Blautia stercoris sp. nov., isolated from human faeces

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Strain GAM6-1^T is a novel, strictly anaerobic, non-spore-forming, Gram-stain-positive bacterium that was isolated from the faeces of a healthy individual. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain GAM6-1^T was most closely related to *Blautia producta* ATCC 27340^T (95.7 % sequence similarity), in the family *Lachnospiraceae*. Strain GAM6-1^T did not exhibit catalase or oxidase activity. The strain's cellular fatty acids were of the straight-chain saturated and mono-unsaturated types, with $C_{14:0}$ (24.10 %), $C_{16:0}$ (19.09 %) and $C_{16:0}$ dimethylacetal (14.35 %) predominant. Strain GAM6-1^T was able to produce acid from various carbohydrates. Glucose fermentation produced acetic acid as the major short-chain fatty acid. The genomic DNA G+C content of strain GAM6-1^T was 35.6 mol%. Based on phenotypic, genotypic and phylogenetic evidence, strain GAM6-1^T (=KCTC 5981^T =JCM 17204^T) is considered to represent a novel species, for which the name *Blautia stercoris* sp. nov. is proposed.

The human gastrointestinal tract represents a complex ecosystem that may be inhabited by any of over a thousand different species of commensal microbes (Tap *et al.*, 2009). Phylogenetic analysis of 16S rRNA gene sequences has been used to examine the diversity of microbiota on the colonic mucosa and in the faeces of humans (Eckburg *et al.*, 2005; Rajilić-Stojanović *et al.*, 2007). Such investigation has revealed that, although the predominant bacterial groups in the gastrointestinal tracts of healthy volunteers are the *Bacteroidetes*, *Firmicutes* and *Actinobacteria* (Andersson *et al.*, 2008; Eckburg *et al.*, 2005; Gill *et al.*, 2006; Kurokawa *et al.*, 2007; Lay *et al.*, 2005; Qin *et al.*, 2010; Tap *et al.*, 2009; Wang *et al.*, 2005; Zoetendal *et al.*, 2002), the microbiota of the human gastrointestinal tract is highly diverse and variable across individuals (Gosalbes *et al.*, 2011).

Recent taxonomic investigations, using a polyphasic approach, have led to one species of *Clostridium*, five species of *Ruminococcus* and several previously misclassified species of ruminococci within the *Firmicutes* being transferred to a novel genus: *Blautia* (Liu *et al.*, 2008). At the time of writing, the genus *Blautia* has eight members, *Blautia coccoides*, *B. glucerasea*, *B. hansenii*, *B. hydrogenophica*, *B. luti*, *B. producta*, *B. schinkii* and *B. wexlerae*, which are Gram-reaction-positive, non-motile bacteria that may be either coccoid or oval and all are obligate anaerobes that produce acetic acid as the major end product of glucose fermentation (Liu *et al.*, 2008). Most species have been isolated from the faeces of humans and other mammals (Liu *et al.*, 2008). Although *Ruminococcus obeum* appears to be more closely related to *Blautia* species than to other species in the genus *Ruminococcus* (Liu *et al.*, 2008; Suau *et al.*, 1999), its reclassification as a *Blautia* species has not been validly published because the criteria in the Bacteriological Code that govern the valid publication of species names and the depositing of type material (i.e. Rules 27 and 30) have not been met (Liu *et al.*, 2008).

As part of an investigation into the microbial diversity of the human intestine, we isolated a novel *Blautia*-like strain from the faeces of an apparently healthy, 28-year-old man in the Republic of Korea. The taxonomy of this isolate, strain GAM6-1^T, was then investigated using a polyphasic approach.

Immediately following its collection, the faecal sample from which strain GAM6-1^T was later isolated was placed into an anaerobic chamber (Bactron II, Sheldon Manufacturing) containing, by volume, 90 % N_2, 5 % H_2 and 5 % CO_2. The sample was serially diluted with 0.1 M PBS (pH 7) before each dilution was cultivated on Gifu anaerobic medium (GAM; Nissui Pharmaceutical; pH 6.2) containing 1.5 % (w/v) agar. Cultures were each grown for 2 or 3 days at 37 °C and several rounds of streaking were performed to obtain a pure culture. The resultant isolate, strain GAM6-1^T, was preserved in 10 % skimmed milk (BBL) and stored at −80 °C.

