Reclassification of the genus *Leuconostoc* and proposals of *Fructobacillus fructosus* gen. nov., comb. nov., *Fructobacillus durionis* comb. nov., *Fructobacillus ficulneus* comb. nov. and *Fructobacillus pseudoficulneus* comb. nov.

Akihito Endo^{1,2,3} and Sanae Okada^{1,4}

Correspondence

Akihito Endo

pegaman@hotmail.co.jp

¹NODAI Culture Collection Center, Tokyo University of Agriculture, 1-1-1 Sakuragaoka, Setagaya, Tokyo 156-8502, Japan

²Division of Pharmacognosy, Phytochemistry and Narcotics, National Institute of Health Sciences, Ministry of Health, Labour and Welfare, 1-18-1 Kamiyoga, Setagaya, Tokyo 158-8501, Japan

³Department of Microbiology, University of Stellenbosch, Stellenbosch 7600, South Africa

⁴Department of Applied Biology and Chemistry, Tokyo University of Agriculture, 1-1-1 Sakuragaoka, Setagaya, Tokyo 156-8502, Japan

A taxonomic study was made of the genus *Leuconostoc*. The species in the genus were divided into three subclusters by phylogenetic analysis based on the 16S rRNA gene sequences. The three subclusters were the *Leuconostoc mesenteroides* subcluster (comprising *L. carnosum*, *L. citreum*, *L. gasicomitatum*, *L. gelidum*, *L. inhae*, *L. kimchii*, *L. lactis*, *L. mesenteroides* and *L. pseudomesenteroides*), the *L. fructosum* subcluster (*L. durionis*, *L. ficulneum*, *L. fructosum* and *L. pseudoficulneum*) and the *L. fallax* subcluster (*L. fallax*). Phylogenetic trees based on the sequences of the 16S–23S rRNA gene intergenic spacer region, the *rpoC* gene or the *recA* gene indicated a good correlation with the phylogenetic tree based on 16S rRNA gene sequences. The species in the *L. fructosum* subcluster were morphologically distinguishable from the species in the *L. mesenteroides* subcluster and *L. fallax* as species in the *L. fructosum* subcluster had rod-shaped cells. In addition, the four species in the *L. fructosum* subcluster needed an electron acceptor for the dissimilation of D-glucose and produced acetic acid from D-glucose rather than ethanol. On the basis of evidence presented in this study, it is proposed that the four species in the *L. fructosum* subcluster, *Leuconostoc durionis*, *Leuconostoc ficulneum*, *Leuconostoc fructosum* and *Leuconostoc pseudoficulneum*, should be transferred to a novel genus, *Fructobacillus* gen. nov., as *Fructobacillus durionis* comb. nov. (type strain D-24^T=LMG 22556^T=CCUG 49949^T), *Fructobacillus ficulneus* comb. nov. (type strain FS-1^T=DSM 13613^T=JCM 12225^T), *Fructobacillus fructosus* comb. nov. (type strain IFO 3516^T=DSM 20349^T=JCM 1119^T=NRIC 1058^T) and *Fructobacillus pseudoficulneus* comb. nov. (type strain LC-51^T=DSM 15468^T=CECT 5759^T). The type species of the genus *Fructobacillus* is *Fructobacillus fructosus* gen. nov., comb. nov.. No significant physiological and biochemical differences were found between the species in the *L. mesenteroides* subcluster and *L. fallax* in the present study and thus *L. fallax* remains as a member of the genus *Leuconostoc*.

Abbreviations: ISR, intergenic spacer region; MLSA, multilocus sequence analysis; SEM, scanning electron microscope.

The GenBank/EMBL/DDBJ accession numbers for the sequences of the ISR and *recA* genes of *L. carnosum* NRIC 1722^T, *L. citreum* NRIC 1776^T, *L. gasicomitatum* JCM 12535^T, *L. gelidum* NRIC 1778^T, *L. inhae* NRIC 0562^T, *L. lactis* NRIC 1540^T, *L. mesenteroides* NRIC 1541^T, *L. pseudomesenteroides* NRIC 1777^T, *F. durionis* LMG 22556^T, *F. ficulneus* JCM 12225^T, *F. fructosus* NRIC 1058^T, *F. pseudoficulneus* DSM 15468^T and *L. fallax* NRIC 0210^T are AB290436–AB29448 and AB354939–AB354951, respectively. The GenBank/EMBL/DDBJ accession numbers for the sequences of the *rpoC* genes of *L. inhae* NRIC 0562^T, *L. lactis* NRIC 1540^T, *F. durionis* LMG 22556^T, *L. gasicomitatum* JCM 12535^T and *L. carnosum* NRIC 1722^T are AB354934–AB354938, respectively.

Additional phylogenetic trees derived from 16S rRNA gene sequence analysis and constructed by using the maximum-likelihood and maximum-parsimony methods and growth curves in FYP, GYP and FGYP broth for the *Leuconostoc* species used in this study are available with the online version of this paper.

The genus *Leuconostoc* was described by Van Tieghem (1878), and at the present time the genus consists of the following 14 species (excluding reclassified and synonymous species): *Leuconostoc mesenteroides* (type species), *L. carnosum*, *L. citreum*, *L. durionis*, *L. fallax*, *L. ficulneum*, *L. fructosum*, *L. gasicomitatum*, *L. gelidum*, *L. inhae*, *L. kimchii*, *L. lactis*, *L. pseudoficulneum* and *L. pseudomesenteroides* (Antunes *et al.*, 2002; Björkroth *et al.*, 2000, 2001; Chambel *et al.*, 2006; Farrow *et al.*, 1989; Garvie, 1960; Kim *et al.*, 2000, 2003; Leisner *et al.*, 2005; Martinez-Murcia & Collins, 1991, 1992; Shaw & Harding, 1989; Skerman *et al.*, 1980; Van Tieghem, 1878). *Leuconostoc argentinum* was recently reclassified as a later synonym of *L. lactis* (Vancanneyt *et al.*, 2006). Of the 14 species, *L. durionis*, *L. fallax*, *L. ficulneum*, *L. fructosum* and *L. pseudoficulneum* have been reported as atypical species in the genus *Leuconostoc* on the basis of their biochemical characteristics and/or phylogenetic position as determined by 16S rRNA gene sequence analysis (Antunes *et al.*, 2002; Chambel *et al.*, 2006; Leisner *et al.*, 2005; Martinez-Murcia & Collins, 1991). Moreover, cells of *L. durionis*, *L. ficulneum* and *L. fructosum* have been reported to be rod-shaped (Antunes *et al.*, 2002; Leisner *et al.*, 2005) and the morphological characteristics of these species disagreed with those of members of the genus *Leuconostoc sensu strictu*, which were coccoid or elongated (Dellaglio *et al.*, 1995). Therefore, the genus *Leuconostoc* is considered to be heterogeneous and a taxonomic re-evaluation is needed. This work reports the results of a taxonomic study of the species in the genus *Leuconostoc* and concludes that the genus *Leuconostoc* should be divided into two genera.

