

## *Lactobacillus suntoryeus* Cachat and Priest 2005 is a later synonym of *Lactobacillus helveticus* (Orla-Jensen 1919) Bergey et al. 1925 (Approved Lists 1980)

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Strain R0052, isolated from a North American dairy starter culture, was initially identified as *Lactobacillus acidophilus* based on phenotypic analyses. However, upon sequencing the 16S rRNA gene, it became clear that the isolate was very highly related to *Lactobacillus suntoryeus*, *Lactobacillus helveticus* and *Lactobacillus gallinarum*, as similarities ranging from 99.3 to 99.8% were observed. As an initial screening test to investigate the relatedness of strain R0052 and reference strains of *L. suntoryeus*, *L. helveticus* and *L. gallinarum*, the partial sequences for the genes encoding the alpha subunit of ATP synthase (*atpA*), RNA polymerase alpha subunit (*rpoA*), phenylalanyl-tRNA synthase alpha subunit (*pheS*), the translational elongation factor Tu (*tuf*), a surface-layer protein (*slp*) and the Hsp60 chaperonins (*groEL*) were determined and they revealed high relatedness between all of the strains. The determination of the 16S–23S rRNA internally transcribed spacer (ITS) sequences revealed 98.3–100% similarity between *L. suntoryeus* and *L. helveticus* strains. SDS-PAGE of whole-cell proteins did not distinguish between these species. Fluorescent amplified fragment length polymorphism (FAFLP) could distinguish between these taxa, but they still constituted a single cluster within the *L. acidophilus* group. Finally, DNA–DNA hybridization experiments between strain R0052 and the type strains of *L. helveticus* and *L. suntoryeus* yielded reassocia-tion values above 70% and confirmed that these names are synonyms.

*Lactobacillus suntoryeus*, isolated from Japanese and Scottish malt whisky distilleries, has been described recently by Cachat & Priest (2005). The type strain SA<sup>T</sup> (= LMG 22464<sup>T</sup>), strain Y10 and strain M4 (= LMG 22465) had identical 16S rRNA gene sequences and the topology of the phylogenetic

tree confirmed that they were members of the *Lactobacillus acidophilus* group. The 16S rRNA gene sequences of these strains showed 99.3 and 98.1% similarity to *Lactobacillus helveticus* and *Lactobacillus gallinarum*, respectively. DNA–DNA hybridization values of less than 43% were observed

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**Abbreviations:** FAFLP, fluorescent amplified fragment length polymorphism; ITS, internally transcribed spacer.

The GenBank/EMBL/DDBJ accession numbers for the sequences reported in this paper are: 16S rRNA partial gene sequences for strains LMG 11445 and R0052, DQ123572 and DQ123580; *atpA* partial gene sequences for strains LMG 6413<sup>T</sup>, LMG 22464<sup>T</sup>, R0052, LMG 6904<sup>T</sup>, LMG 9435<sup>T</sup>, LMG 11445, LMG 13522 and LMG 22465, AM086429–AM086431, AM157788–AM157792, respectively; *pheS* partial gene sequences for strains LMG 6413<sup>T</sup>, LMG 22464<sup>T</sup>, R0052, LMG 6904<sup>T</sup>, LMG 9435<sup>T</sup>, LMG 18225, LMG 11445, LMG 11447, LMG 13522 and LMG 22465, AM086432–AM086434, AM157781–AM157787, respectively; *rpoA* partial gene sequences for strains LMG 6413<sup>T</sup>, LMG 22464<sup>T</sup>, R0052, LMG 9435<sup>T</sup>, LMG 18225, LMG 6904<sup>T</sup>, LMG 11445, LMG 11447, LMG 13522 and LMG 22465, AM086435–AM086437, AM157774–AM157780, respectively; *tuf* partial gene sequences for strains LMG 11445, LMG 22465 and R0052, DQ123571, DQ123578 and DQ123584, respectively; *groEL* partial gene sequences for strains LMG 11445, LMG 22464<sup>T</sup>, LMG 22465 and R0052, DQ123573, DQ123576, DQ123579 and DQ123581, respectively; 16S–23S ITS gene sequences for strains LMG 11445, LMG 6413<sup>T</sup>, LMG 22464<sup>T</sup>, LMG 22465 and R0052, DQ123570, DQ123574, DQ123575, DQ123577 and DQ123582, respectively; *slp* partial gene sequence for strain R0052, DQ123583.

