Isolation of bacteria from the ileal mucosa of TNF^{deltaARE} mice and description of *Enterorhabdus mucosicola* gen. nov., sp. nov.

Thomas Clavel,[1] Cédric Charrier,[2] Annett Braune,[3] Mareike Wenning,[4] Michael Blaut[3] and Dirk Haller[1]

Correspondence
Thomas Clavel
thomas.clavel@wzw.tum.de

[1]Biofunctionality, ZIEL – Research Center for Nutrition and Food Science, Technische Universität München (TUM), 85350 Freising Weihenstephan, Germany

[2]NovaBiotics Ltd, Aberdeen AB21 9TR, UK

[3]Gastrointestinal Microbiology, German Institute of Human Nutrition Potsdam-Rehbrücke (DIfE), 14558 Nuthetal, Germany

[4]Microbiology, Nutrition and Food Research Centre (ZIEL), Technische Universität München (TUM), 85350 Freising Weihenstephan, Germany

The diversity of bacteria associated with inflamed mucosa was investigated by culturing ileal samples from TNF^{deltaARE} mice on a selective medium containing mucin. Among eight isolates, two strains (Mt1B3 and Mt1B8^T) belonged to bacterial groups not yet cultured from the mouse intestine. Whereas strain Mt1B3 was identified as a member of the family *Planococcaceae* and is closely related to *Sporosarcina* species and *Filibacter limicola* DSM 13886^T, strain Mt1B8^T was a novel bacterium. Based on phylogenetic analysis, strain Mt1B8^T is a member of the family *Coriobacteriaceae*. The closest relatives with validly published names were *Asaccharobacter celatus*, *Adlercreutzia equolifaciens* (<96 % similarity) and *Eggerthella* species (<92 %). With respect to *Asaccharobacter celatus* and *Eggerthella*, the phylogenetic position of strain Mt1B8^T was confirmed at the chemotaxonomic level by Fourier-transform infrared spectroscopic analysis. The major fatty acid of strain Mt1B8^T is $C_{16:0}$ (23.9 %). Menaquinones were monomethylated. DNA–DNA relatedness between strain Mt1B8^T and *Asaccharobacter celatus* DSM 18785^T was 28 %. Strain Mt1B8^T is a Gram-positive-staining rod that does not form spores and has a high DNA G+C content (64.2 mol%). Cells are aerotolerant but grow only under strictly anoxic conditions. They are sensitive to cefotaxime, clarithromycin, erythromycin, metronidazole, tetracycline, tobramycin and vancomycin. API and VITEK analysis showed the ability of strain Mt1B8^T to convert a variety of amino acid derivatives. According to these findings, it is proposed to create a novel genus and species, *Enterorhabdus mucosicola* gen. nov., sp. nov., to accommodate strain Mt1B8^T. The type strain of *Enterorhabdus mucosicola* is Mt1B8^T (=DSM 19490^T =CCUG 54980^T).

The pioneering work by Dubos & Schaedler (1960) demonstrated the importance of intestinal bacteria to host physiology. Since then, it has been acknowledged that intestinal bacteria play a crucial role in inflammatory bowel diseases (IBD) (Manichanh *et al.*, 2006). Taking into account that the intestinal epithelium is the first line of defence against luminal stimuli and is important for the regulation of innate and adaptive immune responses (Clavel & Haller, 2007), bacteria living in contact with the intestinal mucosa may be of particular interest (Conte *et al.*, 2006; Derrien *et al.*, 2004). Although *Mus musculus* is one of the most widely used laboratory animals, knowledge of its intestinal microbiota is scant. Recent advances in molecular microbiology have revealed the presence of uncultured novel bacterial groups in the mouse intestine (Apajalahti *et al.*, 2002; Salzman *et al.*, 2002). To date, little

Abbreviations: FT-IR, Fourier-transform infrared; IBD, inflammatory bowel diseases.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences obtained in this study are AM747811 for strain Mt1B8^T and AM944637–AM944643 for strains Mt1B1–Mt1B7, and those for the *gyrB* gene sequences are EU594341 for strain Mt1B8^T and EU594342 for *Eggerthella lenta* DSM 2243^T.

