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Polyphasic characterization of two novel *Lactobacillus* spp. isolated from blown salami packages: Description of *Lactobacillus halodurans* sp. nov. and *Lactobacillus salsicarnum* sp. nov.

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ABSTRACT

Microbiota analysis of blown pack spoiled salami revealed five distinguishable *Lactobacillus* isolates we could not assign to a known species. Two of the isolates (TMW 1.2172^T and TMW 1.1920) are rod-shaped, whilst three isolates (TMW 1.2098^T, TMW 1.2118 and TMW 1.2188) appear coccus shaped or as short rods. All isolates are Gram-stain positive, facultative anaerobic, catalase and oxidase negative, non-motile and non-sporulating. Phylogenetic analysis of the 16S rRNA, *dna*K, *phe*S and *rpo*A gene sequences revealed two distinct lineages within the genus *Lactobacillus (L.)*. The isolates are members of the *Lactobacillus alimentarius* group with *Lactobacillus ginsenosidimutans* DSM 24154^T (99.4% 16S similarity*)*, *Lactobacillus versmoldensis* DSM 14857^T (97.9%) and *Lactobacillus furfuricola* DSM 27174^T (97.7%) as phylogenetic closest related species and *L. alimentarius* DSM 20249^T (97.7%) and *Lactobacillus paralimentarius* DSM 13961^T (97.5%) as closest relatives, respectively. Average Nucleotide Identity (ANI) and digital DNA-DNA hybridization (dDDH) values between the isolates and their close related type strains are lower than 80% and 25%, respectively. For both designated type strains, the peptidoglycan type is A4α L-Lys-D-Asp and the major fatty acids are $C_{16:0}$, $C_{18:1\omega9c}$ and summed feature 7. Based on phylogenetic, phenotypic and chemotaxonomic analysis we demonstrated that the investigated isolates belong to two novel *Lactobacillus* species for which we propose the names *Lactobacillus salsicarnum* with the type strain TMW 1.2098^T = DSM 109451^T = LMG 31401^T *and Lactobacillus halodurans* with the type strain TMW 1.2172^T = DSM 109452^T = LMG 31402^T.

Introduction

The genus *Lactobacillus* consists currently of more than 250 validly described species including a large number of organisms, which are important in food microbiology and human nutrition [17]. Amongst others, *L.* strains are used as starter cultures for the ripening process of raw fermented sausages like salami, which is important to maintain a constant, high product quality. Within lactobacilli *Lactobacillus sakei, Lactobacillus curvatus, Lactobacillus plantarum* and *Lactobacillus pentosus* have shown to possess the best properties for this purpose [1,6]. In addition to technological parameters, the ability to assert itself against the autochthonous microbiota of meat is an essential characteristic of a good starter culture. Selected strains and/or mixtures thereof are usually distributed as commercial salami starter cultures. In industrial production pre-sliced dry fermented meat products are commonly sold as modified atmosphere packages with an increased shelf life. However, during extended storage periods and/or suboptimal storage conditions e.g. an increased storage temperature after final ripening of the product, the microbiota may shift and other than starter microorganisms can become metabolically active. A typical form of spoilage is excessive gas formation leading to unintended blowing of the package. In this study we describe the novel species *Lactobacillus salsicarnum* sp. nov. and *Lactobacillus halodurans* sp. nov, which were isolated from blown salami packages and characterized on a polyphasic approach using phylogenetic, phenotypic and chemo-taxonomic methods.

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Material and methods

Bacterial strains, media and culture conditions

Different packages with pre-sliced pork meat salami (200 g content, produced by different manufacturers in Germany) were bought in local food markets and stored 3–4 weeks beyond the indicated expiration date. The microbiota of packages showing visible inflation were analyzed using standard microbiological methods. Colonies grown on MRS agar plates incubated for 48 h at 30 °C under anoxic conditions were analyzed by MALDI-TOF MS performed on a Microflex LT spectrometer (Bruker Corporation, USA). Isolates not identified as typical starter culture species and even not attributable to any known species were selected for further analyses. Five isolates were added to the strain collection (TMW) and designated TMW 1.1920 from producer A, TMW 1.2098^T and TMW 1.2118 from producer B, TMW 1.2172^T and TMW 1.2188 from producer C. Reference strains were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ) and included *L. ginsenosidimutans* DSM 24154^T, *L. versmoldensis* DSM 14857^T, *L. furfuricola* DSM 27174^T, *L. alimentarius* DSM 20249^T and *L. paralimentarius* DSM 13961^T. Bacteria were grown at 30 °C under anaerobic conditions using MRS-agar or -broth [4]. Short term storage was carried out at 4 °C (up to 14 days), long term storage at −80 °C, using 50% glycerol as cryoprotectant.

