Lactobacillus gastricus sp. nov., *Lactobacillus antri* sp. nov., *Lactobacillus kalixensis* sp. nov. and *Lactobacillus ultunensis* sp. nov., isolated from human stomach mucosa

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In an attempt to study the composition of the *Lactobacillus* flora from mucosa of human stomach, 16S rRNA gene sequences of 129 isolates were analysed. For 15 of these, the results differed significantly from known sequences, and additional tests were performed to determine whether these isolates represented as yet unrecognized species. Phenotypic and genetic characteristics revealed that these isolates represented four novel *Lactobacillus* species. Two belong to the *Lactobacillus reuteri* and the other two to the *Lactobacillus delbrueckii* subgroup of *Lactobacillus*. The names *Lactobacillus gastricus* sp. nov., *Lactobacillus antri* sp. nov., *Lactobacillus kalixensis* sp. nov. and *Lactobacillus ultunensis* sp. nov. are proposed, with the respective type strains Kx156A7^T (=LMG 22113^T=DSM 16045^T=CCUG 48454^T), Kx146A4^T (=LMG 22111^T=DSM 16041^T=CCUG 48456^T), Kx127A2^T (=LMG 22115^T=DSM 16043^T=CCUG 48459^T) and Kx146C1^T (=LMG 22117^T=DSM 16047^T=CCUG 48460^T).

In a study of the *Lactobacillus* flora of the human stomach, the almost complete 16S rRNA gene sequences of 129 isolates from this environment were analysed. For 15 of these isolates, the results differed significantly from known sequences and additional tests were performed to determine whether these isolates represented novel *Lactobacillus* species.

The strains were isolated from human gastric mucosa obtained from healthy individuals from Kalix, a town in northern Sweden. Primary isolates were produced by spreading homogenates of biopsies from both the antrum and the corpus region of the stomach on Rogosa agar (Merck). The plates were incubated for 3 days in anaerobic jars under a CO₂ + N₂ atmosphere (GasPak System, BBL) at 37 °C. All further cultivation was performed at 37 °C in anaerobic jars on MRS agar or in MRS broth (Oxoid) if not otherwise stated. Strains Kx127A2^T (=LMG 22115^T=DSM 16043^T=CCUG 48459^T), Kx146A4^T (=LMG 22111^T=DSM 16041^T=CCUG 48456^T), Kx146C1^T

(=LMG 22117^T=DSM 16047^T=CCUG 48460^T), Kx146C2 (=LMG 22489=DSM 16044=CCUG 48458), Kx156A7^T (=LMG 22113^T=DSM 16045^T=CCUG 48454^T), Kx293C1 (=LMG 22490=DSM 16048=CCUG 48461), Kx293C4 (=LMG 22488=DSM 16046=CCUG 48455) and Kx329A2 (=LMG 22112=DSM 16042=CCUG 48457) have been deposited with the BCCM, DSMZ and CCUG.

Bacterial DNA was isolated using the DNeasy Tissue kit (Qiagen). The almost complete 16S rRNA gene sequences for the strains were amplified by PCR with domain *Bacteria*-specific primers (Weizenegger *et al.*, 1992). The resulting PCR products were purified by using the Qiagen PCR purification kit. The first part (about 500 bp) of the purified fragments was sequenced according to standard methods. For some strains, the whole fragment was sequenced. The primers used for amplification, together with additional internal primers, were used for sequencing of the PCR products. The sequences obtained from the isolates were used for searches in the GenBank database and levels of DNA similarity were analysed with the SIMILARITY MATRIX tool at Ribosomal Database Project II (<http://rdp.cme.msu.edu/html>). 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

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Lactobacillus gastricus* Kx156A7^T, *Lactobacillus antri* Kx146A4^T, *Lactobacillus kalixensis* Kx127A2^T and *Lactobacillus ultunensis* Kx146C1^T are AY253658, AY253659, AY253657 and AY253660, respectively.