The type strains of 13 of the species in the genus *Leuconostoc* were used in the present study (Table 1). The type strain of *L. kimchii* could not be obtained from any culture collection or from co-authors due to a patent which covers the strain. The strains used in this study were obtained from the NODAI Culture Collection Center, Tokyo University of Agriculture, Tokyo, and the JCM, DSMZ and LMG culture

collections. The culture broths and temperatures used for culturing the type strains are listed in Table 1. GYP broth was composed of (l^{-1}): 20 g D-glucose, 10 g yeast extract, 5 g polypeptone, 2 g sodium acetate, 0.5 g Tween 80, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.01 g $MnSO_4 \cdot 4H_2O$, 0.01 g $FeSO_4 \cdot 7H_2O$ and 0.01 g NaCl, at pH 6.8. FYP broth was composed of GYP broth in which 20 g D-glucose (l^{-1}) was replaced with 20 g D-fructose (l^{-1}).

To determine the phylogenetic relationships among the species of the genus *Leuconostoc*, the sequences of the 16S rRNA gene and the 16S-23S rRNA gene intergenic spacer region (ISR) were analysed. In addition, multilocus sequence analysis (MLSA) using two housekeeping genes was applied. MLSA was recently used for the classification of lactic acid bacteria and related taxa (Jian & Dong, 2002; Naser *et al.*, 2005, 2006).

For the phylogenetic analysis based on 16S rRNA gene sequences, the sequences of the type strains of the species in the genus *Leuconostoc* and related taxa were retrieved from the DDBJ database. Multiple alignments of the sequences were performed with the CLUSTAL_X program version 1.18 (Thompson *et al.*, 1997). Distance matrices for the aligned sequences were calculated by using Kimura's two-parameter method (Kimura, 1980). The neighbour-joining method was used to construct a phylogenetic tree (Saitou & Nei, 1987). The robustness of individual branches was estimated by using bootstrapping with 1000 replicates (Felsenstein, 1985). Phylogenetic trees were also constructed by using the maximum-likelihood (Cavalli-Sforza & Edwards, 1967) and maximum-parsimony (Kluge & Farris, 1969) methods with PHYLIP version 3.65 (Felsenstein, 2005). *Lactococcus lactis* was used as an outgroup. Approximately 1430 bp of the 16S rRNA gene sequences of the tested strains were used to construct phylogenetic trees.

The phylogenetic tree constructed with the neighbour-joining method revealed that the genus *Leuconostoc* was

Table 1. Bacterial strains used in the present study

Species	Type strain	Revised nomenclature	Culture medium	Temperature (°C)
<i>L. carnosum</i>	NRIC 1722 ^T	<i>L. carnosum</i>	GYP	30
<i>L. citreum</i>	NRIC 1776 ^T	<i>L. citreum</i>	GYP	30
<i>L. durionis</i>	LMG 22556 ^T	<i>Fructobacillus durionis</i> comb. nov.	FYP	30
<i>L. fallax</i>	NRIC 0210 ^T	<i>L. fallax</i>	GYP	30
<i>L. ficulneum</i>	JCM 12225 ^T	<i>Fructobacillus ficulneus</i> comb. nov.	FYP	30
<i>L. fructosum</i>	NRIC 1058 ^T	<i>Fructobacillus fructosus</i> comb. nov.	FYP	30
<i>L. gasicomitatum</i>	JCM 12535 ^T	<i>L. gasicomitatum</i>	GYP	25
<i>L. gelidum</i>	NRIC 1778 ^T	<i>L. gelidum</i>	GYP	25
<i>L. inhae</i>	NRIC 0562 ^T	<i>L. inhae</i>	GYP	25
<i>L. lactis</i>	NRIC 1540 ^T	<i>L. lactis</i>	GYP	30
<i>L. mesenteroides</i>	NRIC 1541 ^T	<i>L. mesenteroides</i>	GYP	30
<i>L. pseudoficulneum</i>	DSM 15468 ^T	<i>Fructobacillus pseudoficulneus</i> comb. nov.	FYP	30
<i>L. pseudomesenteroides</i>	NRIC 1777 ^T	<i>L. pseudomesenteroides</i>	GYP	30

divided into three subclusters; the *L. mesenteroides* subcluster (comprising *L. carnosum*, *L. citreum*, *L. gasicomitatum*, *L. gelidum*, *L. inhae*, *L. kimchii*, *L. lactis*, *L. mesenteroides* and *L. pseudomesenteroides*), the *L. fructosum* subcluster (comprising *L. durionis*, *L. ficulneum*, *L. fructosum* and *L. pseudoficulneum*) and the *L. fallax* subcluster (*L. fallax*) (Fig. 1). Identical tree topologies were obtained from the phylogenetic trees constructed by using maximum-likelihood and maximum-parsimony methods (see Supplementary Figs S1 and S2 in IJSEM Online). When the sequences of the type strains were aligned by using CLUSTAL_X, it was apparent that *L. fallax* was genetically distinct from the other *Leuconostoc* species based on the sequence of the V1 region of the 16S rRNA gene. The V1 region was based on the secondary structure model of SSU rRNA described by Van de Peer *et al.* (2000). The sequence of the V1 region of *L. fallax* had large gaps (approx. 16 bp) compared with those of other *Leuconostoc* species and was similar to those of species of the genera *Weissella* and *Oenococcus* (data not shown). The differences between *L. fallax* and other species of the genus *Leuconostoc* may be due to differences in the secondary structure of the V1 region of the 16S rRNA gene. The gene sequence similarities of the species within the *L. mesenteroides* subcluster ranged from 97.1 to 99.5%. Species in the *L. mesenteroides* subcluster had gene sequence similarities of 91.0–94.4% and 91.1–93.1% with species of the *L. fructosum* subcluster and *L. fallax* subcluster, respectively. The sequence similarities of the species within the *L.*

fructosum subcluster ranged from 94.2 to 98.0% and showed 90.4–91.7% sequence similarity with *L. fallax*. Recently, the genera *Pilibacter* and *Paralactobacillus* were described as novel genera of lactic acid bacteria (Higashiguchi *et al.*, 2006; Leisner *et al.*, 2000). The sequence similarity between the genus *Pilibacter* and the closest phylogenetic relative, the genus *Enterococcus*, was less than 92.8% (Higashiguchi *et al.*, 2006) and that between the genus *Paralactobacillus* and the closest phylogenetic relatives, species in the *Lactobacillus casei* group (Collins *et al.*, 1991), was less than 91.7% (Leisner *et al.*, 2000). These values are close to the sequence similarities found among the three subclusters of the genus *Leuconostoc* mentioned above.