Phenotypic characterization

Gram staining, catalase and oxidase activities as well as motility were investigated using standard methods [3]. Growth characteristics were determined after 7 days under the following conditions: Temperature: 6 °C, 10 °C, 15 °C, 20 °C, 30 °C, 37 °C, 42 °C and 45 °C; pH: 3, 3.5, 4, 4.5, 5, (buffer system: 0.1 M citric acid/0.1 M sodium citrate) 6, 7, 8, 8.5 (buffer system: 0.1 M KH_2PO_4/0.1 M NaOH) and 9 (buffer system: 0.1 M $NaHCO_3$/0.1 M Na_2CO_3); NaCl: 2%, 4%, 6%, 8% 10%, 12%, 14% and 16% (w/v). API 50 CH (Biomerieux®, France) was used to evaluate carbohydrate fermentation (incubation: 30 °C for 48 h, anaerobic), API ZYM (Biomerieux®, France) was used to determine enzymatic activity (incubation: 30 °C for 5 h). D-Lactate and L-lactate were assessed enzymatically (Megazyme International, Ireland).

DNA extraction and genome sequencing

DNA was extracted from 2 ml aliquots (overnight cultures) using the E.N.Z.A® Bacterial DNA Kit (Omega bio-tek, USA). DNA quantity and quality were evaluated spectrophotometric (NanoDrop®, ThermoFisher Scientific, USA) and by gel electrophoresis. Whole genome shotgun sequencing (WGS) was carried out by BayBioMS (Freising, Germany) using Illumina MiSeq technology (Illumina, USA). Reads were assembled using SPAdes 3.0 [2]. WGS sequences were annotated using the NCBI Prokaryotic Genomes Automatic Annotation Pipeline and deposed in the NCBI public database under the GenBank Accession No's. VDFL00000000, VDFM00000000, VDFN00000000, VDFO00000000 and VDFP00000000.

RAPD-fingerprinting

RAPD PCR was carried out in 50 μl reaction volume, containing 10–200 ng DNA, 1× reaction mix, 5 mM MgCl, 200 μM dNTPs, 0.5 μM Primer M13V (5′-GTT TTC CCA GTC ACG AC-3′) and 2 U TAQ-polymerase (all PCR reagents from Thermo Fisher Scientific, USA). Electrophoresis was carried out at 80 V for 2.5 h using 15 μl PCR product, loaded on a 1.4% agarose gel. After electrophoresis RAPD patterns were visualized using dimidium bromide staining and UV-light.

Phylogenetic analysis

Almost complete 16S rRNA gene sequences (>1400 bp) and partial *dnaK* (544 bp), *pheS* (369 bp) and *rpoA* (590 bp) sequences were either retrieved from the NCBI nucleotide collection (reference type strains) or from the genome sequences (reference type strains and TMW isolates). 16S rRNA gene sequences of the TMW isolates were determined by Sanger sequencing (Eurofins, Martinsried, DE) using the primers 27F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1507R (5′-TAC CTT GTT ACG ACT TCA CCC CAG-3′) for both, PCR and sequencing. The Sequences were deposed in the NCBI Nucleotide collection (GenBank accession-numbers: MK968445-MK968449). Sequence alignment and phylogenetic tree construction was performed on the MEGA 7.0 software [10] using the minimum evolution-, maximum likelihood- and neighbor joining method. ANI values were calculated using the JSpeciesWS online tool [16]. dDDH values were determined using the DSMZ Genome to Genome Distance Calculator (GGDC) [13].

Cellular fatty acid composition and analysis of the peptidoglycan structure

Analysis of the cellular fatty acid composition was carried out as described previously [11,14] with minor modifications using a Sherlock MIS (MIDI Inc, Newark, USA) system. Analysis of the peptidoglycan structure was performed according to established protocols [18]. Both analyses were carried out by the DSMZ identification service.

Scanning electron microscopy

For scanning electron microscopy (SEM), cells were picked from MRS-Agar plates and resuspended in a small amount of fixation buffer containing 2.5% glutaraldehyde in 75 mM cacodylate buffer including 2 mM $MgCl_2$ (pH 7.0). These cells were then applied to a glass slide, covered with a cover slip and frozen in liquid nitrogen. After detaching the cover slip, the glass slide was further fixed in fixation buffer over night. After five washing steps with buffer (5, 25, 45, 50, 120 min), the cells were post-fixed with 0.2% OsO_4 in buffer for 30 min. This was followed by another washing step with buffer (15 min) and 3 washing steps with double distilled water (10, 15, 25 min). After dehydration in a graded acetone series, the cells were critical-point-dried, mounted on aluminum stubs and sputter-coated with platinum. Scanning electron microscopy was performed on a Zeiss Auriga dual-beam microscope (Zeiss Oberkochen, Germany) at an acceleration voltage of 2 kV.

