Molecular analysis of sourdough reveals *Lactobacillus mindensis* sp. nov.

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Genotypic fingerprinting to analyse the bacterial flora of an industrial sourdough revealed a coherent group of strains which could not be associated with a valid species. Comparative 16S rDNA sequence analysis showed that these strains formed a homogeneous cluster distinct from their closest relatives, *Lactobacillus farciminis*, *Lactobacillus alimentarius* and *Lactobacillus kimchii*. To characterize them further, physiological (sugar fermentation, formation of DL-lactate, hydrolysis of arginine, growth temperature, CO₂ production) and chemotaxonomic properties have been determined. The DNA G + C content was 37.5 ± 0.2 mol%. The peptidoglycan was of the lysine-D-iso-asparagine (L-Lys-D-Asp) type. The strains were homofermentative, Gram-positive, catalase-negative, non-spore-forming, non-motile rods. They were found as a major stable component of a rye flour sourdough fermentation. Physiological, biochemical as well as genotypic data suggested them to be a new species of the genus *Lactobacillus*. This was confirmed by DNA–DNA hybridization of genomic DNA, and the name *Lactobacillus mindensis* is proposed. The type strain of this species is DSM 14500^T (=LMG 21508^T).

INTRODUCTION

Amongst bacteria, the lactic acid bacteria (LAB) group plays the obviously most important role in human and animal nutrition and maintenance of health (Hammes & Vogel, 1995; Herrero *et al.*, 1996). From an ecological point of view, food fermentations represent special niches where communities of highly specialized organisms have been established. As in cereal fermentations educts cannot be subjected to heat sterilization, the occurrence of micro-organisms as well as their numbers are strictly dependent on substrates and technological parameters (Salovaara, 1998). With few exceptions, in sourdough fermentation for rye bread production lactobacilli were shown to be mainly responsible for acidification, inhibition of rye amylases, bread volume, texture and nutritional value or increased shelf life and flavour (Vogel *et al.*, 1996, 1999). Depending on the tradition in production parameters of the sourdough, isolates were assigned to the obligately homofermentative species *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* and *Lactobacillus farciminis*, the facultatively heterofermentative *Lactobacillus alimentarius*, *Lactobacillus casei*, *Lactobacillus paralimentarius* and *Lactobacillus plantarum*, and the heterofermentative *Lactobacillus brevis*, *Lactobacillus buchneri*, *Lactobacillus fermentum*, *Lactobacillus fructivorans* and

Lactobacillus sanfranciscensis. The consequent application of molecular techniques led to the identification of many new species.

Both *Lactobacillus pontis* and its phylogenetic relative *Lactobacillus panis* were described as endemic members in batters with an extended fermentation period and higher temperatures (Vogel *et al.*, 1994; Wiese *et al.*, 1996). The most recently described species were *Lactobacillus frumenti* (Müller *et al.*, 2000) and *L. paralimentarius* (Cai *et al.*, 1999).

Recently, we isolated an organism, not assignable to an hitherto known species, that occurred in small numbers along with dominating strains of *L. sanfranciscensis* in a commercial sourdough starter preparation. As this organism was also shown to persist after multiple consecutive propagations over 6 months in a bakery's sourdough, we considered it to be a relevant member of the sourdough flora. According to phenotypic and genotypic results, the purpose of the present study was to describe this *Lactobacillus* as a new species for which we propose the name *Lactobacillus mindensis*.

METHODS

Strains, medium and culture conditions. Strain TMW 1.80^T was isolated from an industrial rye sourdough starter preparation (BRS). Strains TMW 1.1201, TMW 1.1199 and TMW 1.1206 originate from a bakery sourdough initiated with BRS and propagated by back-slopping (repeated cyclic reinoculation) for 6 months without additional inoculation with BRS. Samples were subjected to serial

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The EMBL accession number for the 16S rDNA sequence of *L. mindensis* DSM 14500^T is AJ313530.

dilutions and plated on modified MRS medium as described by Vogel *et al.* (1994). Strains of *L. mindensis* were incubated at 30 °C. Other lactobacilli were grown on regular MRS and incubated at the temperature recommended by the respective strain collection. Solid media were incubated under a modified atmosphere (N₂:CO₂ 90%:10%, v/v). Cultures were maintained at -80 °C in glycerol stocks. The purity of cultures was checked microscopically. Strains have been deposited at the DSMZ (Braunschweig, Germany), the type strain being DSM 14500^T.

