Reclassification of *Paralactobacillus selangorensis* Leisner *et al.* 2000 as *Lactobacillus selangorensis* comb. nov.

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The taxonomic status of *Paralactobacillus selangorensis* is described and, based on evidence presented, transfer of the species to the genus *Lactobacillus* with the name *Lactobacillus selangorensis* comb. nov. is proposed. This reclassification is supported by multilocus sequence analysis of the 16S rRNA gene and portions of the *cpn60*, *pheS* and *rpoA* genes. Mode of cell division and existing phenotypic information also show that *P. selangorensis* cannot be differentiated from the genus *Lactobacillus*. The type strain of *Lactobacillus selangorensis* comb. nov. is ATCC BAA-66^T (=LMG 17710^T =CIP 106482^T).

INTRODUCTION

Consisting of a single species, the genus *Paralactobacillus* was first mentioned in 1997 (Leisner *et al.*, 1997) and the genus name was validly published in 2000 (Leisner *et al.*, 2000). At the time of description, *Paralactobacillus selangorensis* was only compared to select subsets of species of the genus *Lactobacillus*, thereby giving the appearance that it was a separate genus (Leisner *et al.*, 1997, 1999, 2000). Specifically, phenotypic comparison was done with only seven and fifteen species from the *Lactobacillus casei*/*Pediococcus* and *Lactobacillus delbrueckii* groups, respectively, thus ignoring more than one hundred other species of the genus *Lactobacillus* described at that time (Leisner *et al.*, 2000). Moreover, in the same study, the 16S rRNA gene phylogeny that was done included only three species for each of the same two comparative *Lactobacillus* groups. Other authors have since shown by 16S rRNA gene phylogeny that *P. selangorensis*, in fact, resides in the midst of the genus *Lactobacillus* (Ennahar *et al.*, 2003; Hammes & Hertel, 2006; Ljungh & Wadstrom, 2009), but no formal request for reclassification has been made. Here we show that by conducting phylogenetic analysis based on four conserved genetic loci, and by including species of the genus *Lactobacillus* representative of all major *Lactobacillus* groups (as defined by Hammes & Hertel, 2006), the genus *Paralactobacillus* does not warrant separate genus status, but rather the single species of the genus belongs in the genus *Lactobacillus*.

Abbreviation: MLSA, Multilocus sequence analysis.

The GenBank/EMBL/DBJ accession numbers for the *cpn60*, *rpoA* and *pheS* gene sequences of strain ATCC BAA-66^T are HM214469, HM214470 and HM214471, respectively.

A supplementary figure is available with the online version of this paper.

METHODS

Multilocus sequence analysis (MLSA). Although 16S rRNA gene sequences are commonly used to elucidate phylogenetic relationships, using only a single gene to infer phylogeny has been criticized as it assumes that one molecule can reflect organismal evolutionary history (Fox *et al.*, 1992). In the same manner as that applied to clarify the taxonomic position of *Pediococcus dextrinicus* within the genus *Lactobacillus* (Haakensen *et al.*, 2009), we used a MLSA to assess the relationship of *P. selangorensis* to species of the genus *Lactobacillus* and species of related genera. In addition to the full-length 16S rRNA gene, we analysed portions of the conserved genes *cpn60* (552 bp), *pheS* (455 bp) and *rpoA* (533 bp).

Phylogenetic trees were reconstructed using GenBank accessible sequences for type strains of the genus *Lactobacillus*. *cpn60*, *pheS* and *rpoA* gene sequences were aligned by using CLUSTAL X (Thompson *et al.*, 1997) and 16S rRNA gene sequences were aligned using the tools available in the myRDP section of the Ribosomal Database Project (RDP) website (Cole *et al.*, 2007, 2009). DNA extracted from *P. selangorensis* ATCC BAA-66^T was used for amplification and sequencing of *cpn60*, *pheS* and *rpoA* gene sequences as previously described (Haakensen *et al.*, 2009). Tree topology was evaluated using minimum-evolution, maximum-parsimony, neighbour-joining and unweighted pair group method of arithmetic means algorithms, with all methods producing similar overall topologies (data not shown). Trees shown in Fig. 1(a–d) were inferred by using the neighbour-joining maximum composite likelihood method (Saitou & Nei, 1987) for the 16S rRNA, *cpn60*, *pheS* and *rpoA* genes and are representative of the topology reconstructed by all four algorithms. Due to space constraints, the 16S rRNA gene tree (Fig. 1a) was reconstructed using only sequences from two representatives from 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 seven groups, the overall structure of the 16S rRNA gene tree remains the same as that shown in more extensive investigations regarding taxonomy of the genus *Lactobacillus* (Collins *et al.*, 1991; Stiles & Holzapfel, 1997; Klein *et al.*, 1998; Holzapfel *et al.*, 2001; Hammes & Hertel, 2006; Felis & Dellaglio, 2007; Naser *et al.*, 2007; Supplementary Fig. S1, available in IJSEM Online, which contains the 16S rRNA gene phylogenetic tree reconstructed using RDP data

