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ORIGINAL PAPER

Acetatifactor muris gen. nov., sp. nov., a novel bacterium isolated from the intestine of an obese mouse

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Abstract We used selective agar media for culturing bacteria from the caecum of mice fed a high calorie diet. In addition to the isolation of *Enterobacteriaceae* growing on a medium containing cholesterol and bile salts, we focused on the characterization of strain CT-m2^T, which, based on 16S rDNA analysis, did not appear to correspond to any currently described organisms. The isolate belongs to the *Clostridium* cluster XIV and is most closely related to members of the *Lachnospiraceae*, including the genera *Anaerostipes*, *Blautia*, *Butyrivibrio*, *Clostridium*, *Coprococcus*, *Eubacterium*, *Robinsoniella*, *Roseburia*, *Ruminococcus* and *Syntrophococcus* (≤90 % similarity). Strain CT-m2^T is a non-motile Gram-positive rod that does not form spores and has a G + C content of DNA of 48.5 %. Cells grow under strictly anoxic conditions (100 % N_2) and produce acetate and butyrate after growth in reduced WCA broth. In contrast to related species, the new bacterium does not metabolize glucose and is positive for phenylalanine arylamidase, and its major cellular fatty acid is $C_{14:0}$. Based on phylogenetic and phenotypic studies, the isolate merits recognition as a member of a novel genus and species, for which the name *Acetatifactor muris* is proposed. The type strain is CT-m2^T (= DSM 23669^T = ATCC BAA-2170^T).

Keywords Mouse intestinal microbiota · Diet-induced obesity · *Firmicutes* · *Clostridia* · *Acetatifactor muris*

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The GenBank accession number of the 16S rRNA gene sequence of strain CT-m2^T is HM989805.

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Introduction

The mammalian gut microbiota is dominated by over 1,000 species of strictly anaerobic bacteria, predominantly Gram-positive type cells belonging to the phylum *Firmicutes* (Ley et al. 2008). The study of such highly diverse ecosystems is partly hampered by the fact that most gut bacteria (>60 %) have no representative strains yet in culture (Goodman et al. 2011), highlighting the importance of isolating novel bacterial species for further in vitro

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characterization of functions of interest, including metabolic features and regulation of host cell stress responses (Alain and Querellou 2009). Ultimately, the isolation of new bacteria is also essential for refining taxonomic definitions of complex and heterogeneous groups of bacteria, such as the *Clostridia*. Several members of the *Firmicutes* encode functions of relevance to host health, including production of the short-chain fatty acid butyrate or conversion of dietary components such as polyphenols (Clavel et al. 2006; Louis and Flint 2009). Moreover, it has been recently shown that the occurrence of *Firmicutes*, especially members of the family *Erysipelotrichaceae*, is markedly increased in mouse models of diet-induced obesity (Turnbaugh et al. 2009; Fleissner et al. 2010). In that context, we aimed at isolating bacteria from the caecum of obese mice and focused on the description of one novel bacterial genus within the *Firmicutes*.

Materials and methods

Sampling

Male C57BL/6NCrl mice were fed a standard (STD) or a cafeteria (CT) diet ($n = 5$ mice/group). The feeding protocol and the diets are given in the Electronic Supplementary Material. All procedures were conducted according to the German guidelines for animal care and approved by the state ethics committee (ref. no. 209.1/211-2531-41/03). After 81 days on the experimental diets, mice were killed by cervical dislocation. Caecal contents were collected, weighed using a precision balance (TB-215D, Denver Instrument) and immediately used for culturing.

Media

Two agar media were used for isolation, referred to as CBS and CT agar hereafter. The cholesterol and bile salts (CBS) agar is described in the Electronic Supplementary Material. The selective CT medium was used to mimic dietary intake in mice fed the CT diet. It was prepared using 10.4 g pellets of cocoa-flavoured, 7.4 g coconut-flavoured and 2.2 g peanut-flavoured diet (Suppl. Table S1), which were ground and solved in distilled water (1 l) by mixing for 30 min at 50 °C. After addition of 2 % (w/v) agar, the medium was autoclaved (121 °C, 15 min). The sterile medium (55 °C) was supplemented with the following filter-sterilized solutions prior to pouring into Petri dishes (final concentrations in CT agar are given in brackets): DTT (0.02 %), cysteine (0.05 %), taurine (15 mM) (Sigma, cat. no. T0625), ammonium iron citrate (0.05 %) (Sigma, cat. no. 09713), erythromycin (10 μg/ml) (Sigma, cat. no. 45673) and polymyxin B (50 μg/ml) (AppliChem, cat no. A0890). Antibiotics were used to select for anaerobic Gram-positive bacteria other than streptococci, staphylococci and bacilli.

