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Lactiplantibacillus carotarum AMBF275^T sp. nov. isolated from carrot juice fermentation

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Abstract

A novel strain of the genus *Lactiplantibacillus*, named AMBF275^T, was isolated from fermented carrot juice, a salted fermented beverage dominated by lactic acid bacteria. The results of phylogenetic analysis indicated that the 16S rRNA gene of AMBF275^T is most similar to the 16S rRNA gene of *Lactiplantibacillus garii* FI11369^T with a sequence similarity of 99.4%. However, a genome-wide comparison using average nucleotide identity (ANI) revealed that AMBF275^T and *L. garii* FI11369^T have an ANI of only 82.35%. ANI values between AMBF275^T and other representative strains of species of the genus *Lactiplantibacillus* from the Genome Taxonomy Database (GTDB) were even lower than this 82.35%, indicating that AMBF275^T represents a distinct species. We thus propose the name *Lactiplantibacillus carotarum* sp. nov. for this novel species, with AMBF275^T (=LMG 32885^T, =CECT 30757^T) as the type strain.

INTRODUCTION

In a previous citizen science project, named 'Ferme Pekes' https://www.uantwerpen.be/nl/projecten/ferme-pekes/, we identified the bacteria dominating carrot juice fermentations under laboratory and home-made conditions carried out by 40 participants [1]. These vegetable fermentations are characterised by a salt concentration (2.5% NaCl) and absence of oxygen, resulting in a pH lower than 4.6 after 3 days for the majority of the fermentations [1]. The results of sequencing of 16S rRNA genes indicated that these vegetable fermentations are initially dominated by members of the genus *Leuconostoc,* generally after 1–3 days, followed by members of the genus *Lactiplantibacillus* after 3–10 days in the majority of fermentations studied. In addition, different isolates have been cultured from these vegetable fermentations and stored for further analyses.

The genus *Lactiplantibacillus,* members of which dominate carrot juice fermentations and other plant-based fermentations [2], was until recently known as the *Lactobacillus plantarum* group as part of the genus *Lactobacillus*. However, in 2020, then genus *Lactobacillus* was split into 25 separate genera due to its heterogeneity and paraphyly [3]. One of this novel genera was *Lactiplantibacillus*. Species of this genus are commonly detected in various habitats by culture-dependent [4] and -independent [5, 6] methods. A key habitat of members of the genus *Lactiplantibacillus* seems to be fermented products [7] such as fermented vegetables [8, 9], cereals [10], meat [4] and dairy [4] products where they can dominate these ecosystems, although it is unclear to what extent these are their natural habitats. Additionally, members of this genus are commonly found in a wide range of plant, environmental, vertebrate and invertebrate habitats [11]. Considering all these habitats, it is not surprising that this genus has been classified as a nomadic genus [12]. Besides having a natural habitat in these different environments, different strains of members of the genus *Lactiplantibacillus* are also deliberately added to dairy and other fermentations as starter cultures or probiotics. Many species of this genus have been assigned the generally recognized as safe (GRAS) status [13] by the Food and Drug Administration (FDA) and the qualified presumption of safety (QPS) status by the European Food Standards Agency (EFSA) [14].

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Abbreviations: ANI, average nucleotide identity; ENA, European Nucleotide Archive; GTDB, genome taxonomy database; meso-DAP, meso-2,6-diaminopimelicacid; MRS, de Man, Rogosa and Sharpe.
The EMBL-EBI accession numbers of the 16S rRNA gene and the genome sequence of AMBF275T are OX442439 and SAMEA112195921, respectively. (https://www.ebi.ac.uk/ena/browser/view/SAMEA112195921?dataType=&show=related-records)
One supplementary figure is available with the online version of this article.

In our 'Ferme Pekes' citizen-science project, one strain, isolate AMBF275^T, was isolated from a carrot juice fermentation at day 30 of a home fermentation. Here, we identify this strain as representing a novel species, describe its relationship to other species of its genus, and characterise some of its genotypic and phenotypic properties.

