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Terrisporobacter hibernicus sp. nov., isolated from bovine faeces in Northern Ireland

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Abstract

A new species of *Terrisporobacter*, a Gram-positive, spore-forming anaerobic group, proposed name *Terrisporobacter hibernicus* sp. nov., was isolated in Northern Ireland from bovine faeces collected in 2016. Designated as MCA3^T, cells of *T. hibernicus* sp. nov. are rod shaped and motile. Cells tolerate NaCl from 0.5 to 5.5% (w/v), with a pH tolerance between pH 6 and 9. The optimal temperature for growth is 35–40 °C, and temperatures from 20 to 30 °C are tolerated. The polar lipid profile displays diphosphatidylglycerol, phosphatidylglycerol, two aminoglycolipids, one glycophospholipid, one aminolipid, three glycolipids, five phospholipids and one lipid. No respiratory quinones are detected. The predominant fatty acid profile includes $C_{16:0}$ at 22.8%. Strain MCA3^T is positive for glucose and maltose acidification, as well as glycerol and sorbitol. The biochemical results from a VITEK2 assay of strain MCA3^T, *Terrisporobacter petrolearius* LAM0A37^T and *Terrisporobacter mayombei* DSM 6539^T are also included for the first time. The closed and complete genome of strain MCA3^T from a hybrid Oxford Nanopore Technology MinION/Illumina assembly reveals no evidence for known virulence genes. Draft genome sequencing of *T. mayombei* DSM 6539^T and *T. petrolearius* LAM0A37^T, as performed by Illumina MiSeq, provides reference genomes for these respective species of *Terrisporobacter* for the first time. DNA–DNA hybridization values (d_4) of MCA3^T to *Terrisporobacter glycolicus* ATCC 14880^T, *T. petrolearius* LAM0A37^T and *T. mayombei* DSM 6539^T are 48.8, 67.4 and 46.3 %, with cutoff value at 70%. The type strain for *T. hibernicus* sp. nov. is MCA3^T (=NCTC 14625^T=LMG 32430^T).

BACKGROUND AND BRIEF LITERATURE REVIEW ON *TERRISPOROBACTER* SPECIES

The *Terrisporobacter* (basonym *Clostridium*) is a Gram-positive, spore-forming, anaerobic bacterial genus typically found in soil [1, 2]. Currently, this genus has three validly published species, *Terrisporobacter glycolicus*, *Terrisporobacter mayombei* and *Terrisporobacter petrolearius* and the not validly published '*Terrisporobacter othiniensis*'. The type species of the genus *Terrisporobacter* was originally described in 1963 as a member of the genus *Clostridium* when a novel species, *Clostridium glycolicum,* was isolated from mud and was found to be genetically distinct from other *Clostridium* species [3]. Closely related *Clostridium mayombei* was isolated in 1991 from an African soil-feeding termite [4]. This species has since been isolated in oil mill wastewaters, a bone marrow transplant, a wound infection, a case of an infected otogenic brain abscess, and as the sole causative agent in a cholecystitis (gallbladder infection) case [5–7]. Both *C. glycolicum* and *C. mayombei* were officially renamed as *T. glycolicus* and *T. mayombei*, respectively, in 2014 following a comparison of 16S rRNA gene sequences [2]. *T. petrolearius* was isolated in a

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Keywords: Illumina MiSeq; ONT MinION; phylogeny; *Terrisporobacter*; *Terrisporobacter hibernicus*; WGS.
Abbreviations: ANI, average nucleotide identity; BHIB, brain–heart infusion broth; CBA, Columbia blood agar; dDDH, digital DNA–DNA hybridization; MALDI-TOF, matrix-assisted laser desorption ionization–time of flight; OGRI, overall genome related index; TSA, tryptone soya agar; TYGS, Type Strain Genome Server.
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The completed genomes of the chromosome (accession CP081135) and plasmid (accession CP081136) are deposited in NCBI GenBank. The ribosomal 16S sequence is deposited in GenBank under the accession MZ497377. BioProject: PRJNA702263 *T. hibernicus* CP081135–CP081136, BioSample: SAMN17935209 16S gene sequence: Seq1 MZ497377 *T. mayombei* JAHZMP000000000, BioSample: SAMN20427643 *T. petrolearius* JAHZMO000000000, BioSample: SAMN20427644.
Seven supplementary figures and six supplementary tables are available with the online version of this article.

