

Lutispora saccharofermentans sp. nov., a mesophilic, non-spore-forming bacterium isolated from a lab-scale methanogenic landfill bioreactor digesting anaerobic sludge, and emendation of the genus Lutispora to include species which are non-spore-forming and mesophilic

El Houari, Abdelaziz; Carpenter, Morgan; Chaplin, Daniel; Golyshin, Peter; McDonald, James E

International Journal of Systematic Evolutionary Microbiology

DOI:
[10.1099/ijsem.0.005683](https://doi.org/10.1099/ijsem.0.005683)

Published: 01/02/2023

Publisher's PDF, also known as Version of record

[Cyswllt i'r cyhoeddiad / Link to publication](#)

Dyfyniad o'r fersiwn a gyhoeddwyd / Citation for published version (APA):
El Houari, A., Carpenter, M., Chaplin, D., Golyshin, P., & McDonald, J. E. (2023). Lutispora saccharofermentans sp. nov., a mesophilic, non-spore-forming bacterium isolated from a lab-scale methanogenic landfill bioreactor digesting anaerobic sludge, and emendation of the genus Lutispora to include species which are non-spore-forming and mesophilic. *International Journal of Systematic Evolutionary Microbiology*, 73(1), [005683]. <https://doi.org/10.1099/ijsem.0.005683>

Hawliau Cyffredinol / General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Lutispora saccharofermentans sp. nov., a mesophilic, non-spore-forming bacterium isolated from a lab-scale methanogenic landfill bioreactor digesting anaerobic sludge, and emendation of the genus *Lutispora* to include species which are non-spore-forming and mesophilic

Abdelaziz El Houari^{1,*}, Morgan Carpenter¹, Daniel Chaplin², Peter Golyshin² and James E. McDonald^{1,3,*}

Abstract

A novel anaerobic, mesophilic, non-spore-forming bacterium (strain m25^T) was isolated from methanogenic enrichment cultures obtained from a lab-scale methanogenic landfill bioreactor containing anaerobic digester sludge. Cells were Gram-stain-negative, catalase-positive, oxidase-negative, rod-shaped, and motile by means of a flagellum. The genomic DNA G+C content was 40.11 mol%. The optimal NaCl concentration, temperature and pH for growth were 2.5 g l⁻¹, 35 °C and at pH 7.0, respectively. Strain m25^T was able to grow in the absence of yeast extract on glycerol, pyruvate, arginine and cysteine. In the presence of 0.2% yeast extract, strain m25^T grew on carbohydrates and was able to use glucose, cellobiose, fructose, raffinose and galactose. The novel strain could utilize glycerol, urea, pyruvate, peptone and tryptone. The major fatty acids were iso-C_{15:0}, C_{14:0}, C_{16:0} DMA (dimethyl acetal) and iso-C_{15:0} DMA. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the new isolate was closely related to *Lutispora thermophila* EBR46^T (95.02% 16S rRNA gene sequence similarity). Genome relatedness was determined using both average nucleotide identity and amino acid identity analyses, the results of which both strongly supported that strain m25^T belongs to the genus *Lutispora*. Based on its unique phylogenetic features, strain m25^T is considered to represent a novel species within the genus *Lutispora*. Moreover, based on its unique physiologic features, mainly the lack of spore formation, a proposal to amend the genus *Lutispora* is also provided to include the non-spore-forming and mesophilic species. *Lutispora saccharofermentans* sp. nov. is proposed. The type strain of the species is m25^T (=DSM 112749^T=ATCC TSD-268^T).

Landfill sites are engineered environments used for the disposal of municipal solid waste (MSW), agricultural and industrial waste, where communities of hydrolytic, fermentative, acetogenic and methanogenic micro-organisms convert waste biomass into methane-rich biogas [1]. Due to geographical and societal variation, the composition of landfill waste is highly heterogeneous [2], supporting diverse consortia of degradative microorganisms. Cellulose and hemicellulose are the major biodegradable components of MSW and their conversion to biogas in landfills is well documented [3, 4]. The hydrolytic potential of landfill microbiota is also well known [5]; however, the taxonomic and functional diversity, and physiology, of landfill microbiota is still poorly understood [6, 7].

Given the scale of waste biomass generated by society (household and green waste), agriculture (livestock and crop residues) and industrial processes, the biotransformation of lignocellulosic biomass to biogas and other bio-based materials is an important

Author affiliations: ¹School of Natural Sciences, Environment Center Wales, Bangor University, UK; ²Centre for Environmental Biotechnology (CEB), Bangor University, UK; ³Current address: School of Biosciences, University of Birmingham, Birmingham, UK.

***Correspondence:** Abdelaziz El Houari, a.elhouari@bangor.ac.uk; James E. McDonald, j.e.mcdonald@bham.ac.uk

Keywords: AD sludge bioreactor; *Clostridiaceae*; landfill; methanogens enrichment.

Abbreviations: AAI, amino acid identity; AD, anaerobic digestion; ANI, average nucleotide identity; CHES, N-cyclohexyl-2-aminoethanesulfonic acid; CMC, carboxymethylcellulose; dDDH, digital DNA–DNA hybridization; DMA, dimethyl acetal; FAB, fastidious anaerobic broth; GBDP, genome blast distance phylogeny; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; LSM, lab-scale methanogenic; MES, 2-(N-morpholino) ethanesulfonic acid; MSW, municipal solid waste; NA, data not available.

The GenBank accession numbers for both 16S rRNA gene and genome sequences of strain m25^T are MW962255 and JAJEKE000000000, respectively

One supplementary figure and four supplementary tables are available with the online version of this article.

005683 © 2023 The Authors



This is an open-access article distributed under the terms of the Creative Commons Attribution License. This article was made open access via a Publish and Read agreement between the Microbiology Society and the corresponding author's institution.

feature of a future sustainable bio-based economy [8–12]. However, the complex structure of lignocellulosic plant biomass makes their biotransformation, in particular their hydrolysis, a challenge [13].

Due to difficulties in obtaining decomposing waste samples from landfill sites, studies of landfill microbiology are often limited to leachate samples [14, 15], which could miss key microbiota associated with the solid waste fraction. However, recent advancements in technologies for the sampling and analysis of landfill microbiota have driven improvements in our knowledge of landfill microbial diversity and thus increased interest in characterising previously uncultivated micro-organisms from landfills [6]. Among these microbial populations, the *Clostridiaceae*-type family represents a large fraction of phylum *Firmicutes* and are well established as key members of anaerobic digestion (AD) treatment systems and known for their hydrolytic and fermentative potential [16–19].

