

Reclassification of *Clostridium coccooides*, *Ruminococcus hansenii*, *Ruminococcus hydrogenotrophicus*, *Ruminococcus luti*, *Ruminococcus productus* and *Ruminococcus schinkii* as *Blautia coccooides* gen. nov., comb. nov., *Blautia hansenii* comb. nov., *Blautia hydrogenotrophica* comb. nov., *Blautia luti* comb. nov., *Blautia producta* comb. nov., *Blautia schinkii* comb. nov. and description of *Blautia wexlerae* sp. nov., isolated from human faeces

Chengxu Liu,¹ Sydney M. Finegold,^{2,3,4} Yuli Song¹ and Paul A. Lawson⁵

Correspondence

Chengxu Liu
chengxu66@yahoo.com

¹Research Service, VA Medical Center West Los Angeles, CA, USA

²Infectious Diseases Section VA Medical Center West Los Angeles, CA, USA

³Department of Medicine, UCLA School of Medicine, CA, USA

⁴Department of Microbiology, Immunology, and Molecular Genetics, UCLA School of Medicine, CA, USA

⁵Department of Botany and Microbiology, University of Oklahoma, Norman, OK, USA

Phenotypic and phylogenetic studies were performed on 15 isolates of an unidentified Gram-positive, anaerobic, non-sporulating coccobacillus-shaped bacterium isolated from human faeces. The novel organisms were catalase-negative, indole-negative and produced acetate and succinate as end products of metabolism. Comparative 16S rRNA gene sequencing demonstrated that the 15 isolates were highly related to each other and formed a hitherto unknown subline within the clostridial rRNA cluster XIVa. The novel isolates formed a robust phylogenetic group with a number of organisms which included *Clostridium coccooides*, *Ruminococcus luti*, *Ruminococcus obeum* and a number of other misclassified ruminococci. On the basis of these studies, a novel genus, *Blautia* gen. nov., is proposed. It is suggested that *Clostridium coccooides*, *Ruminococcus hansenii*, *Ruminococcus hydrogenotrophicus*, *Ruminococcus luti*, *Ruminococcus productus*, and *Ruminococcus schinkii* are transferred to this genus as *Blautia coccooides* gen. nov., comb. nov., *Blautia hansenii* comb. nov., *Blautia hydrogenotrophica* comb. nov., *Blautia luti* comb. nov., *Blautia producta* comb. nov. and *Blautia schinkii* comb. nov. One of the new isolates, the hitherto unknown coccus-shaped bacterial strain WAL 14507^T (=ATCC BAA-1564^T=DSM 19850^T) is proposed as representing the type strain of a novel species, *Blautia wexlerae* sp. nov.

Abbreviations: PRAS, pre-reduced, anaerobically sterilized; WAL, Wadsworth Anaerobic Laboratory.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain WAL 14507^T is EF036467.

A phylogenetic tree constructed by the maximum-likelihood method using 16S rRNA gene sequences of strain WAL 14507^T and related organisms is available with the online version of this paper.

It is becoming increasingly clear that humans have a symbiotic relationship with their intestinal microbial community. This community enjoys a stable nutrient-rich environment with limited host immune responses, and in turn, it facilitates the normal functions of the body. Although this community has been studied using culture techniques, culture-independent investigations based on sequence analysis of rRNA genes suggest that 40 to 80 % of

the total 16S rRNA gene sequences retrieved represent as yet uncultivated species (Suau *et al.*, 1999). It is recognized that 16S rRNA gene sequencing used in conjunction with culturing techniques represents a powerful approach for discerning novel species diversity within the human gut and faeces (Barcenilla *et al.*, 2000; Suau *et al.*, 1999). In the present study, a combination of culture-based and molecular-based methodologies was used to facilitate the identification of 15 isolates of a hitherto unknown species within the *Clostridium coccooides* rRNA XIVa cluster. On the basis of an investigation using a polyphasic taxonomic, it is proposed that these strains be classified, alongside a number of misclassified ruminococci, in a novel genus.

