Mucosa-Adherent Lactobacilli: Commensal and Pathogenic Characteristics

Sanna Edelman

General Microbiology
Faculty of Biosciences
and
Viikki Graduate School in Biosciences
University of Helsinki

Academic Dissertation in General Microbiology

To be presented, with the permission of the Faculty of Biosciences,
Department of Biological and Environmental Sciences,
University of Helsinki,
for public criticism in the Auditorium 1041, Viikinkaari 5
on the 1st of April, at 12 o´clock noon.

Helsinki 2005

Supervisor Professor Timo K. Korhonen, PhD

General Microbiology
Department of Biological and Environmental Sciences
University of Helsinki

Reviewers Docent Pentti Kuusela, MD, PhD

Department of Bacteriology and Immunology
Haartman Institute
University of Helsinki
and
Division of Clinical Microbiology
Helsinki University Central Hospital, Helsinki, Finland

Professor Airi Palva, PhD

Division of Microbiology and Epidemiology
Department of Basic Veterinary Sciences
University of Helsinki

Cover figure: Immuno-EM image of the subcellular localization of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and enolase on *Lactobacillus crispatus.*

ISSN 1239-9469
ISBN 952-10-2345-7 (paperpack)
ISBN 952-10-2346-5 (pdf)
http://ethesis.helsinki.fi

Yliopistopaino, Helsinki 2005

TO HARRY AND MARTIN

CONTENTS

LIST OF ORIGINAL ARTICLES

This thesis is based on the following published articles and manuscripts, which in the text are referred to by their roman numerals. The articles I and II are reprinted by the kind permission from the publishers.

I **Edelman, S., Westerlund-Wikström, B., Leskelä, S., Kettunen, H., Rautonen, N., Apajalahti, J. and Korhonen, T.K.** 2002 In vitro adhesion specificity of indigenous lactobacilli within the avian intestinal tract. *Appl Environ Microbiol* **68**: 5155-5159

II **Edelman, S., Leskelä, S., Ron, E., Apajalahti, J. and Korhonen, T.K.** 2003 In vitro adhesion of an avian pathogenic *Escherichia coli* O78 strain to surfaces of the chicken intestinal tract and to ileal mucus. *Vet Microbiol* **91**:41-56

III **Edelman, S., Pirilä, R., Apajalahti, J. and Korhonen T.K.** Identification of LEA-1, a *Lactobacillus* adhesin with affinity for stratified squamous epithelium. Manuscript

IV **Edelman, S., Kuparinen, V., Lähteenmäki, K., Baumann, M. and Korhonen T.K.** Interaction of lactobacilli with the mammalian plasminogen system. Manuscript

ABBREVIATIONS

AC/I	avian *Escherichia coli* I fimbriae
APEC	avian pathogenic *Escherichia coli*
ATCC	American type culture collection
α2AP	alpha-2 antiplasmin
BM	basement membrane
EACA	ε-aminocaproic acid
ECM	extracellular matrix
ERIC	enterobacterial repetitive intergenic consensus sequences
FAE	follicle-associated epithelium
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GAS	group A streptococcus
GI	gastrointestinal
GRAS	generally regarded as safe
MALDI-TOF	Matrix-Assisted Laser Desorption Ionization - Time of Flight mass spectroscopy
M-cells	membranous epithelial cells
MMP	matrix metalloprotease
MRS	de Man, Rogosa and Sharpe broth
LBS	*Lactobacillus* selection agar
Lpf	long polar fimbriae
PA	plasminogen activator
PAI	plasminogen activator inhibitor
PAM	plasminogen-binding group A streptococcal M protein
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
Plg	plasminogen
PlgR	plasminogen receptor
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
tPA	tissue-type plasminogen activator
uPA	urokinase

SUMMARY

Lactobacilli belong to the commensal gastrointestinal and urogenital microbiota of man and animals and are proposed to endow the host with several beneficial health effects. Adhesion is considered important for bacterial colonization at the host mucosal surfaces as well as for bacteria-host interactions both in health and disease. In this thesis work, the *in vitro* tissue tropism in adhesiveness of lactobacilli to the mucosal structures of the alimentary tract of the host and the underlying molecular bases of the adhesion were studied. To characterize the potential health effects of the adhesion of lactobacilli, the ability of lactobacilli to prevent *in vitro* adhesion of bacterial pathogens was tested. In addition, the ability of lactobacilli to interact with the mammalian plasminogen system, known to be harnessed by several invasive bacterial pathogens, was demonstrated.

Lactobacillus ssp. isolated from the avian alimentary tract exhibited strain-specific cell and tissue tropism in their *in vitro* adhesion to chicken intestinal tissue structures. In general, adhesion to crop epithelium was a common trait in well-colonizing strains. *Lactobacillus crispatus* strain ST1, characterized as a potential colonizer, showed a strong adhesion to the epithelial areas of crop as well as small and large intestine. Notably, ST1 also adhered efficiently to the immunologically important follicle-associated epithelium in ileum.

The avian pathogenic *E. coli* (APEC) and *Salmonella* strains adhered via their type-1 fimbriae essentially to the same tissue areas in the chicken intestinal mucosa as did the adhesive lactobacilli. The strongly adhesive *L. crispatus* ST1 efficiently inhibited the adhesion of APEC at the crop and the follicle-associated epithelia, whereas the poorly adhesive *L. crispatus* 134mi inhibited the adhesion of pathogens only partially.

Adhesive surface proteins from *L. crispatus* ST1 were isolated by mutanolysin treatment. A 39-kDa peptide bound to singular intraepithelial and lamina propria cells of chicken intestine and was identified as glyceraldehyde-3-phosphate dehydrogenase (GAPDH) by N-terminal amino acid sequenzing and protein

database analysis. The polypeptides with apparent molecular sizes of 50 and 55 kDa bound to basement membrane areas in chicken tissue. Their N-terminal amino acid sequences showed no close similarity to those in databases. A high molecular weight protein (280 kDa) that efficiently bound to the stratum corneum of stratified squamous epithelium of chicken crop and to the epithelial cells of human vagina was named as *Lactobacillus* epithelium adhesin-1 (LEA-1). To our knowledge LEA-1 is the first stratified squamous epithelial adhesin identified on lactobacilli.

Surface-expressed GAPDH is known as a plasmin(ogen)-binding protein on pathogenic streptococci. Intestinal, dairy and probiotic strains of *Lactobacillus* and *Lactococcus* enhanced the tissue-type plasminogen activator (tPA)-catalyzed plasminogen (Plg) activation. The activation was efficiently inhibited by a lysine analog, ε-aminocaproic acid, suggesting that lysine residues have a role in the interaction. The activation was also efficiently inhibited by the main plasmin inhibitor, α2-antiplasmin, as well as by serine protease inhibitor, aprotinin. Binding of Plg and plasmin on the surface of lactobacilli was low, and *L. crispatus* ST1 was not able to retain the bacterium-bound plasmin activity on cell surface. The Plg molecule was further modified in the presence of *L. crispatus* and *Lactobacillus johnsonii* cells into internal plasminogen fragments which included angiostatin known to suppress endothelial cell proliferation and tumor metastasis. The glycolytic enzymes, GAPDH and enolase, were indentified as plasminogen receptors on *L. crispatus* ST1. The genes encoding GAPDH and enolase were cloned and sequenzed and expressed in *Escherichia coli*. The purified recombinant proteins bound plasminogen and plasmin in a lysine inhibitable manner. This is the first report on active plasminogen receptors on commensal bacteria and indicates that bacteria-Plg interaction may have a function for indigenous microbiota of humans.

1. INTRODUCTION

The members of the genus *Lactobacillus* are Gram-positive organisms that belong to the large and heterogenous group of aerotolerant anaerobic Lactic Acid Bacteria (LAB). They form lactic acid as the major end product of their carbohydrate fermentation and possess low DNA base composition (32-53% G+C) (Axelsson, 1998). More than 50 species of *Lactobacillus* are recognized and divided into three metabolic groups: obligate homofermentative, facultative heterofermentative and obligate heterofermentative, based on differences in sugar metabolism caused by the presence or absence of fructose-1,6-diphosphate aldolase and phosphoketolase (Axelsson, 1998). Homofermentative bacteria produce lactic acid, and heterofermentative ones produce lactic acid, CO_2 and ethanol and/or acetic acid, as main fermentation end products. Based on DNA-DNA-hybridization studies, the large *Lactobacillus acidophilus* group is divided into six homology groups, A1-B2, that correspond to previously assigned species, *Lactobacillus acidophilus* (A1), *Lactobacillus crispatus* (A2), *Lactobacillus amylovorus* (A3), *Lactobacillus gallinarum* (A4), *Lactobacillus gasseri* (B1) and *Lactobacillus johnsonii* (B2) (Johnson *et al*., 1980; Lauer *et al*., 1980; Fujisawa *et al*., 1992). Recent EcoRI ribotyping data further suggests that the group should be devided into 14 genotypes, A1-A11, B1-B3, and gives evidence that some of the previously identified *Lactobacillus* strains would require reclassification as different species (Ryo *et al*., 2001). LAB are frequent in the intestinal and urogenital tracts of man and animals and are also widely distributed in nature, in plant materials and in sewage. The interaction of LAB with food stuffs caught the enthusiastic attention of early bacteriologists and led to identification of lactic acid fermentation by Louis Pasteur in 1857 and isolation of *Bacterium lactis* by Lister in 1873. The use of starter cultures for cheese and sour milk production was introduced at those days by Weigmann in Kiel and by Stoch in Copenhagen. Fermentative LAB have been used in food products for centuries, well before the scientific basis of the bacterial fermentation processes became established. The importance of LAB as industrial microbes today is next only to that of the common baker´s yeast, *Saccharomyces cerevisiae*. The interest in LAB as health-promoting organisms dates back to the early 20th-century, when Elie Metchnikoff suggested that consumption of LAB

contributes to the prolonging of life by modifying the intestinal microbiota. The production and consumption of health-promoting probiotics for human and animal use have widely increased during the last decades, and the molecular mechanisms of LAB interactions with the host both in health and disease represent a growing field in bacterial research today.

1.1. LACTOBACILLI IN THE NORMAL MICROBIOTA

Lactobacilli constitute an important part of the normal indigenous alimentary and urogenital tract microbiota of man and animals. The indigenous microbiota is a natural resistance factor against potential pathogenic microorganisms and provides colonization resistance by promoting gut maturation and integrity, occupying available niches, producing autogenic regulation factors (e.g. organic acids, hydrogen peroxide and bacteriocins), and modulating non-specific immune systems of the host to maintain intestinal immune homeostasis (Havenaar and Huis in´t Veld, 1992; Schiffrin and Blum, 2002). Several reports indicate that imbalanced normal microbiota (e.g. due to disease or use of broad spectrum antibiotics) is associated with an abnormally high presence of microbial species, such as *Escherichia coli*, *Salmonella*, streptococci, *Bacteroides*, *Gardnerella* and yeasts, often associated with infective diseases, as well as with reduced levels of lactobacilli (Mitsuoka, 1992; Sarra *et al*., 1992; Hay, 2004; Reid and Bocking, 2003).

Former methods to identify bacterial species in the normal microbiota were based on bacterial cultivation, and it has been suggested that only 20-40 % of the bacteria from intestinal samples have been characterized, due to lack of knowledge of proper culture conditions (Suau *et al*., 1999). Indeed, the diversity of intestinal microbiota is tremendous, and 10^{13}-10^{14} bacteria of different species and subspecies are estimated to colonize the human alimentary canal (Hao and Lee, 2004). The new culture-independent techniques, such as pulse field gel electrophoresis (PFGE), ribotyping, 16S rDNA restriction fragment length polymorphism (RFLP), multiplex polymerase chain reaction (PCR), arbitrary primed (AP) PCR or triplet arbitrary primed (TAP) PCR (O'Sullivan, 2000), provide

more specific methods for detailed investigations at the species and the strain levels and are valuable tools for ecological *Lactobacillus* studies as well.

1.1.1. ALIMENTARY TRACT AS AN ECOLOGICAL NICHE FOR LACTOBACILLI

Lactobacilli belong to the normal microbiota of human oral cavity and human gastrointestinal (GI) tract (Mitsuoka, 1992; Lidbeck and Nord, 1993; Vaughan *et al*., 2002). The most frequent *Lactobacillus* species colonizing the human alimentary canal are *L. acidophilus, L. crispatus*, *L. brevis, L. casei, L. delbrueckii, L. fermentum, L. gasseri*, *L. johnsonii, L. paracasei, L. plantarum, L. rhamnosus, L. reuteri, L. ruminis, L. sakei*, *L salivarius*, *L. vaginalis*, *L. curvatus* and *L. fructovorans* (Mitsuoka, 1999; Vaughan *et al*., 2002). Several factors, such as pH, peristalsis, redox potential, bacterial adhesion, bacteria-bacteria interactions, mucus and bile secretion, immunoglobulins, intestinal enzymes, exfoliated epithelial cells, nutrient availability, diet, as well as bacterial antagonism, affect the prevalence of bacteria in different parts of the GI tract (Holzapfer *et al*., 1998; Tannock, 1999; Hao and Lee, 2004). In general, the alimentary bacterial numbers are highly dependent on intestinal pH and peristalsis. In the neutral pH of oral cavity, bacteria reach high numbers (up to 10^9 per ml of fluids). In the stomach and the upper two-thirds of the small intestine (e.g. duodenum and jejunum) only 10^3-10^5 bacteria per g of contents are present, probably because of the acidic conditions of stomach and the fast peristaltic movement in the upper parts of the alimentary canal. In the distal small intestine, ileum and the large intestine, the pH rises and the peristaltic movement decreases, and the bacterial numbers are higher again. The colon is the largest bacterial reservoir in the body and contains up to 10^{12} bacteria per g of contents (Mitsuoka, 1992; Hao and Lee, 2004). The acid-tolerant lactobacilli are one of the dominating species in the oral cavity, stomach and duodenum and jejunum, with 10^3-10^7 bacteria per g of contents (Mitsuoka, 1992; Lidbeck and Nord, 1993). In the colon, lactobacilli are frequent (10^7 bacteria per g of contests) but not the major bacterial species (Mitsuoka, 1992).

The presence and composition lactobacilli in the microbiota of the GI tract of mammalian animals closely resemble those found in humans, although some variations at the species level occur depending on the host (Mitsuoka, 1992; Tannock, 1992). Also, the anatomical differences of the alimentary canals influence the microbiota: the non-secreting stratified squamous epithelia in the fore-stomach of pigs, mice, rats and horses are efficiently colonized by lactobacilli (Tannock, 1992; Yuki et al., 2000). In the fowl intestinal tract, the dominating *Lactobacillus* species are *L. crispatus, L. gallinarum, L. johnsonii, L. salivarius* and *L. reuteri* (Fuller and Turvey, 1971; Mitsuoka, 1992; Sarra et al., 1992; Guan et al., 2003; Lan et al., 2002). Lactobacilli efficiently colonize the stratified squamous epithelium lining the crop, which functions as a food storage pouch in the middle of esophagus. Due to their fermentation, the colonizing lactobacilli secrete acid and lower the pH of crop to 4.5-6 (Sarra et al., 1992).

For successful colonization, intestinal bacteria, including lactobacilli, have been suggested to resist the peristaltic movement by adhering to intestinal epithelia and/or mucus, particularly in the upper parts of the alimentary canal (Fuller, 1989; Tannock, 1992, Rojas and Conway, 1996). The topic has remained controversial since there is also evidence that *in vitro* adhesion ability of lactobacilli to epithelia is not a prerequisite for *in vivo* colonization in an animal model (Pedersen and Tannock, 1989). Most probably, successful colonization by intestinal bacteria is dependent on several bacterial factors, and adhesiveness can be one of them.

1.1.2. LACTOBACILLI IN UROGENITAL TRACT

Lactobacilli are the dominating bacteria (10^7 to 10^8 cells per g of fluids) in vagina of healthy premenopausal females (Redondo-Lopez *et al*., 1990; Paavonen, 1983). The most prevalent species in vagina are *L. crispatus*, *L. gasseri*, *L. acidophilus*, *L. jensenii*, and *L. iners* (Song *et al*., 1999; Vásquez *et al*., 2002; Silvester and Dicks, 2003; Zhou *et al*., 2004). Microbial colonization is affected by glycogen content, pH and hormone levels and/or treatments (Galask, 1988). The antagonism of lactobacilli against urogenital infections, e.g. yeast vaginitis, bacterial vaginosis or urinary tract infections, has been demonstrated to depend on production of antimicrobial compounds such as H_2O_2 and bacteriocins, on low pH and on high

redox potential. Also, adherence of lactobacilli to and colonization at the stratified squamous epithelium of vagina has a protective role against pathogens (McLean and Rosenstein, 2000; Barbés and Boris, 1999; Pabich *et al*., 2003; St. Amant *et al*., 2002; Marelli *et al*., 2004).

1.2 LACTOBACILLI AS PROBIOTICS

The findings that colonization by lactobacilli and other lactic acid bacteria, e.g. bifidobacteria, improves infection resistance of the host, have let to the production and consumption of probiotics. Probiotics are live microbial cultures or cultured dairy products which beneficially influence the health and nutrition of the host (Salminen, 1996). To date, several health-promoting effects of probiotics have been proposed (Table 1, for reviews, see Reid and Burton, 2002; Sanders, 2003; Mercenier *et al*., 2003; Vaarala, 2003; Tuohy *et al*., 2003). However, the mechanisms underlying the health effects and the host-probiotic communication in prophylactic and/or therapeutic treatments have remained poorly characterized. The probiotic strains are expected to fulfil several health-promoting characteristics and safety criteria (Table 2).

Table 1. Proposed beneficial effects of probiotics (Modified from Klaenhammer, 2001; Mercenier *et al*., 2003 and Sanders, 2003)

Alleviation of lactose intolerance
Prevention of gastric, intestinal and urogenital infections
Modulation of intestinal immune system
Reduction of inflammatory or allergenic reactions
Anticarcinogenic effects
Antihypertensive effects
Reduction of serum cholesterol level
Regulation of gut motility

Table 2. Expected characteristics and safety criteria of probiotics (Mercenier *et al*., 2003)

Non toxic and non pathogenic
Accurate taxonomic identification
Normal inhabitant of the targeted species
Capability to survive, proliferate and be metabolically active in the targeted site, which implies:
resistance to gastric juice and bile
ability to persist in the GI tract
ability to adhere
ability to compete with the resident microbiota
Production of antimicrobial substances
Antagonism towards pathogenic bacteria
Ability to modulate immune responses
Ability to exert at least one clinically documented health benefit
Genetically stable
Amenability of the strain and stability of the desired characteristics during processing, storage and delivery
Viability at high populations
Desirable organoleptic and technological properties when included in industrial prosesses

1.3 LACTOBACILLI AS OPPORTUNISTIC PATHOGENS

Lactobacilli are Generally Regarded As Safe (GRAS) and non-pathogenic (Adams and Marteau, 1995). Occasionally, isolates of *Lactobacillus* are associated with opportunistic infectious diseases in humans, such as infective endocarditis (IE), bacteremia, urinary tract infections, dental caries, chorioamnionitis, endometritis, meningitis, deep abscesses and empyema (Husni *et al*., 1997; Aguirre and Collins, 1993; Brouqui and Raoult, 2001). The *Lactobacillus*-associated infections have often been polymicrobial, and no direct indication of a primary role for lactobacilli in the infection has been found. The portal of entry for lactobacilli into circulation has remained unresolved in most cases; it has been suggested that the bacteria from the microbiota of the oral cavity or the GI tract can be introduced into the blood circulation as a result of poor dental hygiene, dental manipulation, gastrointestinal lesions or surgery (Husni *et al*., 1997; Aguirre and Collins, 1993). Also, sepsis has been reported to induce translocation of indigenous intestinal bacteria from intestine to underlying host tissues in mouse model (Naaber *et al*., 2000). However, the molecular mechanisms that contribute to the opportunistic

pathogenicity of commensal lactobacilli have remained unknown. The wide-spread ability of lactobacilli to aggregate platelets coupled with their ability to bind fibrinogen, fibronectin and collagen, in particular type V collagen demonstrated at the sites of endothelial damage (Kerényi *et al*., 1985), as well as their proteolytic activities are so far the only suggested pathogenic factors of lactobacilli (Harty *et al*., 1993; Harty *et al*., 1994; Oakey *et al*., 1995). These characteristics are common in all *Lactobacillus* strains and their role in lactobacilli-associated infectious diseases remains unclear. In most cases the infections associated with lactobacilli have preceded predisposing microbial infections (Husni *et al*., 1997; Aguirre and Collins, 1993; Salminen *et al*., 2004), and the pathogenic potential of lactobacilli in a healthy host is considered very low, particularly in a view of the ubiquitous presence of these bacteria in the hosts and the environment (Adams and Marteau, 1995).

2. ADHESION PROTEINS OF LACTOBACILLI

2.1 ADHERENCE TO CELLS AND TISSUE COMPONENTS

Lactobacilli have been frequently observed to bind to epithelial cells and dissected tissue samples of the alimentary canal from humans and animals (Conway and Adams, 1989; Henriksson *et al*., 1991; Yuki *et al*., 2000; Sarem-Damerdji *et al*., 1995; Fuller, 1973 and 1978; Jin *et al*., 1996; Mäyra-Mäkinen *et al*., 1983; Kotarsky and Savage, 1979; Conway *et al*., 1987), to human vaginal epithelial cells (Andreu *et al*., 1995; Osset *et al*., 2001; McLean and Rosenstein, 2000; Redondo-Lopez *et al*., 1990), to intestinal mucus (Rojas and Conway, 1996; Matsumura *et al*., 1999; Kirjavainen *et al*., 1998 and 1999; Tuomola *et al*., 1999; Roos *et al*., 2000; Roos and Jonsson, 2002; Gusils *et al*., 2003), to cultured human carcinomal intestinal cell lines (Adlerberth *et al*., 1996; Kirjavainen *et al*., 1999; Granato *et al*., 1999; Todoroki *et al*., 2001) and to the components of the extracellular matrix (ECM) (Aleljung *et al*., 1991; Nagy *et al*., 1992, Harty *et al*., 1994; Toba *et al*., 1995; McGrady *et al*., 1995; Styriak *et al*., 2003). The reports on the adherence of lactobacilli are numerous, but detailed knowledge of the adhesion mechanisms is very limited. Species-specificity in the adherence of

lactobacilli has also been suggested (Fuller, 1973; Mäyrä-Mäkinen *et al*., 1983; Yuki *et al*., 2000), but the topic has remained controversial, since intestinal and environmental lactobacilli adhere to non-host tissue targets as well (Kotarsky and Savage, 1979; Lin and Savage, 1984; Conway *et al*., 1987; Jacobsen *et al*., 1999; Todoroki *et al*., 2001; Sarem-Damerdji *et al*., 1995).

2.2 ADHESINS

The reduced adhesiveness of lactobacilli treated with proteinases has led to the hypothesis that proteinaceous molecules mediate the adhesion of lactobacilli in the host intestine (Fuller, 1975; Henriksson *et al*., 1991; Reid *et al*., 1993; Greene and Klaenhammer, 1994). The involvement of carbohydrates and lipoteichoic acids in the adherence of lactobacilli to intestinal and genital epithelia has also been reported (Fuller, 1975; Adlerberth *et al*., 1996; Henriksson *et al*., 1991; Coconnier *et al*., 1992; Greene and Klaenhammer, 1994; Ahrné *et al*., 1998; Boris *et al*., 1998; Granato *et al*., 1999; Neeser *et al*., 2000), but the adhesive structures have not been identified. Overall, the various results suggest that lactobacilli adhere to host tissues via mechanisms that vary in different species.

Few adhesins of lactobacilli have been characterized at the molecular level. These include the collagen binding CnBP of *L. reuteri* (Aleljung *et al*., 1994; Roos *et al*., 1996), the collagen and laminin-binding CbsA of *L. crispatus* (Sillanpää *et al*., 2000; Antikainen *et al*., 2002), fibronectin binding SlpA of *L. brevis* (Hynönen *et al*., 2002), and the pig and hen mucus-binding Mub of *L. reuteri* (Roos and Jonsson, 2002). CnBP is a 29 kDa-sized surface protein that is encoded in an ABC transporter operon in *L. reuteri.* The native CnBP and recombinant CnBP produced in *E. coli* bind solubilized type I collagen (Roos *et al*., 1996). From *L. reuteri,* another collagen binding protein with 31 kDa molecular mass has been purified. The protein showed immunocrossreactivity with CnBP, but the N-terminal peptide sequences of these proteins were not related (Roos *et al*., 1996). CbsA is a 43-kDa S-layer protein, which constitutes the major cellular protein and consists of single subunit which forms a regular crystalline array surrounding the cell (Toba *et al*., 1995). CbsA carries adhesive sequences for collagen type I and IV as well as laminin in its N-terminal domain and binds to bacterial cell wall by its C-terminal

domain (Antikainen *et al*., 2002). SlpA is also a S-layer protein that binds via its N-terminal domain to immobilized fibronectin and cultivated human intestinal cell lines possible via fibronectin bridging mechanism (Hynönen *et al*., 2002). Mub has a high molecular weigh (358 kDa) and contains 14 approximately 200 amino acid-long sequences and regions typical for other cell surface proteins in Gram positive bacteria, such as an N-terminal secretion signal peptide, a cell wall anchoring motif (LPXTG), a putative membrane-spanning region and a cell-membrane anchor. It was also detected by immunogical methods in the growth medium, suggesting secretion and/or release of the protein from bacterial surface (Roos and Jonsson, 2002).

Further, a 29-kDa surface protein of *L. fermentum* that binds to pig small intestinal mucus and gastric mucins has been identified. The N-terminal amino acid sequence of this protein shows no similarity to peptide sequences in databases (Rojas *et al*., 2002) and further investigations are needed to characterize the adhesin. In preliminary studies, *L. acidophilus* was observed to express a 15-kDa protein that binds to fibronectin and 45-kDa and 58-kDa proteins that bind to collagen type I (Lorca *et al*., 2002).

