***Lactobacillus mucosae* sp. nov., a new species with *in vitro* mucus-binding activity isolated from pig intestine**

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A new *Lactobacillus* species from pig small intestine has been identified. In an attempt to isolate *Lactobacillus reuteri* strains carrying the putative colonization-factor gene (*mub*, for mucus binding) a *mub*-derived gene probe was used to screen pig intestinal material. A number of isolates were obtained and primary characterization showed that they were Gram-positive, catalase-negative, non-spore-forming, non-motile rods. Growth occurred at 45 °C but not at 15 °C and the DNA G+C content was 46 mol %. Cell wall analysis together with DNA–DNA hybridization and analysis of the 16S rRNA sequence revealed that the new isolates represent a previously undescribed *Lactobacillus* species closely related to *L. reuteri*, *Lactobacillus fermentum* and *Lactobacillus pontis*. The name *Lactobacillus mucosae* is proposed for this species and the type strain is S32^T.

Keywords: *Lactobacillus mucosae*, new species, mucus binding

INTRODUCTION

The use of lactic acid bacteria (LAB) in the production and preservation of food and animal feed dates back several thousand years. Today this use is manifested by various dairy products such as cheese and yoghurt, fermented sausages, vegetables and silage for animal feed. Recently, a great deal of interest has been focused on some members of the LAB with regard to their use as probiotics (Fuller, 1989; Marteau & Rambaud, 1993; Salminen *et al.*, 1996). The term ‘probiotic’ refers to live organisms that are administered to animals or humans via feed or food products and are in some way beneficial to health (Fuller, 1989). The LAB that are currently used as probiotics are primarily species of *Lactobacillus* and *Bifidobacterium*. In this context, bifidobacteria are often included in the LAB group since they have many features in common with this group. However, in contrast to the LAB group in general, bifidobacteria belong to the high G+C group of Gram-positive bacteria. The positive effects attributed to these probiotic organisms include stabilization of the normal microflora, protection against pathogens, lowering of cholesterol levels,

immune stimulation and protection against certain forms of cancer (Elmer *et al.*, 1996; Isolauri *et al.*, 1998; Lichtenstein & Goldin, 1998). One of the basic assumptions regarding the important features of probiotic micro-organisms is the need for colonization ability (Huis in’t Veld *et al.*, 1994; Brassart & Schiffrin, 1997). In contrast to the case of many pathogenic bacteria, little is known about the mechanisms by which LAB interact with host components in the intestinal tract. However, in recent years several reports have begun to establish a knowledge base on how lactobacilli adhere to the intestinal mucosa (Adlerberth *et al.*, 1996; Roos *et al.*, 1996; Yamamoto *et al.*, 1996; Granato *et al.*, 1999).

We have recently cloned and sequenced an extremely large gene, *mub*, from the pig intestinal isolate *Lactobacillus reuteri* strain 1063, encoding a cell-surface protein with mucus-binding activity (S. Roos & H. Jonsson, unpublished results). This protein, termed Mub, has a molecular mass of 358 kDa and contains two types of large amino acid repeats. The parental strain, 1063, has very good binding activity against crude pig mucus *in vitro*. Furthermore, both the native and the recombinant Mub protein were shown to interact with immobilized mucus material. In order to clarify the correlation between the presence of *mub* and the ability to adhere to mucus material, we screened pig small-intestinal mucosa for bacteria harbouring

Abbreviations: LAB, lactic acid bacteria; RDP, Ribosomal Database Project.

The GenBank/EMBL/DBJ accession number for the 16S rDNA gene sequence of strain S32^T is AF126738.

this gene. In this work we describe the isolation of a number of strains that are reactive with a gene probe derived from *mub* and that exhibit binding to mucus material *in vitro*. Further characterization of these isolates showed that they represent a new *Lactobacillus* species that is closely related to *L. reuteri*, *Lactobacillus fermentum* and *Lactobacillus pontis*. Interestingly, three strains of lactobacilli previously isolated from pig small intestine (Axelsson & Lindgren, 1987; Wadström *et al.*, 1987) were shown to belong to this new species. These isolates also harbour *mub* and possess the ability to adhere to pig mucus *in vitro*. An oligonucleotide probe that can be used for rapid identification of *Lactobacillus mucosae* sp. nov. is also described.