Fig. 1. Phylogenetic trees illustrating the evolutionary relationship of *Paralactobacillus selangorensis* to species representative of the major clades of the genus *Lactobacillus*. Where sequences were available, *Lactococcus lactis*, *Leuconostoc lactis*, *Leuconostoc mesenteroides*, *Oenococcus oeni* and *Weissella viridescens* have been included as outliers. GenBank accession numbers are given in parentheses. Bootstrap values >50 % are given at nodes as a percentage of 1000 replicates. (a) 16S rRNA gene; bar, 2 % divergence; (b) *cpn60*; bar, 5 % divergence; (c) *pheS*; bar, 5 % divergence; (d) *rpoA*; bar, 5 % divergence.

accessed 1 December 2010 for the type strains of all species of the genera *Lactobacillus* and *Pediococcus*, as well as for the type strain of *Paralactobacillus selangorensis* and one other isolate). The *cpn60*, *pheS* and *rpoA* trees (Fig. 1b–d) were reconstructed using available sequences for the isolates of the species of the genus *Lactobacillus* used to create the 16S rRNA gene tree. Also, when available, sequences of isolates from the closely related genera *Lactococcus*, *Leuconostoc*, *Oenococcus* and *Weissella* were included as outliers for each tree. All phylogenetic trees were produced and visualized using MEGA version 4.0 (Tamura *et al.*, 2007). The percentage of replicate trees in which the associated taxa clustered together (>50 %) in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985).

Comparison of phenotypic properties. Multiple and diverse phenotypic properties have been published for species of the genus *Lactobacillus*. Excellent summaries of these phenotypes can be found in books and book chapters by Dellaglio & Torriani (1986), Hammes & Hertel (2006) and Ljungh & Wadstrom (2009). For purposes of comparison in this paper, phenotypic traits previously published for *P. selangorensis* (Leisner *et al.*, 1997, 1999, 2000) were compared to known phenotypes of the genus *Lactobacillus* (Table 1).

RESULTS AND DISCUSSION

The description of *Paralactobacillus selangorensis* was based on phenotypic comparisons and 16S rRNA gene phylogeny involving only a restricted number of species of the genera *Lactobacillus* and *Pediococcus* (Leisner *et al.*, 2000). In addition, the species chosen for the comparative analyses represented only two of the major *Lactobacillus* groups

currently defined (Hammes & Hertel, 2006). Given how the genus *Paralactobacillus* was established, our goal was to determine whether this genus truly is a separate genus, or whether it should be renamed to reflect classification within an existing genus.

Phylogeny of the 16S rRNA gene has previously shown that *P. selangorensis* resides taxonomically within the genus *Lactobacillus* (Ennahar *et al.*, 2003; Hammes & Hertel, 2006; Ljungh & Wadstrom, 2009). To confirm and extend these findings, we applied a MLSA approach to further assess the taxonomic relationship of the genus *Paralactobacillus* with the genus *Lactobacillus*. Phylogenetic trees were reconstructed by using sequences of the 16S rRNA gene and portions of the *cpn60*, *pheS* and *rpoA* genes (Fig. 1a–d, respectively). As shown in both Fig. 1(a) and in the comprehensive 16S rRNA gene phylogenetic tree found in Supplementary Fig. S1, *P. selangorensis* is located within the genus *Lactobacillus*. The phylogenetic trees reconstructed with portions of the *cpn60*, *pheS* and *rpoA* genes also place *P. selangorensis* firmly within the genus *Lactobacillus* (Fig. 1b–d). This taxonomic placement for *P. selangorensis* is congruent with the previously published 16S rRNA gene sequence analyses of the genus *Lactobacillus* which have included *P. selangorensis* (Ennahar *et al.*, 2003; Hammes & Hertel, 2006; Ljungh & Wadstrom, 2009). As well, when phenotypic data are assessed, *P. selangorensis* cannot be differentiated from accepted phenotypic variances already recognized in species of the genus *Lactobacillus* (Table 1).

Table 1. Phenotypic characteristics of *P. selangorensis* and the genus *Lactobacillus*

+, Positive; −, negative; +/−, genus includes species that are both positive and negative for that phenotype. Data adapted from Dellaglio & Torriani (1986), Hammes & Hertel (2006) and Leisner *et al.* (1997, 1999, 2000).

Characteristic	<i>Paralactobacillus</i>	<i>Lactobacillus</i>
Production of acid from:		
Starch	−	+/−
Mannitol	−	+/−
Raffinose	−	+/−
Configuration of lactate	DL	D, L(+), DL
Growth with/at:		
6.5 % NaCl	−	+/−
pH 4.5	+	+/−
45 °C	−	+/−
Strictly anaerobic	−	+/−

Our MLSA findings (Fig. 1a–d) and available phenotypic information (Table 1) show that the genus *Paralactobacillus* does not warrant status as an independent genus. Rather, the data presented support the proposal that the sole species of this genus, *P. selangorensis*, represents a novel species of the genus *Lactobacillus*. Through reclassification of this organism, a better understanding of the characteristics and relationships of members of the genus *Lactobacillus* should be facilitated.

Description of *Lactobacillus selangorensis* comb. nov.

Lactobacillus selangorensis (sel.an.gor'en.sis. M.L. adj. *selangorensis* belonging to the province of Selangor, Malaysia).

Basonym: *Paralactobacillus selangorensis* Leisner *et al.* 2000.

The description for the species remains as described by Leisner *et al.* (2000), with the description of the genus *Paralactobacillus* (Leisner *et al.*, 2000) being the description of *Lactobacillus selangorensis*.

The type strain is ATCC BAA-66^T (=LMG 17710^T =CIP 106482^T).

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