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TAXONOMIC DESCRIPTION

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Romboutsia hominis sp. nov., the first human gut-derived representative of the genus *Romboutsia*, isolated from ileostoma effluent

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Abstract

A Gram-stain-positive, motile, rod-shaped, obligately anaerobic bacterium, designated FRIFI^T, was isolated from human ileostoma effluent and characterized. On the basis of 16S rRNA gene sequence similarity, strain FRIFI^T was most closely related to the species *Romboutsia ilealis* CRIB^T (97.7 %), *Romboutsia lituseburensis* DSM 797^T (97.6 %) and *Romboutsia sedimentorum* LAM201^T (96.6 %). The level of DNA–DNA relatedness between strain FRIFI^T and *R. ilealis* CRIB^T was 13.9 ±3.3 % based on DNA–DNA hybridization. Whole genome sequence-based average nucleotide identity between strain FRIFI^T and closely related *Romboutsia* strains ranged from 78.4–79.1 %. The genomic DNA G+C content of strain FRIFI^T was 27.8 mol%. The major cellular fatty acids of strain FRIFI^T were saturated and unsaturated straight-chain C12–C19 fatty acids as well as cyclopropane fatty acids, with $C_{16:0}$ being the predominant fatty acid. The polar lipid profile comprised five phospholipids and six glycolipids. These results, together with differences in phenotypic features, support the proposal that strain FRIFI^T represents a novel species within the genus *Romboutsia*, for which the name *Romboutsia hominis* sp. nov. is proposed. The type strain is FRIFI^T (=DSM 28814^T=KCTC 15553^T).

Recently, the genus *Romboutsia* was created to allocate the newly isolated species *Romboutsia ilealis* as well as *Romboutsia lituseburensis*, which was previously named *Clostridium lituseburense* [1]. Since the creation of the genus *Romboutsia*, the novel species *Romboutsia sedimentorum* has been added to the genus as well [2]. Members of the *Peptostreptococcaceae* are commonly found in diverse environments, but sequence-based studies have reported the presence of *Romboutsia*-like 16S rRNA gene sequences predominantly in samples of mammalian intestinal origin [3–6]. Nevertheless, the roles that members of *Romboutsia* species play especially in the (small) intestinal tract remain largely unknown because of the still limited availability of cultured representatives. To gain more insight in metabolic and functional capabilities of the genus *Romboutsia* we set out to isolate *Romboutsia* strains of human small intestinal origin, resulting in the isolation of strain FRIFI^T from ileostoma effluent of a human adult. To determine the taxonomic position of this isolate, it was subjected to further characterization in accordance with the recommendations provided by Kämpfer *et al.* [7] and Tindall *et al.* [8]. On the basis of phenotypic and genetic studies, we propose strain FRIFI^T to be the type strain for a novel *Romboutsia* species for which we propose the name *Romboutsia hominis* sp. nov.

Isolation of strain FRIFI^T was performed by serial dilution of an ileostoma effluent sample in liquid anoxic bicarbonate-buffered CRIB medium (pH 7.0) prepared as previously described [1]. The sample was obtained from a female human volunteer who was otherwise healthy. The study was approved by the local medical committee of the University Medical Centre St. Radboud (Nijmegen, The Netherlands).

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Keywords: *Romboutsia*; *Clostridium*; Peptostreptococcaceae; ileostoma effluent; human intestine.

Abbreviations: ANI, average nucleotide identity; DDH, DNA–DNA hybridization; ECL, equivalent chain length; MIS, microbial identification system; PY, peptone–yeast extract; PYG, peptone–yeast extract–glucose.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain FRIFI^T is KT372679. All genome sequence data have been deposited under BioProject accession numbers PRJEB7106 (for strain FRIFI^T) and PRJEB7306 (for strain DSM 797^T).

One supplementary figure is available with the online version of this article.

A waiver for informed consent was given due to the non-invasive nature of the study. The sample was collected by taking material directly from the ileostomy bag and preserved as glycerol suspension (25 % v/v) for subsequent cultivation. Enrichment was achieved by repeated serial dilutions. Cell pellets were collected of 1 ml samples taken from the enrichment cultures and total DNA was extracted using the FastDNA Spin kit for soil (MP Biomedicals) according to the manufacturer's instructions. The increase in relative abundance of the target phylotype was followed in the subsequent serial dilutions using quantitative PCR and the *R. ilealis*-specific primer pair CRIB-61F/CRIB-235R as described previously [9].