Isolation of bacteria

Anaerobic work was carried out in a VA500 workstation (Don Whitley Scientific) containing 90 % (v/v) N_2 and 10 % H_2. The atmosphere was kept at 37 °C and 75 % humidity. It was tested for anaerobic conditions using Anaerotest® (Merck, cat. no. 1.15112.0001). All materials, including agar media and buffered solutions, were brought into the workstation approximately 24 h prior to the sample preparation. Immediately after the collection and weight determination, caecal samples were brought into the anaerobic workstation and suspended in filter-sterilized phosphate-buffered saline (PBS) solution (per litre dH_2O: NaCl, 8.60 g; Na_2HPO_4, 0.86 g; KH_2PO_4, 0.40 g, pH 7.2) supplemented with 0.02 % peptone from meat (Roth, cat. no. 2366) and 0.05 % L-cysteine (PBS/PC). Tenfold serial dilutions were prepared for each sample. Bacterial suspensions (100 μl) were spread on CBS and CT agar using sterile glass beads. Counts were determined after 6 days of incubation. Only plates with 10–200 colonies per plate were taken into account for cell density determination. Weighted means were calculated if two successive dilutions gave rise to appropriate numbers of colonies. Results were expressed as mean ± SD of colony-forming units (CFU) per gram of wet caecal content or as logarithmic values thereof. Data were analysed statistically (*t* test) using the SigmaStat software, version 3.10 (Systat Software Inc.). All single-colony morphology types observed after 6 days of growth were streaked onto CBS or CT agar to ensure purity. Thereafter, the non-selective medium used for sub-culturing isolates was reduced WCA, that is, Wilkins-Chalgren-Anaerobe bouillon (Oxoid, cat. no. CM0643) supplemented with cysteine and DTT and prepared using strictly anaerobic techniques (100 % N_2) (Attebery and Finegold 1969). Culture purity was examined by observing cell morphology after Gram-staining and colony morphology. Cryo-stocks (100 μl) were stored at −80 °C after mixing bacterial suspensions with equal volumes of Tris-buffered aqueous solution (60 mM) containing 40 % glycerol.

Phylogenetic and DNA-based analyses

DNA was extracted from washed bacterial cell pellets using the DNeasy Blood & Tissue kit (Qiagen), following the instructions for pretreatment of Gram-positive bacteria. The 16S rRNA genes were amplified using primer 27F 5′-AGA GTT TGA TCC TGG CTC AG and 1492R 5′-GGT TAC CTT GTT ACG ACT T (Kageyama et al. 1999). The annealing temperature was 56 °C. Amplicons were purified using the Wizard SV Gel and PCR Clean-Up System

(Promega) and sequenced with primer 27F using the Qiagen Genomic Services. The 16S rRNA gene of strain CT-m2^T was further sequenced using primer 1492R. Sequences of organisms closely related to the isolated strains were obtained using the BLAST function of the NCBI server (Altschul et al. 1990) and The All-Species Living Tree project (Yarza et al. 2008). Ribosomal sequences were checked for anomalies using the greengenes web application (DeSantis et al. 2006a, b). All sequences were aligned using the BioEdit software, version 7.0.5.3 (Hall 1999). Percentages of similarity were calculated after unambiguous alignment of each isolated sequence with those of the most closely related species, using the DNA Distance Matrix function of the BioEdit software. The G + C content of DNA of strain CT-m2^T was determined at the German Collection of Microorganisms and Cell Cultures by using high-performance liquid chromatography (www.dsmz.de).