Table 1. Genomic characteristics of *Terrisporobacter petrolearius* LAM0A37^T, *Terrisporobacter mayombei* DSM 6539^T and strain MCA3^T

Annotations were completed using the NCBI Prokaryotic Genome pipeline.

Strain	GenBank Accession	Genome size (Mbp)	G+C content (mol%)	Total contigs	Total genes	Total CDS
Terrisporobacter petrolearius LAM0A37^T	JAHZMO000000000	4.0	28.8	269	4130	3992
Terrisporobacter mayombei DSM 6539^T	JAHZMP000000000	4.1	29.1	326	4351	4214
MCA3^T	CP081135, CP081136	4.1	28.8	2	4016	3883

petroleum reservoir and '*T. othiniensis*' was isolated from a blood culture wound [2, 8, 9]. To date, there are few references to the genus *Terrisporobacter* in the literature with no concrete information on growth, maintenance and pathogenicity, beyond that described by Chamkha *et al.* [6] and Gerritsen *et al.* [2].

INTRODUCTION

Terrisporobacter are Gram-positive, spore forming anaerobic bacteria, and a potential opportunistic pathogen [5, 7, 9–11]. A new species of *Terrisporobacter* was isolated in 2016 from bovine (*Bos taurus*) faeces in Northern Ireland. The 1 g faecal sample was incubated in 5 ml brain heart infusion broth supplemented with moxalactam (32 µg ml^{-1}), norfloxacin (12 µg ml^{-1}) and 0.4% (w/v) sodium taurocholate and was then incubated at 37 °C anaerobically for 24 h before being shocked with absolute ethanol (50% [v/v]) for 1 h to kill vegetative cells. Then 200 µl of alcohol-shocked broth was plated onto Brazier's medium (Fannin) and incubated anaerobically at 37 °C for 48 h. Brazier's agar plates were then sub-cultured onto fastidious anaerobe agar with horse blood (FAABL) with a metronidazole disc (5 µg). After anaerobic incubation at 37 °C for a further 24 h, isolates were frozen in glycerol stocks. Following genomic analysis and 16S rRNA gene analysis, the isolate was identified as a novel taxon belonging to the genus *Terrisporobacter*. Comparative average nucleotide identity (ANI) of MCA3^T and type strains of *Terrisporobacter* species recorded values lower than 96%; 92.51% to *T. glycolicus* ATCC 14880^T and 86.60% to '*T. othiniensis*' 08–306576. Two other *Terrisporobacter* species, *T. mayombei* and *T. petrolearius*, which at the time had not yet been sequenced, were also sequenced in this study and compared to the novel strain. These data suggested that the study strain represented a novel species with values of both ANI and digital DNA–DNA hybridization below species genomic thresholds when compared to *T. petrolearius* LAM0A37^T and *T. mayombei* DSM 6539^T. The proposed novel species, named *Terrisporobacter hibernicus* sp. nov., represents the first identification of *Terrisporobacter* species in Ireland. Information about strain MCA3^T, *T. petrolearius* LAM0A37^T and *T. mayombei* DSM 6539^T pertaining to phenotypic and genomic characteristics are described herein.

GENOME FEATURES

Strain MCA3^T was grown anaerobically at 37 °C, *T. petrolearius* at 35 °C and *T. mayombei* at 30 °C for 48 h on Columbia blood agar (CBA; Oxoid) supplemented with 5% (v/v) defibrinated horse blood. A single colony was used to inoculate brain–heart infusion broth (BHI-B; Sigma Aldrich), supplemented with 0.5% (w/v) Bacto yeast extract (BD), which was incubated for 48 h in anaerobic conditions at 37, 35 and 30 °C respectively. Genomic DNA (gDNA) was extracted from broth culture using the DNeasy

