

## Proposal of *Roseburia faecis* sp. nov., *Roseburia hominis* sp. nov. and *Roseburia inulinivorans* sp. nov., based on isolates from human faeces

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Seven recently cultured bacterial isolates, although similar in their 16S rRNA gene sequences to *Roseburia intestinalis* L1-82<sup>T</sup> (DSM 14610<sup>T</sup>), were not sufficiently related for inclusion within existing species, forming three separate clusters in a 16S rRNA gene phylogenetic tree. The isolates, which were obtained from human stools, were Gram-variable or Gram-negative, strictly anaerobic, slightly curved rods; cells from all strains measured approximately 0.5 × 1.5–5.0 µm and were motile. Two strains belonging to one cluster (A2-181 and A2-183<sup>T</sup>) were the only strains that were able to grow on glycerol and that failed to grow on any of the complex substrates tested (inulin, xylan and amylopectin). Strains belonging to a second cluster (represented by M6/1 and M72/1<sup>T</sup>) differed from the other isolates in their ability to grow on sorbitol. Isolates belonging to a third cluster (L1-83 and A2-194<sup>T</sup>) were the only strains that failed to grow on xylose and that gave good growth on inulin (strains M6/1 and M72/1<sup>T</sup> gave weak growth). All strains were net acetate utilizers. The DNA G + C contents of representative *Roseburia* strains A2-183<sup>T</sup>, A2-194<sup>T</sup>, M72/1<sup>T</sup> and *R. intestinalis* L1-82<sup>T</sup> were 47.4, 41.4, 42.0 and 42.6 mol%, respectively. Based on 16S rRNA gene sequence similarity, three novel *Roseburia* species are proposed, with the names *Roseburia hominis* sp. nov. (type strain A2-183<sup>T</sup> = DSM 16839<sup>T</sup> = NCIMB 14029<sup>T</sup>), *Roseburia inulinivorans* sp. nov. (type strain A2-194<sup>T</sup> = DSM 16841<sup>T</sup> = NCIMB 14030<sup>T</sup>) and *Roseburia faecis* sp. nov. (type strain M72/1<sup>T</sup> = DSM 16840<sup>T</sup> = NCIMB 14031<sup>T</sup>).

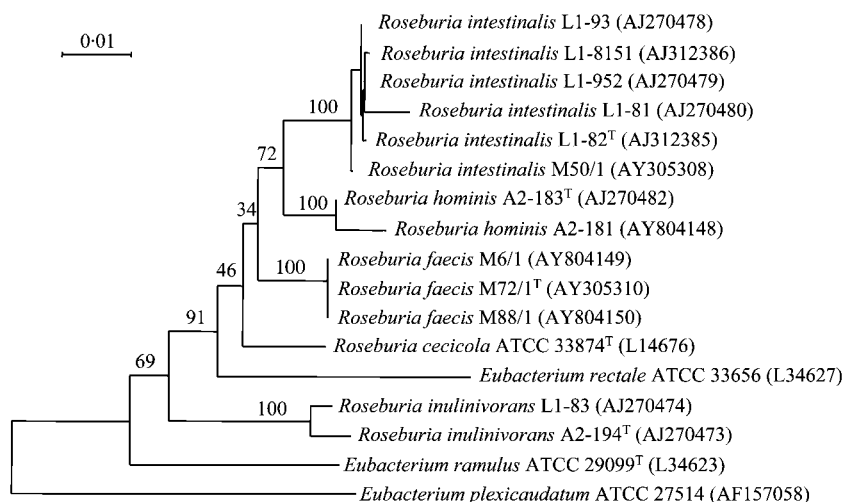
Strict anaerobes that produced butyrate as a major product were isolated from human faecal samples. Most butyrate producers from human faeces were found previously to belong to two main groups, clostridial cluster IV (Duncan *et al.*, 2002b) and cluster XIVa (Hold *et al.*, 2003; Barcenilla *et al.*, 2000). Particularly prevalent among the latter group were bacteria related to *Roseburia cecicola*. *Roseburia intestinalis* was proposed previously based on isolates from human faeces (Duncan *et al.*, 2002a) and the purpose of the present paper is to propose three novel species of the genus *Roseburia*.