To date of writing, the family *Clostridiaceae* comprises a total of 77 child taxa, among them 49 were taxonomically named and validly published (<https://lpsn.dsmz.de/family/clostridiaceae>). Here, we report the isolation and the characterization of a novel member of the family *Clostridiaceae* belonging to the genus *Lutispora*, strain m25^T, which was isolated from a lab-scale methanogenic (LSM) bioreactor inoculated with microbiota from landfill leachate and drilled waste. To our knowledge, currently *Lutispora* includes only one validly published species, *Lutispora thermophila*, isolated from thermophilic methanogenic sludge [20]. Both phylogenetic and physiologic characteristics of the newly isolated strain reported in this study indicate that strain m25^T represents a novel species within the genus *Lutispora*.

To obtain a representative microbial community from municipal solid waste landfill sites, drilled solid waste and landfill leachate samples were collected from two different locations. Drilled solid waste was obtained from Ruaben landfill site (Rouaben, UK) and the landfill leachate was sampled from Hafod landfill site (Wrexham, UK). Leachate and drilled waste samples were collected and stored in hermetically sealed containers (30l plastic carboy bottles). The containers were filled to the top to displace headspace air and sealed immediately to preserve anaerobic conditions. Samples were transported on the same day to the laboratory within 3 h. On arrival, both fresh drilled solid waste and leachate were mixed (10% w/v, respectively) anaerobically under a stream of oxygen-free N₂ and used as an inoculum to set up the LSM bioreactors containing 2% of AD sludge used as feedstock for the microbial communities.

Duran stirred reactor bioreactors were used to construct the lab-scale landfill microbiome reactors. The connections (2×GL14 and 2×GL18) available on the top of the bottles allowed the addition or the removal of samples from the bottle during the mixing process using the stirrer shaft on the top of each Duran GLS 80 laboratory glass bottles (1000 ml), and therefore providing notably improved mixing in comparison with standard magnetic stir bars. The whole unit was autoclaved before inoculation. The LSM bioreactors were incubated at 35 °C under agitation (150 r.p.m.) and monitored for 28 days. At day 21 (maximum production of biogas), sludge was sampled from the bioreactors and used to inoculate methanogen-enrichment cultures (at 10% v/v: final volume of 40 ml). Methanogenic enrichment cultures were produced in basal medium [21] supplemented with formate, methanol, methylamine, acetate (10 mM each as final concentration) and in the presence of 2 g l⁻¹ yeast extract, 0.5 g l⁻¹ penicillin and under 1 Bar of N₂/H₂/CO₂ (80/10/10%) atmosphere.

The basal medium composition consisted of (per 1 litre of distilled water): 0.5 g K₂HPO₄, 0.5 g KH₂PO₄, 0.5 g NH₄Cl, 0.4 g KCl, 0.05 g CaCl₂·2H₂O, 0.3 g MgCl₂·6H₂O, 0.4 g NaCl, 0.25 g HCl-cysteine, 1 ml trace element mineral solution [22] modified by Imhoff-Stuckle and Pfennig [23] and 1 ml resazurin 0.1% (w/v). For medium preparation, the medium was heated to boiling until the medium turned colourless, then cooled and dispatched into 120 ml penicillin flasks at room temperature under a stream of O₂-free nitrogen. The medium was subsequently sterilized by autoclaving for 20 min at 121 °C. After autoclaving, sterile solutions (autoclaved separately) of NaHCO₃ (2 g l⁻¹) and Na₂S·9H₂O (0.4 g l⁻¹; pH adjusted at pH 7.1), were added to the medium in addition to 1 ml filter-sterilized (0.22 µm) vitamin solution [24]. The final pH after adding solutions was pH 7.1.

Methanogenic enrichments were incubated for 4 weeks at 35 °C. The growth of the methanogenic enrichment cultures was monitored by gas composition in terms of the analysis of CH₄ and CO₂ production. The microbial community structure of both LSM bioreactors and methanogenic enrichments was monitored using 16S rRNA gene community profiling using the Illumina MiSeq platform (results not shown). After three subcultures under the same conditions, isolation procedures were carried out using high-throughput isolation in microplates. Prior to isolation, serial decimal dilutions of the enrichment were conducted (10% v/v of inoculation; final volume of 100 ml), to determine the optimal dilution, for which half of the wells in the microplates contained positive growth that had theoretically grown only from the introduction of one cell per well. The optimum dilution was then dispatched in 96-well microplates (300 µl per well). The microplates were sealed as soon as possible with an Alumaseal film and incubated in Gaspack bags. The entire isolation procedure and incubation were carried out inside a Whitley DG250 Anaerobic Workstation (Don Whitley Scientific), under N₂/H₂/CO₂ (80/10/10%) atmosphere. After 1 week of incubation, two growth positive wells from the highest dilution (10⁻⁶), corresponding to two pure isolates, m25^T and m26, were transferred to 5 ml liquid medium for purity check (by both phase-contrast microscopy observation and by growth streaking on agar Petri dishes) and for identification by 16S rRNA gene sequencing. Both strains, m25^T and m26, very similar in their morphological characteristics, were obtained and subsequently shown to be identical by comparing their 16S rRNA gene sequences (100% sequence similarity). The strain designated m25^T was characterized in detail.

Strain m25^T grew optimally on basal medium supplemented by 30 g l⁻¹ of fastidious anaerobic broth (modified-FAB medium). Therefore, unless stated otherwise, modified-FAB medium was used to maintain cultures. Cellular characteristics of the strain (shape, size, mobility, sporulation, Gram-staining) and flagella examination were observed by both phase contrast microscopy (Zeiss Axioplan 2 Fluorescence microscope FluoArc 100) and transmission electron microscopy performed at the Animal and Plant Health Agency (<http://apha.defra.gov.uk/apha-scientific/index.htm>), respectively. Gram staining was carried out using the standard Gram reaction method and confirmed by using the NaOH method [25]. Cells of strain m25^T were rod shaped, slightly curved and motile. Cells were variable in size; approximately 0.5–0.7 µm in diameter and 2.1–5.5 µm long (Fig. 1a). Cells of strain m25^T stained Gram-negative and transmission electron microscopy revealed cells forming flagellum (Fig. 1b) confirming the mobility of strain m25^T to as observed for *L. thermophila* EBR46^T [20] motile by lateral flagella. Growth of strain m25^T on modified-FAB agar medium showed pale brown spherical colonies (1–2 mm in diameter) after 1 week of incubation. The ability of strain m25^T to grow in aerobic conditions was assessed using solid and liquid aerobic medium without HCl-cysteine and Na₂S·9H₂O prepared and incubated under aerobic conditions. No growth of strain m25^T was observed in aerobic conditions.