3. AVIAN PATHOGENIC *E. coli* (APEC) AND SALMONELLA

Avian colisepticaemia caused by virulent strains of *E. coli* O1, O2 or O78 serotypes, is an important cause of morbidity and mortality in poultry (Dho-Moulin and Fairbrother, 1999). Poultry is also a major source of food-born infections in humans, particularly in cases of enterocolitis caused by pathogenic salmonella (Humphries *et al*., 2001; Jordan Lin *et al*., 1997). *E. coli* and *Salmonella* ssp. are present in the normal microbiota of the lower intestinal tract of birds with 10^3-10^7 bacteria per gram of contents (Dho-Moulin and Fairbrother, 1999; Fedorka-Cray *et al*., 2001).

Adherence of pathogenic bacteria to host tissues has been considered to be a crucial first step in the infection process (Finlay and Cossard, 1997; Finlay and Falkow, 1997; Klemm and Schembri, 2000). The avian colisepticaemia begins by

bacterial adherence and invasion at the upper respiratory tract and later develops into systemic infection (Dho-Moulin and Fairbrother, 1999). Adherence and subsequent invasion through intestinal epithelium also play a critical role during diseases caused by *Salmonella* serovars (Humphries *et al*., 2001). Overall, the bacterial adherence to and persistence on intestinal mucosa offers an intestinal reservoir for shedding and horizontal transmission of bacteria via environment. This may influence the frequency of infections in chickens (Cerquett and Gherardi, 2000; Carlson and Whenham, 1968).

3.1 FIMBRIAL ADHESINS

E. coli and salmonella frequently express fimbriae, thin, proteinaceous, polymeric surface appendages that mediate binding to host tissues structures (Stordeur *et al*., 2002; Humphries *et al*., 2001). In APEC, the most frequent and important fimbria are type-1, AC/I and P fimbriae as well as curli (Janben *et al*., 2001; Stordeur *et al*., 2002; Yerushalmi *et al*., 1990). According to genome analysis, *Salmonella enterica* serovars have 14 putative fimbrial operons in their genome; only few of them have been characterized (Townsend *et al*., 2001). This indicates that our understanding of fimbrial functions in salmonellosis is inadequate at the present. Also, flagella, the bacterial motility elements, have shown to play a role in bacterial adhesion to mucus-protected epithelia *in vitro* and invasion *in vivo* (Allen-Vercoe and Woodward, 1999a; La Ragione *et al*., 2000 and 2003).

The type-1 fimbriae that binds to oligomannoside chains of glycoproteins (Neser *et al*., 1986), have been observed to be more often present on APEC than on non-pathogenic strains isolated from chickens (Dozois *et al*., 1992; Wooley *et al*., 1992; Janben *et al*., 2001) In *in vitro* and *in vivo* studies, the type-1 fimbriae have been shown to mediate *E. coli* adherence to avian pharyngeal and tracheal enterocytes (Naveh *et al*., 1984; Dho and Lafont, 1984; Vidotto *et al*., 1997) and the fimbriated strains are also better colonizers of chicken trachea as well as intestine (Dho and Lafont, 1982; Marc *et al*., 1998). The adherence of *E. coli* to mucosal enterocytes of human oral cavity was also shown to be mannose sensitive (Ofek *et al*., 1977). In salmonella, the type-1 fimbriae have been observed to be important in adhesion to rat, mouse and pig small intestinal enterocytes as well as to HeLa and Hep-2

cell lines (Lindquist *et al*., 1987; Thankavel *et al*., 1999; Hancox *et al*., 1998; Althouse *et al*., 2003) In chickens, D-mannose abolished the adhesion of *S. enterica* serovar Typhimurium to intestinal enterocytes *in vitro,* and the mannose-fed chickens were significantly less colonized by *S. enterica* serovar Typhimurium *in vivo* (Oyofo *et al*., 1989 a and b). These results indicate that type-1 fimbrial receptor epitopes most likely are present in the host intestinal mucosa and contribute to bacterial adhesion and colonization, however the role of type-1 fimbriae in the infectious diseases is still unclear (Marc *et al*., 1998; Dho-Moulin and Fairbrother, 1999; La Ragione and Woodward, 2002).

AC/I fimbriae are expressed on APEC O78 serotypes with prevalence of 50 % (Babai *et al*., 1997 and 2000) and belong to the S- fimbriae family, although they do not exhibit the sialyl oligosaccharide-sensitive hemagglutination mediated by S-fimbriae (Yerushalmi *et al*., 1990). AC/I have been shown to mediate *E. coli* adhesion to chicken tracheal and intestinal epithelial cells (Yerushalmi *et al*., 1990), but its role in the virulence is not known. The P fimbriae are expressed *in vivo* in the later stages of APEC infections (Pourbakhsh *et al*., 1997 a and b) and do not the mediate *in vitro* adhesion to primary infections sites, i.e. trachea or pharynx (van den Bosch *et al*., 1993; Vidotto *et al*., 1997; Dozois *et al*., 1994). These results suggest that P fimbriae do not have role in the early stages of APEC pathogenicity in chickens. The specific binding of curli to ECM and serum proteins (Olsen *et al*., 1989) may contribute to bacterial adherence and colonization in the initial stages of infection, and in bacterial internalization into human epithelial cells (Gophna *et al*., 2001) although, the overall role of curli in the pathogenesis of APEC has not yet been elucidated (La Ragione and Woodward, 2002).

One of the characterized mucosal adhesins of salmonella associated with intestinal infections is the long polar fimbria (Lpf) which mediates specific adherence to murine intestinal membranous epithelial cells (M-cells) (Bäumler *et al*., 1996). The M-cells are specialized epithelial cells that occur in the lymphoid follicle associated epithelia (FAE) and by transepithelial transport deliver luminal samples to the underlying lymphoid tissues aggregated in ileum (Peyer´s patches) and scattered in other parts of small and large intestine (Sieber and Finlay, 1996; Gebert *et al*., 1996). Following transport through M cells, salmonella are

phagocytosed by resident macrophages in which they are able to multiply and are subsequently disseminated to secondary infection sites (Jepson and Clark, 2001; Santos and Bäumler, 2004). The specific receptor epitope for salmonella Lpf is not known and its role in the virulence has remained unclear since oral admistration of Lpf deficient salmonella show only limited reduction in their virulence in mice (Bäumler *et al*., 1996).

3.2 EXCLUSION OF PATHOGENIC BACTERIA BY LACTOBACILLI

One of the physiological effects of lactobacilli in the host has proposed to be the antagonism against pathogens. Lactobacilli have been shown to affect the prevalence of several intestinal and urogenital pathogens *in vitro* and *in vivo* (Reid *et al*., 1988; Coconnier *et al*., 1998; Boris *et al*., 1998; Jin *et al*., 1996; Pascual *et al*., 1999; Mack *et al*., 1999; McLean *et al*., 2000; Osset *et al*., 2001; La Ragione *et al*., 2004). The detailed molecular mechanisms underlying this phenomenon remain poorly characterized, and the reports vary in degrees of successful inhibition depending on the strains used as competitive exclusion agents, the pathogens as well as the methods of assessment.

The importance of adhesion in pathogen exclusion at chicken ileal epithelia has been shown *in vitro* with *L. acidophilus* against *Salmonella pullorum* (Jin *et al*., 1996) but *L. acidophilus* failed to exclude *Salmonella enterica* serovar Enteritidis and *Salmonella enterica* serovar Typhimurium and APEC (Jin *et al*., 1996 and 1998). At human vaginal epithelia, adherent *Lactobacillus* ssp. diminished adhesion of *Candida albicans*, bacterial vaginosis associated species and uropathogens (Boris *et al*., 1998; McLean *et al*., 2000; Ossest *et al*., 2001). Also, exclusion of *E. coli* and salmonalla by lactobacilli in human and piglet mucus have been reported (Blomberg *et al*., 1993; Lee *et al*., 2003), but lactobacilli failed to inhibit the adhesion of *Salmonella* ssp. to chicken mucus (Gusils *et al*., 2003). *L. crispatus* and its collagen binding S-layer protein inhibit the adherence of *E. coli* to the components of basement membrane (Horie *et al*., 2002) and a 29-kDa surface protein from *L. fermentum* inhibited adhesion of uropathogenic *Enterococcus faecalis* to polystyrene (Heinemann *et al*., 2000).

4. BACTERIAL INTERACTION WITH THE PLASMINOGEN SYSTEM

4.1 THE HUMAN PLASMINOGEN SYSTEM

Plasminogen (Plg) is a proenzyme of the serine protease plasmin, which is involved in several physiological and pathological processes such as fibrinolysis, degradation of the extracellular matrix (ECM), eukaryotic cell migration, tissue remodelling, embryonic development, inflammation and tumor metastasis (Mignatti and Rifkin, 1993; Lijnen and Collen, 1995; Plow *et al*., 1999; Boyle and Lottenberg, 1997; Berger, 2002; Oh *et al*., 2003, Myöhänen and Vaheri, 2004). Plg circulates at high concentrations in human blood and is abundant in other body fluids, such as saliva, urine and breast milk as well (Chen *et al*., 1980; Moody, 1982; Lijnen and Collen, 1995; Heegaard *et al*., 1997). Plasmin is important for cell migration as it directly degrades laminin, the major glycoprotein in basement membranes, and indirectly enhances tissue damage by activating latent matrix metalloproteases (MMPs) which are capable of degrading collagens and other constituents of ECM (Zucker and Vacirca, 2004). The basement membranes form important tissue barriers and also offer a milieu where components of the Plg system are present and can be activated (Lähteenmäki *et al*., 2001), thus the proteolytic activity of plasmin is efficiently targeted to these physiological barrier structures. Notably, the metastatic tumor cells have shown to adhere to the components of ECM as well as bind and activate Plg (Schwartz, 1996; Berger, 2002), which subsequently enables focal tissue degradation and remodelling.

Plasmin(ogen) binds to fibrin, components of ECM and to lysine-containing receptors that are broadly distributed on mammalian cells. Plg receptors (PlgRs) include proteins with C-terminal lysines, e.g. α-enolase, proteins rich in internal lysines, e.g. amphoterin, and non-protein receptors, e.g. gangliosides and glycosaminoglycans (Plow *et al*., 1995). Binding of Plg to lysine-containing receptors leads to conformational changes which render it more susceptible to cleavage by Plg activators (PAs)(Mangel *et al*., 1990). Mammalians have two PAs, the tissue-type plasminogen activator (tPA) and urokinase (uPA) (Rijken, 1995), which cleave a peptide bond at Arg561-Val562 of the Plg molecule. The resulting heavy (65 kDa) and light (25 kDa) chains of plasmin are held together via two

disulphide bridges (Miyashita *et al*., 1988) (Fig. 1). The active site in the light chain of plasmin possesses typical characteristics of serine proteases, the catalytic triad of amino acids His603, Asp 646 and Ser741, while the heavy chain is composed of five kringle domains (K1-K5) that contain the lysine binding sites. The intact 90-kDa Plg (Glu-Plg) contains an amino-terminal glutamic acid and is cleaved by serine proteases into an 82-kDa N-terminally truncated form of the zymogen Plg (Lys-Plg). The formation of Lys-Plg enables stronger interaction of Plg with its receptor molecules and enhances the activation of Plg into plasmin (Miyashita *et al*., 1988; Gong *et al*., 2001).

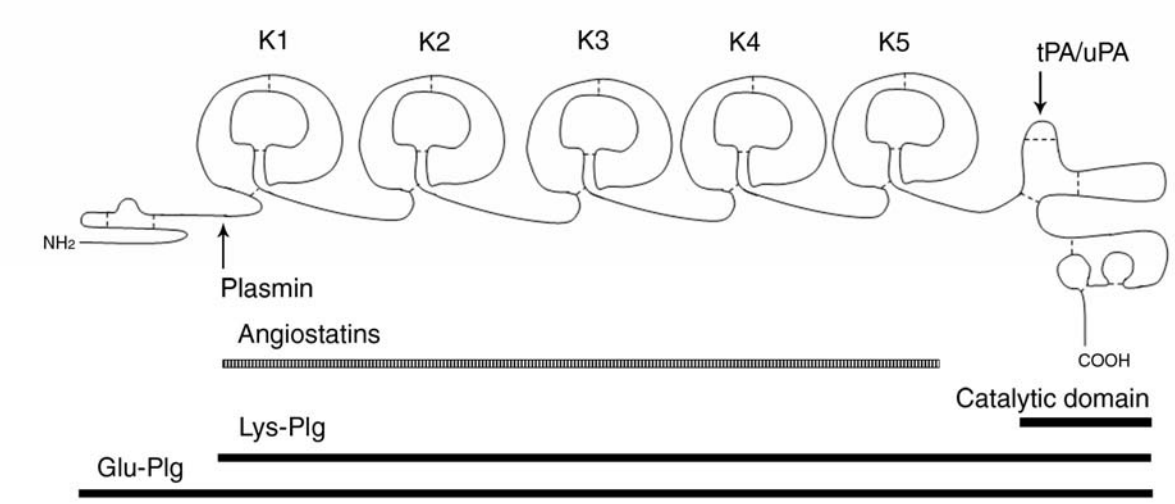

Fig. 1. Schematic structure of human plaminogen. The five kringle domains that contain the lysine-binding sites are marked as K1, K2, K3, K4, and K5. The molecule is stabilized by disulphide bridges (dashed lines). Arrows indicate the cleavage sites for plasminogen activators (tPA/uPA) and plasmin. The solid bars indicate the molecular domains that are present in Glu-Plg, Lys-Plg and in the catalytic chain. The striped bar indicates the kringle domains that consist of the varying combinations of the reported angiostatin molecules, i.e. K1, K4, K5, K2-K3, K1-K3, K1-K4, and K1-K5. Modified from Miyashita *et al*., 1988; Cao and Xue, 2004.

Due to the highly potent proteolytic activity of plasmin, the Plg system is tightly regulated at the levels of produced Plg activators and plasmin activity. The PAs and their inhibitors, plasminogen activator inhibitors (PAIs), are secreted by vascular endothelium and other cell types in response to various physiological and pathological stimuli (Lijnen and Collen, 1995; Mignatti and Rifkin, 1993; Berger, 2002). α2-antiplasmin (α2AP) is the main physiological inhibitor of plasmin, and α2-macroglobulin inhibits plasmin along other proteases (Travis and Salvesen, 1982). Since the α2AP binds to the same lysine-binding sites of Plg as do the

receptors, the receptor-bound Plg is protected from the inhibitor. On the contrary, free circulating plasmin is rapidly inactivated by the antiprotease.

The proteolytic cleavage of Plg can further lead to formation of internal Plg fragments, collectively called as angiostatins, which lack the catalytic light chain and vary in the number of kringle domains (Cao, 1999; Cao and Xue, 2004) (Fig. 1). The internal Plg fragments inhibit the endothelial cell proliferation and act as natural regulators of physiological and pathological angiogenesis (O´Reilly *et al*., 1994; Cao, 1999; Cao and Xue 2004). The Plg proteolysis into internal fragments has been shown with variety of physiological proteases, such as members of the matrix metalloprotease (MMP) family and elastase (Dong *et al*., 1997; Patterson and Sang, 1997; Cao and Xue, 2004). Reductases secreted by tumor cells (Stathakis *et al*., 1997 and 1999; Lay *et al*., 2000) generate angiostatin from plasmin and also, tPA, uPA and the streptococcal PA streptokinase cleave Plg to angiostatin in the presence of free sulfhydryl donors (Gately *et al*., 1997). Also, autoproteolytic conversion of membrane-associated plasmin into angiostatin on tumor cells has been reported (Wang *et al*., 2004). Fragmentation of the Plg into angiostatin has been characterized with fungi-derived nonlysine triprenyl phenol metabolites (Ohyama *et al*., 2004), but bacterial cells have not been reported to modulate Plg fragmentation.

4.2 BACTERIAL INTERVENTION WITH THE PLASMINOGEN SYSTEM

Invasive bacterial pathogens intervene with the Plg system by expressing PAs and/or PlgRs (Fig. 2) which enables the pathogens to gain surface-bound proteolytic activity (Lähteenmäki *et al*., 2005). Also, the production levels of mammalian PAs, uPA-receptor (uPAR), PAIs and MMPs have been reported to be affected by bacteria and/or bacteria-derived components, such as endotoxin (Suffredini *et al*., 1989; Brandtzaeg *et al*., 1990; Behera *et al*., 2005). The central role of bacteria-Plg interaction in bacterial invasiveness has been so far documented *in vivo* in infections caused by *Yersinia pestis*, *Borrelia* species, *Streptococcus pneumoniae* and group A streptococci (GAS) (Sodeinde *et al*., 1992; Gebbia *et al*., 1999; Nordstrand *et al*., 2001; Sun *et al*., 2004; Bergmann *et al*., 2003). These studies have highlighted the importance of synergistic action of

PlgRs and PAs in bacterial invasion from intravenous primary infection site into circulation or secondary infection sites. A considerably higher number of pathogenic bacteria has been found to express PlgRs (Lähteenmäki *et al*., 2001 and 2005). Many *in vitro* studies have demonstrated that the bacterial capture of the host-derived proteolytic plasmin activity enables bacterial invasiveness through tissue structures and cell layers (Coleman and Benach, 1999) indicating that utilization of the Plg system by bacteria can be more general. The combined attributes of bacterial adherence to components of the extracellular matrix (Westerlund and Korhonen, 1993) and engagement of the plasminogen system, mimic the mechanisms in metastasis of tumor cells (Mignatti and Rifkin, 1993; Schwartz, 1996; Berger, 2002).

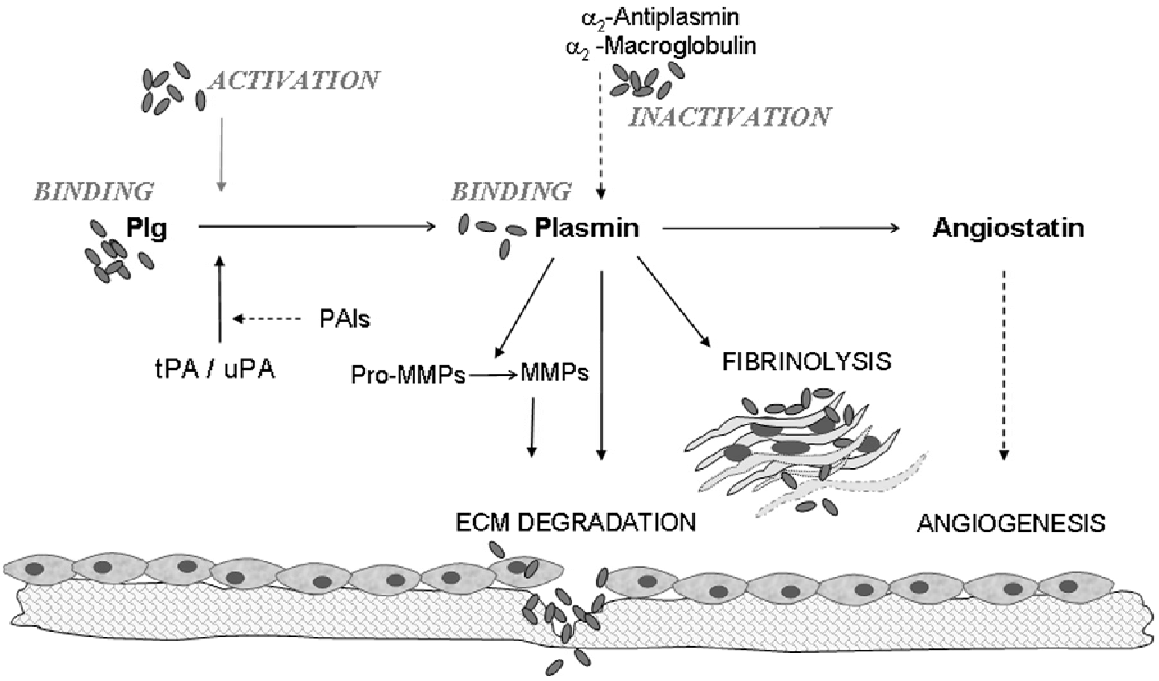

Fig. 2. Schematic overview of the bacterial intervenience with the plasminogen system. The proenzyme plasminogen (Plg) is activated by tissue-type plasminogen activator (tPA) or urokinase (uPA). The active plasmin degrades fibrin clots (fibrinolysis), laminin of basement membrane and activates precursors of matrix metalloproteases (Pro-MMPs) which in active form can further degrade components of basement membranes and extracellular matrix (ECM). Plasmin can be also proteolytically cleaved into angiostatin. Dashed lines indicate inhibition: plasminogen activator inhibitors (PAIs) inhibit the plasminogen activators, α2-antiplasmin and α_2-macroglobulin inhibit plasmin and angiostatin inhibits endothelial cell proliferation and angiogenesis. Bacteria intervene (shown in *italics*) with the system by binding Plg, plasmin and tPA on their surface, which enhances the activation of Plg by tPA and protects plasmin from the main inhibitor, α2-antiplasmin. Few bacterial species are able to activate Plg or inactivate antiproteases. The host-derived proteolysis can be targeted on bacteria to degradation of fibrin clots and by bacterial adherence to basement membranes and ECM for focused invasion though tissue barriers. Modified from Lähteenmäki *et al*., 2005; Berger, 2002.

4.2.1. BACTERIAL PLASMINOGEN RECEPTORS

The best characterized PlgRs in Gram-positive bacteria are the plasminogen-binding group A streptococcal M protein (PAM) as well as streptococcal glycolytic enzymes, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and α-enolase (Wistedt *et al*., 1995; Winram and Lottenberg, 1998; Pancholi and Fischetti, 1998). In Gram negative bacterial species, such as *E. coli* and salmonella, the filamentous surface appendages, fimbriae and flagella, act as PlgR molecules (Parkkinen and Korhonen, 1989; Lähteenmäki *et al*, 1993; Kukkonen *et al*., 1998; Sjöbring *et al*., 1994). Also, aspartase in *Haemophilus influenzae*, outer surface protein A (OspA) and a 70-kDa surface protein OppA in *Borrelia burgdorferi* as well as the surface proteins PgbA and PgbB in *Helicobacter pylori* have been characterized as PlgRs (Sjöström *et al*., 1997; Fuchs *et al*., 1994; Hu *et al*., 1997; Jönssön *et al*., 2004).

PAM is associated with GAS strains that cause skin infections in humans (Svensson *et al*., 1999) and binds Plg via lysine residues present in two N-terminally located repeat regions of 13 amino acids. Inactivation of the PAM-encoding *emm53* gene abolishes the Plg binding *in vitro* and leads to attenuated infection in an experimental animal model of infection *in vivo* (Svensson *et al*., 2002; Sun *et al*., 2004).

Streptococcal GAPDH, also called as streptococcal surface hydrogenase (SDH), has been shown to be surface localized and to bind human Plg and plasmin (Pancholi and Fischetti, 1992; Lottenberg *et al*., 1992; Winram and Lottenberg, 1998; Bergmann *et al*., 2004). The C-terminal lysine residue of GAPDH seems to be important in the Plg binding, since substitution of this amino acid to leucine abolished the binding (Winram and Lottenberg, 1998). However, finding that the recombinant bacteria with mutated GAPDH still retained the Plg-binding ability, emphasizes the importance of other PlgRs in streptococci (Winram and Lottenberg, 1998; Jobin *et al*., 2004). The GAPDH protein has also been observed to possess multifunctional activity, such as binding to fibronectin, lysozyme, cytoskeletal proteins as well as displaying auto-ribosylating and phosphorylating

activities (Pancholi and Fischetti, 1992 and 1993), but the *in vivo* role of these multiple characteristics are not known.

The surface-localized α-enolase is reported to be the main PlgR in streptococci and binds Plg and plasmin in a lysine dependent manner (Pancholi and Fischetti, 1998; Bergmann *et al*., 2001). Similar to GAPDH, α-enolase binds with greater affinity to Lys-Plg and plasmin than to Glu-Plg (Winram and Lottenberg, 1998; Derbise *et al*., 2004). The C-terminal lysine residues have been shown to be important in the PlgR function of GAS and pneumococci (Pancholi and Fischetti, 1998; Bergmann *et al*., 2001; Derbise *et al*., 2004), although recent report of the 3D structure of pneumococcal α-enolase suggests that the internal binding epitope ^{248}FYDKERKVY^{256} (Bergmann *et al*., 2003), provides the major binding site for Plg (Ehinger *et al*., 2004). Substituting of lysines at this sequence reduces Plg-binding and attenuates the bacterium in a mouse model of intranasal infection (Bergmann *et al*., 2003). The α-enolase performs also several other functions in addition to glycolytic activity and plasmin(ogen) binding, e.g. binding to laminin, salivary mucin and DNA. Enolase is also a heat-shock protein and a constructing component of turtle cellular lenses and playes a role in autoimmunity disorders (Williams *et al*., 1985; Wistow *et al*., 1988; al-Giery and Brewe, 1992; Aaronson *et al*., 1995; Pancholi, 2001; Carneiro *et al*., 2004; Ge *et al*., 2004).

The mechanisms that contribute to surface localization of GAPDH and α-enolase have remained unknown. These house-keeping molecules are traditionally considered as cytoplasmic enzymes and they do not possess known signal sequences, membrane-anchoring motifs or hydrophobic membrane-spanning regions; however, they have been reported to be surface-localized or secreted in several prokaryotic and eukaryotic organisms (Table 3).

Table 3. Surface localization and/or secretion of GAPDH and α-enolase by prokaryotic and eukaryotic cells.

