Antibacterial treatment of *Staphylococcus aureus*: Response and resistance to the lantibiotic mersacidin and evaluation of endolysins as a biofilm treatment strategy

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Knowledge must come through action;
you can have no test which is not fanciful, save by trial.

Sophocles (496 BC - 406 BC)

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1 Abstract

Staphylococcus aureus, both a commensal organism and an important human pathogen, has been the objective of basic and clinical research for decades. *S. aureus* is the leading cause for a broad range of diseases, such as pneumonia, endocarditis or toxic shock syndrome. Especially nosocomial and community-acquired infections by methicillin-resistant *S. aureus* (MRSA) have become a major health issue. Since there has been an increased emergence of microorganisms resistant to various antibiotics, the development of new treatment strategies has become a key issue of modern biological and medical science and triggered the need for a fundamental knowledge on how these bacteria gain resistance to antibiotics.

In the first part of this work, the response and putative resistance strategies of *S. aureus* to the lantibiotic mersacidin were studied. Mersacidin is an antimicrobial peptide of 20 amino acids that is ribosomally produced by *Bacillus* sp. strain HIL Y-85,54728. Mersacidin acts by complexing the sugar phosphate head group of the peptidoglycan precursor lipid II, thereby inhibiting the transglycosylation reaction of peptidoglycan biosynthesis. First, the growth of *S. aureus* in the presence of subinhibitory concentrations of mersacidin was analyzed. Transcriptional data revealed an extensive induction of the cell wall stress response which is partly controlled by the two-component regulatory system (TCRS) VraSR and which predominantly included the transcription of cell wall biosynthesis genes. In contrast to other cell wall-active antibiotics, such as the glycopeptide vancomycin, lower concentrations of mersacidin were sufficient for induction, probably, because the efficacy of mersacidin is not affected by an increased cell wall thickness. However, the cell wall stress response was equally induced in the more resistant *S. aureus* strains SA137/93A and SA137/93G as well as in the highly susceptible strain SG511-Berlin. Therefore, the cell wall stress response may not account for the different susceptibilities of these strains to mersacidin, but it appears to be a general accelerator system of cell wall biosynthesis, thereby contributing to a common resistance strategy of *S. aureus* to cell wall-active antibiotics. Since the transcription of the VraDE ABC transporter genes was induced up to 1700-fold in these experiments, the role of VraDE in the response to mersacidin was examined. However, a *vraE* knock-out phenotype did not exhibit an increased susceptibility to mersacidin compared to the wild type strain.

In order to gain further insights into the mechanisms that *S. aureus* uses to counteract antimicrobials like mersacidin, the features of *S. aureus* SG511-Berlin were identified that contribute to its high susceptibility to antimicrobial peptides (AMPs) compared to other *S. aureus* strains. The fairly susceptible strain SG511-Berlin has been extensively used in the field of basic research on staphylococci and has represented a standard strain for antimicrobial

susceptibility testing for many years. Comparative expression profiling of *S. aureus* SG511-Berlin versus the more resistant *S. aureus* strain SA137/93A revealed a divergent regulation of the *dltB*, *mprF* and *vraFG* genes, which are under the control of the TCRS GraRS. These transcripts showed significantly lower abundance in strain SG511-Berlin. Sequence analysis of *graS* in strain SG511-Berlin revealed a native nucleotide insertion that generates a stop codon at position 64 of the sensor histidine kinase GraS, thereby deleting the entire cytoplasmic part of the protein. Quantitative RT-PCR and determination of the whole cell surface charge of *graS* complemented *S. aureus* SG511-Berlin directly linked its decreased *dltB* transcript level and the resulting increased negative cell surface charge to the nucleotide insertion in *graS*. MIC determinations identified the GraRS TCRS as a resistance factor to the lantibiotics mersacidin, nisin and Pep5. In conclusion, mersacidin appears to be a strong inducer of the cell wall stress response of *S. aureus* at very low concentrations, which reflects its general mode of action as a cell wall-active peptide as well as its use of a unique target site on lipid II. Additionally, mersacidin appeared not to be a substrate for the ABC transporter VraDE and therefore may provide directions for the design of future antimicrobials that circumvent the action of resistance transporters. Furthermore, the GraRS system represents an important resistance factor of *S. aureus* to counteract AMPs and, due to these findings, the use of *S. aureus* SG511-Berlin for research purposes should be carefully considered, since this strain does not reflect the normal response of *S. aureus* against antibiotics.

In the second part of this work, the lysis genes of the bacteriophages ϕ11 and ϕ12 of *S. aureus* NCTC8325 were characterized to evaluate the potential of endolysins as a novel treatment strategy for *S. aureus* biofilms. Knowledge about the lytic activities of both endolysins is limited. Their nucleotide sequences have been published and the ϕ11 endolysin has been shown to possess a D-alanyl-glycyl endopeptidase and an N-acetylmuramyl-L-alanine amidase activity on crude cell walls of *S. aureus* OS2. In this approach, the lytic activities of heterologously overexpressed enzymes and their single subdomains were tested on isolated cell walls, whole cells and biofilms of staphylococci. The recombinant ϕ11 endolysin hydrolyzed heat-killed staphylococci as well as staphylococcal biofilms. Cell wall targeting appeared to be a prerequisite for lysis of whole cells and the combined action of the endopeptidase and amidase domains was necessary for maximum activity. In contrast, the ϕ12 endolysin was inactive and caused aggregation of the cells. Thus, endolysins may provide directions for the development of new biofilm treatment strategies to combat *S. aureus* nosocomial infections.

2 Introduction

2.1 The human pathogen *Staphylococcus aureus*

In 1884, Friedrich Julius Rosenbach described two differently pigmented colony types of staphylococci, namely *Staphylococcus aureus* (yellow) and *Staphylococcus albus* (white) which had been isolated from bacteriological cultures of the human nose and skin. The latter species comprises a growing number of coagulase-negative staphylococci including *Staphylococcus epidermidis.* The designation of the genus *Staphylococcus* is derived from its characteristical form of growth. The spherical cells, which are about 1 µm in diameter, occur in microscopic clusters resembling a bunch of grapes (in Greek: *staphyle*) because staphylococci divide in two planes (Fig. 2.1) (Peters and Pulverer, 2001). The configuration of the cocci helps to distinguish staphylococci from streptococci, since streptococci only divide in one plane, thereby forming slightly oblong cells that usually grow in chains. *S. aureus* is an immotile, non-sporulating bacterium with low GC-content that taxonomically belongs to the bacterial family of *Staphylococcaceae* within the phylum of the Gram-positive *Firmicutes*. The best-known of its nearby phylogenetic relatives are the members of the genus *Bacillus* in the family *Bacillaceae*. *S. aureus* can grow at a temperature range of 15 to 45°C and at NaCl concentrations as high as 15%. However, the growth optimum of *S. aureus* is 30-37°C which correlates with the body temperature of the human host. *S. aureus* is a facultative anaerobe that grows by fermentation which yields principally lactic acid. It forms a fairly large yellow colony on rich medium and is often hemolytic on blood agar (Hahn et al., 2004).

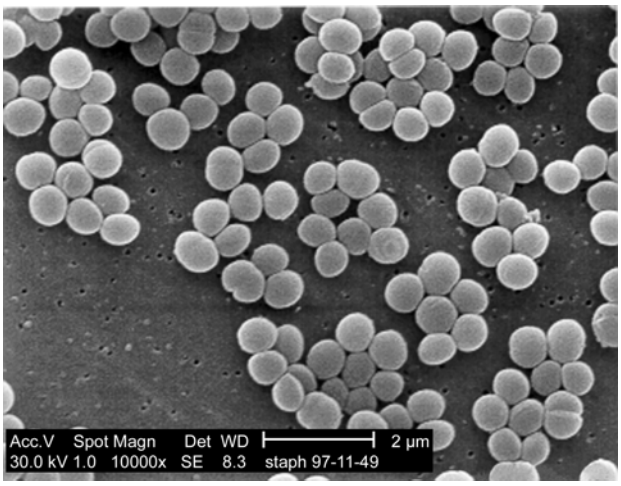

Figure 2.1: Scanning electron microscope (SEM) image of *Staphylococcus aureus*. Source: Centers for Disease Control and Prevention's Public Health Image Library (PHIL, identification number #6486). Photo credit: Janice Carr; content providers: CDC/ Matthew J. Arduino, DRPH; Janice Carr.

Although more than 30 species of *Staphylococcus* are described (Götz et al., 2007), especially *S. aureus* and *S. epidermidis* appear to be important in their interactions with humans. *S. aureus* colonizes mainly the human nasal passages with a carrier rate of up to 40% (Noble, 1997), but it may also be found regularly in most other anatomical areas. Noteworthy, *S. epidermidis* is an inhabitant of the human skin. Since *S. aureus* is relatively tolerant towards dehumidified conditions and disinfectants, it also persists outside the human host and therefore can be transmitted by smear infections. Actually, *S. aureus* is the leading cause for a broad spectrum of diseases that ranges from superficial skin lesions (furunculosis) and invasive inflammations (e.g. otitis and sinusitis) to life-threatening diseases such as pneumonia, endocarditis, meningitis or toxic shock syndrome (Hahn et al., 2004). The latter belongs to a class of toxin-mediated diseases caused by *S. aureus* that evokes multisystem disorders in the human host due to the staphylococcal toxic shock syndrome toxin (TSST-1). Additionally, *S. aureus* can cause food poisoning by releasing heat-stable enterotoxins (A, B, C1-3, D, E, G, H and TSST-1) into food that results in diarrhea and emesis. The pathogenesis of *S. aureus* infections may be divided into three essential steps comprising the adherence to the host cell tissue, the invasion of the host and strategies to overcome the host defenses. Here, numerous surface proteins of *S. aureus*, that belong to the MSCRAMM (*m*icrobial *s*urface *c*omponents *r*ecognizing *a*dhesive *m*atrix *m*olecules) protein family, promote the attachment to the host tissue, e.g. the clumping factors A and B (fibrinogen receptors, ClfA and ClfB) as well as the fibronectin-binding proteins A and B (FnBPA, FnBPB) of *S. aureus* function as adhesins, thereby initiating the colonization of a new host. The invasion of the host tissue involves several extracellular factors produced by *S. aureus* including membrane-damaging toxins, e.g. the α-toxin (α-hemolysin) and the β-toxin. Additionally, *S. aureus* produces coagulase and staphylokinase which take part in host invasion and help to survive the host defense mechanisms. Coagulase binds to prothrombin forming a complex called staphylothrombin that converts fibrinogen into fibrin, thereby coating the bacterial surface with fibrin that avoids phagocytosis. Nearly all strains of *S. aureus* produce coagulase, which is also an essential factor to distinguish between *S. aureus* (coagulase-positive) and *S. epidermidis*, since nearly all strains of *S. epidermidis* lack this enzyme (coagulase-negative). The staphylokinase lyses fibrin allowing the bacteria to escape from the fibrin meshwork that is often formed to keep an infection localized. The polysaccaride microcapsule as well as the protein A are further prominent factors that contribute to the virulence of *S. aureus*, since they prevent the opsonisation of *S. aureus*. While protein A is a surface protein that binds to the Fc region of immunoglobulins which is usually recognized by the Fc receptor of phagocytes, the

polysaccaride microcapsule masks the cells to inhibit phagocytic engulfment (Peters and Pulverer, 2001). *S. aureus* is also able to form small-colony variants (SCVs), which persist inside the host cells without causing host-cell damage or being affected by antibiotic treatment. Later those SCVs can revert to a more virulent phenotype and therefore contribute to persistent and recurrent infection (Proctor and Peters, 1998; Proctor et al., 1998).

2.2 Staphylococcal biofilms

Bacterial biofilms are currently defined as matrix-enclosed sessile populations of microorganisms that adhere to biological or non-biological surfaces which form structurally and dynamically complex biological systems. Biofilm formation is part of an intrinsic bacterial survival strategy that is usually induced upon suboptimal environmental conditions, e.g. by the limitation of nutrients, the presence of lethal concentrations of antibiotics or other conditions of stress. The overall development of bacterial biofilms can be divided into different stages. The initial stages generally involve the loose and transient association of bacteria with the surface and subsequent adhesion. Next, the cells aggregate into microcolonies, the structural units of biofilms, followed by growth and maturation. Here, the use of scanning electron microscopy and confocal laser scanning microscope techniques allowed the *in situ* examination of vital biofilms (Fig. 2.2). Bacterial biofilms were observed to be highly heterogenous and dynamic in structure showing defined cell clusters and water channels that permit a hydrodynamic flow through the biofilm which is necessary for an adequate supply of nutrients and the removal of waste (Lewandowski, 2000; Dunne, Jr., 2002). The last stage is a discrete process that is important for the dispersal and structural development of biofilms and is defined by transient motility and the detachment of biofilm cells (Hall-Stoodley et al., 2004). The bacteria that grow in biofilms are characterized by a modified phenotype compared to planktonic cells which includes an altered growth rate and the expression of biofilm-specific genes. The initial cell attachment of bacteria triggers the transcription of genes controlling the production of bacterial compounds necessary for adhesion, biofilm formation and the dispersal of biofilm cells (Donlan and Costerton, 2002). Especially regulated cell death and lysis appear to be essential for the development of bacterial biofilms, since the genomic DNA, that is released by lysed bacteria, is an important structural component of bacterial biofilms and therefore takes part in cellular adhesion and biofilm stability (Bayles, 2007; Rice et al., 2007). Bacterial biofilms commonly occur in natural aquatic ecosystems and industrial water systems. However, the most serious appearance of bacterial biofilms involves device-related and chronic infections. Particularly

S. aureus and *S. epidermidis* are frequently associated with the colonization of medical devices like intravenous catheters, joint protheses, cardiac pacemakers or prosthetic heart valves (Götz, 2002; Peters et al., 1981; Marrie et al., 1982; von Eiff et al., 1999). Hence, biofilm formation may be defined as a virulence factor of *S. aureus* that contributes to infection. The most characteristic feature of staphylococcal biofilms is the extracellular matrix that is produced by the bacteria itself and that predominantly consists of polysaccharides or proteins. The most common matrix compound represents PIA (*p*olysaccharide *i*ntercellular *a*dhesion) which is composed of positively or negatively charged, β-1,6-linked N-acetylglucosamines that can be deacetylated, phosphorylated or contain esters of succinate (Mack et al., 1996a). Interestingly, most of the PIA producers also form a biofilm (Mack et al., 1996b). PIA synthesis is regulated by the *ica* gene cluster (Götz, 2002), a cluster that is conserved among staphylococci (Cramton et al., 1999). Most probably, PIA contributes to cell aggregation and accumulation of biofilm cells, since mutagenesis of the *ica* genes prevented biofilm formation (Heilmann et al., 1996a; Heilmann et al., 1996b). Additionally, several other essential factors have been identified to take part in staphylococcal biofilm formation, e.g. the AtlE murein hydrolase as well as the net charge of teichoic acids (Heilmann et al., 1997; Gross et al., 2001).

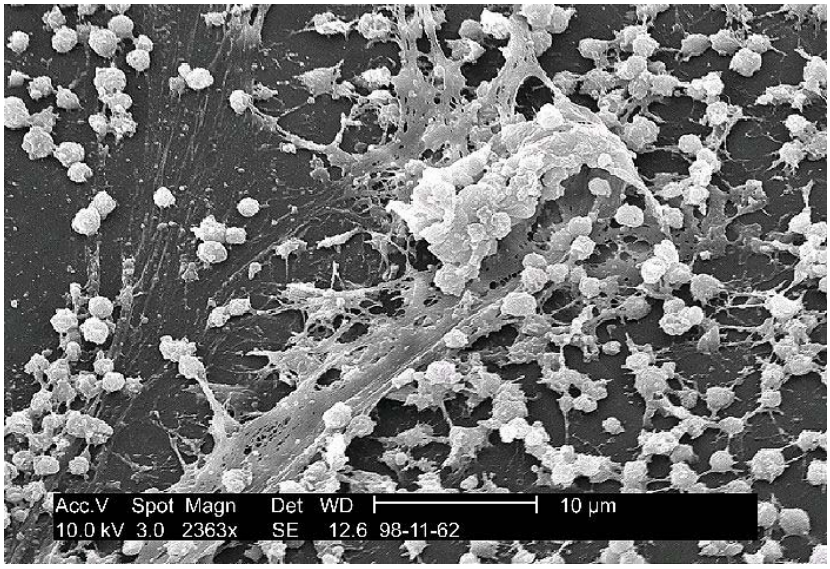

Figure 2.2: Scanning electron micrograph (SEM) of the polysaccharide matrix of a *Staphylococcus* biofilm. Here, the staphylococcal biofilm is located on the inner surface of a needleless connector. Biofilms are characterized by the presence of an extracellular matrix consisting of polymeric substances, primarily polysaccharides, that surround and encase the cells. Source: Hardin Library for the Health Sciences, University of Iowa (http://www.lib.uiowa.edu/ hardin/md/cdc/staph/).

Furthermore, the surface proteins Bap (biofilm-associated protein), AAP (accumulation-associated protein) and Eap (extracellular adherence protein) have been implicated in a protein-associated biofilm of *S. aureus* (Cucarella et al., 2001; Hussain et al., 1997; Hussain et al., 2002). Beside the colonization of artificial surfaces, staphylococci also settle on the extracellular matrix of the host tissue. To this end, receptors of the bacterial cell surface recognize host molecules like glycoprotein components and mediate the adhesion to host cell tissue (Götz, 2002). These receptors, called adhesins, belong to the family of staphylococcal MSCRAMMs which include FnBPA and FnBPB, the collagen-binding protein (Cna) as well as ClfA and ClfB. Furthermore, the α-toxin of *S. aureus* seems to contribute to cell-cell interactions during biofilm formation (Caiazza and O'Toole, 2003).

An intrinsic feature of staphylococcal biofilms is the increased resistance to antibiotic treatment, disinfectants and host defenses which contributes to the persistance of biofilm infections (Mah and O'Toole, 2001; Stewart, 2002). A combination of several factors like poor antibiotic penetration, an altered microenvironment as well as adaptive responses appears to account for the protective mechanism of cells living in a biofilm. In this context, the extracellular matrix of biofilms might decelerate the diffusion rate of antimicrobial agents through the biofilm and antibiotic-matrix interactions may affect the activity of antibiotic compounds (Duguid et al., 1992a; Suci et al., 1994; Souli and Giamarellou, 1998). Furthermore, an altered growth rate of biofilm cells has an impact on the susceptibility to antibiotics (Stewart, 2002). Biofilm-associated cells are characterized by a decreased growth rate compared to planktonic cells which concomitantly leads to a decelerated uptake of antimicrobial substances and a decreased efficiency of specific antibiotics, e.g. β-lactams, that particularly act on growing cells. Indeed, a decreased growth rate had a direct influence on the susceptibility of *S. epidermidis* biofilms to fluorchinolones (Duguid et al., 1992b). Especially persister cells, which represent a phenotypic variant of microorganisms living in a biofilm, appear to contribute to antibiotic resistance (Anderson and O'Toole, 2008). In regions of substrate limitation, normal cells fail to grow and slowly convert to the persister state which is assumed to be incapable of growth. Persisters are generated at a fixed rate, which is not dependend on the presence of antimicrobial agents, and they are able to revert from the persister state after antibiotic treatment and allow the biofilm to regrow (Roberts and Stewart, 2005; Shah et al., 2006). As a consequence of the increased antibiotic resistance, staphylococcal biofilms are of severe clinical relevance and about 65% of the nosokomial infections are due to the formation of biofilms (Mah and O'Toole, 2001). Biofilms are involved in various human infections like endocarditis, otitis media, osteomyelitis and

prostatitis as well as the colonization of medical devices like catheters and artificial heart valves. Considering the increased resistance of biofilms to conventionally used antibiotics, there is an urgent need to develop new strategies to combat biofilm infections.

2.3 The cell wall envelope of *S. aureus*

The cell wall of Gram-positive bacteria constitutes a structural entity that is located outside the bacterial plasma membrane and completely covers the cell. The bacterial cell wall comprises a multi-layer heteropolymer that predominantly consists of the sugar-peptide polymer peptidoglycan (up to 70%) with attached accessory molecules such as teichoic acids, teichuronic acids, polyphosphates, carbohydrates and proteins. The main structural features of the peptidoglycan are linear glycan chains of alternating units of β-1,4 linked N-acetyl-glucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc) interlinked by short peptides (Fig. 2.3). These peptide side chains are bound to the D-lactyl moiety of the MurNAc and are additionally cross-linked with peptides of neighboring glycan strands. The peptide side chain of *S. aureus* consists of L-alanine (L-Ala), D-glutamic acid (D-Glu), L-lysin (L-Lys) and D-alanine (D-Ala), in which the ε amino group of L-Lys is further modified by the addition of five glycine residues that form a characteristical interpeptide bridge to neighboring peptides. Thereby, a three-dimensional, elastic molecular network is generated that is intimately involved in the cell division process as well as the maintenance of the cell shape and the cell integrity by withstanding the internal osmotic pressure (Navarre and Schneewind, 1999; van Heijenoort, 2001).

Cell wall biosynthesis occurs in three distinct subcellular compartments, the cytoplasm, the membrane and the cell wall itself (Fig. 2.3). In the first stage, several cytoplasmic steps lead to the formation of the soluble UDP-MurNAc-pentapeptide precursor (Park`s nucleotide). The assembly of UDP-MurNAc from UDP-GlcNAc and phosphoenolpyruvate as well as the consecutive attachment of the pentapeptide side chain (L-Ala, D-Glu, L-Lys and the D-Ala-D-Ala dipeptide) are mediated by MurA to MurF. In the second stage, the MurNAc-pentapeptide is phosphodiester linked via MraY to the membrane-bound undecaprenyl-phosphate carrier molecule generating lipid I. Subsequently, MurG and FemXAB mediate the linkage of GlcNAc and five glycine residues, respectively, to produce the disaccharide precursor lipid II (van Heijenoort, 2001; Navarre and Schneewind, 1999). Finally, lipid II is translocated across the cytoplasmic membrane to the outside surface by a yet unknown mechanism. The third stage of cell wall biosynthesis comprises the incorporation of lipid II into the nascent peptidoglycan which is catalyzed by penicillin-binding proteins (PBPs).

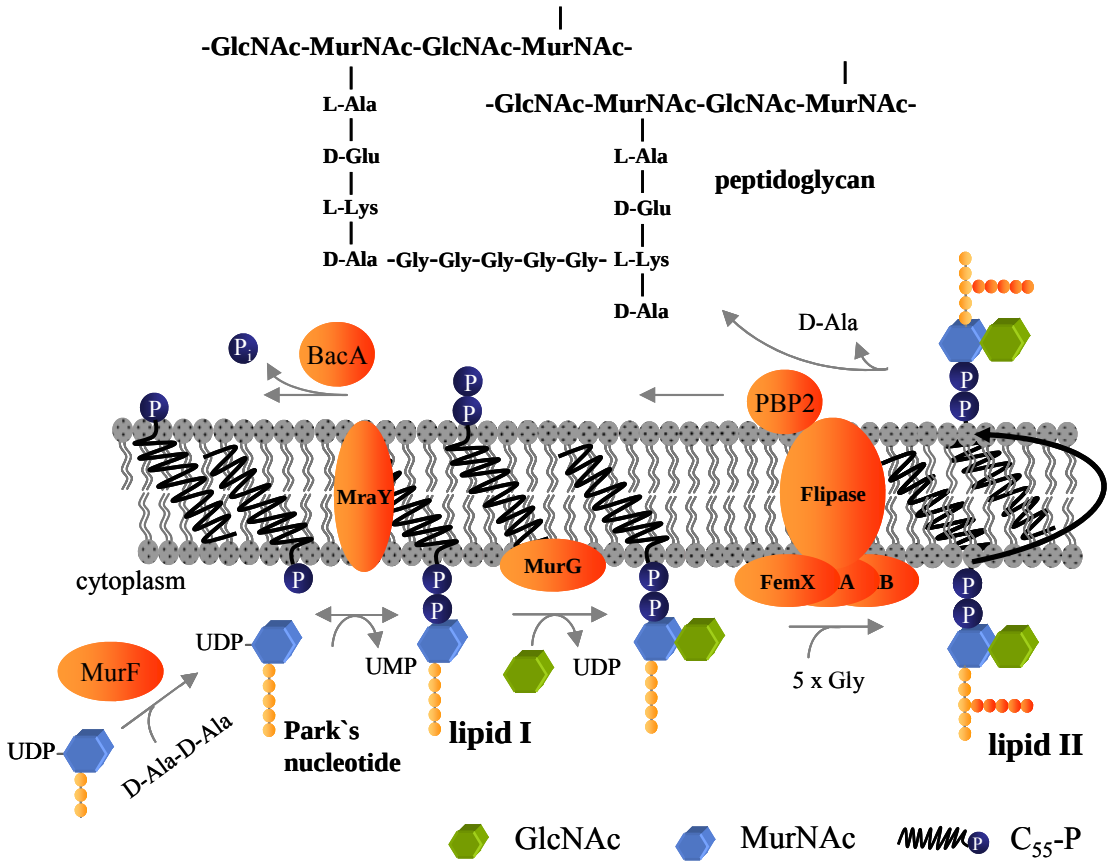

Figure 2.3: *S. aureus* peptidoglycan structure and biosynthesis pathway of cell wall assembly. The original diagram was designed by Dr. T. Schneider and has been modified by P. Sass.

The bifunctional PBPs promote both the polymerization of glycan from the lipid II precursors as well as the transpeptidation (cross-linkage) of the peptide side chains which results in the removal of a D-Ala at the C-terminal end of the pentapeptide (van Heijenoort, 2001; Navarre and Schneewind, 1999). The peptidoglycan is a structure that is unique for bacteria and therefore represents an effective target site for antimicrobial agents, e.g. β-lactam antibiotics imitate the structure of the D-alanyl-D-alanine and bind to the active site of PBPs, whereby the polymerization of new peptidoglycan is inhibited and the cell wall becomes vulnerable to lysis (Yocum et al., 1979; Yocum et al., 1980).

2.4 Role of *S. aureus* in hospital-associated infections – MRSA / VISA / VRSA

Infections with *S. aureus* are normally endogenous, i.e. the causative organism originates from the flora of the patient itself. However, hospital-aquired infections are often exogenous and are promoted by the extensive use of anti-infective agents among post-operative and immune suppressed patients. Here, the widespread use of anti-infective agents can lead to the

dissemination of multiresistant organisms that are spread among the patients via the medical and nursing staff. In fact, *S. aureus* is probably the most dangerous multiresistant bacterial organism in hospital settings which is due to its ability to easily acquire resistance to antibiotics. The accessory genome represents 25% of the whole genome of *S. aureus* and includes mobile elements, e.g. plasmids, bacteriophages, transposons, cassette chromosomes and genetic islands, which harbour genetic determinants for antibiotic resistance and virulence (Lindsay and Holden, 2004).

In the 1940s, the first β-lactam resistant *S. aureus* strains have been described shortly after the implementation of penicillin treatment in hospitals (Barber, 1947; North and Christie, 1946). Today, more than 80% of *S. aureus* produce penicillin-degrading enzymes, the so-called β-lactamases (Peters and Pulverer, 2001). The introduction of semi-synthetic, β-lactamase-stable penicillin derivatives, e.g. methicillin and oxacillin, was followed by the isolation of the first methicillin-resistant *S. aureus* (MRSA) in the early 1960s (Barber, 1961). Methicillin resistance is based on the acquisition of the staphylococcal cassette chromosome *mec* (SCC*mec*) by horizontal transfer from a yet unidentified donor (Gordon and Lowy, 2008). SCC*mec* contains the *mecA* gene encoding an additional PBP2, namely PBP2a. PBP2a is characterized by a decreased affinity for β-lactam antibiotics and thus it ensures the cross-linking of the bacterial peptidoglycan when the endogenous PBP2 is inhibited by methicillin. Actually, hospital-acquired MRSA strains (HA-MRSA) represent a serious problem in hospital settings due to the eventuality of antibiotic treatment failure with severe consequences for the patient. Currently, 20% of the hospital isolates of *S. aureus* in Germany are MRSA (Hahn et al., 2004) and even 40-60% of MRSA were recorded in the United States and Japan (Lindsay and Holden, 2004). More recently, community-acquired MRSA (CA-MRSA) infections occurred among individuals in Australia and in the United States without health care contact (Udo et al., 1993; Tenover et al., 2006) and the number of CA-MRSA infections appears to be increasing (Vandenesch et al., 2003). From 1997 to 1999, four children died in the United States because of aggressive CA-MRSA infections caused by *S. aureus* strain MW2 (USA400) which were rapidly fatal involving necrotizing pneumonia or pulmonary abscesses and sepsis (Gordon and Lowy, 2008). The increased emergence of CA-MRSA has been proposed to be due to numerous factors like increased fitness, improved evasion of the host immune system and unique toxin production compared to HA-MRSA (Gordon and Lowy, 2008).

Infections with MRSA are currently treated with vancomycin, a glycopeptide antibiotic that inhibits bacterial cell wall biosynthesis by binding to the D-alanyl-D-alanine terminus of the

cell wall precursor lipid II. However, in the last years, strains with decreased susceptibility to vancomycin (VISA – "vancomycin-intermediate resistance", MIC 4-16 µg/ml) have emerged worldwide (Ruef, 2004) that are associated with clinical treatment failure of glycopeptide therapy (Song et al., 2004; Woods et al., 2004; Bierbaum et al., 1999). The VISA phenotype is variable and the genotypes of VISA strains have not yet been well understood. A common feature of VISA is a thickened cell wall as a cause of an elevated cell wall metabolism (Cui et al., 2000; Cui et al., 2003; Reipert et al., 2003; Cui et al., 2006; Hanaki et al., 1998a). In some strains, the cell wall cross-linking is decreased, resulting in a higher concentration of free D-alanyl-D-alanine termini in the cell wall that are able to complex vancomycin (Cui et al., 2000; Hanaki et al., 1998b; Sieradzki and Tomasz, 2003; Reipert et al., 2003). Many strains are characterized by a decreased activity of autolysins, which may contribute to the increased cell wall thickness (Utaida et al., 2006; Boyle-Vavra et al., 2003; Koehl et al., 2004; Sakoulas et al., 2005). Alterations of the teichoic acids, which are able to complex vancomycin in an ionic interaction, may also augment vancomycin resistance (Peschel et al., 2000). For the development of high resistance, the loss of SSC*mec* has been described (Sieradzki and Tomasz, 1999; Reipert et al., 2003; Adhikari et al., 2004). In 2002, the first high level vancomycin resistant *S. aureus* strains (VRSA, MIC >32µg/ml) were isolated in the United States (Miller et al., 2002; Sievert et al., 2002). Probably, VRSA emerged by the acquisition of the *vanA* operon from an *Enterococcus* faecalis strain that colonized the same location (Chang et al., 2003). VanA-type resistance is due to the synthesis of modified cell wall precursors which do not complex vancomycin. However, high level vancomycin resistance is rare in *S. aureus* and appears to be incompatible with methicillin resistance (Perichon and Courvalin, 2006). In this context, there is a demand to establish new effective treatment strategies to treat MRSA.

2.5 *S. aureus* strain SG511

S. aureus strain SG511 is rather susceptible to most antibiotics and has been extensively used in the field of basic research on staphylococci and the screening for new antimicrobial agents for many years. Widespread use of strain SG511 in the first half of the last century resulted in the appearance of several strains named SG511 which could be divided into three different lysotypes (Pöhn, 1959), namely type "Berlin" (lysotype I), type "Hoechst" (lysotype II) and type "Duesseldorf" (lysotype III). Despite the differences concerning their lysotypes, pigmentation and hemolysis, all three subtypes displayed a concordant spectrum of susceptibility to antibiotics, which might be one of the reasons for their common designation

SG511 (Pulverer and Grün, 1960). Especially, the type "Berlin", from the Robert Koch institute (Berlin, Germany), and the type "Hoechst", originating from the strain collection of the "Farbwerke Hoechst AG" (Frankfurt am Main, Germany), have experienced intensive use in antibiotic susceptibility testing of new antimicrobial substances and basic microbiological research (Chatterjee et al., 1992a; Wink, 2002; Lampilas and Vogel, 1999; Garrard and Lascelles, 1968; Maidhof et al., 1989; de Jonge et al., 1993; Sass et al., 2008b; Pag et al., 2008; Brötz et al., 1997).

2.6 Lantibiotics – *lan*thionine containing peptide an*tibiotics*

Antimicrobial peptides (AMPs) are produced by a large variety of organisms as a part of their first line of defense. AMPs are generally regarded to be relatively short in length (up to 100 amino acids) and have been isolated from microorganisms, insects, plants, amphibians, birds, fish and mammals, including humans (Jenssen et al., 2006). Some AMPs have been shown to be potent antimicrobial agents (Jack et al., 1995), however, these peptides also seem to play an important role as effector molecules of the innate immune system, e.g. by enhancing phagocytosis or recruitment and accumulation of various immune cells at inflammatory sites (Bowdish et al., 2005; Yang et al., 2002). The lantibiotics form a particular group among the AMPs and are characterized by unique structural features. These result from extensive posttranslational modifications that are mostly based on the amino acids serine (Ser), threonine (Thr) and cysteine (Cys) of the ribosomally produced precursor peptide. Here, the selective dehydration of serine and threonine yields the α,β-unsaturated amino acids didehydroalanine (Dha) and didehyrobutyrine (Dhb), respectively. Dha and Dhb are usually targets for the nucleophilic addition of the SH-groups of suitable positioned Cys residues that results in the formation of the ring forming thioether amino acids lanthionine (Lan, from Dha) and 3-methyllanthionine (MeLan, from Dhb) (Fig. 2.4). In fact, the designation "lantibiotics" is derived from "*lan*thionine containing peptide an*tibiotics*". Since the number of suitable reaction partners is limited, mature lantibiotics usually contain one or more dehydroamino acids that did not undergo nucleophilic addition. Furthermore, Cys residues located at the C-terminus may be oxidized and decarboxylated before the addition of Dha or Dhb resulting in 2-aminovinyl-D-cysteine (AviCys) or 2-aminovinyl-3-methyl-D-cysteine (AviMeCys) in the case of mersacidin (Sahl and Bierbaum, 1998). The ring structures contribute to enhanced resistance towards proteolysis (Bierbaum et al., 1996) and to increased tolerance to oxidizing conditions (Sahl et al., 1995). Mature lantibiotics are synthesized from gene encoded precursor peptides. The structural gene encoding the prepeptide (*lanA*) as well as the genes

coding for the postranslational modification of the prepeptide (*lanM* or *lanB* and *lanC*), export of the prepeptide and subsequent activation by cleavage of its N-terminal leader sequence (*lanP*, *lanT*), the regulation of the biosynthesis (*lanK*, *lanQ*, *lanR*) and the genes encoding producer self protection (*lanE*, *lanF*, *lanG*, *lanI*) are organized in gene clusters (Sahl and Bierbaum, 1998). So far (November, 2008), more than 60 lantibiotics have been described (Table 2.1) all of which are produced by and predominantly act against Gram-positive bacteria, thereby exerting multiple modes of action like pore formation and/or inhibition of cell wall biosynthesis (Chatterjee et al., 1992b; Willey and van der Donk, 2007; Bierbaum and Sahl, 2009).

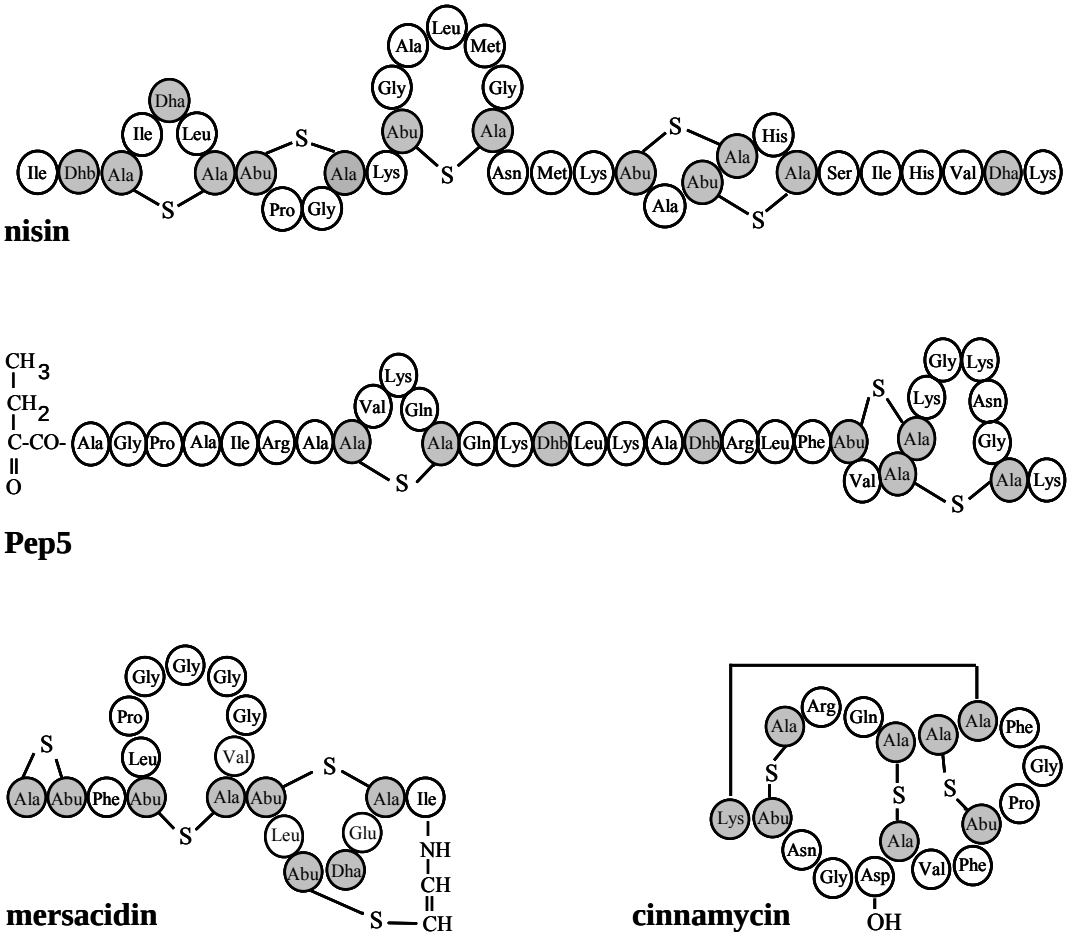

Figure 2.4: Structure of the lantibiotics nisin, Pep5, mersacidin and cinnamycin. Nisin and Pep5 represent type-AI lantibiotics with elongated and flexible configurations. Mersacidin and cinnamycin belong to the type-B lantibiotics that are characterized by a rather globular structure.

Table 2.1: Overview of lantibiotics

Lantibiotic	Mass (Da)	Producer strain	Reference
Type-AI lantibiotics			
nisin group			
nisin A	3353	*Lactococcus lactis*	Gross & Morell (1971)
nisin Z	3330	*Lactococcus lactis*	Mulders et al. 1991
nisin Q	3327	*Lactococcus lactis*	Zendo et al (2003)
nisin U	3029	*Streptococcus uberis*	Wirawan et al (2006)
nisin F	*not determined*	*Lactococcus lactis*	de Kwaadsteniet et al (2008)
subtilin	3317	*Bacillus subtilis*	Gross et al (1973)
ericin A	2986	*Bacillus subtilis*	Stein et al (2002)
ericin S	3442	*Bacillus subtilis*	Stein et al (2002)
microbisporicin	2246/2230	*Microbiospora sp.*	Castiglione et al. (2008)
epidermin group			
epidermin	2164	*Staphylococcus epidermidis*	Allgaier et al. (1986)
[Val1, Leu6]-epidermin	2151	*Staphylococcus epidermidis*	Israil et al. (1996)
gallidermin	2164	*Staphylococcus gallinarum*	Kellner et al. (1988)
staphylococcin T	2166	*Staphylococcus cohnii*	Furmanek et al. (1999)
mutacin B-Ny266	2270	*Streptococcus mutans*	Mota-Meira et al. (1997)
mutacin 1140	2263	*Streptococcus mutans*	Hillman et al. (1998)
mutacin I	2364	*Streptococcus mutans*	Qi et al. (2000)
mutacin III	2266	*Streptococcus mutans*	Qi et al. (1999)
streptin	2424	*Streptococcus pyogenes*	Wescombe & Tagg (2003)
Pep5 group			
Pep5	3488	*Staphylococcus epidermidis*	Kaletta et al. (1989)
epilancin K7	3032	*Staphylococcus epidermidis*	van de Kamp et al. (1995)
epicidin 280	3133	*Staphylococcus epidermidis*	Heidrich et al. (1998)
epilancin 15X	3173	*Staphylococcus epidermidis*	Ekkelenkamp et al. (2005)
Type-AII lantibiotics			
lacticin 481 group			
lacticin 481	2901	*Lactococcus lactis*	Piard et al. (1993)
mutacin II	3245	*Streptococcus mutans*	Novak et al. (1994)
streptococcin A-FF22	2795	*Streptococcus pyogenes*	Jack et al. (1994)
salivaricin A	2315	*Streptococcus salivarius*	Ross et al. (1993)
salivaricin A1	2321	*Streptococcus salivarius*	Simpson et al. (1995)
salivaricin A2	2364	*Streptococcus salivarius*	Wescombe et al. (2006)
salivaricin A3	2312	*Streptococcus salivarius*	Wescombe et al. (2006)
salivaricin A4	2340	*Streptococcus salivarius*	Wescombe et al. (2006)
salivaricin A5	2328	*Streptococcus salivarius*	Wescombe et al. (2006)
salivaricin B	2740	*Streptococcus salivarius*	Hyink et al. (2007)
variacin	2658	*Micrococcus varians*	Pridmore et al. (1996)
macedocin	2795	*Streptococcus macedonicus*	Georgalaki et al. (2002)
nukacin ISK-1	2960	*Staphylococcus warneri*	Sashihara et al. (2000)
nukacin KQU-131	3004	*Staphylococcus hominis*	Wilaipun et al. (2008)
plantaricin C	2880	*Lactobacillus plantarum*	Turner et al. (1999)
mutacin K8	2734	*Streptococcus mutans*	Robson et al. (2007)
butyrivibriocin	*not determined*	*Butyrivibrio fibrisolvens*	Kalmokoff et al. (1999)

continued on following page

Table 2.1 - *Continued*

Lantibiotic	Mass (Da)	Producer strain	Reference
ruminococcin A	2675	*Ruminococcus gnavus*	Dabard et al. (2001)
Type-B lantibiotics			
mersacidin group			
mersacidin	1825	*Bacillus* ssp.	Chatterjee et al. (1992)
actagardine	1890	*Actinoplanes liguriae*	Zimmermann et al. (1995)
Ala(O)-actagardine	1961	*Actinoplanes liguriae*	Vertesy et al. (1999)
michiganin A	2145	*Clavibacter michiganensis*	Holtsmark et al. (2006)
cinnamycin group			
cinnamycin	2042	*Streptomyces cinnamoneus*	Fredenhagen et al. (1990)
duramycin	2014	*Streptomyces cinnamoneus*	Fredenhagen et al. (1990)
duramycin B	1951	*Streptoverticillium* sp.	Fredenhagen et al. (1990)
duramycin C	2008	*Streptomyces griseoluteus*	Fredenhagen et al. (1990)
ancovenin	1959	*Streptomyces* ssp.	Kido et al. (1983)
Others			
class III morphogenic peptides			
SapB	2026	*Streptomyces coelicolor*	Kodani et al. (2004)
SapT	2032	*Streptomyces tendae*	Kodani et al. (2005)
AmfS	*not determined*	*Streptomyces griseus*	Ueda et al. (2002)
two peptide lantibiotics			
lacticin 3147	3322/2847	*Lactococcus lactis* ssp.	Martin et al. (2004)
staphylococcin C55	3339/2993	*Staphylococcus aureus* C55	Navaratna et al. (1998)
plantaricin W	3223/3099	*Lactobacillus plantarum*	Holo et al. (2001)
haloduracin	2332/3046	*Bacillus halodurans*	McClerren et al. (2006)
Smb	not determined	*Streptococcus mutans* GS5	Yonezawa & Kiramitsu (2005)
BHT	3375/2802	*Streptococcus ratus* BHT	Hyink et al. (2005)
cytolysin L_L/L_S	4164/2631	*Enterococcus faecalis*	Booth et al. (1996)
peptides which have not yet been assigned to a group			
planosporicin	2194	*Planomonospora sp.*	Castiglione et al., 2007
cypemycin	2094	*Streptomyces ssp.*	Komiyama et al. (1993)
lactocin S	3764	*Lactobacillus sake*	Skaugen et al. (1994)
sublancin 168	3877	*Bacillus subtilis*	Paik et al. (1998)
pediocin PD-1	2866	*Pediococcus damnosus*	Bauer et al. (2005)
carnocin UI 49	4635	*Carnobacterium piscicola*	Stoffels et al. (1993)
bovicin HJ50	3428	*Streptococcus bovis* HJ50	Xiao et al. (2004)
paenibacillin	2983	*Paenibacillus polymyxa*	He et al. (2007)

According to their configuration and mode of action, the lantibiotics can be subdivided into several types. Nisin, subtilin, epidermin and Pep5 belong to the type-AI lantibiotics with an elongated and flexible configuration that predominantly disturb bacterial membranes. Nisin probably represents the most prominent lantibiotic which is commonly employed as a food preservative (Galvez et al., 2007). Nisin is biologically active in nanomolar concentrations. It binds specifically to lipid II and causes pore formation in bacterial membranes as well as

inhibition of cell wall biosynthesis (Wiedemann et al., 2001). The type-B lantibiotics comprise rather globular peptides including mersacidin that act by inhibition of the cell wall biosynthesis since they tightly bind to lipid II. The type-AII lantibiotics combine features of the type-AI and type-B lantibiotics, e.g. lacticin 481 is characterized by a linear N-terminus and a globular C-terminus and its modification system is analogous to the type-B lantibiotics. Furthermore, there are lantibiotics that cannot be classified into the established types. Some lantibiotics like SapB exert a morphogenic rather than a bactericidal effect (Kodani et al., 2004) and others function optimally as a consequence of the synergistic activity of two peptides (Lawton et al., 2007).

2.7 The lantibiotic mersacidin

Mersacidin is the smallest lantibiotic known so far (1825 Da) and belongs to the type-B lantibiotics. It is an uncharged molecule of 20 amino acids forming four intramolecular thioether bridges (3 x MeLan, 1 x AviMeCys) which confer a globular structure to the peptide (Fig. 2.4) (Prasch et al., 1997). The mersacidin gene cluster (12.3 kb) is located on the chromosome of the producer *Bacillus* sp. strain HIL Y-85,54728. For the biosynthesis of biologically active mersacidin, a precursor peptide consisting of the N-terminal leader peptide and the C-terminal propeptide is ribosomally synthesized from its structural gene *mrsA* (Bierbaum et al., 1995). The posttranslational modification of the precursor peptide is catalyzed by two distinct enzymes, namely MrsM and MrsD. MrsM consists of 1062 amino acids and confers the dehydration of the serine and threonine residues as well as the formation of the thioether bridges, thereby generating the characteristic MeLan residues. MrsD is a FAD-containing enzyme of 194 amino acids that oxidatively decarboxylizes the C-terminal Cys residue to produce AviMeCys (Altena et al., 2000; Majer et al., 2002). Proteolytic activation of the modified precurser peptide by cleavage of the leader peptide is performed concomitantly with export by the ABC transporter MrsT that contains an N-terminal protease domain (Havarstein et al., 1995; Altena et al., 2000). Producer self-protection is also mediated by an ABC transporter (MrsFEG) that confers immunity to exogenous mersacidin and prohibits binding of mersacidin to the producer strain. MrsE and MrsG both form membrane-spanning subunits while MrsF contains an ATP binding site that is responsible for ATP hydrolysis and drives the active extrusion of mersacidin (Guder et al., 2002). The active transport of antibiotics away from the bacterial cell by membrane-associated ABC transporters, or so-called drug resistance transporters, is a mechanism that is often utilized by bacteria to escape from the effects of antimicrobial agents. Hence, intrinsic and aquired

resistance transporters play an important role in the antimicrobial resistance of several pathogens, including *S. aureus*. Drug resistance transporters usually respond to specific classes of antimicrobial compounds and decrease their concentration at the bacterial membrane below a critical level to prevent killing of the cell (Putman et al., 2000). Since ABC transporters appear to be energy consuming systems, they are subject to tight modulation by regulatory pathways including two-component regulatory systems (TCRS). The TCRS constitutes a type of phosphotransfer-mediated signalling pathway and consists of two conserved modular proteins: a sensor histidine protein kinase and a corresponding response regulator. Upon specific environmental stimuli, the histidine kinase undergoes autophosphorylation at a conserved histidine residue followed by transfer of the phosphoryl group to a conserved aspartate residue of the regulatory domain of the cognate response regulator resulting in its activation. The response regulator again is regulated by the phosphatase activity of the histidine kinase or the response regulator itself (Mascher et al., 2006; Mascher, 2006). Commonly, the activated response regulator functions as transcription regulator of specific target genes. In the case of mersacidin biosynthesis, the immunity transporter MrsFGE is controlled by the TCRS MrsR2/K2 in which MrsR2 belongs to the family of OmpR/PhoB winged helix binding domain response regulators and MrsK2 represents the sensor histidine kinase (Guder et al., 2002; Altena et al., 2000). The biosynthesis cluster of mersacidin comprises a second regulatory protein with lower similarity to the Omp/PhoB family of response regulators, namely MrsR1, which appears to be essential for the biosynthesis of mersacidin (Guder et al., 2002). However, a gene encoding a corresponding histidine kinase is not present (Altena et al., 2000). Further, the biosynthesis of mersacidin seems to be regulated by an autoinducing mechanism (Schmitz et al., 2006).

