Characterization of *Romboutsia ilealis* gen. nov., sp. nov., isolated from the gastro-intestinal tract of a rat, and proposal for the reclassification of five closely related members of the genus *Clostridium* into the genera *Romboutsia* gen. nov., *Intestinibacter* gen. nov., *Terrisporobacter* gen. nov. and *Asaccharospora* gen. nov.

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A Gram-positive staining, rod-shaped, non-motile, spore-forming obligately anaerobic bacterium, designated CRIBT, was isolated from the gastro-intestinal tract of a rat and characterized. The major cellular fatty acids of strain CRIBT were saturated and unsaturated straight-chain C12–C19 fatty acids, with C16:0 being the predominant fatty acid. The polar lipid profile comprised six glycolipids, four phospholipids and one lipid that did not stain with any of the specific spray reagents used. The only quinone was MK-6. The predominating cell-wall sugars were glucose and galactose. The peptidoglycan type of strain CRIBT was A1s lantionine-direct. The genomic DNA G+C content of strain CRIBT was 28.1 mol%. On the basis of 16S rRNA gene sequence similarity, strain CRIBT was most closely related to a number of species of the genus *Clostridium*, including *Clostridium lituseburense* (97.2 %), *Clostridium glycolicum* (96.2 %), *Clostridium mayombei* (96.2 %), *Clostridium bartlettii* (96.0 %) and *Clostridium irregular* (95.5 %). All these species show very low 16S rRNA gene sequence similarity (<85%) to the type strain of *Clostridium butyricum*, the type species of the genus *Clostridium*. DNA–DNA hybridization with

Abbreviations: DDH, DNA–DNA hybridization; DMA, dimethyl acetal. ECL, equivalent chain length; MIS, microbial identification system; PY, peptone-yeast extract; PYG, peptone-yeast extract-glucose; PVX, PolyViteX; qPCR, quantitative PCR.

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The GenBank/EMBL/DDJB accession number for the 16S rRNA gene sequence of strain CRIBT is JN381505.

Four supplementary figures are available with the online version of this paper.
The gastro-intestinal tract of both humans and animals contains an enormous diversity of microbial species (Gerritsen et al., 2011a; Ley et al., 2008), of which many still remain to be cultured and characterized. Here we describe a novel isolate obtained from the gastro-intestinal tract of rats. This isolate is the first representative of a novel bacterial phylotype (referred to as ‘CRIB’), which was recently reported by Gerritsen et al. (2011b) to be associated with probiotic-induced changes in the gut microbiota of rats in an experimental model for acute pancreatitis. To determine the taxonomic position of this isolate, it was subjected to further characterization in accordance with the recommendations provided by Kämpfer et al. (2003) and Tindall et al. (2010).

Strain CRIB\textsuperscript{T} was isolated from an ileal digesta sample obtained from a healthy Sprague–Dawley rat that was selected based on high relative abundance of the target phylotype as determined using quantitative PCR and the primer set CRIB-61F/CRIB-235R as described previously (Gerritsen et al., 2011b). Isolation was performed by serial dilution of the sample in liquid anoxic bicarbonate-buffered medium, further referred to as CRIB medium. CRIB medium consisted of a basal bicarbonate-buffered medium, further referred to as CRIB medium. CRIB medium consisted of a basal bicarbonate-buffered medium (Stams et al., 1993) supplemented with (per litre distilled water): 30 g bacteriological peptone, 5 g yeast extract, 5 g beef extract, 4 g glucose, 1 g cellobose, 1 g maltose, 1 g soluble starch, 0.5 g L-cysteine hydrochloride, 0.4 g bile salts, 0.25 mg haemin, 0.0001 % (v/v) vitamin K\textsubscript{1} and 0.5 % (v/v) clarified, sterile rumen fluid. The final pH of the medium was pH 7.0. The increase in relative abundance of the target phylotype was followed in the subsequent serial dilutions using quantitative PCR as described previously (Gerritsen et al., 2011b). After primary isolation, accomplished by repeated rounds of fast transfers (within 24 h), the strain was purified by repeated inoculation and subculturing on solid CRIB medium [containing 1.5 % (w/v) Bacto agar (BD)] using the anaerobic roll tube method. The purified strain was cultured routinely in liquid CRIB medium at 37 °C (pH 7.0), and stored as a glycerol suspension (25 %, v/v) at −80 °C. Unless indicated otherwise, morphological, physiological, molecular and chemotaxonomic studies were performed with cells grown on liquid anoxic basal peptone-yeast extract (PY) medium (Holdeman et al., 1977) supplemented with 0.5 % (w/v) glucose at 37 °C (pH 7.0), further referred to as PYG medium. Solid PYG or CRIB medium was prepared by supplementation of liquid medium with 0.8 % Gelrite (Carl Roth).

Total DNA was extracted for phylogenetic analysis of strain CRIB\textsuperscript{T} using the FastDNA SPIN kit for Soil (MP Biomedicals). The 16S rRNA gene was amplified by PCR using the universal primers 8F (Lane, 1991) and 1492R (Suzuki et al., 2000), and PCR products were purified using the High Pure PCR Cleanup Micro kit (Roche Diagnostics). In order to obtain an almost complete 16S rRNA gene sequence of strain CRIB\textsuperscript{T}, purified PCR products were cloned in Escherichia coli using the pGEM-T easy vector system (Promega) according to the manufacturer’s instructions. Plasmid DNA was isolated from two transformant cultures using the QIAprep Spin Miniprep kit (Qiagen) and used as template for sequence analysis with an ABI 3730XL DNA analyser (BaseClear) using insert-flanking T7 and SP6 promoter-targeted primers (Promega). In order to merge the sequences of the two clones and the different primers and to identify sequencing errors in individual reads, sequences were aligned using tools available in the DNA STAR package, and the alignment was corrected manually. An almost complete 16S rRNA gene sequence (1466 bp) of strain CRIB\textsuperscript{T} was obtained. The 16S rRNA gene sequence of strain CRIB\textsuperscript{T} and other members of the family Peptostreptococcaceae were aligned using the SINA aligner (http://www.arb-silva.de/aligner/) (Pruesse et al., 2012). Phylogenetic trees were reconstructed using MEGA 6 software (Tamura et al., 2013). Clustering was determined with the neighbour-joining method and bootstrap values were calculated based on 1000 replications. Tree topology was also confirmed using maximum-likelihood and maximum-parsimony methods. For pairwise 16S rRNA gene nucleotide sequence alignments, the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/) was used (Kim et al., 2012) to generate the pairwise similarity values.