Additional neighbour-joining trees based on the sequences of six genes and ITS sequences are available as supplementary figures in IJSEM Online.

between the distillery strains and their closest neighbours, *L. helveticus* and *L. gallinarum*. Further, genomic and phenotypic data demonstrated that *L. suntoryeus* represented a novel species (Cachat & Priest, 2005).

*L. helveticus*, isolated from sour milk, cheese starter cultures and cheese, was first described by Orla-Jensen in 1919 (Kandler & Weiss, 1986; Hammes & Vogel, 1995). *L. helveticus* represents a homofermentative, thermophilic lactic acid bacterium, which ferments hexoses to lactic acid (Holzapfel *et al.*, 2001). *L. helveticus* is an important food-associated species adapted to sour whey and is traditionally used in the manufacture of Swiss-type and long-ripened Italian cheeses, such as Emmental, Gruyère and Provolone. In particular, *L. helveticus* is the most prevalent species recovered from natural lactic starter cultures used to produce typical Italian cheeses (Giraffa *et al.*, 2000; Stiles & Holzapfel, 1997). *L. helveticus* is distinct from *Lactobacillus delbrueckii*, the type species of the genus *Lactobacillus*, but is closely related to *Lactobacillus acidophilus* with respect to DNA–DNA hybridization, biochemical features and 16S rRNA gene sequences (Kandler & Weiss, 1986; Klein *et al.*, 1998). Accordingly, *L. acidophilus*, *L. gallinarum*, *Lactobacillus crispatus* and *L. helveticus* form a cluster of closely related species.

Strain R0052 was isolated from a North American dairy starter culture in March 1990 by Institut Rosell, Canada, and was initially typed as *L. acidophilus* based on its API profile and D-lactate production. In addition to strain R0052, several reference strains of *L. helveticus*, LMG 6413<sup>T</sup>, LMG 11445, LMG 11447, LMG 11448, LMG 13522 and LMG 18225, were selected for further comparative study. Two *L. suntoryeus* strains, LMG 22464<sup>T</sup> and LMG 22465 (supplied by F. Priest), were included in the study. All strains studied were cultivated and maintained on de Man, Rogosa and Sharpe (MRS) medium and incubated anaerobically at 37 °C, unless indicated otherwise.

The 16S rRNA genes of R0052 and LMG 11445 were obtained using primers Lb16a (Guan *et al.*, 2003), Lb16b (5'-CGGTGTGTACAAGGCCCG-3'), 16Smidford (degenerate form of 16Smidfor, Requena *et al.*, 2002; 5'-GKCYG-YWACTGACGCTGAG-3') and 16Smidrev (5'-GCRTG-GACTACCAGGGTATC-3') at an annealing temperature of 56 °C with 0.2 mM dNTPs, 1 pmol primer  $\mu\text{l}^{-1}$  and 0.05 U *Taq* DNA polymerase  $\mu\text{l}^{-1}$ , which were used to amplify genomic DNA extracted by bead beating (Walter *et al.*, 2001). PCR products were purified using a QIAGEN PCR purification kit following the manufacturer's instructions. Sequencing of the purified PCR products was performed with an Amersham DYEnamic ET terminator cycle sequencing kit following the guidelines provided by the DNA Sequencing Facility at the Biotechnology Research Institute (Montreal, Canada). The R0052 sequence was compared with the 16S rRNA gene sequences of *L. helveticus*, *L. gallinarum* and *L. suntoryeus* as available on GenBank and with the sequence obtained for strain LMG 11445 and strain R0052 was found to share 99.8% similarity with *L.*

*suntoryeus* strains LMG 22464<sup>T</sup> and LMG 22465. The sequence was also 99.5% similar to the 16S rRNA gene sequence of *L. gallinarum* ATCC 33199<sup>T</sup> and shared 99.3% and 99.7% similarity with *L. helveticus* strains LMG 6413<sup>T</sup> and LMG 11445, suggesting that strain R0052 could belong to any of these three species.