For the determination of ISR sequences of the type strains of the *Leuconostoc* species, amplification of the ISR was performed by using primers 16S/p2 (5'-CTGTGACACACGCCCGTC) and 23S/p7 (5'-GGTACTTAGATGTTTCAGTTC) as described by Rachman *et al.* (2003). The primers 16S/p2 and 23S/p7 anneal at positions 1388–1406 of the 16S rRNA gene and at positions 207–189 of the 23S rRNA gene; position numbers were based on the *Escherichia coli* numbering system (GenBank accession no. V00348; Brosius *et al.*, 1981). An amplification product of approximately 750 bp was obtained for each of the strains (data not shown). Purification and sequencing of the product were performed as described previously (Endo & Okada, 2005). After sequencing, the

Fig. 1. Phylogenetic relationships of the genera *Leuconostoc*, *Fructobacillus* gen. nov. and related taxa based on 16S rRNA gene sequences. The tree was constructed by the neighbour-joining method. *Lactococcus lactis* was used as an outgroup. Bootstrap values are given at branching points; values above 70% are indicated. Bar, 0.01 K_{nuc} .

sequences of the 16S rRNA gene and the 23S rRNA gene attached to the ISR were deleted and only the sequences of the ISR were used for the phylogenetic analysis. Phylogenetic analysis was performed as described above and a phylogenetic tree was constructed according to the neighbour-joining method. *Oenococcus oeni* was used as an outgroup. Species of the genus *Weissella* were not used in this study as they possessed different length ISRs in each chromosomal DNA (Zavaleta *et al.*, 1996).

The phylogenetic tree based on ISR sequences showed that the genus *Leuconostoc* was divided into three ISR-subclusters (Fig. 2). The *L. mesenteroides* ISR-subcluster consisted of eight species (*L. carnosum*, *L. citreum*, *L. gasicomitatum*, *L. gelidum*, *L. inhae*, *L. lactis*, *L. mesenteroides* and *L. pseudomesenteroides*), the *L. fructosum* ISR-subcluster consisted of four species (*L. durionis*, *L. ficulneum*, *L. fructosum* and *L. pseudoficulneum*) and the *L. fallax* ISR-subcluster consisted of only *L. fallax* (Fig. 2). The ISR-subclusters correlated well with the subclusters based on the 16S rRNA gene sequences (Fig. 1). The sequence similarities of the species within the *L. mesenteroides* ISR-subcluster ranged from 80.2 to 99.5 %. Species in the *L. mesenteroides* ISR-subcluster showed 69.2–80.1 % and 73.7–83.6 % to the species in the *L. fructosum* ISR-subcluster and *L. fallax*, respectively. Sequence similarities of the species within the *L. fructosum* ISR-subcluster ranged from 81.3 to 92.4 %. Species in the *L. fructosum* ISR-

subcluster showed 71.1–73.1 % sequence similarity to *L. fallax*.

The *rpoC* gene, encoding the β' subunit of the DNA-dependent RNA polymerase, and the *recA* gene, encoding a recombinase A protein, were used for MLSA in this study. Recombinase A is a protein implicated in homologous DNA recombination, SOS induction and DNA damage-induced mutagenesis (Eisen, 1995). These two genes have often been used previously as markers for phylogenetic analysis and for the differentiation of lactic acid bacteria (Chelo *et al.*, 2007; Felis *et al.*, 2001; Morse *et al.*, 1996; Torriani *et al.*, 2001). Partial *rpoC* gene sequences were determined for the type strains of *L. carnosum*, *L. durionis*, *L. gasicomitatum*, *L. inhae* and *L. lactis* and partial *recA* gene sequences were determined for the type strains of the genus *Leuconostoc* listed in Table 1. The sequences of both genes from related taxa and those of the *rpoC* gene for other *Leuconostoc* species were retrieved from the DDBJ database. Partial *rpoC* genes of the tested strains were amplified by the method described by Morse *et al.* (1996), but the *rpoC*1053R primer (CAAGAAAGGSCCAACAT-CRATAAC; Chelo *et al.*, 2007) was used as a reverse primer. An amplification of the *recA* genes was performed according to the method described by Dellaglio *et al.* (2005). Purification and sequencing of the amplification products were carried out as described previously (Endo & Okada, 2005). Phylogenetic analysis was conducted as

Fig. 2. Phylogenetic relationships of the genera *Leuconostoc* and *Fructobacillus* gen. nov. based on the sequences of the 16S-23S rRNA gene ISR. The tree was constructed by the neighbour-joining method. *Oenococcus oeni* was used as an outgroup. Bootstrap values are given at branching points; values above 70 % are indicated. Bar, 0.01 K_{nuc} .

described above and phylogenetic trees were constructed by the neighbour-joining method. *Lactococcus lactis* was used as an outgroup.

The phylogenetic tree based on the *rpoC* gene sequences revealed that the genus *Leuconostoc* produced three subclusters: the *L. mesenteroides* *rpoC*-subcluster, the *L. fructosum* *rpoC*-subcluster and the *L. fallax* *rpoC*-subcluster (Fig. 3). The three *rpoC*-subclusters showed a good correlation with the subclusters identified on the basis of the 16S rRNA gene or the ISR sequences (Figs 1 and 2). The *L. fructosum* *rpoC*-subcluster was related to the *Weissella* *rpoC*-cluster and *L. fallax* was distantly positioned with regard to all of the species used in this study (Fig. 3). Based on the *recA* gene sequences, the genus *Leuconostoc* was divided into two subclusters: the *L. mesenteroides* *recA*-subcluster and the *L. fructosum* *recA*-subcluster (Fig. 4). Unlike the other phylogenetic trees (Figs 1, 2 and 3), *L. fallax* was located inside the *L. mesenteroides* *recA*-subcluster (Fig. 4). The different phylogenetic positions of *L. fallax* among the three phylogenetic trees may be due to the fact that it had no phylogenetic neighbours (Fig. 1).

In our phylogenetic analysis, three of the four phylogenetic trees, based on the sequences of the 16S rRNA gene, ISR, *rpoC* gene or *recA* gene, confirmed the formation of three subclusters in the genus *Leuconostoc* with a good

correlation and high bootstrap values. Thus, we conclude that the genus *Leuconostoc* is phylogenetically divided into three groups: the *L. mesenteroides* group (comprising *L. carnosum*, *L. citreum*, *L. gasicomitatum*, *L. gelidum*, *L. inhae*, *L. kimchii*, *L. lactis*, *L. mesenteroides* and *L. pseudomesenteroides*), the *L. fructosum* group (comprising *L. durionis*, *L. ficulneum*, *L. fructosum* and *L. pseudoficulneum*) and the *L. fallax* group (*L. fallax*). This finding correlated well with the MLSA data obtained by using four housekeeping genes of three genera of the *Leuconostoc* group, *Leuconostoc*, *Oenococcus* and *Weissella*, as determined by Chelo *et al.* (2007). Disagreements have often been found between phylogenetic analyses based on housekeeping genes and the 16S rRNA gene (Naser *et al.*, 2005; Chelo *et al.*, 2007).