Physiological characterization. Sugar fermentation patterns were determined by using a microtitre plate assay as described by Müller *et al.* (2000). The reproducibility was verified by repeated analyses. A pH-dependent change of the indicator was documented after 24, 48 and 168 h. Only definitive turnovers were rated as positive. Additional experiments were carried out using the API 50 CH kit (bioMérieux).

The formation of lactate isomers in fermented broth was determined enzymically using the DL-lactate test kit (Boehringer). Arginine hydrolysis was determined according to the methods described by Sharpe (1979).

Morphological characteristics. Cell morphology was studied by phase-contrast microscopy. Gram determination was performed using the KOH method of Gregersen (1978).

DNA base composition. The mol% G+C content of strain TMW 1.80^T was determined by a HPLC analytical method. The experiments were performed by the DSMZ (Germany). They were carried out using the protocol previously described by Tamaoka & Komagata (1984). The G+C content (mol%) was determined after Mesbah *et al.* (1989). Wild-type lambda phage DNA was used as standard.

Cell wall. The peptidoglycan structure of the cell wall was determined by the DSMZ (Germany). The absence of teichoic acids was determined as described by Baddiley & Davison (1961).

DNA isolation. DNA was isolated according to Marmur (1961) with some modifications. One hour before cells were harvested, penicillin G (Sigma) was added to inhibit the synthesis of cross-linking of the cell wall, and therefore to facilitate the lysis. A wet weight of 70 mg cells was used for the DNA isolation. Following the protocol, lysis was completed within 45–90 min after the addition of lysozyme and mutanolysin. For some strains a more effective lysis was obtained by an overnight lysis at 4 °C with a subsequent proteinase K treatment at 60 °C for 1 h, and then continuing the protocol. The purified and vacuum-dried DNA was dissolved in 2 × SSC (0.3 M NaCl, 0.03 M Na₃citrate.2H₂O, pH 7.0). This DNA preparation served for DNA–DNA hybridization experiments as well as for 16S rDNA amplification. DNA used for RAPD analyses was prepared in small-scale preparations as described by Lewington *et al.* (1987).

RAPD-PCR. The colonies subjected to RAPD-PCR were picked randomly. PCR was carried out with the oligonucleotide primer M13V (5'-GTT TTC CCA GTC ACG AC-3'). All reactions were performed in TopYield Strips (Nunc) with oil overlay (50 µl) and TECAN sealing (Tecan). The conditions for PCR amplification were as follows: 1 µl genomic DNA, 5 µl 10 × reaction buffer, 5 mM MgCl₂, 200 nM of each of the four deoxynucleotides, 1.5 U *Taq* polymerase (all from Amersham Pharmacia Biotech) and 20 pmol primer M13V. The PCR reactions were carried out on a Hybaid OmniGene thermocycler equipped with heated lid (MWG-Biotech). The cycling program was: 3 cycles of 96 °C for 3 min, 35 °C for 5 min and 75 °C for 5 min; 32 cycles of 96 °C for 1 min, 55 °C for 2 min and 75 °C for 3 min. Amplicons were electrophoretically separated on 1.5% TBE agarose gels.

