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Allobaculum mucilyticum sp. nov. and *Allobaculum fili* sp. nov., isolated from the human intestinal tract

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Abstract

As part of a culturomics study to identify bacterial species associated with inflammatory bowel disease, a large collection of bacteria was isolated from patients with ulcerative colitis. Two of these isolates were tentatively identified as members of the family *Erysipelotrichaceae*. Following phylogenetic analysis based on 16S rRNA gene sequence and genome sequences, both strain 128^T and 539^T were found to be most closely related to *Allobaculum stercoricanis*, with G+C contents of 48.6 and 50.5 mol%, respectively, and the genome sizes of 2864314 and 2580362 base pairs, respectively. Strains 128^T and 539^T were strict anaerobe rods that grew in long chains between 37 and 42 °C. Scanning electron microscopy did not reveal flagella, fimbriae or visible endospores. Biochemical analysis showed nearly identical results for both strains with enzymatic activity of C4 and C8 esterases, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-glucuronidase, *N*-acetyl-β-glucosaminidase and arginine arylamidase. In addition, both strains produced indole and reduced nitrate. Major fatty acids were identified as $C_{18:1}$ ω9*c* (oleic acid, 64.06% in 128^T and 74.35% in 539^T), $C_{18:1}$ ω7*c*/$C_{18:1}$ ω9t/$C_{18:1}$ ω12t/UN17.834 (16.18% in 128^T and 6.22% in 539^T) and $C_{16:0}$ (6.23% in 128^T and 7.37% in 538^T). Based on these analyses two novel species are proposed, *Allobaculum mucilyticum* sp. nov. with the type strain 128^T (=NCTC 14626^T=DSM 112815^T) and *Allobaculum fili* sp. nov. with the type strain 539^T (=NCTC 14627^T=DSM 112814^T).

INTRODUCTION

The family *Erysipelotrichaceae* was originally proposed by Verbarg *et al.* [1] in 2004 and named after the family's original genus *Erysipelothrix*—best known for the member *Erysipelothrix rhusiopathiae*, which is an animal and zoonotic pathogen [2]. To date, the family *Erysipelotrichaceae* has expanded to more than 30 (proposed) genera according to the List of Prokaryotic names with Standing in Nomenclature (LPSN, http://www.bacterio.net), including *Allobaculum* [3], *Bulleidia* [4], *Catenisphaera* [5], *Dubosiella* [6], *Faecalibaculum* [7], *Faecalicoccus* [8], *Holdemania* [9], *Ileibacterium* [6] and *Solobacterium* [10]. Members of the family *Erysipelotrichaceae* are Gram-positive, anaerobic, non-motile, slender rods. In addition, several members have been described to have the tendency to form long filaments and contain a B-type peptidoglycan with a unique peptide cross-linking [1]. The vast majority of *Erysipelotrichaceae* family members have been isolated from the faeces or the intestinal tract of humans or animals and, with the recent advances in microbiota research, have been increasingly associated with metabolic syndrome and intestinal inflammatory disorders in both humans and mice. *Erysipelotrichaceae* therefore are an interesting but functionally still largely understudied family of bacteria.

The genus *Allobaculum* ('the other small rod') currently consists of the sole species *Allobaculum stercoricanis*, described by Greetham *et al.* in 2004 after being isolated from the faeces of a dog [3]. *A. stercoricanis* grows strictly anaerobic in pairs or chains, is non-spore-forming and non-motile, and was shown to be present in the intestinal tract of all dogs examined, suggesting it is

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Keywords: *Allobaculum*; *Erysipelotrichaceae*; IBD; intestine; microbiota.
Abbreviations: ANI, average sequence identity of shared genes; CDS, coding sequences; GMM, gut microbiota medium; IBD, inflammatory bowel disease; POCP, percentage of conserved proteins.
Repositories: *Allobaculum mucilyticum* 128^T 16S rRNA gene GenBank accession number: MZ153114 *Allobaculum mucilyticum* 128^T WGS NCBI accession number: JAHUZH000000000 *Allobaculum fili* 539^T 16S rRNA gene GenBank accession number: MZ153115 *Allobaculum fili* 539^T 16S NCBI WGS gene accession number: JAHDSX000000000.
†These authors contributed equally to this work
One supplementary table is available with the online version of this article.