Supplementary figures showing an extended 16S rRNA gene sequence phylogeny, polar lipid analyses and data on daidzein and genistein conversion for strain Mt1B8^T are available with the online version of this paper.

is known about the intestinal microbiota in mouse models of IBD and work has been focused mainly on interleukin-2- and interleukin-10-deficient mice (Bibiloni *et al.*, 2005; Duck *et al.*, 2007; Pena *et al.*, 2004; Schuppler *et al.*, 2004; Swidsinski *et al.*, 2005; Ye *et al.*, 2008). Only two of these studies used culture-based techniques (Duck *et al.*, 2007; Pena *et al.*, 2004) and three included analysis of mucosa-associated bacteria (Schuppler *et al.*, 2004; Swidsinski *et al.*, 2005; Ye *et al.*, 2008). In that context, the aim of the present study was to isolate mucosa-associated bacteria from inflamed ileal samples obtained from $TNF^{deltaARE}$ mice (Kontoyiannis *et al.*, 1999). Furthermore, we focused on the genotypic and phenotypic description of the novel bacterium Mt1B8^T.

Eight bacterial strains (Mt1B1–Mt1B7 and Mt1B8^T) were isolated on a selective mucin-containing medium (Mt1) from two distal ileal samples obtained from $TNF^{deltaARE}$ C57BL/6 mice. Animal use was approved by the local institution in charge (approval no. 55.2-1-54-2531-74-06; Regierung von Oberbayern). Mice were fed a standard animal diet (V1534-000 R/M-H; Ssniff, Soest). Two female $TNF^{deltaARE}$ mice were sacrificed by neck dislocation at the age of 12 weeks. Previous experiments performed in our laboratory showed that $TNF^{deltaARE}$ mice develop mild inflammation in the distal ileum at the age of 12 weeks (mean histological score 5.2 ± 1.3, $n=3$). Histological scores, ranging from 0 (not inflamed) to 12 (highly inflamed), were determined by assigning points to specific pathological criteria, as described by Katakura *et al.* (2005). One distal ileal segment (approx. 3 mm long) from each mouse was stored for 30 min on ice in filter-sterilized PBS (l^{-1}: 8.60 g NaCl, 0.87 g Na_2HPO_4, 0.40 g KH_2PO_4, pH 7.2) supplemented with 0.02 % (w/v) peptone from meat (2366; Roth) and 0.05 % (w/v) L-cysteine (PBS-PC). Samples were prepared as described by Conte *et al.* (2006). Four washing steps prevented analysis of luminal bacteria. All steps were carried out under sterile conditions. Briefly, PBS-DTT was removed using a pipette and samples were vortexed in 500 µl PBS supplemented with DTT (0.016 %, w/v) for 5 min at room temperature using a Vortex-Genie 2 (Scientific Industries) set to 'Shake 1'. Subsequently, the PBS-DTT was removed by pipette and replaced by a volume of 500 µl PBS-PC. Eppendorf tubes were shaken by hand for about 15 s. After removal of the PBS-PC, two additional washing steps in PBS-PC were performed. Finally, samples were vortexed in distilled water for 30 min using a Vortex-Genie 2 set to 'Vortex 4'. Undiluted and 10-fold dilution series (10^{-1}–10^{-3}) of the cell suspensions (100 µl each) were plated in triplicate on the selective medium Mt1 [l^{-1}: 5 g mucin (M1778; Sigma), 500 mg L-cysteine, 1 mg yeast extract, 20 µg folic acid, 20 µg vitamin B_{12}, 50 mmol $NaHCO_3$, 10 mmol sodium acetate, 5 mmol Na_2HPO_4, 5 mmol NaCl, 3 mmol KH_2PO_4, 1 mmol $CaCl_2$, 1 mmol $MgCl_2$, 10 µmol $FeCl_3$, 1 % (w/v) agar]. The pH of the medium was 7.7 prior to autoclaving (121 °C, 15 min). Mucin was first dissolved in 100 ml of 5 % (v/v) ethanol in distilled water. The final concentration of ethanol in Mt1 was 0.5 % (v/v). For each animal and each dilution, each of the triplicate plates was incubated at 37 °C under oxic conditions in a humidified atmosphere containing 5 % CO_2, at 30 °C, under microaerophilic conditions or at 37 °C under anaerobic conditions in jars using CampyGen or AnaeroGen catalysers (Oxoid). All colony morphology types observed after 9 days of growth were streaked on brain heart infusion (BHI) agar (211059; BD) supplemented with (l^{-1}) 2 g yeast extract and 2 g glucose and 0.05 % (w/v) cysteine (GY-BHI-c) to support better growth and ensure purity. Culture purity was assessed as described previously (Clavel *et al.*, 2007). Cryo-stocks (100 µl) were prepared by mixing bacterial suspensions with equal volumes of Tris-buffered aqueous solution (60 mM) containing 40 % glycerol. Cryo-stocks were stored at −80 °C after snap-freezing in liquid nitrogen. For characterization of strain Mt1B8^T, unless otherwise stated, bacteria were grown in GY-BHI-c at 37 °C under strictly anoxic conditions (Attebery & Finegold, 1969). The gas phase was 100 % N_2.