Enrichment of the strain in culture was accomplished by repeated rounds of fast transfers (8 h incubation), giving this fast-growing strain a competitive advantage over other anaerobes present in the original inoculum. After enrichment, strain FRIFI^T was purified by repeated plating of single colonies on solid DSMZ 104b medium containing 0.8 % gelrite (Carl Roth). This medium consisted of (per litre distilled water): 5 g trypticase peptone, 5 g peptone from meat (pepsin-digested), 10 g yeast extract, 5 glucose, 1 mg resazurin, 40 ml salts solution [10] and 0.5 g L-cysteine hydrochloride. The purified strain was stored as glycerol suspension (25 % v/v) at –80 °C. Strain FRIFI^T, *R. lituseburensis* DSM 797^T and *R. ilealis* CRIB^T (DSM 25109) were routinely grown in liquid CRIB medium.

Total genomic DNA was extracted from cells of strain FRIFI^T, and near full-length 16S rRNA gene fragments were amplified by PCR and sequenced as previously described [1]. The obtained sequence was aligned with reference sequences using the online SINA aligner [11]. Phylogenetic trees were reconstructed using the MEGA6 software [12]. The neighbour-joining tree confirmed the clustering of strain FRIFI^T within the genus *Romboutsia* (Fig. 1). Tree topology was confirmed using maximum-parsimony and maximum-likelihood methods. In order to generate pairwise 16S rRNA gene nucleotide sequence similarity values, pairwise alignments were performed using the EzTaxon server [13]. Pairwise nucleotide sequence similarities of the 16S rRNA gene indicated that the closest known relatives of strain FRIFI^T are *Romboutsia ilealis* (97.7 %), *R. lituseburensis* (97.6 %) and *R. sedimentorum* (96.6 %). Recently three other novel *Romboutsia* species have been proposed to allocate '*R. maritimum*' strain CCRI-22766^T [14], '*R. timonensis*' strain Marseille-P326 [15], and '*R. weinsteinii*' strain CCRI-19649 [16]. Based on the available 16S rRNA gene sequences, all three strains were found to be highly similar to validly described *Romboutsia* species (>97 %). However, thus far the names for the species '*R. maritimum*', '*R. timonensis*' and '*R. weinsteinii*' have not been validly published and therefore presently have no standing in the literature, and a thorough characterization should be performed for these isolates to justify their position as type strains of novel species. Therefore '*R. maritimum*', '*R. timonensis*' and '*R. weinsteinii*' were not included in the *Romboutsia* species comparisons described here.

To further support the novel status of strain FRIFI^T, genotypic characterization was carried out. For DNA–DNA hybridization (DDH) analyses, strain FRIFI^T and *R. ilealis* CRIB^T were grown overnight at 37 °C, and cells were sent to the Leibniz-Institute DSMZ – German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). Cells were disrupted using a French press, and the DNA in the crude cell lysate was purified by chromatography on hydroxyapatite as described by Cashion *et al.* [17]. DDH was carried out as previously described [18] under consideration of the modifications described by Huss *et al.* [19]. Strain FRIFI^T showed low DNA–DNA relatedness (mean % reassociation ±SD, n=2) to *R. ilealis* CRIB^T (13.9 ±3.3 %). This value is well below the threshold of 70 % for species delineation that was recommended by [20] and thereby confirms that strain FRIFI^T represents a novel species.

Whole genome-based comparisons have demonstrated their potential for phylogenetic analyses, as was recently shown by a phylogenomic analysis of the family *Peptostreptococcaceae* [21]. Whole genome sequencing of strain FRIFI^T (BioProject PRJEB7106), *R. ilealis* CRIB^T (BioProject PRJEB4727) and *R. lituseburensis* DSM 797^T (BioProject PRJEB7306) was carried out as described elsewhere [22]. Based on the whole genome sequence of strain FRIFI^T, the genomic DNA G+C content of strain FRIFI^T was determined to be 27.8 mol%, which is similar to the values determined for the other *Romboutsia* species (Table 1). Average nucleotide identity (ANI) values were calculated with JSpecies version 1.2.1 [23] by pairwise comparisons of the complete genomes. In all cases the ANI values for pairwise comparisons of the different *Romboutsia* strains amounted to 78.4–79.1 %, which is well below the cut-off point of 95–96 % that is generally accepted to be the ANI threshold for species delineation [23–25], further affirming the novel species assignment of *R. hominis* FRIFI^T. Based on 16S rRNA gene sequence and whole genome-based comparisons we conclude that *R. hominis* FRIFI^T represents a novel species within the genus *Romboutsia*.

