INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

TAXONOMIC DESCRIPTION

Schnürer *et al.*, *Int. J. Syst. Evol. Microbiol.* 2022;72:005328

DOI 10.1099/ijsem.0.005328

MICROBIOLOGY SOCIETY

OPEN ACCESS

Miniphocaeibacter halophilus sp. nov., an ammonium-tolerant acetate-producing bacterium isolated from a biogas system

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Abstract

An anaerobic bacterial strain, designated AMB_01^T, recovered from mesophilic propionate enrichment of a high-ammonia biogas digester, was characterised using phenotypic and molecular taxonomic methods. Cells of AMB_01^T are coccus-shaped and often occur arranged as diplococci or sarcina. Growth occurred at 20–45 °C, initial pH 5.5–8.5 and with up to 0.7 M NH_4Cl, with optimum growth at 37–42 °C and pH 8.0. AMB_01^T achieved high cell density and highest acetate production when grown on carbohydrates, including monomers, disaccharides and polysaccharides, such as glucose, maltose, cellobiose and starch. The strain was also able to use amino acids and some organic acids and alcoholic compounds for growth. Acetate was formed as the main product and yeast was not required for growth. The major cellular fatty acids were summed feature 4 (iso-$C_{17:1}$I and/or anteiso-$C_{17:1}$B), $C_{18:1}\omega7$, $C_{14:0}$, $C_{16:0}$ and summed feature 3 ($C_{16:1}\omega7$ and/or iso-$C_{15:0}$ 2OH). The highest 16S rRNA gene sequence similarity found was with *Miniphocaeibacter massiliensis* (96.6%), within the family *Peptoniphilaceae*, phylum Bacillota (Firmicutes). The genomic DNA G+C content was 29.0 mol%. An almost complete set of genes for the acetyl-CoA pathway was found. Genome comparisons between AMB_01^T and close relatives showed highest digital DNA–DNA hybridisation to *Finegoldia magna* (23%), highest average nucleotide identity with genome nucleotide and amino acid sequences to *M. massiliensis* (72 and 73%, respectively) and highest average nucleotide identity (87%) with *Schnuerera ultunensis*, indicating that AMB_01^T represents a novel species. Analysis of genomic, chemotaxonomic, biochemical and physiological data confirmed that strain AMB_01^T represents a novel species, for which the name *Miniphocaeibacter halophilus* sp. nov. is proposed. The type strain is AMB_01^T (=DSM 110247^T=JCM 39107 ^{T}).

Anaerobic degradation for production of biogas or green chemicals offers ways to develop resource-conserving waste treatment methods concurrently with efforts to diminish dependency on fossil fuels and mineral fertiliser. The key to successful anaerobic digestion is balanced, efficient microbial activity in the complex multi-step sequence of substrate hydrolysis, acidogenesis, acetogenesis and methanogenesis [1]. Proteinaceous waste is an abundant organic material worldwide that is suitable as feedstock for the biogas system. Proteinaceous waste has high biogas potential, while anaerobic processing of this waste also yields a residue with good fertiliser value. However, anaerobic degradation of proteins can produce high levels of ammonia, which is toxic for many microbial species [2]. Common indirect causes of biological process inhibition by ammonia are decreased biogas production and accumulation of degradation intermediates (such as acetate and propionate) [3]. Through microbial adaptation, some processes can still function at high ammonia levels, but microbe management is needed in order to increase the productivity and stability of these processes [2]. Thorough characterising and obtaining a greater understanding of ammonia-tolerant microbial populations with key roles in degradation processes, it is possible to increase insights and better inform the design and operation of biogas reactors treating proteinaceous waste material. Identification and characterisation of novel species from anaerobic degradation systems may also be beneficial in development of biobased technologies for production of green products, such as acetate and renewable hydrogen . We report here on isolation of an ammonia-tolerant, acetate-producing species obtained by enrichment of a

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Keywords: *Peptoniphilaceae*; ammonia-tolerant; anaerobic digestion; hydrogen-producing.

Abbreviations: AAI, average amino-acid identity; ANI, average nucleotide-acid identity; dDDH, digital DNA–DNA hybridisation; GTR, generalised time-reversible; HPLC, high-performance liquid chromatography; ML-NNI, maximum likelihood nearest neighbour interchange; POCP, percentage of conserved proteins; SPR, subtree-prune-regraft; STAG, species tree inference from all genes; STRIDE, species tree root inference from gene duplication events.

The 16S rRNA gene sequence of AMB_01^T has been assigned the accession number MN365126 in the GenBank database of the NCBI. The draft genome sequence of AMB_01^T has been deposited under the accession number CP066744.

Six supplementary figures and three supplementary tables are available with the online version of this article.

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biogas community on propionate. The isolated species described in the present study is a member of the genus *Miniphocaeibacter*. The name of this genus has recently been modified [4] and contains a mesophilic bacterium isolated from human gut [5].