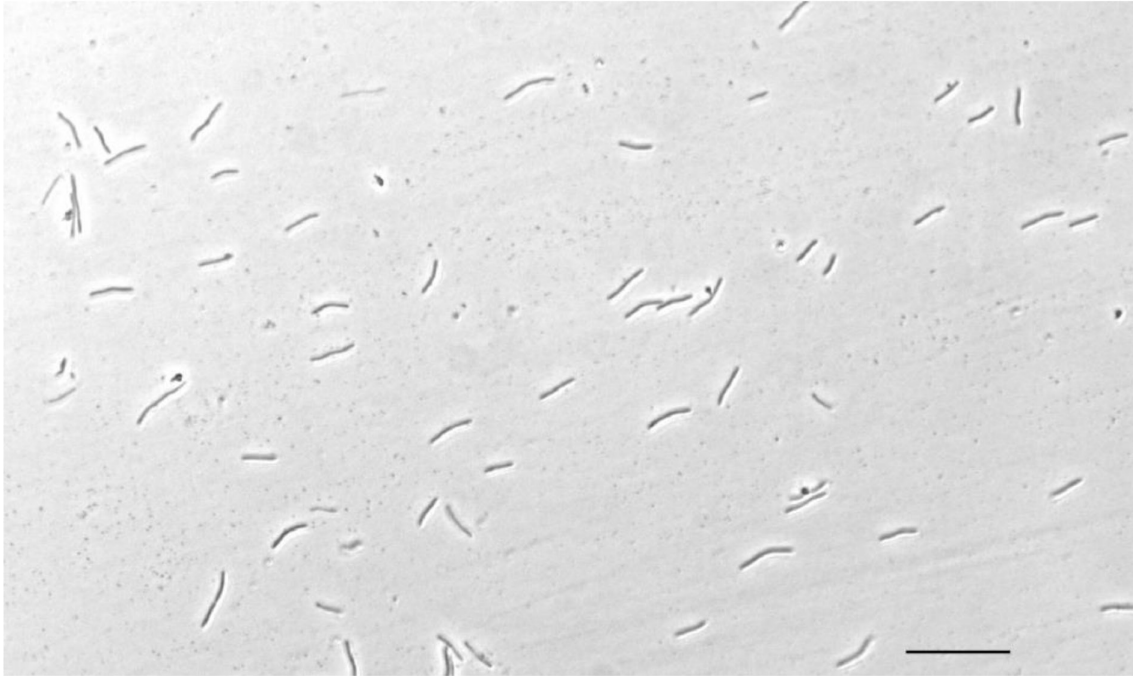
Phase-contrast observation was used to check the presence of endospores and confirmed by sporulation test. The sporulation test was performed using exponential growing cells of strain m25^T incubated on the following conditions and alternated by a thermal shock (incubation in ice for 5 min): 10 min at 80 °C, 20 min at 80 °C, 10 min at 90 °C. Cells were then subcultured in a new culture medium. No spores or other forms of heat resistance was observed. Sporulating and non-sporulating strains were used as a control. No growth was observed after 1 week of re-incubation in the optimal temperature. *L. thermophila* EBR46^T was originally described as spore-forming bacterium isolated from thermophilic methanogenic sludge [20], hence the name *L. thermophila*. However, the non-spore-forming feature is the one of the main physiological features that distinguishes strain m25^T from the closest related *L. thermophila* EBR46^T. In addition, the strain m25^T was isolated from a mesophilic lab-scale methanogenic landfill bioreactor. Consequently, amending the genus *Lutispora* is proposed to include the non-spore-forming and mesophilic species.

Genomic DNA extraction from strain m25^T cultures was carried out using the DNeasy UltraClean Microbial Kit (Qiagen) according to the supplier's recommendations. Exponential growth culture (1.8 ml) was centrifuged, and the cell pellet was used for DNA extraction. The extracted DNA was eluted in nuclease-free EB buffer (10 mM Tris-HCl pH 8.0). The integrity of the genomic DNA was assessed using 1% (w/v) agarose gel electrophoresis with a 1 Kb molecular weight ladder. DNA quantification was carried out using the Qubit dsDNA Quantification Kit following the manufacture recommendations (Invitrogen Qubit 3 Fluorometer, Fisher Scientific). The extracted DNA (2 µl) was used for the 16S rRNA gene amplification in a final volume of 50 µl containing 2×MyTaq Red Mix (Meridian Bioscience), with the primers 27F (5'-TGAGCCATGATCAAACCTCT-3') and 1492R (5'-GGWTACCTTGTTACG-3') [26] at final concentrations of 0.8 µM each. The PCR amplification reactions were carried out according to the following programme: 1 cycle of 5 min at 94 °C to denature the nucleic acids, 35 cycles of three steps: denaturation 45 s at 94 °C, annealing 45 s at 55 °C and elongation 1 min 30 s at 72 °C, then a final elongation cycle of 10 min at 72 °C. 16S rRNA gene amplicons were purified using the QIAquick PCR Purification Kit (Qiagen) prior to Sanger DNA sequencing by Genewiz facilities (Takeley, UK). After quality clipping sequences, the 16S rRNA gene sequences of isolated strains (1385 bp) was compared with reference sequences from the GenBank NCBI database for identification (www.ncbi.nlm.nih.gov/BLAST/). The closest GenBank relatives of strain m25^T were an uncultured *Lutispora* sp. (clones SCTB017, SCTB017 and SCTB027 with 16S rRNA gene sequence similarities of 99.78%; GenBank accession nos. JN650234, JN650240 and JN650246, respectively) derived from a cellar, and an uncultured bacterium clone CM5 (99.35% identity; GenBank accession no. KF275152) derived from a dechlorinating anaerobic microcosm inoculated with soil.

The 16S rRNA gene sequences of strain m25^T and m26 were aligned with reference sequences belonging to the family *Clostridiaceae* and obtained from the NCBI database. Sequences were trimmed at the minimum length (1385 bp) and phylogenetic analyses were then conducted using the maximum composite likelihood model [27] within 1000 bootstrap replicates. This analysis involved 20 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). Evolutionary analyses were conducted in MEGA X [28]. Phylogenetic analyses based on the full 16S rRNA gene sequences revealed that strain m25^T was related to the representatives of the genera *Lutispora* and *Gracilibacter* belonged to the *Clostridiaceae* family (Fig. 2). Within both genera, strain m25^T was distantly related to *L. thermophila* EBR46^T (95.02% sequence similarity) isolated from an enrichment culture derived from an anaerobic thermophilic methanogenic bioreactor treating artificial solid wastes [20] and *Gracilibacter thermotolerans* (89.33% similarity) isolated from a constructed wetland receiving acid sulphate water [29].

