**Pediococcus argentinicus** sp. nov. from Argentinean fermented wheat flour and identification of *Pediococcus* species by *pheS*, *rpoA* and *atpA* sequence analysis

Katrien De Bruyne,1 Charles M. A. P. Franz,2 Marc Vancanneyt,3 Ulrich Schillinger,2 Fernanda Mozzi,4 Graciela Font de Valdez,4 Luc De Vuyst5 and Peter Vandamme1

Correspondence
Katrien De Bruyne
Katrien.DeBruyne@UGent.be

1Laboratory of Microbiology, Ghent University, Ledeganckstraat 35, B-9000 Ghent, Belgium
2Federal Research Centre for Nutrition and Food, Institute of Hygiene and Toxicology, Haid-und-Neu-Strasse 9, D-76131 Karlsruhe, Germany
3BCCM/LMG Bacteria Collection, Ghent University, Ledeganckstraat 35, B-9000 Ghent, Belgium
4Centro de Referencia para Lactobacilos (CERELA)-CONICET, Chacabuco 145, 4000 San Miguel de Tucumán, Argentina
5Research Group of Industrial Microbiology and Food Biotechnology (IMDO), Department of Applied Biological Sciences and Engineering, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium

A Gram-positive, small coccus-shaped lactic acid bacterium, strain LMG 23999T, was isolated from Argentinean wheat flour. 16S rRNA gene sequence analysis revealed that the phylogenetic position of the novel strain was within the genus *Pediococcus*, with *Pediococcus stilesii*, *Pediococcus pentosaceus* and *Pediococcus acidilactici* as its closest relatives (97.7, 97.3 and 96.9 % gene sequence similarity, respectively). Fluorescent amplified fragment length polymorphism fingerprinting of whole genomes and whole-cell protein electrophoresis confirmed the unique taxonomic status of the novel strain. DNA–DNA hybridizations, DNA G+C content determination, comparative sequence analysis of the *pheS*, *rpoA* and *atpA* genes and physiological and biochemical characterization demonstrated that strain LMG 23999T (≡CCUG 54535≡CRL 776) represents a novel species for which the name *Pediococcus argentinicus* sp. nov. is proposed. Multi-locus sequence analysis based on *pheS*, *rpoA* and *atpA* genes was found to be a suitable method for the identification of species of the genus *Pediococcus*.

**Abbreviations:** FAFLP, fluorescent amplified fragment length polymorphism; LAB, lactic acid bacteria; T<sub>OR</sub>, temperature of optimal renaturation.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of LMG 23999T is AM709786. The accession numbers for the *pheS*, *rpoA* and *atpA* gene sequences reported in this paper are AM899805–AM899946, as indicated in Supplementary Fig. S2a–c.

Supplementary figures showing a cluster analysis of whole cell protein profiles with a UPGMA dendrogram and additional neighbour-joining phylogenetic trees based on *pheS*, *rpoA* and *atpA* gene sequences are available with the online version of this paper. A supplementary table giving details of the genomes used for the design of primers rpo-21-F* and rpoA-23-R* is also provided.
(atpA) was developed as an alternative approach for the identification of species of the genus *Pediococcus*. Special attention was give to *Pediococcus dextrinicus* as it represents a distantly related species. Konstantinidis *et al.* (2006) considered that three was the minimum number of genes needed for multilocus, sequence-based differentiation between species. This number of genes is required in order to exclude possible horizontal gene transfer or recombination events. The three genes used in this study have already given satisfactory results with several genera of lactic acid bacteria (De Bruyne *et al.*, 2007; Naser *et al.*, 2005a, b, 2007).

Strain LMG 23999\(^T\) (=CRL 776\(^T\)) was isolated in 1990 in the Centro de Referencia para Lactobacilos (CERELA, CONICET), San Miguel de Tucumán, Argentina. Wheat flour was mixed with warm sterilized tap water and after a fermentation of 4–6 days at 30 °C, samples of the dough were taken. Samples were diluted to 10\(^{-6}\) in peptone water (0.1 % w/v) and plated onto LAP\(\_\)Tg agar using the pour plate technique. Plates were incubated aerobically at 37 °C for 48 h. The strain was subcultured onto MRS agar at 37 °C, unless indicated otherwise.