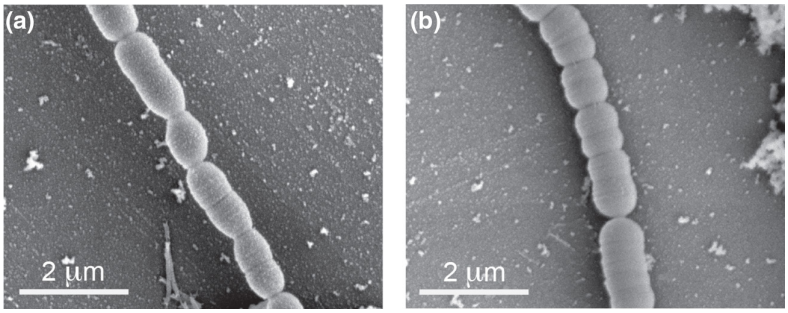

Fig. 1. Scanning electron microscope images of strains 128^T (a) and 539^T (b).

widely dispersed without an obvious association with canine pathogenesis. Since then, 16S rRNA gene profiling of the microbiota of humans, mice and rats has often identified the genus *Allobaculum* in the context of dietary interventions [11], ageing [12], intestinal inflammation [13] or experimental autoimmune encephalitis [14], but thus far no new additions to the genus have been proposed.

In this article, we describe two novel, strictly anaerobic *Allobaculum* strains 128^T and 539^T isolated from the faeces of humans with inflammatory bowel disease (IBD). We have previously described *Allobaculum* strain 128^T to produce a large array of human intestinal mucin degrading carbohydrate active enzymes [15]; however, further phylogenetic and chemotaxonomic characterization of these strains is warranted. While being closely related to *A. stercoricanis*, the 16S rRNA gene sequences of isolates 128^T and 539^T show 91.95 and 93.25% similarity (95.49% similar to each other) and therefore phylogenetically cluster separately. We propose to designate 128^T as *Allobaculum mucilyticum* sp. nov. and 539^T as *Allobaculum fili* sp. nov.

ISOLATION AND MORPHOLOGY

Strains 128^T and 539^T were isolated from faecal samples of two patients with inflammatory bowel disease as part of an IBD culturomics study. Samples were stored at −20 °C, thawed in the anaerobic chamber, diluted in deoxygenated phosphate-buffered saline (pH 7.4) and cultured under strict anoxic culturing conditions in a vinyl anaerobic chamber (Coy Labs) with a gas mixture containing 5% H_2, 10% CO_2 and 85% N_2 using nonselective gut microbiota medium (GMM) [16] culture plates, on which visible bacteria appeared after 2–3 days as opaque to white, mainly round, flat colonies with undulating margins and a dry mucoid appearance. GMM broth and plates were prereduced to deplete oxygen for at least 24 h prior to bacterial culturing. Strains 128^T and 539^T are strict anaerobes as no growth was observed when grown on GMM plates in the presence of 1%, 5% or 20% oxygen in jars from the Anoxomat Mark II system (Advanced Instruments). Both strains showed limited growth at 30 °C but grew readily between 37 and 42 °C. To assess the generation time, static liquid GMM cultures were inoculated with three separate bacterial colonies each and grown for 16 h at 37 °C in the anaerobic chamber. Bacteria were diluted to a starting OD_{600} of 0.01 in liquid GMM and absorbance values were recorded using a FLUOstar Omega plate reader (BMG Labtech). Both strains 128^T and 539^T had a generation time of ~130 min in GMM broth at 37 °C under anoxic conditions. Both strains grew between pH 6.0–8.0 in GMM, with an optimum between pH 6.5–7.0. Gifu anaerobic medium supported moderate growth as compared to GMM medium, while Brain Heart Infusion medium did not support growth at all. Scanning electron microscopy (SEM) of strains 128^T and 539^T, cultured in GMM broth for 48 h under anoxic conditions at 37 °C, revealed rods with tapering ends and varying lengths (~1–2×0.7 µm) that grew in long chains and appeared to have one or more symmetrical ring-like structures that resemble Z-rings that drive septum constriction (Fig. 1). SEM did not reveal flagella or fimbriae. No visible endospores were observed, similar to what has been previously reported for other members of the family *Erysipelotrichaceae* [1, 17].

