SUMMARY

Lactobacilli, generally known as lactic acid bacteria, inhabit the gastrointestinal tract of healthy mammals and birds and are the major indigenous microorganisms inhabiting the mammalian urogenital tract. More than a century ago, researchers began to link the presence of lactobacilli in the gastrointestinal tract to the maintenance of a healthy host. In the 1890s, Metchnikoff was one of the first to acknowledge the importance of bacterial interference, i.e. the interaction of the protective indigenous microorganisms with invading pathogens, in keeping the host from infection. He propagated the ingestion of lactobacilli for restoration of the normal gastrointestinal microflora. The first report of *Lactobacillus* therapy for the treatment of bladder infection dates from 1915 by Newman.

The interest in bacteriotherapy faded with the advent of antibiotics in the 1940s. However, once it was realized in the 1960s that antibiotics alone are not the solution to infectious diseases, research into the use of live lactobacilli and other probiotic microorganisms for the prevention and treatment of infections revived. Currently, evidence is accumulating that the administration of selected microorganisms is beneficial in the treatment and prevention of certain intestinal and, possibly, urogenital infections. The mechanisms by which autochthonous lactobacilli can interfere with uropathogen adhesion to uroepithelial cells and catheter materials and, in this way, protect the host from acquiring urinary tract infection are discussed in **Chapter 1**. One possible mechanism of bacterial interference in microbial adhesion to surfaces that has recently been suggested in a few studies includes the involvement of so-called biosurfactants, surface-active compounds released by microorganisms. Based on these studies, we came to the following formulation of the goals of this thesis. Firstly, we wanted to establish whether lactobacilli release biosurfactants and, as this proved to be true, to provide a physico-chemical and biochemical characterization of the compounds released. Secondly, we intended to determine the potential role of *Lactobacillus* biosurfactants as antiadhesive, non-antibiotic coatings on catheter surfaces, such as silicone rubber.

In **Chapter 2**, 15 *Lactobacillus* isolates were screened for biosurfactant production by ADSA-P (axisymmetric drop shape analysis by profile), a rapid technique for measuring microbial biosurfactant production, based on a shape analysis of an axisymmetric droplet of a microbial suspension on a hydrophobic substratum. All strains were found to produce biosurfactants in the mid-exponential and stationary growth phases. The stationary phase biosurfactants from *Lactobacillus casei* subsp.

rhamnosus 36 and ATCC 7469, *Lactobacillus fermentum* B54, and *Lactobacillus acidophilus* RC14 were investigated further to determine their capacity to inhibit the initial adhesion of uropathogenic *Enterococcus faecalis* 1131. Using a parallel-plate flow chamber, *E. faecalis* adhesion to glass with adsorbed biosurfactant layers was followed *in situ* by automated image analysis. Adsorbed biosurfactant layers from *L. acidophilus* RC14 and *L. fermentum* B54 on glass significantly inhibited the initial deposition rate and number of adhering *E. faecalis* after 4 h of adhesion, whereas those from *L. casei* subsp. *rhamnosus* 36 and ATCC 7469 did not. Fourier transform infrared spectroscopy and X-ray photoelectron spectroscopy showed that the freeze-dried biosurfactants from *L. acidophilus* RC14 and *L. fermentum* B54 were richest in protein, while those from *L. casei* subsp. *rhamnosus* 36 and ATCC 7469 had relatively high polysaccharide and phosphate contents. This suggests that the substance responsible for the inhibition of *E. faecalis* adhesion in these biosurfactants is protein-like.

In **Chapter 3**, the biosurfactants from *L. casei* subsp. *rhamnosus* 36 and ATCC 7469, *L. fermentum* B54 and *L. acidophilus* RC14 were isolated from bacteria in the mid-exponential (4-5 h) and stationary growth phases (18 h) and physico-chemical and biochemical properties of the freeze-dried biosurfactants from both growth phases were compared. The mid-exponential and stationary phase biosurfactants were similar in their surface activities, but the latter had a lower and better defined critical micelle concentration. In particular, the stationary phase biosurfactant from *L. acidophilus* RC14 reached a low liquid surface tension of 39 mJ m^{-2} in phosphate-buffered saline, with a critical micelle concentration of 1.0 mg ml^{-1}. All biosurfactants consisted of a mixture of protein and polysaccharides, possibly containing bound phosphate-groups, but the stationary phase biosurfactants were richest in protein, as concluded from Fourier transform infrared spectroscopy on biosurfactants in KBr-pellets and from X-ray photoelectron spectroscopy on biosurfactants deposited on gold-coated glass slides. For the protein-rich stationary phase biosurfactants that are released by certain *Lactobacillus* strains, such as *L. fermentum* B54 and *L. acidophilus* RC14, and that have been demonstrated to interfere with enterococcal adhesion to glass (**Chapter 2**), the name "surlactin" was proposed.