Fifteen isolates were recovered from stool specimens from control children and children with late onset autism at the Rush Children's Hospital, Chicago, USA (Finegold *et al.*, 2002). The strains were characterized biochemically by using a combination of conventional tests as described previously in the Wadsworth and VPI anaerobic bacteriology manuals (Holdeman *et al.*, 1977; Jousimies-Somer *et al.*, 2002) and the API ZYM and Rapid ID32A systems (bioMérieux) according to the manufacturer's instructions. All biochemical tests were performed in duplicate. Carbohydrate fermentation tests were conducted using pre-reduced, anaerobically sterilized (PRAS) peptone-yeast-carbohydrate broth tubes (Anaerobe Systems). The strains were grown in peptone-yeast (PY) broth and peptone-yeast-glucose (PYG) broth (Anaerobe Systems) for metabolic end product (short-chain volatile and non-volatile fatty acids) analysis by GLC (Jousimies-Somer *et al.*, 2002). Long-chain cellular fatty acids were analysed as previously described (Wexler *et al.*, 1996). The 16S rRNA genes were amplified by PCR using universal primers 8UA (positions 8–28, *Escherichia coli* numbering) and 1485B (positions 1485–1507) as described previously (Brosius *et al.*, 1978). The amplified product was purified by using QIAamp PCR purification kit (Qiagen, Inc.) and directly sequenced with an ABI 3100 Avant genetic system (Applied Biosystems). The closest known relatives of the new isolates were determined by performing database searches using the FASTA program (Pearson & Lipman, 1988). These sequences and those of other known related strains were retrieved from GenBank and aligned with the newly determined sequence using the ARB program (Ludwig *et al.*, 2004). Phylogenetic analysis was performed on 1297 shared nucleotides using the maximum-likelihood and maximum-parsimony algorithms and the stability of the groupings was estimated by bootstrap analysis (1000 replications) using the same programs.

The isolates originating from the faecal material were strictly anaerobic, Gram-positive, non-spore forming, 1.0–1.5 × 1.0–3.0 µm in size and coccobacillus-shaped. After 48 h of anaerobic incubation at 37 °C under a gas phase of N₂ (86%), H₂ (7%) and CO₂ (7%), colonies on *Brucella* blood agar plates were 1–2 mm in diameter, grey with a white centre, entire edges, umbonate and opaque. The isolates grew well anaerobically, but no growth occurred

following subculture in an atmosphere of 2% or 6% O₂. All of the isolates were negative for lecithinase, lipase, catalase and indole. They were capable of hydrolysing aesculin, but not gelatin. All of the isolates utilized arabinose, glucose, mannose, ribose, xylose, but not glycogen as substrates when grown in PRAS carbohydrate broths. The utilization of amygdalin, cellobiose, fructose, lactose, maltose, mannitol, melezitose, melibiose, raffinose, rhamnose, salicin, sorbitol, sucrose and trehalose was variable. Analysis of end products by GLC from peptone-yeast-glucose broth revealed acetate and succinate as the major end products of metabolism. The long-chain cellular fatty acids of the isolates were of the straight-chain saturated and monounsaturated types, with C_{16:0} (major component), C_{14:0}, and C_{16:0} dimethyl acetal fatty acids predominating. Using the API ZYM and Rapid ID32A systems, all 15 isolates produced a similar profile. Positive reactions were obtained for α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase and α-fucosidase by both systems. With the Rapid ID32A system, α-arabinosidase activity showed as positive and acid phosphatase was detected by the API ZYM system. No activity was detected for N-acetyl-β-glucosaminidase, alkaline phosphatase, α-chymotrypsin, cystine arylamidase, β-glucuronidase, lipase (C14), leucine arylamidase, α-mannosidase, trypsin or valine arylamidase. Urease results were variable when tested by the Rapid ID32A system and esterase (C4), esterase lipase (C8), and naphthol-AS-BI-phosphohydrolase were variable as tested by the API ZYM system. The isolates were sensitive to vancomycin (5 µg) and kanamycin (1000 µg), but resistant to colistin (10 µg) identification discs.

To determine the phylogenetic affinities of the novel isolates, their 16S rRNA genes were amplified by PCR and sequenced. The 15 isolates were highly related to each other with 99.5–100% 16S rRNA gene sequence similarity. Sequence searches of GenBank and Ribosomal Database Project libraries revealed that the isolates were members of the phylum *Firmicutes*. It was evident from treeing analysis that the novel isolates represented a previously unknown lineage within the clostridial rRNA cluster XIVa subgroup and, in particular, shared a close relationship with *Ruminococcus luti* and *Ruminococcus obeum*. Pairwise comparison revealed approximately 5.0% sequence divergence between the novel isolates and the type strains of the closest recognized species, *R. luti* and *R. obeum*, based on the almost full-length 16S rRNA gene sequences (=1400 nt). A phylogenetic tree, constructed by the maximum-parsimony method, depicting the phylogenetic affinity of the novel bacterium as exemplified by strain WAL 14507^T is shown in Fig. 1. Strain WAL 14507^T formed a distinct lineage within a small subcluster of species which included several recognized ruminococcal species (*Ruminococcus hanseni*, *Ruminococcus hydrogenotrophicus*, *R. luti*, *R. obeum*, *Ruminococcus productus*, *Ruminococcus schinkii*) and *Clostridium coccooides*. The major branching orders were confirmed by using the