To sequence the whole genome of strain m25^T, the same extracted DNA used for 16S rRNA gene PCR above was used to prepare libraries for whole genome sequencing on the Illumina Novaseq platform with 2×250 bp paired-end reads by MicrobesNG (Birmingham, UK). Raw reads were adapter trimmed using Trimmomatic 0.30 with a sliding window quality cutoff of Q15 [30]. *De novo* assembly was then performed using SPAdes version 3.12 [31], and finally the contigs were annotated using Prokka 1.14 [32]. Scaffolds below to 1500 bp were filtered and the final assembled genome of strains m25^T have been deposited in the GenBank database under the accession number JAJEKE000000000. The G+C content of the genomic DNA determined from the genome sequences was 40.11 mol%.

(a)



(b)



Fig. 1. Microscope images of strain m25^T cells grown in fastidious anaerobic broth medium. (a) Phase-contrast microscope image (bar, 10 μm) and (b) transmission electron microscope image of cells forming monotrichous flagellum (bar, 1 μm).

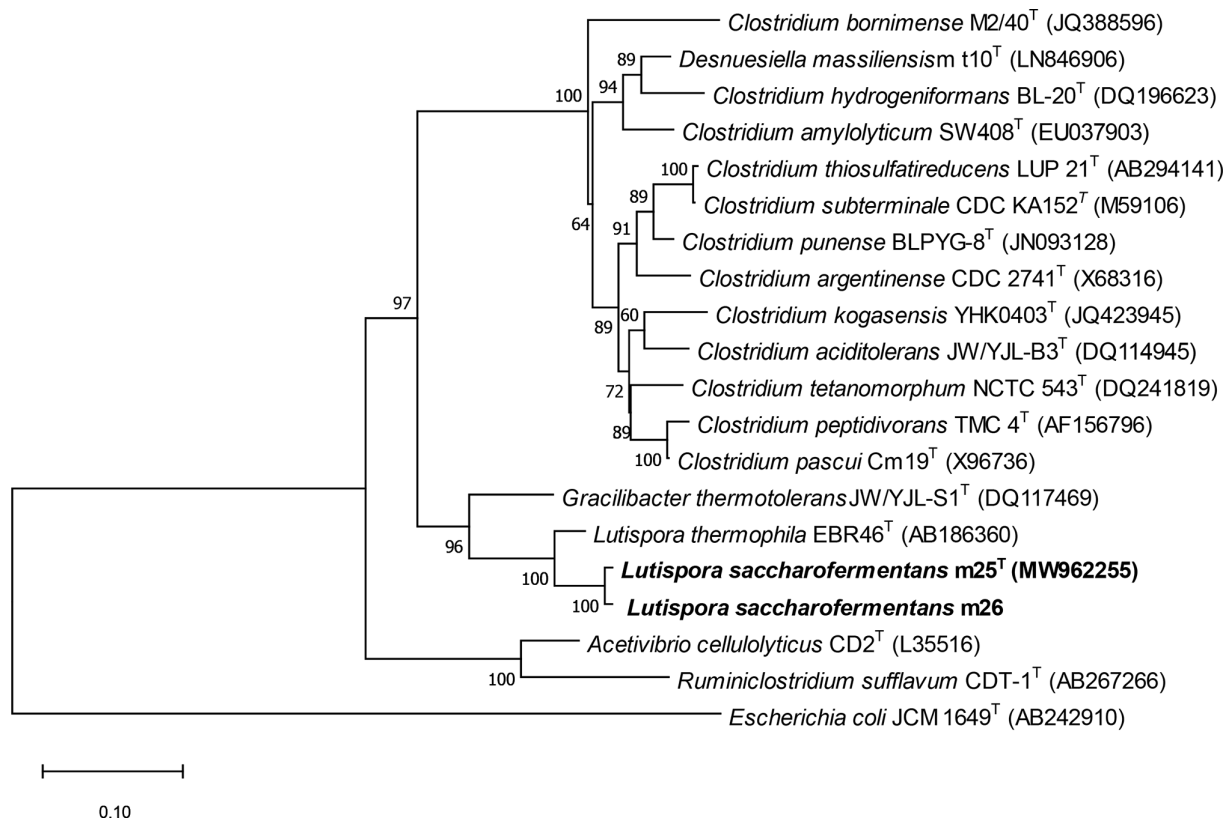


Fig. 2. Phylogenetic evolutionary dendrogram reconstructed by the maximum-likelihood method showing the affiliation of *Lutispora saccharofermentans* sp. nov. (strains m25^T and m26) 16S rRNA gene sequences (1385 bp) to their closest relative reference sequences obtained from NCBI GenBank database (accession numbers in brackets). *Escherichia coli* JCM1649^T (AB242910) was used as outgroup to root the tree. Bootstrap values are represented at nodes. Bar represents 0.10 substitutions per site. The isolated strains are represented in in bold format.

The draft genome size of strain m25^T is 4321915 bp from 37 scaffolds and has an N50 scaffold length of 170981. There were 3988 protein-coding genes, 51 tRNA and four rRNA gene copies predicted. CheckM analysis showed that the assembled genome completeness of strain m25^T was 99.19%. Digital DNA–DNA hybridization (dDDH) analysis was performed using the DSMZ Genome-to-Genome Distance Calculator platform (<http://ggdc.dsmz.de/distcalc2.php>). The dDDH value between strain m25^T and the closest relative genome of *L. thermophila* EBR46^T was 15.1% and therefore below to DDH values ($\leq 70\%$) which are considered as an indication that both strains belong to a different species [33–35]. Genome relatedness was computed between strain m25^T and the closest relative species reference genomes publicly available from the family *Clostridiaceae* (Fig. 2). The genomes were aligned using GTDB-Tk version 2.1.1 [36] using the defaults parameters, then the phylogenetic tree was created using RAXML-NG version 0.9.0 [37]. In addition, the average nucleotide identity (ANI) and amino acid identity (AAI) values were calculated between strain m25^T and the reference genomes using pyani version 0.2.8 (<https://github.com/widdowquinn/pyani>) and CompareM version 0.1.2 (<https://github.com/dparks1134/CompareM>), respectively. Results of the analysis of the phylogenetic relationship of the strain m25^T genome and its closest relative species reference genomes (Fig. 3) were consistent with those obtained by phylogenetic analyses based on the full 16S rRNA gene sequences (Fig. 2) revealing that strain m25^T is closely related to the strain *L. thermophila* DSM 19022 [20]. These findings were confirmed by the ANI (Fig. S1) and AAI (Fig. S2) values of strain m25^T showing average values of 82.49–93.24% and 50.93–74.4%, respectively. Strain m25^T shared an ANI of 82.49% and an AAI of 74.4% to its closely relative species *L. thermophila* EBR46^T.