The phylogenetic position of strain LMG 23999\(^T\) was first determined by analysis of its 16S rRNA gene sequence, as described by Vancanneyt *et al.* (2004) using the following modifications. The PCR-amplified 16S rRNA gene was purified by using a NucleoFast 96 PCR Clean-up kit (Macherey-Nagel). Sequencing reactions were purified using a Montage SEQ96 Sequencing Reaction Clean-up kit (Millipore). Electrophoresis of sequence reaction products was performed by using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems). FASTA analysis of the 16S rRNA gene sequence of strain LMG 23999\(^T\) (a continuous stretch of 1492 bp) revealed that *Pediococcus stilesii*, *Pediococcus pentosaceus* and *Pediococcus acidilactici* were the closest relatives (with 97.7, 97.3 and 96.9 % sequence similarity, respectively). The 16S rRNA gene sequence of strain LMG 23999\(^T\) and sequences of reference strains (retrieved from EMBL) were aligned using CLUSTAL\_X. A neighbour-joining phylogenetic tree was constructed using the BioNumerics software package, version 4.61 (Applied Maths). The statistical reliability of the tree topology was evaluated by bootstrapping analysis (Fig. 1). The phylogenetic tree of the genus *Pediococcus* consisted of two separate clades. The clade containing strain LMG 23999\(^T\) also included *Pediococcus clausenii*, *P. stilesii*, *P. acidilactici* and *P. pentosaceus*. The second clade comprised *Pediococcus ethanolidurans*, *Pediococcus siamensis*, *Pediococcus celliloca*, *Pediococcus damnosus*, *Pediococcus inopinatus* and *Pediococcus parvulus*. The species *P. dextrinicus* represented a divergent line which was not surprising as this species is atypical of the genus *Pediococcus* and it has been suggested that *P. dextrinicus* may represent a novel genus (Holzapfel *et al.*, 2005).

SDS-PAGE of whole-cell proteins and fluorescent amplified fragment length polymorphism (FAFLP) analysis were used to compare strain LMG 23999\(^T\) with other strains of the genus *Pediococcus*, especially strains belonging to its nearest phylogenetic neighbours *P. stilesii*, *P. pentosaceus* and *P. acidilactici*. The protein profiles of all reference strains used were from a previous study (Franz *et al.*, 2006). SDS-PAGE of cellular proteins from strain LMG 23999\(^T\) was performed as described by Pot *et al.* (1994). Densitometric analysis, normalization and interpolation of protein profiles and numerical analysis were performed by the use of BioNumerics software package, version 4.61 (Applied Maths). When compared with those of the *Pediococcus* reference strains, the whole-cell protein profile of strain LMG 23999\(^T\) was well separated from *P. stilesii*, *P. pentosaceus* and *P. acidilactici*, its nearest phylogenetic

\[
\begin{align*}
P. clausenii & \text{ DSM 14800}^T (AJ621555) \\
P. argentinicus & \text{ sp. nov. LMG 23999}^T (AM709786) \\
P. stilesii & \text{ LMG 23082}^T (AJ973157) \\
P. acidilactici & \text{ LMG 11384}^T (AJ305320) \\
P. pentosaceus & \text{ LMG 11488}^T (AJ930321) \\
P. ethanolidurans & \text{ Z-9}^T (AY956789) \\
P. siamensis & \text{ JCM 13997}^T (AB258357) \\
P. cellicola & \text{ LMG 22956}^T (AY956788) \\
P. damnosus & \text{ LMG 11484}^T (AJ318414) \\
P. inopinatus & \text{ LMG 11409}^T (AJ271383) \\
P. parvulus & \text{ LMG 11486}^T (D88528) \\
P. dextrinicus & \text{ LMG 11485}^T (D87579) \\
\text{Lactobacillus delbrueckii subsp. delbrueckii} & \text{ LMG 6412}^T (AY050172)
\end{align*}
\]