To further support the description of strain FRIFI^T as type strain of a novel species, more detailed phenotypic characterization was performed. Unless indicated otherwise, morphological, physiological and chemotaxonomic studies were performed with cells grown on liquid anoxic basal peptone–yeast extract (PY) medium [10] supplemented with 0.5 % (w/v) glucose at 37 °C (pH 7.0), further referred to as PYG medium.

Cell morphology of strain FRIFI^T was examined using a phase-contrast microscope (DM2000, Leica Microsystems) at ×1000 magnification, with cells grown for 6 h at 37 °C in liquid CRIB or DSMZ 104b medium (Fig. S1, available in the online version of this article). Gram-staining was performed using a four-step Gram stain kit (BD) on cells from

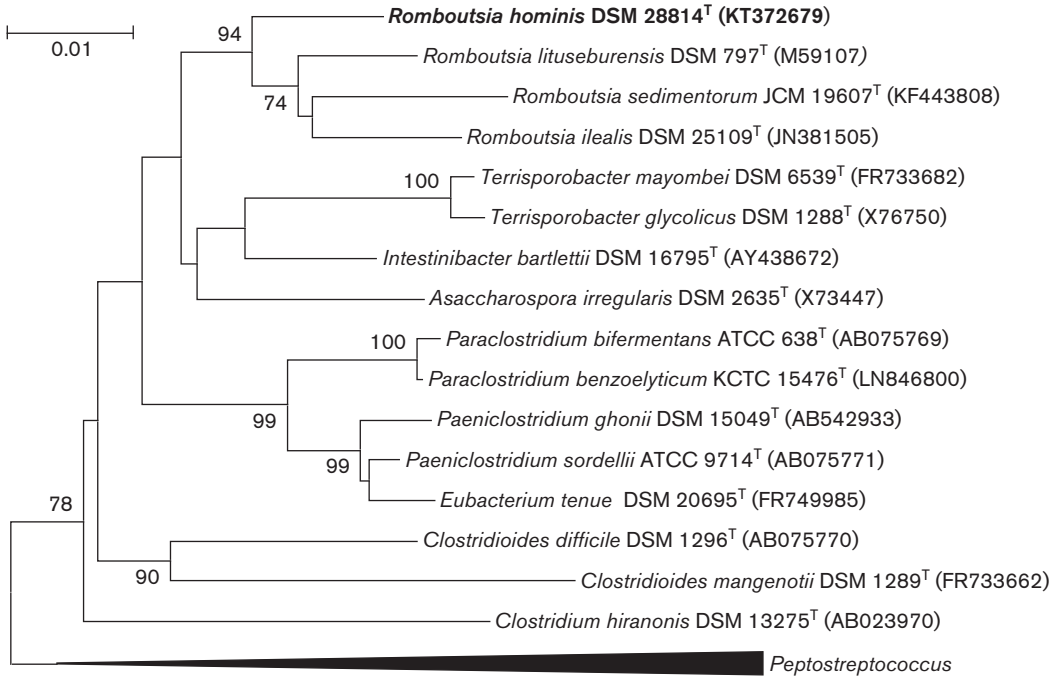

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences, showing the phylogenetic position of strain FRIFI^T compared to other members of the genus *Romboutsia* and some other members of the family *Peptostreptococcaceae*. The 16S rRNA gene sequences were aligned using the SINA aligner [11]. The tree was reconstructed using MEGA6 software [12] with Kimura's two-parameter model as substitution model. Only bootstrap values ≥70 % are shown at branch nodes. Bootstrap values were calculated based on 1000 replications. The reference bar indicates 1 % sequence divergence. GenBank accession numbers are given in parentheses. The tree was rooted using the 16S rRNA gene sequences of *Peptostreptococcus* species, representing the type genus of the family *Peptostreptococcaceae*.