In **Chapter 4**, the potency of the *L. acidophilus* RC14 biosurfactant surlactin to reduce the initial adhesion of uropathogenic *E. faecalis* 1131 was investigated on a hydrophilic and a hydrophobic substratum in a parallel-plate flow chamber, using phosphate-buffered saline and pooled human urine as a suspending fluid. A parallel-plate flow chamber with a glass or silicone rubber bottom plate, was filled with different biosurfactant solutions of 0, 0.01, 0.1 or 1.0 mg ml^{-1} for overnight

adsorption (18 h). Subsequently, the adhesion of *E. faecalis* on the prepared biosurfactant layers was observed *in situ* by automated image analysis. Adsorbed biosurfactant layers caused an important, dose-related inhibition of the initial deposition rate of *E. faecalis* and the number of adherent bacteria after 4 h on both hydrophilic glass and hydrophobic silicone rubber, although this effect was stronger in buffer than in urine. For the experiments carried out in urine, the inhibitory effect of the biosurfactant layer was largest when silicone rubber was used rather than glass, while no influence of the substratum hydrophobicity on the inhibition of *E. faecalis* adhesion was noticed for experiments performed in buffer.

In **Chapter 5**, the ability of the *L. acidophilus* RC14 biosurfactant surlactin to inhibit initial microbial adhesion to silicone rubber was tested against a variety of uropathogenic bacteria and two yeast strains in a parallel-plate flow chamber in the presence of filter-sterilized pooled human urine. A parallel-plate flow chamber with a silicone rubber bottom plate was filled with a 1.0 mg ml^{-1} biosurfactant solution for adsorption overnight (18 h). Subsequently, microbial adhesion from a urine suspension to the prepared biosurfactant layers was followed *in situ* by automated image analysis. Controls were carried out on clean silicone rubber. Surlactin layers caused a marked inhibition of the initial deposition rates and adhesion numbers after 4 h for the majority of the bacteria (11 out of 15 strains tested) and this inhibition was particularly effective against *E. faecalis*, *Escherichia coli*, and *Staphylococcus epidermidis* species. Although the initial deposition rates of the two *Candida albicans* strains were reduced by approximately 50% in comparison with the controls, the numbers of adhering yeast cells after 4 h were nearly equal. This study shows that the biosurfactant surlactin has a potential as an antiadhesive coating for catheter materials by delaying the onset of biofilm growth, and the results support the idea of fighting infections by the reintroduction of indigenous biosurfactant-releasing lactobacilli into their natural environment.

In **Chapter 6**, the influence of the biosurfactant-releasing strain *L. fermentum* B54 on the development of a uropathogenic biofilm on silicone rubber disks was examined in urine from 9 healthy volunteers of both sexes. Per test person, 2 sterile containers with 3 silicone rubber disks were filled with either fresh mid-stream morning urine (control), or with urine supplemented with 10^{10} cells ml^{-1} of *L. fermentum* B54. The containers were incubated at 37°C in an atmosphere containing 5% CO_2 to stimulate *Lactobacillus* growth. Urine and lactobacilli were replaced daily. After 2, 4, and 8 days of incubation, a control and a treated silicone rubber disk were isolated and the numbers and types of uropathogenic bacteria growing on each disk were determined by dilution plating and microscopic evaluation respectively. For all 5 men included in

the study, the numbers of uropathogens recovered from the silicone rubber disks after 8 days were markedly decreased by *L. fermentum* B54, albeit to various extents. A complete inhibition was seen in 2 cases, while for the other 3 men the inhibition was partial. In contrast, no clear reduction in uropathogen growth was observed when female urine was used. From thè fact that the inhibition of uropathogen growth by *L. fermentum* B54 was different for male and female urine, it can be concluded that besides the properties of the *Lactobacillus* strain itself also other factors influence its potential to interfere with pathogenic species.

In the **General discussion**, we have discussed a couple of questions that arose during the project and that are worth being further investigated, such as for instance the stability of the *Lactobacillus* biosurfactants under clinical conditions.

In conclusion, this thesis has demonstrated that surlactin, a proteinaceous biosurfactant released by certain strains of lactobacilli, can inhibit the adhesion of various uropathogens to hydrophobic (silicone rubber) and hydrophilic (glass) substrata. We therefore feel that surlactin can play a role in the development of antiadhesive coatings for catheter surfaces by delaying the onset of biofilm growth. In addition, the results support the use of probiotic biosurfactant-releasing *Lactobacillus* strains in the development of non-antibiotic alternatives to the treatment or prevention of urinary tract infection.