Mersacidin exerts bactericidal activity by the selective inhibition of the transglycosylation reaction of bacterial cell wall biosynthesis. To this end, it complexes the sugar phosphate head group of the peptidoglycan precursor lipid II (Fig. 2.3), thereby preventing the incorporation of the peptidoglycan precursor molecule into the nascent peptidoglycan (Brötz et al., 1998). Interestingly, the efficiency of mersacidin is dependent on the presence of divalent cations, since Ca^{2+}-ions dramatically increase the bactericidal effect of mersacidin *in vitro* (Barrett et al., 1992). Furthermore, mersacidin undergoes conformational changes upon substrate binding which is due to a hinge region (Ala-12 and Abu-13) in between the second and the third ring structure (Hsu et al., 2003). Probably, mersacidin binds to a Ca^{2+}-ion located at the diphosphate group of lipid II which is followed by the enclosure of the lipid II disaccharide domain as a result of conformational reconfiguration of mersacidin. In addition, the lipid II-

binding mechanism of mersacidin is assumed to depend on electrostatic interactions, since the carboxylic acid residue at position Glu-17 of the third ring structure appeared to be essential for biological activity (Szekat et al., 2003). In fact, this ring represents the putative lipid II-binding motif and it is conserved among other lantibiotics, e.g the lacticin 481 group lantibiotics, the two-peptide lantibiotics and plantaricin C (Willey and van der Donk, 2007). Importantly, mersacidin uses a target binding site that is different from any other clinically applied antibiotic. It has been shown to successfully inhibit the growth of Gram-positive bacteria including MRSA *in vitro* and *in vivo* (Hoffmann et al., 2002; Kruszewska et al., 2004; Chatterjee et al., 1992a) as well as enterococci expressing the VanA vancomycin resistance phenotype (Brötz et al., 1997). Considering the emergence of VISA since the late 1990s (Ruef, 2004), new effective treatment strategies for MRSA are urgently needed. In this context, lantibiotics could represent alternatives for clinical applications (Willey and van der Donk, 2007) and mersacidin might be a blueprint for the development of new antibiotics to control nosocomial infections (Brötz et al., 1998; Kruszewska et al., 2004).

2.8 Antibacterial activities of phage-encoded cell wall hydrolases

Temperate bacteriophages, which integrate as prophages into the genome of the bacterial host without propagation and induction of host lysis, represent a common feature of bacteria including *S. aureus*. Upon specific environmental conditions, the prophage is able to switch from the lysogenic stage to the lytic cycle which involves the production of new phage particles and lysis of the host cell. Newly synthesized phage particles are usually released from bacterial host cells by the synergistic action of phage-encoded two-component lysis systems. Here, a hydrophobic membrane protein, termed holin, oligomerizes in the bacterial membrane to form disruptive lesions, thereby promoting the access of a cell wall hydrolase (endolysin) to its substrate, the peptidoglycan of the bacterial host, at a programmed time of the phage's life cycle (Young et al., 2000; Ronda-Lain et al., 1977).

Usually, endolysins are multi-domain proteins that belong to the late gene products which are produced towards the end of the phage's lytic cycle. An example represents the temperate bacteriophage ϕ11 of *S. aureus* NCTC8325 (Iandolo et al., 2002). The ϕ11 endolysin consists of an N-terminal, a central and a C-terminal domain which give rise to multiple enzymatic activities that cleave the bacterial peptidoglycan at two distinct sites. The N-terminal domain displays D-alanyl-glycyl endopeptidase activity and therefore cleaves the peptide bond between the D-Ala of the pentapeptide and the glycine of the interpeptide bridge. The central domain represents an N-acetylmuramyl-L-alanine amidase which hydrolyzes the peptide bond

between N-acetylmuramyl and L-Ala (Navarre et al., 1999). The C-terminus is homologous to the cell wall binding domain of lysostaphin (Loessner et al., 1998), a staphylolytic bacteriocin secreted by *Staphylococcus simulans* biovar *staphylolyticus* (Schindler and Schuhardt, 1964) with glycyl-glycine endopeptidase activity. The cell wall binding domain directs the endolysin to its target in the bacterial cell wall. Since the cell wall binding domain has been shown to distinguish between different peptidoglycan cross-bridges (Lu et al., 2006), it confers high specificity, in this case to *S. aureus*, which appears to be a common characteristic among endolysins. Phage ϕ12 is another temperate bacteriophage of *S. aureus* NCTC8325 (Iandolo et al., 2002) that has not yet been studied in detail.

Due to the antibacterial properties of endolysins, endolysin therapy is intensively discussed as an alternative to common antibiotic therapy for clinical treatment or the prevention of infectious disease (Parisien et al., 2008). Endolysins possess several important features, e.g. a narrow antibacterial spectrum, activity against bacteria regardless of their antibiotic sensitivity, a low probability of developing resistance and a novel mode of action. In fact, first clinical studies indicated that the therapeutic use of endolysins should not be prevented by potential problems with endolysin therapy, e.g. their immunogenicity or the release of proinflammatory components during bacteriolysis (Borysowski et al., 2006). Additionally, the specificity of endolysins makes them more appealing today compared to broad-spectrum antibiotics, since they should not affect the microbial flora of the host.

2.9 Objectives of this work

In times of increased antibiotic treatment failure, which can lead to severe clinical infections, there is an obvious need to understand how bacteria respond to the presence of antibacterial compounds and develop resistance. Additionally, new treatment strategies need to be explored to counteract microorganisms that are multi-resistant to conventionally applied antibiotics.

The first part of this work was conceived to analyze the response and to identify putative resistance mechanisms of *S. aureus* to the lantibiotic mersacidin, an antimicrobial peptide with potential for clinical application. To this end, gene expression profiling by using full genome *S. aureus* microarrays and quantitative Real-Time PCR techniques was conducted to elucidate the transcriptional response of *S. aureus* to subinhibitory concentrations of mersacidin. For this purpose, three *S. aureus* strains providing varying susceptibility characteristics were employed, namely the vancomycin- and methicillin-susceptible (VSSA / MSSA) strain *S. aureus* SG511-Berlin as well as the heterogeneous VISA / MRSA strain

S. aureus SA137/93A and the closely related VISA / MSSA strain *S. aureus* SA137/93G (Reipert et al., 2003; Maki et al., 2004). To further identify probable constitutive resistance mechanisms of *S. aureus* to mersacidin and other antimicrobials, the gene expression profile of the susceptible strain SG511-Berlin was compared to that of the more resistant *S. aureus* strain SA137/93A in the absence of antibiotics.

In the second part of this work, the *in vitro* activity of endolysins was evaluated to hydrolyze staphylococcal biofilms. Since conventional antibiotics predominantly affect the metabolism of bacteria, they have no effect on non-growing persister cells of bacterial biofilms. In contrast, endolysins are enzymes that hydrolyze the bacterial peptidoglycan and, thus, should target growing and non-growing cells. For this purpose, the endolysins of the staphylococcal bacteriophages ϕ11 and ϕ12 were heterologously overexpressed in *Escherichia coli*, purified via immobilized metal affinity chromatography (IMAC) and subsequently applied to staphylococcal cells and biofilms growing on polystyrene surfaces. Additionally, the lytic activities of the single subdomains of the ϕ11 endolysin were further characterized in order to gain deeper insights into the mode of action of endolysins.

3 Materials and methods

3.1 Bacterial strains, plasmids and growth conditions

Bacterial strains used in this thesis are listed in Table 3.1. Staphylococci were cultured in tryptic soy broth (TSB; Oxoid, Wesel, Germany), brain heart infusion (BHI) medium (Becton-Dickinson GmbH, Heidelberg, Germany) or half concentrated BHI as indicated. *Escherichia coli* was cultivated in lysogeny broth (LB; 10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl (Bertani, 2004; Bertani, 1951)). The growth conditions were 37°C with aeration unless indicated otherwise. Plasmids used in this thesis are listed in Table 3.2. *S. aureus* strains harbouring the pTX15 vector, which comprises a xylose-inducible promoter, or its derivatives were grown in the presence of 12.5 µg/ml tetracycline and 0.5% of xylose was routinely used to induce expression from the plasmid. For the maintenance of pCU1 derivatives, *S. aureus* cultures were grown with 20 µg/ml chloramphenicol. *E. coli* strains carrying pUC19, pCU1 or the pET22b derivatives were grown with 40 µg/ml ampicillin.

3.2 *S. aureus* typing methods

3.2.1 Pulsed field gel electrophoresis (PFGE)

Purification of chromosomal DNA for the *Sma*I restriction digest and PFGE were performed as described previously (Goering and Duensing, 1990). PFGE was carried out on the Chef DRIII System (BioRad, München, Germany) using Pulsed Field Certified Agarose (1%) (Bio-Rad), 6 V/cm, a field angle of 120°, and switch times of 5-15 s for 7 h and 15-60 s for a further 19 h. A chromosomal DNA *Sma*I digest of *S. aureus* NCTC8325 served as mass standard.

3.2.2 Multi locus sequence typing (MLST)

MLST of *S. aureus* strain SG511-Berlin and strain SA137/93A was performed as previously described (Enright et al., 2000). The sequence types (ST) were determined using the MLST database via http://saureus.mlst.net/.

3.2.3 Phage typing

The identity of *S. aureus* SG511-Berlin was confirmed by phage typing. Phage typing was conducted using the international set for phage typing employed at the routine test dilution and 100 times the routine test dilution in accordance with the standard rules agreed on by the

International Union of Microbiological Societies Subcommittee on Phage Typing of Staphylococci. Phage typing was kindly performed by M. Oedenkoven (IMMIP).

Table 3.1: Bacterial strains and bacteriophages used in this thesis.

Strain / phage	Relevant characteristic(s)	Reference(s) or source
Staphylococcus aureus		
SG511-Berlin	Susceptible control strain	RKI Berlin, Germany
SG511ΔvraE	*vraE*-defective mutant of strain SG511-Berlin	Sass V. et al. (2008)
SA137/93A	Clinical hVISA isolate; MET^r, Northern German epidemic MRSA	Reipert et al. (2003)
SA137/93G	Spontaneous mutant of SA137/93A, ΔSCC*mec* (MET^s), Δ*tcaA*	Reipert et al. (2003), Maki et al. (2004)
NCTC8325	Biofilm-positiv, laboratory strain with defect *rsbU*, lysogenic for phages ϕ11, ϕ12and ϕ13	Iandolo et al. 2002
RN4220	Restriction-negative derivative of *S. aureus* NCTC8325	Kreiswirth et al. (1983)
RN1"HG"	*S. aureus* NCTC8325 with repaired *rsbU*	F. Götz, Tübingen
MRSA252	U.K. hospital-acquired MRSA strain	Holden et al. (2004)
N315	Japan hospital-acquired MRSA strain	Kuroda et al. (2001)
Cowan I	MSSA, catheter infections by binding of fibrinogen and fibronectin	Juuti et al. (2004)
Newman	MSSA	Wolz et al. (1996)
Wood 46	Protein A-deficient, alpha-toxin producing strain	Delmi et al. (1994)
Staphylococcus simulans		
22	Penicillin-resistant, lysostaphin susceptible	Kloos and Schleifer (1975)
Staphylococcus epidermidis		
O-47	Biofilm-positiv, clinical isolate causing central venous catheter-associated infections	Rupp et al. (2001)
Escherichia coli		
K12 JM109	Subcloning host	Yanisch-Perron et al. (1985)
Bl21(DE3)	λDE3 lysogen harbouring the T7 RNA-polymerase gene; protein expression host	Studier and Moffatt (1986)
Bacteriophages		
ϕ11	Siphoviridae; temperate bacteriophage of *S. aureus* NCTC8325	Iandolo et al. 2002
ϕ12	Siphoviridae; temperate bacteriophage of *S. aureus* NCTC8325	Iandolo et al. 2002

Abbreviations: hVISA, heterogeneous vancomycin intermediately resistant *S. aureus*; MRSA, methicillin-resistant *S. aureus*; MET, methicillin; r, resistant; s, susceptible.

Table 3.2: Plasmids used in this thesis.

Plasmid	Relevant characteristic(s)	Reference(s) or source
pTX15	Xylose-inducible staphylococcal expression vector; Tet^r	Peschel et al. (1996)
pTX16	Derivative of pTX15 lacking the lipase gene downstream of *xylAR*	Peschel et al. (1996)
pTXgraS (MRSA252)	pTX15 derivative with the *graS* gene of *S. aureus* MRSA252 (SAR0670) under the control of *xylAR*	This thesis
pTXgraS (N315)	pTX15 derivative with the *graS* gene of *S. aureus* N315 (SA0615) under the control of *xylAR*	This thesis
pCU1	high copy shuttle vector, Amp^r, Cm^r, MCS-*lacZ*`	Augustin et al. (1992)
pCU1graXRS (MRSA252)	pCU1 derivative carrying the SAR0668-70 genes of *S. aureus* MRSA252	This thesis
pCU1graXRS (N315)	pCU1 derivative carrying the SA0613-15 genes of *S. aureus* N315	This thesis
pUC19	high copy vector, Amp^r, MCS-*lacZ*`	Vieira and Messing (1982)
pUC19gyrB	pUC19 (Amp^r), carrying a 560 bp internal fragment of *gyrB* (SA0005); external plasmid standard for qRT-PCR	Jansen et al. (2007)
pUC19dltB	pUC19 (Amp^r), carrying a 1354 bp internal fragment of *dltB* (SA0794); external plasmid standard for qRT-PCR	This thesis
pUC19vraF	pUC19 (Amp^r), carrying a 761 bp internal fragment of *vraF* (SA0616); external plasmid standard for qRT-PCR	This thesis
pET22b	C-terminal 6xHis-tag expression vector, T7 *lac* promoter, Amp^r	Studier and Moffatt (1986)
pET22bΔpelB	pET22b without *pelB*-leader tag	Sass, 2005
pETerΔ11	pET22b + ORF 53 of ϕ11 (aa 1-490, 56.5 kDa) [I/II]	Sass, 2005
pETendo/ami11	pET22b + ORF 53 of ϕ11 (aa 1-371, 43.2 kDa) [I/IX]	This thesis
pETendo11	pET22b + ORF 53 of ϕ11 (aa 1-180, 22.1 kDa) [I/V]	This thesis
pETendoCBD11	pET22b + ORF 53 of ϕ11 (Δaa 181-370, 35.2 kDa) [I/V; VI/VII]	This thesis
pETami11	pET22b + ORF 53 of ϕ11 (aa 180-371, 22.8 kDa) [VIII/IX]	This thesis
pETamiCBD11	pET22b + ORF 53 of ϕ11 (aa 180-490, 36.1 kDa) [VIII/II]	This thesis
pETerΔ12	pET22b + ORF 49 of ϕ12 (aa 1-484, 55.3 kDa) [III/IV]	Sass, 2005
pETerΔ12mut	mutated pETerΔ12 for the expression of T780G substituted ϕ12mut endolysin	This thesis

Abbreviations: Tet, tetracycline; Amp, ampicillin; Cm, chloramphenicol; r, resistant; s, susceptible.

Roman numerals in parentheses are primer pairs (see Table 3.3) used for amplification of ORFs [for/rev].

3.3 Antimicrobial susceptibility testing

Determination of the minimal inhibitory concentration (MIC) was performed in polystyrene round bottom microtiter plates (Greiner, Frickenhausen, Germany) using BHI broth or half concentrated BHI broth as indicated. An inoculum of 5×10^5 CFU/ml was employed in the

arithmetic broth microdilution method. All experiments were done in triplicate. For MIC testing of mersacidin (Hoechst, Frankfurt am Main, Germany), $CaCl_2$ was added to all cultures to a final concentration of 1 mM unless indicated otherwise, since Ca^{2+}-ions enhance the bactericidal effect of mersacidin (Barrett et al., 1992; Schneider et al., 2000).

3.4 Growth conditions of *S. aureus* for the preparation of total RNA

For the analysis of the mersacidin induced transcriptional response of *S. aureus*, the cultures were diluted 200-fold from overnight precultures and grown to exponential phase until they reached an optical density at 600 nm (OD_{600}) of 0.5 in BHI broth. Then, mersacidin was added as indicated, and the cultures were further grown to an OD_{600} of 1.0 for total RNA preparations (~30 minutes) or longer to assess growth behavior.

For the comparative analysis of *S. aureus* SG511-Berlin with *S. aureus* SA137/93A via DNA microarrays, 10 ml cultures were diluted 200-fold from fresh BHI broth overnight precultures and were further incubated until they reached an OD_{600} of 1.0.

For the extraction of total RNA for *dltB* and *vraF* expression analysis, overnight cultures were diluted 200-fold into 10 ml half concentrated BHI and grown to exponential phase until they reached an OD_{600} of 0.5. Then, the cationic antimicrobial peptide (CAMP) indolicidin was added to a final concentration of 0.5 µg/ml in order to induce *dltB* and *vraF* expression via the GraRS two-component regulatory system (Li et al., 2007a). The cultures were further grown for 30 minutes to an OD_{600} of approximately 1.0.

3.5 Preparation of total RNA

For RNA preparation, *S. aureus* cultures were grown as aforementioned and aliquots of 10 ml were stabilized by incubation with two volumes of prewarmed RNAprotect Bacteria Reagent (Qiagen, Hilden, Germany) for 5 min at 37°C. The culture was subsequently harvested by centrifugation and the pellets were shock-frozen in liquid nitrogen and kept at -70°C. The cells were lysed in the presence of 400 µg/ml lysostaphin (Genmedics, Reutlingen, Germany) and total RNA was extracted using the PrestoSpin R bug kit including DNase I treatment (Molzym, Bremen, Germany) following the manufacturer's instructions. Quality and quantity of total RNA were determined by agarose gel electrophoresis and measured by using the Nanodrop spectrophotometer (Nanodrop Technologies, Wilmington, USA).

3.6 Synthesis of CyDye-3 and CyDye-5 labeled cDNA for microarray experiments

3.6.1 Direct cDNA labeling

For the analysis of the mersacidin induced response of *S. aureus*, fluorescence-labeled single-stranded cDNA was obtained by reverse transcription of total RNA. To this end, aliquots of total RNA preparations from three different cultures of each condition were pooled to a total amount of 9 µg (3 µg each) and transcribed into cDNA using 100 units (U) of BioScript reverse transcriptase (Bioline, Luckenwalde, Germany) following the manufacturer's instructions. The total reaction volume of 40 µl contained 75 µg/ml pd(N)6 random hexamers (GE Healthcare, Freiburg, Germany), 0.1 mM CyDye3- or CyDye5-dCTPs (GE Healthcare) aside from 0.2 mM dCTP, 0.5 mM dATP, 0.5 mM dTTP, 0.5 mM dGTP and 25 U/ml RNase-OUT (Invitrogen, Karlsruhe, Germany). RNA was degraded by alkaline hydrolysis at 65°C and fluorescence-labeled cDNA was purified using the MinElute PCR purification kit (Qiagen). cDNA synthesis and CyDye3 / CyDye5 incorporation were verified by using the Nanodrop spectrophotometer (Nanodrop Technologies).

3.6.2 Indirect cDNA labeling

For the comparative analysis of *S. aureus* SG511-Berlin and *S. aureus* SA137/93A, fluorescence-labeled DNA was obtained by reverse transcription of total RNA. Therefore, total RNA preparations (3 µg) were transcribed into cDNA using BioScript reverse transcriptase (Bioline) and pd(N)6 random hexamers (GE Healthcare) following the manufacturers' instructions. For fluorescence-labeling, three different aliquots of cDNA preparations from separate cultures of the respective experiment were pooled to a total amount of 300 ng (100 ng each) and were subsequently used in the labeling reaction using the BioPrime(R) DNA Labeling System (Invitrogen) according to the instruction manual using pd(N)6 random hexamers (Invitrogen), 0.1 mM CyDye3- or CyDye5-dCTPs aside from 0.2 mM dCTP, 0.5 mM dATP, 0.5 mM dTTP, 0.5 mM dGTP (GE Healthcare).

3.7 Microarray hybridization and analysis

Microarray-based transcriptional profiling by competitive hybridization of fluorescence-labeled cDNA was performed by using the custom PCR product full-genome chip *sciTracer* (Scienion, Berlin, Germany) containing 2338 unique open reading frames representing 90% of the *S. aureus* N315 genome (NC_002745). Additional information on the microarray platform has been deposited in NCBI's Gene Expression Omnibus (see below). Each

experiment was performed 4 times including a dye swap resulting in four chips per competitive comparison to increase reproducibility. *S. aureus* SA137/93G incubated with 0.15 x MIC of mersacidin for the analysis of the mersacidin induced response of *S. aureus* as well as the microarray experiments for the comparative analysis of *S. aureus* SG511-Berlin with *S. aureus* SA137/93A were only reproduced in duplicate. All hybridizations were done with equal amounts of cDNA probes displaying similar picomoles of incorporated dye. Fluorescence-labeled cDNA probes were mixed in hybridization buffer (Scienion) in a total volume of 55 µl, denatured at 95°C for 2 min and subsequently applied to the microarray slide followed by incubation at 42°C for 72 hours under humidified conditions according to the manufacturer's instructions. Hybridized microarrays were washed at room temperature in SSC buffer with decreasing salt concentrations (1 x SSC / 0.3% SDS for 5 min, 0.2 x SSC for 5 min, 0.06 x SSC for 30s). For image capture, the microarrays were scanned with a GenePix 4000B scanner (Axon Instruments / Distribution by Biozyme Scientific GmbH, Hessisch Oldendorf, Germany). The TIFF images were analyzed with GenePixPro4.1 software (Axon Instruments). The actual signal intensity was calculated by using the mean of the "median of ratios" of the individual spot. The data sets were then normalized by using Acuity 3.1 software (Axon Instruments) and by applying the LOWESS algorithm. For the analysis of the mersacidin induced response of *S. aureus*, significant changes of gene expression were determined by implementing SAM (significance analysis of microarrays; http://www-stat.stanford.edu/~tibs/SAM/) (Tusher et al., 2001) using the one class response and a false discovery rate of <1% with a medium number of falsely called significant genes of <1.

3.8 Microarray data accession number

Additional information on the microarray platform as well as the processed and raw microarray data of this thesis have been deposited in NCBI's Gene Expression Omnibus (GEO) (http://www.ncbi.nlm.nih.gov/geo/) to be found under the GEO Series accession numbers GSE9261 and GSE12016.

3.9 Transcript quantification by Real-Time PCR (qRT-PCR)

The LightCycler instrument (Roche Diagnostics, Mannheim, Germany) was employed to generate quantitative transcription data by measuring sample amplification during the log-linear phase of the PCR. Therefore, total RNA preparations (3 µg) were transcribed into cDNA using BioScript reverse transcriptase (Bioline) and pd(N)6 random hexamers (GE Healthcare) following the manufacturers' instructions. Quantitative RT-PCR was

performed by using the LightCycler FastStart DNA Master SYBR Green I kit (Roche Diagnostics) according to the manufacturer's instructions. For all experiments, the amount of transcripts was determined from the appropriate standard curve and the target concentration was expressed in relation to the concentration of the constitutively expressed housekeeping gene *gyrB*. Each standard curve was generated by assaying gene specific PCR product or plasmid templates. The specific primers, which were used for the synthesis of external LightCycler standards or for qRT-PCR of the target genes and the endogenous control *gyrB*, are listed in Table 3.3. To control quality and reproducibility of the qRT-PCR data, at least two different cDNA probes were synthesized employing RNA preparations from independent cultures for every condition. The PCR products were verified by melting curve analysis and ethidium bromide staining on agarose gels.

3.10 Nucleotide sequence analysis of *vraDE*, *vraS* and *graXRS*

The nucleotide sequences of *vraDE* and *vraS* as well as *graXRS* (SA0613-5, of the genomic sequence of *S. aureus* N315) in *S. aureus* SG511-Berlin, SA137/93A and SA137/93G were determined from PCR fragments, which were amplified from genomic DNA using a standard PCR protocol and the primers described in Table 3.3. For *graXRS* sequencing of strain SG511-Berlin, the PCR products were amplified from at least two different long-term glycerol stock cultures for each strain. The nucleotide sequences were obtained by Sanger sequencing including quality editing of the automatically determined sequence (Sequiserve, Vaterstetten, Germany). *In silico* analysis of sequence data was performed using world wide web-based online analysis tools (http://molbiol-tools.ca/).

3.11 Molecular cloning procedures

3.11.1 Isolation of genomic DNA

For genomic DNA preparations of *S. aureus*, 2-4 ml overnight cultures were harvested and lysed in the presence of 125 µg/ml lysostaphin (Genmedics) followed by genomic DNA extraction using the PrestoSpin D bug kit (Molzym) according to the manufacturer's instructions. Quality and quantity of genomic DNA were determined by agarose gel electrophoresis and measured by using the Nanodrop spectrophotometer (Nanodrop Technologies).

Table 3.3: Oligonucleotide primers used in this thesis.

Primer	Primer sequence 5'-3'	Anneal. Temp.	Reference(s) or source
a) Oligonucleotide primers used for the synthesis of external *vraE* and *vraS* qRT-PCR standards			
vraE-1	TCTCATATGACATTTAACCATATCGTTTTC (*Nde*I)	64.5°C	This thesis
vraE-2	TAACTCGAGAATGGTTTTCTTAATCAATTTGTTTG (*Xho*I)	64.5°C	This thesis
vraS-1	TTACATATGAACCACTACATTAGAACAAT (*Nde*I)	63.2°C	This thesis
vraS-2	AATAAGCTTATCGTCATACGAATCCTCCT (*Hin*dIII)	63.2°C	This thesis
b) Oligonucleotide primers used to construct the external qRT-PCR standards pUC19dltB and pUC19vraF			
dltBfor-Xba1	AAATCTAGATGCAGAGGTAATTAACGGATGA (*Xba*1)	62°C	This thesis
dltBrev-Pst1	ATACTGCAGTCTTCAAAAATTTCTACGTCTGGA (*Pst*1)	62°C	This thesis
vraFfor-Xba1	AAATCTAGAGGTAAGCGTGCCAAATTGAAT (*Xba*1)	62°C	This thesis
vraFrev-Pst1	TTACTGCAGTGCTATGCGTATATTTTAGTGCT (*Pst*1)	62°C	This thesis
c) Oligonucleotide primers used for qRT-PCR			
gyrB-297	TTAGTGTGGGAAATTGTCGATAAT	52°C	Goerke et al. (2006)
gyrB-547	AGTCTTGTGACAATGCGTTTACA	52°C	Goerke et al. (2006)
vraE-1-RT2	GTAACTGTATTGTGTTTCGCGGC	52°C	This thesis
vraE-2-RT2	TGATGGCATTGTTGCCTGTTACC	52°C	This thesis
vraS-1-RT	GTTGGTTCGGTACTCGCATA	52°C	This thesis
vraS-2-RT	CTCGAGCTAGTCTTTGACGTTC	52°C	This thesis
dltB-RT1	CATGGTTAGGTGGACATCAGA	52°C	This thesis
dltB-RT2	CGATATTCATTGCCTGTTGG	52°C	This thesis
vraF-RT1	TCCATCCGAATTGTCTGGT	52°C	This thesis
vraF-RT2	CCGTGAATATTTGCCCATC	52°C	This thesis
d) Oligonucleotide primers used for *vraDE* sequencing			
vraDprom_for	TCCAATCAACCAGGTAATAGCA	62°C	This thesis
vraDint_rev	TTGGAAGATGAAACCTAATGATTCT	62°C	This thesis
vraEfor	CAATCAGTATTAGGTGGTGTC	62°C	This thesis
vraEint_rev	GCGTTTCACGAATAAATGATTG	62°C	This thesis
vraErev	CTCAATGAAGCATCTTTTAATCG	62°C	This thesis
e) Oligonucleotide primers used for *vraS* sequencing			
vraSfor	CGTTTATCGGAGACGTAGAG	62°C	This thesis
vraSrev	GTACCATTTCATGATCATCCAC	62°C	This thesis
f) Oligonucleotide primers used for *graXRS* (SA0613-5) sequencing			
SA0613_for	CTAACTCTACGTATAATATGGGC	64°C	This thesis
SA0613int_rev	GTGTTATTTTGGCAGAATTCTTTGT	64°C	This thesis

continued on following page

Table 3.3 - *Continued*

Primer	Primer sequence 5'-3'	Anneal. Temp.	Reference(s) or source
graRint_rev	ATACCAGCAACATTAAAATCCCATT	64°C	This thesis
graSintern_rev	GCGCTGAGATTTGACGATATAAATAA	64°C	This thesis
graS_rev	CAATTCTAAGTAACAAAACGCATG	64°C	This thesis
g) Oligonucleotide primers used for the construction of pTXgraS			
graSpTX-BamH1	GTAGGATCCGGATATATGGCACATGAATAATTTG (*Bam*HI)	64°C	This thesis
graSpTX-Mlu1	ATGACGCGTCAATTCTAAGTAACAAAACGCATG (*Mlu*1)	64°C	This thesis
h) Oligonucleotide primers used for the construction of pCU1graXRS			
SA0613pCU-Xba1	TCTTCTAGACTAACTCTACGTATAATATGGGC (*Xba*I)	64°C	This thesis
graSpCU-Pst1	ATGCTGCAGCAATTCTAAGTAACAAAACGCATG (*Pst*I)	64°C	This thesis
i) Oligonucleotide primers for the mapping of Tn*551* transposon insertion			
Tn*551*-P1	TCCGAGAGTGATTGGTCTTG	60°C	This thesis
Tn*551*-P2	AAATTTCTCGTAGGCGCTCG	60°C	This thesis
Tn*551*-P2.2	GTTTGGATCTCGCAATACACG	62°C	This thesis
k) Oligonucleotide primers used for the construction of pET22b derivatives			
I. phi11F	TTTGGATCCAATGAGTATCATCATGGAGGTG (*Bam*HI)	62°C	This thesis
II. phi11R	GTCAAGCTTACTGATTTCTCCCCATAAGTCA (*Hind*III)	62°C	This thesis
III. phi12F	GTGGGATCCAATGTTGATAACAAAAAACCAA (*Bam*HI)	62°C	This thesis
IV. phi12R	TAAAAGCTTAATCGTGCTAAACTTACCAAAAC (*Hind*III)	62°C	This thesis
V. endo11R	TTGAAGCTTAGCTGTTTCTTTTTTAGGT (*Hind*III)	62°C	This thesis
VI. CBD11F	ATGAAGCTTAAAATACCGGTTGCCACTGT (*Hind*III)	69.3°C	This thesis
VII. CBD11R	GTCCTCGAGACTGATTTCTCCCCATAAGTCA (*Xho*I)	69.3°C	This thesis
VIII. ami11F	GAACATATGAAGCCACAACCTAAAGCAGTAG (*Nde*I)	64°C	This thesis
IX. ami11R	CGGAAGCTTACCATCCATGTACGCCCTAA (*Hind*III)	64°C	This thesis
l) Oligonucleotide primers used for the construction of mutated ϕ12 endolysin			
phi12mutFor	"Phos"-ACA*G*GGGTATGACATTGTTCTAGAGA	62°C	This thesis
phi12mutRev	"Phos"-GATTTAACCCAATATAAGCCATAA	62°C	This thesis

Restriction sites are underlined and in brackets. Mutated primer nucleotides are underlined and in italics.

3.11.2 Isolation of plasmid DNA

For plasmid DNA preparations, 2-4 ml overnight cultures were harvested followed by plasmid DNA extraction using the QIAprep® Spin Miniprep kit (Qiagen) according to the manufacturer's instructions. Lysis of *S. aureus* cultures was supported by the presence of 125 µg/ml lysostaphin (Genmedics). Quality and quantity of genomic DNA were determined by

agarose gel electrophoresis and measured by using the Nanodrop spectrophotometer (Nanodrop Technologies).

3.11.3 Purification of DNA fragments and extraction from agarose gels

DNA fragments were excised from agarose gels under UV light using a scalpel and purified by employing the MinElute™ PCR Purification kit (Qiagen) or the QIAquick™ Gel Extraction kit (Qiagen) depending on the target's size and according to the manufacturer's instructions.

3.11.4 Agarose gel electrophoresis

The electrophoretic separation of RNA and DNA fragments was performed on a horizontal electrophoresis apparatus (Peqlab, Erlangen, Germany) using Top Vision™ LE GQ agarose (Fermentas, St. Leon-Rot, Germany) with concentrations of 0.8-2% depending on the molecular weight of the specific fragments. TAE (Tris-Acetate-EDTA) buffer was used for the preparation of agarose gels and as an electrophoresis running buffer according to standard procedures (Sambrook et al., 1989). RNA and DNA fragments were visiualized by ethidium bromide staining and documented using the ImageMaster VDS system (GE Healthcare/ Pharmacia).

3.11.5 Polymerase chain reaction (PCR)

PCR was performed on the PCRexpress Thermal Cycler (Thermo Fisher Scientific, Waltham, USA; formerly Hybaid) or the SensoQuest Labcycler (Sensoquest, Göttingen, Germany). GoTaq DNA polymerase (Promega, Mannheim, Germany) or Phusion™ HF DNA polymerase (NEB, Frankfurt am Main, Germany) were used for DNA amplification following the manufacturers' instructions and according to standard PCR procedures. The primers used for PCR are listed in table 3.3.

3.11.6 Enzymatic modification of DNA

Digestion of DNA by FastDigest® restriction enzymes (Fermentas), vector dephosphorylation using FastAP™ thermosensitive alkaline phosphatase (Fermentas), filling of 5`-protruding ends for blunt end DNA ligation using Klenow fragment (Fermentas) and ligation of DNA fragments using T4-ligase (Roche) were performed following the manufacturers' instructions and according to standard procedures (Sambrook et al., 1989).

3.11.7 Site-directed mutagenesis

The Phusion™ Site-Directed Mutagenesis kit (NEB) was employed to introduce single point mutations into the coding sequence of target genes located on the pET22b plasmid (Fig. 3.1). According to the manufacturer's instructions, PCR amplification of the target plasmid was performed using two 5`-phosphorylated primers, comprising the desired mutation, which anneal back to back to the plasmid. Circularization of the mutated PCR products was achieved by blunt end ligation followed by transformation of the mutated plasmid into *E. coli* by electroporation (see below).

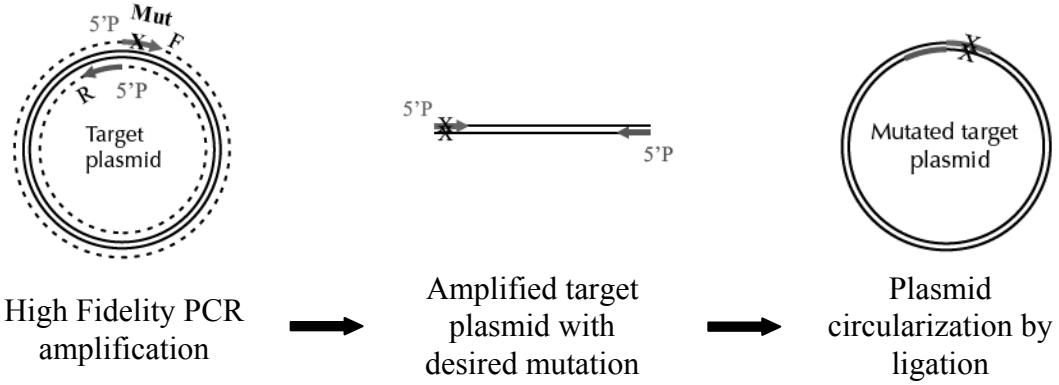

Figure 3.1: Schematic representation of site-directed mutagenesis. Derived from www.finnzymes.com.

3.11.8 Mapping of Tn*551* transposon insertion

Chromosomal DNA of *S. simulans* 22 Tn*551*-7 was isolated as aforementioned and digested with *Hpa*I (Roche). The digest was purified using the E.Z.N.A.® Cycle Pure kit (Peqlab), diluted 1:5 and religated. A PCR was performed employing the outgoing primers Tn*551*-P1 and Tn*551*-P2 (Table 3.3) that anneal within the 5`-terminal 278 bp *Hpa*I fragment of Tn*551* and Phusion™ HF DNA polymerase (NEB) using a standard PCR protocol. The PCR products were analyzed on 1% agarose gels and new bands appearing compared to the wild type were extracted from the gel, purified and sequenced (Sequiserve).

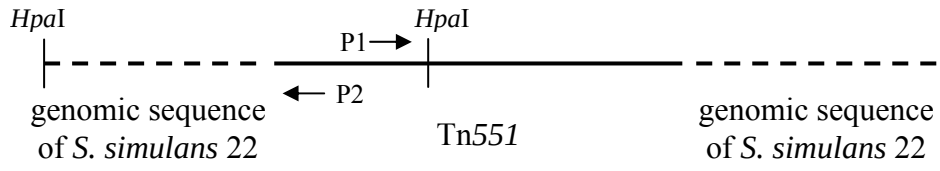

Figure 3.2: Schematic representation of mapping the Tn*551* transposon insertion.

3.11.9 Transformation of *E. coli* and *S. aureus* by electroporation

The transformation of bacteria by electroporation is based on a significant increase of electrical conductivity and permeability of the cell plasma membrane caused by an externally applied electrical field which allows the introduction of DNA into the cell. For the preparation of electrocompetent cells, the bacteria were grown to an OD_{600} of 0.5 and were subsequently washed several times with destilled water and resuspended in 10% glycerol for cryoprotection. Electroporation was performed as described previously for *S. aureus* (Schenk and Laddaga, 1992) and *E. coli* (Sass, 2005).

3.11.10 Transformation of *S. aureus* by phage transduction

The pCU1 vector derivatives were transduced into *S. aureus* SG511-Berlin by phage transduction using bacteriophage 85 as described previously (Berger-Bächi and Kohler, 1983) including a heat shock at 52°C for 2 min to inactivate the host restriction system.

3.12 Cytochrome c assay

For the determination of the whole-cell surface charge, stationary-phase bacteria (OD_{600} of 3-5) were harvested and washed twice with 20 mM 3-[N-morpholino] propanesulfonic acid (MOPS; pH 7.0; Sigma-Aldrich, Taufkirchen, Germany). Six absorbance units (e.g. bacteria from 2 ml of a culture with an OD_{600} of 3) were resuspended in 500 µl equine heart cytochrome c (0.5 mg/ml; Sigma) dissolved in MOPS buffer. After an incubation for 10 min at room temperature, the samples were pelleted and the supernatant was assayed photometrically at 530 nm using a control (buffer without cells) and a cytochrome c standard curve as a reference. The data represent the mean values of at least three independent studies unless indicated otherwise.

3.13 C-terminal six-His-tagged proteins

3.13.1 The pET22b C-terminal six-His-tag protein expression vector

The pET22b C-terminal six-His-tag protein expression vector belongs to the pET system family (Merck-Novagen, Darmstadt, Germany) which are employed for the cloning and expression of recombinant proteins in *E. coli* (Fig. 3.3). Target genes are cloned into pET22b under the control of the strong bacteriophage T7*lac* promoter and the *lac* operator. In the pET22b vector, the coding sequence of six histidine residues is located downstream the multiple cloning site (mcs) that enables the C-terminal fusion of a six-His-tag to any target

gene which allows its purification via Ni-NTA affinity chromatography. Expression is induced by providing a source of T7 RNA polymerase in the host cell and by the addition of isopropyl-β-D-thiogalactopyranoside (IPTG) to the bacterial culture. The expression host *E. coli* BL21(DE3) is lysogenic for bacteriophage DE3 and therefore carries a copy of the T7 RNA polymerase gene under the control of the lacUV5 promoter. *E. coli* BL21(DE3) also harbours the *lac* repressor gene *lacI* which blocks the transcription of the T7 RNA polymerase gene and the target gene on pET22b until IPTG induction. Furthermore, the pET22b vector offers the *pelB* signal sequence, which is co-transcribed with the target gene and mediates the transport of the six-His-tagged protein into the periplasm of *E. coli*.

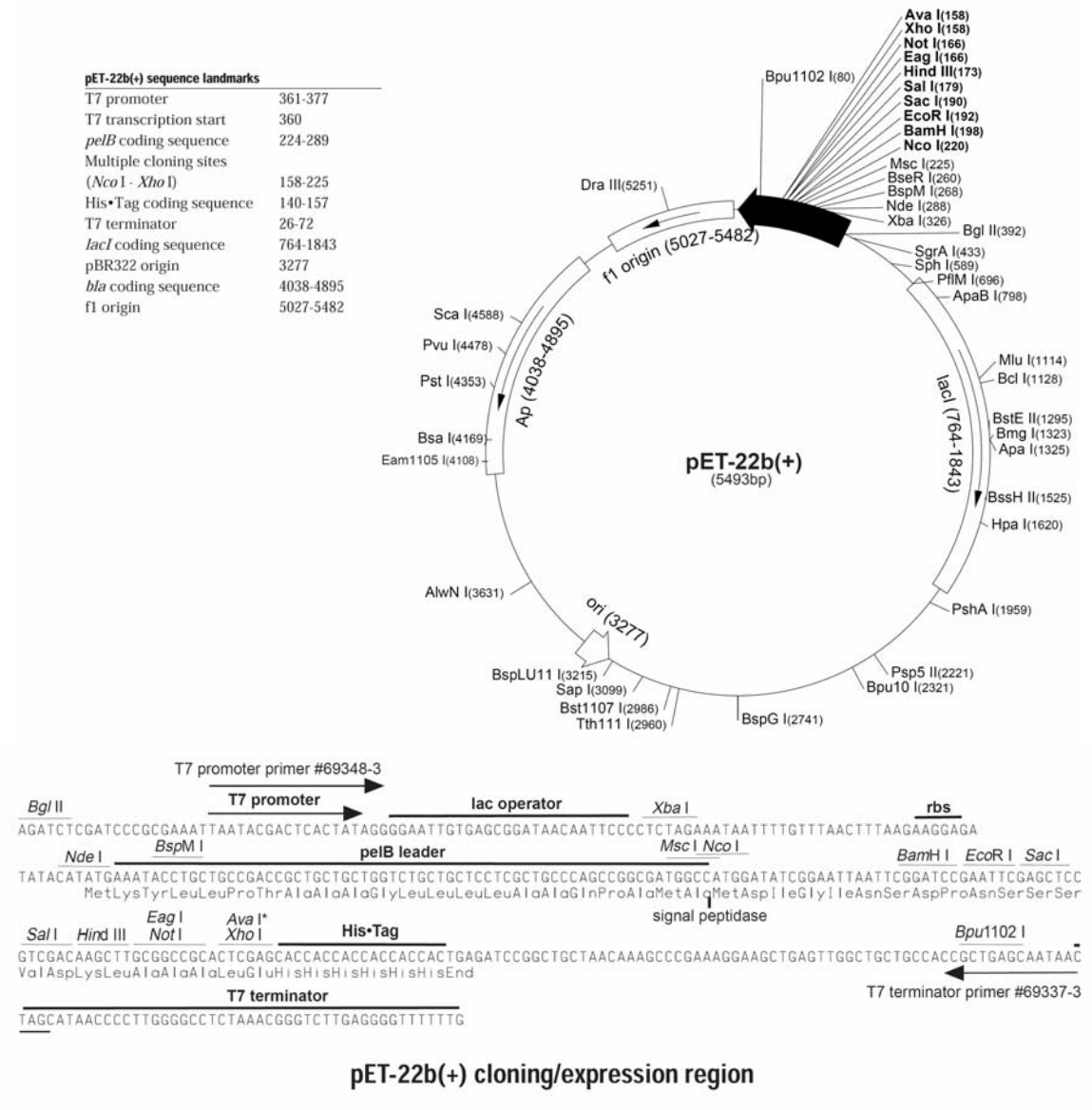

Figure 3.3: The pET22b C-terminal six-His-tag protein expression vector. Source: www.novagen.com

3.13.2 Expression and purification of C-terminal six-His-tagged proteins

Expression cultures (1000 ml) were grown at 30°C in LB broth containing ampicillin (40 µg/ml) to an OD_{600} of 0.6. Then protein expression was induced by addition of IPTG to a final concentration of 1 mM. Expression cultures were harvested after 4 hours followed by protein purification steps under native conditions via Ni-NTA affinity chromatography as described previously (Sass, 2005). Protein purification was also performed with cells harbouring the empty vector and the eluate served as control in the activity tests.

3.13.3 Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-Page)

The six-His-tagged proteins were analyzed via SDS-Page as described previously (Laemmli, 1970) on the Mini Protean III vertical electrophoresis apparatus (BioRad).

Chemicals used for SDS-Page:

Acrylamide:	40% acrylamide/bisacrylamide-solution (19:1)
Solution A:	3 M Tris/HCl; pH 8.5
Solution D:	0.96 M Tris
	0.8% (w/v) SDS
Ammonium persulfate (APS):	0.21%
SDS:	20%
5x SDS loading dye:	0.225 M Tris/HCl
	50% (w/v) glycerol
	5% (w/v) SDS
	0.05% (w/v) bromphenol blue
	0.25 M DTT; pH 6.8
Running buffer:	25 mM Tris
	192 mM glycine
	0.1% (w/v) SDS; pH 8.5
Staining solution:	PageBlue™ Protein Staining Solution (Fermentas)

Table 3.4: Chemical ingredients of resolving and stacking gels.

Chemicals	Resolving gel (12%)	Stacking gel (4%)
Acrylamide/bisacrylamide (19:1)	2.25 ml	0.238 ml
Solution A	1.25 ml	-------
Solution D	-------	0.3 ml
APS	2.0 ml	0.8 ml
Distilled water	2.7 ml	1.03 ml
SDS (20%)	50 µl	-------
Tetramethylethylendiamin (TEMED)	5 µl	5 µl

3.13.4 Determination of protein concentrations according to Bradford

The sample protein concentration was determined by the Bradford method (Bradford, 1976) using the Protein Assay Reagent (BioRad) as described previously (Sass, 2005).

3.14 Activity testing of C-terminal six-His-tagged proteins

3.14.1 Photometric assay

A photometric assay was employed to analyze the lytic activities of the six-His-tagged endolysins (each applied in a concentration of 20 µg/ml). To this end, the turbidity of purified SDS cell walls of *S. simulans* 22 (Bierbaum and Sahl, 1987) or heat-inactivated staphylococci (pasteurized for 10 minutes at 80°C), both resuspended in incubation buffer [50 mM Tris-HCl, 100 mM NaCl, pH 7.5] to an OD_{600} of ~0.3, was measured over time. All lysis experiments were performed in triplicates.

3.14.2 Biofilm assay

The influence of six-His-tagged endolysins on staphylococcal biofilms was determined by a modified biofilm plate assay (Wu et al., 2003). To obtain staphylococcal biofilms, an overnight culture of the desired strain was diluted 1:200 in TSB medium supplemented with 0.25% D-(+)-glucose to a final volume of 200 µl in each well of a flat-bottomed 96-well polystyrene microtiter plate (Nunc, Wiesbaden, Germany). The plates were incubated for 24 h or 48 h at 37°C under aerobic conditions or in 5% CO_2 incubation atmosphere, respectively. Afterwards, the wells were washed twice with incubation buffer [50 mM Tris-HCl, 100 mM NaCl, pH 7.5]. The biofilm-containing wells were then filled with incubation buffer plus endolysin. Fresh medium and incubation buffer alone or incubation buffer plus lysostaphin or lysozyme were employed as controls. Lysostaphin was used as a positive control, since it is able to lyse *S. aureus* and *S. epidermidis* biofilms (Wu et al., 2003). Following incubation for two hours at 37°C, the wells were washed again and stained with 0.1% safranin.