The simultaneous use of several housekeeping genes in bacterial taxonomy offers a higher resolution than 16S rRNA gene sequence data at the species level as it integrates information from different molecular markers from throughout the bacterial chromosome (Stackebrandt *et al.*, 2002; Zeigler, 2003). The partial sequences for the genes encoding the alpha subunit of ATP synthase (*atpA*), RNA polymerase alpha subunit (*rpoA*) and phenylalanyl-tRNA synthase alpha subunit (*pheS*) were used as an initial screening test to investigate the relatedness between the *L. helveticus* strains LMG 6413<sup>T</sup>, LMG 11445, LMG 11447, LMG 13522 and LMG 18225, the *L. suntoryeus* strains LMG 22464<sup>T</sup> and LMG 22465, *L. gallinarum* LMG 9435<sup>T</sup> and strain R0052. Primer sequences, amplification conditions and sequencing reactions were performed as described by Naser *et al.* (2005a, b). Neighbour-joining trees of the *atpA*, *rpoA* and *pheS* gene sequences revealed high relatedness between the investigated strains of *L. helveticus* and *L. suntoryeus*, with at least 99.5% similarity in the *atpA*, *rpoA* and *pheS* gene sequences. Significantly lower values, in the range of 95–98%, were found with the nearest neighbour *L. gallinarum*, indicating that *L. helveticus* and *L. suntoryeus* may represent the same species (see Supplementary Figs S1, S2 and S3 in IJSEM Online). Strain R0052 showed high levels of similarity to strains of both *L. helveticus* and *L. suntoryeus* (>99.5% *atpA*, *rpoA* and *pheS* gene sequence similarities).

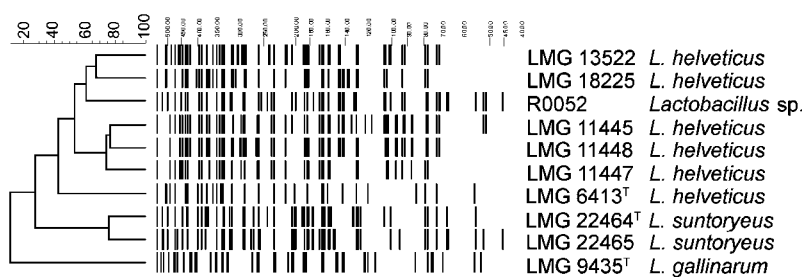
The translational elongation factor Tu (*tuf*) and Hsp60 chaperonin (*groEL*) genes were also analysed. DNA from strain R0052, *L. helveticus* LMG 11445 and *L. suntoryeus* LMG 22465 was extracted by bead beating as described above for 16S rRNA gene sequence analysis. Amplification and sequencing of *tuf* genes, using primers TUF-1 and TUF-2, was performed as described by Ventura *et al.* (2003). A comparison was made with the sequences available from GenBank for the strains *L. helveticus* LMG 6413<sup>T</sup>, *L. suntoryeus* LMG 22464<sup>T</sup> and *L. gallinarum* LMG 9435<sup>T</sup>. The *L. suntoryeus*, *L. helveticus* and *L. gallinarum* strains were highly similar (>98% sequence similarity; see Supplementary Fig. S4). Strain R0052 had >98.8% similarity with the *L. helveticus* and *L. suntoryeus* strains and 98.1% similarity to the *L. gallinarum* type strain. DNA from strains R0052, *L. helveticus* LMG 11445 and *L. suntoryeus* strains LMG 22464<sup>T</sup> and LMG 22465 was further used as template for *groEL* gene sequencing using primers groEL1F (5'-GAAGGNATGAAGAAYGTBAC-3') and groEL1R (5'-AATGTHCCACGVATCTTG-3') at an annealing temperature of 47 °C with 0.2 mM dNTPs, 1 pmol primer  $\mu\text{l}^{-1}$  and 0.05 U *Taq* DNA polymerase  $\mu\text{l}^{-1}$ . A comparison was made with the sequences available from GenBank for