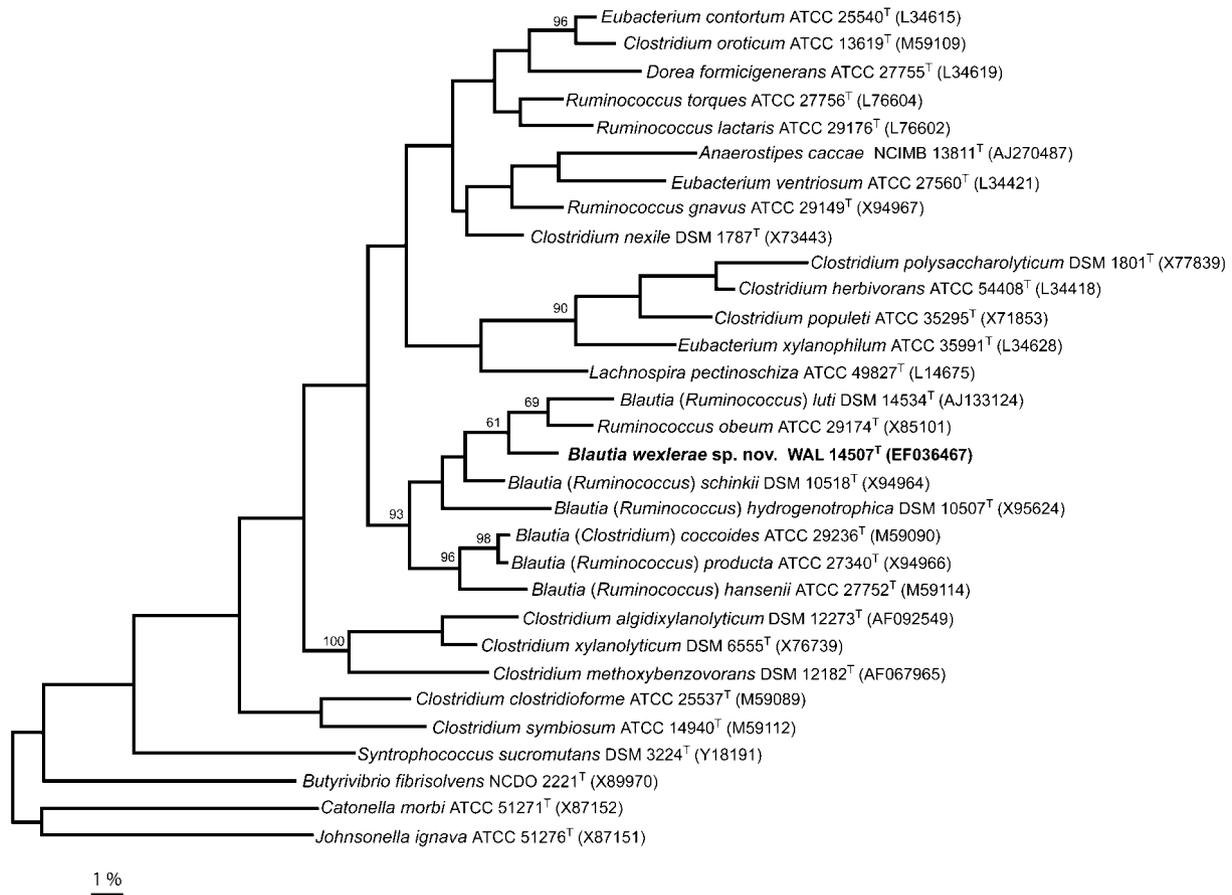


Fig. 1. Unrooted phylogenetic tree showing the inter-relationships of *Blautia wexlerae* sp. nov. with some of its nearest relatives within the rRNA cluster XIVa. Phylogenetic analyses were performed on 1297 shared nucleotides using the parsimony method within the ARB software packages. Major branching orders and bootstrap values expressed as a percentage of 1000 replications are given at the branching points and were confirmed by the same software package. Bar, 1% sequence variation.

maximum-likelihood method (see Supplementary Fig. S1 in IJSEM Online). Although there is no precise correlation between the 16S rRNA gene sequence divergence value and species delineation, it is generally recognized that similarity values of 97% or less are significant (Stackebrandt & Goebel, 1994). However, Stackebrandt & Ebers (2006) have recently made the recommendation that this value should be increased to 98.7–99% without sacrificing the quality and precision of a ‘species’ description and as an aid to taxonomists. This followed observations that with a threshold value of 98.5% gene sequence similarity, the corresponding DNA–DNA reassociation values were always lower than 70%, the accepted value for a species. It is apparent that the 16S rRNA gene, although a powerful tool in phylogenetic reconstructions, has limitations in discriminating recent speciation events. This is reflected in an increasing number of species now being described within the literature with very high rRNA gene sequence similarities to their nearest relatives. For example, *Clostridium carboxidivorans* displayed 99.7% and 99.8% to *Clostridium scatologenes* and *Clostridium drakei*, respectively

