic results (16S RNA, dnaK, groEL, atpA genes), the isolates are considered to represent a novel species of a new genus of the family Lachnospiraceae, for which the name *Agathobacter ruminis* gen. nov., sp. nov. is proposed (type strain JK623 T=DSM 29029 T=LMG 28559 T). We also propose the transfer of *Eubacterium rectale* to the new genus as *Agathobacter rectalis* gen. nov., comb nov. This new genus represents saccharoclastic, chemo-organotrophic and obligatory anaerobic, non-spore-forming rods with Gram-positive membrane. The main fermentation products on peptone yeast glucose (PYG) medium were butyrate, acetate, hydrogen and lactate. The type species of the genus is *Agathobacter rectalis* gen. nov., comb nov. (Prévot, 1938) with type strain ATCC 33656 T (JCM 17463 T).

The genus *Eubacterium* (family *Eubacteriaceae*, order *Clostridiales*, class *Clostridia*, phylum *Firmicutes*) is highly heterogeneous, including 46 phylogenetically distant species and subspecies widely distributed among the phylum *Firmicutes* (Wade, 2009). According to Bergey's Manual, species of the genus *Eubacterium* can be divided into three subgroups: those producing butyric acid (usually in combination with other volatile fatty acids); those producing combinations of lactate, acetate and formate, together with hydrogen; and those producing little or no fermentation acid (Wade, 2009).
Butyrate-producing bacteria represent an important component of the mammalian gastrointestinal tract microbiome. Butyrate is the major source of energy to the colonic mucosa, an important regulator of gene expression, inflammation, differentiation and apoptosis in host cells and plays a special role in bacterial energy metabolism (Louis & Flint, 2009). The functional group of butyrate producers is, however, phylogenetically incoherent, with the majority and numerically significant species belonging to clostridial cluster XIVa (Roseburia spp., Eubacterium rectale, Butyryrivibrio and Pseudobutyryrivibrio) and cluster IV (Faecalibacterium prausnitzii-related bacteria) of families Lachnospiraceae and Ruminococcaceae, respectively (Louis et al., 2010). Newly described species Pseudobutyryrivibrio xylanivorans and Butyryrivibrio hungatiae (Kopečný et al., 2003), Roseburia faecis, R. inulinivorans, R. intestinalis and R. hominis (Duncan et al., 2006), as well as Butyryrivibrio proteoclasticus (Moon et al., 2008) have significantly improved the taxonomy of anaerobic butyrate producers from the digestive tract.

During our studies of rumen butyrate-producing isolates, bacteria closely related to E. rectale but embedded between the Pseudobutyryrivibrio and Roseburia/Eubacterium clusters have been detected. Strains JK623^T and JK633 from sheep and strain Mz3 from cow rumen contents were isolated under anaerobic atmosphere (N_2 70 %, CO_2 20 %, H_2 5 %), using medium M10 enriched by 10 % (v/v) rumen fluid containing 10 mM Tris/HCl (pH 8.3), 50 mM KCl, 200 mM of each dNTP; 0.5 μM of each primer, 1 U Taq polymerase (Top-Bio) and 10–100 ng template DNA isolated from the evaluated strains using the PrepMan Ultra (Applied Biosystems) according to the manufacturer’s protocol. The PCR conditions were optimized using a TProfessional Gradient 96 thermocycler (Bioter). The amplification program for the partial atpA gene consisted of one cycle at 95 °C for 5 min; 32 cycles at 95 °C for 45 s, 52 °C for 50 s, 72 °C for 45 s; and a final step at 72 °C for 6 min. The amplification program for the partial groEL gene was as follows: one cycle at 95 °C for 5 min; 32 cycles at 95 °C for 40 s, 56 °C for 40 s, 72 °C for 1 min; and a final step at 72 °C for 6 min. Partial sequences of dnaK were amplified under these conditions: one cycle at 95 °C for 5 min; 32 cycles at 95 °C for 45 s, 56 °C for 50 s, 72 °C for 1 min 40 s; and a final step at 72 °C for 8 min. The PCR products were sequenced, checked and edited using the BioEdit v.7.2.5 program (http://www.mbio.ncsu.edu/bioedit/bioedit.html) and deposited in the GenBank database using the BanKlt application (http://www.ncbi.nlm.nih.gov/WebSub/?tool=genbank).