Isolation

Isolate AMBF275^T was cultured from spontaneous carrot (*Daucus carota*) juice fermentations [1]. To prepare the carrot juice, carrot juice was made with a Solis Juice Fountain Pro (Solis, Switzerland) and 25 g l^{-1} NaCl was added to a Weck jar. Fermentations were sampled on day 1, 3 and 30 and plated on de Man, Rogosa and Sharpe (MRS) mdium containing 15 g l^{-1} bacteriological agar. Isolate AMBF275^T was isolated on day 30 (27 October 2015) of this carrot juice fermentation on MRS agar. Pure cultures were obtained by inoculating multiple times and stored in 25% v/v glycerol in −80 °C for long-term storage.

Genome features

For whole-genome sequencing, isolate AMBF275^T was grown overnight in MRS medium and its DNA was extracted and short-read sequenced according to the methods described previously [8]. Briefly, the genome was sequenced using the NexteraXT DNA Sample Preparation kit (Illumina, United States of America) and the MiSeq platform (Illumina, United States of America) using 2×250 cycles at the Laboratory of Medical Microbiology (University of Antwerp, Belgium). In addition to short-read sequencing, nanopore sequencing was also performed on the MinION Mk1C (Oxford Nanopore Technologies, United Kingdom). DNA was extracted using the MagAttract HMW DNA kit (Qiagen, Germany) and sequenced using the ligation sequencing kit (LSK-109) with an additional Native Barcoding Expansion 1–12 (EXP-NBD104) on a R9 chemistry flowcell (FLO-MIN106D) (Oxford Nanopore Technologies, United Kingdom). Long reads were assembled using Flye [15] and subsequently polished three consecutive times using the Illumina reads with Polypolish [16]. This analysis resulted in a complete assembly of the genome Fig. S1. The assembly is available at the European Nucleotide Archive (ENA) with the biosample number SAMEA112195921. The genome of *L. carotarum* AMBF275^T has a total length of 3.45 Mbp and a DNA G+C content of 47.56 mol% with an estimated completeness of 98.30% and contamination of 3.09% as assessed with checkM [17]. The genome contains a chromosome of 3.27 Mb (DNA G+C content of 48.0 mol%) and eight plasmids ranging in size from 7.7Kb to 45 Kb (DNA G+C content ranging from 36.8 mol% to 42.1 mol%) (Fig. S1, available in the online version of this article). Annotation with Prokka [18] identified in total 3801 genes with 3715 CDSs and 19 rRNA, 66 tRNA and 1 tmRNA genes. The 16S rRNA gene sequence was checked with EzBiocloud [19] and it was found that it was positioned within the genus *Lactiplantibacillus*.

Position within the genus *Lactiplantibacillus*

To classify AMBF275^T using the 16S rRNA gene, the rRNA genes of AMBF275^T and all type strains of species of the genus *Lactiplantibacillus* were extracted from their genomes using Barrnap and an in-house script. To check the relatedness to species for which whole genomes are available but that do not yet have validly published names, three additional genomes were included that represent species in the Genome Taxonomy Database (GTDB) with placeholder names (sp002970915, sp002970935 and plantarum_A). However, we note that *Lactiplantibacillus argentoratensis* is represented in this work by *L. plantarum* due to its high ANI of 95.5%. Throughout this paper, we refer to this collection as the GTDB representative strains of the genus *Lactiplantibacillus*. The genomes of the representative strains were downloaded from the GTDB. The 16S rRNA genes were then compared using BLASTN [20]. This method confirmed that the 16S rRNA of AMBF275^T was most similar to the 16S rRNA gene of *Lactiplantibacillus garii* FI11369^{T,} (99.424%) (Table 1).

