Sutterella parvirubra sp. nov. and *Megamonas funiformis* sp. nov., isolated from human faeces

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Three strains of anaerobic, non-spore-forming, Gram-negative coccobacilli (YIT 11816^T, YIT 11817 and YIT 11818) were isolated from human faeces. On the basis of 16S rRNA gene sequence similarity, these strains were shown to belong to the family *Alcaligenaceae* and to be related to the type strain of *Sutterella stercoricanis* (94.9%) and to *Sutterella wadsworthensis* WAL 7877 (94.3%); the similarity to strains of any other species with a validly published name within the family *Alcaligenaceae* was less than 92%. Biochemical data supported the affiliation of these strains to the genus *Sutterella*. These strains therefore represent a novel species, for which the name *Sutterella parvirubra* sp. nov. is proposed; the type strain is YIT 11816^T (=DSM 19354^T =JCM 14724^T). The cells of another isolate, strain YIT 11815^T, were non-spore-forming, Gram-negative, very large rods, 1×5–200 µm in size, with or without a central, subterminal or terminal swelling of 2–4 µm diameter when grown in a broth medium supplemented with glucose. Based on comparative 16S rRNA gene sequencing, this bacterium is a member of the family *Acidaminococcaceae*, and most closely related to *Megamonas hypermegale* (95.3% similarity to the type strain). Interestingly, the 16S rRNA gene sequence of strain YIT 11815^T showed 99% similarity to sequences of uncultured colonic bacteria. A 16S rRNA gene sequence divergence value of >3% from known cultured species suggested that isolate YIT 11815^T represents a novel species, for which the name *Megamonas funiformis* sp. nov. is proposed; the type strain is YIT 11815^T (=DSM 19343^T =JCM 14723^T).

The human intestinal flora is a complex ecosystem containing hundreds of microbial species, of which a substantial proportion have not yet been cultured. For example, Eckburg *et al.* (2005) examined 13 355 prokaryotic rRNA gene sequences from colonic mucosa and faeces of healthy subjects. Of the 395 bacterial phylotypes they detected, 244 (62%) were novel, and 80% represented sequences from species that have not been cultivated (Eckburg *et al.*, 2005). This observation can be attributed to the lack of appropriate cultivation techniques.

While investigating succinate-utilizing human intestinal microbiota, we have isolated four strains with unique biological and morphological characteristics, YIT 11816^T, YIT 11817, YIT 11818 and YIT 11815^T, by dilution of faeces in anaerobic medium containing succinate as the sole carbon source. Although these strains were not succinate-utilizing bacteria, phylogenetic analysis based on comparative 16S

rRNA gene sequencing showed that the isolates represent previously uncultured sublines within the genera *Sutterella* (YIT 11816^T, YIT 11817 and YIT 11818) and *Megamonas* (YIT 11815^T).

Faecal samples were collected from three healthy Japanese males (27–35 years old) and transferred immediately to anaerobic conditions. Each sample was weighed and diluted with pre-reduced 0.1 M PBS (pH 7) in an anaerobic glove box (Coy Laboratory Products, Inc.) which contained 88% nitrogen, 7% hydrogen and 5% carbon dioxide. Each dilution was then spread on medium 10 (Caldwell & Bryant, 1966) supplemented with 40 mM succinic acid as the sole carbon source, from which the other basal carbon sources (glucose, cellobiose, soluble starch and volatile fatty acid mixture) had been excluded. Plates were incubated at 37 °C for 3 days in the same anaerobic cabinet. Single colonies were picked and streaked out until single cultures were obtained on modified Gifu anaerobic medium (GAM) agar (Nissui Pharmaceutical). The composition of the modified GAM agar was as follows (l⁻¹): peptone, 5.0 g; soy peptone, 3.0 g; proteose peptone, 5.0 g; serum powder, 10.0 g; yeast extract, 2.5 g; beef extract powder, 2.2 g; liver extract powder, 1.2 g; glucose, 0.5 g; soluble starch, 5.0 g; L-cysteine monohydrochloride,

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains YIT 11815^T and YIT 11816^T are AB300988 and AB300989, respectively.

Results of RAPD-PCR fingerprinting comparing the three novel isolates of the genus *Sutterella* are available as supplementary material with the online version of this paper.

