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 mali* subgroup of lactobacilli. The name *Lactobacillus saerimneri* sp. nov. is proposed (type strain GDA154^T=LMG 22087^T=DSM 16049^T=CCUG 48462^T).

The seven strains GDA154^T, 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faeces in a study to determine the effect of different diets on the diversity of lactobacilli (C. Pedersen and others, unpublished results).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 GDA154^T 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 GDA154^T, 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

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Abbreviation: *m*-DAP, *meso*-diaminopimelic acid.

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of *Lactobacillus saerimneri* GDA154^T is AY255802.

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*.

chromatography. 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^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 salivarius*, *Lactobacillus aviarius* and *Lactobacillus mali*, with 95.0, 94.8 and 94.6 % similarity, respectively. The sequence was then aligned with the

sequences of all members of the *L. mali* subgroup and some other representatives of the *Lactobacillus casei*–*Pediococcus* group of lactobacilli, and this alignment was then used to construct a phylogenetic tree. This clearly placed the bacterium in the *L. mali* subgroup and confirmed the relationship to *L. salivarius* and *L. aviarius* (Fig. 1). The mean G+C content of the DNA of strain GDA154^T was 42.9 mol% and the peptidoglycan type was A1 γ meso-diaminopimelic acid (*m*-DAP)-direct. The considerable difference in 16S rRNA gene sequence compared to all recognized lactobacilli shows that the isolated strains represent a novel species. This is confirmed by comparing the G+C content and peptidoglycan type with other members of the *L. mali* subgroup (Table 1). The strains

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. aviarius* (Hammes *et al.*, 1992); 7, *L. acidipiscis* (Tanasupawat *et al.*, 2000); 8, *L. cypricasei* (Lawson *et al.*, 2001); 9, *L. ruminus* (Kandler & Weiss, 1986); 10, *L. agilis* (Kandler & Weiss, 1986); 11, *L. equi* (Morotomi *et al.*, 2002); 12, *L. animalis* (Kandler & Weiss, 1986); 13, *L. murinus* (Kandler & Weiss, 1986). +, ≥ 90 % strains positive; –, ≥ 90 % strains negative; d, 11–89 % strains positive; w, weakly positive; NA, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13
Acid from:													
L-Arabinose	–	+	–	+	–	NA	–	d	–	–	–	d	+
Ribose	–	+	–	NA	–	NA	+	–	–	+	d	–	+
Galactose	–	+	+	+	+	d	+	+	+	+	+	+	+
Salicin	–	+	+	+	d	+	w	+	+	+	d	+	d
Cellobiose	–	d	+	d	–	+	–	+	+	+	–	+	+
Melibiose	–	d	–	–	+	d	–	–	+	+	+	+	+
Sucrose	+	–	–	+	+	+	–	–	+	+	–	+	+
Trehalose	+	+	+	+	+	+	d	d	–	+	–	–	d
D-Raffinose	–	–	–	–	+	+	–	–	+	+	–	–	+
Hydrolysis of aesculin	–	+	+	NA	d	NA	+	+	+	+	+	+	+
Growth at 45 °C	+	–	+	NA	+	NA	–	–	d	+	+	+	+
Lactic acid configuration	DL	L	DL	L	L	DL	L	NA	L	L	DL	L	L
G + C content (mol%)	42.9	36–37	NA	32–34	34–36	39–43	39–42	NA	44–47	43–44	38–40	41–44	43–44
Peptidoglycan type	<i>m</i> -DAP	<i>m</i> -DAP	NA	<i>m</i> -DAP	Lys-D-Asp	Lys-D-Asp	Lys-D-Asp	NA	<i>m</i> -DAP	<i>m</i> -DAP	NA	Lys-D-Asp	Lys-D-Asp

Fig. 2. Dendrogram derived from SDS-PAGE protein pattern analysis of *Lactobacillus saerimneri* sp. nov. and representative strains within the *Lactobacillus mali* subgroup of lactobacilli.

represent a novel species, for which we propose the name *Lactobacillus saerimneri* sp. nov., with strain GDA154^T as the type strain.