Results and discussion

Based on the analysis of the 16S rRNA gene sequences the strains TMW 1.2098^T, TMW 1.2118 and TMW 1.2188 were identical among each other, but different to the type strains of *L. ginsenosidimutans* (99.4% similarity), *L. versmoldensis* (97.9%) and *L. furfuricola* (97.7%) as closest related neighbors. Equally the strains TMW 1.1920 and TMW 1.2172^T share identical 16S gene sequences, but were different to *L. alimentarius* (97.7%) and *L. paralimentarius* (97.5%) as closest related species. Therefore, all isolates are placed within the *L. alimentarius* group [5,17]. The Phylogenetic relationship was investigated using the minimal evolution method (Fig. 1). Phylogenetic positions were confirmed using neighbor joining method (Supplementary Fig. S1) and the maximum likelihood method (Supplementary Fig. S2). Since at least two additional phylogenetic markers are recommended for precise species identification [12],

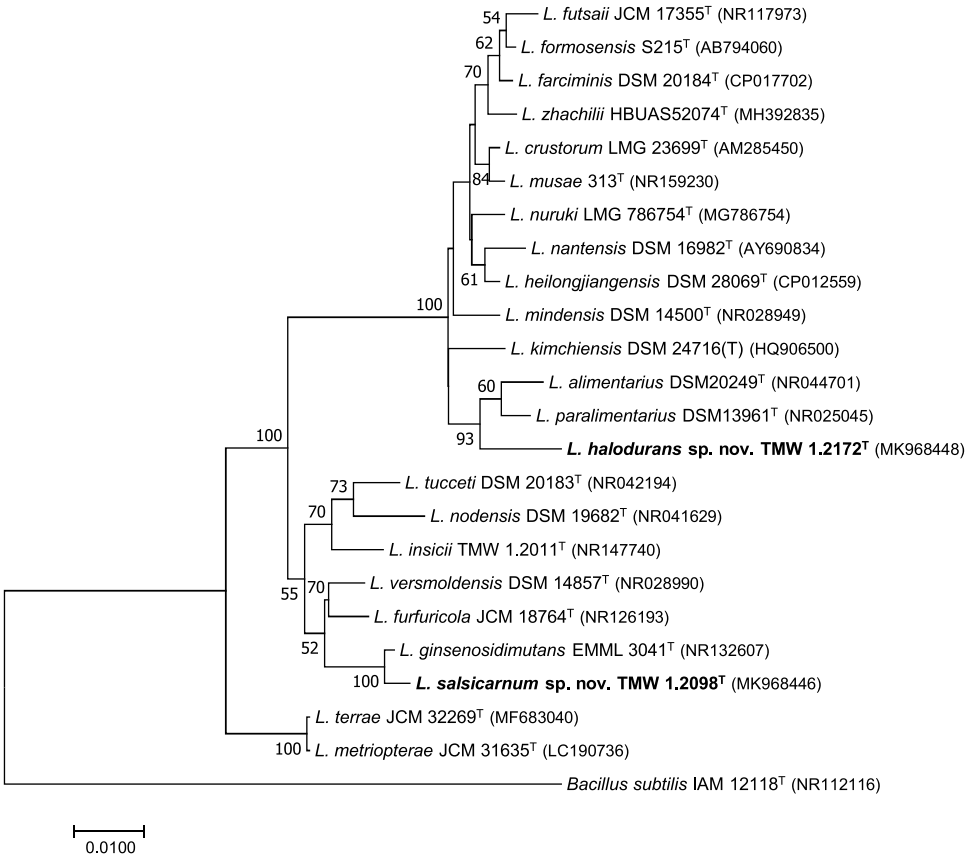

Fig. 1. Phylogenetic tree based on almost complete 16S rRNA gene sequences (>1400 bp) of novel isolates and closely related type strains, using *Bacillus subtilis* as outgroup. GenBank accession numbers are shown in brackets. The tree was constructed by the minimum evolution method. Bootstrap values >50%, based on 100 replications, are shown at branch nodes. Scale bar represents 1% sequence divergence.

Table 1
Sequence similaritys for the 16S rRNA (>1400 nt), dnaK (544 nt), pheS (369 nt) and rpoA (590 nt) genes and the concatenated MLSA sequence (1503 nt) of the novel *Lactobacillus* isolates and their closely related type strains.

Strain	Sequence similarity [%] with *L. salsicarnum* sp. nov. TMW12098^T				
	16S rRNA	dnaK	pheS	rpoA	MLSA
L. salsicarnum sp. nov. TMW12118	100	98.2	99.2	99.7	99.1
L. salsicarnum sp. nov. TMW12188	100	98.5	99.7	99.8	99.3
L. ginsenosidimutans EMML 3041^T	99.4	93.1	84.7	91.8	89.8
L. versmoldensis DSM 14857^T	97.9	90.3	83.3	89.5	88.2
L furfuricola JCM 18764^T	97.7	88.2	85.2	87.2	86.6
Strain	Sequence similarity [%] with *L. halodurans* sp. nov. TMW1.2172^T				
	16S rRNA	dnaK	pheS	rpoA	MLSA
L. halodurans sp. nov. TMW 1.1920	100	100	100	100	100
L. alimentarius DSM 20249^T	97.7	89.2	86.2	94.4	90.3
L. paralimentarius DSM 13961^T	97.5	91.4	86.5	94.4	90.7
L. kimchiensis DSM24716^T	97.1	91.4	85.1	95.1	90.5
L. mindensis DSM14500^T	97.3	89.8	85.9	94.1	89.9

