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Isolation of surface (S-) layer protein carrying *Lactobacillus* species from porcine intestine and faeces and characterization of their adhesion properties to different host tissues

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Abstract

Surface layer proteins (Slps) of lactobacilli have been shown to confer tissue adherence. This study aimed to isolate and identify Slps carrying *Lactobacillus* species from the porcine intestine and faeces and to characterize these S-layer expressing strains for their ability to adhere to the pig and human intestinal cells and to extracellular matrix (ECM) proteins. In total 99 strains, putatively belonging to the genus *Lactobacillus,* were isolated as pure cultures. SDS-PAGE and a gene probe specific for the *Lactobacillus brevis* ATCC 8287 S-layer protein gene (*slpA*) were used to screen the presence of strains possessing putative Slps. Eight of the 99 pure cultures exhibited Slps according to the SDS-PAGE analyses. In these strains the presence of genes encoding Slps was confirmed by PCR and partial sequencing. Only one isolate of the 99 strains gave a positive hybridization signal with the *L. brevis slpA* probe but did not appear to produce S-layer protein. Their taxonomic identification, based on phenotyping and the 16S rRNA sequences, revealed that the eight S-layer protein-producing strains were closely related to *Lactobacillus amylovorus, Lactobacillus sobrius* and *Lactobacillus crispatus.* The strain with the *slpA* positive hybridization result was identified as *Lactobacillus mucosae*. The SDS-extractable protein profile, the size of the putative S-layer protein and binding capability of the strains varied greatly, even among the isolates belonging to the same *Lactobacillus* cluster. Removal of the intact Slps from the bacterial surface by extraction with guanidine hydrochloride reduced the adhesion of some strains to fibronectin and laminin, whereas, the adhesiveness to laminin increased with some strains.

Keywords: Porcine lactobacilli, S-layer, Adhesion, ECM-components

1. Introduction

Lactobacilli, identified as common members of porcine intestinal microbiota, have been considered to be an important group of bacteria in maintaining the stability of the gastrointestinal tract (GIT), in preventing intestinal infections and, generally, in supporting intestinal health. Since several species of lactobacilli have the GRAS (generally regarded as safe) status and some of them have the ability to interact with intestinal epithelial cells, their possible applications as a mucosal vaccine vector (Mercenier et al., 2000; Seegers, 2002) and probiotics (Mulder et al., 1997) have aroused interest.

Several factors contribute to the interaction of lactobacilli with the host tissues, such as cell surface hydrophobicity and autoaggregation (Kos et al., 2003), lipoteichoic acids (Granato et al., 1999) and cell surface proteins. Several cell surface proteins have been reported to bind to epithelial cells, mucus and/or ECM components. These include the mucus binding proteins from *L. reuteri* (Roos et al., 2002, Båth et al., 2005), *L. acidophilus* (Buck et al., 2005) and *L. plantarum* (Boeckhorst et al., 2006a). In addition to the high MW mucus binding protein of *L. reuteri* (Roos at al., 2002), mucus/collagen binding proteins with sequence similarity to solute binding proteins of ABC transporters have been described for *L. reuteri* strains (Miyoshi et al., 2006). Furthermore, genome analysis of *L. plantarum* has revealed several cell surface proteins with adhesive domain structures (Bockhorst et al., 2006b). Another group of proteins identified as putative adhesins in some *Lactobacillus* are the S-layer proteins. *Lactobacillus* species, such as *L. brevis, L. helveticus, L. acidophilus, L. crispatus, L. amylovorus* and *L. gallinarum* possess surface-layer proteins (Slps) (reviewed in Åvall-Jääskeläinen and Palva, 2005). S-layers are crystalline arrays of proteinaceous subunits located at the outermost part of the cell wall. Due to the high number of Slp subunits required to cover the entire cell surface, S-layer proteins represent approximately 10 % of the total cellular proteins (Åvall-Jääskeläinen and Palva, 2005).

The functions of Slps are not yet completely revealed but it has been proposed that these structures protect the microbe from hostile environmental agents and aid in maintaining cellular integrity (Åvall-Jääskeläinen and Palva, 2005). Slps of several lactobacilli**,** including *L. crispatus* and *L. acidophilus,* whose ability to bind to host epithelial cells is decreased after removal or disruption of the S-layer

proteins (Sillanpää et al., 2000; Frece et al., 2005; Buck et al., 2005) have been shown to confer tissue adherence. In addition, S-layer protein extracts from *L. helveticus* have been reported to inhibit enterohaemorrhagic *E. coli* adhesion to host epithelial cells (Johnson-Henry et al., 2006). The *L. brevis* ATCC 8287 SlpA protein has been shown to possess an affinity for human intestinal epithelial cell lines, urinary bladder, endothelial cells and fibronectin (Hynönen et al., 2002). Recently, by using surface plasmon resonance, SlpA was found to interact with fibronectin and laminin (de Leeuw et al., 2006). In some lactobacilli, carrying several *slp* genes, the S-layer may be subjected to variation. These bacteria may express alternative S-layer protein genes, most likely to adapt to different stress factors such as drastic changes in the environmental conditions (Boot and Pouwels, 1996; Jakava-Viljanen et al., 2002; Hagen et al., 2005).

In this study, our aim was to isolate new S-layer positive lactobacilli from the intestine and faeces of pigs for later use as vaccine vectors and/or probiotics for pigs, identify the lactobacilli found by using phenotypic and molecular tests and to test putative S-layer expressing strains for their ability to adhere to pig and human intestinal cells and to ECM components, collagen, laminin and fibronectin.