![Fig. 1. Phylogenetic neighbour-joining tree based on 16S RNA gene sequence analysis showing the phylogenetic relationships of *Pediococcus argentinicus* sp. nov. LMG 23999\(^T\) within the genus *Pediococcus*. *Lactobacillus delbrueckii* subsp. *delbrueckii* LMG 6412\(^T\) was used as the outgroup organism. Bootstrap percentage values (>50) based on 500 tree replications are indicated at the branching points. Bar, 1 % sequence divergence.](image-url)
neighbours (see Supplementary Fig. S1, available in IJSEM Online). The unique position of strain LMG 23999<sup>T</sup> was confirmed by FAFLP analysis. Already available FAFLP fingerprint patterns of <i>Pediococcus</i> reference strains were used (Franz et al., 2006) and additional patterns for <i>P. ethanolidurans</i>, <i>P. siamensis</i> and <i>P. argentinicus</i> were generated as described by Franz et al. (2006). The resulting electrophoretic patterns were tracked and normalized using the GENESCAN 3.1 software package (Applera). Normalized tables of peaks were transferred into the BioNumerics software package, version 4.61 (Applied Maths). The FAFLP fingerprint of strain LMG 23999<sup>T</sup> was compared with reference profiles of its closest phylogenetic neighbours and with those of the type strains of all recognized species within the genus <i>Pediococcus</i> (Fig. 2). The results confirmed that strain LMG 23999<sup>T</sup> was very different from its nearest neighbours and from other recognized species of the genus <i>Pediococcus</i>.

Genomic DNA of strain LMG 23999<sup>T</sup> and of <i>P. stilesii</i> LMG 23082<sup>T</sup>, <i>P. pentosaceus</i> LMG 11488<sup>T</sup> and <i>P. acidilactici</i> LMG 11384<sup>T</sup> was isolated according to Marmur (1961), as modified by Stackebrandt & Kandler (1979). DNA–DNA hybridizations were performed using the spectrophotometric method as described by De Ley et al. (1970). Hybridization values of strain LMG 23999<sup>T</sup> towards strains LMG 23082<sup>T</sup> (<i>T<sub>OR</sub></i>: 68.4 °C), LMG 11488<sup>T</sup> (<i>T<sub>OR</sub></i>: 67.8 °C) and LMG 11384<sup>T</sup> (<i>T<sub>OR</sub></i>: 69.7 °C) were 24, 25 and 21%, respectively, confirming that strain LMG 23999<sup>T</sup> represents a novel species (Wayne et al., 1987). The DNA G+C content of strain LMG 23999<sup>T</sup> was determined as described by Mesbah et al. (1989) using a Waters Breeze HPLC system and XBridge Shield RP18 column. The solvent used was 0.02 M NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> (pH 4.0), 1.5% (v/v) acetonitrile. Non-methylated lambda phage (Sigma) and <i>Escherichia coli</i> LMG 2093 DNA were used as the calibration reference and control, respectively. The DNA G+C content of strain LMG 23999<sup>T</sup> was 40.8 mol%. This value was consistent with the DNA G+C contents observed in the genus <i>Pediococcus</i>, which are 37–42 mol% (Dobson et al., 2002; Liu et al., 2006; Sneath et al., 1986; Tanasupawat et al., 2007; Zhang et al., 2005).

As already investigated for the genera <i>Lactobacillus</i> (Naser et al., 2007), <i>Enterococcus</i> (Naser et al., 2005a, b) and <i>Leuconostoc</i> (De Bruyne et al., 2007), we evaluated whether pheS, rpoA and atpA gene sequence analysis could be used to differentiate species of the genus <i>Pediococcus</i>. In order to assess inter- and intra-species variability among the loci, a total of 44 <i>Pediococcus</i> strains was examined: 18 reference strains and 26 field isolates which were additionally identified through AFLP and SDS-PAGE of whole-cell proteins. Several representative strains per species were included when possible. Bacterial strains, depositors and sources are listed in Table 1. The design of the primers, amplification conditions and sequencing reactions were as described by Naser et al. (2005a, b). The primer combinations pheS-21-F/pheS-23-R, rpoA-21-F/rpoA-23-R and atpA-20-F/atpA-26-R amplified the target genes of most strains. When no amplification product for the <i>atpA</i> gene was obtained, an alternative primer set atpA-22-F/atpA-26-R was used. For the amplification of the <i>rpoA</i> genes of <i>P. pentosaceus</i> strains LMG 13561, LMG 13562,
Table 1. List of *Pediococcus* strains used in this study

