Lactobacillus plantarum subsp. *argentoratensis* subsp. nov., isolated from vegetable matrices

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Fourteen strains isolated from vegetable sources and identified as belonging to *Lactobacillus plantarum* presented an atypical pattern of amplification with a species-specific multiplex-PCR assay. Phylogenetic analysis of two protein-encoding genes, *recA* (encoding the recombinase A protein) and *cpn60* (encoding the GroEL chaperonin), as well as phenotypic and genomic traits revealed a homogeneous group of very closely related strains for which subspecies status is proposed, with the name *Lactobacillus plantarum* subsp. *argentoratensis*. The type strain is DKO 22^T (= CIP 108320^T = DSM 16365^T).

Lactobacillus plantarum belongs to the *Lactobacillus casei*–*Pediococcus* phylogenetic group of the genus *Lactobacillus* (Hammes & Vogel, 1995) and in particular to the *L. plantarum* phylogenetic subgroup (Hammes & Hertel, 2003). This species is characterized by many metabolic activities that allow it to colonize different environments such as dairy products, pickled vegetables, fish products, silage, wine as well as mammal intestinal tracts (Vescovo *et al.*, 1993). The species is highly heterogeneous (Dellaglio *et al.*, 1975). Several studies, which have tried to elucidate intraspecific relationships, led to the description of two novel species: *Lactobacillus pentosus* and *Lactobacillus paraplantarum* (Zanoni *et al.*, 1987; Curk *et al.*, 1996). These three closely related species cannot be distinguished by 16S rRNA gene sequence analysis because they show 99 % sequence similarity. They also have very similar

fermentation abilities: *L. pentosus* can be differentiated from *L. plantarum* only by its capacity to ferment glycerol and xylose (Zanoni *et al.*, 1987). Some exceptions, however, exist: some *L. plantarum* strains are able to metabolize glycerol as with *L. pentosus* and not all *L. pentosus* strains can metabolize xylose (Bringel *et al.*, 1996). Fermentation differences between *L. plantarum* and *L. paraplantarum* are even less helpful: the main difference is that strains of the latter species metabolize methyl α -D-glucopyranoside, whereas 66 % of *L. plantarum* strains cannot (Bringel *et al.*, 1996).

With regard to molecular techniques, *L. plantarum*, *L. pentosus* and *L. paraplantarum* can be rapidly distinguished using PCR amplification targeting the 16S/23S rRNA gene spacer region (Berthier & Ehrlich, 1998) or the *recA* gene as described by Torriani *et al.* (2001). Moreover, a polyphasic approach with a combination of RAPD (randomly amplified polymorphic DNA)-PCR, Southern hybridization and phenotypic traits not only distinguished the three species *L. plantarum*, *L. paraplantarum* and *L. pentosus*, but also revealed the presence of two groups of strains in the species *L. plantarum* (Bringel *et al.*, 2001): 90 % of these were closely linked to the *L. plantarum* type strain, ATCC 14917^T, and 10 % (14 strains) formed a distinct group. We report here the analyses performed on those 14 ‘atypical’ strains and, on the basis of collected data, a novel subspecies of *L. plantarum* is proposed.

The studied bacterial strains listed in Table 1 were grown in MRS at 37 °C. After genomic DNA extraction (Marmur, 1961), the *recA*-nested multiplex-PCR assay was performed as described by Torriani *et al.* (2001). This multiplex-PCR

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Abbreviation: RAPD, randomly amplified polymorphic DNA.

The GenBank/EMBL/DDBJ accession numbers obtained in the present study are: 16S rRNA gene sequence of *L. plantarum* subsp. *argentoratensis* DKO 22^T, AJ640078; complete *recA* gene sequences of *L. plantarum* subsp. *plantarum* ATCC 14917^T, AJ621668; *L. plantarum* subsp. *argentoratensis* DKO 22^T, AJ640079; *L. plantarum* subsp. *argentoratensis* A7, AJ640080; *L. pentosus* LMG 10755^T, AJ621666; *L. paraplantarum* LMG 16673^T, AJ621662; partial *cpn60* gene sequences of *L. plantarum* subsp. *argentoratensis* DKO 22^T, AJ640081; *L. plantarum* subsp. *plantarum* ATCC 14917^T, AJ640082.

Positions of the primers of species-specific multiplex-PCR assay based on *recA* gene sequences are shown in a supplementary figure available in IJSEM Online.