Phylogenetic trees based on partial 16S rRNA gene, atpA, groEL and dnaK sequences were reconstructed for evaluation of phylogenetic relationships among the studied strains JK623^T, Mz3, E. rectale ATCC 33656^T and A1-86^NT and type strain sequences representing the families Lachnospiraceae and Eubacteriaceae, obtained from the GenBank database (http://www.ncbi.nlm.nih.gov/genome/; http://www.ncbi.nlm.nih.gov/nuccore/). Sequences were aligned using the CLUSTALW algorithm within the BioEdit.
v.7.2.5 program (Larkin et al., 2007). All alignments were then improved by removing hypervariable positions using the Gblocks application (http://molevol.cmima.csic.es/castresana/Gblocks_server.html) under the default conditions (Castresana, 2000). Tree reconstructions were performed using the maximum-likelihood method and the Juke–Cantor model with bootstrapping (1000 datasets) in the MEGA 6 program (Tamura et al., 2013). Only the phylogenetic tree based on amino acid sequences was reconstructed using the Jones–Taylor–Thornton model (Whelan & Goldman, 2001). All phylogenetic trees were reconstructed as unrooted.

Phylogenetic trees based on partial 16S rRNA gene (Fig. 1), atpA (Fig. S3), groEL (Fig. S4), dnaK (Fig. S5), and concatenated atpA, groEL and dnaK (Fig. S6) sequences show separate clusterings of the novel strains and their nearest relatives, indicating a novel genus within the family Lachnospiraceae. This result is supported by the phylogenetic tree reconstructed using concatenated amino acid sequences derived from the groEL and dnaK genes (Fig. S7).

The DNA G + C content was determined by the HPLC method of Mesbah et al. (1989) using a RP-C18 column (Supelco), as described previously by Killer et al. (2011). The mean DNA G + C content of strain JK623T was 41.4 mol% (Table 1). This result is close to the G + C content of E. rectale A1-86NT (41.2 mol%), determined by Duncan & Flint (2008) and replicated in this study (41.0 mol%).

Fermentation end-products (Table 1) of hexose catabolism were analysed in overnight peptone yeast glucose (PYG) cultures on an Agilent GC 6890N gas chromatograph with an FFAP capillary column.
Strain JK623T produced butyrate (3–7 mM) and acetate (4–10 mM) from glucose. Lactate was produced in a lower amount (1–3 mM); propionate and valerate were not detected. Acetate utilization in Medium M10 produced in a lower amount (1–3 mM); propionate and valerate were not detected. Acetate utilization in Medium M10 produced in a lower amount (1–3 mM); propionate and valerate were not detected. Acetate utilization in Medium M10 produced in a lower amount (1–3 mM); propionate and valerate were not detected.

The substrate utilization and enzyme activities of strains JK623T, Mz3, E. rectale ATCC 33656T and E. rectale A1-86NT were determined under anaerobic conditions using API 50 CHL, Rapid ID 20A and 32A test strips (bioMérieux) and compared with patterns of R. intestinalis and E. limosum (Table 1).

The ability to grow in aerobic, microaerophilic and anaerobic environments at different temperatures was tested by methods previously described (Killer et al., 2013). Most strains grew anaerobically at temperatures ranging from 25 to 40 °C in 2 days culture.

Cellular fatty acid profiling of strains JK623T, Mz3, E. rectale ATCC 33656T, E. rectale A1-86NT and related R. intestinalis L1-82T as well as E. limosum ATCC 8486T was performed by the method described previously by Killer et al. (2009). Results summarized in Table S1 show that isolate JK623T contains unsaturated C14 : 1 (15.1 %), C14 : 0 (10.8 %), C16 : 0 (10.7 %), and C16 : 1 (10.5 %), similar to the isolate Mz3, with a dominance of C14 and C16 isomers. Both novel strains showed a prevalence of unsaturated fatty acids over saturated fatty acids and produced traces of C22 : 2 and C23 : 0. On the contrary, both E. rectale isolates, ATCC 33656T and A1-86NT, showed prevalence of saturated fatty acids. In addition to C13 to C16 isomers, C17 : 0, C17 : 1 and C18 : 0 were also detected. This profile differed from that of R. intestinalis L1-82T with C14 : 0 and C15 : 0 dominating, and from E. limosum ATCC 8486T which produced major amounts of C12 : 0, C13 : 0 and C15 : 0.

