

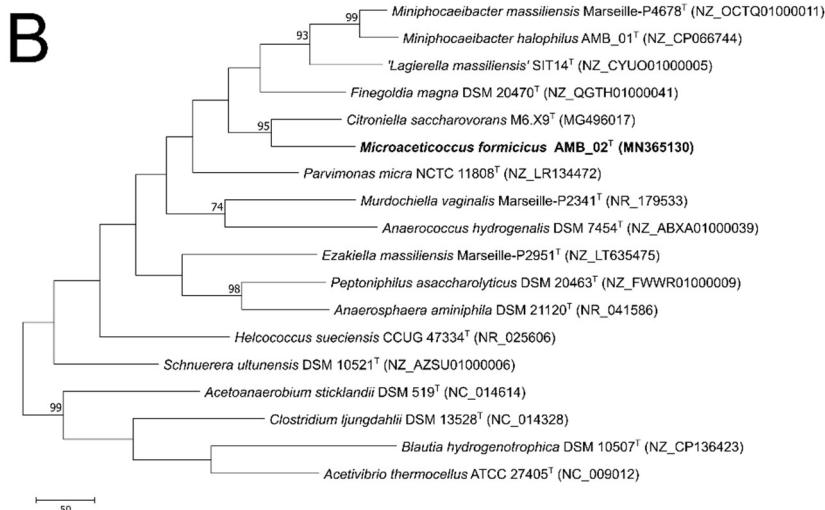
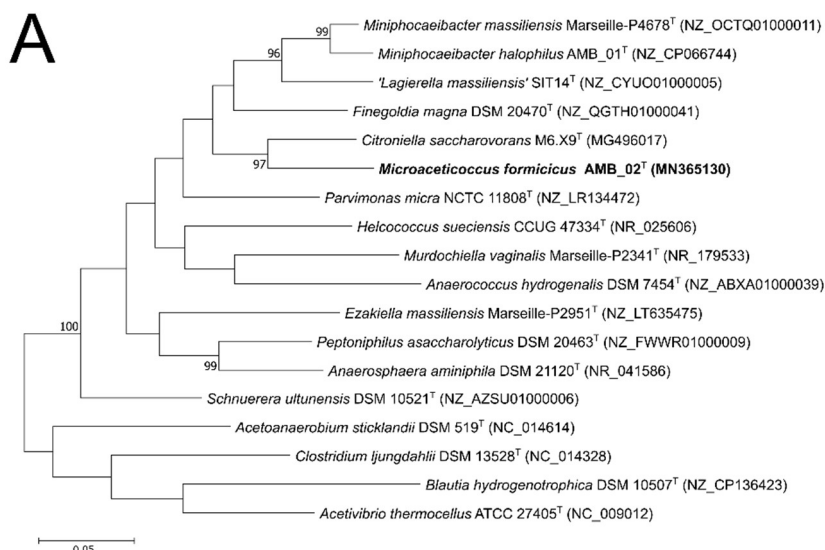


Fig. S1 Maximum-likelihood tree (A) and maximum-parsimony tree (B) based on 16S rRNA gene sequences of AMB_02^T and closely related species. Bootstrap values above 70% (based on 1000 iterations) are presented at the branch points, with bar 0.05 substitutions per nucleotide site (A) and bar with 50 nucleotide substitutions across branch length (B).