Table 1. Strains tested

ATCC, American Type Culture Collection, Manassas, VA, USA; CIP, Collection of bacterial strains of Institut Pasteur, Paris, France; CNRZ, Centre National de Recherches Zootechniques, INRA Jouy-en-Josas, France; LMG, BCCM/LMG Bacteria Collection, Laboratorium voor Microbiologie, Universiteit Gent, Belgium; NCFB, National Collection of Food Bacteria, Reading, UK; NCIMB, National Collection of Industrial and Marine Bacteria, Aberdeen, UK.

Strain	Isolation source
<i>L. plantarum</i> subsp. <i>plantarum</i>	
ATCC 14917 ^T	Pickled cabbage
A12	Cassava, Colombia
ATCC 10012	Unknown
B41	Silage, Italy
CNRZ 1220	Cheese, Egypt
CNRZ 1246	Domiat cheese, Egypt
LAB R1M3	Monte Veronese cheese, Italy
LMF 415	Silage
LMG 18035	Milk, Egypt
NCFB 340	Silage, UK
NCIMB 8826	Human saliva
NS26	Monte Veronese cheese, Italy
ZW20	Cheese, Switzerland
817	Cheese, Italy
38AA	Cassava, Colombia
<i>L. plantarum</i> subsp. <i>argentoratensis</i>	
A1	Cassava, Colombia
DK 19	White maize, Nigeria
NCIMB 12120	Fermented cereals (ogi), Nigeria
13	Cow udder, Algeria
57.2	Cassava, Colombia
A4	Cassava, Colombia
A7	Cassava, Colombia
CNRZ 1889	Fermented cereals (ogi), Nigeria
CNRZ 1890	Fermented millet (baba), Nigeria
DKO 22 ^T	Sour cassava, Nigeria
DK 9	Fermented cucumber, Nigeria
DK 36	Tapioca, Nigeria
LP85-2	Silage, France
SF2A35B	Sour cassava, South America
<i>L. paraplantarum</i>	
LMG 16673 ^T	Beer, France
<i>L. pentosus</i>	
LMG 10755 ^T	Sawdust fermentation

assay uses a reverse primer (pRev) in combination with three species-specific forward primers (planF, pentF and paraF) to distinguish the closely related species of the *L. plantarum* group. Three species-specific amplification products of different length were obtained: 318 bp for *L. plantarum*, 218 bp for *L. pentosus* and 107 bp for *L. paraplantarum*. The 14 atypical strains, isolated from vegetable sources, displayed an unexpected pattern characterized by two amplification bands, i.e. the 318 bp band

typical of *L. plantarum*, and an additional 120 bp band (see Fig. 1). To evaluate the origin and the specificity of this new band, further PCR amplifications were performed on the 14 strains and on *L. plantarum* ATCC 14917^T. Each of the three species-targeted forward primers was assayed in combination with the reverse primer pRev in separate PCR assays. As expected, a unique 318 bp band was obtained when the primer specific for *L. plantarum* species was used. By contrast, in the presence of the primer pentF, specific for *L. pentosus*, a single amplification product of 120 bp was obtained from all 14 strains (data not shown), but no amplification was detected with the type strain of *L. plantarum*. Sequencing confirmed that both amplicons are *recA* gene fragments. Moreover, the *recA* gene sequences of the 14 strains were more similar to the *recA* gene of *L. plantarum* ATCC 14917^T, confirming that they belong to the species *L. plantarum*. The positions of the multiplex-PCR primers on the *recA* gene sequence of *L. plantarum* ATCC 14917^T (GenBank accession no. AJ621668), *L. plantarum* DKO 22^T (AJ640079), *L. pentosus* LMG 10755^T (AJ621666) and *L. paraplantarum* LMG 16673^T (AJ621662) are given in Supplementary Fig. S1 available in IJSEM Online.