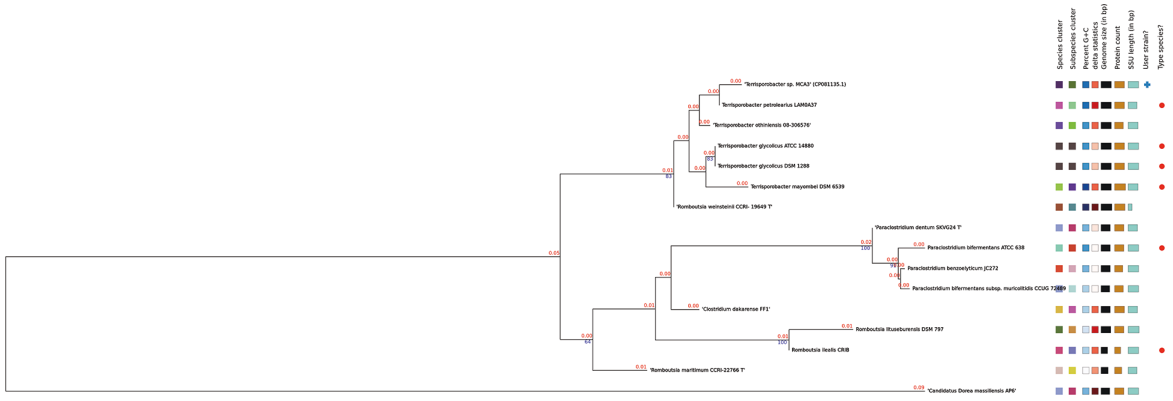

Fig. 1. TYGS 16S rRNA gene tree of the genus *Terrisporobacter* and closely related species [23]. Accessions associated with this graph are outlined in Table S2.

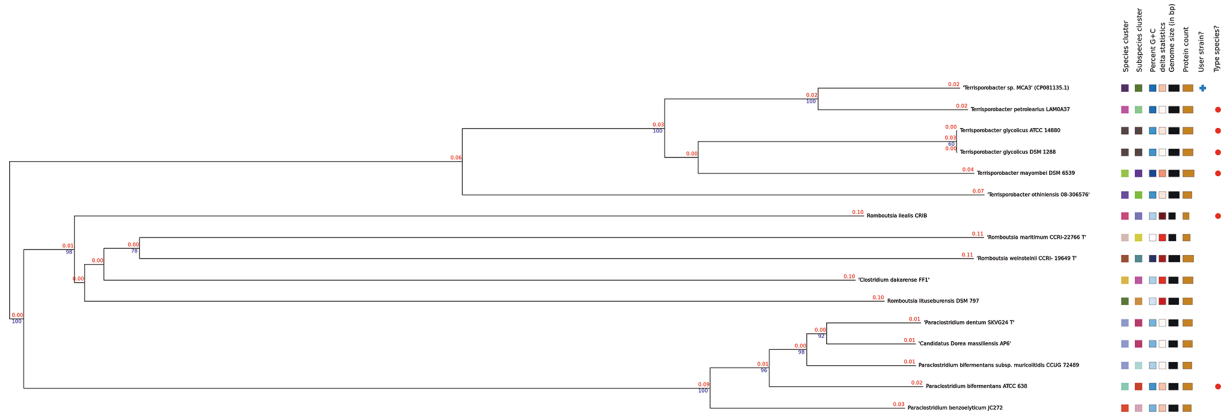

Fig. 2. TYGS whole-genome sequence tree of the genus *Terrisporobacter*and closely related species [23]. Accessions associated with this graph are outlined in Table S2.

UltraClean Microbial Kit (Qiagen). Library preparation was completed using the NEBNext Ultra II FS DNA Library Prep Kit, following the NEBNext Ultra II protocol for large fragment sizes (>550 bp; New England BioLabs) and subsequently sequenced on the Illumina MiSeq platform. Following 72 h sequencing run time, fastq files were quality checked using both FastQC (version 0.11.9) and MultiQC (version 1.9), trimmed with default parameters on fastp (version 0.20.0) [12–14]. *De novo* assembly was completed using SPAdes (version 3.13.1) [15].