Phenotypic characterization

Strain CT-m2^T was characterized as described previously (Clavel et al. 2009; Clavel et al. 2010). For all tests, bacteria were grown in reduced WCA medium. Peptidoglycan, whole-cell sugars, polar lipids and cellular fatty acids were analysed by the Identification Service of the DSMZ (Braunschweig, Germany) according to the standard procedures. For these analyses, cell mass was obtained as follows: batch cultures (1.5 l) grown under anoxic conditions for 3 days in reduced WCA broth were centrifuged (5525 $\times g$, 20 min, RT) in 500-ml containers using a 4K15C centrifuge (Sigma). Pellets were re-suspended in sterile PBS, and supernatants were centrifuged again as above. The pellets were pooled with the first ones in 50-ml Falcon tubes, and bacterial suspensions were centrifuged (5525 $\times g$, 15 min, RT). Supernatants were discarded by inverting the tubes. Cell pellets were stored at −80 °C and dried by lyophilization overnight (Alpha 1-4 LDplus, Christ) prior to shipment. To determine enzymatic features, bacterial suspensions were analysed with the rapid ID 32A test and with ANI cards using the VITEK® system following the manufacturer's instructions (Biomérieux, Marcy-l'Etoile, France). For both tests, two cell suspensions in PBS (>Mc Farland Standard no. 3) were prepared from two independent batch cultures grown for 48 h. Data obtained with the rapid ID 32A were compared using apiweb (https://apiweb.biomerieux.com). For VITEK® analysis, ANI cards were inoculated automatically using the VITEK filling module and were incubated aerobically for 4 h at 37 °C. The cards were read manually against a comparator template provided by the manufacturer. Data were analysed using the VITEK program. To measure the production of short-chain fatty acids, cells were grown for 72 h in reduced WCA broth. Acetate, butyrate, propionate, valerate and isovalerate were measured with an HP 5890 series II gas chromatograph (Hewlett-Packard, Waldbronn, Germany) equipped with an HP-20 M column and a flame ionization detector. Supernatants (200 µl) collected at 0, 24, 48 and 72 h after inoculation were mixed with 23.6 µl of isobutyric acid (12 mM), 270 µl of NaOH (1 M) and 280 µl of $HClO_4$ (0.36 M). Freeze-dried mixtures were re-suspended in 400 µl of acetone and 100 µl of formic acid (5 M). After centrifugation, supernatants (1 µl) were injected twice into the chromatograph.

Results and discussion

Isolation and identification of bacterial isolates

The density of caecal bacteria able to grow on CBS agar containing cholesterol and bile salts tended to be higher in mice fed the CT diet. Counts were 5.4 ± 1.0 versus 4.0 ± 1.0 $\log_{10}$ (CFU/g wet weight) for the CT and STD group, respectively ($p = 0.098$). Further description of CBS isolates is given in the Electronic Supplementary Material. On the selective CT agar, the number of colonies was too low (<10 per plate) to provide reliable counts. Representatives of each of the four types of colonies observed were picked and isolates ($n = 7$) were identified by 16S rRNA gene sequencing. Apart from members of the genera *Proteus* and *Enterococcus* and the species *Parabacteroides gordonii*, we found that one bacterium, strain CT-m2^T, isolated from CT diet-fed mouse no. 2, belonged to a novel taxon, based on phylogenetic and phenotypic evidence.

Genotypic characterization of strain CT-m2^T

Phylogenetic analysis based on partial 16S rRNA gene sequencing showed that the new isolate is a member of the order *Clostridiales* (Fig. 1 and Suppl. Fig. S1). Indeed, strain CT-m2^T was very distantly related to bacteria from classes other than *Clostridia* within the *Firmicutes*, namely *Bacilli* (*e.g.* enterococci and lactobacilli), *Erysipelotrichi* (*Clostridium cylindroides, Clostridium ramosum* and related species), *Negativicutes* (*e.g. Clostridium quercicolum* and *Veillonella parvula*) and *Thermolithobacteria* (*e.g. Thermolithobacter ferrireducens*). Moreover, the new strain fell into the *Clostridium* cluster XIV, as defined by Collins et al. (Collins et al. 1994), the members of which belong almost exclusively to the family *Clostridiaceae*, *Eubacteriaceae*, *Lachnospiraceae* and *Ruminococcaceae* within the *Clostridiales* (Rainey 2009a, b). According to BLAST analysis, the partial 16S rRNA gene sequence of strain CT-m2^T (1 376 bp) (HM989805) was ≥98 % similar to already published sequences of uncultured bacteria obtained from the mouse caecum, including lean mice