16S RRNA AND GENOME-BASED PHYLOGENY

The near-complete 16S rRNA gene sequences of strain 128^T and 539^T were obtained by PCR using the universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-CGGTTACCTTGTTACGACTT-3′), Sanger-sequenced (Macrogen) and deposited under GenBank accession numbers MZ153114 and MZ153115, respectively. The EzBioCloud 16S-based ID database [18] was searched for the closest homologues for strains 128^T and 539^T, which was an uncultured bacterium from the faeces of a rat for both strains (accession FJ880578, 95.10 and 99.72% similarity, respectively). Based on 16S rRNA sequence similarity alone this uncultured bacterial clone FJ880578, designated '*Allobaculum*' in the EzBioCloud 16S Database, likely belongs to the same species as strain 539^T. The 16S rRNA gene sequence similarity between strain 128^T and 539^T was 95.49% (EzBioCloud Pairwise Nucleotide Sequence Alignment for Taxonomy tool), suggesting that they are distinct species from the same genus. When the

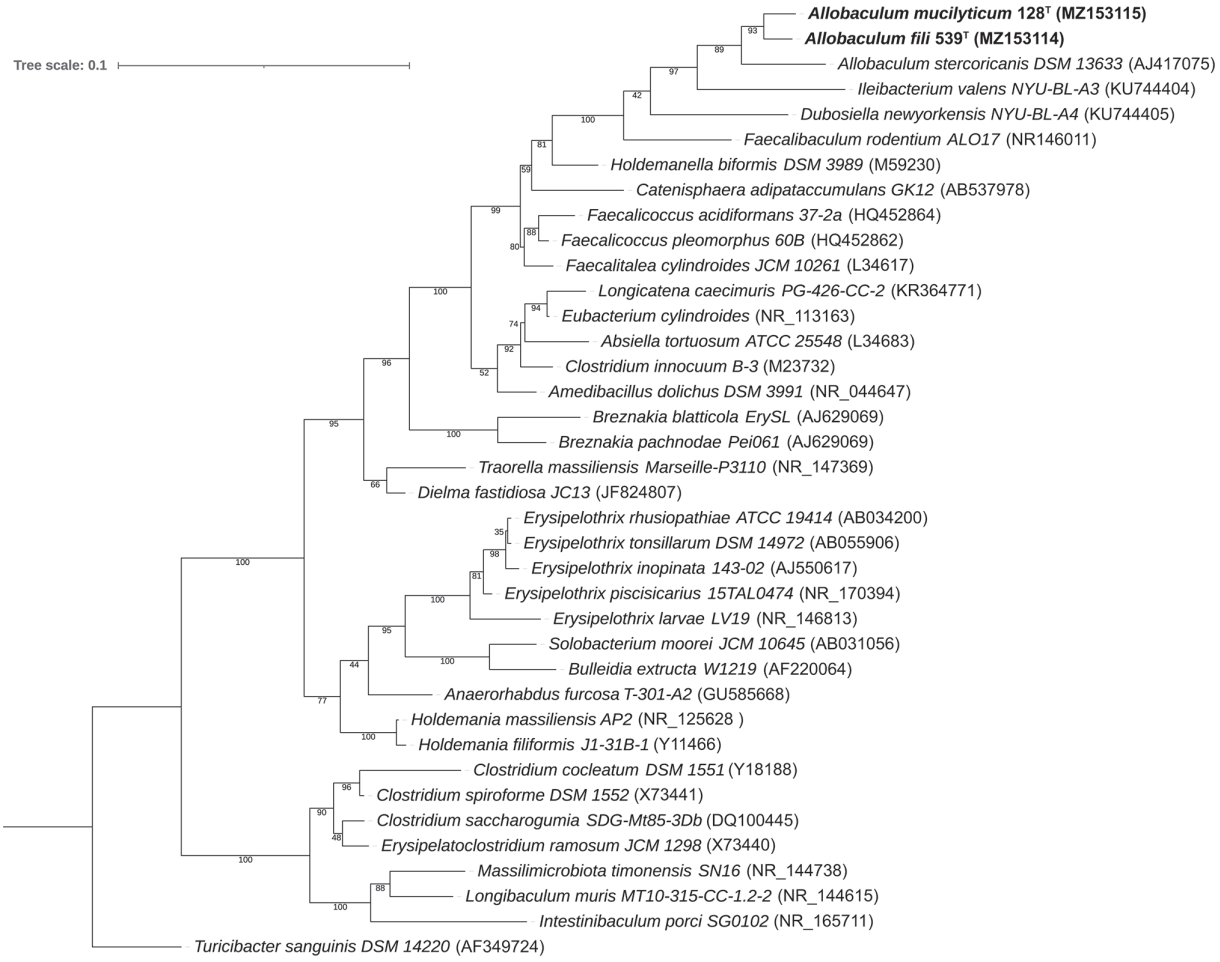

Fig. 2. Maximum-likelihood phylogenetic dendrogram based on 16S rRNA gene sequences of strains 128^T and 538^T and all *Erysipelotrichaceae* family member 16S rRNA gene RefSeq sequences. 16S rRNA gene sequences accession numbers are given in parentheses. The sequences were aligned using the SINA aligner (version 1.2.11) using FastTree (GTR model) [19] and visualized using iTOL (https://itol.embl.de). The percentage of 500 bootstrap tests are shown at the branching point with branch lengths measured in the number of base substitutions per site. Bar represents 0.05 substitutions per nucleotide position. *Escherichia coli* M25588.1 was used as an outgroup.

16S rRNA gene sequence similarity of strain 128^T and 539^T was compared to *A. stercoricanis* DSM 13633^T, the only previously classified *Allobaculum* species, 128^T had a similarity of 91.95%, while 539^T was 93.25% similar.

