Description of two novel members of the family Erysipelotrichaceae: Ileibacterium valens gen. nov., sp. nov. and Dubosiella newyorkensis, gen. nov., sp. nov., from the murine intestine, and emendation to the description of Faecalibacterium rodentium

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Abstract

To better characterize murine intestinal microbiota, a large number (187) of Gram-positive-staining, rod- and coccoid-shaped, and facultatively or strictly anaerobic bacteria were isolated from small and large intestinal contents from mice. Based on 16S rRNA gene sequencing, a total 115 isolates formed three phylogenetically distinct clusters located within the family Erysipelotrichaceae. Group 1, as represented by strain NYU-BL-A³¹, was most closely related to Allobaculum stercoricanis, with 16S rRNA gene sequence similarity values of 87.7 %. A second group, represented by NYU-BL-A⁴Τ, was most closely related to Faecalibaculum rodentium, with 86.6 % 16S rRNA gene sequence similarity. A third group had a nearly identical 16S rRNA gene sequence (99.9 %) compared with the recently described Faecalibaculum rodentium, also recovered from a laboratory mouse; however, this strain had a few differences in biochemical characteristics, which are detailed in an emended description. The predominant (>10 %) cellular fatty acids of strain NYU-BL-A³¹ were C₁₆:₀ and C₁₈:₀, and those of strain NYU-BL-A⁴Τ were C₁₀:₀, C₁₆:₀, C₁₈:₀ and C₁₈:ω₉c. The two groups could also be distinguished by multiple biochemical reactions, with the group represented by NYU-BL-A⁴Τ being considerably more active. Based on phylogenetic, biochemical and chemotaxonomic criteria, two novel genera are proposed, Ileibacterium valens gen. nov., sp. nov. with NYU-BL-A³¹ (=ATCC TSD-6³¹=DSM 103668¹) as the type strain and Dubosiella newyorkensis gen. nov., sp. nov. with NYU-BL-A⁴Τ (=ATCC TSD-6⁴Τ=DSM 103457¹) as the type strain.

High-throughput sequencing technology has markedly accelerated research on microbes in complex ecosystems, including host-associated bacteria, collectively known as the microbiota, that have roles in shaping health and disease [1–3]. With untested potential microbe–host associations being identified on a regular basis, there is increasing need to culture and characterize members of novel bacterial taxa to further hypothesis-driven research. In particular, the family Erysipelotrichaceae is emerging as a group of bacteria that may influence host metabolism and inflammatory diseases [4, 5], and closely related species have been associated with a high-fat diet [6], promotion of obesity [7] or protection from weight gain on a high fat diet [4].

The family Erysipelotrichaceae was first described by Verbarg et al. [8] to include a number of Gram-stain-positive, slender or filamentous rods with a β-cross-linking type peptidoglycan belonging to the genus Erysipelothrix, with Erysipelothrix rhusiopathiae listed as the type species [8]. While the family was originally described to have facultatively anaerobic and microaerophilic organisms, it has recently been emended by Tegtmeier et al. [9] to include strictly anaerobic organisms. Recent additions to the family Erysipelotrichaceae include

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Keywords: Ileibacterium valens; Dubosiella newyorkensis; Erysipelotrichaceae; anaerobe.

Abbreviations: DAP, diaminopimelic acid; PRAS, pre-reduced anaerobically sterilized.

The GenBank/EMBL/DBJ accession numbers for the 16S rRNA gene sequences of strains Ileibacterium valens NYU-BL-A³¹ and Dubosiella newyorkensis NYU-BL-A⁴Τ are KU744404 and KU744405, respectively. Whole-genome sequences have been deposited in NCBI under bioproject numbers PRJNA352999 and PRJNA353002, respectively.

Three supplementary tables and one supplementary figure are available with the online supplementary Material.
Breznakia blatticola and Breznakia pachnodae [9], and Catenisphaera adipataccumulans [10]. In addition, the genera Faecalibacillus, Holdemanella and Faecalitalea were created to resolve the misclassified Streptococcus pleomorphus, Eubacterium biforme and Eubacterium cylindroides, respectively [11].