The morphological characteristics of the *Leuconostoc* type strains were determined by using a scanning electron microscope (SEM). The strains were cultured for 1–2 days under the conditions shown in Table 1. After culture, cells were washed with 0.2 M phosphate buffer (pH 7.0), fixed with 2 % glutaraldehyde for 2 h at 4 °C, post-fixed with 1 % OsO₄ for 3 h at 4 °C, dehydrated with a series of increasing ethanol concentrations (70 %, 80 %, 90 %, 95 % and twice at 100 % for 15 min each) and soaked in 3-methylbutyl acetate for 1 day. The prepared cells were subsequently critical-point dried in a drying unit (model HCP-2; Hitachi), sputtered with Pt/Pd (model E-102;

Fig. 3. Phylogenetic relationships of the genera *Leuconostoc*, *Fructobacillus* gen. nov. and related taxa based on the *rpoC* gene sequences. *Lactococcus lactis* was used as an outgroup. Bootstrap values are given at branching points; values above 70 % are indicated. Bar, 0.1 K_{nuc} .

Fig. 4. Phylogenetic relationships of the genera *Leuconostoc*, *Fructobacillus* gen. nov. and related taxa based on *recA* gene sequences. *Lactococcus lactis* was used as an outgroup. Bootstrap values are given at branching points; values above 70 % are indicated. Bar, 0.1 K_{nuc} .

Hitachi), and observed with a SEM (model S-4000; Hitachi). The SEM observations revealed various morphological characteristics for the strains examined. Cells of *L. carnosum*, *L. citreum*, *L. gasicomitatum*, *L. gelidum*, *L. inhae*, *L. lactis*, *L. mesenteroides* and *L. pseudomesenteroides*, the species of the *L. mesenteroides* subcluster (Figs 1, 2, 3 and 4), were spherical, oval or coccobacillary (data not shown) and the cell shape was consistent with that previously given for the genus *Leuconostoc* (Sneath *et al.*, 1986). However, cells of *L. durionis*, *L. ficulneum*, *L. fructosum* and *L. pseudoficulneum*, the species of the *L. fructosum* subcluster (Figs 1, 2, 3 and 4), were rod-shaped (Fig. 5) and resembled species of the genus *Lactobacillus*. This observation disagreed with the morphological characteristics of the genus *Leuconostoc* as described previously (Sneath *et al.*, 1986). *L. fructosum* was formerly classified as *Lactobacillus fructosus* (Kodama, 1956) and was reclassified as *L. fructosum* by Antunes *et al.* (2002). Therefore, cells of *L. fructosum* were known to be rod-shaped. Cells of *L. durionis* and *L. ficulneum* have also been reported to be rod-shaped (Antunes *et al.*, 2002; Leisner *et al.*, 2005). Cells of *L. pseudoficulneum* were previously reported to be ovoid (Chambel *et al.*, 2006), but our SEM observations clearly indicated that *L. pseudoficulneum* had rod-shaped cells. Six strains of *L. pseudoficulneum* were originally isolated from a ripe fig (Chambel *et al.*, 2006) and so we determined the morphological characteristics of these additional strains, LC47, LC 48, LC 49, LC 50 and LC 52, which were provided by Dr Rogério Tenreiro (Universidade de

Lisboa). Our microscopic observations confirmed that these five additional strains of *L. pseudoficulneum* had rod-shaped cells rather than ovoid cells, and it is not clear why the cells were originally described as ovoid by Chambel *et al.* (2006). *L. fallax* had spherical cells (Fig. 5) and coccobacillary cells were not detected.

Biochemical and physiological characteristics such as acid production from carbohydrates, growth in a broth containing D-glucose and/or fructose as substrate(s), organic acids, ethanol produced from D-glucose and sugar tolerance were determined for the *Leuconostoc* type strains. Acid production from carbohydrates was determined by a previously described method (Endo & Okada, 2005). The data are presented in Table 2. All the strains produced acid from D-glucose and D-fructose, but the type strain of *L. lactis* did not produce acid from D-fructose. Interestingly, the type strains of *L. durionis*, *L. ficulneum*, *L. fructosum* and *L. pseudoficulneum* produced acid from only a limited number of carbohydrates, two for *L. ficulneum*, *L. fructosum* and *L. pseudoficulneum*, and three for *L. durionis*. This number of carbohydrates was much less than that found for the type strains of other *Leuconostoc* species (Table 2). The type strains of *L. ficulneum*, *L. fructosum* and *L. pseudoficulneum* and *L. durionis* produced acid from D-glucose and D-fructose and showed a delayed reaction for D-mannitol.

Growth in GYP broth, FYP broth and FGYP broth was studied for the determination of the most suitable growth

Fig. 5. Scanning electron micrographs of *F. durionis* LMG 22556^T (a), *F. ficulneus* JCM 12225^T (b), *F. fructosus* NRIC 1058^T (c), *F. pseudoficulneus* DSM 15468^T (d) and *L. fallax* NRIC 0210^T (e). Bars, 1 µm.

Table 2. Acid production from carbohydrates of the type strains of the species in the genera *Leuconostoc* and *Fructobacillus* gen. nov.

Taxa: 1, *L. carnosum*; 2, *L. citreum*; 3, *L. gasicomitatum*; 4, *L. gelidum*; 5, *L. inhae*; 6, *L. lactis*; 7, *L. mesenteroides*; 8, *L. pseudomesenteroides*; 9, *Fructobacillus durionis*; 10, *Fructobacillus ficulneus*; 11, *Fructobacillus fructosus*; 12, *Fructobacillus pseudoficulneus*; 13, *L. fallax*. +, Positive; –, negative; D, delay. All values are for the type strains of the species. All strains showed a positive reaction for D-glucose, but were negative for D-rhamnose, D-melezitose, D-sorbitol and starch.

Carbohydrate	1	2	3	4	5	6	7	8	9	10	11	12	13
L-Arabinose	–	+	+	+	D	–	D	+	–	–	–	–	–
Cellobiose	–	+	+	+	+	–	+	D	–	–	–	–	–
D-Fructose	+	+	+	+	+	–	+	+	+	+	+	+	+
D-Galactose	–	–	–	D	–	+	+	+	–	–	–	–	–
D-Gluconate	–	–	D	D	–	–	–	+	–	–	–	–	–
Lactose	–	–	–	+	–	–	+	+	–	–	–	–	–
Maltose	D	+	+	D	+	+	+	+	D	–	–	–	–
D-Mannitol	–	D	–	–	–	–	D	–	D	D	D	D	+
D-Mannose	+	+	+	+	+	–	+	+	–	–	–	–	+
Melibiose	–	–	+	+	–	–	+	+	–	–	–	–	–
Raffinose	–	–	+	+	–	–	+	+	–	–	–	–	–
D-Ribose	–	–	D	D	D	–	–	+	–	–	–	–	–
D-Salicin	–	+	–	–	–	–	+	–	–	–	–	–	–
Sucrose	+	+	+	+	+	+	+	+	+	–	–	–	+
D-Trehalose	+	+	+	+	+	–	+	+	D	D	–	–	–
D-Xylose	–	–	+	+	–	–	–	+	–	–	–	–	–