active cultures. Cells were examined using a light microscope at ×1000 magnification (Primo Star, Carl Zeiss MicroImaging) and stained Gram-positive. For induction of sporulation, the strain was grown in Duncan–Strong medium [26] and Difco cooked meat medium (BD). Sporulation was not observed in any condition, also not after extended incubation times of up to 2 months, and no viable cells could be recovered from cultures exposed to 80 °C for 10 min. In addition, endospore staining performed according to Schaeffer–Fulton's method [27] using 5 % (w/v) malachite green and 2 % safranin as counterstain, did not reveal sporulating cells or free spores.

Colony morphology was examined after 24 and 72 h of growth at 37 °C under anoxic conditions on solid DSMZ 104b medium containing 0.8 % gelrite. After 24 h incubation surface colonies were white-grey in colour, 1–2 mm in diameter, with a translucent margin and small convex elevation. Colonies were mucoid and circular in form with an undulate margin. After 72 h, the colonies were larger (2–4 mm), and they appeared flat.

Cell motility of strain FRIFI^T was examined by detection of turbidity throughout stab-inoculated tubes containing semi-solid DSMZ 104b medium after 72 h of growth at 37 °C. Growth in this medium resulted in abundant gas production, as visible by the cracks in the agar occurring after 72 h of growth. Strain FRIFI^T was demonstrated to be motile, since growth was observed extending from the stab line (Table 1). Cell motility was confirmed by examining the growth of strain FRIFI^T in semi-solid SIM medium [28]. Semi-solid SIM medium consisted of (per litre distilled water): 20 g tryptone, 6.1 g peptone, 0.2 g ferrous ammonium sulfate, 0.2 g sodium thiosulfate and 0.4 % (w/v) Gelrite. After 5 h of incubation at 37 °C growth extending from the stab line was observed. In addition, complete blackening of the medium was observed after 24 h indicating abundant sulphide production.

Substrate utilization properties of strain FRIFI^T were examined using the API 50 CH and API 20 A systems (bioMérieux) according to the manufacturer's instructions except that liquid PY medium was used for inoculation, and the strips were incubated anoxically. *R. ilealis* CRIB^T and *R. lituseburensis* DSM 797^T were used for comparison. In addition to the API tests, substrate utilization of strain FRIFI^T was confirmed by adding one of the following compounds to liquid PY medium in culture bottles to a final concentration of 0.5 % (w/v) incubated under anoxic conditions: D-fructose, L-fucose, D-galactose, D-glucose, glycerol, maltose, D-ribose and sucrose. Growth was determined

Table 1. Differential characteristics of strain FRIFI^T and the type strains of other *Romboutsia* species

Strains: 1, *Romboutsia hominis* sp. nov. FRIFI^T; 2, *R. ilealis* CRIB^T; 3, *R. lituseburensis* DSM 797^T; 4, *R. sedimentorum* LAM201^T. +, Positive; w, moderately positive; –, negative; ND, no data available. Data were obtained in this study, unless indicated otherwise. With the API 50 CH and API 20 A systems (bioMérieux), strains 1, 2 and 3 were negative for growth on n-acetylglucosamine, D-adonitol, starch, amygdalin, L-arabinose, D-arabitol, L-arabitol, arbutin, cellobiose, dulcitol, erythritol, D-fucose, gentiobiose, glycogen, inositol, inulin, lactose, D-lyxose, D-mannitol, D-mannose, melezitose, melibiose, methyl α-D-glucopyranoside, methyl α-D-mannopyranoside, methyl β-D-xylopyranoside, potassium 2-ketogluconate, potassium 5-ketogluconate, potassium gluconate, L-rhamnose, salicin, L-sorbose, D-tagatose, turanose, xylitol, D-xylose and L-xylose, catalase and urease activity, and indole formation.