Currently, the family Erysipelotrichaceae comprises the following genera: Allobaculum, Breznakia, Bulleidia, Catenibacterium, Catenisphaera, Coprobacillus, Eggerthia, Erysipelothrix, Faecalibaculum, Faecalitalea, Holdemanella, Holdemania, Kandleria, Solobacterium and Turicibacter [8, 11–13]. In addition, several other species related to the genera Clostridium and Eubacterium having 16S rRNA gene sequence relatedness to the family Erysipelotrichaceae have been proposed to be incorporated as well [9, 13].

Our cultivation studies designed to isolate species of the genus Allobaculum yielded 115 organisms that, when screened via 16S rRNA gene sequencing, corresponded to three phylogenetically distinct clusters located in the family Erysipelotrichaceae. One cluster had 16S rRNA gene sequences nearly identical (99.9 % similar) to that of Faecalibaculum rodentium [14, 15], which was also recovered from a laboratory mouse. A second cluster was most closely related to Allobaculum stercoricanis, with 16S rRNA gene sequence similarity values of 87.7 %, and a third cluster was most closely related to Faecalibaculum rodentium, with 16S rRNA gene sequence similarity of 86.6 %. The present study expands our knowledge of the relatively understudied family Erysipelotrichaceae by describing two novel genera and characterizing additional strains of Faecalibaculum rodentium.

Several steps were undertaken to ensure viability of anaerobic organisms throughout specimen collection, processing, cultivation and isolation. Four intestinal samples from three different gut locations (caecum, small intestine and large intestine) were obtained from humanely euthanized female C57BL6j mice and homogenized in pre-reduced anaerobically sterilized (PRAS) saline (Anaerobe Systems). Specimen 1 represented caecal microbiota from an adult mouse that had never received antibiotics and was being maintained on a high-fat diet (diet-induced obesity diet D12451, Research Diets) for 18 weeks. Two sets of flame-sterilized instruments were used to prevent contamination from mouse skin, hair or environmental microbiota. Two-thirds of the caecal contents were transferred to a sterile tube, frozen on dry ice and stored at −80 °C for 2 years. Specimen 2 was a pool of caecal microbiota from a pregnant female mouse; specimens were collected in liquid dental transport medium (Anaerobe Systems), homogenized, with PRAS saline and stored at −80 °C for several months. Further specimens were collected from an adult female mouse at sacrifice, from which all tissues were removed aseptically, and all of the large (specimen 3) and small intestine (specimen 4) were placed into sterile PRAS chopped meat medium (Anaerobe Systems) tubes and vortex-mixed to homogenize the sample. Immediately following their collection, specimens 3 and 4 were inoculated to the plates listed below. Specimen processing, inoculation and isolation was performed in an anaerobic chamber under an atmosphere of 90 % nitrogen, 5 % hydrogen and 5 % carbon dioxide. For each of the four specimens, 10 µl of each intestinal suspension was plated on five different types of enriched (Brucella and MTGE-anaerobic enrichment agar), selective, [phenylethyl alcohol (PEA) and laked blood-kanamycin-vancomycin (LKV)] and selective-differential (Bacteroides bile aesculin – BBE) media (Anaerobe Systems). Plates were observed daily for 7 days, and isolates were sub-cultured onto Brucella or MTGE agar to obtain pure cultures. Isolates were preserved by suspending 1–2 plates of pure culture growth in four 0.5 ml aliquots of filter-sterilized powdered milk and frozen at −80 °C.

The oxygen requirements were tested by incubating strains A3, A4 and K8 in an anaerobic chamber, under microaerobic conditions (5–12 % CO2, 5–15 % O2, balance N2 and other trace gases from ambient air) (GasPak EZ Campy Container System, BD) and aerobic conditions enriched with 5 % CO2. Growth was only observed in strictly anaerobic conditions. Temperature testing was carried out at 30, 37 and 42 °C. All strains grew optimally at 37 °C, with weak growth detected at 30 °C and no growth detected at 42 °C. Lipase activity was tested on egg yolk agar (Anaerobe Systems), and results were read at 24, 48 and 72 h for strains A3 and A4; all tests were negative.