(Liou *et al.*, 2005), with DNA–DNA reassociation values demonstrating that all three strains represented distinct species. When such high 16S rRNA gene sequence similarity values are used for describing novel species, they must always be accompanied by appropriate phenotypic observations that can be used to distinguish the novel taxa. Support for the distinctiveness of the novel bacterium isolated from human faeces from the related bacterial species is also provided from the phenotypic characterization. Although 16S rRNA gene sequence analysis showed the closest phylogenetic relatives to the novel bacteria are *R. luti* and *R. obeum*, strain WAL 14507^T could be distinguished easily from *R. luti* and *R. obeum* by several features. In contrast to *R. luti*, the novel strain did not produce β -*N*-acetylglucosaminidase, alkaline phosphatase or α -mannosidase. Similarly, strain WAL 14507^T could be differentiated from *R. obeum* by the production of acetate and succinate as major fermentation products, but not ethanol and also by its ability to strongly hydrolyse aesculin and produce significant amounts of α -arabinosidase and α -fucosidase. The characteristics that distinguish the novel

strains from their most closely related species are summarized in Table 1.

In several studies, a number of uncultured bacteria represented by sequences derived from faecal 16S rRNA gene clone libraries were found to be closely related to *R. obeum* (Suau *et al.*, 1999; Zoetendal *et al.*, 1998). Zoetendal *et al.* (2002) followed up on these studies using a combination of fluorescent *in situ* hybridization and flow cytometry. Several probes, one of which was designated Urobe63, were specific for these *R. obeum*-like sequences. Their data showed that approximately 16% of the total community belonged to the cluster XIVa (*C. coccoides* group) and of this 2.5% belonged to *R. obeum*-like organisms, although this value varied between 1 and 6% for different individuals. A comparison of the nucleotide sequence of the Urobe63 probe and the 16S rRNA gene sequence of our novel organism showed that the probe sequence was present within the 16S rRNA genes of the novel bacterium. Furthermore, two of the sequences that were used by Zoetendal *et al.* (2002) to validate and test the probes were found to be 98–99% related to our novel organism and it is highly likely that these sequences correspond to strains of this hitherto unknown bacterium. Therefore it can be reasonably assumed that some proportion of the organisms enumerated by the Urobe63 probe represented our novel organism and this suggests that the novel bacterium (and other closely related *R. obeum*-like strains) comprises a significant fraction of the faecal community.

A number of studies have shown that the genus *Ruminococcus* is not monophyletic and is phylogenetically heterogeneous (Rainey & Janssen, 1995; Willems & Collins, 1995; Rieu-Lesme *et al.*, 1996). The type species, *Ruminococcus flavefaciens*, is a member of a cluster designated clostridial rRNA cluster IV (Collins *et al.*, 1994) and this cluster is also referred to as the *Clostridium leptum* group of organisms. *Ruminococcus albus*,

Ruminococcus callidus and *Ruminococcus bromii* are also members of this suprageneric cluster, although the latter species is somewhat removed from the former species. Phylogenetically, the remainder of the ruminococci are members of the rRNA cluster XIVa (Collins *et al.*, 1994) and therefore should not be considered true phylogenetic members of the genus *Ruminococcus sensu stricto*. This grouping is often referred to as the *Clostridium coccoides* group, although this label is misleading due to this large supra-generic cluster embracing taxa that bear a plethora of generic names. Currently the grouping embraces almost 20 genera such as *Acetitomaculum*, *Anaerostipes*, *Bryantella*, *Butyrivibrio*, *Catonella*, *Clostridium*, *Coproccoccus*, *Dorea*, *Eubacterium*, *Hespellia*, *Johnsonella*, *Lachnospira*, *Lachnobacterium*, *Pseudobutyrvibrio*, *Roseburia*, *Ruminococcus*, *Shuttleworthia*, *Sporobacterium* and *Syntrophococcus* and many misclassified clostridial species. It is now recognized that members of this cluster are in need of extensive taxonomic revision with Collins *et al.* (1994) proposing to retain the true clostridia in cluster I which bears *Clostridium butyricum* as the type species.