METHODS

Origin of the strains. Strains 1028, 1031 and 1035 were isolated from pig small intestine and described earlier (Axelsson & Lindgren, 1987; Wadström *et al.*, 1987). Strains S5, S14, S15, S17 and S32^T were isolated in this work. Small intestine from a newly slaughtered pig was collected from the slaughterhouse. In the laboratory, the intestine was sectioned and 15-cm-long sections were cut open and rinsed with ice-cold PBS (8.0 g NaCl, 0.2 g KCl, 1.44 g Na₂HPO₄ · 2 H₂O and 0.2 g KH₂PO₄ per 1000 ml dH₂O) in order to remove loosely associated intestinal material. Mucosal material was then released by gently scraping the intestine with a spatula. The released material was collected in a tube with ice-cold PBS and subsequently diluted and used to inoculate agar plates. *L. reuteri* DSM 20016^T, *L. fermentum* DSM 20052^T and *L. pontis* DSM 8475^T were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany, and *Lactobacillus acidophilus* ATCC 4356^T was obtained from the American Type Culture Collection (ATCC), Manassas, VA, USA.

Culture conditions. Primary isolation was done on *Lactobacillus* Selective Agar (BBL) in anaerobic jars under a CO₂ + N₂ atmosphere (GasPak System, BBL) at 37 °C. All further cultivation was done at 37 °C on De Man–Rogosa–Sharpe (MRS) agar (Oxoid), in anaerobic jars or in MRS broth (Oxoid) unless stated otherwise.

Morphological characteristics. Cell morphology was observed using phase-contrast microscopy. Gram determination was performed using both Gram-staining and the KOH method of Gregersen (1978).

Physiological and chemical characterization. Sugar-fermentation patterns were determined using the API 50 CH system (BioMérieux). Cell wall analysis was performed at DSMZ. The preparation of cell walls and the determination of peptidoglycan structure were carried out using the methods described by Schleifer & Kandler (1972), with the modification that TLC was performed on cellulose sheets instead of paper. The lactic acid configuration was determined using a commercial test kit (Boehringer Mannheim). Arginine cleavage was determined using the method of Collins & Lyne (1970). Catalase activity was determined by transferring fresh colonies from MRS agar to a glass slide and adding 5% H₂O₂.

DNA base composition. The G + C content of the DNA (mol %) was determined at DSMZ. The DNA was isolated by chromatography on hydroxyapatite using the procedure of Cashion *et al.* (1977). The G + C content of the DNA was

determined using HPLC, as described by Mesbah *et al.* (1989).

DNA–DNA hybridization. DNA–DNA hybridization was performed at DSMZ, Braunschweig, Germany. DNA hybridization was carried out according to De Ley *et al.* (1970) with modifications as described by Escara & Hutton (1980) and Huß *et al.* (1983), using a Gilford System 2600 spectrophotometer equipped with a Gilford 2527-R thermo-programmer and plotter. Renaturation rates were calculated by the program TRANSFER: BAS (Jahnke, 1992).

Colony hybridization. The original isolates were recultivated and single colonies were inoculated on Protran BA85 nitrocellulose filters (Schleicher & Schuell) placed on top of MRS-agar plates and grown overnight in anaerobic jars at 37 °C. Colony hybridization was performed according to Sambrook *et al.* (1989) with a modified protocol for lysis of the cells which includes a prolonged incubation with SDS (30 min). An internal 1100 bp restriction fragment from the *mub* gene of *L. reuteri* strain 1063 (S. Roos & H. Jonsson, unpublished results) was labelled with ³²P, with the Multi-prime DNA labelling system (Amersham), and used as a probe in the hybridizations.