To further examine the phylogeny of type strains 128^T and 539^T, a maximum-likelihood phylogenetic tree was reconstructed with the SINA aligner version 1.2.11 (www.arb-silva.de/aligner/) using FastTree (GTR model) [19] and visualized using iTOL (https://itol.embl.de). RefSeq 16S rRNA gene sequences of all *Erysipelotrichaceae* family members, after removal of duplicate sequences, were used as input for the aligment with bootstrap values based on 500 repetitions (Fig. 2). The 16S rRNA gene sequence of *Turicibacter sanguinis* strain DSM 14220^T was used as an outgroup to root the phylogenetic tree. Strains 128^T and 539^T were placed within the family *Erysipelotrichaceae* and were most closely related to *A. stercoricanis* (91.95 and 93.25% sequence similarity, respectively) and *Ileibacterium valens* (89.56 and 90.25%, respectively) (Fig. 3). Guidelines for sequence-based grouping of taxa into the same genus are not strictly defined and can vary greatly among different taxa. This may pose a particular challenge for the family of *Erysipelotrichaceae*, which displays high phylogenetic heterogeneity and is characterized by many monotypic genera [20]. For instance, >94.5% similarity based on 16S rRNA gene sequence was recently proposed as a genus cutoff [21], which would place strains 128^T and 539^T in the same genus but outside the genus *Allobaculum* when compared to *A. stercoricanis*. Notably, with 93.25% similarity strain 539^T is phylogenetically markedly closer to the genus *Allobaculum* than strain 128^T while both strains genetically belong to the same genus, highlighting the limits of using cutoff for genus classification based on 16S rRNA gene sequences.

We next used the genome sequences of type strains 128^T and 539^T for phylogenomic analysis using the Genome Taxonomy Database Toolkit (GTDB-Tk, version 2.1.1) [22, 23]. Based on topological placement (strains 128^T) and topological placement