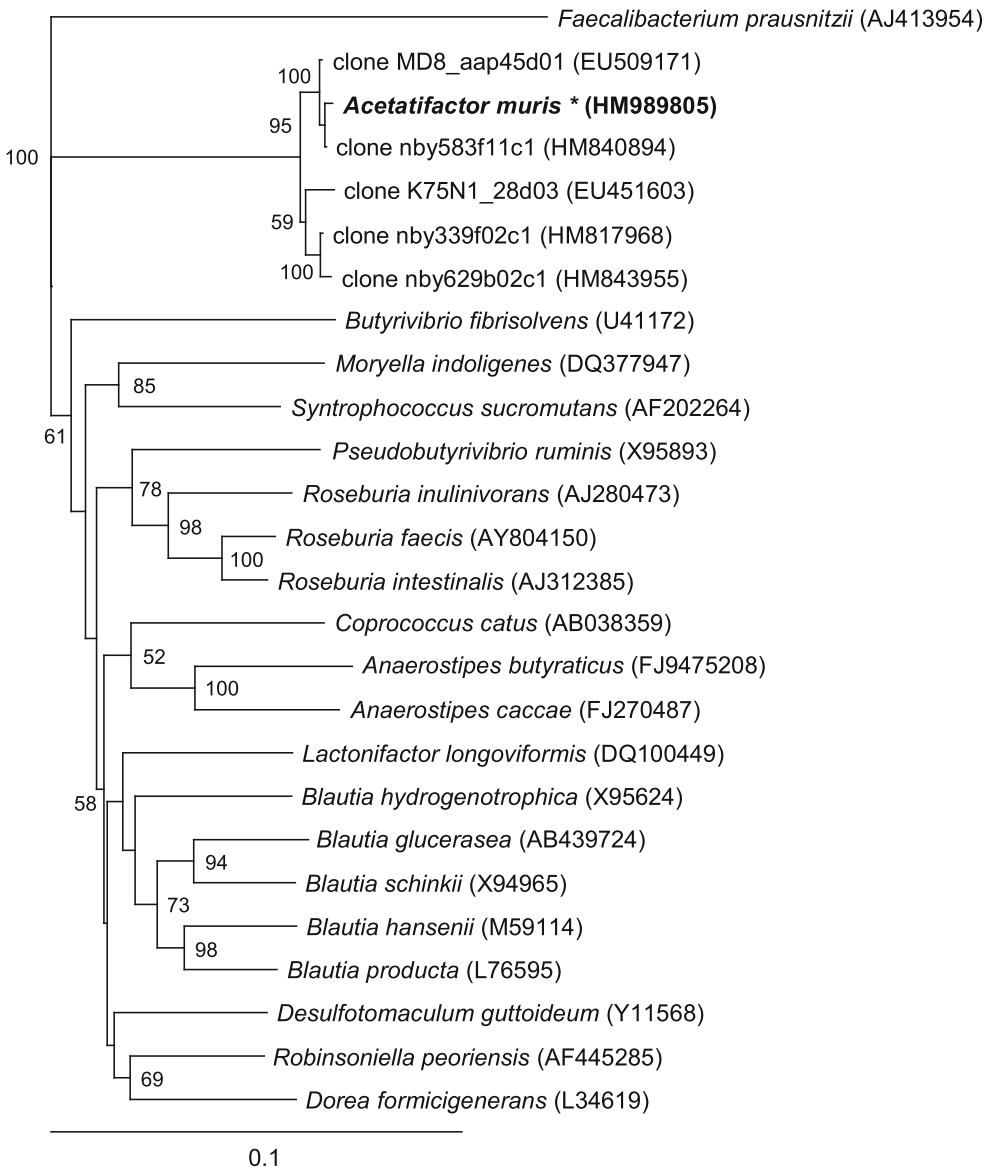

Fig. 1 Phylogenetic tree showing the position of strain CT-m2^T within members of the *Lachnospiraceae*. The GenBank accession numbers of the 16S rRNA gene sequences used to construct the tree are indicated in *brackets*. Sequences were aligned using the BioEdit software, and the rooted tree was constructed with Clustal X 2.0 using the neighbour-joining method with bootstrap values calculated from 1,000 trees. *Faecalibacterium prausnitzii*, a member of the family *Ruminococcaceae* within the *Clostridium* cluster IV, was used as outgroup to root the tree