Western blot analysis. Bacteria were grown for 48 h at 37 °C in 100 ml *Lactobacillus* Defined Medium II (Kotarski & Savage, 1979) supplemented with 1% glucose instead of sucrose. The cells were sedimented by centrifugation and the growth medium was sterile-filtered through a 45 µm filter and dialysed against 1 mM EDTA overnight with several changes of buffer. The material was then lyophilized and dissolved in 1.5 ml dH₂O. The protein preparations were mixed 1:1 with sample buffer containing SDS and mercapto-ethanol and separated by SDS-PAGE. The electrophoresis and staining of the gel with Coomassie blue was performed with the PhastSystem (Pharmacia Biotech) according to the manufacturer's instructions. The proteins were blotted to a Hybond-C nitrocellulose membrane (Amersham Life Science) by diffusion at 65 °C for 45 min. The membrane was blocked in PBST (PBS supplemented with 0.05% Tween 20, pH 7.3) for 1 h at 37 °C and then incubated overnight at 4 °C with 10 µg ml⁻¹ antibodies against extracellular proteins from *L. reuteri* strain 1063 (S. Roos & H. Jonsson, unpublished results) which had been preadsorbed with an *Escherichia coli* lysate according to Sambrook *et al.* (1989). After being washed with PBST, the membrane was incubated with HRP-conjugated goat anti-rabbit IgG (Bio-Rad), diluted 1:1000, at 37 °C for 1 h. After being washed, the membranes were finally developed with 4-chloro-1-naphthol as substrate.

Assay of mucus binding. Mucus was prepared from the same intestine that was used for isolation of bacteria. By scraping the inside of the intestine with a spatula, material was removed and collected in 200 ml ice-cold PBS. The resulting suspension was centrifuged first at 11 000 g for 10 min and then at 26 000 g for 15 min in order to remove cells and particulate matter. The crude mucus preparation was stored at 20 °C. The mucus material was diluted to approximately 100 µg ml⁻¹ in 50 mM Na₂CO₃ buffer, pH 9.7, and incubated overnight in microtiter wells (Greiner) (150 µl per well) at 4 °C with slow rotation. The wells were blocked with PBS with 1% Tween 20 for 1 h and thereafter washed with PBST. The bacteria were grown in MRS broth for 16 h at 37 °C, washed once in PBST and diluted to OD₆₀₀ = 0.5 (1 cm cuvette; Beckman DU650) in the same buffer. Bacterial suspension (150 µl) was added to each well and incubated for 1 h at room temperature. The wells were washed with PBST and binding was examined with an

inverted microscope. The buffer was poured off and, after the wells had dried, the OD₄₅₀ was measured in an ELISA plate reader (EL309 Autoreader; BIO-TEK Instruments).

16S rRNA gene sequencing. The almost complete 16S rRNA gene was amplified by PCR by using slightly modified domain *Bacteria*-specific primers according to Weizenegger *et al.* (1992). The primer sequences were 5'-AGAGTTTG-ATYMTGGC-3' and 5'-AGAAAGGAGGTGATCC-3'. PCR reactions involved 35 cycles under the following conditions: 94 °C for 30 s, 54 °C for 30 s and 72 °C for 80 s; the resulting PCR products were purified from agarose gels. Both strands of the purified fragments were sequenced using the Thermo Sequenase dye terminator cycle sequencing premix (Amersham) and the automated sequence analyser ABI PRISM 377XL (Perkin Elmer). The same primers that were used for the amplification were used (together with additional customized internal primers) to sequence the PCR products.

Phylogenetic analysis of the sequence data. The sequences achieved from the new isolates were used for searching in the public databases (GenBank and the Ribosomal Database Project, RDP). The sequences representing the best matches were retrieved and aligned using the CLUSTAL W program (Thompson *et al.*, 1994). The sequences were manually modified before the alignment and approximately 1450 nucleotides covered by all sequences were used. The following sequences from the type strains of the respective species were used: *L. fermentum*, M58819; *L. reuteri*, X76328; *L. pontis*, X76329; *Lactobacillus vaginalis*, X61136; *Lactobacillus oris*, X94229; *Lactobacillus panis*, X94230; *Lactobacillus sakei*, M58829; *Lactobacillus casei*, X61135; *Lactobacillus brevis*, X61134; *Lactobacillus salivarius* subsp. *salivarius*, AF089108; *Lactobacillus delbrueckii* subsp. *delbrueckii*, M58814; *Lactobacillus fructivorans*, X76330; *Pediococcus acidilactici*, M58833. A distance matrix was calculated with the DNADIST program of the PHYLIP package (Felsenstein, 1993), using the Kimura 2-parameter model, and a phylogenetic tree was constructed with the NEIGHBOR program (PHYLIP package) using the neighbour-joining method. The statistical significance of the grouping was estimated by bootstrapping (100 replicates) using the programs SEQBOOT, DNADIST, NEIGHBOR and CONSENSE (all from the PHYLIP package).