DNA–DNA hybridization. The determination of DNA homology values was carried out using a modified procedure as described by Cardinali *et al.* (2000). Hydroxyapatite [HTP; 100 mg (Bio-Rad)] was suspended in 1 ml 100 mM sodium phosphate buffer (NPB), pH 6.7, heated for 10 min at 65 °C and centrifuged (14 000 g) for 30 s at 4 °C. The HTP pellet was resuspended with the DNA solution already equilibrated at 65 °C, incubated at 65 °C for 15 min and then centrifuged (14 000 g) for 30 s at 4 °C. HTP-bound DNA was washed twice with 600 µl 120 mM NPB and once with 600 µl 180 mM NPB. Finally, DNA was resuspended in 400 µl 300 mM potassium/sodium phosphate buffer (NPPB; pH 7.2) incubated for 15 min at 65 °C and then centrifuged for 30 s at 14 000 g.

Desalination was carried out with NAP-5 columns (Amersham Pharmacia Biotech).

DNA was diluted in water to reach a final concentration of 10 ng µl⁻¹ (A₂₆₀ 0.200 ± 5%). DNA was stored at -18 °C.

For dot-blotting of DNA, samples were diluted in 0.4 M NaOH to a final concentration of 1 ng µl⁻¹ and incubated for 30 min at room temperature. DNA samples (10 ng) were transferred by using a dot-blot apparatus (Stratagene) on nylon Hybaid-N+ membrane (Amersham Pharmacia Biotech). Fixation of DNA on the membrane was achieved by incubation at 80 °C for 1 h.

For quantification, a serial dilution (10, 8, 6, 4, 2 ng) of unlabelled DNA was dotted.

DNAs used for probes were labelled using the non-radioactive ECL random prime labelling and detection system (Amersham Pharmacia Biotech) according to the manufacturer's instructions. Prehybridization, hybridization and stringency washings (0.5 M NaCl) were carried out at 42 °C.

Membranes were exposed to a Kodak X-Omat film (Sigma-Aldrich). The spot intensity was calculated with the Image Master 2D Elite software (Amersham Pharmacia Biotech).

16S rDNA amplification and sequencing. PCR-mediated amplification of the complete 16S rDNA was carried out in a Gradient Master Thermocycler (Eppendorf). All reagents if not indicated otherwise were from Amersham Pharmacia Biotech. The amplification conditions were as follows: 1 µl genomic DNA, 10 µl 10 × reaction buffer, 200 nM each of the four deoxynucleotides, 1.5 U *Taq* polymerase, 20 pmol each primer (Interactiva) (616V, 5'-AGAGTTTGATYMTGGCTCAG-3'; 630R, 5'-CAKAAAG-GAGGTGATCC-3'), dH₂O to a final volume of 100 µl. The PCR conditions were: (94 °C/2 min)1 ×, (94 °C/45 s, 52 °C/1 min, 72 °C/30 s) 30 ×, (94 °C/1 min, 72 °C/4 min) 1 ×. PCR products were purified by the QIAquick PCR purification kit (Qiagen) and were eluted with 60 µl elution buffer. DNA sequences were determined by the chain-termination method (Sanger *et al.*, 1977) using the ABI Prism Dye Terminator Cycle Sequencing Kit (Perkin Elmer) on an ABI 373 stretch sequencing system by a commercial service (SequiServe). For sequencing, the amplification primer 616V together with the internal primers 609R [5'-ACT AC(CT) (AGC)GG GTA TCT AA(GT) CC-3'], 612R [5'-GTA AGG TT(CT) T(AGCT)C GCG T-3'], 607R (5'-ACG TGT GTA GCC C-3'), 606R [5'-T(AG)A CGG (GC)C(AG) GTG TGT ACA-3'] and 607V (5'-GGG CTA CAC ACG TGC-3') were used.

Phylogenetic analysis. The complete 16S rDNA sequence of *L. mindensis* DSM 14500^T was fitted into alignments of almost complete primary structures available in public databases (Ludwig, 1995). Additional sequences were obtained from the Ribosomal Database Project (Maidak *et al.*, 2001). Distance matrix, maximum-parsimony and maximum-likelihood methods were applied for tree

reconstructions as implemented in the ARB software package (Ludwig & Strunk, 1997). Different datasets varying with respect to included outgroup reference sequences as well as alignment positions were analysed. To exclude highly variable regions, a filter with 50 % invariance was applied.

