**Lactobacillus cypricasei** Lawson et al. 2001 is a later heterotypic synonym of *Lactobacillus acidipiscis* Tanasupawat et al. 2000

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The applicability of a multilocus sequence analysis (MLSA)-based identification system for lactobacilli was evaluated. Two housekeeping genes that code for the phenylalanyl-tRNA synthase \(\alpha\)-subunit (pheS) and RNA polymerase \(\alpha\)-subunit (rpoA) were sequenced and analysed for members of the *Lactobacillus salivarius* species group. The type strains of *Lactobacillus acidipiscis* and *Lactobacillus cypricasei* were investigated further using a third gene that encodes the \(\alpha\)-subunit of ATP synthase (atpA). The MLSA data revealed close relatedness between *L. acidipiscis* and *L. cypricasei*, with 99·8–100% pheS, rpoA and atpA gene sequence similarities. Comparison of the 16S rRNA gene sequences of the type strains of the two species confirmed the close relatedness (99·8% gene sequence similarity) between the two taxa. Similar phenotypes and high DNA–DNA binding values in the range of 84 to 97·5% confirmed that *L. acidipiscis* and *L. cypricasei* are synonymous species. On the basis of the present study, it is proposed that *Lactobacillus cypricasei* is a later heterotypic synonym of *Lactobacillus acidipiscis*.

*Lactobacillus acidipiscis* was described by Tanasupawat et al. (2000) based on 11 strains isolated from fermented fish (pla-ra and pla-chom) in Thailand. *L. acidipiscis* utilizes D-glucose homfermentatively and produces L-lactic acid from glucose without the production of gas. *L. acidipiscis* strains do not grow at pH 4·0 or 8·5 and grow in 10% NaCl. Phylogenetic analysis based on 16S rRNA gene sequences showed that *L. acidipiscis* strains are positioned in a monophyletic cluster consisting of *Lactobacillus salivarius*, *Lactobacillus aviarius*, *Lactobacillus ruminis*, *Lactobacillus agilis*, *Lactobacillus murinus*, *Lactobacillus animalis* and *Lactobacillus mali*. The DNA–DNA relatedness of *L. acidipiscis* strains and other related *Lactobacillus* species was found to be in the range of 3·6 to 26·7% (Tanasupawat et al., 2000). One year later, *Lactobacillus cypricasei* was described by Lawson et al. (2001) based on four strains isolated from Halloumi, a cheese produced in Cyprus. The strains were Gram-positive, non-spore-forming, facultatively anaerobic and catalase- and oxidase-negative. No growth was observed at 15 or 45°C. Like *L. acidipiscis*, *L. cypricasei* strains formed a distinct branch within the *L. salivarius* species group, with *L. salivarius* and *L. aviarius* as the nearest neighbours. However, Lawson et al. (2001) did not include the related recognized species *L. acidipiscis* in comparisons with other species. In the present study, the relatedness between *L. acidipiscis* and *L. cypricasei* strains was investigated and revealed synonymy between the two taxa.

Two reference strains of *L. acidipiscis*, LMG 19820\(^2\) and LMG 23135, and four reference strains of *L. cypricasei*, LMG 21592\(^2\), CCUG 42959, CCUG 42960 and CCUG 42962, were selected for further comparative study. *L. cypricasei* LMG 21592\(^2\) was cultivated and maintained on de Man, Rogosa and Sharpe medium (MRS; Difco). All other strains studied were cultivated and maintained on MRS (Oxoid) medium and incubated anaerobically at 37°C, unless otherwise indicated.

The use of protein-coding gene sequence data for the determination of genomic relatedness at the bacterial species and genus levels has recently been advocated because of its ability to provide higher taxonomic resolution, since 16S rRNA
gene sequence analysis may not be sufficiently specific to discriminate between closely related species (Chavagnat et al., 2002; Stackebrandt et al., 2002; Torriani et al., 2001; Ventura et al., 2003; Zeigler, 2003). Recently, the application of multilocus sequence analysis (MLSA) using the genes that code for the z-subunit of bacterial phenylalanyl-tRNA synthase (pheS), the z-subunit of RNA polymerase (rpoA) and the z-subunit of ATP synthase (atpA) provided a robust system for the identification of Enterococcus species (Naser et al., 2005a, b). The MLSA-based identification system has proved to be valuable for the detection of synonymous species names within the genus Enterococcus (Naser et al., 2006b). In addition, relatedness between strains of Lactobacillus helveticus and Lactobacillus surnyoryeus was initially evaluated using the same MLSA loci. Naser et al. (2006a) demonstrated that Lactobacillus surnyoryeus is a later synonym of Lactobacillus helveticus. The pheS and rpoA genes were subsequently used for the analysis of lactobacilli of the L. salivarius species group, including the two strains of L. acidipiscis and the four reference strains of L. cypricasei.