ISOLATION

Strain AMB_01^T was isolated from a propionate-degrading, methane-producing culture enriched from a laboratory-scale mesophilic (37 °C) biogas digester (Uppsala, Sweden, 5.4 g NH_4^+-N l^{-1}, 0.6–0.9 g NH_3 l^{-1}). Enrichment was performed in a reactor continuously fed bicarbonate-buffered medium containing yeast (0.2 g l^{-1}), sodium propionate (9.6 g l^{-1}) and NH_4Cl (16 g l^{-1}), as described previously [6]. The reactor had been operated under stable conditions for over 6 months at the point of sampling. Isolation was conducted using agar shakes, as described previously [7], with pyruvate (10 mM) added as the carbon source. The aim in the isolation step was to isolate propionate-oxidising bacteria, and pyruvate was added since this is a common substrate used by syntrophic microorganisms in pure cultivation. Greyish spherical colonies were transferred to anaerobic bottles containing basal medium and pyruvate, as described previously [7]. Unless otherwise stated, AMB_01^T was incubated in darkness, without shaking, at 37 °C, with an initial pH of 7.3.

MORPHOLOGY

Cell morphology was checked by phase-contrast microscopy (DMI 4000 microscope, Leica) and images were taken with a DFC360 FX (Leica, C-mount adapter: 0.7×) monochrome fluorescence camera. Gram reaction was determined with conventional staining as described previously [8] and by KOH (3%) test under anaerobic conditions also as described previously [9], with the modification the cells were grown in liquid medium and the cell pellet obtained after centrifugation was used instead of a colony from a solid medium. Cells of the isolate were non-motile, stained Gram-variable but showed Gram-positive characteristics in the KOH test (no treading) and had spherical morphology, with a diameter of about 0.5 µm (Fig. S1, available in the online version of this article). The isolate grew as free-living cells, but under suboptimal growth conditions (pH >8) the cells aggregated. Motility was not observed. Harvested cells from cultures were unpigmented.

CELLULAR FATTY ACIDS

Cellular fatty acids analysis was performed commercially by the Identification Service of the DSMZ (Braunschweig, Germany). In short, the cellular fatty acids were analysed after conversion into fatty acid methyl esters by saponification, methylation and extraction using minor modifications of published methods [10, 11]. The fatty acid methyl esters mixtures were separated by gas chromatography and detected by a flame ionisation detector using Sherlock Microbial Identification System (MIS) (MIDI TSBA40, Microbial ID). Peaks were automatically integrated and fatty acid names and percentages were calculated using the MIS Standard Software. In summary, the cellular fatty acid patters of AMB_01^T resembled the profile of the its relative, *Miniphocaeibacter massiliensis*, with the exception that the most abundant fatty acid was summed feature 4 (iso-$C_{17:1}$I and/or anteiso-$C_{17:1}$B) for AMB_01^T and $C_{16:0}$ for *M. massiliensis* (Table 1).

GROWTH CHARACTERISTICS

Anaerobic cultivation was performed in serum bottles (Nordic pack) sealed with rubber stoppers (Rubber Bv) containing 20 ml bicarbonate-buffered basal media with yeast extract (0.2 g l^{-1}) prepared as described previously [7]. The initial pH of the media was 7.3. The cultures were incubated in darkness, without shaking, at 37 °C. Substrate utilisation was tested at a concentration of 10 mM, unless otherwise stated. A control without substrate (or without added external electron acceptor) was prepared simultaneously. Growth on different substrates were assessed by visual examination of turbidity and finally confirmed after 4 months of incubation by analyses of degradation products by high-performance liquid chromatography (HPLC), as described previously [7]. Hydrogen was measured by gas chromatography with a reducing compound (HgO bed) photometer (Peak Performer Reduced Gas Analyzer PP1, Peak Laboratories), as described previously [12]. The isolate, designated AMB_01^T, grew on yeast extract and the amount of acetate (0.2 g l^{-1}), which was the sole product, formed from yeast was subtracted when calculating acetate formation during the substrate utilisation test. The isolate utilised a wide spectrum of substrates, including fumarate, pyruvate, malate, 1,2-propandiol, glycerol, glucose, fructose, galactose, mannose, maltose, cellobiose, ribose, betaine, cysteine, serine and tryptophan. Acetate was formed as the main product (0.4–1.8 g l^{-1}) from all substrates. In the degradation of ethanol and serine, low levels (0.2–0.3 g l^{-1}) of butyrate was formed in addition to acetate. Hydrogen was formed (1.9 kPa from 10 mM fructose). Low levels of acetate formation (0.1–0.3 g l^{-1}) were obtained from raffinose, dimethylamine, asparagine, casamino acids and tryptone. The isolate did not utilise acetate (25 mM), formate, citrate, lactate, methanol, 2-propanol, 1-butanol, 2,3-butandiol, mannitol, sorbitol, acetoin, benzoic acid, lactose, sucrose, arabinose, leucine, proline, acetoin, histidine, methionine, ethylene glycol (5 mM), syringate (2 mM), vanillate (3 mM), xylose (2 mM) or H_2/CO_2 (80:20; 0.8 atm). Utilisation of electron acceptors was tested with acetate (25 mM) or propionate (25 mM) as the electron donor. None of the following compounds was used as an electron acceptor: sulphate, sulphur, fumarate, nitrate