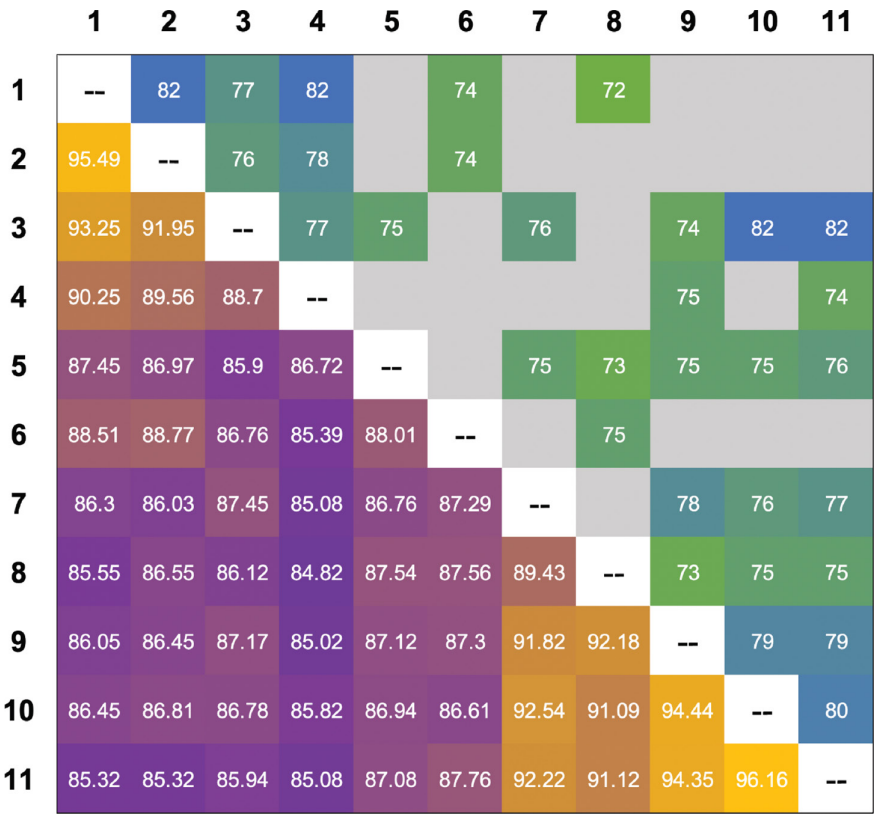

Fig. 3. 16S rRNA gene sequence similaries were calculated using EzBioCloud's Pairwise Nucleotide Sequence Alignment for Taxonomy Tool (www.ezbiocloud.net/tools/pairAlign) and shown in the bottom half of the matrix in orange and purple. Average sequence identities of shared genes (ANI) as calculated using the ANI/AAI-Matrix Genome-based distance calculator [23] are shown in the top half of the matrix in blue and green. Grey boxes has values below the cutt-off. Strains: 1, Allobaculum *fili* 539^T; 2, Allobaculum *mucilyticum* 128^T; 3, *Allobaculum stercoricanis* DSM 13633^T; 4, *Ileibacterium valens* NYU-BL-A3^T; 5, *Dubosiella newyorkensis* NYU-BL-A4^T; 6, *Faecalibaculum rodentium* ALO17; 7, *Holdemanella biformis* MGYG-HGUT-01711; 8, *Catenisphaera adipataccumulans* DSM 25799^T; 9, *Faecalitalea cylindroides* strain An64; 10, *Faecalicoccus acidiformans* DSM 26963^T; 11, *Faecalicoccus pleomorphus* NCTC 11087.

and ANI (strain 539^T), both strains were placed within the genus *Allobaculum* and were classified as separate species, confirming the results obtained with 16S rRNA gene phylogeny (Table S1, available in the online version of this article). We next used the 128^T and 539^T genome sequences and closely related *Erysipelotrichaceae* family members (based on the 16S phylogenetic analysis depicted in Fig. 2) to calculate average sequence identities of shared genes (ANI) using an ANI/AAI-matrix genome-based distance calculator [24] (Fig. 3). These calculations again show that strains 128^T and 539^T do not belong to the same species and do not cluster with other known *Erysipelotrichaceae* species, as all ANI values fell outside of the proposed 83–95% range [25]; 128^T and 539^T had ANI values of 82% when compare to each other, and 77 and 76% when compared to *A. stercoricanis*, respectively. An alternative method to delineate genera and estimate their evolutionary and phenotypic distance uses the percentage of conserved proteins (POCP) [26]. POCP values between 128^T and *A. stercoricanis*, and 539^T and *A. stercoricanis* were 54.5 and 57.2%, respectively, which is well above the proposed genus boundary of 50%. We therefore conclude that, while the 16S rRNA gene sequence of strains 128^T and 539^T differ significantly from that of *A. stercoricanis*, both strains belong to the genus *Allobaculum* as based on the POCP values.