Near-full-length 16S rRNA genes were amplified using the 8F and 1510R primers, as described Gao et al. [16], and sequences were determined by the Sanger method (Macrogen, New York, NY). Abi files were converted to fastq by the Emboss script seqret (www.ebi.ac.uk/Tools/sfc/emboss_seqret/). When Phred Q score was <30, the 5 ends were trimmed by PrinSeq [17]. Paired-end reads were assembled using fastq-join from Ea-Utils (http://code.google.com/p/ea-utils) with match ≥50 % and overlap of ≥100 base pairs. The closest known relatives of the new isolates based on the 16S rRNA gene sequences were identified using the basic local alignment search tool (BLAST) [18]. Of the 187 isolates recovered, 115 isolates closely matched strains within the family Erysipelotrichaceae, and formed three separated clusters. Thirteen isolates were nearly identical to Faecalibaculum rodentium (99.9 %) and included strain NYU-BL-K8, 84 isolates had closest matches to Allobaculum stercoricanis (87.7 %) and included NYU-BL-A3T, and 18 isolates had closest matches to Faecalibaculum rodentium (86.6 %) and included NYU-BL-A4T. Six or seven strains from each of the three phylogenetic lineages were selected for further chemotaxonomic characterization, and phylogenetic analysis was done with the type strain of each group, which was selected based on the first named isolate of each group.

To determine the precise relationships with members of the family Erysipelotrichaceae and close relatives, a multiple alignment was created. Several strains each from groups 1–3 were characterized using 16S rRNA gene sequencing. Two subregions of the 16S rRNA gene were amplified and then sequenced, using the two sets of primer pairs: 8F–907R and 774F–1485R (underline indicates sequencing primer).
Purified DNA was sequenced directly (Laguna Scientific Laboratory, Laguna Beach, CA) with a DYEEnamic ET Terminator Kit z-BIGDYEv3 (Amersham Biosciences) on an ABI 3730xl sequencer (Applied Biosystems). The two subregions were imported into the Alignment Explorer Tool in MEGA7 [20], and a consensus alignment was created using native implementation of CLUSTALW [20]. A consensus of the almost-complete 16S rRNA gene sequence was created for each novel strain by manual inspection using MEGA7 and FinchTV (Geospiza) to correct base-pair discrepancies (NYU-BL-A3\textsuperscript{T}, 1459 bp, and NYU-BL-A4\textsuperscript{T}, 1460 bp). Finally, a multiple alignment was created to the consensus sequences of the novel strains as well as type sequences of other members of the family Erysipelotrichaceae obtained from NCBI GenBank; the analysis involved a total of 33 nucleotide sequences (Fig. 1). The evolutionary history was inferred using the neighbour-joining method [21]. The percentage of replicate trees in which the associated taxa clustered together was tested by the bootstrap method with 500 replicates [22]. The evolutionary distances were computed using the Kimura 2-parameter method [23]. All ambiguous positions were removed for each sequence pair. There were a total of 1556 positions in the final dataset.

Evolutionary analyses were conducted in MEGA7 [19]. For phylogenetic placement, type strains NYU-BL-A3\textsuperscript{T} and NYU-BL-A4\textsuperscript{T} were compared with members of the family Erysipelotrichaceae and other closely related bacteria (Fig. 1). Strain NYU-BL-A3\textsuperscript{T} was most closely related to Allobaculum stercoricans (87.7 % sequence similarity), Faecalibaculum rodentium (83.8 %), Faecalicoccus acidiformans (84.3 %), Faecalibacterium prausnitzii (82.7 %), Catenisphaera adipataccumulans (82.9 %), Holdemanaella biforans (83.0 %), Eubacterium tortuosum (82.6 %), Eubacterium dolichum (82.1 %), Clostridium innocuum (80.8 %), Erysipelothrix rhusiopathiae (78.1 %) and other closely related strains with less than 80 % similarity (Table S1, available in online Supplementary Material). Notably, two uncultured clones were nearly identical to NYU-BL-A3, one from a mouse caecal sample [24], and the other from a human ileal sample [25]. Strain NYU-BL-A4\textsuperscript{T} was related to Faecalibaculum rodentium (86.6 %), Allobaculum stercoricans (83.6 %), Faecalicoccus acidiformans (84.6 %), Faecalibacterium prausnitzii (84.5 %), Faecalibacterium prausnitzii (84.5 %), Catenisphaera adipataccumulans (85.5 %), Holdemanaella biforans (84.8 %), Eubacterium tortuosum (81.7 %), Eubacterium dolichum (81.6 %), Clostridium innocuum (82.4 %), Erysipelothrix rhusiopathiae (79.8 %) and other closely related strains with less than 80 % similarity (Table S1). Based on 16S rRNA gene sequence phylogenetic analysis, the novel strains NYU-BL-A3\textsuperscript{T} and NYU-BL-A4\textsuperscript{T} were placed within the family Erysipelotrichaceae but are distant from all members of this family. Pairwise similarities with the type strains of all other members of the family were all <90 % (Table S1), and while there are no strict rules for defining new genera based on 16S rRNA gene sequence homology, it has been suggested that taxa should be grouped into a genus if they share >95 % 16S rRNA gene sequence [26].