*L. helveticus* LMG 6413<sup>T</sup> and *L. gallinarum* LMG 9435<sup>T</sup>. The partial sequence of *groEL* of strain R0052 showed >99% similarity with the sequences of the investigated *L. helveticus* and *L. suntoryeus* strains and a significantly lower similarity (96%) with the *L. gallinarum* type strain (see Supplementary Fig. S5 in IJSEM Online). The *tuf* and *groEL* sequences indicated that *L. suntoryeus* and *L. helveticus* cannot be differentiated and, in particular, *groEL* sequence analysis demonstrated a more distant relatedness of the species to their nearest neighbour, *L. gallinarum*.

The 16S–23S rRNA internally transcribed spacer (ITS) sequences were amplified from template DNA (prepared as described above) from strain R0052, *L. helveticus* strains LMG 6413<sup>T</sup> and LMG 11445 and *L. suntoryeus* strains LMG 22464<sup>T</sup> and LMG 22465 with primers 16-1Ad (a degenerate form of 16-1A, 5'-GBYGGARTCGCTAGTAATCG-3') and 23-1B as described in Tannock *et al.* (1999). A comparison was made with the sequences available from GenBank for the *L. gallinarum* type strain. It was found that strain R0052 shared 100% similarity with both *L. suntoryeus* strains and 99 and 98.5% similarity with *L. helveticus* strains LMG 6413<sup>T</sup> and LMG 11445, respectively. The first 60 nucleotides of the *L. gallinarum* ITS sequence available from GenBank showed only 91.7% similarity with R0052, supporting the indications that strain R0052 is a member of one of the closely related species *L. suntoryeus* or *L. helveticus* (Supplementary Fig. S6 in IJSEM Online).

*Lactobacillus* sp. strain R0052, *L. helveticus* strains LMG 6413<sup>T</sup>, LMG 11445, LMG 11447, LMG 11448, LMG 13522 and LMG 18225 and two *L. suntoryeus* strains, LMG 22464<sup>T</sup> and LMG 22465, were further investigated using PAGE of whole-cell proteins. Whole-cell protein extracts were prepared and SDS-PAGE was performed as described by Pot *et al.* (1994). Densitometric analysis, normalization and interpolation of protein profiles and numerical analysis were performed by using the GELCOMP software package, versions 3.1 and 4.0, respectively (Applied Maths). The five *L. helveticus* strains, the type strain of *L. suntoryeus* and strain R0052 constituted a homogeneous cluster, with the exception being *L. suntoryeus* LMG 22465 (Fig. 1). This aberrant position may be due to strain-specific variations of dominant protein bands in the molecular mass range of 35–42 kDa, perhaps due to differences in the surface layer (S-layer) proteins located on the outer surface of the bacterial cell (Boot *et al.*, 1996).