we performed multi locus sequence analyses (MLSA) of the three housekeeping genes *dna*K, *phe*S and *rpo*A, which are commonly used for the identification and description of lactobacilli [7,8,15]. MLSA similarity calculated from 1503 aligned nucleotides, points up the difference between our strains and their closely related type strains (Table 1): MLSA similarity between TMW 1.2098^T and TMW 1.2118, respectively TMW 1.2188 ranges from 99.1% to 99.3%, between TMW 1.2098^T and the close related type strains from 86.6% to 89.8%. MLSA is identical between TMW 1.2172^T and TMW 1.1920 and ranges from 89.9% to 90.7% to closely related type strains. A multi locus sequence tree of the concatenated *dna*K (544 nt), *phe*S (369 nt) and *rpo*A (590 nt) sequences (1503 nucleotides) was constructed using the minimum evolution (Fig. 2), neighbor joining (Supplementary Fig. S3) and maximum likelihood (Supplementary Fig. S4). The topology was recovered in all calculated trees and suggests that our isolates may represent two novel species within the *L. alimentarius*-group.

RAPD-PCR fingerprinting

Since RAPD-PCR provides a fast, reliable and widely accepted method for the detection of genetic variances within and across

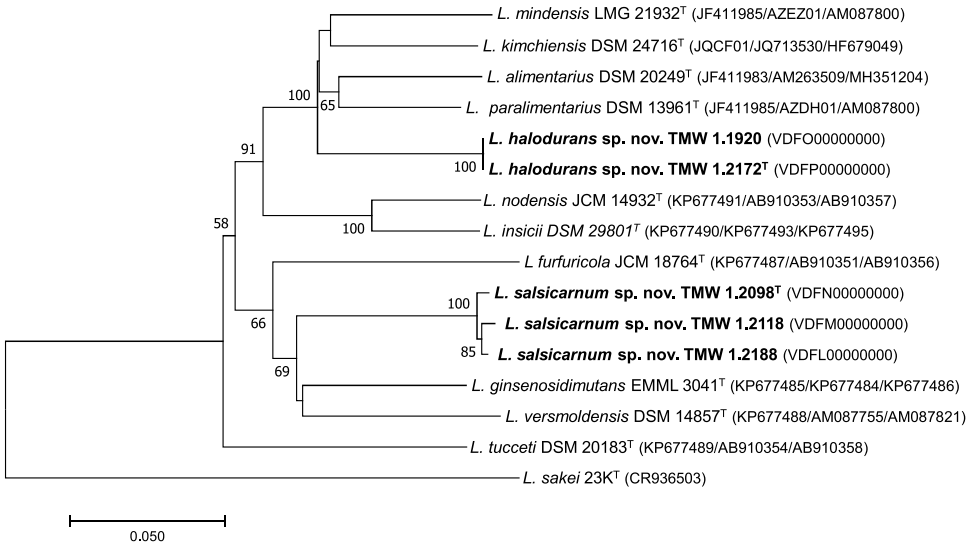

Fig. 2. Phylogenetic tree based on the concatenated partial housekeeping gene sequences *dna*K, *phe*S and *rpo*A of novel isolates and closely related type strains, using *L. sakei* as outgroup. GenBank accession numbers are shown in brackets. The tree was constructed by the minimum evolution method. Bootstrap values >50%, based on 100 replications, are shown at branch nodes. Scale bar represents 5% sequence divergence.

