Reclassification of *Lactobacillus cellobiosus* Rogosa *et al.* 1953 as a later synonym of *Lactobacillus fermentum* Beijerinck 1901

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The name *Lactobacillus cellobiosus* is validly published, but the species is often neglected in taxonomic studies, due to its high similarity to *Lactobacillus fermentum*. In the present paper, literature data concerning the two species were reviewed. Phylogenetic placement of *L. cellobiosus* was obtained based on 16S rDNA sequences, and genetic similarity was further investigated by comparing partial *recA* gene sequences for the type strains of *L. cellobiosus* and *L. fermentum*. Based on the high identity values for 16S rDNA (99 %) and *recA* gene (98 %) sequences, the results of DNA–DNA hybridization assays and phenotypic traits available from the literature, it is proposed that *L. cellobiosus* be reclassified and, as a rule of priority, renamed as *L. fermentum*, the first described species.

The first description of *Lactobacillus fermentum* can be traced back to Beijerinck (1901), as a heterofermentative *Lactobacillus* species which could be isolated from milk products, sourdough, fermenting plant material, manure, sewage and the mouth and faeces of man. *Lactobacillus cellobiosus* was first described by Rogosa *et al.* (1953) and is another heterofermentative *Lactobacillus* species. They share very similar phenotypic properties and both belong to the subgenus ‘*Betabacterium*’ Orla-Jensen of *Lactobacillus*.

Since the 1970s, strains belonging to *L. cellobiosus* and *L. fermentum* have been subjected to DNA–DNA hybridization studies, which proved their high relatedness (Vescovo *et al.*, 1979). Despite the evidence that strains ATCC 11739^T and ATCC 11740 of *L. cellobiosus* showed hybridization values higher than 70 % with *L. fermentum* ATCC 14932^T, the two species were both included in the Approved List of Bacterial Names (Skerman *et al.*, 1980). A later DNA–DNA hybridization study (Sriranganathan *et al.*, 1985), in which the strains *L. fermentum* NCDO 215 and *L. cellobiosus* NCDO 927 were examined, confirmed the findings of Vescovo *et al.* (1979), but no reclassification was proposed. On the basis of DNA-relatedness data, Kandler & Weiss (1989) reported *L. cellobiosus* as a biotype of *L. fermentum* in *Bergey’s Manual of Systematic Bacteriology*. As a consequence, *L. cellobiosus* was omitted from the phylogenetic analysis of the genus *Lactobacillus* (Collins *et al.*, 1991), and the 16S rDNA sequence of *L. cellobiosus* has been deposited

only very recently, under the designation *L. fermentum* (AJ575812).

Considering other literature, it is clear that the treatment of *L. cellobiosus* is inconsistent. Pot *et al.* (1994) reported that the name *L. cellobiosus* was invalid, while Dellaglio *et al.* (1994) presented it as a taxon awaiting reclassification, and Hammes & Vogel (1995) did not include it in a discussion of the *Lactobacillus* species. Nevertheless, no formal reclassification proposal has been presented so far, despite the availability of data supporting the close relationship between *L. fermentum* and *L. cellobiosus*. The latter name is validly published, as described in online taxonomic resources (List of Bacterial Names with Standing in Nomenclature, <http://www.bacterio.cict.fr/>; Bacterial Nomenclature Up-to-date, <http://www.dsmz.de/bactnom/bactname.htm>).

Strain depositions in major culture collections further complicate the study of the status of the species *L. cellobiosus*. In the ATCC (<http://www.atcc.org>), only two strains are available, ATCC 11739^T and ATCC 11740, but they are registered as ‘*Lactobacillus fermentum* Beijerinck deposited as *Lactobacillus cellobiosus* Rogosa *et al.*’. In the DSMZ (<http://www.dsmz.de>), only strain DSM 20055^T is available, with the comment ‘pro synon., *Lactobacillus fermentum*, never formally stated’. The Belgian Co-ordinated Collections of Microorganisms, Bacteria Collection (BCCM/LMG; <http://www.belspo.be/bccm>), has two strains, LMG 9846^T and LMG 11441, corresponding to ATCC 11739^T and ATCC 11740, respectively, with the former registered as *L. cellobiosus* and the latter as *L. fermentum*. Finally, in the Japan Collection of Microorganisms (JCM; <http://www.jcm.riken.go.jp/>), four strains are available: JCM 1137,

The GenBank/EMBL/DBJ accession numbers for the partial *recA* sequences of *Lactobacillus cellobiosus* and *Lactobacillus fermentum* are AJ579535 and AJ579534, respectively.

JCM 2766, JCM 2767 and JCM 2768 – all registered as *L. fermentum*.

In the present study, the phylogenetic placement of *L. cellobiosus* was determined by the analysis of the 16S rDNA sequences available for the most closely related species. Moreover, for the type strains of *L. fermentum* and *L. cellobiosus*, partial sequences for the *recA* gene were obtained and compared to evaluate better the relatedness of the two species.

Lactobacillus fermentum LMG 6902^T and *L. cellobiosus* DSM 20055^T were grown in MRS at 37 and 30 °C, respectively. Cultures were checked for purity, and DNA was extracted by the procedure of Marmur (1961).

