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Staphylococcus epidermidis is a nosocomial pathogen, associated with infections of indwelling medical devices. The ability to adhere and form biofilms on biomaterial surfaces contributes to its virulence. Biofilm formation of *S. epidermidis* is influenced by the presence of polysaccharide intercellular adhesin, synthesized by proteins encoded in the *icaADBC* operon.

Regulatory network controlling expression of *icaADBC* and other factors that are reportedly involved in biofilm formation by *S. epidermidis* have been described in **Chapter 1**. Further, the role of physico-chemical surfaces properties and biocides in adhesion and biofilm formation are discussed. The main aim of this thesis was to investigate the regulation of *icaADBC* gene expression during biofilm formation of clinical *S. epidermidis* isolates on different biomaterials and how environmental signals affect this regulation.

Expression of *ica* is subject to phenotypic variation, which can result in heterogeneity in surface characteristics of individual bacteria in axenic cultures. In **Chapter 2**, the relationship between phenotypic variation of clinical isolates of *S. epidermidis*, biofilm formation and electrophoretic mobility distribution was investigated. Five clinical *S. epidermidis* isolates demonstrated phenotypic variation, i.e. both black and red colonies on Congo Red Agar. Black colonies displayed bi-modal electrophoretic mobility distributions at pH 2, but such phenotypic variation was absent in red colonies of the same strain as well as in control strains without phenotypic variation. All red colonies had lost *ica* and the ability to form biofilms, in contrast to black colonies of the same strain. Real time PCR targeting *icaA* indicated a reduction in gene copy number within cultures exhibiting phenotypic variation, which correlated quantitatively with phenotypic variations on Congo Red Agar and electrophoretic mobility distribution. Strikingly, loss of *ica* was irreversible and independent of the mobile element IS256, indicating a new, unknown mechanism of phenotypic variation in clinical *S. epidermidis* isolates.

Phenotypic variation of *S. epidermidis* involving the slime related *ica*-operon results in heterogeneity in surface characteristics of individual bacteria in axenic cultures. As described in **Chapter 2**, we found that in clinical *S. epidermidis* isolates, loss of *ica* was irreversible and independent of the mobile element IS256. Therefore in **Chapter 3**, we investigated the role of LexA and RecA in the observed irreversible switching from ica-positive to *ica*-negative in clinical *S. epidermidis* isolates. In high frequency *S. epidermidis* switching strains, spontaneous mutations in *lexA* were found which resulted in deregulation of *recA* expression, as shown by real time PCR. RecA is involved in genetic deletions and rearrangements and we postulate a model representing a new mechanism of phenotypic variation in clinical isolates of *S. epidermidis*. This is the first report of *S. epidermidis* strains irreversibly switching from *ica*-positive to *ica*-negative phenotype by spontaneous deletion of *icaADBC*, which represents a new mechanism of phenotypic variation.

S. epidermidis is notorious for its biofilm formation on medical devices and novel approaches to prevent and kill *S. epidermidis* biofilms are desired. In **Chapter 4**, the effect of cinnamon oil on planktonic and biofilm cultures of clinical *S. epidermidis* isolates was evaluated. Initially, susceptibility to cinnamon oil in planktonic cultures was compared to commonly-used antimicrobial agents: chlorhexidine, triclosan and gentamicin. The minimal inhibitory concentration (MIC) of cinnamon oil, defined as the lowest concentration able to inhibit visible microbial growth, and the minimal bactericidal concentration (MBC), the lowest concentration required to kill 99.9 % of the bacteria were determined using the microbroth dilution method and plating on agar. A chequerboard assay was used to evaluate a possible synergy between cinnamon oil and the other antimicrobial agents. The effect of cinnamon oil on biofilm growth was studied in 96 wells plates and with confocal laser scanning microscopy (CLSM). Biofilm susceptibility was determined using a metabolic MTT-assay. Real time PCR analysis was performed to determine the effect of sub-MIC concentrations of cinnamon oil on expression of the biofilm related gene, *icaA*. Cinnamon oil showed antimicrobial activity against

both planktonic and biofilm cultures of clinical *S. epidermidis* strains. There was only a small difference between planktonic and biofilm MIC, ranging from 0.5-1% and 1-2%, respectively. CLSM images indicated that cinnamon oil is able to detach and kill existing biofilms. Thus, cinnamon oil is an effective antimicrobial agent to combat *S. epidermidis* biofilms.

The influence of the *ica*-operon in *S. epidermidis* strains on its cell surface hydrophobicity, thermodynamics of adhesion, and biofilm formation was evaluated in **Chapter 5**. Lifshitz-Van der Waals and acid-base surface free energies of the bacterial and TC-PS (tissue-culture-polystyrene) surfaces were determined using contact angles, while biofilm formation was assayed using crystal violet staining. *Ica*-positive strains were more hydrophobic due to a smaller electron-donating surface free energy parameter than *ica*-negative strains. In addition, interaction of *ica*-positive strains with the TC-PS surface was energetically favourable ($\Delta G_{adh} < 0$), in contrast to the interaction of *ica*-negative strains, which was energetically unfavourable ($\Delta G_{adh} > 0$). In line, more biofilm was formed by the *ica*-positive strains than by the *ica*-negative strains, although CLSM analysis showed that slime was not necessarily directly involved in contact with the TC-PS.

S. epidermidis generates a polysaccharide intercellular adhesin (PIA), that facilitates bacterial cell aggregation and colonisation of biomaterial implants, which is regulated by the *icaADBC* operon. *Ica*-expression depends on environmental conditions that may include the implant biomaterial and the presence of antibiotics. **Chapter 6** is aimed to evaluate biofilm formation and *ica*-expression of four *S. epidermidis* strains on different biomaterials involved in total hip- and knee arthroplasty (polyethylene: PE, polymethylmethacrylate: PMMA and stainless steel: SS). Secondly, *ica*-expression in biofilms on the different biomaterial surfaces was related to the susceptibility of the biofilms to gentamicin. *Ica*-expression, assayed using real-time RT-PCR, was highest on PE, as confirmed using CLSM. Yet biofilm formation by *S. epidermidis* was most extensive on SS, with less slime production. *Ica*-expression and slime production were minimal on PMMA. After 3 h continued growth of 24 h old biofilms in the presence of gentamicin, biofilms on PE showed

lower susceptibility to gentamicin relative to the other materials, presumably as a result of the stronger *ica*-expression. A higher gentamicin concentration further decreased metabolic activity on all biomaterials. It is concluded that the level of biomaterial-induced *ica*-expression does not correlate with the amount of biofilm formed, but initially aids bacteria in surviving antibiotic attacks. Once antibiotic treatment has started however, also the antibiotic itself induces slime production and if its concentration is high enough, killing results. Results suggest that biomaterial-associated infections in orthopedics by *S. epidermidis* on PE may be more difficult to eradicate than on PMMA or SS.

The results of this thesis are discussed in **Chapter 7**. Here we present an explanation of why a large proportion of clinical *S. epidermidis* strains isolated from biofilm-associated infections appears *ica*-negative once grown planktonically. Additionally, the presence of gentamicin, as well as cinnamon oil, is suggested to be a co-regulator of *ica*-expression and slime production in *S. epidermidis* isolates together with the substratum. Therefore, development of a modified biomaterial is proposed that does not induce *ica*-expression, reduces the tendency for staphylococci to form a biofilm and the level of antibiotic resistance.