The type strains of L. acidipiscis and L. cypricasei were further investigated using the atpA gene. The primer sequences, amplification conditions and sequencing reactions were as described by Naser et al. (2005a, b). Raw sequence data were transferred to GeneBuilder (Applied Maths) where consensus sequences were determined. Consensus sequences were imported into BioNumerics 4.0 software (Applied Maths). The determined partial pheS and rpoA gene sequences (453 and 402 bp, respectively) were compared for the two L. acidipiscis strains (LMG 19820T and LMG 23135), four L. cypricasei strains (LMG 21592T, CCUG 42959, CCUG 42960 and CCUG 42962) and other members of the L. salivarius species group. Comparison of the sequences of the strains of L. cypricasei and L. acidipiscis revealed pheS and rpoA gene sequence similarities in the range of 99.8 to 100%. The atpA partial gene sequences of L. cypricasei LMG 21592T (GenBank accession no. AM168423) and L. acidipiscis LMG 19820T (AM168424) were also compared (976 bp) and showed high relatedness. Strains of the two species had a maximum of 78% pheS gene sequence similarity with L. mali and L. murinus, 82% rpoA gene sequence similarity with L. agilis and 80% atpA gene sequence similarity with L. murinus (Fig. 1). Consequently, the MLSA data indicated that L. acidipiscis and L. cypricasei might represent a single species.

The phylogenetic relatedness between L. acidipiscis FS60-1T and L. cypricasei CCUG 42961T was investigated by comparing the available 16S rRNA gene sequences (L. acidipiscis GenBank accession no. AB023836, 1406 bp; L. cypricasei GenBank accession no. AJ251560, 1456 bp). The latter sequences and those of related species were retrieved from GenBank and aligned. A phylogenetic tree was constructed by the neighbour-joining method using BioNumerics software, version 4.0 (Applied Maths). Unknown bases were discarded for the analyses. Comparison of the sequences of the type strains of L. cypricasei and L. acidipiscis revealed 99.8% 16S rRNA gene sequence similarity.

In the original description of L. cypricasei, the authors did not determine the DNA G+C content. In the present study, we investigated this characteristic for the type strain of L. cypricasei. Cells were cultivated in MRS broth at 37ºC for 24 h. DNA was extracted from 0.5–0.75 g (wet weight) by using the protocol described by Marmur (1961) with the following modifications: (i) cells were suspended overnight in Tris/HCl buffer that contained lysozyme (8 mg ml⁻¹) before the addition of SDS and (ii) lysed cells were treated with protease K (360 mg l⁻¹; Merck) at 37ºC for 2 h. For determination of the DNA G+C content, DNA was degraded enzymically into nucleosides as described by Mesbah et al. (1989). The nucleoside mixture was then separated by HPLC using a SymmetryShield RP8 column (Waters) maintained at 37ºC. The solvent was 0.02 M NH₄H₂PO₄ (pH 4.0) with 1.5% acetonitrile. Non-methylated λ-phage DNA (Sigma) was used as the calibration reference. The DNA G+C content of L. cypricasei LMG 21592T was 40.1 mol%. In comparison, the DNA G+C content of L. acidipiscis LMG 19820T was 38.7 mol%.

**Fig. 1.** Neighbour-joining tree based on the partial pheS gene sequences of L. cypricasei and L. acidipiscis strains. Carnobacterium maltaromaticum LMG 6903T was included as an outgroup. Bootstrap percentages (≥ 50%) after 500 simulations are shown. Bar, 2% sequence divergence.
Finally, DNA–DNA hybridizations were performed between L. cypricasei strains LMG 21592<sup>T</sup> and CCUG 42960 and L. acidipiscis strains LMG 19820<sup>T</sup> and LMG 23135. Genomic DNA was prepared according to the protocol of Pitcher et al. (1989) with the following modifications: the cell pellet was resuspended and lysed in a buffer (10 mM Tris-HCl, 100 mM EDTA, pH 8.0) that contained RNase (200 µg ml<sup>−1</sup>; Sigma), mutanolysin (100 U ml<sup>−1</sup>; Sigma) and lysozyme (25 mg ml<sup>−1</sup>; SERVA) for 1 h at 37 °C. The microplate method was used as described by Ezaki et al. (1989) and Goris et al. (1998) using a HTS7000 Bio Assay Reader (Perkin Elmer) for the fluorescence measurements. Biotinylated DNA was hybridized with unlabelled ssDNA, which was bound non-covalently to microplate wells. Hybridizations were performed at 36 °C in hybridization mixture (2 × SSC, 5 × Denhardt’s solution, 2-5% dextran sulfate, 50% formamide, 100 ng biotinylated probe DNA ml<sup>−1</sup>). Reciprocal reactions (e.g. A × B and B × A) were performed. The DNA–DNA binding values reported were the mean values of a minimum of four hybridization experiments, including the reciprocal reactions. L. acidipiscis strains LMG 19820<sup>T</sup> and LMG 23135 and L. cypricasei strains LMG 21592<sup>T</sup> and CCUG 42960 showed high DNA–DNA binding values in the range of 84 to 97%, indicating clearly that the two taxa represent the same species. A DNA–DNA hybridization value of 93% was found between the type strains of the two species.

On the basis of the evidence presented, it is concluded that Lactobacillus cypricasei is a later heterotypic synonym of Lactobacillus acidipiscis. It is proposed that the two species be united under the same name. As a rule of priority (Rules 38 and 42 of the Bacteriological Code; Lapage et al., 1992), the name Lactobacillus acidipiscis should be retained and strains of Lactobacillus cypricasei should be reclassified as such. The description of L. acidipiscis remains essentially the same except for some strain-dependent reactions, such as the production of acid from mannitol and D-ribose and the production of arginine dihydrolase.

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References