Table 1. Cellular fatty acid patterns of AMB_01^T (1) and the closely related species *Miniphocaeibacter massiliensis* Marseille-P4678^T (2 [5]). Major fatty acid values (>10% of the total) are indicted by bold type. Values are percentages of total fatty acids

Fatty acid*	1	2
$C_{10:0}$	TR	–
$C_{12:0}$	6.9	2.0
$C_{13:0}$	TR	TR
$C_{14:0}$	**14.1**	**10.8**
$C_{15:0}$	TR	1.6
iso-$C_{15:1}$I and/or $C_{13:0}$ 3OH	TR	–
$C_{16:0}$	9.9	**51.9**
$C_{16:1}\omega7$ and/or iso-$C_{15:0}$ 2OH	7.9	1.5
iso-$C_{17:1}$I and/or anteiso-$C_{17:1}$B	**34.7**	–
$C_{18:0}$	1.1	–
$C_{18:1}\omega7$	**17.0**	**22.3**
$C_{18:2}\omega6$	–	6.8

*–, Not detected, TR, fatty acids detected in trace amount (<1.0% of the total amount)

(10 mM), $FeCl_3$ (0.1 M) or thiosulphate (20 mM). Substrate utilization were also evaluated by biochemical analyses (API 50CH and 20 A; bioMérieux) according to manufacturer's protocol with the following modification; the cells were grown in the bicarbonate-buffered basal media [7] with fructose (10 mM). A 10–20 ml sample of the culture liquid was centrifuged and the obtained cell pellet was resuspended in the API medium to reach turbidity according to the manufacturer's protocols. The strips were incubated anaerobically in an anaerobic glove box (A45, Whitley) for 24 h. For API 20A, positive results were obtained for D-glucose, maltose, trehalose, salicin and gelatin. For API 50CH, positive reactions were observed with D-fructose, maltose, trehalose, *N*-acetylglucosamine, arbutin, aesculin, salicin and D-fucose. The relatively wide spectrum of substrates used by AMB_01^T differentiated the isolated strain from the related species *Parvimonas micra*. This species appears to be more specialized, using primarily amino acids and purines (Table 2). The substrate pattern of the other closely related species has not been evaluated to the same extent and cannot be well compared. However, evaluation of *M. massiliensis* using API 50CH showed positive reactions with D-ribose, potassium gluconate, *N*-acetylglucosamine and D-fructose ([5], Table 2). No positive reactions were reported for API 20A in the original description of this bacterium [5] but when again evaluated in the present study results were consistent with those obtained for strain AMB_01^T, e. g. positive reactions for D-glucose, D-maltose, D-trehalose and salicin. In addition, a positive result for *M massiliensis* was also obtained for esculin using API 50CH.

Conditions supporting growth were assessed in anaerobic cultivation flasks and growth was determined through visualisation and formation of degradation products. The level of product formation from growth on yeast extract as sole substrate was subtracted from the results. For characterisation of growth optima, AMB_01^T was cultivated in anaerobic glass tubes with glucose as sole carbon and energy source. Growth was assessed based on differences in OD_{600} compared with uninoculated controls. The pH and temperature were set to increase at intervals of 0.5 pH units and 5 °C, respectively, as described by Westerholm *et al.* [12]. Ammonia tolerance and NaCl tolerance were determined by consecutive transfer with an increasing gradient of 0.5 M NH_4Cl at pH 7.3 until growth stopped. Optimal growth of AMB_01^T was obtained at pH 7.5–8.0 (Fig. S2), but weak growth was observed at initial pH 5.5–8.5. Growth was not observed at initial pH 5.0 or 9.0. This pH ranges for growth resembled the ranges obtained from the closest relative, *M. massiliensis*, but the minimum pH level was considerably lower than was observed for *Finegoldia magna* (Table 2). Temperature optimum was 37–42 °C, determined by incubation at between 35 and 45 °C at increments of 2–3 °C. The isolate grew at temperatures between 20 and 45 °C, but acetate was not produced at 15 and 48 °C. This temperature range is similar to the growth ranges of closely related species (Table 2). NaCl concentrations above 35 g l^{-1} (0.6 M) inhibited growth (Table 2). Thus, the salt tolerance of AMB_01^T was lower than that of its closest relative *M. massiliensis*, which can tolerate NaCl up to 100 g l^{-1} (1.7 M) [4, 5]. AMB_01^T was able to grow at ammonium concentrations up to 13 g NH_4^+ l^{-1} (0.05 g NH_3 l^{-1} at pH 6.6). The ammonia tolerance has not been determined for *M. massiliensis*. Addition of oxygen representing 10% of the gas volume in the anaerobic bottle completely inhibited growth of AMB_01^T (Fig. S3).