gDNA for MCA3^T was also extracted from broth culture using the Wizard Genomic DNA Purification Kit (Promega). Sequencing was performed using the Rapid Sequencing Protocol (SQK-RAD004) on a FLO-MIN106 instrument (Oxford Nanopore Technologies). Basecalling was completed using Guppy with default parameters (version 5.0.11+2b6 dbff). Raw fastq files were trimmed

	T. glycolicus ATCC14880	*T. hibernicus* MCA3	*T. hibernicus* FS03	*T. hibernicus* KPPR-9	*T. hibernicus* MGYG-HGUT-00005	*T. mayombei* DSM6539	*"T. othiniensis"* 08-306576	*T. petrolearius* LAM0A37
T. glycolicus ATCC14880	100							
T. hibernicus MCA3	92.27	100						
T. hibernicus FS03	92.29	97.94	100					
T. hibernicus KPPR-9	92.28	98.39	97.83	100				
T. hibernicus MGYG-HGUT-00005	92.29	98.69	97.93	98.37	100			
T. mayombei DSM6539	92.76	91.48	91.51	91.52	91.51	100		
"T. othiniensis" 08-306576	87.03	86.5	86.41	86.44	86.46	87.15	100	
T. petrolearius LAM0A37	92.32	95.62	95.6	95.74	95.8	91.6	86.51	100

Fig. 3. The comparative fastANI (version 1.32) for strain MCA3^T against all other *Terrisporobacter* species. A 95–96% ANI cut-off value was used as proposed by Chun *et al.* [22, 26].

Table 2. dDDH values calculated for *T. hibernicus* MCA3^T against all other *Terrisporobacter* strains using the TYGS with the 70% dDDH cutoff [23]

Species	Strain	dDDH (d4 in %)	Model C.I.
T. petrolearius	LAM0A37^T	67.4	[64.4–70.3]
T. mayombei	DSM 6539^T	46.3	[43.7–48.8]
T. hibernicus sp. nov.	MGYG-HGUT-00005	88.9	[86.4–90.9]
T. hibernicus sp. nov.	KPPR-9	87.2	[84.6–89.4]
T. hibernicus sp. nov.	FS03	82.8	[80.0–85.3]
T. glycolicus	ATCC 14880^T	48.8	[46.2–51.4]
'T. otheniensis'	08–306576	32.9	[30.5–35.4]

using Porechop (version 0.2.3_seqan2.1.1) [16]. The genome of MCA3^T was assembled using Trycycler (version 0.4.1) and Flye (version 2.8.3-b1695) using both Illumina and MinION reads [17, 18]. The resulting consensus genomes were polished with three iterative rounds of Pilon (version 1.24) to generate a chromosome of 4095141 bp and a plasmid 21696 bp in length [19]. IDEEL was used to confirm that the MCA3^T genome has minimal indels as a result of polishing (Fig. S1, available in the online version of this article) [20].

Genome size, G+C content (mol%), as well as the number of contigs, genes and coding sequences of all three strains is available in Table 1. The NCBI GenBank accession number for LAM0A37^T is JAHZMO000000000 and for DSM 6539^T it is JAHZMP000000000. The NCBI Prokaryotic Genome Annotation Pipeline analysis of strain MCA3^T identified 4016 genes, 3845 coding genes, 36 rRNA genes, four non-coding RNA genes and 133 RNA genes. The full 16S rRNA gene of strain MCA3^T was queried against EzBioCloud's 16S database with 16S rRNA gene percent similarity greater than 99% (Table S1) [21, 22]. As the 16S rRNA gene of *T. hibernicus* was greater than 98.7% identical to other type species in *Terrisporobacter*, the overall genomic related indices (OGRIs) were queried [22]. 16S rRNA gene and whole genome phylogenetic trees were created using the Type Strain Genome Server (TYGS; Figs. 1–2) [23]. The 16S rRNA gene tree displays MCA3^T closely clustering with *T. petrolearius* and '*T. othiniensis*', and more distantly with *T. glycolicus* (Fig. 1), though clustering of MCA3^T to other *Terrisporobacter* species, including *T. glycolicus*, can be seen in Fig. 2 [23]. kSNP3 (version 3.1) with parameters set to -k 21 and -ML was used to create a parsimony phylogeny tree of *Terrisporobacter* and closely related *Intestinibacter* to show grouped *T. hibernicus* strains. The iTOL visualizer (version 5.7) was used to create Fig. S2 from the kSNP3 parsimony tree [24, 25].

