**Pediococcus lolii** DSM 19927\(^T\) and JCM 15055\(^T\) are strains of *Pediococcus acidilactici*  

Anneleen Wieme,\(^1,2\) Ilse Cleenwerck,\(^3\) Anita Van Landschoot\(^1,2\) and Peter Vandamme\(^2\)

\(^1\)Laboratory of Biochemistry and Brewing, Faculty Applied Bioscience Engineering, University College Ghent, Schooneeenstraat 52, B-9000 Ghent, Belgium  
\(^2\)Laboratory of Microbiology, Faculty of Science, Ghent University, K. L. Ledeganckstraat 35, B-9000 Ghent, Belgium  
\(^3\)BCCM/LMG Bacteria Collection, Faculty of Science, Ghent University, K. L. Ledeganckstraat 35, B-9000 Ghent, Belgium

Strain NGRI 05100\(^T\), isolated from ryegrass silage, was recently classified as a representative of a novel *Pediococcus* species, *Pediococcus lolii* Doi et al. 2009. It was deposited in the DSMZ and JCM culture collections as DSM 19927\(^T\) and JCM 15055\(^T\), respectively. A polyphasic taxonomic study, including matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, phe\(S\) and 16S rRNA gene sequence analysis, fluorescent amplified fragment length polymorphism and DNA–DNA hybridization, was used to prove that both subcultures of the type, and only, strain of this species are strains of *Pediococcus acidilactici*.

In 2009, Doi and colleagues reported on a novel strain belonging to the genus *Pediococcus*, NGRI 05100\(^T\), for which they proposed the name *Pediococcus lolii* (Doi et al., 2009). The description was based on a single strain isolated from ryegrass silage that was deposited in the DSMZ and JCM culture collections as DSM 19927\(^T\) and JCM 15055\(^T\), respectively. Their study revealed that the strain exhibited distinct phenotypic characteristics, divergent sequences of the 16S rRNA gene and the 16S–23S rRNA intergenic spacer region, and low rates of DNA–DNA hybridization in comparison with the type strains of *Pediococcus acidilactici* DSM 20284\(^T\) (=LMG 11384\(^T\)) and *Pediococcus pentosaceus* DSM 20336\(^T\) (=LMG 11488\(^T\)).

The present study was initiated upon analysis of the *P. lolii* type strain accessioned from the JCM culture collection, JCM 15055\(^T\) (=LMG 25667\(^T\)), by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), which failed to discriminate *P. lolii* LMG 25667\(^T\) from *P. acidilactici* strains. Subsequently, DSM 19927\(^T\) (=LMG 27029\(^T\)) was accessioned from the DSMZ culture collection and the polyphasic taxonomic study described below was performed.

All strains were grown on MRS agar (Oxoid) at 28 °C in anaerobic atmosphere, except strain *P. acidilactici* LMG 11384\(^T\), which was cultured in an aerobic atmosphere. Prior to MALDI-TOF MS analysis, strains were subcultured twice. Five to ten milligrams of wet cells were suspended in MilliQ-water comprising 75 % pure ethanol. Subsequently, formic acid and acetonitrile were added in a 1 : 1 (v/v) ratio to the bacterial cell pellet. After shaking vigorously, 1 μl supernatant (=the cell extract) was spotted onto a MALDI-TOF MS stainless steel target plate. Spots were overlaid with 1 μl matrix, which consisted of 5 mg x-cyano-4-hydroxycinnamic acid dissolved in 1 ml acetonitrile/trifluoroacetic acid/MilliQ-solvent (50 : 2 : 48). Prior to analysis, the mass spectrometer was externally calibrated using a peptide mixture of adrenocorticotropin hormone (fragment 18–39) (Sigma-Aldrich), insulin (Sigma-Aldrich), ubiquitin (Sigma-Aldrich), cytochrome C (Sigma-Aldrich) and myoglobin (Sigma-Aldrich). A 4800 Plus MALDI TOF/TOF Analyser (Applied Biosystems) was used in linear mode and covered a mass range of 2–20 kDa. The mass spectrometer used a 200 Hz frequency tripled Nd:YAG laser, operating at a wavelength of 355 nm. Generated ions were accelerated at 20 kV through a grid at 19.2 kV into a short, linear, field-free drift region onto the detector. For each spot, 50 subspectra for each of 40 randomized positions within the spot were collected and presented as one main spectrum. The laser intensity was set between 4300 and 5100. Spectral profiles were retrieved via the 4000 Series Explorer software (Applied Biosystems) and objectively scored for several parameters (signal quality, intensity and the number of peaks). Data Explorer 4.0 software (Applied Biosystems) was used to convert the profiles into .txt files to import them into a

---

**Abbreviations:** MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; MLSA, multi-locus sequence analysis; FAFLP, fluorescent amplified fragment length polymorphism.

The GenBank/EMBL/DDBJ accession numbers for the phe\(S\) gene sequences of LMG 25667\(^T\) and LMG 27029\(^T\) are HE962540 and HE962541, respectively.
BioNumerics 5.1 database (Applied Maths, Belgium). Spectral profiles were compared using the Pearson product moment correlation coefficient and a dendrogram was built using the unweighted pair group method with arithmetic mean (UPGMA)-cluster algorithm (data not shown). Both subcultures, LMG 25667<sup>T</sup> and LMG 27029<sup>T</sup>, clustered together with the <i>P. acidilactici</i> reference strains present in the database. The database used included a set of 36 <i>Pediococcus</i> reference strains representing all established species, examined previously by means of multi-locus sequence analysis (MLSA) (De Bruyne et al., 2008). The MALDI-TOF MS profiles of

**Fig. 1.** Comparison of the MALDI-TOF MS profiles of both <i>P. lolii</i> subcultures LMG 25667<sup>T</sup> and LMG 27029<sup>T</sup>, <i>P. acidilactici</i> LMG 11384<sup>T</sup> and <i>P. pentosaceus</i> LMG 11488<sup>T</sup> using the mMass 5.1.0 software (Strohalm et al., 2010).