Small subunit (16S) rDNA sequences were aligned with the CLUSTAL X program (Thompson *et al.*, 1997). Positions ambiguously aligned, not available or not identified (N in the sequence) were removed from all the sequences. Phylogenetic analysis was performed on the remaining 1366 positions with the TREECON program (Van de Peer & De Wachter, 1994) using Galtier and Gouy distance (Galtier & Gouy, 1995). The phylogenetic tree inferred for the considered species is shown in Fig. 1. The close relationship of *L. cellobiosus* and *L. fermentum* is supported by sequence identity: the two strains share 1361 of 1366 bp, confirming the high genetic relatedness suggested by DNA-hybridization data (Vescovo *et al.*, 1979).

Nevertheless, it is known that 16S rDNA sequence identity is not sufficient to guarantee species identity (Fox *et al.*,

1992). Protein-coding genes show greater variability, and *recA* has proved particularly useful in the differentiation of closely related species with almost identical 16S rDNA sequences, including lactic acid bacteria. Examples are *Lactobacillus plantarum*, *Lactobacillus paraplantarum* and *Lactobacillus pentosus* (Torriani *et al.*, 2001), and *Lactobacillus casei*, *Lactobacillus rhamnosus* and *Lactobacillus zeae* (Felis *et al.*, 2001). Accordingly, partial *recA* amplicons of about 730 bp were obtained by PCR with the degenerate primers recEXT-f (5'-GGC TAT GAA ACA AAT TGA AAA ACA ATW YGG NAA RGG-3') and recEXT1-r (5'-TGT TTA AAC GGT GGA GCA ACT TTR TTY TTN AC-3'). The PCR mixture (50 µl) was composed of 1 × reaction buffer, 2 mM magnesium chloride, 100 µM dNTPs, 1 µM both primers, 0.08 U *Taq* polymerase µl⁻¹, 5 % (v/v) DMSO and 300 ng template DNA. After an initial denaturation of 5 min at 94 °C, 30 cycles of 1 min at 94 °C, 1 min at 50 °C and 1 min at 72 °C were carried out. A final extension at 72 °C for 7 min was performed. Amplification products of the expected length of about 730 bp were obtained from both type strains. Sequencing reactions were performed at the Biomolecular Research Centre at the University of Padua. Regions of primer annealing were removed and 659 bp were used in further comparisons. A 98 % DNA sequence similarity was obtained, confirming that the two type strains belong to the same taxon. Data previously obtained for the *L. casei* species group (Felis *et al.*, 2001) suggest that *recA*-sequence variability within a single *Lactobacillus* species is very low; therefore, we assumed that similarity values obtained for the type strains of *L. cellobiosus* and *L. fermentum* can be extended to all strains belonging to the two species.

Fig. 1. Phylogenetic tree for *Lactobacillus* species, based on partial 16S rDNA sequences. Bar, 1 % sequence divergence.

Table 1. Phenotypic traits that differentiate strains of *L. fermentum* and *L. cellobiosus*

Data reported from Rogosa *et al.* (1953), Rogosa & Hansen (1971) and Dellaglio *et al.* (1994). v, Variable.

Characteristic	Species	
	<i>L. cellobiosus</i>	<i>L. fermentum</i>
Growth at:		
15 °C	v	—
45 °C	—	+
Fermentation of:		
Aesculin	+	—
Amygdalin	v	—
Arabinose	+	v
Cellobiose	+	v
Melibiose	v	+
Salicin	±	—
Trehalose	—	+
Teichoic acid type	Glycerol	—
Lactic acid isomer	L or DL	L

Despite the high genetic relatedness, literature data (Rogosa *et al.*, 1953; Rogosa & Hansen, 1971; Dellaglio *et al.*, 1994) suggest that the two taxa differ in several phenotypic traits, as shown in Table 1. This heterogeneity could be an indication of an infraspecific subdivision, which was not deepened due to the unclear attribution and scarcity of strains in culture collections, as explained above.

Based on the reviewed data and the additional results presented in this paper, it is proposed that *L. cellobiosus* and *L. fermentum* 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. fermentum* is the earlier synonym and the name *L. cellobiosus* is the later synonym.

Emended description of *Lactobacillus fermentum* Beijerinck 1901

Cells are rods, 0.5–0.9 µm thick and highly variable in length, occurring singly or in pairs. Heterofermentative strains; they ferment fructose, galactose, glucose, gluconate, lactose, maltose, mannose, melibiose, raffinose, ribose and sucrose. No acid is produced from mannitol, melezitose, rhamnose, salicin or sorbitol. Some strains may produce acid from amygdalin, arabinose, cellobiose, aesculin, trehalose and xylose. Genome G+C content is 52–54 mol%. They produce L- or DL-lactic acid and NH₃ from arginine, and may grow at 15 or 45 °C. Isolated from milk products, sourdough, fermenting plant material, manure, sewage and human mouth and faeces.

The type strain is ATCC 14931^T (= DSM 20052^T = NCDO 1750^T = LMG 6902^T).

Acknowledgements

We are grateful to Professor M. Vescovo for kindly providing old literature.

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