Since the 16S rRNA gene showed high similarity to that of *L. garii*, the average nucleotide identity (ANI) between the *L. carotarum* AMBF275^T genome and all representative strains of members of the genus *Lactiplantibacillus* was calculated using fastANI [21] (Table 1). The results of this analysis indicated that *L. garii* and *L. xiangfangensis* were most similar to our strain AMBF275^T, but with low ANI values of 82.35 and 81.83%, respectively. This is lower than the 94–95% threshold, thus indicating that AMBF275^T represents a novel species [22, 23]. We propose the name *Lactiplantibacillus carotarum* sp. nov. for this novel species. The difference between the high 16S rRNA percentage identity and the low ANI to strains of existing species shows the limitations of the identification of species based solely on the 16S rRNA gene.

To gain a better understanding of the position of this novel species within the phylogeny of the genus *Lactiplantibacillus*, a 16S rRNA gene tree (Fig. 1) and a core genome tree (Fig. 2) were reconstructed. The core genome tree was inferred using all reference strains of members of the family *Lactobacillaceae* downloaded from GTDB [24]. Genes were predicted with Prodigal [25] and single copy core genes were determined, aligned and concatenated with SCARAP [26]. This resulted in 410 single-copy core genes. The extracted 16S rRNA genes and 410 single-copy core genes were aligned using MAFFT [27] and a maximum likelihood tree was reconstructed with IQ-TREE with the general time reversible with finite sites (GTR+F) substitution model and the Le and Gascuel with optimised frequencies and four gamma categories (LG+F+G4) amino acid substitution model, respectively [28] The 16S rRNA and core genome trees were both rooted with tidygenomes [29] using *Levilactobacillus brevis* ATCC 14869 (GCF_001433855.1) as the outgroup, and visualised using ggtree [30]. The differences between the 16S rRNA gene tree (Fig. 1) and the core genome tree (Fig. 2) indicate that relying solely on the 16S rRNA gene tree is not suitable for establishing the phylogenetic

Table 1. Comparison of average nucleotide identity (ANI) and the 16S rRNA sequence identity of *L. carotarum* AMBF275^T and the GTDB reference genomes from species of the genus *Lactiplantibacillus* with their corresponding accession numbers, species and NCBI strain designation. note: sometimes there is an _A suffix, which indicates cases where the GTDB has split a species because one or more genomes is/are too dissimilar to the type strain $^{(T)}$ (as determined by ANI) to be part of the same species

species	NCBI strain designation	Accession number	ANI (%)	16S rRNA identity(%)
Lactiplantibacillus garii	FI11369^T	GCF_003885105.1	82.3515	99.424
Lactiplantibacillus xiangfangensis	LMG 26013^T	GCF_001438845.1	81.8377	99.232
Lactiplantibacillus paraplantarum	DSM 10667^T	GCF_003641145.1	81.2653	99.04
Lactiplantibacillus plajomi	NBRC 107333^T	GCF_005405405.1	80.9206	99.296
Lactiplantibacillus pentosus	DSM 20314^T	GCF_003641185.1	80.904	99.232
Lactiplantibacillus plantarum	DSM 20174^T	GCF_014131735.1	80.7666	99.296
Lactiplantibacillus modestisalitolerans	NBRC 107235^T	GCF_005405425.1	80.4521	99.104
Lactiplantibacillus plantarum_A	EGD-AQ4	GCF_000463075.2	80.3831	99.296
Lactiplantibacillus fabifermentans	DSM 21115^T	GCF_000498955.2	80.3669	98.464
Lactiplantibacillus sp002970915	CBA3605	GCF_002970915.1	80.2762	97.963
Lactiplantibacillus daowaiensis	203-3^T	GCF_005405085.1	80.1609	98.528
Lactiplantibacillus sp002970935	CBA3606	GCF_002970935.1	80.1205	97.963
Lactiplantibacillus nangangensis	381–7^T	GCF_005405065.1	80.0915	98.528
Lactiplantibacillus herbarum	TCF032-E4^T	GCF_001039045.1	80.0142	98.529
Lactiplantibacillus songbeiensis	398-2^T	GCF_005405125.1	79.9891	97.836
Lactiplantibacillus daoliensis	116-1A^T	GCF_005405005.1	79.7769	98.592
Lactiplantibacillus dongliensis	218-3^T	GCF_005405105.1	79.7659	97.836
Lactiplantibacillus mudanjiangensis	11050^T	GCF_005405385.1	79.5004	97.836
Lactiplantibacillus pingfangensis	382-1^T	GCF_005404945.1	79.4793	98.558

relationships between closely related species. The species *L. garii* and *L. xiangfangensis* were shown to be most closely related to *L. carotarum*.