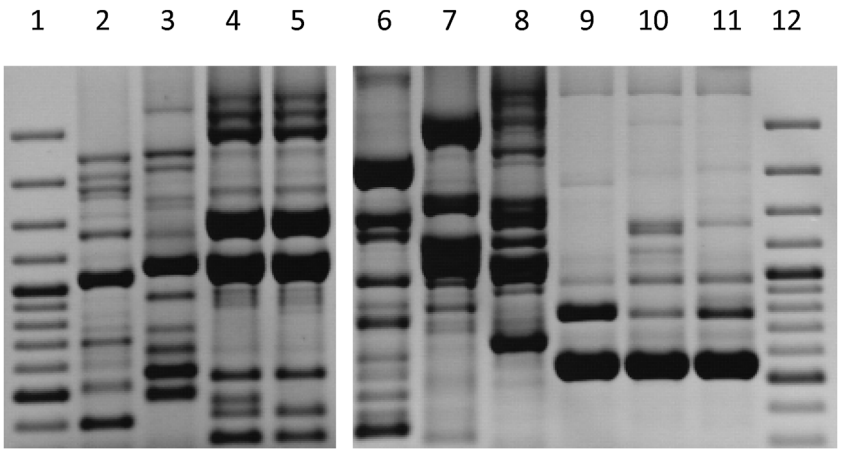

Fig. 3. M13V RAPD-fingerprints of novel isolates and closely related type strains. Lanes: 1 and 12: 100 bp ladder; 2: *L. alimentarius* DSM 20249^T; 3: *L. paralimentarius* DSM 13961^T; 4: *L. halodurans* TMW 1.2172^T; 5: *L. halodurans* TMW 1.1920; 6: *L. ginsenosidimutans* DSM 24154^T; 7: *L. versmoldensis* DSM 14857^T; 8: *L. furfuricola* DSM 27174^T; 9: *L. salsicarnum* TMW 1.2098^T; 10: *L. salsicarnum* TMW 1.2118; 11: *L. salsicarnum* TMW 1.2188.

Table 2
Genomic characteristics of novel *Lactobacillus* strains.

Attribute	TMW 1.2098^T	TMW 1.2118	TMW 1.2188	TMW 1.1920	TMW 1.2172^T
Accession no.	VDFN00000000	VDFM00000000	VDFL00000000	VDFO00000000	VDFP00000000
Genome size (bp)	2,428,248	2,408,828	2,352,088	2,534,442	2,844,764
GC Content (%)	36.6	36.6	36.6	35.8	35.8
Genome coverage	238	165	198	136	117
N50	192,844	120,427	388,963	52,763	50,257
No. of contigs	35	38	17	91	306
Total genes	2350	2327	2239	2469	2878
No. of CDS[a]	2293	2273	2183	2407	2812
Genes (RNA)	57	54	56	62	66
rRNAs (5S, 16S, 23S)	2, 2, 2	2, 2, 1	3, 1, 1	5, 1, 2	5, 3, 3
Complete	2, 1 (5S, 16S)	2, 1 (5S, 23S)	3, 1, 1 (5S, 16S, 23S)	5 (5S)	5, 1 (5S, 16S)
Partial	1, 2 (16S, 23S)	2 (16S)	–	1, 2 (16S, 23S)	2, 3 (16S, 23S)
tRNAs	48	46	48	51	52
ncRNAs[b]	3	3	3	3	3
Pseudogenes	29	19	17	48	98

[a] CDS: coding sequence.
[b] ncRNAs: noncoding RNAs.

Table 3
Average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) values between novel *Lactobacillus* isolates and their closely related type strains.

	Strain	Accession-no.	1	2	3	4	5	6	7	8	9	10	11
1	*L. salsicarnum* sp. nov. TMW 1.2098^T	VDFN00000000	100										
2	*L. salsicarnum* sp. nov. TMW1.2118	VDFM00000000	**97.2/77.7**	100									
3	*L. salsicarnum* sp. nov. TMW12188	VDFL00000000	**97.0/76.4**	**97.0/76.3**	100								
4	*L. ginsenosidimutans* EMML 3041^T	CP012034	76.7/21.1	76.2/20.5	76.5/21.0	100							
5	*L. versmoldensis* DSM 14857^T	AZFA01	74.5/19.7	74.3/19.6	74.2/19.5	76.0/22.2	100						
6	*L furfuricola* JCM 18764^T	RHNT01	74.4/19.8	74.2/19.5	74.3/19.6	76.1/20.9	76.1/20.70	100					
7	*L. halodurans* sp. nov. TMW 1.2172^T	VDFP00000000	72.8/n.d.	72.5/n.d.	72.3/n.d.	72.3/n.d.	72.6/n.d.	72.29/n.d.	100				
8	*L. halodurans* sp. nov. TMW 1.1920	VDFO00000000	72.7/n.d.	72.4/n.d.	72.3/n.d.	72.1/n.d.	72.3/n.d.	72.26/n.d.	**99.67/97.2**	100			
9	*L. alimentarius* DSM 20249^T	CP018867	72.4/n.d.	72.4/n.d.	72.2/n.d.	72.2/n.d.	72.2/n.d.	71.92/n.d.	77.6/22.3	77.7/22.2	100		
10	*L. paralimentarius* DSM 13961^T	AZDH01	72.4/n.d.	72.5/n.d.	72.3/n.d.	72.3/n.d.	72.38/n.d.	71.9/n.d.	77.6/22.3	77.7/22.3	80.4/24.2	100	
11	*L. kimchiensis* DSM 24716^T	JQCF01	72.5/n.d.	72.6/n.d.	72.3/n.d.	72.3/n.d.	72.06/n.d.	72.0/n.d.	78.5/22.9	78.5/22.8	77.8/22.1	77.7/22.5	100
12	*L. mindensis* DSM 14500^T	AZEZ01	72.3/n.d.	72.3/n.d.	72.2/n.d.	71.9/n.d.	72.21/n.d.	72.1/n.d.	78.1/22.8	78.1/23.0	77.3/21.8	77.3/22.0	79.9/24.5

Bold: Values >95% (ANI), respectively >70% (dDDH).
n.d.: not determined.