Characteristic	1	2	3	4
Cell size:				
Width (μm)	1.1–2.1	1.0–2.0[a]§	1.4–1.7[b]	1.2–2.0[c]
Length (μm)	2.7–16.3	1.0–5.3[a]	3.1–6.3[b]	2.3–10.0[c]
Motility	+	–[a]	+[a]	–[c]
Gelatin hydrolysis	–	–[a]	+[a]	–[c]
Acid produced from (API systems):				
D-Arabinose	–	w[a]	–[a]	–[c]
D-Fructose	+	–[a]	+[a]	+
L-Fucose	–	+[a]	–[a]	ND
D-Galactose	–	w[a]	–[a]	–[c]
D-Glucose	+	+[a]	+[a]	+[c]
Glycerol	+	–[a]	W	ND
Maltose	–	–[a]	+[a]	+[c]
raffinose	–	+[a]	–[a]	ND
D-ribose	–	–[a]	+[a]/–	–[c]
D-sorbitol	–	–[a]	–[a]	+[c]
Sucrose	–	+[a]	+[a]	–[c]
Trehalose	–	–[a]	–[a]	+[c]
Products from PYG*	A, F, l	A, F, l (p)[a]	A, F, l, p (b, iv) [a]‡	A, ib, iv[c]
Polar lipids†	5 PL, 6 GL	5 PL, 5 GL	5 PL, 6 GL, 2 PN	4 GL, 5 PL[c]
Predominant cellular fatty acids	$C_{16:0}$	$C_{16:0}$	$C_{16:0}$	$C_{16:0}$[c]
		$C_{17:0}$	$C_{17:0}$	$C_{18:0}$
			$C_{18:1}\omega7c$	
DNA G+C content (mol%)	27.8	28.1[a]/27.9[d]	27[b]/28.2	32[c]

*Products (listed in the order usually detected): A, acetic acid; B, butyric acid; F, formic acid; IB, iso-butyric acid, IV, iso-valeric acid, L, lactic acid; P; propionic acid. Upper case letters indicate major components, lower case minor components (<than 20 % of total measured metabolic end product production). Products in parentheses are not detected uniformly.

†GL, Glycolipid; PL, phospholipid; PN, phosphoaminolipid.

‡Butyric acid and iso-valeric acid are produced in the presence of casamino acids.

§Data were taken from: *a*, Gerritsen *et al.* [1]; *b*, Rainey *et al.* [34]; *c*, Wang *et al.* [2]; *d*, Gerritsen *et al.* [22].

spectrophotometrically by measuring optical density at 600 nm. Growth on a substrate was defined by comparing the increase in OD_{600nm} in liquid PY medium with additional substrate to the increase in OD_{600} in liquid PY medium lacking additional substrate (control). A more than twofold increase in OD_{600nm} over the control was considered to reflect growth on the substrate. The carbohydrate utilization pattern of strain FRIFI^T was clearly different from that of *R. ilealis* CRIB^T, *R. lituseburensis* DSM 797^T and *R. sedimentorum* LAM201^T (Table 1). Strain FRIFI^T was able to utilize D-fructose, D-glucose and glycerol similarly to *R. lituseburensis* DSM 797^T. However, strain FRIFI^T was not able to utilize maltose, D-ribose and sucrose, carbohydrates that *R. lituseburensis* DSM 797^T is able to utilize.

For analysis of metabolites produced during growth, strain FRIFI^T was grown in liquid PYG medium. Growth was determined spectrophotometrically by measuring optical density at 600 nm. Metabolites (including short chain fatty acids) were analysed by high-performance liquid chromatography using a Metacarb 67 h column (Varian). Acetate, butyrate, ethanol, formate, lactate, propionate, iso-butyrate, valerate, succinate, 1,2-propanediol and 1,3-propanediol were identified and quantified with standard curves prepared for each individual compound. Metabolites produced during growth in PYG medium were (within 24 h of growth) acetate (20 mM), formate (10 mM), ethanol (11 mM) and small amounts of lactate (1 mM).