Morphology, arrangement and peptidoglycan cell wall structure

The strains were able to grow at a temperature range between 17 and 45 °C with an optimal temperature of 30 °C in MRS broth. When plated on MRS agar and grown overnight at 37 °C, the bacteria formed round, pulvinate, white colonies. Upon Gram staining and examination under a light microscope (CX41, Olympus, Japan) at a magnification of 1000×, the bacteria were observed to be Gram-stain-positive, short, fat rods that were organised in clusters or chains. For a clear image, this is depicted in Fig. 3 using the DMi8 microscope (Leica, Germany). No spores were found after spore staining and examination under the microscope. Analysis of the presence of *meso*-2,6-diaminopimelic acid (meso-DAP) was carried out by the DSMZ Services, Leibniz-Institut DSMZ- Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany) according to the protocol described previously [31]. This confirmed the presence of meso-DAP, consistent with the genome predictions of the presence of an *asnB* gene, known to be involved in the meso-DAP amidation in *L. plantarum* [32, 33].

Growth optimum and physiological properties

L. carotarum AMBF275^T was isolated on MRS media. The strain was able to grow in a microaerobic atmosphere with 5.0% CO_2, but also grew well under both anaerobic and aerobic conditions. The optimal pH was determined to be 7, but it was able to grow at a pH range between 3.5 and 9.5. The strain was also able to grow with salt concentrations between 0 and 8% with an optimal salt concentration of 0%. Catalase activity was determined under four conditions, as described previously [34]. These conditions were a combination of aerobic and anaerobic, with and without menaquinone supplementation. To explore whether AMBV1719^T is catalase-positive, the degradation of H_2O_2 was monitored. A 2 µl aliquot of the overnight culture was inoculated onto a microscope slide and a few droplets of 3% H_2O_2 solution were added. No reaction was observed for the four conditions, indicating that isolate AMBF275^T is catalase-negative.

