Lactobacillus saerimneri sp. nov., isolated from pig faeces

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In studying the composition of the Lactobacillus flora of faeces from pigs fed different diets, isolates with notable differences in their 16S rRNA gene sequence compared to recognized species were found. Phenotypic characteristics together with 16S rRNA gene sequences revealed that the isolates represented a novel species belonging to the Lactobacillus malii subgroup of lactobacilli. The name Lactobacillus saerimneri sp. nov. is proposed (type strain GDA154T = LMG 22087T = DSM 16049T = CCUG 48462T).

The seven strains GDA154T, GDA158, GDA159, GDA160, GDA164, GDA166 and GDA170 were isolated from pig faeces in a study to determine the effect of different diets on the diversity of lactobacilli (C. Pedersen and others, unpublished results). The first four strains originated from one animal and the latter three from a second animal. Strains GDA154T and GDA164 have been deposited in the Belgian Co-ordinated Collections of Microorganisms (BCCM, Gent, Belgium), the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany) and the Culture Collection, University of Göteborg (CCUG, Gothenburg, Sweden). Primary isolation was on Rogosa agar (Merck) in anaerobic jars under a CO₂ + N₂ atmosphere (GasPak Plus system; BBL) at 37°C. All further cultivation was performed at 37°C in anaerobic jars on MRS agar (Oxoid) or in MRS broth (Oxoid) unless otherwise stated.

Bacterial DNA was isolated using the DNeasy Tissue kit (Qiagen). The almost complete 16S rRNA gene for the strains was amplified using PCR with domain Bacteria-specific primers (Weizenegger et al., 1992). The resulting PCR products were purified using the Qiagen PCR Purification kit. The first part (approximately 500 bp) of the purified fragments was sequenced according to standard methods. For strain GDA154T, the whole fragment was sequenced. Primers that were used for amplification, together with additional internal primers, were also used for sequencing of the PCR products. The sequences determined from the novel isolates were used for searches in the GenBank database (http://www.ncbi.nlm.nih.gov/). Sequences representing the closest matches were retrieved and then aligned using the CLUSTAL W program (Thompson et al., 1994). For all sequences, approximately 1450 nt were used. A distance matrix was calculated using the DNADIST program of the PHYLIP package (Felsenstein, 1993) with the F84 parameter model, and a phylogenetic tree was constructed with the NEIGHBOR program. Statistical significance of the grouping was estimated by bootstrapping (100 replicates) using the programs SEQBOOT, DNADIST, NEIGHBOR and CONSENSE, all of which are from the PHYLIP package. The phylogenetic tree was displayed using the TREEVIEW program (Page, 1996).

Cell morphologies of the bacteria were observed by phase-contrast microscopy. Determination of Gram reactions was performed using the KOH method of Gregersen (1978). Sugar fermentation patterns and aesculin were determined using the API 50 CHL system (bioMérieux) in duplicate at 37°C. Lactic acid configuration was determined using a test kit from Boehringer Mannheim. Catalase activity was determined by transferring fresh colonies from MRS agar to a glass slide and adding 5% H₂O₂. Production of gas from glucose was assayed by growing the bacteria in MRS tubes containing Durham tubes. Electrophoretic analysis of whole-cell proteins was performed by the BCCM. Preparation of whole-cell protein extracts and SDS-PAGE analysis were performed as described by Pot et al. (1994). Normalized and digitized patterns were numerically analysed and clustered with the reference profiles in the SDS-PAGE protein database at the BCCM Bacteria Collection. Cell-wall analysis was performed at the DSMZ. Preparation of cell walls and determination of peptidoglycan structure were carried out using the methods described by Schleifer (1985) and Schleifer & Kandler (1972) with the modification that TLC on cellulose sheets was used instead of paper
The G+C content of the DNA was determined at the DSMZ. DNA was isolated by chromatography on hydroxyapatite by the procedure of Cashion et al. (1977) and the G+C content was determined by HPLC as described by Mesbah et al. (1989).

The partial 16S rRNA sequences (i.e. the first 500 bp) from all strains showed 100% identity. The complete 16S rRNA sequence of GDA154\textsuperscript{T} was analysed with the SIMILARITY MATRIX tool at Ribosomal Database Project II (http://rdp.cme.msu.edu/html); the highest similarity values were found to Lactobacillus mali, Lactobacillus salivarius and some other representatives of the Lactobacillus casei–Pediococcus group of lactobacilli. The sequence of Leuconostoc (Leu.) mesenteroides was used as an outgroup representative. Approximately 1450 nt from each sequence were used for the alignment. Bar, 1% estimated sequence divergence. Numbers indicate bootstrap values for branch points. P., Pediococcus.