Pairwise nucleotide sequence similarities of the 16S rRNA gene indicated that the closest known relatives of strain CRIB\textsuperscript{T} are Clostridium lituseburensense (97.2 %), Clostridium glycolicum (96.2 %), Clostridium mayombei (96.2 %), Clostridium bartlettii (96.0 %) and Clostridium irregularare (95.5 %). However, further phylogenetic analyses indicated that strain CRIB\textsuperscript{T} together with the species listed above closely related reference strains indicated reassociation values below 32 %. On the basis of phenotypic and genetic studies, a novel genus, Romboutsia gen. nov., is proposed. The novel isolate CRIB\textsuperscript{T} (=DSM 25109\textsuperscript{T} =NIZO 4048\textsuperscript{T}) is proposed as the type strain of the type species, Romboutsia ilealis gen. nov., sp. nov., of the proposed novel genus. It is proposed that C. lituseburensense is transferred to this genus as Romboutsia lituseburensis comb. nov. Furthermore, the reclassification into novel genera is proposed for C. bartlettii, as Intestinibacter bartlettii gen. nov., comb. nov. (type species of the genus), C. glycolicum, as Terrisporobacter glycolicus gen. nov., comb. nov. (type species of the genus), C. mayombei, as Terrisporobacter mayombei gen. nov., comb. nov., and C. irregularare, as Asaccharospora irregularis gen. nov., comb. nov. (type species of the genus), on the basis of additional data collected in this study. In addition, an emendation of the species Peptostreptococcus anaerobius and the order Eubacteriales is provided.
were all members of the family Peptostreptococcaceae Ezaki 2010 (Fig. 1 and Figs S1 and S2 available in the online Supplementary Material). It is recognized that species belonging to the genus Clostridium form a large and taxonomically heterogeneous group. Many species were originally assigned to this genus on the basis of a relatively small set of phenotypic characteristics such as Gram-positive staining, rod-shape, anaerobic growth and the ability to form spores (Gottschalk et al., 1981; Rainey et al., 2009). However, this has resulted in the expansion of the genus Clostridium to more than 200 species, forming a very heterogeneous taxon that is long overdue for taxonomic rearrangements (Collins et al., 1994; Rainey et al., 2009; Stackebrandt et al., 1999; Wiegel et al., 2006; Yutin & Galperin, 2013). This is clearly reflected by the fact that many of the current species of the genus Clostridium show less than 90 % 16S rRNA gene sequence similarity to the type strain of the type species of the genus, Clostridium butyricum (Collins et al., 1994; Rainey et al., 2009). Strain CRIB\textsuperscript{T} and its closest known relatives also have low 16S rRNA gene sequence similarities (<85 %) to the type strain of C. butyricum. It has been well known for decades that there are problems with the taxonomic structure of the genus Clostridium, and important steps have already been undertaken to clarify some of these issues. Collins and colleagues have proposed the grouping of the presently known species of the genus Clostridium into a number of distinct phylogenetic clusters based on a large-scale comparison of 16S rRNA gene sequences (Collins et al., 1994). These clusters also include species from a range of other genera, indicating that the taxonomy of the genus Clostridium is not unambiguous. It has been proposed that only species classified into cluster I are true members of the genus Clostridium (Clostridium sensu stricto) (Wiegel et al., 2006). However, this is problematic since this also includes the genus Sarcina, and this name would have priority over the genus name Clostridium if all members of cluster I were to be placed in a single genus (Willems & Collins, 1994). Some of the species previously classified in the genus Clostridium have been transferred to novel genera within the family Clostridiaceae and to novel families within the phylum Firmicutes (Wiegel, 2009). However, many species of the genus Clostridium still remain misclassified if one considers that the genus Clostridium should be restricted as proposed by Collins et al. (1994). This also holds true for members of Clostridium cluster XI (Collins et al., 1994), including the closest known relatives of strain CRIB\textsuperscript{T}, which although formally not currently assigned to the family Peptostreptococcaceae Ezaki 2010 (Ezaki, 2009, 2010) group with the members of this family based on the 16S rRNA gene sequence analysis (Fig. 1); the classification of the family Peptostreptococcaceae will have to be further revised in the future.

In order to substantiate that strain CRIB\textsuperscript{T} represents a novel species, the closest known relatives of strain CRIB\textsuperscript{T}, defined by >95 % 16S rRNA gene sequence similarity, were included for further taxonomic characterization. Strain CRIB\textsuperscript{T} and the type strains of five species of the genus Clostridium (C. lituseburense DSM 797\textsuperscript{T}, C. bartlettii DSM 16795\textsuperscript{T}, C. glycycinum DSM 1288\textsuperscript{T}, C. mayonii DSM 6539\textsuperscript{T} and C. irregularis DSM 2635\textsuperscript{T}) were also compared to the type strain of the type species of the type genus of the family Peptostreptococcaceae, Peptostreptococcus anaerobius DSM 2949\textsuperscript{T}, in order to investigate whether these species should be included in the genus Peptostreptococcus. This study represents a step further in the clarification of the taxonomy at the genus level of a small number of members of the class Clostridia.

To further support the description of strain CRIB\textsuperscript{T} as representative of a novel species, genotypic characterization was carried out by the Leibniz-Institut DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany). Cells of strain CRIB\textsuperscript{T} were cultivated in liquid CRIB medium for 15 h at 37 °C for analysis of the genomic DNA G+C content and for DNA–DNA hybridizations (DDH). For DDH experiments, the type strains of three phylogenetically related and presumably misclassified species of the genus Clostridium [C. lituseburense (Romboutsia lituseburensis comb. nov.) DSM 797\textsuperscript{T}, C. bartlettii (Intestinibacter bartlettii comb. nov.) DSM 16795\textsuperscript{T} and C. irregularis (Asacharospora irregularis comb. nov.) DSM 2635\textsuperscript{T}] were obtained from the Leibniz-Institut DSMZ and cultured in the media suggested in the Leibniz-Institut DSMZ online catalogue (www.dsmz.de). Cells were disrupted by using a French pressure cell (Thermo Spectronic), and the DNA in the crude cell lysate was purified by chromatography on hydroxypatite as described by Cashion et al. (1977). The genomic DNA G+C content was determined by HPLC according to the method of Mesbah et al. (1989). The genomic DNA G+C content of strain CRIB\textsuperscript{T} was determined to be 28.1 mol%, which demonstrates strain CRIB\textsuperscript{T} is a bacterium with a low G+C content. DDH experiments were carried out at 61 °C by the liquid renaturation method as described previously (De Ley et al., 1970) and as modified by Huss et al. (1983). Strain CRIB\textsuperscript{T} showed low DNA–DNA relatedness (mean percent reassociation ± SD, n=2) to the following type strains: 15.5 ± 0.8 % with C. lituseburense (R. lituseburensis comb. nov.) DSM 797\textsuperscript{T}, 20.4 ± 3.5 % with C. bartlettii (I. bartlettii comb. nov.) DSM 16795\textsuperscript{T} and 18.1 ± 1.3 % with C. irregularis (A. irregularis comb. nov.) DSM 2635\textsuperscript{T}. These reassociation values were well below the cut-off point of 70 % for species delineation that was recommended by Wayne et al. (1987) and thereby confirm that strain CRIB\textsuperscript{T} represents a novel species. This is also consistent with the 16S rRNA gene sequence similarity values where more recent work has indicated that a 16S rRNA gene sequence similarity of 98.2–99.0 % may be a more appropriate threshold above which DDH experiments should be carried out than the previously used value of 97 % (Meier-Kolthoff et al., 2013; Stackebrandt & Ebers, 2006).

Cell morphology of strain CRIB\textsuperscript{T} was examined using a phase-contrast microscope (DM2000, Leica Microsystems) at ×1000 magnification, with cells grown for 24 h or 48 h.
Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequence data, showing the phylogenetic position of strain CRIBT and other (misclassified) members of the family Peptostreptococcaceae. The 16S rRNA gene sequences were aligned using the SINA aligner (http://www.arb-silva.de/aligner/). The phylogenetic tree was reconstructed using MEGA 6 software (Tamura et al., 2013) with Kimura’s two-parameter model as the substitution model. Only bootstrap values $\geq 70\%$ are shown at branch nodes. Bar, 1% sequence divergence. GenBank accession numbers are given in parentheses. The 16S rRNA gene sequence from Clostridium butyricum ATCC 19398T (accession no. AB075768), the type strain of the type species of the genus Clostridium, was used as an outgroup.
at 37 °C in liquid CRIB or PYG medium (Fig. S3a, b). Gram staining was performed using a 4-step Gram stain kit (BD) on cells from active cultures. Endospore staining was performed according to Schaeffer–Fulton’s method (Smibert & Krieg, 1994) using 5% (w/v) malachite green and 2% safranin as counterstain. Stained cells were examined using a light microscope at ×1000 magnification (Primo Star, Carl Zeiss MicroImaging). Sporulating cells that were visualized with the malachite green endospore staining were seen occasionally after prolonged incubation (Fig. S4). However, no free spores were observed, and no viable cells could be recovered from sporulating cultures exposed to 80 °C for 10 min. The use of media that has been previously demonstrated to be able to support the sporulation of species belonging to the class Clostridia (Brown et al., 1957; Duncan & Strong, 1968; Holdeman et al., 1977; Sacks & Thompson, 1977) did not result in an increased induction of sporulation in strain CRIB<sup>T</sup>. Although the process of sporulation appears to be initiated in strain CRIB<sup>T</sup>, it was not completed under the experimental conditions examined since free mature spores were not observed. This was distinct from <i>C. lituseburensis</i> (R. lituseburensis comb. nov.) DSM 797<sup>T</sup> that was able to sporulate in most of the media used and high numbers of free mature spores were observed (Fig. S4).