For an additional whole genome-based taxonomic analysis, the genome sequence of strain m25^T was uploaded to the Type (Strain) Genome Server (<https://tygs.dsmz.de>). The genome BLAST distance phylogeny (GBDP) analysis was restricted to the strain m25^T and closely related species reference genomes publicly available from of the family *Clostridiaceae*. The GBDP analysis was inferred using the amino acid sequences of the entire proteome, promising a better-resolved phylogeny in datasets of only remotely related strains [38]. The type-based species clustering using a 70% dDDH radius around each of the closely related species was done as previously described [39]. Subspecies clustering was done using a 79% dDDH threshold as previously introduced [40]. The resulting species and subspecies clusters are listed in Table S3, whereas the taxonomic identification of the strain based on pairwise dDDH values is found in Table S4. Briefly, the clustering yielded 14 species clusters and strain m25^T formed a separated

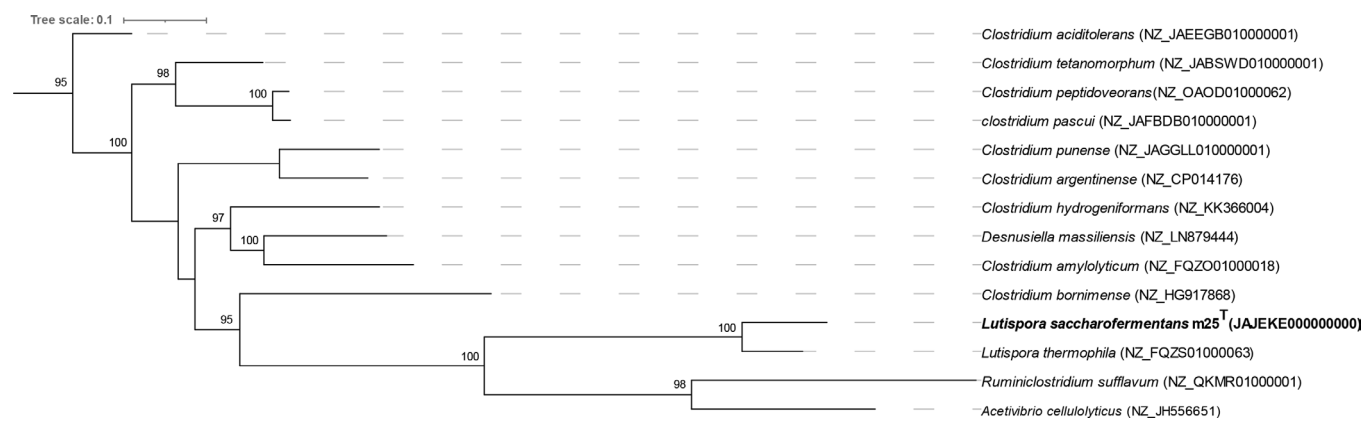


Fig. 3. Whole genome-based phylogenetic tree showing the affiliation of the *Lutispora saccharofermentans* sp. nov. (strain m25^T) genome sequence to its closest relative reference genomes (GenBank accession numbers in brackets) publicly available from the family *Clostridiaceae*. The genomes were aligned using GTDB-Tk and the phylogenetic tree was reconstructed using RAxML-NG. *Clostridium aciditolerans* was used as outgroup to root the tree. Bootstrap values are represented at nodes. Bar represents 0.1 substitutions per site. The isolated strain m25^T (present study) is represented in bold format.

cluster (Fig. S1a) and was assigned to *Clostridium aciditolerans* (NZ_JH556651) [41]. However, the results obtained by whole-proteome-based GBDP distance phylogenetic analysis (Fig. S3b) were consistent with the those obtained by 16S rRNA gene phylogeny analysis (Fig. 2) where strain m25^T was most related to *L. thermophila* (NZ_FQZS010000063).

Temperature, pH and NaCl growth tests for strain m25^T were performed in triplicate at 35 °C (except for temperature-range experiments) on FAB medium (final volume of 10 ml) and using headspace sampler 20 ml vials (QUMA) sealed with rubber stoppers and crimped aluminium seals. Temperatures were tested from 4–60 °C. For pH studies, the media was adjusted with sterile anaerobic solutions of HCl (1 M) or NaOH (1 M). The pH ranges 4–6, 6–8 and 8–10 were obtained in the presence of the buffers (10 mM each) 2-(*N*-morpholino)ethanesulfonic acid (MES), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and *N*-cyclohexyl-2-aminoethanesulfonic acid (CHES), respectively. For salt requirements, NaCl was tested from 0 to 10% (w/v). NaCl was weighed directly into the vials and the medium was dispensed into tubes as described above. Positive growth was determined by monitoring changes in OD_{600nm} using a spectrophotometer (Jenway 7300). The optimum temperature for growth at pH 7.1 was 35 °C. No growth was observed at temperatures below 25 °C or above 45 °C. The optimum temperature of growth corresponds with the mesophilic temperature conditions of LSM bioreactors from which strain m25^T was isolated. However, the growth temperature of strain m25^T was different from the thermophilic growth optimums of the most closely related type strains and thus discriminates strain m25^T from *L. thermophila* EBR46^T [20] and *G. thermotolerans* JW/YJL-S1^T [29], both reported as thermophilic bacterial strains. The pH range for growth was pH 6.0–9.0, with optimum growth occurring at pH 7.0. Strain m25^T did not require NaCl for growth but tolerated up to 0.8% NaCl with an optimum at 0.25% NaCl.

