

Reclassification of *Pediococcus dextrinicus* (Coster and White 1964) Back 1978 (Approved Lists 1980) as *Lactobacillus dextrinicus* comb. nov., and emended description of the genus *Lactobacillus*

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The taxonomic status of *Pediococcus dextrinicus* is described and transfer of the species to the genus *Lactobacillus*, with the name *Lactobacillus dextrinicus* comb. nov., is proposed. This reclassification is supported by multilocus sequence analysis of the 16S rRNA gene and Cpn60, PheS, RecA and RpoA proteins. The mode of cell division and existing phenotypic information also show that *P. dextrinicus* does not belong to the genus *Pediococcus*, but rather to the genus *Lactobacillus*. As such, we propose that *Pediococcus dextrinicus* is reclassified as *Lactobacillus dextrinicus* comb. nov. (type strain ATCC 33087^T=DSM 20335^T=JCM 5887^T=LMG 11485^T=NCDO 1561^T).

The genus *Pediococcus* currently consists of 13 species (correct at the time of writing), including *Pediococcus acidilactici*, *Pediococcus clausenii* (Dobson *et al.*, 2002), *Pediococcus cellicola* (Zhang *et al.*, 2005), *Pediococcus damnosus*, *Pediococcus dextrinicus*, *Pediococcus ethanolidurans* (Liu *et al.*, 2006), *Pediococcus inopinatus*, *Pediococcus parvulus*, *Pediococcus pentosaceus*, *Pediococcus siamensis* (Tanasupawat *et al.*, 2007) and *Pediococcus stilesii* (Franz *et al.*, 2006). Two additional species originally classified as members of the genus *Pediococcus* have been transferred to other genera. *Pediococci* are homofermentative and produce DL-lactate from glucose; the exceptions being *P. clausenii* and *P. dextrinicus* which produce only (+)-L-lactic acid (Dobson *et al.*, 2002; Franz *et al.*, 2006; Pederson, 1949).

The species *P. dextrinicus* was included within the genus *Pediococcus* based upon morphology, cell-wall composition, homofermentative lactic acid production and nutritional requirements (Coster & White, 1964). It should be noted, however, that many of these characteristics are also shared by other lactic acid bacteria, including the related genera *Aerococcus*, *Lactobacillus* and *Tetragenococcus*. Gunther & White (1961a) first introduced the term 'Group III' for what is now *P. dextrinicus*, but unlike *Pediococcus* Groups I and II, Group III was referred to as the 'Possible Group III' as it was less well defined and

differed from Groups I and II (true pediococci) in many phenotypic properties. Group III organisms most resembled *Pediococcus halophilus* (now reclassified as *Tetragenococcus halophilus*), except for salt tolerance. Gunther & White (1961a) went further to say 'it is felt that insufficient evidence is at present available on which to base the establishment of this group at specific rank'. The original descriptions of *P. dextrinicus* (originally referred to as 'Group III *Pediococcus*' by Gunther & White, 1961a, b, and later as '*Pediococcus cerevisiae* subsp. *dextrinicus*' by Coster & White, 1964), describe *P. dextrinicus* as being very different from all other *Pediococcus* isolates and their findings indicated that *P. dextrinicus* may in fact be more closely related to members of the genus *Staphylococcus* (Coster & White, 1964). It was mentioned that *P. dextrinicus* strains 'are not related antigenically to *P. cerevisiae* (Group I) and *P. parvulus* (Group II). They may possibly represent a separate serological group' (Gunther & White, 1961b). In 1964, Coster & White noticed that 'Group III (now *P. dextrinicus*) extracts did not react with antisera prepared against strains other than group III', yet antisera produced to isolates found in Groups I and II show extensive cross-reactivity with bacteria in both Groups. Serological data thus indicate that *P. dextrinicus* does not group with other pediococci.