Species-specific detection by PCR. The specific primer PmindR (5'-AAC AGT GAT CAT GTG AAG AC-3') was checked for its specificity against other bacterial 16S rRNA sequences by using the probe-checking software provided in the Ribosomal Database Project (Maidak *et al.*, 2001). PmindR was applied in combination with primer 616V in the PCR assay. The amplification conditions were as follows: 1 µl genomic DNA, 5 µl 10 × reaction buffer, 1.5 µl DMSO, 200 nM each of the four deoxynucleotides, 1.5 U *Taq* polymerase, 20 pmol each primer (616V, PminR), deionized H₂O to a final volume of 50 µl. The PCR program used was: (94°C/2 min) 1 ×, (94°C/45 s, 62.5°C/30 s, 72°C/30 s) 30 ×. A control PCR to check the accessibility of DNA with universal primers 616V and 609R was performed as described previously (Garriga *et al.*, 1998).

RESULTS AND DISCUSSION

Typical constituents found in traditional three-stage processed doughs were obligately heterofermentative species like *L. sanfranciscensis*, *Lactobacillus brevis*, *L. fermentum* and *L. fructivorans* (Stolz, 1995), amongst which *L. sanfranciscensis* was the numerically dominant organism (Böcker *et al.*, 1990; Spicher & Schröder, 1978).

The composition of the bacterial flora of the sourdough described in Methods was analysed by the use of the RAPD technique. A database of RAPD reference patterns of lactobacilli isolated from food fermentations was generated and shown to allow differentiation at the species level (Fig. 1). The total bacterial cell count in the sourdough under investigation was 10.3×10^8 c.f.u. g⁻¹, whereas the concentration of yeasts was 6×10^7 c.f.u. g⁻¹.

DNAs isolated from 117 randomly selected bacterial colonies were compared to the RAPD database. According to pattern similarity we found that 61 % were *L. sanfranciscensis* and 36 % of strains shared identical patterns with *Lactobacillus* sp. TMW 1.80^T. The latter strain was previously isolated from an industrial sourdough starter preparation and characterized as *Lactobacillus* species only.

The remaining 3 % consisted of other lactic acid bacteria whose RAPD patterns could not be assigned to any *Lactobacillus* species available in our RAPD database (data not shown). The strains of the '36 %' group, TMW 1.1201, TMW 1.119, TMW 1.1206 and the previous isolate TMW 1.80^T, were subjected to a further taxonomic characterization.

Phylogenetic analysis

The complete sequence (1544 bp) of the 16S rRNA gene of strain TMW 1.80^T was determined. It was aligned with all available sequences of low G+C content Gram-positive organisms. The analysis placed the representative strain TMW 1.80^T within the *L. plantarum* group of the

Fig. 1. RAPD patterns of various lactobacilli often described as typical organisms in sourdough fermentations.

heterogeneous *L. casei* group as defined by Schleifer & Ludwig (1995). It represents a cluster of related species consisting of *L. alimentarius*, *L. farciminis*, the recently described *L. paralimentarius* (Cai *et al.*, 1999) and *L. kimchii* (Yoon *et al.*, 2000). Except for *L. kimchii*, all the above-mentioned species have already been isolated from sourdoughs. The closest relatives were *L. kimchii* (98.7 %), *L. alimentarius* (97.5 %) and *L. paralimentarius* (97.2 %). The phylogenetic position is shown in Fig. 2. Positions determined by the parsimony algorithm were identical with those obtained with the maximum-likelihood approach. Minor differences in branching points were found by application of the neighbour-joining method (data not shown).