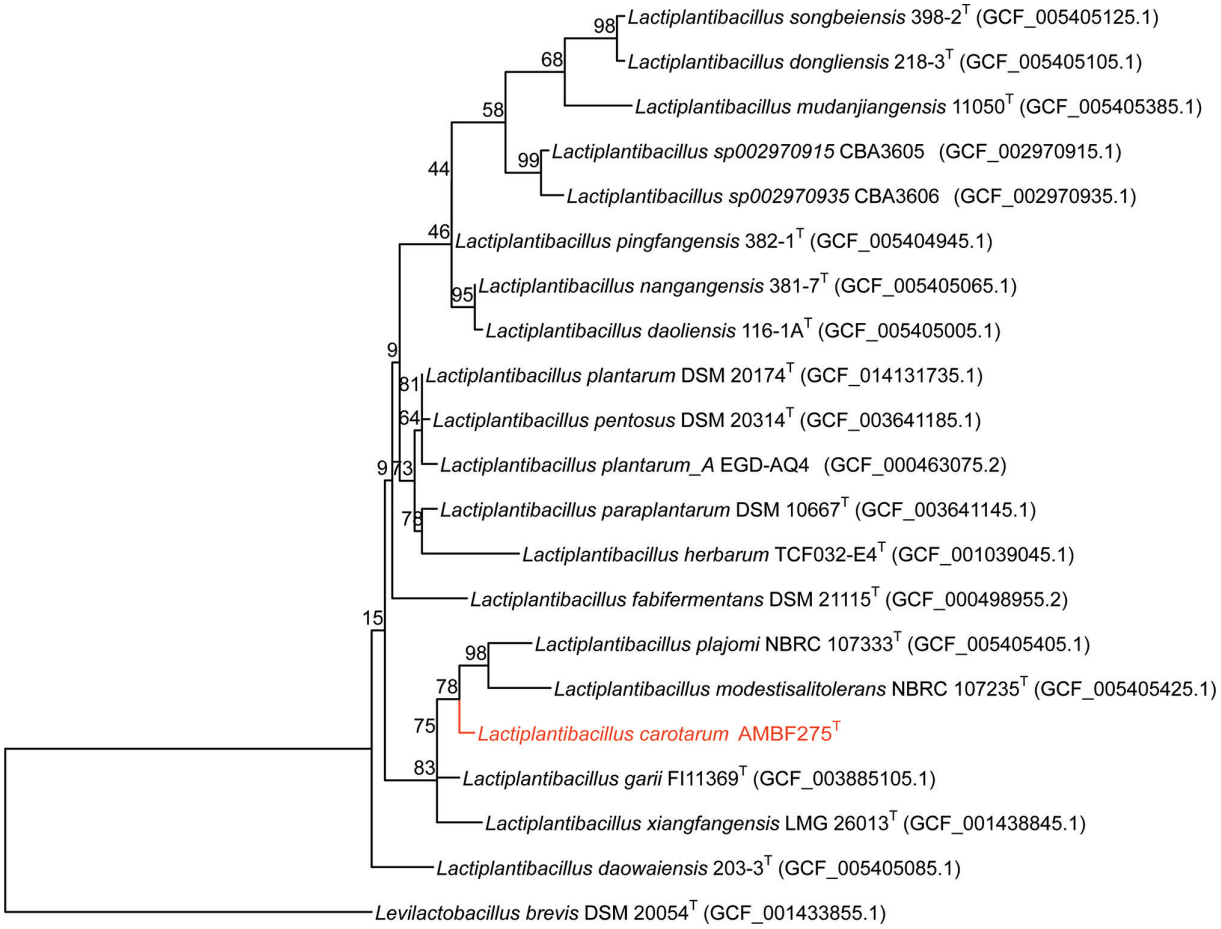

Fig. 1. 16S rRNA tree of all GTDB representative strains of species of the genus *Lactiplantibacillus* and the outgroup L*evilactobacillus brevis*. Note: the species *Lactiplantibacillus argentoratensis* is represented by *L. plantarum*, since the ANI is higher than 94%. All GTDB representative strains (Table 1) were downloaded from the GTDB [24]. 16S rRNA genes were extracted from the genomes using Barrnap (github.com/tseemann/barrnap) and aligned using MAFFT [27]. A maximum likelihood tree was reconstructed with IQ-TREE with the GTR+F substitution model [28]. The tree was rooted with tidygenomes [29] and visualised with ggtree [30].

Carbon-source utilisation

Carbon-source utilisation abilities of *L. carotarum* AMBF275^T and *L. xiangfangensis* LMG 26013^T were determined using the API 50 CH system test (bioMérieux, France). Colonies of each strain were inoculated onto MRS agar and grown overnight, and then some colonies were inoculated in the API 50 CHL medium. The strips were incubated at 37 °C and were read after 48 h and results are shown in Table 2. *L. carotarum* AMBF275^T was found to be able to ferment several carbohydrates that *L. xiangfangensis* LMG 26013^T and *L. garii* FI11369^T could not, including glycerol, erythritol, D- and L-arabinose, D-ribose, L-xylose, D-adonitol, L-sorbose, dulcitol, inositol, methyl α-D-mannopyranoside, methyl α-D-glucoside, melibiose, inulin, glycogen, xylitol, turanose, D-lyxose and potassium gluconate. *L. carotarum* AMBF275^T and *L. xiangfangensis* LMG 26013^T were able to ferment four carbohydrates that *L. garii* FI11369^T could not, L-rhamnose, lactose, melezitose and starch. D-xylose, D-mannitol and D-sorbitol could be metabolised by *L. carotarum* AMBF275^T and *L. garii* FI11369^T but not by *L. xiangfangensis* LMG 26013^T. D-tagatose was only fermented by *L. xiangfangensis* LMG 26013^T and aesculin ferric citrate was only fermented by *L. garii* FI11369^T. All three species were able to ferment D-galactose, D-glucose, D-fructose, D-mannose, *N*-acetylglucosamine, amygdalin, arbutin, salicin, cellobiose, maltose, sucrose, trehalose and gentiobiose. The ability of *L. carotarum* AMBF275^T to ferment D-ribose, L-arabinose and D-arabinose, as well as the presence of all the necessary genes for the Emden–Meyerhof pathway [35], indicates that it is a facultatively heterofermentative strain.