For phylogenetic analyses, DNA was extracted from bacterial cell pellets using the QIAamp DNA Stool Mini kit (Qiagen) according to the protocol for isolation of DNA from stools for pathogen detection. When required, the lysis temperature was increased to 95 °C. The 16S rRNA genes were amplified as described previously (Clavel *et al.*, 2005). Amplicons were purified using the Wizard SV Gel and PCR Clean-Up System (Promega) and sequenced with primer 27f (Clavel *et al.*, 2005) using the Qiagen sequencing service. The 16S rRNA gene of strain Mt1B8^T was further sequenced using primers 338f, 338r and 1492r (Clavel *et al.*, 2005). Gyrase B (*gyrB*) genes were amplified as described by Santos & Ochman (2004). Amplicons were purified as described above and sequenced using the primer gyrBBNDN1 (5′-CCGTCCACGTCGGCRTCN-GYCAT-3′). 16S rRNA gene sequences of organisms closely related to the isolated strains (>90 % similarity) were obtained using the BLAST function of the NCBI server (Altschul *et al.*, 1990) and the Ribosomal Database Project (Cole *et al.*, 2003). Sequences were aligned using 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 DNADIST DNA distance matrix function.

The partial 16S rRNA gene sequence of strain Mt1B8^T (1336 bp) showed 97.6 % similarity with the sequence of bacterium B7 (Duck *et al.*, 2007), 95.9 % similarity with *Adlercreutzia equolifaciens* FJC-B9^T (Maruo *et al.*, 2008) and *Asaccharobacter celatus* do03^T (Minamida *et al.*, 2006, 2008), 95.7 % similarity with strain Julong732 (Wang *et al.*, 2005) and <92 % similarity with *Eggerthella* species (Kageyama *et al.*, 1999; Lau *et al.*, 2004; Wade *et al.*, 1999).

A rooted phylogenetic tree (Fig. 1) was constructed with CLUSTAL X 1.8 using the neighbour-joining method with bootstrap values calculated from 1000 resamplings. Groupings were confirmed using the maximum-parsimony