Butyrate-producing strains were isolated from the highest countable dilution of faecal samples from a healthy infant and from four healthy adults. Ethical approval was obtained from Grampian Research Ethics Committee (project number 00/00133). The isolations were made from roll

tubes of anaerobic M2GSC medium (Miyazaki *et al.*, 1997) or a medium designed to select for *Selenomonas*-like bacteria, as described previously (Louis *et al.*, 2004). All media were prepared and maintained anaerobically using oxygen-free carbon dioxide. The isolates were maintained routinely by growth for 16–18 h at 37 °C in 7.5 ml aliquots of M2GSC medium.

DNA was extracted and purified from 24 h-old cultures grown on M2GSC medium following the method of Ausubel *et al.* (1994). A universal primer set was used for amplification of the 16S rRNA gene (Weisburg *et al.*, 1991). PCR conditions were as described by Wood *et al.* (1998). On-line similarity analysis of the 16S rRNA gene sequences was performed with the BLAST program at NCBI and EMBL and with the Ribosomal Database Project (Maidak *et al.*, 2001). Nucleotide sequences were aligned with reference 16S rRNA gene sequences using the CLUSTAL\_X program (Thompson *et al.*, 1997). Phylogenetic analyses were performed using neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein & Churchill, 1996)

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Roseburia* strains determined in this study are given in Fig. 1.



**Fig. 1.** Phylogenetic tree constructed by using the maximum-likelihood method, based on 16S rRNA gene sequences of *Roseburia*-related strains. 1150 unambiguous nucleotide positions were used in the phylogenetic reconstruction; positions with gaps were omitted from the analysis. Numbers above nodes are confidence levels generated from 100 bootstrap trees. The 16S rRNA gene sequence of *Eubacterium plexicaudatum* was used as an outgroup. Bar, 0.01 substitutions per nucleotide position. GenBank accession numbers are given in parentheses.

methods in the PHYLIP package (Felsenstein, 1989) and parsimony analysis with the PAUP package (Swofford, 2002). Statistical validation of tree branching was done by bootstrap analysis (Felsenstein, 1989), involving 1000 resampled trees in the neighbour-joining and parsimony methods and 100 trees in the maximum-likelihood analysis.

Fig. 1 shows a phylogenetic tree based on 16S rRNA gene sequences for 13 isolates, obtained from three individuals. Six sequences of isolates from two individuals clustered with *R. intestinalis*. The remaining seven sequences, however, did not correspond to recognized species and fell into three clusters within the tree (Fig. 1). None of the isolates was close enough to *R. cecicola*, originally isolated from the mouse gut (Stanton & Savage, 1983), for inclusion in this species. Based on their phylogenetic placement and phenotypic characteristics (reported below), we propose that each of the three clusters represents a novel species, with A2-183<sup>T</sup>, M72/1<sup>T</sup> and A2-194<sup>T</sup> as the representative type strains. A matrix of 16S rRNA gene sequence comparisons showed that strains A2-183<sup>T</sup>, M72/1<sup>T</sup> and A2-194<sup>T</sup> shared 97.3, 95.4 and 92.7 % sequence similarity, respectively, with *R. intestinalis* L1-82<sup>T</sup>. Strains A2-183<sup>T</sup> and M72/1<sup>T</sup> shared 95.4 % similarity with each other and 93 % similarity with strain A2-194<sup>T</sup>. Strains clustering with A2-183<sup>T</sup> and M72/1<sup>T</sup> shared >97 % similarity with the relevant type strain; however, strain L1-83 shared 95.5 % similarity with strain A2-194<sup>T</sup>.