0.3 g; sodium thioglycolate, 0.3 g; potassium dihydrogen phosphate, 2.5 g; sodium chloride, 3.0 g; L-tryptophan, 0.2 g; L-arginine, 1.0 g; vitamin K₁, 5 mg; haemin, 10 mg; agar, 15.0 g. The end products of bacterial metabolism of glucose in pre-reduced peptone-yeast extract-glucose (PYG) medium (Holdeman *et al.*, 1977) were analysed by HPLC according to a previously described procedure (Chonan *et al.*, 1995). Cellular morphology was recorded after Gram staining of 3 day plate or 1 day broth cultures. Haemolysis was tested on anaerobic 5% horse-blood GAM agar.

Biochemical characteristics were determined using the API Rapid ID 32A, API ZYM and API 20A systems (bioMérieux) according to the manufacturer's instructions. The DNA G+C content was determined by hydrolysing the DNA enzymically and quantifying the nucleosides by HPLC by the method of Ezaki *et al.* (1990). Chromosomal DNA to be used as template for RAPD-PCR and 16S rRNA gene amplification was extracted from colonies using the FastDNA SPIN kit and the FastPrep Instrument (Qbiogene). PCR-based RAPD fingerprinting was carried out by the method of Akopyanz *et al.* (1992), using three primers (5'-CCGCAGCCAA, 5'-AACGCGCAAC and 5'-GTGGATGCGA). The PCR products were subjected to 1.5% agarose gel electrophoresis and the gels were stained with ethidium bromide and photographed under UV light.

The 16S rRNA genes of the isolate were amplified by a PCR using universal primers 8F (5'-AGAGTTTGTATCMTGG-CTCAG-3'; Miyake *et al.*, 1998) and 15R (5'-AAGGA-GGTGATCCARCCGCA-3'; Yuki *et al.*, 2000) and direct sequencing of the amplified DNA fragments was performed. Details of these procedures have been described previously (Miyake *et al.*, 1998). Closely related sequences were

retrieved from DDBJ using the program FASTA (Pearson & Lipman, 1985). The sequences were aligned and used to produce an unrooted phylogenetic tree by the neighbour-joining method (Saitou & Nei, 1987) using CLUSTAL_X (version 1.83) (Thompson *et al.*, 1997). Stability of the groupings was estimated by bootstrap analysis (1000 replications) in CLUSTAL_X. The trees were visualized by using the program TreeView (version 1.6.6) (Page, 1996). The minimal evolution method (1000 bootstrap replicates) in MEGA4 (Tamura *et al.*, 2007) was used to confirm the phylogenetic placement of the aligned sequences.

Isolates YIT 11816^T, YIT 11817 and YIT 11818 were non-spore-forming, catalase- and oxidase-negative, non-motile, Gram-negative coccobacilli. Typical cells were 0.4–1 µm wide and 0.4–2 µm long. The organisms grew under anaerobic conditions, but not under microaerobic conditions created by use of the CampyPack microaerophilic system (Becton Dickinson). They were asaccharolytic in API test systems. In the API Rapid ID 32A and API ZYM test systems, the isolates were very unreactive; apart from weak activities for arginine arylamidase, ester lipase C8 and naphthol-AS-BI-phosphohydrolase (YIT 11816^T and YIT 11817), all other tests were negative (Table 1). As shown in Table 1, these isolates differed from the two existing species of *Sutterella* in not reducing nitrate. API results indicated weak, but distinct differences between the isolates and existing strains. Especially, an activity of ester lipase C8 was discernible.

Approximately 1400 bp of the 16S rRNA genes for these isolates were sequenced, revealing identical 16S rRNA gene sequences. These could be discriminated by RAPD fingerprinting when the primer 5'-CCGCAGCCAA was used (Supplementary Fig. S1 in IJSEM Online). Sequence database searches revealed highest 16S rRNA gene sequence

Table 1. Major characteristics of strains YIT 11816^T, YIT 11817 and YIT 11818 and some other related organisms

Taxa: 1, strain YIT 11816^T; 2, YIT 11817; 3, YIT 11818; 4, *S. wadsworthensis*; 5, *S. stercoricanis*. Data for *S. wadsworthensis* and *S. stercoricanis* are from Wexler *et al.* (1996) and Greetham *et al.* (2004), respectively. All these organisms are asaccharolytic. Strains YIT 11816^T, YIT 11817 and YIT 11818 gave negative results for all tests of the API Rapid ID 32A, API ZYM and API 20A systems except for those listed in this table. +, Positive; –, negative; w, weakly positive; NA, no data available.