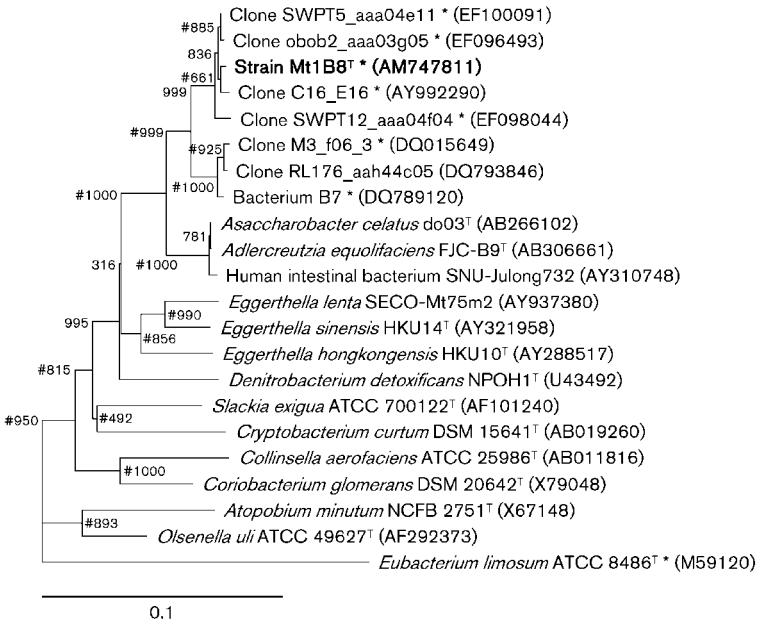

Fig. 1. Phylogenetic position of strain Mt1B8^T among members of the family *Coriobacteriaceae* based on neighbour-joining analysis of 16S rRNA gene sequences. Numbers at nodes are bootstrap values based on 1000 resamplings. The sequence of *Eubacterium limosum* ATCC 8486^T was used as the outgroup. *, Mouse intestinal microbiota; #, nodes conserved with the maximum-parsimony method. Bar, 10 substitutions per 100 nucleotides.

method. Of note, *Adlercreutzia equolifaciens*, *Asaccharobacter celatus* and strain Julong732 cluster together, whereas the sequence of strain Mt1B8^T is similar to cloned sequences originating from the mouse intestine (Ley *et al.*, 2005, 2006; Turnbaugh *et al.*, 2006).

Supplementary Fig. S1 (available in IJSEM Online) shows the phylogenetic position of all of the eight newly isolated strains with closely related species and known strains of the mouse intestinal microbiota. Strains Mt1B1 and Mt1B2 and strains Mt1B4–Mt1B7 showed >98 % similarity with *Escherichia coli* ATCC 25922 and *Lactobacillus murinus* ASF361, respectively, while Mt1B3 and Mt1B8 belonged to so-far unidentified or uncultured bacterial groups within the mouse intestine. Based on partial 16S rRNA gene sequence analysis (714 bp), strain Mt1B3 was >99 % similar to cloned sequences originating from volcanic soil (GenBank accession no. DQ455577), compost (DQ345490) and sewage sludge (AB241601) (results not shown) and >98 % similar to *Sporosarcina soli* I80^T and *Sporosarcina koreensis* F73^T (Kwon *et al.*, 2007), *Sporosarcina aquimarina* SAFN-008 (Yoon *et al.*, 2001), *Sporosarcina saromensis* HG711 (An *et al.*, 2007) and *Filibacter limicola* DSM 13886^T (Maiden & Jones, 1984). Based on a partial sequence analysis of *gyrB* genes (547 bp), strain Mt1B8^T was 79.4 % similar to *Eggerthella lenta* DSM 2243^T. The next most closely related *gyrB* gene sequences available in the NCBI database were those of *Streptomyces* species (<60 % similarity) (Hatano *et al.*, 2003). Apart from *Eggerthella* species, *Asaccharobacter celatus* DSM 18785^T was the only described and related bacterium available as a pure culture. Thus, further analyses for description of strain Mt1B8^T were performed using *Asaccharobacter celatus* DSM 18785^T, *Eggerthella lenta* DSM 2243^T and *Eggerthella hongkongensis* DSM 16106^T.