2. Materials and methods

2.1. Bacterial strains, culture conditions and isolation of genomic DNA

The bacterial strains used in this study are listed in Table 1. To isolate *Lactobacillus* species, the small intestine (duodenum, jejunum and ileum) of newly slaughtered pigs was removed and sealed at both ends with a piece of band. The intestine was chilled in ice-cooled physiological saline and brought to the laboratory within 30 min. Part of the strains used in this study were isolated from sow faeces. The samples were cultured anaerobically in Lactobacilli MRS or Rogosa SL broth (Difco, USA) at 37°C for 48 h. Colonies were randomly isolated and pure cultured on Lactobacilli MRS or Rogosa SL agar (Difco, USA). The bacteria were investigated by Gram staining and stored at -70 °C in MRS containing 15% glycerol. For the isolation of genomic DNA, routine molecular biology techniques were used (Sambrook and Russell, 2001). The DNA was isolated by using the Qiagen

column purification kit (Qiagen, USA), and the concentration was determined with a Versafluor fluorometer (Bio-Rad, USA).

2.2. Detection of S-layer proteins

Pure culture strains were grown in MRS broth for 24 h followed by harvesting of cells by centrifugation at 16,000 x g for 2 min. Each cell pellet, equivalent to 1 ml of culture of equal OD, was dissolved directly in 50 µl of Laemmli sample buffer, boiled for 5 minutes and samples of 15 µl were analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970). After electrophoresis, the gels were stained with Coomassie brilliant blue.

2.3. Dot blot hybridization

Chromosomal DNA samples from 99 lactobacilli isolates were denatured using alkali and heat and transferred to nylon membranes (Boehringer Mannheim, Germany). Dot blot hybridisation was carried out under non-stringent conditions essentially as described before (Sambrook and Russell, 2001). An *L. brevis* ATCC 8287 *slpA* probe was PCR amplified with primers 421 (slpA (+) AAACGATATGCAATCAAGTTTAAAGAAATC) and
422 (slpA (-) AGTAGGATCCGTTGAACCAAGTAGTACCGTTAG) and labelled with digoxigenin using the DIG-High Prime labelling system (Boehringer Mannheim). The Molecular Imager System and the Quantity One program (Bio-Rad, USA) were used for detection and quantification of the hybridisation signals.

2.4. Isolation of S-protein genes by PCR

The universal primers Usl-1 and Usl-2 used for isolation of S-protein genes were based on the *L. gallinarum* S-protein (*lsg*) genes (Hagen et al, 2005). PCR was carried out as described earlier (Hagen et al., 2005). Sequencing was performed with an ABI 310 DNA sequencer (PE Biosystems, USA) and sequence editing was performed with the Sequencher, version 3.0, program (Gene Codes Corporation, Ann Arbor, Mich.). Homology searches of the databases were done with the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST). Sequence alignments were performed using ClustalW.

2.5. PCR-ELISA

A polymerase chain reaction-enzyme-linked immunosorbent assay (PCR-ELISA) was performed according to the method developed by Laitinen et al. (2002). PCR reactions were carried out and amplification of bacterial 16S and 23S rRNA genes was performed with *Lactobacillus* genus-specific primers (Ehrmann et al., 1994). The products were labelled using the DIG-high Prime kit (Boehringer Mannheim, Germany) according to the instructions of the manufacturer. The hybridisation was performed in commercial streptavidin-coated microtiter plates (Labsystems, Finland). Denatured samples were transferred into microtiter wells and hybridised with streptavidin-bound oligonucleotide probes for 2 hours at 44°C. Hybridised DNA products are detected via digoxigenin targeted antibodies linked with an enzyme capable of producing a colorimetric signal when brought together with a substrate. The results were analyzed by measuring the absorbance at 450 nm (iEMS Reader MF, Labsystems, Finland).

2.6. Identification of S-layer carrying lactobacilli

For preliminary species-specific identification, bacterial strains were subjected to API50 CHL (bioMérieux, France) assay. The samples were tested with the API strips according to the manufacture's instructions and the results were read after 24 h and 48 h of incubation at 37°C under aerobic conditions. The species identifications obtained from the biochemical profiles were confirmed with identification software (bioMérieux, France).

The 16S rRNA gene sequence of the selected isolates was amplified by PCR using 16S rDNA 3' (ACGGGCGGTGTGTAC) and 5' (AGAGTTTGATNNTGGCTCAG) universal primers and directly sequenced using the Microseq, 16S rDNA bacterial Identification Gene kit (PE Biosystems, USA) according to the manufacture's instructions. Sequencing was performed with an ABI 310 DNA sequencer (PE Biosystems, USA). The closest known relatives of the isolates were determined by performing database searches. The GenBank/EMBL accession numbers of the sequences obtained in this study are EF120367-120376.

2.7. Adhesion assays

The Intestine 407 cell line for adhesion tests was obtained from the American Type Culture Collection (ATCC). Adhesion assays were performed as described before (Hynönen et al., 2002). The Intestine 407 cell monolayers were prepared by inoculating eight-well microscope slides (Knittel, Germany) with 25 µl of a freshly diluted (in growth medium) cell suspension, incubating them for 1 h at 37°C, and then covering the slides with growth medium and further incubating them for 2 days at 37°C. The slides were washed once with phosphate-buffered saline (PBS) before the adhesion assays. The overnight broth cultures of lactobacilli were centrifuged at 3000 *g*, resuspended in PBS (pH 7.3) and diluted to a concentration of 2 x 10^8/ ml cells using PBS. The slides were covered with the bacterial suspension and incubated for 1 h at 37°C. After incubation, the mixtures were washed three times with sterile PBS. The slides were then fixed with methanol, stained with Giemsa stain (Merck, Germany), and the number of bacteria adhering to epithelial cells was estimated using light microscopy.