The 16S rRNA gene of strain GAM6-1^T was amplified by colony PCR, using a PCR pre-mix (SolGent) and the previously described primers 8F, 518R, 968F and 1492R

The GenBank accession number for the 16S rRNA gene sequence of strain GAM6-1^T is HM626177.

A supplementary table is available with the online version of this paper.

(Baker *et al.*, 2003). The DNA in the reaction mixture was sequenced in an automated system (PRISM 3730XL, Applied Biosystems). After the fragments of the 16S rRNA gene sequence were assembled using the SeqMan software package (DNASTAR), the EzTaxon server (Chun *et al.*, 2007) was used to compare the 16S rRNA gene sequence of the isolate with the corresponding sequences of other bacteria. The results indicated clear affinity between strain GAM6-1^T and not only established species in the genus *Blautia*, particularly *B. producta* ATCC 27340^T (95.7 % sequence similarity), *B. coccoides* ATCC 29236^T (95.5 %), *B. hansenii* DSM 20583^T (95.4 %) and *B. luti* BInIX^T (95.1 %), but also *R. obeum* ATCC 29174^T (94.1 %). The multiple sequence alignment program CLUSTAL_X (Thompson *et al.*, 1997) was subsequently used to align the 16S rRNA gene sequences of strain GAM6-1^T and related species within the family *Lachnospiraceae*. A consensus phylogenetic tree (Fig. 1) was then generated with the MEGA4 package (Tamura *et al.*, 2007), using 1000 randomly chosen bootstrap replications and the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Kluge & Farris, 1969) algorithms.

In the other investigations, four type strains from the DSMZ (*B. coccoides* DSM 935^T, *B. producta* DSM 2950^T, *B. hansenii* DSM 20586^T and *B. schinkii* DSM 10518^T) and one from the American Type Culture Collection (*R. obeum* ATCC 29174^T) were used for comparative purposes. Prior to their investigation, these bacterial strains and strain GAM6-1^T were maintained in GAM medium (pH 6.2) for at least 2 days at 37 °C.

A light microscope (ECLIPSE 50*i*, Nikon) was used to observe cell morphology. Gram staining was performed using a Gram-staining kit (bioMérieux), according to the manufacturer's instructions. Oxidase and catalase activities were assessed with 1 % (v/v) *p*-tetramethyl phenylenediamine (bioMérieux) and 3 % (v/v) hydrogen peroxide, respectively. Malachite green dye was used to determine the presence of spore formation, and motility was examined with a stab into GAM medium containing 0.4 % (w/w) agar. API ZYM and Rapid ID 32A (both bioMérieux) were employed for the determination of biochemical characteristics and enzyme activities, and API 50CHB/E (bioMérieux) was used to identify acid production from various carbohydrates. Table 1 shows a comparison of the biochemical characteristics of strain GAM6-1^T with those of the other strains investigated.

The cellular fatty acid contents of strain GAM6-1^T, *B. coccoides* DSM 935^T, *B. producta* DSM 2950^T and *B. hansenii* DSM 20586^T were compared (see Table S1, available in