Fatty acid analysis

To obtain the cellular fatty acid composition (Table 3), standard protocols were followed as described in the MIDI Microbial Identification System [36] and performed by the Spanish Type Culture Collection (CECT). The cellular fatty acid content was

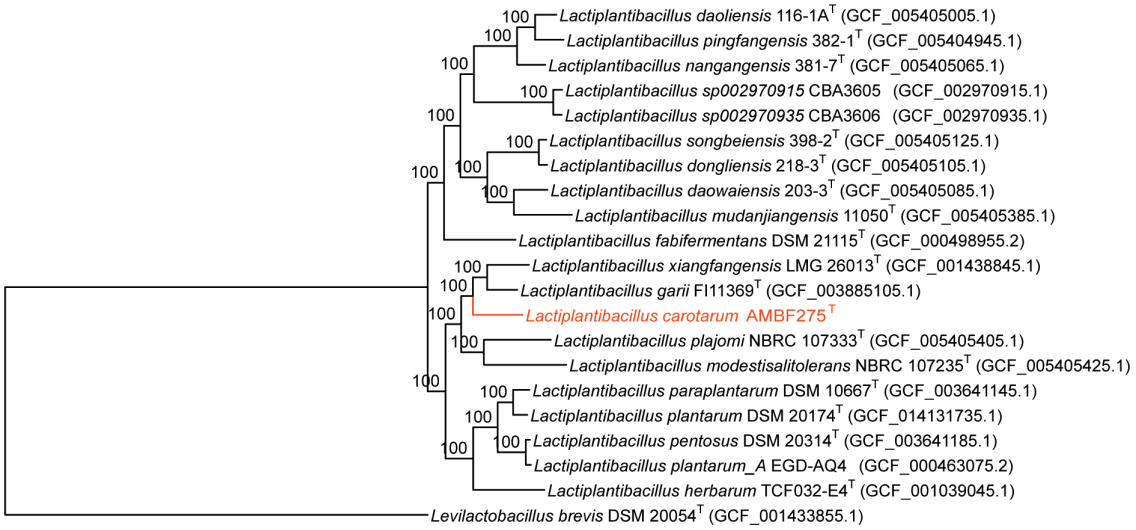

Fig. 2. Core genome phylogenetic tree of the genus *Lactiplantibacillus* with the outgroup *Levilactobacillus brevis* based on all single copy core genes of members of the family *Lactobacillaceae*. All GTDB reference strains of members of this family were downloaded [24] and genes were predicted with Prodigal [25]. Note: the species *L. argentoratensis* is represented by *L. plantarum*, since the ANI is higher than 94%. Core genes were determined, aligned and concatenated with SCARAP [26]. 410 core genes from all representatives were aligned using MAFFT [27] and a maximum likelihood tree was reconstructed with IQ-TREE with the LG+F+G4 amino acid substitution model [28]. This phylogenetic tree was subsetted to include the genus *Lactiplantibacillus* and the outgroup *Levilactobacillus brevis*. The tree was rooted with tidygenomes [29] and visualised with ggtree [30].

