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**Lactobacillus fuchuensis** sp. nov., isolated from vacuum-packaged refrigerated beef

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Four strains of a hitherto unknown bacterium isolated from vacuum-packaged refrigerated beef were characterized by using phenotypic and phylogenetic methods. The novel strains were Gram-positive, catalase-negative, psychrophilic, rod-shaped bacteria with lactic acid-homofermentative mechanism. Comparative 16S rDNA gene sequencing demonstrated that the unknown strains represent a novel subline within the genus *Lactobacillus*, close to but distinct from *Lactobacillus curvatus* and *Lactobacillus sakei*. The unknown strains were readily distinguished from all currently described members of the genus *Lactobacillus* by biochemical properties and SDS-PAGE whole-cell protein profiles. Based on phylogenetic and phenotypic evidence, it is proposed that the unknown bacterium be classified as *Lactobacillus fuchuensis* sp. nov. The type strain is strain B5M10T (= JCM 11249<sup>T</sup> = DSM 14340<sup>T</sup>).

**Keywords:** *Lactobacillus fuchuensis*, lactic acid bacteria, SDS-PAGE, 16S rDNA, vacuum-packaged beef

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Lactic acid bacteria are the predominant group of micro-organisms isolated from vacuum-packaged meat and meat products (Hitchener et al., 1982; Shaw & Harding, 1984; Schillinger & Lucke, 1986; Borch & Molin, 1988). The predominant organisms include *Carnobacterium divergens*, *Carnobacterium piscicola*, *Lactobacillus sakei*, *Lactobacillus curvatus*, *Leuconostoc gelidum*, *Leuconostoc carnosum* and *Brochothrix thermosphaacta* (Shaw & Harding, 1984, 1989; Morishita & Shiromizu, 1986; Schillinger & Lucke, 1986; Borch & Molin, 1988; Hammes et al., 1991b). In earlier studies, characterization of organisms from vacuum-packaged beef was rather difficult, resulting in the description of the strains as ‘atypical’ lactic acid bacteria (Hitchener et al., 1982; Holzapfel & Gerber, 1983; Shaw & Harding, 1984). Development of 16S rRNA/DNA gene sequencing and subsequent comparative phylogenetic analysis has made genetic characterization and identification of atypical organisms easier (Collins et al., 1991, 1993). Recently, the use of 16S rDNA gene sequencing resulted in the description of *Lactobacillus algidus* sp. nov. (Kato et al., 2000), isolated from vacuum-packaged beef.

During the course of a study of the spoilage microflora of vacuum-packaged refrigerated beef, organisms corresponding to *Lactobacillus algidus*, *Leuconostoc gelidum*, *Lactococcus piscium*, *Carnobacterium divergens*, *Carnobacterium piscicola* and *Brochothrix thermosphacta* were recovered as the predominant members of the microflora. In addition, a hitherto unknown *Lactobacillus*-like bacterium was isolated. In this article, we report the characteristics of this unknown lactic acid bacterium and the results of the polyphasic taxonomic study. Based on these results, a novel species, *Lactobacillus fuchuensis* sp. nov., is described.

Four strains, D1M9 (= DSM 14342), E1T8, B4M16 (= DSM 14341) and B5M10<sup>T</sup> (= JCM 11249<sup>T</sup> = DSM 14340<sup>T</sup>), were isolated from vacuum-packaged beef stored at 2 °C. Vacuum-packaged beef was homogenized with PBS at weekly intervals and incubated anaerobically on MRS agar (Oxoid) at 7 °C for 14 days and aerobically on trypticase soy agar (TS; Nissui) at 7 °C for 10 days. The organisms were
characterized biochemically using the Rapid ID32S and API 50CH systems according to the manufacturer’s instructions (bioMérieux). Additional biochemical and physiological tests were performed as described by Kato et al. (2000). To assess the overall phenotypic resemblance of the isolates to reference meso-diaminopimelic acid (m-DAP)-negative, psychrophilic Lactobacillus species, a comparative analysis of whole-cell protein profiles by SDS-PAGE was performed. The protein profiles were analysed using the Phoretix advanced program (Phoretix International, Newcastle upon Tyne, UK). The G+C contents (mol%) of strains B5M10T and B4M16 were determined by HPLC as described by Tamaoka & Komagata (1984). For phylogenetic analysis, the 16S rDNA gene of strain B5M10T was amplified by PCR and sequenced directly using thermostoquences (Amersham) on a 1 dye–4 lane DNA sequencer (DSQ 2000S; Shimadzu) according to the manufacturer’s instructions. A phylogenetic tree was constructed according to the neighbour-joining method and the stability of the groupings was estimated by bootstrap analysis.

The cellular morphology and general biochemical characteristics of the isolates were consistent with their assignment to the genus Lactobacillus, but did not correspond to any currently recognized species. Details are given in the species description below.

To assess the overall phenotypic resemblance of the isolates to reference m-DAP-negative, psychrophilic Lactobacillus species, a comparative analysis of whole-cell protein profiles by SDS-PAGE was performed. Fig. 1 shows that the isolates clustered closely together (r > 0.75) but were distinct from the rest of the Lactobacillus species used (r < 0.52). The G+C contents of strains B5M10T and B4M16 were respectively 41.0 and 41.7 mol%. These values are within the normal range for members of the genus Lactobacillus (Hamme et al., 1991a). To ascertain the phylogenetic relationships of the isolates, the 16S rDNA gene of B5M10T was sequenced and subjected to a comparative analysis. Approximately 1350 bases were determined and sequence database searches showed that the strain was most closely related to Lactobacillus curvatus JCM 1096T (96% sequence similarity) of the group of Lactobacillus sakei/Pediococcus group of the genus Lactobacillus (Collins et al., 1991). A phylogenetic tree showing the relationship of the strain to some Lactobacillus species is shown in Fig. 2.

The unknown Gram-positive rods were found to be morphologically and biochemically consistent with their assignment to the genus Lactobacillus. The strains formed a phylogenetic cluster with Lactobacillus curvatus and Lactobacillus sakei, but sequence divergence values of 4 and 5%, respectively, between strain B5M10T and these two species demonstrated that the relationship is that of phylogenetically closely related but nevertheless quite separate species (Stackebrandt & Goebel, 1994). SDS-PAGE analysis of whole-cell proteins confirmed the phenotypic homogeneity of the isolates and their separateness from other Lactobacillus species. Thus, based on the phylogenetic evidence presented and its distinctive biochemical characteristics, we propose that the unidentified organism from vacuum-packaged beef is assigned to the genus Lactobacillus as Lactobacillus fuchuensis sp. nov. The novel species from vacuum-packaged beef is biochemically unique and can be distinguished from all described members of the Lactobacillus casei/Pediococcus group of Lactobacillus. Lactobacillus fuchuensis could be differentiated from Lactobacillus curvatus and Lactobacillus sakei, its closest
Lactobacillus fuchuensis sp. nov.

Phylogenetic analysis of the genus Lactobacillus and related lactic acid bacteria from prepacked meat and meat products. Antonie van Leeuwenhoek, 54, 301–323.


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