Table 2. Differential characteristics of AMB_01^T (1) from the most closely related species identified by 16S rRNA gene similarity. *Miniphocaeibacter massiliensis* Marseille-P4678^T (2 [5]), *Parvimonas micra* VPI 5464^T (3, [17]), *Finegoldia magna* 11804^T (4 [5],) and '*Lagierella massiliensis*' SIT14^T (5, [19])

Characteristic*	1	2	3	4	5
Cell size (µm)	0.5	0.7	0.3–0.7	0.5–0.9	0.7–0.9
Cell morphology	Cocci	Cocci	Cocci	Cocci	Cocci
Source of isolate	High-ammonia biogas system	Human gut	Human	Chicken faeces	Human gut
Temperature for growth (°C)					
Optimum	37–42	37	NA	NA	37
Range	20–45	25–37	NA	20–40	25–37
Initial pH for growth					
Optimum	7.5–8.0	NA	NA	7.5	NA
Range	5.5–8.5	6–8.5	NA	7.0–9.0	6.0–8.5
NaCl tolerance	35 g l^{-1} (0.6 M)	100 g l^{-1} (1.7 M)	NA	NA	NA
Ammonia tolerance	0.85 M	NA	NA	NA	NA
Substrate utilised†	Monomers, disaccharides, polysaccharides, amino acids, organic acids, alcohols	NA	Peptones, amino acids	NA	NA
Biochemical tests‡	D-glucose, maltose, trehalose, salicin and gelatin, D-fructose, *N*-acetylglucosamine, arbutin, aesculin and D-fucose	α-galactosidase, valine arylamidase, trypsin, naphtol-AS-BI-phosphohydrolase, lipase, esterase lipase, cystine arylamidase, D-ribose, potassium gluconate, *N*-acetylglucosamine, aesculin and D-fructose	NA	NA	ribose, adonitol, erythritol, galactose, mannose, glucose, sorbose, fructose, dulcitol, rhamnose, mannitol, inositol, arbutin, amygdalin, maltose, lactose, sucrose, trehalose and melezitose
Main product†	Acetate	NA	Acetate	NA	NA

*NA, not available
†Determined during cultivation in liquid medium.
‡API 20A and API 50CH.

SEQUENCING, METAGENOMICS AND PHYLOGENY

Genomic DNA extraction and sequencing of partial 16S rRNA gene with universal bacterial primers 27F/1492R was performed as previously reported [6]. The almost complete 16S rRNA gene (1414 bp) was obtained and submitted to the EzTaxon-e server database (https://www.ezbiocloud.net) for identification of closely related species based on the 16S rRNA gene. The 16S rRNA sequences of related strains (Table S1) were aligned using MAFFT [13] (10 retree and 1000 global pair iterations) and phylogenetic tree reconstruction was done with FastTree [14] using a generalised time-reversible (GTR) model with category (CAT) approximation, gamma rate heterogeneity and BioNJ distance optimisation. The tree topology refinement parameters used in FastTree were slow 1000 resampling bootstrap, seed value of 100 and 50 rounds each of subtree-prune–regraft (SPR) and maximum likelihood nearest neighbour interchange (ML-NNI). Visualisation and annotation of the 16S rRNA gene tree was done in Figtree [15].

Taxonomic analyses using the complete 16S rRNA gene sequence (1530 bp) placed AMB_01^T within the family Peptoniphilaceae ([16], Fig. 1). The closest relatives based on sequence similarity were *M. massiliensis* (96.6%, [5]), *P. micra* (90%, [17, 18]), *F. magna* (89% [17]) and '*Lagierella massiliensis*' (92% [19], for which the name is effectively but not validly published, Table S2). Comparison with available 16S rRNA genes previously found in biogas processes was made in order to search for presence in anaerobic systems. The comparison revealed 98% gene similarity (based on 450 bp) with a highly abundant species (representing 46% of the total bacterial community) detected in a mesophilic (38 °C) process fed grass and wheat stillage. That process operated at moderate levels of ammonia (3.5 g NH_4^+-N l^{-1}, 0.2 g NH_3 l^{-1}) and had long hydraulic retention time (70 days) [20].