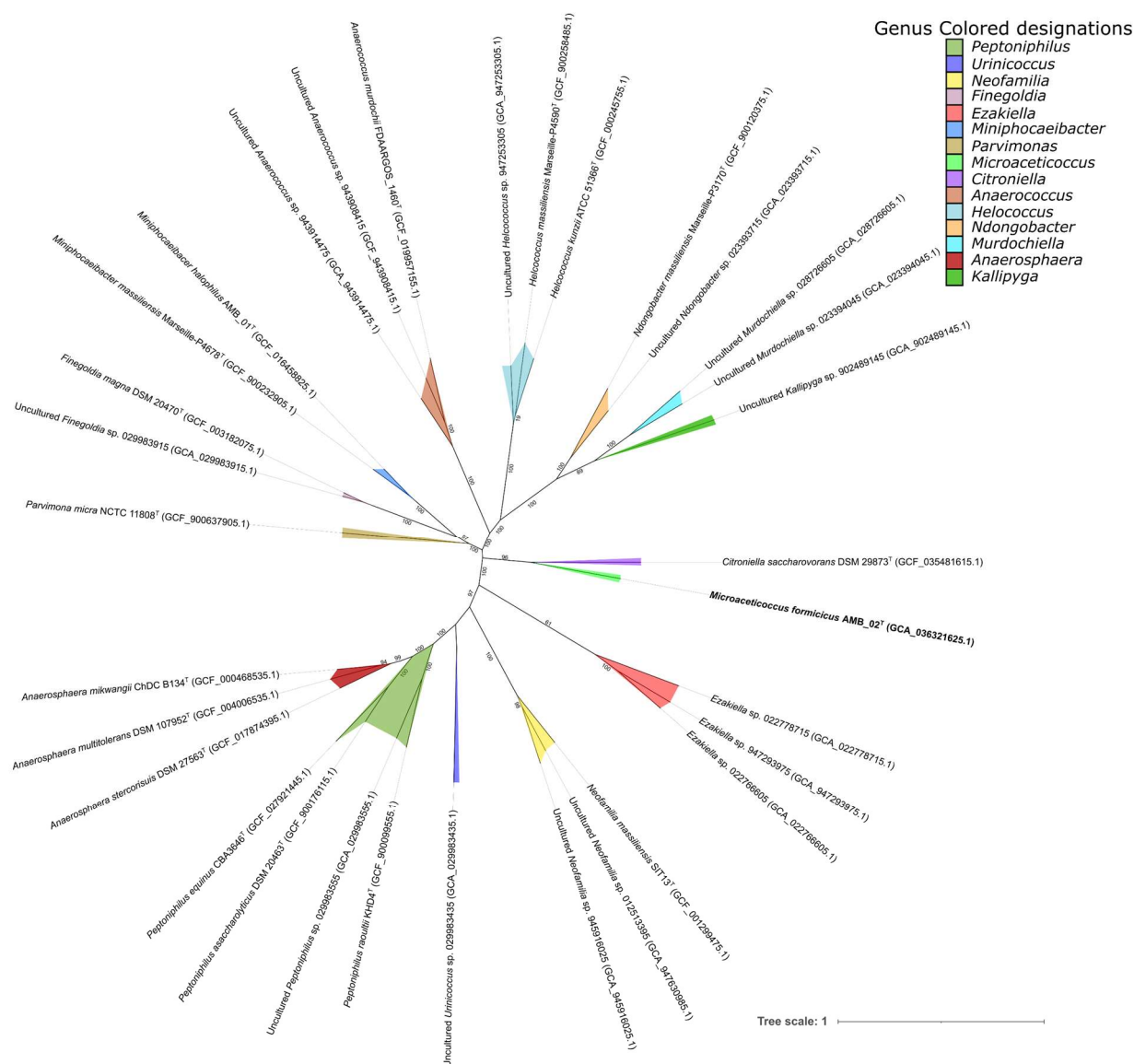


Fig. S2. Reconstructed GTTree species tree from sequence alignment of representatives from the *Peptoniphilaceae* family using 119 marker genes specific to the phylum *Bacillota* (formerly *Firmicutes*), to determine the tentative placement of strain AMB_02^T.