(EU509171 and EU451603) (Wen et al. 2008) and from mouse skin samples (HM817968, HM840894 and HM843955) (Grice et al. 2010). The closest relatives of strain CT-m2^T (percentages of similarity between 88 and 90 %) with validly published names included *Anaerostipes caccae* (AB243985), *Bacteroides pectinophilus* (DQ497993), *Blautia producta* (X94966), *Butyrivibrio fibrisolvens* (U77341), *Clostridium aldenense* (DQ279736), *Clostridium celerecrescens* (X71848), *Clostridium sphenoides* (AB075772), *Coprococcus catus* (EU266552), *Dorea longicatena* (HQ259728), *Eubacterium plexicaudatum* (AF157058), *Desulfotomaculum guttoideum* (NR_026409), *Roseburia intestinalis* (AJ312386), *Ruminococcus gnavus* (X94967) and *Syntrophococcus sucromutans* (AF202264). Furthermore, local BLAST search against a set of 16 528 16S rRNA gene sequences (300–500 bp) from the caecum of five wild-type C57BL/6 mice (Werner et al. 2011) suggests that the novel bacterium is a subdominant member of mouse caecal microbiota, that is, we did not find any full-length hit corresponding to the sequence of strain CT-m2^T. The isolate's G + C content of DNA is 48.5 mol %, which differs substantially from the G + C content of some members of phylogenetically related genera, including *Blautia* spp. (37–47 mol %), *Butyrivibrio fibrisolvens* (42 mol %), *Clostridium butyricum* (28 mol %), *Clostridium celerecrescens* (38 mol %), *Coprococcus catus* (39 to 41 mol %), *Desulfotomaculum gutteoideum* (54 mol %), *Dorea* spp. (40–46 mol %), *Eubacterium ramulus* (39 mol %), *Roseburia intestinalis* (29–31 mol %) and *Ruminococcus hansenii* (38 mol %).

Phenotypic characterization of strain CT-m2^T

Strain CT-m2^T formed long and thin straight rods occurring as single cells, in pairs or small chains. Cells stained Gram-variable, yet the KOH test was negative, which is consistent with a Gram-positive cell wall. Taking into account that strain CT-m2^T belongs to the *Firmicutes*, the presence of *meso*-diaminopimelic acid as a diagnostic component of the peptidoglycan suggested that strain CT-m2^T is characterized by the peptidoglycan type A1γ. Enzymatic tests using the rapid ID 32 A and VITEK® system showed that strain CT-m2^T was positive for α-arabinosidase, α-fucosidase, α- and β-galactosidase, α-glucosidase, N-acetyl-glucosaminidase, phenylalanine arylamidase as well as arabinose and xylose metabolism. Whole-cell sugars were glucose, ribose, galactose and traces of xylose. Polar lipids in strain CT-m2^T included diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphoglycolipid and one each aminolipid and aminoglycolipid (Suppl. Fig. S2). The cellular fatty acid composition of cells grown for 72 h in reduced WCA under anaerobic conditions was (% total fatty acids): $C_{10:0}$ (2.4 %), $C_{11:0\ DMA}$ (2.3 %), $C_{12:0}$ (2.6 %), $C_{14:0}$ (48.7 %), $C_{14:0\ DMA}$ (14.6 %), $C_{16:0}$ (8.8 %), $C_{17:0\ DMA}$ (2.5 %), $C_{18:0}$ (5.2 %), $C_{18:1}$ w9c (5.2 %), $C_{18:1\ w9c\ DMA}$ (4.5 %) and unidentified CFA (3.3 %). Strain CT-m2^T grew best at 30–37 °C between pH 7.0 and 8.0 in reduced WCA broth under anaerobic conditions (100 % N_2). Growth was not inhibited by erythromycin up to ca. 200 µg/ml. Spores have not been observed, and cells did not survive heat treatment under anoxic conditions (60 °C, 20 min and 70 °C, 10 min). After 72 h of growth in reduced WCA, strain CT-m2^T produced acetate (1 370 ± 290 µmol/l medium), butyrate (530 ± 30 µmol/l) and trace amounts of propionate (<70 ± 50 µmol/l) ($n = 2$ independent cultures). Valerate and isovalerate were not detected. So far, approximately 20 butyrate-producing bacteria have been isolated from gut samples and many of them belong to the *Clostridium* cluster XIV (Duncan et al. 2004; Louis and Flint 2009; Eeckhaut et al. 2010). The fact that most of these bacteria produce high concentrations of butyrate (>10 mM) when compared with strain CT-m2^T is likely due to the slow growth rate of our isolate or to the absence of intermediate substrates (*e.g.* acetate or lactate) in the reduced WCA broth originally used to assess butyrate production (Pacaud et al. 1986; Duncan et al. 2004). Addition of arabinose (4 mM), xylose (10 mM), lithium lactate (10 mM) and sodium acetate (10 mM) to the medium did not markedly increase turbidity and butyrate production (680 ± 30 µmol/l) after 72 h of growth.