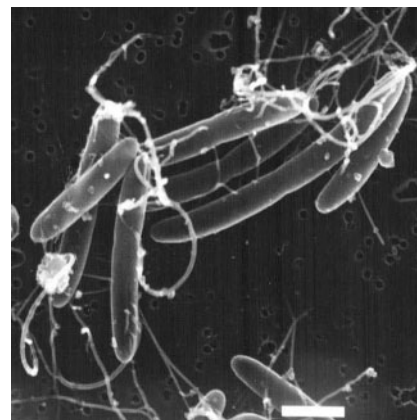
The DNA G+C content was determined using high-performance liquid chromatography (at DSMZ, Germany) (Tamaoka & Komagata, 1984; Mesbah *et al.*, 1989); values for representative strains A2-183<sup>T</sup>, A2-194<sup>T</sup> and M72/1<sup>T</sup> were 47.4, 41.4 and 42.0 mol%, respectively. The G+C content of *R. intestinalis* L1-82<sup>T</sup>, determined here for comparison using the same method, was 42.6 mol%.

The cellular morphology of all the isolates was observed under an Olympus BX50 light microscope at  $\times 1000$  magnification, following Gram-staining of exponential

and stationary phase cultures grown at 37 °C on M2GSC medium, as described by Holdeman *et al.* (1977).

Following incubation of the cultures on complex growth medium in roll tubes at 37 °C for 48 h, single colonies of three representative strains (A2-183<sup>T</sup>, M72/1<sup>T</sup> and A2-194<sup>T</sup>) were small (1–3 mm in diameter), creamy white and translucent with entire edges. All 13 strains studied here were Gram-variable, slightly curved rods and all were motile. *R. intestinalis* L1-82<sup>T</sup> had been shown previously to be motile by the presence of multiple flagella located subterminally (Duncan *et al.*, 2002a) and strain A2-183<sup>T</sup> also possessed a multiple flagellar bundle (Fig. 2). All representative strains failed to grow at 4 or 20 °C, all showed weak growth at 30 °C and optimum growth at 37 °C.

Eight strains were tested for tolerance to oxygen by spreading late exponential cultures on the surface of pre-reduced agar plates and exposing the plates to air for different periods of time. *R. intestinalis* L1-82<sup>T</sup>, L1-952 and L1-8151 and strains



**Fig. 2.** Scanning electron micrograph of *Roseburia hominis* sp. nov. strain A2-183<sup>T</sup>, showing a flagellar bundle. Bar, 1  $\mu$ m.

A2-181, A2-183<sup>T</sup>, M6/1, M72/1<sup>T</sup> and A2-194<sup>T</sup> all failed to grow after a minimum exposure time of 2 min to air, whereas the control plates that remained in the glove box (in an atmosphere of 80 % nitrogen, 10 % carbon dioxide and 10 % hydrogen) gave good growth with viable counts of approximately 10<sup>8</sup> c.f.u. ml<sup>-1</sup> (data not shown).

Substrate utilization was determined by addition of a final concentration of 0.5 % of stock (10 %, w/v) filter-sterilized sugar solutions to YCFA medium (Duncan *et al.*, 2002b), dispensed in 7.5 ml amounts in Hungate tubes. YCFA medium supplemented with a carbon source provided a convenient alternative to rumen fluid medium for the cultivation of the strains in this study. Growth was measured spectrophotometrically as absorbance at 650 nm.

Eleven strains representing *R. intestinalis* (five strains) and the three additional *Roseburia*-related clusters were tested for their ability to grow on a range of carbohydrate substrates in liquid medium (Table 1). Only *R. intestinalis* and strains A2-181 and A2-183<sup>T</sup> were able to grow with arabinose as the sole added energy source. In common with *R. cecicola*, strains clustering with *R. intestinalis* and a second cluster comprising strains L1-83 and A2-194<sup>T</sup> were able to grow (weakly) with sucrose as energy source. The other strains tested, belonging to two other distinct clusters, failed to grow on sucrose. In common with *R. cecicola*, but in contrast to the other three *Roseburia* species, strains A2-181 and A2-183<sup>T</sup> were able to grow with glycerol. Only the two strains M6/1 and M72/1<sup>T</sup>, in common with *R. cecicola*, grew on sorbitol.

Previous observations revealed variation in the ability of *Roseburia*-related strains to utilize polysaccharides that occur in the human diet (Duncan *et al.*, 2003). As summarized in Table 1, only strains M6/1, M72/1<sup>T</sup>, A2-194<sup>T</sup> and L1-83 were able to grow with inulin (dahlia) as substrate. *R. intestinalis* strains in particular grew well with oat spelt xylan as substrate, whereas strains M6/1 and M72/1<sup>T</sup> grew weakly on this substrate. Starch utilization was widespread, with only two strains (A2-181 and A2-183<sup>T</sup>) failing to grow well with amylopectin (from potato starch; Fluka) as substrate.