At that point, the genus *Pediococcus* was too diverse to exclude *P. dextrinicus* as the genus still contained bacteria

Abbreviation: MLSA, multilocus sequence analysis.

that have since been reclassified to the genera *Aerococcus* and *Tetragenococcus*. As such, *P. dextrinicus* was elevated to species status not based upon properties that included it within the genus *Pediococcus*, but based on the fact that it could not be included with any of the other species that had arisen from the original grouping of '*Pediococcus cerevisiae*' (Back, 1978). In addition to the serological data presented by Gunther & White (1961b) and Coster & White (1964), several phenotypic properties also distinguish *P. dextrinicus* from other pediococci, including the lack of acid production from growth on trehalose, production of CO₂ from gluconate, lack of growth at pH 4.5 and the ability to hydrolyse starch and dextrin (Dellaglio & Torriani, 2006; Franz *et al.*, 2006; Holzapfel *et al.*, 2006; Simpson & Taguchi, 1995; Weiss, 1992). The phenotypic differences between *P. dextrinicus* and other pediococci are summarized in Table 1. For comparative purposes, the genera *Aerococcus* and *Tetragenococcus* are included in Table 1 as these bacteria were previously classified as pediococci. While *P. dextrinicus* can be excluded from the genus *Pediococcus* based upon the phenotypic properties in Table 1, these data also show that there are no phenotypic properties which can distinguish *P. dextrinicus* from members of the genus *Lactobacillus*.

Although the mode of division of *P. dextrinicus* has been listed as conforming to that of the genus *Pediococcus*, there is no published microscopy work documenting this and, interestingly, the original description of *P. dextrinicus* did

Table 1. Phenotypic characteristics that differentiate *P. dextrinicus* from related genera, but not from the genus *Lactobacillus*

Taxa: 1, *Aerococcus*; 2, *Lactobacillus*; 3, *P. dextrinicus*; 4, *Pediococcus*; 5, *Tetragenococcus*. +, Positive; -, negative; +/-, genus includes species that are both positive and negative. Data adapted from Dellaglio & Torriani (2006), Franz *et al.* (2006), Hammes & Hertel (2006), Simpson & Taguchi (1995) and Weiss (1992).

| Characteristic | 1* | 2 | 3 | 4† | 5* |
|--------------------------|-------|--------------|-------|-----|-------|
| Production of acid from: | | | | | |
| Starch | - | +/- | + | - | - |
| Dextrin | + | +/- | + | - | - |
| Trehalose | + | +/- | - | + | + |
| Mannose | + | +/- | - | + | + |
| Gas from gluconate | - | +/- | + | - | - |
| Configuration of lactate | (+)-L | D, (+)-L, DL | (+)-L | DL‡ | (+)-L |
| Growth in/at: | | | | | |
| 6.5 % NaCl | + | +/- | - | +§ | + |
| pH 4.5 | - | +/- | - | + | - |

*The genera *Aerococcus* and *Tetragenococcus* both contain isolates that were once classified as *Pediococcus* species.

†Includes current species of the genus *Pediococcus*, except for *P. dextrinicus*.

‡All except *P. clausenii* which produces only (+)-L lactate.

§All except *P. damnosus*.

not include the mode of division (Gunther & White, 1961a, b; Coster & White, 1964). It was not until *P. dextrinicus* was elevated to species status by Back (1978) that the description of the organism was expanded to include 'occurring predominantly in pairs and tetrads, occasionally in short chains and irregular clusters'. This ability to form chains differentiates *P. dextrinicus* from the descriptions of all other species of the genus *Pediococcus* (Simpson & Taguchi, 1995). Clarification of the mode of division of *P. dextrinicus* is clearly necessary for a proper description of this bacteria.

In addition to these differences in phenotype and mode of division, it has been shown based upon 16S rRNA gene, *cpn60* gene and protein and 16S-23S rRNA interspacer sequences that *P. dextrinicus* is phylogenetically distant to other pediococci, belonging instead within the genus *Lactobacillus* (Collins *et al.*, 1991; Dobson *et al.*, 2002; Franz *et al.*, 2006). Despite the extensive documentation showing that *P. dextrinicus* are not pediococci, no formal reclassification of *P. dextrinicus* has been proposed. As such, the aim of the present study is the evaluation and clarification of the taxonomic position of this species.