Transmission electron microscopy was carried out as previously described by van Niftrik et al. (2008). Cells of strain CRIB<sup>T</sup> were cryofixed by high-pressure freezing (Leica EMHPF, Leica Microsystems) and freeze-substituted in acetone containing 2% osmium tetroxide or 2% osmium tetroxide, 0.2% uranyl acetate and 1% water (Walther & Ziegler, 2002) in an automatic freeze-substitution unit (AFS, Leica Microsystems). The cells were then embedded in Epon resin (Mollenhauer, 1964), sectioned using an ultramicrotome (Reichert Ultracut E, Leica Microsystems) and post-stained with 20% uranyl acetate in 70% methanol for 4 min and Reynolds’ lead citrate staining (Reynolds, 1963) for 2 min. The ultrathin (70 nm) sections were investigated at 60–120 kV in a transmission electron microscope (Tecnai12, FEI). Images were recorded using a charge-coupled device (CCD) camera (MegaView II, Olympus) and the AnalySIS software (Olympus). Transmission electron micrographs confirmed the typical cell-wall morphology of Gram-positive-staining cells (Fig. S3c, d). No flagella or other cell-wall extensions were observed under these conditions with these methods.

Colony morphology was examined after 24 h and 72 h of growth at 37 °C under anoxic conditions on solid PYG or CRIB medium or commercial chocolate agar supplemented with PolyViteX (bioMérieux).

Cell motility of strain CRIB<sup>T</sup> was examined by detection of turbidity throughout stab-inoculated tubes containing semi-solid PYG medium (Leifson, 1960) after 72 h of growth at 37 °C. In addition, motility of strain CRIB<sup>T</sup> was compared to that of representatives of three closely related (misclassified) species of the genus Clostridium: <i>C. lituseburensis</i> (R. lituseburensis comb. nov.) DSM 797<sup>T</sup>, <i>C. bartlettii</i> (I. bartlettii comb. nov.) DSM 16795<sup>T</sup> and <i>C. irregularis</i> (A. irregularis comb. nov.) DSM 2635<sup>T</sup>. Cell motility was examined after 48 h of growth at 37 °C in semi-solid SIM medium. 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. In semi-solid PYG and SIM medium, growth of strain CRIB<sup>T</sup> was only observed in a distinct zone directly along the stab, indicating that CRIB<sup>T</sup> is non-motile. This was distinct from <i>C. lituseburensis</i> (R. lituseburensis comb. nov.) DSM 797<sup>T</sup> that was demonstrated to be motile with this method, since growth was observed extending from the stab line (Table 1).

Substrate utilization properties of strain CRIB<sup>T</sup> were compared to those of three closely related (misclassified) species of the genus Clostridium 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. <i>C. lituseburensis</i> (R. lituseburensis comb. nov.) DSM 797<sup>T</sup>, <i>C. bartlettii</i> (I. bartlettii comb. nov.) DSM 16795<sup>T</sup> and <i>C. irregularis</i> (A. irregularis comb. nov.) DSM 2635<sup>T</sup> were used for comparison.

In addition to the API tests, substrate utilization of strain CRIB<sup>T</sup> 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) and incubating under anoxic conditions: D-adonitol, D-arabinose, L-arabinose, D-arabitol, L-arabitol, cellobiose, erythritol, D-fructose, D-fucose, L-fucose, D-galactose, D-glucose, glycerol, glycogen, inositol, inulin, lactose, maltose, D-mannitol, D-mannose, melibiose, methyl x-D-glucopyranoside, methyl D-mannopyranoside, raffinose, L-rhamnose, D-ribose, sucrose, D-sorbitol, L-sorbose, trehalose, turanose, xylitol and L-xylene. In addition to these substrates, which were all also previously examined in the API 50 CH and API 20 A systems, utilization of cellulose, soluble starch and D-xylene (final concentration of 0.5%, w/v, for all substrates) was also examined. Growth was determined spectrophotometrically by measuring optical density at 600 nm. Growth on a substrate was defined by comparing the increase in OD<sub>600</sub> in liquid PY medium with additional substrate to the increase in OD<sub>600</sub> in liquid PY medium lacking additional substrate. A more than twofold increase in OD<sub>600</sub> was considered to reflect good growth, a 1.5- to twofold increase was considered to reflect moderate growth, and a one- to 1.5-fold increase over the control was considered to reflect weak growth. Acid formation was observed by measuring the pH of the media at regular intervals until 30 days of incubation. The carbohydrate utilization pattern of strain CRIB<sup>T</sup> was clearly different from that of <i>C. lituseburensis</i> (R. lituseburensis comb. nov.) DSM 797<sup>T</sup> and representatives of the other (misclassified) species of the genus Clostridium (Table 1). Strain CRIB<sup>T</sup> was able to utilize D-arabinose, L-fucose, D-galactose and raffinose, which are all carbohydrates that the other type strains were not able to utilize.

The metabolic end products produced during growth in liquid PYG medium (including short chain fatty acids)
Table 1. Differential characteristics of strain CRIB$^T$ and the type strains of the five species proposed to be reclassified in the novel genera *Romboutsia* gen. nov., *Intestinibacter* gen. nov., *Terrisporobacter* gen. nov. and *Asaccharospora* gen. nov., compared to those of the type strain of the type species of the genus *Clostridium*, *C. butyricum*.

Strains: 1, *Romboutsia ilealis* gen. nov., sp. nov. CRIB$^T$; 2, *Lituseburensae* (*R. lituseburensae* comb. nov.) DSM 797$^T$; 3, *Bartlettii* (*L. bartlettii* comb. nov.) DSM 16795$^T$; 4, *C. glycolicum* (*T. glycolicus* comb. nov.) DSM 1288$^T$; 5, *Mayomybei* (*T. mayomybei* comb. nov.) DSM 6539$^T$; 6, *C. irregularare* (*A. irregularis* comb. nov.) DSM 2635$^T$; 7, *C. butyricum* DSM 10702$^T$. +, Positive; −, negative; ND, no data available. For strains 1, 2, 5 and 6, data were obtained in this study, unless indicated otherwise. With the API 50 CH and API 20 A systems (bioMérieux), strains 1, 2, 5 and 6 were negative for growth on D-adenitol, starch, amygdalin, L-arabinose, D-arabitol, L-arabitol, arbutin, cellobiose, dulcitol, erythritol, D-fucose, glycerol, glycophenen, inositol, inulin, lactose, D-lyxose, D-mannose, melezitose, melibiose, methyl a-D-glucopyranoside, methyl a-D-mannopyranoside, methyl butyl glycerol, potassium 2-ketogluconate, potassium 5-ketogluconate, potassium gluconate, L-rhamnose, salicin, D-sorbose, trehalose, turanose, xylitol, D-xylose and L-xylose, catalase and urease activity, and indole formation.