All the *Roseburia*-related strains formed butyrate as a major fermentation product when grown on M2GSC medium at 37 °C for 24 h and utilized acetate (Table 2). All strains formed between 14 and 24 mM butyrate with the exception of two strains (L1-83 and A2-194<sup>T</sup>) that formed less than 7.3 mM butyrate and also utilized the lowest concentrations of acetate. All strains formed formate and lactate.

Two strains belonging to one cluster (A2-181 and A2-183<sup>T</sup>) had the highest G + C content (47.4 mol%) and shared 97 % sequence similarity with *R. intestinalis* L1-82<sup>T</sup>; these were the only strains that failed to grow on all the complex carbohydrate substrates tested (inulin, xylan and amylopectin). Strains belonging to a second cluster (M6/1 and M72/1<sup>T</sup>) differed from strains A2-181 and A2-183<sup>T</sup> in their ability to grow on sorbitol and amylopectin. Isolates belonging to a third cluster (L1-83 and A2-194<sup>T</sup>) shared approximately 93 % sequence similarity with *R. intestinalis* L1-82<sup>T</sup> and in addition were the only strains to give good

**Table 1.** Substrate utilization by strains belonging to the genus *Roseburia*

Growth was tested in liquid cultures with 0.5 % (w/v) substrate, by comparison with controls that received no added carbohydrate. All strains are positive for fermentation of cellobiose, fructose, maltose and glucose. ΔOD<sub>650</sub> values at 24 h: +, >0.4, w, >0.15 and <0.4. For *R. cecicola*, + indicates a significant reduction in culture pH. –, Negative; NR, not recorded.

Substrate	<i>R. intestinalis</i> (5 strains)*	<i>R. hominis</i> (2 strains)†	<i>R. faecis</i> (2 strains)‡	<i>R. inulinivorans</i> (2 strains)§	<i>R. cecicola</i>
Arabinose	+	+	–	–	NR
Raffinose	+	–/w	+	–	+
Sucrose	+	–	–	+/w	+
Xylose	+	+	+	–	+
Glycerol	–	+	–	–	+
Sorbitol	–	–	+	–	+
Melibiose	w	+/w	+	w	NR
Inulin (dahlia)	–	–	w	+	NR
Xylan (oat spelt)	+	–	w	–	NR
Starch (amylopectin)	+	–	+	+	NR

\*L1-81, L1-93, L1-952, L1-8151 and L1-82<sup>T</sup>.

†A2-181 and A2-183<sup>T</sup>.

‡M6/1 and M72/1<sup>T</sup>.

§L1-83 and A2-194<sup>T</sup>.

||Data from Stanton & Savage (1983) based on measurement of final culture pH rather than optical density; this strain is no longer extant and direct comparison with the newer isolates was not possible.

**Table 2.** Range of short chain fatty acids (mM) formed and utilized by *Roseburia* strains on M2GSC medium

n, Number of strains studied.

Compound	<i>R. intestinalis</i> (n=4)*	<i>R. faecis</i> (n=3)†	<i>R. hominis</i> (n=2)	<i>R. inulinivorans</i> (n=2)
Acetate utilized	9.1–12.3	3.6–8.3	8.2–11.5	2.3–3.5
Butyrate formed	16.0–21.3	13.9–24.0	16.8–20.0	6.5–7.3
Formate formed	6.9–8.4	9.2–15.7	6.9–7.6	4.4–4.7
Lactate formed	0–7.1	0–2.3	2.2–9.1	2.1–7.1

\*Strains as in Table 1, but without L1–81.

†Strains as in Table 1, with the addition of M88/1.

growth on inulin (strains M6/1 and M72/1<sup>T</sup> gave weak growth).

The latest description of the genus *Roseburia* includes the species *R. intestinalis* and *R. cecicola*, the latter being the type species. The genus description can be taken to apply to the three novel species proposed below.