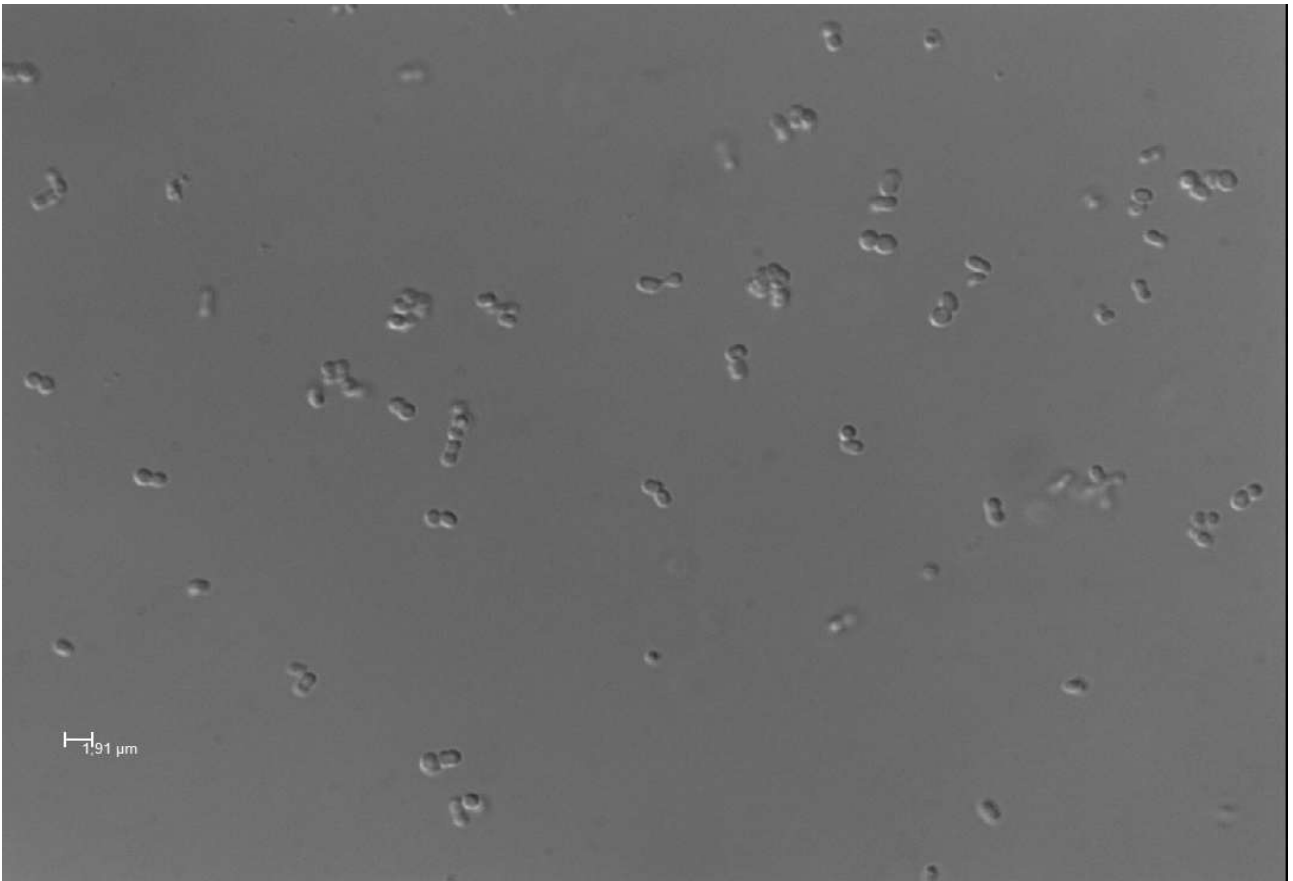


Fig. S3. Microscopy of AMB_02^T. The cells were spherical with a diameter about 0.5μm and had demonstrated motility.