Mode of division

We compared Gram-stains of *P. dextrinicus* with other species of the genus *Pediococcus* by counting the number of cells present as singles, pairs, tetrads and clusters (Table 2). There were no statistically significant differences found for the cell arrangements between: fields of view, isolates of the same species, growth phase or medium (*t*-test for

Table 2. Results of cell division

| Species* | Cell arrangement† | | | |
|------------------------|-------------------|------|---------|----------|
| | Single | Pair | Tetrad‡ | Cluster§ |
| <i>P. acidilactici</i> | 2 | 12 | 76 | 0 |
| <i>P. clausenii</i> | 2 | 2 | 96 | 0 |
| <i>P. dextrinicus</i> | 13 | 20 | 0 | 67 |
| <i>P. inopinatus</i> | 4 | 4 | 92 | 0 |
| <i>P. parvulus</i> | 12 | 20 | 68 | 0 |
| <i>P. pentosaceus</i> | 4 | 56 | 40 | 0 |

*Isolates used: *P. acidilactici* ATCC 8042, ATCC 25740; *P. clausenii* ATCC BAA-344^T, CCC B1099; *P. dextrinicus* ATCC 33087^T, ATCC 700477; *P. inopinatus* ATCC 49902^T; *P. parvulus* ATCC 43013, Spain 2.6; *P. pentosaceus* ATCC 33314, ATCC 8081.

†There was no statistically significant difference found between: fields of view, isolates of the same species, growth phase or type of medium (*t*-test; all *P*-values >0.05); consequently, numbers given are the mean number determined by counting 100 bacteria in each of five separate fields of view, from colonies taken from agar plates, and also at both log- and plateau-phase from broth medium.

‡Symmetrical groups of four cells with two perpendicular lines of division.

§Three or more bacteria that were not symmetrical. Clumps of >10 bacteria were frequently observed.

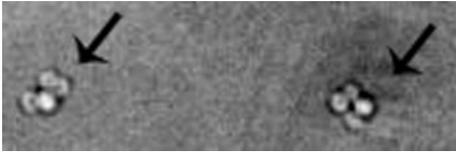


Fig. 1. Clusters of four cells of *P. dextrinicus* ATCC 33087^T produced after division from a single cell. Image taken by phase-contrast microscope.

independent samples; all *P*-values >0.05). In this assessment of cell division, clusters were defined as groups of three or more cells that were not symmetrical and tetrads were defined as symmetrical groups of four cells (Simpson, 1994). This microscopy work strongly indicated that *P. dextrinicus* does not divide using a process like that of other pediococci. To confirm this, single cells of *P. clausenii* ATCC BAA-344^T and *P. dextrinicus* ATCC 33087^T were followed through a minimum of two cycles of division (division from one to four cells) using phase-contrast microscopy. Cells of *P. clausenii* ATCC BAA-344^T divided in two perpendicular directions on a single plane and, once a tetrad was achieved, the four cells split into two doublets along the first line of division. In contrast, cells resulting from the two cell divisions of *P. dextrinicus* were not perpendicular to each other nor were they in a single plane. Moreover, *P. dextrinicus* cells did not separate upon becoming a group of four, but continued to divide, creating large irregular clusters, many of which contained >10 cells. Fig. 1 shows the result of division from one to four cells for two *P. dextrinicus* cells, while Fig. 2 represents *P. dextrinicus* cell division schematically compared with that given in the genus description

for *Pediococcus* (Simpson, 1994). While these results suggest a different arrangement and mode of division for *P. dextrinicus* as compared with other pediococci (i.e. non-perpendicular cell arrangement and non-division after tetrad formation but formation of larger clumps), the exact mode of division needs to be further examined, including studies using scanning electron microscopy.