Hybridization with a specific rDNA-targeted probe. Bacteria were grown and lysed on filters as described for the colony hybridization. The oligonucleotide LM16, with the sequence 5'-GTAAACCAACGTCAGTCC-3', was designed to be specific for 16S rRNA and 16S rDNA from the new isolates by comparing sequences from different species. The PROBE-CHECK program at RDP was used to confirm the specificity of the probe. The customized oligonucleotide was labelled with ³²P using T4 polynucleotide kinase, according to Sambrook *et al.* (1989). The prehybridization, hybridization and washing were performed according to Sambrook *et al.* (1989). The temperature during hybridization and washing was 42 °C.

RESULTS

Identification of colonies reactive with the *mub* probe, producing Mub protein and possessing mucus-binding activity

In an attempt to isolate *L. reuteri* strains carrying the gene for the mucus-binding protein (Mub) (S. Roos & H. Jonsson, unpublished results), we used a probe

derived from this gene from *L. reuteri* strain 1063 for screening lactobacilli isolated from pig small-intestine mucosa. Approximately 100 colonies were screened with colony hybridization, of which five (S5, S14, S15, S17 and S32^T) reacted with the probe. Fifty additional *Lactobacillus* strains previously isolated from pig small intestine [and characterized by Wadström *et al.* (1987) and Axelsson and Lindgren (1987)] were also screened with the probe: three of them (1028, 1031 and 1035) gave positive signals. Strains reacting with the probe were recultivated from the master plate and checked by SDS-PAGE and Western blotting for production of the mucus-binding protein, Mub. Since this protein is very large (358 kDa), bands located at the top of the gel and reactive with antibodies against extracellular proteins from *L. reuteri* strain 1063 were considered to be Mub or a related protein. All strains that were reactive with the *mub* probe also produced a large protein reacting with the antibodies. The same set of strains was also positive in the assay of mucus binding (data not shown).

Colony and cell morphology

Colonies of strains S5, S14, S15, S17, S32^T, 1028, 1031 and 1035 were white, smooth and convex. After anaerobic growth for 2 d on MRS agar at 37 °C, the colonies were 1–2 mm in diameter. Cells of all strains were non-spore-forming, non-motile rods 1 × 2–4 µm in size. The Gram-reaction was positive.

DNA base composition

The mean G + C content of strain S32^T DNA, based on three determinations, was 46.5 ± 0.2 mol%. The G + C content of strains 1028, 1031 and 1035 was determined previously (Axelsson & Lindgren (1987): the values for these three strains were 49, 47 and 49 mol%, respectively.

DNA–DNA hybridization

On the basis of the 16S rRNA sequence analysis, three reference strains were chosen for DNA–DNA hybridization experiments with strain S32^T. These were *L. reuteri* DSM 20016^T, *L. fermentum* DSM 20052^T and *L. pontis* DSM 8475^T. The results showed homology values of 40.5, 41.5 and 52.2%, respectively, between these strains and *L. mucosae* S32^T.

Physiological and chemical characterization

Analysis of the cell wall of *L. mucosae* strain S32^T revealed the presence of ornithine and aspartic acid, which is consistent with an Orn-D-Asp peptidoglycan type. All strains grew well at 45 °C but not at 15 °C. Gas was produced from glucose. All strains grew well in liquid and on solid MRS media in anaerobic jars. Weak growth also occurred on solid MRS media in the presence of air. Arginine was cleaved by all strains. Catalase activity was negative in all strains. D-Lactate and L-lactate were produced by all strains. The

Table 1. Differential characteristics of *Lactobacillus mucosae* sp. nov. and closely related lactobacilli

ND, Not determined; v, variable reaction.