GENOMIC CHARACTERIZATION

Genome sequence of strains 128^T and 539^T were obtained using Illumina MiSeq sequencing with a 2×250 bp paired-end configuration. The resulting sequencing reads were assembled using SPAdes version 3.11.1 [27] using k-mer lengths of 57, 97 and 127. Quality control of the genome assemblies was performed using QUAST [28]. The genome sizes of strains 128^T and 539^T were 2864314 base pairs (412 contigs) and 2 580 362 base pairs (201 contigs), respectively. The DNA G+C content was calculated at 48.6 mol% for 128^T and 50.5 mol% for 539^T, which was markedly higher than *A. stercoricanis* DSM 13633 (37.8 mol%) and the closely related *Ileibacterium valens* NYU-BL-A3^T (41.1 mol%) and *Dubosiella newyorkensis* NYU-BL-A4^T (42.5 mol%), but slightly lower than *Faecalibaculum rodentium* NYU-BL-K8 (53.7 mol%) [6]. Genomes were annotated using PROKKA 1.14.6 [29], which

Table 1. Characteristics of strains 128^T and 539^T compared to the closely related *Erysipelotrichaceae* family members *Allobaculum stercoricanis* and *Ileibacterium valens*

Data for *A. stercoricanis* from Verbarg *et al.* [20]. Data for *I. valens* from Cox *et al.* [6]. +, Positive; –. negative; w, weakly positive; v, variable; ND, not determined.

Characteristic	128^T	539^T	DSM 13633*A. stercoricanis*^{T}	NYU-BL-A3^T*I. valens*
Alkaline phosphatase	–	–	+	–
Esterase (C4)	w	w	+	ND
Esterase lipase (C8)	w	w	+	ND
Lipase (C14)	–	–	–	ND
Arginine arylamidase	w	+	+	–
Leucine arylamidase	–	–	–	–
Proline arylamidase	–	–	–	–
Leucyl glycine arylamidase	–	–	–	–
Phenylalanine arylamidase	–	–	–	–
Pyroglutamic acid arylamidase	–	–	–	–
Tyrosine arylamidase	–	–	–	–
Alanine arylamidase	–	–	–	–
Glycine arylamidase	–	–	–	–
Histidine arylamidase	–	–	–	–
Glutamyl glutamic acid arylamidase	–	–	–	–
Serine arylamidase	–	–	–	–
Valine arylamidase	–	–	–	ND
Cystine arylamidase	–	–	ND	ND
Trypsin	–	–	–	ND
α-Chymotrypsin	–	–	–	ND
Acid phosphatase	+	+	+	ND
Naphthol-AS-BI-phosphohydrolase	+	+	ND	ND
α-Galactosidase	–	–	–	+
β-Galactosidase	+	+	+	+
β-Galactosidase-6-phosphate	–	–	–	–
β-Glucuronidase	+	w	–	–
α-Glucosidase	–	–	+	–/w
β-Glucosidase	–	–	–	+
α-Arabinosidase	–	–	–	–
N-Acetyl-β-glucosaminidase	+	+	w	–
α-Mannosidase	–	–	–	ND
α-Fucosidase	–	–	–	–
Urease	–	–	–	–
Glutamic acid decarboxylase	–	–	–	–
Arginine dihydrolase	–	–	–	–
Mannose fermentation	–	–	–	v
Raffinose fermentation	–	–	–	v
Indole production	w	w	–	–
Nitrate reduction	w	w	–	–

Table 2. Fatty acid composition of strains 128^T and 539^T compared to the closely related *Erysipelotrichaceae* family members *Allobaculum stercoricanis* and *Ileibacterium valens*

Data from *A. stercoricanis* from Verbarg *et al.* [20]. Data from *I. valens* from Cox *et al.* [6]. Summed features are fatty acids that cannot be resolved reliably from another fatty acid using the chromatographic conditions chosen. The MIDI system groups these fatty acids together as one feature with a single percentage of the total. UNx, unknown fatty acid of ECLx; DMA, dimethyl acetal.