Table 1. Differential characteristics of species in the Lactobacillus mali subgroup of lactobacilli

Species: 1, L. saerimneri sp. nov.; 2, L. algidus (data from Kato et al., 2000); 3, L. nagelii (Edwards et al., 2000); 4, L. mali (Kato et al., 2000; Hammes et al., 1992); 5, L. salivarius (Kandler & Weiss, 1986); 6, L. avius (Hammes et al., 1992); 7, L. acidipiscis (Tanupawat et al., 2000); 8, L. cyprocasis (Lawson et al., 2001); 9, L. ruminus (Kandler & Weiss, 1986); 10, L. aligis (Kandler & Weiss, 1986); 11, L. equi (Morotomi et al., 2002); 12, L. animalis (Kandler & Weiss, 1986); 13, L. mali (Kandler & Weiss, 1986). +, 90 % strains positive; –, 90 % strains negative; d, 11–89 % strains positive; W, weakly positive; NA, no data available.

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Fig. 1. Unrooted phylogenetic tree derived from 16S rRNA gene sequence analysis showing the relationship of Lactobacillus saerimneri sp. nov. to all members of the Lactobacillus mali subgroup and some other representatives of the Lactobacillus casei–Pediococcus group of lactobacilli. The sequence of Leuconostoc (Leu.) mesenteroides was used as an outgroup representative. Approximately 1450 nt from each sequence were used for the alignment. Bar, 1% estimated sequence divergence. Numbers indicate bootstrap values for branch points. P., Pediococcus.
represent a novel species, for which we propose the name *Lactobacillus saerimneri* sp. nov., with strain GDA154\textsuperscript{T} as the type strain.

Strains GDA154\textsuperscript{T}, GDA158 and GDA164 showed identical physiological properties; these are listed under the species description. These physiological properties clearly distinguish *L. saerimneri* from closely related species. Differential characteristics for members of the *L. mali* subgroup of lactobacilli are summarized in Table 1. Furthermore, electrophoretic analysis of whole-cell proteins showed that strain GDA154\textsuperscript{T} is distinct from other species in the *L. mali* subgroup of lactobacilli (Fig. 2).

The gastrointestinal microbiota of pigs has been analysed using 16S rRNA gene sequencing by Pryde et al. (1999) and Leser et al. (2002). Although many lactobacilli were detected, none has a 16S rRNA gene sequence similar to *L. saerimneri*. This indicates that the bacterium is a minor component in the gastrointestinal microbiota of pigs.

**Description of Lactobacillus saerimneri** sp. nov.

*Lactobacillus saerimneri* (sae.rim’ne ri. N.L. gen. masc. n. saerimneri of Saerimner, a pig occurring in Nordic mythology, because the organism was isolated from pigs).

Gram-positive, non-motile, non-spore-forming, catalase-negative rods, 1×1.5–4 μm in size and occurring as single cells or in pairs. After anaerobic growth at 37 °C for 48 h, colonies on MRS agar are 2–3 mm in diameter; they are white with an opaque border, smooth and convex. Growth on MRS agar also occurs under aerobic conditions, but at a considerably lower rate. In MRS broth growth occurs between 15 (weak) and 45 °C. Both D- (75 %) and L-lactate (25 %) are produced. Gas is not produced from glucose. Acid is produced from D-glucose, D-fructose, D-mannose, N-acetylglucosamine (delayed reaction), sucrose, trehalose and D-turanose. Acid is not produced from glycerol, erythritol, D-arabinose, L-arabinose, ribose, D-xyllose, L-xyllose, adonitol, methyl β-D-xylulose, galactose, L-sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl α-D-mannoside, methyl α-D-glucoside, amygdalin, arbutin, salicin, cellobiose, maltose, melibiose, lactose, inulin, melizitose, D-raffinose, starch, glycogen, xyitol, β-gentiobiose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, 2-ketogluconate or 5-ketogluconate. Aesculin is not hydrolysed. The DNA G+C content of strain GDA154\textsuperscript{T} is 42-9 mol% and the peptidoglycan type is A1\textsubscript{c} m-DAP-direct. Phylogenetic analysis of the 16S rRNA gene sequence places the species in the *L. mali* subgroup of lactobacilli.

The type strain is GDA154\textsuperscript{T} (=LMG 22087\textsuperscript{T} =DSM 16049\textsuperscript{T} =CCUG 48462\textsuperscript{T}).

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**References**