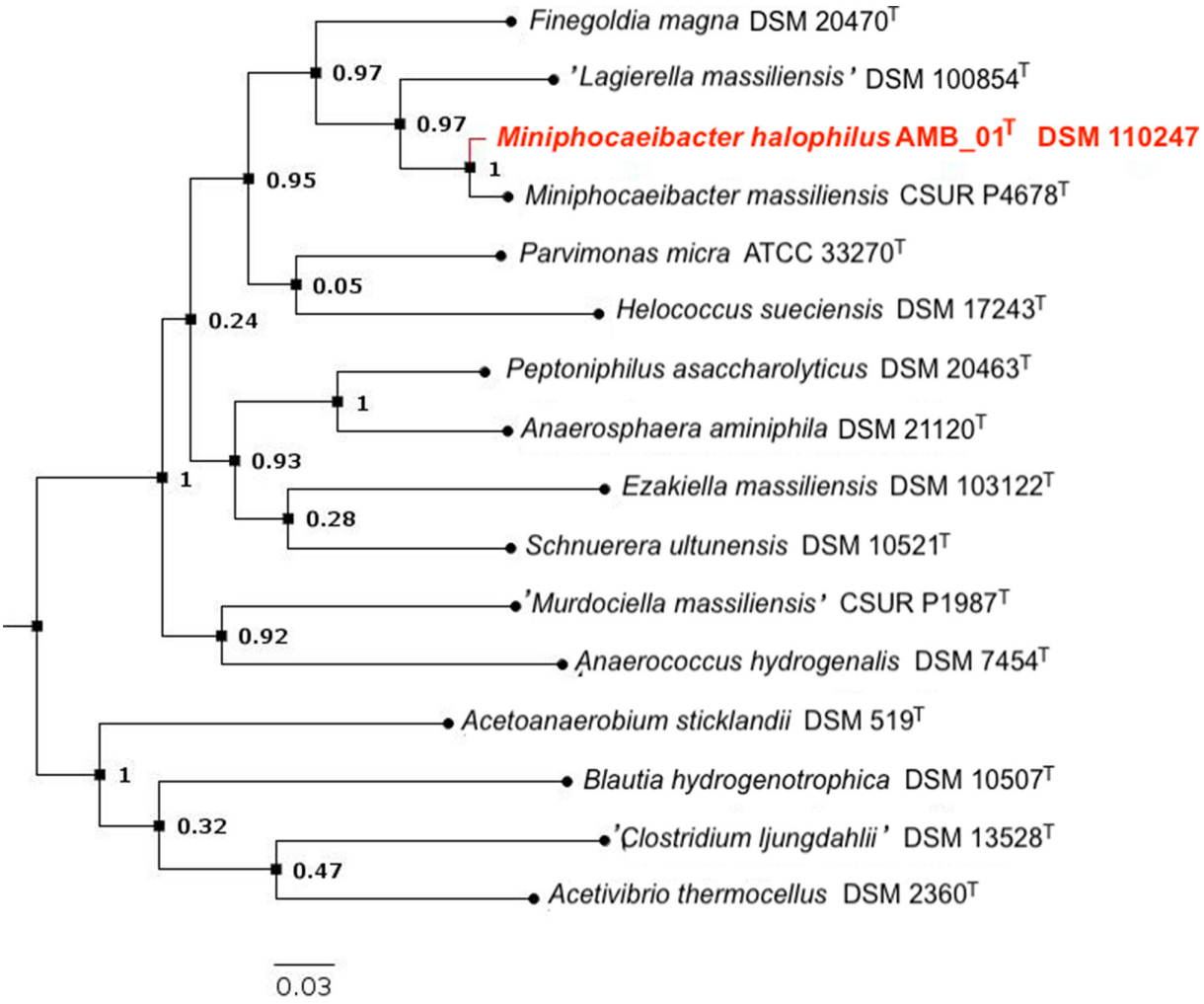

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences containing the novel species *Miniphocaeibacter halophilus* and closely related species. Please refer to table S1 for the full coordinates of the 16S rRNA genes used to construct the tree. Bar, 0.03 substitutions per nucleotide position.

For whole-genome sequencing, genomic DNA was extracted using a NucleoBond kit (Macharey Nagal) according to the protocol described by Sun *et al.* [21], with the modification that steps 1–6 were replaced with centrifugation of 80 ml of culture with an OD_{600}(λ=600 nm) of 0.2. The DNA was cleaned up with AMPure XP beads (Beckman Coulter) and eluted in 50 µl of sterile water. The DNA concentration was quantified by Qubit dsHS DNA assay (Thermo Fisher Scientific) and DNA size was visualised by agarose gel electrophoresis. PCR adapters from Oxford Nanopore Technology were ligated to the DNA strains using the LSK-109 Ligation Sequencing kit. Long-read sequencing was performed using a MinION device (Oxford Nanopore Technologies) until no sequencing activity was observed (approximately 72 h), using a R4.9.1 flow cell (FLO-MIN106) and the MinKNOW software with active channel selection enabled and base calling deactivated. A 'flow cell-refuel' step was performed after approximately 18–20 h of runtime, by adding 75 µl of a 1:1 water–SQB buffer mixture (LSK109 kit) to the flow cell via the SpotON port.