Multilocus sequence analysis

Although 16S rRNA gene sequences are commonly used to elucidate phylogenetic relationships (Woese, 1987), using 16S rRNA gene sequences to infer phylogeny has been criticized as it assumes that one molecule can reflect organismal evolutionary history (Fox *et al.*, 1992). As such, instead of using just the 16S rRNA gene to infer phylogeny, we used a multilocus sequence analysis (MLSA) to assess the relationship of *P. dextrinicus* to other species of the genus *Pediococcus*, species of the genus *Lactobacillus* and species of related genera. In addition to the full-length 16S rRNA gene, we also analysed portions of the following conserved genes: *cpn60* (552 bp), *recA* (531 bp), *pheS* (455 bp) and *rpoA* (533 bp). The usefulness of these five regions in assessing phylogenetic relationships has been shown previously (Dobson *et al.*, 2002; Eisen, 1995; Felis *et al.*, 2001; Jian *et al.*, 2001; Kwok & Chow, 2003; Lloyd & Sharp, 1993; Naser *et al.*, 2005, 2007; Vandamme *et al.*, 1996). We believe that the congruent data obtained through our MLSA approach creates a solid representation of the phylogenetic relationships among the bacterial species analysed, thus clarifying the taxonomic position of *P. dextrinicus*.

Phylogenetic trees were constructed using sequences from, in most cases, the type strains of two representatives from

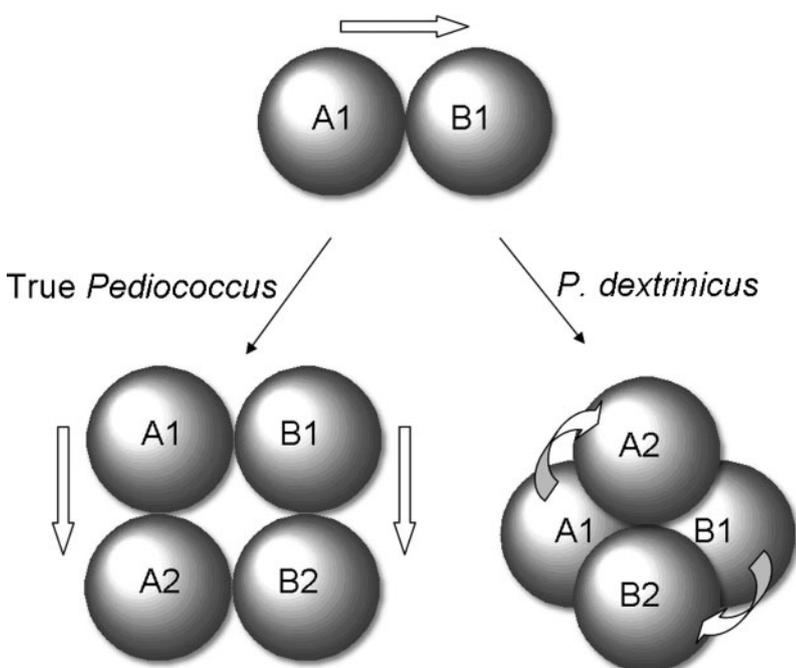


Fig. 2. Schematic representation of cell division from one to four cells for *P. dextrinicus* ATCC 33087^T compared with true pediococci as followed by phase-contrast microscopy. The division of true pediococci is in two perpendicular directions in a single plane at right angles, while the division of *P. dextrinicus* cells is not in two perpendicular directions at right angles, nor is it in a single plane.