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<td>Products from PYG$§$</td>
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<td>A, F, I, p (b, iv)$^m$</td>
<td>A, F, I, p (b, iv)$^m$</td>
<td>A, I (p)</td>
<td>A, I, p (f, l)$^d$</td>
<td>B, A, F (l)$^a$</td>
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<td>25.6</td>
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*Result the same as that reported in the literature.
† Compared to the absence of acid production in the API systems, there was weak growth of strain CRIB$^T$ on this substrate when grown in liquid PY medium.
‡ Data were taken from: a, Rainey et al. (2009); b, Song et al. (2004); c, Chamkha et al. (2001); d, Kane et al. (1991); e, Gaston and Stadtman (1963); f, Biebl & Sproer (2002).
§ Products (listed in the order usually detected): A, acetic acid; B, butyric acid; F, formic acid; IB, iso-butyratic acid; IV, iso-valeric acid; L, lactic acid; P, propionic acid. Upper-case letters indicate major components, lower-case minor components (<20% of total measured metabolic end product production). Products in parentheses are not detected uniformly.
¶ Butyric acid and iso-valeric acid are produced in the presence of Casamino acids.
∥ Iso-butyratic acid is produced in the presence of Casamino acids and the absence of glucose.
# GL, glycolipid; PL, phospholipid; L, lipid.
** The polar lipids of *C. butyricum* comprise phosphatidylglycerol, diradyl (i.e. diacylglycerol and 1-O-alk-1′-enyl-2-acyl glycerol) derivatives of phosphatidylglycerol, diphasophatidylglycerol, phosphatidylethanolamine, a glycerol acetal derivative of the plasmanalgen form of phosphatidylethanolamine and a phosphatidylglycerol acetal derivative of the plasmanalgen form of phosphatidylethanolamine (Goldfine & Johnston, 2005; Johnston & Goldfine, 1983).
were analysed by HPLC using a Metacarb 67H column (Varian). H₂ production was measured by GC using a Shimadzu GC 14B fitted with a Molsieve 13x column (Varian). CO₂ production was measured by GC using a Shimadzu GC 2014 fitted with a CP-PorapLOT Q column (Varian). The metabolic end products of strain CRIBᵀ were compared to those of the three closely related and presumably misclassified species of the genus Clostridium. Acetate was the major end product formed in all species. However, compared to *C. lituseburense* (*R. lituseburensis* comb. nov.) DSM 797ᵀ and the other type strains of *Clostridium* included analysis of peptidoglycan structure, cell-wall lipids, respiratory lipoquinones, and fatty acid methyl esters. The peptidoglycans were hydrolysed in 4 M HCl (16 h at 100 °C). The amino acids and peptides in the cell-wall hydrolysates were analysed by two-dimensional TLC on cellulose plates using the solvent system described by Rhuland *et al.* (1955). After derivatization (MacKenzie, 1987), the molar ratios of amino acids were determined by GC. The N-heptfluorobutyryl amino acid isobutyl esters obtained by derivatization were subjected to GC-MS to determine the identity of the components not identified by previous analyses.

For cell-wall sugar analysis the peptidoglycan of strain CRIBᵀ was hydrolysed in 0.5 M H₂SO₄ (2 h at 100 °C). H₂SO₄ was removed by adding 20% N,N-dioctylmethyamine in chloroform according to the protocol of Whiton *et al.* (1985) and the sugars in the hydrolysate were analysed by TLC on cellulose plates according to the method of Staneck & Roberts (1974).

Respiratory lipoquinones were extracted from lyophilized biomass of strain CRIBᵀ using the two-stage method described by Tindall (1990a, b) using methanol/hexane, followed by phase separation into hexane. UV-absorbing bands were removed from the plate and further analysed by HPLC.

To support the (re)classification into novel genera, analysis of the polar lipid profiles and cellular fatty acid composition was done for the following strains: *Romboutsia ilealis* gen. nov., sp. nov. CRIBᵀ, *C. lituseburense* (*R. lituseburensis* comb. nov.) DSM 797ᵀ, *C. bartlettii* (*I. bartlettii* comb. nov.) DSM 16795ᵀ, *C. glycolicum* (*T. glycolicus* comb. nov.) DSM 1288ᵀ, *C. mayombei* (*T. mayombei* comb. nov.) DSM 6539ᵀ, *C. irregularare* (*A. irregularis* comb. nov.) DSM 2635ᵀ and for comparison *Peptostreptococcus anaeobius* DSM 2949ᵀ. Cultivation of the strains and subsequent analyses were performed by the Leibniz-Institut DSMZ. All strains were grown under identical conditions in liquid DSM medium 104b. This medium consisted of (per litre distilled water): 5 g trypticase peptone, 5 g peptone from meat (pepsin-digested), 10 g yeast extract, 5 g glucose, 1 mg resazurin, 40 ml salts solution (Holdeman *et al.*, 1977) and 0.5 g l-cysteine hydrochloride. Cells were harvested in mid-exponential to end-exponential phase. Polar lipids were extracted from lyophilized biomass using the two-stage method described by Tindall (1990a, b) and separated by two-dimensional silica gel TLC. All polar lipids were detected by spraying the plates with 5% ethanol molybophosphoric acid followed by heating, while head groups were detected using specific staining reagents as described previously (Tindall, 1990a, b). Since chromatography conditions were identical, comparison of the TLC plates was possible and spots were labelled according to their staining behaviour and *Rₚ* value. 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 the methods described by Kuykendall *et al.* (1988) and Miller (1982). Fatty acid methyl ester mixtures were separated by GC and analysed using the Sherlock Microbial Identification System (MIS) as described by Adachi *et al.* (2007). Peaks were automatically integrated, and fatty acid
identification (using the MOORE6 peak-naming database) and relative concentrations were calculated. In the case of anaerobes that synthesize plasmalogens, cleavage of these compounds gives rise to the corresponding aldehydes and dimethyl acetal (DMA) derivatives (Feulgen & Voit, 1924; Frosolono & Rapport, 1969; Morrison & Smith, 1964). Detailed results of the polar lipid and cellular fatty acid analyses can be found in Fig. 2 and Table 2, respectively.

The polar lipid profiles of all species examined were dominated by glycolipids and phospholipids (Fig. 2). In some species, lipids were also detected that did not react with any of the specific spray reagents used. The polar lipid profiles of strain CRIB° and the type strains of the five (misclassified) species of the genus Clostridium all clearly differed from that of Peptostreptococcus anaerobius, the type species of the type genus of the family Peptostreptococcaceae. Based on published information on a limited number of species within Clostridium cluster I (including members of the type species, C. butyricum) the polar lipid composition is clearly different from that of the strains examined here. In particular the wide diversity of glycolipids documented here is not present in those strains assigned to Clostridium cluster I. In addition, the presence of phosphatidylethanolamine in all the strains of Clostridium cluster I examined in previous studies (Goldfine & Johnston, 2005; Guan et al., 2011, 2012, 2013; Johnston & Goldfine, 1983; Johnston et al., 2004, 2010; Oulevey et al., 1986; Thiele et al., 1985; Tian et al., 2013) clearly distinguishes them from the strains examined here that do not synthesize phosphatidylethanolamine, which was supported by the absence of phospholipids with free amino groups in the polar lipid analyses. Care should, however, be taken with regard to the conclusions drawn in some of these publications because links are made to some strains that in the past were incorrectly identified. The potential to synthesize phosphatidylethanolamine was further investigated by examining the genomes available for the presence of psd genes encoding the enzyme (phosphatidylserine decarboxylase) responsible for the formation of phosphatidylethanolamine via decarboxylation of phosphatidylserine (Borkenhagen et al., 1961; Kanfer & Kennedy, 1964). The protein sequence ABK62661 extracted from the genome of Clostridium novyi strain NT (CP000382) was used to ‘seed’ a BLAST search of the available genomes of members of the family Peptostreptococcaceae held on the Joint Genome Institute.