The 14 atypical *L. plantarum* strains were also compared by using physiological tests to a panel of 15 *L. plantarum* strains, including *L. plantarum* ATCC 14917^T, and to the type strains of *L. paraplantarum* and *L. pentosus*. Table 2 lists the fermentation abilities of the 14 strains evaluated using the API-50 CHL detection kit as described by the manufacturer (bioMérieux). All the *L. plantarum* strains and the type strains of *L. pentosus* and *L. paraplantarum* fermented galactose, D-glucose, D-fructose, D-mannose, mannitol, N-acetylglucosamine, amygdalin, aesculin, salicin, cellobiose, maltose, sucrose and β -gentiobiose. None of the strains was able to ferment L-sorbitol, inulin, D-lyxose, erythritol, D-arabinose, L-xylose, adonitol, methyl β -xyloside, inositol, D-tagatose, DL-fucose, L-arabitol, 2-ketogluconate or 5-ketogluconate. By contrast, different fermentation trends were observed between the two groups of *L. plantarum* strains for five carbohydrates (Table 2). All strains were negative for catalase activity (Freny *et al.*, 1999). Growth at 15 °C (positive) and 45 °C (negative) was tested in MRS broth. Hydrolysis of arginine determined according to the method described by Sharpe (1979) was not positive for any strain. Lactic acid isomers were assayed enzymically using the DL-lactate test kit (Boehringer) after growth in MRS. The 14 atypical *L. plantarum* strains formed both isomers, with the D isomer accounting for 65–70 % of the total lactic acid produced.

The partial 16S rRNA gene sequence was determined from strain DKO 22^T, one of the 14 atypical *L. plantarum* strains, using primers Lac16S-for (5'-AATGAGAGTTTGATCCTGGCT-3') and Lac16S-rev (5'-GAGGTGATCCAGCCG-CAGGTT-3'). The reaction mixture (20 μ l) contained 30 ng template DNA, 1.5 mM MgCl₂, 0.2 mM dNTPs, 1 μ M each primer and 1 U *Taq* DNA polymerase (Polymed)

Fig. 1. Multiplex-PCR amplification patterns. Lanes: M, marker 1 kb plus DNA ladder; 1, *L. plantarum* ATCC 14917^T; 2, *L. pentosus* LMG 10755^T; 3, *L. paraplantarum* LMG 16673^T; 4–31, *L. plantarum* NCFB 340, B41, CNRZ 1246, 38AA, CNRZ 1220, A12, NCIMB 8826, CNRZ 1889, CNRZ 1890, DKO 22^T, DK 9, DK 36, LP85-2, SF2A35B, LMG 18035, LAB R1M3, ZW20, NS26, LMF 415, 817, ATCC 10012, A1, DK 19, NCIMB 12120, 13, 57.2, A4 and A7.

in a standard reaction buffer. After an initial denaturation of 4 min at 94 °C, 25 cycles of 1 min at 94 °C, 1.5 min at 50 °C, 2 min at 72 °C and a final extension at 72 °C for 7 min were performed. The 1.6 kb amplification product was extracted from agarose gel (Promega elution kit) and sequenced at the Biomolecular Research (BMR) Center at Padua University (Italy). The sequence obtained was aligned with the 16S rRNA gene sequences of other *Lactobacillus* species using the CLUSTAL_X program (Thompson *et al.*, 1997). Phylogenetic analysis was performed with the MEGA version 2.1 program (Kumar *et al.*, 2001): the distance matrix was obtained with Tamura's three-parameter model and tree reconstruction was obtained with minimum evolution clustering. An

analysis of the robustness of the tree was obtained by using a bootstrap approach with 1000 replicates. A maximum-parsimony method was also applied with default options. Maximum-likelihood analysis was performed with the DNAML program in the PHYLIP software package. Very low divergence of sequences (similarity of 99 %) in the phylogenetic trees confirmed strongly that strain DKO 22^T belongs to the *L. plantarum* group (data not shown). Sequence analysis of the 16S rRNA gene did not allow us to distinguish very closely related species whereas protein-encoding genes did (Torriani *et al.*, 2001). Therefore, the complete *recA* gene sequences of selected *L. plantarum* strains (ATCC 14917^T, DKO 22^T, A7) and of the *L. pentosus*

Table 2. Fermentation activities of the 14 atypical *Lactobacillus plantarum* strains and their incidence (percentage values) in the two groups of *L. plantarum* strains