3.15 Sodium metaperiodate and proteinase K treatment of staphylococcal biofilms

Staphylococcal biofilms were obtained as aforementioned and treated with either 40 mM sodium periodate or 1 mg/ml proteinase K dissolved in incubation buffer [50 mM Tris-HCl, 100 mM NaCl, pH 7.5] for 24 h. Distilled water was used as a control.

4 Results

Part I – Response and resistance of *S. aureus* to the lantibiotic mersacidin

4.1 Antimicrobial susceptibility testing of *S. aureus* SA137/93A, *S. aureus* SA137/93G and *S. aureus* SG511-Berlin to mersacidin

In this thesis, three *S. aureus* strains with different susceptibility characteristics were employed, namely the vancomycin- and methicillin-susceptible (VSSA / MSSA) strain *S. aureus* SG511-Berlin as well as the heterogeneous VISA / MRSA strain *S. aureus* SA137/93A and the closely related VISA / MSSA strain *S. aureus* SA137/93G (Reipert et al., 2003; Maki et al., 2004). The three strains displayed considerable differences in their susceptibilities to mersacidin. While the growth of *S. aureus* SG511-Berlin was already inhibited by 1 µg/ml mersacidin in BHI, the minimal inhibitory concentrations (MIC) of mersacidin against SA137/93A and SA137/93G were 35 µg/ml and 30 µg/ml in BHI broth, respectively. To assess suitable mersacidin concentrations for microarray experiments, the lantibiotic was added to an exponentially growing *S. aureus* culture at an OD_{600} of ~0.5 (time point "a"; Fig. 4.1). In the microarray experiments, the cells were harvested at an OD_{600} of ~1 (time point "b"; Fig. 4.1) before the reduced growth rates of the cultures became visible. The slight variation of the MIC values between the two VISA strains was also reflected by the growth curve of strain SA137/93G showing a stronger decrease of its growth rate after addition of 16 µg/ml mersacidin.

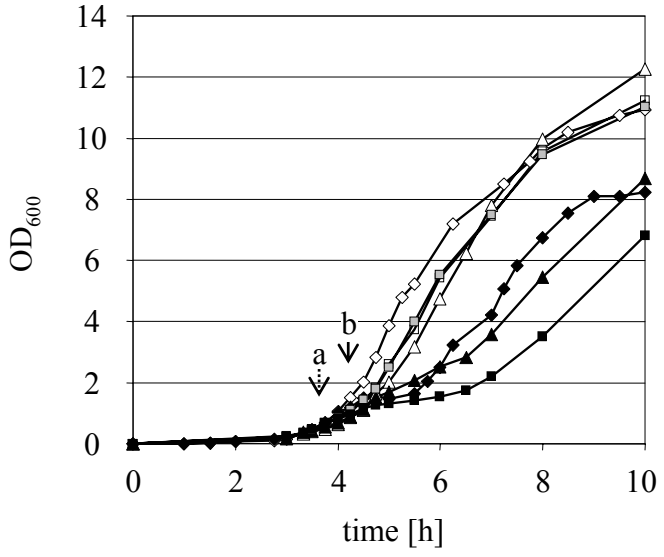

Figure 4.1: *In vitro* growth of *S. aureus* in the presence of mersacidin. *S. aureus* SG511-Berlin (◆ 1 µg/ml, ◇ control); *S. aureus* SA137/93A (▲ 16 µg/ml, △ control); *S. aureus* SA137/93G (■ 16 µg/ml, ■ 4 µg/ml, □ control).

4.2 PFGE, MLST and phage typing of *S. aureus* strains SA137/93A and SG511-Berlin

Since the PFGE patterns and lysotypes of *S. aureus* SA137/93A and SA137/93G have been previously described (Bierbaum et al., 1999; Reipert et al., 2003), *S. aureus* SG511-Berlin was also analyzed via PFGE (Fig. 4.2). However, the PFGE pattern of *S. aureus* SG511-Berlin was dissimilar from the patterns of the strains SA137/93A, SA137/93G and all other *S. aureus* strains typed via PFGE at the reference center for staphylococci at the Institute for Medical Microbiology in Bonn (Marion Oedenkoven, IMMIP; personal communication). This might be due to the fact that PFGE is commonly used to study the international spread of MRSA epidemic clones rather than MSSA strains.

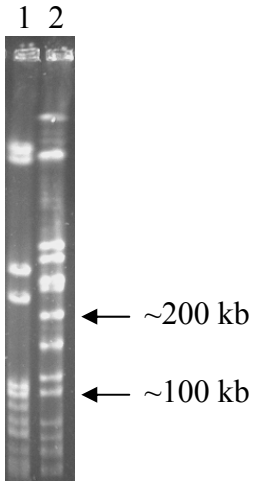

Figure 4.2: Pulsed field gel electrophoresis (PFGE) of *S. aureus* SG511-Berlin. (1) *S. aureus* SG511-Berlin; (2) *S. aureus* NCTC8325 (reference strain).

To further characterize *S. aureus* strain SA137/93A and strain SG511-Berlin, the sequence type (ST) of each strain was determined by MLST (Fig. 4.3 and Fig. 4.4). Since *S. aureus* SA137/93G is isogenic to strain SA137/93A (Reipert et al., 2003), this strain was not tested. MLST is a molecular typing technique that has become the predominant method to study the epidemiology of *S. aureus* because of its high discrimination and inter-laboratory reproducibility (Enright et al., 2000). MLST of *S. aureus* involves sequencing DNA fragments (~500-600 bp) of seven housekeeping genes, namely *arcC* (carbamate kinase), *aroE* (shikimate dehydrogenase), *glpF* (putative glycerol uptake facilitator protein SAR1274), *gmk* (guanylate kinase), *pta* (phosphate acetyltransferase), *tpiA* (triosephosphate isomerase) and *yqi* (acetyle coenzyme A acetyltransferase). The nucleotide sequences of these genes are then trimmed to standard sequence lengths (trimmed sequences) and are compared to known

alleles at each locus via the MLST database (http://saureus.mlst.net/). Here, every isolate is described by a seven-integer allelic profile that defines a sequence type.

S. aureus SA137/93A could be assigned to the allele type 3-3-1-12-4-4-16 (ST247) and is therefore closely related to the Northern German epidemic MRSA strain SA1450/94 (Cookson et al., 2007). *S. aureus* SG511-Berlin had the allele type 2-2-2-2-6-3-2 (ST30) which is considered to be the ancestral type of the MRSA clonal complex (CC) 30 (Robinson and Enright, 2003) and is therefore closely related to *S. aureus* MRSA252 (ST-36) (Johnson et al., 2001; Enright et al., 2000).

S. aureus SA137/93A – *arcC* – allele number 3
TTATTAATCCAACAAGCTAAATCGAACAGTGACACAACGCCGGCAATGCCATTGGATACTTGTGGTGCAATGTCACAGGGTATGATAGGCTATT
GGTTGGAAACTGAAATCAATCGCATTTTAACTGAAATGAATAGTGATAGAACTGTAGGCACAATCGTTACACGTGTGGAAGTAGATAAAGATGA
TCCACGATTTGATAACCCAACTAAACCAATTGGTCCTTTTTATACGAAAGAAGAAGTTGAAGAATTACAAAAAGAACAGCCAGACTCAGTCTTT
AAAGAAGATGCAGGACGTGGTTATAGAAAAGTAGTTGCGTCACCACTACCTCAATCTATACTAGAACACCAGTTAATTCGAACTTTAGCAGACG
GTAAAAATATTGTCATTGCATGCGGTGGTGGCGGTATTCCAGTTATAAAAAAAGAAAATACCTATGAAGGTGTTGAAGCG

S. aureus SA137/93A – *aroE* – allele number 3
AATTTTAATTCTTTAGGATTAGATGATACTTATGAAGCTTTAAATATTCCAATTGAAGATTTTCATTTAATTAAAGAAATTATTTCGAAAAAAG
AATTAGAAGGCTTTAATATCACAATTCCTCATAAAGAACGTATCATACCGTATTTAGATTATGTTGATGAACAAGCGATTAATGCAGGTGCAGT
TAACACTGTTTTGATAAAAGATGGCAAGTGGATAGGGTATAATACAGATGGTATTGGTTATGTTAAAGGATTGCACAGCGTTTATCCAGATTTA
GAAAATGCATACATTTTAATTTTGGGCGCAGGTGGTGCAAGTAAAGGTATTGCTTATGAATTAGCAAAATTTGTAAAGCCCAAATTAACTGTTG
CGAATAGAACGATGGCTCGTTTTGAATCTTGGAATTTAAATATAAACCAAATTTCATTAGCAGATGCTGAAAAGTATTTA

S. aureus SA137/93A – *glpF* – allele number 1
GGTGCTGATTGGATTGTCATCACAGCTGGATGGGGATTAGCGGTTACAATGGGTGTGTTTGCTGTCGGTCAATTCTCAGGTGCACATTTAAACC
CAGCGGTGTCTTTAGCTCTTGCATTAGACGGAAGTTTTGATTGGTCATTAGTTCCTGGTTATATTGTTGCTCAAATGTTAGGTGCAATTGTCGG
AGCAACAATTGTATGGTTAATGTACTTGCCACATTGGAAAGCGACAGAAGAAGCTGGCGCGAAATTAGGTGTTTTCTCTACAGCACCGGCTATT
AAGAATTACTTTGCCAACTTTTTAAGTGAGATTATCGGAACAATGGCATTAACTTTAGGTATTTTATTTATCGGTGTAAACAAAATTGCCGATG
GTTTAAATCCTTTAATTGTCGGAGCATTAATTGTTGCAATCGGATTAAGTTTAGGCGGTGCTACTGGTTATGCAATCAACCCAGCACGT

S. aureus SA137/93A – *gmk* – allele number 12
CGAATATTTGAAGATCCAAGTACATCATATAAGTATTCTATTTCAATGACAACACGTCAAATGCGTGAAGGTGAAGTTGATGGCGTAGATTACT
TTTTTAAAACTAGGGATGCGTTTGAAGCTTTAATCAAAGATGACCAATTTATAGAATATGCTGAATATGTAGGCAACTATTATGGTACACCAGT
TCAATATGTTAAAGATACAATGGACGAAGGTCATGATGTATTTTTAGAAATTGAAGTAGAAGGTGCAAAGCAAGTTAGAAAGAAATTTTCAGAT
GCGCTATTTATTTTCTTAGCACCTCCAAGTTTAGAACACTTGAGAGAGCGATTAGTAGGTAGAGGAACAGAATCTGATGAGAAAATACAAAGTC
GTATTAACGAAGCGCGTAAAGAAGTTGAAATGATGAATTTATACGATTACGTT

S. aureus SA137/93A – *pta* – allele number 4
GCAACACAATTACAAGCAACAGATTATGTTACACCAATCGTGTTAGGTGATGAGACTAAGGTTCAATCTTTAGCGCAAAAACTTGATCTTGATA
TTTCTAATATTGAATTAATTAATCCTGCGACAAGTGAATTGAAAGCTGAATTAGTTCAATCATTTGTTGAACGACGTAAAGGTAAAGCGACTGA
AGAACAAGCACAAGAATTATTAAACAATGTGAACTACTTCGGTACAATGCTTGTTTATGCTGGTAAAGCAGATGGTTTAGTTAGTGGTGCAGCA
CATTCAACAGGCGACACTGTGCGTCCAGCTTTACAAATCATCAAAACGAAACCAGGTGTATCAAGAACATCAGGTATCTTCTTTATGATTAAAG
GTGATGAACAATACATCTTTGGTGATTGTGCAATCAATCCAGAACTTGATTCACAAGGACTTGCAGAAATTGCAGTAGAAAGTGCAAAATCAGC
ATTA

S. aureus SA137/93A – *tpiA* – allele number 4
CACGAAACAGATGAAGAAATTAACAAAAAAAGCGCACGCTATTTTCAAACATGGAATGACTCCAATTATATGTGTTGGTGAAACAGACGAAGAGC
GTGAAAGTGGTAAAGCTAACGATGTTGTAGGTGAGCAAGTTAAGAAAGCTGTTGCAGGTTTATCTGAAGATCAACTTAAATCAGTTGTAATTGC
TTATGAACCAATCTGGGCAATCGGAACTGGTAAATCATCAACATCTGAAGATGCAAATGAAATGTGTGCATTTGTACGTCAAACTATTGCTGAC
TTATCAAGCAAAGAAGTATCAGAAGCAACTCGTATTCAATATGGTGGTAGTGTTAAACCTAACAACATTAAAGAATACATGGCACAAACTGATA
TTGATGGGGCATTAGTAGGTGGCGCA

S. aureus SA137/93A – *yqi* – allele number 16
GCGTTTAAAGACGTGCCAGCCTATGATTTAGGTGCGACTTTAATAGAACATATTATTAAAGAGACGGGTTTGAATCCAAGTGAGATTGATGAAG
TTATCATCGGTAACGTACTACAAGCAGGACAAGGACAAAATCCAGCACGAATTGCTGCTATGAAAGGTGGCTTGCCAGAAACAGTACCTGCATT
TACGGTGAATAAAGTATGTGGTTCTGGGTTAAAGTCGATTCAATTAGCATATCAATCTATTGTGACTGGTGAAAATGACATCGTGCTAGCTGGC
GGTATGGAGAATATGTCTCAATCACCAATGCTTGTCAACAACAGTCGCTTTGGTTTTAAAATGGGACATCAATCAATGGTTGATAGCATGGTAT
ATGATGGTTTAACAGATGTATTTAATCAATATCATATGGGTATTACTGCTGAAAATTTAGCAGAGCAATATGGTATTTCAAGAGAAGAACAAGA
TACATTTGCTGTAAACTCACAACAAAAAGCAGTACGTGCACAGCAA

Figure 4.3: Trimmed nucleotide sequences for MLST analysis of *S. aureus* SA137/93A.

S. aureus SG511-Berlin – *arcC* – allele number 2
TTATTAATCCAACAAGCTAAATCGAACAGTGACACAACGCCGGCAATGCCATTGGATACTTGTGGTGCAATGTCACAAGGTATGATAGGCTATT
GGTTGGAAACTGAAATCAATCGCATTTTAACTGAAATGAATAGTGATAGAACTGTAGGCACAATCGTAACACGTGTGGAAGTAGATAAAGATGA
TCCACGATTTGATAACCCAACTAAACCAATTGGTCCTTTTTATACGAAAGAAGAAGTTGAAGAATTACAAAAAGAACAGCCAGGCTCAGTCTTT
AAAGAAGATGCAGGACGTGGTTATAGAAAAGTAGTTGCGTCACCACTACCTCAATCTATACTAGAACACCAGTTAATTCGAACTTTAGCAGACG
GTAAAAATATTGTCATTGCATGCGGTGGTGGCGGTATTCCAGTTATAAAAAAAGAAAATACCTATGAAGGTGTTGAAGCG

S. aureus SG511-Berlin – *aroE* – allele number 2
AATTTTAATTCTTTAGGATTAGATGATACTTATGAAGCTTTAAATATTCCAATTGAAGATTTTCATTTAATTAAAGAAATTATTTCAAAAAAAG
AATTAGATGGCTTTAATATCACAATTCCTCATAAAGAGCGTATCATACCGTATTTAGATCATGTTGATGAACAAGCGATTAATGCAGGTGCAGT
TAACACTGTTTTGATAAAAGATGGCAAGTGGATAGGGTATAATACAGATGGTATTGGTTATGTTAAAGGATTGCACAGCGTTTATCCAGATTTA
GAAAATGCATACATTTTAATTTTGGGAGCAGGTGGTGCAAGTAAAGGTATTGCTTATGAATTAGCAAAATTTGTAAAGCCCAAATTAACTGTTG
CGAATAGAACGATGGCTCGTTTTGAATCTTGGAATTTAAATATAAACCAAATTTCATTGGCAGATGCTGAAAAGTATTTA

S. aureus SG511-Berlin – *glpF* – allele number 2
GGTGCTGATTGGATTGTCATCACAGCTGGATGGGGATTAGCGGTTACAATGGGTGTGTATGCTGTTGGTCAATTCTCAGGTGCACATTTAAACC
CAGCGGTGTCTTTAGCTCTTGCATTAGACGGAAGTTTTGATTGGTCATTAGTTCCTGGTTATATTGTTGCTCAAATGTTAGGTGCAATTGTCGG
AGCAACAATTGTATGGTTAATGTACTTGCCACATTGGAAAGCGACAGAAGAAGCTGGCGCGAAATTAGGTGTTTTCTCTACAGCACCGGCTATT
AAGAATTACTTTGCCAACTTTTTAAGTGAAATTATCGGAACAATGGCATTAACTTTAGGTATTTTATTTATCGGTGTAAACAAAATTGCTGATG
GTTTAAATCCTTTAATTGTCGGAGCATTAATTGTTGCAATCGGATTAAGTTTAGGCGGTGCTACTGGTTATGCAATCAACCCAGCACGT

S. aureus SG511-Berlin – *gmk* – allele number 2
CGAATATTTGAAGATCCAAGTACATCATATAAGTATTCTATTTCAATGACAACACGTCAAATGCGTGAAGGTGAAGTTGATGGCGTAGATTACT
TTTTTAAAACTAGGGATGCGTTTGAAGCTTTAATTAAAGATGACCAATTTATAGAATATGCTGAATATGTAGGCAACTATTATGGTACACCAGT
TCAATATGTTAAAGATACAATGGACGAAGGTCATGATGTATTTTTAGAAATTGAAGTAGAAGGTGCAAAGCAAGTTAGAAAGAAATTTCCAGAT
GCGTTATTTATTTTCTTAGCACCTCCAAGTTTAGATCACTTGAGAGAGCGATTAGTAGGTAGAGGAACAGAATCTGATGAGAAAATACAAAGTC
GTATTAACGAAGCACGTAAAGAAGTCGAAATGATGAATTTATACGATTACGTT

S. aureus SG511-Berlin – *pta* – allele number 6
GCAACACAATTACAAGCAACAGATTATGTTACACCAATCGTGTTAGGTGATGAGACTAAGGTTCAATCTTTAGCGCAAAAACTTAATCTTGATA
TTTCTAATATTGAATTAATTAATCCTGCGACAAGTGAATTGAAAGCTGAATTAGTTCAATCATTTGTTGAACGACGTAAAGGTAAAGCGACTGA
AGAACAAGCACAAGAATTATTAAACAATGTGAACTACTTCGGTACAATGCTTGTTTATGCTGGTAAAGCAGATGGTTTAGTTAGTGGTGCAGCA
CATTCAACAGGCGACACTGTGCGTCCAGCTTTACAAATCATCAAAACGAAACCAGGTGTATCAAGAACATCAGGTATCTTCTTTATGATTAAAG
GTGATGAACAGTACATCTTTGGTGATTGTGCAATCAATCCAGAACTTGATTCACAAGGACTTGCAGAAATTGCAGTAGAAAGTGCAAAATCAGC
ATTA

S. aureus SG511-Berlin – *tpiA* – allele number 3
CACGAAACAGATGAAGAAATTAACAAAAAAGCGCACGCTATTTTCAAACATGGAATGACTCCAATTATTTGTGTTGGTGAAACAGACGAAGAGC
GTGAAAGTGGTAAAGCTAACGATGTTGTAGGTGAGCAAGTTAAGAAAGCTGTTGCAGGTTTATCTGAAGATCAACTTAAATCAGTTGTAATTGC
TTATGAACCAATCTGGGCAATCGGAACTGGTAAATCATCAACATCTGAAGATGCGAATGAAATGTGTGCATTTGTACGTCAAACTATTGCTGAC
TTATCAAGCAAAGAAGTATCAGAAGCAACTCGTATTCAATATGGTGGTAGTGTTAAACCTAACAACATTAAAGAATACATGGCACAAACTGATA
TTGATGGGGCATTAGTAGGTGGCGCA

S. aureus SG511-Berlin – *yqi* – allele number 2
GCGTTTAAAGACGTGCCAGCCTATGATTTAGGTGCGACTTTAATAGAACATATTATTAAAGAGACGGGTTTGAATCCAAGTGAGATTAATGAAG
TCATCATCGGTAACGTACTACAAGCAGGACAAGGACAAAATCCAGCACGAATTGCTGCTATGAAAGGTGGCTTGCCAGAAACAGTACCTGCATT
TACAGTGAATAAAGTATGTGGTTCTGGGTTAAAGTCGATTCAATTAGCATATCAATCTATTGTGACTGGTGAAAATGACATCGTGCTAGCTGGC
GGTATGGAGAATATGTCTCAATCACCAATGCTTGTCAACAACAGTCGCTTTGGTTTTAAAATGGGACATCAATCAATGGTTGATAGCATGGTAT
ATGATGGTTTAACAGATGTATTTAATCAATATCATATGGGTATTACTGCTGAAAATTTAGTAGAGCAATATGGTATTTCAAGAGAAGAACAAGA
TACATTTGCTGTAAACTCACAACAAAAAGCAGTACGTGCACAGCAA

Figure 4.4: Trimmed nucleotide sequences for MLST analysis of *S. aureus* SG511-Berlin.

Phage typing was used to confirm the identity of *S. aureus* SG511-Berlin, since this strain has been previously assigned to lysotype I (Pöhn, 1959). The identity of strain SG511-Berlin could be confirmed using the international set for phage typing, since its lysis pattern comprised the phages 29/52/52A/80. Strain SG511-Berlin additionally reacted with the phages 42E/77/84/85 of lysotype III, phage 96 of lysotype V and phage 95 of lysotype M.

4.3 Transcriptional response of the *S. aureus* strains SA137/93A, SA137/93G and SG511-Berlin in the presence of mersacidin using DNA-microarrays

S. aureus SA137/93A and SA137/93G were grown in BHI broth to an OD_{600} of 0.5, then subinhibitory concentrations of mersacidin (16 µg/ml, 0.5 x MIC) were added. Samples were taken 30 minutes after addition of mersacidin (OD_{600} of ~1), before the decreased growth rate became apparent (Fig. 4.1). *S. aureus* SG511-Berlin was grown with 1 x MIC of mersacidin to induce a mersacidin-dependent transcriptional response, since the profile of the growth curve of *S. aureus* SG511-Berlin at 1 x MIC was comparable to that recorded for the strains SA137/93A and SA137/93G at 0.5 x MIC (Fig. 4.1). Additionally, the transcriptional response of *S. aureus* SA137/93G was recorded at even lower concentrations of mersacidin (4 µg/ml, 0.15 x MIC; Table 5.1 and Table S1 in the supplemental material). Gene expression profiles of the mersacidin-treated *S. aureus* strains were compared to non-treated control cells and displayed extensive changes in gene expression. These changes were similar for all three tested strains and involved the transcript levels of 380 genes that were significantly changed (>2.5-fold) upon mersacidin treatment in at least one of the tested strains, with 207 genes exhibiting increased transcript levels (Table 4.1) and 173 genes showing decreased transcript levels (Table 4.2). Mersacidin-treatment resulted in the considerable regulation of genes referring to different cellular processes like cell wall biosynthesis, adaptation to atypical conditions, membrane bioenergetics, metabolism-related functions, pathogenic factors, protein and RNA synthesis, protein transport and binding as well as signal transduction.

4.3.1 Regulation of cell wall biosynthesis genes

Significance analysis of the microarray data (SAM) revealed an extensive induction of a subset of genes that has been previously described as the "cell wall stress stimulon" of *S. aureus* that is partly triggered by the VraSR two-component regulatory system (TCRS). This system coordinates the induction of cell wall biosynthesis genes upon treatment with cell-wall-active antibiotics like bacitracin, D-cycloserine, oxacillin and vancomycin (Utaida et al., 2003; Kuroda et al., 2003). Accordingly, *vraSR* gene transcription (SA1700/SA1701) was induced up to 17-fold among all tested strains in the presence of mersacidin. Additionally, the cell wall metabolism related genes *murZ*, *uppS*, *bacA*, *pbp2*, *sgtB*, the proline/betaine transporter homologue gene *proP*, the *tcaA* gene, the protein folding catalyst gene *prsA*, the autolysin gene *atl* and the drug responsive protein 35 gene (*drp35*) were significantly regulated in response to mersacidin treatment. The genes coding for the TcaA protein and the drug resistance protein 35 (*drp35*) were shown to be involved in the development of

teicoplanin and β-lactam resistance (Brandenberger et al., 2000; Murakami et al., 1999), while their definitive physiological function remains unknown yet. Gene expression for cell wall lytic enzymes (*atl*, SA0423, SA2100) was negatively affected.

4.3.2 Regulation of genes belonging to the adaptation to atypical conditions

Several chaperone/protease genes of the Clp-family (caseinolytic proteases) were found to be upregulated like *clpC*, *clpP*, *cplB* and *clpL* which are also part of the *S. aureus* response to heat-shock, vancomycin stress and acid shock (Anderson et al., 2006; Kuroda et al., 2003; Bore et al., 2007) and may play a role in targeting heat-denatured proteins for proteolysis. Suitably, the cold shock protein gene *cspC* and the alkaline shock protein gene *asp23* were significantly downregulated in strain SG511-Berlin.

4.3.3 Regulation of genes with metabolism-related functions

Here, a strong induction of the acetyl-CoA c-transferase gene *vraB* was observed, which is involved in the biosynthesis of lipids and degradation of amino acids to feed catabolic pathways. In other studies, *vraB* was also induced upon mupirocin treatment (stringent response) and in response to D-cycloserine (inhibitor of alanine racemase) (Anderson et al., 2006; Utaida et al., 2003). Furthermore, mersacidin treatment induced the transcription of genes involved in the amino acid biosynthesis of glutamate (*gltB*, *rocA*), lysine (*asd*, *dapAB*, *dhoM*, *lysA*), threonine (*thrBC*), cysteine (*cysK*), serine (*serA*) and valine/isoleucine (*ilvE*). The metabolism of carbohydrates was affected especially in strain SG511-Berlin, where the succinate dehydrogenase genes *sdhBC* were downregulated.

4.3.4 Regulation of genes related to the metabolism of nucleotides.

The pyrimidine synthesis gene cluster SA1043-48 was extremely downregulated in strain SG511-Berlin (up to 33-fold) but remained rather unaffected in strains SA137/93A and SA137/93G. The repression of the pyrimidine synthesis genes was also observed in studies on the *S. aureus* response to acid stress and modulations on the cell wall biosynthesis gene *murF* (Bore et al., 2007; Sobral et al., 2007). Interestingly, the putative GTP-pyrophosphokinase gene SA2297, which catalyses the formation of guanosine 3'-diphosphate 5'-triphosphate, was upregulated over 9-fold in all strains which is reported to be also upregulated by vancomycin and oxacillin treatment (Kuroda et al., 2003; Utaida et al., 2003) and downregulated by the GraRS TCRS (Herbert et al., 2007) and upon stringent response (Anderson et al., 2006).

Table 4.1: Genes with significantly increased expression upon mersacidin treatment[1]

N315 ORF	Gene	Gene product function	Fold change[2] 137/93A (0.5xMIC)	137/93G (0.5xMIC)	SG511 (1xMIC)
Adaptation to atypical conditions					
SA0483	*clpC*	endopeptidase	1.2	**2.7**	**6.6**
SA0659		hypothetical protein, similar to CsbB stress response protein	2.1	**3.5**	3.0
SA0723	*clpP*	ATP-dependent Clp protease proteolytic subunit homologue	1.3	**3.0**	**3.4**
SA0755		hypothetical protein, similar to general stress protein 170	1.7	**4.0**	**2.8**
SA0835	*clpB*	ClpB chaperone homologue	1.5	6.9	**16.4**
SA1146	*bsaA*	glutathione peroxidase	2.1	**2.7**	2.7
SA1410	*grpE*	GrpE protein	0.9	3.1	**5.3**
SA1549		hypothetical protein, similar to serine proteinase Do, heat-shock protein htrA	**7.1**	**4.6**	**5.6**
SA2175		hypothetical protein, similar to small heat shock protein	2.4	**3.0**	3.1
SA2336	*clpL*	ATP-dependent Clp proteinase chain clpL	0.9	**4.0**	1.3
Cell division					
SA1023	ftsL	cell division protein	**2.4**	1.6	**3.0**
Cell wall related genes					
SA0205		hypothetical protein, similar to lysostaphin precursor	**6.0**	**4.5**	**13.6**
SA0244		hypothetical protein, similar to teichoic acid biosynthesis protein F	1.6	1.4	**2.8**
SA0265	*lytM*	peptidoglycan hydrolase	**4.0**	**2.1**	1.7
SA0638	*bacA*	bacitracin resistance protein (putative undecaprenol kinase) homologue	**3.0**	**1.9**	1.8
SA1103	*uppS*	undecaprenyl pyrophosphate synthetase	**2.5**	**3.0**	2.0
SA1266		hypothetical protein, similar to cell wall enzyme EbsB	3.4	1.9	**3.7**
SA1283	*pbp2*	penicillin binding protein 2	3.4	2.3	**3.5**
SA1691	*sgtB*	hypothetical protein, similar to penicillin-binding protein 1A/1B	**13.1**	**8.8**	**9.7**
SA1926	*murZ*	UDP-N-acetylglucosamine 1-carboxylvinyl transferase 2	3.2	**4.4**	**6.1**
SA2481		conserved hypothetical protein	**2.4**	**3.5**	**7.0**
Detoxification					
SA0128	*sodM*	superoxide dismutase	1.0	-	1.0
SA0681		hypothetical protein, similar to multidrug resistance protein	1.2	**3.1**	1.0
SA1170	*katA*	Catalase	1.6	**2.7**	1.9
SA1238		hypothetical protein, similar to tellurite resistance protein	**2.6**	**3.2**	**3.3**
SA1382	*sodA*	superoxide dismutase SodA	1.1	**3.0**	1.8
DNA replication, modification, recombination and repair					
SA1282	*recU*	recombination protein U homologue	**3.6**	2.5	**2.5**
SA1313		probable ATP-dependent DNA helicase RecQ	2.0	**2.2**	**2.7**
SA1485	*radC*	truncated hypothetical protein, similar to DNA repair protein	1.5	1.4	**6.3**
SA1711		hypothetical protein, similar to DNA-damage inducible protein P	2.2	**1.6**	**3.4**
SA2335	*adaB*	probable methylated DNA-protein cysteine methyltransferase	1.7	**2.8**	2.2
Membrane bioenergetics (electron transport chain and ATP synthase)					
SA0211		hypothetical protein, similar to NADH-dependent dehydrogenase	2.2	1.3	**4.0**
SA0411	*ndhF*	NADH dehydrogenase subunit 5	1.6	1.0	**2.9**
SA0817		hypothetical protein, similar to NADH-dependent flavin oxidoreductase	2.1	**3.4**	**2.4**
SA1221		thioredoxine reductase	1.2	**2.6**	1.2
SA2324		hypothetical protein, similar to thioredoxin	1.3	**2.6**	**3.0**
Metabolism of amino acids					
SA0011		hypothetical protein, similar to homoserine-o-acetyltransferase	**1.6**	**2.0**	**2.8**
SA0430	*gltB*	glutamate synthase large subunit	**2.8**	**2.0**	1.3
SA0471	*cysK*	cysteine synthase (o-acetylserine sulfhydrylase) homologue	1.6	**3.4**	1.8
SA0512	*ilvE*	branched-chain amino acid aminotransferase homologue	2.1	**2.5**	2.1
SA0829		hypothetical protein, similar to 5-oxo-1,2,5-tricarboxilic-3-penten acid decarboxylase	2.1	**3.2**	**3.1**
SA0902		HisC homologue	2.2	1.8	**4.2**
SA0942	*pdf1*	formylmethionine deformylase homologue	1.7	1.6	**2.9**
SA1164	*dhoM*	homoserine dehydrogenase	**2.5**	**2.7**	**6.2**
SA1165	*thrC*	threonine synthase	1.6	2.0	**3.9**

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Table 4.1 - *Continued*

N315 ORF	Gene	Gene product function	Fold change[2] 137/93A (0.5xMIC)	137/93G (0.5xMIC)	SG511 (1xMIC)
SA1166	*thrB*	homoserine kinase homologue	1.4	1.7	**3.9**
SA1216		hypothetical protein, similar to oligoendopeptidase	1.8	**1.7**	**2.8**
SA1226	*asd*	aspartate semialdehyde dehydrogenase	2.2	**3.8**	2.2
SA1227	*dapA*	dihydrodipicolinate synthase	**2.3**	**3.0**	2.0
SA1228	*dapB*	dihydrodipicolinate reductase	**2.3**	**2.6**	1.9
SA1232	*lysA*	diaminopimelate decarboxylase	**2.1**	**2.4**	**2.7**
SA1531	*ald*	alanine dehydrogenase	2.1	**1.5**	**4.2**
SA1545	*serA*	D-3-phosphoglycerate dehydrogenase	2.1	**1.6**	**2.8**
SA1861	*ilvC*	alpha-keto-beta-hydroxylacil reductoisomerase	**1.9**	**2.5**	1.2
SA2341	*rocA*	1-pyrroline-5-carboxylate dehydrogenase	1.2	1.4	**3.1**
Metabolism of carbohydrates					
SA0528		hypothetical protein, similar to hexulose-6-phosphate synthase	1.5	**3.4**	3.3
SA0658		hypothetical protein, similar to plant-metabolite dehydrogenases	1.5	**3.3**	**2.6**
SA0958		myo-inositol-1(or 4)-monophosphatase homologue	1.8	**3.1**	**3.1**
SA1142	*glpD*	aerobic glycerol-3-phosphate dehydrogenase	1.4	**2.0**	**3.8**
SA1566		endo-1,4-beta-glucanase homologue	**2.0**	**2.9**	2.4
SA1599		hypothetical protein, similar to transaldolase	1.7	**2.7**	**3.1**
SA1736	*aldH*	aldehyde dehydrogenase	2.0	**2.3**	**3.3**
SA1924		hypothetical protein, similar to aldehyde dehydrogenase	1.3	**3.4**	2.4
SA1925		conserved hypothetical protein	**3.5**	**3.8**	**4.7**
SA2104		hypothetical protein, similar to suppressor protein SuhB	1.4	**2.0**	**2.9**
SA2266		hypothetical protein, similar to oxidoreductase	**2.0**	**3.4**	1.6
SA2346		hypothetical protein, similar to D-specific D-2-hydroxyacid dehydrogenase ddh homologue	1.9	**2.2**	**3.8**
SA2490		hypothetical protein, similar to N-hydroxyarylamine O-acetyltransferase	**3.6**	**6.0**	**5.4**
Metabolism of coenzymes and prosthetic groups					
SA0915	*folD*	FolD bifunctional protein	1.3	**2.7**	1.8
SA2412		hypothetical protein, similar to uroporphyrin-III C-methyltransferase	1.9	**2.3**	**2.7**
SA2438		hypothetical protein, similar to N-carbamoylsarcosine amidohydrolase	**2.3**	**2.7**	**3.7**
Metabolism of lipids					
SA0534	*vraB*	acetyl-CoA c-acetyltransferase	**18.5**	**8.5**	**12.9**
SA0572		hypothetical protein, similar to esterase/lipase	0.9	**3.3**	1.8
SA1435		hypothetical protein, similar to acetyl-CoA carboxylase (biotin carboxyl carrier subunit), *accB* homologue	0.9	1.3	**3.4**
SA1542		hypothetical protein, similar to glycerophosphoryl diester phosphodiesterase	2.3	**2.8**	**4.6**
SA2240		hypothetical protein, similar to para-nitrobenzyl esterase chain A	1.8	**4.1**	2.2
Metabolism of nucleotides and nucleic acids					
SA0864		GTP pyrophosphokinase	1.6	1.8	**2.8**
SA1172		hypothetical protein, similar to GMP reductase	1.8	**3.0**	2.4
SA1308		30S ribosomal protein S1	2.0	**3.5**	**3.2**
SA2297		hypothetical protein, similar to GTP-pyrophosphokinase	**9.1**	**14.1**	**10.3**
Metabolism of phosphate					
SA1237		hypothetical protein, similar to 5-bromo-4-chloroindolyl phosphate hydrolysis protein XpaC	**2.5**	**4.1**	**6.0**
SA2301		hypothetical protein, similar to alkaline phosphatase	2.7	**2.7**	3.3
Pathogenic factors (toxins and colonization factors)					
SA0382	*set6*	exotoxin 6 [Pathogenicity island SaPIn2]	2.4	**2.7**	**5.1**
SA0610		hypothetical protein, similar to lipase LipA	1.2	**0.7**	**3.9**
SA0909	*fmtA*	FmtA, autolysis and methicillin resistant-related protein	**9.3**	3.8	**6.3**
SA2006		hypothetical protein, similar to MHC class II analogue	**3.4**	**7.3**	**4.4**
SA2323		conserved hypothetical protein	1.9	**2.4**	**2.9**
Phage-related functions					
SA1760		holin homologue [Bacteriophage phiN315]	**3.8**	3.2	**17.5**

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Table 4.1 - *Continued*

N315 ORF	Gene	Gene product function	Fold change[2] 137/93A (0.5xMIC)	137/93G (0.5xMIC)	SG511 (1xMIC)
Protein modification, folding and secretion					
SA0825	*spsA*	type-I signal peptidase	**2.2**	1.4	**3.9**
SA0826	*spsB*	type-1 signal peptidase 1B	**2.4**	1.5	**2.9**
SA1257		peptide methionine sulfoxide reductase MsrA2	**6.0**	**11.0**	**17.1**
SA1530		Xaa-Pro dipeptidase homologue	1.8	**3.1**	2.2
SA1409	*dnaK*	DnaK protein	0.9	**2.1**	**4.6**
SA1659	*prsA*	peptidyl-prolyl cis/trans isomerase homologue	**10.8**	**18.1**	**33.3**
SA1836	*groEL*	GroEL protein	1.2	**3.9**	4.5
Protein transport and binding					
SA0166		hypothetical protein, similar to nitrate transporter	2.0	**2.4**	**2.6**
SA0531	*proP*	proline/betaine transporter homologue	2.4	**2.7**	0.6
SA0640		hypothetical protein, similar to ABC transporter required for expression of cytochrome bd	1.6	1.5	**2.6**
SA0677		hypothetical protein, similar to choline transport ATP-binding protein	1.2	1.1	**2.8**
SA0769		ABC transporter ATP-binding protein homologue	0.9	1.4	**3.6**
SA0845	*oppB*	oligopeptide transport system permease protein	1.4	**3.6**	**3.5**
SA0846		hypothetical protein, similar to oligopeptide transport system permease protein OppC	**1.8**	**3.1**	**3.4**
SA0847	*oppD*	oligopeptide transport system ATP-binding protein OppD homologue	1.5	**3.0**	**2.6**
SA0848	*oppF*	oligopeptide transport system ATP-binding protein OppF homologue	2.3	**3.9**	2.1
SA1255		PTS system, glucose-specific enzyme IIA component	**4.7**	**10.7**	**13.1**
SA1478		hypothetical protein, similar to transporter PAB2175 from *Pyrococcus abyssi*	**3.8**	**3.7**	**4.9**
SA1654		hypothetical protein, similar to ABC transporter EcsB	2.3	**2.0**	**2.9**
SA1655		ABC transporter EcsA homologue	1.5	**2.4**	**5.1**
SA1848	*nrgA*	probabale ammonium transporter	1.4	1.4	**4.1**
SA1948	*czrB*	cation-efflux system membrane protein homologue	0.9	**1.9**	**2.7**
SA2112		hypothetical protein, similar to sodium-dependent transporter	1.9	1.3	**2.9**
SA2114	*glvC*	PTS system, arbutin-like IIBC component	**3.7**	**5.6**	**4.7**
SA2148		hypothetical protein, similar to membrane protein	1.4	1.6	**4.1**
SA2396		hypothetical protein, similar to amino acid transporter	1.9	**1.3**	**3.0**
SA2416		hypothetical protein, similar to ABC transporter (ATP-binding protein)	**2.3**	**2.3**	**2.7**
SA2492	*vraD*	hypothetical protein, similar to ABC transporter	**59.4**	**72.2**	**52.7**
SA2493	*vraE*	hypothetical protein, similar to ABC transporter (permease)	**52.7**	**39.6**	**42.4**
RNA synthesis - regulation					
SA0480	*ctsR*	transcription repressor of class III stress genes homologue	1.1	**4.2**	**5.0**
SA1195	*msrR*	peptide methionine sulfoxide reductase regulator MsrR	**4.2**	**3.6**	**5.5**
SA1411	*hrcA*	Heat-inducible transcriptional repressor	1.3	**3.3**	**5.1**
SA1700	*vraR*	two-component response regulator	**13.3**	**8.0**	**17.3**
SA1947	*czrA*	repressor protein	1.2	**2.5**	**3.5**
SA2103		hypothetical protein, similar to lyt divergon expression attenuator LytR	**6.4**	**6.1**	**8.0**
SA2159		hypothetical protein, similar to transcription repressor of sporulation, septation and degradation PaiA	1.8	**2.1**	**3.4**
SA2296		hypothetical protein, similar to transcriptional regulator, MerR family	**8.1**	**10.4**	**5.5**
SA2458	*icaR*	*ica* operon transcriptional regulator IcaR	1.4	**2.6**	1.6
Sensors (signal transduction)					
SA1701	*vraS*	two-component sensor histidine kinase	**11.7**	**8.9**	**17.0**
Transformation competence					
SA0857		hypothetical protein, similar to negative regulator of genetic competence MecA	3.0	3.4	**7.0**
Hypothetical proteins					
SA0165		hypothetical protein, similar to alpha-helical coiled-coil protein SrpF	1.8	**3.4**	**3.5**
SA0192		hypothetical protein, similar to ABC transporter ATP-binding protein	**23.6**	**31.7**	**26.6**
SA0358		conserved hypothetical protein	**10.6**	**15.4**	**39.3**
SA0359		conserved hypothetical protein	1.9	**6.1**	**4.3**

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Table 4.1 - *Continued*

N315 ORF	Gene	Gene product function	Fold change[2]		
			137/93A (0.5xMIC)	137/93G (0.5xMIC)	SG511 (1xMIC)
SA0481		conserved hypothetical protein	1.8	**5.3**	**8.0**
SA0482		hypothetical protein, similar to creatine kinase	1.2	**4.5**	**5.8**
SA0509		conserved hypothetical protein	1.1	**2.8**	0.8
SA0529		conserved hypothetical protein	1.4	**3.0**	**3.7**
SA0530		hypothetical protein, similar to indigoidine synthesis protein	**2.5**	**2.4**	1.3
SA0535	*vraC*	hypothetical protein	**25.7**	**11.3**	**18.9**
SA0536		hypothetical protein	**30.2**	**17.1**	**26.6**
SA0591		hypothetical protein	**11.3**	**8.9**	**10.9**
SA0608		hypothetical protein	**2.7**	**3.8**	**9.2**
SA0611		hypothetical protein	1.2	**0.8**	**4.6**
SA0612		conserved hypothetical protein	2.0	**2.5**	2.4
SA0637		conserved hypothetical protein	2.5	**4.0**	1.5
SA0647		hypothetical protein	2.3	1.2	**3.6**
SA0648		conserved hypothetical protein	**2.4**	1.5	**3.6**
SA0707		conserved hypothetical protein	**4.8**	**9.7**	2.5
SA0721		conserved hypothetical protein	1.0	**3.1**	1.9
SA0725		conserved hypothetical protein	2.9	**2.9**	1.8
SA0750		conserved hypothetical protein	1.9	1.7	**4.9**
SA0770		conserved hypothetical protein	0.8	1.2	**3.8**
SA0771		conserved hypothetical protein	1.2	1.4	**2.8**
SA0772		conserved hypothetical protein	2.4	**4.5**	0.8
SA0782		conserved hypothetical protein	2.1	1.6	**2.9**
SA0824		conserved hypothetical protein	**4.1**	**2.4**	**3.9**
SA0833		conserved hypothetical protein	1.8	**2.6**	1.8
SA0856		conserved hypothetical protein	2.0	**2.6**	2.1
SA0903		conserved hypothetical protein	3.2	1.4	**5.3**
SA0908		conserved hypothetical protein	2.2	1.5	**3.1**
SA0914		hypothetical protein, similar to chitinase B	**14.2**	**3.9**	**15.3**
SA0931		hypothetical protein	**4.9**	**3.2**	**6.2**
SA0943		conserved hypothetical protein	**3.2**	**3.2**	**4.5**
SA0957		conserved hypothetical protein	1.8	2.2	**3.2**
SA0962		conserved hypothetical protein	1.8	1.4	**2.8**
SA1018		conserved hypothetical protein	1.7	**2.2**	**3.0**
SA1019		conserved hypothetical protein	**3.9**	**6.9**	**6.5**
SA1022		conserved hypothetical protein	2.2	2.1	**2.8**
SA1050		conserved hypothetical protein	2.0	**2.6**	1.1
SA1173		conserved hypothetical protein	**2.7**	**2.8**	**3.7**
SA1185		conserved hypothetical protein	2.0	**1.9**	**3.2**
SA1186		conserved hypothetical protein	1.9	1.4	**3.4**
SA1256		conserved hypothetical protein	**7.9**	**16.1**	**18.8**
SA1280		conserved hypothetical protein	1.7	**2.8**	**2.8**
SA1281		conserved hypothetical protein	**1.9**	**3.0**	**2.9**
SA1293		conserved hypothetical protein	1.7	**2.3**	**3.5**
SA1419		conserved hypothetical protein	1.3	1.4	**4.6**
SA1436		conserved hypothetical protein	0.7	1.0	**3.4**
SA1476		hypothetical protein	**11.5**	**11.3**	**15.9**
SA1532		conserved hypothetical protein	2.5	**9.1**	**5.5**
SA1543		conserved hypothetical protein	2.8	**3.9**	**6.0**
SA1544		hypothetical protein, similar to soluble hydrogenase 42 kD subunit	1.8	**2.0**	**4.9**
SA1546		hypothetical protein	**2.3**	**1.7**	**3.2**
SA1606		plant metabolite dehydrogenase homologue	1.9	**3.5**	**2.6**
SA1649		conserved hypothetical protein	1.9	1.1	**3.0**

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Table 4.1 - *Continued*

N315 ORF	Gene	Gene product function	Fold change[2]		
			137/93A (0.5xMIC)	137/93G (0.5xMIC)	SG511 (1xMIC)
SA1680		conserved hypothetical protein	**2.3**	**2.7**	**3.0**
SA1682		conserved hypothetical protein	2.4	**2.9**	1.8
SA1686		conserved hypothetical protein	2.1	**2.1**	**3.5**
SA1690		conserved hypothetical protein	**2.0**	**2.1**	**4.0**
SA1692		conserved hypothetical protein	1.3	**3.1**	0.8
SA1702		conserved hypothetical protein	**10.4**	**9.4**	**12.2**
SA1703		hypothetical protein	**31.6**	**24.8**	**45.4**
SA1712		conserved hypothetical protein	**24.7**	**21.0**	**29.4**
SA1743		hypothetical protein	2.7	**4.3**	2.6
SA1942		conserved hypothetical protein	1.7	**3.7**	2.5
SA1990		conserved hypothetical protein	1.9	**2.6**	**3.9**
SA2004		conserved hypothetical protein	2.1	2.2	**2.5**
SA2049		hypothetical protein	4.3	**5.8**	6.5
SA2113		hypothetical protein	**14.9**	**11.8**	**11.4**
SA2138		conserved hypothetical protein	3.1	**4.2**	5.3
SA2139		hypothetical protein	2.7	**2.8**	3.4
SA2146	*tcaA*	TcaA protein	**3.3**	1.6	**4.0**
SA2158		hypothetical protein, similar to TpgX protein	2.1	**2.7**	**2.3**
SA2220		conserved hypothetical protein	**7.0**	**12.2**	**21.4**
SA2221		hypothetical protein	**25.6**	**18.3**	**42.7**
SA2366		conserved hypothetical protein	0.9	**2.7**	1.1
SA2367		conserevd hypothetical protein	1.0	**3.3**	1.1
SA2480	*drp35*	drug responsive protein 35	2.7	**3.4**	**2.8**

[1] Significant changes of gene expression were determined by implementing SAM (significance analysis of microarrays; http://www-stat.stanford.edu/~tibs/SAM/) (Tusher et al., 2001).