It has recently been proposed that 86.7 % 16S rRNA gene sequence similarity could be used as a cut-off for grouping bacteria into families [26]; however, there are other considerations, including physiological characteristics. There has been considerable reorganization within the phylum Firmicutes, and it has recently been proposed to split the families Coprobacillaceae and Turicibacteraceae from the family Erysipelotrichaceae [9, 13]; however, these proposals have not yet been validly published. Moreover, several additional misclassified species are related to members of the family Erysipelotrichaceae. Clostridium innocuum, Eubacterium dolichum and Eubacterium tortuosum cluster with the genus Allobaculum branch, Anaerorhabdus furcosa clusters with the genus Erysipelothrix branch, and Sharpea azabuensis, Clostridium spiroforme, Clostridium saccharogumia, Clostridium cocleatum and Clostridium ramosum cluster with the genus Coprobacillus branch (Fig. 1 and [13]). In light of the flux of this family and ongoing taxonomic uncertainty, we will refrain from creating a novel family in this manuscript, but further work is clearly warranted to reclassify members within the order Erysipelotrichales.

Genomic DNA G+C content (mol%) was calculated from whole-genome sequences. DNA from strains NYU-BL-A3\textsuperscript{T}, NYU-BL-A4\textsuperscript{T}, NYU-BL-E8, NYU-BL-F16 and NYU-BL-K8 was sequenced by 150×150 paired-end reads on the Illumina MiSeq platform. Individual genomes were assembled using SPAdes [27], using the parameters recommended for longer Illumina reads: k-mer sizes of 21, 33, 55, 77, 99 and 127, with the number of mismatches and indels reduced by Mismatch-Corrector in SPAdes. Genome assembly quality control and DNA G+C content (mol%) calculation were performed using the quality assessment tool for genome assemblies (QUAST) [28]. The DNA G+C content was 41 mol% for all three strains within the NYU-BL-A3\textsuperscript{T} group (41.1, 41.2 and 41.0 for NYU-BL-A3\textsuperscript{T}, NYU-BL-E8 and NYU-BL-F16, respectively), and the genome size was 2,946,774 base pairs. The DNA G+C content was 42.5 mol% for NYU-BL-A4\textsuperscript{T} and 53.7 mol% for NYU-BL-K8 (Fig. S1), and the genome size was 2,387,755 base pairs.

Except as stated for morphological observations and biochemical characterizations, strains were grown on Brucella agar supplemented with 5 % defibrinated sheep blood (Anaerobe Systems) at 37 °C for 48 h. Cells were examined with an Olympus CX41 microscope using phase contrast at ×1000 magnification. Growth was tested in chipped meat broth (CM), Brucella broth (BB), thioglycolate broth(TB) and MTGE broth (Anaerobe Systems, Morgan Hill, CA). No growth was detected inCM, BB or TB within 5 days, but turbid growth was detected within 48 h in MTGE for all strains.