Strain	Dulcitol	Melezitose	Methyl α -D-mannoside	L-Arabinose	D-Turanose
A1	+	–	–	–	–
DK 19	+	–	–	–	–
NCIMB 12120	–	–	–	+	–
13	–	–	–	–	+
57.2	+	–	–	–	–
A4	+	–	–	–	–
A7	+	–	–	–	–
CNRZ 1889	–	–	–	+	+
CNRZ 1890	–	–	–	+	+
DKO 22 ^T	–	–	–	–	–
DK 9	–	–	–	–	–
DK 36	–	–	–	–	–
LP85-2	–	–	–	–	+
SF2A35B	+	–	–	–	–
Fermentation abilities of the 14 atypical <i>L. plantarum</i> strains	+ (43 %)	– (100 %)	– (100 %)	– (79 %)	– (71 %)
Fermentation abilities of ATCC 14917 ^T and the other <i>L. plantarum</i> strains tested (14 strains)	– (100 %)	+	+	+	+

and *L. paraplantarum* type strains were obtained. The corresponding *recA* genes were PCR-amplified using primers annealing the flanking gene sequences, which were conserved in the sequenced genomes (<http://www.tigr.org>) of bacteria belonging to the phylum *Firmicutes* (data not shown). The upstream *cinA* gene (accession no. NT01LP2031 in *L. plantarum* WCFS1, according to TIGR annotation) encodes a putative competence-damage protein. The downstream gene (accession no. NT01LP2029 in *L. plantarum* WCFS1, according to TIGR annotation) encodes a conserved protein with unknown function. The *recA*-flanking gene sequences were used to design degenerate primers to amplify two overlapping fragments that included *recA*. A 1.4 kb fragment corresponding to the 5'-region was amplified with degenerate primers *cinA*-f (5'-CGAGTTTTCGCGTTTGTGGNATHGGNGA-3') and AN-r (5'-CRATTTTTCACGRATYTG-3'). The reaction mixture contained 300 ng DNA template, 2 mM MgCl₂, 0.2 mM dNTPs, 1 µM each primer and 2.5 U *Taq* DNA polymerase (Sigma) in a standard reaction buffer. After an initial denaturation of 5 min at 94 °C, 30 cycles of 1 min at 94 °C, 1.5 min at 44 °C, 1.5 min at 72 °C and a final extension at 72 °C for 5 min were performed. The 1.1 kb 3'-end fragment was amplified using AN-f (5'-AAATYGAWGGNGARATGGG-3') and HDIG-r (5'-TGTTTCACGAAAAATTTGATTTTCTTCYTTNGCYTC-3'). Reaction mixtures contained 300 ng DNA template, 2 mM MgCl₂, 0.1 mM dNTPs, 0.5 µM each primer and 1.5 U *Taq* DNA polymerase (Sigma) in a standard reaction buffer. Reaction conditions were the same as those described above, except primer annealing was performed at 42 °C rather than 44 °C. The amplified fragments from *L. plantarum* DKO 22^T, A7 and ATCC 14917^T, *L. pentosus* LMG 10755^T and *L. paraplantarum* LMG 16673^T were sequenced. The *recA* gene sequence of *L. plantarum* WCFS1, available in the EMBL database (GenBank/EMBL accession no. AL935258), was retrieved from its complete genome sequence (Kleerebezem *et al.*, 2003). The sequenced strain WCFS1 is isogenic to strain NCIMB 8826, which was included in the present study (Table 1) and gave a single 318 bp multiplex-PCR assay amplification product as obtained with the *L. plantarum* type strain (see Fig. 1). The *recA* gene was longer in *L. plantarum* (1143 bp) than in *L. pentosus* and in *L. paraplantarum* (both 1137 bp). Alignments of the *L. plantarum* *recA* nucleotide sequences revealed 100 % similarity between strains DKO 22^T and A7, 99.7 % similarity between strains ATCC 14917^T and WCFS1, but only 93 % similarity between the two *L. plantarum* *recA* gene clusters (strains DKO 22^T/A7 versus ATCC 14917^T/WCFS1). *L. plantarum* strains shared about 81–82 % sequence similarity with *L. pentosus* and *L. paraplantarum* when similarity values were calculated on matching positions in *L. pentosus* and *L. paraplantarum* 1134 bp *recA* genes (without the stop codon). Thus, *recA* gene sequence analysis not only clearly distinguished between *L. plantarum*, *L. paraplantarum* and *L. pentosus*, but also identified two separate groups within the *L. plantarum* species. A phylogenetic tree based on the complete