a phylogenetic tree was constructed with the NEIGHBOR program. Statistical significance of groupings was estimated by bootstrapping (1000 replicates) using the programs SEQBOOT, DNADIST, NEIGHBOR and CONSENSE, all of which are from the PHYLIP package. The phylogenetic tree was displayed by 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 hydrolysis were assessed 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. The 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. The preparation of whole-cell protein extracts and SDS-PAGE analysis were performed as described by Pot *et al.* (1994). The normalized and digitized patterns were numerically analysed and clustered with reference profiles in the SDS-PAGE protein database of the BCCM. Cell-wall analysis was performed at the DSMZ. Preparation of cell walls and determination of peptidoglycan structure were carried out by the methods described by Schleifer (1985) and Schleifer & Kandler (1972) with the modification that TLC on cellulose sheets was used instead of paper chromatography. The G + C content of the DNA and DNA–DNA relatedness values were

determined at the DSMZ. DNA was isolated by chromatography on hydroxyapatite by the procedure of Cashion *et al.* (1977). The G + C content was thereafter determined by HPLC as described by Mesbah *et al.* (1989) and DNA–DNA hybridizations were carried out as described by De Ley *et al.* (1970), with the modifications described by Huß *et al.* (1983), using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-controlled 6 × 6 multicell changer and a temperature controller with *in-situ* temperature probe (Varian).

Analysis of the 16S rRNA gene sequences of the 15 isolates differing from previously described lactobacilli revealed that they could be divided into four groups. Group G1 contained six isolates from three individuals, G2 contained two isolates from two individuals, G3 contained five isolates from two individuals and G4 contained two isolates from two individuals. Two representative isolates from each group were chosen for further analysis: G1, Kx156A7^T and Kx293C4; G2, Kx146A4^T and Kx329A2; G3, Kx127A2^T and Kx146C2; G4, Kx146C1^T and Kx293C1.

Group G1

The complete 16S rRNA gene sequence of Kx156A7^T showed the highest levels of similarity to *Lactobacillus mucosae*, *Lactobacillus thermotolerans* and *Lactobacillus ingluviei*, all members of the *Lactobacillus reuteri* subgroup of lactobacilli, with 96.4, 95.4 and 95.3 % similarity, respectively. A 425 bp sequence from the 16S rRNA gene of Kx293C4 revealed 100 % similarity to that of Kx156A7^T. The low level of 16S rRNA gene sequence similarity with

Table 1. Some differential characteristics of the species in the *L. reuteri* subgroup of lactobacilli

Species/strains: 1, *L. gastricus* sp. nov. Kx156A7^T; 2, *L. gastricus* sp. nov. Kx293C4; 3, *L. antri* sp. nov. Kx146A4^T; 4, *L. antri* sp. nov. Kx329A2; 5, *L. coleohominis* (data from Nikolaitchouk *et al.*, 2001); 6, *L. fermentum* (Kandler & Weiss, 1986); 7, *L. frumenti* (Müller *et al.*, 2000); 8, *L. ingluviei* (Baele *et al.*, 2003); 9, *L. mucosae* (Roos *et al.*, 2000); 10, *L. oris* (Wiese *et al.*, 1996); 11, *L. panis* (Wiese *et al.*, 1996); 12, *L. pontis* (Vogel *et al.*, 1994); 13, *L. reuteri* (Kandler & Weiss, 1986); 14, *L. thermotolerans* (Niamsup *et al.*, 2003); 15, *L. vaginalis* (Wiese *et al.*, 1996). Symbols: +, ≥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	14	15
Acid from:															
L-Arabinose	−	+	+	+	−	−	d	+	d	+	+	−	+	+	−
Ribose	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−
D-Xylose	−	−	+	−	NA	−	NA	+	+	+	+	−	−	+	−
Mannitol	−	−	w	w	−	−	+	−	−	NA	−	−	−	NA	NA
Salicin	+	+	−	−	NA	−	+	−	−	−	+	−	−	NA	−
Lactose	+	+	−	−	−	+	+	−	d	+	+	−	+	−	+
Melibiose	+	+	+	+	−	+	+	−	d	+	+	−	+	d	+
Trehalose	+	+	−	−	−	d	+	−	−	NA	−	NA	−	NA	NA
Hydrolysis of aesculin	+	+	w	w	−	+	+	+	+	+	+	−	−	+	+
Growth at 45 °C	−	−	+	+	+	+	+	NA	+	+	+	+	+	+	+
DNA G + C content (mol%)	41.3	NA	44.9	NA	NA	52–54	43.3	49	46–49	49–51	48	53	40–42	49–51	38–41
Peptidoglycan type	Orn− D-Asp	NA	Lys− D-Asp	NA	meso- DAP	Orn− D-Asp	Lys− D-Asp	NA	Orn− D-Asp	Lys− D-Asp	Lys− D-Asp	Orn− D-Asp	Lys− D-Asp	NA	Orn− D-Asp