Table 2. Cellular fatty acid profiles of strain FRIFI^T and the type strains of other *Romboutsia* species

Strains: 1, *Romboutsia hominis* sp. nov. FRIFI^T; 2, *R. ilealis* CRIB^T; 3, *R. lituseburensis* DSM 797^T; 4, *R. sedimentorum* LAM201^T. Fatty acid methyl esters (and other components that included aldehyde and dimethyl acetal cleavage products of plasmalogen containing lipids) were separated by GC and detected by flame ionisation, using the MIDI Sherlock Microbial Identification System (MIS) and the Anaerobic Bacteria Library (MOORE6) for peak identification. Data are presented as percentages of the total fatty acid content. Only fatty acids with an abundance >1.5 % in at least one of the strains, are shown. For each strain the predominant fatty acid(s) (≥10 %) are indicated in bold type. All strains were grown in DSM medium 104b at 37 °C and cells were harvested in mid-exponential to end-exponential phase. DSM medium 104b does not contain either Tween (80) or animal serum, both of which may be sources of 18 : 1ω9*c* in strains grown in the presence of these materials.

Fatty acid	1	2*	3*	4
Saturated straight-chains:				
$C_{12:0}$	0.7	1.6/2.2	0.6/0.8	2.4
$C_{14:0}$	3.2	3.8/3.6	1.5/3.2	5.4
$C_{15:0}$	2.8	6.2/7.1	5.5/6.4	4.2
$C_{16:0}$	**34.9**	**31.3/21.9**	**10.7/19.0**	**26.7**
$C_{17:0}$	5.5	**10.0/10.1**	**16.8/16.3**	8.0
$C_{18:0}$	9.7	6.6/4.1	4.6/6.8	**11.2**
Unsaturated straight-chains:				
$C_{16:1}\omega7c$	4.9	9.1/9.3	4.1/3.1	3.7
$C_{16:1}\omega9c$	9.0	6.5/7.7	4.0/4.7	4.5
$C_{17:1}\omega6c$	–	3.3/5.8	7.1/4.1	0.6
$C_{18:1}\omega5c$	3.0	0.7/1.1	–	0.2
$C_{18:1}\omega9c$	6.4	3.4/2.9	8.1/7.8	7.4
$C_{19:1}\omega12c$	–	–	2.1/1.3	–
Cyclopropane:				
C_{17} cyclopropane	4.7	–	–	–
C_{19} cyclopropane 9,10	2.5	0.8/1.5	1.6/0.9	–
C_{19} cyclopropane 11,12	2.6	–	–	–
Unknown†:				
Summed feature 4 ($C_{15:1}\omega8c$)‡	0.5	1.1/2.7	1.6/1.0	0.2
Summed feature 7§	1.7	–	7.9/6.0	4.8
Summed feature 8‖	1.2	5.3/9.0	8.9/4.7	0.3
Summed feature 10 ($C_{18:1}\omega7c$)¶	5.0	8.3/7.6	**10.7**/9.1	**10.0**
Total	**98.2**	**98.0/96.5**	**95.9/95.2**	**89.5**

*Data obtained from Gerritsen *et al.* [1]/this study.

†Summed features represent groups of two or more fatty acids that are grouped together for the purpose of evaluation by the MIDI system. In some cases peaks may be identified on the basis of their separate equivalent chain length (ECL) while in other cases the ECLs are almost identical, making an unambiguous identification difficult.

‡Listed as summed feature 4 comprising $C_{15:1}\omega8c$, $C_{15:2}$ and/or an unknown $C_{15:2}$, however the ECL indicates that the main peak is $C_{15:1}\omega8c$.

§Listed as summed feature 7 comprises $C_{17:1}\omega9c$ and/or $C_{17:2,}$ the ECLs are sufficiently close together making an unambiguous identification difficult, however biochemical considerations suggest that the synthesis of a $C_{17:2}$ may require oxygen.

‖Listed as summed feature 8 comprising $C_{17:1}\omega8c$ and/or $C_{17:2}$, the ECLs are sufficiently close together making an unambiguous identification difficult, however biochemical considerations suggest that the synthesis of a $C_{17:2}$ may require oxygen.

¶Listed as summed feature 10 comprising $C_{18:1}\omega7c$ and/or an unknown fatty acid, however the ECL indicates that the main peak is $C_{18:1}\omega7c$.