Characteristic	1	2	3	4	5
Isolation source	Human faeces	Human faeces	Human faeces	Infections of gastro-intestinal tract	Faeces of dog
Morphology	Coccobacilli	Coccobacilli	Coccobacilli	Straight rods	Short, straight rods
Cell size (µm)	0.4–1 × 0.4–2	0.4–1 × 0.4–2	0.4–1 × 0.4–2	0.5–1 × 1–3	0.8 × 1.5–2
DNA G+C content (mol%)	64.4	64.5	64.1	NA	60
Nitrate reduction	–	–	–	+	+
API ZYM tests					
Ester lipase C8	w	w	w	–	–
Naphthol-AS-BI-phosphohydrolase	w	w	–	–	NA
Rapid ID 32A API tests					
Arginine arylamidase	w	w	w	NA	–
Glycine arylamidase	–	–	–	NA	w

Fig. 1. Phylogenetic tree showing the positions of *Sutterella parvirubra* sp. nov. YIT 11816^T and other strains belonging to the order *Burkholderiales* based on 16S rRNA gene sequence similarities. The tree was rooted with *Escherichia coli* ATCC 11775^T and constructed by using the neighbour-joining method, with bootstrap values expressed as percentages of 1000 replications. For each strain, the GenBank/EMBL/DDBJ accession number is shown in parentheses.

similarity to *Sutterella wadsworthensis* WAL 7877 (94.3 % sequence similarity) and *Sutterella stercoricanis* CCUG 47620^T (94.9 %). Phylogenetic analysis of these and other related sequences was performed and confirmed that strains YIT 11816^T, YIT 11817 and YIT 11818 were phylogenetically most closely associated with *S. wadsworthensis* and *S. stercoricanis* (Fig. 1); other taxa (e.g. *Alcaligenes*, *Achromobacter* and *Bordetella* species) were more distantly related. Currently, only two species of the genus *Sutterella* are known, namely *S. wadsworthensis* (Wexler *et al.*, 1996) and *S. stercoricanis* (Greetham *et al.*, 2004). The genus *Sutterella* belongs to the family *Alcaligenaceae* within the *Betaproteobacteria*. *S. stercoricanis* was isolated from canine faeces. To date, *S. wadsworthensis* has been isolated from patients with a variety of gastrointestinal infections (Wexler *et al.*, 1996). There is evidence that *S. wadsworthensis* occurs in human faeces as a common member of the human indigenous microflora, because many uncultured bacteria with highly similar 16S rRNA gene sequences have been reported to DDBJ, for example HuCC33 (Hold *et al.*, 2002; DDBJ accession no. AY684426) and LO17 (Eckburg *et al.*, 2005; DDBJ accession no. AY980798). It is evident from the results of the taxonomic study that the Gram-negative coccobacillus strain YIT 11816^T recovered from human faeces represents a hitherto unknown species.

Cells of strain YIT 11815^T were Gram-negative, non-spore-forming, non-motile, obligately anaerobic, large rods. Cells grown on agar media were relatively uniform, 1 µm wide and 5–10 µm long. In broth cultures containing glucose (e.g. PYG broth), cells are filamentous, 1 × 5–200 µm in size with or without a central, subterminal or terminal swelling of 2–4 µm diameter (Fig. 2). Cells were heat-sensitive (60 °C, 15 min) and no spore-like structures were observed by transmission electron microscopy (Fig. 2e). The organism grew under anaerobic conditions but not under microaerobic conditions created by use of the CampyPack microaerophilic system. On horse-blood agar,

the isolate exhibited weak β-haemolysis. Glucose was fermented with the production of acid and gas. Analysis of metabolic end products by HPLC from PYG broth revealed propionic and acetic acids and small amounts of lactic and succinic acids. Results based on the API Rapid ID 32A, 20A and API ZYM systems are summarized in Table 2. Many of the characteristics were similar to those of *Megamonas hypermegale*.