Phylogenetic analysis employing a number of software programs (neighbour-joining, maximum-likelihood and parsimony) using only matched nucleotides demonstrated that the novel faecal organism formed a robust group with a number of misclassified ruminococci (*R. hansenii*, *R. hydrogenotrophicus*, *R. luti*, *R. obeum*, *R. productus*, *R. schinkii*) together with *C. coccoides* (Fig. 1). It is pertinent to point out that *C. coccoides*, the only species isolated from animal sources (mice), produces endospores unlike all other species in this clade. It may be argued that this organism should not be included in any new genus that contains these *Ruminococcus* species. However, *C. coccoides* and *R. productus* share a very high 16S rRNA gene sequence similarity (99.7%) and an examination of the literature demonstrates that the biochemical profiles, products of glucose metabolism and DNA G+C content of these two organisms are almost identical (Kaneuchi *et al.*, 1976; Ezaki *et al.*, 1994). Although some internal structure is present (see Table 2 and Fig. 1), with *C. coccoides*, *R. hansenii*, and *R. productus* forming a somewhat separate subcluster, the biochemical, morphological and phylogenetic characteristics of these organisms are consistent with generic unity. From a practical standpoint, taxonomy should be a practical subject embracing groups of organisms with unifying features. The proliferation of taxa based solely on a few traits should be resisted. In this diverse group of organisms (cluster XIVa), an accurate identification relies to a great extent upon molecular genetic techniques such as 16S rRNA gene sequence comparisons. Once the sole domain of specialized research facilities, these high throughput methodologies are becoming increasingly automated, reducing costs and making them routinely accessible. The availability of these methods facilitates more rapid and accurate identification of hitherto unknown or misclassified taxa. In addition to the above-mentioned taxa, the species *Ruminococcus*

Table 1. Some distinguishing characteristics of *Blautia wexlerae* sp. nov. and its closest relatives

Taxa: 1, *Blautia wexlerae* sp. nov. (n=15); 2, *Blautia (Ruminococcus) luti* DSM 14534^T; 3, *Ruminococcus obeum* ATCC 29174^T. Data are from Simmering *et al.* (2002) and this study. Enzyme activities were tested by the API ZYM or the Rapid ID32A systems. +, Positive; –, negative; A, acetic acid; S, succinic acid; E, ethanol.

Characteristic	1	2	3
Hydrolysis of aesculin	+	–	–
Enzyme activities:			
β-N-acetylglucosaminidase	–	+	–
Alkaline phosphatase	–	+	+
α-Arabinosidase	+	+	–
α-Fucosidase	+	+	–
α-Mannosidase	–	+	–
Major end products of glucose metabolism	A, S	A, S, H ₂	A, E

Table 2. Biochemical characteristics that can be used to distinguish species of the genus *Blautia* and *Ruminococcus obeum*

Taxa: 1, *Blautia wexlerae* sp. nov. ($n=15$); 2, *Blautia* (*Ruminococcus*) *coccoides*; 3, *Blautia hydrogenotrophica* (*Ruminococcus hydrogenotrophicus*); 4, *Blautia* (*Ruminococcus*) *hansenii*; 5, *Ruminococcus obeum*; 6, *Blautia producta* (*Ruminococcus productus*); 7, *Blautia* (*Ruminococcus*) *schinkii*. Data refer to type strains apart from *B. wexlerae* sp. nov. +, Positive; w, weakly positive; -, negative; D, different among strains; ND, no data; A, acetic acid; S, succinic acid; L, lactate. Data are from Ezaki *et al.* (2006), Simmering *et al.* (2002) and this study. Characteristics were tested by the API ZYM or the Rapid ID32A systems.

Characteristic	1	2	3	4	5	6	7
End products of metabolism	A, S	A, S	A	L, A	A	L, A	A
Fermentation of:							
Aesculin	+	+	ND	D	D	+	ND
Arabinose	+	+	-	-	+	+	+
Cellobiose	D	+	+	-	+	+	+
Glucose	+	+	+	+	+	+	+
Lactose	D	+	ND	+	+	+	ND
Maltose	D	+	ND	+	+	+	+
Mannitol	D	w	-	-	-	-	ND
Mannose	+	+	D	-	+	+	+
Raffinose	D	+	ND	+	+	+	+
Sucrose	D	+	-	-	-	-	+
Xylose	+	+	ND	-	+	+	+

gnavus, *Ruminococcus lactaris* and *Ruminococcus torques* are also present in the XIVa suprageneric cluster although these species are phylogenetically removed from the cluster which contains *C. coccoides*, *R. hansenii*, *R. hydrogenotrophicus*, *R. luti*, *R. obeum*, *R. productus*, *R. schinkii* and the novel faecal isolate. It is not the purpose of this article to reclassify all misclassified ruminococci. We believe that the most appropriate time to reclassify these remaining three species will be when additional novel strains are isolated and can be assigned along with these species to form the nuclei of new genera.