The strains were characterized phenotypically using standard methods [29, 30] that included conventional tests such as spot indole, catalase, motility, spore-formation and PRAS biochemicals for carbohydrate fermentation (Anaerobe Systems). API Rapid ID 32A (bioMérieux,) and Rapid ANA II (Remel) kits were used according to the manufacturers’ instructions.
For chemotaxonomic characterization, cells from the novel type strains as well as the reference strain *Allobaculum stercorisican* DSM 13633T were incubated for 72 h at 37 °C on *Brucella* agar with 5% sheep blood added. Conditions were chosen to allow comparison with values available in the literature. Analysis was performed at the Center for Microbial Identification and Taxonomy (University of Oklahoma, Norman, OK, USA). Fatty acid methyl esters were extracted using the Sherlock Microbial Identification System (MIDI) version 6.1, as described by Kömpfer and Kroppenstedt [31] and Sasser [32]. Fatty acids were identified and expressed in the form of percentages using the SMOORE6 peak-naming database. The major (>10%) fatty acids of strain NYU-BL-A3T consisted of C16:0 and C18:0, those of strain NYU-BL-A4T consisted of C10:0, C12:0, C14:0, C16:0, C18:1ω9c, and C18:1ω9c, and those of strain NYU-BL-K8 consisted of C10:0, C12:0, C14:0, C16:0, C18:2ω6,9c/anteiso-C18:0, and C18:1ω9c. Minor products are listed in Table 1. The fatty acid profile of NYU-BL-K8 matches very closely with that previously reported for *Faecalibaculum rodentium* [14] with C16:0, C18:0, C18:2ω6,9c/anteiso-C18:0 being the major products. The major products of C16:0, C18:0, and C18:1ω9c are consistent with other members of the family *Erysipelotrichaceae* [12, 13].

Whole-cell hydrolysates were examined by TLC for the presence of 2,6-diaminopimelic acid (DAP) isomers by the method of Schumann [33]. The diagnostic diamino acid was determined to be DAP in each of NYU-BL-A3T, NYU-BL-A4T, and NYU-BL-K8. The major products of C16:0, C18:0, and C18:1ω9c, and those of strain NYU-BL-K8 consisted of C10:0, C12:0, C14:0, C16:0, C18:2ω6,9c/anteiso-C18:0, and C18:1ω9c. Minor products are listed in Table 1. The fatty acid profile of NYU-BL-K8 matches very closely with that previously reported for *Faecalibaculum rodentium* [14] with C16:0, C18:0, C18:2ω6,9c/anteiso-C18:0 being the major products. The major products of C16:0, C18:0, and C18:1ω9c are consistent with other members of the family *Erysipelotrichaceae* [12, 13].
by its positive α-galactosidase, β-galactosidase and β-glucosidase activity as well as its inability to produce 6-phospho-β-galactosidase, β-glucuronidase, arginine arylamidase, histidine arylamidase, leucine arylamidase, pyroglutamic acid arylamidase, glycine arylamidase and leucyl-glycine arylamidase, as shown using the API rapid 32A system. NYU-BL-A4\(^T\) could be distinguished from the other members of the family Erysipelotrichaceae based on positive activity for 6-phospho-β-galactosidase, β-glucosidase, β-glucuronidase, arginine arylamidase, leucine arylamidase, leucyl-glycine arylamidase, pyroglutamic acid arylamidase, glycine arylamidase and histidine arylamidase and a negative reaction for β-galactosidase. Faecalibaculum rodentium strains from NYU have similar reactions to the type strain of Faecalibaculum rodentium, except 50 % of NYU strains are weakly positive for α-galactosidase and 100 % are positive for β-glucosidase, whereas the type strain is negative. The NYU Faecalibaculum rodentium strains are negative for arginine arylamidase and leucyl-glycine arylamidase, and 66 % of the strains are negative for utilization of D-raffinose, whereas the type strain of Faecalibaculum rodentium is positive.

The RapID ANA II System was also very useful in the differentiation of the groups represented by strains NYU-BL-A3\(^T\) and NYU-BL-A4\(^T\). The former was negative for α-glucosidase, leucyl-glycine arylamidase, glycine arylamidase, phenylalanine arylamidase, arginine arylamidase, serine arylamidase and pyrrolidonyl arylamidase, whereas the latter was positive. Furthermore, strain NYU-BL-A3\(^T\) produced <5 % C\(_{10:0}\) \(\alpha\)-major component of the fatty acid profile of NYU-BL-A4\(^T\).

Based on phylogenetic, biochemical and chemotaxonomic criteria, two novel genera are proposed, with *Ileibacterium valens* gen. nov., sp. nov. and *Dubosiella newyorkensis* gen. nov., sp. nov. as the type species.

### DESCRIPTION OF *ILEIBACTERIUM* GEN. NOV.

*Ileibacterium* (I.le.i.bac.ter’i.um. N.L. neut. n. *ileum* the distal part of the small intestine; L. neut. n. *bacterium* a small rod; N.L. neut. *Ileibacterium* a rod from the ileum).