Strains GDA154^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^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, $1 \times 1.5\text{--}4\ \mu\text{m}$ 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-xylose, L-xylose, adonitol, methyl β -D-xyloside, galactose, L-sorbose, rhamnose,

dulcitol, inositol, mannitol, sorbitol, methyl α -D-mannoside, methyl α -D-glucoside, amygdalin, arbutin, salicin, cellobiose, maltose, melibiose, lactose, inulin, melezitose, D-raffinose, starch, glycogen, xylitol, 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^T is 42.9 mol% and the peptidoglycan type is A1 γ 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^T (=LMG 22087^T=DSM 16049^T=CCUG 48462^T).

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References

- Cashion, P., Holder-Franklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for the base ratio determination of bacterial DNA. *Anal Biochem* **81**, 461–466.
- Edwards, C. G., Collins, M. D., Lawson, P. A. & Rodriguez, A. V. (2000). *Lactobacillus nagelii* sp. nov., an organism isolated from a partially fermented wine. *Int J Syst Evol Microbiol* **50**, 699–702.
- Felsenstein, J. (1993). PHYLIP (Phylogeny Inference Package), version 3.5c. Distributed by the author. Department of Genetics, University of Washington, Seattle, USA.
- Gregersen, T. (1978). A rapid method for distinction of Gram-negative from Gram-positive bacteria. *Eur J Appl Microbiol Biotechnol* **5**, 123–127.
- Hammes, W. P., Weiss, N. & Holzapfel, W. (1992). The genera *Lactobacillus* and *Carnobacterium*. In *The Prokaryotes*, 2nd edn, pp. 1535–1594. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K.-H. Schleifer. New York: Springer.

- Kandler, O. & Weiss, N. (1986).** Genus *Lactobacillus* Beijerinck 1901, 212^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 2, pp. 1209–1234. Edited by P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.
- Kato, Y., Sakala, R. M., Hayashidani, H., Kiuchi, A., Kaneuchi, C. & Ogawa, M. (2000).** *Lactobacillus algidus* sp. nov., a psychrophilic lactic acid bacterium isolated from vacuum-packaged refrigerated beef. *Int J Syst Evol Microbiol* **50**, 1143–1149.
- Lawson, P. A., Papademas, P., Wachter, C., Falsen, E., Robison, R. & Collins, M. D. (2001).** *Lactobacillus cypricaei* sp. nov., isolated from Halloumi cheese. *Int J Syst Evol Microbiol* **51**, 45–49.
- Leser, T. D., Amenuvor, J. Z., Jensen, T. K., Lindecrone, R. H., Boye, M. & Møller, K. (2002).** Culture-independent analysis of gut bacteria: the pig gastrointestinal tract microbiota revisited. *Appl Environ Microbiol* **68**, 673–690.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989).** Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Morotomi, M., Yuki, N., Kado, Y., Kushiro, A., Shimazaki, T., Watanabe, K. & Yuyama, T. (2002).** *Lactobacillus equi* sp. nov., a predominant intestinal *Lactobacillus* species of the horse isolated from faeces of healthy horses. *Int J Syst Evol Microbiol* **52**, 211–214.
- Page, R. D. M. (1996).** TREEVIEW: an application to display phylogenetic trees on personal computers. *Comput Appl Biosci* **12**, 357–358.
- Pot, B., Vandamme, P. & Kersters, K. (1994).** Analysis of electrophoretic whole-organism protein fingerprints. In *Chemical Methods in Prokaryotic Systematics*, pp. 493–521. Edited by M. Goodfellow & A. G. O'Donnell. Chichester: Wiley.
- Pryde, S. E., Richardson, A. J., Stewart, C. S. & Flint, H. J. (1999).** Molecular analysis of the microbial diversity present in the colonic wall, colonic lumen, and cecal lumen of a pig. *Appl Environ Microbiol* **65**, 5372–5377.
- Schleifer, K. H. (1985).** Analysis of the chemical composition and primary structure of murein. *Methods Microbiol* **18**, 123–156.
- Schleifer, K. H. & Kandler, O. (1972).** Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.
- Tanasupawat, S., Shida, O., Okada, S. & Komagata, K. (2000).** *Lactobacillus acidipiscis* sp. nov. and *Weissella thailandensis* sp. nov., isolated from fermented fish in Thailand. *Int J Syst Evol Microbiol* **50**, 1479–1485.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994).** CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.
- Weizenegger, M., Neumann, M., Stackebrandt, E., Weiss, N. & Ludwig, W. (1992).** *Eubacterium alactolyticum* phylogenetically groups with *Eubacterium limosum*, *Acetobactericum woodii* and *Clostridium barkeri*. *Syst Appl Microbiol* **15**, 32–36.