Therefore, based on morphological, phenotypic and phylogenetic considerations, we believe that *C. coccoides* and a number of misclassified ruminococci merit classification in a novel genus for which we propose the name *Blautia* gen. nov. The novel coccobacillus-shaped organisms isolated from human faeces are proposed as a distinct species within this genus as *Blautia wexlerae* sp. nov. *R. obeum* is clearly a member of the genus *Blautia*, as currently defined. The rules of the Bacteriological Code currently require that the type strains of all new species and subspecies (including new combinations) be deposited in two different collections in two different countries. The type strain of *R. obeum* is currently only deposited in the ATCC and a second independent deposit, as required by the Code, has not been possible (requests to the ATCC

were made to enable this but could not be accommodated satisfactorily). Consequently, the transfer of this species to the genus *Blautia* cannot be made because the resulting species name would not conform to the Rules of the Code governing the valid publication of species names and deposit of type material (Rules 27 and 30) and consequently would not be considered to be validly published.

Description of *Blautia* gen. nov.

Blautia (Blau'ti.a. N.L. fem. n. *Blautia* in honour of Michael Blaut, a German microbiologist, in recognition of his many contributions to human gastrointestinal microbiology).

Gram-positive staining, non-motile. Coccoid or oval-shaped, pointed ends are often observed. Spores are not normally observed, but may be produced by some strains. Chemo-organotrophic and obligately anaerobic having a fermentative type of catabolism. Some species use H_2/CO_2 as major energy sources. The major end products of glucose metabolism are acetate, ethanol, hydrogen, lactate and succinate. The G+C content of the DNA is 37–47 mol%. Isolated from animal and human faeces. The type species of the genus is *Blautia coccoides* (Kaneuchi, Benno & Mitsuoka, 1976).

Description of *Blautia coccoides* comb. nov.

Blautia coccoides (coc.coi'des. Gr. n. *coccus* a berry; Gr. n. *eidos* shape; N.L. adj. *coccoides* berry shaped).

Basonym: *Clostridium coccoides* Kaneuchi *et al.* 1976.

The description of *Blautia coccoides* is identical to that proposed for *C. coccoides* (Kaneuchi *et al.*, 1976). The type strain is ATCC 29236^T (=DSM 935^T=JCM 1395^T=NCTC 11035^T).

Description of *Blautia hansenii* comb. nov.

Blautia hansenii (han.sen'i.i. N.L. gen. n. *hansenii* of Hansen, in honour of P. Arne Hansen, a Danish–American bacteriologist).

Basonym: *Streptococcus hansenii* Holdeman and Moore 1974 (Approved Lists 1980).

Other synonym: *Ruminococcus hansenii* (Holdeman and Moore 1974) Ezaki *et al.* 1994.

The description of *Blautia hansenii* is identical to that proposed for *Ruminococcus hansenii* (Holdeman & Moore, 1974). The type strain is ATCC 27752^T (=CIP 104219^T=DSM 20583^T=JCM 14655).

Description of *Blautia hydrogenotrophica* comb. nov.

Blautia hydrogenotrophica (hy.dro.gen.o.tro'phi.ca. N.L. n. *hydrogenum* hydrogen; Gr. adj. *trophikos* nursing, tending

or feeding; N.L. fem. adj. *hydrogenotrophica* (sic) feeding on hydrogen).

Basonym: *Ruminococcus hydrogenotrophicus* Bernalier *et al.* 1996.

The description of *Blautia hydrogenotrophica* is identical to that proposed for *Ruminococcus hydrogenotrophicus* by Bernalier *et al.*, 1996. The type strain is DSM 10507^T (=JCM 14656^T).

Description of *Blautia luti* comb. nov.

Blautia luti (lu.t.i. L. gen. n. *luti* of mud).

Basonym: *Ruminococcus luti* Simmering *et al.* 2002.

The description of *Blautia luti* is identical to that proposed for *Ruminococcus luti* Simmering *et al.*, 2002. The type strain is DSM 14534^T (=CCUG 45635^T).

Description of *Blautia producta* comb. nov.

Blautia producta (pro'duc.ta. L. adj. *producta* -us -um produces).

Basonym: *Peptostreptococcus productus* (Prévot 1941) Smith 1957 (Approved Lists 1980).

Other synonyms: '*Streptococcus productus*' Prévot 1941; *Ruminococcus productus* (Prévot 1941) Ezaki *et al.* 1994.

The description of *Blautia producta* is identical to that proposed for *Ruminococcus productus* (Ezaki *et al.*, 1994). The type strain is ATCC 27340^T (=CCUG 9990^T=CCUG 10976^T=DSM 2950^T=JCM 1471^T).

Description of *Blautia schinkii* comb. nov.

Blautia schinkii (schin'ki.i. N.L. gen. n. *schinkii* of Schink, named after Bernard Schink).

Basonym: *Ruminococcus schinkii* Rieu-Lesme *et al.* 1997.

The description of *Blautia schinkii* is identical to that proposed for *Ruminococcus schinkii* Rieu-Lesme *et al.*, 1996). The type strain is CIP 105464^T (=CCUG 53897^T=DSM 10518^T).