**Fig. 2.** Two-dimensional thin-layer chromatograms showing total polar lipid profiles of strain CRIB° and the type strains of the five species proposed to be reclassified in the novel genera Romboutsia gen. nov., Intestinibacter gen. nov., Terrisporobacter gen. nov. and Asaccharospora gen. nov. Profiles are compared to the polar lipid profile derived from the type strain of the type species of the type genus of the family Peptostreptococcaceae, *P. anaerobius* DSM 2949°. (a) *R. ilealis* gen. nov. sp. nov. CRIB°; (b) *C. lituseburense* (R. lituseburensis comb. nov.) DSM 797°; (c) *C. bartlettii* (L. bartlettii comb. nov.) DSM 16795°; (d) *C. glycolicum* (T. glycolicum comb. nov.) DSM 1289°; (e) *C. mayombei* (T. mayombei comb. nov.) DSM 6599°; (f) *C. irregularis* (A. irregularis comb. nov.) DSM 2635°; (g) *P. anaerobius* DSM 2949°. Separation of the polar lipids was performed by two dimensional silica gel TLC, with chloroform/methanol/water (65 : 25 : 4, by vol.) in the first direction, and chloroform/methanol/acetic acid/water (80 : 12 : 15 : 4, by vol.) 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 and their *R*° values. GL, glycolipid; PL, phospholipid; L, lipid. PL4 is most probably identified as diphosphatidylglycerol and PL7 as phosphatidylglycerol.
Table 2. Cellular fatty acid profiles of strain CRIBT and the type strains of the five species proposed to be reclassified in the novel genera *Romboutsia* gen. nov., *Intestinibacter* gen. nov., *Terrisporobacter* gen. nov. and *Asaccharospora* gen. nov., compared to the cellular fatty acid profile derived from the type strain of the type species of the type genus of the family *Peptostreptococcaceae*, *Peptostreptococcus* *anaerobius*.

Strains: 1, *Romboutsia ilealis* gen. nov., sp. nov. CRIBT; 2, *C. lituseburense* (*R. lituseburensis* comb. nov.) DSM 797T; 3, *C. bartlettii* (*I. bartlettii* comb. nov.) DSM 16795T; 4, *C. glycolicum* (*T. glycolicus* comb. nov.) DSM 1288T; 5, *C. mayombei* (*T. mayombei* comb. nov.) DSM 6539T; 6, *C. irregulare* (*A. irregularis* comb. nov.) DSM 2635T; 7, *P. anaerobius* DSM 2949T. 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 ionization, 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 $\geq 1.5\%$ in at least one of the strains, are shown. For each strain the predominant fatty acid(s) ($\geq 10\%$) 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 C18 : 1v9c in strains grown in the presence of these materials.

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*J. Gerritsen and others*
Table 2. cont.

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<td>–</td>
</tr>
<tr>
<td>Summed feature 10#</td>
<td>C18:1o7c</td>
<td>8.34</td>
<td>10.66</td>
<td>1.05</td>
<td>2.86</td>
<td>3.98</td>
<td>2.49</td>
</tr>
<tr>
<td>Summed feature 1**</td>
<td>–</td>
<td>–</td>
<td>0.24</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>3.20</td>
</tr>
<tr>
<td>Total</td>
<td>97.29</td>
<td>95.90</td>
<td>95.38</td>
<td>93.00</td>
<td>93.75</td>
<td>95.18</td>
<td>95.80</td>
</tr>
</tbody>
</table>

*Dimethyl acetal and aldehydes are cleavage products of plasmalogens (Feulgen & Voit, 1924; Frosolono & Rapport, 1969; Morrison & Smith, 1964). In the absence of experimental data on the effect of the hydrolysis/methylation conditions used in the MIDI system it is not clear whether the presence of both compounds with the same chain lengths is due to incomplete conversion of plasmalogens to the corresponding dimethyl acetal, or an equilibrium in the ratio of aldehydes/dimethyl acetals released from the plasmalogens. Consequently where both the aldehyde and dimethyl acetal of the same chain length occur together they have also been listed as the sum of the aldehydes/dimethyl acetals. It should also be noted that while plasmalogens appear to be synthesized by an oxygen-dependent pathway in eukaryotes they appear to be synthesized in anaerobic prokaryotes by an oxygen-independent mechanism (Guan et al., 2011).

‡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 C15:1o8c, C15:2 and/or an unknown C15:2; however, the ECL indicates that the main peak is C15:1o8c.

¶Listed as summed feature 5 comprising C15:0 DMA and/or C14:0 3-OH; however, the ECL indicates that the main peak is C15:0 DMA.

‖Listed as summed feature 7 comprising C17:1o9c and/or C17:2; the ECLs are sufficiently close together to make an unambiguous identification difficult; however, biochemical considerations suggest that the synthesis of a C17:2 may require oxygen.

¶Listed as summed feature 8 comprising C17:1o8c and/or C17:2; the ECLs are sufficiently close together to make an unambiguous identification difficult; however, biochemical considerations suggest that the synthesis of a C17:2 may require oxygen.

#Listed as summed feature 10 comprising C18:1o7c and/or an unknown fatty acid; however, the ECL indicates that the main peak is C18:1o7c.

**Listed as summed feature 13 comprising C14:0 2-OH and/or C15:0 anteiso DMA, the ECLs are sufficiently close together to make an unambiguous identification difficult; however, biochemical considerations suggest that the synthesis of a 2-OH fatty acid may require oxygen.

(JGI) Integrated Microbial Genomes (IMG) website (http://img.jgi.doe.gov/) on 10 September 2013. No matches were found in any of the genomes, indicating that homologues of this enzyme do not appear to be present in the genomes, which would also explain the absence of the corresponding polar lipid (phosphatidylethanolamine). Based on currently available data it would appear that all members of Clostridium cluster I (Collins et al., 1994; Johnson & Francis, 1975) produce phosphatidylethanolamine, whereas none of the strains examined here produce this phospholipid.

With respect to the cellular fatty acids, the profiles of strain CRIBT and the type strains of the five (misclassified) species of the genus Clostridium were compared to the type strain of the type species of the type genus of the family Peptostreptococcaceae, Peptostreptococcus anaerobius DSM 2949T. Other studies have reported cellular fatty acid profiles of Peptostreptococcus anaerobius and other species belonging to the genus Peptostreptococcus (Ezaki et al., 1983; Whitehead et al., 2011). However, since there are large discrepancies in the data reported in these studies and because results were carried out under different growth conditions, we performed an additional analysis of the cellular fatty acid profile of Peptostreptococcus anaerobius. The profile of Peptostreptococcus anaerobius presented in this study was determined under identical growth conditions as strain CRIBT and the type strains of the five (misclassified) species of the genus Clostridium. We found that the cellular fatty acid profile of Peptostreptococcus anaerobius is characterized by an almost complete absence of unsaturated straight-chain fatty acids, which is clearly distinct from the cellular fatty acid profiles of strain CRIBT and the type strains of the five (misclassified) species of the genus Clostridium (Table 2).

Based on the polar lipid and cellular fatty acid profiles we can conclude that strain CRIBT and the type strains of the five (misclassified) species of the genus Clostridium do not belong to either the genus Peptostreptococcus or the genus Clostridium [i.e. members of cluster I as defined by Collins et al. (1994)] and should therefore be transferred to novel genera. Based on a combination of polar lipid and cellular fatty acid analyses, strain CRIBT and the type strains of the five (misclassified) species of the genus Clostridium could be divided into four groups, which we propose should be reclassified into four novel genera. The first group to be reclassified as a novel genus, and for which we propose the name Romboutsia gen. nov., consists of strain CRIBT and Romboutsia lituseburensis comb. nov. (C. lituseburensis) DSM 797T. The genus Romboutsia is characterized by the predominance of straight-chain saturated and unsaturated...
fatty acids (mainly C16–C17) and the absence of branched-chain fatty acids, dimethyl acetal and aldehydes. In addition, C19 cyclopropane 9,10 was detected in low abundance in both species within this group (Table 2). The second group, for which we propose the name Intestinibacter gen. nov., comprises Intestinibacter bartlettii comb. nov. (C. bartlettii) DSM 16795T. The genus Intestinibacter is, compared to the other proposed genera, characterized by a low abundance of C16 fatty acids. In turn, branched-chain saturated fatty acids (mainly C15) and dimethyl acetal (mainly C16) predominate in this group. The third group, for which we propose the name Terrisporobacter gen. nov., consists of Terrisporobacter glycolicus comb. nov. (C. glycolicum) DSM 1288T and Terrisporobacter mayombei comb. nov. (C. mayombei) DSM 6539T. This group is characterized by the dominance of (straight and branched chain) saturated and unsaturated fatty acids (mainly C16–C17). In addition, dimethyl acetal (mainly C16 and its corresponding aldehyde) predominate. The fourth group, for which the name Asaccharospora gen. nov. is proposed, comprises Asaccharospora irregularis comb. nov. (C. irregularis) DSM 2635T. This group is characterized by the dominance of (straight and branched chain) saturated and unsaturated fatty acids (mainly C16). Dimethyl acetal and aldehydes are almost absent. Both the polar lipid profiles and the phylogenetic clustering based on the 16S rRNA gene sequence are consistent with the reclassification of strain CRIB2 and the type strains of the five (misclassified) species of the genus Clostridium into four groups that we propose represent novel genera (Figs 1 and 2).