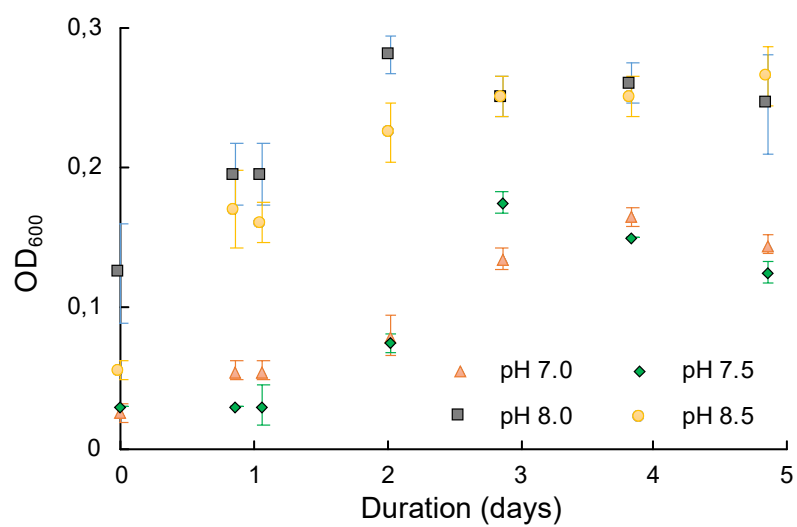


Fig. S4. Growth curves of strain AMB_02^T at different pH levels at 37°C

Table S1 Detailed description of bicarbonate-buffered basal media (BM) prepared as described by Zehnder et al. 1980 [1] with modification as described in Westerholm et al. 2011 [2]. Additional instructions for pH adjustment with the addition of HCl and Na₂CO₃.

Compounds	g/L
KH ₂ PO ₄	0.408
Na ₂ HPO ₄	0.426
Resazurin C ₁₂ H ₆ NaNO ₃	0.5 10 ⁻³
Na ₂ WO ₄ ·2H ₂ O	32.9 10 ⁻⁶
Na ₂ SeO ₃ ·5H ₂ O	26.3 10 ⁻⁶
NaHCO ₃	3.9
Yeast extract	0.2
Trace elements solution	
FeCl ₂ 4H ₂ O	2 10 ⁻³
H ₃ BO ₃	50 10 ⁻⁶
ZnCl ₂	50 10 ⁻⁶
CuCl ₂ 2H ₂ O	38 10 ⁻⁶
MnCl ₂ 2H ₂ O	41 10 ⁻⁶
(NH ₄) ₆ Mo ₇ O ₂₄ 4H ₂ O	50 10 ⁻⁶
AlCl ₃	50 10 ⁻⁶
CoCl ₂ ·6H ₂ O	50 10 ⁻⁶
NiCl ₂ ·6H ₂ O	80 10 ⁻⁶
EDTA	
C ₁₀ H ₁₄ N ₂ Na ₂ O ₈ ·2H ₂ O	500 10 ⁻⁶
Conc. HCl	1 10 ⁻⁶
Mineral salt solution	
NH ₄ Cl	0.30
NaCl	0.30
CaCl ₂ ·2H ₂ O	0.11
MgCl ₂ ·6H ₂ O	0.10
Reducing agent	
Cystein-HCl	0.5
Na ₂ S·9H ₂ O	0.24
Vitamin solution	
Pyridoxamine	2.5 10 ⁻⁴
Nicotinic acid	1 10 ⁻⁴
Nicotinamide	1 10 ⁻⁴
DL-panthothenic acid	5 10 ⁻⁵
Vitamin B ₁₂	5 10 ⁻⁵
<i>p</i> -aminobenzoic acid	5 10 ⁻⁵
Pyridoxine hydrochloride	1 10 ⁻⁴
Biotin	2 10 ⁻⁵
Thioctic acid	5 10 ⁻⁵
Folic acid	2 10 ⁻⁵
Riboflavin	5 10 ⁻⁵
Thiamine hydrochloride	1 10 ⁻⁴

pH adjustment

To avoid pH drift during growth at different pH, the isolate was inoculated in media with the following modifications, KH₂PO₄ or Na₂HPO₄ was increased to 40 g/L for inoculation at low or high pH respectively. Minor pH adjustments were achieved by adding the required volume of HCl (1 M) for pH 6.0 to 7.0 or Na₂CO₃ (1 M) for pH 7.5 to 9.0 and the pH was verified with additional measurements.