<table>
<thead>
<tr>
<th>Strains</th>
<th>Depositor*</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. argentinicus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LMG 23999&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Own isolate</td>
<td>Commercial wheat flour (1990, Argentina)</td>
</tr>
<tr>
<td><em>P. acidilactici</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LMG 6411</td>
<td>DSMZ</td>
<td>Not known</td>
</tr>
<tr>
<td>LMG 10635</td>
<td>A. Ledeboer</td>
<td>Plant</td>
</tr>
<tr>
<td>LMG 11384&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Rijkszuivelstation, Melle</td>
<td>Barley</td>
</tr>
<tr>
<td>LMG 13362</td>
<td>NV Radar, Deinze</td>
<td>Grass sample (Belgium)</td>
</tr>
<tr>
<td>LMG 13369</td>
<td>NV Radar, Deinze</td>
<td>Grass sample (Belgium)</td>
</tr>
<tr>
<td>LMG 17680</td>
<td>G. Rusul</td>
<td>Chili bo (Malaysia)</td>
</tr>
<tr>
<td><em>P. cellicola</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LMG 22956&lt;sup&gt;T&lt;/sup&gt;</td>
<td>CGMCC</td>
<td>Distilled-spirit-fermenting cellar wall (2004, China)</td>
</tr>
<tr>
<td><em>P. clausenii</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LMG 21948&lt;sup&gt;T&lt;/sup&gt;</td>
<td>DSMZ</td>
<td>Spoiled beer (Canada)</td>
</tr>
<tr>
<td>LAB 990</td>
<td>Heineken</td>
<td>Beer (1994, The Netherlands)</td>
</tr>
<tr>
<td>LAB 994</td>
<td>Heineken</td>
<td>Beer (1994, The Netherlands)</td>
</tr>
<tr>
<td>LAB 1004</td>
<td>Heineken</td>
<td>Beer (1994, The Netherlands)</td>
</tr>
<tr>
<td>LAB 1014</td>
<td>Heineken</td>
<td>Beer (1994, The Netherlands)</td>
</tr>
<tr>
<td>LAB 1231</td>
<td>Heineken</td>
<td>Beer (1994, The Netherlands)</td>
</tr>
<tr>
<td>LAB 1232</td>
<td>Heineken</td>
<td>Beer (1994, The Netherlands)</td>
</tr>
<tr>
<td>LAB 1329</td>
<td>Heineken</td>
<td>Beer (1994, The Netherlands)</td>
</tr>
<tr>
<td><em>P. damnosus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LMG 11484&lt;sup&gt;T&lt;/sup&gt;</td>
<td>NCFB</td>
<td>Lager beer yeast</td>
</tr>
<tr>
<td>LAB 1330</td>
<td>Heineken</td>
<td>Beer (1994, Belgium)</td>
</tr>
<tr>
<td>LAB 1362</td>
<td>W. Simpson</td>
<td>Not known</td>
</tr>
<tr>
<td>LAB 1449</td>
<td>I. Bohack</td>
<td>Not known</td>
</tr>
<tr>
<td><em>P. dextrinicus</em></td>
<td></td>
<td></td>
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<tr>
<td>LMG 11485&lt;sup&gt;T&lt;/sup&gt;</td>
<td>NCFB</td>
<td>Silage</td>
</tr>
<tr>
<td><em>P. ethanolidurans</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LMG 23957&lt;sup&gt;T&lt;/sup&gt;</td>
<td>CGMCC</td>
<td>Distilled-spirit-fermenting cellar wall (2004, China)</td>
</tr>
<tr>
<td>LMG 13386</td>
<td>NV Radar, Deinze</td>
<td>Grass sample (Belgium)</td>
</tr>
<tr>
<td>LMG 13387</td>
<td>NV Radar, Deinze</td>
<td>Grass sample (Belgium)</td>
</tr>
<tr>
<td><em>P. inopinatus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LMG 11400&lt;sup&gt;T&lt;/sup&gt;</td>
<td>DSMZ</td>
<td>Brewery yeast</td>
</tr>
<tr>
<td>LMG 11410</td>
<td>DSMZ</td>
<td>Beer</td>
</tr>
<tr>
<td>LMG 22104</td>
<td>J. Leisner</td>
<td>Beer (Czech Republic)</td>
</tr>
<tr>
<td>LMG 22105</td>
<td>J. Leisner</td>
<td>Beer (Czech Republic)</td>
</tr>
<tr>
<td>LAB 1451</td>
