Ia. Summary

Biofilm production is an important step in the pathogenesis of *S. epidermidis* polymer-associated infections and depends on the expression of the *ica*ADBC operon leading to the synthesis of a polysaccharide intercellular adhesin (PIA). The PIA represents a sugar polymer consisting of ß-1,6 linked N-acetyl glucosaminoglycans and mediates the intercellular adherence of the bacteria to each other and the accumulation of a multilayered biofilm. Epidemiological and experimental studies strongly suggest that PIA-production and subsequently biofilm formation contributes significantly to the virulence of specific *S. epidermidis* strains.

This work aimed on the investigation of external factors regulating the *ica* expression in *S. epidermidis*. For this purpose, a reporter gene fusion between the *ica* promoter and the beta-galactosidase gene *lac*Z from *E. coli* was constructed and integrated into the chromosome of an *ica* positive *S. epidermidis* clinical isolate. The reporter gene fusion was used to investigate the influence of external factors and of sub-MICs of different antibiotics on the *ica* expression. It was shown that the *S. epidermidis* biofilm formation is growth phase dependent with a maximum expression in the late logarithmic and early stationary growth phase. The optimal expression was recorded at 42 °C at a neutral pH ranging from 7.0 to 7.5. The glucose content of the medium was found to be essential for biofilm formation, since concentrations of 1.5 to 2 % glucose induced the *ica* expression. In addition, external stress factors as high osmolarity (mediated by 3 to 5 % sodium chloride), and sub-lethal concentrations of detergents, ethanol, hydrogene peroxide, and urea significantly enhanced the biofilm production.

Subinhibitory concentrations of tetracyline, the semisynthetic streptogramin quinupristin/dalfopristin and the streptogramin growth promoter virginiamycin were found to enhance the *ica* expression 8 to 11-fold, respectively, whereas penicillin, oxacillin, gentamicin, clindamycin, vancomycin, teicoplanin, ofloxacin, and chloramphenicol had no effects. A weak induction was recorded for sub-MICs of erythromycin. Both quinupristin/ dalfopristin and tetracyline exhibited a strong postexposure effect on the *S. epidermidis ica* expression, respectively, even when the substances were immediately removed from the growth medium. The results were confirmed by Northern blot analysis of the *ica* transcription and quantitative analysis of biofilm formation in a colorimetric assay.

Expression of the *ica*$_{prom}$*::lacZ* reporter gene plasmid in *Bacillus subtilis* and *S. epidermidis* revealed that the *ica* induction by sub-MICs of streptogramins and tetracycline might depend on unidentified regulatory elements which are specific for the staphylococcal cell. In contrast, the activation by external stress signals seems to be mediated by factors which are present both in Staphylococci and in *Bacillus subtilis*. Construction and analysis of an *agr*-mutant in a biofilm-forming *S. epidermidis* strain excluded the possibility that the Agr-quorum-sensing system significantly contributes to the *ica* expression in the stationary growth phase. However, clear evidence was provided that in *S. aureus* the *ica* transcription depends on the expression of the alternative transcription factor sigmaB, which represents a global regulator of the stress response in *S. aureus* as well as in *B. subtilis*. For this purpose, a sigB knockout mutant had been constructed in a biofilm-forming *S. aureus*. This mutant showed a markedly decrease of the *ica* transcription and biofilm-production, whereas a complement strain carrying the *sig*B gene on an expression vector completely restored the biofilm-forming phenotype of the *S. aureus* wild type.

Southern blot analysis indicated that the the *sig*B gene is also present in *S. epidermidis* and Northern analyses of the *sig*B and the *ica* transcription revealed that both genes are activated under identical conditions (i. e. in the stationary growth phase and by external stress factors) suggesting a similar regulatory pathway as in *S. aureus*. However, since neither in *S. aureus* nor in *S. epidermidis* the *ica* promoter has obvious similiarities to known SigB-dependent promotoer sequences it is tempting to speculate that the *ica* activation is not directely mediated by SigB, but might be indirectely controlled by other SigB-dependent regulatory elements which remain to be elucidated.