Fatty acid	128^T	539^T	DSM 13633*A. stercoricanis*^{T}	NYU-BL-A3^T*I. valens*
$C_{9:0}$	–	–	1.3	2.6
$C_{10:0}$	–	0.1	9.8	4.5
$C_{11:0}$	–	–	–	2.3
$C_{12:0}$	–	–	2.7	4.9
$C_{13:0}$	–	–	–	2.2
$C_{14:0}$	0.3	0.4	5.8	6.0
$C_{15:0}$	–	–	1.0	2.8
$C_{16:0}$	6.23	7.4	33.7	34.2
$C_{17:0}$	–	–	1.0	3.4
$C_{18:0}$	3.6	3.8	12.0	18.9
$C_{16:1}$ *ω7c*	1.6	1.7	–	–
$C_{16:1}$ *ω7c*/ω6*c*	–	–	1.5	–
$C_{18:1}$ *ω9c*	64.1	74.4	15.2	9.4
$C_{18:1}$ *ω7c*	–	–	3.5	–
$C_{20:1}$ *ω9c*	0.5	0.4	–	–
$C_{18:2}$ *ω*6,9*c* and/or $C_{18:0}$ anteiso	–	–	12.5	2.8
UN18.199 and/or $C_{18:2}$ *ω7c*/ω9*c* DMA	2.3	2.1	–	–
$C_{18:1}$ *ω7c* DMA	2.4	1.8	–	–
$C_{18:1}$ *ω7c*/ω9t/ω12t and/or UN17.834*	16.2	6.2	–	–
$C_{17:1}$ *ω*8*c* and/or $C_{17:2}$†	–	0.3	–	–
UN18.622 and/or $C_{19:0}$ iso‡	2.7	1.5	–	–

*MIDI analysis designation 'summed in feature 10', which contains $C_{18:1}$ *ω7c*/*ω*9t/*ω*12t and/or an unknown fatty acid of ECL 17.834.
†MIDI analysis designation 'summed in feature 8', which contains $C_{17:1}$ *ω*8c and/or $C_{17:2}$ and/or an unknown fatty acid of ECL 16.801.
‡MIDI analysis designation 'summed feature 12', which contains $C_{19:0}$ iso and/or an unknown fatty acid of ECL 18.622.

yielded a predicted 2253 coding sequences (CDS) for strain 128^T and 2049 CDS for strain 539^T. The NCBI accession numbers of strain 128^T and 539^T are JAHUZH000000000 and JAHDSX000000000, respectively.

PHYSIOLOGY AND CHEMOTAXONOMY

Allobaculum strains 128^T and 539^T were characterized phenotypically using API Rapid ID32A and API ZYM (bioMérieux) according to the manufacturers' instructions (Table 1). Similar to the closest relative *A. stercoricanis*, both strains 128^T and 539^T exhibited weak C4 and C8 esterases activity, acid phosphatase activity and lacked most arylamidase activity except for arginine arylamidase, which was present in strains 128^T and 539^T, as well as in *A. stercoricanis*. *β*-galactosidase, *β*-glucosidase and *N*-acetyl-*β*-glucosaminidase was also present in all strains. In contrast to *A. stercoricanis*, strains 128^T and 539^T showed *β*-glucuronidase activity and were able to reduce nitrate and produce indole, but did not exhibit alkaline phosphatase activity. Overall, strains 128^T and 539^T were very similar despite minor variations in detected levels of enzymatic activity.

The fatty acids contents from bacterial cultures grown for 48 h in GMM media at 37 °C under anoxic conditions were identified by the Sherlock Microbial Identification System (MIDI) using gas chromatographic analysis of fatty acid methyl esters [30] carried out by the Identification Service, Leibniz-Institut DSMZ – Deutsche Sammlung von Microorganismen und Zellkulturen GmbH (Braunschweig, Germany; Table 2). The major fatty acids of strain 128^T were $C_{18:1}$ *ω9c* (oleic acid, 64.1%), $C_{18:1}$ *ω7c*/$C_{18:1}$ *ω*9t/$C_{18:1}$ *ω*12t/UN17.834 (16.2%) and $C_{16:0}$ (6.2%). For strain 539^T the major fatty acids were $C_{18:1}$ *ω9c* (oleic acid, 74.4%), $C_{16:0}$ (7.4%)

and $C_{18:1}$ *ω7c*/$C_{18:1}$ *ω*9t/$C_{18:1}$ *ω*12t/UN17.834 (6.2%). Minor fatty acids present in strains 128^T and 539^T are shown in Table 2. The major fatty acids in the membrane of *A. stercoricanis* were reported to be $C_{16:0}$, $C_{18:1}$ *ω9c* and $C_{18:0}$, which were also detected in strains 128^T and 539^T, and $C_{18:2}$ n-6 (linoleic acid), which was absent from both strains. Both strains had notably high levels of membrane oleic acid; while *Dakotella fusiforme* was shown to contain 29.8% oleic acid [17], lower amounts of membrane oleic acid have been reported for other *Erysipelotrichaceae* family members, including *Ileibacterium valens*, *Dubosiella newyorkensis* [6], *Erysipelothrix rhusiopathiae*, *Coprobacillus cateniformis* and *Turicibacter sanguinis* [20].