Fig. 1. Dendrogram derived from SDS-PAGE protein pattern analysis of *Lactobacillus gastricus* sp. nov., *Lactobacillus antri* sp. nov. and representative strains within the *L. reuteri* subgroup of lactobacilli.

recognized lactobacilli strongly suggests that the isolates in G1 represent a previously undescribed species. Comparison of DNA G+C content, peptidoglycan type and other characteristics with the members of the *L. reuteri* subgroup (Table 1) confirmed this suggestion. Furthermore, electrophoretic analysis of whole-cell proteins showed that strains Kx156A7^T and Kx293C4 are distinct from the other species in this subgroup (Fig. 1). We propose that the isolates be classified in the genus *Lactobacillus* as *Lactobacillus gastricus* sp. nov. Additional characteristics of strains Kx156A7^T and Kx293C4 are given under the species description.

Group G2

The complete 16S rRNA gene sequence of Kx146A4^T showed the highest levels of similarity to *Lactobacillus oris*, *Lactobacillus panis* and *Lactobacillus frumenti*, all members of the *L. reuteri* subgroup of lactobacilli, with 98.8, 98.0 and 96.6% similarity, respectively. A 578 bp sequence from the 16S rRNA gene of Kx329A2 showed 100% similarity to that of Kx146A4^T. Owing to the relatively small difference between the sequences of Kx146A4^T and the closest related lactobacilli, DNA–DNA hybridization was performed. Levels of DNA–DNA relatedness between Kx146A4^T and *L. oris* DSM 4864^T and *L. panis* DSM 6035^T were 56 and 59%, respectively. This is clearly below the threshold of 70% recommended as the lowest value for isolates allocated to the same species (Wayne *et al.*, 1987), indicating that Kx146A4^T represents a novel species. Comparison of DNA G+C content, peptidoglycan type and other characteristics with members of the *L. reuteri* subgroup (Table 1) confirmed this suggestion. Electrophoretic analysis of whole-cell proteins also showed that strains Kx146A4^T and Kx329A2 are distinct from the other species in this subgroup (Fig. 1). We propose that the isolates be classified in the genus *Lactobacillus* as *Lactobacillus antri* sp. nov. Additional characteristics of strains Kx146A4^T and Kx329A2 are given under the species description.

Group G3

The complete 16S rRNA gene sequence of Kx127A2^T showed the highest levels of similarity to *Lactobacillus intestinalis*, *Lactobacillus amylolyticus* and *Lactobacillus crispatus*, all members of the *Lactobacillus delbrueckii* subgroup of lactobacilli, with 96.4, 95.7 and 95.6% similarity, respectively. A 518 bp sequence from the 16S rRNA gene of Kx146C2 showed 100% similarity to that of Kx127A2^T. The low levels of 16S rRNA gene sequence similarity with recognized lactobacilli strongly suggests that the isolates in G3 represent a previously undescribed species. Comparison of DNA G+C content, peptidoglycan type and other characteristics with the closest related members of the *L. delbrueckii* subgroup (Table 2) confirmed this suggestion. Furthermore, electrophoretic analysis of whole-cell proteins showed that strains Kx127A2^T and Kx146C2 are distinct from the other species in this subgroup (Fig. 2). We propose that the isolates be classified in the genus *Lactobacillus* as *Lactobacillus kalixensis* sp. nov. Additional characteristics of strains Kx127A2^T and Kx146C2 are given under the species description.