Enzyme	Bacteria	Fungi	Parasites	Mammalian
GAPDH	Group A, B, C, E, G, H and L streptococci[1] *Staphylococcus aureus*[2] *Staphylococcus epidermidis*[2] *Mycobacteria*[3] *Neisseria meningitidis*[4] *Neisseria lactimica*[4] enteropathogenic *E. coli*[5]	*Candida albicans*[10] *Saccharomyces cerevisiae*[11] *Kluyveromyces marxianus*[12]	*Schistosoma mansoni*[15] *Trypanosoma*[16] *Leishmania*[16] *Fasciola hepatica*[17]	erythroid[19]
α-enolase	Group A, B, C, D, E, F, G, H and L streptococci[6] *Staphylococcus aureus*[7] *Aeromonas hydrophila*[8] *Actinobacillus actinomycetemcomitans*[9]	*Candida albicans*[13], *Pneumocystis carinii*[14]	*Onchocerca volvulus*[18] *F. hepatica*[17]	hematopoietic[20] pharyngeal[21] carcinomal[22] endothelial[23] neuronal[24] muscular[25]

(Pancholi and Fischetti, 1992[1]; Modun and Williams, 1999[2]; Bermudez *et al*., 1996[3]; Grifanttini *et al*., 2002[4]; Kenny and Finlay, 1995[5]; Pancholi and Fischetti, 1998[6]; Mölkänen *et al*., 2002[7]; Sha *et al*., 2003[8]; Hara *et al*., 2000[9]; Gil-Navarro *et al*., 1997[10]; Crowe *et al*., 2003[10]; Delgado *et al*., 2001[11]; Fernandes *et al*., 1992[12]; Angiolella *et al*., 1996[13]; Fox and Smulian, 2001[14]; Goudot-Crozel *et al*., 1989[15]; Pancholi and Chhatwal, 2003[16]; Bernal *et al*., 2004[17]; Jolodar *et al*., 2003[18]; Allen *et al*., 1987[19]; Miles *et al*., 1991[20]; Redlitz *et al*., 1995[20]; Pancholi *et al*., 2003[21]; López-Alemany *et al*., 1994[22]; Dudani *et al*., 1993[23]; Nakajima *et al*., 1994[24]; Lopez-Alemany *et al*., 2003[25]).

4.2.2. BACTERIAL PLASMINOGEN ACTIVATORS

Few invasive bacterial species (e.g. streptococci, staphylococci, *Yersinia pestis* and salmonella) have PAs that can be functionally divided into two groups: secreted non-enzymatic activators and surface localized protease activators. Streptokinase (SK) and staphylokinase (SAK) belong to the former group and form molecular complexes with Plg and plasmin which leads to changes in conformation and specificity of Plg (Rabijns *et al*., 1997; Wang *et al*., 1998). SK and SAK share little sequence homology but have a similar protein folding (Parry *et al*., 2000). The binary SAK-Plg complex remains proteolytically inactive and requires additional activation by PAs (Schlott *et al*., 1997). SK activates Plg by forming first a binary complex with Plg and subsequently ternary Plg-SK-Plg complex in which the catalytic triad of Plg is functional without cleavage at Arg561-Val562 (Young *et al*., 1998). The SKs produced by strains of human and non-human origin differ structurally and form ternary complexes with Plg mainly in a host species-specific

manner (Caballero *et al*., 1999). Expression of *ska* gene encoding SK has been shown to be increased in GAS isolated from spleens of infected mice (Rezcallah *et al*., 2004). In a mouse model of human skin impetigo inactivation of *ska* reduced bacterial virulence after subcutaneous infection of conventional as well as transgenic mice expressing human Plg (Svensson *et al*., 2002; Khil *et al*., 2003; Sun *et al*., 2004); thus the synergistic action of bacterial PAs and PlgRs is important in the virulence of GAS (Sun *et al*., 2004). The protection of receptor–associated plasmin from α2AP is observed also *in vitro* in SAK-promoted activation of staphylococci-immobilized Plg (Mölkänen *et al*., 2002). The PauA of *Streptococcus uberis* also is a secreted PA (Leigh, 1994; Leigh and Lincoln, 1997) that has a low sequence similarity but high predicted structural analogy with SK (Rosey, *et al*., 1999; Ward *et al*., 2004) and activates bovine, ovine and equine, but not human Plg via SK-like activation (Rosey, *et al*., 1999; Ward *et al*., 2004). A few *Streptococcus uberis* strains associated with clinical bovine mastitis are also found to secrete a novel broad host spectrum PA, PauB (Ward and Leigh, 2002 and 2004), which activates Plg by unknown mechanism.

The Pla of *Y. pestis* and PgtE of enteropathogenic salmonella belong to the beta-barrel surface proteases of the omptin family (Sodeinde and Goguen, 1988). Pla activates Plg by cleaving at the same Arg561-Val562 site as do tPA and uPA, and thus resemble the mammalian PAs in function (Sodeinde *et al*., 1992). The Plg activation by PgtE seems similar in mechanisms to Pla, but is significantly less efficient (Kukkonen *et al*., 2004). Notably, both proteases are highly capable of inactivating α2AP and thus creating uncontrolled plasmin proteolysis (Kukkonen *et al*., 2001; Lähteenmäki *et al*, 2004). The functions of omptins are strongly influenced by lipopolysaccharide (LPS) on bacteria: the activities of Pla and PgtE are sterically inhibited by O-antigen repeats present in smooth LPS, whereas rough LPS allows full activity (Kukkonen *et al*., 2004). LPS in *Y. pestis* is rough whereas the clinical isolates of salmonella nearly constantly possess smooth LPS (Skurnik *et al*., 2000; Kukkonen *et al*., 2004). The importance of Pla for *Y. pestis* virulence is convincingly demonstrated *in vivo* (Sodeinde *et al*., 1992) and elevated expression of PgtE and alterations of LPS O-chain in salmonella cells from intracellular vacuoles of infected macrophages (Lähteenmäki *et al*., 2004) suggest that PgtE has a role in salmonellosis.

5. AIMS OF THE STUDY

The bacterial adhesion to the epithelium is considered important for colonization as well as for bacteria-host crosstalk at host mucosal surfaces. While the molecular bases of adhesion of bacterial pathogens have been well characterized, much less is known about the surface proteins and adhesion mechanisms of commensal lactobacilli. These bacteria, however, form a major prokaryotic group colonizing the human and animal mucosa, and knowledge on their adhesion mechanisms is important both for basic bacterial ecology and for a more optimal use of lactobacilli as probiotics. Overall, the probiotic as well as opportunistic pathogenic mechanisms of intestinal lactobacilli have remained poorly understood.

This study was initiated to characterize the adhesive properties of a chicken intestinal isolate of *Lactobacillus crispatus* within the alimentary tract of its host and to identify the molecules mediating the adhesion. The effects of lactobacilli on adhesiveness of avian pathogenic *E. coli* to chicken tissues were also studied. As it became evident that commensal lactobacilli possess a surface-expressed glycolytic enzyme, GAPDH, which is known to act as plasminogen receptor on pathogenic streptococci, hence we also studied the interaction of lactobacilli with the human plasminogen system.

6. MATERIALS AND METHODS

Bacterial strains and plasmids used in this study are listed in Table 4. The methods are described in detail in the original published and manuscript articles and are listed in Table 5.

Table 4. Bacterial strains and plasmids used in this study

Bacterial strain or plasmid	Origin / relevant property	Article	Reference
Lactic acid bacteria			
Lactobacillus acidophilus E507	dairy strain	IV	Miettinen *et al.,* 1996
Lactobacillus casei ATCC393	cheese	I	Hansen and Lessel, 1971
Lactobacillus casei E506	dairy strain	IV	Miettinen *et al*., 1996
Lactobacillus crispatus A33	chicken crop	I, III	This study
Lactobacillus crispatus ATCC33820	not known	I, III	Skerman *et al*., 1980
Lactobacillus crispatus ST1	chicken crop	I-IV	This study
Lactobacillus crispatus 134mi	chicken ileum	I, II, III	This study
Lactobacillus gasseri ATCC33323	not known	I	Lauer and Kandler, 1980
Lactobacillus gasseri CT5	chicken crop	I	This study
Lactobacillus johnsonii F133	calf faeces	IV	Fujisawa *et al*., 1992
Lactobacillus reuteri ATCC53609	not known	I	Kandler *et al*., 1980
Lactobacillus reuteri CT7	chicken crop	I	This study
Lactobacillus reuteri 1063	pig small intestine	I	Roos and Jonsson, 2002
Lactobacillus rhamnosus ATCC53103	human faeces	IV	Miettinen *et al.,* 1996
Lactococcus lactis E523	dairy strain	IV	Miettinen *et al.,* 1996
Laboratory host strains			
E. coli 392	ATCC non-fimbriated K-12 strain	II	Sambrook *et al.,* 1989
E. coli M15(pREP4)	Qiagen GmbH	IV	
Pathogenic bacteria			
E. coli 789	chicken septicaemia AC/I and type 1-fimbriated	II	Babai *et al.,* 1997 and 2000
E. coli IHE3034	newborn meningitis	IV	Selander *et al*., 1986
Haemophilus influenzae 23459	clinical isolate	IV	Ullberg *et al*., 1990
Salmonella enterica serovar Typhimurium IR715	chicken heart and liver type-1 -fimbriated	II	Stojiljkovic *et al.,* 1995
Salmonella enterica serovar Enteritidis TN2 (CDCSSU7998)	not known type-1 fimbria negative	II	Norris and Bäumler, 1999 Boyd *et al*., 1993
Streptococcus anginosus 753	infective endocarditis	IV	Allen *et al*., 2002
Streptococcus mutans 7120	infective endocarditis	IV	Allen *et al*., 2002
Streptococcus pyogenes IHE30430	bacteremia	IV	Miettinen *et al*., 1998
Plasmids			
pBR322	Fermentas Life Sciences cloning vector	II	
pMK25	*fim* genes from *S. enterica* in pUC19	II	Kukkonen *et al*., 1998
pPKL4	*fim* genes from *E. coli* in pBR322	II	Klemm *et al*., 1985 Pouttu *et al*., 1999
pUC19	Fermentas Life Sciences cloning vector	II	
pQE30	Qiagen GmbH cloning vector	IV	
pRAC221	*fac* genes from *E. coli* 789 in pBR322	II	Babai *et al*., 2000

Table 5. Methods used in this study

Method	Described and used in
Adhesion studies with bacteria and purified proteins	
Adhesion to chicken intestinal tissue sections	I, II, III
Adhesion to vaginal epithelial cells	III
Adhesion to ileal mucus	I, II
Exclusion of adhesion of *E. coli* by lactobacilli	II
Interaction studies of bacteria and purified proteins with the plasminogen	
Kinetic plasminogen activation assay	IV
Binding of ^{125}I-labeled Plg, plasmin and tPA to bacteria	IV
Binding of Plg and plasmin on purified Plg-receptors	IV
Bacteria-bound plasmin activity measurement	IV
Genetic methods	
Sequencing of chromosomal DNA	IV
Cloning and sequencing the Plg-receptors from lactobacilli	IV
Protein work	
Extraction of surface proteins from lactobacilli	III
Expression and purification of the Plg-receptors of *L. crispatus* ST1 in *E. coli*	IV
N-terminal sequencing	III, IV
MALDI-TOF analysis	IV
Immunological methods	
Production of antisera	III, IV
Western-blotting	III, IV
Indirect immunofluorescence	I, II, III
Immuno-EM	IV

7. RESULTS AND DISCUSSION

This study was initiated to characterize molecular mechanisms important for adhesion and colonization of lactobacilli at host intestinal tissues. We used the chicken as a host model since lactobacilli are frequent and adhesive in their alimentary canal (Fuller and Turvey, 1971; Fuller, 1973; Brooker and Fuller, 1975; Mitsuoka, 1992; Sarra *et al*., 1992; Guan *et al*., 2003; Lan *et al*., 2002) and as tissue samples from the animals are easily available.

7.1. Identification of *Lactobacillus crispatus* strains with colonization potential (unpublished)

Our first aim was to isolate a *Lactobacillus* stain with colonization and adhesion potential, and we used a chicken colonization trial for this task. The *Lactobacillus* strains A33 and 134mi were isolated from chicken crop and ileum, respectively, and identified as *L. crispatus* by their 16S rRNA-gene sequences. The PCR amplification of genomic DNA with primers designed for Enterobacterial Repetitive Intergenic Consensus (ERIC) sequences that have been used in analysis of various bacterial genera and species (Sharples *et al*., 1990; Versalovic *et al*., 1991; de Bruijn, 1992), gave different patterns for both strains (data not shown) verifying identification of separate strains. In primary adhesion tests, the strain A33 was found highly adhesive to frozen tissue sections of chicken intestinal tract, whereas the strain 134mi was less efficient in adhesion (see 7.2.). As a comparison, the type strain of *L. crispatus* from culture collection, ATCC33820, was also tested for its adherence and found to be poorly adhesive. For colonization trials, we inoculated newly hatched ross 208 chickens orally with $6x10^7$ bacteria per strains in two pairs of lactobacilli, A33 + ATCC33820 or 134mi + ATCC33820, to compare colonization of adhesive and poorly-adhesive strains. Lactobacilli at days 2, 5 and 12 postinoculum were isolated by cultivating on *Lactobacillus* selection (LBS) agar from crop and ileum of inoculated chickens as well as from chickens that received no exogenous bacteria. Within two days after hatching, the total number of lactobacilli isolates reached similar high levels both in crop (mean $4.1x10^8$ cells per crop) and ileum ($2.6x10^8$ cells per ileum) with no significant difference between the three test groups. The cut-off level of detection

was 10^5 bacteria per intestinal sample. Genomic DNA of 120 randomly chosen *Lactobacillus* colonies from each time point were subjected to PCR amplification with ERIC primers, and 67 isolates representing the most frequent PCR fingerprint patterns as well as isolates yielding patterns indistinguishable from those obtained with A33 and 134mi, were subjected to 16S rRNA-gene sequencing. Based on the 16S rRNA-gene sequences, the isolates belonged to the species *L. crispatus, L. gasseri* and *L. reuteri*. Based on PRC profiling, both A33 and 134mi also persisted in the chicken intestine. A bacterial colony representing the most commonly detected PCR profile within an identified species was chosen for the adherence tests. This selection gave the strains *L. crispatus* ST1, *L. gasseri* CT5, and *L. reuteri* CT7, which were considered capable of colonizing young chickens as they outgrow the challenge lactobacilli in the host intestine. Lactobacilli with the same PCR profiles were not detected among bacteria isolated from chicken egg shells, and it seems probable that ST1, CT5 and CT7 originated from the air of the brooder room or the diet of chickens similar to observations by Fuller (1973).

7.2. Tissue tropisms of indigenous lactobacilli in the avian intestinal tract (I)

Histological analysis of bacterial colonization in the chicken intestine has revealed that the major sites of bacterial colonization are crop, ileum and caecum (Fuller and Turvey, 1971). The alimentary canal consists of multiple tissue structures that vary in physiological functions. Therefore tissue sections rather than isolated epithelial cells or tissue homogenates were used in this PhD work to reveal possible tissue tropism in the adherence of lactobacilli. We used frozen sections from crop, duodenum, jejunum, ileum, caecum and colon to cover the whole alimentary canal.

The adherence of lactobacilli to the tissue domains in the avian alimentary canal is summarized in Table 1; I. *L. crispatus* ST1 adhered efficiently to the non-secreting stratified squamous epithelium of chicken crop, to the apical poles of mature enterocytes at tip of villi, to basolateral poles of enterocytes along the villus-crypt axis as well as to FAE and underlying lymphoid follicles. Also, efficient adherence to lamina propria composed of connective tissue, blood vessels and lymphoid cells was observed. (Fig. 1 and 2; I). The strain A33 showed similar and strong

adhesion profile as did the strain ST1. Instead, the strain 134mi adhered only poorly to the epithelium of crop and to FAE. The adhesiveness of *L. gasseri* CT5 was targeted to the same tissue structures as the strains ST1 and A33, but CT5 showed lower adhesion efficiency to apical surfaces of enterocytes and, importantly, to FAE. The *L. reuteri* CT7 adhered only to the epithelium of crop, and the type culture collection strains *L. crispatus* ATCC33820, *L. gasseri* ATCC33323, *L. reuteri* ATCC53609 *L. casei* ATCC393, showed no adhesion to chicken tissues. These results demonstrate that fresh isolates of lactobacilli exhibit considerable cell and tissue tropism in their adherence to the tissue domains in chicken intestine.

None of the *Lactobacillus* strains adhered to mucus-producing goblet cells on tissue sections, and only A33 and ATCC 53609 showed adherence to secreted ileal mucus; this, however, was low when compared to that of *L. reuteri* strain 1063 reported to bind to hen mucus (Roos and Jonsson, 2002) (Fig. 3; I). To estimate the role of abundant intestinal mucus layer as a colonization niche for lactobacilli, we tested the ability of *L. crispatus* strains ST1, A33, 134mi and ATCC33820, *L. reuteri* CT7, *L. gasseri* CT5 and *L. casei* 393 to grow in a minimal M9 salt medium supplemented with ileal mucus as a carbon source (data not shown). For comparison, the growth of lactobacilli in MRS medium was also tested. Bacteria ($1x10^5$ cfu) were inoculated to 1 ml of the media. After 16-hours cultivation, lactobacilli reached average numbers of $2x10^5$ cells per ml in mucus medium and $1x10^9$ cells per ml in MRS medium.

Our results on the *in vitro* cell specificity of the adherence of lactobacilli are in agreement with the findings of Fuller and Turvey (1971) and Brooker and Fuller (1975) on bacterial colonization and *in vivo* adhesion. Thus there appears to be a similar tissue distribution of the *in vitro* adhesion and the *in vivo* colonization by lactobacilli in the chicken intestine. Furthermore, our adhesion and colonization data support the idea that adhesion to the stratified squamous epithelium of crop is an important factor in successful colonization of lactobacilli to chicken intestine (Fuller, 1973). This idea is highlighted by the fact that strain CT7 adhered *in vitro* only to crop epithelium and was found to be persistent *in vivo* in the intestine during the colonization trial.

In the small and large intestine, the epithelium is simple columnar and serves the functions of secretion and absorption, and undergoes continuous and rapid renewal. The epithelial stem cells are located at the crypts of villi and give rise to mature enterocytes, mucus-producing goblet cells, paneth cells and entero endocrine cells (Huet *et al*., 1987; McCracken and Lorenz, 2001). Our finding that lactobacilli preferentially adhere to the apical poles of mature enterocytes at villus tips is in line with the observation of Fuller and Turvey (1971) that colonizing bacteria were only rarely detected below the top third of the villi. The crypt-villus migration of intestinal enterocytes involves changes in expression of ECM receptors (Louvard *et al*., 1992) but much less is known about the distribution of surface antigens in the polarized epithelium. The mature enterocytes possess apical brush border with well organized microvilli and underlying terminal web, while the immature enterocytes contain only few surface microvilli and lack well-formed terminal web. The maturating process is known to involve changes in the levels of luminal membrane proteins (Burgess *et al*., 1989; Fath *et al*., 1990; Shibayama *et al*., 1987; Weiser *et al*., 1986). Also, the intestinal brush-border associated enzymes as well as laminin expression and basement membrane (BM) organizations are known to differ in healthy vs. colorectal conditions (Real *et al*., 1992; Rémy *et al*., 1992). The enterocyte receptors for adhering lactobacilli remain to be elucidated.

The frozen tissue sections fixed with paraformaldehyde have been successfully used to determine tissue tropism of variety of bacterial adhesins (Korhonen *et al*., 1990; Sillanpää *et al*., 2000). The intact protective mucus layer, however, may be lost under these experimental procedures (Deplancke and Gaskins, 2001). We did not detect bacterial adherence to mucus-producing goblet cells in the tissue sections and hence wanted to confirm the adherence with freshly isolated intestinal mucus. Isolates of *Lactobacillus* have been reported to bind to pig and hen intestinal mucus and human faecal mucus (Rojas and Conway, 1996; Roos *et al*., 2000; Roos and Jonsson, 2002; Tuomola *et al*., 1999; Gusils *et al*., 2003). No adhesion to secreted mucus was observed with *Lactobacillus* strain tested in this study, expect for the strains A33 and ATCC53609 that showed poor adhesion. Further, in contrast to pathogenic *E. coli* that are able to grow in intestinal mucus

(Rang *et al*., 1999; Wadolkowski *et al*., 1988), the lactobacilli tested in this study were not able to grow efficiently in a minimal medium supplemented with salts and mucus. However, Rojas and Conway (1996) reported that *Lactobacillus* ssp. were able to grow in pig ileal mucus suggesting that some lactobacilli are able to occupy the mucus-layer niche in the intestine. The mucus is observed to prevent bacterial adhesion onto epithelial cells *in vitro* (Jin *et al*., 1996; Todoroki *et al*., 2001) and hence promote removal of bacteria from the intestine (Weiser *et al*., 1986). Lactobacilli have also been observed to inhibit adhesion of enteropathogenic *E. coli* to cultured intestinal cell line by inducing mucin gene expression (Mack *et al*., 1999). On the other hand, commensal bacteria alter surface mucus and diminish its deposition in caecum (McCracken and Lorenz, 2001) and enhanced adherence to intestinal mucus *in vitro* has been reported by *L. reuteri* strains grown in the presence of intestinal mucus (Jonsson *et al*., 2001). Overall, the role of mucus in bacterial and in particular *Lactobacillus* colonization in the GI remains controversial (Deplancke and Gaskins, 2001). However, our results suggest that in chicken intestine, the adherence to stratified squamous epithelium of crop which is not secreting mucus (Hodges, 1974) may be important for colonization by lactobacilli. In the colonization trial, the outgrowth of *L. crispatus* strain ST1 over the strain A33, which both adhered to crop epithelium, might be explained by the difference of the strains to adhere to the mucus layer.

Lymphoid follicles are scattered throughout the intestinal mucosa and form lymphatic aggregates i.e. Peyer's patches in the distal small intestine (Ross and Romrell, 1989). We found that the *L. crispatus* strains were adherent to FAE and over the entire area of the lymphoid follicles, including the B-cell-rich germinal centers and the surrounding T-cell-rich dome area. Notably, a sharp contrast was seen in the adherence of ST1 cells onto the FAE and onto the apical enterocyte surface of the same ileal region, where bacteria selectively bound to the FAE. FAE contains M cells that are important for pathogen invasion into the circulation and for presentation of antigens to the immune system (Neutra *et al*., 1999). Our findings are in line with the reports of association of certain lactobacilli with Peyer's patches of the mouse (Ma *et al*., 1990; Perdigón *et al*., 2000). The light microscopic techniques do not allow identification of individual M cells in the chicken ileum (Clark *et al*., 1993; Kitagawa *et al*., 2000; Buda *et al*., 2005), and the

possible interaction of lactobacilli with M cells requires more detailed studies. Interaction of lactic acid bacteria with M cells *in vivo* in the mouse intestine was recently reported on the basis of immune reactions against orally inoculated lactic acid bacteria (Perdigón *et al*., 1999). It was also proposed that lactobacilli are selectively internalized *in vivo* into Peyer's patches or lamina propria of the mouse intestine and that these interactions may be involved in different secretory immunoglobulin A and $CD4^+$ T-cell responses induced by the lactic acid bacteria (Ma *et al*., 1990; Perdigón *et al*., 1999, 2000 and 2001; Plant and Conway, 2001). Our *in vitro* adhesion studies suggest that adherent lactobacilli may indeed make contact with M cells and the underlying Peyer's patches, and the adherence of lactobacilli to FAE observed in this study may denote to presentation of lactobacilli to the immune cells.

7.3. Adhesion of APEC and salmonella in avian intestinal tract (II)

The shedding and horizontal transmission of APEC and salmonella between avian hosts and subsequently to humans causes annually considerable health problems and economical losses both in animal production and public health (Gross, 1994; Gomez *et al*., 1997; Cerquetti and Gherardi, 2000). Not much is known about the role of fimbrial expression on the tissue tropism of APEC and salmonella in chicken intestine. We utilized the tissue sections to screen tissue specificity in fimbriae-mediated adhesion of APEC and salmonella along the host intestinal mucosa. This was done as the first step in evaluating the use of adhesive lactobacilli in exclusion of pathogens in the chicken intestine.

The wild type (wt) APEC and salmonella and the recombinant strains expressing mannose-binding type-1 fimbriae were observed to adhere to the stratified squamous epithelium of crop (Fig. 1; II) as well as to apical and basolateral poles of simple columnar epithelia of small and large intestine and ileal FAE (Fig. 2 and 4; II). Also, adhesion to submucosal connective tissue areas was observed. Notably, poor adherence to the mucus-producing goblet cells but strong adherence to secreted ileal mucus was observed with type-1 fimbriated strains except for *S. enterica* serovar Typhimurium, which did not bind to either of these samples (Fig. 3 and 5; II). The adherence was efficiently inhibited by α-methyl-D-

mannoside, in accordance with the role of type-1 fimbriae in the binding (Fig. 2; II). Adhesion to intestinal goblet cells was detected only with AC/I-fimbriated wt and recombinant strains (Fig. 3; II).

The frequent expression of type-1 fimbriae by APEC and salmonella (Dozois *et al*., 1994; Babai *et al*., 1997; Humphries *et al*., 2001) and the efficient type-1 fimbriae mediated adhesion to chicken intestinal epithelial surfaces observed in this study, support the previous findings (Oyofo *et al*., 1989 a and b; Marc *et al*., 1998) that type-1 fimbriae may have a role in the colonization of enteropathogens in chicken intestine. Prevention of this mannose-dependent adhesion of APEC and salmonella thus may interfere with the fecal-oral transmission and subsequently reduce the frequency of colibacillosis and salmonellosis in the avian and the human hosts.