Table S2 Medium 2 recipe adapted from McSweeney et al. 2005 [3]. Glucose, maltose, and cellobiose were omitted for the substrate test of AMB_02^T and *Citroniella saccharovorans* strain DSM 29873^T. Ingredients are in grams (g) unless specified otherwise and distilled H₂O is added to a final volume of 100mL.

Ingredients	Grams (g)
Casitone	1.0
Yeast extract	0.25
Sodium lactate (70% w/v)	1.0
Glucose	0.20
Maltose	0.20
Cellobiose	0.20
NaHCO ₃	0.4
Solution 1	15 mL
Solution 2	15 mL
Resazurin	0.1 mg
Reducing Agent	
Cystein-HCl	0.05
Solution 1	
K ₂ HPO ₄	6.0 g/L
Solution 2	
CaCl ₂ ·H ₂ O	1.6 g/L
KH ₂ PO ₄	6.0 g/L
NaCl	12.0 g/L
(NH ₄) ₂ SO ₄	6.0 g/L
MgSO ₄ ·7H ₂ O	2.5 g/L

Table S3. Accession numbers of genomes and 16S rRNA gene sequences that were used for phylogenetic tree reconstruction.

Name	Strain ¹	Type Strain ²	Genome Assembly Accession ³	GenBank Accession	16S rRNA gene coordinates ⁴	16S rRNA gene length (bp)
<i>Acetoanaerobium sticklandii</i>	DSM 519	Y	GCF_000196455.1 [♦]	NC_014614	NC_014614.1:1096814-1098446 [#]	1632
<i>Blautia hydrogenotrophica</i>	DSM 10507	Y	GCF_034356035.1 [♦]	NZ_CP136423	NZ_CP136423.1:32445-33978 [#]	1533
<i>Miniphocaeibacter halophilus</i>	AMB_01	Y	GCF_016458825.1 [♦]	NZ_CP066744	NZ_CP066744.1:3895-5424 [#]	1529
<i>Clostridium ljungdahlii</i>	DSM 13528	Y	GCF_000143685.1 [♦]	NC_014328	NC_014328.1:9417-10926 [#]	1509
<i>Helcococcus sueciensis</i>	DSM 17243 ⁺ CCUG 47334*	Y	GCF_000423145.1 ^{♦+}	NR_025606*	NR_025606.1 ^{!*}	1512
<i>Acetivibrio thermocellus</i>	ATCC 27405	Y	GCF_000015865.1 [♦]	NC_009012	NC_009012.1:3427586-3429113 [#]	1527
' <i>Lagierella massiliensis</i> '	SIT14	Y	GCF_001407835.1 [♦]	NZ_CYUO00000005	NZ_CYUO01000005.1:107890-109418 [#]	1528
<i>Miniphocaeibacter massiliensis</i>	Marseille-P4678	Y	GCF_900232905.1 [♦]	NZ_OCTQ01000011	NZ_OCTQ01000011.1:3750-5174 [#]	1424
<i>Parvimonas micra</i>	NCTC 11808	Y	GCF_900637905.1 [♦]	NZ_LR134472	NZ_LR134472.1:1150216-1151741 [#]	1525
<i>Schnuerera ultunensis</i>	DSM 10521	Y	GCF_000511955.1 [♦]	NZ_AZSU00000006	NZ_AZSU01000006.1:3503-5030 [#]	1527
<i>Anaerococcus hydrogenalis</i>	DSM 7454	Y	GCF_000173355.1 [♦]	NZ_ABXA01000039	NZ_ABXA01000039.1:533-2059 [#]	1526
<i>Anaerosphaera aminiphila</i>	DSM 21120	Y	GCF_900129925.1 [♦]	NR_041586	NR_041586.1 [!]	1472
<i>Ezakiella massiliensis</i>	Marseille-P2951	Y	GCF_900120165.1 [♦]	NZ_LT635475	NZ_LT635475.1:1740235-1741776 [#]	1541
<i>Finegoldia magna</i>	DSM 20470	Y	GCF_003182075.1 [♦]	NZ_QGTH01000041	NZ_QGTH01000041.1:3362-4883 [#]	1521
<i>Murdochiella vaginalis</i>	Marseille-P2341	Y	GCF_900119705.1 [♦]	NR_179533	NR_179533.1 [!]	1488
<i>Peptoniphilus asaccharolyticus</i>	DSM 20463	Y	GCF_900176115.1 [♦]	NZ_FWWR00000009	NZ_FWWR01000009.1:938432-939960 [#]	1528
<i>Citroniella saccharovorans</i>	DSM 29873 ⁺	Y	GCF_035481615.1 ^{♦+}	MG496017*	MG496017.1 ^{!*}	1413