Raw nanopore sequencing data were base called and demultiplexed by guppy (v. 4.0.15–1--bb42e40) and filtered using filtlong (v. 0.2.0). Genome reconstruction was performed using flye (v.2.8) and subsequently polished using racon (v. 1.4.13) and medaka (v. 1.0.3). Read mapping for polishing was performed with minmap2 (v. 2.17). Genome annotation was conducted using PROKKA (v. 1.14.6) [22]. Assemblies were submitted to NCBI. The average per-base coverage depth (excluding deletions) was 1663, with 100% of the genome covered by at least 40 reads. Physical coverage (per read, with deletions) was higher, with an average depth of 1663 and 100% of the genome covered to at least 40×. Furthermore, 100% of the genome was covered by at least one read and 0% of the genome had per-base coverage of between 40 and 100. The DNA base composition (DNA G+C content) was calculated from genome sequence data. The AMB_01^T genome was composed of 2 394 777 bp and the genomic DNA G+C content was 29 mol%. A total of 2391 genes were annotated, which of 96% were protein coding (Table 3). Four 16S rRNA gene copies (with

Table 3. General features of the genome of AMB_01^T (1) and its closest relative *Miniphocaeibacter massiliensis* Marseille-P4678^T (2)

	1	**2**
Genome size (bp)	2394777	2077648
DNA G+C content (mol%)	29.0	28.2
Number of genes (total)	2391	2227
Protein coding genes	2287	2150
Number of copies		
5S rRNA gene	4	4
16S rRNA gene	4	3
23S rRNA gene	4	1
tRNA	45	44

99.8% pairwise identity) and 45 tRNAs were identified. The genome of AMB_01^T is available from the NCBI database with the accession number PRJNA642799.

The taxonomy of the target genome was approximated based on concatenated multiple sequence alignment of 120 marker genes as implemented in 'GTDB-Tk' (v1.4.1, https://github.com/Ecogenomics/GTDBTk) [23]. For comparison, all genomes of the same family were selected from a comprehensive genome collection of type strains [24]. After dereplication using a custom script (v0.1, https://github.com/rrwick/Assembly-Dereplicator), a phylogenetic tree was reconstructed using 119 marker genes selected to cover the phylum *Bacillota* (*Firmicutes,* 'GToTree', v1.5.47, https://github.com/AstrobioMike/GToTree) [25]. The tree was visualised using 'iTol' (v4, https://itol.embl.de/) [26]. To further explore the taxonomic placement of the species in relation to closely related species (Table S1), a species tree was reconstructed with 159 single copy orthologous genes using species tree inference from all genes (STAG) and species tree root inference from gene duplication events (STRIDE) methods implemented in Orthofinder2 [27–30]. Species tree visualisation and annotation was done in Figtree [15]. Both analyses of the phylogenetic relationship of the novel isolate revealed highest relatedness to *M. massiliensis* and '*L. massiliensis*' (Fig. 2 and S4).

Digital DNA–DNA hybridisation (dDDH) and average nucleotide/amino-acid identity (ANI/AAI) analyses of AMB_01^T genome and available genomes of closely related species were conducted using the Genome-to-Genome Distance Calculator for dDDH (https://ggdc.dsmz.de/ [31], and Pyani/CompareM [32, 33], respectively. Genome comparison between AMB_01^T and close relatives yielded highest dDDH to *F. magna* (23%), highest AAI from nucleotide and amino acid sequences to *M. massiliensis* (72 and 72%, respectively) (Table S3, Fig. S5a, b) and highest ANI value (87%) from nucleotide sequences to *Schnuerera ultunensis* (Fig. S5c). The percentage of conserved proteins (POCP) was calculated using a Ruby implementation of the formula given by Qin *et al.* [34] (available at https://github.com/hoelzer/pocp; v1.1.1). First, protein-coding genes of all genomes were annotated with PROKKA v1.14.6 using default parameters. Next, the annotated proteins were used as input for the POCP calculation. A POCP metric of the AMB_01^T genome compared with closely related genomes indicated that AMB_01^T shared less than 50% with all genomes compared except that of *M. massiliensis*, for which 67% of the proteins were shared (Fig. S6). It has been suggested that prokaryotic species with all pairwise POCP values higher than 50% may represent members of the same genus [34].