Cells are Gram-stain-positive, non-spore-forming, short rods or cocci, non-motile and catalase- and lipase-negative. The organism is strictly anaerobic. Major fatty acids (>10 %) are C\(_{16:0}\) and C\(_{18:0}\). The diagnostic diamino acid of the peptidoglycan is meso-DAP. Strains have been isolated from murine intestinal contents. The type species is *Ileibacterium valens*.

### ILEIBACTERIUM VALENS SP. NOV.

*Ileibacterium valens* (va’len’s. L. pres. part. *valens* healthy).

The population of this species is associated with metabolic health, vigorously responds to dietary changes and can increase up to 10-fold following introduction of a high-fat, high sucrose diet.

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#### Table 1. Cellular fatty acid compositions of strains NYU-BL-A3\(^T\), NYU-BL-A4\(^T\), NYU-BL-K8 and Allobaculum stercoricans

All data obtained from the present study. Predominant products are shown in bold type. \(-\): Values <1 %.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>NYU-BL-A3(^T)</th>
<th>NYU-BL-A4(^T)</th>
<th>NYU-BL-K8</th>
<th>Allobaculum stercoricans DSM 13633(^T)</th>
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<tbody>
<tr>
<td>C(_{16:0})</td>
<td>2.6</td>
<td>3.3</td>
<td>3.6</td>
<td>1.3</td>
</tr>
<tr>
<td>C(_{10:0})</td>
<td>4.5</td>
<td>19.3</td>
<td>12.5</td>
<td>9.8</td>
</tr>
<tr>
<td>C(_{12:0})</td>
<td>2.3</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C(_{14:0})</td>
<td>4.9</td>
<td>3.6</td>
<td>4.4</td>
<td>2.7</td>
</tr>
<tr>
<td>C(_{15:0})</td>
<td>2.2</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C(_{14:0}) ‐OH</td>
<td>6</td>
<td>6.1</td>
<td>7.0</td>
<td>5.8</td>
</tr>
<tr>
<td>C(_{13:0})</td>
<td>2.8</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
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<td>C(_{16:1}) (\omega6c)</td>
<td>–</td>
<td>1.4</td>
<td>1.6</td>
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</tr>
<tr>
<td>C(_{18:0})</td>
<td>34.2</td>
<td>31.4</td>
<td>34.9</td>
<td>33.7</td>
</tr>
<tr>
<td>C(_{17:0})</td>
<td>3.4</td>
<td>–</td>
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<td>1</td>
</tr>
<tr>
<td>C(_{16:0}) (\omega2)‐OH</td>
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<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C(_{18:0})</td>
<td>18.9</td>
<td>12.0</td>
<td>11.7</td>
<td>12.0</td>
</tr>
<tr>
<td>C(<em>{18:1}) (\omega6c) anteiso-C(</em>{15:0})</td>
<td>2.8</td>
<td>9.8</td>
<td>10.5</td>
<td>12.5</td>
</tr>
<tr>
<td>C(_{18:1}) (\omega9c)</td>
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<td>13.0</td>
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<tr>
<td>C(_{18:1}) (\omega7c)</td>
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<td>–</td>
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BL-A4\(^T\) and NYU-BL-K8, which is consistent with *Faecalibaculum rodentium* and *Allobaculum stercoricans* that are also members of this phylogenetic cluster (Fig. 1). Although the type genus of the family Erysipelotrichaceae, *Erysipelothrix*, contains peptidoglycan belonging to the β-cross-linking type, it is now accepted that members affiliated to this family vary widely in the composition of the peptidoglycan [12, 13].

Strains NYU-BL-A3\(^T\) and NYU-BL-A4\(^T\) are Gram-stain-positive rods, strictly anaerobic, non-motile in semi-solid agar medium and by microscopy, and non-spore-forming by the ethanol spore test. These novel isolates from the murine gut were found to possess biochemical and chemotaxonomic traits consistent with organisms belonging to the family Erysipelotrichaceae including a fermentative metabolism and a similar fatty-acid composition. Although most species in the family Erysipelotrichaceae are reported as microaerophilic or facultatively anaerobic, in the recent emendation of the family by Tegtmeier et al. [9], the family description now includes strictly anaerobic or aerotolerant organisms; our strains were strictly anaerobic also. Other traits, such as the predominant fatty acids C\(_{16:0}\), C\(_{18:0}\) and C\(_{18:1}\) \(\omega9c\) and peptidoglycan type, are consistent with traits found within the family but the novel strains could be clearly distinguished from their nearest phylogenetic relatives using the characteristics shown in Tables 1, 2, S2 and S3, thus strongly supporting the formation of novel genera.