analysed using gas chromatography with a 6850 chromatographic unit (Agilent, United States of America), following the MIDI Microbial Identification System's TSBA6 method. The results were then identified with the Microbial Identification Sherlock software package [37]. Prior to analysis, *L. carotarum* AMBF275^T was incubated on MRS medium at 30 °C for 48 h. The unsaturated fatty acid $C_{18:1}\omega 9c$ was the most predominant, followed by the saturated $C_{16:0}$. *L. carotarum* AMBF275^T had a slightly higher relative abundance of saturated fatty acid $C_{14:0}$ compared with the two most closely related species, *L. garii* FI11369^T and *L. xiangfangensis* DSM 27103^T

Proposal of *Lactiplantibacillus carotarum* sp. nov

On the basis of the low average nucleotide identity (ANI) value between *L carotarum* AMBF275^T and *Lactiplantibacillus garii* FI11369^T, the most similar reference strain of a known species, we propose that strain AMBF275^T represents a novel species. The proposed name for this novel species is *Lactiplantibacillus carotarum* sp. nov.

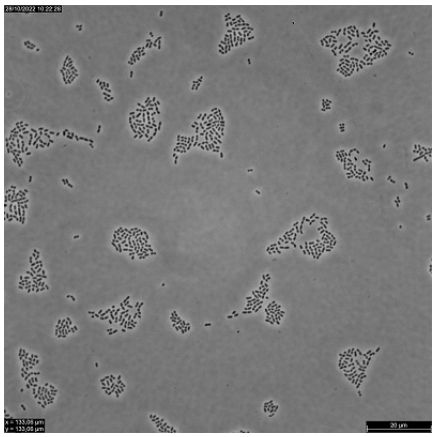

Fig. 3. Light microscope picture (DMi8, Leica) at a magnification of 1000× showing AMBF275^T bacteria grown for 24 h at 37 °C in MRS media.

Table 2. Overview of the ability of carbon source utilisation after 48 h of (1) *L. carotarum* AMBF275^T, (2) *L. xiangfangensis* LMG 26013^T (data from this study) and (3) *L. garii* FI11369^T [38] measured using the API (BioMérieux) assay which shows acidification of the medium based on a colour reaction where yellow corresponds with complete acidification (+), green with partial acidification (+/−), and blue with no growth on this carbon source (−)

		1	2	3			1	2	3
0	Control	−	−	−	25	Aesculin ferric citrate	−	−	+
1	Glycerol	+/−	−	−	26	Salicin	+	+	+
2	Erythritol	+/−	−	−	27	Cellobiose	+	+	+
3	D-arabinose	+/−	−	−	28	Maltose	+	+	+
4	L-arabinose	+	−	−	29	Lactose	+	+	−
5	D-ribose	+	−	−	30	Melibiose	+	−	−
6	D-xylose	+	−	+/−	31	Sucrose	+	+	+
7	L-xylose	+/−	−	−	32	Trehalose	+	+	+
8	D-adonitol	+/−	−	−	33	Inulin	+	−	−
9	methyl β-D-xylopyranoside	−	−	−	34	Melezitose	+	+	−
10	D-galactose	+	+	+/−	35	Raffinose	−	−	−
11	D-glucose	+	+	+	36	Starch	+/−	+	−
12	D-fructose	+	+	+	37	Glycogen	+/−	−	−
13	D-mannose	+	+	+	38	Xylitol	+/−	−	−
14	L-sorbose	+/−	−	−	39	Gentiobiose	+/−	+	+
15	L-rhamnose	+	+/−	−	40	Turanose	+	−	−
16	Dulcitol	+/−	−	−	41	D-lyxose	+/−	−	−
17	Inositol	+/−	−	−	42	D-tagatose	−	+	−
18	D-mannitol	+	−	+	43	D-fucose	−	−	−
19	D-sorbitol	+	−	+	44	L-fucose	−	−	−
20	Methyl α-D-mannopyranoside	+	−	−	45	D-arabitol	−	−	−
21	Methyl α-D-glucoside	+	−	−	46	L-arabitol	−	−	−
22	*N*-acetylglucosamine	+	+	+	47	Potassium gluconate	+/−	−	−
23	Amygdalin	+	+	+	48	Potassium 2-keto-gluconate	−	−	−
24	Arbutin	+	+/−	+	49	Potassium 5-keto-gluconate	−	−	−