substrate for the *Leuconostoc* type strains. FGYP broth was based on GYP broth and contained 10 g D-glucose l⁻¹ and 10 g D-fructose l⁻¹ as the growth substrates. Each strain was pre-cultured under the conditions described in Table 1. Cells were washed twice with saline, washed cells were diluted 100-fold with saline and the dilutions were inoculated in the GYP, FYP and FGYP broths. The broths were cultured for 5 days at the temperatures given in Table 1. Growth was monitored by optical density at 660 nm with a spectrophotometer (NovaspecII; Biochrom Ltd). The type strains of *L. carnosum*, *L. citreum*, *L. gasicomitatum*, *L. gelidum*, *L. inhae*, *L. lactis*, *L. mesenteroides*, *L. pseudomesenteroides* and *L. fallax* grew well in the three broths (see Supplementary Figs S3a, S3b and S3c in IJSEM Online), but the type strain of *L. lactis* did not grow in FYP broth (Supplementary Fig. S3b). The reason that the type strain of *L. lactis* did not grow in FYP broth was due to a deficiency in D-fructose dissimilation (Table 2). In contrast, the type strains of *L. durionis*, *L. ficulneum*, *L. fructosum* and *L. pseudoficulneum* all grew well in the FYP and FGYP broths (Supplementary Figs S3b, S3c), but they grew poorly in GYP broth (Supplementary Fig. S3a). D-Glucose is the most common substrate for the cultivation of lactic acid bacteria and thus the poor growth in GYP broth and good growth in FYP broth of the type strains of *L. durionis*, *L. ficulneum*, *L. fructosum* and *L. pseudoficulneum* is unusual. Interestingly, these four species were originally isolated from fruits, flowers or a fermented food derived from fruit (Antunes *et al.*, 2002; Chambel *et al.*, 2006; Kodama, 1956; Leisner *et al.*, 2005) which may have contained large amounts of D-fructose. This could suggest that these four species have become adapted to survive in such environments. Thus, the isolation of lactic acid bacteria from fructose-rich environments, such as flowers, fruits or their related products, should be performed by using D-glucose and D-fructose as growth substrates. It was reported by Leisner *et al.* (2005) that *L. durionis* and *L. fructosum* produced large amounts of D-mannitol when D-glucose and D-fructose were used as growth substrates. We obtained similar results from the type strains of *L. durionis*, *L. ficulneum*, *L. fructosum* and *L. pseudoficulneum* (data not shown). This may indicate that these four strains need an electron acceptor for growth and are able to utilize D-fructose as an electron acceptor for dissimilation of D-glucose in FGYP broth. In fact, these four strains grew poorly in GYP broth but grew well in GYP broth containing 10 g pyruvate l⁻¹ as an electron acceptor (data not shown). In addition, the growth of these four type strains in GYP broth was enhanced by aerobic culturing, and the aerobic growth in GYP broth was comparable with growth in FYP broth as shown in Supplementary Fig. S3b. The surface growth of these four strains on GYP agar was also enhanced under aerobic conditions compared with growth under anaerobic conditions [anaerobic jars (GasPak System, BBL); data not shown]. This may indicate that these four type strains can also use atmospheric O₂ as an electron acceptor. Sakamoto & Komagata (1996) demonstrated that some strains of the genus *Lactobacillus* and

Pediococcus acidilactici and *L. mesenteroides* grew better under aerobic conditions than under anaerobic conditions.

The amounts of organic acids and ethanol produced from D-glucose in GYP broth under stationary conditions were determined for the *Leuconostoc* type strains by using a HPLC (Alliance PDA System; Waters) or the F-kit ethanol (Roche Diagnostics). The type strains of *L. carnosum*, *L. citreum*, *L. gasicomitatum*, *L. gelidum*, *L. inhae*, *L. lactis*, *L. mesenteroides*, *L. pseudomesenteroides* and *L. fallax* produced equimolar amounts of lactic acid and ethanol and trace amounts of acetic acid from D-glucose and this was consistent with the characteristics of the genus *Leuconostoc* as described previously (Dellaglio *et al.*, 1995). In contrast, the type strains of *L. durionis*, *L. ficulneum*, *L. fructosum* and *L. pseudoficulneum* produced equimolar amounts of lactic acid and acetic acid but ethanol was not detected. The production of equimolar amounts of acids produced from D-glucose under stationary conditions has not previously been reported for any lactic acid bacteria, including the species in the genus *Leuconostoc*. Generally, species of the genus *Leuconostoc* metabolize D-glucose via a combination of the hexose monophosphate pathway and the pentose phosphate pathway (Dellaglio *et al.*, 1995; DeMoss *et al.*, 1951; Sneath *et al.*, 1986), and ethanol is produced by a reduction of acetyl-phosphate via acetyl-CoA and acetaldehyde. However, our results suggest that *L. durionis*, *L. ficulneum*, *L. fructosum* and *L. pseudoficulneum* convert acetyl-phosphate to acetic acid and not to acetyl-CoA. On this pathway, the oxidation of NADPH is not sufficient for NADP/NADPH cycling, as *Leuconostoc* species usually oxidize NADPH to NADP by lactate dehydrogenase, acetaldehyde dehydrogenase and alcohol dehydrogenase (Condon, 1987). Consequently, if the type strains of *L. durionis*, *L. ficulneum*, *L. fructosum* and *L. pseudoficulneum* produce acetic acid by this pathway, an additional electron acceptor is needed for the oxidation of NADPH. This finding correlated well with the results of the requirement for electron acceptors, such as pyruvate and O₂, on D-glucose dissimilation for *L. durionis*, *L. ficulneum*, *L. fructosum* and *L. pseudoficulneum*. In this pathway, it is well known that twice the amount of ATP is produced as compared to the reduction pathway of acetyl-phosphate to ethanol (Johnson & McCleskey, 1957) and species of the genus *Leuconostoc* usually use this pathway under aerobic conditions (Condon, 1987; Dellaglio *et al.*, 1995; Keenan, 1968; Lucey & Condon, 1986).

Sugar tolerance was determined by the growth of the type strains in a broth containing 2%, 10%, 20%, 30%, 40% or 50% (w/v) substrate. D-Glucose was used as the substrate for the type strains of *L. carnosum*, *L. citreum*, *L. gasicomitatum*, *L. gelidum*, *L. inhae*, *L. lactis*, *L. mesenteroides*, *L. pseudomesenteroides* and *L. fallax*, and D-fructose was used for the type strains of *L. durionis*, *L. ficulneum*, *L. fructosum* and *L. pseudoficulneum*. Most strains tested grew on concentrations of 30% substrate, and the type strains of *L. durionis*, *L. fallax*, *L. ficulneum*, *L. fructosum*, *L. pseudoficulneum* and *L. pseudomesenteroides*

were able to grow on 40 % substrate. Furthermore, the type strains of *L. durionis*, *L. ficulneum*, *L. fructosum* and *L. pseudoficulneum* grew poorly on a 50 % substrate. The data indicated that these four species are xerotolerant bacteria. Antunes *et al.* (2002) obtained similar results for *L. ficulneum* and *L. fructosum*. The type strains of *L. durionis*, *L. ficulneum*, *L. fructosum* and *L. pseudoficulneum* had moderate tolerance to NaCl, and these four strains grew well on 5 % (w/v) NaCl, but poorly on 7.5 % NaCl.