The type-1 fimbriae were observed to mediate the adherence of APEC and salmonella to the immunologically important FAE area in the chicken ileum. On the other hand, the specific adherence of salmonella to ileal FAE M-cells in the mouse was reported to be mediated by Lpf fimbriae (Bäumler *et al*., 1996). In lack of specific markers for chicken M-cells, these cells could not be identified on the sections used in the light microscopic analysis and the role of host specificity in the differences of results remains open. Lpf-deficient salmonella have shown only limited reduction in their virulence in mice (Bäumler *et al*., 1996) and translocation of unspecific luminal particles and microorganisms to underlying lymphoid areas by M-cells is also reported (Jepson *et al*., 1996; Owen, 1999), suggesting that close association of bacteria with M-cells might affect their translocation frequency. Hence, the type-1 fimbriae mediated adhesion of APEC and salmonella to chicken FAE may also contribute to their translocation to underlying tissues.

The type-1 fimbriae were also observed to mediate adhesion of APEC and salmonella to ileal mucus but not to the goblet cells. The intestinal mucus layer is composed of secreted mucins mainly synthesized by intestinal goblet cells to form a protective barrier over the epithelium (Deplancke and Gaskins, 2001). The bacterial entrapment into the mucus is regarded as an antimicrobial and defensive function which removes luminal bacteria by peristalsis (Weiser *et al*., 1986).

Hence, it would not be beneficial for enteric bacteria to express type-1 fimbriae *in vivo* in the intestinal lumen. Bacterial motility is reported to be important *in vitro* and *in vivo* for colonization and invasion of the host intestinal mucosa by salmonella (Dibb-Fuller *et al*., 1999; Allen-Vercoe and Woodward, 1999 a and b; Allen-Vercoe *et al*., 1999). Penetration of mucus layer by *E. coli* strain with “on” phase-locked type-1 fimbrial expression was slower than that observed with the strain lacking type-1 fimbriae (McCormick *et al*., 1993), indicating that type-1 fimbria may promote bacterial entrapment into intestinal mucus in mice. Also, the phase-locked mutant showed reduced colonization ability in mouse large intestine when compared to the strain lacking type-1 fimbriae (McCormick *et al*., 1993). On the other hand, mucus may serve as a nutrient source for the growth of enteric pathogens, and hence contribute to their colonization and virulence in mice (Rang *et al*., 1999; Wadolkowski *et al*., 1988). The efficient growth of both APEC and salmonella in chicken ileal mucus supplemented with salts was also observed in this study (Table 1; II), whereas intestinal isolates of *L. crispatus, L. gasseri,* and *L. reuteri* did not multiply under the same experimental conditions (see 7.2). The ability of motile enteropathogens to grow in the mucus may resist peristaltic exhaustion and increase bacterial persistence within the host intestine. For non-motile lactobacilli, on the other hand, the adhesion to mucus might on the contrary be unfavourable.

Opposite to type-1 fimbriae, the AC/I fimbria preferentially bound to goblet cell-associated mucus rather than to the secreted mucus. The difference may be due to variation in the molecular composition of the mucins in the secreted or the cell-associated forms (Deplancke and Gaskins, 2001), to bacterial modification of the mucins (McCracken and Lorenz, 2001) or to different technical conditions in the two experiments (McCracken and Lorenz, 2001). Our results disagree with previously reported AC/I fimbriae-mediated adhesion of APEC to isolated avian intestinal epithelia cells (Yerushalmi *et al*., 1990) in that we did not observe AC/I-mediated bacterial adherence to the intestinal enterocytes of tissues sections. The reasons for the differences remain open, however, our results do not support a role for AC/I fimbriae in colonization of chicken intestine.

7.4. Exclusion of pathogens by lactobacilli (II)

The prevention of intestinal tract infections is one of the health benefits attributed to lactobacilli. The adherence of lactobacilli to intestinal epithelium could prevent pathogen colonization by steric hindrance or competition for epithelial receptors (McGroarty, 1993). The colocalization of the type-1 fimbriae-mediated adhesion of pathogenic *E. coli* and salmonella to the same epithelial areas that were recognized by adhering lactobacilli, encouraged us to test the potential of lactobacilli to exclude the pathogens at sites important for colonization and invasion, i.e. crop and FAE. The strongly adhering *L. crispatus* strain ST1 inhibited the adhesion of APEC strain 789 to the epithelium of crop and FAE, while the poorly adhesive *L. crispatus* strain 134mi inhibited the pathogen adhesion only partially (Fig. 4; II). These results demonstrate that adhesion may be a factor contributing to exclusion of pathogens from intestinal surfaces.

Adhesion is considered to be an important step for pathogens in their invasion (Finlay and Cossard, 1997; Finlay and Falkow, 1997; Dho-Moulin and Fairbrother, 1999; Neutra *et al*., 1999; Klemm and Schembi, 2000), and hence the adhesion-based exclusion of pathogens by commensal bacteria might be important in prevention of bacterial invasion particularly at the area of FAE. In many *in vivo* animal feeding experiments, the supplementation of hosts with *Lactobacillus* ssp. has decreased the colonization and infection ability of enteric pathogens (Cole and Fuller, 1984; Hudault *et al*., 1997; Pascual *et al*., 1999; Zhao *et al*., 1998; Perdigón *et al*., 2001; Sgouras *et al*., 2004), although results with no significant effects have also been reported (Adler and DaMassa, 1980; Hinton and Mead, 1991; Stavric *et al*., 1992). In human trials, the most promising results have been obtained with *Lactobacillus* treatments to decrease rotavirus infections and prevention of pathogen overgrowth during antibiotic therapy (Hilton *et al*., 1997; Isolauri *et al*., 1991 and 1994; Kaila *et al*., 1992; Majamaa *et al*., 1995; Biller *et al*., 1995; Gorbach *et al*., 1987; Siitonen *et al*., 1990; Arvola *et al*., 1999). These studies, however, did not address the mechanisms of exclusion of pathogens by lactobacilli. In the chicken crop, lactobacilli reduce the luminal pH to 4,5-6 (Sarra *et al*., 1992), which most probably affects the viability of enteric pathogens. This has been seen *in vitro* with *Lactobacillus salivarius* strains against *E. coli* and *S.*

enterica serovar Enteritidis (Garriga *et al*., 1998). However, Fuller (1977) reported that the bacteriostatic property of lactobacilli in crop is due to low pH, but the bactericidal activity could not be accounted by pH alone. In human vagina, the production of H_2O_2 rather than alteration of pH by *Lactobacillus* isolates has been observed to decrease gonococcal growth (St. Amant *et al*., 2002), while low pH alone acts against uropathogenic *E. coli* (Juárez Tomás *et al*., 2003). Coaggregation of lactobacilli with type-1 fimbriated uropathogenic *E. coli* has also been demonstrated, and unlike the bacterial coaggregation in oral cavity leading to formation of dental plague, the finding was not related to onset of urogenital disease but rather to prevention of infections (Reid *et al*., 1988). Since we did not observe coaggragation of *L. crispatus* isolates with type-1 fimbriated APEC strain, the mechanisms of inhibition of pathogen adhesion in crop and FAE was most likely due to unspecific steric hindrance of adhesion to tissue receptors and to the strong affinity of the adhesion of lactobacilli. The idea of steric hindrance is in line with the report of mannose sensitive adhesion of *L. plantarum* and type-1 fimbriated *E. coli* to the human colonic cell line HT-29. Due to different reactivity to periodate treatment, the prefered receptor epitopes on HT-29 cells were however, suggested not to be identical for lactobacilli and *E. coli* (Adlerberth *et al*., 1996).

7.5. Isolation of *L. crispatus* adhesins (III)

L. crispatus ST1 was chosen for the isolation and characterization of adhesins due to its strong adhesion ability to several tissue domains in chicken intestine. The strain A33 was also included as an adhesive strain and strains 134mi and ATCC33820 as poorly adhesive comparative strains. The removal of S-layer-protein from the surface of ST1 cells by chaotropic treatment with guanidine hydrochloride (GnHCl) did not affect the adherence of ST1 cells to chicken intestinal tissues (Fig 1; III) and we could not detect adherence with the extracted S-layer proteins released from ST1 (data not shown). By treating the bacteria with peptidoglycan-cleaving bacterial autolysin, mutanolysin, the adhesion was abolished, yielding detached proteins that retained their binding ability (Fig. 1; III). Extracts from the adhesive and poorly-adhesive strains differed only with two protein bands in SDS-PAGE (Fig. 2; III). After fractionating the released surface proteins of STI, three different protein fractions with binding affinity to the stratum

corneum of stratified squamous epithelium of crop, basement membrane areas or singular intraepithelial and lamina propria cells were isolated (Fig. 3; III).

The *Lactobacillus* surface protein that binds to stratum corneum layer of stratified squamous epithelium of crop was named as *Lactobacillus* epithelium adhesin-1 (LEA-1). To our knowledge, this is the first stratified squamous epithelial adhesin isolated form lactobacilli. *Lactobacillus* strains also show adhesion to other epithelial surfaces, indicating a likely existence of other epithelial adhesins. LEA-1 is a high molecular weight protein with an apparent molecular size of approx. 280 kDa and may be a glycoprotein since it bound the conacavalin A lectin. The N-terminal sequencing of LEA-1 was not successful. The finding that LEA-1 and a related proteins could be detected on the surface of the adhesive *L. crispatus* stains ST1 and A33, but not on the poorly adhering strains 134mi and ATCC33820 (Fig. 2; III), also suggest a role in adhesion of lactobacilli to crop epithelium. Different ability of the strains ST1 and 134mi to inhibit pathogen adhesion onto the outermost surface of crop epithelium may also involve different expression of LEA-1 by the strains. Since lactobacilli are also frequent and dominating in human oral cavity and vagina which are lined with stratified squamous epithelia (Mitsuoka, 1992; Redondo-Lopez *et al*., 1990; Paavonen, 1983), we tested whether LEA-1 could mediate adhesion to these tissue structures as well. ST1 cells were found to be adhesive to buccal and vaginal cells freshly isolated from a healthy host, whereas the purified LEA-1 adhered to ca. half of the isolated vaginal cells (Fig. 4; III) but not at all to the buccal cells. The cells isolated from vagina probably contain epithelial cells from stratum corneum as well as from underlying epithelial layers, which might explain the selective binding of LEA-1 to only a fraction of the cell population. However, ST1 cells were observed to bind throughout the stratified squamous epithelium of crop in tissue sections and to all isolated vaginal and buccal cells, suggesting that other adhesins, not identified in this study, also participate in the epithelial adhesion.

Binding to the basement membrane was observed with the protein fraction containing peptides with apparent molecular sizes of 50 and 55 kDa (50/55 kDa) that could not be separated from each other by gel filtration or ion exchange chromatography. N-terminal sequencing of these proteins gave amino acid

sequences that showed no close similarity to those in databases, including the previously characterized collagen and laminin-binding proteins of lactobacilli, CnBP or CbsA (Aleljung *et al*., 1994; Roos *et al*., 1996; Sillanpää *et al.,* 2000). Hence, our results give evidence that *L. crispatus* possessess yet a novel basement membrane-binding adhesin(s). The 50/55 kDa adhesins or their receptor proteins were not determined in this study and further investigations are required to fully characterize these adhesins. Adhesion of several pathogenic bacteria to ECM proteins is thought to contribute to bacterial invasiveness (Westerlund and Korhonen, 1993). Lactobacilli commonly adhere to the components of ECM and BM (Aleljung *et al*., 1991; Nagy *et al*., 1992; Harty *et al*., 1994; Toba *et al*., 1995; McGrady *et al*., 1995; Styriak *et al*., 2003), and the activity has been proposed to have both protective (Horie *et al*., 2002) and pathogenic functions (Harty *et al*., 1993 and 1994), but overall, the biological functions of the adhesiveness of lactobacilli to ECM remain open.

The protein binding to singular intraepithelial and lamina propria cells was putatively identified by N-terminal sequencing and peptide database analysis as GAPDH. The 20 amino acid long N-terminal sequence of the protein shows 100% identity with *L. delbrueckii* and *L. johnsonii* GAPDH sequences (Pridmore *et al*., 2004; Branny *et al*., 1998). The gene encoding GAPDH was cloned into an expression vector and the recombinant GAPDH protein was expressed and purified from *E. coli* (see 7.7). The extracted and the purified recombinant GAPDH were found to bind with similar characteristics to chicken tissues. Histologically the cells recognized by GAPDH resembled lymphoid cells that are numerous in lamina propria and scattered in intraepithelial regions (Ross and Romrell, 1989; McCracken and Lorenz, 2001). Double staining of tissue sections with specific anti-chicken T-cell receptor 2 antibodies (Chen *et al*., 1988) showed a partial colocalization with GAPDH-binding. However, due to lack of specific markers for chicken lymphoid cells, the receptor cells for GAPDH could not be identified. The surface-localization of the glycolytic enzyme GAPDH is well characterized in several prokaryotic and eukaryotic species (Pancholi and Chhatwal, 2003) (Table 3.), and our results indicate that this is valid for lactobacilli also. The sequence of GAPDH from *L. crispatus* ST1 contains no features associated with secretion of proteins from Gram positive bacteria, and its secretion mechanisms remain open.

GAPDH has a variety of functions seemingly unrelated to glycolysis (Pancholi and Fischetti, 1992 and 1993) and it has been observed to bind Plg (Pancholi and Fischetti, 1992; Lottenberg *et al*., 1992). Binding of the GAPDH of lactobacilli to intraepithelial and lamina propria cells in chicken intestine may represent a novel function for bacterial GAPDHs.

The results described above indicate that a single strain of *Lactobacillus* possesses several adhesins with affinity to distinct tissue domains in the host intestine. However, as ST1 bacteria exhibited even broader tissue tropisms than the adhesins identified here, more adhesion factors are likely to have a role in the adhesiveness of ST1. The detailed knowledge of lactobacilli-host interaction at molecular level could be important for developing efficiently targeted probiotic products or efficient oral vaccine carriers. The results from lactobacilli as oral tetanus toxin carriers have shown that the immunization efficiency is dependent on the expression levels of the antigens, the *Lactobacillus* strain used as carrier, and the viability and persistence of the carrier strain in the host GI tract (Grangette *et al*., 2002; Seegers, 2002).

7.6. Interaction of commensal lactobacilli with the plasminogen system (IV)

Several pathogenic bacterial species intervene with the human proteolytic plasminogen system to enhance their invasion and migration through tissue barriers within the human host (Lähteenmäki *et al*., 2005). The Plg-binding has so far been linked to bacterial pathogenesis in invasive infections (Coleman and Benach, 1999; Lähteenmäki *et al*., 2001). Our results indicated that GAPDH, which is a well characterized PlgR on pathogenic streptococci (Pancholi and Fischetti, 1992; Seifert *et al*., 2003; Jobin *et al*., 2004; Bergmann *et al*., 2004), is surface-exposed also on lactobacilli. Lactobacilli are GRAS microbes (Adams and Marteau, 1995) and have been associated only with sporadic cases of infective diseases (Husni *et al*., 1997; Aguirre and Collins, 1993; Brouqui and Raoult, 2001; Salminen *et al*., 2004). We begun to study the possible interaction of lactobacilli with Plg as a possible mechanism contributing to the opportunistic pathogenesis of lactobacilli; however, in view of the range of physiological functions of plasmin, this

interaction might have non-pathogenic functions as well. The methods we first used have successfully been applied in studies of bacterial pathogens.

7.6.1. Plasminogen receptor function in lactobacilli (IV)

The ability of lactobacilli and lactococci to enhance Plg activation was tested in a kinetic assay by incubating the bacteria with human Plg in the presence or absence of the physiological PA, tPA, and the formed plasmin activity was measured as the breakdown of a choromogenic substrate of plasmin. In inhibition tests, the lysine analog ε-aminocaproic acid, EACA, the plasmin inhibitor α2-antiplasmin (α2AP), and the serine protease inhibitor aprotinin, were included to evaluate the role of lysine residues in bacteria-Plg interaction and the level of bacteria-bound plasmin. The bacteria were highly efficient in enhancing the tPA-catalyzed Plg-activation (Fig. 1; IV). The strains represented different species and origins of isolation or use: intestinal, dairy and probiotic. Hence the interaction with Plg-system seems to be a common characteristic for lactobacilli and lactococci. For comparison, pathogenic bacteria isolated from systemic infections, i.e. GAS, *Streptococcus anginosus, Streptococcus mutans, E. coli* and *Haemophilus influenzae*, and earlier found to express PlgR activity (Pancholi and Fischetti, 1992 and 1998; Urdaneta *et al*., 2004; Lähteenmäki *et al*., 1993; Virkola *et al*., 1996) were tested under the same conditions. These bacteria exhibited a significantly less efficient enhancement of Plg activation that did the lactic acid bacteria (Fig. 1; IV). Comparison of these results with previous reports on enhancement of Plg activation by pathogenic bacteria e.g. *E. coli* (Lähteenmäki *et al*., 1993; Kukkonen *et al*., 1998) is complicated due to different experimental conditions, such as the ratio of the concentrations of Plg and tPA. The Plg activation on lactic acid bacteria was strongly inhibited by EACA suggesting lysine-dependent interaction of bacteria with the Plg. Also, α2AP efficiently inhibited the activation, indicating that only a small proportion of the formed plasmin activity was bacterium-bound. Aprotinin consistently inhibited the plasmin activity formed in the experiment (Fig. 1; IV). No plasmin activity could be measured in the absence of tPA, suggesting no release of PAs from lactobacilli under these experimental conditions (data not shown).

We next tested the binding of Plg, plasmin and tPA, onto the bacteria. The strains *L. crispatus* ST1, *L. rhamnosus* GG were chosen to represent non-pathogenic bacteria, and the strains *S. anginosus* 753 and *S. mutans* 7120 were chosen to represent pathogenic species. Bacteria were first incubated with excess of ^{125}I-labeled proteins to allow binding, and after washing, the radioactivity remaining bacteria-bound was measured (Fig. 2; IV). The binding of Plg and plasmin to bacteria was poor (0,7-4,3%) when compared to the values reported in binding of Plg and plasmin onto pathogenic GAS*, S. aureus*, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *H. influenzae*, *E. coli*, *Branhamella catarrhalis* and strains of *Proteus* species (Broeseker et al., 1988; Ullberg *et al*., 1990 and 1992; Kuusela and Saksela, 1990). The binding was inhibited by EACA, which is in line with the role of lysine residues in the binding. No binding of tPA to bacterial strains tested here was observed. Previously tPA has been reported to bind only to curli and fimbriae of *E. coli* and *S. enterica* (Sjöbring *et al*., 1994).

To further investigate the binding of plasmin(ogen) to lactobacilli, we analysed the ability of these bacteria to obtain and retain plasmin activity on their surface. Plasminogen was allowed to bind to *L. crispatus* ST1 cells and after washing the excess Plg, tPA was added to catalyze the activation. After incubation for 1, 2 and 3 hours, the cells and the supernatants were separated, and the plasmin activity was measured with the chromogenic substrate of plasmin. The plasmin activity on the surface of *L. crispatus* ST1 was low and rapidly lost into the incubation supernatant (Fig. 5; IV). The subsequent increase of plasmin activity in the supernatant suggests cleavage of plasmin from bacterial surface or low affinity in the binding. Release of plasmin from bacterial surface of group A, C and G streptococci has also been suggested (Kuusela *et al*., 1992), but the importance of this phenomenon in bacteria-host interaction has remained unknown.

Our results show that the bacterial interaction with the Plg is not restricted to pathogenic bacteria but also commensal, dairy and probiotic lactobacilli and lactococci express Plg receptors. The data from our and previous studies demonstrate that lactobacilli are able to both adhere to components of ECM (Aleljung *et al*., 1991; Nagy *et al*., 1992; Harty et al., 1994; Toba *et al*., 1995; McGrady *et al*., 1995; Styriak *et al*., 2003) and bind Plg. These are common

characteristics for metastatic cancer cells and for several invasive bacterial pathogens (Mignatti and Rifkin, 1993; Myöhänen and Vaheri, 2004; Westerlund and Korhonen, 1993; Lähteenmäki *et al*., 2005) and might hence also enhance the opportunistic pathogenic potential of lactobacilli. However, lactobacilli have been associated with only sporadic cases of infective diseases in humans (Husni *et al*., 1997; Aguirre and Collins, 1993; Brouqui and Raoult, 2001), and these cases have usually preceded other severe predisposing diseases (Husni *et al*., 1997; Salminen *et al*., 2004). Healthy persons get exceedingly rarely infectious diseases associated with lactobacilli even though these bacteria are highly ubiquitous in the indigenous microbiota of the hosts and in the environment. The risks of lactobacilli to cause septicaemia and endocarditis have so far been evaluated by their ability to trigger platelet aggregation, to participate in the platelet-fibrin-clot formation on endothelial surface and to bind collagens *in vitro,* as well as by their proteolytic activities (Harty *et al*., 1993 and 1994; Oakey *et al*., 1995). None of these characteristics are restricted to strains associated with infective diseases and thus their role in the virulence of lactobacilli remains open. Our data show that although lactobacilli and lactococci were highly efficient in enhancing the tPA-catalyzed Plg activation, the binding of Plg and plasmin onto the bacterial cells was poor. Further, lactobacilli were not able to retain the cell-bound plasmin activity which was further susceptible to physiological antiproteases, suggesting that they might not be able to take advantage of the Plg system for proteolysis in a manner similar to what pathogenic bacteria and tumor cells do (Lähteenmäki *et al*., 2005; Berger, 2002). In a view of our results the expression of PlgRs alone seems not to be sufficient for bacterial virulence but might rather be a general property utilized for bacteria-host interactions and in predisposing conditions for opportunistic pathogenicity. The PlgR function of lactobacilli may also be utilized for nutritional demands and persistence in the host e.g. oral cavity and intestine or the environment e.g. milk.

7.6.2. Modulation of the plasminogen molecule by lactobacilli (IV)

It is known that in presence of tumor cells, Plg is cleaved into a small internal Plg fragment, angiostatin, which is known to suppress endothelial proliferation and tumor metastasis (O'Reilly *et al*., 1994; Cao and Xue, 2004). We allowed *L.*

crispatus ST1 bacteria to interact with Plg in PBS in the presence or absence of tPA for 6, 12 and 24 hours and analysed the proteolytic cleavage of the Plg molecule into smaller fragments by Western-blotting, N-terminal sequencing and MALDI-TOF.

In the presence of tPA, ST1 enhanced the fragmentation of Plg to molecules corresponding in size to plasmin and angiostatin (Fig. 6; IV). Plasmin has been reported to possess autoproteolytic nature (Gately *et al*., 1997) which was also observed here when incubating Plg in presence of tPA in plain PBS for 24 h. However, the presence of *L. crispatus* ST1 significantly enhanced the Plg fragmentation. N-terminal sequencing and MALDI-TOF analysis showed that in 24-h incubation of ST1 with Plg and tPA, the main fragments modified from Plg were K78-W783, corresponding to Lys-plasmin, and K78-R474 and V79-R493, corresponding to angiostatin molecules (Fig. 6; IV)(Cao, 1999). Also, fragments V79-W685 and K78-P670 lacking the S741 of the catalytic triad of plasmin were generated. The fragments V79-W685 and K78-P670 had also been hydrolysed at the position R474-A475 but the resulting molecules were still held together via the disulphide bond C462-C541.

In the 24-h incubation in the absence of tPA, the *L. crispatus* ST1 and *L. johnsonii* F133 were the only strains able to promote generation of internal Plg fragments. The formed molecules resembled plasmin and angiostatin in molecular size (Fig. 6; IV). Based on N-terminal sequencing and MALDI-TOF data, the proteolytic processing of Glu-Plg in the presence of *L. crispatus* ST1 yielded the internal fragments of Plg, V79-W685 and K78-P670, as well as angiostatins, K78-R474 and V79-R493. Fragment corresponding to the Lys-plasmin was not detected from the sample lacking tPA. We did not detect plasmin activity in the 24-h samples in the kinetic plasminogen activation assay. The generated internal Plg fragments were however, N-terminally cleaved at K77-K78 or K78-V79 sites, known to be recognized by plasmin and other serine proteases as well as other trypsin/chymotrypsin enzymes. Also, the fragmentation was efficiently inhibited with α2AP and aprotinin, further suggesting trypsin-like cleavage of Plg. The interaction of lactobacilli with Plg might involve bacterial cell envelope-associated serine proteases (Kunji *et al*., 1996; Fira *et al*., 2001) that could initiate the N-

terminal cleavage of Plg at K77-K78 or K78-V79 and subsequently promote fragmentation of Plg. The mechanimsm of Plg fragmentation by lactobacilli might also involve action of surface exposed of secreted reductases similar to those in tumor cells (Stathakis *et al*., 1997 and 1999; Lay *et al*., 2000), or autoproteolysis catalyzed by free sulfhydryl donors or cell surface receptors (Gately *et al*., 1997; Wang *et al*., 2004). Finally, secreted bacterial PAs such as streptokinase and staphylokinase (Wang *et al*., 1998; Rabijns *et al*., 1997) have been shown to alter the conformation of Plg to make it more susceptible for cleavage. We did not find any indication of secreted PAs in the kinetic activation assays performed with the bacteria or their supernatants containing secreted PlgRs under the experimental conditions and the potential expression of PAs by lactobacilli need to be studied in the future.

These results demonstrate that *Lactobacillus* cells promote modulation of Plg to potential antitumorigenic angiostatin molecules both in the presence or absence of tPA. The formation of internal Plg fragments and angiostatin could suppress tumor growth or inhibit pathological plasmin-mediated cell invasion (Ji *et al*., 1998; Stack *et al*., 1999; Gonzalez-Gronow *et al*., 2005). Commensal and probiotic lactobacilli have been proposed to have an anti-colon cancer effect (Mercenier *et al*., 2003), but the mechanisms of the tumor suppression have remained poorly known. In acute toxoplasmosis, the infection-induced resistance to tumors has been shown to result from suppression of tumor neovascularization rather than antitumor cytotoxic signalling (Hunter *et al*., 2001). Characterization of *Lactobacillus* factor(s) responsible for modulating Plg into angiostatin and their potential to suppress intestinal tumor neovascularization or plasmin mediated tumor metastasis would be of great importance for understanding the influence of indigenous microbiota on the host in health and disease as well as for efficient targeting of health-promoting probiotics.