The in vitro test for adhesion to porcine intestinal cells was performed as described previously by Mäyrä-Mäkinen et al. (1983) with slight modifications. Segments of duodenum, jejunum and ileum were opened, held in PBS at 4°C for 30 min in order to loosen the surface mucus, and washed three times with PBS. The enterocytes of the small intestine were scraped off gently with a spoon and the scrapings were suspended in PBS. All cell suspensions were pre-examined microscopically to ensure that adhered commensal bacteria were removed. The cells were diluted to a concentration of approximately 5 x 10^6 cells /ml. The enterocyte suspension was used within 1 h for binding studies. The overnight broth cultures of lactobacilli were centrifuged at 3000 *g*, resuspended in PBS and diluted to a concentration of 1 x 10^9 cells /ml using PBS. The suspensions of lactobacilli (0.5 ml) and enterocytes (0.5 ml) were mixed and incubated with shaking at 37°C for 60 min. After incubation, the mixtures were centrifuged and washed three times with sterile PBS. The pellet was resuspended to half of the initial volume. The slides were then fixed with methanol, stained with Giemsa stain (Merck, Germany), and the number of bacteria adhering to an epithelial cell was estimated using light microscopy.

Bacterial adherence to individual proteins of the mammalian ECM was tested on glass slides essentially as described earlier (Antikainen et al., 2002; Åvall-Jääskeläinen et al., 2003), with the following modifications. The bacteria were tested at a concentration of 5 x 10^8 cells per ml. The surface concentration of human type IV collagen (Sigma, USA), mouse laminin (Upstate Biotechnology Inc., USA) and human fibronectin (Collaborative Biomedical Products, USA) used was 1.25 pmol per well and the slides were blocked with 1% (wt/vol) blocking reagent (Roche) in PBS and washed twice with 0.25% blocking reagent in PBS and once with PBS. The time of incubation with bacteria in PBS was 2 h, and nonadherent bacteria were removed by washing 3 x 10 min in PBS. The slides were then fixed, stained with methyleneblue, and the number of bacteria adhering to different ECM proteins was calculated using light microscopy.

The statistical significance of the adherence capabilities of the bacterial cells was evaluated by one-way analysis of variance, and pairwise differences between the means of groups were determined by the Tukey HSD test for post-analysis of variance pairwise comparisons (available at http://faculty.vassar.edu/lowry /VassarStats.html). Differences were considered significant when *P* values were less than 0.01.

2.8. Extraction of S-layer proteins

The putative S-layer proteins were extracted from the bacterial strains with 2 M guanidine hydrochloride (GHCl) as described earlier (Åvall-Jääskeläinen et al., 2003). Cells from 5 ml of MRS broth were collected, washed, and then incubated for 2 h at 37°C in 1.8 ml of 2 M GHCl. When GHCl-treated cells were used for adhesion tests, fresh cells were treated with GHCl, collected by centrifugation followed by washing with PBS three times. The treated cells were resuspended in PBS and used for the adhesion assay as described earlier. The GHCl extracts were filtered through an 0.2 µm filter and exhaustively dialysed against distilled water. The dialysed extracts were centrifuged (16 000 g, 20 minutes, 4°C) and the precipitates were suspended in Laemmli sample buffer. The removal of the putative S-layer protein from cells and the precipitates formed during dialysis were analyzed in SDS-PAGE.

3. Results

3.1. Identification of Lactobacillus species

Ninety nine pure cultures with the ability to grow on *Lactobacillus* MRS medium were isolated from the pig intestine and faeces and identified at the genus level in this study by using a PCR-ELISA assay developed and verified earlier for the analysis of the genus *Lactobacillus* (Laitinen et al., 2002). To distinguish between a *Lactobacillus* negative and positive identity, a cut-off value of >0.3 was used in the PCR-ELISA, resulting in the verification of 91 isolates of the 99 tested as strains belonging to the genus *Lactobacillus*.

3.2. Screening for the presence of S-layer proteins and their extraction with GHCl

The presence of a putative S-layer on the bacterial cell surface can be deduced from the occurrence of a dominant protein band in the protein profile of non-lysed bacteria. The *Lactobacillus* isolates of this study were analysed in SDS-PAGE. Dominant protein bands of 45-62 kDa were present in 8 of the 99 isolates, suggesting the presence of S-layer proteins in these strains (Fig.1.). The putative S-layer carrying strains were extracted with 2 M GHCl to remove the putative S-layer protein. In 6 out of 8 strains this resulted in effective removal of the S-layer protein (Fig.1.g).In strain LAB2, removal was only partial but, as this strain showed no affinity to fibronectin and negative response to laminin (Fig.5.), no harsher extraction conditions were applied. In strain LAB31, there appears to be two S-layer protein bands in SDS-PAGE. The GHCl extraction partially removed the upper band and the lower band completely (Fig.1). When the GHCl extracts were dialysed, a white precipitate was formed containing the putative S-layer proteins as expected (Fig.1.d).