Although there is, so far, no example to show that strains with less than 97 % 16S rRNA gene sequence similarity may exceed the proposed threshold of 70 % DNA–DNA relatedness for distinguishing species (Gevers *et al.*, 2005), DNA–DNA hybridization experiments were carried out at the DSMZ according to standard methods (Cashion *et al.*, 1977; De Ley *et al.*, 1970; Huß *et al.*, 1983; Mesbah *et al.*, 1989; Tamaoka & Komagata, 1984; Visuvanathan *et al.*, 1989). Hybridization of DNA from strain Mt1B8^T with DNA from *Asaccharobacter celatus* DSM 18785^T revealed relatedness values of 28 ± 2.1 % from duplicate experiments. These data support the hypothesis that strain Mt1B8^T represents a novel bacterial genus. The DNA G+C content (64.2 mol%) is comparable to values reported in the literature for *Eggerthella lenta* and *Asaccharobacter celatus*.

Fourier-transform infrared (FT-IR) spectroscopy was used to evaluate phenotypic differences between strain Mt1B8^T, *Asaccharobacter celatus* DSM 18785^T, *Eggerthella lenta* DSM 2243^T and *Eggerthella hongkongensis* DSM 16106^T. As a whole-cell fingerprinting technique, FT-IR spectroscopy gives information on the overall biochemical composition of cells, and is a useful tool for species identification and strain typing (Oberreuter *et al.*, 2002; Wenning *et al.*, 2006, 2008). After revival from cryo-stocks, bacteria were cultured twice in 10 ml GY-BHI-c for 24 h. Prior to spectrometric analysis, cells were washed twice (8000 ***g***, 3 min, room temperature) with filter-sterilized distilled water. Two independent experiments with different batches of medium were performed, each experiment with two independent cultures of each bacterium. Spectra were recorded using an IFS 28B spectrometer (Bruker Optics), as described by Kummerle *et al.* (1998). Spectral similarities were assessed by hierarchical cluster analysis using

OPUS version 5.5 (Bruker). FT-IR data showed clearly that strain Mt1B8^T is related less distantly to *Asaccharobacter celatus* than to *Eggerthella lenta* and *Eggerthella hongkongensis* (Fig. 2) and supports the phylogenetic analysis. Additional chemotaxonomic analyses included the determination of whole-cell fatty acid composition by gas chromatography. For that purpose, bacteria were grown anaerobically at 37 °C on chocolate agar (C376; CCUG). Conditions for preparation of cell extracts and gas chromatography analysis are detailed in Sasser (1990) and detailed experimental information is available online (http://www.ccug.se). Fatty acid determination was done in duplicate. Comparison analyses with reference strains were done as described by Eerola & Lehtonen (1988). The complete cellular fatty acid profiles of strain Mt1B8^T and related organisms are available online (http://www.ccug.se). Cellular fatty acid analysis revealed that strain Mt1B8^T has a unique fatty acid profile within the family *Coriobacteriaceae* (92 % similarity with *Eggerthella* species). The major fatty acid was $C_{16:0}$ (23.9 ± 1.7 % of total fatty acids) (Table 1). In contrast, the major fatty acids in *Adlercreutzia equolifaciens* and *Asaccharobacter celatus* were $C_{17:1}$ iso I (24.9 %) and $C_{18:1}\omega 9c$ (54.0 %), respectively. Finally, whole-cell sugar, peptidoglycan, polar lipid and menaquinone analysis were performed at the DSMZ according to standard procedures (Rhuland *et al.*, 1955; Staneck & Roberts, 1974; Whiton *et al.*, 1985). The diamino acid in the peptidoglycan was identified as LL-diaminopimelic acid. Galactose and ribose were detected as whole-cell sugars. The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol, three unidentified phospholipids and four unidentified glycolipids (Supplementary Fig. S2). The major menaquinone was monomethylmenaquinone-6 (MMK-6) (100 %).