An almost-complete 16S rRNA gene sequence of strain YIT 11815^T was determined (1341 bp). The most similar 16S rRNA gene sequences, which were derived from studies of uncultured colonic bacteria [e.g. RL304_aa176e09 (Ley *et al.*, 2006; DDBJ accession no. DQ824482) and SJTU_A2_05_79 (M. Li and others; unpublished DDBJ accession no. EF403718)] were 99 % identical to that of strain YIT 11815^T. The cultured bacterium most closely related to strain YIT 11815^T was the type strain of *M. hypermegale*, and this was only distantly related (95.3 % similarity). A tree showing the phylogenetic relationships of the unknown bacterium is depicted in Fig. 3 and shows that the strain YIT 11815^T is related to the genus *Megamonas*. Recently we proposed that genus *Megamonas* should be placed in the lineage of *Firmicutes*; *Clostridia*; *Clostridiales*; *Acidaminococcaceae*; *Megamonas* (Morotomi *et al.*, 2007). Thus, as shown in Fig. 3, strain YIT 11815^T belongs to this lineage.

In this report, we described the isolation of representatives of two novel species from human faeces. Their respective phenotypic properties correspond to earlier studies of the genera *Sutterella* and *Megamonas*. Based on their phylogenetic distinctiveness, it is considered that strains YIT 11816^T, YIT 11817 and YIT 11818 represent a novel species in the genus *Sutterella*, for which we propose the name *Sutterella parvirubra* sp. nov. Likewise, on the basis of the findings presented, we propose that strain YIT 11815^T represents a novel species of the genus *Megamonas*, *Megamonas funiformis* sp. nov.

Fig. 2. Morphology of strain YIT 11815^T. (a, b) Light micrographs of cells grown on trypticase soy agar (a) and in PYG broth (b). (c–e) Scanning electron micrographs (c, d) and transmission electron micrograph (e) of cells grown in PYG broth. Bars, 2.5 μm (a–d) and 1 μm (e).

Description of *Sutterella parvirubra* sp. nov.

Sutterella parvirubra (par.vi.rub'ra. L. adj. *parvus* small; L. adj. *ruber* -bra -brum red; N.L. fem. adj. *parvirubra* small and red, referring to cell size and cell mass colour).

Cells are non-motile, non-spore-forming and coccoid to coccobacillary, approximately $0.4\text{--}1 \times 0.4\text{--}2\text{ }\mu\text{m}$, which stain Gram-negative. Colonies on GAM agar after 2 days anaerobic incubation are 0.2–1.1 mm in diameter, circular, flat and translucent. Oxidase and catalase are negative. Aesculin and gelatin are not hydrolysed and nitrate is not reduced. Indole is not produced. Asaccharolytic. Weak activity for arginine arylamidase, ester lipase C8 and naphthol-AS-BI-phosphohydrolase may be detected. Using commercially available API test systems, no activity is detected for acid phosphatase, alkaline phosphatase, alanine arylamidase, α -arabinosidase, arginine arylamidase, arginine dihydrolase, esterase C4, α -chymotrypsin, cystine arylamidase, α -fucosidase, α -glucosidase, β -glucosidase, β -glucuronidase, α -galactosidase, β -galactosidase, β -galactosidase-6-phosphate, glutamic acid decarboxylase, glutamyl glutamic acid arylamidase, glycine arylamidase, histidine arylamidase, leucine arylamidase, leucyl glycine arylamidase, lipase C4, α -mannosidase, *N*-acetyl- β -glucosaminidase, proline arylamidase, phenylalanine arylamidase, phosphoamidase, pyroglutamic acid arylamidase, serine arylamidase, trypsin, valine arylamidase, urease or tyrosine arylamidase. The DNA G + C content of the type strain is 64.4 mol%.

The type strain is YIT 11816^T (=DSM 19354^T =JCM 14724^T), isolated from human faeces. Strains YIT 11817 and YIT 11818 are also assigned to this species.

Description of *Megamonas funiformis* sp. nov.

Megamonas funiformis (fu.ni.for'mis. L. masc. n. *funis* string; L. fem. adj. suffix -*formis* shaped, N.L. fem. adj. *funiformis* string-shaped, referring to the cell shape).