In addition to its unique 16S rRNA gene sequence, NYU-BL-A3\(^T\) could be distinguished from NYU-BL-A4\(^T\) and other members of the family Erysipelotrichaceae (Table 2)
Table 2. Characteristics that distinguish strains NYU-BL-A3T and NYU-BL-A4T from the type strains of related species in the family Erysipelotrichaceae

Strains: 1, NYU-BL-A3T; 2, NYU-BL-A4T; 3, NYU-BL-KB; 4, Allobaculum stercoricanis DSM 13633T (data from Verbang et al. [13]); 5, Faecalibaculum rodentium KCTC 15484T [14]; 6, Faecalibaculum acidifermentans LMG 27248T [11]; 7, Faecalibaculum pleomorphic KCTC 3656T [13]; 8, Faecalitalia cylindroides ATCC 27803T [13]; 9, Holdemanella bifors KCTC 5969T [13]; 10, Catenibacteria adipatacumulans DSM 25799T [10]. All strains were negative for urease, arginine dihydrolase, α-arabinosidase, glutamic acid deaminase, α-fucosidase, nitrate reduction, indole production, proline arylamidase, phenylalanine arylamidase, tyrosine arylamidase, alanine arylamidase, glutamyl-glutamic acid arylamidase and serine arylamidase. +, Positive; −, negative; w, weakly positive; v, variable.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<td>−</td>
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<td>−</td>
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<td>w</td>
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<td>Major fatty acids</td>
<td>C16:0, C16:1</td>
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<td>C16:0, C16:1</td>
<td>C16:0, C16:1</td>
<td>C16:0, C16:1</td>
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<td>C16:0, C16:1</td>
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<td>53.8</td>
<td>36.9</td>
<td>52.3</td>
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<td>38.5</td>
<td>33.3</td>
<td>32.1</td>
<td>47.7</td>
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In addition to the characteristics provided in the genus description, cells may be short rods or cocci singly, in pairs and chains, non-motile and catalase-negative. When grown on Brucella agar supplemented with blood, grey-coloured colonies are formed and grow up to 0.5 mm in 24 h and 2–3 mm in 48–72 h. The organism will grow in MTGE broth, but not in Brucella broth, chopped meat broth or thioglycollate broth. Growth is optimal at 37 °C; weak growth occurs at 30 °C but not at 42 °C. Using the API Rapid ID 32A test system, positive reactions are observed for α-galactosidase, β-galactosidase and β-glucosidase (1 strain weakly). Negative reactions are obtained for assimilation of D-mannose and raffinose. Using the RapID ANA II System, a positive reaction is only obtained with β-galactosidase. Negative reactions are obtained with alkaline phosphatase, α-arabinosidase, arginine arylamidase, α-fucosidase, α-glucosidase, glycine arylamidase, indole, leucyl-glycine aminopeptidase, p-nitrophenyl β-disaccharide, ortho-nitrophenyl-β-galactosidase, phenylalanine arylamidase, proline arylamidase and urease. Pyroglutamic acid arylamidase and serine arylamidase give negative or very weakly positive reactions. A variable reaction is obtained with N-acetyl-β-D-glucosaminidase. The major cellular fatty acids consist of C16:0 and C18:0, and minor products are C9:0, C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C17:0, C18:1ω9c and C18:ω7c anteiso-C18:0.

The type strain is NYU-BL-A3T (=ATCC TSD-63T=DSM 103668T), isolated from murine intestinal contents. The G+C content of the DNA of the type strain is 41.1 mol%, and the genome size is 2 946 774 base pairs.
**DUBOSIELLA GEN. NOV**

*Dubosia* [Du.bos.i.a. N.L. fem. dim. n. Dubosiella named after the late French-born American microbiologist René Dubos (1901–1982) for his numerous important contributions to the field of microbiology and ecology and his early discoveries of antibiotics].