Genes encoding enzymes involved in energy metabolism included Na^+-translocating NADH-quinine reductase (*nqr*B-F), V-type Na^+ ATPase (*ntp*A-D, G, K) and Na^+-translocating ferredoxin:NAD^+ (*rnf*A–E, G). For energy metabolism, a complete set of genes for glycolysis, pyruvate oxidation and the citrate cycle was found. For starch degradation, the genome contained *mal*Q encoding 4-α-glucanotransferase. Genes involved in the acetyl-CoA pathway were found, including those for formate dehydrogenase (*fdh*), formate-tetrahydrofolate ligase (*fhs*), 5,10-methylenetetrahydrofolate reductase (*met*), carbon monoxide dehydrogenase (*cut*), acetate kinase (*ack*), dihydropteroate synthase (*fol*), methionine synthase (*met*) phosphate acetyltransferase (*pta*) and acetate production from acetyl-CoA to acetate (*pta*, *ack*). The results of BLASTX analysis against the AcetoBase protein database indicated that the *fhs* gene of AMB_01^T was similar to that of *M. massiliensis* (91%), '*L. massiliensis*' (84%) and *S. ultunensis* (75%) [35]. The genome also contained the gene for pyruvate ferredoxin oxidoreductase, which links heterotrophic metabolism to the acetyl-CoA pathway by catalysing oxidative decarboxylation of pyruvate to acetyl-CoA and CO_2 [36]. This genomic repertoire, together with the finding that acetate was the main product, indicate that AMB_01^T is an acetogenic bacteria.

The results of further analyses indicated that AMB_01^T shares a phylogenetic relationship with genera in the family Peptoniphilaceae. The results for phenotypic characteristics (including growth in NaCl, pH range and temperature), genome analysis and characterisation of morphological and chemotaxis traits indicated that AMB_01^T represents a novel species