Cells are anaerobic, non-motile and non-spore-forming. In young plate cultures, cells consist of fairly homogeneous-sized, straight rods, approximately $1 \times 5\text{--}10\text{ }\mu\text{m}$, which stain Gram-negative. In broth cultures containing glucose (e.g. PYG broth), cells are filamentous, $1 \times 5\text{--}200\text{ }\mu\text{m}$ in size, with or without a central, subterminal or terminal swelling of 2–4 μm diameter. Colonies on GAM agar are approximately 0.5–1 cm in diameter, rough, convex, rhizoid with an erose margin. Colonies are weakly β -haemolytic on horse-blood agar. In API systems, oxidase and catalase are negative. Aesculin and gelatin are not hydrolysed and nitrate is not reduced. Indole is not produced. L-Arabinose, glucose, lactose, maltose, D-mannitol, D-mannose, raffinose, salicin, D-sorbitol, sucrose, trehalose and D-xylose are utilized. Cellobiose, glycerol, melezitose and L-rhamnose are not utilized. Positive reactions are obtained for α -galactosidase, β -galactosidase and α -glucosidase. Weakly positive reactions are obtained for *N*-acetyl- β -glucosaminidase, acid phosphatase, esterase (C4) and naphthol-AS-BI-phosphohydrolase. Activity is not detected for alanine arylamidase, alkaline phosphatase, α -arabinosidase, arginine arylamidase, arginine dihydrolase, chymotrypsin, cystine arylamidase, esterase lipase (C8), α -fucosidase, β -galactosidase-6-phosphate, β -glucosidase, β -glucuronidase, glutamic acid decarboxylase, glutamyl glutamic acid arylamidase, glycine arylamidase, histidine arylamidase, leucine arylamidase, leucyl glycine

Table 2. Major characteristics that differentiate strain YIT 11815^T from its phylogenetic relative *Megamonas hypermegale*

As indicated in the text, acid production from sugars, hydrolysis of aesculin and α -mannosidase activity of strain YIT 11815^T were determined by using the API system. Data for *M. hypermegale* are from Shah & Collins (1982), except for the G+C content, which was taken from Barnes & Impey (1968). Results of acid production from melibiose and cellobiose were determined by the API system. Other characteristics were examined by conventional biochemical methods. Both organisms are positive for acid production from glucose, maltose, lactose, mannose, arabinose, xylose and raffinose. +, Positive; –, negative; w, weakly positive.

Characteristic	Strain YIT 11815 ^T	<i>M. hypermegale</i>
Isolation source	Human faeces	Intestines of turkeys
Morphology	Very large rods	Very large, round ends
Cell size (μm)	1 $\times$ 5–200	0.8–3 $\times$ 3–20
DNA G+C content (mol%)	32.9	34.8
Acid production from:		
Melibiose	–	+
Amygdalin	–	w
Cellobiose	–	+
Inositol	–	w
Glycerol	–	w
Melezitose	–	w
Rhamnose	–	w
Salicin	+	w
Hydrolysis of aesculin	–	+
α -Mannosidase	–	+
Haemolysis	Weak β (horse blood)	–

Fig. 3. Neighbour-joining phylogenetic tree showing the position of *Megamonas funiformis* sp. nov. YIT 11815^T among selected clones or strains belonging to the family Acidaminococcaceae. For further details see the legend to Fig. 1.

arylamidase, lipase (C4), α -mannosidase, phenylalanine arylamidase, proline arylamidase, pyroglutamic acid arylamidase, serine arylamidase, trypsin, tyrosine arylamidase, urease or valine arylamidase. End products of metabolism from PYG broth are propionic and acetic acids and small amounts of lactic and succinic acids. The DNA G+C content of the type strain is 32.9 mol%.

The type strain, YIT 11815^T (=DSM 19343^T =JCM 14723^T), was isolated from human faeces.

Acknowledgements

We are grateful to Professor Dr Hans G. Trüper, University of Bonn, Germany, for his suggestions regarding the etymology of the species epithets. We thank K. Watanabe, T. Matsuki, J. Fujimoto and H. Makino for advice and help in the phylogenetic analysis. We also thank M. Ando for helping with the electron micrographs.

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