<td>I. Bohack</td>
<td>Not known</td>
</tr>
<tr>
<td>LAB 1454</td>
<td>I. Bohack</td>
<td>Not known</td>
</tr>
<tr>
<td><em>P. parvulus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LMG 11486&lt;sup&gt;T&lt;/sup&gt;</td>
<td>NCFB</td>
<td>Silage</td>
</tr>
<tr>
<td>LMG 16740</td>
<td>ATCC</td>
<td>Wine (Australia)</td>
</tr>
<tr>
<td>LAB 194</td>
<td>R. Vogel</td>
<td>Wine</td>
</tr>
<tr>
<td><em>P. pentosaceus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LMG 10634</td>
<td>Unilever, Vlaardingen</td>
<td>Fermented milk</td>
</tr>
<tr>
<td>LMG 11488&lt;sup&gt;T&lt;/sup&gt;</td>
<td>NCFB</td>
<td>Dried American beer yeast (1931)</td>
</tr>
<tr>
<td>LMG 13392</td>
<td>NV Radar, Deinze</td>
<td>Grass sample (Belgium)</td>
</tr>
<tr>
<td>LMG 13561</td>
<td>TNO-Voeding, Zeist</td>
<td>Not known</td>
</tr>
<tr>
<td>LMG 13562</td>
<td>TNO-Voeding, Zeist</td>
<td>Not known</td>
</tr>
<tr>
<td>LMG 13652</td>
<td>L. A. Devriese</td>
<td>Cat (Belgium)</td>
</tr>
<tr>
<td>LMG 13377</td>
<td>NV Radar, Deinze</td>
<td>Grass sample (Belgium)</td>
</tr>
<tr>
<td>LMG 17236</td>
<td>L. A. Devriese</td>
<td>Sow (Belgium)</td>
</tr>
<tr>
<td>LMG 17237</td>
<td>L. A. Devriese</td>
<td>Sow (Belgium)</td>
</tr>
<tr>
<td><em>P. siamensis</em></td>
<td></td>
<td></td>
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<tr>
<td>LMG 24279&lt;sup&gt;T&lt;/sup&gt;</td>
<td>JCM</td>
<td>Fermented tea leaves (2007, Thailand)</td>
</tr>
<tr>
<td><em>P. stilesii</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LMG 23082&lt;sup&gt;T&lt;/sup&gt;</td>
<td>W. Holzapfel</td>
<td>White maize grains (1997, Nigeria)</td>
</tr>
</tbody>
</table>
Oenococcus strains and one strain of Lactococcus containing 32 genomes (see Supplementary Table S1 in IJSEM Online). 21-F and rpoA-23-R were evaluated against 53 LAB was solved by designing new primers. The primers rpoA-21-F and rpoA-23-R were evaluated against 53 LAB genomes (see Supplementary Table S1 in IJSEM Online) containing 32 Streptococcus strains, two Lactobacillus strains, three Lactococcus strains, two Enterococcus strains, two Oenococcus strains and one strain of Leuconostoc mesenteroides subsp. mesenteroides and, most important for the present study, *P. pentosaceus* ATCC 25745 (GenBank accession no. CP000422). The evaluation was performed using the genomic BLAST tool of NCBI (http://www.ncbi.nlm.nih.gov/sutils/genom_table.cgi). Starting from the rpoA amino acid sequence of *Leuconostoc mesenteroides* subsp. mesenteroides LMG 6893T translated from the gene sequence GenBank accession no. AM711294, a BLASTP sequence similarity search was performed against protein databases of genomes of all the members of the order Lactobacillales in this study (Fig. 3), an observation that strongly supported the two options already suggested in the literature: to transfer *P. dextrinicus* to a new genus (Holzapfel et al., 1990, 1991; Stiles & Holzapfel, 1997), sequence data of the type strains of all species of the genus *Lactobacillus casei* group were included in the sequence analyses (Fig. 3, Supplementary Fig. S2a–c). *P. dextrinicus* represented the most divergent lineage in each of the analyses. This observation strongly supported the two