Description of *Lactiplantibacillus carotarum* sp. nov

Lactiplantibacillus carotarum (ca.ro.ta'rum. L. gen. pl. n. *carotarum*, of carrots)

Bacteria from the species *Lactiplantibacillus carotarum* are facultatively heterofermentative, Gram-positive, non-motile, non-spore forming, rod-shaped organisms that can form long chains. Able to grow in MRS media both aerobically and anaerobically, at a temperature range of 17–45 °C with an optimal temperature of 30 °C. Can also grow at any pH between 3.5 and 9.5 with an optimal pH of 7 and in salt concentrations ranging from 0 to 8%, with an optimal salt concentration of 0%. When grown on MRS agar, forms round, pulvinate, entire, shiny, white colonies. Able to ferment a wide range of carbohydrates, including L-arabinose, D-ribose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, D-mannitol, D-sorbitol, methyl α-D-mannopyroside, methyl α-D-glucoside, *N*-acetylglucosamine, amygdalin, arbutin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, inulin, melezitose and turanose. In addition, also able to weakly ferment glycerol, erythritol, D-arabinose, L-xylose, D-adonitol, L-sorbose, dulcitol, inositol, starch, glycogen, xylitol, gentiobiose, D-lyxose and gluconate. Unable to ferment a number of other compounds including methyl β-xylopyranoside, aesculin ferric citrate, raffinose, D-tagatose, D- and L-fucose, D- and L-arabitol, 2-ketogluconate, and 5-ketogluconate. Can be differentiated from the two most closely related species as it is more versatile in carbon source usage, for example the metabolisation of glycerol, erythritol, D- and L-arabinose, D-ribose,

Table 3. The relative fatty acid content (percentages) of AMBF275^T (1) and the closely related species *Lactiplantibacillus xiangfangensis* DSM 27103^T (2) and *Lactiplantibacillus garii* FI11369^T [3, 38]. Some fatty acids were not detected and are indicated with a dash "–"

Fatty acid	1	*2*	*3*
Saturated:			
$C_{9:0}$	–	–	
$C_{12:0}$	–	–	
$C_{14:0}$	4.1	0.7	0.8
$C_{16:0}$	15.1	18.2	20.0
$C_{18:0}$	3.3	2.9	2.5
$C_{19:0}$ iso	–	2.0	–
Unsaturated:			
$C_{18:1}\omega9c$	45.6	43.8	28.1
Ambiguous peaks:			
$C_{16:1}\omega7c/C_{16:1}\omega6c$	2.0	1.1	0.9
$C_{18:1}\omega7c/C_{18:1}\omega6c$	5.7	8.8	6.9
$C_{19:0}$cyclo $\omega10c/C_{19:1}\omega6c$	24.3	22.5	40.8

L-xylose, D-adonitol, L-sorbose, dulcitol, inositol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, melibiose, inulin, glycogen, xylitol, turanose, D-lyxose and gluconate. The peptidoglycan-layer is linked with *meso*-2,6-diaminopimelic acid.

The type strain, AMBF275^T (=LMG 32885^T, =CECT 30757^T), was isolated from a spontaneous carrot juice fermentation. The genome size of the type strain is 3.45 Mbp and the DNA G+C content is 47.56 mol%. The EMBL-EBI accession numbers of the 16S rRNA gene and the genome sequence are OX442439 and SAMEA112195921, respectively.

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Author contributions
T.E.: conceptualisation, investigation, writing – original draft, review and editing, project administration, visualisation. J.D.: Nanopore sequencing, writing – review and editing. N.V.D.V: writing – editing, practical work. S.W.: providing pangenome tools, supervision, methodology, writing – review and editing. S.L.: conceptualisation, funding acquisition, resources, supervision, writing – review and editing.

Conflicts of interest
No conflict related to this work. S.L. is an academic board member of ISAPP (https://isappscience.org) and chairperson and cofounder of YUN (yun.be), but these organisations were not involved in this work.

Reference

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