Substrate utilization was investigated in triplicate using exponentially growing cells resuspended in pre-reduced basal medium without L-cysteine hydrochloride anhydrous, in both absence and presence of 0.2% (w/v) yeast extract under N₂ as the gas phase. Substrate utilization was determined for the following substrates (20 mM each): sugars, amino acids, organic acids and alcohols. Furthermore, the strain was tested for its capacity to utilize complex substrates (1% each) such as yeast extract, tryptone, peptone, casaminoacids, starch, carboxymethylcellulose (CMC), crystalline cellulose (Avicel), xylan and casein. More details on substrate utilization are provided in the species description below. The main characteristics and substrate utilization observations that discriminate strain m25^T from the most closely related type from the *Clostridiaceae* family strains are shown in Table 1. The genomic DNA G+C content of strain m25^T was 40.11 mol% and differs from the most related type strains *L. thermophila* EBR46^T and *G. thermotolerans* JW/YJL-S1^T (G+C of 35.66 mol% and 42.8 mol%, respectively). Strain m25^T was able to grow in the absence of yeast extract, on glycerol, pyruvate, arginine and cysteine. Therefore, this capacity is considered as the main physiological feature that distinguishes strain m25^T from its nearest relatives requiring the presence of yeast extract for their growth. However, the presence of yeast extract on the medium enhances the growth of the strain m25^T, a common characteristic for closely described species. Compared to its closely related species, *L. thermophila* EBR46^T and *G. thermotolerans* JW/YJL-S1^T, strain m25^T differs by its ability to ferment D-glucose, cellobiose, D-fructose, D-galactose, glycerol, raffinose and urea. Strain m25^T fermented pyruvate, yeast extract, peptone and tryptone, as observed in its closely related species [20, 29]. Poor growth was observed in acetate and butyrate. Furthermore, strain m25^T could not grow on gelatin in contrast to *L. thermophila* EBR46^T [20]. Vitamins were not required for the growth of strain m25^T. Nitrate (10 mM), nitrite (5 mM), fumarate (10 mM) and oxygen were tested as electron acceptors. None of the tested electron acceptors were used by strain m25^T or enhanced growth. Additional biochemical characterizations were also performed using API 20E and API 20NE systems (bioMérieux) incubated under anoxic

Table 1. Major characteristics that discriminate *Lutispora saccharofermentans* sp. nov. m25^T from the most closely related type strains belonging to the subphylum *Clostridia* (*Firmicutes*)

Strains: 1, m25^T (present study); 2, *Lutispora thermophila* EBR46^T [20]; 3, *Gracilibacter thermotolerans* JW/YJL-S1^T [29]. Optimum values are given in parentheses. –, Negative; +, positive; (+), weak growth or result; NA, data not available.

Characteristics	1	2	3
Habitat	Laboratory-scale biogas reactors	Methanogenic bioreactor	Constructed wetland system sediment
Cell shape	Rod	Rod	Straight to curved rods
Cell size (µm)	0.5–0.7×2.1–5.5	0.6–0.8×5.0–8.5	0.2–0.4×2.0–7.0
Gram type	Negative	Negative	Negative
Spore formation	–	+	–
Mobility	+	+	+
Temperature range (optimum) for growth (°C)	25–45 (35)	40–60 (55–58)	25–54 (42.5–46.5)
Salinity range (optimum) for growth (g l ⁻¹)	0–8 (2.5)	0–20 (NA)	0–15 (5)
pH range (optimum) for growth	6–9 (7)	6–9 (7.5–8.0)	6.0–8.25 (6.8–7.75)
DNA G+C content (mol%)	40.29	36.2	42.8
Substrate utilization:			
D-Glucose	+	–	+
Cellobiose	+	–	–
D-Fructose	+	–	+
D-Galactose	+	–	+
Glycerol	+	–	NA
Raffinose	+	–	–
Acetate	(+)	–	–
Butyrate	(+)	–	NA
Pyruvate	+	+	–
Urea	+	–	NA
Yeast extract	+	+	+
Peptone	+	+	+
Tryptone	+	+	+
Gelatin	–	(+)	NA
End-products:			
Glucose	Acetate and lactate.	Substrate not used	Acetate, lactate and ethanol
Tryptone	Acetate, lactate, propionate, <i>iso</i> -butyrate and <i>iso</i> -valerate.	Acetate, <i>iso</i> -butyrate, propionate, and <i>iso</i> -valerate.	NA

conditions. Strain m25^T was positive for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, urea hydrolysis, and aesculin hydrolysis. Strain m25^T was catalase positive and oxidase negative. The production of hydrogen sulphides was negative.

The monitoring and quantification of certain metabolic products (volatile fatty acids, sugars and alcohols) was carried out by high performance liquid chromatography on a Shimadzu LC-2030C 3D Plus. Organic acids, sugars and alcohols were analysed using an Aminex HPX-87H column (300×7.8 mm, particle size of 9 µm), with 0.005 N sulphuric acid as eluent (elution rate of 0.6 ml

Table 2. Whole-cell fatty acid profiles (percentage of total) of *Lutispora saccharofermentans*, sp. nov. m25^T and the closely related strains *Lutispora thermophila* EBR46^T [20] and *Gracilibacter thermotolerans* JW/YJL-S1^T [29]

DMA, dimethyl acetal; –, Absent

Fatty acids	Strain m25 ^T	<i>L. thermophila</i> EBR46 ^T	<i>G. thermotolerans</i> JW/YJL-S1 ^T
C _{12:0}	–	0.6	–
iso-C _{13:0}	1.8	0.6	–
C _{14:0}	15.7	21.4	2.3
C _{14:0} DMA	4.43	6.7	–
iso-C _{15:0}	34.3	25.6	23.8
anteiso-C _{15:0}	4.5	3.6	4.9
C _{15:0}	1.3	0.9	0.5
iso-C _{15:0} DMA	7.5	6.7	–
iso-C _{16:0}	4.4	0.1	0.6
C _{16:1}	–	–	5.4
C _{16:1} ω7c	1.4	2.6	–
C _{16:0}	4.4	3.9	29.0
C _{16:0} DMA	5.2	10.7	–
iso-C _{17:0}	1.1	0.3	15.4
C _{17:1}	–	–	5.4
C _{17:0}	1.1	–	2.5
C _{18:0}	1	0.4	3.5
C _{18:1}	–	–	0.8
C _{18:1} ω9c	–	0.8	1.6
C _{18:1} ω9c DMA	–	0.3	–

min⁻¹) and at an operating temperature of 35 °C for 60 min. The UV detector (Shimadzu) set at 210 nm was used to detect acids, and a Shimadzu RID was used to detect alcohols. Everything was connected to a computer allowing the processing of results by software (LabSolutions version 5.92). The collected samples were centrifuged for 5 min at 10000 g and 600 µl supernatant are recovered for the sample changer, which injects 20 µl onto the column. Strain m25^T produced acetate, butyrate, propionate and valerate from tryptone. However, the growth on glucose produced acetate, lactate and ethanol. These fermentation products from tryptone were consistent with end-products previously reported for its closely related species *L. thermophila* EBR46^T [20]. However, strain m25^T differed in the production of lactate as an additional end-product from tryptone. Strain m25^T produced acetate and lactate as end-products from glucose. These results were consistent with end-products reported for its closely related species *G. thermotolerans* JW/YJL-S1^T [29], but strain m25^T did not produce ethanol as end-product from glucose.