Antunes *et al.* (2002) mentioned that *L. ficulneum*, *L. fructosum* and *L. fallax* could be assigned to novel genera by phylogenetic analysis based on the 16S rRNA gene sequences, but there were no noteworthy biochemical or physiological differences to distinguish these three species from the other species of the genus *Leuconostoc*. Our phylogenetic trees confirmed the division of the genus *Leuconostoc* into three groups, the *L. mesenteroides* group, the *L. fructosum* group and *L. fallax*. In addition, the species in the *L. fructosum* group, *L. durionis*, *L. ficulneum*, *L. fructosum* and *L. pseudoficulneum*, were clearly distinguished from the other species of the genus *Leuconostoc* by morphological and biochemical characteristics. Cell morphology and the inability to produce ethanol from D-glucose were key features that enabled the separation of these four species from the genus *Leuconostoc*. These four species also needed an electron acceptor for D-glucose dissimilation. These are noteworthy differential characteristics for the reclassification of the genus *Leuconostoc* as compared with the genera of lactic acid bacteria described recently. The genera *Pilibacter* and *Paralactobacillus* were established recently as novel genera of lactic acid bacteria by the differentiation of biochemical and morphological characteristics or their phylogenetic position as compared with the genera *Enterococcus* and *Lactobacillus* (Higashiguchi *et al.*, 2006; Leisner *et al.*, 2000). Therefore it is proposed that the species *Leuconostoc durionis*, *Leuconostoc ficulneum*, *Leuconostoc fructosum* and *Leuconostoc pseudoficulneum* should be transferred to a novel genus, *Fructobacillus* gen. nov., as *Fructobacillus durionis* comb. nov., *Fructobacillus ficulneus* comb. nov., *Fructobacillus fructosus* comb. nov. and *Fructobacillus pseudoficulneus* comb. nov. The type species of the genus *Fructobacillus* is *F. fructosus*.

L. fallax was distinguished from *L. durionis*, *L. ficulneum*, *L. fructosum* and *L. pseudoficulneum* and the other species of the genus *Leuconostoc* as determined by on phylogenetic analysis based on the sequences of the 16S rRNA gene, the ISR and two housekeeping genes, but not the *recA* gene. However, no noteworthy physiological and biochemical differences were found between *L. fallax* and the other species of the genus *Leuconostoc* in the present study, and thus *L. fallax* must remain in the genus *Leuconostoc*. The species will probably be reclassified in a novel genus when a phylogenetic relative of this species is found and a significant physiological or biochemical difference is determined between *L. fallax* and the remaining species of the genus *Leuconostoc*. A novel species of the genus *Leuconostoc*, *Leuconostoc holzapfelii*, was described recently (De Bruyne *et al.*, 2007). Our independent

phylogenetic analysis based on 16S rRNA gene sequences showed that *L. holzapfelii* was a member of the *L. mesenteroides* subcluster (data not shown) and this correlated well with the results of De Bruyne *et al.* (2007). Therefore, a reclassification of *L. holzapfelii* is not needed.

Description of *Fructobacillus* gen. nov.

Fructobacillus (Fruc.to.ba.cil'lus. N.L. masc. n. *Fructobacillus* arbitrarily derived from fructose and *Lactobacillus*, intended to mean fructose-loving lactic acid-producing bacillus).

Cells are Gram-positive and non-motile rods. Cells usually occur singly or in pairs and in chains. Facultatively anaerobic. Active growth is observed in a broth containing D-fructose as a substrate, and poor growth occurs on D-glucose. Growth in GYP broth is enhanced under aerobic conditions. Colonies are beige, smooth and approximately 1–2 mm in diameter on FYP agar after incubation for 2 days and are less than 1 mm in diameter on GYP agar after incubation for 5 days under anaerobic conditions. Growth is enhanced under aerobic conditions on GYP agar and colony size is approximately 1–2 mm in diameter after incubation for 2 days. Heterofermentative and produce lactic acid, carbon dioxide and acetic acid from D-glucose or D-fructose. Ethanol is not produced. Major lactic acid produced is of the D-isomer. Nitrate is not reduced. Acid is produced from a limited number of carbohydrates, D-glucose and D-fructose. Acid production from D-mannitol is delayed. The optimum temperature for growth is approximately 30 °C, and the optimum pH for growth is approximately 6.5. Xerotolerant. Cells grow in FYP broth containing 5 % (w/v) NaCl and poorly on 7.5 % (w/v) NaCl. Cells grow in a broth containing 40 % (w/v) D-fructose and poorly on 50 % (w/v) D-fructose. The DNA G+C content ranges from 42 to 45 mol%. The type species is *Fructobacillus fructosus*.

Description of *Fructobacillus fructosus* comb. nov.

Fructobacillus fructosus (fruc.to'sus. N.L. masc. adj. *fructosus* pertaining to fructose).

Basonym: *Lactobacillus fructosus* Kodama 1956 (Approved Lists 1980).

Other synonym: *Leuconostoc fructosum* (Kodama 1956) Antunes *et al.* 2002.

The description of *Fructobacillus fructosus* is the same as that given for *Leuconostoc fructosum* by Antunes *et al.* (2002).

The type strain is IFO 3516^T (=JCM 1119^T=DSM 20349^T=NRIC 1058^T).

Description of *Fructobacillus durionis* comb. nov.

Fructobacillus durionis (du.ri.o'nis. N.L. gen. n. *durionis* of *Durio*, the generic name of *Durio zibenthinus*, the durian fruit).

Basonym: *Leuconostoc durionis* Leisner *et al.* 2005.

The description of *Fructobacillus durionis* is the same as that given for *Leuconostoc durionis* by Leisner *et al.* (2005).

The type strain is D-24^T (=LMG 22556^T=CCUG 49949^T).

Description of *Fructobacillus ficulneus* comb. nov.

Fructobacillus ficulneus (fi.cul'ne.us. L. masc. adj. *ficulneus* of the fig tree).

Basonym: *Leuconostoc ficulneum* Antunes *et al.* 2002.

The description of *Fructobacillus ficulneus* is the same as that given for *Leuconostoc ficulneum* by Antunes *et al.* (2002).

The type strain is FS-1^T (=DSM 13613^T=JCM 12225^T).

Description of *Fructobacillus pseudoficulneus* comb. nov.