Cells are Gram-stain-positive, non-spore-forming, short rods and cocci, non-motile and catalase- and lipase-negative. The organism is strictly anaerobic. Major fatty acids (>10 %) are C16:0 and C18:0. The diagnostic diamino acid of the peptidoglycan is meso-DAP. Strains have been isolated from murine intestinal contents. The type species is *Dubosia newyorkensis*.

**DUBOSIELLA NEWYORKENSIS SP. NOV.**

*Dubosia newyorkensis* (new.york.en’sis. N.L. fem. adj. newyorkensis of or belonging to the state of New York in the USA, where the first isolate was obtained.)

In addition to the characteristics provided in the genus description, cells may be short rods or cocci, appearing singly, in pairs and in chains, and non-motile. When grown on Brucella agar supplemented with blood, grey-coloured colonies are formed and grow up to 0.5 mm in 24 h and 2–3 mm in 48–72 h. The organism will grow in MTGE broth, but not in Brucella broth, chopped meat medium broth or thioglycolate broth. Growth is optimal at 37°C; weak growth occurs at 30°C but not at 42°C. Using the API Rapid 32A test system, positive reactions are observed for arginine arylamidase, 6-phospho-β-galactosidase, β-glucosidase, β-glucuronidase, glycine arylamidase, histidine arylamidase, leucine arylamidase, leucyl-glycine arylamidase and pyroglutamic acid arylamidase. Negative reactions are obtained for alanine arylamidase, alkaline phosphatase, α-arabinosidase, arginine dihydrolase, glutamyl-glutamic acid, α-fucosidase, β-galactosidase, glutamic acid decarboxylase, indole production, nitrate reduction, phenylalanine arylamidase, proline arylamidase, serine arylamidase, tyrosine arylamidase and urease. α-Glucosidase is either negative or weakly positive. Variable reactions are obtained for N-acetyl-β-glucosaminidase, α-galactosidase, and assimilation of d-mannose and raffinose. Using the rapid RapID-ANA II System, positive reactions are obtained with β-N-acetyl-α-glucosaminidase, α-glucosidase, β-glucosidase, α-galactosidase, leucyl-glycine aminopeptidase, glycine arylamidase, phenylalanine arylamidase and proline arylamidase. Negative reactions are obtained with alanine phosphatase, arginine arylamidase, arginine arylamidase, α-fucosidase, indole production, p-nitrophenyl β-disaccharide, ortho-nitrophenyl-β-galactosidase, serine arylamidase and urease. Pyroglutamic acid arylamidase is variable. The major fatty acids consist of C10:0, C16:0, C18:1ω9c and C18:2ω6c/ω9c. Minor products include C9:0, C12:0, C14:0, C16:1ω7c/C16:1ω6c and C18:2ω6c/ω9c and anteiso-C18:0.

The type strain is NYU-BL-A4T (=ATCC TSD-64T=DSM 103457T), isolated from murine intestinal contents. The G+C content of the DNA of the type strain is 42.5 mol%, and the genome size is 2,387,755 base pairs.

**EMENDED DESCRIPTION OF FAECALIBACULUM RODENTIUM CHANG ET AL. 2015**

The description is that of Chang et al. [14] except that strains may be weakly positive for α-galactosidase and positive for β-glucosidase, whereas the type strain of *Faecalibaculum rodentium* (JCM 30274T) is negative. Also, some strains may be negative for arginine arylamidase and leucyl-glycine arylamidase activity and raffinose assimilation, whereas, the type strain is positive.

Funding information

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Conflicts of interest

The authors declare that there are no conflicts of interest.

References

11. de Maesschalck C, van Immerseel F, Eckhardt V, de Baere S, Cronkaert M et al. Faecalibacillus acidiformans gen. nov., sp. nov., isolated from the chicken Caecum, and reclassification of Streptococcus pleomorphus (Barnes et al. 1977), Eubacterium bifforme (Eggerth 1939) and Eubacterium cylindroides (Cato et al. 1974) as Faecalibacillus pleomorphus comb. nov., Holdemanella biformis gen. nov., comb. nov. and Faecalitalea cylindroides gen. nov., comb. nov.