Fig. 2. Phylogenetic tree demonstrating the relationship of *L. mindensis* to the closest related lactobacilli. The tree was constructed by using the parsimony approach. The tree was based on a dataset that included only positions which are present in at least 50 % of all available *Lactobacillus* 16S rRNA sequences. Bar, 5 % estimated sequence divergence.

The G + C content of the 16S rDNA was 51.2 mol%.

The DNA–DNA hybridization studies were performed according to the method of Cardinali *et al.* (2000). For each hybridization experiment, a calibration by serial dilutions of homologous DNA was applied on the same membrane. The relationship between spot intensity and amount of blotted DNA was highly linear with correlation values (r^2) equal or over 95 % (data not shown).

Whereas DNA–DNA relatedness between strains TMW 1.80T, TMW 1201, TMW 1.1199 and TMW 1206 of *L. mindensis* revealed by DNA–DNA hybridization experiments was greater than 85 %, hybridizations of these strains against DNA from all relevant type strains showed values below 30 % (Fig. 3).

As these data revealed a phylogenetically homogeneous group of strains that is separate from other known species, we propose a new species, *L. mindensis*. The type strain is TMW 1.80T deposited at the DSMZ as *L. mindensis* DSM 14500T.

Colony and cell morphology

Colonies of *L. mindensis* DSM 14500T appeared white with a regular sharp edge and after 3 days of growth they were 1 mm in diameter. Colonies older than 3 days appeared frayed at the edges.

Cells were non-spore-forming, non-motile rods that occurred singly or in pairs, seldom in chains. Under the phase-contrast microscope, cells grown in liquid culture appeared as straight rods measuring 0.3–0.7 × 3–5 µm (in

Fig. 3. DNA–DNA hybridization experiment between DNA of *L. mindensis* and other lactobacilli. Genomic DNA of *L. mindensis* TMW 1.1201 was used as a probe and 10 ng DNA of the following strains was blotted. Row A: 1, *L. mindensis* TMW 1.80T; 2, *L. mindensis* TMW 1.1201; 3, *L. mindensis* TMW 1.1199; 4, *L. mindensis* TMW 1.1206; 5, *L. sanfranciscensis* TMW 1.1203; 6, *L. farciminis* DSM 20184T; 7, *L. alimentarius* DSM 20249T; 8, *L. sanfranciscensis* DSM 20451T; 9, *L. pontis* DSM 8475T; 10, *Lactobacillus oris* DSM 4864; 11, *L. panis* DSM 6035; 12, *L. panis* DSM 6036. Row B: 1, *L. frumenti* DSM 13143; 2, *L. frumenti* DSM 13145T; 3, *L. buchneri* DSM 20057T; 4, *Lactobacillus kefir* DSM 20587T; 5, *Lactobacillus malefermentans* DSM 2017; 6, *L. plantarum* DSM 20174T; 7, *L. brevis* DSM 20451T; 8, *L. fermentum* DSM 20052T; 9, *Lactobacillus lindneri* DSM 20690T; 10, *Lactobacillus vaginalis* LMG 1289; 11, *Lactobacillus hilgardii* DSM 20176; 12, *Lactobacillus parabuchneri* DSM 5707T. Row C: 1, *Lactobacillus amylolyticus* DSM 11664T; 2, *L. pontis* TMW 1.675; 3, *L. plantarum* TMW 1.535.

Fig. 4. Phase-contrast micrographs of cells of *Lactobacillus mindensis* DSM 14500^T. Cells were grown on MRS. Left-hand panel, cells grown in liquid culture; right-hand panel, cells grown on solid medium. Bar, 10 µm.

mid- to late-exponential growth phase). Cells on solid media were observed to elongate filamentously with a length between 5 and 20 µm (Fig. 4). The KOH test indicated a Gram-positive behaviour.

Physiological and biochemical properties

The sugar fermentation patterns and further physiological and biochemical characteristics of *L. mindensis* strains and the reference organisms are listed in Table 1. Lactate is produced in a ratio of 96 % L-lactate to 4 % D-lactate. Gas was not produced from glucose. Acid was produced from glucose, fructose, mannose, aesculin, salicin and cellobiose by all strains tested. Only weak fermentation of maltose was detected. Strain TMW 1.1206 did not ferment amygdalin.