[2] Fold change in transcript level of at least 2.5-fold is indicated as mean of the "median of ratios" compared to control cells. Fold change in bold = classified as "significantly" regulated in this strain by SAM.

4.3.5 Regulation of genes related to protein synthesis

Mersacidin treatment reduced the transcription of 30 genes coding for 30S and 50S ribosomal proteins. To date, only acid shock treatment of *S. aureus* cells has been shown to induce such a response (Bore et al., 2007). The loss of ribosomal proteins and pyrimidines (see section 4.3.4) may be one of the reasons for the retarded growth of *S. aureus* after addition of mersacidin. Furthermore, the chaperone genes *groEL*, *prsA* and *dnaK* and the signal peptidase genes *spsAB* were upregulated.

4.3.6 Regulation of genes related to signal transduction and global regulators

Besides the induction of the VraSR TCRS, the global regulators *pyrR*, *rot*, *agrA*, *agrC* and *sarR* were downregulated. Further, the GraRS antimicrobial peptide-sensing system of *S. aureus* (Li et al., 2007a) was not induced upon mersacidin treatment, as many genes reported to be positively controlled by GraRS were downregulated, like *dltABC* and *vraFG* (Li et al., 2007a; Herbert et al., 2007; Meehl et al., 2007).

Table 4.2: Genes with significantly decreased expression upon mersacidin treatment[1]

N315 ORF	Gene	Gene product function	Fold change[2] 137/93A (0.5xMIC)	137/93G (0.5xMIC)	SG511 (1xMIC)
Adaptation to atypical conditions					
SA0747	*cspC*	cold-shock protein C	0.65	0.84	**0.40**
SA1984	*asp23*	alkaline shock protein 23, ASP23	0.64	2.24	**0.32**
Cell division					
SA0905	*atl*	autolysin	**0.42**	0.49	**0.18**
SA2499	*gidB*	glucose inhibited division protein B	0.57	**0.36**	0.56
SA2500	*gidA*	glucose inhibited division protein A	**0.55**	**0.34**	**0.51**
Cell wall related genes					
SA0423		hypothetical protein, similar to autolysin	0.58	**0.20**	**0.20**
SA0793	*dltA*	D-alanine-D-alanyl carrier protein ligase	0.63	**0.22**	**0.24**
SA2100		hypothetical protein, similar to autolysin E	**0.46**	**0.32**	**0.30**
SA2354		hypothetical protein, similar to acyltransferase	0.73	**0.37**	**0.47**
Membrane bioenergetics (electron transport chain and ATP synthase)					
SA0910		hypothetical protein, similar to quinol oxidase polypeptide IV QoxD	**0.41**	**0.20**	**0.12**
SA0911	*qoxC*	Quinol oxidase polypeptide III QoxC	**0.34**	**0.19**	**0.14**
SA0912	*qoxB*	Quinol oxidase polypeptide I QoxB	**0.37**	**0.21**	**0.11**
SA0913		hypothetical protein, similar to quinol oxidase polypeptide II QoxA	**0.40**	**0.24**	**0.19**
SA0965	*ctaB*	cytochrome caa3 oxidase (assembly factor) homologue	0.57	**0.40**	**0.35**
SA1909	*atpF*	ATP synthase B chain	0.72	**0.38**	0.49
SA1910	*atpE*	ATP synthase C chain	0.79	0.43	**0.38**
SA1911	*atpB*	ATP synthase A chain	0.66	0.43	**0.39**
Metabolism of amino acids					
SA0180		hypothetical protein, similar to branched-chain amino acid transport system carrier protein	0.54	**0.34**	**0.33**
SA2095		hypothetical protein, similar to D-octopine dehydrogenase	**0.45**	**0.49**	**0.33**
Metabolism of carbohydrates					
SA0562	*adh1*	alcohol dehydrogenase I	2.23	1.59	**0.30**
SA0994	*sdhC*	succinate dehydrogenase cytochrome b-558	**0.58**	0.62	**0.35**
SA0996	*sdhB*	succinate dehydrogenase iron-sulfur protein subunit	0.62	0.58	**0.32**
SA2008	*alsS*	alpha-acetolactate synthase	0.73	**0.39**	**0.29**
SA2102		formate dehydrogenase homologue	0.47	0.86	**0.23**
Metabolism of coenzymes and prosthetic groups					
SA0665		coenzyme PQQ synthesis homologue	0.73	**0.38**	**0.54**
SA0666		hypothetical protein 6-pyruvoyl tetrahydrobipterin synthase homologue	0.61	0.34	**0.38**
SA1494	*hemC*	porphobilinogen deaminase	0.65	**0.33**	**0.42**
SA1495	*hemX*	HemA concentration negative effector HemX	0.68	**0.37**	**0.37**
SA1537		hypothetical protein, similar to thiamine biosynthesis protein ThiI	0.65	**0.35**	**0.43**
SA1538		hypothetical protein, similar to iron-sulfur cofactor synthesis protein NifZ	**0.52**	**0.31**	**0.48**
SA1919		hypothetical protein, similar to protoporphyrinogen oxidase (HemK)	**0.44**	**0.39**	**0.39**
Metabolism of lipids					
SA0820	*glpQ*	glycerophosphoryl diester phosphodiesterase	1.11	**0.47**	**0.29**
Metabolism of nucleotides and nucleic acids					
SA0373	*xprT*	xanthine phosphoribosyltransferase	**0.41**	**0.13**	**0.43**
SA0511		hypothetical protein, similar to UDP-glucose 4-epimerase related protein	0.57	**0.52**	**0.33**
SA0927		conserved hypothetical protein	0.75	0.69	**0.22**
SA1043	*pyrB*	aspartate transcarbamoylase chain A	0.92	2.22	**0.05**
SA1044	*pyrC*	dihydroorotase	0.98	**2.06**	**0.03**
SA1045	*pyrAA*	carbamoyl-phosphate synthase small chain	1.05	1.58	**0.07**
SA1046	*pyrAB*	carbamoyl-phosphate synthase large chain	0.87	1.58	**0.08**
SA1047	*pyrF*	orotidine-5-phosphate decarboxylase	0.81	1.65	**0.05**
SA1048	*pyrE*	orotate phosphoribosyltransferase	0.87	1.43	**0.07**
SA1461	*apt*	adenine phosphoribosyl transferase	0.71	0.62	**0.39**
SA1921	*tdk*	thymidine kinase	0.61	**0.50**	**0.39**

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Table 4.2 - *Continued*

N315 ORF	Gene	Gene product function	Fold change[2] 137/93A (0.5xMIC)	137/93G (0.5xMIC)	SG511 (1xMIC)
SA2027	*adk*	adenylate kinase	0.53	0.31	**0.33**
Pathogenic factors (toxins and colonization factors)					
SA0270		hypothetical protein, similar to secretory antigen precursor SsaA	0.67	**0.51**	**0.13**
SA0276		conserved hypothetical protein, similar to diarrheal toxin	0.84	**0.34**	0.99
SA0519	*sdrC*	Ser-Asp rich fibrinogen-binding, bone sialoprotein-binding protein	0.53	**0.27**	**0.08**
SA0521	*sdrE*	Ser-Asp rich fibrinogen-binding, bone sialoprotein-binding protein	1.74	1.40	**0.32**
SA0587		lipoprotein, streptococcal adhesin PsaA homologue	0.76	1.21	**0.25**
SA0742	*clfA*	fibrinogen-binding protein A, clumping factor	0.80	1.21	**0.22**
SA0746		staphylococcal nuclease	0.99	**0.26**	0.65
SA1645	*yent1*	enterotoxin Yent1 [Pathogenicity island SaPIn3]	1.28	1.30	**0.39**
SA1647	*sem*	enterotoxin SEM [Pathogenicity island SaPIn3]	1.47	1.31	**0.36**
SA2093	*ssaA*	secretory antigen precursor SsaA homologue	0.57	**0.23**	**0.34**
SA2206	*sbi*	IgG-binding protein SBI	0.87	**0.33**	1.30
SA2290	*fnbB*	fibronectin-binding protein homologue	0.49	**0.27**	1.29
SA2291	*fnb*	fibronectin-binding protein homologue	0.51	**0.32**	1.15
SA2353		hypothetical protein, similar to secretory antigen precursor SsaA	0.69	**0.38**	0.78
SA2356	*isaA*	immunodominant antigen A	0.73	0.51	**0.36**
SA2423	*clfB*	clumping factor B	0.81	0.90	**0.17**
Phage-related functions					
SA1798		hypothetical protein [Bacteriophage phiN315]	0.57	**0.38**	**0.36**
Protein modification, folding and secretion					
SA2028	*secY*	preprotein translocase SecY subunit	0.58	0.32	**0.34**
SA2446		hypothetical protein, similar to preprotein translocase SecY	0.87	**0.39**	1.08
Protein synthesis - initation and termination					
SA1504	*infC*	translation initiation factor IF-3 InfC	0.72	**0.40**	**0.53**
SA1920	*prfA*	peptide chain release factor 1	0.47	**0.37**	**0.41**
Protein synthesis - ribosomal proteins and aminoacyl-tRNA synthases					
SA0354	*rpsR*	30S ribosomal protein S18	0.73	**0.48**	**0.29**
SA0486	*gltX*	glutamyl-tRNA synthetase	0.57	**0.35**	0.53
SA0497	*rplJ*	50S ribosomal protein L10 (BL5)	0.48	0.28	**0.32**
SA0498	*rplL*	50S ribosomal protein L7/L12	0.51	0.37	**0.30**
SA0503	*rpsL*	30S ribosomal protein S12	0.48	**0.28**	**0.40**
SA0504	*rpsG*	30S ribosomal protein S7	**0.42**	**0.28**	**0.44**
SA1081	*rpsP*	30S ribosomal protein S16	0.55	**0.52**	**0.31**
SA1471	*rpmA*	50S ribosomal protein L27	0.46	**0.37**	0.59
SA1922	*rpmE*	50S ribosomal protein L31	0.61	**0.48**	**0.38**
SA2022	*rplQ*	50S ribosomal protein L17	0.45	**0.22**	**0.26**
SA2024	*rpsK*	30S ribosomal protein S11	0.44	0.31	**0.30**
SA2029	*rplO*	50S ribosomal protein L15	0.50	0.29	**0.31**
SA2030	*rpmD*	50S ribosomal protein L30	0.53	0.25	**0.32**
SA2031	*rpsE*	30S ribosomal protein S5	0.66	**0.29**	**0.39**
SA2032	*rplR*	50S ribosomal protein L18	0.49	0.25	**0.29**
SA2033	*rplF*	50S ribosomal protein L6	0.43	0.26	**0.28**
SA2034	*rpsH*	30S ribosomal protein S8	0.50	0.26	**0.35**
SA2035	*rplE*	50S ribosomal protein L5	0.47	0.24	**0.26**
SA2036	*rplX*	50S ribosomal protein L24	0.41	0.25	**0.26**
SA2037	*rplN*	50S ribosomal protein L14	0.43	0.22	**0.28**
SA2038	*rpsQ*	30S ribosomal protein S17	0.42	**0.24**	**0.25**
SA2039	*rpmC*	50S ribosomal protein L29	0.41	**0.22**	**0.25**
SA2040	*rplP*	50S ribosomal protein L16	0.53	0.24	**0.31**
SA2041	*rpsC*	30S ribosomal protein S3	0.43	0.25	**0.24**
SA2042	*rplV*	50S ribosomal protein L22	0.58	0.31	**0.31**
SA2043	*rpsS*	30S ribosomal protein S19	0.54	0.28	**0.29**

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Table 4.2 - *Continued*

N315 ORF	Gene	Gene product function	Fold change[2]		
			137/93A (0.5xMIC)	137/93G (0.5xMIC)	SG511 (1xMIC)
SA2045	*rplW*	50S ribosomal protein L23	0.46	0.26	**0.36**
SA2046	*rplD*	50S ribosomal protein L4	0.46	0.27	**0.36**
SA2048	*rpsJ*	30S ribosomal protein S10	0.43	**0.31**	**0.48**
SAS052	*rpsD*	30S ribosomal protein S4	0.71	**0.47**	**0.38**
SAS079	*rpsN*	30S ribosomal protein S14	0.51	0.27	**0.27**
Protein transport and binding					
SA0183	*glcA*	PTS enzyme II, glucose-specific, factor IIA homologue	**0.34**	**0.21**	**0.09**
SA0272		hypothetical protein, similar to transmembrane protein Tmp7	**0.47**	**0.33**	0.97
SA0295		hypothetical protein, similar to outer membrane protein precursor	0.65	**0.31**	**0.26**
SA0374	*pbuX*	xanthine permease	0.52	**0.11**	**0.50**
SA0479	*nupC*	pyrimidine nucleoside transport protein	**0.33**	**0.24**	**0.10**
SA0616	*vraF*	ABC transporter ATP-binding protein	0.64	**0.36**	1.46
SA0617	*vraG*	ABC transporter permease	0.92	**0.38**	1.49
SA0688		hypothetical protein, similar to ferrichrome ABC transporter permease	1.26	0.75	**0.32**
SA0689		hypothetical protein, similar to ferrichrome ABC transporter permease	0.88	**0.69**	**0.26**
SA0690		hypothetical protein, similar to ferrichrome ABC transporter ATP-binding protein	**0.44**	**0.52**	**0.37**
SA0794	*dltB*	DltB membrane protein	0.79	**0.26**	**0.31**
SA0796	*dltD*	poly D-alanine transfer protein	0.75	0.24	**0.30**
SA0928		hypothetical protein, similar to cation ABC transporter	0.90	0.90	**0.23**
SA0950	*potA*	spermidine/putrescine ABC transporter, ATP-binding protein homologue	0.62	**0.31**	**0.30**
SA0951	*potB*		0.58	**0.44**	**0.28**
SA0952	*potC*	spermidine/putrescine ABC transporter homologue	0.51	**0.23**	**0.28**
SA1042	*pyrP*	uracil permease	1.16	**2.02**	**0.07**
SA1224		ABC transporter (ATP-binding protein) homologue	**0.48**	**0.35**	**0.36**
SA1979		hypothetical protein, similar to ferrichrome ABC transporter (binding protein)	0.46	**0.55**	**0.32**
SA2094		hypothetical protein, similar to Na+/H+ antiporter	**0.46**	**0.33**	0.81
SA2117		hypothetical protein, similar to Na+/H+ antiporter	0.82	**0.36**	0.76
SA2132		hypothetical protein, similar to ABC transporter (ATP-binding protein)	**0.34**	**0.22**	**0.21**
SA2135		hypothetical protein, similar to sodium/glutamate symporter	0.71	0.65	**0.23**
SA2142		hypothetical protein, similar to multidrug resistance protein	**0.40**	**0.42**	**0.23**
SA2191		hypothetical protein, similar to NirC	0.54	**0.31**	0.67
SA2300		hypothetical protein, similar to glucarate transporter	0.81	0.83	**0.22**
SA2302		hypothetical protein, similar to ABC transporter	1.12	0.83	**0.27**
SA2303		hypothetical protein, similar to membrane spanning protein	1.15	0.65	**0.18**
SA2326	*ptsG*	PTS system, glucose-specific IIABC component	0.64	**0.42**	**0.32**
RNA synthesis - regulation, elongation and modification					
SA0501	*rpoC*	RNA polymerase beta-prime chain	0.81	**0.39**	0.54
SA1041	*pyrR*	pyrimidine operon repressor chain A	0.72	1.28	**0.13**
SA1082	*rimM*	probable 16S rRNA processing protein	**0.40**	**0.31**	**0.32**
SA1083	*trmD*	tRNA-(guanine-N1)-methyltransferase	**0.44**	**0.32**	**0.38**
SA1583	*rot*	repressor of toxins Rot	0.55	0.64	**0.23**
SA1844	*agrA*	accessory gene regulator A	**0.33**	**0.22**	**0.53**
SA2023	*rpoA*	DNA-directed RNA polymerase alpha chain	0.43	**0.23**	**0.25**
SA2089	*sarR*	staphylococcal accessory regulator A homologue	0.53	**0.36**	**0.31**
Sensors (signal transduction)					
SA1843	*agrC*	accessory gene regulator C	**0.51**	**0.27**	0.70
Hypothetical proteins					
SA0121		hypothetical protein	0.64	-	**0.34**
SA0213		conserved hypothetical protein	**0.33**	**0.39**	**0.37**
SA0231		hypothetical protein, similar to flavohemoprotein	0.90	0.90	**0.36**
SA0262		hypothetical protein	0.51	**0.47**	**0.20**

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Table 4.2 - *Continued*

N315 ORF	Gene	Gene product function	Fold change[2]		
			137/93A (0.5xMIC)	137/93G (0.5xMIC)	SG511 (1xMIC)
SA0266		conserved hypothetical protein	1.47	1.03	**0.34**
SA0267		hypothetical protein	1.13	0.78	**0.35**
SA0268		hypothetical protein	1.05	0.90	**0.39**
SA0269		hypothetical protein	1.10	1.18	**0.03**
SA0291		hypothetical protein	**0.31**	**0.36**	**0.11**
SA0292		hypothetical protein	**0.26**	**0.23**	**0.07**
SA0406		hypothetical protein	0.70	0.98	**0.35**
SA0499		conserved hypothetical protein	0.54	**0.28**	**0.53**
SA0555		conserved hypothetical protein	0.74	**0.53**	**0.27**
SA0588		conserved hypothetical protein	0.63	1.26	**0.30**
SA0667		conserved hypothetical protein	0.79	**0.21**	**0.40**
SA0739		conserved hypothetical protein	**0.27**	**0.29**	**0.04**
SA0890		conserved hypothetical protein	0.42	**0.20**	0.58
SA0929		conserved hypothetical protein	0.73	0.94	**0.34**
SA0949		conserved hypothetical protein	0.73	**0.31**	0.52
SA1002		hypothetical protein	1.01	**0.55**	**0.20**
SA1049		hypothetical protein	1.11	1.44	**0.13**
SA1056		hypothetical protein	0.62	**0.53**	**0.35**
SA1265		conserved hypothetical protein	**0.49**	**0.19**	**0.36**
SA1275		conserved hypothetical protein	0.64	**0.57**	**0.39**
SA1536		conserved hypothetical protein	**0.55**	**0.35**	0.53
SA1693		conserved hypothetical protein	0.73	**0.60**	**0.35**
SA1840		conserved hypothetical protein	0.62	**0.39**	0.57
SA1912		hypothetical protein	0.55	0.45	**0.33**
SA1985		hypothetical protein	0.53	1.74	**0.20**
SA1986		hypothetical protein	0.49	1.56	**0.19**
SA2050		conserved hypothetical protein	0.59	**0.29**	**0.44**
SA2133		conserved hypothetical protein	0.51	**0.31**	**0.21**
SA2143		conserved hypothetical protein	**0.46**	**0.53**	**0.30**
SA2328		conserved hypothetical protein	0.90	1.38	**0.37**
SA2329		conserved hypothetical protein	**0.26**	**0.22**	**0.21**
SA2355		conserved hypothetical protein	0.80	**0.39**	0.72
SA2371		conserved hypothetical protein	0.69	0.80	**0.29**
SA2372		hypothetical protein	0.58	0.74	**0.34**
SA2443		hypothetical protein	0.84	**0.37**	1.00

[1] Significant changes of gene expression were determined by implementing SAM (significance analysis of microarrays; http://www-stat.stanford.edu/~tibs/SAM/) (Tusher et al., 2001).

[2] Fold change in transcript level of at least 2.5-fold is indicated as mean of the "median of ratios" compared to control cells. Fold change in bold = classified as "significantly" regulated in this strain by SAM.

4.3.7 Regulation of genes related to protein transport and binding

In this thesis, the most prominent event of gene regulation comprised the upregulation of the genes coding for the hypothetical ABC transporter VraDE in the presence of mersacidin. The *vraSR* genes were induced 40 to 70-fold in the microarray studies in all three strains tested. This transporter has been previously shown to be induced by vancomycin and cationic AMPs (Kuroda et al., 2000; Li et al., 2007a) and seems to be involved in the increased resistance towards bacitracin and the human β-defensin 3 (hBD3) in *S. aureus* (Sass et al., 2008b).

Furthermore, the *oppBCDF* gene cluster coding for an oligopeptide transport system and the components of the phospho-transferase system (PTS), notably ORF SA1255 (specific to glucose), *glvC*, as well as *glcA* and *ptsG* (both specific to glucose), displayed increased expression. Only few genes coding for transport proteins were differently expressed in the VISA strains SA137/93A and SA137/93G compared to the sensitive strain SG511-Berlin. Among these, the proline/betaine transporter homologue gene *proP*, coding for a transporter of osmoprotectants, was upregulated in both VISA strains while downregulated in strain SG511-Berlin. Interestingly, the genes coding for the ABC transporter VraFG, which have recently been shown to be positively controlled by the GraRS TCRS and seem to support vancomycin-intermediate resistance (Meehl et al., 2007) as well as resistance to cationic AMPs (Li et al., 2007a), were slightly downregulated in strains SA137/93A and SA137/93G compared to strain SG511-Berlin.

4.4 Quantitative Real-Time PCR (qRT-PCR) of *vraS* and *vraE* marker gene transcription in response to mersacidin

Quantitative RT-PCR of the sensor histidine kinase gene *vraS* was employed to further analyze the induction of the VraSR-triggered cell wall stress stimulon (Fig. 4.5A). In the presence of 0.5 x MIC of mersacidin, a strong induction of *vraS* transcription (16 to 26-fold) was recorded for all strains tested. Even 0.15 x MIC of mersacidin considerably induced *vraS* transcription in strain SA137/93G (5.6-fold; incubated with 4 µg/ml of mersacidin corresponding to 2.175 µM) and strain SG511-Berlin (3.4-fold; incubated with 0.2 µg/ml of mersacidin corresponding to 0.116 µM) (strain SA137/93A not tested). As a control, strain SA137/93G was incubated with subinhibitory concentrations of vancomycin (4 µg/ml, 2.75 µM, 0.5 x MIC). Here, no induction was visible by qRT-PCR. However, the transcript levels of untreated or treated conditions, respectively, did not vary significantly between the tested strains. Interestingly, *vraS* transcription was not significantly affected in strain SA137/93G when incubated with 0.5 µg/ml that corresponds to 0.02 x MIC mersacidin for this strain.

The induction of *vraDE* gene transcription was also verified using qRT-PCR (Fig. 4.5B). Upon incubation with 0.5 x MIC of mersacidin, *vraDE* transcription was induced 76 to 79-fold in strain SA137/93A and SA137/93G, respectively. In the susceptible strain SG511-Berlin, the transcript level of the *vraDE* genes rose almost 1700-fold upon incubation with mersacidin.

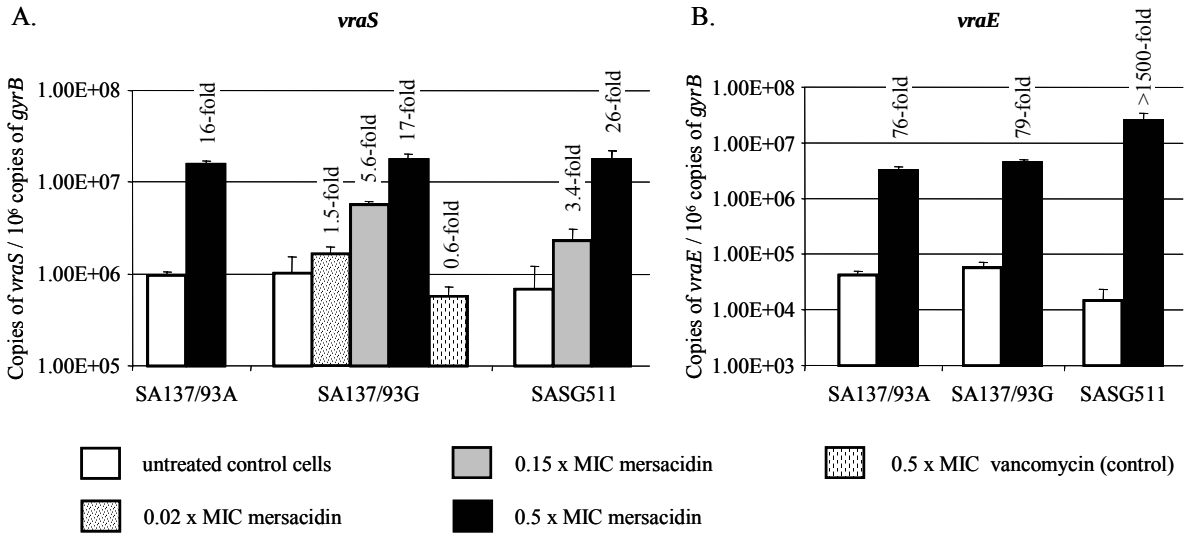

Figure 4.5: Gene regulatory response of the *S. aureus* strains SA137/93A, SA137/93G and SG511-Berlin to the lantibiotic mersacidin. A. qRT-PCR of *vraS* gene induction in response to subinhibitory concentrations of mersacidin and vancomycin (control). **B.** qRT-PCR of *vraE* gene expression upon mersacidin treatment. The qRT-PCR values represent the mean of at least two independent experiments. Quantitative data are presented in relation to 10^6 copies of the housekeeping gene *gyrB*. Fold changes were calculated in relation to the untreated control cells, i.e. transcription levels in the absence of mersacidin.

4.5 Susceptibility testing of a *vraDE* deletion mutant of *S. aureus* SG511-Berlin against mersacidin

In this thesis, microarray profiling and qRT-PCR of different *S. aureus* strains revealed the extensive upregulation of the genes coding for the hypothetical ABC transporter VraDE in response to mersacidin treatment. In the producer strain of mersacidin, resistance is conferred by an ABC transporter (Guder et al., 2002). For this reason, the participation of an ABC transporter in the decreased susceptibility of *S. aureus* seemed to be likely. To test whether VraDE is able to transport mersacidin out of the cell membrane and therefore supports a resistance phenotype, the growth behaviour and MICs of a *vraE* knock-out mutant of *S. aureus* SG511-Berlin (Sass et al., 2008b) and its parent strain were examined in the presence of the lantibiotic. Unexpectedly, *S. aureus* SG511ΔvraE was characterized by a lowered growth rate but did not show increased susceptibility towards mersacidin neither in growth curve recordings (Fig. 4.6) nor in MIC studies (MIC of SG511ΔvraE: 1 µg/ml; SG511-Berlin: 1 µg/ml).

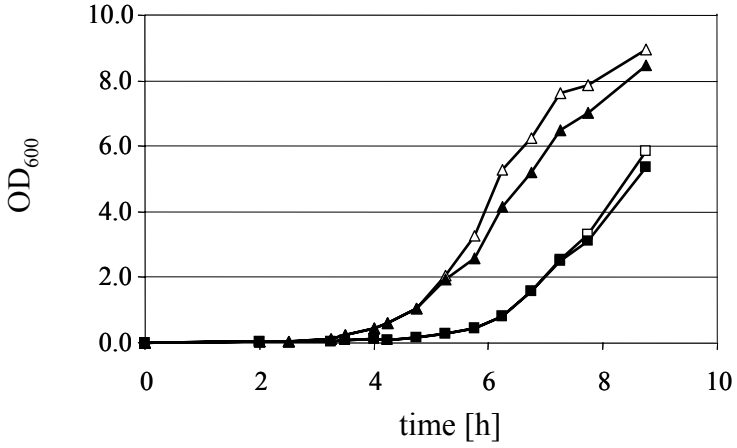

Figure 4.6: *In vitro* growth curve recordings of *S. aureus* SG511ΔvraE in the presence of mersacidin. *S. aureus* SG511-Berlin wild type (▲ 0.5 µg/ml, △ control); *S. aureus* SG511ΔvraE (■ 0.5 µg/ml, □ control).

4.6 Nucleotide sequence analysis of *vraDE* and *vraS*

For further characterization of VraDE and VraS in *S. aureus* SA137/93A and *S. aureus* SG511-Berlin, the nucleotide sequences of the *vraDE* genes plus the corresponding promoter region as well as the *vraS* gene were determined in both strains. Sequencing of the *vraDE* genes revealed single differences in the overall nucleotide sequence, however, the -35 and the -10 region, the ribosomal binding site (Fig. 4.7) as well as the *vraD* gene (for protein sequence alignment see Fig. 4.8) were highly conserved in strains SA137/93A and SG511-Berlin showing 100% sequence identity. The *vraE* gene still showed 95.7% sequence identity with mostly conservative amino acid substitutions (for protein sequence alignment see Fig. 4.8). Noteworthy, the *vraDE* sequence of *S. aureus* SA137/93G was identical to that of strain SA137/93A (data not shown). *In silico* analysis of putative transmembrane helices of VraE using online analysis tools (http://www.cbs.dtu.dk/services/TMHMM-2.0/) assumed that these amino acid substitutions predominantly occurred in the C-terminal loop of VraE that is located in the periplasmic space between the plasma membrane and the thick peptidoglycan layer of *S. aureus*. Protein sequence analysis of VraDE via Basic Local Alignment Search Tool (BLAST; http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to determine the homology of VraDE of strain SA137/93A and strain SG511-Berlin to other *S. aureus* species. Conserved domain analysis using BLAST linked VraDE to the SalXY-like ABC_ATPase superfamily. Here, VraD of *S. aureus* SA137/93A and strain SG511-Berlin showed 100% sequence identity to all other *S. aureus* VraD protein sequences of the NCBI database (http://www.ncbi.nlm.nih.gov/) except for *S. aureus* MRSA252 which was

characterized by a single conservative amino acid substitution (E236K). The VraE protein sequence of *S. aureus* SA137/93A also showed 100% sequence identity to the VraE sequences of *S. aureus* strain COL, strain USA300 and strain Newman. Less homology was noted for *S. aureus* strain NCTC8325 (2 conservative amino acid (aa) substitutions), strain Mu50, strain N315, strain JH9 (each 1 conservative and 4 non-conservative aa substitutions), strain MW2, strain MSSA476, (each 1 conservative and 5 non-conservative aa substitutions), strain RF122 (8 conservative and 12 non-conservative aa substitutions) and strain MRSA252 (15 conservative and 13 non-conservative aa substitutions). Interestingly, the homology pattern of *S. aureus* SG511-Berlin was reversed. The closest homology was detected with *S. aureus* strain MRSA252 (1 non-conservative aa substitution; S441I), followed by strain RF122 (9 conservative and 7 non-conservative aa substitutions), strain MW2, strain MSSA476 (each 14 conservative and 9 non-conservative aa substitutions), strain NCTC8325 (15 conservative and 12 non-conservative aa substitutions), strain Mu50, strain N315, strain JH9 (each 17 conservative and 11 non-conservative aa substitutions), strain COL, strain Newman and strain USA300 (each 15 conservative and 12 non-conservative aa substitutions). The protein sequence of the histidine sensor kinase VraS of *S. aureus* strain SA137/93A, strain SA137/93G and strain SG511-Berlin showed 100% sequence identity to all other *S. aureus* protein sequences of the NCBI database (http://www.ncbi.nlm.nih.gov/) except for *S. aureus* strain Mu50 and strain Mu3 which were characterized by a single non-conservative amino acid substitution (I5N; Fig. 4.9).

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SA137/93A           AACAATCAATTCCTTTCAAATAAAACCCTCTTTTAATTTTTACAAAAAAT-TTATTATAC 59
SG511-Berlin        AACAATCATTTCCTTTCAAACAACGCCTTCTTTATATTTTAATTAGTGTCACTATTATTC 60
                    ******** *********** **  ** *****  ***** *  *       ****** *

SA137/93A           AATAATTAAATTCCCTTTTCTCAATTTACACAAACATCATCACTTAGAAAGCACCAT--T 117
SG511-Berlin        ATTGTTTAAATTCCCTCATCTTAACTTATAGAAACATTTCCTCAGTGCAAATACTGTGAT 120
                    * *  ***********  *** ** *** * ******   * *   * **  **  *  *

SA137/93A           GCT----TTTGTCTAA-TATGTACGATGAATCTGTATCCTCACTCAAAAATCTACTAAGT 172
SG511-Berlin        GCTCAAATTAGTTGAACTGCTTTCCAATAATCCAAATTCACCCGAGAAAATC-ACGATAT 179
                    ***    ** **  ** *   * * *  ****   ** * * *   ****** ** *  *
                                                                  -35 region
SA137/93A           AGCGATAGCTTTCAACTCTGTAAGGTTCATTCATTGAATTGTAAGTTTAGATAAAGGCTA 232
SG511-Berlin        ATTAATATCTTTCAACTATGTAAGATTCAAACATTGAATTGTAAGTTTAGATAAAGGCTA 239
                    *   *** ********* ****** ****  ***************************
                                  -10 region              rbs        start codon vraD
SA137/93A           CTACTCCAAACATTATTTAAAATGAAATTAAATACAAAAGGAGTGAGACTATG 285
SG511-Berlin        CTGTTCTACTTATTATTTAAAATGAATTTAAATACAAAAGGAGTGATACTATG 292
                    **  ** *   *************** ******************* ******
```

Figure 4.7: Nucleotide sequence alignment of the promoter region of *vraDE* in *S. aureus* SA137/93A and *S. aureus* SG511-Berlin. The multiple sequence alignment was performed using the online analysis tool "ClustalW" (http://www.ebi.ac.uk/Tools/clustalw2/index.html). The -35 region (consensus sequence: TTGACA), the -10 region (consensus sequence: TATAAT) and the ribosomal binding site (rbs/shine dalgarno consensus sequence: AGGAGGU) are underlined. "*" indicates identical nucleotides.

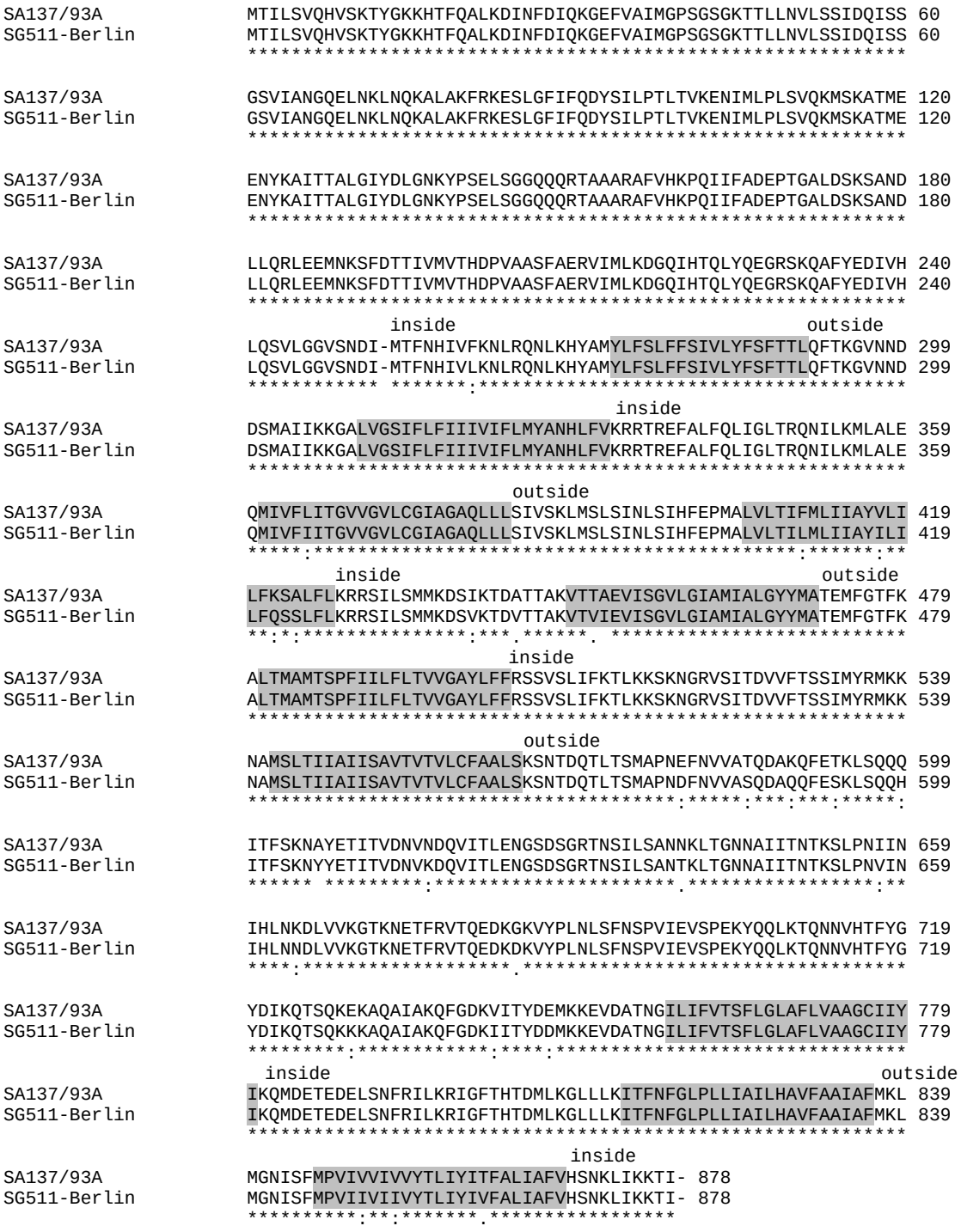

Figure 4.8: Amino acid sequence alignment of VraDE in *S. aureus* SA137/93A and *S. aureus* SG511-Berlin. Multiple sequence alignment and determination of putative transmembrane helices were performed using the online analysis tool "ClustalW" (http://www.ebi.ac.uk/Tools/clustalw2/index.html) and the TMHMM server v2.0 (http://www.cbs.dtu.dk/services/TMHMM-2.0/). Putative transmembrane helices are highlighted in grey. "*" indicates identical amino acids (aa), ":" conservative aa substitution, "." semi-conservative aa substitution.

```
SA137/93A         MNHYIRTIGSMLILVYSMLAAFLFIDKVFVNIIYFQGMFYTQIFGIPVFLFLNLIIILLC 60
SA137/93G         MNHYIRTIGSMLILVYSMLAAFLFIDKVFVNIIYFQGMFYTQIFGIPVFLFLNLIIILLC 60
SG511-Berlin      MNHYIRTIGSMLILVYSMLAAFLFIDKVFVNIIYFQGMFYTQIFGIPVFLFLNLIIILLC 60
Mu50              MNHYNRTIGSMLILVYSMLAAFLFIDKVFVNIIYFQGMFYTQIFGIPVFLFLNLIIILLC 60
                  **** ************************************************

SA137/93A         IIVGSVLAYKINQQNDWIKTQIERSMEGETVGINDQNIEIYSETLDLYHTLVPLNQELHK 120
SA137/93G         IIVGSVLAYKINQQNDWIKTQIERSMEGETVGINDQNIEIYSETLDLYHTLVPLNQELHK 120
SG511-Berlin      IIVGSVLAYKINQQNDWIKTQIERSMEGETVGINDQNIEIYSETLDLYHTLVPLNQELHK 120
Mu50              IIVGSVLAYKINQQNDWIKTQIERSMEGETVGINDQNIEIYSETLDLYHTLVPLNQELHK 120
                  ************************************************************

SA137/93A         LRLKTQNLTNENYNINDVKVKKIIEDERQRLARELHDSVSQQLFAASMMLSAIKETKLEP 180
SA137/93G         LRLKTQNLTNENYNINDVKVKKIIEDERQRLARELHDSVSQQLFAASMMLSAIKETKLEP 180
SG511-Berlin      LRLKTQNLTNENYNINDVKVKKIIEDERQRLARELHDSVSQQLFAASMMLSAIKETKLEP 180
Mu50              LRLKTQNLTNENYNINDVKVKKIIEDERQRLARELHDSVSQQLFAASMMLSAIKETKLEP 180
                  ************************************************************

SA137/93A         PLDQQIPILEKMVQDSQLEMRALLLHLRPLGLKDKSLGEGIKDLVIDLQKKVPMKVVHEI 240
SA137/93G         PLDQQIPILEKMVQDSQLEMRALLLHLRPLGLKDKSLGEGIKDLVIDLQKKVPMKVVHEI 240
SG511-Berlin      PLDQQIPILEKMVQDSQLEMRALLLHLRPLGLKDKSLGEGIKDLVIDLQKKVPMKVVHEI 240
Mu50              PLDQQIPILEKMVQDSQLEMRALLLHLRPLGLKDKSLGEGIKDLVIDLQKKVPMKVVHEI 240
                  ************************************************************

SA137/93A         QDFKVPKGIEDHLFRITQEAISNTLRHSNGTKVTVELFNKDDYLLLRIQDNGKGFNVDEK 300
SA137/93G         QDFKVPKGIEDHLFRITQEAISNTLRHSNGTKVTVELFNKDDYLLLRIQDNGKGFNVDEK 300
SG511-Berlin      QDFKVPKGIEDHLFRITQEAISNTLRHSNGTKVTVELFNKDDYLLLRIQDNGKGFNVDEK 300
Mu50              QDFKVPKGIEDHLFRITQEAISNTLRHSNGTKVTVELFNKDDYLLLRIQDNGKGFNVDEK 300
                  ************************************************************

SA137/93A         LEQSYGLKNMRERALEIGATFHIVSLPDSGTRIEVKAPLNKEDSYDD- 347
SA137/93G         LEQSYGLKNMRERALEIGATFHIVSLPDSGTRIEVKAPLNKEDSYDD- 347
SG511-Berlin      LEQSYGLKNMRERALEIGATFHIVSLPDSGTRIEVKAPLNKEDSYDD- 347
Mu50              LEQSYGLKNMRERALEIGATFHIVSLPDSGTRIEVKAPLNKEDSYDD- 347
                  *********************************************
```

Figure 4.9: Amino acid sequence alignment of VraS of *S. aureus* strain SA137/93A, strain SA137/93G, strain SG511-Berlin and strain Mu50. The I5N amino acid substitution in VraS of *S. aureus* Mu50 is shaded in grey. "*" indicates identical amino acids.

4.7 Comparative transcriptomics of *S. aureus* SG511-Berlin and *S. aureus* SA137/93A

Since the expression pattern of *S. aureus* SG511-Berlin and *S. aureus* SA137/93A in the presence of mersacidin did not reveal significant differences among theses strains which could explain their divergent susceptibilities to mersacidin, the expression profiles of both strains were compared to each other in the absence of antibiotics using *S. aureus* microarrays. Here, mannifold differences in their transcriptional patterns were observed (Table 4.3 and Table S2 in the supplemental material). Due to the susceptibility of strain SG511-Berlin to methicillin, kanamycin, neomycin and erythromycin, resulting from the absence of the respective resistance determinants SCC*mec*, *aadD* and *ermA*, the transcripts of these genes were virtually absent in this strain compared to *S. aureus* SA137/93A. Furthermore, the divergent regulation of factors involved in biofilm formation and colonization was observed, e.g. *atl* and *sirC* (0.38- and 0.21-fold, respectively). Additionally, the transcript levels of the genes coding for the biosynthesis of the Cap5/8 microcapsule (<0.35-fold) and the urease synthesis cluster (<0.15-fold) were lower in SG511-Berlin compared to SA137/39A. In terms of global regulators, the *sigB* (0.3-fold) and *agrC* (0.08-fold) transcripts showed a lower level

in strain SG511-Berlin, while the level of *rot* gene transcript (4.32-fold) was higher compared to SA137/93A. The latter fact might explain the decreased expression of the urease genes in strain SG511-Berlin, since Rot has been shown to be a negative regulator for this gene cluster (Said-Salim et al., 2003). In agreement with earlier observations (Jansen et al., 2007), the genes of the YycFG TCRS showed a lower expression in SG511-Berlin (0.11-fold), which has been linked to the presence of the insertion element IS*256* in the promoter region of *yycFG* in SA137/93A, thereby generating a stronger hybrid promoter.

Table 4.3: Transcriptional profiling of *S. aureus* SG511-Berlin versus strain SA137/93A. The data represent differentially expressed genes associated with antimicrobial resistance and regulation by GraRS.

N315 ORF	Gene	Gene product function	Value[1]
SA0017	*yycF*	response regulator	0.11
SA0018	*yycG*	two-component sensor histidine kinase	0.11
SA0033	*aadD*	kanamycin nucleotidyltransferase	0.02
SA0038	*mecA*	penicillin binding protein 2a	0.11
SA0109	*sirC*	lipoprotein	0.21
SA0144	*capA*	capsular polysaccharide synthesis enzyme Cap5A	0.26
SA0145	*capB*	capsular polysaccharide synthesis enzyme Cap5B	0.18
SA0146	*capC*	capsular polysaccharide synthesis enzyme Cap8C	0.22
SA0147	*capD*	capsular polysaccharide synthesis enzyme Cap5D	0.19
SA0148	*capE*	capsular polysaccharide synthesis enzyme Cap8E	0.25
SA0150	*capG*	capsular polysaccharide synthesis enzyme Cap5G	0.35
SA0152	*capI*	capsular polysaccharide synthesis enzyme Cap5I	0.30
SA0153	*capJ*	capsular polysaccharide synthesis enzyme Cap5J	0.26
SA0154	*capK*	capsular polysaccharide synthesis enzyme Cap5K	0.22
SA0616	*vraF*	ABC transporter ATP-binding protein	0.11
SA0617	*vraG*	ABC transporter permease	0.21
SA0650	*norA*	quinolone resistance protein	0.12
SA0794	*dltB*	DltB membrane protein	0.49
SA0905	*atl*	autolysin	0.38
SA1193	*mprF*	lysyltransferase (MprF or FmtC)	0.40
SA1583	*rot*	repressor of toxins Rot	4.32
SA1843	*agrC*	accessory gene regulator C	0.08
SA1869	*sigB*	sigma factor B	0.30
SA1984	*asp23*	alkaline shock protein 23	0.15
SA2082	*ureA*	urease gamma subunit	0.07
SA2083	*ureB*	urease beta subunit	0.04
SA2084	*ureC*	urease alpha subunit	0.07
SA2085	*ureE*	urease accessory protein UreE	0.11
SA2086	*ureF*	urease accessory protein UreF	0.08
SA2087	*ureG*	urease accessory protein UreG	0.13
SA2088	*ureD*	urease accessory protein UreD	0.15
SA2384	*ermA*	rRNA methylase	0.02

[1] Relative transcript levels of *S. aureus* SG511-Berlin compared to *S. aureus* SA137/93A.

Finally, a lower expression of the genes coding for the membrane protein DltB (0.49-fold), the lysyltransferase MprF (0.4-fold) and the ABC transporter VraFG (<0.21-fold) was observed in strain SG511-Berlin. The *dltB*, *mprF* and *vraFG* genes are controlled by the GraRS TCRS, that has been proven to influence the susceptibility of *S. aureus* to antimicrobial substances (Li et al., 2007a; Herbert et al., 2007; Meehl et al., 2007). In *S. epidermidis*, the *graRS* (also: *apsRS*; SA0614/15, of the genomic sequence of *S. aureus* N315) and SA0613 (*graX*; also: *apsX*) homologues have been shown to constitute a three-component regulatory system and have been referred to as antimicrobial peptide sensing system (aps). The role of GraX is unclear, however, the protein is essential for signal transduction in *S. epidermidis* (Li et al., 2007b); its essentiality in *S. aureus* has not yet been ascertained by inactivation.

4.8 Sequence analysis of *graXRS* of *S. aureus* SG511-Berlin and *S. aureus* SA137/93A

In order to analyze the reasons for the divergent expression levels of *dltB*, *mprF* and *vraFG* in the *S. aureus* strains SG511-Berlin and SA137/93A, a 2.8 kb fragment of *graXRS* (SA0613-5) was sequenced in both strains. Besides several, mostly silent nucleotide exchanges, a thymine was inserted at position 190 of the sensor histidine kinase gene *graS* of strain SG511-Berlin compared to *S. aureus* SA137/93A and other *S. aureus* genomes of the NCBI database (http://www.ncbi.nlm.nih.gov/). As a direct consequence, this nucleotide insertion generates a stop codon at the amino acid (aa) position 64 (Fig. 4.11), thereby eliminating 283 aa of GraS, which is the entire cytoplasmic part of the membrane protein (Li et al., 2007a) that is responsible for the activation of the response regulator GraR. A defect GraS sensor histidine kinase, followed by an impaired signal transduction, might well be the reason for an altered *dltB*, *mprF* and *vraFG* expression and hence might have a direct influence on the susceptibility of *S. aureus* SG511-Berlin to various antimicrobials. Noteworthy, the *graXRS* sequence of *S. aureus* SA137/93A was identical to that of strain SA137/93G (data not shown) which was most homologous to the *graXRS* sequences of the *S. aureus* strains COL, USA300, Newman and NCTC8325. Aside from the nucleotide insertion, the *graS* sequence of SG511-Berlin showed 100% sequence identity to the *graS* gene of *S. aureus* MRSA252 (Holden et al., 2004). Since strain SG511-Berlin seems to be closely related to *S. aureus* MRSA252 (see section 4.2 and 4.6), *S. aureus* MRSA252 was regarded as the appropriate control to strain SG511-Berlin in the following experiments.