In addition to large variability in phenotypic properties, many species belonging to Clostridium cluster XI show low 16S rRNA gene sequence similarity to one another (Fig. 1). Based on these observations, it is very likely that the closely related and also presumably misclassified species Clostridium bifermantes, Clostridium sordellii, Clostridium ghonii and Eubacterium tenue will form a separate novel genus within the Peptostreptococcaceae. However, additional taxonomic characterization experiments (such as comparative cellular fatty acid and polar lipid analyses) are necessary to confirm this. Based on the data presented here the placement of C. lituseburensis, C. glycolicum, C. mayombei, C. bartlettii and C. irregularare in novel genera also has implications for the nomenclature of the important human pathogen Clostridium difficile where it would seem unavoidable that within the foreseeable future this species will also have to be placed in a novel genus. Drucker et al. (1996) and Korachi et al. (2002) reported on a single main phospholipid present in C. difficile (phosphatidilylycerol) and the absence of glycolipids, suggesting very clear differences from the taxa examined in this study. Yutin & Galperin (2013) have proposed that C. difficile be transferred to a novel genus, ‘Peptoclostridium’. While they also suggest that C. bartlettii, C. glycolicum, C. irregularare, C. lituseburensis and C. mayombei should be transferred to this genus, their paper seems to lack a formal proposal for the creation of new combinations. However, based on the data presented here there would be no inconsistency between their evaluation of the data and the inclusion of additional chemical data that we interpret as indicating the presence of several genera and not just one.

A similar situation arises with members of other taxa that share a high degree of 16S rRNA gene sequence similarity with C. difficile, including Clostridium mangenotii and Clostridium hiranonis that either are members of the same novel genus or may need to be placed in other novel genera. It is beyond the purpose of this article to reclassify all misclassified species of the genus Clostridium. Nevertheless, the transfer of the five (misclassified) species of the genus Clostridium as proposed here is another important step in resolving some of the taxonomic problems associated with the genus Clostridium.

Based on phenotypic and genetic characterization we conclude that strain CRIB3, a rod-shaped organism isolated from the gastro-intestinal tract of rats, clearly represents a novel species. In addition, we propose, based on phenotypic (including comparative cellular fatty acid and polar lipid analyses) and genetic considerations, that a number of (misclassified) species of the genus Clostridium be reclassified in four novel genera. We propose a novel genus, Romboutsia gen. nov., to include Romboutsia lituseburensis comb. nov. The novel isolate CRIB1 (=DSM 25109T=NIZO 4048T) is proposed as the type strain of the type species, Romboutsia ilealis gen. nov., sp. nov. of the proposed novel genus. In addition, we propose a second novel genus, Intestinibacter gen. nov., to include Intestinibacter bartlettii comb. nov. (type species). Furthermore, we propose a third novel genus, Terrisporobacter gen. nov., to include Terrisporobacter glycolicus comb. nov. (type species) and Terrisporobacter mayombei comb. nov. We propose a fourth novel genus, Asaccharospora gen. nov., to include Asaccharospora irregularis comb. nov. (type species).

**Description of Romboutsia gen. nov.**

*Romboutsia* (Rom.bout’sia. N.L. fem. n. Romboutsia in honour of the Dutch microbiologist Frans M. Rombouts, in recognition of his contributions to food microbiology and probiotic research).

Members of the genus are anaerobic. Cells stain Gram-positive or Gram-variable. Cells are straight or slightly curved rods, which often form chains. All species are capable of spore formation, but free spore formation can be very sparse. The major end products of metabolism are acetate and formate. Cells contain both phospholipids and glycolipids. The major phospholipids are labelled PL4, PL5, PL7 and PL8 and the major glycolipids comprise GL2, GL5, GL7 and GL9, the Rf values of which can be determined by reference to Fig. 2 and also serve as reference points for future work on the elucidation of these structures. Phospholipids with free amino groups (e.g. phosphatidylethanolamine) are absent. The fatty acids present in the hydrolysis products of whole cells are straight-chain.
saturated and unsaturated (Table 2). Dimethyl acetal (i.e. plasmalogens) are not present. The genomic DNA G+C content is 27–28.1 mol%. The type species is Romboutsia ilealis.

Description of Romboutsia ilealis sp. nov.

Romboutsia ilealis (i’le.a’lis N.L. fem. adj. ilealis pertaining to the ileum).

Cells are obligately anaerobic, non-motile, spore-forming rods. Typical cells are 1.0–2.0 μm × 1.0–5.3 μm in size and occur primarily in chains; however, single cells and pairs are observed as well. Cells stain Gram-positive; however, they stain Gram-negative as cultures reach stationary phase. Sporulation is seen occasionally after prolonged incubation. Growth occurs both on liquid and solid CRIB or PYG medium. Surface colonies on solid PYG and CRIB medium, and PolyViteX agar plates, incubated under anoxic conditions for 24 h, are white or light grey, circular, 0.5–1 mm in diameter with a shiny and smooth surface, a moist texture and an entire margin, and the elevation is flat to raised. After incubation for 72 h the colonies are larger (2–4 mm) and have translucent and undulate margins. Temperature range for growth is 30–45 °C with an optimum temperature of 37 °C. The pH range for growth is pH 6.5–8.0 with an optimum pH of pH 7.0–7.5. Growth occurs at NaCl concentrations of 0–1% (w/v). Growth is inhibited by 5–20% bile salts. Growth is stimulated by addition of 0.01% Tween 80 (v/v) to liquid PYG medium and shaking of the cultures at 100 r.p.m. Cultures in liquid PYG medium are turbid with a smooth sediment and pH 5.5–6.0 after incubation for 1 week. Abundant gas occurs at NaCl concentrations of 0–1% (w/v). Growth is reduced by 5–20% bile salts.

The peptidoglycan is of the A1L-lanthionine-direct type, which has, to our knowledge, not been previously described for species belonging to the class Clostridia. The cell-wall sugars are glucose and galactose. The only respiratory lipid that does not stain with any of the specific spray reagents used (Fig. 2). Phospholipids with free amino groups (e.g. phosphatidylethanolamine) are absent.

The type strain, CRIBT (=DSM 25109T =NIZO 4048T), was isolated from the gastro-intestinal tract of a Sprague–Dawley rat at Wageningen University, The Netherlands. The genomic DNA G+C content of strain CRIBT is 28.1 mol%. Strain CRIBT shows low DNA–DNA relatedness (percentage reassociation) to the type strains Romboutsia lituseburensis (Clostridium lituseburensis) DSM 797T (15.5 ± 0.8%), Intestinibacter bartlettii (Clostridium bartlettii) DSM 16795T (20.4 ± 3.5%) and Asaccharospora irregularis (Clostridium irregularare) DSM 2635T (18.1 ± 1.3%).