Description of *Blautia wexlerae* sp. nov.

Blautia wexlerae (wex'ler.ae. N.L. fem. gen. n. *wexlerae* of Wexler, in honour of the American microbiologist Hannah M. Wexler, who has contributed significantly to our knowledge of anaerobic bacteria, particularly with regard to antimicrobial susceptibility testing and studies of mechanisms of antimicrobial resistance).

Cells are Gram-positive, non-spore-forming coccobacilli that are 1.0–1.5 × 1–3 µm in size. Strictly anaerobic. After 48 h of incubation at 37 °C under N₂ and CO₂ (80 : 20, v/v) gas phase, colonies on *Brucella* blood agar plates are 1–2 mm in diameter, grey with a white centre, umbonate and

opaque with entire edges. Grows well anaerobically, but no growth occurs in an atmosphere of 2 % or 6 % O₂. Isolates are negative in tests for lecithinase, lipase, catalase and indole, but are positive for urease. Aesculin is hydrolysed, but gelatin is not. Arabinose, glucose, mannose, ribose, and xylose are utilized as substrates when grown in PRAS carbohydrate broths, but glycogen is not utilized. Reactions for amygdalin, cellobiose, fructose, lactose, maltose, mannitol, melezitose, melibiose, raffinose, rhamnose, salicin, sorbitol, sucrose and trehalose are variable. End products of metabolism from peptone-yeast-glucose broth are acetate and succinate. Long-chain cellular fatty acids are of the straight-chain saturated and monounsaturated types, with C_{16:0} (major component), and C_{14:0}, C_{16:0} dimethyl acetal acids predominating. Using the API ZYM and Rapid ID32A systems, all isolates produce a similar profile. Positive reactions are obtained for α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase and α-fucosidase by both systems. With the Rapid ID32A system, α-arabinosidase is positive and acid phosphatase is detected by the API ZYM system. No activity was detected for N-acetyl-β-glucosaminidase, alkaline phosphatase, α-chymotrypsin, cystine arylamidase, β-glucuronidase, lipase (C14), leucine arylamidase, α-mannosidase, trypsin or valine arylamidase. Urease results are variable by the Rapid ID32A system. Esterase (C4), esterase lipase (C8) and naphthol-AS-BI-phosphohydrolase are variable using the API ZYM system. The isolates are sensitive to vancomycin (5 µg) and kanamycin (1000 µg), but resistant to colistin (10 µg) identification discs.

The type strain, WAL 14507^T (=ATCC BAA-1564^T=DSM 19850^T), was isolated from human faeces.

Acknowledgements

This work has been carried out, in part, with financial support from Veterans Administration Merit Review funds. We also thank Hans Trüper for help in the derivation of the genus and species epithets. We also wish to thank Dr B. J. Tindall, chairman of the ICSP Judicial Commission for his invaluable advice on the Rules of the Bacteriological Code.

References

- Barcenilla, A., Pryde, S. E., Martin, J. C., Duncan, S. H., Stewart, C. S., Henderson, C. & Flint, H. J. (2000). Phylogenetic relationships of butyrate-producing bacteria from the human gut. *Appl Environ Microbiol* **66**, 1654–1661.
- Bernalier, A., Willems, A., Leclerc, M., Rochet, V. & Collins, M. D. (1996). *Ruminococcus hydrogenotrophicus* sp. nov., a new H₂/CO₂-utilizing acetogenic bacterium isolated from human feces. *Arch Microbiol* **166**, 176–183.
- Brosius, J., Palmer, M. L., Kennedy, P. J. & Noller, H. F. (1978). Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. *Proc Natl Acad Sci U S A* **75**, 4801–4805.
- Collins, M. D., Lawson, P. A., Willems, A., Cordoba, J. J., Fernandez-Garayzabal, J., Garcia, P., Cai, J. & Farrow, J. A. E. (1994). The phylogeny of the genus *Clostridium*: proposal of five new genera and eleven new species combinations. *Int J Syst Bacteriol* **44**, 812–826.