```
SA0613 (GraX)
SA137/93A          MKPKVLLAGGTGYIGKYLSEVIENDAELFAISKYPDNKKTDDVEMTWIQCDIFHYEQVVA 60
SASG511-Berlin     MKPKVLLAGGTGYIGKYLSEVIENDAELFAISKYPDNKKTDDVEMTWIQCDIFHYEQVVA 60
                   ************************************************************

SA137/93A          AMNQIDIAVFFIDPTKNSAKITQSSARDLTLIAADNFGRAAAINQVKKVIYIPGSRYDNE 120
SASG511-Berlin     AMNQIDIAVFFIDPTKNSAKITQSSARDLTLIAADNFGRAAAINQVKKVIYIPGSRYDNE 120
                   ************************************************************

SA137/93A          TIERLGAYGTPVETTNLVFKRSLVNVELQVSKYDDVRSTMKVVLPKGWTLKNVVNHFIAW 180
SASG511-Berlin     TIERLGAYGTTVETTNLVFKRSLVNVELQVSKYDDVRSTMKVVLPKGWTLKNVVNHFIAW 180
                   **********.*************************************************

SA137/93A          MGYTKGTFVKTEKSHDQFKIYIKNKVRPLAVFKIEETADGIITLILLSGSLVKKYTVNQG 240
SASG511-Berlin     MGYTKGTFVKTEKSHDQFKIYIKNKVRPLAVFKIVETADGIITLILLSGSLVKKYTVNQG 240
                   ********************************* ************************

SA137/93A          KLEFRLIKESAVVYIHLYDYIPRLFWPIYYFIQAPMQKMMIHGFEVDCRIKDFQSRLKSG 300
SASG511-Berlin     KLEFRLIKESSVVYIHLYDYIPRLFWPIYYFIQAPMQKMMIHGFEVDCRIKDFQSRLKSG 300
                   **********:*************************************************

SA137/93A          ENMKYTKstop 307
SASG511-Berlin     ENMKYTKstop 307
                   *******

SA0614 (GraR)
SA137/93A          MQILLVEDDNTLFQELKKELEQWDFNVAGIEDFGKVMDTFESFNPEIVILDVQLPKYDGF 60
SASG511-Berlin     MQILLVEDDNTLFQELKKELEQWDFNVAGIEDFGKVMDTFESFNPEIVILDVQLPKYDGF 60
                   ************************************************************

SA137/93A          YWCRKMREVSNVPILFLSSRDNPMDQVMSMELGADDYMQKPFYTNVLIAKLQAIYRRVYE 120
SASG511-Berlin     YWCRKMREVSNVPILFLSSRDNPMDQVMSMELGADDYMQKPFYTNVLIAKLQAIYRRVYE 120
                   ************************************************************

SA137/93A          FTAEEKRTLTWQDAVVDLSKDSIQKGDQTIFLSKTEMIILEILITKKNQIVSRDTIITAL 180
SASG511-Berlin     FTAEEKRTLTWQDAVIDLSKDSIQKGDQTIFLSKTEMIILEILITKKNQIVSRDTIITAL 180
                   ***************:********************************************

SA137/93A          WDDEAFVSDNTLTVNVNRLRKKLSEISMDSAIETKVGKGYMAHEstop 224
SASG511-Berlin     WDDEAFVSDNTLTVNVNRLRKKLSEIGMDSAIETKVGKGYMAHEstop 224
                   *************************.*****************

SA0615 (GraS)
SA137/93A          MNNLKWVAYFLKSRMNWIFWILFLNFLMLGISLIDYDFPIDSLFYIVSLNLSLTMIFLLL 60
SASG511-Berlin     MNNLKWVVYFLKSRKNWIFWILFLNILMLGISLIDYDFPIDSLFYIVSLNLSLTLIFLIL 60
                   *******.****** **********:****************************:***:*

SA137/93A          TYFKEVKLYKHFDKDKEIEEIKHKDLAETPFQRHTVDYLYRQISAHKEKVVEQQLQLNMH 120
SASG511-Berlin     TFFstop---------------------------------------------------- 63
                   *:*

SA137/93A          EQTITEFVHDIKTPVTAMKLLIDQEKNQERKQALLYEWSRINSMLDTQLYITRLESQRKD 180
SASG511-Berlin     ------------------------------------------------------------


SA137/93A          MYFDYVSLKRMVIDEIQLTRHISQVKGIGFDVDFKVDDYVYTDIKWCRMIIRQILSNALK 240
SASG511-Berlin     ------------------------------------------------------------


SA137/93A          YSENFNIEIGTELNDQHVSLYIKDYGRGISKKDMPRIFERGFTSTANRNETTSSGMGLYL 300
SASG511-Berlin     ------------------------------------------------------------


SA137/93A          VNSVKDQLGIHLQVTSTVGKGTTVRLIFPLQNEIVERMSEVTNLSFstop 346
SASG511-Berlin     -----------------------------------------------
```

Figure 4.11: Amino acid sequence alignment of GraXRS of *S. aureus* SA137/93A and *S. aureus* SG511-Berlin. The first and second transmembrane segments of GraS are underlined and the extracellular loop as postulated by Li et al. (2007a) is presented in bold. In strain SG511-Berlin, the insertion of a thymine at position 190 of *graS* generates a stop codon at the amino acid (aa) position 64 resulting in the deletion of 283 aa of the sensor histidine kinase representing the cytoplasmic, signal transducing part of the membrane protein. "*" indicates identical amino acids (aa), ":" conservative aa substitution, "." semi-conservative aa substitution.

4.9 Complementation of *graS* in *S. aureus* SG511-Berlin

To analyze the effect of the nucleotide insertion in *graS* of strain SG511-Berlin, different mutants of this strain were constructed that carry the *graS* gene *in trans* under the control of a xylose-inducible promoter on the recombinant plasmid pTXgraS. To this end, a 1079 bp PCR fragment of the *graS* gene was amplified from genomic DNA of *S. aureus* MRSA252 (ORF SAR0670) and from genomic DNA of *S. aureus* N315 (ORF SA0615) as a control. The PCR fragments were subsequently inserted into the *Bam*HI and *Mlu*I restriction sites of *Bam*HI/*Mlu*I-digested pTX15, allowing the xylose-controlled expression of *graS* (Fig. 4.12). Ligation assays were transformed into *S. aureus* RN4220 and subsequently into *S. aureus* SG511-Berlin by electroporation (Schenk and Laddaga, 1992). As a second strategy, 2810 bp PCR fragments comprising the *graXRS* genes were amplified from genomic DNA of *S. aureus* MRSA252 (ORF SAR0668-70) and from genomic DNA of *S. aureus* N315 (ORF SA0613-5), inserted into the *Xba*I and *Pst*I restriction sites of *Xba*I/*Pst*I-digested pCU1, transformed into *S. aureus* RN4220 by electroporation and finally transduced into *S. aureus* SG511-Berlin by phage transduction. Since *S. aureus* SG511-Berlin harbouring the pCU1 derivatives or pTXgraS(N315) showed similar results in preliminary MIC testings towards mersacidin, only *S. aureus* SG511-Berlin carrying pTXgraS(MRSA252) was used for follow up experiments.

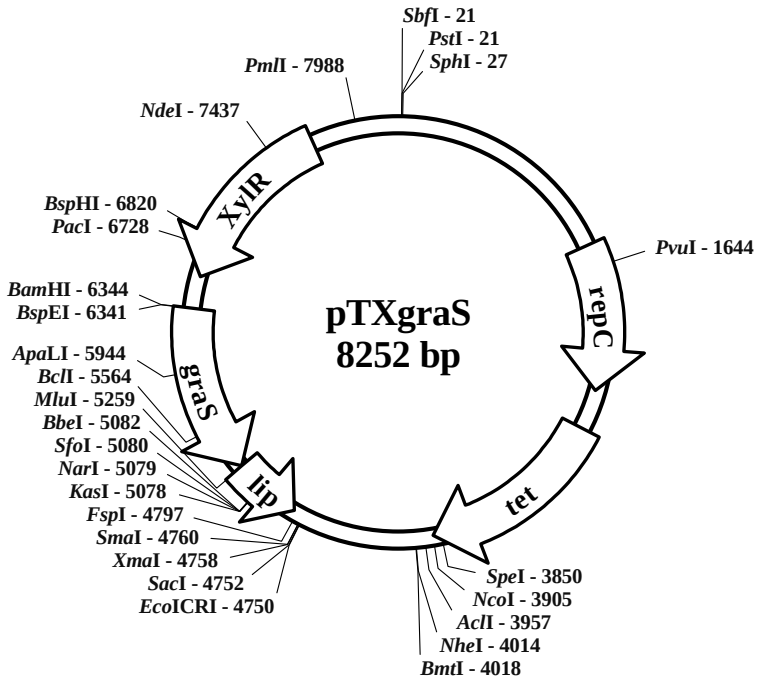

Figure 4.12: Construction of the pTXgraS(MRSA252) expression plasmid. The expression plasmid pTXgraS(MRSA252) was generated by insertion of SAR0670 (*graS*) of *S. aureus* MRSA252 via the single *BamH*I and *Mlu*I restriction sites into the xylose-inducible expression vector pTX15.

4.10 Analysis of *dltB* and *vraF* gene expression by qRT-PCR in *graS* complemented *S. aureus* SG511-Berlin

Since *dltB* and *vraF* expression of *S. aureus* has been shown to be dependent on the activation of GraS (Herbert et al., 2007; Li et al., 2007a; Meehl et al., 2007), qRT-PCR studies were performed to control the *graS*-dependent *dltB* and *vraF* expression in the wildtype of *S. aureus* SG511-Berlin, the empty vector control (pTX16) as well as the complemented mutant carrying pTXgraS(MRSA252). For the construction of the external qRT-PCR plasmid standards pUC19dltB and pUC19vraF, PCR fragments of *dltB* (1354bp, SA0794) and vraF (761 bp, SA0616) were amplified by PCR from *S. aureus* MRSA252 genomic DNA and cloned via single *Xba*I and *Pst*I sites into the *Xba*I/*Pst*I-digested pUC19 vector (Fig. 4.13).

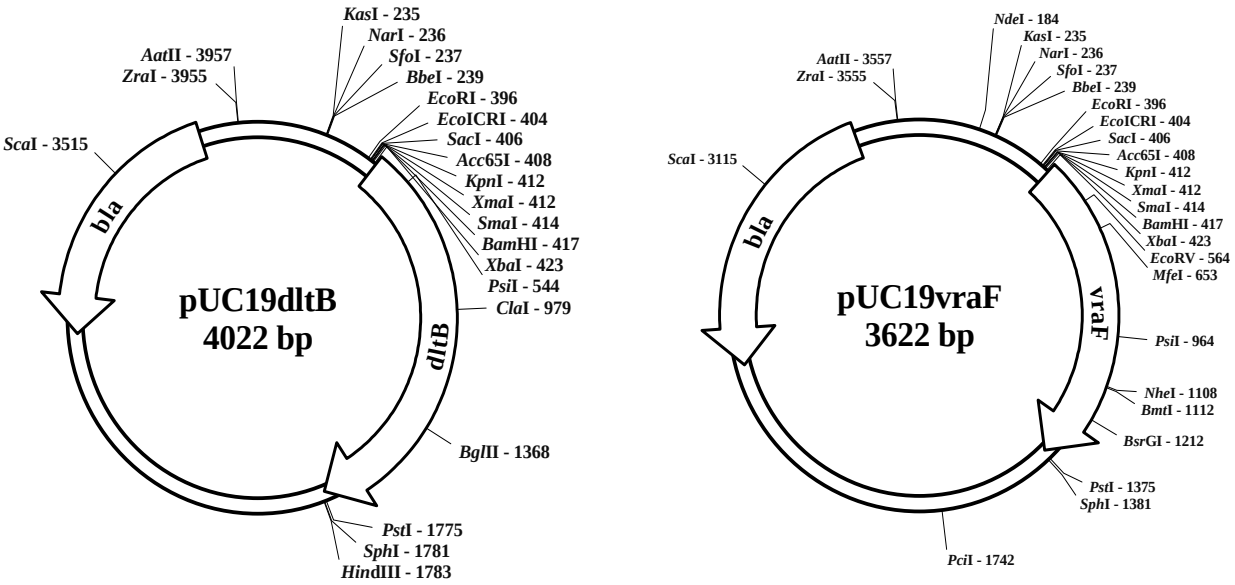

Figure 4.13: Construction of the qRT-PCR standards pUC19dltB and pUC19vraF.

The RNA used for *dltB* and *vraF* gene expression analysis was obtained under inducing conditions using the cationic AMP indolicidin to ensure a proper induction of GraS (Li et al., 2007a). Here, the complementation of *graS* resulted in a significant increase of *dltB* transcripts in *S. aureus* SG511-Berlin carrying pTXgraS(MRSA252) of more than fourfold compared to the wildtype strain (Fig. 4.14A), indicating the functionality of the complemented system. Further, *vraF* transcription was induced 1.5-fold compared to the wildtype strain (Fig. 4.14B).

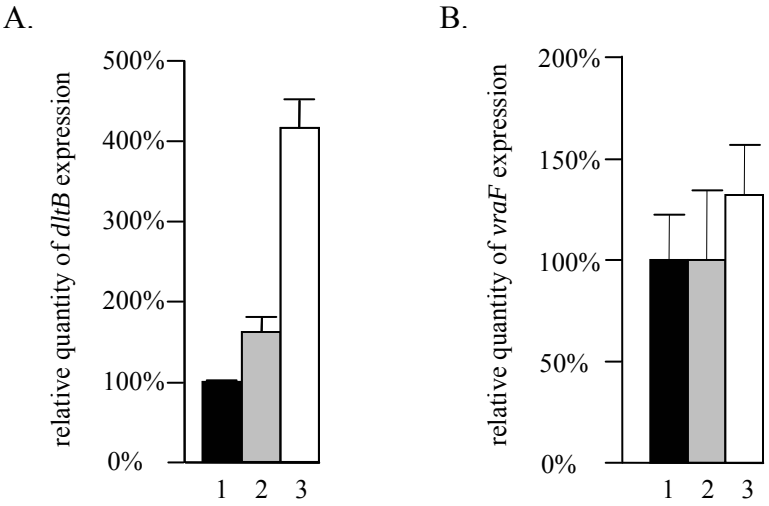

Figure 4.14: Induction of the *graS*-dependent *dltB* and *vraF* expression. Relative transcription levels of indolicidin-induced *dltB* (A) and *vraF* (B) expression in the wildtype *S. aureus* SG511-Berlin (1), the pTX16 control (2) and the pTXgraS(MRSA252) complemented strain (3). Expression of *dltB* and *vraF* is compared to the transcript levels of the wildtype strain. Error bars represent the maximal deviations of the measurements from the respective mean value.

4.11 Determination of the whole-cell surface charge in *graS* complemented *S. aureus* SG511-Berlin

The effect of the nucleotide insertion in *graS* on the phenotype of strain SG511-Berlin was further analyzed by measuring the whole-cell surface charge using a cytochrome c assay. Since the DltB membrane protein, whose expression is controlled by GraRS, confers a less negative charge to the cell surface of *S. aureus* by catalyzing the alanylation of teichoic acids in the bacterial cell wall (Peschel et al., 1999), a higher negative cell surface charge would be expected for cells with an impaired GraS protein. Cytochrome c is a highly positively charged protein (pI = 10; 12 kDa) and can be detected at 530 nm (the absorption maximum of the prosthetic group). Cytochrome c binding is dependent on the net negative cell surface charge of *S. aureus*, thereby allowing a comparison of the whole-cell surface charge of different strains. In fact, the wildtype of strain SG511-Berlin bound a much higher amount of the available cytochrome c (>65%) compared to the control strain MRSA252 (<30%) (Fig. 4.15). These data correspond to the decreased *dltB* expression of strain SG511-Berlin in the qRT-PCR study (Fig. 4.14). The result for the control strain is consistent with other studies, where cytochrome c binding was found to be relatively low in *S. aureus* due to the *graS*-controlled expression of *dltB* (Kraus et al., 2008; Peschel et al., 1999). The complementation of *graS* in

strain SG511-Berlin resulted in a less negative cell surface charge compared to the wildtype that was indicated by a significantly lower amount of bound cytochrome c (~45%). Since the charge of the cell surface and the functionality of the GraRS TCRS have a crucial effect on the susceptibility of *S. aureus* to antimicrobial peptides (Peschel et al., 1999; Li et al., 2007a; Herbert et al., 2007; Meehl et al., 2007), the nucleotide insertion in *graS* of strain SG511-Berlin might account at least partly for its extensive susceptibility to antimicrobial agents.

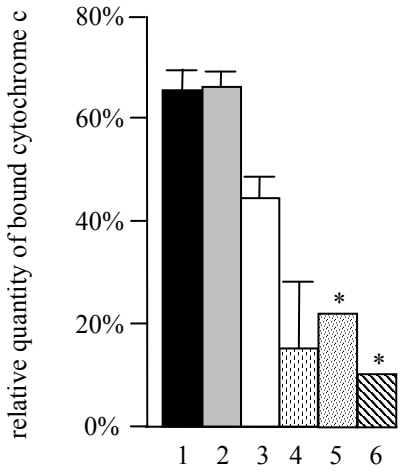

Figure 4.15: Determination of the whole-cell surface charge. The whole-cell surface charge was measured using a cytochrome c binding assay. The data show the relative amount of cytochrome c bound to the cell wall of the wildtype *S. aureus* SG511-Berlin (1), the pTX16 control (2), the pTXgraS(MRSA252) complemented strain (3) as well as *S. aureus* strain MRSA252 (4), strain RN1"HG" (5) and strain N315 (6) as additional controls. The data was calculated from the appropriate standard curve. Error bars represent the maximal deviations of the measurements from the respective mean value. *The data was obtained by a single measurement in LB broth.

4.12 MIC determination of *graS* complemented *S. aureus* SG511-Berlin

To analyze the role of the nucleotide insertion in *graS* in the susceptibility of *S. aureus* strain SG511-Berlin, the MICs of several antimicrobial agents were determined (Table 4.4). In order to maintain the experimental conditions of the *dltB* expression studies and the determination of the whole-cell surface charge, the bacteria were grown in half concentrated BHI broth. MIC values were read after 20 hours of growth. Here, the wildtype of strain SG511-Berlin was more susceptible to most of the tested substances compared to the control strain MRSA252. Interestingly, the complementation of *graS* in SG511-Berlin resulted in a significantly decreased susceptibility to the lantibiotics mersacidin, Pep5 and nisin, whereas the susceptibility to vancomycin, indolicidin and bacitracin was hardly changed.

Table 4.4: MIC determination of the wildtype *S. aureus* SG511-Berlin, the pTX16 control, the pTXgraS(MRSA252) complemented strain and of *S. aureus* MRSA252 (additional control). Values [µg/ml] correspond to the mean of at least three independent MIC determinations.

	SG511 wildtype	SG511 pTX16	SG511 pTXgraS	MRSA 252
Mersacidin	1	1	2.5	3.35
Pep5	0.0095	0.0095	0.0205	0.2
Nisin	2	2	4	4
Vancomycin	0.55	0.58	0.6	0.4
Indolicidin	1.33	1.33	1.83	>2
Bacitracin	5	5	5	13

4.13 Analysis of Tn*551* insertion mutants with decreased susceptibility to mersacidin

Compared to *S. aureus*, *Staphylococcus simulans* 22 is rather susceptible to antibiotics including mersacidin. In order to identify putative determinants that influence the susceptibility of different staphylococci to mersacidin and other antimicrobial agents, a library of Tn*551* transposon insertion mutants of *S. simulans* 22 had been constructed earlier (Gabriele Bierbaum, IMMIP; unpublished data). In the present thesis, antimicrobial susceptibility testing and mapping of Tn*551* transposon insertion was performed employing "clone 7" of the Tn*551* insertion library of *S. simulans* 22. The MIC cultures were supplemented with erythromycin (20 µg/ml) to maintain the insertion of Tn*551* but did not contain additional $CaCl_2$ to increase the range of the bactericidal effect of mersacidin. Here, *S. simulans* 22 Tn*551*-7 was characterized by a significantly increased MIC in half concentrated Mueller-Hinton broth. After 24 hours of incubation, the MIC of *S. simulans* 22 Tn*551*-7 was 50 µg/ml of mersacidin, whereas the control strain *S. simulans* 22 pI258 (vector control) had a MIC of 30 µg/ml. Interestingly, no effect was detectable when erythromycin was omitted. Mapping of the Tn*551* transposon insertion in *S. simulans* 22 Tn*551*-7 revealed the location of Tn*551* in the C-terminal region of a gene that is homologous to the hypothetical gene SSP0470 of the genome of *Staphylococcus saprophyticus* ATCC15305 (NC007350) respectively SH0294 of the genome of *Staphylococcus haemolyticus* JCSC1435 (NC007168) (Fig. 4.10). However, a gene homologous to SSP0470/SH0294 was not present in any sequenced *S. aureus* strain of the NCBI database and the function of SSP0470/SH0294 has not yet been identified. BLAST analysis characterized SSP0470/SH0294 as a potential

membrane protein and the C-terminal region of SSP0470/SH0294 showed ~50% homology to a permease domain of streptococci (data not shown). In *S. saprophyticus* ATCC15305 and *S. haemolyticus* JCSC1435, SSP0470/SH0294 are located directly upstream of the pyrrolidone carboxylate peptidase gene *pcp*, which might therefore also be affected in *S. simulans* 22 Tn*551*-7.

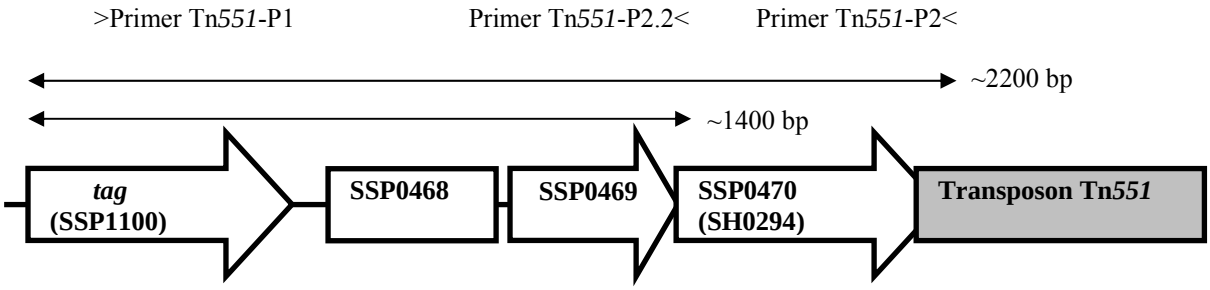

Figure 4.10: Mapping of Tn*551* transposon insertion into *S. simulans* 22. For primers see table 3.3.

Part II – Evaluation of endolysins as novel biofilm treatment strategy

4.14 Sequence comparison of ϕ11 and ϕ12 endolysins

The endolysin sequences of the bacteriophages ϕ11 (ORF 53, 1473 bp, accession number NC_004615) and ϕ12 (ORF 49, 1455 bp, accession number NC_004616) were blasted against the NCBI protein database. Both endolysins are modular enzymes which consist of three distinct domains coding for an N-terminal CHAP-domain with hydrolytic function (*c*ysteine, *h*istidine-dependent *a*midohydrolases/*p*eptidases), a central amidase-domain (*N*-acetyl-muramyl-L-alanine amidase) and a C-terminal SH3b domain, which is involved in cell wall recognition (Baba and Schneewind, 1996) (Fig. 4.16). In spite of their similar domain architecture, these endolysins show low sequence identity and similarity (26.9% and 39% respectively; Fig. 4.17). Additionally, the protein sequences of the ϕ11 and ϕ12 endolysins were further analyzed using BLAST to identify homologous proteins of other bacteriophages (Table 4.5). Both endolysins were characterized by high protein sequence identities with mostly conservative amino acid substitutions compared to homologous genes of other bacteriophages (Sass, 2005). However, the protein sequence of the ϕ12 endolysin contained a unique amino acid exchange at position 260 in the amidase domain which introduces a histidine and therefore an additional positive charge in a position that is occupied by glutamine, glutamate or asparagine in other staphylococcal phage enzymes (Fig. 4.18).

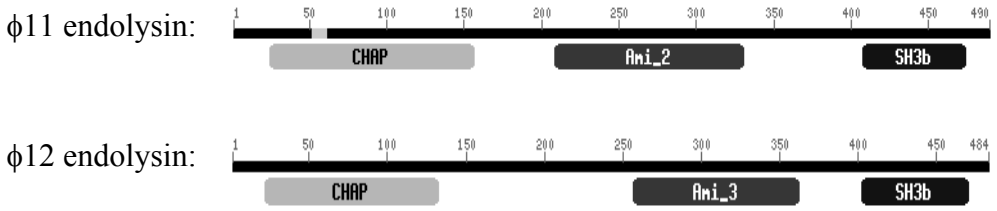

Figure 4.16: Schematic representation of the protein domain architecture of the ϕ11 and ϕ12 endolysins. The protein sequences of both endolysins were analyzed using the online analysis tool "CDART" (Conserved Domain Architecture Retrieval Tool; http://www.ncbi.nlm.nih.gov/Structure/lexington/lexington.cgi). Each endolysin consists of three conserved domains.

```
ϕ11-ORF53      MSIIMEVATMQAKLTKNEFIEWLKTSEGKQFNVDLWYGFQCFDYANAGWKVLFGLLLKGL 60
ϕ12-ORF49      -----------MLITKNQAEKWFDNSLGKQFNPDLFYGFQCYDYANMFFMIATGERLQGL 49
                          :***:  :*:..* ***** **:*****:****  : :  *  *:**

ϕ11-ORF53      GAKDIPFAN--NFDGLATVYQNTPDFLAQPGDMVVFGSNYGAGYGHVAWVIEATLDYIIV 118
ϕ12-ORF49      YAYNIPFDNKARIEKYGQIIKNYDSFLPQKLDIVVFPSKYGGGAGHVEIVESANLNTFTS 109
                * :*** *  .::  . : :*  .**.*  *:*** *:**.* ***  * .*.*: :

ϕ11-ORF53      YEQNWLGGGWTDGIEQPGWGWEKVTRRQHAYDFPMWFIRPNFKSETAPRSVQSPTQAPKK 178
ϕ12-ORF49      FGQNWNGKGWTNGVAQPGWGPETVTRHVHYYDDPMYFIRLNFPDKVSVG--DKAKSVIKQ 167
               : *** * ***:*: ***** *.***: * ** **:*** ** .:.:    :..... *:

ϕ11-ORF53      ETAKPQPKAVELKIIKDVVKGYDLPKRGSNPKGIVIHNDAGSKGATAEAYRNGLVNAPLS 238
ϕ12-ORF49      ATAKKQAVIKPKKIMLVAGHGYNDPGAVGNGTNERDFIRKYITPNIAKYLRHAGHEVALY 227
                *** *.     **:  . :**: *   .* ..   .     .   *:  *:.  :..*

ϕ11-ORF53      RLEAGIAHSYVSGNTVWQALDESQVGWHTANQIGNKYYYGIEVCQSMGADNATFLKNEQA 298
ϕ12-ORF49      GGSSQSQDMYQDTAYGVNVGNNKDYGLYWVKSHGYDIVLEIHLDAAGESASGGHVIISSQ 287
                 .:   . * .     :. ::.: * : .:. * .    *.:  :  : .. .:  ..

ϕ11-ORF53      TFQECARLLKKWGLPANRNTIRLHNEFTSTSCPHRSSVLHTGFDPVTRGLLPEDKRLQLK 358
ϕ12-ORF49      FNADTIDKSIQDVIKNNLGQIRGVTPRNDLLNVNVSAEININYRLSELGFITNKNDMDWI 347
                  :      :  :  * . **  .  ..    : *: :: .:     *::.:.: ::

ϕ11-ORF53      ----DYFIKQIRAYMDGKIPVATVSN--ESSASSNTVKPVASAWKRNKYGTYYMEESARF 412
ϕ12-ORF49      KKNYDLYSKLIAGAIHGKPIGGLVAGNVKTSAKNQKNPPVPAGYTLDKNNVPYKKETGYY 407
                   * : * * . :.**   . *:.  ::**..:.  **.:.:. :* .. * :*:. :

ϕ11-ORF53      TNGNQPITVRKVGPFLSCPVGYQFQPGGYCDYTEVMLQDGHVWVGYTWE-GQRYYLPIRT 471
ϕ12-ORF49      TVANVKGNNVRDGYSTNSRITGVLPNNATIKYDGAYCINGYRWITYIANSGQRRYIATGE 467
               * .*   .  : *   .. :   :  ..  .*  .   :*: *: *  : *** *:.

ϕ11-ORF53      WNGSAPPNQILGDLWGEIS 490
ϕ12-ORF49      VDKAGNRISSFGKFSTI-- 484
                : :.   . :*.:
```

Figure 4.17: Amino acid sequence alignment of the ϕ11 and ϕ12 endolysins. The protein sequence alignment of both endolysin sequences reveals a low sequence identity of 26.9%. "*" indicates identical amino acids (aa), ":" conservative aa substitution, "." semi-conservative aa substitution.

Table 4.5: Bacteriophage proteins homologous to the ϕ11 or the ϕ12 endolysin.

Bacteriophage proteins homologous to the ϕ11 endolysin:

1. ORF 007 of staphylococcus phage 29 (AAX91726); protein sequence identity of 99%
2. ORF NWMN1039 of staphylococcus phage phiNM2 (BAF67311); protein sequence identity of 98%
3. ORF 007 of staphylococcus phage 69 (AAX90762); protein sequence identity of 98%
4. ORF 006 of staphylococcus phage 92 (AAX91937); protein sequence identity of 98%
5. ORF 007 of staphylococcus phage 55 (AAX91649); protein sequence identity of 98%
6. ORF 007 of staphylococcus phage 52a (AAX91801); protein sequence identity of 98%
7. ORF SAV0913 of the prophage ϕMu1 of *S. aureus* Mu50 (BAB57075); protein sequence identity of 97%
8. ORF 006 of staphylococcus phage 88 (AAX91865); protein sequence identity of 97%
9. Amidase of staphylococcus phage 80alpha (AAB39699); protein sequence identity of 91%

Bacteriophage proteins homologous to the ϕ12 endolysin:

1. ORF 007 of staphylococcus phage 47 (AAX91198); protein sequence identity of 100%
2. Amidase of phage ϕSA2MW of *S. aureus* MW2 (BAB95245); protein sequence identity of 98%
3. Amidase of phage MSSA476 (CAG42730); protein sequence identity of 97%
4. Amidase of phage L54a of *S. aureus* COL (AAW38858); protein sequence identity of 95%
5. Amidase of phage ϕSLT of *S. aureus* MRSA252 (YP040898); protein sequence identity of 95%

```
ϕ12              241 AYGVNVGNNKDYGLYWVKSHGYDIVLEIHLDAAGESASGGHVIISSQFNADTIDKSIQDV 300
ϕSA2MW           241 AYGVNVGNNKDYGLYWVKSQGYDIVLEIHLDAAGESASGGHVIISSQFNADTIDKSIQDV 300
phageMSSA476     241 AYGVNVGNNKDYGLYWVKSQGYDIVLEIHLDAAGENASGGHVIISSQFNADTIDKSIQDV 300
L54a             241 AYGVNVGNKKDYGLYWVKSQGYDIVLEIHLDAAGESASGGHVIISSQFNADTIDKSIQDV 300
ϕSLT             241 AYGVNVGNKKDYGLYWVKSQGYDIVLEIHLDAAGESASGGHVIISSQFNADTIDKSIQDV 300
                     ********:**********:***************.**********************
```

Figure 4.18: Amino acid substitution at position 260 of the ϕ12 endolysin. "*" indicates identical amino acids (aa), ":" conservative aa substitution, "." semi-conservative aa substitution. The aa substitution of the ϕ12 endolysin at position 260 is shaded in grey.

4.15 Cloning and overexpression of C-terminal six-His-tagged ϕ11 and ϕ12 endolysins

The endolysin genes of the bacteriophages ϕ11 and ϕ12 were amplified by PCR from genomic DNA of *S. aureus* NCTC8325, using the primers listed in table 3.3. In order to test the activity of the ϕ11 endolysin subunits, the endopeptidase unit (ϕ11endo, amino acid [aa] 1-180) and the amidase unit (ϕ11ami, aa 180-371) as well as each unit plus the cell wall binding domain (ϕ11endo/CBD, aa 1-180/371-490, and ϕ11ami/CBD, aa 180-490) were constructed separately (Fig. 4.19A). In addition, the cell wall binding module was deleted

from the ϕ11 endolysin (ϕ11endo/ami, aa 1-371). The amplification products were cloned into the multiple cloning site of the expression vector pET22bΔpelB (Sass, 2005), a pET22b derivative that has been deleted of the *pelB* leader sequence to inhibit protein transport to the periplasm of the expression host, except for ϕ11ami and ϕ11ami/CBD which were cloned into pET22b using the *Nde*I restriction site and thereby deleting the *pelB* leader sequence of pET22b. The resulting plasmids, pETerΔ11 and pETerΔ12 (Sass, 2005) as well as pETendo11, pETendoCBD11, pETami11, pETamiCBD11 and pETendo/ami11 were used to overexpress each endolysin and the single subunits of the ϕ11 enzyme as C-terminal six-His-tagged fusion protein. After subcloning of the plasmids in *E. coli* JM109, *E. coli* Bl21(DE3) was used as a host for expression of each six-His-tagged endolysin. Expression cultures were grown in LB broth containing ampicillin (40 µg/ml) to an OD_{600} of 0.6. Then protein expression was induced by addition of IPTG to a final concentration of 1 mM. Expression cultures were harvested after 4 hours followed by protein purification steps under native conditions via Ni-NTA affinity chromatography. Purity of the protein eluates was controlled by SDS-PAGE analysis (Fig. 4.20). Protein purification was also performed with cells harbouring the empty vector and the eluate served as control in the activity tests.

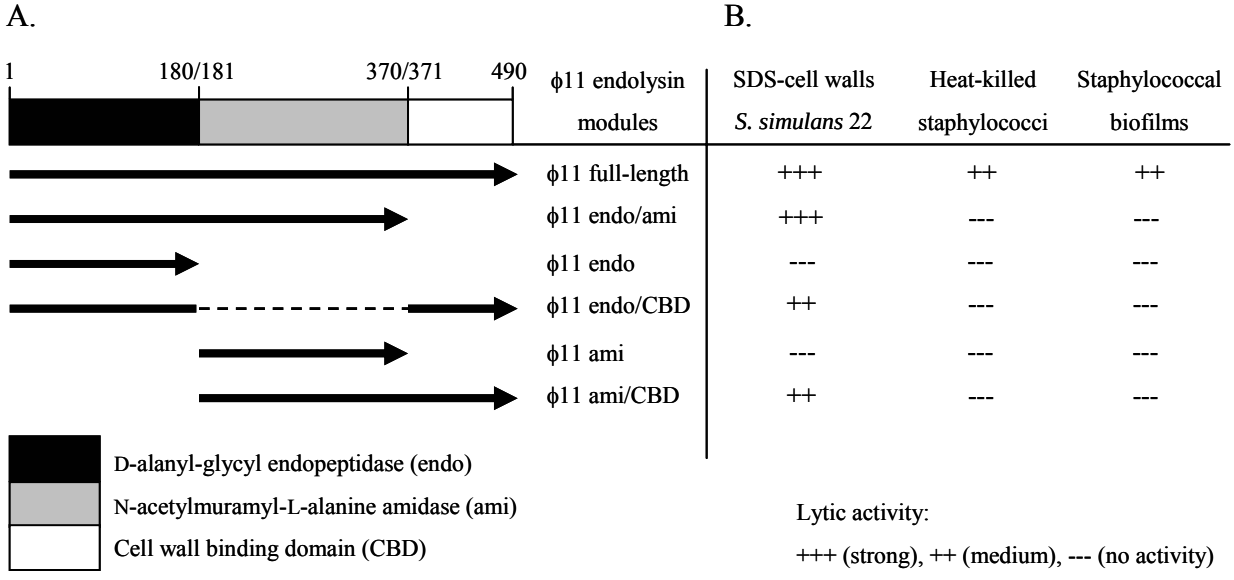

Figure 4.19: Recombinant murein hydrolases of the staphylococcal phage ϕ11. A. Schematic overview of the ϕ11 endolysin modules illustrating the constructs tested in this thesis. The ϕ11 endolysin features a modular design which consists of an N-terminal endopeptidase domain (endo), a central amidase domain (ami) and a C-terminal cell wall binding domain (CBD). The full-length and deletion constructs of the ϕ11 endolysin were overexpressed as six-His-fusion proteins. **B.** Lytic activity of the staphylococcal phage ϕ11 endolysin domains.

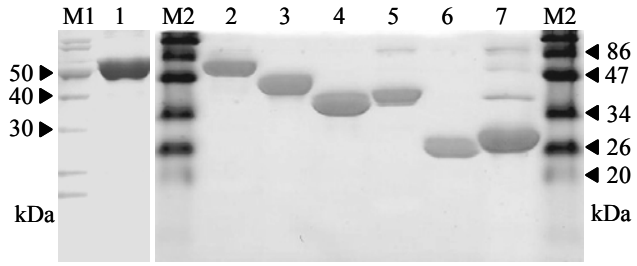

Figure 4.20: SDS-PAGE analysis containing ϕ12 and ϕ11 endolysins and derived proteins purified by Ni-NTA affinity chromatography. The purified proteins were analyzed by 15% SDS-PAGE and stained with PageBlue™ protein staining solution (Fermentas) according to the manufacturer`s instructions. Lane M1, Fermentas Page Ruler™ unstained protein ladder; lane M2, Fermentas prestained protein molecular weight marker; lane 1, ϕ12 endolysin (full-length); lane 2, ϕ11 endolysin (full-length); lane 3, ϕ11endo/ami; lane 4, ϕ11 endo/CBD; lane 5, ϕ11 ami/CBD; lane 6, ϕ11 endo; lane 7, ϕ11 ami.

4.16 Analysis of the lytic activities of the ϕ11 endolysin modules

The lytic activities of the full-length ϕ11 endolysin and its deletion variants (each applied in a concentration of 20 µg/ml) were examined photometrically at 600 nm employing purified SDS cell walls of *S. simulans* 22 (Bierbaum and Sahl, 1987) resuspended in incubation buffer [50 mM Tris-HCl, 100 mM NaCl; pH 7.5] to an OD_{600} of ~0.3 (Fig. 4.21). All lysis experiments were performed in triplicate. The full-length enzyme showed efficient lysis of the peptidoglycan compared to the control (empty vector eluate) and both, the endopeptidase and the amidase module plus cell wall binding domain (ϕ11endo/CBD and ϕ11ami/CBD), were active and able to lyse cell walls. Furthermore, the ϕ11 enzyme without the cell wall binding domain hydrolyzed *S. simulans* 22 cell walls. However, the full length enzyme was more active than the isolated subdomains and only the combination of ϕ11endo/CBD and ϕ11ami/CBD (20 µg/ml each) restored full lytic activity. The effect of the truncation was even more pronounced with whole cells. To this end, cells of *S. aureus* NCTC8325 were grown overnight, diluted in incubation buffer to an OD_{600} of approximately 0.3 and pasteurized for 10 minutes at 80°C. After addition of equivalent amounts of endolysin (20 µg/ml), the lytic activity was determined as before. In contrast to the full-length protein (Fig. 4.22), the deletion variants of the ϕ11 enzyme were hardly active against heat-killed cells.

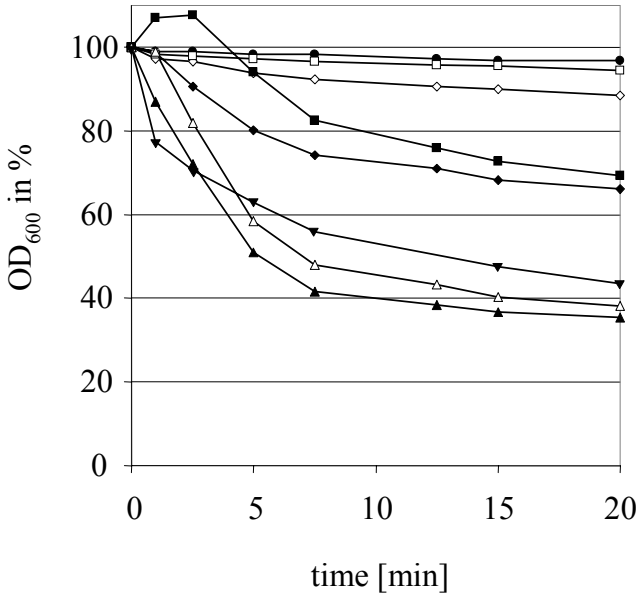

Figure 4.21: Activity of the domains of the multifunctional ϕ11 endolysin. The lytic activities of the purified enzyme samples of the full-length and truncated ϕ11 endolysin (20 µg/ml each) were examined using purified cell walls of *S. simulans* 22. The full-length enzyme (▲) as well as the ϕ11 enzyme without the cell wall binding domain (ϕ11endo/ami; ▼) were able to lyse the substrate efficiently compared to the protein eluate of the empty vector control (●). The truncated enzymes ϕ11endo (□) and ϕ11ami (◊) did not show noteworthy lytic activity. The truncated enzymes containing the cell wall binding domain ϕ11endoCBD (■) and ϕ11amiCBD (♦) showed enhanced lytic activity but still worked less effective than the full length enzyme. The lytic activity could be restored by the parallel use of ϕ11endoCBD and ϕ11amiCBD (Δ).

4.17 Activities of the ϕ11 and ϕ12 endolysins on different staphylococcal strains

Next, the lytic activities of the ϕ11 and ϕ12 endolysins on whole cells of several staphylococcal strains were analyzed. In addition to *S. aureus* NCTC8325, the full-length ϕ11 endolysin rapidly lysed heat-killed cells of *S. aureus* Wood 46, *S. aureus* Cowan I, *S. aureus* Newman, *S. epidermidis* O-47 and *S. simulans* 22 (Fig. 4.22). Its lytic activity was equivalent to that of highly purified lysostaphin (5 µg/ml). Interestingly, the ϕ11 enzyme without cell wall binding domain (ϕ11endo/ami) did not hydrolyze whole staphylococcal cells. Unlike ϕ11, the ϕ12 endolysin was not able to hydrolyze heat-killed cells of *S. aureus* Wood 46, *S. aureus* Cowan I or *S. aureus* Newman. However, after addition of the ϕ12 endolysin an increase in optical density of the cultures was observed (Sass, 2005). This was accompanied and most probably caused by an aggregation of the cells that was macroscopically visible. This phenomenon was earlier described by Takano et al. (2000) who investigated the influence of synthetic peptides derived from the *S. aureus* major autolysin Atl on autolysis.

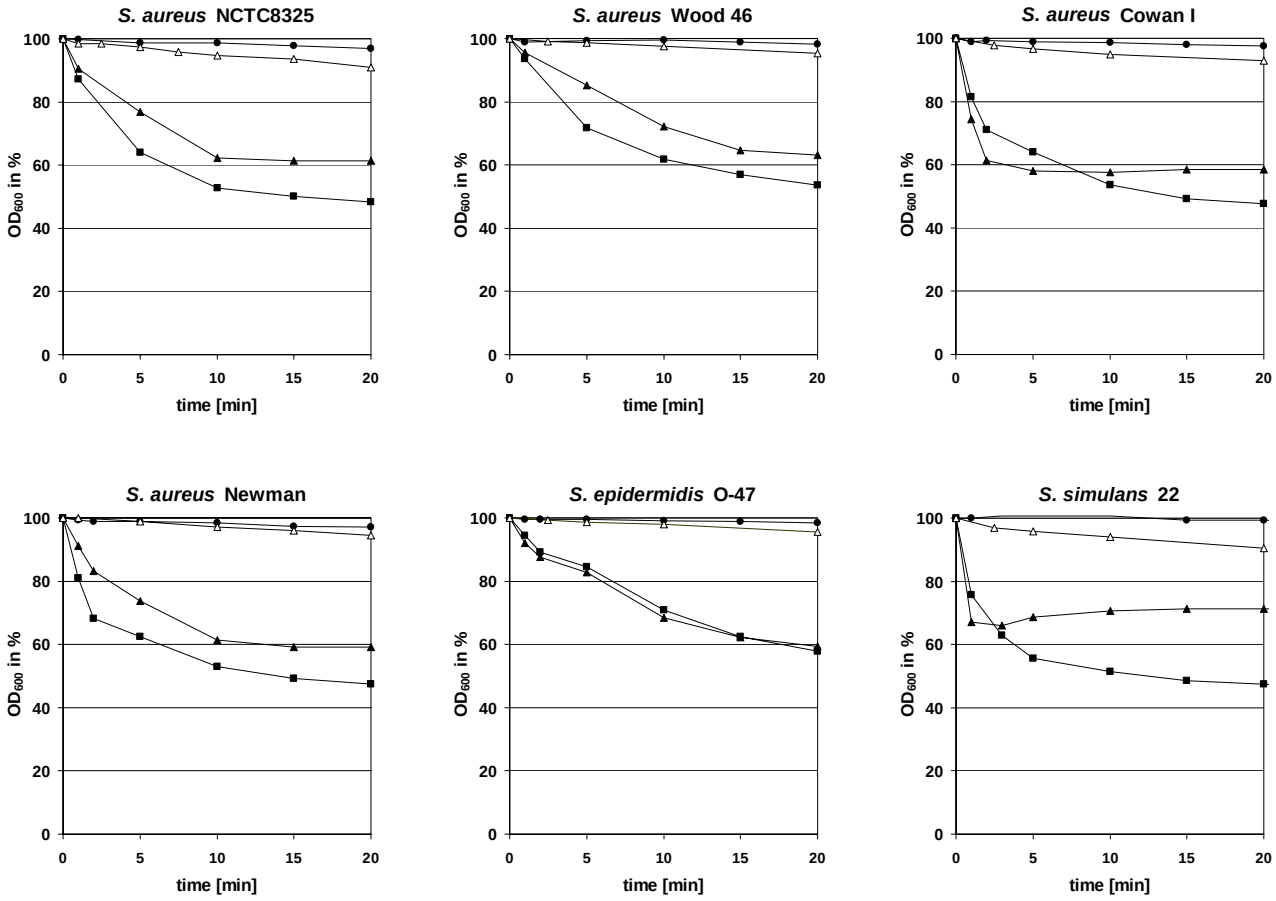

Figure 4.22: The ϕ11 endolysin lyses whole cells of several staphylococci. Light scattering data showed that the ϕ11 endolysin (▲; 20 µg/ml) rapidly decreased the optical density of heat-inactivated *S. aureus* NCTC8325, *S. aureus* Wood 46, *S. aureus* Cowan I, *S. aureus* Newman, *S. epidermidis* O-47 and *S. simulans* 22 cultures compared to the protein eluate of the empty vector control (●). Its lytic activity was comparable to the effect observed after addition of lysostaphin (■; 5 µg/ml), which is a highly efficient glycyl-glycine endopeptidase. After deletion of the cell wall targeting domain, the ϕ11 enzyme (ϕ11endo/ami, Δ; 20 µg/ml) showed a significantly reduced activity, which indicates a fundamental role of the CBD for efficient cell lysis.

4.18 Site-directed mutagenesis and overexpression of mutated ϕ12 endolysin

Since the ϕ12 endolysin was characterized by an amino acid substitution at position 260 in the amidase domain, a single point mutation (T780G) was introduced into the nucleotide sequence of pETerΔ12 by site-directed mutagenesis to generate pETerΔ12mut (Fig. 4.23). This T780G nucleotide exchange results in a mutated ϕ12 endolysin (ϕ12mut) in which the histidine of the ϕ12 endolysin at position 260 is exchanged to the glutamine present in homologous sequences from the NCBI database (Fig. 4.18). The pETerΔ12mut plasmid was then used to overexpress the ϕ12mut endolysin as C-terminal six-His-tagged fusion protein (Fig. 4.24) as aforementioned. The lytic activity of the purified ϕ12mut endolysin (applied in a concentration of 15 µg/ml) was examined photometrically at 600 nm employing heat-

inactivated *S. aureus* NCTC8325 cells (Fig. 4.25) as described above. Here, the ϕ12mut endolysin was not able to hydrolyze heat-inactivated cells. Thus, the H260Q amino acid exchange of the ϕ12mut endolysin does not provide lytic activity on whole cells of *S. aureus*.