Group G4

The complete 16S rRNA gene sequence of Kx146C1^T showed the highest levels of similarity to *L. crispatus*, *Lactobacillus helveticus*, *Lactobacillus amylovorus* and *Lactobacillus acidophilus*, all members of the *L. delbrueckii* subgroup of lactobacilli, with 98.2, 97.9, 97.2 and 96.6% similarity, respectively. A 560 bp sequence from the 16S rRNA gene of Kx293C1 showed 100% similarity to that of Kx146C1^T. Because of the relatively small difference between the sequence of Kx146C1 and those of the closest related lactobacilli, DNA–DNA hybridization was performed. Levels of DNA–DNA relatedness between Kx146C1^T and *L. crispatus* DSM 20584^T, *L. helveticus* DSM 20075^T, *L. amylovorus* DSM 20531^T and *L. acidophilus* DSM 20079^T were 53, 54, 40 and 47%, respectively. These are below the threshold of 70% recommended as the lowest value for isolates allocated to the same species (Wayne *et al.*, 1987), indicating that Kx146C1^T represents a previously undescribed species. Comparison of DNA G+C content, peptidoglycan type and other characteristics with the closest related members of the *L. delbrueckii* subgroup (Table 2) confirmed this suggestion. In addition, electrophoretic analysis of whole-cell proteins showed that strains Kx146C1^T and Kx293C1 are distinct from the other species in this subgroup (Fig. 2). We propose that the isolates be classified in the genus *Lactobacillus* as *Lactobacillus ultunensis* sp. nov. Additional characteristics of strains Kx146C1^T and Kx293C1 are given under the species description.

The 16S rRNA gene sequences of the four novel species were aligned with those of all members of the *L. reuteri* subgroup and the most relevant members of the *L. delbrueckii* subgroup. This alignment was then used for a tree analysis. The analysis placed the novel species in

Table 2. Some differential characteristics of the species in the *L. delbrueckii* subgroup of lactobacilli

Species/strains: 1, *L. kalixensis* sp. nov. Kx127A2^T; 2, *L. kalixensis* sp. nov. Kx146C2; 3, *L. ultunensis* sp. nov. Kx146C1^T; 4, *L. ultunensis* sp. nov. Kx293C1; 5, *L. acidophilus* (data from Kandler & Weiss, 1986); 6, *L. amylolyticus* (Bohak *et al.*, 1998); 7, *L. amylovorus* (Kandler & Weiss, 1986); 8, *L. crispatus* (Kandler & Weiss, 1986); 9, *L. delbrueckii* subsp. *delbrueckii* (Kandler & Weiss, 1986); 10, *L. gallinarum* (Fujisawa *et al.*, 1992); 11, *L. gasseri* (Kandler & Weiss, 1986); 12, *L. hamsteri* (Hammes *et al.*, 1992); 13, *L. helveticus* (Kandler & Weiss, 1986); 14, *L. intestinalis* (Fujisawa *et al.*, 1990). Symbols: +, ≥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	14
Acid from:														
Mannitol	−	+	−	−	−	−	−	−	−	−	−	−	−	+
Amygdalin	+	+	−	w	+	d	+	+	−	+	+	+	−	−
Salicin	+	+	+	+	+	d	+	+	−	+	+	+	−	−
Lactose	+	+	+	+	+	−	−	+	−	d	d	+	+	d
Melibiose	+	+	−	w	d	d	−	−	−	+	d	+	+	d
Trehalose	+	+	+	+	d	−	+	−	d	−	d	d	d	−
D-Raffinose	+	+	−	+	d	d	−	−	−	+	d	+	−	d
Hydrolysis of aesculin	+	+	+	+	+	d	+	+	−	+	+	NA	−	−
DNA G+C content (mol%)	35.5	NA	35.7	NA	34–37	39	40–41	35–38	49–51	36–37	33–35	33–35	38–40	32–35
Peptidoglycan type	Lys−	NA	Lys−	Lys−	Lys−	Lys−	Lys−	Lys−	Lys−	NA	Lys−	Lys−	Lys−	NA
	D-Asp		D-Asp	D-Asp	D-Asp	D-Asp	D-Asp	D-Asp	D-Asp		D-Asp	D-Asp	D-Asp	

positions of the phylogenetic tree that correspond to what was found in the similarity rank analyses (Fig. 3).