For the phenotypic characterization of strain Mt1B8^T in liquid media, growth was monitored using McFarland turbidity standards and by measuring optical density at 600 nm. Cell morphology was determined by light microscopy after Gram-staining. The Gram-staining result

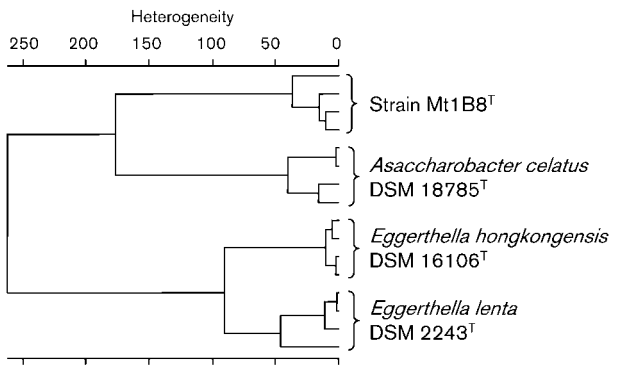

Fig. 2. Cluster analysis of FT-IR spectra calculated using Ward's algorithm and vector-normalized first derivatives of the spectra in the ranges 3000–2800 and 1800–700 cm^{-1}.

was confirmed using the KOH lysis test (Gregersen, 1978). To assess the temperature range for growth, cultures were incubated, in duplicate, at 25–45 °C, at intervals of 5 °C. The pH range for growth was tested, in duplicate, at initial pH values ranging from 5.0 to 9.5 at intervals of 0.5 pH units. The pH of GY-BHI-c was adjusted using aqueous solutions of HCl and NaOH and the medium was filter-sterilized. Spore formation and motility were examined as described previously (Clavel *et al.*, 2007). For spore formation, strain Mt1B3 was used as a positive control. To determine enzymic features, bacterial suspensions were analysed with the Rapid ID 32A test and with ANI cards using the VITEK system (bioMérieux), following the manufacturer's instructions. For both tests, cell suspensions were prepared from two independent batch cultures. Catalase, coagulase, indole and oxidase activities were tested using commercial reagents (Bactident and James; bioMérieux). Most phenotypic traits of strain Mt1B8^T are given below in the species description. In addition, strain Mt1B8^T grew on Mt1 agar, in contrast to *Asaccharobacter celatus* DSM 18785^T. However, strain Mt1B8^T did not grow in GY-BHI-c in the presence of 0.5 % (v/v) bile salts (48305; Fluka). *Eggerthella lenta* DSM 2243^T was used as a positive control. The procedure that was used for isolation implied that strain Mt1B8^T could survive for at least 30 min at room temperature in the presence of atmospheric oxygen. However, strain Mt1B8^T did not survive after ethanol or heat treatment. In contrast, strain Mt1B3 survived after ethanol treatment and after 20 min at 60 °C and 10 min at 80 °C. Spores were not observed by microscopic analysis of cells of strain Mt1B8^T stained according to Schaeffer & Fulton (1933). The API and VITEK analyses showed that the only substrates that gave positive signals with strain Mt1B8^T were *p*-nitroanilide and *β*-naphthylamide derivatives of the following amino acids: L-alanine, L-glycine, L-histidine, L-leucine, L-lysine, L-phenylalanine, L-proline, L-serine and L-tyrosine. The test for arginine dihydrolase was also positive.

The sensitivity of strain Mt1B8^T was tested towards 10 antimicrobial agents. All chemicals were obtained from Sigma and Oxoid. The medium was GY-BHI-c supplemented with 0.05 % (w/v) arginine and 2 % (w/v) agar. MICs were determined after 48 h at 37 °C in a miniMACS anaerobic workstation using Etest strips (Bio-Stat Diagnostics) according to the approved standard M11-A7 of the CLSI and standard BSAC methods (Andrews, 2007). Each antibiotic was tested in duplicate in three independent experiments and the MIC breakpoint was expressed as the mean of the six replicates. The MIC breakpoints were (μg ml^{-1}): cefotaxime, 1.250 ± 0.112; ciprofloxacin, >32; clarithromycin, <0.016; colistin, >256; erythromycin, 0.048 ± 0.007; metronidazole, 0.034 ± 0.004; oxacillin, 4.667 ± 0.667; tetracycline, 0.115 ± 0.007; tobramycin, 2.667 ± 0.211; and vancomycin, 1.333 ± 0.105. Thus, strain Mt1B8^T is resistant to colistin, which interacts with the cytoplasmic membrane of Gram-negative bacteria. It is also resistant to ciprofloxacin, a broad-spectrum antibiotic