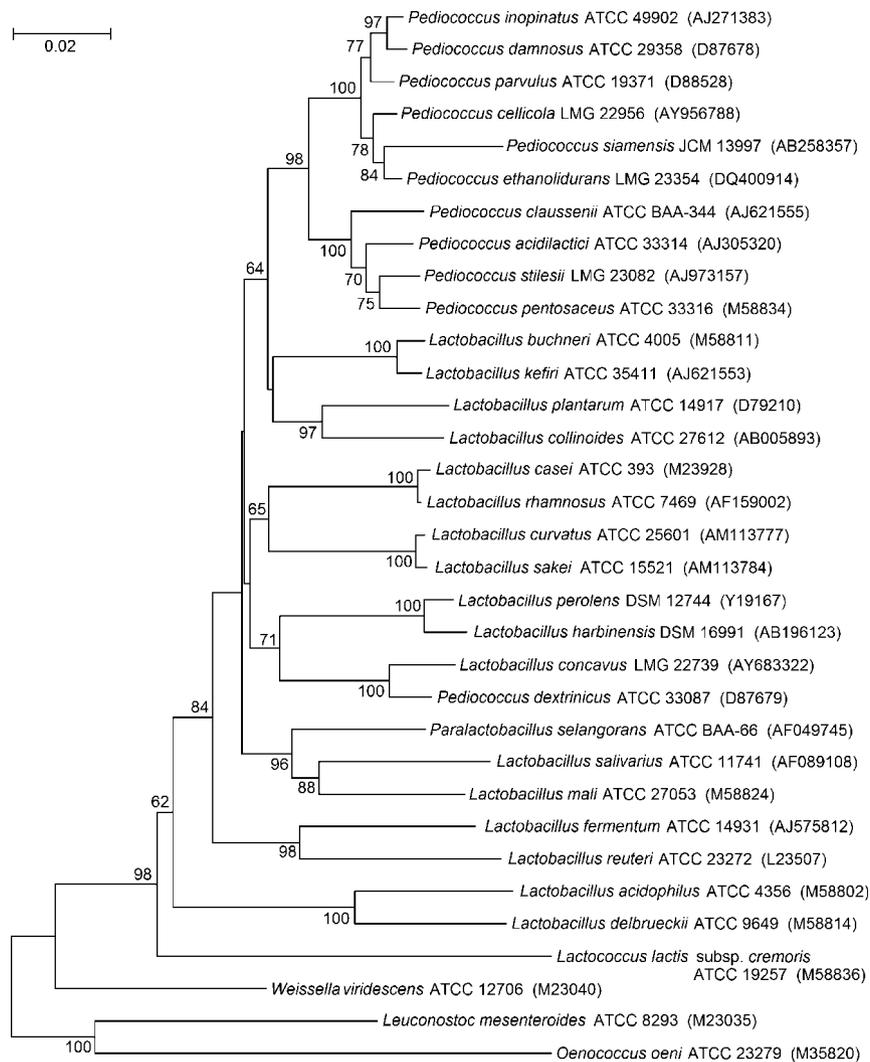


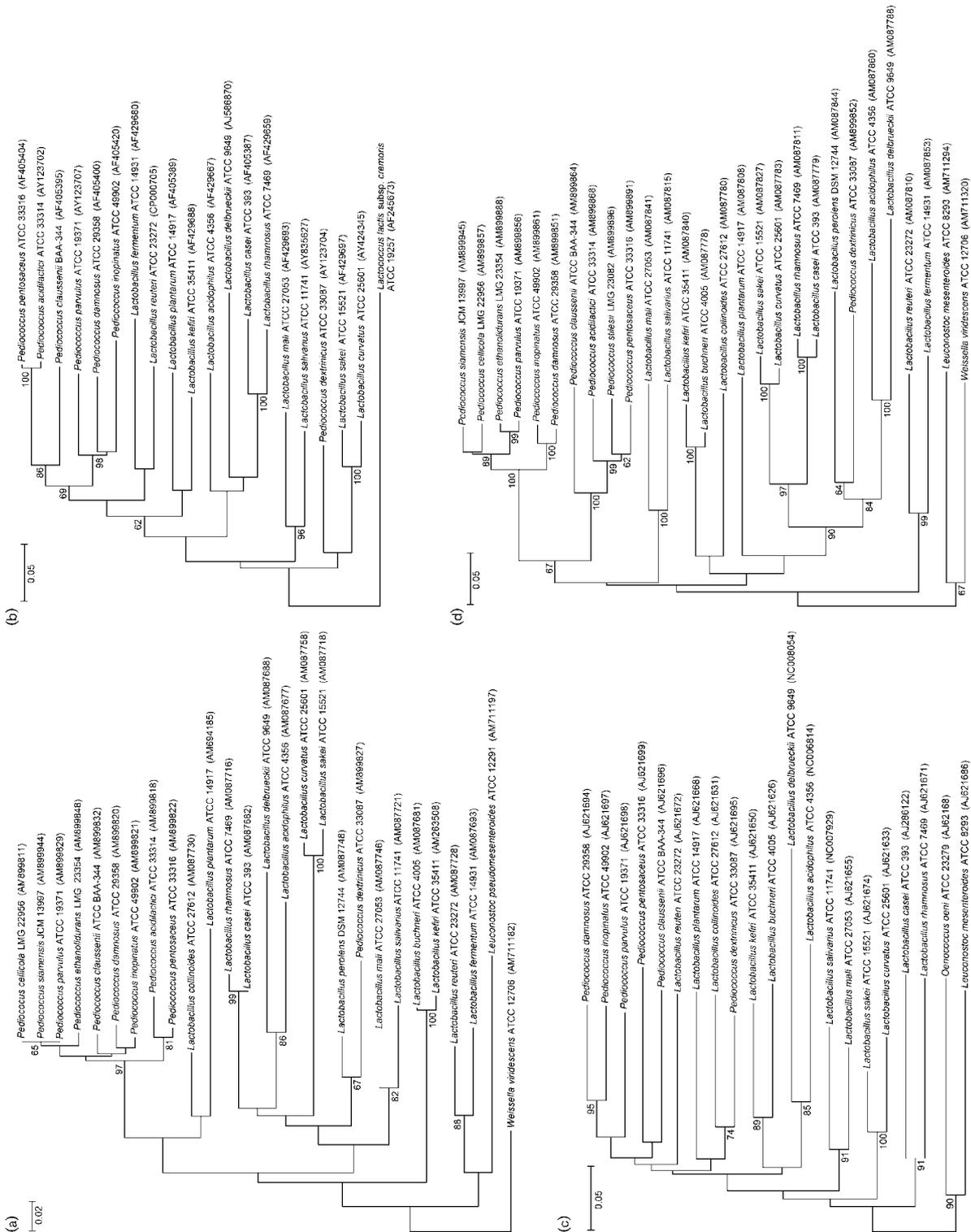
Fig. 3. Phylogenetic tree based on 16S rRNA gene sequences illustrating the evolutionary relationship of *Pediococcus dextrinicus* with all other species of the genus *Pediococcus* and species that are representative of the major clades of the genus *Lactobacillus*. GenBank accession numbers are given in parentheses. Except for *Lactobacillus fermentum*, *P. acidilacti* and *P. ethanolidurans*, all strains are type strains. Bootstrap values >60% are given at nodes as a percentage of 1000 replicates. Bar, 2% divergence.

each of the seven groups of the genus *Lactobacillus* as described in *The Prokaryotes* (Hammes & Hertel, 2006). By using two representatives from each of these groups, the overall structure of the 16S rRNA gene tree remains the same as that shown in other publications regarding the current taxonomy of the genus *Lactobacillus* (Collins *et al.*, 1991; Hammes & Hertel, 2006; Holzapfel *et al.*, 2001; Klein *et al.*, 1998; Naser *et al.*, 2007; Stiles & Holzapfel, 1997). The best GenBank matches to *P.*

dextrinicus (i.e. *Lactobacillus concavus*, *Lactobacillus harbinensis* and *Lactobacillus perolens*) were also included in the MLSA where sequences were available. The type strain sequences of the closely related genera *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Paralactobacillus* and *Weissella* are included as outliers, again, where sequences were available.

Sequences were aligned with CLUSTAL_X (Thompson *et al.*, 1997) and 16S rRNA gene alignments were visualized and

Fig. 4. Phylogenetic trees illustrating the evolutionary relationship of *Pediococcus dextrinicus* with all other species of the genus *Pediococcus* and species that are representative of the major clades of the genus *Lactobacillus*. GenBank accession numbers are given in parentheses. The strains used are the same as those in Fig. 3. Bootstrap values >60% are given at nodes as a percentage of 1000 replicates. (a) PheS protein; (b) Cpn60 protein; (c) RecA protein; (d) RpoA protein. Bars, 2% divergence (a); 5% divergence (b, c, d).