Description of *Lactobacillus gastricus* sp. nov.

Lactobacillus gastricus (gas'tri.cus. N.L. masc. adj. *gastricus* from Gr. adj. *gastrikos* of the stomach).

Cells are Gram-positive, non-motile, non-spore-forming, catalase-negative rods, 0.9 × 1.2 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 mm in diameter; they are white, smooth and convex. Growth on MRS agar under aerobic conditions is very weak. In MRS broth, growth occurs at 25 and 42 °C, but not at 20 or 45 °C. Both D- and L-lactate are produced. Gas is produced from glucose. Acid is produced from L-arabinose (one of two strains), ribose (one of two strains), galactose, D-glucose, D-fructose, D-mannose, methyl α-D-glucopyranoside, N-acetylglucosamine (one of two strains), amygdalin, arbutin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, D-raffinose, β-gentiobiose, D-turanose and gluconate. Acid is not produced from glycerol, erythritol, D-arabinose, D-xylose, L-xylose, adonitol, methyl β-D-xyloside, L-sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl α-D-mannoside, inulin, melezitose, starch, glycogen, xylitol, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, 2-ketogluconate or 5-ketogluconate. Aesculin is not hydrolysed. The DNA G+C content of strain Kx156A7^T is 41.3 mol% and the peptidoglycan type is A4β L-Orn-D-Asp. Phylogenetic analysis of the 16S rRNA gene sequence places the species in the *L. reuteri* subgroup of lactobacilli.

The type strain is Kx156A7^T (=LMG 22113^T=DSM 16045^T=CCUG 48454^T), isolated from a biopsy of the healthy human gastric mucosa.

Description of *Lactobacillus antri* sp. nov.

Lactobacillus antri [an'tri. L. gen. n. *antri* of a cave (the antrum region of the stomach)].

Cells are Gram-positive, non-motile, non-spore-forming, catalase-negative rods, 1 × 1.2–2 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 2–3.5 mm in diameter;

Fig. 2. Dendrogram derived from SDS-PAGE protein pattern analysis of *Lactobacillus kalixensis* sp. nov., *Lactobacillus ultunensis* sp. nov. and representative strains within the *L. delbrueckii* subgroup of lactobacilli.

Fig. 3. Unrooted phylogenetic tree derived from 16S rRNA gene sequence analysis showing the relationship of the novel species to members of the *Lactobacillus reuteri* and *L. delbrueckii* subgroups of lactobacilli. The sequence of *Leuconostoc mesenteroides* (*Leu. mesenteroides*) was used as an outgroup representative. Approximately 1500 nt from each sequence were used for the alignment. Bar, 1 % estimated sequence divergence. Numbers indicate bootstrap values for branch points.

they are white, smooth and slightly convex. Growth on MRS agar under aerobic conditions is very weak. In MRS broth, growth occurs at 25 and 45 °C, but not 20 °C. Both D- and L-lactate are produced. Gas is produced from glucose. Acid is produced from L-arabinose, ribose, D-xylose (one of two strains), galactose, D-glucose, D-fructose, mannitol (weak reaction), methyl α -D-glucopyranoside (weak reaction), maltose, melibiose, sucrose, D-raffinose, D-turanose (weak reaction), D-arabitol and gluconate (weak reaction). Acid is not produced from glycerol, erythritol, D-arabinose, L-xylose, adonitol, methyl β -D-xyloside, D-mannose, L-sorbose, rhamnose, dulcitol, inositol, sorbitol, methyl α -D-mannoside, N-acetylglucosamine, amygdalin, arbutin, salicin, cellobiose, lactose, trehalose, inulin, melezitose, starch, glycogen, xylitol, β -gentiobiose, D-lyxose, D-tagatose, D-fucose, L-fucose, L-arabitol, 2-ketogluconate or 5-ketogluconate. Aesculin is hydrolysed (weak reaction). The DNA G+C content of strain Kx146A4^T is 44.9 mol% and the peptidoglycan type is A4 α L-Lys-D-Asp. Phylogenetic analysis of the 16S rRNA gene sequence places the species in the *L. reuteri* subgroup of lactobacilli.