FastANI (with the --fragLen 5000 flag, version 1.32) was used to query all *Terrisporobacter* assemblies from NCBI RefSeq against each other. ANI results for strain MCA3^T indicated less than 96% identity to all other type strains of *Terrisporobacter*, suggesting that strain MCA3^T is part of a novel genospecies (Fig. 3) [22, 26]. dDDH values were also calculated using the TYGS with the recommended formula d_4 (Table 2) [23]. Strain MCA3^T showed dDDH values of 67.4%, below the 70% dDDH threshold for the same species, when compared to other *Terrisporobacter* species (Table 2) [22]. Based on dDDH values (Table 2), ANI values (Fig. 3) and the whole genome sequence phylogenetic tree generated by TYGS (Fig. 2) the three *T. glycolicus* genomes on NCBI RefSeq, strains MGYG-HGUT-00005 (NZ_CABIWC000000000), KPPR-9 (NZ_FORW00000000) and FS03 (NZ_JAFLEP000000000), should be renamed to the appropriate species, *T. hibernicus* sp. nov. According to metadata from NCBI, strain MGYG-HGUT-00005 was isolated from human gut and strain FS03 was collected from a dairy farm in Manawatu, New Zealand.

Table 3. VITEK2 results for strain MCA3^T, T. petrolearius LAM0A37^T and *T. mayombei* DSM 6539^T including the recommendations for further tests to differentiate the species and contradicting biopatterns

ND, No data available; ELLM, Ellman; LIP, lipase. Full results in Table S5.

Species	Positive	Confidence	Analysis organisms and tests to separate	Contradicting typical biopattern
T. petrolearius	ELLM, L-proline (ProA)	Low discrimination	*Clostridium bifermentans* [LIP (0)] *Clostridium sporogenes* [LIP (100)] *Terrisporobacter glycolicus* [LIP (10)]	*Clostridium bifermentans* [ELLM (24)] *Terrisporobacter glycolicus* [phosphatase (88)]
T. hibernicus	L-Proline (ProA)	Low discrimination	*Clostridium bifermentans* [D-fructose (10)] *Clostridioides difficile* [D-fructose (90)] *Clostridium sporogenes* [D-fructose (0)]	ND
T. mayombei	ELLM, L-Proline (ProA)	Low discrimination	*Clostridium bifermentans* [LIP (0)] *Clostridium sporogenes* [LIP (100)] *Terrisporobacter glycolicus* [LIP (10)]	*Clostridium bifermentans* [ELLM (24)] *Terrisporobacter glycolicus* [phosphatase (88)]

Table 4. Phenotypic and biochemical characteristics of *Terrisporobacter petrolearius* LAM0A37^T, *Terrisporobacter glycolicus* JCM 1401^T, *Terrisporobacter mayombei* DSM 6539^T and Terrisporobacter *hibernicus* MCA3^T

+, Positive; –, negative; ND, no data available. Adapted from Deng *et al*. [8].

Characteristic	LAM0A37^T	JCM 1401^T	DSM 6539^T	MCA3^T
Cell size (µm)	0.3–0.6×1.8–5.0‡	0.3–1.3×1.8–15.4*	1–1.12×2–6†	0.5×2.75–3.5
pH tolerance	7.0–7.5‡	ND*	ND‡	6–9
NaCl range for growth (g l^{-1})	0–30‡	0–50*	ND‡	0–55
Growth temperature (°C):				
Range	15–45‡	20–42*	15–45†	20–40
Optimum	40‡	37*	33†	37
Sulphite reduction	+‡	–*	ND‡	ND
Polar lipids	5 GL, 6 PL, 2 L‡	7 GL, 6 PL, 1 L‡	6 GL, 6 PL‡	3 GL, 5 PL, 1 L
API 20A results:				
+	GLU, MAL, XYL, GEL, ESC, GLY, SOR	ND	GLU, MAL, SAL, XYL, GEL, ESC, GLY, SOR	GLU, MAL, GEL, ESC, GLY, SOR
–	IND, URE, MAN, LAC, SAC, SAL, ARA, CEL, MNE, MLZ, RAF, RHA, TRE, CAT	ND	IND, URE, MAN, LAC, SAC, ARA, CEL, MNE, MLZ, RAF, RHA, TRE, CAT	IND, URE, MAN, LAC, SAC, SAL, ARA, CEL, MNE, MLZ, RAF, RHA, TRE, CAT
Inconclusive	ND	ND	ND	XYL