Fructobacillus pseudoficulneus (pseu.do.fi.cul'ne.us. Gr. adj. *pseudes* false; L. adj. *ficulneus* -a -um of the fig-tree and also a bacterial specific epithet; N.L. masc. adj. *pseudoficulneus* not the true *Leuconostoc ficulneum*/*Fructobacillus ficulneus*).

Basonym: *Leuconostoc pseudoficulneum* Chambel *et al.* 2006.

The description of *Fructobacillus pseudoficulneus* is based on the description of *Leuconostoc pseudoficulneum* as given by Chambel *et al.* (2006), with the following modification. Cells are rods measuring 0.4–0.6 × 2–5 µm and occur singly or in pairs and in chains.

The type strain is LC-51^T (=DSM 15468^T=CECT 5759^T).

Acknowledgements

We are grateful to K. Komagata and L. M. T. Dicks (Department of Microbiology, University of Stellenbosch) for valuable discussions, Y. Yaguchi (Electron Microscope Center, Tokyo University of Agriculture) for advice about the procedures for SEM observations, J. P. Euzéby (Société de Bactériologie Systématique et Vétérinaire and École Nationale Vétérinaire de Toulouse) for advice on naming a novel genus in Latin, B. J. Tindall (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) for advice regarding the treatment of a patent strain and R. Tenreiro (Universidade de Lisboa) for the kind donation of *L. pseudoficulneum* strains. Thanks are also due to K. Sonobe (NODAI Culture Collection Center, Tokyo University of Agriculture) for her technical assistance.

References

- Antunes, A., Rainey, F. A., Nobre, M. F., Schumann, P., Ferreira, A. M., Ramos, A., Santos, H. & da Costa, M. S. (2002). *Leuconostoc ficulneum* sp. nov., a novel lactic acid bacterium isolated from a ripe fig, and reclassification of *Lactobacillus fructosus* as *Leuconostoc fructosum* comb. nov. *Int J Syst Evol Microbiol* **52**, 647–655.
- Björkroth, K. J., Geisen, R., Schillinger, U., Weiss, N., De Vos, P., Holzapfel, W. H., Korkeala, H. J. & Vandamme, P. (2000).

Characterization of *Leuconostoc gasicomitatum* sp. nov., associated with spoiled raw tomato-marinated broiler meat strips packaged under modified-atmosphere conditions. *Appl Environ Microbiol* **66**, 3764–3772.

Björkroth, K. J., Geisen, R., Schillinger, U., Weiss, N., De Vos, P., Holzapfel, W. H., Korkeala, H. J. & Vandamme, P. (2001). *Leuconostoc gasicomitatum* sp. nov. In *Validation of Publication of New Names and New Combinations Previously Effectively Published Outside the IJSEM*, List no. 79. *Int J Syst Evol Microbiol* **51**, 263–265.

Brosius, J., Dull, T. J., Sleeter, D. D. & Noller, H. F. (1981). Gene organization and primary structure of a ribosomal RNA operon from *Escherichia coli*. *J Mol Biol* **148**, 107–127.

Cavalli-Sforza, L. L. & Edwards, A. W. F. (1967). Phylogenetic analysis models and estimation procedures. *Am J Hum Genet* **19**, 233–257.

Chambel, L., Chelo, I. M., Zé, Zé, L., Pedro, L. G., Santos, M. A. & Tenreiro, R. (2006). *Leuconostoc pseudoficulneum* sp. nov., isolated from a ripe fig. *Int J Syst Evol Microbiol* **56**, 1375–1381.

Chelo, I. M., Zé-Zé, L. & Tenreiro, R. (2007). Congruence of evolutionary relationships inside the *Leuconostoc*–*Oenococcus*–*Weissella* clade assessed by phylogenetic analysis of the 16S rRNA gene, *dnaA*, *gyrB*, *rpoC* and *dnaK*. *Int J Syst Evol Microbiol* **57**, 276–286.

Collins, M. D., Rodrigues, U. M., Ash, C., Aguirre, M., Farrow, J. A. E., Martinez-Murcia, A., Philips, B. A., Williams, A. M. & Wallbanks, S. (1991). Phylogenetic analysis of the genus *Lactobacillus* and related lactic acid bacteria as determined by reverse transcriptase sequencing of 16S rRNA. *FEMS Microbiol Lett* **77**, 5–12.

Condon, S. (1987). Responses of lactic acid bacteria to oxygen. *FEMS Microbiol Rev* **46**, 269–280.

De Bruyne, K., Schillinger, U., Caroline, L., Böhringer, B., Cleenwerck, I., Vancanneeyt, M., De Vuyst, L., Franz, C. M. A. P. & Vandamme, P. (2007). *Leuconostoc holzapfelii* sp. nov., isolated from Ethiopian coffee fermentation and assessment of sequence analysis of housekeeping genes for delineation of *Leuconostoc* species. *Int J Syst Evol Microbiol* **57**, 2952–2959.

Dellaglio, F., Dicks, L. M. T. & Torriani, S. (1995). The genus *Leuconostoc*. In *Lactic Acid Bacteria*, vol. 2. *The Genera of Lactic Acid Bacteria*, pp. 235–238. Edited by B. J. B. Wood & W. H. Holzapfel. Blackie Academic & Professional.

Dellaglio, F., Felis, G. E., Castioni, A., Torriani, S. & Germond, J. E. (2005). *Lactobacillus delbrueckii* subsp. *indicus* subsp. nov., isolated from Indian dairy products. *Int J Syst Evol Microbiol* **55**, 401–404.

DeMoss, R. D., Bard, R. C. & Gunsalus, I. C. (1951). The mechanism of heterolactic fermentation: a new route of ethanol fermentation. *J Bacteriol* **62**, 499–511.

Eisen, J. A. (1995). The RecA protein as a model molecule for molecular systematic studies of bacteria: comparison of trees of RecAs and 16S rRNAs from the same species. *J Mol Evol* **41**, 1105–1123.

Endo, A. & Okada, S. (2005). *Lactobacillus satsumensis* sp. nov., isolated from mashes of shochu, a traditional Japanese distilled spirit made from fermented rice and other starchy materials. *Int J Syst Evol Microbiol* **55**, 83–85.

Farrow, J. A. E., Facklam, R. R. & Collins, M. D. (1989). Nucleic acid homologies of some vancomycin-resistant leuconostocs and description of *Leuconostoc citreum* sp. nov. and *Leuconostoc pseudomesenteroides* sp. nov. *Int J Syst Bacteriol* **39**, 279–283.