Chemotaxonomic characterization of strain FRIFI^T included analysis of the polar lipid profile and cellular fatty acid composition of strain FRIFI^T in comparison to closely related *Romboutsia* species (performed by the Leibniz-Institute DSMZ). The following strains were grown under identical conditions in liquid DSMZ 104b medium: strain FRIFI^T, *R. ilealis* CRIB^T and *R. lituseburensis* DSM 797^T. Cells were harvested in mid-exponential to end-exponential phase. For determination of cellular fatty acid composition, fatty acid methyl esters were obtained from fresh cells by saponification, methylation and extraction using minor modifications of previously described methods [29, 30]. Fatty acid methyl ester mixtures were separated by gas chromatography (GC) and analysed using the Sherlock Microbial Identification System (MIS) as previously described [31]. Peaks were automatically integrated, and fatty acid identification (using the MOORE6 peak naming database) and relative concentrations were calculated. For polar lipid analysis, polar lipids were extracted from lyophilized biomass using the two-stage method described by Tindall [32]

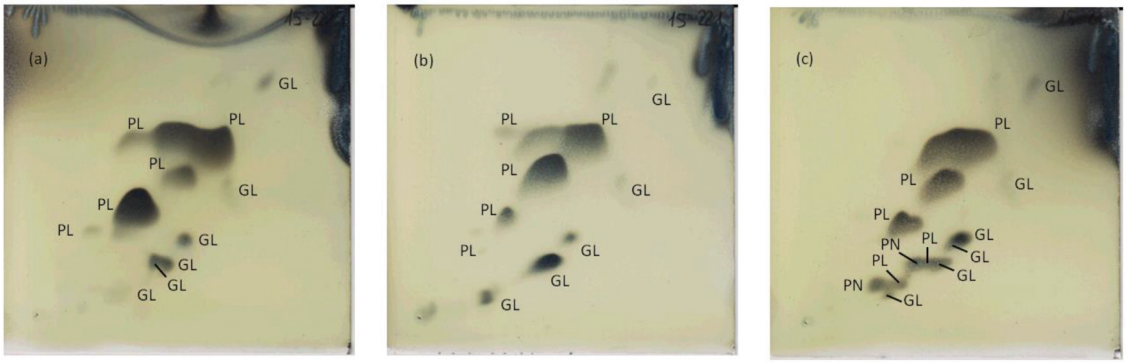

Fig. 2. Two-dimensional thin-layer chromatograms showing total polar lipid profiles of (a) *Romboutsia hominis* sp. nov. FRIFI^T, (b) *R. ilealis* CRIB^T and (c) *R. lituseburensis* DSM 797^T. Separation of the polar lipids was performed by two-dimensional silica gel TLC, with chloroform–: methanol–water (65 : 25 : 4, v/v/v) in the first direction, and chloroform–methanol–acetic acid–water (80 : 12 : 15 : 4, v/v/v/v) in the second direction. Plates were stained with 5 % ethanolic molybdophosphoric acid to show all lipids. Polar lipids were labelled according to their staining behaviour. GL, glycolipid; PL, phospholipid; PN, phosphoaminolipid.

and separated by two-dimensional silica gel thin-layer chromatography (TLC). All polar lipids were detected by spraying the plates with 5 % ethanolic molybdophosphoric acid followed by heating, while head groups were detected using specific staining reagents as described previously by Tindall [32]. Since chromatography conditions were identical, comparison of the TLC plates was possibly and spots were labelled according to their staining behaviour and R_f value.

The genus *Romboutsia* was previously found to be characterized by the predominance of straight-chain saturated and unsaturated fatty acids (mainly C_{16}-C_{17}) and the absence of branched chain fatty acids, dimethyl acetals and aldehydes [1]. For strains *R. ilealis* CRIB^T and *R. lituseburensis* DSM 797^T similar cellular fatty acid profiles were found as reported previously (Table 2). For strain FRIFI^T the major cellular fatty acids were found to be C_{14}-C_{19} straight-chain saturated and unsaturated fatty acids, with $C_{16:0}$ being the predominant fatty acid. Compared to other *Romboutsia* species, a higher diversity of cyclopropane fatty acids was found [1, 2].