	M6.X9*					
<i>Microaceticoccus</i> <i>formicus</i> AMB_02 ^T	AMB_02	Y	GCA_036321625.1 ^o	MN365130	MN365130.1 [!]	1420

1 * - Corresponding strain to accession number for 16S rRNA gene, + - Corresponding strain to accession number for genome assembly

2 Y – yes, X – no

3 ♦ - RefSeq assembly, o - Genbank assembly

4 # - Extracted from assemblies, ! - Downloaded from NCBI

Table S4. Substrate growth characteristics comparison for 1, AMB_02^T; 2, *Citroniella saccharovorans* DSM 29873^T [4]; 3, *Finegoldia magna* DSM 20470^T [5]; 4, *Miniphocaeibacter halophilus* DSM 110247^T [6]; 5, *Parvimonas micra* ATCC 33270^T [7]. ++strong growth, +weak growth, -no reaction, ND no data. Information of medium used can be found in the corresponding references.

Substrate	1 [!]	2*	3*	4 [!]	5*
Acetate	-	ND	ND	-	ND
Acetoin	-	ND	ND	-	ND
Arabinose	-	ND	ND	-	ND
Asparagine	++	++	ND	++	ND
Benzoic Acid	-	ND	ND	-	ND
Betaine	++	ND	ND	++	ND
1-Butanol	-	ND	ND	-	ND
2,3-Butandiol	-	ND	ND	-	ND
Casaamino Acid	++	ND	ND	-	ND
Cellobiose	-	++	ND	++	ND
Citrate	-	ND	ND	-	ND
L-Cysteine	+	ND	ND	++	++
Dimethylamine	-	ND	ND	-	ND
D-Galactose	++	+	ND	++	ND
D-Glucose	++	++	+	++	ND
Glycerol	-	ND	ND	++	ND
Ethanol	-	ND	ND	+	ND
Ethanolamin	-	ND	ND	-	ND
Ethylene glycol	-	ND	ND	-	ND
Formate	++	ND	ND	-	ND
Fructose	++	+	+	++	++
Fumaric acid	-	ND	ND	++	ND
Histidine	++	ND	+	-	ND
Lactose	-	ND	ND	-	ND
Lactate	-	ND	ND	-	ND
Leucine	-	ND	++	-	ND
Malic Acid	-	ND	ND	++	ND
Maltose	+	++	ND	++	ND
Mannitol	-	ND	ND	-	ND
Mannose	-	-	ND	++	ND
Methanol	-	ND	ND	-	ND
Methionine	-	ND	ND	-	ND
Methylamine	-	ND	ND	-	ND
Phenylalanine	-	ND	ND	-	ND
Proline	-	ND	-	-	ND
1,2-Propanediol	-	ND	ND	++	ND
2-Propanol	-	ND	ND	-	ND
Pyruvate	-	++	ND	++	ND
Raffinose	-	ND	ND	-	ND
Ribose	++	ND	ND	++	++
Salicin	-	ND	ND	-	ND
Serine	++	++	+	++	ND
Sorbitol	-	ND	ND	-	ND
Sucrose	-	ND	ND	-	ND
Syngate	-	ND	ND	-	ND
Tryptone	++	ND	ND	-	ND
Tryptophan	+	ND	ND	++	ND
Vanillic Acid	-	ND	ND	-	ND
Xylose	-	ND	ND	-	ND

! - substrate tests performed on bicarbonate-buffered basal media (BM).

*- for information on medium used please refer to the corresponding references.

Reference

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