```
          251  D   Y   G   L   Y   W   V   K   S   H   G   Y   D   I   V   L   E  267
ϕ12       761 GAT TAT GGC TTA TAT TGG GTT AAA TCA CAT GGG TAT GAC ATT GTT CTA GAG 801
ϕ12mut    761 GAT TAT GGC TTA TAT TGG GTT AAA TCA CAG GGG TAT GAC ATT GTT CTA GAG 801
              *** *** *** *** *** *** *** *** ***  **  *** *** *** *** *** *** ***
          251  D   Y   G   L   Y   W   V   K   S   Q   G   Y   D   I   V   L   E  267
```

Figure 4.23: Site-directed mutagenesis of the ϕ12 endolysin. "*" indicates identical nucleotides. The T780G nucleotide exchange and the resulting H260Q amino acid substitution of the ϕ12 endolysin are indicated in bold.

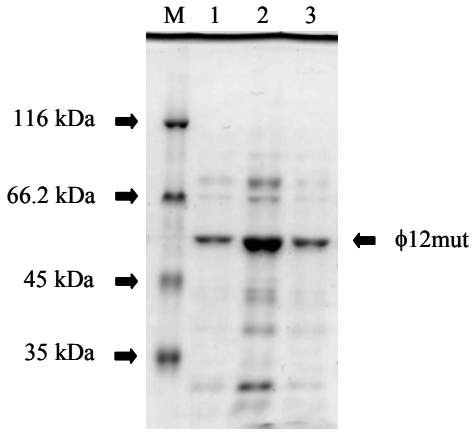

Figure 4.24: SDS-PAGE analysis of the ϕ12mut endolysin purified by Ni-NTA affinity chromatography. Lane M, unstained protein molecular weight marker; lane 1, ϕ12mut endolysin (eluate 1); lane 2, ϕ12mut endolysin (eluate 2); lane 3, ϕ12mut endolysin (eluate 3).

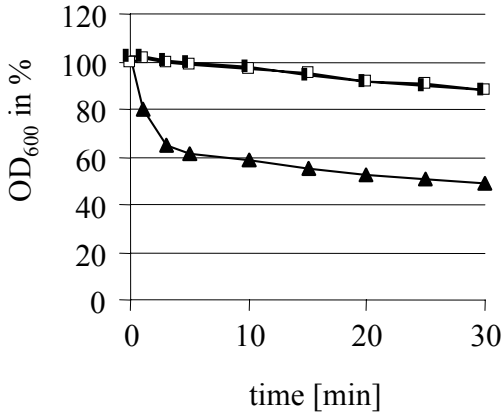

Figure 4.25: Lysis assay of the ϕ12mut endolysin. The lysis assay was performed using heat-inactivated cells of *S. aureus* NCTC8325. The ϕ12mut endolysin (■) and the ϕ11 endolysin (▲; positive control) were applied in a concentration of 15 µg/ml and lysis was examined photometrically at 600 nm. The protein eluate of the empty vector control (□) was used as a negative control.

4.19 Staphylococcal biofilm assay on artificial surfaces

To determine the influence of the ϕ11 and ϕ12 endolysins on staphylococcal biofilms, a modified biofilm plate assay was employed (Fig. 4.19B and Fig. 4.26). Here, the ϕ12 endolysin showed no hydrolytic activity on biofilms of *S. aureus* NCTC8325 and *S. aureus* Newman. In contrast, after addition of the ϕ12 endolysin an enhanced staining of the biofilms was noticed. This effect is consistent with the results of the experiments performed with heat-inactivated cells and may be explained by a murein hydrolase-mediated adhesion of staphylococci (Heilmann et al., 1997; Heilmann et al., 2003). In contrast, the purified ϕ11 endolysin was able to eliminate biofilms of *S. aureus* NCTC8325 and *S. aureus* Newman with an efficiency that was comparable to or slightly lower than that of lysostaphin. However, there was no effect on biofilms of *S. epidermidis* O-47.

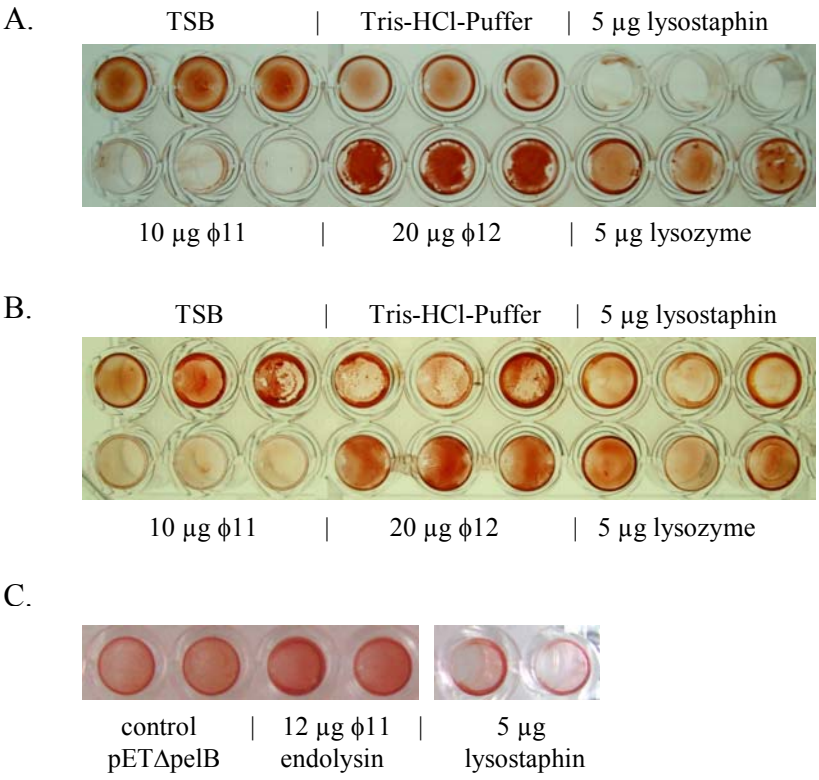

Figure 4.26: Biofilm plate assay of *S. aureus* NCTC8325 (A) (Sass, 2005), *S. aureus* Newman (B) (Sass, 2005) and *S. epidermidis* O-47 (C). The ϕ11 endolysin lysed biofilms of *S. aureus* NCTC8325 and *S. aureus* Newman with an efficiency comparable to that of lysostaphin. However, biofilms of *S. epidermidis* O-47 were not dissolved by the ϕ11 endolysin. In contrast, addition of the ϕ12 endolysin resulted in enhanced staining of the *S. aureus* biofilms.

4.20 Analysis of the biofilm nature of *S. aureus* NCTC8325 and *S. epidermidis* O-47

The ϕ11 endolysin was able to hydrolyze heat-killed cells of *S. aureus* NCTC8325 and *S. epidermidis* O-47 (Fig. 4.22). However, it was only able to dissolve biofilms of *S. aureus* NCTC8325 while *S. epidermidis* O-47 biofilms remained unaffected (Fig. 4.26). To explore the reasons for this differentiation, the nature of *S. aureus* NCTC8325 and *S. epidermidis* O-47 biofilms was analyzed by incubation with proteinase K or sodium metaperiodate in the modified biofilm plate assay (Fig. 4.27). Biofilms that predominantly consist of PIA (β-1,6-linked N-acetylglucosamine) are dissolved by incubation with sodium metaperiodate and protein biofilms are dissolved by proteinase K treatment. *S. epidermidis* O-47 biofilms were only dissolved by sodium metaperiodate treatment which indicates that this biofilm is exclusively polysaccharide mediated. In contrast, neither sodium metaperiodate nor proteinase K treatment could completely eradicate biofilms of *S. aureus* NCTC8325 grown in TSB broth which suggests that an elevated portion of proteinogenous biofilm is present along with a biofilm mediated by the characteristic polysaccaride PIA.

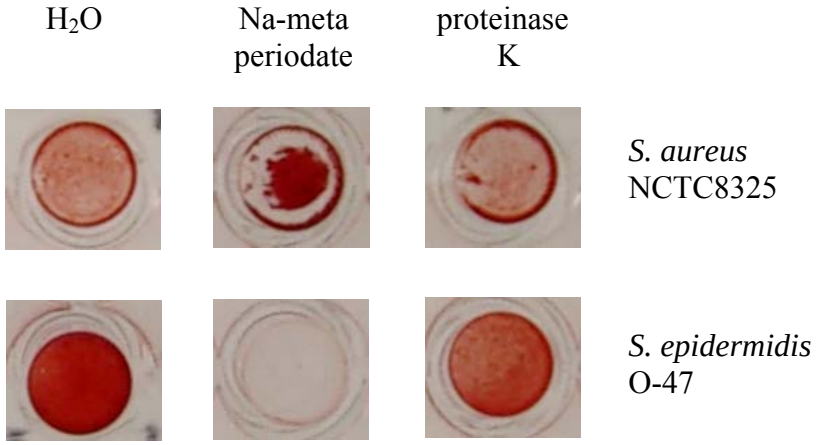

Figure 4.27: Biofilm nature of *S. aureus* NCTC8325 and *S. epidermidis* O-47. Neither sodium metaperiodate nor proteinase K treatment could completely eradicate biofilms of *S. aureus* NCTC8325 grown in TSB broth. In contrast, *S. epidermidis* O-47 biofilms were dissolved by sodium metaperiodate treatment.

5 Discussion

Infections caused by *S. aureus* and *S. epidermidis* still play a major role in human and animal disease. Considering the increased emergence of staphylococci and other microorganisms that are resistant to current treatment strategies, there is an urgent need to expand our knowledge on how bacteria respond to antibiotic treatment and develop resistance mechanisms that help to persist during host infection. To ensure the control of bacterial infections in future, new treatment strategies need to be explored that target actively growing and non-growing cells.

5.1 The lantibiotic mersacidin is a strong inducer of the cell wall stress response of *Staphylococcus aureus*

One objective of this work was to study the response of *S. aureus* to the lantibiotic mersacidin, in order to reveal insights into the mechanisms that *S. aureus* might use to establish reduced susceptibility towards this antimicrobial peptide. To this end, the transcriptional responses of the *S. aureus* strains SA137/93A (heterogenic VISA / MRSA) and SA137/93G (VISA / MSSA, a laboratory mutant of strain SA137/93A) (Reipert et al., 2003) as well as of the susceptible strain *S. aureus* SG511-Berlin were studied.

Previous gene expression studies of Kuroda et al. and Utaida et al. concerning the *S. aureus* responses to the cell wall-active substances vancomycin (Kuroda et al., 2003), oxacillin, bacitracin and D-cycloserine (Utaida et al., 2003) had identified a cell wall stress stimulon, which seems to be predominantly regulated by the VraSR two-component regulatory system (TCRS). Members of this stimulon comprise the *vraSR* genes together with genes related to the cell wall metabolism of *S. aureus* like *murZ*, *uppS*, *bacA*, *pbp2*, *sgtB* and genes related to protein metabolism. Recently, McAleese et al. described a core cell wall stress stimulon of 17 genes by merging their own results, that had been recorded in the presence of vancomycin, together with the results of Kuroda et al. and Utaida et al. (Kuroda et al., 2003; Utaida et al., 2003; McAleese et al., 2006). In conclusion, the cell wall stress stimulon is characterized through a comprehensive response that involves manifold cellular processes. This general cell wall stress response seems to be conserved among Gram-positive bacteria (Jordan et al., 2008). Most recently, the transcriptional profile of the *S. aureus* response to the lantibiotic nisin has become available (Muthaiyan et al., 2008). Nisin acts by lipid II-based pore formation and inhibition of cell wall biosynthesis (Wiedemann et al., 2001). The *S. aureus* response to nisin (~3 x MIC) was relatively moderate and an induction of the cell wall stress stimulon was not observed.

In the presence of the lantibiotic mersacidin, the induction of the VraSR-triggered cell wall stress stimulon was one of the most striking events of gene regulation (Table 4.1 and Table 4.2) and included 71 genes of the 161 cell wall stress stimulon members (Utaida et al., 2003). Similarly, 35 genes out of the 46 genes of the VraSR regulon (Kuroda et al., 2003) were upregulated after exposure to mersacidin (Table 5.1). Furthermore, 16 out of the 17 members of the core cell wall stress stimulon (McAleese et al., 2006) were differentially expressed after mersacidin treatment. The *vraSR* genes and several cell wall biosynthesis genes were strongly upregulated, which included the putative monofunctional glycosyltransferase gene *sgtB*, the transpeptidase/transglycosylase gene *pbp2* and the UDP-N-acetylglucosamine 1-carboxylvinyl transferase gene *murZ*, as well as *tcaA* and *drp35*. The predominant induction of the glutamate (*gltB*, *rocA*) and lysine (*asd*, *dapAB*, *dhoM*, *lysA*) amino acid biosynthesis pathways might also support cell wall biosynthesis, since glutamate and lysine represent essential components of the peptidoglycan precursor lipid II. In fact, the disruption of the *dap* operon or the *lysA* gene has been shown to be involved in a decrease of oxacillin resistance and growth attenuation of *S. aureus* (de Lencastre et al., 1999; Wiltshire and Foster, 2001). Especially in strain SA137/93A, mersacidin treatment induced the transcription of the *bacA* and *uppS* genes, which are involved in lipid II carrier regeneration and synthesis. Expecting the carrier level to be a limiting factor when lipid II is blocked by mersacidin, an enhanced availability of carriers might be beneficial for peptidoglycan synthesis. In accordance with peptidoglycan biosynthesis inhibition, gene expression for cell wall lytic enzymes (*atl*, SA0423, SA2100) was negatively affected. In terms of membrane trafficking, the oligopeptide transport system genes *oppBCDF* and the components of the phosphotransferase system (PTS) were upregulated. While an increased expression of the PTS may boost the import of glucose to supply additional energy, the induction of OppBCDF might support cell wall biosynthesis by the acquisition of essential amino acids.

Another prominent category of the cell wall stress stimulon is formed by genes related to protein metabolism (Utaida et al., 2003). Upregulation was recorded for the chaperone genes *prsA*, *groEL* and *dnaK* as well as the chaperone/protease genes of the Clp-family (caseinolytic proteases) like *clpC*, *clpP*, *cplB* and *clpL*, which are also part of the *S. aureus* response to heat-shock, vancomycin stress and acid shock (Anderson et al., 2006; Kuroda et al., 2003; Bore et al., 2007). The upregulation of genes related to protein metabolism might indicate an accumulation of damaged or inactive proteins upon mersacidin treatment which has also been observed after treatment with oxacillin or bacitracin (Utaida et al., 2003).

Table 5.1: Significant induction of the VraSR-dependent cell wall stress response of *S. aureus*[1] by mersacidin

N315 ORF	Gene	Gene product	Product function	Fold change[2]			
				SA137/93A (0.5xMIC)	SA137/93G (0.5xMIC)	(0.15xMIC)	SG511 (1xMIC)
SA0531	*proP*	proline/betaine transporter homologue	Protein transport and binding	**2.4**	**2.7**		0.6
SA0536		hypothetical protein		**30.2**	**17.1**	**8.5**	**26.6**
SA0608		hypothetical protein		**2.7**	**3.8**		**9.2**
SA0825	*spsA*	type-I signal peptidase	Protein secretion	**2.2**	1.4		**3.9**
SA0909	*fmtA*	FmtA, autolysis and methicillin resistance-related protein	Pathogenic factors	**9.3**	**3.8**	**6.3**	**6.3**
SA0914		hypothetical protein, similar to chitinase B		**14.2**	**3.9**		**15.3**
SA0931		hypothetical protein		**4.9**	**3.2**		**6.2**
SA1183	*opuD*	glycine betaine transporter	Protein transport and binding	0.9	**0.5**		**0.5**
SA1255		PTS system, glucose-specific enzyme IIA component	Protein transport and binding	**4.7**	**10.7**	**2.6**	**13.1**
SA1282	*recU*	recombination protein U homologue	DNA recombination	**3.6**	2.5	2.2	**2.5**
SA1283	*pbp2*	penicillin-binding protein 2	Cell wall related genes	3.4	2.3	2.7	**3.5**
SA1476		hypothetical protein		**11.5**	**11.3**	**6.5**	**15.9**
SA1548		hypothetical protein, similar to acylglycerol-3-phosphate-O-acyltransferase homologue	Metabolism of lipids	0.7	**0.5**		0.6
SA1549		heat-shock protein homologue, similar to serine proteinase	Adaptation to atypical conditions	**7.1**	**4.6**	**3.9**	**5.6**
SA1657		conserved hypothetical protein		1.3	**2.3**		0.9
SA1659	*prsA*	peptidyl-prolyl *cis/trans* isomerase homologue	Protein folding	**10.8**	**18.1**	**10.0**	**33.3**
SA1691	*sgtB*	hypothetical protein, similar to penicillin-binding protein 1A/1B	Cell wall related genes	**13.1**	**8.8**	**4.8**	**9.7**
SA1700	*vraR*	two-component response regulator	RNA synthesis - Regulation	**13.3**	**8.0**	**7.3**	**17.3**
SA1701	*vraS*	two-component sensor histidine kinase	Sensors (signal transduction)	**11.7**	**8.9**	**5.8**	**17.0**
SA1702		conserved hypothetical protein		**10.4**	**9.4**	**4.4**	**12.2**
SA1703		hypothetical protein		**31.6**	**24.8**	**6.8**	**45.4**
SA1711		hypothetical protein, similar to DNA-damage inducible protein P	DNA replication, modification, repair	2.2	**1.6**		**3.4**
SA1712		conserved hypothetical protein		**24.7**	**21.0**	**5.0**	**29.4**
SA1926	*murZ*	UDP-N-acetylglucosamine 1-carboxylvinyl transferase 2	Cell wall related genes	3.2	**4.4**	3.9	**6.1**
SA2103		hypothetical protein, similar to *lyt* divergon expression attenuator LytR	RNA synthesis - Regulation	**6.4**	**6.1**	4.4	**8.0**
SA2113		hypothetical protein		**14.9**	**11.8**	**4.9**	**11.4**
SA2146	*tcaA*	TcaA protein		**3.3**	1.6	**2.2**	**4.0**
SA2220		conserved hypothetical protein		**7.0**	**12.2**	**3.7**	**21.4**
SA2221		hypothetical protein		**25.6**	**18.3**	**5.2**	**42.7**
SA2222		hypothetical protein, similar to TcaB	Protein transport and binding	1.2	**0.6**		1.0
SA2296		hypothetical protein, similar to transcriptional regulator MerR	RNA synthesis - Regulation	**8.1**	**10.4**	**3.9**	**5.5**
SA2297		hypothetical protein, similar to GTP-pyrophosphokinase	Nucleotide, nucleic acid metabolism	**9.1**	**14.1**	**3.9**	**10.3**
SA2298		conserved hypothetical protein		1.8	**2.2**		1.9
SA2413		sulfite reductase flavoprotein (NADPH)	Metabolism of sulfur	1.7	2.2		**2.3**
SA2480	*drp35*	drug responsive protein 35		2.7	**3.4**	**2.8**	**2.8**

[1] Kuroda et al. (2003)

[2] Significant changes of gene expression were determined by implementing SAM (significance analysis of microarrays; http://www-stat.stanford.edu/~tibs/SAM/) (Tusher et al., 2001). Fold change in transcript level indicated as mean of the “median of ratios” compared to control cells. Fold change in bold = classified as "significantly" regulated in this strain by SAM.

In fact, increased degradation of aberrant proteins may provide additional amino acids to support a cell wall damage-induced response. The strong induction of the acetyl-CoA C-acetyltransferase gene *vraB*, which is involved in the degradation of amino acids and in lipid metabolism, might be an additional indicator in this context.

Since incubation with 0.5 x MIC of mersacidin was sufficient to massively induce the cell wall stress response, the gene expression profile of strain SA137/93G was further examined at even lower concentrations using DNA microarrays (Table 5.1 and Table S2 in the supplemental material). Incubation with 0.15 x MIC (4 µg/ml, 2.175 µM) of mersacidin was sufficient to significantly alter the expression of 19 out of 46 genes of the VraSR regulon (Kuroda et al., 2003) (Table 5.1) and 12 out of 17 genes belonging to the core cell wall stress stimulon (McAleese et al., 2006). These results show, that inhibitory antibiotic concentrations are not an obligatory requirement for the induction of the cell wall stress stimulon as it is the case for e.g. vancomycin and oxacillin (McAleese et al., 2006; Wootton et al., 2005; McCallum et al., 2006). Compared to 0.5 x MIC, the response was reduced after employing 0.15 x MIC in strain SA137/93G, suggesting a dose-dependent effect on gene expression.

To verify the induction of the cell wall stress response by the autoregulatory VraSR TCRS (Yin et al., 2006), overexpression of the sensor histidine kinase gene *vraS* was further analyzed via qRT-PCR (Fig. 4.5A). In the presence of 0.5 x MIC of mersacidin, a strong induction of *vraS* transcription (16- to 26-fold) was observed in all strains tested. Even 0.15 x MIC of mersacidin considerably induced *vraS* gene transcription in strain SA137/93G (5.6-fold) and strain SG511-Berlin (3.4-fold) (strain SA137/93A not tested). As a control, strain SA137/93G was incubated with subinhibitory concentrations of vancomycin (4 µg/ml, 2.75 µM, 0.5 x MIC). Here, no induction was visible by qRT-PCR. This is also confirmed by earlier studies of other groups who employed multiple MICs (8 to 10 x MICs) of cell wall affecting agents like vancomycin, oxacillin or bacitracin to induce the cell wall stress response (Kuroda et al., 2003; Utaida et al., 2003; McAleese et al., 2006), while low inhibitory or subinhibitory concentrations of vancomycin or oxacillin did not lead to an induction (McAleese et al., 2006; Wootton et al., 2005; McCallum et al., 2006). Likewise, an induction of the cell wall stress response could not be observed for the lantibiotic nisin at inhibitory concentrations (Muthaiyan et al., 2008). Nisin acts by fast depolarization of the cell membrane as a cause of lipid II-based pore formation rather than by inhibition of cell wall biosynthesis (Wiedemann et al., 2004; Wiedemann et al., 2001). Thus, the bacteria will be rapidly inactivated by nisin which prevents an adequate response to cell wall damage through the VraSR-stimulon. Hence, mersacidin turned out to be a strong inducer of the cell wall

stress response in *S. aureus* compared to other substances like vancomycin, oxacillin, bacitracin and nisin.

The VISA strains SA137/93A and SA137/93G are characterized by a cell wall with an increased thickness, which is also formed in the absence of antibiotics (Reipert et al., 2003). Actually, vancomycin intermediate resistance partly relies on an increase in cell wall material (Cui et al., 2000; Cui et al., 2003; Reipert et al., 2003), which might result from an activated cell wall metabolism. In previous studies by Christiane Szekat and Gabriele Bierbaum (Sass et al., 2008a), the influence of an increased cell wall thickness on the action of mersacidin was tested (Fig. 5.1). To this end, *S. aureus* cells with increased amounts of peptidoglycan were obtained by incubation in resting medium (RM) for two hours. Cell wall resting medium allows the biosynthesis of excess cell wall material, while the absence of essential amino acids prohibits growth. Controls were performed in RM devoid of glucose (RM-g), which is necessary for synthesis of extra cell wall material (Cui et al., 2000; Shockman et al., 1961). After incubation of the cells in RM or RM-g, the susceptibility to mersacidin was tested. Vancomycin served as control. In the case of mersacidin, the incubation in the presence of glucose did not decrease the susceptibility of *S. aureus* SG511-Berlin and of the VISA strains SA137/93A and SA137/93G (Fig. 5.1), whereas the efficacy of vancomycin was always lower against cells that had been incubated in the presence of glucose. In strain SG511-Berlin, mersacidin was even more effective than vancomycin, which was not the case for the strains SA137/93A and SA137/93G. These results demonstrate that the cell wall thickness does not impact on the antibiotic efficacy of mersacidin. Thus, the induction of the cell wall stress response at low mersacidin concentrations may be partly explained by the differences that characterize the interactions of mersacidin and vancomycin with the cell envelope: While mersacidin and vancomycin both target lipid II, vancomycin acts by binding to the D-alanyl-D-alanine terminus of the peptide side chain of lipid II. In a thickened cell wall, increased amounts of free D-alanyl-D-alanine termini provide false target sites for vancomycin binding. This results in a decreased diffusion velocity of vancomycin through the cell wall, the "clogging effect" (Cui et al., 2000), and only those vancomycin molecules that are not trapped in the cell wall will reach lipid II. In contrast to vancomycin, mersacidin complexes the sugar phosphate head group of the peptidoglycan precursor lipid II, which is only present in the membrane and experiments with radiolabelled mersacidin have confirmed that it does not bind to isolated cell walls (Brötz et al., 1998). Additionally, in *in vitro* cell wall biosynthesis systems, the effective concentration of mersacidin is in the same range as that of vancomycin (Brötz et al., 1997).

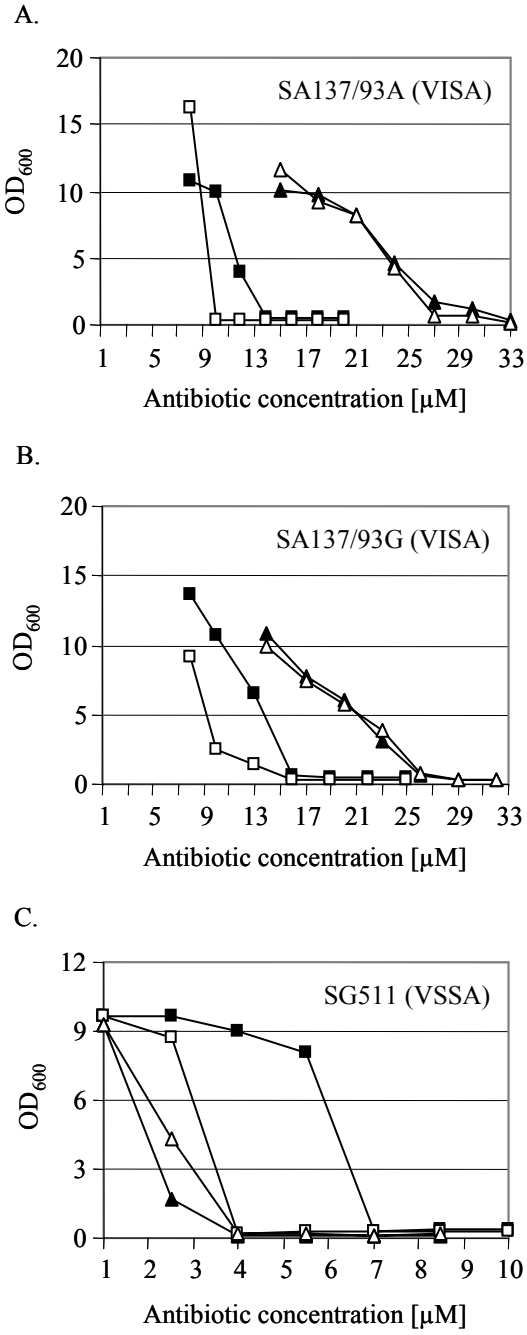

Figure 5.1: Influence of the cell wall thickness on the efficacy of mersacidin. The influence of an increased cell wall thickness on the efficacy of mersacidin was studied for the *S. aureus* strains SA137/93A (A), SA137/93G (B) and SG511-Berlin (C) (Sass et al., 2008a). To this end, *S. aureus* cells were incubated in resting medium supplemented with glucose (RM+g), which allows the synthesis of increased amounts of peptidoglycan, or in the absence of glucose (RM-g), which prevents synthesis of extra cell wall material. The data represent the optical density (600 nm) of RM-preincubated *S. aureus* cells after treatment with different concentrations of mersacidin. Vancomycin served as a control. (▲) mersacidin/RM+g; (△) mersacidin/RM-g; (■) vancomycin/RM+g; (□) vancomycin/RM-g.

Therefore, although nearly similar molar concentrations (2.175 µM mersacidin (0.15 x MIC) and 2.75 µM vancomycin (0.5 x MIC)) of the two antibiotics were used in the induction assays, less vancomycin than mersacidin molecules may have been available at the cell membrane for the binding of lipid II. This effect could explain why higher concentrations of vancomycin have to be employed in order to induce the cell wall stress response and why lower initial concentrations of mersacidin may be sufficient for an effective induction of VraSR.

A comparison of the transcript levels of *vraSR* of all tested strains showed that the levels did not vary significantly between the strains (Fig. 4.5A). Since the *vraSR* genes have been found to be more highly expressed in *S. aureus* Mu50 (VISA) compared to VSSA (Kuroda et al. 2003), which has been linked to the I5N substitution of the *vraS* gene (Fig. 4.9), the VraSR TCRS has been proposed to be involved in the VISA-type resistance mechanism via contribution to cell wall thickening (Kuroda et al., 2000; McAleese et al., 2006) and the deletion of the *vraSR* locus has been described to influence the development of resistance towards glycopeptides and β-lactams (Kuroda et al., 2003). However, the cell wall stress response was similarly induced upon mersacidin treatment in the VISA strains SA137/93A and SA137/93G as well as in the susceptible strain SG511-Berlin in our studies, all strains showed equal basal transcript levels and the *vraS* nucleotide sequences of these strains showed 100% identity. Interestingly, the induction of VraSR did not depend on the absolute concentration of mersacidin in the medium but seemed to be triggered by the stress itself, since 0.5 µg/ml of mersacidin massively induced the cell wall stress response in the susceptible strain SG511-Berlin (here 0.5 µg/ml correspond to 0.5 x MIC mersacidin; Fig. 4.5A), while *vraS* transcription levels were not significantly affected in strain SA137/93G (for this strain 0.5 µg/ml correspond to 0.02 x MIC mersacidin). Therefore, the VraSR TCRS might have evolved as a damage-sensing system that is indirectly induced as a cause of cell wall damage rather than a drug sensing system that recognizes the drug itself at defined concentrations. This is also indicated by findings showing that VraSR responds to various cell wall-active antibiotics as well as other conditions of cell wall stress like suboptimal transcript levels of *murF* and *pbp2* (Sobral et al., 2007; Gardete et al., 2006). In this regard, the bacitracin-dependent induction of the LiaRS TCRS in *Bacillus subtilis*, a close homologue of VraSR, also indicated a damage-sensing mechanism (Rietkötter et al., 2008). This supports the idea of VraSR being a common accelerator system of peptidoglycan synthesis (Kuroda et al., 2003), which reacts to perturbations of cell wall integrity.

In conclusion, mersacidin strongly induces the cell wall stress response of *S. aureus* even at very low concentrations as compared to other antibiotics like nisin, vancomycin, bacitracin or oxacillin, thereby reflecting its unique mode of action. The results presented here underline the important role of lipid II in this context and demonstrate that the induction of the cell wall stress response is not in every case dependent on inhibitory concentrations of cell wall-active substances. Interestingly, the cell wall stress response was similarly induced in *S. aureus* strains with different susceptibilities to antimicrobial agents, e.g. mersacidin, and therefore other mechanisms may account for the altered resistance of certain strains to mersacidin and similar antimicrobial peptides. Nevertheless, since the cell wall stress response comprises the increased expression of cell wall biosynthesis genes, it appears to be an inherent mechanism of growing cells to counteract the effect of antibiotics like mersacidin that target cell wall biosynthesis.

5.2 Effect of the VraDE ABC transporter on the decreased susceptibility to mersacidin

The presence of mersacidin significantly influenced the regulation of several genes coding for hypothetical ABC transporters such as SA0192, *vraFG* and *vraDE* (Table 4.1 and Table 4.2). Indeed, the most prominent event of gene regulation involved the upregulation of *vraDE* (40 to 70-fold in the microarray studies) (Table 4.1). The induction of *vraDE* gene transcription was further verified via qRT-PCR (Fig. 4.5B). Upon incubation with 0.5 x MIC, *vraDE* expression was induced 76 to 79-fold in strain SA137/93A and SA137/93G, respectively. In the susceptible strain SG511-Berlin, the transcript level of the *vraDE* genes rose almost 1700-fold upon incubation with mersacidin. This transporter has been previously shown to be inducible by vancomycin and cationic AMPs (Kuroda et al., 2000; Li et al., 2007a; Sass et al., 2008b) and seems to be involved in the increased resistance towards bacitracin and the human β-defensin 3 (hBD3) in *S. aureus* (Sass et al., 2008b). However, the regulation underlying *vraDE* induction has not been identified yet. The VraSR TCRS does not seem to be solely involved in the upregulation of *vraDE* transcription as knock-out mutations of the respective gene locus did not lead to an altered expression of the *vraDE* genes (Kuroda et al., 2003). The upregulation to such an extent might indicate that VraDE plays a critical role in the staphylococcal defense against mersacidin, especially since resistance is conferred by an ABC transporter in the producer strain of mersacidin (Guder et al., 2002).

To test whether VraDE is able to transport mersacidin out of the cell membrane and therefore supports a resistance phenotype, the growth behaviour and MICs of a *vraE* knock-out mutant of *S. aureus* SG511-Berlin (Sass et al., 2008b) and its parent strain were examined in the

presence of the lantibiotic. Since *S. aureus* SG511ΔvraE had displayed significantly reduced resistance to bacitracin and the lantibiotics nisin and Pep5 (Sass et al., 2008b), VraDE was considered to be functional in the parent strain. Unexpectedly, *S. aureus* SG511ΔvraE did not show increased susceptibility towards mersacidin neither in growth curve recordings nor in MIC studies. Sequencing of the *vraDE* genes including the promoter region revealed, that the -35 and the -10 region as well as the ribosomal binding site and the *vraD* gene were highly conserved in strains SA137/93A and SG511-Berlin (100% sequence identity) and that the *vraE* gene showed 95.7% sequence identity with mostly conservative amino acid substitutions. Therefore, VraDE is unlikely to contribute to mersacidin resistance, presumably because it is unable to transport the lantibiotic. This could be due to the properties of mersacidin which is globular, neutral and non-membrane disturbing. Since VraDE has been shown to predominantly transport linear, cationic, membrane interacting compounds, it might be unable to faciliate the transport of other antimicrobials as also shown for chloramphenicol and oxacillin (Sass et al., 2008b). However, it cannot be ruled out that the knockout phenotype is indiscernible through the activity of another ABC transporter. In conclusion, mersacidin represents a molecule that seems not to be a substrate for the multidrug resistance transporter VraDE which apparently functions in the first line bacterial counter strategy against bacitracin and cationic toxic compounds.

5.3 A native *graS* mutation supports the susceptibility of *Staphylococcus aureus* strain SG511-Berlin to antimicrobial peptides

The rather susceptible *S. aureus* strain SG511-Berlin represents a standard strain for antimicrobial susceptibility testing and has been used as a model for basic research on staphylococci (Brötz et al., 1997; Maidhof et al., 1989; Pag et al., 2008; Sass et al., 2008b; de Jonge et al., 1993; Naumann and Labischinski, 1990). While studying the influence of the lantibiotic mersacidin on the expression profiles of *S. aureus* (see section 5.1), strain SG511-Berlin was characterized by an increased susceptibility to mersacidin, which, however, could not be traced to differences in the transcriptional response pattern of strain SG511-Berlin upon mersacidin treatment compared to the other *S. aureus* strains tested.

In order to identify the factors which are responsible for the increased susceptibility of strain SG511-Berlin to antimicrobials like mersacidin, the expression profile of strain SG511-Berlin was compared with the more resistant strain SA137/93A in the absence of antibiotics. Here, a different expression of the genes coding for the membrane proteine DltB, the lysyltransferase MprF and the ABC transport system VraFG was observed. DltB contributes to the alanylation

of the cell wall by the transport and incorporation of activated D-alanine into the teichoic acids, while MprF catalyses the lysinylation of phosphatidylglycerol. Both, DltB and MprF, confer a more positive net charge to the cell surface, thereby preventing the binding of cationic AMPs to the cytoplasmic membrane (Peschel et al., 1999; Peschel et al., 2001). Furthermore, the *dltB*, *mprF* and *vraFG* genes have been shown to be positively controlled by the TCRS GraRS in *S. aureus* (Herbert et al., 2007; Meehl et al., 2007). The *vraFG* genes are even encoded downstream of *graRS* and therefore constitute a typical bacteriocin immunity regulon of grampositive bacteria comprising a TCRS with an adjacent ABC transporter (Jordan et al., 2008). Actually, the GraRS regulatory unit has recently been proposed as an antimicrobial peptide sensing system (*aps*) in *S. aureus* and *S. epidermidis* (Li et al., 2007a; Li et al., 2007b) and in fact its influence on the susceptibility of *S. aureus* to antimicrobials has been described (Peschel et al., 2000; Peschel et al., 1999; Meehl et al., 2007; Kraus et al., 2008; Howden et al., 2008; Neoh et al., 2008). In addition, GraRS seems to interact with other global regulators of *S. aureus* to influence factors involved in biofilm formation (*dltB*, *atl*) and colonization (*atl*, *sirC, cap*5/8) (Herbert et al., 2007). Interestingly, our results showed that these proteins were also characterized by a lower expression level in *S. aureus* SG511-Berlin compared to *S. aureus* SA137/93A.

Sequence analysis of the *graS* gene in strain SG511-Berlin revealed a native nucleotide insertion that generates a stop codon at amino acid position 64 which leads to the deletion of 82% of GraS, which is the entire cytoplasmic part of the sensor histidine kinase. As expected, this deletion had a direct influence on the expression of *dltB* and is accompanied by a more negative surface charge of strain SG511-Berlin compared to other *S. aureus* strains that could be reversed by the reconstitution of *graS*. Finally, the *graS*-reconstituted strain SG511-Berlin also showed significantly decreased susceptibility to the lantibiotics mersacidin, nisin and Pep5. Although the GraRS TCRS is not induced upon mersacidin treatment (see section 4.3.6), these results demonstrate that GraS contributes to an inherent defense mechanism of *S. aureus* towards mersacidin. A similar phenomenon has been shown for human beta defensin 3, which, although it does not induce GraRS in *S. aureus* significantly, is susceptible to the resistance mechanisms controlled by the GraRS system (Li et al., 2007a). GraRS is activated by positively charged peptides (Li et al., 2007a) as e.g. nisin and indolicidin. The cationic peptides nisin and Pep5 accumulate in the cell wall by binding to the teichoic and lipoteichoic acids by an ionic interaction. At least with Pep5, the inhibition of this accumulation results in complete loss of activity (Sahl et al., 1985; Bierbaum and Sahl, 1987; Sahl and Brandis, 1983). Therefore, the activity of these peptides is diminished by the

incorporation of positively charged D-alanine residues into the teichoic acids that is due to DltB expression (Peschel et al., 1999). In contrast, mersacidin, which prevents the transglycosylation reaction of peptidoglycan biosynthesis by complexing the peptidoglycan precursor lipid II (Brötz et al., 1998), does not carry a net positive charge and does not bind to isolated cell walls in buffer (Brötz et al., 1998). However, the activity of mersacidin is enhanced in the presence of calcium ions (Barrett et al., 1992; Schneider et al., 2000), and the binding of calcium ions may impart a positive net charge to the complex which would facilitate the interaction with the negatively charged cell wall polymers as well as with the negatively charged head group of lipid II (Hsu et al., 2003). Furthermore, although the specific transport functions of VraFG have not yet been ascertained, mersacidin or the mersacidin calcium complex could also be removed from the bacterial cell and membrane via this transport system, thereby preventing its accumulation at the site of peptidoglycan biosynthesis. Further studies are needed to define the mode by which GraRS controls resistance towards mersacidin.

The GraRS system has additionally been implicated to have a central role in conferring resistance to the glycopeptide antibiotic vancomycin in *S. aureus* (Meehl et al., 2007) and even single nucleotide exchanges in *graR* as well as *graS* have been demonstrated to support resistance to vancomycin (Howden et al., 2008; Neoh et al., 2008). However, a significant increase in vancomycin resistance after complementation of *graS* in *S. aureus* SG511-Berlin could not be detected. This could be due to the genetic background of strain SG511-Berlin that might not be adequate for establishing a significant gain in vancomycin resistance, since the closely related *S. aureus* MRSA252, which bears a functional GraS, shows an even lower vancomycin MIC.

In conclusion, a native *graS* mutation directly influences the cell surface properties and concomitantly the susceptibility of *S. aureus* SG511-Berlin to antimicrobial substances like mersacidin, nisin and Pep5. Besides the VraSR system (see section 5.1), the GraRS system represents a second intrinsic resistance mechanism of actively growing cells that reacts to antimicrobial agents. Taking this into account, it is desireable to develop new antibacterial treatment strategies that circumvent the influence of the intrinsic resistance mechanisms of actively growing cells. Noteworthy, GraRS is more than an antimicrobial peptide sensing system as it also interacts with global regulators and influences biofilm formation, colonization and autolysis, i.e. it represents an important factor in controlling the vital functions of *S. aureus*. These facts should be considered upon further use of strain SG511-Berlin as a standard strain for antimicrobial susceptibility testing and basic research purposes.

5.4 Role of "SSP0470:Tn*551*" in the increased resistance of *S. simulans* 22 to mersacidin

Tn*551* transposon mutagenesis of *S. simulans* 22 had been previously performed by Gabriele Bierbaum (IMMIP; unpublished data) to identify factors which potentially influence the susceptibility of staphylococci to antimicrobials like mersacidin. By this means, a Tn*551* insertion mutant of *S. simulans* 22 had been isolated that showed a significant difference in its susceptibility to mersacidin compared to the wild type strain. In the present work, mapping of the Tn*551* transposon insertion in the genome of *S. simulans* 22 revealed its location in the C-terminal region of the hypothetical gene SSP0470 (of the genomic sequence of *S. saprophyticus* ATCC15305). A BLAST analysis was performed and characterized SSP0470 as a potential membrane protein with low homology to streptococcal permeases. Curiously, in contrast to other described resistance markers, the susceptibility of *S. simulans* 22 to mersacidin decreases when SSP0470 is disrupted by Tn*551*. This is similar to previous observations concerning the *S. aureus* gene *tcaA* (Maki et al., 2004). These studies linked an increased glycopeptide resistance of selected *S. aureus* strains, including strain SA137/93G, to the inactivation of the *tcaA* gene. TcaA is a membrane protein whose biochemical functions still need to be determined. Maki et al. presumed that induction of *tcaA* transcription in the presence of cell wall-active antibiotics, as described by Utaida et al. (2003), would increase the susceptibility to teicoplanin, thereby creating a selective pressure for the inactivation of *tcaA* in the presence of the glycopeptide teicoplanin. The mode by which SSP0470 influences the susceptibility to mersacidin is yet unclear and further studies are needed to characterize its role as a resistance determinant. Interestingly, a gene homologous to SSP0470 is not present in any sequenced *S. aureus* strain of the NCBI database to date. Compared to *S. aureus*, *S. simulans* 22 is quite susceptible to antimicrobial agents and therefore is a favoured organism for antimicrobial susceptibility testing and routine examinations. Perhaps, SSP0470 is one of the factors that account for the different susceptibility characteristics among the different staphylococci.

5.5 Lytic activity of recombinant phage ϕ11 and ϕ12 endolysins on whole cells and biofilms of *Staphylococcus aureus*

Staphylococcal biofilms on indwelling devices are difficult to treat due to their inherent antibiotic resistance (Costerton et al., 1987; Donlan and Costerton, 2002; O'Grady et al., 2002; Geisel et al., 2001; Mah and O'Toole, 2001; Stewart, 2002). Especially, persister cells contribute to biofilm resistance (Roberts and Stewart, 2005; Anderson and O'Toole, 2008), since these non-growing cells appear to escape from the action of antibiotics that target the

bacterial metabolism. In this context, it is important to develop alternative treatment strategies that target actively growing cells as well as non-growing persister cells to combat staphylococcal biofilm infections in future. Phage lysins, or endolysins, have received noticeable attention as possible antimicrobial agents against Gram-positive bacteria and have been applied to a variety of pathogens, such as *Bacillus anthracis* (Low et al., 2005), *Streptococcus pneumoniae* (Entenza et al., 2005) and *S. aureus* (O'Flaherty et al., 2005). Endolysins are active against non-growing and dead cells as well as living, planktonic cells (O'Flaherty et al., 2005; Entenza et al., 2005), however, their ability to lyse the complex structure of staphylococcal biofilms has not yet been investigated. In this approach, the lysis genes of the bacteriophages ϕ11 and ϕ12 of *S. aureus* NCTC8325 were cloned and heterologously overexpressed in *E. coli* for subsequent analysis of the lytic activity of the enzymes and their single subdomains on cell walls, whole cells and biofilms of staphylococci (Sass and Bierbaum, 2007). Here, the full-length ϕ11 endolysin efficiently hydrolyzed purified peptidoglycan of *S. simulans* 22 and both, the endopeptidase and the amidase module plus cell wall binding domain (ϕ11endo/CBD and ϕ11ami/CBD), were active and able to lyse cell walls (Fig. 4.21). However, the full length ϕ11 endolysin was more active than the isolated subdomains and only the combination of ϕ11endo/CBD and ϕ11ami/CBD restored full lytic activity. Hence, the two catalytic domains of the enzyme have to be combined with each other to cleave the cell wall of intact cells. Thus, the ϕ11 endolysin seems to belong to a group of endolysins which act as multifunctional hydrolases (Bateman and Rawlings, 2003). Recently, the lytic enzyme of phage ϕWMY of *Staphylococcus warneri* M (LysWMY) was reported to show strong similarities in its domain architecture to the ϕ11 endolysin (Yokoi et al., 2005). In contrast to the ϕ11 endolysin, LysWMY retained its full activity even when both, the amidase and cell wall binding domain had been deleted. This result indicates that a combined action of different domains does not seem to be an obligatory characteristic even among closely related murein hydrolases. Noteworthy, purified cell walls were a much more sensitive substrate for the enzymes than heat-killed cells, since they showed the residual activities of the single subdomains much more clearly. In spite of this, the ϕ12 endolysin did not show any activity with *S. simulans* 22 cell walls (Sass, 2005).

Furthermore, substrate recognition mediated by the cell wall binding domain, which is homologous to the C-terminal domain of lysostaphin (Loessner et al., 1998), appeared to be necessary for high catalytic efficiency of the ϕ11 endolysin. Lytic activity of the ϕ11 endolysin on heat-killed staphylococcal cells was abolished after deletion of the cell wall binding domain (ϕ11endo/ami), while it retained nearly all of its lytic activity on SDS cell

walls. The single domains, ϕ11endo and ϕ11ami, showed significantly reduced lytic activity on SDS cell walls in the absence of the cell wall binding domain. These findings are similar to the results obtained with lysostaphin and ALE-1, a glycyl-glycine endopeptidase homologous to lysostaphin. After the deletion of their C-terminal cell wall targeting domains (Baba and Schneewind, 1996; Lu et al., 2006), which mediate the binding to cross-linked peptidoglycan and recognise the $[Gly]_5$ interpeptide crossbridge (Lu et al., 2006; Gründling and Schneewind, 2006), lysostaphin and ALE-1 showed a significant reduction in lytic activity on autoclaved staphylococci or viable cells, respectively. Our results suggest that a similar targeting mechanism exists for the ϕ11 endolysin and that, in this respect, the phage enzyme closely resembles its staphylococcal counterparts.