A more detailed study was performed on the S-layer proteins. Partial sequences of the S-layer protein (or *slp*) of strain R0052 were obtained with primers Usl-1 and Usl-2 (Hagen *et al.*, 2005). The PCR product was purified as described above for 16S rRNA gene sequencing and ligated into cloning vector pGEM-T Easy (Promega) which was transformed into *Escherichia coli* JM109 competent cells (Promega) according to the manufacturer's instructions. Clones were grown on Luria–Bertani (LB) agar containing 100 µg ampicillin ml<sup>-1</sup>, 80 µg X-Gal ml<sup>-1</sup> (5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside) and 0.5 mM IPTG (isopropyl-beta-D-thiogalactopyranoside) at 37 °C overnight. Eight blue transformants were picked and grown in 2YT broth (1.6% tryptone, 0.8% yeast extract and 85.6 mM NaCl; Sambrook *et al.*, 1989) overnight at 37 °C with vigorous aeration. A 0.5 ml sample of each clone was stocked in LB with 50% glycerol and stored at –80 °C. The remainder was used for plasmid DNA extraction using the alkaline lysis method with polyethylene glycol (PEG) precipitation (Sambrook *et al.*, 1989). Clones were screened by sequencing the purified plasmid DNA using primers T7 and Sp6. One *slp* gene sequence was obtained and the clone was fully sequenced in both directions using primers R0052slp-1 (5'-TAACGATACTAGCAATGATG-3') and R0052slp-2 (5'-CCGTATTGGTCCAAACTTAC-3'). The sequence of the extreme 3' end of the gene was obtained with primers R0052CTD-5 (5'-AATGGTAAGTCAACTGTTGT-3') and R0052CTD-6 (5'-AAGTTTCAACTCTAACG-3') at an annealing temperature of 44 °C with 0.2 mM dNTPs, 1 pmol primer µl<sup>-1</sup> and 0.05 U *Taq* DNA polymerase µl<sup>-1</sup>. The PCR product was purified and sequenced as described above. A large DNA sequence divergence was observed among S-layer protein genes of strain R0052, *L. helveticus* strains LMG 6413<sup>T</sup> and LMG 11445 and two *L. suntoryeus* strains, LMG 22464<sup>T</sup> and LMG 22465 (see Supplementary Fig. S7 in IJSEM Online). This is not surprising, given the location and function of S-layer proteins, which are highly expressed and form a paracrystalline coat outside of the bacterial cell wall (Sleytr & Sara, 1997). S-layer proteins are thought to mediate tissue adherence (Sillanpää *et al.*, 2000; Antikainen *et al.*, 2002) and may also affect selective nutrient transport (Sára & Sleytr, 2000) or protect from proteases (Lortal, 1993). As these proteins are surface-exposed and play a role in survival and adaptation in the environment, their primary sequence differs considerably between members of the same species (Boot *et al.*, 1996) as can be seen in SDS-PAGE of proteins (Fig. 1). Conversely, Ventura *et al.*



**Fig. 1.** SDS-PAGE protein profiles and the corresponding dendrogram derived from the UPGMA average linkage of correlation coefficients *r* (expressed for convenience as a percentage value) for *L. helveticus* and *L. suntoryeus* strains and related reference strains.

(2000) observed very high similarity between other S-layer proteins of *L. helveticus*.

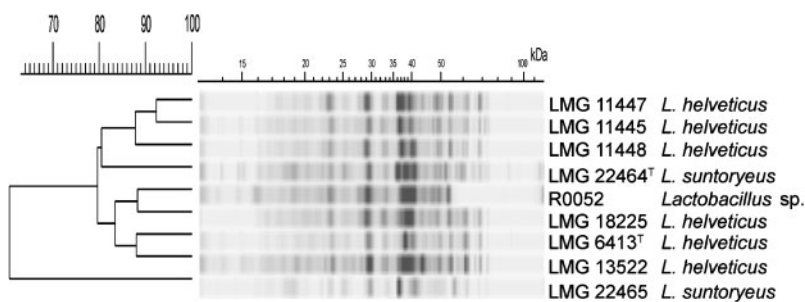
The same set of strains analysed by 1D SDS-PAGE was further investigated using fluorescent amplified fragment length polymorphism (FAFLP) fingerprinting of whole genomes. FAFLP fingerprinting was performed as described by Vancanneyt *et al.* (2005). The FAFLP fingerprints of these strains were compared with reference profiles of lactic acid bacteria taxa as currently available in the database developed at Ghent University, Belgium. FAFLP analysis revealed a high similarity between the *L. helveticus* and *L. suntoryeus* strains. Fig. 2 shows a dendrogram in which strain R0052 grouped with *L. helveticus* reference strains and was separated from *L. suntoryeus* strains and *L. gallinarum*. However, both taxa (*L. helveticus* and *L. suntoryeus*) still constituted a single cluster within the *L. acidophilus* group (data not shown). FAFLP randomly samples the whole bacterial genome and better differentiates closely related strains (i.e. the interpretation of intraspecies relationships) (Dellaglio *et al.*, 2005).