Table 1. Traits of strain Mt1B8^T and related species

Species: 1, *Enterorhabdus mucosicola* gen. nov., sp. nov. (strain Mt1B8^T); 2, *Adlercreutzia equolifaciens* (data from Maruo *et al.*, 2008); 3, *Asaccharobacter celatus* (Minamida *et al.*, 2008); 4, *Eggerthella lenta* (unless indicated, data from Kageyama *et al.*, 1999; Wade *et al.*, 1999). DMA, Dimethylacetal; DMMK, dimethylmenaquinone; MMK, monomethylmenaquinone; +, positive; −, negative; R, resistant; ND, not reported.

Trait	1	2	3	4
Diaminopimelic acid isomer	LL	*meso*	*meso*	LL
Predominant menaquinone	MMK-6	DMMK-6	ND	MMK-6
Isoflavone conversion				
Daidzein	+	+	+	ND
Genistein	+	ND	ND	ND
Response to antibiotics (μg ml^{-1})				
Ciprofloxacin	32 (R)*	ND	ND	ND
Colistin	>256 (R)*	ND	ND	ND
Erythromycin	0.048*	ND	ND	3 (R, *n*=1)†
Tetracycline	0.115*	ND	ND	6 (R, *n*=5)†
Cellular fatty acids (%)‡				
14 : 0	7.6±0.6	7.5	ND	4.4–14.3
15 : 0 anteiso	0.0±0.0	9.4	ND	1.0–5.9
16 : 0	23.9±1.7	1.7	8.4	9.5–10.9
16 : 0 aldehyde	6.4±2.3	18.0	ND	0–7.2
17 : 1 iso I/16 : 0 DMA	15.8±0.4	24.9	ND	29.1–32.4
18 : 0	8.8±0.4	4.4	11.7	0–6.2
18 : 1ω7*c*/ω12*t*/ω9*t*	6.6±1.9	6.3	ND	0–2.5
18 : 1ω9*c*	11.2±0.1	6.0	54.0	3.1–22.5
18 : 1ω9*c* DMA	3.0±3.0	4.5	ND	0
18 : 2ω6,9*c*/18 : 0 anteiso	8.2±0.5	0.0	ND	4.1–5.8
19 : 1 iso I	5.8±0.0	3.8	ND	1.1–4.6
DNA G+C content (mol%)	64	64–67	63	62
Genomic comparison of type strain with strain Mt1B8^T				
16S rRNA gene sequence similarity (%)	(100)	95.9	95.9	91.4
DNA–DNA relatedness (%)§	(100)	10.3±0.3	28±2.1	ND

*MIC breakpoint (μg ml^{-1}).

†Numbers of 12 strains that are resistant to the given antibiotic at the concentration indicated are given in parentheses (data from Moore & Moore, 1986).

‡Data for reference taxa are from *Adlercreutzia equolifaciens* CCUG 54925^T, *Asaccharobacter celatus* DSM 18785^T and *Eggerthella lenta* strains CCUG 33760, CCUG 34779 and CCUG 45577 (fatty acid profile varied between strains; ranges are given) (data for CCUG strains from http://www.ccug.se).

§Comparisons were made in this study with *Adlercreutzia equolifaciens* DSM 19450^T and *Asaccharobacter celatus* DSM 18785^T.

often used in the treatment of IBD (Sartor, 2004). Strain Mt1B8^T was susceptible to most of the other antimicrobial agents tested, with the exception of oxacillin, a narrow-spectrum *β*-lactam antibiotic. In contrast, *Eggerthella hongkongensis* DSM16106^T is resistant to cefotaxime (Lau *et al.*, 2004).