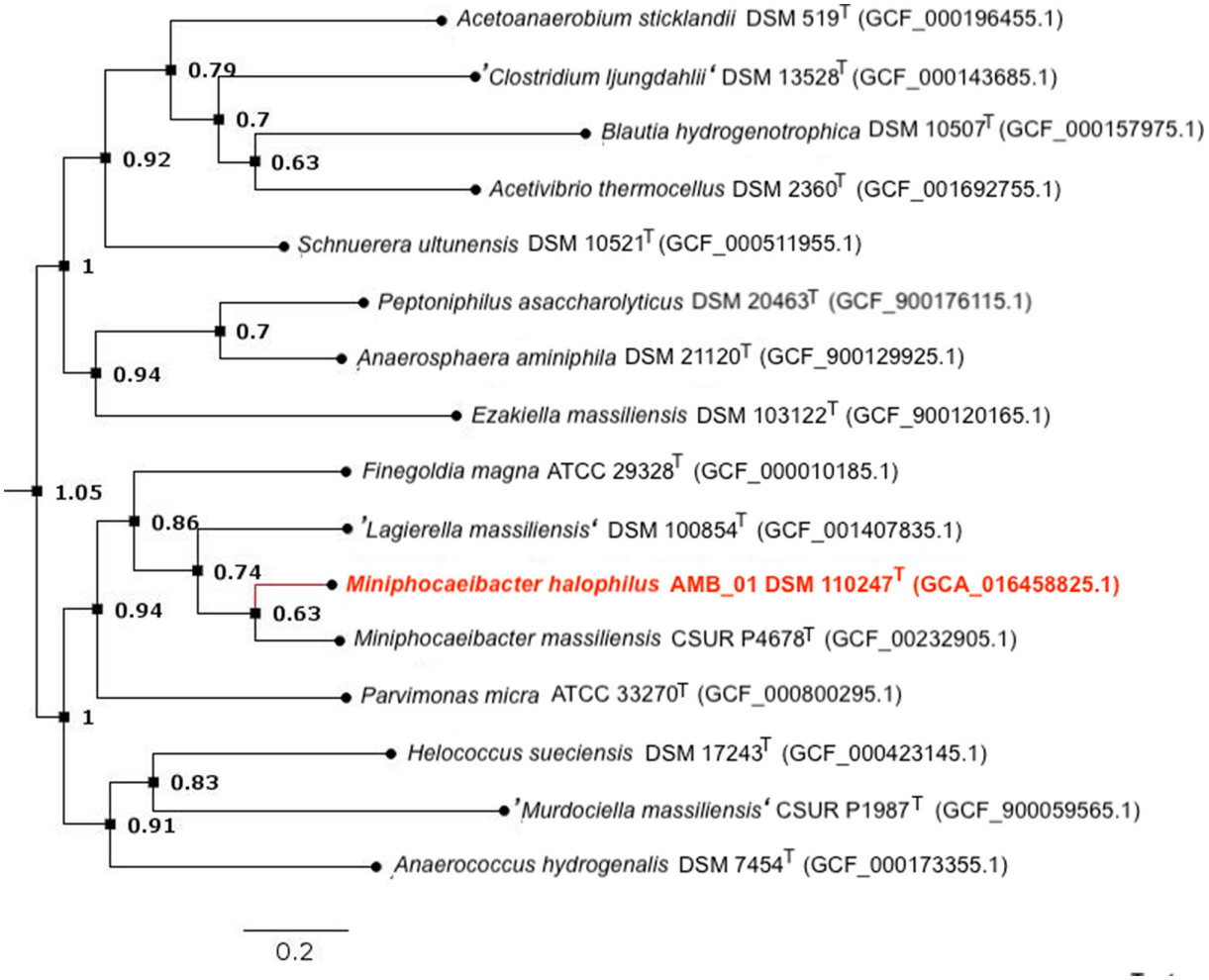

Fig. 2. Species tree based on whole genome sequences with the most closely related species. Bar, 0.2 substitutions per nucleotide position.

within the genus *Miniphocaeibacter*, for which the name *Miniphocaeibacter halophilus* sp. nov. is proposed. The type strain is AMB_01^{T} (=DSM 110247^{T}=JCM 39107 ^{T}).

EMENDED DESCRIPTION OF THE GENUS *MINIPHOCAEIBACTER* BILEN ET *AL.* 2018

The description is given by Bilen at al. 2019 [5] with the following emendation. *Miniphocaeibacter* (Mi.ni.pho.cae.i.bac'ter. L. comp. adj. minor, smaller, inferior; L. fem. n. Phocaea, Latin name of Marseille; N.L. masc. n. bacter, a rod; N.L. masc. n. Miniphocaeibacter, intended to mean a rod from small pygmy people isolated in Marseille).

Cells are Gram-positive/variable cocci (0.5–0.7 µm in diameter), anaerobic and sometimes spore forming. Optimal growth at around 37 °C, pH range 5.5–8.5, moderate halophilic (>35 g l^{-1} NaCl). Sugars and proteinious substrates are used for growth. Genome size 2.1–2.3 million bp with 28–29 mol% DNA G+C content. The genus is comprised of two species *M. massiliensis* and *M. halophillius*, isolated from human gut and an anaerobic bioreactor, respectively. The type species is *M. massiliensis*.

DESCRIPTION OF *MINIPHOCAEIBACTER HALOPHILUS* SP. NOV.

Miniphocaeibacter halophilus [ha.lo'phi.lus. Gr. masc. n. *hals*, *halos*, salt; N.L. masc. adj. *philus* (from Gr. masc. adj. *philos*), friend, loving; N.L. masc. adj. *halophilus* salt-loving]

Has the following properties in addition to those given in the genus description. Cells are free-living, unpigmented, staining Gram-variable but showing Gram-positive characteristics in KOH tests and are non-endospore forming. Yeast is not required for growth in defined media containing one single carbon source for growth. The following carbon sources support growth:

fumarate, pyruvate, malate, 1,2-propandiol, glycerol, glucose, fructose, galactose, mannose, maltose, cellobiose, ribose, betaine, cysteine, serine and tryptophan. Acetate is formed as the main product from all substrates with the exception of ethanol and serine, from which butyrate is produced in trace amounts. The following compounds showed positive reactions in biochemical tests; trehalose, salicin, gelatin, *N*-acetylglucosamine, arbutin, aesculin and D-fucose. Moderately halophilic, thermotolerant and ammonia/salt-tolerant, grows at up to an initial pH of 8.5, 45 °C and with 0.85 M NH_4Cl/ 0.60 M NaCl. Predominant cellular fatty acids are summed feature 4 (iso-$C_{17:1}$I and/or anteiso-$C_{17:1}$B), $C_{18:1}\omega7$ and $C_{14:0}$, while moderate amounts (>7.5 and <10% of the total amount) of $C_{16:0}$ and summed feature 3 ($C_{16:1}\omega7$ and/or iso-$C_{15:0}$ 2OH) are present. Nitrate, sulphate and thiosulphate are not reduced in presence of acetate or propionate as electron donor.

The type strain, AMB_01^T (=DSM 110247^T=JCM 39107 ^{T}), was isolated from a propionate enrichment operating under high-ammonia (5.4 g NH_4^+-N l^{-1}, 0.6–0.9 g NH_3 l^{-1}) and mesophilic (37 °C) conditions. The genome of the type strain is characterised by a size of 2.4 Mb and a DNA G+C content of 29 mol%. The GenBank/EMBL/DDBJ 16S rRNA gene accession number is MN365126. The whole genome sequence has been deposited in GenBank/EMBL/DDBJ, under the accession number CP066744.

Funding information
Research reported in this publication was supported by the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (Formas) under grant number 2012-808 (to M. W.), the Swedish University of Agricultural Sciences (to A. S. and A. S.) and the National Natural Science Foundation of China, number 51778616 (to S. B. and W. Q.).

Acknowledgements
We thank George Cheng for help with some of the cultivation experiments.

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

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