**Fig. 2.** Maximum-likelihood tree based on <i>pheS</i> gene sequences showing the phylogenetic relationships of the two subcultures of <i>P. lolii</i>. Bootstrap percentage values (>50 %), based on 1000 replications, are shown at branch points. The substitution model used is the Tamura 3-parameter model and the aligned sequences have a length of 344 bp. Bar, 0.1 % sequence divergence.
both LMG 25667T and LMG 27029T were indistinguishable from those of 13 P. acidilactici reference strains (data not shown). Fig. 1 was generated using the mMass 5.1.0 software (Strohalm et al., 2010) and shows the high similarity between the MALDI-TOF MS profiles of both P. lolii subcultures and that of P. acidilactici LMG 11384T, in contrast with that of P. pentosaceus LMG 11488T.

Reliable identification of Pediococcus strains and many other lactic acid bacteria is complicated by their ambiguous response in traditional physiological tests and methods. Also the sequence divergence of the 16S rRNA gene fails to discriminate between closely related species and therefore, the use of protein-coding gene sequence data for the determination of genomic relatedness at the species and genus levels is more appropriate (Naser et al., 2005, 2007). MLSA data of the genes encoding the alpha subunit of phenylalanyl-tRNA synthase (pheS), RNA polymerase (rpoA) and ATP synthase (atpA) were generated by De Bruyne et al. (2008) as a superior approach for species level identification of pediococci. In that scheme, the pheS gene is the most variable gene. Therefore, the pheS gene was amplified and sequenced as described previously (De Bruyne et al., 2008). The MEGA package version 5.05 (Tamura et al., 2011) was used to align and analyse the pheS sequences of LMG 25667T, LMG 27029T and all sequences of the type strains of all established Pediococcus species. The neighbour-joining, maximum-parsimony and maximum-likelihood methods were used to analyse the sequences. The statistical reliability of the tree topologies was established by bootstrapping analysis based on 1000 tree replicates. The neighbour-joining tree and maximum-parsimony tree revealed topologies similar to those obtained in a phylogenetic tree based on the maximum-likelihood method (Fig. 2). The pairwise similarity matrix revealed that the strain P. lolii LMG 25667T has 100, 97.7 and 82.0% sequence similarities with P. lolii LMG 27029T, P. acidilactici LMG 11384T and P. pentosaceus LMG 11484T, respectively. The high degree of similarity between the P. lolii and P. acidilactici strains indicates that these strains represent the same species.

Similarly, fluorescent amplified fragment length polymorphism (FAFLP) profiles of P. lolii LMG 25667T and LMG 27029T were generated as described previously and compared with FAFLP profiles of Pediococcus reference strains generated previously (Franz et al., 2006; De Bruyne et al., 2008; Sistek et al., 2012). The resulting electrophoretic patterns were tracked and normalized using the Gene Mapper 4.0 software package (Applera) and normalized tables of peaks were transferred into the BioNumerics software package, version 5.1 (Applied Maths, Belgium). The FAFLP fingerprints of LMG 25667T and LMG 27029T proved to be similar to those from P. acidilactici strains (Fig. 3).

Genomic DNAs of strain P. lolii LMG 25667T, P. acidilactici LMG 11384T and P. pentosaceus LMG 11488T were extracted and purified as described by Marmur (1961) and modified by Stackebrandt & Kandler (1979). DNA–DNA hybridizations were performed using the microplate method, with photobiotin for labelling the DNA (Ezaki et al., 1989), as modified by Goris et al. (1998). The mean DNA–DNA hybridization values of strain LMG 25667T
with P. acidilactici LMG 11384<sup>T</sup> and P. pentosaceus LMG 11488<sup>T</sup> were 87% (reciprocal values were 82% and 92%) and 19% (reciprocal values were 18% and 19%), respectively, confirming that strain P. lolii LMG 25667<sup>T</sup> is a strain of P. acidilactici.

Finally, complete 16S rRNA gene sequence analysis was performed as described previously (Vancanneyt et al., 2004) for both P. lolii subcultures LMG 25667<sup>T</sup> and LMG 27029<sup>T</sup> to check their authenticity. The 16S rRNA gene sequences of LMG 25667<sup>T</sup> and LMG 27029<sup>T</sup> were identical and differed only in 6 nt (99.3% sequence similarity) from the sequence deposited by Doi et al. (2009). Furthermore, they were 99.5% and 98.3% similar to those of P. acidilactici DSM 20284<sup>T</sup> (AJ305320) and P. pentosaceus DSM 20336<sup>T</sup> (AJ305321), respectively.

On the basis of the evidence presented, we conclude that the two P. lolii NGRI 0510Q<sup>T</sup> subcultures deposited in the DSMZ and JCM culture collections as DSM 19927<sup>T</sup> (=LMG 27029<sup>T</sup>) and JCM 15055<sup>T</sup> (=LMG 25667<sup>T</sup>) belong to P. acidilactici. Whether P. lolii should be considered a junior heterotypic synonym of P. acidilactici depends on the availability of biological material corresponding with the original description of P. lolii by Doi et al. (2009).

**Acknowledgements**

We thank the BCCM/LMG Culture Collection for the provision of the bacterial strains. We thank Cindy Snauwaert and Katrien Engelbeen for the technical support. Financial support for this study was granted by the research fund of the University College Ghent.

**References**