Strain	Utilization of:							
	NH ₃ from arginine	Growth at 15/45 °C	Gas from glucose	Glucose	Ribose	L-Arabinose	D-Xylose	Galactose
<i>L. mucosae</i> S5	+	–/+	+	+	+	+	+	+
<i>L. mucosae</i> S14	+	–/+	+	+	+	–	–	–
<i>L. mucosae</i> S15	+	–/+	+	+	v	–	+	–
<i>L. mucosae</i> S17	+	–/+	+	+	+	–	+	+
<i>L. mucosae</i> S32 ^T	+	–/+	+	+	+	–	+	+
<i>L. mucosae</i> 1028	+	–/+	+	+	+	+	+	+
<i>L. mucosae</i> 1031	+	–/+	+	+	+	+	+	–
<i>L. mucosae</i> 1035	+	–/+	+	+	+	+	+	–
<i>L. reuteri</i> DSM 20016 ^T	+	–/+	+	+	+	+	–	+
<i>L. fermentum</i> DSM 20052 ^T	+	–/+	+	+	+	–	–	+
<i>L. pontis</i> DSM 8475 ^{T†}	+	+/+	–	–	+	–	–	–
<i>L. oris</i> DSM 4864 ^T	–	–/+	+	+¶	+	+	+	+¶
<i>L. panis</i> DSM 6035 ^T	–	–/+	+	+	+	+	+	ND
<i>L. vaginalis</i> DSM 583 ^T	+	–/+	+	+¶	–	–	–	+¶
Strain	Utilization of:						Peptidoglycan type	G + C content (mol %)
	D-Fructose	Aesculin	Lactose	Melibiose	D-Raffinose	Gluconate		
<i>L. mucosae</i> S5	–	+	–	+	+	+	ND	ND
<i>L. mucosae</i> S14	–	+	–	–	–	–	ND	ND
<i>L. mucosae</i> S15	–	+	–	+	+	+	ND	ND
<i>L. mucosae</i> S17	–	+	–	+	+	+	ND	ND
<i>L. mucosae</i> S32 ^T	–	+	–	+	+	+	Orn-D-Asp	46.5 ± 0.2
<i>L. mucosae</i> 1028	v	+	+	+	+	+	ND	49*
<i>L. mucosae</i> 1031	–	+	–	+	+	+	ND	47*
<i>L. mucosae</i> 1035	–	+	–	+	+	+	ND	49*
<i>L. reuteri</i> DSM 20016 ^T	+	–	+	+	+	+‡	Lys-D-Asp	40–42‡
<i>L. fermentum</i> DSM 20052 ^T	+	+	+	+	+	+	Orn-D-Asp	52–54‡
<i>L. pontis</i> DSM 8475 ^{T†}	+	–	–	–	+	+	Orn-D-Asp	53§
<i>L. oris</i> DSM 4864 ^T	+	+	+	+	+	+	Lys-D-Asp	49–51#
<i>L. panis</i> DSM 6035 ^T	+	+	+	+	+	+	Lys-D-Asp	48
<i>L. vaginalis</i> DSM 583 ^T	+	+	+	+	+	–	Orn-D-Asp	38–41**

* Data from Axelsson & Lindgren (1987).

† Data from Vogel *et al.* (1994).

‡ Data from Kandler & Weiss (1986). Data are typical for the species, not specifically the type strain.

§ The values for *L. pontis* LTH 2585 and 2586 (Vogel *et al.*, 1994).|| Data from Wiese *et al.* (1996).¶ Data from Hammes *et al.* (1992).

Data from Farrow & Collins (1988).