Table 4
Differential phenotypic characteristics of the novel *Lactobacillus* strains and their closely related type strains.

Strain	1	2	3	4	5	6	7	8	9	10
Growth at/with										
10 °C	+	+	+	+	+	–	w	+	–	+
37 °C	+	+	+	+	–	+	+	+	+	+
42 °C	–	–	–	–	–	–	–	–	–	w
pH 4.0	+	–	+	–	–	+	+	+	+	+
pH 4.5	+	+	+	w	–	+	+	+	+	+
pH 5.0	+	+	+	+	–	+	+	+	+	+
pH 8.5	+	+	+	+	+	+	–	+	+	+
8% NaCl	–	+	+	+	+	+	+	+	+	–
10% NaCl	–	–	–	–	+	+	+	+	+	–
12% NaCl	–	–	–	–	–	–	+	+	–	–
14% NaCl	–	–	–	–	–	–	w	w	–	–
Acid production from:										
D-Ribose	+	+	+	+	+	+	–	–	+	+
D-Lactose	–	–	–	–	–	–	+	+	–	–
Glycerol	–	–	–	+	–	–	–	–	–	–
L-Arabinose	+	+	+	+	–	–	–	–	–	–
D-Galactose	–	–	–	+	+	+	+	+	–	–
D-Mannitol	–	–	+	+	–	–	–	–	–	+
Methyl-αD-glucopyranoside	–	–	–	+	–	–	–	–	–	–
Amygdaline	–	–	–	–	–	–	–	+	+	+
Salicine	w	–	w	+	–	w	+	+	+	+
D-Melibiose	–	–	–	+	+	–	–	–	–	–
D-Saccharose	–	–	–	–	–	–	–	–	+	+
D-Trehalose	+	+	+	–	–	–	–	+	+	+
D-Tagatose	–	–	–	–	–	–	+	+	–	–
Potassium-gluconat	–	–	–	+	–	–	–	–	–	+
Enzymatic activity										
Alkaline phosphatase	+	+	+	+	–	+	–	+	+	–
Esterase	+	+	+	+	+	+	+	w	w	–
α-Galactosidase	–	–	–	+	+	–	–	–	–	–
β-Galactosidase	–	–	–	+	w	–	–	–	+	–
α-Glucosidase	–	–	–	+	+	–	+	+	+	–
β-Glucosidase	+	+	+	+	+	+	+	+	+	–
N-Acetyl-β-glucosaminidase	–	–	–	–	–	–	+	+	+	–

+: positive reaction; –: negative reaction; w: weak reaction.
Strains: 1: *L. salsicarnum* sp. nov. TMW 1.2098^T; 2: *L. salsicarnum* sp. nov. TMW 1.2118; 3: *L. salsicarnum* sp. nov. TMW 1.2188; 4: *L. ginsenosidimutans* DSM24154^T; 5: *L. versmoldensis* DSM14857^T; 6: *L. furfuricola* DSM27174; 7: *L. halodurans* sp. nov. TMW 1.2172^T; 8: *L. halodurans* sp. nov. TMW 1.1920; 9: *L. alimentarius* DSM 20249^T; 10: *L. paralimentarius* DSM 13961^T.

bacterial species, we applied M13V RAPD-PCR to investigate the genetic variance within our isolates and the genetic difference to their closely related type strains. We observed a clear discrimination of our isolates and their closely related type strains as well as an intra species diversity within the isolates TMW 1.2098^T, TMW 1.2118 and TMW 1.2188 (Fig. 3). Although RAPD-patterns of the isolates TMW 1.2172^T and TMW 1.1920 are quite similar, one band distinguished the two strains reproducible amongst different users in our lab. Moreover, various phenotypic characteristics were found to vary between the two isolates (chapter 'Phenotypic and chemotaxonomic characterization'). Therefore, M13V RAPD PCR and phenotypic characterization demonstrated that all of our isolates are non-clonal varieties, which are easy distinguishable to closely related type strains.