*Data from [6].
†Data from [4].
‡Data from [8].
§GL, glycolipid; PL, phospholipid; L, lipid.
ARA, D-arabinose; CAT, catalase CEL, cellobiose; ESC, aesculin; GEL, gelatin; GLU, D-glucose; GLY, glycerol; IND, indole; LAC, lactose; MAL, maltose; MAN, D-mannitol; MLZ, melezitose; MNE, D-mannose; RAF, raffinose; RHA, L-rhamnose; SAC, sucrose; SAL, salicin; SOR, D-sorbitol; TRE, trehalose; URE, urease; XYL, D-xylose.

Due to evidence in literature describing *Terrisporobacter* species as a potential etiological agent for various diseases, all three strains, MCA3^T, *T. petrolearius* LAM0A37^T and *T. mayombei* DSM 6539^T, were analysed for virulence genes through the VFDB (Virulence Factor Database) which resulted in no genes being detected, and antimicrobial resistance genes using ResFinder, MEGARres, CARD, and ARG-ANNOT through ABRicate (version 1.0.0). Results from these databases are outlined in Table S3 [27–32]. The genomes of all three strains were also queried against phage and plasmid databases, including PlasmidFinder (ABRicate version 1.0.0), PLSDB and PHASTER [32–36]. The PHASTER database identified strain MCA3^T as carrying a *Streptococcus* phage (Dp-1; NC_015274(9); 39.8 kbp) and *T. mayombei* DSM 6539^T to have a *Clostridium* phage, phiCD38 (NC_015568(5) 46.6 kbp) [33, 35].

PHENOTYPIC, BIOCHEMICAL AND CHEMOTAXONOMIC CHARACTERIZATION

Cells of strain MCA3^T grew anaerobically on CBA with 5% (v/v) defibrinated horse blood, BHI-B supplemented with 0.5% (w/v) Bacto yeast extract, tryptone soya agar (TSA; Oxoid and in tryptone soya broth (Oxoid) at 37 °C for 48 h producing white-grey opaque cells 0.7 mm in diameter with irregular edges often with spreading in the direction of growth, and similar in morphology to that of other *Clostridium* and *Clostridioides* species (Fig. S3) [8, 11, 37]. MCA3^T cells tolerated NaCl from 0.5 to 5.5% (w/v), with a pH tolerance between pH 6 and 9. The optimal temperature range for growth was 35–40 °C, with tolerance from 20–30 °C. Strain MCA3^T was positive for motility (microscopic) and sporulation and negative for oxidase production (Figs S4-S5). Transmission electron micrograph imaging of a MCA3^T cell was completed by fixing the strain in 2.5% glutaraldehyde in PBS for 1 h prior to washing three times with PBS. Fixed sample was applied to copper grids for 10 min before staining with uranyl acetate for 5 min. Samples were then rinsed with distilled and deionized water before staining with lead citrate for a further 5 min. Grids were again washed with water and dried on filter paper prior to imaging using a JEOL JEM –1400 plus transmission electron microscope (Fig. S6).

Biochemical characteristics of strain MCA3^T, *T. petrolearius* LAM0A37^T and *T. mayombei* DSM 6539^T were determined using API 20A strips (bioMérieux) in triplicate at 37 °C, according to the manufacturer's instructions (Table S4). API 20A results of

strain MCA3^T determined an identification of *Actinomyces israelii*. At 37 °C, MCA3^T cells were positive for D-glucose and maltose acidification, as well as glycerol and D-sorbitol. Cells were also positive for β-glucosidase and protease enzyme activities. At 37 °C, MCA3^T cells were negative for indole formation, urease utilization, and fermentation of D-mannitol, lactose, sucrose, salicin, L-arabinose, cellobiose, D-mannose, melezitose, D-raffinose, L-rhamnose and trehalose, and were catalase-negative.