DESCRIPTION OF *ALLOBACULUM MUCILYTICUM* SP. NOV.

Allobaculum mucilyticum (mu.ci.ly'.ti.cum. L. masc. n. *mucus*, slime; Gr. adj. *lytikos*, causing degradation; N.L. neut. n. *mucilyticum*, mucus-degrading).

Cells are rod-shaped with tapering ends. On average, ~1–2 µm long and ~0.7 µm wide. Gram-stain-positive. Cells grow under strict anoxic conditions between 30 and 42 °C, with an optimal temperature of 37 °C, in long chains, are non-motile and show no visible fimbriae. Optimal growth is observed with gut microbiota medium, while Gifu anaerobic media showed limited growth. Growth was observed at a pH between 6.0–8.0 with an optimal pH of 6.5–7.0. With the API Rapid ID32A and API ZYM systems, positive reactions were observed for C4 and C8 esterases activity (weak activity), acid phosphatase activity, naphthol-AS-BI-phosphohydrolase activity, arginine arylamidase activity, *β*-glucuronidase activity, *N*-acetyl-*β*-glucosaminidase activity, indole production and nitrate reduction. Major fatty acids are $C_{18:1}$ *ω9*c, $C_{18:1}$ *ω7c*/$C1_{8:1}$ *ω9*t/$C1_{8:1}$ *ω12*t/UN17.834 and $C_{16:0}$. The type strain is 128^T (=NCTC 14626^T=DSM 112815^T), isolated from the intestinal tract of a patient with ulcerative colitis. Its G+C content is 48.6 mol% and the genome size is 2864314 base pairs.

DESCRIPTION OF *ALLOBACULUM FILI* SP. NOV.

Allobaculum fili (fi'li. L. gen. n. *fili*, of a string).

Cells stain Gram-positive and are rod-shaped with tapering ends. On average, ~1–2 µm long and ~0.7 µm wide. Cells grow in long chains and are restricted to anoxic conditions with a temperature range between 30 and 42 °C, with an optimal temperature of 37 °C, are non-motile and show no visible fimbriae. Optimal growth is observed with gut microbiota medium, while Gifu anaerobic media showed limited growth. Growth was observed at a pH between 6.0–8.0 with an optimal pH of 6.5–7.0. With the API Rapid ID32A and API ZYM systems, positive reactions were observed for C4 and C8 esterases activity (weakly positive), arginine arylamidase activity, acid phosphatase activity, naphthol-AS-BI-phosphohydrolase activity, *β*-glucuronidase activity, *N*-acetyl-*β*-glucosaminidase activity, indole production and nitrate reduction. Major fatty acids are $C_{18:1}$ *ω9c*, $C_{16:0}$ and $C_{18:1}$ *ω7c*/$C_{18:1}$ *ω*9t/$C_{18:1}$ *ω*12t/UN17.834. The type strain is 539^T (=NCTC 14627^T=DSM 112814^T), isolated from the intestinal tract of a patient with ulcerative colitis. Its G+C content is 50.5 mol% and the genome size is 2580362 base pairs.

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Author contributions
M.R.d.Z., N.W.P., T.A.R., R.A.F. and G.H.v.M. conceived and designed the study and co-wrote the manuscript. M.R.d.Z. and N.W.P. isolated *A. mucilyticum* sp. nov. T.A.R. isolated *A. fili* sp. nov. G.H.v.M performed all experiments.

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

Ethical statement
Strain 128^T was isolated from a faecal sample obtained under human study protocol No.10–1047, which was approved by the Institutional Review Board of the Icahn Medical School at Mount Sinai, New York. Strain 538^T was isolated from a faecal sample obtained under the human study protocol HIC # 1607018104, which was approved by the Institutional Review Board of Yale School of Medicine. Informed consent was obtained from all participants and/or their legal guardians and all methods were performed according to relevant guidelines and regulations.

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