3.3. Isolation of S-layer protein genes

To confirm that the eight putative Slp carrying strains indeed contain S-layer protein genes (*slp*), a PCR analysis was performed. Based on the 16S rRNA analysis, universal primers for the *L. acidophilus* A-type *slp* genes were chosen. With these primers (Usl-1 and Usl-2) a PCR product of

the expected size (~1.2 kb) was obtained from each strain (Fig.2). These PCR products were partially sequenced to reveal homology in the conserved C-terminal domain encoding part in this type of S-layer protein genes. A BLAST-P analysis of a conserved C-terminal fragment indicated that the eight isolated strains contain genes homologous to the *slp* genes of *L. gallinarum* and *L. crispatus* (Fig.3).

3.4. Presence of L. brevis slpA homologs

To test whether the genomes of the 99 isolates shared DNA identity with the *slp* gene of *L. brevis* ATCC 8287, chromosomal DNA was isolated from the 99 strains followed by hybridisation with an *L. brevis slpA* probe of 1.397 kb. Only one of the isolates (LAB87) gave a positive hybridisation signal, suggesting the presence of an *slpA* homolog in this strain, whereas no detectable hybridization signals were obtained between *slpA* and the other isolates.

3.5. Identification of the S-layer protein positive isolates

All the 8 strains expressing a putative S-layer structure and the one strain with a positive hybridisation signal with the *slpA* probe were further identified by using phenotypic and molecular taxonomic methods. The fermentation profile obtained by API 50 CHL test strips for the nine isolates led to their classification as *L. acidophilus, L. crispatus, Lactobacillus fermentum* and *Lactobacillus delbrueckii* subsp*. delbrueckii*. To ascertain the phylogenetic relationships of the strains, the16S rRNA gene sequence of representative isolates were determined (app. 1500 bp) and aligned. The identification results are listed in Table 2. Seven of the isolates, clustered with *L. sobrius* and *L. amylovorus*, were 100% identical with each other and shared 99 - 100% identity with *L. sobrius* DSM 16698^T (AY700063) *and L. amylovorus* DSM 20531^T (AY944408). The isolates identified as *L. crispatus* and *L. mucosae* shared 99% identity with *L. crispatus* AF257097 and *L. mucosae* AF126738, respectively.

3.6. Adherence of the putative S-layer carrying Lactobacillus strains to human epithelial cells, porcine enterocytes and ECM proteins

Adhesiveness of the putative S-layer-expressing strains and S-layer negative strain LAB87 to intestinal tissues was investigated. Microscopic examinations showed that these strains adhered from strongly to moderately to human and porcine small intestine enterocytes (Table 2.). Adhesive strains were attached on the epithelial cells, whereas non-adhesive strains had a uniform distribution around the cells (Fig.4.). To investigate the role of the putative S-layer in the ECM binding, adhesion of S-layer-expressing and S-layer-depleted bacteria to human fibronectin, human laminin, and mouse type IV collagen were also examined. Strong adhesion to fibronectin was observed in the presence of the S-layer proteins in *L. brevis* strain, *L. crispatus* -like strain and five out of the seven *L. amylovorus - L. sobrius* -like strains, whereas the removal of Slps with GHCl from the cell surface abolished the adhesiveness of these bacteria (Fig.5). Adhesion to laminin was observed in seven S-layer carrying lactobacilli strains (Fig.5). Interestingly, the adhesiveness to laminin increased in three strains after the GHCl treatment (Fig.5). With exception to strain LAB16, no adherence to type IV collagen was observed irrespective of the GHCl treatment. In strain LAB16, the collagen binding was also independent of the GHCL treatment, indicating that the adhesion was not mediated by the putative S-layer. The strain LAB87, showing no presence of S-layer subunits but hybridising with the *L. brevis slpA*, did not exhibit any detectable adhesiveness in the binding tests performed.

Discussion

Surface (S-) layers are monomolecular crystalline arrays identified in several *Lactobacillus* species. In some of these bacteria, S-layers have been shown to function as adhesins mediating the binding of *Lactobacillus* cells to the host's epithelial cells and/or ECM. Adhesive properties of S-layers to matrix components have also been linked to protective functions against invasiveness of pathogenic bacteria as well as to the probiotic properties of health-benefiting bacteria (reviewed in Åvall-Jääskeläinen and Palva, 2005). Due to these observed adhesive properties, the possible therapeutic applications of S-layers have gained increasing interest e.g. as targeted live antigen delivery vehicles

to host tissues (Seegers, 2002). It has already been demonstrated that S-layer protein subunits can be modified to carry foreign epitopes as a uniform recombinant S-layer on the *Lactobacillus* cell surface (Åvall-Jääskeläinen et al., 2002; Smit et al., 2002).

In this study, our aim was to isolate and identify porcine-specific S-layer carrying strains for later use as vaccine vectors and/ or probiotics. *Lactobacillus* strains, carrying a putative S-layer or an *L. brevis slpA* homolog, were isolated from the intestine and faeces of pigs using biochemical and genetic screening followed by further characterization of their ability to adhere to porcine and human intestinal cells and to major ECM proteins.

From the 99 isolates tested, 8% of the strains were found to produce S-layer proteins. Due to the lack of prior data, it cannot be evaluated whether the observed 8% frequency is high or low in the porcine intestine. It is, however, interesting that seven out of the eight S-layer carrying *Lactobacillus* strains were finally identified as *L. amylovorus* / *L. sobrius* -like species from all the five pigs and only one *L. cripatus* -like phylotype was found. Moreover, no *L. acidophilus* strains, known to exhibit an S-layer structure and shown to be one of the dominant *Lactobacillus* species in pigs in earlier studies (Pryde et al., 1999; Konstantinov et al., 2006), were found in this study. Our result suggests that either *L. amylovorus* is the dominant S-layer carrying *Lactobacillus* in pigs or this finding may just reflect the effect of feeding constituents on the enrichment of a certain species.