Based on these phenotypic and genotypic data, it is proposed that strain CT-m2^T be designated the type species of a novel bacterial genus, namely *Acetatifactor muris*, and that it belongs to the *Lachnospiraceae* within the *Clostridiales* (Rainey 2009a). Parameters that distinguish the isolate from related taxa are given in Table 1.

Table 1 Characteristics of strain CT-m2^T and phylogenetically related species

	1	2	3	4	5	6	7	8
Origin	Mouse caecum	Dog faeces	Cow rumen	Cow manure	Human faeces	Swine manure	Human faeces	Human faeces
Gram type	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Positive
Motility	–	–	+	+	–	–	+	–
Spore formation	–	+	–	+	n.r.	+	–	–
Major CFA	$C_{14:0}$	$C_{16:0\ DMA}$	$C_{16:0}$	n.r.	n.r.	$C_{16:0}$	n.r.	$C_{16:0}$
α-arabinosidase	+	n.r.	–	n.r.	n.r.	+	n.r.	n.r.
α-glucosidase	+	–	n.r.	n.r.	n.r.	+	n.r.	n.r.
Phe arylamidase	+	n.r.	–	n.r.	n.r.	–	–	n.r.
Arabinose	+	+	+	+	–	+	+	+
Glucose	–	+	+	+	+	+	+	+
Raffinose	–	+	–	+	–	+	+	+
Xylose	+	+	+	+	–	+	+	+
Butyrate	+	–	+	+	+	–	+	–
G + C (mol %)	48.5	40.7	42.1	38.0	39–41	48.7	29–31	43

Taxa: 1, *Acetatifactor muris*; 2, *Blautia glucerasea* (Furuya et al. 2010); 3, *Butyrivibrio fibrisolvens* (Kopecny et al. 2003); 4, *Clostridium celerecrescens* (Palop et al. 1989); 5, *Coprococcus catus* (Holdeman and Moore 1974); 6, *Robinsoniella peoriensis* (Cotta et al. 2009); 7, *Roseburia intestinalis* (Duncan et al. 2002); 8, *Ruminococcus gnavus* (Moore et al. 1976)

Symbols and abbreviations: +, positive reaction; −, negative reaction; *CFA* cellular fatty acid; *n.r.* not reported, *Phe* phenylalanine. Carbohydrate utilization by strain CT-m2^T was tested using the rapid ID 32A test and with ANI cards using the VITEK® system following the manufacturer's instructions (Biomérieux, Marcy-l'Etoile, France)

Description of *Acetatifactor* gen. nov.

(A.ce.ta.ti.fac'tor. N.L. n. *acetas -atis*, acetate; L. masc. n. *factor*, a maker; N.L. masc. n. *Acetatifactor*, acetate-maker).

Bacteria of the genus *Acetatifactor* are strictly anaerobic Gram-positive rods that grow best in a gas phase of 100 % N_2 and produce acetate and butyrate in a ratio of approximately 3:1. Cultures in the stationary growth phase are characterized by a very low turbidity in reduced WCA broth (<0.5 McFarland standard). Spore formation and motility have not been observed. Major cellular fatty acids are $C_{14:0}$ and $C_{14:0\ DMA}$. Galactose, glucose, ribose and traces of xylose are detected as whole-cell sugars. The diamino acid in the peptidoglycan is *meso*-diaminopimelic acid. Major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphoglycolipid and one each aminolipid and aminoglycolipid. Within the *Clostridiales*, members of the genus *Acetatifactor* are distantly related to bacteria belonging to the *Clostridium* cluster XIV, namely members of the genera *Anaerostipes, Blautia, Butyrivibrio, Clostridium, Coprococcus, Dorea, Eubacterium, Lactonifactor, Robinsoniella, Roseburia, Ruminococcus* and *Syntrophococcus* (≤90 % similarity based on partial 16S rRNA gene sequence analysis). The type species is *Acetatifactor muris*.