The type strain is Kx146A4^T (=LMG 22111^T=DSM 16041^T=CCUG 48456^T), isolated from a biopsy of the healthy human gastric mucosa.

Description of *Lactobacillus kalixensis* sp. nov.

Lactobacillus kalixensis (ka.lix.en'sis. N.L. masc. adj. *kalixensis* pertaining to Kalix, a town in northern Sweden, where the gastric biopsies were sampled).

Cells are Gram-positive, non-motile, non-spore-forming, catalase-negative rods, 1 $\times$ 1.5–10 μ m in size and occurring as single cells, pairs or chains. After anaerobic growth at 37 °C for 48 h, colonies on MRS agar are 2 mm in diameter;

they are white, smooth and convex. Growth on MRS agar under aerobic conditions is weak. In MRS broth, growth occurs at 25 and 45 °C, but not at 20 °C. Both D- and L-lactate are produced. Gas is not produced from glucose. Acid is produced from galactose, D-glucose, D-fructose, D-mannose, mannitol (one of two strains), N-acetylglucosamine, amygdalin, arbutin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, D-raffinose, starch and β -gentiobiose. Acid is not produced from glycerol, erythritol, D-arabinose, L-arabinose, ribose, D-xylose, L-xylose, adonitol, methyl β -D-xyloside, L-sorbose, rhamnose, dulcitol, inositol, sorbitol, methyl α -D-mannoside, methyl α -D-glucopyranoside, inulin, melezitose, glycogen, xylitol, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, 2-ketogluconate or 5-ketogluconate. Aesculin is hydrolysed. The DNA G+C content of strain Kx127A2^T is 35.5 mol% and the peptidoglycan type is A4 α L-Lys-D-Asp. Phylogenetic analysis of the 16S rRNA gene sequence places the species in the *L. delbrueckii* subgroup of lactobacilli.

The type strain is Kx127A2^T (=LMG 22115^T=DSM 16043^T=CCUG 48459^T), isolated from a biopsy of the healthy human gastric mucosa.

Description of *Lactobacillus ultunensis* sp. nov.

Lactobacillus ultunensis (ul.tun.en'sis. N.L. masc. adj. *ultunensis* pertaining to Ultuna, the site of Swedish University of Agricultural Sciences in Uppsala, Sweden).

Cells are Gram-positive, non-motile, non-spore-forming, catalase-negative rods, 1 $\times$ 2–30 μ m in size and occurring as single cells, pairs or filaments. After anaerobic growth at 37 °C for 48 h, colonies on MRS agar are 2–3 mm in diameter; they are white, irregular and have a dry appearance. Growth on MRS agar under aerobic conditions is

very weak. In MRS broth, growth occurs at 25 and 42 °C, but not at 20 or 45 °C. Both D- and L-lactate are produced. Gas is not produced from glucose. Acid is produced from galactose, D-glucose, D-fructose, D-mannose, N-acetylglucosamine, arbutin, salicin, cellobiose, maltose, lactose, melibiose (one of two strains, weak reaction), sucrose, trehalose, D-raffinose (one of two strains), starch (one of two strains) and β -gentiobiose. Acid is not produced from glycerol, erythritol, D-arabinose, L-arabinose, ribose, D-xylose, L-xylose, adonitol, methyl β -D-xyloside, L-sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl α -D-mannoside, methyl α -D-glucopyranoside, amygdalin, inulin, melezitose, glycogen, xylitol, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, 2-ketogluconate or 5-ketogluconate. Aesculin is hydrolysed. The DNA G + C content of strain Kx146C1^T is 35.7 mol% and the peptidoglycan type is A4 α L-Lys-D-Asp. Phylogenetic analysis of the 16S rRNA gene sequence places the species in the *L. delbrueckii* subgroup of lactobacilli.