Felis, G. E., Dellaglio, F., Mizzi, L. & Torriani, S. (2001). Comparative sequence analysis of a *recA* gene fragment brings new evidence for a change in the taxonomy of the *Lactobacillus casei* group. *Int J Syst Evol Microbiol* **51**, 2113–2117.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

- Felsenstein, J. (2005). PHYLIP (Phylogeny Inference Package), version 3.6. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Garvie, E. I. (1960). The genus *Leuconostoc* and its nomenclature. *J Dairy Res* 27, 283–292.
- Higashiguchi, D. T., Husseneder, C., Grace, J. K. & Berestecky, J. M. (2006). *Pilibacter termitis* gen. nov., sp. nov., a lactic acid bacterium from the hindgut of the Formosan subterranean termite (*Coptotermes formosanus*). *Int J Syst Evol Microbiol* 56, 15–20.
- Jian, W. & Dong, X. (2002). Transfer of *Bifidobacterium inopinatum* and *Bifidobacterium denticolens* to *Scardovia inopinata* gen. nov., comb. nov., and *Parascardovia denticolens* gen. nov., comb. nov., respectively. *Int J Syst Evol Microbiol* 52, 809–812.
- Johnson, M. K. & McCleskey, C. S. (1957). Studies on the aerobic carbohydrate metabolism of *Leuconostoc mesenteroides*. *J Bacteriol* 74, 22–25.
- Keenan, T. W. (1968). Production of acetic acid and other volatile compounds by *Leuconostoc citrovorum* and *Leuconostoc dextranicum*. *Appl Microbiol* 16, 1881–1885.
- Kim, J., Chun, J. & Han, H. U. (2000). *Leuconostoc kimchii* sp. nov., a new species from kimchi. *Int J Syst Evol Microbiol* 50, 1915–1919.
- Kim, B., Lee, J., Jang, J., Kim, J. & Han, H. (2003). *Leuconostoc inhae* sp. nov., a lactic acid bacterium isolated from kimchi. *Int J Syst Evol Microbiol* 53, 1123–1126.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 16, 111–120.
- Kluge, A. G. & Farris, F. S. (1969). Quantitative phyletics and the evolution of anurans. *Syst Zool* 18, 1–32.
- Kodama, R. (1956). Studies on the nutrition of lactic acid bacteria. Part IV. *Lactobacillus fructosus* nov. sp., a new species of lactic acid bacteria. *J Agric Chem Soc Jpn* 30, 705–708 (in Japanese).
- Leisner, J. J., Vancanneyt, M., Goris, J., Christensen, H. & Rusul, G. (2000). Description of *Paralactobacillus selangorensis* gen. nov., sp. nov., a new lactic acid bacterium isolated from chili bo, a Malaysian food ingredient. *Int J Syst Evol Microbiol* 50, 19–24.
- Leisner, J. J., Vancanneyt, M., van der Meulen, R., Lefebvre, K., Engelbeen, K., Hoste, B., Laursen, B. G., Bay, L., Rusul, G. & other authors (2005). *Leuconostoc durionis* sp. nov., a heterofermenter with no detectable gas production from glucose. *Int J Syst Evol Microbiol* 55, 1267–1270.
- Lucey, C. A. & Condon, S. (1986). Active role of oxygen and NADH oxidase in growth and energy metabolism of *Leuconostoc*. *J Gen Microbiol* 132, 1789–1796.
- Martinez-Murcia, A. J. & Collins, M. D. (1991). A phylogenetic analysis of an atypical leuconostoc: description of *Leuconostoc fallax* sp. nov. *FEMS Microbiol Lett* 82, 55–60.
- Martinez-Murcia, A. J. & Collins, M. D. (1992). *Leuconostoc fallax* sp. nov. In *Validation of the Publication of New Names and New Combinations Previously Effectively Published Outside the IJSB*, List no. 40. *Int J Syst Bacteriol* 42, 191–192.
- Morse, R., Collins, M. D., O'Hanlon, K., Wallbanks, S. & Richardson, P. T. (1996). Analysis of the β' subunit of DNA-dependent RNA polymerase does not support the hypothesis inferred from 16S rRNA analysis that *Oenococcus oeni* (formerly *Leuconostoc oenos*) is a techytelic (fast-evolving) bacterium. *Int J Syst Bacteriol* 46, 1004–1009.
- Naser, S. M., Thompson, F. L., Hoste, B., Gevers, D., Dawyndt, P., Vancanneyt, M. & Swings, J. (2005). Application of multilocus sequence analysis (MLSA) for rapid identification of *Enterococcus* species based on *rpoA* and *pheS* genes. *Microbiology* 151, 2141–2150.
- Naser, S. M., Vancanneyt, M., Hoste, B., Snauwaert, C. & Swings, J. (2006). *Lactobacillus cypricaei* Lawson et al. 2001 is a later heterotypic synonym of *Lactobacillus acidipiscis* Tanasupawat et al. 2000. *Int J Syst Evol Microbiol* 56, 1681–1683.
- Rachman, C. N., Kabadjova, H., Prévost, H. & Dousset, X. (2003). Identification of *Lactobacillus alimentarius* and *Lactobacillus farciminis* with 16S–23S rDNA intergenic spacer region polymorphism and PCR amplification using species-specific oligonucleotide. *J Appl Microbiol* 95, 1207–1216.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4, 406–425.
- Sakamoto, M. & Komagata, K. (1996). Aerobic growth of and activities of NADH oxidase and NADH peroxidase in lactic acid bacteria. *J Ferment Bioeng* 82, 210–216.
- Shaw, B. G. & Harding, C. D. (1989). *Leuconostoc gelidum* sp. nov. and *Leuconostoc carnosum* sp. nov. from chill-stored meats. *Int J Syst Bacteriol* 39, 217–223.
- Skerman, V. B. D., McGowan, V. & Sneath, P. H. A. (editors) (1980). Approved lists of bacterial names. *Int J Syst Bacteriol* 30, 225–420.
- Sneath, P. H. A., Mair, N. S., Sharpe, M. E. & Holt, J. G. (editors) (1986). *Bergey's Manual of Systematic Bacteriology*, vol. 2, Baltimore: Williams & Wilkins.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 25, 4876–4882.
- Torriani, S., Felis, G. E. & Dellaglio, F. (2001). Differentiation of *Lactobacillus plantarum*, *L. pentosus*, and *L. paraplantarum* by *recA* gene sequence analysis and multiplex PCR with *recA* gene-derived primers. *Appl Environ Microbiol* 67, 3450–3454.
- Van de Peer, Y., De Rijk, P., Wuyts, J., Winkelmans, T. & De Wachter, R. (2000). The European small subunit ribosomal RNA database. *Nucleic Acids Res* 28, 175–176.
- Van Tieghem, P. (1878). Sur la gomme du sucre (Leuconostoc mesenteroides). *Ann Sci Nat Bot* 7, 180–203.
- Vancanneyt, M., Zamfir, M., de Wachter, M., Cleenwerck, I., Hoste, B., Rossi, F., Dellaglio, F., de Vuyst, L. & Swings, J. (2006). Reclassification of *Leuconostoc argentinum* as a later synonym of *Leuconostoc lactis*. *Int J Syst Evol Microbiol* 56, 213–216.
- Zavaleta, A. I., Martinez-Murcia, A. J. & Rodriguez-Valera, F. (1996). 16S–23S rDNA intergenic sequences indicate that *Leuconostoc oenos* is phylogenetically homogeneous. *Microbiology* 142, 2105–2114.