Description of *Acetatifactor muris* gen. nov., sp. nov.

(mu'ris. L. gen. n. *muris*, of a mouse).

The species has the features of the genus. Cells are approximately 1.0 μm wide and 2.0 to 5.0 μm long. They grow best in the temperature range from 30 to 37 °C and give rise to pinpoint translucent colonies after 6 days of growth on CT agar. The species is positive for α-arabinosidase, α-fucosidase, α- and β-galactosidase, α-glucosidase, N-acetyl-glucosaminidase, phenylalanine arylamidase as well as arabinose and xylose fermentation. It is negative for β-fucosidase, β-glucosidase, β-glucuronidase, β-lactosidase, alanine, arginine, glutamic acid, glycine, histidine, leucyl glycine, lysine, proline, serine, tryptophan and tyrosine arylamidase, urea hydrolysis as well as glucose, mannose, raffinose and trehalose fermentation. Its G + C content of DNA is 48.5 mol %. The type strain (CT-m2^T = DSM 23669^T = ATCC BAA-2170^T) is resistant to erythromycin and was isolated from the caecal content of a 20-week-old male C57BL/6NCrl mouse fed a high calorie diet.

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References

Alain K, Querellou J (2009) Cultivating the uncultured: limits, advances and future challenges. Extremophiles 13:583–594

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Attebery HR, Finegold SM (1969) Combined screw-cap and rubber-stopper closure for Hungate tubes (pre-reduced anaerobically sterilized roll tubes and liquid media). Appl Microbiol 18:558–561

Clavel T, Henderson G, Engst W, Dore J, Blaut M (2006) Phylogeny of human intestinal bacteria that activate the dietary lignan secoisolariciresinol diglucoside. FEMS Microbiol Ecol 55:471–478

Clavel T, Charrier C, Braune A, Wenning M, Blaut M, Haller D (2009) Isolation of bacteria from the ileal mucosa of TNF^{deltaARE} mice and description of *Enterorhabdus mucosicola* gen. nov., sp. nov. Int J Syst Evol Microbiol 59:1805–1812

Clavel T, Saalfrank A, Charrier C, Haller D (2010) Isolation of bacteria from mouse caecal samples and description of *Bacteroides sartorii* sp. nov. Arch Microbiol 192:427–435

Collins MD, Lawson PA, Willems A, Cordoba JJ, Fernandez-Garayzabal J, Garcia P, Cai J, Hippe H, Farrow JA (1994) The phylogeny of the genus *Clostridium*: proposal of five new genera and eleven new species combinations. Int J Syst Bacteriol 44:812–826

Cotta MA, Whitehead TR, Falsen E, Moore E, Lawson PA (2009) *Robinsoniella peoriensis* gen. nov., sp. nov., isolated from a swine-manure storage pit and a human clinical source. Int J Syst Evol Microbiol 59:150–155

DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, Andersen GL (2006a) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol 72:5069–5072

DeSantis TZ Jr, Hugenholtz P, Keller K, Brodie EL, Larsen N, Piceno YM, Phan R, Andersen GL (2006b) NAST: a multiple sequence alignment server for comparative analysis of 16S rRNA genes. Nucleic Acids Res 34:W394–W399

Duncan SH, Hold GL, Barcenilla A, Stewart CS, Flint HJ (2002) *Roseburia intestinalis* sp. nov., a novel saccharolytic, butyrate-producing bacterium from human faeces. Int J Syst Evol Microbiol 52:1615–1620

Duncan SH, Louis P, Flint HJ (2004) Lactate-utilizing bacteria, isolated from human feces, that produce butyrate as a major fermentation product. Appl Environ Microbiol 70:5810–5817

Eeckhaut V, Van Immerseel F, Pasmans F, De Brandt E, Haesebrouck F, Ducatelle R, Vandamme P (2010) *Anaerostipes butyraticus* sp. nov., an anaerobic, butyrate-producing bacterium from *Clostridium* cluster XIVa isolated from broiler chicken caecal content, and emended description of the genus *Anaerostipes*. Int J Syst Evol Microbiol 60:1108–1112

Fleissner CK, Huebel N, Abd El-Bary MM, Loh G, Klaus S, Blaut M (2010) Absence of intestinal microbiota does not protect mice from diet-induced obesity. Br J Nutr 104:919–929