The type strain is Kx146C1^T (=LMG 22117^T=DSM 16047^T=CCUG 48460^T), isolated from a biopsy of the healthy human gastric mucosa.

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References

- Baele, M., Vancanneyt, M., Devriese, L. A., Lefebvre, K., Swings, J. & Haesebrouck, F. (2003). *Lactobacillus ingluviei* sp. nov., isolated from the intestinal tract of pigeons. *Int J Syst Evol Microbiol* **53**, 133–136.
- Bohak, I., Back, W., Richter, L., Ehrmann, M., Ludwig, W. & Schleifer, K. H. (1998). *Lactobacillus amylolyticus* sp. nov., isolated from beer malt and beer wort. *Syst Appl Microbiol* **21**, 360–364.
- 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.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Felsenstein, J. (1993). PHYLIP (Phylogeny Inference Package), version 3.5c. Distributed by the author. Department of Genetics, University of Washington, Seattle, USA.
- Fujisawa, T., Itoh, K., Benno, Y. & Mitsuoka, T. (1990). *Lactobacillus intestinalis* (ex Hemme 1974) sp. nov., nom. rev., isolated from the intestines of mice and rats. *Int J Syst Bacteriol* **40**, 302–304.
- Fujisawa, T., Benno, Y., Yaeshima, T. & Mitsuoka, T. (1992). Taxonomic study of the *Lactobacillus acidophilus* group, with recognition of *Lactobacillus gallinarum* sp. nov. and *Lactobacillus johnsonii* sp. nov. and synonymy of *Lactobacillus acidophilus* group A3 (Johnson *et al.* 1980) with the type strain of *Lactobacillus amylovorus* (Nakamura 1981). *Int J Syst Bacteriol* **42**, 487–491.
- 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. Springer: New York.
- Huß, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.
- 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.
- 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.
- Müller, M. R. A., Ehrmann, M. A. & Vogel, R. F. (2000). *Lactobacillus frumenti* sp. nov., a new lactic acid bacterium isolated from rye-bran fermentations with a long fermentation period. *Int J Syst Evol Microbiol* **50**, 2127–2133.
- Niamsup, P., Sujaya, I. N., Tanaka, M. & 8 other authors (2003). *Lactobacillus thermotolerans* sp. nov., a novel thermotolerant species isolated from chicken faeces. *Int J Syst Evol Microbiol* **53**, 263–268.
- Nikolaitchouk, N., Wachter, C., Falsen, E., Andersch, B., Collins, M. D. & Lawson, P. A. (2001). *Lactobacillus coleohominis* sp. nov., isolated from human sources. *Int J Syst Evol Microbiol* **51**, 2081–2085.
- 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.
- Roos, S., Karner, F., Axelsson, L. & Jonsson, H. (2000). *Lactobacillus mucosae* sp. nov., a new species with *in vitro* mucus-binding activity isolated from pig intestine. *Int J Syst Evol Microbiol* **50**, 251–258.
- 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.
- 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.
- Vogel, R. F., Böcker, G., Stolz, P. & 7 other authors (1994). Identification of lactobacilli from sourdough and description of *Lactobacillus pontis* sp. nov. *Int J Syst Bacteriol* **44**, 223–229.
- Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 other authors (1987). Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Weizenegger, M., Neumann, M., Stackebrandt, E., Weiss, N. & Ludwig, W. (1992). *Eubacterium alactolyticum* phylogenetically groups with *Eubacterium limosum*, *Acetobacterium woodii* and *Clostridium barkeri*. *Syst Appl Microbiol* **15**, 32–36.
- Wiese, B. G., Strohm, W., Rainey, F. A. & Diekmann, H. (1996). *Lactobacillus panis* sp. nov., from sourdough with a long fermentation period. *Int J Syst Bacteriol* **46**, 449–453.