A VITEK MS was used to query strain MCA3^T, *T. petrolearius* LAM0A37^T and *T. mayombei* DSM 6539^T by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF). MALDI-TOF could accurately identify all three species to genus level only, identifying them as *T. glycolicus* with 99.9% confidence level. The VITEK2 Biochemical ANC card (bioMérieux; no. 2441412403) reported all three strains to be positive for L-proline (ProA) synthesis, and *T. petrolearius* LAM0A37^T and *T. mayombei* DSM 6539^T positive for Ellman's reagent (Table 3 and S5).

The polar lipid profile of MCA3^T displays diphosphatidylglycerol, phosphatidylglycerol, two aminoglycolipids, one glycophospholipid, one aminolipid, three glycolipids, five phospholipids and one lipid (Fig. S7). Fatty acid analysis of MCA3^T determined that the predominant fatty acid profile was $C_{16:0}$ at 22.8%. Results from Deng *et al.* [8] for *Terrisporobacter* species fatty acid analysis via MIDI are merged in Table S6 [8]. These results, as well as other phenotypic and biochemical characteristics, are compiled with data from Deng *et al.* [8] to compare LAM0A37^T, JCM 1401^T, DSM 6539^T and MCA3^T in Table 4 [8]. DSMZ Services, Leibniz-Institute DSMZ - Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany) carried out the following analyses: temperature, pH and NaCl for growth; phenotypic characterization (sporulation, motility, oxidase); fatty acid analysis via MIDI; and respiratory quinone and polar lipid studies.

Based on OGRI evidence from fastANI, dDDH, phenotypic and the biochemical profiles, MCA3^T should be considered a member of a new species in the genus *Terrisporobacter*; therefore, *T. hibernicus* sp. nov. is proposed (Tables 2 and 4, Figs. 1–3) with MCA3^T as the type strain [8, 22, 23, 26].

DESCRIPTION OF *TERRISPOROBACTER HIBERNICUS* SP. NOV.

Terrisporobacter hibernicus (hi.ber'ni.cus. L. masc. adj. *hibernicus*, pertaining to Ireland).

T. hibernicus is a Gram-positive, spore-forming anaerobic bacterium, which produces rod-shaped cells 0.7 mm in diameter. Colonies on TSA at 37 °C after 48 h of incubation are white/opaque. Cells tolerate NaCl from 0.5 to 5.5% (w/v), with a pH tolerance between pH 6 and 9. The optimal temperature for growth is 35–40 °C, with a tolerance range of 20–30 °C. No respiratory quinones (menaquinone, ubiquinone, demethylmenaquinone or dimethylmeaquinone) are detected. VITEK MS identifies *T. hibernicus* as *T. glyocolicus* with a 99.95% confidence level. Cells utilize D-glucose and maltose, and are postive for D-glycerol, D-sorbitol, β-glucosidase and protease enzyme activities. Cells do not utilize indole and urease, and are negative for fermentation of D-mannitol, lactose, sucrose, salicin, L-arabinose, cellobiose, D-mannose, melezitose, D-raffinose, L-rhamnose and trehalose. Catalase negative.

The G+C content of the type strain of *T. hibernicus* MCA3^T was found to be 28.8mol% with a chromosomal length of 4.1 Mbp and a plasmid of 21.7 kbp. The type strain, MCA3^T (=NCTC 14625^T=LMG 32430^T), was isolated in Northern Ireland from a bovine faecal sample.

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Authors and contributors
M.M.: conceptualization, methodology, formal analysis, data curation, writing – original draft, writing – review and editing. S.V.N.: conceptualization, methodology, validation, formal analysis, data curation, writing – original draft, writing – review and editing. M.C.C.: methodology, investigation, resources. D.J.F.: methodology, investigation, resources. O. D.: investigation, resources. H.M.: investigation and resources. L.K.: investigation and resources. G.M.: supervision, investigation and resources. K.S.: resources, supervision. J.W.M.: supervision, project administration. S.F.: validation, writing – original draft, writing – review and editing, supervision, project administration.

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

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