Furuya H, Ide Y, Hamamoto M, Asanuma N, Hino T (2010) Isolation of a novel bacterium, *Blautia glucerasei* sp. nov., hydrolyzing plant glucosylceramide to ceramide. Arch Microbiol 192: 365–372

Goodman AL, Kallstrom G, Faith JJ, Reyes A, Moore A, Dantas G, Gordon JI (2011) Extensive personal human gut microbiota

culture collections characterized and manipulated in gnotobiotic mice. Proc Natl Acad Sci USA 108:6252–6257

Grice EA, Snitkin ES, Yockey LJ, Bermudez DM, Liechty KW, Segre JA (2010) Longitudinal shift in diabetic wound microbiota correlates with prolonged skin defense response. Proc Natl Acad Sci USA 107:14799–14804

Hall TA (1999) Bioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nuc Acids Symp Ser 41:95–98

Holdeman LV, Moore WEC (1974) New genus, *Coprococcus*, twelve new species, and emended descriptions of four previously described species of bacteria from human feces. Int J Syst Bacteriol 24:260–277

Kageyama A, Benno Y, Nakase T (1999) Phylogenetic evidence for the transfer of *Eubacterium lentum* to the genus *Eggerthella* as *Eggerthella lenta* gen. nov., comb. nov. Int J Syst Bacteriol 49:1725–1732

Kopecny J, Zorec M, Mrazek J, Kobayashi Y, Marinsek-Logar R (2003) *Butyrivibrio hungatei* sp. nov. and *Pseudobutyrivibrio xylanivorans* sp. nov., butyrate-producing bacteria from the rumen. Int J Syst Evol Microbiol 53:201–209

Ley RE, Hamady M, Lozupone C, Turnbaugh PJ, Ramey RR, Bircher JS, Schlegel ML, Tucker TA, Schrenzel MD, Knight R, Gordon JI (2008) Evolution of mammals and their gut microbes. Science 320:1647–1651

Louis P, Flint HJ (2009) Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. FEMS Microbiol Lett 294:1–8

Moore WEC, Johnson JL, Holdeman LV (1976) Emendation of *Bacteroidaceae* and *Butyrivibrio* and descriptions of *Desulfomonas* gen. nov. and ten new species in the genera *Desulfomonas*, *Butyrivibrio*, *Eubacterium*, *Clostridium* and *Ruminococcus*. Int J Syst Bacteriol 26:238–252

Pacaud S, Loubiere P, Goma G, Lindley ND (1986) Organic acid production during methylotrophic growth of Eubacterium limosum B 2: displacement towards increased butyric acid yields by supplementing with acetate. Appl Microbiol Biotechnol 23:330–335

Palop M, Valles S, Pinaga F, Flors A (1989) Isolation and characterization of an anaerobic, cellulolytic bacterium, *Clostridium celerecrescens* sp. nov. Int J Syst Bacteriol 39:68–71

Rainey FA (2009a) Family V. *Lachnospiracea*e fam. nov. In: De Vos P, Garrity G, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer K-H, Whitman WB (eds) Bergey;s manual of systematic bacteriology, 2nd edn. Springer, New York, p 921

Rainey FA (2009b) Family VIII. *Ruminococcacea*e fam. nov. In: De Vos P, Garrity G, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer K-H, Whitman WB (eds) Bergey's manual of systematic bacteriology, 2nd edn. Springer, New York, p 1016

Turnbaugh P, Ridaura V, Faith J, Rey F, Knight R, Gordon J (2009) The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. Sci Trans Med 1:6ra14

Wen L, Ley RE, Volchkov PY, Stranges PB, Avanesyan L, Stonebraker AC, Hu C, Wong FS, Szot GL, Bluestone JA, Gordon JI, Chervonsky AV (2008) Innate immunity and intestinal microbiota in the development of Type 1 diabetes. Nature 455:1109–1113

Werner T, Wagner SJ, Martinez I, Walter J, Chang JS, Clavel T, Kisling S, Schuemann K, Haller D (2011) Depletion of luminal iron alters the gut microbiota and prevents Crohn's disease-like ileitis. Gut 60:325–333

Yarza P, Richter M, Peplies J, Euzeby J, Amann R, Schleifer KH, Ludwig W, Glockner FO, Rossello-Mora R (2008) The all-species living tree project: a 16S rRNA-based phylogenetic tree of all sequenced type strains. Syst Appl Microbiol 31:241–250