Due to our research interests in *L. brevis* S-layers, we also screened the isolates directly for *L. brevis slpA* –homologs by hybridization, but no *L. brevis* -like strains could be isolated. The only strain found, with no apparent S-layer protein, was identified as *L. mucosae,* which is not phylogenetically related to *L. brevis*. Thus, one may speculate that the observed homology is not linked to a silent *slp* gene but rather to conserved cell wall binding domains of wall-associated proteins.

Lactobacillus isolates with S-layers have been found to adhere to the intestinal epithelial cell lines derived from their mammalian hosts, intestinal or gastric mucus, and ECM components. Our finding was that the putative S-layer-protein-expressing cells of porcine isolates adhered very efficiently to pig enterocytes. It was significant that some porcine isolates also adhered to human cells with a high

affinity and to ECM preparations from different hosts, such as mouse and human, indicating that the adhesiveness of lactobacilli is not strictly host-specific. Furthermore, it was surprising that the *L amylovorus* isolates exhibited significant binding variability to ECM components. This variability is also reflected by differences in the SDS-extractable protein pattern, which was found to be characteristic for each strain. PCR isolation of S-layer protein genes suggests the presence of more than one *slp* gene, at least in some of the strains. With the possible exception of the strain LAB31, only one putative Slp could be found in SDS-PAGE in each strain. Recent characterization of *slps* from *L.gallinarum* indicates the presence of two *slps* of which only one is expressed in each strain (Hagen et al., 2005). The expressed genes show high sequence variation, thus allowing different strains of the same species to share the same habitat by different adherence targets of the encoded Slps (Hagen et al, 2005). Full sequence analysis of the isolated *L. amylovorus slp* -genes will reveal whether the encoded Slps show high heterogeneity.

Extraction with guanidine hydrochloride (GHCl), as used in this study, due to the lack of isogenic null mutants, is a routine procedure to remove S-layer proteins and did not cause detectable lysis of cells. However, it is obvious that some other cell wall proteins or components, critical to the binding activity, may be simultaneously removed. In addition to peptidoglycan, the rigid cell wall of lactobacilli is composed of secondary cell wall polymers (SCWP) such as teichoic acid, lipoteichoic acids, lipoglycans or neutral or acidic glygans. The adhesion of *L. brevis* ATCC 8287 and some of our porcine *Lactobacillus* isolates to fibronectin and laminin was clearly reduced after the GHCl treatment, whereas some of the isolates possessed better adhesiveness to laminin after abolishment of the S-layer structure. Thus, it is clear that surface structures other than the S-layer protein mediate adhesion in such strains, e.g. lipoteichoic acid has been shown to contribute to the adhesion process. The poor adhesiveness of S-layer-depleted ATCC 8287 cells and some porcine isolates to fibronectin and laminin, however, is in accordance with the observation that S-layer subunits indeed are adhesion proteins and one of the key adhesion factors of the cell.

In conclusion, it is likely that the amount of S-layer carrying porcine lactobacilli is relatively low and that *Lactobacillus* strains carrying putative S-layers, even among isolates representing the same or

closely related homology groups, show different adhesion affinity to various host tissues. The S-layer structures of the isolated *Lactobacillus* strains will be further characterized to assess their potential as antigen surface display platforms.

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FIGURE LEGENDS

Fig.1.

SDS-PAGE of the cell protein content of eight different strains: numbers on the lanes refer to the strains LAB2, LAB7, LAB8, LAB13, LAB16, LAB31, LAB32 and LAB52 before and after (g) treatment of bacterial cells with 2 M GHCl and dialyzed S-protein precipitates (d). The molecular masses on the marker proteins (lane M) are given on the right-hand side.

Fig.2. PCR detection of S-protein genes; Numbers refer to the respective LAB strain. M, molecular weight marker λ Pst1.

Fig.3.

Multiple sequence alignment of deduced amino acid sequences of the LAB strains studied. Part of the SlgB *L. gallinarum* sequence (NCBI identification number AAT99074) and of the *L. crispatus* CsbA sequence (NCBI identification number AAB58734) were used in comparision. Strain numbers are given on the left, and residue numbers on the top. Asterisks indicate identical residues, colons indicate strongly similar residues and dots indicate the weakly similar residues.

Fig.4. Adhering (A) and non-adhering (B) porcine lactobacilli.

Fig.5. Adherence of *Lactobacillus brevis* GRL1 (ATCC 8287) and porcine *Lactobacillus* strains to fibronectin, laminin and type IV collagen is shown before and after (dark) removal of S-layer protein by guanidine hydrochloride (GHCl). The mean numbers and standard deviation of bacteria on 20 randomly chosen microscopic fields are shown. Significantly higher or lower than depletion of S-layer at $P < 0.01$ (*)

Fig.1.