7.7. Identification of GAPDH and enolase as plasminogen receptors in lactobacilli (IV)

We found that GAPDH was surface-localized on lactobacilli (III). GAPDH and another glycolytic enzyme, α-enolase, have been characterized as PlgRs on

pathogenic streptococci (Pancholi and Fischetti, 1992; Seifert *et al*., 2003; Jobin *et al*., 2004; Bergmann *et al*., 2004; Pancholi and Fischetti, 1998; Bergmann *et al*., 2001), and we tested the possible role of both of these proteins in the Plg-binding of lactobacilli. The open reading frames of *gap* encoding the 338 amino acid GAPDH and *eno1* encoding the 428 amino acid enolase-1 were cloned from the *L. crispatus* ST1 genome. Unlike pathogenic GAS and pneumococci which possess only single genes coding for α-enolase in their genomes (Hoskins *et al*., 2001; Ferretti *et al*., 2001), three enolase genes are present in *L. johnsonii* genome and two in *L. plantarum* and *L. lactis* (Pridmore *et al*., 2004; Kleerebezem *et al*., 2003; Bolotin *et al*., 2001). The GAPDH encoding gene exists as one copy both in streptococci and lactobacilli and lactococci (Hoskins *et al*., 2001; Ferretti *et al*., 2001; Pridmore *et al*., 2004; Kleerebezem *et al*., 2003; Bolotin *et al*., 2001). Expression of other possible α-enolases in *L. crispatus* and their potential interactions with Plg remain to be characterized in the future.

The recombinant GAPDH and enolase-1 were expressed and purified from *E. coli* as N-terminal His_6-tag fusion proteins under nonreducing conditions. To identify their function as PlgRs, the proteins were tested in the kinetic plasminogen activation assay at same molar concentrations. GAPDH was slightly more efficient than enolase-1 in enhancing the tPA-catalyzed activation (Fig. 3; IV). The reaction was inhibited by EACA, suggesting that lysine residues have a role in the interaction. Also, α2AP and aprotinin inhibited the reactions. Next, the binding of GAPDH and enolase-1 to Plg and plasmin were tested. Both GAPDH and enolase-1 were observed to bind Plg (Fig. 3; IV) and plasmin in a lysine dependent manner, GAPDH being slightly more efficient in binding than enolase-1. *L. crispatus* ST1 GAPDH shows 57% and enolase-1 51% sequence identity to GAPDH and enolase of pneumococci and GAS. *L. crispatus* ST1 GAPDH and enolase-1 lack C-terminal lysines that are important in streptococcal Plg-binding, but carry internal lysines in areas that are hydrophilic and predictably exposed on protein surface. The 3D structure of the pneumococcal octameric enolase show that the internal Plg-binding regions of the subunits are well exposed on protein surface while the C-terminal lysine residues are only poorly displayed, suggesting that the internal binding-regions are more important in the interaction with Plg (Ehinger *et al*., 2004). The enolase-1 of *L. crispatus* ST1 possesses two lysines,

K251 and K255, at internal region which in sequence is highly similar to the internal Plg-binding epitope of pneumococcal enolase (Bergmann *et al*., 2003).

The GAPDH and α-enolase are traditionally classified as cytoplasmic enzymes and their predicted sequences possess no putative signal sequences, anchoring motifs or membrane-spanning regions (Pancholi and Chhatwal, 2003). The secretion mechanisms are thus undefined. Proteins with these characteristics have been postulated to be present on the cell surface of pathogens which possess either only an outer cell membrane such as parasites, or a membrane and a wall such as Gram positive bacteria, but only rarely on the surface of organisms with two cell membrane layers such as Gram negative bacteria (Pancholi and Chhatwal, 2003). Secretion of GAPDH and α-enolase from Gram positive as well as Gram negative bacteria (Nelson *et al*., 2001; Fluegge *et al*., 2004; Kenny and Finlay, 1995) and human parasites (Bernal *et al*., 2004) has been shown. To assess whether these proteins are surface located in *L. crispatus*, we used immunoelectron microscopy (IEM) to characterize their subcellular localization. In the postcutting IEM detection with antibodies raised against the GAPDH and enolase-1, both proteins were detected in bacterial cell wall and cell membrane as well as in cytoplasm (Fig. 4; IV). The porous nature of bacterial S-layers (Sleytr and Messner, 1988) may allow the PlgRs of lactobacilli to be well exposed on bacterial surface. We also tested possible secretion of GAPDH and enolase-1 from *L. crispatus* ST1. Bacteria were incubated in culture medium or in PBS for 5 and 24 hours, cells were pelleted, and the spent culture media and the spent incubation buffers were analysed in SDS-PAGE and by Western-blotting with anti-GAPDH and anti-enolase-1 antibodies. Both GAPDH and enolase-1 were observed to be secreted from lactobacilli (Fig. 4; IV), the spent incubation buffers enhanced also the tPA-catalyzed plasmin formation. No activation of Plg was observed in the absence of tPA, suggesting that lactobacilli did not secrete a PA under these experimental conditions.

Plg-binding most likely is not the primary function of GAPDH and α-enolase, and we therefore also tested the glycolytic enzyme activities in *Lactobacillus* cells, in the GAPDH and the enolase-1 secreted from ST1 cells, and in the purified recombinant proteins. No GAPDH or α-enolase activities were found in native *L.*

crispatus ST1 cells, but when the S-layer was removed, strong GAPDH activity but no α-enolase activity could be detected. The spent incubation buffers of lactobacilli did not contain proteins with GAPDH or α-enolase activity, which might, however, result from low-level of secreted proteins available in the detection. The purified recombinant GAPDH and enolase-1 on the contrary were glycolytically active. GAPDH and enolase, either when purified as recombinants or located on streptococcal surface, have been reported to be enzymatically active (Pancholi and Fischetti, 1992 and 1998). Tetrameric and octameric structures are required for the enzyme activity of GAPDH and α-enolase (Roitel *et al*., 1999; Brown *et al*., 1998) and at the present, we do not know the oligomerization state of the GAPDH and enolase-1 of lactobacilli.

The results described above demontrate that lactobacilli are not only capable of expressing plasmin(ogen)-binding GAPDH and enolase, but also secreting PlgRs to their environment which could further lead to enhanced plasmin formation and offer proteolytic benefit of shared detached plasmin which is protected from α2AP. The same effect could result from the lactobacilli-released plasmin if still receptor-bound after deassociation from bacterial surface and could hence enhance virulence of lactobacilli. However, these bacteria have not been observed to be invasive in healthy hosts. In our experiments, the plasmin activities were efficiently inhibited by α2AP, suggesting that the lactobacilli-promoted plasmin formation is susceptible to its physiological inhibitor and would not be able to counteract the host´s control mechanisms. The biological functions of the release of PlgRs and surface-bound plasmin by commensal lactobacilli remain open. In contrast to pathogenic bacteria, *L. crispatus* and *L. johnsonii* were also observed to promote modification of Plg into internal fragments, including angiostatin, indicating that the interaction of lactobacilli with Plg is not restricted to formation of highly proteolytic plasmin but instead, may also provide heath-promoting effects for the host.

8. CONCLUSIONS

This work characterized adherence of lactobacilli in the avian alimentary canal and the interaction of lactobacilli with the mammalian proteolytic plasminogen system. The results of this study show that commensal lactobacilli and pathogenic bacterial species possess partially similar adhesion characteristics and express similar surface proteins. Lactobacilli, avian pathogenic *E. coli* and salmonella adhered to the same important tissue areas at the chicken intestine; i.e. to epithelial surface of crop, apical surfaces of enterocytes in small and large intestinal villi and follicle-associated epithelium in ileum as well as to basement membranes along the intestine. The expression of plasminogen receptors, GAPDH and enolase, which are considered as virulence factors in pathogenic streptococci, was also described on lactobacilli.

Our results suggest that the adherence of lactobacilli to crop epithelium may be important for successful colonization as well as for exclusion of pathogenic bacteria by steric hindrance. Hence, the adhesion of lactobacilli to the epithelial surfaces may play a role in the prevention of pathogen colonization and invasion in the host intestine. Indigenous microbiota has been proposed to provide the host with a colonization resistance. This idea is in line with our results and previous reports of successful suppression of intestinal and urogenital pathogens by lactobacilli (Jin *et al*., 1996; Boris *et al*., 1998; McLean *et al*., 2000; Ossest *et al*., 2001) and highlights the importance of efficient adhesion of lactobacilli. We isolated the *Lactobacillus* epithelium adhesin (LEA-1) which was detected on the strongly adhesive strains of *L. crispatus*. Characterization of this adhesin and its receptor(s) remain to be completed; this would provide tools to better evaluate the adhesive characteristics in lactobacilli and to utilize these factors in lactobacilli-host interactions. The adherence of commensal and pathogenic bacteria to mucosal surfaces may be explained by competition of an important colonization niche that may lead to commensal persistence in the intestine or, in the case of pathogenic bacteria, to enhanced virulence.

Bacterial adhesion to the components of basement membrane and engagement of the proteolytic plasminogen system are important for bacterial invasiveness into

circulation or secondary infection sites (Westerlund and Korhonen, 1993; Lähteenmäki *et al*., 2005). The results of the present and previous studies show that the plasminogen receptor function and the adhesiveness to components of ECM are also frequent properties among commensal *Lactobacillus* isolates (Aleljung *et al*., 1991; Nagy *et al*., 1992; Harty *et al*., 1994; Toba *et al*., 1995; Mcgrady *et al*., 1995 and Styriak *et al*., 2003). Lactobacilli are regarded as low-virulent microorganisms and are only sporadically associated with infectious diseases in human hosts. Their mechanisms as opportunistic pathogens have remained unknown. Our results offer a mechanism that may contribute to virulence of lactobacilli in opportunistic diseases such as infective endocarditis, intra-abdominal abscesses and bacteremia. However, the lactobacilli-Plg interaction *in vitro* was susceptible to physiological antiproteases and we hence consider this finding as a low risk factor for healthy hosts. Lactobacilli are frequent in oral and intestinal cavities of the humans (Mitsuoka, 1992). The components of the Plg system are also present and tightly regulated in saliva and oral cavities of healthy hosts (Moody, 1982), whereas less is known about the availability of Plg to intestinal bacteria in the gut lumen under the normal physiological conditions. The predisposing pathological conditions related to lactobacilli-associated infectious diseases (Husni *et al*., 1997; Aguirre and Collins, 1993; Salminen *et al*., 2004) may render the Plg system for intervenience with commensal lactobacilli. The fragmentation of Plg promoted by lactobacilli also offers a potential non-pathogenic function in inhibition of plasmin-mediated invasion of pathogenic bacteria or metastatic tumor cells by internal Plg fragments (Stack *et al*., 1999; Gonzalez-Gronow *et al*., 2005). Also, several endogenous inhibitors of angiogenesis and tumor neovascularization are shown to evolve from proteolytic prosessing of host's large precursor proteins, such as Plg (Cao, 1999). Putative lactobacilli-derived compounds that reduce disulphide bridges in Plg may also inhibit the proteolytic action of plasmin as was reported with denatured β-lactoglobulin that inhibit plasmin-mediated proteolysis of milk casein (Grufferty and Fox, 1986). Overall, the *in vivo* importance of ECM adhesion as well as Plg receptor function in lactobacilli remains to be elucidated and offers an interesting field of research to compare the role of the plasminogen system in bacterial commensalism and pathogenicity.

Based on current results it seems that the more we learn about commensal bacteria the more attributes previously linked to pathogenic bacteria are found also in commensals. The non-pathogenic probiotic *E. coli* strain Nissle1917 has the overall genetic structure homologous to pathogenic strains and possesses also insertion (IS)-elements and transposons homologous to those of pathogenic *E. coli* strains (Grozdanov *et al*., 2004). The sequence similarity in the lysogenic conversion genes of prophages of commensal *L. johnsonii* strain NCC533 and pathogenic streptococci and staphylococci suggests that the genes previously linked to virulence also may be present in commensal bacteria (Ventura *et al*., 2004). Sequence similarity between pneumococcal colonization and virulence factor PspC and a putative surface protein of *Lactobacillus reuteri* DSM20016^{T} further support this idea (Wall *et al*., 2003). These findings together with the adhesion of lactobacilli to ECM and interaction with the plasminogen system reported here raise the possibility that at least part of the previously classified pathogenic characteristics are not virulence factors as such but rather common bacterial properties that may be utilized for competition for the available ecological niches.

9. ACKNOWLEDGEMENTS

This work was carried out at the University of Helsinki, Department of Biological and Environmental Sciences, in the Division of General Microbiology with financial and educational support from the Viikki Graduate School in Biosciences, University of Helsinki, Academy of Finland, and Danisco Innovation, which are gratefully acknowledged. I warmly thank Professor Kielo Haahtela, the Head of the Department, Professor Timo Korhonen, the Head of the Division, and Professor Marja Makarow, the Head of the Viikki Graduate School in Biosciences for providing excellent working and educational facilities.

To my supervisor, Professor Timo Korhonen, I want to express my gratitude for his valuable visions, vast knowledge and criticism that guided me during this thesis work. I have been given an opportunity to work in a research group that provides young scientists with inspiring atmosphere and leads them into an exciting and fascinating world of science. Docent Kaarina Lähteenmäki and Docent Benita Westerlund-Wikström have also been my witty mentors both in my thesis work and in my early academic career. Kaarina as a student advisor had already an influence on me during my undergraduate studies, and at least partly, she may be thanked for me becoming a microbiologist. Kaarina's scientific knowledge on the plasminogen system is also highly appreciated. Benita has given me an enormous support not only on the scientific issues related to the early studies of the thesis but also issues far beyond science. I truly respect her impact on the research group.

Docent Pentti Kuusela and Professor Airi Palva are warmly acknowledged for the efficient and thorough reviewing of the thesis and for their valuable comments as well as encouragement.

I warmly thank all the other co-workers of the project, Juha Apajalahti, Nina Rautonen, Hannele Kettunen, Susanna Leskelä, Eliora Ron, Riikka Pirilä, Veera Kuparinen and Marc Baumann. This work would not have been possible to carry out without your efficient and fruitful collaboration. My special thanks belong to you Veera for your interest in this work and excellent participation in the late phase of

the project and for sharing the ups and downs of the enolase studies. I also want to acknowledge Professor Per Saris, a member of my PhD thesis follow-up group for his critical comments and appreciable encouragement during this work.

I want to thank all the present and the former members of Timå's research group for creating such a pleasant and joyful working atmosphere. The old, green, and ugly sofa in the coffee corner did not flag conversations during coffee breaks, but instead, created a nice and cosy place in which to share and solve the problems of science. Ann-Mari, Hannu, Jarna, Jenni, Jouko, Katariina, Katri, Lena, Maini, Marjo, Päivi, Raili, Riitta, Ritva, Sirkku and Ulla, I have been lucky to have an opportunity to work with you. My warm thanks belong to Jenni for sharing the office with me, for numerous sympathetic conversations, and especially for the friendship. My warm thanks belong also to Marjo for her friendship. Jarna is acknowledged for the instructions during my first summer job period at the division as well as for the hilarious country music played in the lab and for talks that could have been endless. Also, Maini has given a nice companionship especially during the late working hours and weekends. Recently, Katri has served as a model for female scientists how to cope with the tight schedule of the thesis writing and pregnancy.

I am grateful to my parents for the support and encouragement that they have given me during the years of my studies. Finally, I want to thank my family, Harry and Martin for their love and care which have given a backbone for my life. Harry's unfathomable support and faith in me are more appreciated than can be expressed by words.

Helsinki, March 2005

10. REFERENCES

Aaronson, R.M., Graven, K.K., Tucci, M., McDonald, J. and Farber, H.W. (1995) Non-neuronal enolase is an endothelial hypoxic stress protein. *J Biol Chem* **270**:27752-27757

Adams, M.R. and Marteau, P. (1995) On the safety of lactic acid bacteria from food. *Int J Food Microbiol* **27**:263-264

Adler, H.E. and DaMassa, A.J. (1980) Effect of ingested lactobacilli on *Salmonella infantis* and *E. coli* and intestinal flora, pasted vents and chick growth. *Avian Dis* **24**:868-878

Adlerberth, I., Ahrné, S., Johansson, M.-L., Molin, G., Hanson, L.Å. and Wold, A.E. (1996) A mannose-specific adherence mechanism in *Lactobacillus plantarum* conferring binding to the human colonic cell line HT-29. *Appl Environ Microbiol* **62**:2244-2251

Aguirre, M., and Collins, M.D. (1993) Lactic acid bacteria and human clinical infection. *J Appl Bacteriol* **75**:95-107

Ahrné, S., Nobaek, S., Jeppsson, B., Adlerberth, I., Word, A.E. and Molin, G. (1998) The normal *Lactobacillus* flora of healthy human rectal and oral mucosa. *J Appl Microbiol* **85**:88-94

Andreu, A., Stapleton, A.E., Fennell, C.L., Hillier, S.L. and Stamm, W.E. (1995) Hemagglutination, adherence, and surface properties of vaginal *Lactobacillus* species*. J Infect Dis* **171**:1237-1243

Aleljung, P., Paulsson, M., Emödy, L., Andersson, M., Naidu, A.S. and Wadström, T. (1991) Collagen binding by lactobacilli. *Curr Microbiol* **23**:33-38

Aleljung, P., Shen, W., Rozalska, B., Hellman, U., Ljungh, Å. and Wadström T. (1994) Purification of collagen-binding proteins of *Lactobacillus reuteri* NCIB 11951. *Curr Microbiol* **28**:231-236

al-Giery, A.G. and Brewer, J.M. (1992) Characterization of the interaction of yeast enolase with polynucleotides. *Biochim Biophys Acta* **1159**:134-140

Allen, B.L., Katz, B. and Höök, M. (2002) *Streptococcus anginosus* adheres to vascular endothelium basement membrane and purified extracellular matrix proteins. *Microbiol Pathogen* **32**:191-204

Allen, R.W., Trach, K.A. and Hock, J.A. (1987) Identification of the 37-kDa protein displaying a variable interaction with the erythroid cell membrane as glyceraldehyde-3-phosphate dehydrogenase. *J Biol Chem* **262**:649-653

Allen-Vercoe, E., Sayers, A.R. and Woodward, M.J. (1999) Virulence of *Salmonella enterica* serotype Enteritidis aflagellate and afimbriate mutants in a day-old chick model. *Epidemiol Infect* **122**:395-402

Allen-Vercoe, E. and Woodward, M.J. (1999a) The role of flagella, but not fimbriae, in the adherence of *Salmonella enterica* serotype Enteritidis to chick gut explant. *J Med Microbiol* **48**:771-780

Allen-Vercoe, E. and Woodward, M.J. (1999b) Colonization of the chicken caecum by afimbriate and aflagellate derivates of *Salmonella enterica* serotype Enteritidis. *Vet Microbiol* **69**:265-275

Angiolella, L., Facchin, M., Stringaro, A., Maras, B., Simonetti, N. and Cassone, A. (1996) Identification of a glucan-associated enolase as a main cell wall protein of *Candida albicans* and an indirect target of lipopeptide antimycotics. *J Infect Dis* **173**:684-690

Antikainen, J., Anton, L., Sillanpää, J. and Korhonen, T.K. (2002) Domains in the S-layer protein CbsA of *Lactobacillus crispatus* involved in adherence to collagens, laminin and lipoteichoic acids and in self-assebly. *Mol Microbiol* **46**:381-394

Althouse, C., Patterson, S., Fedorka-Cray, P. and Isaacson, R.E. (2003) Type-1 fimbriae of *Salmonella enterica* serovar Typhimurium bind to enterocytes and contribute to colonization of swine *in vivo*. *Infect Immun* **71**:6446-6452

Arvola, T., Laiho, K., Torkkeli, S., Mykkänen, H., Salminen, S., Maunula, L. and Isolauri, E. (1999) Prophylactic *Lactobacillus* GG reduces antibiotic-associated diarrhea in children with respiratory infections: a randomized study. *Pediatrics* **104**:1-4

Axelsson, L. (1998) Lactic Acid Bacteria: classification and physiology. 2nd ed. Lactic acid bacteria: microbiology and functional aspects. (Salminen, S and von Wright, A. eds.) pp.1-72 Marcel Dekker Inc. New York, USA

Babai, R., Blum-Oehler, G., Stern, B.E., Hacker, J. and Ron, E.Z. (1997) Virulence patterns from secticemic *Escherichia coli* O78 strains. *FEMS Microbiol Letters* **149**:99-105

Babai, R., Stern, B.E., Hacker, J. and Ron, E.Z. (2000) New fimbrial gene cluster of S-fimbrial adhesin family. *Infect Immun* **68**:5901-5907

Barbés, C. and Boris, S. (1999) Potential role of lactobacilli as prophylactic agents against genital pathogens. *AIDS Patient Care STDS* **13**:747-751

Behera, A.K., Hildebrand, E., Scagliotti, J., Steere, A.C. and Hu, L.T. (2005) Induction of host matrix metalloproteinases by *Borrelia burgdorferi* differs in human and murine lyme arthritis. *Infect Immun* **73**:126-134

Berger, D.H. (2002) Plasmin/plasminogen system in colorectal cancer. *World J Surg* **26**:767-771

Bergmann, S., Rohde, M., Chhatwal, S. and Hammerschmidt, S. (2001) α-Enolase of *Streptococcus pneumoniae* is a plasmin(ogen)-binding protein displayed on the bacterial cell surface. *Mol Microbiol* **40**:1273-1287

Bergmann, S., Rohde, M. and Hammerschmidt, S. (2004) Glyceraldehyde-3-phosphate dehydrogenase of *Streptococcus pneumoniae* is a surface-displayed plasminogen-binding protein. *Infect Immun* **72**:2416-2419

Bergmann, S., Wild, D., Diekmann, R.F., Bracht, D., Chhatwal, G.S. and Hammerschmidt, S. (2003) Identification of a novel plasmin(ogen)-binding motif in surface displayed α-enolase of *Streptococcus pneumoniae*. *Mol Microbiol* **49**:411-423

Bermudez, L.E., Petrofsky, M. and Shelton, K. (1996) Epidermal growth factor-binding protein in *Mycobacterium avium* and *Mycobacterium tuberculosis*: a possible role in the mechanism of infection. *Infect Immun* **64**:2917-2922

Bernal, D., de la Rubia, J.E., Carrasco-Abad, A.M., Toledo, R., Mas-Coma, S. and Marcilla, A. (2004) Identification of enolase as a plasminogen-binding protein in excretory-secretory products of *Fasciola hepatica*. *FEBS Lett* **563**:203-206

Biller, J.A., Katz, A.J., Flores, A.F., Buie, T.M. and Gorbach, S.L. (1995) Treatment of recurrent *Clostridium difficile* colitis with *Lactobacillus* GG. *J Pediatr Gastroenterol Nutr* **21**:224-226

Blomberg, L., Henriksson, A. and Conway, P.L. (1993) Inhibition of adhesion of *Escherichia coli* K88 to piglet ileal mucus by *Lactobacillus* ssp. *Appl Environ Microbiol* **59**:34-39

Bolotin, A., Wincker, P., Mauger, S., Jailloin, O., Malarme, K., Weissenbach, J., Ehrlich, S.D. and Sorokin, A. (2001) The complete genome sequence of the lactic acid bacterium *Lactococcus lactis* ssp. *lactis* IL1403. *Genome Res* **11**:731-753

Boris, S., Suárez, J.E., Vázquez, F. and Barbés, C. (1998) Adherence of human vaginal lactobacilli to vaginal epithelial cells and interaction with uropathogens. *Infect Immun* **66**:1985-1989

Boyd, E.F., Wang, F.-S., Beltran, P., Plock, S.A., Nelson, K. and Selander, R.K. (1993) Salmonella reference collection B (SARB): strains of 37 serovars of subspecies I. *J Gen Microbiol* **139**:1125-1132

Boyle, M.D.P. and Lottenberg, R. (1997). Plasminogen activation by invasive human pathogens. *Thromb Haemost* **77**:1-10

Brandtzaeg, P., Joo, G.B., Brusletto, B. and Kierulf, P (1990) Plasminogen activator inhibitor 1 and 2, alpha-2-antiplasmin, plasminogen, and endotoxin levels in systemic meningococcal disease. *Thromb Res* **57**:271-278

Branny, P., de la Torre, F. and Garel, J.R. (1998) An operon encoding three glycolytic enzymes in *Lactobacillus delbrueckii* subsp. *bulgaricus*: glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase and triosephosphate isomerase. *Microbiol* **144**:905-914

Broeseker, T.A., Boyle, M.D.P. and Lottenberg, R. (1988) Characterization of the interaction of human plasmin with its specific receptor on a group A streptococcus. *Microbial Pathogenesis* **5**:19-27

Brooker, B.E. and Fuller, R. (1975) Adhesion of lactobacilli to the chicken crop epithelium. *J Ultrastruct Res* **52**:21-31

Brouqui, P. and Raoult, D. (2001) Endocarditis due to rare and fastidious bacteria. *Clin Microbiol Rev* **14**:177-207

Brown, C.K., Kuhlman, P.L., Mattingly, S., Slates, K., Calie, P.J. and Farrer, W.W. (1998) A model of the quaternary structure of enolases, based on the structural and evolutionary analysis of the octameric enolase from *Bacillus subtilis*. *J Prot Chem* **17**:855-866