Fig. 2. Phylogenetic tree based on *recA* gene sequence analysis showing the phylogenetic relationship of *L. plantarum* subsp. *argenteratensis* subsp. nov. with other strains of *L. plantarum* subsp. *plantarum*, *L. pentosus* and *L. paraplantarum*. The distance matrix was calculated with Tamura's three-parameter formula and the tree was inferred by minimum evolution (MEGA2 software; Kumar *et al.*, 2001). Bootstrap values at the nodes were all 100 % and were calculated on 1000 resamplings. Maximum-parsimony and maximum-likelihood analyses gave almost identical results. Bar, 0.02 substitutions per nucleotide position.

recA gene sequences was obtained as described above and is shown in Fig. 2. The two *L. plantarum* clusters deduced from the complete sequence analysis of *recA* genes in four strains was validated in the other *L. plantarum* strains by comparing a 750 bp internal *recA* sequence obtained as described by Dellaglio *et al.* (2004). About 20 ng PCR product was restricted with endonucleases *AfaI*, *BamHI* and *SalI* (Takara; 5 U, 1 h). The same restriction patterns found in *L. plantarum* DKO 22^T were found in 12 of the 14 atypical strains (data not shown). The two exceptions, strains DK 19 and SF2A35B, showed the same restriction pattern as for *L. plantarum* ATCC 14917^T, suggesting sequence divergence in their restriction enzyme recognition sites.

Strains from the two *L. plantarum* groups can be distinguished by the size of the products obtained with a *recA*-nested multiplex-PCR assay and in particular by the amplification product obtained using primer set pentF/pRev (see above and Fig. 1). In DKO 22^T, a C→T point mutation in the *recA* sequence (nucleotide 505 in GenBank accession number AJ640079) was linked to the specific annealing of primer pentF. The same point mutation was also found in strain A7 but not in *L. plantarum* ATCC 14917^T and WCFS1 (see Supplementary Fig. S1). Thus, in the *recA*-nested multiplex-PCR test, the two *L. plantarum* groups can be discriminated on the basis of a C→T sequence divergence.

Comparative sequence analysis on a second protein-encoding gene, *cpn60*, was also performed. Part of the *cpn60* gene was amplified using the degenerate primers *cpn-f* (5'-CTTGGGCCCCAAAAGGCGMNAAYGTNGT-3') and *cpn-r* (5'-CCAACCGTTCTTGAATTTTTCNCKR-TCR-AA-3'). The reaction mixture (20 µl) contained 100 ng template DNA, 2 mM MgCl₂, 0.1 mM dNTPs, 1 µM of each primer and 1.5 U *Taq* polymerase in a standard reaction buffer. After an initial denaturation of 5 min at 94 °C, 30 cycles of 1 min at 94 °C, 1 min at 55 °C, 1.5 min at 72 °C and a final extension at 72 °C for 7 min were performed. Amplicons of the expected length of about 1.0 kb were obtained for all the strains. Amplification products of about 450 bp were sequenced for both *L. plantarum* DKO 22^T and ATCC 14917^T. The two groups of *L. plantarum* could not be distinguished with *cpn60* partial sequence analysis as their sequences were highly similar (99.8 %).

In conclusion, the 14 atypical strains belonged to *L. plantarum* as previously determined with Southern blot analysis with species-specific probes and RAPD-PCR (Bringel *et al.*, 2001), with DNA–DNA hybridization of LP85-2 with the type strain of *L. plantarum* (Bringel *et al.*, 1996) and with multiplex-PCR assay. However, for five carbohydrates, these 14 strains had different fermentation trends from those of the other *L. plantarum* strains tested (Table 2). Moreover, the 14 strains were easily distinguished based on highly standardized, reproducible and independent molecular tests, such as Southern analysis (Bringel *et al.*, 1996), RAPD-PCR analysis (Bringel *et al.*, 2001), *recA* multiplex-PCR assay (Torriani *et al.*, 2001) and *recA* gene sequence analysis. Moreover, comparative analysis of 20 strains of *L. plantarum* using microarrays genotyped *L. plantarum* strains into two clearly distinguishable groups in agreement with the results of this study (unpublished data; personal communication of D. Molenaar, F. Bringel, F. Schuren, W. de Vos, R. Siezen and M. Kleerebezem). These results justify a novel subspecies designation for the 14 atypical strains in the species *L. plantarum*, for which the name *Lactobacillus plantarum* subsp. *argentoratensis* subsp. nov. is proposed. According to Rule 40b of the Bacteriological Code (Lapage *et al.*, 1992), the description of a new subspecies automatically creates the subspecies *L. plantarum* subsp. *plantarum*, which contains the type strain (ATCC 14917^T).