** Data from Embley *et al.* (1989).

Fig. 1. Unrooted phylogenetic tree, derived from 16S rDNA sequence analysis, showing the relationship between *Lactobacillus mucosae* strain S32^T and members of the *Lactobacillus casei*-*Pediococcus* group of lactobacilli. The sequence from *Lactobacillus delbrueckii* was used as an outgroup representative. All strains are type strains. Approximately 1450 nucleotides from each sequence were used for the alignment. Bar, 1% estimated sequence divergence.

following sugars were fermented: glucose, ribose, maltose, saccharose, D-xylose (7 of 8 strains), melibiose (7 of 8), D-raffinose (7 of 8), gluconate (7 of 8), L-arabinose (4 of 8), galactose (4 of 8) and lactose (1 of 8). There was no fermentation of D-fructose, glycerol, erythritol, L-xylose, adonitol, D-mannose, L-sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl α -D-glucoside, N-acetylglucosamine, salicine, cellobiose, trehalose, inuline, melizitose, amidon, glycogen, xylitol, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, 2-keto-gluconate or 5-keto-gluconate. Aesculin was hydrolysed by all strains. The minor differences in the sugar-fermentation patterns of strains 1028, 1031 and 1035 found when data from this work were compared with data presented by Axelsson & Lindgren (1987) cannot be explained at this point.

Relevant characteristics of the *L. mucosae* strains are summarized in Table 1.

16S rRNA sequence and phylogenetic analysis

Almost the complete 16S rDNA sequence was determined for strains S14 and S32^T, while partial sequences from each end of the gene were determined for strains 1028, 1031, 1035, S5, S15 and S17. Analysis of all sequences from the 5'- and 3' ends of the genes showed no differences except for a small number of ambiguities, so we concluded from these data that all isolates belong to the same species. The complete sequences from S14 and S32^T were aligned and found to be identical. The complete S32^T sequence was used to search the RDP and the highest similarity rank was found with *L. reuteri*, *L. pontis* and *L. fermentum*, the values being 95.1, 94.6 and 94.4%, respectively. The new sequence was then aligned with the sequences from members of the *L. reuteri*-*L. fermentum* branch of the *L. casei*-*Pediococcus* group and other representatives of this group and the alignment was used for a tree analysis. This analysis confirmed the close relationship of *L. mucosae* to *L. reuteri* and *L. fermentum* (Fig. 1).

Specific detection of *L. mucosae* with the aid of a rDNA probe

Colony hybridization of the oligonucleotide probe LM16 (5'-GTAAACCAACGTCAGTCC-3') with all eight *L. mucosae* strains, *L. reuteri* DSM 20016^T, *L. fermentum* DSM 20052^T and *L. acidophilus* ATCC 4356^T was performed. The oligonucleotide was selective for the *L. mucosae* strains under the conditions used.

DISCUSSION

The genus *Lactobacillus* currently includes more than 60 species; three of these were described during 1998 (Morlon-Guyot *et al.*, 1998; Edwards *et al.*, 1998; Bohak *et al.*, 1998) and one was described at the beginning of 1999 (Falsen *et al.*, 1999). Members of this genus have been isolated from a large number of habitats, including oral and genital sites and the gastrointestinal tracts of animals and humans. In pigs, a number of *Lactobacillus* species have been identified and *L. reuteri*, *L. fermentum*, *L. acidophilus*, *L. delbrueckii* and *L. salivarius* are commonly reported as being isolated from pig intestine (Stewart, 1997). On two different occasions separated by a 12-year interval, we have isolated strains of lactobacilli from pig small intestine. Many of these isolates are *L. reuteri* and one of them, strain 1063, has been characterized with respect to autoaggregation and mucus-binding ability (Roos *et al.*, 1999; S. Roos & H. Jonsson, unpublished results). Three isolates (1028, 1031 and 1035) were partially described by Axelsson & Lindgren (1987) and these three strains, together with five new isolates (S5,