Genome based taxonomic relationships

The draft genome sequences of the strains TMW 1.2098^T, TMW 1.2118 and TMW 1.2188 range from 2.35 Mb to 2.43 Mb in size with 36.6% GC content. The draft genome sequences of the strains TMW 1.2172^T and TMW 1.1920 are 2.85 Mb, respectively 2.53 Mb in size with 35.8% GC content (Table 2). ANI and dDDH values between the strains TMW 1.2098^T, TMW 1.2118 and TMW 1.2188 ranges from 97.0% to 97.2%, respectively from 76.3% to 77.7%, between TMW 1.2098^T and close related type strains from 74.4% to 76.7%, respectively from 19.7% to 21.1%. The ANI and dDDH value between the strains TMW 1.2172^T and TMW 1.1920 is 99.7%, respectively 97.2%. To the closely related type strains ANI and dDDH values range from 77.6% to 78.5%, respectively 22.3% to 22.9% (Table 3). In both cases ANI and dDDH values between the designated type strains and the closest related type strains are well below the thresholds of 95–96% (ANI) and 70% (dDDH) previously proposed for the delineation of bacterial species [9,19]. These findings confirm that our isolates represent two novel species within the genus *Lactobacillus.*

Phenotypic and chemotaxonomic characterization

After two days of growth (30 °C, MRS-Agar) colonies of are 1–2 mm in diameter for *L. salsicarnum* sp. nov. and 3–5 mm in diameter for *L. halodurans* sp. nov., respectively. The colonies are circular, smooth, flat and greyish–white to pale yellow for both species. Cells of *L. salsicarnum* sp. nov. appear coccus shaped or as short rods (about 0.5–1 μm × 0.5–2 μm in size), whilst *L. halodurans* sp. nov. cells show a classical rod shaped morphology (about 0.5–1 μm × 2–5 μm in size (Fig. 4).

Various phenotypic characteristics distinguishing the two novel species from their close related type strains have been observed. Moreover, some phenotypic characteristics have been observed as variable within the novel species (Table 4). Major fatty acids (>10%) for TMW 1.2098^T and TMW 1.2172 were $C_{16:0}$ (23.1%; 16.1%), $C_{18:1}$ ω9c (26.5%; 56.7%) and summed feature 7 (consists of unknown constituent with equivalent chain length of 18.846/$C_{19:1}$ ω6c and cyclo-$C_{19:0}$ ω10c/19ω6) (36.4%; 12.4%) (Table 5). For both novel species the peptidoglycan type was determined as A4α L-Lys-D-Asp, which is the main peptidoglycan type in the *L. alimentarius* group [17]. D- and L-Lactate are produced from both type strains

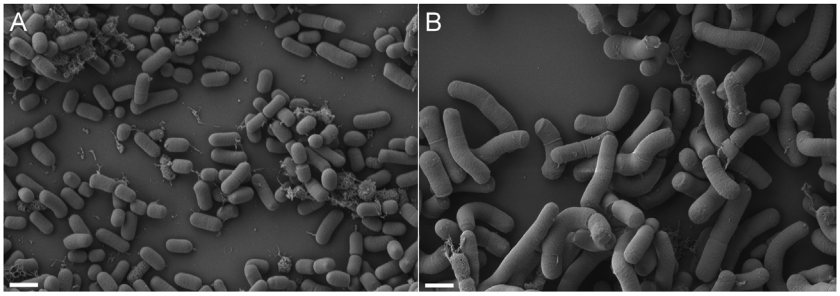

Fig. 4. Scanning electron micrograph of cells depicting the cell morphology of (A): *Lactobacillus salsicarnum* sp. nov. TMW 1.2098^T (= DSM 109451^T), the irregular small and coccoid shape of *Lactobacillus salsicarnum* is clearly visible and (B): *Lactobacillus halodurans* sp. nov. TMW 1.2172^T (= DSM 109452^T); scale bars indicate 1 μm.

Table 5
Cellular fatty acid compositions (%) of the novel type strains.

Fatty acid	TMW 1.2098^T	TMW 1.2172^T
$C_{14:0}$	0.98	0.61
$C_{16:0}$	23.10	16.10
$C_{18:1\omega9c}$	26.54	56.66
$C_{18:0}$	2.55	2.25
Iso-$C_{19:0}$	1.37	1.80
Summed features*		
3	1.38	1.31
7	36.35	12.41
8	7.17	8.49

Values are expressed as percentage of total fatty acids, values <0.5% are not shown.
*Summed feature 3 consisted of $C_{16:1}$ ω6c and $C_{16:1}$ ω7c.
*Summed feature 7 consisted of unknown constituent with equivalent chain length of 18.846/$C_{19:1}$ ω6c and cyclo-$C_{19:0}$ ω10c/19ω6.
*Summed feature 8 consisted of $C_{18:1}$ ω6c and $C_{18:1}$ ω7c.

at a D:L ratio of about 1:3 (TMW 1.2098^T) and 1:1 (TMW 1.2172^T), respectively.

Conclusion

Based on the results obtained from the phenotypic and chemotaxonomic characterization as well as from the genomic and phylogenetic analysis, we conclude that strains TMW 1.2089^T, TMW 1.2118, TMW 1.2188, and strains TMW 1.2172^T and TMW 1.1920 represent two novel species within the *L. alimentarius* group, for which we propose the names *L. salsicarnum* sp. nov. with TMW 1.2098^T (= DSM 109451^T = LMG 31401^T) as type strain and *L. halodurans* sp. nov. with TMW 1.2172^T (= DSM 109452^T = LMG 31402^T) as type strain.

