Adaptive gene expression of endocarditis-causing viridans group streptococci and Staphilococcus aureus
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CHAPTER 1

General Introduction
Infective endocarditis (IE) has long been recognized as an important human endocardial disease (1). In the preantibiotic era this disease always had a fatal outcome. After the discovery of penicillin in the late 1920s the mortality rates have dropped drastically. However, despite the availability of a broad range of different antibiotics and of novel surgical techniques nowadays, morbidity and mortality remain high.

The classification of IE as acute, subacute, or chronic, indicating the interval between the start of the clinical manifestations and death, dates back to the preantibiotic era. Acute endocarditis was defined as a rapid fatal disease with death occurring within several weeks, and was mostly caused by virulent microorganisms, including *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Streptococcus pyogenes*. Subacute and chronic endocarditis, mostly caused by viridans group streptococci, had a more indolent disease progression. However, objections to this classification, including the largely arbitrary division based on the duration of the illness, have resulted in new classification schemes. At present, IE is classified as native valve endocarditis (NVE), prosthetic valve endocarditis (PVE), or endocarditis in intravenous drug abusers (IVDU), and the microorganism involved defines the type of IE. Disease definition is mainly based on clinical and pathological findings, which are gathered into identification schemes like the Von Reyn or the more recent Duke criteria (2,3).

The demographic characteristics of IE have changed over the past decades, with different factors contributing. The age of the patients contracting disease has increased, with a growing number of patients over 60 years. Furthermore, the predominant type of underlying heart disease, as predisposing factor for IE, has changed. Rheumatic heart disease has decreased dramatically as underlying cause, compared to the incidence in the beginning of this century. Congenital heart disease is still a major clinical finding, mainly in pediatric patients with IE. In elderly patients the predisposing endocardial damage is mostly the result of mitral valve prolapse or degenerative heart disease. In addition, the implant of prosthetic heart valves, a surgical intervention applied worldwide nowadays, has been associated with the initiation of IE (PVE). In an increasing number of cases, especially in intravenous drug users, no underlying cardiac disease is present (4). The etiological agents involved in IE have also changed. Although the viridans group streptococci are still the predominant cause of NVE, the frequency of NVE cases caused by *Staphylococcus aureus* is increased over the last decade. This might complicate treatment of IE, as antibiotic resistance is a widespread phenomenon within this latter bacterial species. PVE is predominantly caused by coagulase-negative staphylococci in the first year after surgery.

Over the past century the pathogenesis of IE due to viridans group streptococci has been studied extensively. This has resulted in a detailed insight into the initiation and progression of IE (Fig.1). Initial damage to the endocardial lining or the heart valves leads to accretion of blood components, mainly blood platelets and fibrin, onto the lesion. The resulting thrombus (or vegetation) becomes colonized after adherence of bacteria which are transiently present in the bloodstream. Bacterial growth within such a vegetation induces ongoing local...
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Figure 1.
Schematical depiction of the progressive steps in the pathogenesis of infective endocarditis. Modified from Durack and Beeson (5).

Figure 2.
The interaction of Staphylococcus aureus with human vascular endothelium. The successive events of (1.) bacterial binding, (2.) internalization, (3.) persistence, and (4.) cellular damage followed by platelet and fibrin deposition, result in the onset of infective endocarditis.
coagulation, leading to an increase in the size of the vegetation. Embolic events, major heart failure, and bacterial sepsis are the most often observed complications, causing severe morbidity and even death.

Underlying heart disease appears no strict prerequisite in the onset of IE. Strikingly, \textit{S. aureus} is the predominant pathogen isolated from cases without known prior heart disease or heart abnormality, which is possibly due to the tropism of this microorganism for endovascular tissue (6). Binding of \textit{S. aureus} to vascular endothelial cells is followed by uptake of the bacteria by these cells. \textit{S. aureus} are able to persist in this cellular environment for prolonged periods of time, and eventually their presence leads to cellular damage. The exposure of the subcellular matrix triggers deposition of blood components, which then can lead to IE (Fig.2).

Most IE research over the past decade has either focussed on extracellular bacterial structures that are possibly involved in disease development, or on cellular and immunological host responses (7-11). The adaptive processes of the bacterium necessary for survival in hostile niches in the host, and presumably required for its virulence, remained largely unknown up to now. Recently two novel molecular genetic techniques, IVET (\textit{In Vivo} Expression Technology) and STM (Signature Tagged Mutagenesis), were implemented in IE research (12-16). Both these techniques contemplate the detection of genes that are of importance for bacterial virulence or survival. Using IVET, promoter regions of genes can be identified that are activated under specific physiological conditions, through differential expression of two promoterless selection markers. STM involves the identification of genes that are important for bacterial survival, by detection of tagged transposon mutants which have reduced survival capacities under certain (\textit{in vivo}) conditions.

**Scope and outline of this thesis**

Knowledge on the regulation of bacterial gene expression in specific niches in the human host can result in novel understandings regarding the development of IE. The scope of this thesis is, therefore, to obtain insight in the adaptive response of both viridans group streptococci and \textit{S. aureus} to their environment. Assessment of the adaptive bacterial gene expression involved was carried out by both IVET and transposon-based techniques.

In Chapter 2 of this thesis an overview of the current knowledge on NVE, the virulence characteristics of the most often encountered pathogens, and a description of new research methods in this field are presented. The development of various molecular genetic tools required for the research on viridans group streptococci is described in Chapters 3 and 4. These tools were applied for the identification of genes from \textit{Streptococcus gordonii} CH1 which are activated by specific environmental stimuli encountered in the host during the pathogenesis of IE, including neutral pH and iron limitation (Chapters 4 to 6). In addition, the growth characteristics of different viridans group streptococcal strains within platelet-fibrin thrombi, which might represent major virulence determinants in IE, were studied in Chapter 7.
The interaction of *S. aureus* with human vascular endothelium, and the bacterial response to this niche, are addressed in Chapter 8. Finally, in Chapter 9 the significance of the obtained results in the light of infective endocarditis is discussed.

REFERENCES