Description of Romboutsia lituseburensis

(Laplanche and Saissac 1948) comb. nov.

Romboutsia lituseburensis (li.tus.e.bur.en’ sis. L. n. litus coast; L. n. ebur ivory; N.L. fem. adj. lituseburensis pertaining to Côte d’Ivoire).


The properties of Romboutsia lituseburensis are as given for Clostridium lituseburensis by Holdeman et al. (1977), with the following additions. With the API 50 CH and API 20 A systems, acid is produced from D-fructose, D-glucose, maltose, and melibiose, in addition to moderate growth on D-arabinose and D-galactose and good growth on L-fucose, D-glucose, raffinose and sucrose. No growth is observed on N-acetylglucosamine, D-adonitol, starch, amygdalin, D-arabinose, D-arabitol, D-arabinot, arbutin, cellobiose, cellulose, dulcitol, erythritol, D-fructose, D-fucos, gentiobiase, glycerol, glycogen, inositol, melibiose, ribose, D-xylose, maltose, D-mannitol, D-mannose, melezitos, melibiose, methyl 2,4-diacetamido-5-propionamide, methyl 2,4-diaminopyranoside, methyl β-D-xylpyranoside, potassium 2-ketogluconate, potassium 5-kojiculate, potassium gluconate, L-rhamnose, D-ribose, salicin, soluble starch, D-sorbitol, L-sorbose, D-tagatose, trehalose, turanose, xylitol, D-xyllose and L-xyllose. Negative for indole production, urease and catalase activity, and gelatin and starch hydrolysis. Metabolic products produced from PYG are acetate, formate and lactate. Abundant H₂ and CO₂ are produced. Sulfite is reduced. Sulfate and thiosulfate are not reduced. The type strain is sensitive to clindamycin (MIC 0.125 μg ml⁻¹), penicillin G (MIC < 0.016 μg ml⁻¹) and metronidazole (MIC < 0.016 μg ml⁻¹). The major cellular fatty acids are saturated and unsaturated straight chain C₁₂–C₁₉ fatty acids, with C₁₆:0 being the predominant fatty acid. Details of the fatty acid profile are provided in Table 2, with percentages serving as a guide to the relative abundance of the different compounds. The peptidoglycan contains alanine and glutamic acid (molar ratio 1.4:1.0) and a di-N-heptfluorobutyl-lanthionine-disobutylester is detected by GC-MS indicating that lanthionine is present. The peptidoglycan is of the A1L-lanthionine-direct type, which has, to our knowledge, not been previously described for species belonging to the class Clostridia. The cell-wall sugars are glucose and galactose. The only respiratory lipid that does not stain with any of the specific spray reagents used (Fig. 2). Phospholipids with free amino groups (e.g. phosphatidylethanolamine) are absent.

The type strain, A25KT (=DSM 25109T =NIZO 4048T), was isolated from the gastro-intestinal tract of a Sprague–Dawley rat at Wageningen University, The Netherlands. The genomic DNA G+C content of strain CRIBT is 28.1 mol%. Strain CRIBT shows low DNA–DNA relatedness (percentage reassociation) to the type strains Romboutsia lituseburensis (Clostridium lituseburensis) DSM 797T (15.5 ± 0.8%), Intestinibacter bartlettii (Clostridium bartlettii) DSM 16795T (20.4 ± 3.5%) and Asaccharospora irregularis (Clostridium irregularare) DSM 2635T (18.1 ± 1.3%).
**Description of Intestinibacter gen. nov.**

*Intestinibacter* (In teas’ti.ni.bac’ter. L. neut. n. *intestinum* gut; N.L. masc. n. *bacter* a rod; N.L. masc. n. *Intestinibacter* a rod from the gut).

Members of the genus are anaerobic. Cells stain Gram-positive or Gram-variable. Cells are straight or slightly curved, non-motile rods, which often form chains. Capable of spore formation, but free spore formation can be very sparse. The major end products of metabolism are acetate and formate. Cells contain both phospholipids and glycolipids. The major phospholipids are labelled PL2, PL4, PL5, PL6, PL7 and PL10 and the major glycolipids comprise GL1, GL3, GL6, GL7, GL8 and GL9, the $R_f$ values of which can be determined by reference to Fig. 2 and also serve as reference points for future work on the elucidation of these structures. Phospholipids with free amino groups (e.g. phosphatidylethanolamine) are absent. The fatty acids present in the hydrolysis products of whole cells are both iso- and anteiso-branched as well straight chain saturated and unsaturated (Table 2). Dimethyl acetal (i.e. plasmalogens) are predominantly straight chain with smaller amounts of iso- and anteiso-branched derivatives. The genomic DNA G+C content is 29.8 mol%. The type species is *Intestinibacter bartlettii*.

**Description of Intestinibacter bartlettii (Song et al. 2004) comb. nov.**

*Intestinibacter bartlettii* (bart.let’tii.i. N.L. masc. gen. n. bartlettii to honour John G. Bartlett, for his contributions to the role of intestinal microbiota in disease and to our knowledge of infectious diseases in general).

Basonym: *Clostridium bartlettii* Song et al. 2004 (Song et al., 2004).

The properties of *Intestinibacter bartlettii* are as given for *Clostridium bartlettii* by Song et al. (2004), with the following additions. With the API 50 CH and API 20 A systems, acid is produced from D-fructose, gentiobiose, D-glucose, maltose, D-mannitol, D-ribose, D-sorbitol, sucrose and D-tagatose. Little or no H$_2$ is formed. The polar lipid profile comprises seven glycolipids, four phospholipids and one lipid that does not stain with any of the specific spray reagents used. Phospholipids with free amino groups (e.g. phosphatidylethanolamine) are absent. The predominant cellular fatty acids are iso-C$_{15:0}$ and C$_{16:0}$ DMA. Details of the fatty acid profile are provided in Table 2, with percentages serving as a guide to the relative abundance of the different compounds.

The type strain is WAL 16138$^T$ (=ATCC BAA-827$^T$ = CCUG 48940$^T$ = DSM 16795$^T$).

**Description of Terrisporobacter gen. nov.**

*Terrisporobacter* (Ter.ri spo’ro.bac’ter. L. n. *terra* soil; Gr. fem. n. *spora* a seed, and in biology a spore; N.L. masc. n. *bacter* a rod; N.L. masc. n. *Terrisporobacter* a spore-forming rod found in soil).

Members of the genus are anaerobic. Cells stain Gram-positive or Gram-variable. Cells are straight or slightly curved, motile rods, occurring singly or in pairs. All species are capable of spore formation. The major end product of metabolism is acetate. Cells contain both phospholipids and glycolipids. The major phospholipids are labelled PL2, PL4, PL5, PL6, PL7 and PL10 and the major glycolipids comprise GL1, GL3, GL6, GL7, GL8 and GL9, the $R_f$ values of which can be determined by reference to Fig. 2 and also serve as reference points for future work on the elucidation of these structures. Phospholipids with free amino groups (e.g. phosphatidylethanolamine) are absent. The fatty acids present in the hydrolysis products of whole cells are both straight-chain saturated and unsaturated (which predominate) as well as iso- and anteiso-branched (Table 2). Dimethyl acetal (i.e. plasmalogens) include straight-chain, iso- and anteiso-branched derivatives. The genomic DNA G+C content is 25–30 mol%. The type species is *Terrisporobacter glycolicus*.

**Description of Terrisporobacter glycolicus (Gaston and Stadtmann 1963) comb. nov.**

*Terrisporobacter glycolicus* (gly.co’li.cus. L. adj. suff. -icus related to, belonging to; N.L. masc. adj. *glylicicus* referring to the ability to ferment ethylene glycol).