Cellular fatty acids analysis was performed from freeze-dried cells grown on peptone–yeast extract–glucose in the same medium conditions previously reported [20, 29]. The fatty acid methyl esters were analysed by the DSMZ (German Microorganism Collection und Zellculturen, Braunschweig, Germany) according to the standard protocol of the Sherlock Microbial Identification System (MIDI). The cellular fatty acid compositions are shown in Table 2. The major fatty acids were iso-C_{15:0}, C_{14:0}, iso-C_{15:0} DMA (dimethyl acetal) and C_{16:0} DMA, which accounted for 62.7% of the total fatty acids. These results were similar to those obtained for the fatty acid analysis of *L. thermophila* EBR46^T [20]; however, there were some differences in the cellular fatty acids profile of strain m25^T when compared with its closest recognized relative *L. thermophila* EBR46^T, in terms of the absence of C_{12:0}, C_{18:1} ω9c and C_{18:1} ω9c DMA, and the presence of C_{17:0} (Table 2).

EMENDED DESCRIPTION OF THE GENUS *LUTISPORA* GEN. NOV.

Lutispora (Lu.ti.spo'ra. L. n. *lutum* mud, sludge; Gr. fem. n. *spora* a seed and, in biology, a spore; N.L. fem. n. *Lutispora* a spore-forming organism that lives in anaerobic sludge).

The description is essentially as given by Shiratori and colleagues [20]. However, a proposal to amend the genus *Lutispora* is provided to include the non-spore forming and mesophilic species.

DESCRIPTION OF *LUTISPORA SACCHAROFERMENTANS* SP. NOV.

Lutispora saccharofermentans (sac.cha.ro.fer.men'tans. Gr. fem. n. *sakchâr*, sugar; L. part. adj. *fermentans*, fermenting; N.L. part. adj. *saccharofermentans*, sugar-fermenting).

Cells are Gram negative, oxidase negative and catalase negative. Cells are long, rod-shaped and motile by means of a flagellum. Individual cells are 0.5–0.7 µm wide and 2.1–5.5 µm. Colonies (1–2 mm in diameter) are pale brown and spherical. The major fatty acids are iso-C_{15:0}, C_{14:0}, C_{16:0} DMA and iso-C_{15:0} DMA. It is mesophilic, and obligatory anaerobic. Optimum growth occurs at 35 °C, 2.5 g l⁻¹ NaCl and pH 7.0.

The type strain is able to grow in the absence of yeast extract on glycerol, pyruvate, arginine and cysteine. However, in the presence of yeast extract, it is positive for the utilization of glucose, fructose, arabinose, cellobiose, galactose, inositol, raffinose, sucrose, glycerol, pyruvate, acetate, butyrate, citrate, succinate, gluconate, alanine, arginine, cysteine, tryptophane, methionine, histidine, asparagine and methanol. No growth occurs on lactose, mannitol, maltose, malate, lactate, fumarate, glutamate, hippuric acid, gallic acid, methylamine, tyrosine, glutamine, glycine, leucine, isoleucine and phenylalanine. For complex substrates, the type strain is able to grow on yeast extract, tryptone, peptone, casaminoacids, starch and casein but not on CMC, Avicel and xylan. Major end-products from tryptone and glucose are lactate, acetate, *iso*-butyrate, propionate and *iso*-valerate. Nitrate and nitrite are not used as electron acceptors. In API 20E and API 20NE tests, strain m25^T is positive for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, urea hydrolysis and aesculin hydrolysis.

The DNA G+C content calculated from the genome sequences is 40.11 mol%. The genome size is 4.3 Mbp. The type strain, m25^T (=DSM 112749^T=ATCC TSD-268^T), was isolated from lab-scale methanogenic landfill bioreactor digesting anaerobic sludge.

Funding information

This work was supported by UKRI/BBSRC (Biotechnology and Biological Sciences Research Council) grant BB/T011076/1 as part of the ERACoBioTech (www.cobiotech.eu/) SYNBIOGAS project: Synthetic landfill microbiomes for enhanced anaerobic digestion to biogas.

Acknowledgements

We would like to thank Anthony Roberts (Senior Officer and Landfill Gas Technical Specialist, Natural Resources Wales), Ed Bastow (Material Change, UK) and Andrew Leeding (Infinis, UK) for their help in providing the materials and inocula to set up the bioreactor experiments. We also thank Keith Torr and Ian Craven for providing the landfill materials.

Conflicts of interest

The authors declare that there are no conflicts of interest.

References

1. Palmisano AC, Barlaz MA. *Microbiology of Solid Waste*. CRC press, 2020.
2. Denafas G, Ruzgas T, Martuzevičius D, Shmarin S, Hoffmann M, et al. Seasonal variation of municipal solid waste generation and composition in four East European cities. *Resour Conserv Recycl* 2014;89:22–30.
3. Barlaz MA, Schaefer DM, Ham RK. Bacterial population development and chemical characteristics of refuse decomposition in a simulated sanitary landfill. *Appl Environ Microbiol* 1989;55:55–65.
4. Weaver JE, Wang L, Reyes FL, Barlaz MA. Systems and methods for studying microbial processes and communities in landfills. In: *Understanding Terrestrial Microbial Communities*. Springer, 2019. pp. 129–150.
5. Ransom-Jones E, McCarthy AJ, Haldenby S, Doonan J, McDonald JE. Lignocellulose-degrading microbial communities in landfill sites represent a repository of unexplored biomass-degrading diversity. *mSphere* 2017;2:e00300-17.
6. Meyer-Dombard DR, Bogner JE, Malas J. A review of landfill microbiology and ecology: a call for modernization with "next generation" technology. *Front Microbiol* 2020;11:1127.
7. Stamps BW, Lyles CN, Suflita JM, Masoner JR, Cozzarelli IM, et al. Municipal solid waste landfills harbor distinct microbiomes. *Front Microbiol* 2016;7:534.
8. Feng Q, Lin Y. Integrated processes of anaerobic digestion and pyrolysis for higher bioenergy recovery from lignocellulosic biomass: a brief review. *Renew Sustain Energy Rev* 2017;77:1272–1287.
9. Gogoi G, Hazarika S. Coupling of ionic liquid treatment and membrane filtration for recovery of lignin from lignocellulosic biomass. *Sep Purif Technol* 2017;173:113–120.
10. Hosseini Koupaie E, Dahadha S, Bazayar Lakeh AA, Azizi A, Elbeshbishy E. Enzymatic pretreatment of lignocellulosic biomass for enhanced biomethane production—a review. *J Environ Manage* 2019;233:774–784.
11. Loow Y-L, Wu TY, Yang GH, Ang LY, New EK, et al. Deep eutectic solvent and inorganic salt pretreatment of lignocellulosic biomass for improving xylose recovery. *Bioresour Technol* 2018;249:818–825.
12. Yao Y, Chen S. A novel and simple approach to the good process performance of methane recovery from lignocellulosic biomass alone. *Biotechnol Biofuels* 2016;9:1–9.
13. Ali J, Rasheed T, Afreen M, Anwar MT, Nawaz Z, et al. Modalities for conversion of waste to energy - challenges and perspectives. *Sci Total Environ* 2020;727:138610.
14. Hurst CJ, Crawford RL, Garland JL, Lipson DA, Mills AL. Anaerobic decomposition of refuse in landfills and methane oxidation in landfill covers. In: *Manual of Environmental Microbiology*, Third Edition. American Society of microbiology, . pp. 818–842.
15. McDonald JE, Allison HE, McCarthy AJ. Composition of the landfill microbial community as determined by application of domain- and group-specific 16S and 18S rRNA-targeted oligonucleotide probes. *Appl Environ Microbiol* 2010;76:1301–1306.
16. Burrell PC, O'Sullivan C, Song H, Clarke WP, Blackall LL. Identification, detection, and spatial resolution of *Clostridium* populations responsible for cellulose degradation in a methanogenic landfill leachate bioreactor. *Appl Environ Microbiol* 2004;70:2414–2419.
17. Huang L-N, Zhu S, Zhou H, Qu L-H. Molecular phylogenetic diversity of bacteria associated with the leachate of a closed municipal solid waste landfill. *FEMS Microbiol Lett* 2005;242:297–303.