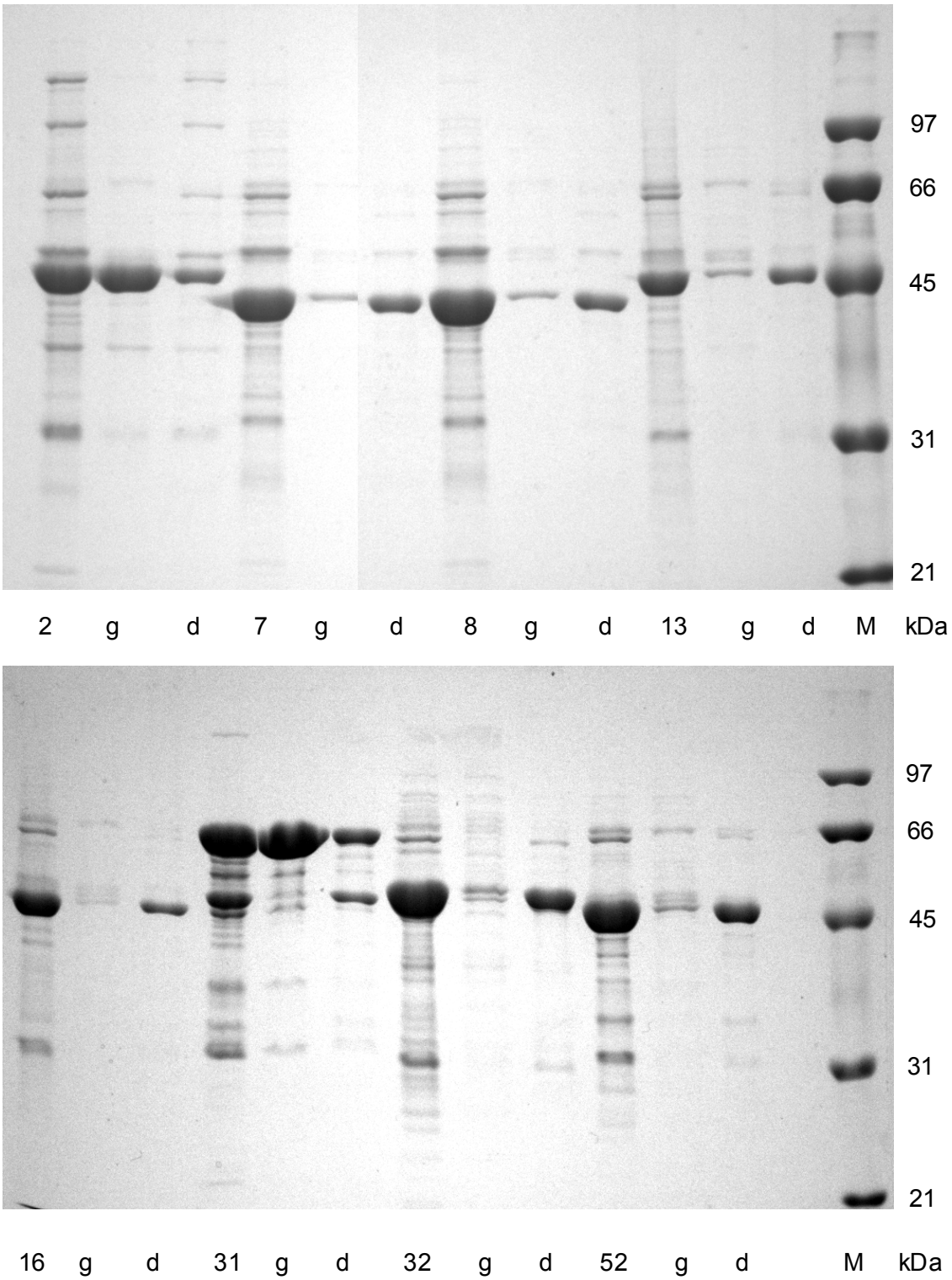


Fig.2.

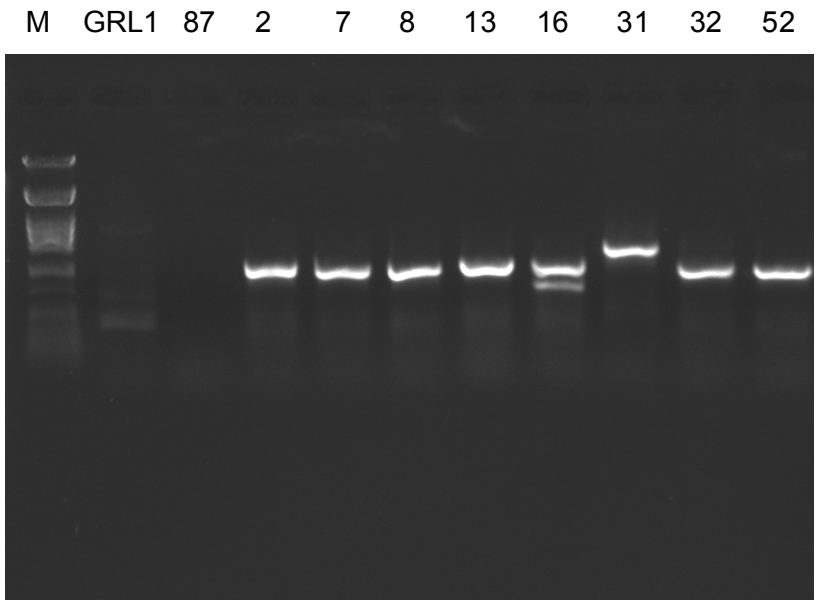


Fig.3.