- Ezaki, T., Li, N., Hashimoto, Y., Miura, H. & Yamamoto, H. (1994).** 16S ribosomal DNA sequences of anaerobic cocci and proposal of *Ruminococcus hansenii* comb. nov. and *Ruminococcus productus* comb. nov. *Int J Syst Bacteriol* **44**, 130–136.
- Ezaki, T., Li, N. & Kawamura, Y. (2006).** The anaerobic gram-positive cocci. In *The Prokaryotes*, vol. 6, pp. 795–808. Edited by M. Dworkin, S. Fallow, E. Rosenberg, K.-H. Schleifer & E. Stackebrandt. New York: Springer-Verlag.
- Finegold, S. M., Molitoris, D., Song, Y. L., Liu, C. X., Vaisanen, M. L., Bolte, E., McTeague, M., Sandler, R., Wexler, H. & other authors (2002).** Gastrointestinal microflora studies in late-onset autism. *Clin Infect Dis* **35** (Suppl. 1), S6–S16.
- Holdeman, L. V., Cato, E. P. & Moore, W. E. C. (1977).** *Anaerobe Laboratory Manual*, 4th edn. Blacksburg, VA: Virginia Polytechnic Institute and State University.
- Holdeman, L. V. & Moore, W. E. C. (1974).** New genus, *Coprococcus*, twelve new species, and emended descriptions of bacteria from human feces. *Int J Syst Bacteriol* **24**, 260–277.
- Jousimies-Somer, H. R., Summanen, P., Citron, D. M., Baron, E. J., Wexler, H. M. & Finegold, S. M. (2002).** *Wadsworth-KTL Anaerobic Bacteriology Manual*, 6th edn. Belmont, CA: Star Publishing Co.
- Kaneuchi, C., Benno, Y. & Mitsuoka, T. (1976).** *Clostridium coccoides*, a new species from the feces of mice. *Int J Syst Bacteriol* **26**, 482–486.
- Liou, J. S.-C., Balkwill, D. L., Drake, G. R. & Tanner, R. S. (2005).** *Clostridium carboxidivorans* sp. nov., a solvent-producing clostridium isolated from an agricultural settling lagoon, and reclassification of the acetogen *Clostridium scatologenes* strain SL-1 as *Clostridium drakei* sp. nov. *Int J Syst Evol Microbiol* **55**, 2085–2091.
- Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, Buchner, A., Lai, T., Steppi, S. & other authors (2004).** ARB: a software environment for sequence data. *Nucleic Acids Res* **32**, 1363–1371.
- Pearson, W. R. & Lipman, D. J. (1988).** Improved tools for biological sequence comparison. *Proc Natl Acad Sci U S A* **85**, 2444–2448.
- Rainey, F. A. & Janssen, P. H. (1995).** Phylogenetic analysis by 16S ribosomal DNA sequence comparison reveals two unrelated groups of species within the genus *Ruminococcus*. *FEMS Microbiol Lett* **129**, 69–73.
- Rieu-Lesme, F., Morvan, B., Collins, M. D., Fonty, G. & Willems, A. (1996).** A new H₂/CO₂-using acetogenic bacterium from the rumen: description of *Ruminococcus schinkii* sp. nov. *FEMS Microbiol Lett* **140**, 281–286.
- Simmering, R., Taras, D., Schwiertz, A., Le Blay, G., Gruhl, B., Lawson, P. A., Collins, M. D. & Blaut, M. (2002).** *Ruminococcus luti* sp. nov., isolated from a human faecal sample. *Syst Appl Microbiol* **25**, 189–193.
- Stackebrandt, E. & Ebers, J. (2006).** Taxonomic parameters revisited: tarnished gold standards. *Microbiol Today* **33**, 152–155.
- Stackebrandt, E. & Goebel, B. M. (1994).** Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Suau, A., Bonnet, R., Sutren, M., Godon, J.-J., Gibson, G. R., Collins, M. D. & Doré, J. (1999).** Direct analysis of genes encoding 16S rRNA from complex communities reveals many novel molecular species within the human gut. *Appl Environ Microbiol* **65**, 4799–4807.
- Wexler, H. M., Reeves, D., Summanen, P. H., Molitoris, E., McTeague, M., Duncan, J., Wilson, K. H. & Finegold, S. M. (1996).** *Sutterella wadsworthensis* gen. nov., sp. nov., bile-resistant micro-aerophilic *Campylobacter gracilis*-like clinical isolates. *Int J Syst Bacteriol* **46**, 252–258.
- Willems, A. & Collins, M. D. (1995).** Phylogenetic analysis of *Ruminococcus flavefaciens*, the type species of the genus *Ruminococcus* does not support the reclassification of *Streptococcus hansenii* and *Peptostreptococcus productus* as ruminococci. *Int J Syst Bacteriol* **45**, 572–575.
- Zoetendal, E. G., Ben-Amor, K., Harmsen, H. J. M., Schut, F., Akkermans, A. D. L. & de Vos, W. M. (2002).** Quantification of uncultured *Ruminococcus obeum*-like bacteria in human fecal samples by fluorescent in situ hybridization and flow cytometry using 16S rRNA-targeted probes. *Appl Environ Microbiol* **68**, 4225–4232.
- Zoetendal, E. G., Akkermans, A. D. L. & de Vos, W. M. (1998).** Temperature gradient gel electrophoresis analysis of 16S rRNA from human fecal samples reveals stable and host-specific communities of active bacteria. *Appl Environ Microbiol* **64**, 3854–3859.