manually edited using the GeneDoc software program (Nicholas *et al.*, 1997) to conform with structural information and inferred locations of conserved and variable regions (Neefs *et al.*, 1993). Tree topology was evaluated using minimum evolution, maximum-parsimony, neighbour-joining and unweighted pair group method of arithmetic means (UPGMA) algorithms, with all methods producing similar overall topologies (data not shown). Fig. 3 and Fig. 4a–d were inferred using the neighbour-joining maximum composite likelihood method (Saitou & Nei, 1987) for the 16S rRNA gene and the PheS, Cpn60, RecA and RpoA proteins and are representative of the topology constructed by all four algorithms. Phylogenetic trees produced from DNA and translated protein sequences also produced similar overall topologies (data not shown). All phylogenetic trees were visualized and produced using MEGA version 4.0 (Tamura *et al.*, 2007). The percentage of replicate trees in which the associated taxa clustered together at levels >60% in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985).

P. dextrinicus consistently branches distant to other species of the genus *Pediococcus* for all five genetic loci (Fig. 3, Fig. 4a–d), grouping instead with members of the genus *Lactobacillus*. Based upon the 16S rRNA gene sequence analysis, the species most similar to *P. dextrinicus* are *Lactobacillus concavus*, *Lactobacillus harbinensis* and *Lactobacillus perolens*. This similarity is reinforced by the fact that the 16S rRNA gene of these *Lactobacillus* species shares 93–97% similarity with that of *P. dextrinicus*, while other members of the genus *Pediococcus* share only 90–92% sequence similarity with *P. dextrinicus*. In contrast, the other ten species of the genus *Pediococcus* exhibit 93–97% inter-species similarity for the 16S rRNA gene.

Using 16S rRNA gene sequences, Collins *et al.* (1991) and Franz *et al.* (2006) both found, as did we (Fig. 3), that *P. dextrinicus* forms a distinct line of descent compared to the rest of the species in the genus *Pediococcus*. Here we have supported this finding with the application of a MLSA using PheS, Cpn60, RecA and RpoA sequences (Fig. 4a–d). This MLSA phylogenetic information, in conjunction with evidence that *P. dextrinicus* does not divide in two perpendicular directions in a single plane (Table 2; Figs 1 and 2) as do true pediococci (Table 2; Fig. 2), reinforces the phenotypic evidence (Table 1) indicating that this species does not belong within the genus *Pediococcus*, but rather does belong within the closely related genus *Lactobacillus*. We therefore propose that *Pediococcus dextrinicus* be reclassified as *Lactobacillus dextrinicus* comb. nov.

Emended description of the genus *Lactobacillus* Beijerinck 1901

This description of the genus *Lactobacillus* is based on that of Beijerinck (1901) as given by Kandler & Weiss (1986), with the addition that cells may be rods, cocci or coccobacilli in shape.

Description of *Lactobacillus dextrinicus* comb. nov.

Lactobacillus dextrinicus (dex.tri'ni.cus. N.L. n. *dextrinum* dextrin; L. suff. *-icus* suffix used with the sense of belonging to; N.L. masc. adj. *dextrinicus* related to dextrin).

Basonym: *Pediococcus dextrinicus* (Coster and White 1964) Back 1978 (Approved Lists 1980).

Gram-positive, non-motile, non-spore-forming, facultative anaerobes. Cells are spherical, never elongated and may occur singly, in pairs or clusters and rarely in chains. Clusters of four may be observed, but not as the result of division in two perpendicular directions at right angles. Division does not occur in a single plane. Catalase-negative. Only (+)-L-lactic acid is produced [from maltose, dextrin and starch, but not from ribose, arabinose, xylose, mannitol, trehalose (10–90% of strains), melezitose]; no ammonia is produced from arginine. Cells grow at pH 7.0, but not pH 4.5 (Dellaglio & Torriani, 2006; Franz *et al.*, 2006; Garvie, 1986; Stiles & Holzapfel, 1997). The optimal temperature for growth is 32 °C (Cai *et al.*, 1999), weak growth is observed at 43–45 °C, but not above 45 °C (Franz *et al.*, 2006). G+C content of the DNA is 40–41 mol% (Cai *et al.*, 1999).

The type strain is ATCC 33087^T (=DSM 20335^T=JCM 5887^T=LMG 11485^T=NCDO 1561^T).

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