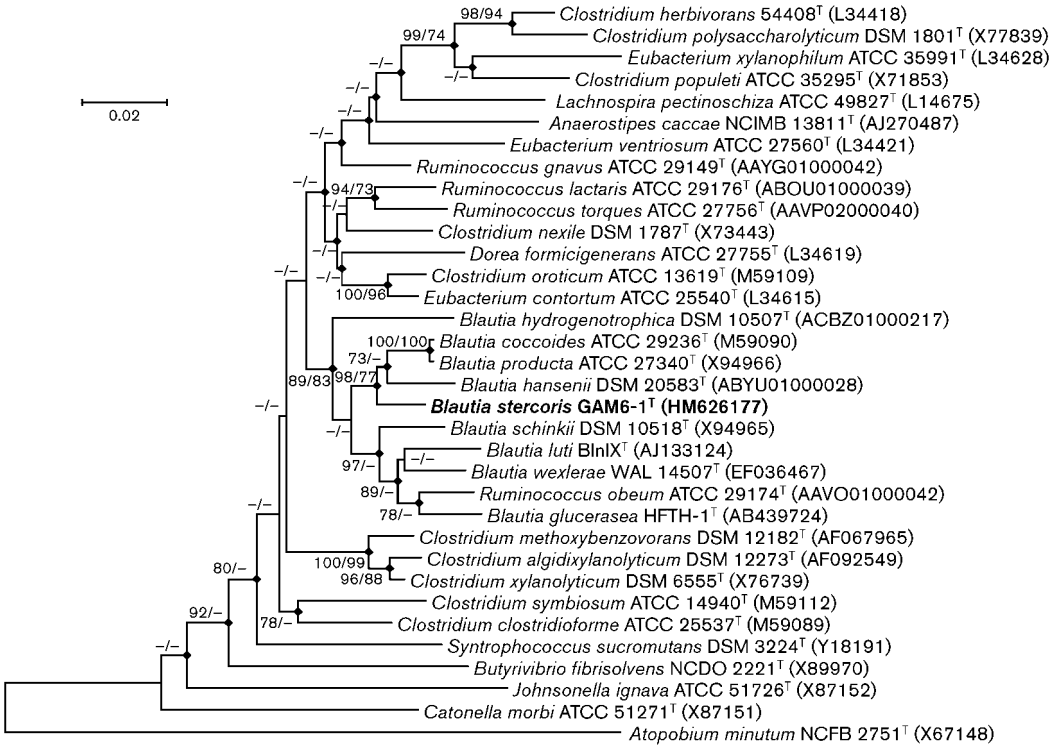

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences from strain GAM6-1^T and close relatives within the family *Lachnospiraceae*. Filled diamonds indicate branches that were recovered using both neighbour-joining and maximum-parsimony algorithms. Numbers at nodes indicate bootstrap values, expressed as percentages of 1000 resampled datasets, calculated by neighbour-joining/maximum-parsimony probabilities; only values greater than 70 % are shown. The sequence of *Atopobium minutum* NCFB 2751^T was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.

Table 1. Characteristics that distinguish strain GAM6-1^T from the type strains of closely related species

Strain: 1, GAM6-1^T; 2, *B. coccoides* DSM 935^T; 3, *B. producta* DSM 2950^T; 4, *B. hansenii* DSM 20583^T; 5, *R. obeum* ATCC 29174^T; 6, *B. schinkii* DSM 10518^T. +, Positive; −, negative; W, weakly positive. All data were obtained in this study.

Characteristic	1	2	3	4	5	6
End products of glucose fermentation*	A	A, S	L, A	L, A	A	A
Enzyme activities (API ZYM)						
Alkaline phosphatase	−	−	−	−	+	−
Esterase (C4)	−	+	−	−	−	−
Acid phosphatase	−	−	+	−	+	+
Naphthol-AS-BI-phosphohydrolase	−	+	+	−	+	+
α-Galactosidase	+	+	+	−	+	+
β-Galactosidase	+	+	+	+	−	−
α-Glucosidase	+	+	+	−	+	+
β-Glucosidase	+	+	+	−	−	+
N-Acetyl-β-glucosaminidase	−	−	−	+	−	−
API Rapid ID32A test results						
Arginine dihydrolase	+	−	−	−	−	+
α-Galactosidase	+	+	+	+	+	W
β-Galactosidase	+	+	+	+	+	W
β-Glucosidase	+	+	W	−	W	+
α-Arabinosidase	+	+	+	−	−	+
N-Acetyl-β-glucosaminidase	−	−	−	+	−	+
Glutamic acid decarboxylase	−	−	+	+	−	−
α-Fucosidase	−	−	−	−	−	W
Mannose fermentation	−	+	+	−	−	−
Raffinose fermentation	−	+	+	−	−	−
Fermentation of (API 50CHB/E):						
Glycerol	+	−	−	−	−	−
Erythritol	+	−	−	−	−	−
D-Arabinose	+	−	−	−	+	+
L-Arabinose	+	−	−	−	−	−
Methyl β-D-xyloside	−	−	−	−	+	−
Methyl α-D-glucoside	−	−	−	−	+	−
N-Acetyl-D-glucosamine	−	−	−	−	+	−
Salicin	−	−	−	−	+	−
Glycogen	−	−	+	−	−	−
Turanose	+	−	−	−	−	−
D-Lyxose	−	−	−	−	+	−
L-Fucose	+	−	−	−	−	−
L-Arabitol	−	−	+	−	−	−

*A, Acetic acid; L, lactic acid; S, succinic acid.