The lytic activities of the ϕ11 and ϕ12 endolysins were also tested on whole cells of *S. aureus* NCTC8325, *S. aureus* Wood 46, *S. aureus* Cowan I, *S. aureus* Newman, *S. epidermidis* O-47 and *S. simulans* 22. Here, the full-length ϕ11 endolysin was characterized by rapid hydrolysis of heat-inactivated staphylococcal cells which was equivalent to the activity of lysostaphin. Unlike ϕ11, the ϕ12 endolysin was not able to hydrolyze heat-inactivated staphylococci. After addition of the ϕ12 endolysin an increase in optical density of the cultures was observed which was accompanied and most probably caused by an aggregation of the cells that was macroscopically visible (Sass, 2005). This phenomenon has been described previously (Takano et al., 2000) by investigating the influence of synthetic peptides derived from the *S. aureus* major autolysin Atl on autolysis. Sequence comparison with the homologous amidase-3 domains of the *S. aureus* phages ϕSA 2MW, L54a, ϕSLT, PVL, 96, 3a, 53, 77, ROSA, ϕETA and amidases of *Staphylococcus haemolyticus* JCSC1435 and *Staphylococcus epidermidis* RP62a identified an amino acid exchange at position 260 in the amidase-3 domain of the ϕ12 endolysin. This exchange introduces a histidine and therefore an additional positive charge in a position that is occupied by glutamine, glutamate or asparagine in the other staphylococcal enzymes. To analyze the reasons for the ineffectiveness of the ϕ12 endolysin, a mutated ϕ12 endolysin was constructed in which the histidine at position 260 was exchanged to glutamine. Unexpectedly, the ϕ12mut endolysin was still incapable to hydrolyze heat-inactivated staphylococci. Thus, in our test systems, the ϕ12 endolysin as well as the ϕ12mut endolysin are unable to exert efficient hydrolytic activity on staphylococcal peptidoglycan, while retaining the ability to bind the cell wall. The substrate binding ability of the enzyme may then lead to the enhanced adhesion between the cells. *In vivo*, the ineffectiveness of the ϕ12 endolysin would mean severe disadvantages for phage ϕ12. A

phage, that owns an endolysin which is incapable to properly hydrolyze the cell wall of the bacterial host, would be dependent on other mechanisms, e.g. other phages, to escape from the bacterial cell after activation of the phage lytic cycle. This could explain, why phage ϕ12 commonly exists as a lysogenic phage in *S. aureus* NCTC8325 together with the phages ϕ11 and ϕ13. Nevertheless, it cannot be excluded that an inadequate folding of the six-His-tagged ϕ12 endolysin is involved in the loss of hydrolytic activity.

In this work, the activity of the endolysins was also tested against staphylococcal biofilms. Bacterial biofilm formation is part of a survival strategy to resist suboptimal environmental conditions such as limited nutrient availability or lethal concentrations of antibiotics. Antimicrobial agents often show significantly reduced effects on biofilms, which is thought to be due to several biofilm-inherent properties. For example, a slow growth rate impedes the action of antibiotics (Duguid et al., 1992b; Stewart, 2002) and evidence is emerging that the sessile cells in biofilms live in an altered metabolic state (Beenken et al., 2004; Resch et al., 2005). Furthermore, the diffusion velocity of antibiotics is limited within biofilms. A reduced rate of antibiotic penetration leads to a gradually increasing concentration of the antibiotic in the deeper layers of a biofilm which permits adaptation of the biofilm cells to the antibiotic by stress-induced metabolic and transcriptional changes (Jefferson et al., 2005). Additionally, non-growing persister cells circumvent the action of conventionally used antibiotics that predominantly act on the metabolism of actively growing cells (Anderson and O'Toole, 2008; Roberts and Stewart, 2005). Here, the purified ϕ11 endolysin was able to eliminate biofilms of *S. aureus* NCTC8325 and *S. aureus* Newman. So far, no other phage lysin has been reported to disrupt staphylococcal biofilms. However, *S. epidermidis* biofilms remained unaffected, although whole cells of *S. epidermidis* were susceptible to the action of the ϕ11 endolysin. The ϕ11 endolysin most probably destabilizes the biofilm structure by fast lysis of sessile cells, which are embedded in the extracellular matrix or at the interface of the matrix and surface, whereupon the biofilm is dissolved. Another possibility could be an influence of the intrinsic nature of the biofilm. Neither Na-meta-periodate nor proteinase K treatment could completely eradicate biofilms of *S. aureus* NCTC8325 which suggests that an elevated portion of proteinogenous biofilm is present along with a biofilm mediated by the characteristic polysaccaride PIA. PIA consists of β-1,6-linked N-acetylglucosamine residues (Götz, 2002) and is not the target of the ϕ11 murein hydrolase. In contrast to *S. aureus* NCTC8325, *S. epidermidis* O-47 biofilms are exclusively polysaccaride-mediated and were not dissolved by the ϕ11 enzyme. Therefore, the different natures of the biofilm matrices tested here could be an explanation for the varying efficacy of the ϕ11 endolysin.

In conclusion, the combined action of the endopeptidase and amidase domains of the ϕ11 endolysin allowed the efficient hydrolysis of whole cells and biofilms of *S. aureus*. Additionally, cell wall targeting appeared to be a prerequisite for maximum activity. Considering the huge clinical relevance of staphylococcal biofilms in terms of human diseases, the endolysins may constitute a novel strategy to treat actively growing cells and non-growing persister cells in order to combat *S. aureus* nosocomial infections that are mediated by biofilm formation on medical devices.

5.6 Perspectives

S. aureus infections remain difficult to treat due to their ability to persist during host invasion. Today, there is an urgent need for a better understanding of how bacteria develop resistance to antibiotics and new treatment strategies need to be explored to control bacterial infections.

In this work, the lantibiotic mersacidin has been characterized as a strong inducer of the cell wall stress response of *S. aureus* and the GraRS (aps) antimicrobial peptide sensing system of *S. aureus* could be identified as a resistance determinant to mersacidin and other lantibiotics. However, further studies are needed to gain deeper insights into the mechanism that *S. aureus* uses to resist antibiotic treatment. In this context, the selective inactivation of the *dltB*, *mprF* and *vraFG* genes of a more resistant strain, e.g. *S. aureus* SA137/93G, could specify the mode by which the GraRS system controls a resistance mechanism against mersacidin. Despite the fact, that the inactivation of the VraDE ABC transporter genes did not have an influence on the susceptibility of *S. aureus* to mersacidin in this work, it cannot be excluded that the differences in mersacidin susceptibility are based on a transport mechanism. Here, biochemical characterization of VraFG, VraDE and related transporters will test this hypothesis and may provide directions for circumventing the activity of resistance transporters through structural modification of antibiotics. However, the factors presented in this work may only be partly responsible for the different susceptibilities among staphylococci and new techniques must be employed to understand the lifestyle of *S. aureus*, its constitutive and inducible resistance factors. This work underlined the importance of a fundamental knowledge on bacterial genomics, since single variations of the nucleotide sequences may have a hugh impact on the bacterial phenotype. In this context, the recent introduction of pyrosequencing techniques now allows for the convenient sequencing of whole bacterial genomes. Combined with elaborate bioinformatic analysis tools, which enable the rapid analysis of entire genomes, *S. aureus* wild type strains or laboratory mutants of interest could be screened for variations in their nucleotide sequences that may influence the

susceptibility of *S. aureus* and other staphylococci to antibiotics. Hereupon, genes of interest should be selectively inactivated or alternatively be overexpressed to study their contribution to antibiotic resistance and to evaluate desireable properties of new antimicrobial agents. Further studies on the response and resistance of *S. aureus* to mersacidin and other antibiotics should also comprise the analysis of the *S. aureus* proteome. While the genomic data provides the building blocks for the *S. aureus* way of life, the bacterial proteome would present a snapshot of the actual state of the cell and, therefore, would allow to understand the mechanisms that *S. aureus* uses to evade antimicrobial treatment.

In this work, the ϕ11 endolysin was used to efficiently treat *S. aureus* biofilms *in vitro*. Nevertheless, further studies need to be conducted to characterize the ϕ11 endolysin and to ascertain its putative contribution to a new biofilm treatment strategy in order to overcome the restrictions of antibiotics that target only actively growing cells. Here, it would also be interesting to analyze the *S. aureus* response to the ϕ11 endolysin on a transcriptional and proteomic level to evaluate a putative gain of resistance towards these group of antimicrobial compounds. The ϕ11 endolysin could further be coupled to GFP (green fluorescent protein) to study the binding mechanism of the protein to staphylococcal cells. Furthermore, the cell wall-binding domain could be exchanged to one of another murein hydrolase to possibly gain activity against cells that were previously not affected by the ϕ11 endolysin, e.g. biofilms of *S. epidermidis*. Additionally, site-directed mutagenesis could be employed to improve the activity of the ϕ11 endolysin or to detect amino acid residues that are essential for its lytic activity. Finally, *in vivo* studies have to be performed to confirm the potential of endolysins to counteract infections caused by *S. aureus*.

6 References

Adhikari, R.P., Scales, G.C., Kobayashi, K., Smith, J.M., Berger-Bächi, B., Cook, G.M., 2004. Vancomycin-induced deletion of the methicillin resistance gene *mecA* in *Staphylococcus aureus*. J. Antimicrob. Chemother. 54, 360-363.

Allgaier, H., Jung, G., Werner, R.G., Schneider, U., Zahner, H., 1986. Epidermin: sequencing of a heterodetic tetracyclic 21-peptide amide antibiotic. Eur. J Biochem. 160, 9-22.

Altena, K., Guder, A., Cramer, C., Bierbaum, G., 2000. Biosynthesis of the lantibiotic mersacidin: organization of a type B lantibiotic gene cluster. Appl. Environ. Microbiol. 66, 2565-2571.

Anderson, G.G., O'Toole, G.A., 2008. Innate and induced resistance mechanisms of bacterial biofilms. Curr. Top. Microbiol. Immunol. 322, 85-105.

Anderson, K.L., Roberts, C., Disz, T., Vonstein, V., Hwang, K., Overbeek, R., Olson, P.D., Projan, S.J., Dunman, P.M., 2006. Characterization of the *Staphylococcus aureus* heat shock, cold shock, stringent, and SOS responses and their effects on log-phase mRNA turnover. J. Bacteriol. 188, 6739-6756.

Augustin, J., Rosenstein, R., Wieland, B., Schneider, U., Schnell, N., Engelke, G., Entian, K.D., Götz, F., 1992. Genetic analysis of epidermin biosynthetic genes and epidermin-negative mutants of *Staphylococcus epidermidis*. Eur. J. Biochem. 204, 1149-1154.

Baba, T., Schneewind, O., 1996. Target cell specificity of a bacteriocin molecule: a C-terminal signal directs lysostaphin to the cell wall of *Staphylococcus aureus*. EMBO J. 15, 4789-4797.

Barber, M., 1947. Staphylococcal infections due to penicillin resistant strains. British Medical Journal 2, 863-865.

Barber, M., 1961. Methicillin-resistant staphylococci. J Clin. Pathol. 14, 385-393.

Barrett, M.S., Wenzel, R.P., Jones, R.N., 1992. In vitro activity of mersacidin (M87-1551), an investigational peptide antibiotic tested against gram-positive bloodstream isolates. Diagn. Microbiol. Infect. Dis. 15, 641-644.

Bateman, A., Rawlings, N.D., 2003. The CHAP domain: a large family of amidases including GSP amidase and peptidoglycan hydrolases. Trends Biochem. Sci. 28, 234-237.

Bauer, R., Chikindas, M.L., Dicks, L.M., 2005. Purification, partial amino acid sequence and mode of action of pediocin PD-1, a bacteriocin produced by *Pediococcus damnosus* NCFB 1832. Int. J Food Microbiol. 101, 17-27.

Bayles, K.W., 2007. The biological role of death and lysis in biofilm development. Nat. Rev. Microbiol. 5, 721-726.

Beenken, K.E., Dunman, P.M., McAleese, F., Macapagal, D., Murphy, E., Projan, S.J., Blevins, J.S., Smeltzer, M.S., 2004. Global gene expression in *Staphylococcus aureus* biofilms. J. Bacteriol. 186, 4665-4684.

Berger-Bächi, B., Kohler, M.L., 1983. A novel site on the chromosome of *Staphylococcus aureus* influencing the level of methicillin resistance: Genetic mapping. FEMS Microbiology Letters. 20, 305-309.

Bertani, G., 1951. Studies on lysogenesis. I. The mode of phage liberation by lysogenic *Escherichia coli*. J. Bacteriol. 62, 293-300.

Bertani, G., 2004. Lysogeny at mid-twentieth century: P1, P2, and other experimental systems. J. Bacteriol. 186, 595-600.

Bierbaum, G., Brötz, H., Koller, K.P., Sahl, H.G., 1995. Cloning, sequencing and production of the lantibiotic mersacidin. FEMS Microbiol. Lett. 127, 121-126.

Bierbaum, G., Fuchs, K., Lenz, W., Szekat, C., Sahl, H.G., 1999. Presence of *Staphylococcus aureus* with reduced susceptibility to vancomycin in Germany. Eur. J. Clin. Microbiol. Infect. Dis. 18, 691-696.

Bierbaum, G., Sahl, H.G., 1987. Autolytic system of *Staphylococcus simulans* 22: influence of cationic peptides on activity of N-acetylmuramoyl-L-alanine amidase. J. Bacteriol. 169, 5452-5458.

Bierbaum, G., Sahl, H.G., 2009. Lantibiotics: Mode of action, biosynthesis and bioengineering. Curr. Pharm. Biotech. 10, in press.

Bierbaum, G., Szekat, C., Josten, M., Heidrich, C., Kempter, C., Jung, G., Sahl, H.G., 1996. Engineering of a novel thioether bridge and role of modified residues in the lantibiotic Pep5. Appl. Environ. Microbiol. 62, 385-392.

Booth, M.C., Bogie, C.P., Sahl, H.G., Siezen, R.J., Hatter, K.L., Gilmore, M.S., 1996. Structural analysis and proteolytic activation of *Enterococcus faecalis* cytolysin, a novel lantibiotic. Mol. Microbiol. 21, 1175-1184.

Bore, E., Langsrud, S., Langsrud, O., Rode, T.M., Holck, A., 2007. Acid-shock responses in *Staphylococcus aureus* investigated by global gene expression analysis. Microbiology 153, 2289-2303.

Borysowski, J., Weber-Dabrowska, B., Gorski, A., 2006. Bacteriophage endolysins as a novel class of antibacterial agents. Exp. Biol. Med (Maywood.) 231, 366-377.

Bowdish, D.M., Davidson, D.J., Lau, Y.E., Lee, K., Scott, M.G., Hancock, R.E., 2005. Impact of LL-37 on anti-infective immunity. J Leukoc. Biol. 77, 451-459.

Boyle-Vavra, S., Challapalli, M., Daum, R.S., 2003. Resistance to autolysis in vancomycin-selected *Staphylococcus aureus* isolates precedes vancomycin-intermediate resistance. Antimicrob. Agents Chemother. 47, 2036-2039.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248-254.

Brandenberger, M., Tschierske, M., Giachino, P., Wada, A., Berger-Bächi, B., 2000. Inactivation of a novel three-cistronic operon *tcaR-tcaA-tcaB* increases teicoplanin resistance in *Staphylococcus aureus*. Biochim. Biophys. Acta 1523, 135-139.

Brötz, H., Bierbaum, G., Leopold, K., Reynolds, P.E., Sahl, H.G., 1998. The lantibiotic mersacidin inhibits peptidoglycan synthesis by targeting lipid II. Antimicrob. Agents Chemother. 42, 154-160.

Brötz, H., Bierbaum, G., Reynolds, P.E., Sahl, H.G., 1997. The lantibiotic mersacidin inhibits peptidoglycan biosynthesis at the level of transglycosylation. Eur. J. Biochem. 246, 193-199.

Caiazza, N.C., O'Toole, G.A., 2003. Alpha-toxin is required for biofilm formation by *Staphylococcus aureus*. J Bacteriol. 185, 3214-3217.

Castiglione, F., Cavaletti, L., Losi, D., Lazzarini, A., Carrano, L., Feroggio, M., Ciciliato, I., Corti, E., Candiani, G., Marinelli, F., Selva, E., 2007. A novel lantibiotic acting on bacterial cell wall synthesis produced by the uncommon actinomycete *Planomonospora* sp. Biochemistry 46, 5884-5895.

Chang, S., Sievert, D.M., Hageman, J.C., Boulton, M.L., Tenover, F.C., Downes, F.P., Shah, S., Rudrik, J.T., Pupp, G.R., Brown, W.J., Cardo, D., Fridkin, S.K., 2003. Infection with vancomycin-resistant *Staphylococcus aureus* containing the *vanA* resistance gene. N. Engl. J Med 348, 1342-1347.

Chatterjee, S., Chatterjee, D.K., Jani, R.H., Blumbach, J., Ganguli, B.N., Klesel, N., Limbert, M., Seibert, G., 1992a. Mersacidin, a new antibiotic from *Bacillus*. *In vitro* and *in vivo* antibacterial activity. J. Antibiot. (Tokyo) 45, 839-845.

Chatterjee, S., Chatterjee, D.K., Lad, S.J., Phansalkar, M.S., Rupp, R.H., Ganguli, B.N., Fehlhaber, H.W., Kogler, H., 1992b. Mersacidin, a new antibiotic from *Bacillus*. Fermentation, isolation, purification and chemical characterization. J. Antibiot. (Tokyo) 45, 832-838.

Cookson, B.D., Robinson, D.A., Monk, A.B., Murchan, S., Deplano, A., de Ryck, R., Struelens, M.J., Scheel, C., Fussing, V., Salmenlinna, S., Vuopio-Varkila, J., Cuny, C., Witte, W., Tassios, P.T., Legakis, N.J., van Leeuwen, W., van Belkum, A., Vindel, A., Garaizar, J., Haeggman, S., Olsson-Liljequist, B., Ransjo, U., Muller-Premru, M., Hryniewicz, W., Rossney, A., O'Connell, B., Short, B.D., Thomas, J., O'Hanlon, S., Enright, M.C., 2007. Evaluation of molecular typing methods in characterizing a European collection of epidemic methicillin-resistant *Staphylococcus aureus* strains: the HARMONY collection. J. Clin. Microbiol. 45, 1830-1837.

Costerton, J.W., Cheng, K.J., Geesey, G.G., Ladd, T.I., Nickel, J.C., Dasgupta, M., Marrie, T.J., 1987. Bacterial biofilms in nature and disease. Annu. Rev. Microbiol. 41:435-64., 435-464.

Cramton, S.E., Gerke, C., Schnell, N.F., Nichols, W.W., Götz, F., 1999. The intercellular adhesion (*ica*) locus is present in *Staphylococcus aureus* and is required for biofilm formation. Infect. Immun. 67, 5427-5433.

Cucarella, C., Solano, C., Valle, J., Amorena, B., Lasa, I., Penades, J.R., 2001. Bap, a *Staphylococcus aureus* surface protein involved in biofilm formation. J Bacteriol. 183, 2888-2896.

Cui, L., Iwamoto, A., Lian, J.Q., Neoh, H.M., Maruyama, T., Horikawa, Y., Hiramatsu, K., 2006. Novel mechanism of antibiotic resistance originating in vancomycin-intermediate *Staphylococcus aureus*. Antimicrob. Agents Chemother. 50, 428-438.

Cui, L., Ma, X., Sato, K., Okuma, K., Tenover, F.C., Mamizuka, E.M., Gemmell, C.G., Kim, M.N., Ploy, M.C., El Solh, N., Ferraz, V., Hiramatsu, K., 2003. Cell wall thickening is a common feature of vancomycin resistance in *Staphylococcus aureus*. J. Clin. Microbiol. 41, 5-14.

Cui, L., Murakami, H., Kuwahara-Arai, K., Hanaki, H., Hiramatsu, K., 2000. Contribution of a thickened cell wall and its glutamine nonamidated component to the vancomycin resistance expressed by *Staphylococcus aureus* Mu50. Antimicrob. Agents Chemother. 44, 2276-2285.

Dabard, J., Bridonneau, C., Phillipe, C., Anglade, P., Molle, D., Nardi, M., Ladire, M., Girardin, H., Marcille, F., Gomez, A., Fons, M., 2001. Ruminococcin A, a new lantibiotic produced by a *Ruminococcus gnavus* strain isolated from human feces. Appl. Environ. Microbiol. 67, 4111-4118.

de Jonge, B.L., Sidow, T., Chang, Y.S., Labischinski, H., Berger-Bächi, B., Gage, D.A., Tomasz, A., 1993. Altered muropeptide composition in *Staphylococcus aureus* strains with an inactivated *femA* locus. J. Bacteriol. 175, 2779-2782.

de Kwaadsteniet, M., Ten Doeschate, K., Dicks, L.M., 2008. Characterization of the structural gene encoding nisin F, a new lantibiotic produced by a *Lactococcus lactis* subsp. lactis isolate from freshwater catfish (*Clarias gariepinus*). Appl. Environ. Microbiol. 74, 547-549.

de Lencastre, H., Wu, S.W., Pinho, M.G., Ludovice, A.M., Filipe, S., Gardete, S., Sobral, R., Gill, S., Chung, M., Tomasz, A., 1999. Antibiotic resistance as a stress response: complete sequencing of a large number of chromosomal loci in *Staphylococcus aureus* strain COL that impact on the expression of resistance to methicillin. Microb. Drug Resist. 5, 163-175.

Delmi, M., Vaudaux, P., Lew, D.P., Vasey, H., 1994. Role of fibronectin in staphylococcal adhesion to metallic surfaces used as models of orthopaedic devices. J. Orthop. Res. 12, 432-438.

Donlan, R.M., Costerton, J.W., 2002. Biofilms: survival mechanisms of clinically relevant microorganisms. Clin. Microbiol. Rev. 15, 167-193.

Duguid, I.G., Evans, E., Brown, M.R., Gilbert, P., 1992a. Effect of biofilm culture upon the susceptibility of *Staphylococcus epidermidis* to tobramycin. J. Antimicrob. Chemother. 30, 803-810.

Duguid, I.G., Evans, E., Brown, M.R., Gilbert, P., 1992b. Growth-rate-independent killing by ciprofloxacin of biofilm-derived *Staphylococcus epidermidis*; evidence for cell-cycle dependency. J. Antimicrob. Chemother. 30, 791-802.

Dunne, W.M., Jr., 2002. Bacterial adhesion: seen any good biofilms lately? Clin. Microbiol. Rev. 15, 155-166.

Ekkelenkamp, M.B., Hanssen, M., Danny Hsu, S.T., de Jong, A., Milatovic, D., Verhoef, J., van Nuland, N.A., 2005. Isolation and structural characterization of epilancin 15X, a novel lantibiotic from a clinical strain of *Staphylococcus epidermidis*. FEBS Lett. 579, 1917-1922.

Enright, M.C., Day, N.P., Davies, C.E., Peacock, S.J., Spratt, B.G., 2000. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J. Clin. Microbiol. 38, 1008-1015.

Entenza, J.M., Loeffler, J.M., Grandgirard, D., Fischetti, V.A., Moreillon, P., 2005. Therapeutic effects of bacteriophage Cpl-1 lysin against *Streptococcus pneumoniae* endocarditis in rats. Antimicrob. Agents Chemother. 49, 4789-4792.

Fredenhagen, A., Fendrich, G., Marki, F., Marki, W., Gruner, J., Raschdorf, F., Peter, H.H., 1990. Duramycins B and C, two new lanthionine containing antibiotics as inhibitors of phospholipase A2. Structural revision of duramycin and cinnamycin. J Antibiot. (Tokyo) 43, 1403-1412.

Furmanek, B., Kaczorowski, T., Bugalski, R., Bielawski, K., Bohdanowicz, J., Podhajska, A.J., 1999. Identification, characterization and purification of the lantibiotic staphylococcin T, a natural gallidermin variant. J Appl. Microbiol. 87, 856-866.

Galvez, A., Abriouel, H., Lopez, R.L., Ben Omar, N., 2007. Bacteriocin-based strategies for food biopreservation. Int. J Food Microbiol. 120, 51-70.

Gardete, S., Wu, S.W., Gill, S., Tomasz, A., 2006. Role of VraSR in antibiotic resistance and antibiotic-induced stress response in *Staphylococcus aureus*. Antimicrob. Agents Chemother. 50, 3424-3434.

Garrard, W., Lascelles, J., 1968. Regulation of *Staphylococcus aureus* lactate dehydrogenase. J. Bacteriol. 95, 152-156.

Geisel, R., Schmitz, F.J., Fluit, A.C., Labischinski, H., 2001. Emergence, mechanism, and clinical implications of reduced glycopeptide susceptibility in *Staphylococcus aureus*. Eur. J. Clin. Microbiol. Infect. Dis. 20, 685-697.

Georgalaki, M.D., Van Den, B.E., Kritikos, D., Devreese, B., Van Beeumen, J., Kalantzopoulos, G., De Vuyst, L., Tsakalidou, E., 2002. Macedocin, a food-grade lantibiotic produced by *Streptococcus macedonicus* ACA-DC 198. Appl. Environ. Microbiol. 68, 5891-5903.

Goering, R.V., Duensing, T.D., 1990. Rapid field inversion gel electrophoresis in combination with an rRNA gene probe in the epidemiological evaluation of staphylococci. J. Clin. Microbiol. 28, 426-429.

Goerke, C., Koller, J., Wolz, C., 2006. Ciprofloxacin and trimethoprim cause phage induction and virulence modulation in *Staphylococcus aureus*. Antimicrob. Agents Chemother. 50, 171-177.

Gordon, R.J., Lowy, F.D., 2008. Pathogenesis of methicillin-resistant *Staphylococcus aureus* infection. Clin. Infect. Dis. 46 Suppl 5, S350-S359.

Götz,F., Bannermann,T., Schleifer,K., 2007. The Genera *Staphylococcus* and *Macrococcus*. In: Dworkin,M., Falkow,S., Rosenberg,E., Schleifer,K., Stackebrandt,E. (Eds.), The Prokaryotes, 4, Springer Verlag, pp. 5-75.

Götz, F., 2002. *Staphylococcus* and biofilms. Mol. Microbiol. 43, 1367-1378.

Gross, E., Kiltz, H.H., Craig, L.C., 1973. Subtilin, II: the amino acid composition of subtilin. Hoppe Seylers. Z. Physiol Chem. 354, 799-801.

Gross, E., Morell, J.L., 1971. The structure of nisin. J Am. Chem. Soc. 93, 4634-4635.

Gross, M., Cramton, S.E., Götz, F., Peschel, A., 2001. Key role of teichoic acid net charge in *Staphylococcus aureus* colonization of artificial surfaces. Infect. Immun. 69, 3423-3426.

Gründling, A., Schneewind, O., 2006. Cross-linked peptidoglycan mediates lysostaphin binding to the cell wall envelope of *Staphylococcus aureus*. J. Bacteriol. 188, 2463-2472.

Guder, A., Schmitter, T., Wiedemann, I., Sahl, H.G., Bierbaum, G., 2002. Role of the single regulator MrsR1 and the two-component system MrsR2/K2 in the regulation of mersacidin production and immunity. Appl. Environ. Microbiol. 68, 106-113.

Hahn,H., Miksits,K., Gatermann,S., 2004. Staphylokokken. In: Hahn, Falke, Kaufmann, Ullmann (Eds.), Medizinische Mikrobiologie und Infektiologie, Springer, Berlin, pp. 187-198.

Hall-Stoodley, L., Costerton, J.W., Stoodley, P., 2004. Bacterial biofilms: from the natural environment to infectious diseases. Nat. Rev. Microbiol. 2, 95-108.

Hanaki, H., Kuwahara-Arai, K., Boyle-Vavra, S., Daum, R.S., Labischinski, H., Hiramatsu, K., 1998a. Activated cell-wall synthesis is associated with vancomycin resistance in methicillin-resistant *Staphylococcus aureus* clinical strains Mu3 and Mu50. J. Antimicrob. Chemother. 42, 199-209.

Hanaki, H., Labischinski, H., Inaba, Y., Kondo, N., Murakami, H., Hiramatsu, K., 1998b. Increase in glutamine-non-amidated muropeptides in the peptidoglycan of vancomycin-resistant *Staphylococcus aureus* strain Mu50. J. Antimicrob. Chemother. 42, 315-320.

Havarstein, L.S., Diep, D.B., Nes, I.F., 1995. A family of bacteriocin ABC transporters carry out proteolytic processing of their substrates concomitant with export. Mol. Microbiol. 16, 229-240.

He, Z., Kisla, D., Zhang, L., Yuan, C., Green-Church, K.B., Yousef, A.E., 2007. Isolation and identification of a *Paenibacillus polymyxa* strain that coproduces a novel lantibiotic and polymyxin. Appl. Environ. Microbiol. 73, 168-178.

Heidrich, C., Pag, U., Josten, M., Metzger, J., Jack, R.W., Bierbaum, G., Jung, G., Sahl, H.G., 1998. Isolation, characterization, and heterologous expression of the novel lantibiotic epicidin 280 and analysis of its biosynthetic gene cluster. Appl. Environ. Microbiol. 64, 3140-3146.

Heilmann, C., Gerke, C., Perdreau-Remington, F., Götz, F., 1996a. Characterization of Tn917 insertion mutants of *Staphylococcus epidermidis* affected in biofilm formation. Infect. Immun. 64, 277-282.

Heilmann, C., Hussain, M., Peters, G., Götz, F., 1997. Evidence for autolysin-mediated primary attachment of *Staphylococcus epidermidis* to a polystyrene surface. Mol. Microbiol. 24, 1013-1024.

Heilmann, C., Schweitzer, O., Gerke, C., Vanittanakom, N., Mack, D., Götz, F., 1996b. Molecular basis of intercellular adhesion in the biofilm-forming *Staphylococcus epidermidis*. Mol. Microbiol. 20, 1083-1091.

Heilmann, C., Thumm, G., Chhatwal, G.S., Hartleib, J., Uekotter, A., Peters, G., 2003. Identification and characterization of a novel autolysin (Aae) with adhesive properties from *Staphylococcus epidermidis*. Microbiology. 149, 2769-2778.

Herbert, S., Bera, A., Nerz, C., Kraus, D., Peschel, A., Goerke, C., Meehl, M., Cheung, A., Götz, F., 2007. Molecular basis of resistance to muramidase and cationic antimicrobial peptide activity of lysozyme in staphylococci. PLoS. Pathog. 3, e102.

Hillman, J.D., Novak, J., Sagura, E., Gutierrez, J.A., Brooks, T.A., Crowley, P.J., Hess, M., Azizi, A., Leung, K., Cvitkovitch, D., Bleiweis, A.S., 1998. Genetic and biochemical analysis of mutacin 1140, a lantibiotic from *Streptococcus mutans*. Infect. Immun. 66, 2743-2749.

Hoffmann, A., Pag, U., Wiedemann, I., Sahl, H.G., 2002. Combination of antibiotic mechanisms in lantibiotics. Farmaco 57, 685-691.

Holden, M.T., Feil, E.J., Lindsay, J.A., Peacock, S.J., Day, N.P., Enright, M.C., Foster, T.J., Moore, C.E., Hurst, L., Atkin, R., Barron, A., Bason, N., Bentley, S.D., Chillingworth, C., Chillingworth, T., Churcher, C., Clark, L., Corton, C., Cronin, A., Doggett, J., Dowd, L., Feltwell, T., Hance, Z., Harris, B., Hauser, H., Holroyd, S., Jagels, K., James, K.D., Lennard, N., Line, A., Mayes, R., Moule, S., Mungall, K., Ormond, D., Quail, M.A., Rabbinowitsch, E., Rutherford, K., Sanders, M., Sharp, S., Simmonds, M., Stevens, K., Whitehead, S., Barrell, B.G., Spratt, B.G., Parkhill, J., 2004. Complete genomes of two clinical *Staphylococcus aureus* strains: evidence for the rapid evolution of virulence and drug resistance. Proc. Natl. Acad. Sci. U. S. A 101, 9786-9791.

Holo, H., Jeknic, Z., Daeschel, M., Stevanovic, S., Nes, I.F., 2001. Plantaricin W from *Lactobacillus plantarum* belongs to a new family of two-peptide lantibiotics. Microbiology 147, 643-651.

Holtsmark, I., Mantzilas, D., Eijsink, V.G., Brurberg, M.B., 2006. Purification, characterization, and gene sequence of michiganin A, an actagardine-like lantibiotic produced by the tomato pathogen *Clavibacter michiganensis* subsp. *michiganensis*. Appl. Environ. Microbiol. 72, 5814-5821.

Howden, B.P., Stinear, T.P., Allen, D.L., Johnson, P.D., Ward, P.B., Davies, J.K., 2008. Genomics reveals a point mutation in the two-component sensor gene *graS* that leads to vancomycin-intermediate resistance in clinical *Staphylococcus aureus*. Antimicrob. Agents Chemother. doi:10.1128/AAC.01613-07.

Hsu, S.T., Breukink, E., Bierbaum, G., Sahl, H.G., de Kruijff, B., Kaptein, R., van Nuland, N.A., Bonvin, A.M., 2003. NMR study of mersacidin and lipid II interaction in dodecylphosphocholine micelles. Conformational changes are a key to antimicrobial activity. J. Biol. Chem. 278, 13110-13117.

Hussain, M., Haggar, A., Heilmann, C., Peters, G., Flock, J.I., Herrmann, M., 2002. Insertional inactivation of *eap* in *Staphylococcus aureus* strain Newman confers reduced staphylococcal binding to fibroblasts. Infect. Immun. 70, 2933-2940.

Hussain, M., Herrmann, M., von Eiff, C., Perdreau-Remington, F., Peters, G., 1997. A 140-kilodalton extracellular protein is essential for the accumulation of *Staphylococcus epidermidis* strains on surfaces. Infect. Immun. 65, 519-524.

Hyink, O., Balakrishnan, M., Tagg, J.R., 2005. *Streptococcus rattus* strain BHT produces both a class I two-component lantibiotic and a class II bacteriocin. FEMS Microbiol. Lett. 252, 235-241.

Hyink, O., Wescombe, P.A., Upton, M., Ragland, N., Burton, J.P., Tagg, J.R., 2007. Salivaricin A2 and the novel lantibiotic salivaricin B are encoded at adjacent loci on a 190-kilobase transmissible megaplasmid in the oral probiotic strain *Streptococcus salivarius* K12. Appl. Environ. Microbiol. 73, 1107-1113.

Iandolo, J.J., Worrell, V., Groicher, K.H., Qian, Y., Tian, R., Kenton, S., Dorman, A., Ji, H., Lin, S., Loh, P., Qi, S., Zhu, H., Roe, B.A., 2002. Comparative analysis of the genomes of the temperate bacteriophages phi 11, phi 12 and phi 13 of *Staphylococcus aureus* 8325. Gene. 289, 109-118.

Israil, A.M., Jack, R.W., Jung, G., Sahl, H.G., 1996. Isolation of a new epidermin variant from two strains of *Staphylococcus epidermidis*-frequency of lantibiotic production in coagulase-negative staphylococci. Zentralbl. Bakteriol. 284, 285-296.

Jack, R.W., Carne, A., Metzger, J., Stefanovic, S., Sahl, H.G., Jung, G., Tagg, J., 1994. Elucidation of the structure of SA-FF22, a lanthionine-containing antibacterial peptide produced by *Streptococcus pyogenes* strain FF22. Eur. J Biochem. 220, 455-462.

Jack, R.W., Tagg, J.R., Ray, B., 1995. Bacteriocins of Gram-positive bacteria. Microbiol. Rev. 59, 171-200.

Jansen, A., Türck, M., Szekat, C., Nagel, M., Clever, I., Bierbaum, G., 2007. Role of insertion elements and *yycFG* in the development of decreased susceptibility to vancomycin in *Staphylococcus aureus*. Int. J. Med. Microbiol. 297, 205-215.

Jefferson, K.K., Goldmann, D.A., Pier, G.B., 2005. Use of confocal microscopy to analyze the rate of vancomycin penetration through *Staphylococcus aureus* biofilms. Antimicrob. Agents Chemother. 49, 2467-2473.

Jenssen, H., Hamill, P., Hancock, R.E., 2006. Peptide antimicrobial agents. Clin. Microbiol. Rev. 19, 491-511.

Johnson, A.P., Aucken, H.M., Cavendish, S., Ganner, M., Wale, M.C., Warner, M., Livermore, D.M., Cookson, B.D., 2001. Dominance of EMRSA-15 and -16 among MRSA causing nosocomial bacteraemia in the UK: analysis of isolates from the

European Antimicrobial Resistance Surveillance System (EARSS). J. Antimicrob. Chemother. 48, 143-144.

Jordan, S., Hutchings, M.I., Mascher, T., 2008. Cell envelope stress response in Gram-positive bacteria. FEMS Microbiol. Rev. 32, 107-146.

Juuti, K.M., Sinha, B., Werbick, C., Peters, G., Kuusela, P.I., 2004. Reduced adherence and host cell invasion by methicillin-resistant *Staphylococcus aureus* expressing the surface protein Pls. J. Infect. Dis. 189, 1574-1584.

Kaletta, C., Entian, K.D., Kellner, R., Jung, G., Reis, M., Sahl, H.G., 1989. Pep5, a new lantibiotic: structural gene isolation and prepeptide sequence. Arch. Microbiol. 152, 16-19.

Kalmokoff, M.L., Lu, D., Whitford, M.F., Teather, R.M., 1999. Evidence for production of a new lantibiotic (butyrivibriocin OR79A) by the ruminal anaerobe *Butyrivibrio fibrisolvens* OR79: characterization of the structural gene encoding butyrivibriocin OR79A. Appl. Environ. Microbiol. 65, 2128-2135.

Kellner, R., Jung, G., Horner, T., Zahner, H., Schnell, N., Entian, K.D., Götz, F., 1988. Gallidermin: a new lanthionine-containing polypeptide antibiotic. Eur. J Biochem. 177, 53-59.

Kido, Y., Hamakado, T., Yoshida, T., Anno, M., Motoki, Y., Wakamiya, T., Shiba, T., 1983. Isolation and characterization of ancovenin, a new inhibitor of angiotensin I converting enzyme, produced by actinomycetes. J Antibiot. (Tokyo) 36, 1295-1299.

Kloos, W.E., Schleifer, K.H., 1975. Isolation and characterization of Staphylococci from human skin. II. Descriptions of four new species: *Staphylococcus warneri*, *Staphylococcus capitis*, *Staphylococcus hominis*, and *Staphylococcus simulans*. International Journal of Systematic Bacteriology 25(1), 62-79.

Kodani, S., Hudson, M.E., Durrant, M.C., Buttner, M.J., Nodwell, J.R., Willey, J.M., 2004. The SapB morphogen is a lantibiotic-like peptide derived from the product of the developmental gene *ramS* in *Streptomyces coelicolor*. Proc. Natl. Acad. Sci. U. S. A 101, 11448-11453.

Kodani, S., Lodato, M.A., Durrant, M.C., Picart, F., Willey, J.M., 2005. SapT, a lanthionine-containing peptide involved in aerial hyphae formation in the streptomycetes. Mol. Microbiol. 58, 1368-1380.

Koehl, J.L., Muthaiyan, A., Jayaswal, R.K., Ehlert, K., Labischinski, H., Wilkinson, B.J., 2004. Cell wall composition and decreased autolytic activity and lysostaphin susceptibility of glycopeptide-intermediate *Staphylococcus aureus*. Antimicrob. Agents Chemother. 48, 3749-3757.

Komiyama, K., Otoguro, K., Segawa, T., Shiomi, K., Yang, H., Takahashi, Y., Hayashi, M., Otani, T., Omura, S., 1993. A new antibiotic, cypemycin. Taxonomy, fermentation, isolation and biological characteristics. J Antibiot. (Tokyo) 46, 1666-1671.

Kraus, D., Herbert, S., Kristian, S.A., Khosravi, A., Nizet, V., Götz, F., Peschel, A., 2008. The GraRS regulatory system controls *Staphylococcus aureus* susceptibility to antimicrobial host defenses. BMC Microbiol. 8, 85.

Kreiswirth, B.N., Lofdahl, S., Betley, M.J., O'Reilly, M., Schlievert, P.M., Bergdoll, M.S., Novick, R.P., 1983. The toxic shock syndrome exotoxin structural gene is not detectably transmitted by a prophage. Nature 305, 709-712.

Kruszewska, D., Sahl, H.G., Bierbaum, G., Pag, U., Hynes, S.O., Ljungh, A., 2004. Mersacidin eradicates methicillin-resistant *Staphylococcus aureus* (MRSA) in a mouse rhinitis model. J. Antimicrob. Chemother. 54, 648-653.

Kuroda, M., Kuroda, H., Oshima, T., Takeuchi, F., Mori, H., Hiramatsu, K., 2003. Two-component system VraSR positively modulates the regulation of cell-wall biosynthesis pathway in *Staphylococcus aureus*. Mol. Microbiol. 49, 807-821.

Kuroda, M., Kuwahara-Arai, K., Hiramatsu, K., 2000. Identification of the up- and down-regulated genes in vancomycin-resistant *Staphylococcus aureus* strains Mu3 and Mu50 by cDNA differential hybridization method. Biochem. Biophys. Res. Commun. 269, 485-490.

Kuroda, M., Ohta, T., Uchiyama, I., Baba, T., Yuzawa, H., Kobayashi, I., Cui, L., Oguchi, A., Aoki, K., Nagai, Y., Lian, J., Ito, T., Kanamori, M., Matsumaru, H., Maruyama, A., Murakami, H., Hosoyama, A., Mizutani-Ui, Y., Takahashi, N.K., Sawano, T., Inoue, R., Kaito, C., Sekimizu, K., Hirakawa, H., Kuhara, S., Goto, S., Yabuzaki, J., Kanehisa, M., Yamashita, A., Oshima, K., Furuya, K., Yoshino, C., Shiba, T., Hattori, M., Ogasawara, N., Hayashi, H., Hiramatsu, K., 2001. Whole genome sequencing of methicillin-resistant *Staphylococcus aureus*. Lancet 357, 1225-1240.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680-685.

Lampilas, M. and Vogel, S., 1999. Moenomycin A derivates, their preparation, and use as antibacterial products. Patent number EP1069130.

Lawton, E.M., Ross, R.P., Hill, C., Cotter, P.D., 2007. Two-peptide lantibiotics: a medical perspective. Mini. Rev. Med Chem. 7, 1236-1247.

Lewandowski,Z., 2000. Structure and function of biofilms. In: Evans,L.V. (Ed.), Biofilms: recent advances in their study and control, Harwood Academic Publishers, Amsterdam, The Netherlands, pp. 1-17.

Li, M., Cha, D.J., Lai, Y., Villaruz, A.E., Sturdevant, D.E., Otto, M., 2007a. The antimicrobial peptide-sensing system *aps* of *Staphylococcus aureus*. Mol. Microbiol. 66, 1136-1147.

Li, M., Lai, Y., Villaruz, A.E., Cha, D.J., Sturdevant, D.E., Otto, M., 2007b. Gram-positive three-component antimicrobial peptide-sensing system. Proc. Natl. Acad. Sci. U. S. A 104, 9469-9474.

Lindsay, J.A., Holden, M.T., 2004. *Staphylococcus aureus*: superbug, super genome? Trends Microbiol. 12, 378-385.

Loessner, M.J., Gaeng, S., Wendlinger, G., Maier, S.K., Scherer, S., 1998. The two-component lysis system of *Staphylococcus aureus* bacteriophage Twort: a large TTG-start holin and an associated amidase endolysin. FEMS Microbiol. Lett. 162, 265-274.

Low, L.Y., Yang, C., Perego, M., Osterman, A., Liddington, R.C., 2005. Structure and lytic activity of a *Bacillus anthracis* prophage endolysin. J. Biol. Chem. 280, 35433-35439.

Lu, J.Z., Fujiwara, T., Komatsuzawa, H., Sugai, M., Sakon, J., 2006. Cell wall-targeting domain of glycylglycine endopeptidase distinguishes among peptidoglycan cross-bridges. J. Biol. Chem. 281, 549-558.

Mack, D., Fischer, W., Krokotsch, A., Leopold, K., Hartmann, R., Egge, H., Laufs, R., 1996a. The intercellular adhesin involved in biofilm accumulation of *Staphylococcus epidermidis* is a linear beta-1,6-linked glucosaminoglycan: purification and structural analysis. J Bacteriol. 178, 175-183.

Mack, D., Haeder, M., Siemssen, N., Laufs, R., 1996b. Association of biofilm production of coagulase-negative staphylococci with expression of a specific polysaccharide intercellular adhesin. J Infect. Dis. 174, 881-884.

Mah, T.F., O'Toole, G.A., 2001. Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol. 9, 34-39.

Maidhof, H., Johannsen, L., Labischinski, H., Giesbrecht, P., 1989. Onset of penicillin-induced bacteriolysis in staphylococci is cell cycle dependent. J. Bacteriol. 171, 2252-2257.

Majer, F., Schmid, D.G., Altena, K., Bierbaum, G., Kupke, T., 2002. The flavoprotein MrsD catalyzes the oxidative decarboxylation reaction involved in formation of the peptidoglycan biosynthesis inhibitor mersacidin. J. Bacteriol. 184, 1234-1243.

Maki, H., McCallum, N., Bischoff, M., Wada, A., Berger-Bächi, B., 2004. *tcaA* inactivation increases glycopeptide resistance in *Staphylococcus aureus*. Antimicrob. Agents Chemother. 48, 1953-1959.

Marrie, T.J., Nelligan, J., Costerton, J.W., 1982. A scanning and transmission electron microscopic study of an infected endocardial pacemaker lead. Circulation 66, 1339-1341.

Martin, N.I., Sprules, T., Carpenter, M.R., Cotter, P.D., Hill, C., Ross, R.P., Vederas, J.C., 2004. Structural characterization of lacticin 3147, a two-peptide lantibiotic with synergistic activity. Biochemistry 43, 3049-3056.

Mascher, T., 2006. Intramembrane-sensing histidine kinases: a new family of cell envelope stress sensors in Firmicutes bacteria. FEMS Microbiol. Lett. 264, 133-144.

Mascher, T., Helmann, J.D., Unden, G., 2006. Stimulus perception in bacterial signal-transducing histidine kinases. Microbiol. Mol. Biol. Rev. 70, 910-938.

McAleese, F., Wu, S.W., Sieradzki, K., Dunman, P., Murphy, E., Projan, S., Tomasz, A., 2006. Overexpression of genes of the cell wall stimulon in clinical isolates of *Staphylococcus aureus* exhibiting vancomycin-intermediate- *S. aureus*-type resistance to vancomycin. J. Bacteriol. 188, 1120-1133.

McCallum, N., Spehar, G., Bischoff, M., Berger-Bächi, B., 2006. Strain dependence of the cell wall-damage induced stimulon in *Staphylococcus aureus*. Biochim. Biophys. Acta 1760, 1475-1481.

McClerren, A.L., Cooper, L.E., Quan, C., Thomas, P.M., Kelleher, N.L., van der Donk, W.A., 2006. Discovery and in vitro biosynthesis of haloduracin, a two-component lantibiotic. Proc. Natl. Acad. Sci. U. S. A 103, 17243-17248.

Meehl, M., Herbert, S., Götz, F., Cheung, A., 2007. Interaction of the GraRS two-component system with the VraFG ABC transporter to support vancomycin-intermediate resistance in *Staphylococcus aureus*. Antimicrob. Agents Chemother. 51, 2679-2689.

Miller, D., Urdaneta, V., Weltman, A., Park, S., 2002. Vancomycin-resistant *Staphylococcus aureus*. Morb. Mortal. Wkly. Rep. 51, 902.