The G + C content of genomic DNA is 37.5 mol% (T_m). Analysis of the cell wall in strain TMW 1.80^T revealed the presence of lysine and aspartic acid, which indicates the Lys-D-Asp peptidoglycan type.

Design of a species-specific PCR detection assay

A diagnostic sequence was identified within the 16S rRNA gene (see Table 2) that allows the identification of strains of *L. mindensis* and differentiation thereof from other relevant lactobacilli when used as target site in a PCR assay (Fig. 5). Primer PmindR, in combination with the 16S rDNA universal primer 616V, generated a 226 bp fragment. No cross-reaction was detected for DNA of other lactobacilli. Accessibility of DNA preparations for amplification was successfully controlled by a simultaneous amplification with 16S rDNA specific universal primers.

The RAPD fingerprint identity of all four strains (TMW 1.80^T, 1.1199, 1.1201, 1.1206), and their DNA-DNA homology values above 80 % with each other and values less than 30 % to other species, provided enough phylogenetic data to propose a separate species status.

The G + C content of 37.5 mol% is within the range of the *L. plantarum* group (34–46 %), and the peptidoglycan

type (L-Lys-D-Asp) fell into line with the majority of lactobacilli.

Taxonomic significance is provided by the lack of fermentation of galactose, arbutin, lactose and trehalose, which allows differentiation from its closest relatives. One difference to *L. paralimentarius* is the lack of ability to ferment ribose, arbutin, sucrose and trehalose. The limited fermentation spectrum is a typical trait as it can also be observed in other sourdough lactobacilli. The moderate fermentation of maltose also observed in some strains of *L. paralimentarius* (Cai *et al.*, 1999) seems to be at first unfavourable for this environment, but may explain the observed coexistence with *L. sanfranciscensis*, which was shown to possess a highly optimized maltose metabolism resulting in production of glucose (Stolz, 1995; Ehrmann & Vogel, 1998).

The increased occurrence of *L. mindensis* in the investigated sourdough may be caused by specific process parameters used in the bakery. Its effect on dough quality and aroma was not investigated in this study.

Description of *Lactobacillus mindensis* sp. nov.

Lactobacillus mindensis (min.den'sis. N.L. adj. *mindensis* pertaining to the city of Minden, Germany, from where the first strain of this species was isolated).

Cells are Gram-positive, non-motile, non-spore-forming rods (0.9–5 µm), occurring singly, in pairs or in chains. Colonies are usually small (2 mm), smooth, low convex and flat with a white colour on MRS agar. Cells are catalase-negative and homofermentative. Growth occurs at 15–30 °C but not above. Growth optimum is at pH 4.6–5.2; no growth at or above pH 6.5. Acid is produced from glucose, maltose, fructose, mannose, N-acetylglucosamine, cellobiose and salicin. Some strains produce acid from amygdalin. Neither acid nor gas is produced from arabinose, dextrin, galactose, lactose, mannitol, melezitose, melibiose, raffinose, rhamnose, ribose, sucrose, sorbitol, trehalose or xylose. Arginine decarboxylase was not detected. Urease and H₂S are not produced. Nitrate is not reduced to nitrite.

Table 1. Physiological and biochemical characteristics of *L. mindensis* and selected reference organisms

1, *L. mindensis* DSM 14500^T TMW 1.80^T; 2, *L. frumenti* DSM 13145^T; 3, *L. kimchii* JCM 10707^T; 4, *L. farciminis* LMG 9200^T; 5, *L. alimentarius* JCM 1095^T; 6, *L. paralimentarius* JCM 10415^T; 7, *L. sanfranciscensis* ATCC 27651^T. ND, Not determined; –, negative reaction; +, positive reaction; w, weak reaction.