| Table 1. cont. |

| ATCC, American Type Culture Collection; I. Bohack, Lehrstuhl für Technologie der Brauerei I, Technische Universität München, Germany; CGMCC, China General Microbiological Culture Collection Centre; L. A. Devriese, Ghent University, Ghent, Belgium; DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen; W. Holzapfel, Institute of Hygiene and Toxicology, Karlsruhe, Germany; JCM, Japan Collection of Microorganisms; A. Ledeboer, Unilever, Vlaardingen, The Netherlands; J. Leisner, Royal Veterinary and Agricultural University, Frederiksberg Copenhagen, Denmark; LMG, Belgian Co-ordinated Collections/Laboratory of Microbiology Ghent, Ghent, Belgium; NCFB, National Collection of Food Bacteria (now NCIMB); NCIMB, National Collections of Industrial, Food and Marine Bacteria, Aberdeen, UK; G. Rusul, University Putra, Malaysia; W. Simpson, BRF International, Surrey, UK; R. Vogel, Universität Hohenheim, Stuttgart, Germany. |

LMG 11488T, LMG 13377 and LMG 17237 and *P. stilesii* LMG 23082T, no amplicon was obtained despite using different sets of primers and amplification conditions as described by Naser et al. (2005b). The objective of identifying a maximum number of lactic acid bacteria (LAB) strains using the same amplification protocol (Naser et al., 2005a, b) has led to the use of a standard protocol, which is probably not the most optimal protocol for some members of the genus *Pediococcus*. Failure due to DNA sequence variability in these genes, which prevented annealing of the PCR primers to their target sequences, was solved by designing new primers. The primers rpoA-21-F and rpoA-23-R were evaluated against 53 LAB genomes (see Supplementary Table S1 in IJSEM Online) containing 32 Streptococcus strains, 12 Lactobacillus strains, three Lactococcus strains, two Enterococcus strains, two Oenococcus strains and one strain of *Leuconostoc mesenteroides* subsp. mesenteroides and, most important for the present study, *P. pentosaceus* ATCC 25745 (GenBank accession no. CP000422). The evaluation was performed using the genomic BLAST tool of NCBI (http://www.ncbi.nlm.nih.gov/sutils/genom_table.cgi). Starting from the rpoA amino acid sequence of *Leuconostoc mesenteroides* subsp. mesenteroides LMG 6893T translated from the gene sequence GenBank accession no. AM711294, a BLASTP sequence similarity search was performed against protein databases of genomes of all the members of the order Lactobacillales present in the database at time of writing (strain information and accession numbers are listed in Supplementary Table S1). The rpoA gene identities obtained were used to retrieve the rpoA nucleotide sequences from the whole genomes. Together with the primer sequences, all rpoA gene sequences were used to perform sequence alignments using CLUSTAL_X. The position of primers within the alignment was verified and substitutions were checked manually. An A/G substitution specific for the *Pediococcus* strains could be found in primer sequence rpoA-21-F and a T/A and a C/G substitution were found in primer sequence rpoA-23-R. Taking into account these substitutions and the information from the other 52 LAB genomes, primers rpoA-21-F and rpoA-23-R were modified to rpoA-21-F* (5’-ATGATYGARTTTGARAAARC-3’) and rpoA-23-R* (5’-ACHTRTRATTACCDGCNCG-3’). Using these modified primers, rpoA products for all the remaining strains were obtained.

The phylogenetic trees constructed for the pheS, rpoA and atpA gene sequences are based on the neighbour-joining method and were obtained by importing the external sequence alignments from CLUSTAL_X into the BioNumerics software package. For each of the genes, the intra-species diversity was smaller than the inter-species diversity and, therefore, numerical analysis yielded species-specific clusters (see Supplementary Figs S2a–c in IJSEM Online). The topologies of the neighbour-joining phylogenetic trees roughly resembled those of the 16S rRNA gene-based phylogeny. To resolve the ambiguous position of *P. dextrinicus*, for which suggestions for it to be reclassified next to the *Lactobacillus casei* group have already been made (Collins et al., 1990, 1991; Stiles & Holzapfel, 1997), sequence data of the type strains of all species of the genus *Lactobacillus casei* group were included in the sequence analyses (Fig. 3, Supplementary Fig. S2a–c). *P. dextrinicus* represented the most divergent lineage in each of the analyses. This observation strongly supported the two options already suggested in the literature: to transfer *P. dextrinicus* to a new genus (Holzapfel et al., 2005) or to reclassify this species as a novel species of the genus *Lactobacillus*, close to the *Lactobacillus casei* group (Collins et al., 1990, 1991; Stiles & Holzapfel, 1997). To date, no official proposal has been made for this change (Dobson et al., 2002). For the remaining pediococci species, both rpoA and atpA gene analysis yielded the same division into two clades, corresponding to the 16S rRNA gene sequence phylogeny. Analysis of the pheS gene showed a slightly different topology; it was however the most discriminatory gene for the identification of species of the genus *Pediococcus*. By integrating information from different molecular markers, the simultaneous use of several housekeeping genes offers a higher reliability for bacterial identification (Fig. 3) (Konstantinidis et al., 2006). Using the concatenated gene sequences, all *Pediococcus* isolates could be classified into species-specific clusters. In addition, two previously unidentified *Pediococcus* isolates from grass silage in Belgium were identified as *P. ethanololituras* in this study (Fig. 3), an observation that was confirmed by AFLP analysis and SDS-PAGE of whole-cell proteins.