IJSEM Online). Cells were grown for 3 days on peptone-yeast extract-glucose (PYG) agar medium (Liu *et al.*, 2008). The cellular fatty acids were then extracted, by following the standard protocol of the Sherlock Microbial Identification Systems (MIDI, 1999), and identified using the Sherlock version 4.0 and BHIBLA 3.80 libraries. Gas chromatography indicated that $C_{14:0}$ and $C_{16:0}$ were the predominant fatty acids in strain GAM6-1^T and that the fatty acid profile of the novel strain was therefore similar to those of recognized *Blautia* species (Furuya *et al.*, 2010; Liu *et al.*, 2008).

To investigate the end products of glucose fermentation, cells were cultivated at 37 °C for 5 days in PYG (Liu *et al.*, 2008), as described by Guerrant *et al.* (1982). A Thermo Surveyor HPLC (TSP-0299) with a Thermo PDA detector (Thermo Scientific) and an Alltech Prevail organic acid column (Grace Davison Discovery Sciences) were used to determine the end products of glucose fermentation. With strain GAM6-1^T, as with recognized *Blautia* species (Liu *et al.*, 2008), the major end product of glucose metabolism was acetic acid.

Genomic DNA was extracted from strain GAM6-1^T using an UltraClean microbial DNA isolation kit (Mo Bio Laboratories Inc.). The G+C content was then evaluated fluorometrically, in triplicate, using SYBR Green I and real-time PCR (Gonzalez & Saiz-Jimenez, 2002). Genomic DNA from three strains that have had their complete genomes sequenced, *Escherichia coli* K-12 (Gonzalez & Saiz-Jimenez, 2002), *Bifidobacterium adolescentis* ATCC 15703^T and *R. obeum* ATCC 29174^T, was used for calibration. Strain GAM6-1^T exhibited a genomic DNA G+C content of 35.6 mol%, which is somewhat lower than the corresponding values reported for recognized members of the genus *Blautia* (Furuya *et al.*, 2010; Liu *et al.*, 2008).

Based on phenotypic, chemical, genotypic and phylogenetic evidence, strain GAM6-1^T is considered to represent a novel species in the genus *Blautia*, for which the name *Blautia stercoris* sp. nov. is proposed.

Description of *Blautia stercoris* sp. nov.

Blautia stercoris (ster.co′ris. L. gen. n. *stercoris* of dung, excrement, ordure).

Cells are strictly anaerobic, coccoid, non-motile and Gram-staining-positive, reaching 1.5–2.5 × 0.5–0.8 μm. The cells do not produce endospores. On GAM agar plates, cells form circular, umbonate, pale grey colonies that have opaque, entire edges. Strict anaerobe at 37 °C. Does not exhibit catalase or oxidase activity. Positive (in the Rapid ID 32A system) for arginine dihydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase and α-arabinosidase, but negative for urease, β-galactosidase-6-phosphate, β-glucuronidase, *N*-acetyl-β-glucosaminidase, mannose fermentation, raffinose fermentation, glutamic acid decarboxylase, α-fucosidase, reduction of nitrates, indole production, alkaline phosphate, arginine arylamidase, proline arylamidase, leucyl glycine arylamidase, phenylalanine arylamidase, leucine arylamidase, pyroglutamic acid arylamidase, tyrosine arylamidase, alanine arylamidase, glycine arylamidase, histidine arylamidase, glutamyl glutamic acid arylamidase and serine arylamidase. Positive (by the API ZYM system) for α-galactosidase, β-galactosidase, α-glucosidase and β-glucosidase activity, but negative for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase,

trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activity. The API 50CHB/E system shows fermentation of glycerol, erythritol, D-arabinose, L-arabinose, ribose, glucose, aesculin, turanose, D-tagatose, L-fucose and 5-ketogluconate, but not of D-xylose, L-xylose, adonitol, methyl β-D-xyloside, galactose, fructose, mannose, sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl α-D-mannoside, methyl α-D-glucoside, *N*-acetyl-D-glucosamine, amygdalin, arbutin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, D-lyxose, D-fucose, D-arabitol, L-arabitol, gluconate and 2-ketogluconate. Cellular fatty acids are of the straight-chain saturated and mono-unsaturated types, with $C_{14:0}$, $C_{16:0}$, $C_{16:0}$ dimethylacetal predominant. In PYG broth, the major end product of glucose fermentation is acetic acid.

The type strain GAM6-1^T (=KCTC 5981^T =JCM 17204^T) was isolated from human faeces. Its genomic DNA G+C content is 35.6 mol%.

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