Buda, A., Sands, C. and Jepson, M.A. (2005) Use of fluorescence imaging to investigate the structure and function of intestinal M cells. *Adv Drug Deliv Rev* **57**:123-134

Burgess, D.R., Jiang, W., Mamajiwalla, S. and Kinsey, W. (1989) Intestinal crypt stem cells possess high levels of cytoskeletal-associated phosphotyrosine-containing proteins and tyrosine kinase activity relative to differentiated enterocytes. *J Cell Biol* **109:** 2139-2144

Bäumler, A.J., Tsolis, R.M. and Heffron, F. (1996) The lpf fimbrial operon mediates adhesion of *Salmonella typhimurium* to murine Peyer's patches. *Proc Natl Acad Sci* **93**:279-283

Caballero, A.R., Lottenberg, R. and Johnston, K.H. (1999) Cloning, expression, sequence analysis, and characterization of streptokinases secreted by porcine and equine isolates of *Streptococcus equisimilis*. *Infect Immun* **67**:6478-6486

Cao, Y. (1999) Therapeutic potential of angiostatin in the treatment of cancer. *Haematol* **84**:643-650

Cao, Y. and Xue, L. (2004) Angiostatin. *Sem Thromb Hemostasis* **30**:83-93

Carlson, H.C. and Whenham, G.R. (1968) Coliform bacteria in chicken broiler house dust and their possible relationship to coli-septicemia. *Avian Dis* **12**:297-302

Carneiro, C.R.W., Postol, E., Nomizo, R., Reis, L.F.L. and Brentani, R.R. (2004) Identification of enolase as a laminin-binding protein on the surface of *Staphylococcus aureus*. *Microbes Infect* **6**:604-608

Cerquetti, M.C. and Gherardi, M.M. (2000) Orally administered attenuated *Salmonella enteritidis* reduces chicken cecal carriage of virulent *Salmonella* challenge organisms. *Vet Microbiol* **76**:185-192

Chen, C.L., Cihak, J., Losch, U. and Cooper, M.D. (1988) Differential expression of two T cell receptors, TcR1 and TcR2, on chicken lymphocytes. *Eur J Immunol* **18**:539-543

Chen, H.F., Nakabayashi, M., Satoh, K. and Sakamoto, S. (1980) Studies on the purification and characterization of human urinary plasminogen and plasmin. *Thromb Haemost* **42**:1536-1547

Clark, M.A., Jepson, M.A., Simmons, N.L., Booth, T.A. and Hirst, B.H. (1993) Differential expression of lectin-binding sites defines mouse intestinal M-cells. *J Histochem Cytochem* **41:**1679-1687

Coconnier, M.-H., Klaenhammer, T.R., Kernéis, S., Bernet, M.-F. and Servin, A.L. (1992) Protein-mediated adhesion of *Lactobacillus acidophilus* BG2FO4 on human enterocyte and mucus-secreting cell lines in culture. *Appl Environ Microbiol* **58**:2034-2039

Coconnier, M.-H., Lieven, V., Hemery, E. and Servin, A.L. (1998) Antagonistic activity against *Helicobacter* infection *in vitro* and *in vivo* by the human *Lactobacillus acidophilus* strain LB. *Appl Environ Microbiol* **64**:4573-4580

Cole, B.B. and Fuller, R. (1984) A note on the effect on host specific fermented milk on the coliform population of the neonatal rat gut. *J Appl Bacteriol* **56**:495-498

Coleman, J.L. and Benach, J.L. (1999) Use of the plasminogen activation system by microorganisms. *J Lab Clin Med* **134**:567-576

Conway, P.L. and Adams, R.F. (1989) Role of erythrosine in the inhibition of adhesion of *Lactobacillus fermentum* strains 737 to mouse stomach tissue. *J Gen Microbiol* **135**:1167-1173

Conway, P.L., Gorbach, S.L. and Golding, B.R. (1987) Survival of lactic acid bacteria in the human stomach and adhesion to intestinal cells. *J Dairy Sci* **70**:1-12

Crowe, J.D., Sievwright, I.S., Auld, G.C., Moore, N.R., Gow, N.A.R. and Booth, N.A. (2003) *Candida albicans* bind human plasminogen: identification of eight plasminogen-binding proteins. *Mol Microbiol* **47**:1637-1651

de Bruijn, F. (1992) Use of repetitive (repetitive extragenic palindromic and enterobactrerial repetitive intergenic consensus) sequences and the polymerase chain reaction to fingerprint the genomes of *Rhizobium meliloti* isolates and other soil bacteria. *Appl Environ Microbiol* **58**:2180-2187.

Delgado, M.L., O´Connor, J.E., Azorín, I., Reuna-Piqueras, J., Gil, M.L. and Gozalbo, D. (2001) The glyceraldehydes-3-phosphate dehydrogenase polypeptides encoded by the *Saccharomyces cerevisiae* TDH1, TDH2 and TDH3 genes are also cell wall proteins*. Microbiol*. **147**:411-417

Deplancke, B. and H.R. Gasskins. (2001) Microbial modulation of innate defense: goblet cells and the intestinal mucus layer. *Am J Clin Nutr* **73:**1131S-1141S

Derbise, A., Song, Y.P., Parikh, S., Fischetti, V.A. and Pancholi, V. (2004) Role of the C-terminal lysine residues of streptococcal surface enolase in Glu- and Lys-plasminogen-binding activities of group A streptococci. *Infect Immun* **72**:94-105

Dho, M. and Lafont, J.P. (1982) *Escherichia coli* colonization of the trachea in poultry: comparison of virulent strains in gnotoxenic chickens. *Avian Dis* **26**:787-797

Dho, M. and Lafont, J.P. (1984) Adhesive properties and iron uptake ability in *Escherichia coli* lethal and nonlethal for chicks. *Avian Dis* **28**:1016-1025

Dho-Moulin, M. and Fairbrother, J.M. (1999) Avian pathogenic *Escherichia coli* (APEC). *Vet Res* **30**:299-316

Dibb-Fuller, M.P., Allen-Vercoe, E., Thorns, C.J. and Woodward, M.J. (1999) Fimbriae- and flagella-mediated association with and invasion of cultured epithelial cells by *Salmonella enteritidis*. *Microbiol* **145**:1023-1031

Dong, Z., Kumar, R., Yang, X. and Fidler, I.J. (1997) Macrophage-derived metalloelastase is responsible for the generation of angiostatin in Lewis lung carcinoma. *Cell* **88**:801-810

Dozois, C.M., Chanteloup, N., Dho-Moulin, M., Brée, A., Desautels, C. and Fairbrother, J.M. (1994) Bacterial colonization and *in vivo* expression of F1 (type 1) fimbrial antigens in chickens experimentally infected with pathogenic *Escherichia coli*. *Avian Dis* **38**:231-239

Dozois, C.M., Fairbrother, J.M., Harel, J. and Bossé, M. (1992) *pap*- and *pil*-related DNA sequences and other virulence determinant associated with *Escherichia coli* isolated from secticemic chickens and turkeys. *Infect Immun* **60**:2648-2656

Dudani, A.K., Cummings, C., Hashemi, S. and Ganz, P.R. (1993) Isolation of a novel 45 kDa plasminogen receptor from human endothelial cells. *Thromb Res* **69**:185-196

Ehinger, S., Schubert, W-D., Bergmann, S., Hammerschmidt, S. and Heinz, D.W. (2004) Plasmi(ogen)-binding α-enolase from *Streptococcus pneumoniae*: crystal structure and evaluation of plasmin(ogen)-binding sites. *J Mol Biol* **343**:997-1005

Fath, K.R., Obenauf, S.D. and Byrgess, D.R. (1990) Cytoskeletal protein and mRNA accumulation during brush border formation in adult chicken enterocytes. *Development* **109:**449-459

Fedorka-Cray, P.J., Ladely, S.R., Bailey, J.S. and Stern, N.J. (2001) Colonization of broiler chicks by *Salmonella typhimurium* definite phage type 104. *J Food Prot* **64**:1698-1704

Fernandes, P.A., Keen, J.N., Findlay, J.B.C. and Moradas-Ferreira, P. (1992) A protein homologous to glyceraldehyde-3-phosphate dehydrogenase is induced in the cell wall of a flocculent *Kluyveromyces marxianus*. *Biochim Biophys Acta* **1159**:67-73

Ferretti, J.J., McShan, W.M. Adjic, D., Savic, D., Savic, G., Lyon K., Primeaux, C., Sezate, S., Suvorov, A.N., Kenton, S., Lai, H.S., Lin, S.P., Qian, Y., Jia, H.G., Najar, F.Z., Ren, Q., Zhu, H., Song, L., White, J., Yuan, X., Clifton, S.W., Roe, B.A. and McLaughlin, R. (2001) Complete genome sequence of an M1 strain of *Streptococcus pyogenes. Proc Natl Acad Sci* **98**:4658-4663

Finlay, B.B. and Cossard, P. (1997) Exploitation of mammalian host cell functions by bacterial pathogens. *Science* **276**:718-725

Finlay, B.B. and Falkow, S. (1997) Common themes in microbial pathogenicity revisited. *Microbiol Mol Biol Rev* **61**:136-169

Fira, D., Kojic, M., Banina, A., Spasojevic, I., Strahinic, I. and Topisirovic, L. (2001) Characterization of cell envelope-associated proteinases of thermophilic lactobacilli. *J Appl Microbiol* **90**:123-130

Fluegge, K., Schweier, O., Schiltz, E., Batsford, S. and Berner, R. (2004) Identification and immunoreactivity of proteins released from *Streptococcus agalactiae*. *Eur J Clin Microbiol Infect Dis* **23**:818-824

Fox, D. and Smulian, A.G. (2001) Plasminogen-binding activity of enolase in the opportunistic pathogen *Pneumocystis carinii*. *Med Mycol* **39**:495-507

Fuchs, H., Wallich, R., Simon, M.M. and Kramer, M.D. (1994) The outer surface protein A of the spirochete *Borrelia burgdorferi* is a plasmin(ogen) receptor. *Proc Natl Acad Sci* **91**:12594-12598

Fujisawa, S., Benno, Y., Yaeshima, T. and 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

Fuller, R. (1973) Ecological studies on the *Lactobacillus* flora associated with the crop epithelium of the fowl. *J Appl Bacteriol* **36**:131-139

Fuller, R. (1975) Nature of the determinant responsible for the adhesion of lactobacilli to chicken crop epithelial cells. *J Gen Microbiol* **87**:245-250

Fuller, R. (1977) The importance of lactobacilli in maintaining normal microbial balance in the crop. *Br Poult Sci* **18**:85-94

Fuller, R. (1978) Epithelial attachment and other factors controlling the colonization of the intestine of the gnotobiotic chicken by lactobacilli. *J Appl Bacteriol* **45**:389-395

Fuller, R. (1989) Probiotics in man and animals *J Appl Bacteriol* **66**:365-378

Fuller, R. and Turvey, A. (1971) Bacteria associated with the intestinal wall of the fowl (*Gallus domesticus*). *J Appl Bacteriol* **34**:617-622

Galask, R.P. (1988) Vaginal colonization by bacteria and yeast. *Am J Obstet Gynecol* **158**:993-995

Garriga, M., Pascual, M., Monfort, J.M. and Hugas, M. (1998) Selection of lactobacilli for chicken probiotic adjuncts. *J Appl Microbiol* **84**:125-132

Gately, S., Twardowski, P., Stack, M.S., Cundiff, D.L., Grella, D., Castello, F.J., Enghild, J., Kwaan, H.C., Lee, F., Kramer, R.A., Volpert, O., Bouck, N. and Soff, G.A. (1997) The mechanism of cancer-mediated concersion of plasminogen to the angiogenesis inhibitor angiostatin. *Proc Natl Acad Sci* **94**:10868-10872

Ge, J., Catt, D.M. and Gregory, R.L. (2004) *Streptococcus mutans* surface α-enolase binds salivary mucin MG2 and human plasminogen. *Infect Immun* **72**:6748-6752

Gebbia, J.A., Monco, J.C., Degen, J.L., Bucce, T.H. and Benach, J.L. (1999) The plasminogen activation system enhances brain and heart invasion in murine relapsing fever borreliosis. *J Clin Invest* **103**:81-87

Gebert, A., Rothkötter, H.J. and Pabst, R. (1996) M cells in Peyer's patches of the intestine. *Int Rev Cytol* **167**:91-159

Gil-Navarro, I., Gil, M.L., Casanova, M., O'Connor, J.E., Martinez, J.P. and Gozalbo, D. (1997) The glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase of *Candida albicans* in a surface antigen. *J Bacteriol* **179**:4992-4999

Gomez, T.M., Motarjemi, Y., Miyagama, S., Kaferstein, F.K. and Stohr, K. (1997) Foodborne salmonellosis. *World Health Stat Q* **50**:81-89

Gong Y., Kim, S.O., Felez, J., Grella, D.K., Castellino, F.J. and Miles, L.A. (2001) Conversion of Glu-plasminogen to Lys-plasminogen is necessary for optimal stimulation of plasminogen activation on the endothelial cell surface. *J Biol Chem* **276**:19078-19083

Gonzalez-Gronow, M., Grenett, H.E., Gawdi, G. and Pizzo, S.V. (2005) Angiostatin directly inhibits human prostate tumor cell invasion by blocking plasminogen binding to its cellular receptor, CD26. *Exp Cell Res* **303**:22-31

Gophna, U., Berlev, M., Seijffers, R., Oelschlager, A., Hacker, J. and Ron, E.Z. (2001) Curli fibers mediate inernalization of *Escherichia coli* by eukaryotic cells. *Infect Immun* **69**:2659-2665

Gorbach, S., Chang, T.W. and Goldin, B. (1987) Successful treatment of relapsing *Clostridium difficile* colitis with *Lactobacillus* GG. *Lancet* **2**:1519

Goudot-Crozel, V., Caillol, D., Djabali, M. and Dessein, A.J. (1989) The major parasite surface antigen associated with human resistance to schistosomiasis is a 37-kDa glyceraldehyde-3P-dehydrogenase. *J Exp Med* **170**:2065-2080

Granato, D., Perotti, F., Masserey, I., Rouvet, M., Golliard, M., Servin, A. and Brassart, D. (1999) Cell surface-associated lipoteichoid acid acts as an adhesion factor for attachment of *Lactobacillus johnsonii* La1 to human enterocyte-like Caco-2 cells. *Appl Environ Microbiol* **65**:1071-1077

Grangette, C., Müller-Alouf, H., Geoffroy, M.-C., Goudercourt, D., Turneer, M. and Mercenier, A. (2002) Protection against tetanus toxin after intragastric administration of two recombinant lactic acid bacteria: impact of strain viability and *in vivo* persistence. *Vaccine* **20**:3304-3309

Greene, J.D. and Klaenhammer, T.R. (1994) Factors involved in adherence of lactobacilli to human Caco-2 cells. *Appl Environ Microbiol* **60**:4487-4494

Grifantini, R., Bartolini, E., Muzzi, A., Draghi, M., Frigimelica, E., Berger, J., Randazzo, F. and Grandi, G. (2002) Gene expression profile in *Neisseria meningitidis* and *Neisseria lactamica* upon host-cell contact: from basic research to vaccine development. *Ann NY Acad Sci* **975**:202-216

Gross, W.G. (1994) Diseases due to *Escherichia coli* in poultry. *In Escherichia coli* in domestic animals and humans. (Gyles, C.L. ed.) pp. 237-259 CAB International, Wallingford, UK

Grozdanov, L., Raasch, C., Schulze, J., Sonnenborn, U., Gottschalk, G., Hacker, J. and Dobrindt, U. (2004) Analysis of the genome structure of the non-pathogenic probiotic *Escherichia coli* strain Nissle 1917. *J Bacteriol* **186**:5432-5441

Grufferty, M.B. and Fox, P.F. (1986) Potassium iodate-induced proteolysis in ultra heat treated milk during storage: the role of β-lactoglobulin and plasmin. *J Dairy Res* **53**:601-613

Guan, L.L., Hagen, K.E., Tannock, G.W., Korver, D.R., Fasenko, G.M. and Allison, G.E. (2003) Detection and identification of *Lactobacillus* species in crops of broilers of different ages by using PCR-denaturing gradient gel electrophoresis and amplified ribosomal DNA restriction analysis. *Appl Environ Microbiol* **69**:6750-6757

Gusils, C., Oppezzo, O., Pizarro, R. and González, S. (2003) Adhesion of probiotic lactobacilli to chick intestinal mucus. *Can J Microbiol* **49**:472-478

Hancox, L.S., Yeh, K.-S. and Clegg, S. (1998) Construction and characterization of type 1 non-fimbriate and non-adhesive mutants of *Salmonella typhimurium*. *FEMS Immun Med Microbiol* **19**:289-296

Hansen, P.A. and Lessel, E.F. (1971) *Lactobacillus casei* (Orla-Jensen) comb. nov. *Int J Syst Bacteriol* **21**:69-71

Hao, W.-L. and Lee, Y.-K. (2004) Microflora of the gastrointestinal tract. *In* Methods in molecular biology. Vol 268. Public health microbiology: methods and protocols pp. 491-502 (Spencer, J.F.T. and Ragout de Spencer, A.L. ed.) Humana Press Inc., Totowa, NJ, USA

Hara, H., Ohta, H., Inoue, T., Ohashi, T., Takashiba, S., Murayama, Y. and Fukui, K. (2000) Cell surface-associated enolase in *Actinobacillus actinomycetemcomitans. Microbiol Immunol* **44**:349-356

Harty, D.W.S., Patrikakis, M. and Knox, K.W. (1993) Identification of *Lactobacillus* strains isolated from patients with infective endocarditis and comparison of their surface-associated properties with those of other strains of same species. *Microbial Ecol Health Disease* **6**:191-201

Harty, D.W.S., Oakey, H.J., Patrikakis, M., Hume, E.B.H. and Knox, K.W. (1994) Pathogenic potential of lactobacilli. *Int J Food Microbiol* **24**:179-189

Havernaar, R. and Huis in´t Veld, J.H.J. (1992) Probiotics: a general view. *In* The lactic acid bacteria. Vol 1. The lactic acid bacteria in health and disease. (Wood, B.J.B. ed.) pp. 151-170 Elsevier Applied Science, NY, USA

Hay, P.E. (2004) Bacterial vaginosis and miscarriage. *Curr Opin Infect Dis* **17**:41-44

Heegaard, C.W., Larsen, L.B., Rasmussen, L.K., Hojberg, K.E., Petersen, T.E. and Andreasen, P.A. (1997) Plasminogen activation system in human milk. *J Pediatr Gastroenterol Nutr* **25**:159-166

Heinemann, C., van Hylcama Vlieg, J.E.T., Janssen, D.B., Busscher, H.J., van der Mei, H.C and Reid, G. (2000) Purification and characterization of a surface-binding protein from *Lactobacillus fermentum* RC-14 that inhibits adhesion of *Enterococcus faecalis* 1131. *FEMS Microbiol Lett* **190**:177-180

Henriksson, A., Szewzyk, R. and Conway, P.L. (1991) Characteristics of adhesive determinants of *Lactobacillus fermentum* 104. *Appl Environ Microbiol* **57**:499-502

Hilton, E., Kolakowski, P., Singer, C. and Smith, M. (1997) Efficacy of *Lactobacillus* GG as a diarrhea preventative. *J Travel Med* **4**:41-43

Hinton, M. and Mead, G.C. (1991) *Salmonella* control in poultry: the need for the satisfactory evaluation of probiotics for this purpose. *Lett Appl Microbiol* **13**:49-50

Hodges, R.D. (1974) The histology of the fowl. Chapter 2: The digestive system. London Academic Press, UK

Holzapfel, W.H., Haberer, P., Snel, J., Schillinger, U. and Huis in't Veld, J.H. (1998) Overview of gut flora and probiotics. *Int J Food Microbiol* **41**:85-101

Hoskins, J.A., Alborn, W. Jr., Arnold, J., Blaszczak, L., Burgett, S., DeHoff, B.S., Estrem, S.T., Fritz, L., Fu, D.J., Fuller, W., Geringer, C., Gilmour, R., Glass, J.S., Khoja, H., Kraft, A.R., Lagace, R.E., LeBlanc, D.J., Lee, L.N., Lefkowitz, E.J., Lu, J., Matsushima, P., McAhren, S.M., McHenney, M., McLeaster, K., Mundy, C.W., Nicas, T.I., Norris, F.H., O'Gara, M., Peery, R.B., Robertson, G.T., Rockey, P., Sun, P.M., Winkler, M.E., Yang, Y., Young-Bellido, M., Zhao, G., Zook, C.A., Baltz, R.H., Jaskunas, S.R., Rosteck, P.R. Jr, Skatrud, P.L. and Glass, J.I. (2001) The genome of the bacterium *Streptococcus pneumoniae* strain R6. *J Bacteriol* **183**:5709-5717

Horie, M., Ishiyama, A., Fujihira-Ueki, Y., Sillanpää, J., Korhonen, T.K. and Toba, T. (2002) Inhibition of the adherence of *Escherichia coli* strains to basement membrane by *Lactobacillus crispatus* expressing an S-layer. *J Appl Microbiol* **92**:396-403

Hu, L.T., Pratt, S.D., Perides, G., Katz, L., Rogers, R.A. and Klempner, M.S. (1997) Isolation, cloning, and expression of a 70-kilodalton plasminogen binding protein of *Borrelia burgdorferi*. *Infect Immun* **65**:4989-4995

Hudault, S., Liévin, V., Bernet-Camard, M.-F. and Servin, A.L. (1997) Antagonistic activity exerted *in vitro* and *in vivo* by *Lactobacillus casei* (strain GG) against *Salmonella typhimurium* C5 infection. *Appl Environ Microbiol* **63**:513-518

Huet, C., Sahuquillo-Merino, C., Coudrier, E. and Louvard, D. (1987) Absorptive and mucus-secreting subclones isolated from a multipotent intestinal cell line (HT-29) provide new models for cell polarity and terminal differentiation. *J Cell Biol* **105**:345-357

Humphries, A.D., Townsend, S.M., Kingsley, A., Nicholson, T.L., Tsoli, R.M. and Bäumler, A.J. (2001) Role of fimbriae as antigens and intestinal colonization factors of *Salmonella* serovars. *FEMS Microbiol Lett* **201**:121-125

Hunter, C.A., Yu, D., Gee, M., Ngo, C.V., Sevignani, C., Goldschmidt, M., Golovkina, T.V., Evans, S., Lee, W.F. and Thomas-Tikhonenko, A. (2001) Cutting edge: systemic inhibition of angiogenesis underlies resistance to tumors during acute toxoplasmosis. *J Immunol* **166**:5878-5881

Husni, R.N., Gordon, S.M., Washington, J.A. and Longworth, D.L. (1997) *Lactobacillus* bacteremia and endocarditis: Review of 45 cases. *Clin Inf Dis* **25**:1048-1055

Hynönen, U., Westerlund-Wikström, B., Palva, A. and Korhonen, T.K. (2002) Identification by flagellum display of an epithelial cell- and fibronectin-binding function in the SlpA surface protein of *Lactobacillus brevis*. *J Bacteriol*. **184**:3360-3367

Isolauri, E., Juntunen, M., Rautanen, T., Sillanaukee, P. and Salminen, S. (1991) A human *Lactobacillus* strain (*Lactobacillus casei* strain GG) promotes recovery from acute diarrhea in children. *Pediatrics* **88**:90-97

Isolauri, E., Kaila, M., Mykkänen, H., Ling, W.H. and Salminen, S. (1994) Oral bacteriotherapy for viral gastroenteritidis. *Dig Dis Sci* **39**:2595-2600

Jacobsen, C.N., Nielsen, V.R., Hayford, A.E., Møller, P.L., Michaelsen, K.F., Pœrregaard, A., Sandtröm, B., Tvede, M. and Jacobsen, M. (1999) Screening of probiotic activities of forty-seven strains of *Lactobacillus* ssp. by *in vitro* techniques and evaluation of the colonization ability of five selected strains in humans. *Appl Environ Microbiol* **65**:4949-4956

Janben, T., Schwarz, C., Preikschat, P., Voss, M., Philipp, H.-C. and Wieler, L.H. (2001) Virulence-associated genes in avian pathogenic *Escherichia coli* (APEC) isolated from internal organs of poultry having diet from colibacillosis. *Int J Med Microbiol* **291**:371-378

Jepson, M.A. and Clark, M.A. (2001) The role of M cells in *Salmonella* infection. *Microbes Infect* **3**:1183-1190

Jepson, M.A., Clark, M.A., Foster, N., Mason, C.M., Bennett, M.K., Simmons, N.L. and Hirst, B.H. (1996) Targeting to intestinal M cells. *J Anat* **189**:507-516

Ji, W.-R., Castellino, F.J., Chang, Y., Deford, M.E., Gray, H., Villarreal, X., Kondri, M.E., Marti, D.N., Llinás, M., Schaller, J., Kramer, R.A. and Trail, P.A. (1998) Characterization of kringle domains of angiostatin as antagonists of endothelial cell migration, an important process in angiogenesis. *FASEB J* **12**:1731-1738

Jin, L.Z., Ho, Y.W., Ali, M.A., Abdullah, N. and Jalaludin, S. (1996) Effect of adherent *Lactobacillus* spp. on *in vitro* adherence of salmonellae to the intestinal epithelial cells of chicken. *J Appl Bacteriol* **81**:201-206

Jin, L.Z., Ho, Y.W., Abdullah, N., Ali, M.A. and Jalaludin, S. (1998) Lack of influence of adherent *Lactobacillus* isolates on the attachment of *Escherichia coli* to the intestinal epithelial cells of chicken *in vitro*. *J Appl Microbiol* **84**:1171-1174