Morphological, physiological and biochemical tests were performed according to Schillinger & Lücke (1987). Lactate enantiomer production was determined using an enzymic test kit (Roche Diagnostics). After 48 h growth, culture...
supernatants comprised more than 90% L-lactate. After a prolonged incubation of 4 days, a DL-lactate mixture was obtained (87.6% L-lactate, 12.4% D-lactate). The API 50 CHL identification system (bioMérieux) was used to determine the carbohydrate fermentation profile. Morphological and physiological characteristics are given in the species description. An overview of the physiological differences between the novel species and the most closely related species is presented in Table 2. It is evident from these summarized physiological data that strain LMG 23999T can be distinguished from related species of the genus *Pediococcus* by a combination of acid production tests from sugars (arabinose, galactose, lactose, maltose, mannitol, methyl α-D-mannopyranoside and xylose), NaCl tolerance tests and by the lactic acid configuration.

The results of the polyphasic analysis demonstrate that strain LMG 23999T represents a novel species within the genus *Pediococcus*, for which we propose the name *Pediococcus argentinicus* sp. nov. Sequence analysis of the
Table 2. Phenotypic characteristics that differentiate Pediococcus argentinicus sp. nov. from the most closely related species of the genus Pediococcus

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<tbody>
<tr>
<td>Growth at 9.0 pH</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>40 °C</td>
<td>+</td>
<td>+</td>
<td>d</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>45 °C</td>
<td>–</td>
<td>+</td>
<td>d</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>48 °C</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Lactic acid configuration</td>
<td>L(+)</td>
<td>DL</td>
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<td>L(+)</td>
<td>DL</td>
</tr>
<tr>
<td>Acid from:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arabinose</td>
<td>–</td>
<td>d</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Galactose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Lactose</td>
<td>–</td>
<td>d</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Maltose</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>d</td>
<td>+</td>
</tr>
<tr>
<td>Mannitol</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>d</td>
<td>–</td>
</tr>
<tr>
<td>Methyl D-mannopyranoside</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Xylose</td>
<td>–</td>
<td>+</td>
<td>d</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Max. NaCl concentration for growth (% w/v)</td>
<td>6</td>
<td>10</td>
<td>10</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>40.8</td>
<td>42.0</td>
<td>38.0</td>
<td>40.5</td>
<td>38.0</td>
</tr>
</tbody>
</table>

*pheS, rpoA and atpA genes proved to be a valuable technique for the differentiation of recognized species of the genus Pediococcus.*

**Description of Pediococcus argentinicus sp. nov.**

*Pediococcus argentinicus* (ar.gen’ti.ni.cus. N.L. masc. adj. argentinicus pertaining to Argentina).

After 24 h growth, cells are small coccis (0.7–1.0 μm) and occur singly or in pairs. They are Gram-positive, do not form spores and no gliding motility is observed. Colonies are greyish white, opaque, smooth and circular with a convex elevation and an entire margin. Both D- and L-lactate are produced as end products of glucose metabolism. Able to grow at pH values of 4–8 and at temperatures up to 40 °C. The maximum NaCl concentration for growth is 6% (w/v). Acid is produced from glucose, ribose, galactose, fructose, mannose, mannotol, methyl D-mannopyranoside, N-acetylglucosamine, amygdalin, arbutin, aesculin, salicin, cellobiose, maltose, sucrose, gentiobiose and tagatose. Acid is not produced from glycerol, erythritol, D-arabinose, D-xylose, L-xyllose, adonitol, methyl β-D-xylpyranoside, sorbose, rhamnose, dulcitol, inositol, sorbitol, methyl D-glucopyranoside, lactose, melibiose, trehalose, inulin, melezitose, raffinose, glycosgen, xylositol, turanose, lyxose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate or potassium 5-ketogluconate.

The type strain, LMG 23999T (=CCUG 54535T=CRM 776T), was isolated from Argentinean fermented wheat flour. The DNA G+C content of the type strain is 40.8 mol%.

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**References**