18. O'Sullivan CA, Burrell PC, Clarke WP, Blackall LL. Structure of a cellulose degrading bacterial community during anaerobic digestion. *Biotechnol Bioeng* 2005;92:871–878.
19. Wiegel J, Tanner R, Rainey FA. An introduction to the family clostridiaceae. *Prokaryotes* 2006;4:654–678.
20. Shiratori H, Ohiwa H, Ikeno H, Ayame S, Kataoka N, et al. *Lutispora thermophila* gen. nov., sp. nov., a thermophilic, spore-forming bacterium isolated from a thermophilic methanogenic bioreactor digesting municipal solid wastes. *Int J Syst Evol Microbiol* 2008;58:964–969.
21. El Houari A, Ranchou-Peyruse M, Ranchou-Peyruse A, Dakdaki A, Guignard M, et al. *Desulfobulbus oligotrophicus* sp. nov., a sulfate-reducing and propionate-oxidizing bacterium isolated from a municipal anaerobic sewage sludge digester. *Int J Syst Evol Microbiol* 2017;67:275–281.
22. Widdel F, Pfennig N. Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids. *Arch Microbiol* 1981;129:395–400.
23. Imhoff-Stuckle D, Pfennig N. Isolation and characterization of a nicotinic acid-degrading sulfate-reducing bacterium, *Desulfococcus niacini* sp. nov. *Arch Microbiol* 1983;136:194–198.
24. Balch WE, Fox GE, Magrum LJ, Woese CR, Wolfe RS. Methanogens: reevaluation of a unique biological group. *Microbiol Rev* 1979;32:260–296.
25. Wada A, Kono M, Kawauchi S, Takagi Y, Morikawa T, et al. Rapid discrimination of Gram-positive and Gram-negative bacteria in liquid samples by using NaOH-sodium dodecyl sulfate solution and flow cytometry. *PLoS ONE* 2012;7:e47093.
26. Lane D J. 16S/23S rRNA sequencing. *Nucleic Acid Tech Bact Syst* 1991:115–175.
27. Tamura K, Nei M, Kumar S. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proc Natl Acad Sci* 2004;101:11030–11035.
28. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Mol Biol Evol* 2018;35:1547–1549.
29. Lee YJ, Romanek CS, Mills GL, Davis RC, Whitman WB, et al. *Gracilibacter thermotolerans* gen. nov., sp. nov., an anaerobic, thermotolerant bacterium from a constructed wetland receiving acid sulfate water. *Int J Syst Evol Microbiol* 2006;56:2089–2093.
30. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 2014;30:2114–2120.
31. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 2012;19:455–477.
32. Seemann T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 2014;30:2068–2069.
33. Auch AF, von Jan M, Klenk H-P, Göker M. Digital DNA-DNA hybridization for microbial species delineation by means of genome-to-genome sequence comparison. *Stand Genomic Sci* 2010;2:117–134.
34. Tindall BJ, Rosselló-Móra R, Busse H-J, Ludwig W, Kämpfer P. Notes on the characterization of prokaryote strains for taxonomic purposes. *Int J Syst Evol Microbiol* 2010;60:249–266.
35. Moore WEC, Stackebrandt E, Kandler O, Colwell RR, Krichevsky MI, et al. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Evol Microbiol* 1987;37:463–464.
36. Chaumeil PA, Mussig AJ, Hugenholtz P, Parks DH. GTDB-Tk: a toolkit to classify genomes with the genome taxonomy database. *Bioinformatics* 2019;36:1925–1927.
37. Kozlov AM, Darriba D, Flouri T, Morel B, Stamatakis A. RAxML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. *Bioinformatics* 2019;35:4453–4455.
38. Meier-Kolthoff JP, Auch AF, Klenk H-P, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 2013;14:60.
39. Meier-Kolthoff JP, Göker M. TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. *Nat Commun* 2019;10:2182.
40. Meier-Kolthoff JP, Hahnke RL, Petersen J, Scheuner C, Michael V, et al. Complete genome sequence of DSM 30083(T), the type strain (U5/41(T)) of *Escherichia coli*, and a proposal for delineating subspecies in microbial taxonomy. *Stand Genomic Sci* 2014;9:2.
41. Lee YJ, Romanek CS, Wiegel J. *Clostridium aciditolerans* sp. nov., an acid-tolerant spore-forming anaerobic bacterium from constructed wetland sediment. *Int J Syst Evol Microbiol* 2007;57:311–315.

Five reasons to publish your next article with a Microbiology Society journal

1. When you submit to our journals, you are supporting Society activities for your community.
2. Experience a fair, transparent process and critical, constructive review.
3. If you are at a Publish and Read institution, you'll enjoy the benefits of Open Access across our journal portfolio.
4. Author feedback says our Editors are 'thorough and fair' and 'patient and caring'.
5. Increase your reach and impact and share your research more widely.

Find out more and submit your article at microbiologyresearch.org.