Jobin, M.-C., Brassard, J., Quessy, S., Gottschalk, M. and Grenier, D. (2004) Acquisition of host plasmin activity by the swine pathogen *Streptococcus suis* serotype 2. *Infect Immun* **72**:606-610

Johnson, J.L., Phelps, C.F., Cummins, C.S., London, J. and Gasser, F. (1980) Taxonomy of the *Lactobacillus acidophilus* group. *Int J Syst Bacteriol* **30**:53-68

Jolodar, A., Fischer, P., Bergmann, S., Buttner, D.W., Hammerschmidt, S. and Brattig, N.W. (2003) Molecular cloning of an alpha-enolase from the human filarial parasite *Onchocerca volvulus* that binds human plasminogen. *Biochim Biophys Acta* **1627**:111-120

Jonsson, H., Ström, E. and Roos, S. (2001) Addition of mucin to the growth medium triggers mucus-binding activity in different strains of *Lactobacillus reuteri in vitro*. *FEMS Microbiol Lett* **204**:19-22

Jordan Lin, C.T., Morales R.A. and Ralston, K. (1997) Raw and under-cooked eggs: a danger of salmonellosis. *Food Rev* **20**:27-32

Juárez Tomás, M.S., Ocaña, V.S., Wiese, B. and Nader-Macías, M.E. (2003) Growth and lactic acid production by vaginal *Lactobacillus acidophilus* CRL 1259, and inhibition of uropathogenic *Escherichia coli*. *J Med Microbiol* **52**:1117-1124

Jönssön, K., Guo, B.P., Monstein, H.J., Mekalanos, J.J. and Kronvall, G. (2004) Molecular cloning and characterization of two *Helicobacter pylori* genes coding for plasminogen-binding proteins. *Proc Natl Acad Sci* **101**:1852-1857

Kaila, M., Isolauri, E., Soppi, E., Viitanen, V., Lane, S. and Arvolommi, H. (1992) Enhancement of circulating antibody secreting cell responce in human diarrhoea by a human *Lactobacillus* strain. *Pediatr Res* **32**:141-144

Kandler, O., Stetter, K.O. and Köhl, R. (1980) *Lactobacillus reuteri* sp. nov., a new species of heterofermentative lactobacilli. *Zbl Bakt Hyg I Abt Orig C* **1**:264-269

Kenny, B. and Finlay, B.B. (1995) Protein secretion by enteropathogenic *Escherichia coli* is essential for transducing signals to epithelial cells. *Proc Natl Acad Sci* **92**:7991-7995

Kerényi, T., Voss, B., Rauterberg, J., Fromme, H.G., Jellinek, H. and Hauss, W.H. (1985) Connective tissue proteins on the injured endothelium of the rat aorta. *Exp Mol Pathol* **43**:151-161

Khil, J., Im, M., Heath, A., Ringdahl, U., Mundada, L., Cary Engleberg, N. and Fay, W.P. (2003) Plasminogen enhances virulence of group A streptococci by streptokinase-dependent and streptokinase-independent mechanisms. *J Infect Dis* **188**:497-505

Kirjavainen, P.V., Ouwehand, A.C., Isolauri, E. and Salminen, S.J. (1998) The ability of probiotic bacteria to bind to human intestinal mucus. *FEMS Microbiol Lett* **167**:185-189

Kirjavainen, P.V, Tuomola, E.M., Crittenden, R.G., Ouwehand, A.C., Harty, D.W.S., Morris, L.F., Rautelin, H., Playne, M.J., Donohue, D.C. and Salminen, S.J. (1999) *In vitro* adhesion and platelet aggregation properties of bacteremia-associated lactobacilli. *Infect Immun* **67**:2653-2655

Kitagawa, H., Shiraishi, S., Imagawa, T. and Uehara, M. (2000) Ultrastructural characteristics and lectin-binding properties of M cells in the follicle-associated epithelium of chicken caecal tonsils. *J Anat* **197:**607-616

Klaenhammer, T.R. (2001) Probiotics and prebiotics. *In* Food microbiology, fundamentals and frontiers 2nd ed. pp. 797-811 (Doyle, M.P., Beuchat, L.R. and Montville, T.J. ed.) ASM Press Washington D.C. USA

Kleerebezem, M., Boekhorst, J., van Kranenburg, R., Molenaar, D., Kuipers, O.P., Leer, R., Tarchini, R., Peters, S.A., Sandbrink, H.M., Fiers, M.W.E.J., Stiekema, W., Lankhorst, R.M.K., Bron, P.A., Hoffer, S.M., Groot, M.N.N., Kerkhoven, R., de Vries, M., Ursing, B., de Vos, W.M. and Sizen, R.J. (2003) Complete genome sequence of *Lactobacillus plantarum* WSFS1. *Proc Natl Acad Sci* **100**:1990-1995

Klemm, P., Jörgensen, B.J., van Die, I., de Ree, H. and Bergmans, H. (1985) The *fim* genes responsible for synthesis of type 1 fimbriae in *Escherichia coli*, cloning and genetic organization. *Mol Gen Genet* **199**:410-414

Klemm, P. and Schembri, M.A. (2000) Bacterial adhesins: function and structure*. Int J Med Microbiol* **290**:27-35

Korhonen, T.K., Virkola R., Westerlund B., Holthöfer H. and Parkkinen J. (1990) Tissue tropism of *Escherichia coli* adhesins in human extraintestinal infections. *Curr Top Microbiol Immunol* **151:**115-127.

Kotarsky, S.F. and Savage, D.C. (1979) Models for study of the specificity by which indigenous lactobacilli adhere to murine gastric epithelium*. Infect Immun* **26**:966-975

Kukkonen, M., Lähteenmäki, K., Suomalainen, M., Kalkkinen, N., Emody, L., Lång, H. and Korhonen, T.K. (2001) Protein regions important for plasminogen activation and inactivation of alpha2-antiplasmin in the surface protease Pla of *Yersinia pestis*. *Mol Microbiol* **40**:1097-1111

Kukkonen, M., Saarela, S., Lähteenmäki, K., Hynönen, U., Westerlund-Wikström, B., Rhen, M., and Korhonen, T.K. (1998) Identification of two laminin-binding fimbriae, the type-1 fimbria of *Salmonella enterica* serovar Typhimurium and the G fimbria of *Escherichia coli*, as plasminogen receptors. *Infect Immun* **66**:4965-4970

Kukkonen, M., Suomalainen, M., Kyllönen, P., Lähteenmäki, K., Lång, H., Virkola, R., Helander, I.M., Holst, O. and Korhonen, T.K. (2004) Lack of O-antigen is essential for plasminogen activation by *Yersinia pestis* and *Salmonella enterica*. *Mol Microbiol* **51**:215-225

Kunji, E.R.S, Mierau, I., Hagting, A., Poolman, B., and Konings, W.N. (1996) The proteolytic system of lactic acid bacteria. *Antonie van Leeuwenhoek* **70**:187-221

Kuusela, P., and Saksela, O. (1990) Binding and activation of plasminogen at the surface of *Staphylococcus aureus*. Increase in affinity after conversion to the Lys form of the ligand. *Eur J Biochem* **193**:759-765

Kuusela, P., Ullberg, M., Saksela, O. and Kronvall, G. (1992) Tissue-type plasminogen activator-mediated activation of plasminogen on the surface of group A, C, and G streptococci. *Infect Immun* **60**:196-201

Lan, P.T.N., Hayashi, H., Sakamoto, M. and Benno, Y. (2002) Phylogenetic analysis of cecal microbiota in chicken by the use of 16S rDNA clone libraries. *Microbiol Immunol* **46**:371-382

La Ragione, R.M., Cooley, W.A., Velge, P., Jepson, M.A. and Woodward, M.J. (2003) Membrane ruffling and invasion of human and avian cell lines is reduced for aflagellate mutants of *Salmonella enterica* serotype *Enteritidis*. *Int J Med Microbiol* **293**:261-272

La Ragione, R.M., Cooley, W.A. and Woodward, M.J. (2000) The role of fimbriae and flagella in the adherence of avian strains of *Escherichia coli* O78:K80 to tissue culture cells and tracheal and gut explants. *J Med Microbiol* **49**:327-338

La Ragione, R.M., Narbad, A., Gasson, M.J. and Woodward, M.J. (2004) *In vivo* characterization of *Lactobacillus johnsonii* FI9785 for use as a defined competitive exclusion agent against bacterial pathogens in poultry. *Lett Appl Microbiol* **38**:197-205

La Ragione, R.M. and Woodward, M.J. (2002) Virulence factors of *Escherichia coli* serotypes associated with avian colisepticaemia. *Res Vet Sci* **73**:27-35

Lauer, E., Helming, C. and Kandler, O. (1980) Heterogeneity of the specied *Lactobacillus acidophilus* (Moro) Hansen and Mocquot as released by biochemical and DNA-DNA hybridization. *Zenralbl Bakteriol Hyg Abt I Orig C* **1**:150-168

Lauer, E. and Kandler, O. (1980) *Lactobacillus gasseri* sp. nov., a new species of the subgenus *Thermobacterium*. *Zentralbl Bakteriol Hyg Abt I Orig C* **1**:75-78

Lay, A.J., Jiang, X.M., Kisker, O., Flynn, E., Underwood, A., Condron, R. and Hogg, P.J. (2000) Phosphoglycerate kinase acts in tumour angiogenesis as a disulphide reductase. *Nature* **408**:869-873

Lee, Y.-K., Puong, K.-Y., Ouwehand, A.C. and Salminen, S. (2003) Displacement of bacterial pathogens from mucus and Caco-2 cell surface by lactobacilli. *J Med Microbiol* **52**:925-930

Leigh, J.A. (1994) Purification of a plasminogen activator from *Streptococcus uberis*. *FEMS Microbiol Lett* **118**:153-158

Leigh, J.A. and Lincoln, R.A. (1997) *Streptococcus uberis* acquires plasmin activity following growth in the presence of bovine plasminogen through the action of its specific plasminogen activator. *FEMS Microbiol Lett* **154**:123-129

Lidbeck, A. and Nord, C.E. (1993) Lactobacilli and the normal human anaerobic microflora. *Clin Infect Dis* **16**:S181-187

Lijnen, H.R. and Collen, D. (1995) Mechanisms of physiological fibrinolysis. *Baillière´s Clin Haemat* **8**:277-290

Lin, J.H.-C. and Savage, D.C. (1984) Host specificity of the colonization of murine gastric epithelium by lactobacilli. *FEMS Microbiol Lett* **24**:67-71

Lindquist, B.L., Lebenthal, E., Lee, P.-C. Stinson, M.W. and Merrick, J.M. (1987) Adherence of *Salmonella typhimurium* to small-intestinal enterocytes of the rat. *Infect Immun* **55**:3044-3050

López-Alemany, R., Correc, P., Camoin, L. and Burtin, P. (1994) Purification of the plasmin receptor from human carcinoma cells and comparison to alpha-enolase. *Thromb Res* **75**:371-381

López-Alemany, R., Suelves, M. and Muñoz-Cánoves, P (2003) Plasmin generation dependent on α-enolase-type plasminogen receptor is required for myogenesis. *Thromb Haemost* **90**:724-733

Lorca, G., Torino, M.I., de Valdez, G.F. and Ljungh, Å. (2002) Lactobacilli express cell surface proteins which mediate binding of immobilized collagen and fibronectin. *FEMS Microbiol Lett* **206**:31-37

Lottenberg, R., Broder, C.C., Boyle, M.D.P., Kain, S.J., Schroeder, B.L. and Curtiss, R. (1992) Cloning, sequence analysis, and expression in *Escherichia coli* of a streptococcal plasmin receptor. *J Bacteriol* **174**:5204-5210

Louvard, D., Keringer, M. and Hauri, H.P. (1992) The differentiating intestinal epithelial cell: Establishment and maintenance of functions through interactions between cellular structures. *Annu Rev Cell Biol* **8:**157-195

Lähteenmäki, K., Westerlund, B., Kuusela, P. and Korhonen, T.K. (1993) Immobilization of plasminogen on *Escherichia coli* flagella. *FEMS Micobiol Lett* **106**:309-314

Lähteenmäki, K., Kuusela, P. and Korhonen, T.K. (2001) Bacterial plasminogen activators and receptors. *FEMS Microbiol Rev* **25**:531-552

Lähteenmäki, K., Kyllönen, P., Partanen, L. and Korhonen, T.K. (2004) Antiprotease interaction by *Salmonella enterica* released from infected macrophages. *Cell Microbiol*, in press, early online doi: 10.1111/j.1462-5822.2004.00483.x

Lähteenmäki, K., Edelman, S. and Korhonen, T.K. (2005) Bacterial metastasis: the host plasminogen system in bacterial invasion. *Trends Microbiol* **13**:79-85

Lähteenmäki, K., Westerlund, B., Kuusela, P. and Korhonen, T.K. (1993) Immobilization of plasminogen on *Escherichia coli* flagella. *FEMS Microbiol Lett* **106**:309-314

Ma, L., Deitch, E., Specian, R., Steffen, E. and Berg, R. (1990) Translocation of *Lactobacillus murinus* from the gastrointestinal tract. *Curr Microbiol* **20:**177-184

Mack, D.R., Michail, S., Wei, S., McDougall, L. and Hollingsworth, M.A. (1999) Probiotics inhibit enteropathogenic *E. coli* adherence *in vitro* by inducing intestinal mucin gene expression. *Am J Physiol* **276**:G941-G950

Majamaa, H., Isolauri, E., Saxelin, M. and Vesikari, T. (1995) Lactic acid bacteria in the treatment of acute rotavirus gastroenteritis. *J Pediatr Gastroenterol Nutr* **20**:333-338

Mangel, W.F., Lin, B. and Ramakrishnan, V. (1990) Characterization of an extremely large, ligand-induced conformational change in plasminogen. *Science* **248**:69-73

Marc, D., Arné, P., Brée, A. and Dho-Moulin, M. (1998) Colonization ability and pathogenic properties of a *fim*$^-$ mutant of an avian strain of *Escherichia coli*. *Res Microbiol* **149**:473-485

Marelli, G., Papaleo, E. and Ferrari, A. (2004) Lactobacilli for prevention of urogenital infections: a review. *Eur Rev Med Pharmacol Sci* **8**:87-95

Matsumura, A., Saito, T., Arakuni, M., Kitazawa, H., Kawai, Y. and Itoh, T. (1999) New binding assay and preparative trials of cell-surface lectin from *Lactobacillus acidophilus* group lactic acid bacteria. *J Dairy Sci* **82**:2525-2529

McCormick, B.A., Klemm, P., Krogfelt, K.A., Burghoff, R.L. Pallesen, L., Laux, D.C. and Cohen, P.S. (1993) *Escherichia coli* F-18 phase locked "on" for expression of type 1 fimbriae is a poor colonizer of the streptomycin-treated mouse large intestine. *Microbial Pathogenesis* **14**:33-43

McCracken, V.J. and Lorenz, R.G. (2001) The gastrointestinal ecosystem: a precarious alliance among epithelium, immunity and microbiota. *Cell Microbiol* **3:**1-11

McCroarty, J.A. (1993) Probiotic use of lactobacilli in the human female urogenital tract. *FEMS Immunol Med Microbiol* **6:**251-264

McGrady, J.A., Butcher, W.G., Beigthon, D. and Switalski, L.M. (1995) Specific and charge interactions mediate collagen binding by oral lactobacilli. *J Dent Res* **74**:649-657

McLean, N.W. and Rosenstein, I.J. (2000) Characterization and selection of a *Lactobacillus* species to re-colonize the vagina of women with recurrent bacterial vaginosis. *J Med Microbiol* **49**:543-552

Mercenier, A., Pavan, S. and Pot, B. (2003) Probiotics as biotherapeutic agents: present knowledge and future prospects. *Curr Pharmaceut Design* **8**:99-110

Miettinen, M., Matikainen, S., Vuopio-Varkila, J., Pirhonen, J., Varkila, K., Kurimoto, M. and Julkunen, I. (1998) Lactobacilli and Streptococci induce interleukin-12 (IL-12), IL-18, and gamma interferon production in human peripheral blood mononuclear cells. *Infect Immun* **66**:6058-6062

Miettinen, M., Vuopio-Varkila, J. and Varkila, K. (1996) Production of human tumor necrosis factor alpha interleucin-6 and interleucin-10 is induced by lactic acid bacteria. *Infect Immun* **64**:5403-5405

Mignatti, P. and Rifkin, D.B. (1993) Biology and biochemistry of proteinases in tumor invasion. *Physiol Rev* **73**:161-195

Miles, L.A., Dahlberg, C.M., Plescia, J., Felez, J., Kato, K. and Plow, E.F. (1991) Role of cell-surface lysines in plasminogen binding to cells: identification of α-enolase as a candidate plasminogen receptor. *Biochem* **30**:1682-1691

Mitsuoka, T. (1992) The human gastrointestinal tract. *In* The lactic acid bacteria. Vol 1. The lactic acid bacteria in health and disease. (Wood, B.J.B. ed.) pp. 69-114 Elsevier Applied Science, NY, USA

Miyashita, C., Wenzel, E. and Heiden, M. (1988) Plasminogen: a brief introduction into its biochemistry and function. *Haemostasis* **18** suppl.:7-13

Modun, B. and Williams, P. (1999) The staphylococcal transferrin-binding protein is a cell wall glyceraldehyde-3-phosphate dehydrogenase. *Infect Immun* **67**:1086-1092

Moody, G.H. (1982) Plasminogen in human saliva. *Int J Oral Surg* **11**:110-114

Myöhänen, H. and Vaheri, A. (2004) Regulation and interactions in the activation of cell-associated plasminogen. *Cell Mol Life Sci* **61**:2840-2858

Mäyrä-Mäkinen, A., Manninen, M. and Gyllenberg, H. (1983) The adherence of lactic acid bacteria to the columnar epithelial cells of pigs and calves. *J Appl Bacteriol* **55**:241-245

Mölkänen, T., Tyynelä, J., Helin, J., Kalkkinen, N. and Kuusela, P. (2002) Enhanced activation of bound plasminogen on *Staphylococcus aureus* by staphylokinase. *FEBS Lett* **517**:72-78

Naaber, P., Smidt, I., Tamme, K., Liigant, A., Tapfer, H., Mikaelsaar, M. and Talvik, R. (2000) Translocation of indigenous microflora in an experimental model of sepsis. *J Med Microbiol* **49**:432-439

Nagy, E., Fröman, G. and Mårdh, P.-A. (1992) Fibronectin binding of *Lactobacillus* species isolated from women with and without bacterial vaginosis. *J Med Microbiol* **37**:38-42

Nakajima, K., Hamanoue, M., Takemoto, N., Hattori, T., Kato, K. and Kohsaka, S. (1994) Plasminogen binds specifically to α-enolase on rat neuronal plasma membrane. *J Neurochem* **63**:2048-2057

Naveh, M.W., Zusman, T., Skutelsky, E. and Ron E.Z. (1984) Adherence pili in avian strains of *Escherichia coli*: effect on pathogenicity. *Avian Dis* **28**:651-661

Neeser, J.-R., Granato, D., Rouvet, M., Servin, A., Teneberg, S. and Karlsson, K.-A. (2000) *Lactobacillus johnsonii* La1 shares carbohydrate-binding specificities with several enteropathogenic bacteria. *Glycobiol* **10**:1193-1199

Nelson, D., Goldstein, J.M., Boatright, K., Harty, D.W.S., Cook, S.L., Hickman, P.J., Potempa, J., Travis, J. and Mayo, J.A. (2001) pH-regulated secretion of a glyceraldehydes-3-phosphate dehydrogenase from *Streptococcus gordonii* FSS2: Purification, characterization and cloning of the gene encoding this enzyme. *J Dent Res* **80**:371-377

Neser, J.-R., Koellreutter, P., Wuersch, P. (1986) Oligomannoside-type glycopeptides inhibiting adhesion of *Escherichia coli* strains mediated by type 1 pili: preparation of potent inhibitors from plant glycoproteins. *Infect Immun* **52**:428-436

Neutra, M.R., Mantis, N.J., Frey, A. and Giannasca, P.J. (1999) The composition and function of M cell apical membranes: Implications for microbial pathogenesis. *Immunol* **11:**171-181

Nordstrand, A., Shamaei-Tousi, A., Ny, A. and Bergström, S. (2001) Delayed invasion of the kidney and brain by *Borrelia crocidurae* in plasminogen-deficient mice. *Infect Immun* **69**:5832-5839

Norris, T.L. and Bäumler, A.J. (1999) Phase variation of the lpf operon is a mechanism to evade cross-immunity between *Salmonella* serotypes. *Proc Natl Acad Sci* **96**:13393-13398

Oakey, H.J., Harty, D.W. and Knox, K.W. (1995) Enzyme production by lactobacilli and the potential link with infective endocarditis. *J Appl Bacteriol* **78**:142-148

Ofek, I., Mirelman, D. and Sharon, N. (1977) Adherence of *Escherichia coli* to human mucosal cells mediated by mannose receptors. *Nature* **265**:623-625

Oh, C.-W., Hoover-Plow, J. and Plow, E.F. (2003) The role of plasminogen in angiogenesis *in vivo*. *J Thromb Haemost* **1**:1683-1687

Ohyama, S., Harada, T., Chikanishi, T., Miura, Y. and Hasumi, K. (2004) Nonlysine-analog plasminogen modulators promote autoproteolytic generation of plasmin(ogen) fragments with angiostatin-like activity. *Eur J Biochem* **271**:809-820

Olsen, A., Jonsson, A. and Normark, S. (1989) Fibronectin binding mediated by a novel class of surface organelles on *Escherichia coli*. *Nature* **338**:652-655

O´Reilly, M.S., Holmgren, L., Shing, Y., Chen, C., Rosenthal, A., Moses, M., Lane, W.S., Cao, Y., Sage, E.H. and Folkman, J. (1994) Angiostatin: a novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma. *Cell* **79**:315-328

Osset, J., Bartolomé, R.M., García, E. and Andreu, A. (2001) Assessment of the capacity of *Lactobacillus* to inhibit the growth of uropathogens and block their adhesion to vaginal epithelial cell. *J Infect Dis* **183**:485-491

O'Sullivan, D.J. (2000) Methods for analysis of the intestinal flora. *Curr Issues Intest Microbiol* **1**:39-50

Owen, R.L (1999) Uptake and transport of intestinal macromolecules and microorganisms by M cells in Peyer's patches -a personal and historical perspective. *Semin Immunol* 11:157-162

Oyofo, B.A., DeLoach, J.R., Corrier, D.E., Norman, J.O., Ziprin, R.L. and Mollenhauer, H.H. (1989a) Prevention of *Salmonella typhimurium* colonization of broilers with D-mannose. *Poultry Sci* **68**:1357-1360

Oyofo, B.A., Droleskey, R.E., Norman, J.O., Mollenhauer, H.H., Ziprin, R.L., Corrier, D.E. and DeLoach, J.R. (1989b) Inhibition by mannose of *in vitro* colonization of chicken small intestine by *Salmonella typhimurium*. *Poultry Sci* **68**:1351-1356

Paavonen, J. (1983) Physiology and ecology of the vagina. *Scand J Infect Dis Suppl* **40**:31-35

Pabich, W.L., Fihn, S.D., Stamm, W.E., Scholes, D., Boyko, E. and Gupta, K. (2003) Prevalence and determinants of vaginal flora alterations in postmenopausal women. *J Infect Dis* **188**:1054-1058

Pancholi, V. (2001) Multifunctional α-enolase: its role in diseases. *Cell Mol Life Sci* **58**:902-920

Pancholi, V. and Chhatwal, G.S. (2003) Housekeeping enzymes as virulence factors for pathogens. *Int J Med Microbiol* **293**:391-401

Pancholi, V. and Fischetti, V.A. (1992) A major surface protein on Group A Streptococci is a glyceraldehydes-3-phosphate-dehydrogenase with multiple binding activity. *J Exp Med* **176**:415-426

Pancholi, V. and Fischetti, V.A. (1993) Glyceraldehyde-3-phosphate dehydrogenase on the surface of group A streptococci is also an ADP-ribosylating enzyme. *Proc Natl Acad Sci* **90**:8154-8158

Pancholi, V. and Fischetti, V.A. (1998) α-enolase, a novel strong plasmin(ogen) binding protein on the surface of pathogenic streptococci *J Biol Chem* **273**:14503-14515

Pancholi, V., Fontan, P. and Jin, H. (2003) Plasminogen-mediated group A streptococcal adherence to and pericellular invasion of human pharyngeal cells. *Microb Pathogen* **35**:293-303

Parkkinen, J. and Korhonen, T.K. (1989) Binding of plasminogen to *Escherichia coli* adhesion proteins. *FEBS Lett* **250**:437-440

Parry, M.A.A., Zhang, X.C. and Bode, W. (2000) Molecular mechanisms of plasminogen activation: bacterial cofactors provide clues. *Trends Biochem Sci* **25**:53-59

Pascual, M., Hugas, M., Badiola, J.I., Monfort, J.M. and Garriga, M. (1999) *Lactobacillus salivarius* CTC2197 prevents *Salmonella enteritidis* colonization in chickens. *Appl Environ Microbiol* **65**:4981-4986

Patterson, B.C. and Sang, Q.A. (1997) Angiostatin-converting enzyme activities of human matrilysin (MMP-7) and gelatinaseB/type IV collagenase (MMP-9). *J Biol Chem* **272**:28823-28825

Plant, L. and Conway, P. (2001) Association of *Lactobacillus* ssp. with Peyer's patches in mice. *Clin Diag Lab Immunol* **8**:320-324

Pedersen, K. and Tannock, G.W. (1989) Colonization of the porcine gastrointestinal tract by lactobacilli. *Appl Environ Microbiol* **55**:279-283

Perdigón, G., Fuller, R. and Raya, R. (2001) Lactic acid bacteria and their effect on the immune system. *Curr Issues Intest Microbiol* **2:**27-42