S14, S15, S17 and S32^T), have been further investigated in this work. The common characteristic of these isolates, which originally attracted our attention, was the reactivity with a gene probe derived from the putative colonization-factor gene *mub* from *L. reuteri* strain 1063 (S. Roos & H. Jonsson, unpublished results). When we further examined these isolates we also found that they exhibited *in vitro* characteristics similar to those of strain 1063, with respect to mucus-binding activity. Primary characterization using sugar-fermentation analysis indicated that these isolates were not *L. reuteri* and we therefore used nucleic acid methods to investigate their taxonomic position in more detail. Thus, 16S rRNA sequence analysis, G + C mol % determination and DNA–DNA hybridization, together with cell wall analysis, showed that they represent a previously undescribed species. On the basis of phylogenetic analysis, the new species can be allocated to the *L. casei*–*Pediococcus* group described by Collins *et al.* (1991). The closest relatives are *L. reuteri*, *L. fermentum* and *L. pontis*, which show 16S rRNA sequence similarity values of 95.1, 94.4 and 94.6%, respectively. The construction of a phylogenetic tree confirmed the close affiliation of the new isolates with the *L. reuteri*–*L. fermentum* group. DNA–DNA hybridization of total DNA from S32^T with the type strains of the three closely related species showed an overall sequence homology in the range 40.5–52.2%, clearly indicating that *L. mucosae* constitutes a new species separate from these others. Our own data and also work by others have shown that *L. reuteri* and *L. mucosae* can be found in the same part of the pig intestine. *L. fermentum*, though frequently reported as being present in the pig intestinal tract, was not found in this niche by Axelsson & Lindgren (1987). The species *L. pontis*, *L. oris*, *L. vaginalis* and *L. panis* have never been reported as colonizing this environment. However, it must be mentioned that few, if any, ecological studies on the gastrointestinal-tract microbiota have employed 16S rDNA sequence determination for identification of the bacteria. The methods that have been used in most studies either fail to detect all types of lactobacilli or fail to distinguish between certain species.

The relationship between *L. reuteri* and *L. mucosae* is further accentuated by the presence of the *mub* gene. Interestingly, the G + C content (mol %) of this gene is close to the overall G + C content of *L. mucosae* and rather different from that of other genes from *L. reuteri*. Although five of the isolates (S5, S14, S15, S17 and S32^T) were primarily selected for harbouring the *mub* gene, this was not the case with the three previously isolated strains (1028, 1031 and 1035) yet the gene was still present. Thus, it seems that strains of *L. mucosae* commonly carry this gene. In contrast, the presence of *mub* is restricted to a limited number of strains of *L. reuteri*. Altogether, this could implicate *L. mucosae* as the source of *mub* and *L. reuteri* as a recipient of the gene at some point during the course of evolution. The presence of a *mub* homologue in more

than one *Lactobacillus* species and the correlation of the presence of this gene with mucus-binding ability argue that it represents an important part of the colonization mechanism for lactobacilli in the pig intestine.

Description of *Lactobacillus mucosae* sp. nov.

Lactobacillus mucosae (mu'co.sae. L. gen. n. *mucosae* of mucosa).

Cells are Gram-positive, non-motile, non-spore-forming, catalase-negative rods with dimensions of $1 \times 2\text{--}4\ \mu\text{m}$. The cells occur singly, in pairs or as short chains and have an Orn-D-Asp peptidoglycan type. After anaerobic growth at 37 °C for 2 d, colonies on MRS agar are 1–2 mm in diameter. They are white, smooth and convex. The cells are obligately heterofermentative and produce D- and L-lactic acid. They grow at 45 °C but not at 15 °C. Acid is produced from glucose, ribose, maltose and saccharose. The majority of strains also ferment D-xylose, melibiose, D-raffinose and gluconate. L-Arabinose, galactose or lactose may be utilized by some strains. D-Fructose, glycerol, erythritol, L-xylose, adonitol, D-mannose, L-sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl α -D-glucoside, N-acetylglucosamine, salicine, cellobiose, trehalose, inuline, melizitose, amidon, glycogen, xylytol, D-turanose, D-lyxose, D-taganose, D-fucose, L-fucose, D-arabitol, 2-keto-gluconate and 5-keto-gluconate are not utilized. Aesculin is hydrolysed. Arginine is cleaved. The DNA G + C content of strain S32^T is 46.5 ± 0.2 mol %. All strains of this species so far described have a homologue to the mucus-binding protein, Mub, and exhibit mucus-binding ability *in vitro*.

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