Recently, a detailed analysis of the cellular lipids of three *Romboutsia* species was published by Guan *et al.* [33]. Many similarities were found in the cellular lipid profiles of strain FRIFI^T, *R. ilealis* CRIB^T and *R. lituseburensis* DSM 797^T with the absence of plasmologens being one of one the main characteristics, which can be used to distinguish *Romboutsia* species from related *Clostridium* species. The polar lipid profile of strain FRIFI^T was found to be the most complex of the three *Romboutsia* species analysed. Tetrahexosyldiacylglycerol and acyltetrahexosyldiacylglycerol were found in strain FRIFI^T and *R. ilealis* CRIB^T, but were found to be absent in *R. lituseburensis* DSM 797^T. In addition, lysyl-phosphatidylglycerol was detected in FRIFI^T and *R. lituseburensis* DSM 797^T, but was found to be absent in *R. ilealis* CRIB^T. The total polar lipid analysis performed by the Leibniz-Institute DSMZ confirmed the highly similar total polar lipid profiles of strain FRIFI^T, *R. ilealis* CRIB^T and *R. lituseburensis* DSM 797^T characterized by the presence of several phospholipids and glycolipids (Fig. 2). These profiles were similar to those previously reported for *R. ilealis* CRIB^T and *R. lituseburensis* DSM 797^T [1], with as main difference the spots identified as a phosphoaminolipid in *R. lituseburensis* DSM 797^T. As also emphasised by Guan *et al.* [33] it should be noted that a cellular lipid analysis provides only a snapshot of cells grown in a single medium and harvested at one time, and the relative amounts of the lipids will undoubtedly change when cells are grown under different conditions and harvested at different stages of growth.

Based on phenotypic and genetic characterization we conclude that strain FRIFI^T, a rod-shaped organism isolated from ileostoma effluent of a female human volunteer, clearly represents a novel species.

DESCRIPTION OF *ROMBOUTSIA HOMINIS* SP. NOV.

Romboutsia hominis (ho′ mi.nis. L. gen. n. *hominis* of a man, the host from which the species was first isolated).

Cells are obligately anaerobic, motile rods. Typical cells are 1.1–2.1 µm×2.7–16.3 µm in size and occur primarily in single cells and pairs, however chains are observed as well especially during incubation in PYG medium. Cells stain Gram-positive. Sporulation is not observed. Growth occurs both on liquid and solid CRIB, PYG or DSMZ 104b medium. Surface colonies on solid DSMZ 104b medium, incubated under anoxic conditions for 24 h, are white or light grey, circular, 1–2 mm in diameter with a mucoid surface, a moist texture, an undulate and translucent margin, and small convex elevation. After incubation for 72 h, the colonies are larger (2–4 mm), and they appear flattened with strong undulate margins.

Weak growth is observed on yeast extract and bacterial peptone as the sole carbon source. Strain FRIFI^T is able to grow on a limited number of carbohydrates. In the API 50 CH and API 20 A systems, acid is produced from D-fructose, D-glucose and glycerol. No acid is produced from N-acetylglucosamine, D-adonitol, starch, amygdalin, L-arabinose, D-arabinose, D-arabitol, L-arabitol, arbutin, cellobiose, dulcitol, erythritol, L-fucose, D-fucose, D-galactose, gentiobiose, glycogen, inositol, inulin, lactose, D-lyxose, maltose, D-mannitol, D-mannose, melezitose, melibiose, methyl α-D-glucopyranoside, methyl α-D-mannopyranoside, methyl ß-D-xylopyranoside, potassium 2-ketogluconate, potassium 5-ketogluconate, potassium gluconate, raffinose, L-rhamnose, D-ribose, salicin, D-sorbitol, L-sorbose, sucrose, D-tagatose, trehalose, turanose, xylitol, D-xylose and L-xylose. The strain is negative for indole production, urease and catalase activity, gelatine and starch hydrolysis. Metabolic products produced from PYG are acetate, formate, ethanol and small amounts of lactate. The major cellular fatty acids of strain FRIFI^T are saturated and unsaturated straight-chain C_{12}-C_{19} fatty acids as well as cyclopropane fatty acids, with $C_{16:0}$ being the predominant fatty acid. Details of the fatty acid profile are provided in Table 2, with percentage compositions serving as a guide to the relative abundance of the different compounds. The polar lipids of strain FRIFI^T comprise five phospholipids and six glycolipids.

The type strain, FRIFI^T (=DSM 28814^T=KCTC 15553^T), was isolated from the ileostoma effluent of an otherwise healthy human volunteer at Wageningen University, The Netherlands. The genomic DNA G+C content of strain FRIFI^T is 27.8 mol%.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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