Table 1. Phenotypic, genetic and metabolic characteristics of Agathobacter ruminis sp. nov. compared with Agathobacter rectalis gen. nov., comb. nov., R. intestinalis and E. limosum

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
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<th>4</th>
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<tr>
<td>DNA G+C (mol%)</td>
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<td>42.2±0.4</td>
<td>41.5</td>
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<td>+/−</td>
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<td>A, (P), B,</td>
<td>A, (P), B,</td>
<td>A, (P), B,</td>
<td>A, (P), B, (iB),</td>
<td>A, (P), B, (iB), (V), (iV),</td>
<td>A, B</td>
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<td>Acetate consumption</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
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</tbody>
</table>

* A, acetic acid; B, butyric acid; iB, iso-butyric acid; iV, iso-valeric acid; P, propionic acid; V, valeric acid. Major products are underlined, while those in parentheses are inconsistently produced.

× 0.25 μm). Strain JK623T produced butyrate (3–7 mM) and acetate (4–10 mM) from glucose. Lactate was produced in a lower amount (1–3 mM); propionate and valerate were not detected. Acetate utilization in Medium M10 with rumen fluid (2–6 mM) was observed. E. rectale ATCC 33656T produced butyrate (5–9 mM), acetate (3–7 mM) and lactate (0.7–3 mM); this finding is in agreement with the published data (Wade, 2009). All strains produced hydrogen.

The substrate utilization and enzyme activities of strains JK623T, Mz3, E. rectale ATCC 33656T and E. rectale A1-86NT were determined under anaerobic conditions using API 50 CHL, Rapid ID 20A and 32A test strips (bioMérieux) and compared with patterns of R. intestinalis and E. limosum (Table 1).

The ability to grow in aerobic, microaerophilic and anaerobic environments at different temperatures was tested by methods previously described (Killer et al., 2013). Most strains grew anaerobically at temperatures ranging from 25 to 40 °C in 2 days culture.

Cellular fatty acid profiling of strains JK623T, Mz3, E. rectale ATCC 33656T, E. rectale A1-86NT and related R. intestinalis L1-82T as well as E. limosum ATCC 8486T was performed by the method described previously by Killer et al. (2009). Results summarized in Table S1 show that isolate JK623T contains unsaturated C14 : 1 (15.1 %), C14 : 0 (10.8 %), C16 : 0 (10.7 %), and C16 : 1 (10.5 %), similar to the isolate Mz3, with a dominance of C14 and C16 isomers. Both novel strains showed a prevalence of unsaturated fatty acids over saturated fatty acids and produced traces of C22 : 2 and C23 : 0. On the contrary, both E. rectale isolates, ATCC 33656T and A1-86NT, showed prevalence of saturated fatty acids. In addition to C13 to C16 isomers, C17 : 0, C17 : 1 and C18 : 0 were also detected. This profile differed from that of R. intestinalis L1-82T with C14 : 0 and C15 : 0 dominating, and from E. limosum ATCC 8486T which produced major amounts of C12 : 0, C13 : 0 and C15 : 0. Peptidoglycan types of bacterial membranes of isolate JK623T and E. rectale A1-86NT were determined by the Identification Service of the Leibniz Institut Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) using the methods of Schumann (2011). Chemical analysis revealed the same peptidoglycan type A1γ (A31)
for both strains, containing diaminopimelic acid (meso-Dpm-direct), which is common in a large number of aerobic and anaerobic bacterial strains (Table 1) but is in sharp contrast with the B4 peptidoglycan type found in E. limosum ATCC 8486T (Wade, 2009).

Comparison of strains JK623T, Mz3, E. rectale ATCC 33656T and E. rectale A1-86NT showed that these strains have a Gram-stain-positive membrane. However, the newly described isolates (JK623T and Mz3) exhibit Gram-negative staining while E. rectale is Gram-stain-positive in late-exponential phase. All strains are strictly anaerobic, butyrate-producing, non-spor-forming, straight to slightly curved rod-shaped and have a single subpolar flagellum. Grown on PYG medium, they produce butyrate, lactate and acetate. In the presence of acetate in the medium, the acid is utilized, indicating butyryl coenzyme A and acetate-coenzyme A transferase in butyrate formation. The major fatty acids are C14:0, C16:0 and C16:1. The type of cell-wall peptidoglycan is A1γ, containing meso-diaminopimelic acid. Most strains were isolated from the digesta of human beings or ruminants.