The properties of *Terrisporobacter glycolicus* are as given for *Clostridium glycolicum* by Holdeman et al. (1977) and in the emendation of Chamkha et al. (2001), with the following additions. The polar lipid profile comprises seven glycolipids, seven phospholipids and one lipid that does not stain with any of the specific spray reagents used. Phospholipids with free amino groups (e.g. phosphatidylethanolamine) are absent. The predominant cellular fatty acid is C$_{16:0}$. Details of the fatty acid profile are provided in Table 2, with percentages serving as a guide to the relative abundance of the different compounds.

The type strain is ATCC 14880$^T$ (=BCRC 14553$^T$ = DSM 1288$^T$ = JCM 1401$^T$ = NCIMB 10632$^T$ = NCTC 13026$^T$).

**Description of Terrisporobacter mayombei (Kane et al. 1992) comb. nov.**


Basonym: *Clostridium mayombei* Kane et al. 1992 (Kane et al., 1992).

Although the epithet *mayombensis* may be considered to be the proper orthographic form, this correction is no longer
The properties of Terrisporobacter mayonbei are as given for Clostridium mayonbei by Kane et al. (1991), with the following additions. The polar lipid profile comprises six glycolipids and six phospholipids. Phospholipids with free amino groups (e.g. phosphatidylethanolamine) are absent. The predominant cellular fatty acid is C_{16:0}. Details of the fatty acid profile are provided in Table 2, with percentages serving as a guide to the relative abundance of the different compounds.

The type strain is SFC-5^{T} (=ATCC 51428^{T}=DSM 6539^{T}).

**Description of Asaccharospora gen. nov.**

Asaccharospora (A.sac.cha.ro.spo’ra. Gr. pref. a not; Gr. n. sakchár sugar; Gr. fem. n. spora a seed, and in biology a spore; N.L. fem. n. Asaccharospora a spore-forming organism that is unable to ferment sugars).

Members of the genus are anaerobic. Cells stain Gram-positive or Gram-variable. Cells are straight or slightly curved, motile rods, which often form chains. Cells may be quite filamentous. Spore formation is observed. Carbohydrates are not fermented. The major end product of metabolism is acetate. Cells contain both phospholipids and glycolipids. The major phospholipids are labelled PL4, PL5, PL7 and PL8 and the major glycolipids comprise GL1, GL3a/b, GL6 and GL9, the R_{f} values of which can be determined by reference to Fig. 2 and also serve as reference points for future work on the elucidation of these structures. Phospholipids with free amino groups (e.g. phosphatidylethanolamine) are absent. The fatty acids present in the hydrolysis products of whole cells are both straight-chain saturated and unsaturated (which predominate) as well as iso- and anteiso-branched (Table 2). Dimethyl acetalts (i.e. plasmalogens) are almost absent. The type species is Asaccharospora irregularis.

**Description of Asaccharospora irregularis (Choukévitch 1911) comb. nov.**

Asaccharospora irregularis (ir.re.gu.lar’is. L. fem. adj. irregularis irregular, referring to pleomorphic, irregular cells).

Basonym: Clostridium irregular (Choukévitch 1911)
Prévote 1938 (Approved Lists 1980) (Choukévitch, 1911; Prévote, 1938b; Skerman et al., 1980).

The properties of Asaccharospora irregularis are as given for Clostridium irregular by Holdeman et al. (1977), with the following additions. With the API 50 CH and API 20 A systems, there is no acid is produced with any of the carbohydrates tested. The polar lipid profile comprises four glycolipids and four phospholipids. The predominant cellular fatty acids are C_{16:0} and C_{16:1o7c}. Details of the fatty acid profile are provided in Table 2, with percentages serving as a guide to the relative abundance of the different compounds.

The type strain is 6VI^{T} (=ATCC 25756^{T}=BCRC 14528^{T}=DSM 2635^{T}=JCM 1425^{T}=NCIMB 11830^{T}).

Based on the results presented here, an emended description of Peptostreptococcus anaerobius would also be appropriate.

**Emended description of Peptostreptococcus anaerobius (Natvig 1905) Kluyver and van Niel 1936 (Approved Lists 1980)**

Peptostreptococcus anaerobius (an.a.e.ro’bi.us. Gr. pref. an not; Gr. n. aer air; Gr. n. bios life; N.L. adj. anaerobius not living in air, anaerobic).


The properties of the species are those given by Holdeman et al. (1977) and Ezaki (2009) (for the species Peptostreptococcus anaerobius) with the following additions. Respiratory lipoquinones have not been reported. Polar lipids comprise phospholipids and glycolipids. The phospholipids present are PL4 and PL7 and the only glycolipids comprise GL1 and GL9, the R_{f} values of which can be determined by reference to Fig. 2 and also serve as reference points for future work on the elucidation of these structures. Phospholipids with free amino groups (e.g. phosphatidylethanolamine) are absent. The fatty acids present in the hydrolysis products of whole cells are predominantly saturated straight-chain and iso- and anteiso-branched chain (Table 2). Dimethyl acetales (i.e. plasmalogens) are predominantly even straight-chain with smaller amounts of iso- and anteiso-branched derivatives. Details of the fatty acid profile are provided in Table 2, with percentages serving as a guide to the relative abundance of the different compounds. The fatty acid data cited by Ezaki (2009) from Ezaki et al. (1983) are not consistent with the results presented here and are not to be included in the emended description.

The type strain is Prévot 4372^{T} (=ATCC 27337^{T}=BCRC 10722^{T}=CCUG 7835^{T}=CIP 104411^{T}=DSM 2949^{T}=KCTC 5182^{T}=LMG 15865^{T}=NCTC 11460^{T}=VTT E-02070^{T}).

The present edition also has to deal with an additional nomenclatural issue. Based on information published in the second edition of Bergey’s Manual of Systematic Bacteriology, the order Clostridiales Prévote 1953 (Rainey, 2009) contains the family Eubacteriaceae Ludwig et al. 2010, which in turn is based on the type genus Eubacterium Prévote 1938 (Approved Lists 1980). The use of the name Clostridiales Prévote 1953 (Approved Lists 1980) (Prévot, 1953; Skerman et al., 1980) is in contradiction to Principles 1 and 8 as well as Rule 23a of the current Code of nomenclature dealing with prokaryotes that requires that the older name Eubacteriales Buchanan 1917 (Approved
Lists 1980) (Buchanan, 1917; Skerman et al., 1980) is to be used for this order (Lapage et al., 1992). This would also require that an emended description of the order Eubacteriales Buchanan 1917 (Approved Lists 1980) is published. We therefore formally emend the circumscription and description of the order Eubacteriales Buchanan 1917 (Approved Lists 1980).

Emendation of the order Eubacteriales Buchanan 1917 (Approved Lists 1980)

The properties of the order are those given by Rainey (2009) under the order name Clostridiales Prévot 1953 (Approved Lists 1980). The type genus is Eubacterium Prévot 1938 (Approved Lists 1980). The order contains the families Caldicoprobacteraceae, Christensenellaceae, Clostridiaceae, Defluviitaleaceae, Eubacteriaceae, Gularibacteriaceae, Heliotacteriaceae, Lachnospiraceae, Peptococcaceae, Peptostreptococcaceae, Ruminococcaceae and Syntrophonadomadaceae.

It should be noted that as long as the type genera of the order Clostridiales Prévot 1953 (Approved Lists 1980) [i.e. Clostridium Prazmowski 1880 (Approved Lists 1980) (Prazmowski, 1880; Skerman et al., 1980)] and the order Eubacteriales Buchanan 1917 (Approved Lists 1980) [i.e. Eubacterium Prévot 1938 (Approved Lists 1980) (Prévot, 1938a; Skerman et al., 1980)] are placed in one order, then the name of the order Eubacteriales Buchanan 1917 (Approved Lists 1980) has priority. However, should these two genera be placed in separate orders, then the names Clostridiales Prévot 1953 (Approved Lists 1980) and Eubacteriales Buchanan 1917 (Approved Lists 1980) may be used for the two orders, but an emendation would be required.

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