Description of L. salsicarnum *sp. nov.*

L. salsicarnum (sal.si.carnúm. L. adj. salsus, salted; L. gen. n. carnis of meat; N.L. gen. n. salsicarnum of salted meat, referring to the source of isolation).The isolates are gram-stain positive, facultative anaerobic, catalase and oxidase negative, non-motile and non-sporulating. The cells are coccoid or short rods, 0.5–1.0 μm × 0.5–2 μm in size and appear as single cells or arranged in pairs or short chains. After two days of growth (MRS-Agar, 30 °C) colonies are about 1–2 mm in diameter, circular, smooth, flat and greyish–white to pale yellow. No gas is produced from glucose. Both L- and D-lactate are produced from glucose at a D:L ratio of about 1:3. On MRS-agar growth occurs at 10 °C–37 °C, but not at 6 °C or 42 °C. Growth occurs at pH 4.5–pH 8.5. For pH 4 growth is variable and no growth occurs at pH 3.5 or pH 9. Growth occurs in the presence of 0–6.0% NaCl and is variable in the presence of 8.0% NaCl. No growth occurs in the presence of 10.0% NaCl. Acid is produced from L-Arabinose, D-ribose, D-glucose, D-fructose, D-mannose, *N*-acetylglucosamine, D-maltose and D-trehalose. Acid production from D-mannitol and salicin is variable and no acid is produced from glycerol, erythritol, D-arabinose, D- and L-xylose, D-adonitol, methyl-β-D-xylopyranoside, D-galactose, L-sorbose, L-rhamnose, dulcitol, inositol, D-sorbitol, methyl-αD-mannopyranoside, methyl-αD-glucopyranoside, amygdaline, arbutin, D-cellobiose, D-lactose, D-melibiose, D-saccharose, Inulin, D-melezitose, D-raffinose, starch, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D- and L-fucose, D- and L-arabitol, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate. Alkaline phosphatase, esterase, lipase (C 8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphtol-AS-BI-phosphohydrolase and β-glucosidase are produced. The peptidoglycan type is A4α L-Lys-D-Asp and the major fatty acids (>10%) are $C_{16:0}$, $C_{18:1\omega9c}$ and summed feature 7. The DNA GC content of the type strain is 35.8%. The type strain is TMW 1.2098^T (= DSM 109451^T = LMG 31401^T) isolated from salami produced in Germany in 2018. Digital Protologue Taxonumber: TA00982.

Description of L. halodurans *sp. nov.*

L. halodurans (ha.lo.du′rans. Gr. n. hals, halos salt; L. pres. part. durans enduring; N.L. part. adj. halodurans salt-enduring, resisting), the name refers to the high salt tolerance of up to 14% NaCl.

The isolates are gram-stain positive, facultative anaerobic, catalase and oxidase negative, non-motile and non-sporulating. The cells are rod shaped, 0.5–1 μm × 2–5 μm in size, occuring as single cells or in pairs. After two days of growth (MRS-Agar, 30 °C) colonies are about 2–5 mm in diameter, circular, smooth, flat and greyish–white to pale yellow. No gas is produced from glucose. Both, L- and D-lactate are produced from glucose at a D:L ratio of 1:1. On MRS-agar growth occurs at 10 °C–37 °C, but not at 6 °C or 42 °C. Growth occurs at pH 4–pH 8 and is variable at pH 8.5. No growth occurs at pH 3.5 or pH 9. Growth occurs in the presence of 0–12.0% NaCl. Growth is weak in the presence of 14.0% NaCl and no growth occurs in the presence of 16.0% NaCl. Acid is produced from, D-ribose, D-galactose, D-glucose, D-fructose, D-mannose, *N*-acetylglucosamine, arbutin, salicin, D-cellobiose, D-lactose, gentiobiose, and D-tagatose. No acid is produced from glycerol, erythritol, L- and D-arabinose, D- and L-xylose, D-adonitol, methyl-βD-xylopyranoside, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl-αD-mannopyranoside, methyl-αD-glucopyranoside, amygdaline, D-maltose, D-melibiose, D-saccharose, D-trehalose, inulin, D-melezitose, D-raffinose, starch, glycogen, xylitol, D-turanose, D-lyxose, D- and L-fucose, D- and L-arabitol, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate. Esterase, lipase (C 14), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphtol-AS-BI-phosphohydrolase, α-glucosidase, β-glucosidase and *N*-acetyl-β-glucosaminidase are produced. The peptidoglycan type is A4α L-Lys-D-Asp and the major fatty acids (>10%) are $C_{16:0}$, $C_{18:1}$ ω9c and summed feature 7. The DNA GC content of the type strain is 35.8%. The type strain is TMW

1.2172^T (= DSM 109452^T = LMG 31402^T) isolated from salami produced in Germany in 2018. Digital Protologue Taxonumber: TA00983.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.syapm.2019.126023.

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