Because a number of bacteria belonging to the family *Coriobacteriaceae* convert phyto-oestrogens (Clavel *et al.*, 2006; Minamida *et al.*, 2006; Wang *et al.*, 2005), we investigated the conversion of the isoflavones daidzein and genistein by strain Mt1B8^T. Daidzein and genistein were purchased from Acros Organics and Roth, respectively. Stock solutions (20 mmol l^{-1}) were prepared in DMSO and filter-sterilized (Millex-GV filter; Millipore). Strain Mt1B8^T was cultured as described above. BHI was not supplemented with glucose and yeast extract and the gas phase was H_2 : CO_2 (80 : 20, v/v). For conversion experiments, 50 μl of the appropriate isoflavone stock solution and 200 μl of an overnight culture of strain Mt1B8^T were added to 10 ml medium. Isoflavones and bacteria were incubated in medium separately as controls. Samples were taken over time with a syringe and centrifuged (14 000 ***g***, 5 min). Supernatants (20 μl) were directly used for reversed-phase HPLC analysis as described by Schoefer *et al.* (2002). The mobile phases were 98 : 2 water/acetic acid (A) and methanol (B) in a gradient mode (5 to 55 % B over 15 min, 55 % B for 10 min, 55–100 % B over 3 min, 100 % B for 4 min). The flow rate was 0.8 ml min^{-1} and compounds were detected at 280 nm. Growing cells of strain Mt1B8^T transformed daidzein completely within 24 h to form two metabolites, DM 1 and DM 2

(Supplementary Fig. S3a). Likewise, genistein was converted completely to GM 1 and GM 2 by growing cells of strain Mt1B8^T within 16 h (Supplementary Fig. S3b). The conversion of both daidzein and genistein has not been reported for *Asaccharobacter celatus*, *Adlercreutzia equolifaciens* or strain Julong732 (Maruo *et al.*, 2008; Minamida *et al.*, 2006; Wang *et al.*, 2005).

Description of *Enterorhabdus* gen. nov.

Enterorhabdus (En.te.ro.rhab′dus. Gr. n. *enteron* intestine; Gr. fem. n. *rhabdos* a rod; N.L. fem. n. *Enterorhabdus* a rod isolated from the intestine).

Gram-positive-staining rods with a high DNA G+C content (64.2 mol% for the type strain of the type species) that belong to the family *Coriobacteriaceae*. They are distantly related to the genera *Eggerthella* (<92 % similarity based on partial 16S rRNA gene sequence analysis), *Adlercreutzia* and *Asaccharobacter* (<96 %). They are mesophilic and grow as single cells under strictly anoxic conditions. Cells are catalase-, coagulase-, indole- and oxidase-negative. Spore formation and motility have not been observed. The major cellular fatty acids are $C_{16:0}$, $C_{17:1}$ iso I and $C_{18:1}\omega9c$. Galactose and ribose are detected as whole-cell sugars. The principal respiratory quinone is MMK-6. The diamino acid in the peptidoglycan is LL-diaminopimelic acid. The type species is *Enterorhabdus mucosicola*.

Description of *Enterorhabdus mucosicola* sp. nov.

Enterorhabdus mucosicola [mu.co.si′co.la. N.L. n. *mucosa* mucosa from L. adj. *mucosus -a -um* mucous; L. suff. *-cola* (from L. n. *incola*) inhabitant, dweller; N.L. n. *mucosicola* inhabitant of the intestinal mucosa].

The bacterium has the aforementioned features of the genus. Cells are approximately 0.5 × 2.0 μm. Grows at pH 5.5–9.5. Cultures in the stationary phase of growth are characterized by stable pH 6.9–7.1 and a typically low turbidity ($\leqslant$0.5 McFarland standard). After 48 h of growth at 37 °C on GY-BHI-c-agar under anoxic conditions, colonies are circular, entire, pinpoint, raised and translucent to slightly opaque. Grows well in the temperature range 30–40 °C. The species is aerotolerant and possesses aminopeptidases but no glycosidases. It is capable of converting the isoflavones daidzein and genistein. The type strain is resistant to colistin and ciprofloxacin. The DNA G+C content of the type strain is 64.2 mol %.

The type strain is Mt1B8^T (=DSM 19490^T =CCUG 54980^T), isolated from the ileal mucosa of a 12-week-old female TNF^{deltaARE} mouse.

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