Characteristic(s)	1	2*	3†	4‡	5§	6§	7
DL-Lactate	DL	DL	DL	DL	DL	ND	DL
NH ₃ from arginine	–	+	–	ND	–	ND	–
Growth at 15 °C/45 °C	+ / –	– / +	+ / –	+ / –	+ / –	ND / +	+ / –
Peptidoglycan type	L-Lys–D-Asp	L-Lys–D-Asp	L-Lys–D-Asp	L-Lys–D-Asp	L-Lys–D-Asp	ND	L-Lys–Ala
DNA G + C content (mol%)	37.5 ± 0.2	43.3 ± 0.2	35.0	34–36	36–37	37.2–38	36–38
Acid production from:							
DL-Arabinose	–	+ / –	+	–	–	–	–
Ribose	–	+	+	–	+	+	+
DL-Xylose	–	–	+	–	+	–	–
Galactose	–	+	w	+	+	–	+ / –
Glucose	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	–
Mannose	+	+	+	+	+	+	–
Mannitol	–	+	ND	–	–	–	–
Sorbitol	–	+	ND	–	–	–	–
N-Acetylglucosamine	+	+	+	ND	+	+	–
Amygdalin	+	+	+	+	+	+	–
Arbutin	–	+	+	ND	+	+	–
Aesculin	+	+	+	+	+	+	–
Salicin	+	+	+	+	+	+	–
Cellobiose	+	+	+	+	+	+	+
Maltose	w	+	+	+	+	+ / –	+
Lactose	–	+	–	+	–	–	–
Melibiose	–	+	ND	–	–	–	–
Sucrose	–	+	+	+	+	+	–
Trehalose	–	+	+	+	+	+	–
Melezitose	–	–	+	–	–	–	–
Raffinose	–	+	ND	–	–	–	–
Gluconate	–	+	+	ND	–	–	–

*Data from Müller *et al.* (2000).

†Data from Yoon *et al.* (2000).

‡Data from Kandler & Weiss (1986).

§Data from Cai *et al.* (1999).

||Data from Vogel *et al.* (1994); Stolz (1995).

Table 2. Diagnostic sequence signatures in the 16S rRNA to differentiate *L. mindensis* from its closest relatives

The sequence used for the specific PCR (primer PmindR) is underlined.

Species	Sequence (5'→3')
<i>L. mindensis</i>	CAACAGTCTTCACATGATCACTGTTTAA
<i>L. kimchii</i>	CAACTTAGATCACATGATCTTTGTTTAA
<i>L. farciminis</i>	CAACTACTTTCACATGATCGTAGCTTGA
<i>L. alimentarius</i>	CAACATTAAACACATNTTNTTTGTTTAA
<i>L. paralimentarius</i>	CAACTTAGATCACATGATCTTTGTTTAA

All strains produce mainly L-lactate (4 % D-lactate and 96 % L-lactate). The peptidoglycan is of the lysine–D-iso-asparagine (L-Lys–D-Asp) type and the cell wall does not contain teichoic acid. The DNA G + C content is 37.5 mol% (T_m). Strains were isolated from commercial sourdough starter preparations and from bakery's sourdough after continuous propagations for long periods. The type strain is DSM 14500^T (= LMG 21508^T).

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Fig. 5. Identification of *L. mindensis* by a specific PCR approach. For the species-specific PCR, primers PmindR and 616V were used, and for control PCR, primers 616V and 609R were used. Specific and control PCRs were performed separately, but run in a single lane. Lanes: 1 and 12, 100 bp ladder (molecular mass standard); 2, *L. frumenti*; 3, *L. sanfranciscensis*; 4, *L. farciminis*; 5, *L. alimentarius*; 6, *L. kimchii*; 7, *L. paralimentarius*; 8, *L. mindensis* TMW 1.1201; 9, *L. mindensis* TMW 1.1199; 10, *L. mindensis* TMW 1.1206; 11, *L. mindensis* DSM 14500^T.

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