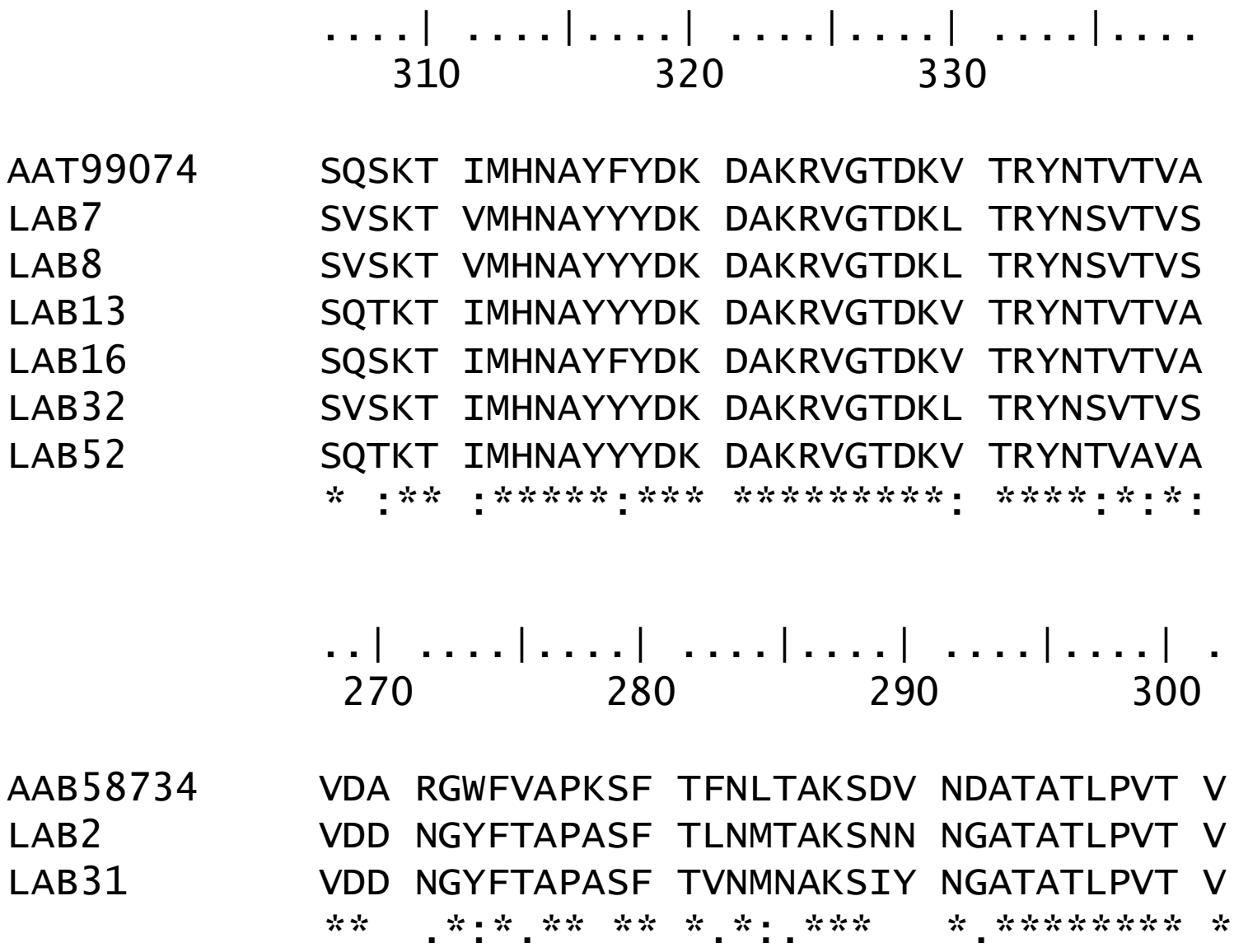


Fig.4.

A

B

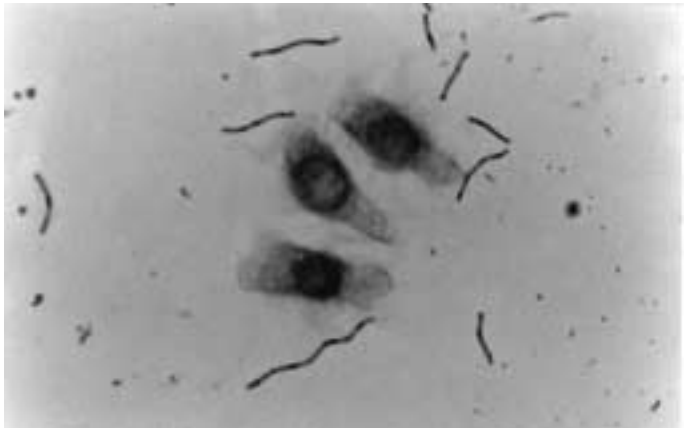
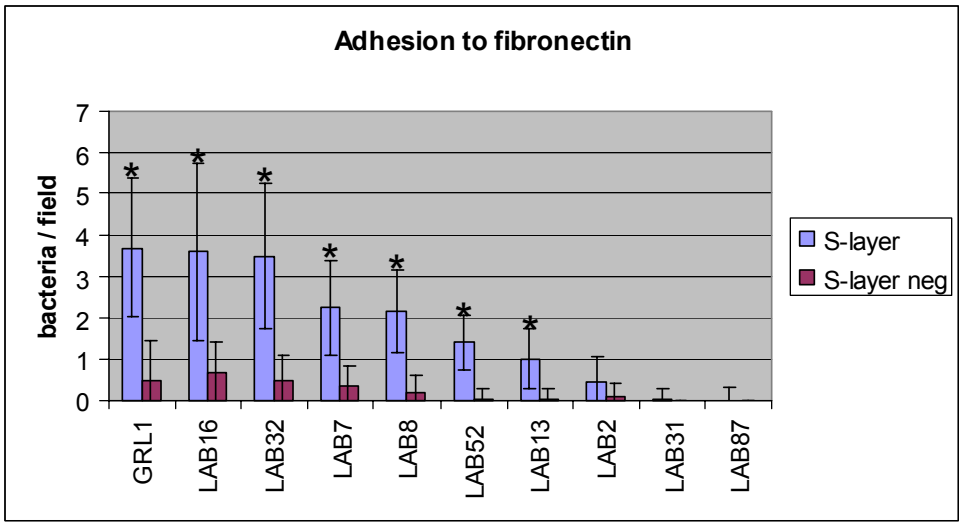
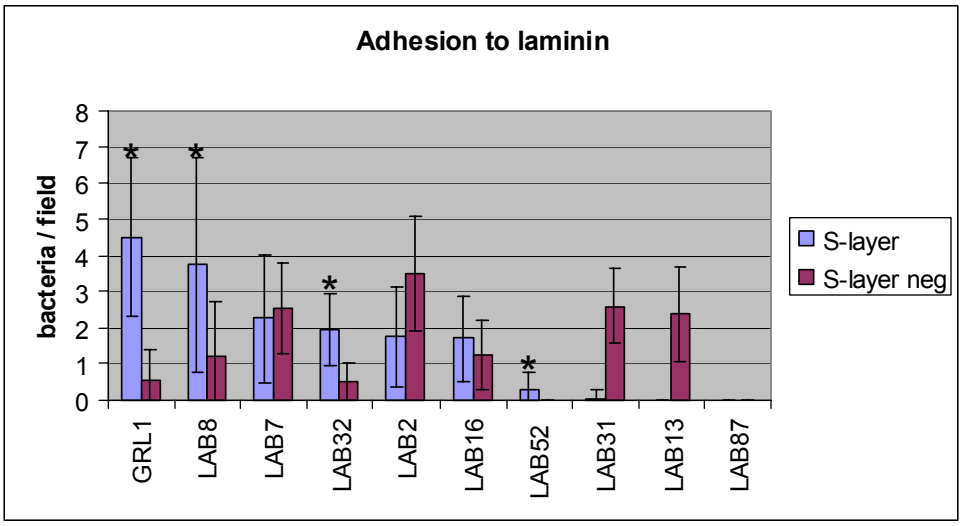