In a final step, DNA–DNA hybridizations were performed between strain R0052, *L. suntoryeus* LMG 22464<sup>T</sup> and *L. helveticus* strains LMG 6413<sup>T</sup> and LMG 11445. *L. acidophilus* LMG 9433<sup>T</sup> and *L. gallinarum* LMG 9435<sup>T</sup> were used as controls. Genomic DNA was prepared according to the protocol of Pitcher *et al.* (1989) with the following modifications: the washed 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 an HTS7000 Bio Assay Reader (Perkin Elmer) for the fluorescence measurements. Biotinylated DNA was hybridized with unlabelled single-stranded DNA, 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 µg denatured salmon sperm DNA ml<sup>-1</sup>, 1250 ng biotinylated probe DNA ml<sup>-1</sup>). Reciprocal reactions (e.g. A × B and B × A) were performed. The DNA–DNA reassociation values reported were the mean values of a minimum of four hybridization experiments, including the reciprocal reactions. *L. helveticus* (LMG 6413<sup>T</sup> and LMG 11445), strain R0052 and *L. suntoryeus* LMG 22464<sup>T</sup> showed

high DNA–DNA binding values (70–90%), indicating clearly that both taxa belong to the same genospecies. In particular, a DNA–DNA hybridization value of 78% was found between *L. helveticus* LMG 6413<sup>T</sup> and *L. suntoryeus* LMG 22464<sup>T</sup>, whereas hybridization values of 80% were obtained between strain R0052 and the type strains of *L. helveticus* and *L. suntoryeus*. The latter results contradict those obtained by Cachat & Priest (2005), who reported a low hybridization value of less than 43% between the type strains of these taxa.

Growth characteristics and biochemical features were investigated for strains R0052, *L. gallinarum* LMG 9435<sup>T</sup>, *L. helveticus* strains LMG 6413<sup>T</sup> and LMG 11445 and *L. suntoryeus* strains LMG 22464<sup>T</sup> and LMG 22465. The strains were grown on MRS agar after 24 h incubation at 37 °C under aerobic conditions. Carbohydrate fermentation tests were carried out using API50 CHL galleries following the manufacturer's instructions (bioMérieux) and the results are presented in Table 1. In particular, the *L. gallinarum* type strain, LMG 9435<sup>T</sup>, utilized D-raffinose and glycogen, whereas the other strains did not. The *L. suntoryeus* strains did not ferment galactose and the *L. helveticus* strains did not ferment sucrose, aesculin, salicin or cellobiose. Strain R0052 was able to metabolize all of these carbohydrates as well as arbutin and β-gentiobiose, and showed the broadest utilization of carbohydrates among the strains tested.

Samples were prepared for API ZYM analysis following the manufacturer's instructions (bioMérieux) except that the cells were resuspended in 0.85% NaCl and no mineral oil was added. The strips were incubated for the time stated in the manufacturer's instructions. Strain R0052 and the *L. suntoryeus* isolates were identified as *L. acidophilus*. All the strains tested negative for alkaline phosphatase, esterase lipase (C8), lipase (C14), trypsin, α-chymotrypsin, β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities. All the strains had leucine, valine and cystine arylamidase activities and acid phosphatase, naphthol-AS-BI-phosphohydrolase and β-galactosidase activities. Weak esterase (C4) activity was observed for all strains, but only the *L. gallinarum* strain showed α-galactosidase activity. Differences in glucosidase production were observed: R0052 and the *L. suntoryeus* strains had α-glucosidase and β-glucosidase activities whereas the *L. helveticus* strains did not.