#### Emended description of *Roseburia intestinalis* Duncan *et al.* 2002

The description is as given by Duncan *et al.* (2002a). The G+C content of the DNA of the type strain (L1–82<sup>T</sup>) is 42.6 mol%.

#### Description of *Roseburia hominis* sp. nov.

*Roseburia hominis* (hom.i'nis. L. gen. n. *hominis*, of a human being, referring to human gut habitat).

Cells are Gram-variable to Gram-negative, slightly curved rods, and motile by means of multiple flagella. Cells measure approximately 0.5 × 1.5–5 µm. Optimum growth temperature is 37 °C. Strictly anaerobic. Good growth occurs on M2GSC agar at 37 °C and after incubation for 48 h forms creamy white translucent colonies with entire edges, approximately 1–3 mm in diameter. Chemo-organotrophic. Utilizes arabinose, fructose, glucose, maltose, cellobiose, xylose and glycerol as energy sources for growth. Weak/no growth occurs with raffinose or melibiose as energy source. Sucrose, sorbitol, oat spelt xylan, amylopectin starch and inulin (dahlia) are not utilized for growth. Butyrate and formate are major products and lactate a minor product from glucose (0.2%), with net consumption of acetate present in the medium. Catalase-negative. The G+C content of the DNA of the type strain is 47.4 mol%.

Isolated from human faeces in Aberdeen, Scotland. The type strain is A2-183<sup>T</sup> (=DSM 16839<sup>T</sup>=NCIMB 14029<sup>T</sup>).

#### Description of *Roseburia inulinivorans* sp. nov.

*Roseburia inulinivorans* (in.ul'in.i'vor.ans. N.L. n. *inulinum*, inulin; L. part. adj. *vorans*, devouring; N.L. part. adj.

*inulinivorans* inulin-devouring, referring to the ability to use inulin as a growth substrate).

Cells are Gram-variable to Gram-negative, motile, slightly curved rods. Cells measure approximately 0.5 × 1.5–5 µm. Optimum growth temperature is 37 °C. Strictly anaerobic. Good growth occurs on M2GSC agar at 37 °C and after incubation for 48 h forms creamy white translucent colonies with entire edges, approximately 1–3 mm in diameter. Chemo-organotrophic. Utilizes fructose, glucose, maltose, cellobiose, inulin (dahlia) and amylopectin starch as energy sources for growth. Weak growth occurs with sucrose or melibiose as energy source. Arabinose, raffinose, xylose, glycerol, sorbitol and oat spelt xylan are not utilized for growth. Butyrate, formate and some lactate are produced from glucose, with net consumption of acetate present in the medium. Catalase-negative. The G+C content of the DNA of the type strain is 41.4 mol%.

Isolated from human faeces in Aberdeen, Scotland. The type strain is A2-194<sup>T</sup> (=DSM 16841<sup>T</sup>=NCIMB 14030<sup>T</sup>).

#### Description of *Roseburia faecis* sp. nov.

*Roseburia faecis* (fae'cis. L. gen. n. *faecis* referring to faecal origin).

Gram-variable, motile, slightly curved rods. Cells measure approximately 0.5 × 1.5–5 µm. Optimum growth temperature is 37 °C. Strictly anaerobic. Good growth occurs on M2GSC agar at 37 °C and after incubation for 48 h forms creamy white translucent colonies with entire edges, approximately 1–3 mm in diameter. Chemo-organotrophic. Utilizes fructose, glucose, maltose, cellobiose, raffinose, xylose, sorbitol, melibiose and amylopectin starch as energy sources for growth. Weak growth occurs with inulin or oat spelt xylan as energy source. Arabinose, sucrose and glycerol are not utilized for growth. Butyrate and formate are major products and lactate a minor product from glucose (0.2%), with net consumption of acetate present in the medium. Catalase-negative. The G+C content of the DNA of the type strain is 42.0 mol%.

Isolated from human faeces in Aberdeen, Scotland. The type strain is M72/1<sup>T</sup> (=DSM 16840<sup>T</sup>=NCIMB 14031<sup>T</sup>).

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