Description of *Lactobacillus plantarum* subsp. *plantarum* subsp. nov.

Lactobacillus plantarum subsp. *plantarum* (plan.ta'rum. L. fem. n. *planta* a sprout; M.L. n. *planta* a plant; M.L. gen. pl. n. *plantarum* of plants).

Gram-positive, non-motile, non-spore-forming straight rods with rounded ends, usually 0.9–1.2 µm by 3–8 µm, that occur singly, in pairs or in short chains. Facultatively anaerobic; growth occurs at 15 °C but not at 45 °C. Both isomers of lactic acid are produced. Gas from glucose is not produced. Catalase is not produced, but some strains may

exhibit pseudocatalase activity especially if grown under glucose limitation. Amygdalin, cellobiose, aesculin, D-fructose, galactose, β-gentiobiose, N-acetylglucosamine, D-glucose, lactose, maltose, mannitol, D-mannose, melibiose, trehalose, salicin and sucrose are fermented. Adonitol, D-arabinose, L-arabitol, erythritol, DL-fucose, 5-ketogluconate, methyl β-xylose, 2-ketogluconate, inositol, inulin, D-lyxose, L-sorbitol, D-tagatose and DL-xylose are not fermented. Arginine is not deaminated. *meso*-Diaminopimelic acid is present in the cell wall and ribitol or glycerol have been described to be present as teichoic acids. G + C content of the DNA is 44–46 mol%. Considering phylogenetic placement based on 16S rRNA gene sequence analysis, *L. plantarum* belongs to the *L. plantarum* phylogenetic subgroup (Hammes & Hertel, 2003), very closely related to *L. paraplantarum* and *L. pentosus*. The three species are easily distinguishable by means of molecular techniques: Southern hybridization with a *pyr* probe on *Bgl*I digestion of chromosomal DNA (Bringel *et al.*, 1996, 2001), multiplex species-specific PCR analysis (Torriani *et al.*, 2001) and *recA* gene sequencing. Such techniques also allow the differentiation of the two subspecies of *L. plantarum*, *L. plantarum* subsp. *plantarum* and *L. plantarum* subsp. *argentoratensis*. When analysed by a specific multiplex-PCR approach (Torriani *et al.*, 2001), DNA of strains belonging to *L. plantarum* subsp. *plantarum* produce a single amplification product of 318 bp. A rough separation of the two subspecies of *L. plantarum* may also be obtained based on fermentation profiles of dulcitol, melezitose, methyl α-D-mannoside, L-arabinose, D-turanose (Table 2): usually, *L. plantarum* subsp. *plantarum* strains do not ferment dulcitol but do metabolize melezitose, methyl α-D-mannoside, L-arabinose and D-turanose.

The type strain is ATCC 14917^T (= NCDO 1752^T = DSM 20174^T).

Description of *Lactobacillus plantarum* subsp. *argentoratensis* subsp. nov.

Lactobacillus plantarum subsp. *argentoratensis* (ar.gen.to.ra.ten'sis. L. masc. adj. *argentoratensis* of or pertaining to Argentorātus, the Roman name of the city of Strasbourg in Alsace, France, where the 14 strains were first collected and analysed).

Morphological, physiological and biochemical characteristics are the same as those of *L. plantarum* subsp. *plantarum*, with only a few exceptions: strains belonging to *L. plantarum* subsp. *argentoratensis* are not able to metabolize either melezitose or methyl α-D-mannoside. Strains of this subspecies may be reliably differentiated from strains of *L. plantarum* subsp. *plantarum* by Southern hybridization with a *pyr* probe on *Bgl*I digestion of chromosomal DNA (Bringel *et al.*, 1996, 2001), by multiplex species-specific PCR analysis (Torriani *et al.*, 2001) and by *recA* gene sequencing, as reported in the present paper. When analysed by a specific multiplex-PCR approach (Torriani *et al.*, 2001), DNA of strains belonging to this subspecies produce two

amplification products, of 318 bp (specific for the species *L. plantarum*) and 120 bp (specific for the *L. plantarum* subsp. *argenteratensis* subspecies).

The type strain is DKO 22^T (=CIP 108320^T=DSM 16365^T).

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