On the basis of phenotypic, chemotaxonomic and phylogenetic considerations, E. rectale merits reclassification in a novel genus of the family Lachnospiraceae as Agathobacter rectalis gen. nov., comb. nov. Strains JK623T, JK633 and Mz3 are considered to represent strains of a distinct species of the same genus, for which the name Agathobacter ruminis sp. nov. is proposed.

**Description of Agathobacter gen. nov.**

Agathobacter (A.ga.tho.bac’ter. Gr. adj. agathos good; N.L. masc. n. bacter a rod; N.L. masc. n. Agathobacter beneficial bacterium).

The genus represents species closely related to the former E. rectale. Cells are uniform, non-spor-forming rods, with Gram-positive membrane, obligately anaerobic, motile or non-motile. All motile strains are subpolar monoflagellated. Flagella diameter is less than in other bacteria and is closer to the diameter of Archaea flagella (Trachtenberg & Cohen-Krausz, 2006). Saccharolactic chemo-organotrophs. Main fermentation products on PYG medium are butyrate, acetate, hydrogen and lactate. Peptidoglycan in all species is of A1γ type. The DNA G+C content is in the range of 41.6 ± 0.6 mol%.

The type species is Agathobacter rectalis (Hauduroy et al. 1937) Prévol 1938.

**Description of Agathobacter rectalis comb. nov.**

Agathobacter rectalis (rec.ta’le, N.L. n. rectum the straight bowel; N.L. masc. adj. rectalis rectal).

Basonym: Eubacterium rectale (Hauduroy et al. 1937) Prévol 1938.

The description of Agathobacter rectalis is identical to that proposed for E. rectale (Prévol 1938), with phenotypic description by Prévol et al. (1967), Holdeman & Moore (1974) and Moore & Holdeman Moore (1986). The type strain is ATCC 33656T (=DSM 3377T).

**Description of Agathobacter ruminis sp. nov.**

Agathobacter ruminis (ru’mi.nis. L. gen. n. ruminis of the rumen).

Membrane stains Gram-negative, but cells have a very thin cell-wall with a Gram-positive ultrastructure. Cells are non-spor-forming, straight-to-slightly-curved motile rods (0.3–0.4 μm wide and 1.5–2.1 μm long) with single polar or subpolar flagellum (diameter 16.1 ± 0.1 nm). Colonies on M10 agar medium with cellobiose under aerobic conditions after incubation for 48 h at 39 °C are 3–4 mm in diameter, white, lentil-shaped and smooth. Growth is observed at pH 6–8 (optimally at pH 7) and 25–45 °C (optimally at 35–39 °C). Negative for urease and catalase activities, positive for gelatin and aesculin hydrolysis. Able to produce acid from amygdalin, L-arabinose, arbutin, cellobiose, cellobioside, D-fructose, α-galactoside, gentiobiose, D-glucose, glycerogen, inulin, maltose, rhamnose and sucrose, but not from N-acetylglucosamine, D-adenitol, D-arabinose, D-arabitol, L-arabitol, dulcitol, erythritol, D-fucose, L-fucose, potassium gluconate, glycerol, D-glucoside, inositol, lactose, D-lxose, D-mannitol, D-mannose, D-mannoside, melezitose, melibiose, raffinose, D-ribose, salicin, D-sorbitol, L-sorbose, D-tagatose, turanose, xyitol, L-xyllose and D-xylulose. The predominant fatty acids are C14:0, C14:0, C15:0, C16:1, C16:0 and C17:1 (Table S1). The cell-wall diamino acid is meso-diaminopimelic acid.

The type strain is JK623T (=DSM 29029T = LMG 28559T) isolated from the rumen fluid of sheep grazing in a semi-natural meadow in central Bohemia, Czech Republic. The 16S RNA sequence of the type strain was deposited in NCBI database under accession no. KT229569. Similar strains were isolated in Bohemia and Slovenia, and the following strains were shown to belong to this species: Mz3 (GenBank accession no. KT229570) and JK633 (KM507173). The DNA G+C content of the isolates is 41–42 mol% (Table 1).

**Acknowledgements**

Neotype strain E. rectale A1-86NT (=DSM 17629NT) was kindly provided by Dr H. Flint (University of Aberdeen, UK). This work was supported by the FP7 project: Ruminomics (no. 289319), the Czech Science Foundation project (no. 304/11/1252), the Ministry of Education, Youth and Sports of the Czech Republic project (LO1509) and by the Operational Program Prague Competitiveness project (CZ.2.16/3.1.00/24023), supported by the European Union.

**References**