Perdigón, G. Medina, M., Vitiñi, E. and Valdez, J.C. (2000) Intestinal pathway of internalization of lactic acid bacteria and gut mucosal immunostimulation. *Int J Immunopath Pharmacol* **13**:141-150

Perdigón, G., Vintiñi, E., Alvaraz, S., Medina, M. and Medici, M. (1999) Study of the possible mechanisms involved in the mucosal immune system activation by lactic acid bacteria. *J Dairy Sci* **82:**1108-1124

Plow, E.F., Herren, T., Redlitz, A., Miles, L.A. and Hoover-Plow, J.L. (1995) The cell biology of the plasminogen system. *FASEB J* **9**:939-945

Plow, E.F., Ploplis, V.A., Carmeliet, P. and Colen, D. (1999) Plasminogen and cell migration *in vivo*. *Fibrinolys Proteolys* **13**:49-53

Pourbakhsh, S.A., Boulianne, M., Martineau-Doizé, B. and Fairbrother, J.M. (1997a) Virulence mechanisms of avian fimbriated *Escherichia coli* in experimentally inoculated chickens. *Vet Microbiol* **58**:195-213

Pourbakhsh, S.A., Dho-Moulin, M., Brée, A., Desautels, C., Martineau-Doizé, B. and Fairbrother, J.M. (1997b) Localization of the in vivo expression of P and F1 fimbriae in chickens experimentally inoculated with pathogenic *Escherichia coli*. *Microb Pathog* **22**:331-341

Pouttu, R., Puustinen, T., Virkola, R., Hacker, J., Klemm, P. and Korhonen, T.K. (1999) Amino acid residue Ala-62 in the FimH fimbrial adhesin is critical for the adhesiveness of meningitis-associated *Escherichia coli* to collagens. *Mol Microbiol* **31**:1747-1757

Pridmore, R.D., Berger, B., Desiere, F., Vilanova, D., Barretto, C., Pittet, A.-C., Zwahlen, M.-C., Rouvet, M., Altermann, E., Barrangou, R., Mollet, B., Mercenier, A., Klaenhammer, T., Arigoni, F. and Schell, M.A. (2004) The genome sequence of the probiotic intestinal bacterium *Lactobacillus johnsonii* NCC 533. *Proc Natl Acad Sci* **101**:2512-2517

Rabijns, A., De Bondt, H.L. and De Ranter, C. (1997) Three-dimensional structure of staphylokinase, a plasminogen activator with therapeutic potential. *Nature Struct Biol* **4**:357-360

Rang, C.U., Licht, T.R., Midvedt, T., Conway, P.L., Chao, L., Krogfelt, K.A., Cohen, P.S. and Molin, S. (1999) Estimation of growth rates of *Escherichia coli* BJ4 in streptomycin-treated and previously germfree mice by in situ rRNA hybridization. *Clin Diagn Lab Immunol* **6**:434-436

Real, F.X., Xu, M., Vilá, M.A. and de Bolós, C. (1992) Intestinal brush-border-associated enzymes: co-ordinated expression in colorectal cancer. *Int J Cancer* **51**:173-181

Redlitz, A., Fowler, B.J., Plow, E.F. and Miles, L.A. (1995) The role of an enolase-related molecule in plasminogen binding to cells. *Eur J Biochem* **227**:407-415

Redondo-Lopez, V., Cook, R.L. and Sobel, J.D. (1990) Emerging role of lactobacilli in the control and maintainance of the vaginal bacterial microflora. *Rev Infect Dis* **12**:856-872

Reid, G. and Bocking, A. (2003) The potential for probiotics to prevent bacterial vaginosis and preterm labor. *Am J Obstet Gynecol* **189**:1202-1208

Reid, G., and Burton, J. (2002) Use of *Lactobacillus* to prevent infection by pathogenic bacteria. *Microbes Infect* **4**:319-324

Reid, G., McGroarty, J.A., Angotti, R. and Cook, R.L. (1988) Lactobacillus inhibitor production against *Escherichia coli* and coaggregation ability with uropathogens. *Can J Microbiol* **34**:344-351

Reid, G., Servin, A.L., Bruce, A.W. and Busscher, H. (1993) Adhesion of three *Lactobacillus* strains to human urinary and epithelial cells. *Microbios* **75**:57-65

Rémy, L., Lissitzky. J.-C., Daemi, N., Jacquier, M.F., Bailly, M., Martin, P.M., Bignon, C. and Doré, J.F. (1992) Laminin expression by two clones isolated from the colon carcinoma cell line LoVo that differ in metastatic potential and basement-membrane organization. *Int J Cancer* **51**:204-212

Rezcallah, M.S., Boyle, M.D. and Sledjeski, D.D. (2004) Mouse skin passage of *Streptococcus pyogenes* results in increased streptokinase expression and activity. *Microbiol* **150**:365-371

Rijken, D.C. (1995) Plasminogen activators and plasminogen activator inhibitors: biochemical aspects. *Baillière's Clin Haematol* **8**:291-312

Roitel, O., Sergienko, E. and Branland, G. (1999) Dimers generated from tetrameric phosphorylating glyceraldehydes-3-phosphate dehydrogenase from *Bacillus stearothermophilus* are inactive but exhibit cooperativity in NAD binding. *Biochem* **38**:16084-16091

Rojas, M., Ascencio, F. and Conway, P.L. (2002) Purification and characterization of a surface protein from *Lactobacillus fermentum* 104R that binds to porcine small intestinal mucus and gastric mucin. *Appl Environ Microbiol* **68**:2330-2336

Rojas, M. and Conway, P.L. (1996) Colonization by lactobacilli of piglet small intestinal mucus. *J Appl Bacteriol* **81**:474-480

Roos, S., Aleljung, P., Robert, N., Lee, B., Wadström, T., Lindberg, M. and Jonsson, H. (1996) A collagen binding protein from *Lactobacillus reuteri* is part of an ABC transporter system? *FEMS Microbiol Lett* **144**:33-38

Roos, S. and Jonsson, H. (2002) A high-molecular-mass cell-surface protein from *Lactobacillus reuteri* 1063 adheres to mucus components. *Microbiol* **148**:433-442

Roos, S., Karner, F., Axelsson, L. and 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

Rosey, E.L., Lincoln, R.A., Ward, P.N., Yancey, R.J. Jr and Leigh, J.A. (1999) PauA: a novel plasminogen activator from *Streptococcus uberis*. *FEMS Microbiol Lett* **178**:27-33

Ross, M.H. and Romrell, L.J. (1989) Histology: A text and atlas. 2nd edit. Williams&Wilkins, Baltimore, USA

Ryu, C.S., Czajka, J.W., Sakamoto, M. and Benno, Y. (2001) Characterization of the *Lactobacillus casei* group and the *Lactobacillus acidophilus* group by automated ribotyping. *Microbiol Immunol* **45**:271-275

Salminen, S. (1996) Uniqueness of probiotic bacteria. *IDF Nutrition Newsletter* **145**:18-19

Salminen, M.K., Rautelin, H., Tynkkynen, S., Poussa, T., Saxelin, M., Valtonen, V. and Järvinen, A. (2004) *Lactobacillus* bacteremia, clinical significance and patient outcome, with special focus on probiotic *L. rhamnosus* GG. *Clin Inf Dis* **38**:62-69

Sambrook, J., Fritsch, E.F., Maniatis, T. (1989) Molecular Cloning: a Laboratory Manual, 2nd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Sanders, M.E. (2003) Probiotics: considerations for human health. *Nutr Rev* **61**:91-99

Santos, R.L. and Bäumler, A.J. (2004) Cell tropism of *Salmonella enterica. Int J Med Microbiol* **294**:225-233

Sarem-Damerdji, L., Sarem, F., Marchal, L. and Nicolas, J.-P. (1995) *In vitro* colonization ability of human colon mucosa by exogenous *Lactobacillus* strains. *FEMS Microbiol Lett* **131**:133-137

Sarra, P.G., Morelli, L. and Bottazzi, V. (1992) The lactic microflora of fowl. Vol 1. The lactic acid bacteria in health and disease. (Wood, B.J.B. ed.) pp. 3-19 Elsevier Applied Science, NY, USA

Schiffrin, E. and Blum, S. (2002) Interactions between the microbiota and the intestinal mucosa. *Eur J Clin Nutr* **56**:S60-S64

Schlott, B., Gührs, K.H., Hartmann, M., Röcker, A. and Collen, D. (1997) Staphylokinase requires NH_2-terminal proteolysis for plasminogen activation. *J Biol Chem* **28**:6067-6072

Schwartz, G.L. (1996) Invasion and metastasis in gastric cancer: *in vitro* and *in vivo* models with clinical correlations. *Sem Oncol* **23**:316-324

Seegers, J.F.M.L. (2002) Lactobacilli as live vaccine delivery vectors: progress and prospects. *Trends Microbiol* **20**:508-515

Seifert, K.N., McArthur, W.P., Bleiweis, A.S. and Brady, L.J. (2003) Characterization of group B streptococcal glyceraldehyde-3-phosphate dehydrogenase: surface localization, enzymatic activity, and protein-protein interactions. *Can J Microbiol* **49**:350-356

Selander, R.K., Korhonen, T.K., Väisänen-Rhen, V., Williams, P.H., Pattison, P.A. and Caugant, D.A. (1986) Genetic relationship and clonal structure of strains of *Escherichia coli* causing neonatal septicaemia and meningitis. *Infect Immun* **52**:213-222

Sgouras, D., Maragkoudakis, P., Petraki, K., Martinez-Gonzalez, B., Eriotou, E., Michopoulos, S., Kalantzopoulos, G., Tsakalidou, E. and Mentis, A. (2004) *In vitro* and *in vivo* inhibition of *Helicobacter pylori* by *Lactobacillus casei* strain Shirota. *Appl Environ Microbiol* **70**:518-526

Sha, J., Galindo, C.L., Pancholi, V., Popov, V.L., Zhao, Y., Houston, C.W. and Chopra, A.K. (2003) Differential expression of the enolase gene under *in vivo* versus *in vitro* growth conditions of *Aeromonas hydrophila*. *Microb Pathog* **34**:195-204

Sharples, G.J. and Lloyd, R.G. (1990) A novel repeated DNA sequence located in the intergenic regions of bacterial chromosomes. *Nuc.Acids Res* **18:** 6503-6508

Shibayama, T., Carboni, J.M. and Mooseker, M.S. (1987) Assembly of the intestinal brush border: Appearance and redistribution of microvillar core proteins in developing chick enterocytes. *J Cell Biol* **105:**335-344

Sieber, A. and Finlay, B.B. (1996) M cells and the pathogenesis of mucosal and systemic infections. *Trends Microbiol* **4**:22-29

Siitonen, S., Vapaatalo, H., Salminen, S., Goldin, A., Saxelin, M., Wikberg, R. and Kirkkola, A.L. (1990) Effect of *Lactobacillus* GG yoghurt in prevention of antibiotic associated diarrhea. *Ann Med* **22**:57-59

Sillanpää, J., Martínez, B., Antikainen, J., Toba, T., Kalkkinen, N., Tankka, S., Lounatmaa, K., Keränen, J., Hook, M., Westerlund-Wikström, B., Pouwels, P.H. and Korhonen, T.K. (2000) Characterization of the collagen-binding S-layer protein CbsA of *Lactobacillus crispatus. J Bacteriol* **182**:6440-6450

Silvester, M.E. and Dicks, L.M.T. (2003) Identification of lactic acid bacteria isolated from human vaginal secretions. *Antonie van Leeuwenhoek* **83**:117-123

Sjöbring, U., Pohl, G. and Olsén, A. (1994) Plasminogen, absorbed by *Escherichia coli* expressing curli or by *Salmonella enteritidis* expressing thin aggregative fimbriae, can be activated by simultaneously captured tissue-type plasminogen activator (tPA). *Mol Microbiol* **14**:443-452

Sjöström, I., Gröndahl, H., Falk, G., Kronvall, G. and Ullberg, M. (1997) Purification and characterisation of a plasminogen-binding protein from *Haemophilus influenzae*. Sequence determination reveals identity with aspartase. *Biochem Biophys Acta* **1324**:182-190

Skerman, V. B., McGowan, D.V. and Sneath, P.A.H. (1980) Approved lists of bacterial names. *Int J Syst Bacteriol* **30**:225-420

Skurnik, M., Peippo, A. and Ervelä, E. (2000) Characterization of the O-antigen gene cluster of *Yersinia pestis* shows that the plague bacillus is most closely related to and has evolved from *Y. pseudotuberculosis* serotype O:1b. *Mol Microbiol* **37**:316-330

Sleytr, U.B. and Messner, P. (1988) Crystalline surface layers in procaryoties. *J Bacteriol* **170**:2891-2897

Sodeinde, O.A. and Goguen, J.D. (1988) Genetic analysis of the 9.5-kilobase virulence plasmid of *Yersinia pestis*. *Infect Immun* **56**:2743-2748

Sodeinde, O.A. Subrahmanyam, Y.V.B.K., Stark, K., Quan, T., Bao, Y. and Goguen, J.D. (1992) A surface protease and the invasive character of plague. *Science* **258**:1004-1007

Song, Y.-L., Kato, N., Matsumiya, Y., Liu, C.-X., Kato, H. and Watanabe, K. (1999) Identification of and hydrogen peroxide production by fecal and vaginal lactobacilli isolated from Japanese women and newborn infants. *J Clin Microbiol* **37**:3062-3064

Stack, M.S., Gately, S., Bafetti, L.M., Enghild, J.J. and Soff, G.A. (1999) Angiostatin inhibits endothelial and melanoma cellular invasion by blocking matrix-enhanced plasminogen activation. *Biochem J* **340**:77-84

St. Amant, D.C., Valentin-Bon, I.E. and Jerse, A.E. (2002) Inhibition of *Neisseria gonorrhoeae* by *Lactobacillus* species that are commonly isolated from the female genital tract *Infect Immun* **70**:7169-7171

Stathakis, P., Fitzgerald, M., Matthias, L.J., Chesterman, C.N. and Hogg, P.J. (1997) Generation of angiostatin by reduction and proteolysis of plasmin. *J Biol Chem* **272**:20641-20645

Stathakis, P., Lay, A.J., Fitzgerald, M., Schlieker, C., Matthias, L.J. and Hogg, P.J. (1999) Angiostatin formation involves disulfide bond reduction and proteolysis in kringle 5 of plasmin. *J Biol Chem* **274**:8910-8916

Stavric, S., Gleeson, T.M., Buchanan, B. and Blanchfield, B. (1992) Experience of the use of probiotics for *Salmonella* control in poultry. *Lett Appl Microbiol* **14**:69-71

Stojiljkovic, I., Bäumler, A.J. and Heffron, F. (1995) Enthanolamine utilization in *Salmonella typhimurium*: nucleotide sequence, protein expression, and mutational analysis of the cchA cchB eutE eutH gene cluster. *J Bacteriol* **177**:1357-1366

Stordeur, P., Marlier, D., Blanco, J., Oswald, E., Biet, F., Dho-Moulin, M. and Mainil, J. (2002) Examination of *Escherichia coli* from poultry for selected adhesin genes important in disease caused by mammalian pathogenic *E. coli*. *Vet Microbiol* **84**:231-241

Styriak, I., Nemcová, R., Chang, Y.-H. and Ljungh, Å. (2003) Binding of extracellular matrix molecules by probiotic bacteria. *Lett Appl Microbiol* **37**:329-333

Suau, A., Bonnet, R., Sutren, M., Godon, J.-J., Gibson, G.R., Collins, M.D. and Dore, J. (1999) Direct analysis of genes encoding 16S rRNA from complex communities reveals many molecular species within the human gut. *Appl Environ Microbiol* **65**:4799-4807

Suffredini, A.F., Halper, P.C. and Parrillo, J.E. (1989) Promotion and subsequent inhibition of plasminogen activation after admistration of intravenous endotoxin to normal subjects. *New Engl J Med* **320**:1165-1172

Sun, H., Ringdahl, U., Homeister, J.W., Fay, W.P., Engleberg, N.C., Yang, A.Y., Rozek, L.S., Wang, X., Sjöbring, U. and Ginsburg, D. (2004) Plasminogen is a critical host pathogenicity factor for group A streptococcal infection. *Science* **305**:1283-1286

Svensson, M.D., Sjöbring, U. and Bessen, D.E. (1999) Selective distribution of a high-affinity plasminogen-binding site among group A streptococci associated with impetigo. *Infect Immun* **67**:3915-3920

Svensson, M.D., Sjöbring, U., Luo, F. and Bessen, D.E. (2002) Roles of the plasminogen activator streptokinase and the plasminogen-associated M protein in an experimental model for streptococcal impetigo. *Microbiol* **148**:3933-3945

Tannock, G.W. (1992) The lactic microflora of pigs, mice and rats. *In* The lactic acid bacteria. Vol 1. The lactic acid bacteria in health and disease. (Wood, B.J.B. ed.) pp. 21-48 Elsevier Applied Science, NY, USA

Tannock, G.W. (1999) Analysis of the intestinal microflora: a renaissance. *Antonie van Leeuwenhok* **76**:265-278

Thankavel, K., Shah, A.H., Cohen, M.S., Ikeda, T., Lorenz, R.G., Curtiss, R. and Abraham, S.N. (1999) Molecular basis for the enterocyte tropism exhibited by *Salmonella typhimurium* type 1 fimbriae. *J Biol Chem* **274**:5797-5809

Toba, T., Virkola, R., Westerlund, B., Björkman, Y., Sillanpää, J., Vartio, T., Kalkkinen, N. and Korhonen, T.K. (1995) A collagen-binding S-layer protein in *Lactobacillus crispatus. Appl Environ Microbiol* **61**:2467-2471

Todoroki, K., Mukai, T., Soto, S. and Toba, T. (2001) Inhibition of adhesion of food-borne pathogens to Caco-2 cells by *Lactobacillus* strains. *J Appl Microbiol* **91**:1-6

Townsend, S.M., Kramer, N.E., Edwards, R., Baker, S., Hamlin, N., Simmonds, M., Stevens, K., Maloy, S., Parkhill, J., Dougan, G. and Bäumler, A.J. (2001) *Salmonella enterica* serotype Typhi possesses a unique repertoire of fimbrial gene sequences. *Infect Immun* **69**:2894-2901

Travis, J. and Salvesen, G.S. (1982) Human plasma proteinase inhibitors. *Ann Rev Biochem* **52**:655-709

Tuohy, K.M., Probert, H.M., Smejkal, C.W. and Gibson, G.R. (2003) Using probiotics and prebiotics to improve gut healt. *Drug Disc Today* **8**:692-700

Tuomola, E.M., Ouwehand, A.C. and Salminen, S.J. (1999) Human ileostomy glycoproteins as a model for small intestinal mucus to investigate adhesion of probiotics. *Lett Appl Microbiol* **28**:159-163

Ullberg, M., Kronvall, G., Karlsson, I. and Wiman, B. (1990) Receptors for human plasminogen on Gram-negative bacteria. *Infect Immun* **58**:21-25

Ullberg, M., Kuusela, P., Kristiansen, B.E. and Kronvall, G. (1992) Binding of plasminogen to *Neisseria meningitidis* and *Neisseria gonorrhoeae* and formation of surface-associated plasmin. *J Infect Dis* **166**:1329-1334

Urdaneta, L., Vanegas, G., Premoli, G. and Avilan, L. (2004) Plasminogen interaction and activation on *Streptococcus mutans* surface. *Oral Microbiol Immunol* **19**:257-261

Vaarala, O. (2003) Immunological effects of probiotics with special reference to lactobacilli. *Clin Exp Allergy* **33**:1634-1640

Van den Bosch, J.F., Hendriks, J.H., Gladigau, I., Willems, H.M., Storm, P.K. and deGraaf, F.K. (1993) Identification of F11 fimbriae on chicken *Escherichia coli* strains. *Infect Immun* **61**:800-806

Vásquez, A., Jakobsson, T., Ahrné, S., Forsum, U. and Molin, G. (2002) Vaginal *Lactobacillus* flora of healthy Swedish women. *J Clin Microbiol* **40**:2746-2749

Vaughan, E.E., de Vries, M.C., Zoeteldal, E.G., Ben-Amor, K., Akkermans, A.D.L. and de Vos, W.M. (2002) The intestinal LABs. *Antonie van Leeuwenhoek* **82**:341-352

Ventura, M., Canchaya, C., Pridmore, D. and Brüssow, H. (2004) The prophage of *Lactobacillus johnsonii* NCC 533: comparative genomics and transcriptional analysis. *Virology* **320**:229-242

Versalovic, J., Koeuth, T. and Lupski, R. (1991) Distribution of repetitive DNA sequences in eubacteria and application to fingerprint of bacterial genomes. *Nuc Acid Res* **19**:6823-6831

Vidotto, M.C., Navarro, H.R. and Gaziri, L.C.J. (1997) Adherence pili of pathogenic strains of avian *Escherichia coli*. *Vet Microbiol* **59**:79-87

Virkola, R., Lähteenmäki, K., Eberhard, T., Kuusela, P., van Alphen, L., Ullberg, M. and Korhonen, T.K. (1996) Interaction of *Haemophilus influenzae* with the mammalian extracellular matrix. *J Infect Dis* **173**:1137-1147

Wadolkowski, E.A.., Laux, D.C. and Cohen, P.S. (1988) Colonization of the streptomycin-treated mouse large intestine by a human fecal *Escherichia coli* strain: role of adhesion to mucosal receptors. *Infect Immun* **56:**1036-1043

Wall, T., Roos, S., Jacobsson, K., Rosander, A. and Jonsson, H. (2003) Phage display revelas 52 novel extracellular and transmembrane proteins from *Lactobacillus reuteri* DSM20016^{T}. *Microbiol* **149**:3493-3505

Wang, X., Lin, X., Loy, J.A., Tang, J. and Zhang, X.C. (1998) Crystal structure of the catalytic domain of human plasmin complexed with streptokinase. *Science* **281**:1662-1665

Wang, H., Schultz, R., Hong, J., Cundiff, D.L., Jiang, K. and Soff, G.A. (2004) Cell surface-dependent generation of angiostatin4.5. *Cancer Res* **64**:162-168

Ward, P.N., Field, T.R., Rosey, E.L., Abu-Median, A.-B., Lincoln, R.A. and Leigh, J.A. (2004) Complex interactions between bovine plasminogen and streptococcal plasminogen activator PauA. *J Mol Biol* **342**:1101-1114

Ward, P.N. and Leigh, J.A. (2002) Characterization of PauB, a novel broad-spectrum plasminogen activator from *Streptococcus uberis*. *J Bacteriol* **184**:119-125

Ward, P.N. and Leigh, J.A. (2004) Genetic analysis of *Streptococcus uberis* plasminogen activators. *Indian J Med Res* **119**S:136-140

Williams, L.A., Ding, L., Horwitz, J. and Piatigorsky, J. (1985) tau-Crystallin from the turtle lens: purification and partial characterization. *Exp Eye Res* **40**:741-749

Winram, S.B. and Lottenberg, R. (1998) Site-directed mutagenesis of streptococcal plasmin receptor protein (Plr) identifies the C-terminal Lys^{334} as essential for plasmin binding, but mutation of the *plr* gene does not reduce plasmin binding to group A streptococci. *Microbiol* **144**:2025-2035

Wistedt, A.C., Ringdahl, U., Müller-Esterl, W. and Sjöbring, U. (1995) Identification of a plasminogen-binding motif in PAM, a bacterial surface protein. *Mol Microbiol* **18**:569-578

Wistow, G.J., Lietman, T., Williams, L.A., Stapel, S.O., de Jong, W.W., Horwitz, J. and Piatigorsky, J. (1988) τ-Crystallin/α-enolase: one gene encodes both an enzyme and a lens structural protein. *J Cell Biol* **107**:2729-2736

Weiser, M.M., Walters, J.R.F. and Wilson, J.R. (1986) Intestinal cell membranes. *Int Rev Cytol* **101**:1-57

Westerlund, B. and Korhonen T.K. (1993) Bacterial proteins binding to the mammalian extracellular matrix. *Mol Microbiol* **9**:687-694

Wooley, R.E., Spears, K.R., Brown, J., Nolan, L.K. and Fletcher, O.J. (1992) Relationship of complement resistance and selected virulence factors in pathogenic avian *Escherichia coli*. *Avian Dis* **36**:679-684

Yerushalmi, Z., Smorodinsky, N.I., Naveh, M.W. and Ron, E.Z. (1990) Adherence pili of avian strains of *Escherichia coli* O78. *Infect Immun* **58**:1129-1131

Young, K.C., Shi, G.Y., Wu, D.H., Chang, L.C., Chang, B.I., Ou, C.P. and Wu, H.L. (1998) Plasminogen activation by streptokinase via unique mechanisms. *J Biol Chem* **273**:3110-3116

Yuki, N., Shimazaki, T., Kushiro, A., Watanabe, K., Uchida, K., Yuyama, T. and Morotomi, M. (2000) Colonization of the stratified squamous epithelium of the nonsecreting area of horse stomach by lactobacilli. *Appl Environ Microbiol* **66**:5030-5034

Zhao, T., Doyle, M.P., Harmon, B.G., Brown, C.A., Mueller, P.O.E. and Parks, A.H. (1998) Reduction of carriage of enteric *Escherichia coli* O157:H7 in cattle by inoculation with probiotic bacteria. *J Clin Microbiol* **36**:641-647

Zhou, X., Bent, S.J., Schneider, M.G., Davis, C.C., Islam, M.R. and Forney, L.J. (2004) Characterization of vaginal microbial communities in adult healthy women using cultivation-independent methods. *Microbiol* **150**:2565-2573

Zucker, S. and Vacirca, J. (2004) Role of matrix metalloproteinases (MMPs) in colorectal cancer. *Cancer Metastasis Rev* **23**:101-117