Fig.5.





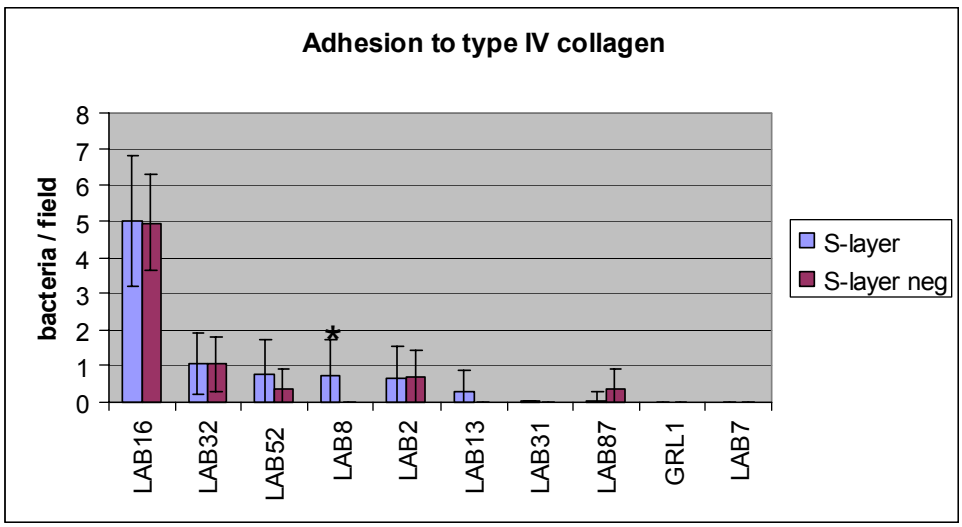


Table 1.

List of strains used
The table lists the sources, isolated medium used and results ofthe cluster analyses of SDS-PAGE patterns (see also Fig.1.)

Strains	Source	Medium	Swine	SDS-PAGE (kDa)
LAB2	porcine faeces	MRS	sow 1 (Lyyli)	47
LAB7	porcine faeces	MRS	sow 2 (Laima)	45
LAB8	porcine faeces	MRS	sow 2 (Laima)	45
LAB13	ileum	MRS	1	47
LAB16	jejunum	MRS	1	50
LAB31	jejunum	SL	2	55 + 62
LAB32	jejunum	SL	2	55
LAB52	ileum	MRS	3	47
LAB87	ileum	SL	4	none

Table 2.

Identification of *Lactobacillus* species isolated from pig intestine and faeces and adhesion of these bacteria to human and porcine epithelial cells of the intestine in vitro

Strain	16S rRNA sequencing	In vitro adhesion[a] Henle 407	Pig
ATCC 8287	*not done*	+++	+
LAB2	*L. sobrius / L. amylovorus*	+++	++
LAB7	*L. sobrius / L. amylovorus*	+++	++
LAB8	*L. sobrius / L. amylovorus*	+++	++
LAB13	*L. sobrius / L. amylovorus*	+	+++
LAB16	*L. sobrius / L. amylovorus*	+++	++
LAB31	*L. sobrius / L. amylovorus*	+++	++
LAB32	*L. crispatus*	++	++
LAB52	*L. sobrius / L. amylovorus*	+	+++
LAB87	*L. mucosae*	++	Not done

[a] The number of bacteria attached per epithel cells. Score: +++, >100; ++, 10 to 100; +, 1to 10; -,0. Details are as described in Materials and Methods.