**Fig. 2.** FAFLP patterns and the corresponding dendrogram, derived from the UPGMA linkage of Dice coefficients (expressed as a percentage value for convenience) of *L. helveticus* and *L. suntoryeus* strains and related reference strains.

**Table 1.** Differential phenotypic features between strains R0052, *L. helveticus* strains LMG 6413<sup>T</sup> and LMG 11445, *L. suntoryeus* strains LMG 22464<sup>T</sup> and LMG 22465 and *L. gallinarum* LMG 9435<sup>T</sup>

All strains tested positive for utilization of D-glucose and D-mannose. Other sugars were not metabolized by the assayed strains. No growth was observed in the absence of sugars. –, Negative; +, positive; ±, weak reaction.

| Characteristic      | R0052 | <i>L. helveticus</i>  |           | <i>L. suntoryeus</i>   |           | <i>L. gallinarum</i>  |
|---------------------|-------|-----------------------|-----------|------------------------|-----------|-----------------------|
|                     |       | LMG 6413 <sup>T</sup> | LMG 11445 | LMG 22464 <sup>T</sup> | LMG 22465 | LMG 9435 <sup>T</sup> |
| N-Acetylglucosamine | +     | +                     | ±         | –                      | +         | +                     |
| Aesculin            | +     | –                     | –         | +                      | +         | +                     |
| Amygdalin           | +     | –                     | –         | –                      | +         | –                     |
| Arbutin             | +     | –                     | –         | –                      | –         | –                     |
| Cellobiose          | +     | –                     | –         | +                      | +         | +                     |
| D-Fructose          | +     | +                     | –         | +                      | +         | +                     |
| Galactose           | +     | +                     | +         | –                      | –         | +                     |
| β-Gentiobiose       | +     | –                     | –         | –                      | –         | –                     |
| Glycogen            | –     | –                     | –         | –                      | –         | +                     |
| Lactose             | +     | +                     | –         | –                      | –         | +                     |
| Maltose             | +     | +                     | –         | +                      | +         | +                     |
| D-Raffinose         | –     | –                     | –         | –                      | –         | +                     |
| Salicin             | +     | –                     | –         | +                      | +         | +                     |
| Sucrose             | +     | –                     | –         | +                      | +         | +                     |
| Trehalose           | +     | +                     | –         | –                      | –         | –                     |

The strains were also tested for the production of D- and L-lactic acid and acetate by the D-/L-lactic acid kit (Megazyme) and the acetic acid kit (Megazyme). For the lactic acid assays, an overnight culture of each strain was centrifuged at 3000 g for 10 min and the supernatant was collected. The pH of the supernatant was adjusted to a value between 8 and 10, then the supernatant was incubated at room temperature for 15 min and then assayed with the kit according to the manufacturer's instructions. For the acetate assay, the same procedure was performed except that the pH was adjusted to 7.5. All strains tested produced D- and L-lactic acid at a ratio of approximately 45:55, with the exception of R0052 which had a ratio of 60:40. Additionally, all strains produced small amounts of acetate (mean 0.29 g l<sup>-1</sup>).

Taken together, these phenotypic data support the observation that sugar utilization is insufficient for the identification of closely related lactobacilli (Fujisawa *et al.*, 1992), thus necessitating DNA–DNA hybridization tests.

On the basis of the evidence presented, it is proposed that the species *L. suntoryeus* and *L. helveticus* 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 *L. helveticus* should be retained and strains of *L. suntoryeus* should be reclassified as such.

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