Mota-Meira, M., Lacroix, C., LaPointe, G., Lavoie, M.C., 1997. Purification and structure of mutacin B-Ny266: a new lantibiotic produced by *Streptococcus mutans*. FEBS Lett. 410, 275-279.

Mulders, J.W., Boerrigter, I.J., Rollema, H.S., Siezen, R.J., de Vos, W.M., 1991. Identification and characterization of the lantibiotic nisin Z, a natural nisin variant. Eur. J Biochem. 201, 581-584.

Murakami, H., Matsumaru, H., Kanamori, M., Hayashi, H., Ohta, T., 1999. Cell wall-affecting antibiotics induce expression of a novel gene, *drp35*, in *Staphylococcus aureus*. Biochem. Biophys. Res. Commun. 264, 348-351.

Muthaiyan, A., Silverman, J.A., Jayaswal, R.K., Wilkinson, B.J., 2008. Transcriptional profiling reveals that daptomycin induces the *Staphylococcus aureus* cell wall stress stimulon and genes responsive to membrane depolarization. Antimicrob. Agents Chemother. 52, 980-990.

Naumann, D. and Labischinski, H., 1990. Process and device for rapid testing of the effect of agents on micro-organisms. Patent number EP1069130.

Navaratna, M.A., Sahl, H.G., Tagg, J.R., 1998. Two-component anti-*Staphylococcus aureus* lantibiotic activity produced by *Staphylococcus aureus* C55. Appl. Environ. Microbiol. 64, 4803-4808.

Navarre, W.W., Schneewind, O., 1999. Surface proteins of gram-positive bacteria and mechanisms of their targeting to the cell wall envelope. Microbiol. Mol. Biol. Rev. 63, 174-229.

Navarre, W.W., Ton-That, H., Faull, K.F., Schneewind, O., 1999. Multiple enzymatic activities of the murein hydrolase from staphylococcal phage phi11. Identification of a D-alanyl-glycine endopeptidase activity. J. Biol. Chem. 274, 15847-15856.

Neoh, H.M., Cui, L., Yuzawa, H., Takeuchi, F., Matsuo, M., Hiramatsu, K., 2008. Mutated response regulator *graR* is responsible for phenotypic conversion of *Staphylococcus aureus* from heterogeneous vancomycin-intermediate resistance to vancomycin-intermediate resistance. Antimicrob. Agents Chemother. 52, 45-53.

Noble,W., 1997. Staphylococcal carriage and skin and soft tissue infection. In: Crossley,K.B., Archer,G.L. (Eds.), Staphylococcal carriage and skin and soft tissue infection, New York: Churchill Livingston, pp. 1-38.

North, E.A., Christie, R., 1946. Acquired resistance of staphylococci to the action of penicillin. Med J Australia 1, 176-179.

Novak, J., Caufield, P.W., Miller, E.J., 1994. Isolation and biochemical characterization of a novel lantibiotic mutacin from *Streptococcus mutans*. J Bacteriol. 176, 4316-4320.

O'Flaherty, S., Coffey, A., Meaney, W., Fitzgerald, G.F., Ross, R.P., 2005. The recombinant phage lysin LysK has a broad spectrum of lytic activity against clinically relevant staphylococci, including methicillin-resistant *Staphylococcus aureus*. J. Bacteriol. 187, 7161-7164.

O'Grady, N.P., Alexander, M., Dellinger, E.P., Gerberding, J.L., Heard, S.O., Maki, D.G., Masur, H., McCormick, R.D., Mermel, L.A., Pearson, M.L., Raad, I.I., Randolph, A., Weinstein, R.A., 2002. Guidelines for the prevention of intravascular catheter-related infections. The Hospital Infection Control Practices Advisory Committee, Center for Disese Control and Prevention, u.s. Pediatrics. 110, e51.

Pag, U., Oedenkoven, M., Sass, V., Shai, Y., Shamova, O., Antcheva, N., Tossi, A., Sahl, H.G., 2008. Analysis of in vitro activities and modes of action of synthetic antimicrobial peptides derived from an alpha-helical 'sequence template'. J. Antimicrob. Chemother. 61, 341-352.

Paik, S.H., Chakicherla, A., Hansen, J.N., 1998. Identification and characterization of the structural and transporter genes for, and the chemical and biological properties of, sublancin 168, a novel lantibiotic produced by *Bacillus subtilis* 168. J Biol. Chem. 273, 23134-23142.

Parisien, A., Allain, B., Zhang, J., Mandeville, R., Lan, C.Q., 2008. Novel alternatives to antibiotics: bacteriophages, bacterial cell wall hydrolases, and antimicrobial peptides. J Appl. Microbiol. 104, 1-13.

Perichon, B., Courvalin, P., 2006. Synergism between beta-lactams and glycopeptides against VanA-type methicillin-resistant *Staphylococcus aureus* and heterologous expression of the *vanA* operon. Antimicrob. Agents Chemother. 50, 3622-3630.

Peschel, A., Jack, R.W., Otto, M., Collins, L.V., Staubitz, P., Nicholson, G., Kalbacher, H., Nieuwenhuizen, W.F., Jung, G., Tarkowski, A., van Kessel, K.P., van Strijp, J.A., 2001. *Staphylococcus aureus* resistance to human defensins and evasion of neutrophil killing via the novel virulence factor MprF is based on modification of membrane lipids with l-lysine. J Exp. Med 193, 1067-1076.

Peschel, A., Ottenwalder, B., Götz, F., 1996. Inducible production and cellular location of the epidermin biosynthetic enzyme EpiB using an improved staphylococcal expression system. FEMS Microbiol. Lett. 137, 279-284.

Peschel, A., Otto, M., Jack, R.W., Kalbacher, H., Jung, G., Götz, F., 1999. Inactivation of the *dlt* operon in *Staphylococcus aureus* confers sensitivity to defensins, protegrins, and other antimicrobial peptides. J. Biol. Chem. 274, 8405-8410.

Peschel, A., Vuong, C., Otto, M., Götz, F., 2000. The D-alanine residues of *Staphylococcus aureus* teichoic acids alter the susceptibility to vancomycin and the activity of autolytic enzymes. Antimicrob. Agents Chemother. 44, 2845-2847.

Peters, G., Locci, R., Pulverer, G., 1981. Microbial colonization of prosthetic devices. II. Scanning electron microscopy of naturally infected intravenous catheters. Zentralbl. Bakteriol. Mikrobiol. Hyg. [B] 173, 293-299.

Peters,G., Pulverer,G., 2001. Die Familie der Micrococcaceae. In: Köhler, Eggers, Fleischer, Marre, Pfister, Pulverer (Eds.), Medizinische Mikrobiologie, Urban & Fischer, Jena, pp. 250-260.

Piard, J.C., Kuipers, O.P., Rollema, H.S., Desmazeaud, M.J., de Vos, W.M., 1993. Structure, organization, and expression of the *lct* gene for lacticin 481, a novel lantibiotic produced by *Lactococcus lactis*. J Biol. Chem. 268, 16361-16368.

Pöhn, H.-P., 1959. Zur Frage der Einheitlichkeit des Staphylokokken-Stammes "SG 511". Zentralbl. Bakteriol. Abt. Orig. 177, 432.

Prasch, T., Naumann, T., Markert, R.L., Sattler, M., Schubert, W., Schaal, S., Bauch, M., Kogler, H., Griesinger, C., 1997. Constitution and solution conformation of the antibiotic mersacidin determined by NMR and molecular dynamics. Eur. J. Biochem. 244, 501-512.

Pridmore, D., Rekhif, N., Pittet, A.C., Suri, B., Mollet, B., 1996. Variacin, a new lanthionine-containing bacteriocin produced by *Micrococcus varians*: comparison to lacticin 481 of *Lactococcus lactis*. Appl. Environ. Microbiol. 62, 1799-1802.

Proctor, R.A., Kahl, B., von Eiff, C., Vaudaux, P.E., Lew, D.P., Peters, G., 1998. Staphylococcal small colony variants have novel mechanisms for antibiotic resistance. Clin. Infect. Dis. 27 Suppl 1, S68-S74.

Proctor, R.A., Peters, G., 1998. Small colony variants in staphylococcal infections: diagnostic and therapeutic implications. Clin. Infect. Dis. 27, 419-422.

Pulverer, G., Grün, L., 1960. Studies on the differentiation of the *Staphylococcus* standard strain "SG 511". Z. Hyg. Infektionskr. 146, 537-543.

Putman, M., van Veen, H.W., Konings, W.N., 2000. Molecular properties of bacterial multidrug transporters. Microbiol. Mol. Biol. Rev. 64, 672-693.

Qi, F., Chen, P., Caufield, P.W., 1999. Functional analyses of the promoters in the lantibiotic mutacin II biosynthetic locus in *Streptococcus mutans*. Appl. Environ. Microbiol. 65, 652-658.

Qi, F., Chen, P., Caufield, P.W., 2000. Purification and biochemical characterization of mutacin I from the group I strain of *Streptococcus mutans*, CH43, and genetic analysis of mutacin I biosynthesis genes. Appl. Environ. Microbiol. 66, 3221-3229.

Reipert, A., Ehlert, K., Kast, T., Bierbaum, G., 2003. Morphological and genetic differences in two isogenic *Staphylococcus aureus* strains with decreased susceptibilities to vancomycin. Antimicrob. Agents Chemother. 47, 568-576.

Resch, A., Rosenstein, R., Nerz, C., Götz, F., 2005. Differential gene expression profiling of *Staphylococcus aureus* cultivated under biofilm and planktonic conditions. Appl. Environ. Microbiol. 71, 2663-2676.

Rice, K.C., Mann, E.E., Endres, J.L., Weiss, E.C., Cassat, J.E., Smeltzer, M.S., Bayles, K.W., 2007. The *cidA* murein hydrolase regulator contributes to DNA release and biofilm development in *Staphylococcus aureus*. Proc. Natl. Acad. Sci. U. S. A 104, 8113-8118.

Rietkötter, E., Hoyer, D., Mascher, T., 2008. Bacitracin sensing in *Bacillus subtilis*. Mol. Microbiol. 68, 768-785.

Roberts, M.E., Stewart, P.S., 2005. Modelling protection from antimicrobial agents in biofilms through the formation of persister cells. Microbiology 151, 75-80.

Robinson, D.A., Enright, M.C., 2003. Evolutionary models of the emergence of methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. 47, 3926-3934.

Robson, C.L., Wescombe, P.A., Klesse, N.A., Tagg, J.R., 2007. Isolation and partial characterization of the *Streptococcus mutans* type AII lantibiotic mutacin K8. Microbiology 153, 1631-1641.

Ronda-Lain, C., Lopez, R., Tapia, A., Tomasz, A., 1977. Role of the pneumococcal autolysin (murein hydrolase) in the release of progeny bacteriophage and in the bacteriophage-induced lysis of the host cells. J Virol. 21, 366-374.

Ross, K.F., Ronson, C.W., Tagg, J.R., 1993. Isolation and characterization of the lantibiotic salivaricin A and its structural gene *salA* from *Streptococcus salivarius* 20P3. Appl. Environ. Microbiol. 59, 2014-2021.

Ruef, C., 2004. Epidemiology and clinical impact of glycopeptide resistance in *Staphylococcus aureus*. Infection 32, 315-327.

Rupp, M.E., Fey, P.D., Heilmann, C., Götz, F., 2001. Characterization of the importance of *Staphylococcus epidermidis* autolysin and polysaccharide intercellular adhesin in the pathogenesis of intravascular catheter-associated infection in a rat model. J. Infect. Dis. 183, 1038-1042.

Sahl, H.G., Brandis, H., 1983. Efflux of low-Mr substances from the cytoplasm of sensitive cells caused by the staphylococcin-like agent Pep5. FEMS Microbiol. Lett. 16, 75-79.

Sahl, H.G., Bierbaum, G., 1998. Lantibiotics: biosynthesis and biological activities of uniquely modified peptides from gram-positive bacteria. Annu. Rev. Microbiol. 52, 41-79.

Sahl, H.G., Hahn, C., Brandis, H., 1985. Interaction of the staphylococcin-like peptide Pep 5 with cell walls and isolated cell wall components of Gram-positive bacteria. Zentralbl. Bakteriol. Mikrobiol. Hyg. [A] 260, 197-205.

Sahl, H.G., Jack, R.W., Bierbaum, G., 1995. Biosynthesis and biological activities of lantibiotics with unique post-translational modifications. Eur. J. Biochem. 230, 827-853.

Said-Salim, B., Dunman, P.M., McAleese, F.M., Macapagal, D., Murphy, E., McNamara, P.J., Arvidson, S., Foster, T.J., Projan, S.J., Kreiswirth, B.N., 2003. Global regulation of *Staphylococcus aureus* genes by Rot. J. Bacteriol. 185, 610-619.

Sakoulas, G., Eliopoulos, G.M., Fowler, V.G., Jr., Moellering, R.C., Jr., Novick, R.P., Lucindo, N., Yeaman, M.R., Bayer, A.S., 2005. Reduced susceptibility of *Staphylococcus aureus* to vancomycin and platelet microbicidal protein correlates with defective autolysis and loss of accessory gene regulator (*agr*) function. Antimicrob. Agents Chemother. 49, 2687-2692.

Sambrook, J., Fritsch, E. F., Maniatis, T., 1989. Molecular Cloning. A laboratory manual., Cold Spring Harbor, NY.

Sashihara, T., Kimura, H., Higuchi, T., Adachi, A., Matsusaki, H., Sonomoto, K., Ishizaki, A., 2000. A novel lantibiotic, nukacin ISK-1, of *Staphylococcus warneri* ISK-1: cloning of the structural gene and identification of the structure. Biosci. Biotechnol. Biochem. 64, 2420-2428.

Sass, P., 2005. Klonierung, Expression und Aktivitätsanalyse von Endolysinen aus *Staphylococcus aureus* NCTC8325 mit Untersuchungen zu deren Wirkung auf Staphylokokken-Biofilme. Diplomarbeit, Institut für Medizinische Mikrobiologie der Universität Bonn.

Sass, P., Bierbaum, G., 2007. Lytic activity of recombinant bacteriophage phi11 and phi12 endolysins on whole cells and biofilms of *Staphylococcus aureus*. Appl. Environ. Microbiol. 73, 347-352.

Sass, P., Jansen, A., Szekat, C., Sass, V., Sahl, H.G., Bierbaum, G., 2008a. The lantibiotic mersacidin is a strong inducer of the cell wall stress response of *Staphylococcus aureus*. BMC microbiology 8, 186.

Sass, V., Pag, U., Tossi, A., Bierbaum, G., Sahl H.G., 2008b. Mode of action of human beta-defensin 3 (hBD3) against *Staphylococcus aureus* and transcriptional analysis of responses to defensin challenge. Int. J. Med. Microbiol. 298, 619-633.

Schenk, S., Laddaga, R.A., 1992. Improved method for electroporation of *Staphylococcus aureus*. FEMS Microbiol. Lett. 73, 133-138.

Schindler, C.A., Schuhardt, V.T., 1964. Lysostaphin: a new bacteriolytic agent for the *Staphylococcus*. Proc. Natl. Acad. Sci. U. S. A 51, 414-421.

Schmitz, S., Hoffmann, A., Szekat, C., Rudd, B., Bierbaum, G., 2006. The lantibiotic mersacidin is an autoinducing peptide. Appl. Environ. Microbiol. 72, 7270-7277.

Schneider, T.R., Karcher, J., Pohl, E., Lubini, P., Sheldrick, G.M., 2000. *Ab initio* structure determination of the lantibiotic mersacidin. Acta Crystallogr. D. Biol. Crystallogr. 56, 705-713.

Shah, D., Zhang, Z., Khodursky, A., Kaldalu, N., Kurg, K., Lewis, K., 2006. Persisters: a distinct physiological state of *E. coli*. BMC Microbiol. 6, 53.

Shockman, G.D., Conover, M.J., Kolb, J.J., Riley, L.S., Toennies, G., 1961. Nutritional requirements for bacterial cell wall synthesis. J. Bacteriol. 81, 44-50.

Sieradzki, K., Tomasz, A., 1999. Gradual alterations in cell wall structure and metabolism in vancomycin-resistant mutants of *Staphylococcus aureus*. J. Bacteriol. 181, 7566-7570.

Sieradzki, K., Tomasz, A., 2003. Alterations of cell wall structure and metabolism accompany reduced susceptibility to vancomycin in an isogenic series of clinical isolates of *Staphylococcus aureus*. J. Bacteriol. 185, 7103-7110.

Sievert, D.M., Boulton, M.L., Stolman, G., Johnson, D., Stobierski, M.G., Downes, F.P., Somsel, P.A., Rudrik, T.J., Brown, W., Hafeez, W., Lundstrom, T., Flanagan, E., Johnson, R., Mitchell, J., Chang, S., 2002. *Staphylococcus aureus* resistant to vancomycin. Morb. Mortal. Wkly. Rep. 51, 565-567.

Simpson, W.J., Ragland, N.L., Ronson, C.W., Tagg, J.R., 1995. A lantibiotic gene family widely distributed in *Streptococcus salivarius* and *Streptococcus pyogenes*. Dev. Biol. Stand. 85, 639-643.

Skaugen, M., Nissen-Meyer, J., Jung, G., Stevanovic, S., Sletten, K., Inger, C., Abildgaard, M., Nes, I.F., 1994. *In vivo* conversion of L-serine to D-alanine in a ribosomally synthesized polypeptide. J Biol. Chem. 269, 27183-27185.

Sobral, R.G., Jones, A.E., Des Etages, S.G., Dougherty, T.J., Peitzsch, R.M., Gaasterland, T., Ludovice, A.M., de Lencastre, H., Tomasz, A., 2007. Extensive and genome-wide changes in the transcription profile of *Staphylococcus aureus* induced by modulating the transcription of the cell wall synthesis gene *murF*. J. Bacteriol. 189, 2376-2391.

Song, J.H., Hiramatsu, K., Suh, J.Y., Ko, K.S., Ito, T., Kapi, M., Kiem, S., Kim, Y.S., Oh, W.S., Peck, K.R., Lee, N.Y., 2004. Emergence in Asian countries of *Staphylococcus aureus* with reduced susceptibility to vancomycin. Antimicrob. Agents Chemother. 48, 4926-4928.

Souli, M., Giamarellou, H., 1998. Effects of slime produced by clinical isolates of coagulase-negative staphylococci on activities of various antimicrobial agents. Antimicrob. Agents Chemother. 42, 939-941.

Stein, T., Borchert, S., Conrad, B., Feesche, J., Hofemeister, B., Hofemeister, J., Entian, K.D., 2002. Two different lantibiotic-like peptides originate from the ericin gene cluster of *Bacillus subtilis* A1/3. J Bacteriol. 184, 1703-1711.

Stewart, P.S., 2002. Mechanisms of antibiotic resistance in bacterial biofilms. Int. J. Med. Microbiol. 292, 107-113.

Stoffels, G., Sahl, H.G., Gudmundsdottir, A., 1993. Carnocin UI49, a potential biopreservative produced by *Carnobacterium piscicola*: large scale purification and activity against various gram-positive bacteria including *Listeria* sp. Int. J Food Microbiol. 20, 199-210.

Studier, F.W., Moffatt, B.A., 1986. Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. J. Mol. Biol. 189, 113-130.

Suci, P.A., Mittelman, M.W., Yu, F.P., Geesey, G.G., 1994. Investigation of ciprofloxacin penetration into *Pseudomonas aeruginosa* biofilms. Antimicrob. Agents Chemother. 38, 2125-2133.

Szekat, C., Jack, R.W., Skutlarek, D., Farber, H., Bierbaum, G., 2003. Construction of an expression system for site-directed mutagenesis of the lantibiotic mersacidin. Appl. Environ. Microbiol. 69, 3777-3783.

Takano, M., Oshida, T., Yasojima, A., Yamada, M., Okagaki, C., Sugai, M., Suginaka, H., Matsushita, T., 2000. Modification of autolysis by synthetic peptides derived from the presumptive binding domain of *Staphylococcus aureus* autolysin. Microbiol. Immunol. 44, 463-472.

Tenover, F.C., McDougal, L.K., Goering, R.V., Killgore, G., Projan, S.J., Patel, J.B., Dunman, P.M., 2006. Characterization of a strain of community-associated methicillin-resistant *Staphylococcus aureus* widely disseminated in the United States. J Clin. Microbiol. 44, 108-118.

Turner, D.L., Brennan, L., Meyer, H.E., Lohaus, C., Siethoff, C., Costa, H.S., Gonzalez, B., Santos, H., Suarez, J.E., 1999. Solution structure of plantaricin C, a novel lantibiotic. Eur. J Biochem. 264, 833-839.

Tusher, V.G., Tibshirani, R., Chu, G., 2001. Significance analysis of microarrays applied to the ionizing radiation response. Proc. Natl. Acad. Sci. U. S. A 98, 5116-5121.

Udo, E.E., Pearman, J.W., Grubb, W.B., 1993. Genetic analysis of community isolates of methicillin-resistant *Staphylococcus aureus* in Western Australia. J Hosp. Infect. 25, 97-108.

Ueda, K., Oinuma, K., Ikeda, G., Hosono, K., Ohnishi, Y., Horinouchi, S., Beppu, T., 2002. AmfS, an extracellular peptidic morphogen in *Streptomyces griseus*. J Bacteriol. 184, 1488-1492.

Utaida, S., Dunman, P.M., Macapagal, D., Murphy, E., Projan, S.J., Singh, V.K., Jayaswal, R.K., Wilkinson, B.J., 2003. Genome-wide transcriptional profiling of the response of *Staphylococcus aureus* to cell-wall-active antibiotics reveals a cell-wall-stress stimulon. Microbiology 149, 2719-2732.

Utaida, S., Pfeltz, R.F., Jayaswal, R.K., Wilkinson, B.J., 2006. Autolytic properties of glycopeptide-intermediate *Staphylococcus aureus* Mu50. Antimicrob. Agents Chemother. 50, 1541-1545.

van de, K.M., van den Hooven, H.W., Konings, R.N., Bierbaum, G., Sahl, H.G., Kuipers, O.P., Siezen, R.J., de Vos, W.M., Hilbers, C.W., van de Ven, F.J., 1995. Elucidation of the primary structure of the lantibiotic epilancin K7 from *Staphylococcus epidermidis* K7. Cloning and characterisation of the epilancin-K7-encoding gene and NMR analysis of mature epilancin K7. Eur. J Biochem. 230, 587-600.

van Heijenoort, J., 2001. Formation of the glycan chains in the synthesis of bacterial peptidoglycan. Glycobiology 11, 25-36.

Vandenesch, F., Naimi, T., Enright, M.C., Lina, G., Nimmo, G.R., Heffernan, H., Liassine, N., Bes, M., Greenland, T., Reverdy, M.E., Etienne, J., 2003. Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerg. Infect. Dis. 9, 978-984.

Vertesy, L., Aretz, W., Bonnefoy, A., Ehlers, E., Kurz, M., Markus, A., Schiell, M., Vogel, M., Wink, J., Kogler, H., 1999. Ala(0)-actagardine, a new lantibiotic from cultures of *Actinoplanes liguriae* ATCC 31048. J Antibiot. (Tokyo) 52, 730-741.

Vieira, J., Messing, J., 1982. The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene 19, 259-268.

von Eiff, C., Heilmann, C., Herrmann, M., Peters, G., 1999. Basic aspects of the pathogenesis of staphylococcal polymer-associated infections. Infection 27 Suppl 1, S7-10.

Wescombe, P.A., Tagg, J.R., 2003. Purification and characterization of streptin, a type A1 lantibiotic produced by *Streptococcus pyogenes*. Appl. Environ. Microbiol. 69, 2737-2747.

Wescombe, P.A., Upton, M., Dierksen, K.P., Ragland, N.L., Sivabalan, S., Wirawan, R.E., Inglis, M.A., Moore, C.J., Walker, G.V., Chilcott, C.N., Jenkinson, H.F., Tagg, J.R., 2006. Production of the lantibiotic salivaricin A and its variants by oral streptococci and use of a specific induction assay to detect their presence in human saliva. Appl. Environ. Microbiol. 72, 1459-1466.

Wiedemann, I., Benz, R., Sahl, H.G., 2004. Lipid II-mediated pore formation by the peptide antibiotic nisin: a black lipid membrane study. J. Bacteriol. 186, 3259-3261.

Wiedemann, I., Breukink, E., van Kraaij, C., Kuipers, O.P., Bierbaum, G., de Kruijff, B., Sahl, H.G., 2001. Specific binding of nisin to the peptidoglycan precursor lipid II combines pore formation and inhibition of cell wall biosynthesis for potent antibiotic activity. J. Biol. Chem. 276, 1772-1779.

Wilaipun, P., Zendo, T., Okuda, K., Nakayama, J., Sonomoto, K., 2008. Identification of the nukacin KQU-131, a new type-A(II) lantibiotic produced by *Staphylococcus hominis* KQU-131 isolated from Thai fermented fish product (Pla-ra). Biosci. Biotechnol. Biochem. 72, 2232-2235.

Willey, J.M., van der Donk, W.A., 2007. Lantibiotics: peptides of diverse structure and function. Annu. Rev. Microbiol. 61, 477-501.

Wiltshire, M.D., Foster, S.J., 2001. Identification and analysis of *Staphylococcus aureus* components expressed by a model system of growth in serum. Infect. Immun. 69, 5198-5202.

Wink, J., 2002. Vancoresmycin, a process for its production and its use as a pharmaceutical. Patent number EP1129208.

Wirawan, R.E., Klesse, N.A., Jack, R.W., Tagg, J.R., 2006. Molecular and genetic characterization of a novel nisin variant produced by *Streptococcus uberis*. Appl. Environ. Microbiol. 72, 1148-1156.

Wolz, C., McDevitt, D., Foster, T.J., Cheung, A.L., 1996. Influence of agr on fibrinogen binding in *Staphylococcus aureus* Newman. Infect. Immun. 64, 3142-3147.

Woods, C.W., Cheng, A.C., Fowler, V.G., Jr., Moorefield, M., Frederick, J., Sakoulas, G., Meka, V.G., Tenover, F.C., Zwadyk, P., Wilson, K.H., 2004. Endocarditis caused by *Staphylococcus aureus* with reduced susceptibility to vancomycin. Clin. Infect. Dis. 38, 1188-1191.

Wootton, M., Macgowan, A.P., Walsh, T.R., 2005. Expression of *tcaA* and *mprF* and glycopeptide resistance in clinical glycopeptide-intermediate *Staphylococcus aureus* (GISA) and heteroGISA strains. Biochim. Biophys. Acta 1726, 326-327.

Wu, J.A., Kusuma, C., Mond, J.J., Kokai-Kun, J.F., 2003. Lysostaphin disrupts Staphylococcus aureus and Staphylococcus epidermidis biofilms on artificial surfaces. Antimicrob. Agents Chemother. 47, 3407-3414.

Xiao, H., Chen, X., Chen, M., Tang, S., Zhao, X., Huan, L., 2004. Bovicin HJ50, a novel lantibiotic produced by *Streptococcus bovis* HJ50. Microbiology 150, 103-108.

Yang, D., Biragyn, A., Kwak, L.W., Oppenheim, J.J., 2002. Mammalian defensins in immunity: more than just microbicidal. Trends Immunol. 23, 291-296.

Yanisch-Perron, C., Vieira, J., Messing, J., 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene. 33, 103-119.

Yin, S., Daum, R.S., Boyle-Vavra, S., 2006. VraSR two-component regulatory system and its role in induction of *pbp2* and *vraSR* expression by cell wall antimicrobials in *Staphylococcus aureus*. Antimicrob. Agents Chemother. 50, 336-343.

Yocum, R.R., Rasmussen, J.R., Strominger, J.L., 1980. The mechanism of action of penicillin. Penicillin acylates the active site of *Bacillus stearothermophilus* D-alanine carboxypeptidase. J Biol. Chem. 255, 3977-3986.

Yocum, R.R., Waxman, D.J., Rasmussen, J.R., Strominger, J.L., 1979. Mechanism of penicillin action: penicillin and substrate bind covalently to the same active site serine in two bacterial D-alanine carboxypeptidases. Proc. Natl. Acad. Sci. U. S. A 76, 2730-2734.

Yokoi, K.J., Kawahigashi, N., Uchida, M., Sugahara, K., Shinohara, M., Kawasaki, K., Nakamura, S., Taketo, A., Kodaira, K., 2005. The two-component cell lysis genes holWMY and lysWMY of the *Staphylococcus warneri* M phage varphiWMY: cloning, sequencing, expression, and mutational analysis in *Escherichia coli*. Gene. 351, 97-108.

Yonezawa, H., Kuramitsu, H.K., 2005. Genetic analysis of a unique bacteriocin, Smb, produced by *Streptococcus mutans* GS5. Antimicrob. Agents Chemother. 49, 541-548.

Young, I., Wang, I., Roof, W.D., 2000. Phages will out: strategies of host cell lysis. Trends Microbiol. 8, 120-128.

Zendo, T., Fukao, M., Ueda, K., Higuchi, T., Nakayama, J., Sonomoto, K., 2003. Identification of the lantibiotic nisin Q, a new natural nisin variant produced by *Lactococcus lactis* 61-14 isolated from a river in Japan. Biosci. Biotechnol. Biochem. 67, 1616-1619.

Zimmermann, N., Metzger, J.W., Jung, G., 1995. The tetracyclic lantibiotic actagardine. 1H-NMR and 13C-NMR assignments and revised primary structure. Eur. J Biochem. 228, 786-797.

7 Supplemental material

Table S1: Genes with significantly altered expression of *S. aureus* SA137/93G upon mersacidin treatment[1]

N315 ORF	Gene	Gene product function	Fold change[2] SA137/93G (0.15 x MIC)
Cell wall			
SA0205		hypothetical protein, similar to lysostaphin precursor	2.16
SA1691	*sgtB*	hypothetical protein, similar to penicillin-binding protein 1A/1B	4.79
SA2481		conserved hypothetical protein	2.57
Transport/binding proteins and lipoproteins			
SA0192		hypothetical protein, similar to ABC transporter ATP-binding protein	11.96
SA0567		hypothetical protein, similar to iron (III) ABC transporter permease protein	0.27
SA1255		PTS system, glucose-specific enzyme II, A component	2.60
SA1478		hypothetical protein, similar to transporter PAB2175 from *Pyrococcus abyssi*	3.19
SA2112		hypothetical protein, similar to sodium-dependent transporter	0.37
SA2492	*vraD*	hypothetical protein, similar to ABC transporter	34.28
SA2493	*vraE*	hypothetical protein, similar to ABC transporter (permease)	35.07
Sensors (signal transduction)			
SA1701	*vraS*	two-component sensor histidine kinase	5.84
Metabolism related genes			
SA0515		hypothetical protein, similar to deoxypurine kinase	0.18
SA0534	*vraB*	acetyl-CoA c-acetyltransferase	4.62
SA1301	*ndk*	nucleoside diphosphate kinase	0.44
SA1925		conserved hypothetical protein	3.50
SA2297		hypothetical protein, similar to GTP-pyrophosphokinase	3.91
DNA replication, RNA and protein synthesis			
SA0442	*holB*	probable DNA polymerase III, delta prime subunit	0.22
SA1195	*msrR*	peptide methionine sulfoxide reductase regulator MsrR	3.63
SA1659	*prsA*	peptidyl-prolyl cis/trans isomerase homologue	9.97
SA1700	*vraR*	two-component response regulator	7.27
SA2296		hypothetical protein, similar to transcriptional regulator, MerR family	3.94
Adaption to atypical conditions			
SA1549		hypothetical protein, similar to serine proteinase, heat-shock protein *htrA*	3.94
Pathogenic factors (toxins and colonization factors)			
SA0519	*sdrC*	Ser-Asp rich fibrinogen-binding, bone sialoprotein-binding protein	0.50
SA0909	*fmtA*	FmtA, autolysis and methicillin resistant-related protein	6.28
SA2006		hypothetical protein, similar to MHC class II analog	2.76
Hypothetical proteins			
SA0275		conserved hypothetical protein	0.61
SA0329		conserved hypothetical protein	0.36
SA0358		conserved hypothetical protein	11.08
SA0364		hypothetical protein	0.28
SA0467		conserved hypothetical protein	0.36
SA0517		conserved hypothetical protein	0.43
SA0535	*vraC*	hypothetical protein	4.24
SA0536		hypothetical protein	8.58
SA0591		hypothetical protein	4.15
SA1256		conserved hypothetical protein	2.83
SA1419		conserved hypothetical protein	0.37
SA1476		hypothetical protein	6.52
SA1702		conserved hypothetical protein	4.42
SA1703		hypothetical protein	6.82

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Table S1 - *Continued*

N315 ORF	Gene	Gene product function	Fold change[2] SA137/93G (0.15 x MIC)
SA1712		conserved hypothetical protein	5.03
SA2113		hypothetical protein	4.91
SA2146	*tcaA*	TcaA protein	2.20
SA2195		conserved hypothetical protein	1.75
SA2220		conserved hypothetical protein	3.66
SA2221		hypothetical protein	5.24
SA2238		conserved hypothetical protein	1.99
SA2480	*drp35*	Drp35	2.84
SAS025		hypothetical protein	1.91

[1] Significant changes of gene expression were determined by implementing SAM (significance analysis of microarrays; http://www-stat.stanford.edu/~tibs/SAM/) (Tusher et al., 2001).

[2] Fold change in transcript level indicated as mean of the "median of ratios" compared to control cells.

Table S2: Comparative gene expression analysis of *S. aureus* strain SG511-Berlin versus strain SA137/93A

N315 ORF	Gene	Gene product function	Fold change SG511-Berlin/ SA137/93A [1]
Cell wall			
SA0038	*mecA*	penicillin binding protein 2a	0.11
SA0265	*lytM*	peptidoglycan hydrolase	0.38
SA1935	*hmrA*	similar to amidase (HmrA)	0.40
SA2199	*fmhA*	*fmhA* protein	7.00
Transformation competence			
SA0705		hypothetical protein, similar to *comF* operon protein 1	0.48
Transport/binding proteins and lipoproteins			
SA0099		hypothetical protein, similar to transmembrane efflux pump protein	0.14
SA0106	*lctP*	L-lactate permease homologue	0.16
SA0109	*sirC*	lipoprotein	0.21
SA0183	*glcA*	PTS enzyme II (EC 2.7.1.69), glucose-specific, factor IIA homologue	4.27
SA0197		hypothetical protein, similar to ABC transporter ATP-binding protein	0.18
SA0198	*oppF*	oligopeptide transport ATP-binding protein	2.05
SA0294		hypothetical protein, similar to branched-chain amino acid uptake carrier	0.45
SA0295		hypothetical protein, similar to outer membrane protein precursor	2.97
SA0325	*glpT*	glycerol-3-phosphate transporter	2.45
SA0368		hypothetical protein, similar to proton/sodium-glutamate symport protein	5.14
SA0420		hypothetical protein, similar to ABC transporter ATP-binding protein	0.21
SA0421		hypothetical protein, similar to ABC transporter permease protein	0.18
SA0422		hypothetical protein, similar to lactococcal lipoprotein	0.22
SA0531	*proP*	proline/betaine transporter homologue	0.35
SA0541		hypothetical protein, similar to cationic amino acid transporter	0.32
SA0566		hypothetical protein, similar to iron-binding protein	2.34
SA0589		hypothetical protein, similar to ABC transporter ATP-binding protein	8.99
SA0616	*vraF*	ABC transporter ATP-binding protein	0.11
SA0617	*vraG*	ABC transporter permease	0.21
SA0691		lipoprotein, similar to ferrichrome ABC transporter	0.33
SA0769		ABC transporter ATP-binding protein homologue	0.32
SA0794	*dltB*	DltB membrane protein	0.49
SA0845	*oppB*	oligopeptide transport system permease protein	0.47
SA0848	*oppF*	oligopeptide transport system ATP-binding protein OppF homologue	0.43
SA0928		hypothetical protein, similar to cation ABC transporter	17.55
SA0956		hypothetical protein, similar to Mn^{2+}-transport protein	0.11
SA1042	*pyrP*	uracil permease	7.49
SA1140	*glpF*	glycerol uptake facilitator	2.89
SA1183	*opuD*	glycine betaine transporter	2.16
SA1341		hypothetical protein, similar to export protein SpcT protein	2.46
SA1505	*lysP*	lysine-specific permease	2.12
SA1592		aesenical pump membrane protein homologue	2.30
SA1634		truncated hypothetical protein [Pathogenicity island SaPIn3]	0.31
SA1699		hypothetical protein, similar to transporter	0.23
SA1958		hypothetical protein, similar to transposase for IS*232*	0.40
SA1962	*mtlA*	PTS system, mannitol specific IIA component	0.17
SA1972		hypothetical protein, similar to multidrug transporter	0.20
SA1978		hypothetical protein, similar to ferrichrome ABC transporter (permease)	0.43
SA1987		glycine betaine transporter *opuD* homologue	0.18
SA2061		hypothetical protein	2.20
SA2081		hypothetical protein, similar to urea transporter	0.32
SA2094		hypothetical protein, similar to Na^+/H^+ antiporter	0.26

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Table S2 - *Continued*

N315 ORF	Gene	Gene product function	Fold change SG511-Berlin/ SA137/93A [1]
SA2135		hypothetical protein, similar to sodium/glutamate symporter	4.34
SA2148		hypothetical protein, similar to membrane protein	0.23
SA2167	*scrA*	PTS system, sucrose-specific IIBC component	2.86
SA2200		hypothetical protein, similar to ABC transporter, ATP binding subunit	3.93
SA2201		hypothetical protein, similar to ABC transporter, permease protein	2.91
SA2202		hypothetical protein, similar to ABC transporter	3.30
SA2242		conserved hypothetical protein	0.19
SA2243		hypothetical protein, similar to ABC transporter ATP-binding protein	0.16
SA2300		hypothetical protein, similar to glucarate transporter	4.03
SA2302		hypothetical protein, similar to ABC transporter	4.48
SA2303		hypothetical protein, similar to membrane spanning protein	5.44
SA2314		hypothetical protein, similar to ABC transporter ATP-binding protein	0.45
SA2326	*ptsG*	PTS system, glucose-specific IIABC component	3.40
SA2339		hypothetical protein, similar to antibiotic transport-associated protein	5.64
SA2345		hypothetical protein, similar to mercuric ion-binding protein	2.20
SA2396		hypothetical protein, similar to amino acid transporter	0.40
SA2489		hypothetical protein, similar to high-affinity nickel-transport protein	0.31
Sensors (signal transduction)			
SA0018	*vicK*	two-component sensor histidine kinase	0.11
SA0039	*mecR1*	methicillin resistance protein	0.08
SA0615	*graS*	hypothetical protein, similar to two-component sensor histidine kinase	0.36
SA1158		hypothetical protein, similar to two-component sensor histidine kinase	0.36
SA1653	*TRAP*	signal transduction protein TRAP	0.06
SA1843	*agrC*	accessory gene regulator C	0.08
Membrane bioenergetics (electron transport chain and ATP synthase)			
SA0366	*ahpC*	alkyl hydroperoxide reductase subunit C	2.19
SA0578		hypothetical protein, similar to NADH dehydrogenase	0.39
SA0579		hypothetical protein, similar to Na+/H+ antiporter	0.33
SA0580		hypothetical protein, similar to Na+/H+ antiporter	0.27
SA0583		hypothetical protein, similar to Na+/H+ antiporter	0.35
SA0799		hypothetical protein, similar to NADH dehydrogenase	2.06
SA0910		hypothetical protein, similar to quinol oxidase polypeptide IV QoxD	3.53
SA0911	*qoxC*	Quinol oxidase polypeptide III QoxC	2.95
SA0912	*qoxB*	Quinol oxidase polypeptide I QoxB	3.20
SA0913		hypothetical protein, similar to quinol oxidase polypeptide II QoxA	2.59
SA1904	*atpC*	FoF1-ATP synthase epsilon subunit	2.02
Cell division			
SA0249	*scdA*	cell division and morphogenesis-related protein	2.59
SA0724		hypothetical protein, similar to cell-division inhibitor	0.25
SA0905	*atl*	autolysin, N-acetylmuramyl-L-alanine amidase and glucosaminidase	0.38
Sporulation			
SA2498		hypothetical protein, similar to DNA-binding protein Spo0J-like homologue	2.76
Metabolism of carbohydrates and related molecules - Specific pathways			
SA0242		hypothetical protein, similar to xylitol dehydrogenase	2.19
SA0510	*araB*	probable L-ribulokinase	2.01
SA0528		hypothetical protein, similar to hexulose-6-phosphate synthase	0.31
SA0562	*adh1*	alcohol dehydrogenase I	46.70
SA0605		hypothetical protein, similar to dihydroxyacetone kinase	2.43
SA0658		hypothetical protein, similar to plant-metabolite dehydrogenases	0.41
SA0669		hypothetical protein, similar to para-aminobenzoate synthase component I	0.23
SA0670		hypothetical protein, similar to para-aminobenzoate synthase component I	0.27

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Table S2 - *Continued*

N315 ORF	Gene	Gene product function	Fold change SG511-Berlin/ SA137/93A [1]
SA0946	*pdhD*	dihydrolipoamide dehydrogenase component of pyruvate dehydrogenase E3	0.30
SA0994	*sdhC*	succinate dehydrogenase cytochrome b-558	2.07
SA0995	*sdhA*	succinate dehydrogenase flavoprotein subunit	2.35
SA1736	*aldH*	aldehyde dehydrogenase	0.21
SA1913	*mnaA*	UDP-GlcNAc 2-epimerase	0.41
SA1924		hypothetical protein, similar to aldehyde dehydrogenase	0.40
SA1963	*mtlD*	mannitol-1-phosphate 5-dehydrogenase	0.22
SA1965	*glmM*	phosphoglucosamine-mutase	0.43
SA2102		formate dehydrogenase homologue	0.29
SA2119		hypothetical protein, similar to dehydrogenase	0.21
SA2155		hypothetical protein, similar to malate:quinone oxidoreductase	2.01
SA2204		phosphoglycerate mutase, *pgm* homologue	5.58
SA2260		hypothetical protein, similar to glucose 1-dehydrogenase	0.28
SA2342		hypothetical protein, similar to O-acetyltransferase	4.73
SA2346		hypothetical protein, similar to D-specific D-2-hydroxyacid dehydrogenase *ddh*	0.33
SA2394		hypothetical protein, similar to alpha-acetolactate decarboxylase	2.12
SA2395		L-lactate dehydrogenase	2.24
SA2402		acetate-CoA ligase	0.42
Metabolism of amino acids and related molecules			
SA0098		hypothetical protein, similar to aminoacylase	0.14
SA0176		hypothetical protein, similar to N-acetylglutamate 5-phosphotransferase	0.42
SA0344	*metE*	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase	0.27
SA0346		hypothetical protein, similar to cystathionine beta-lyase	0.39
SA0347		hypothetical protein, similar to cystathionine gamma-synthase	0.36
SA0418	*cysM*	cysteine synthase homologue	0.36
SA0471	*cysK*	cysteine synthase (o-acetylserine sulfhydrylase) homologue	3.27
SA0508		hypothetical protein, similar to glycine C-acetyltransferase	2.14
SA0668		hypothetical protein, similar to anthranilate synthase component II	0.26
SA0672		hypothetical protein, similar to urea amidolyase	0.40
SA0902		HisC homologue	0.49
SA1164	*dhoM*	homoserine dehydrogenase	0.40
SA1165	*thrC*	threonine synthase	0.44
SA1200	*trpG*	anthranilate synthase component II	0.39
SA1201	*trpD*	anthranilate phosphoribosyltransferase	0.35
SA1202	*trpC*	indole-3-glycerol phosphate synthase	0.45
SA1203	*trpF*	phosphoriborylanthranilate isomerase	0.45
SA1204	*trpB*	tryptophan synthase beta chain	0.46
SA1205	*trpA*	tryptophan synthase alpha chain	0.47
SA1343		hypothetical protein, similar to tripeptidase	0.42
SA1365		glycine dehydrogenase (decarboxylating) subunit 2 homologue	0.40
SA1545	*serA*	D-3-phosphoglycerate dehydrogenase	0.47
SA1585		proline dehydrohenase homologue	0.47
SA1814		hypothetical protein, similar to succinyl-diaminopimelate desuccinylase	0.32
SA1859	*ilvB*	acetolactate synthase large subunit	0.40
SA1861	*ilvC*	alpha-keto-beta-hydroxylacil reductoisomerase	0.28
SA1862	*leuA*	2-isopropylmalate synthase	0.38
SA1863	*leuB*	3-isopropylmalate dehydrogenase	0.43
SA1864	*leuC*	3-isopropylmalate dehydratase large subunit	0.38
SA1865	*leuD*	3-isopropylmalate dehydratase small subunit	0.41
SA2082	*ureA*	urease gamma subunit	0.07
SA2083	*ureB*	urease beta subunit	0.04

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Table S2 - *Continued*

N315 ORF	Gene	Gene product function	Fold change SG511-Berlin/ SA137/93A [1]
SA2084	*ureC*	urease alpha subunit	0.07
SA2085	*ureE*	urease accessory protein UreE	0.11
SA2086	*ureF*	urease accessory protein UreF	0.08
SA2087	*ureG*	urease accessory protein UreG	0.13
SA2088	*ureD*	urease accessory protein UreD	0.15
SA2095		hypothetical protein, similar to D-octopine dehydrogenase	0.40
SA2098		hypothetical protein, similar to glycerate dehydrogenase	2.59
SA2125		hypothetical protein, similar to formiminoglutamase	0.27
SA2341	*rocA*	1-pyrroline-5-carboxylate dehydrogenase	2.51
SA2347		hypothetical protein, similar to aspartate aminotransferase	0.38
SA2425	*arcC*	carbamate kinase	3.17
Metabolism of nucleotides and nucleic acids			
SA0016	*purA*	adenylosuccinate synthase	2.34
SA0022		hypothetical protein, similar to 5'-nucleotidase	2.56
SA0687	*nrdF*	ribonucleoside-diphosphate reductase minor subunit	0.41
SA0917	*purK*	phosphoribosylaminoimidazole carboxylase carbon dioxide-fixation chain PurK	0.36
SA0918	*purC*	phosphoribosylaminoimidazolesuccinocarboxamide synthetase homologue	0.40
SA0920	*purQ*	phosphoribosylformylglycinamidine synthase I PurQ	0.20
SA0921	*purL*	phosphoribosylformylglycinamidine synthetase PurL	0.23
SA0922	*purF*	phosphoribosylpyrophosphate amidotransferase PurF	0.25
SA0923	*purM*	phosphoribosylformylglycinamidine cyclo-ligase PurM	0.23
SA0924	*purN*	phosphoribosylglycinamide formyltransferase	0.26
SA0925	*purH*	bifunctional purine biosynthesis protein PurH	0.19
SA0926	*purD*	phosphoribosylamine--glycine ligase PurD	0.39
SA0927		conserved hypothetical protein	16.03
SA1043	*pyrB*	aspartate transcarbamoylase chain A	8.36
SA1044	*pyrC*	dihydroorotase	10.04
SA1045	*pyrAA*	carbamoyl-phosphate synthase small chain	8.50
SA1046	*pyrAB*	carbamoyl-phosphate synthase large chain	8.64
SA1047	*pyrF*	orotidine-5-phosphate decarboxylase	8.68
SA1048	*pyrE*	orotate phosphoribosyltransferase	8.93
SA1172		hypothetical protein, similar to GMP reductase	2.06
SA1260	*thyA*	thymidylate synthase	0.47
SA1938	*pdp*	pyrimidine nucleoside phosphorylase	3.45
SA2127		hypothetical protein, similar to ribose 5-phosphate isomerase (*rpi*)	2.70
Metabolism of lipids			
SA0036		glycerophosphoryldiester phosphodiesterase homologue	0.12
SA0572		hypothetical protein, similar to esterase/lipase	0.15
SA0869	*fabI*	trans-2-enoyl-ACP reductase	0.40
SA0969		hypothetical protein, similar to glycerophosphoryl diester phosphodiesterase	0.49
SA1434		acetyl-CoA carboxylase (biotin carboxylase subunit), *accC* homologue	0.25
SA1435		hypothetical protein, similar to acetyl-CoA carboxylase, *accB* homologue	0.25
SA1542		hypothetical protein, similar to glycerophosphoryl diester phosphodiesterase	0.43
SA2333	*mvaA*	hydroxymethylglutaryl-CoA reductase	2.27
SA2349	*crtM*	squalene synthase	0.39
SAS044		4-oxalocrotonate tautomerase	2.23
Metabolism of coenzymes and prosthetic groups			
SA0241		hypothetical protein, similar to 4-diphosphocytidyl-2C-methyl-D-erythritol synthase	2.20
SA0317		hypothetical protein, similar to dihydroflavonol-4-reductase	0.47
SA0898	*menB*	naphthoate synthase	2.05
SA1491	*hemL*	glutamate-1-semialdehyde 2,1-aminomutase	2.18

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Table S2 - *Continued*

N315 ORF	Gene	Gene product function	Fold change SG511-Berlin/ SA137/93A [1]
SA1492	*hemB*	delta-aminolevulinic acid dehydratase	2.20
SA1493	*hemD*	uroporphyrinogen III synthase	3.32
SA1495	*hemX*	*hemA* concentration negative effector *hemX*	3.19
SA1496	*hemA*	glutamyl-tRNA reductase	2.24
SA1586	*ribH*	6,7-dimethyl-8-ribityllumazine synthase	0.40
SA1587	*ribA*	riboflavin biosynthesis protein	0.36
SA1588	*ribB*	riboflavin synthase alpha chain	0.36
SA2070	*moaB*	molybdopterin precursor biosynthesis *moaB*	0.48
DNA replication, modification and repair, recombination, packaging and segregation			
SA0027	*repB*	truncated replication protein for plasmid	0.08
SA0028	*repB*	truncated replication protein for pUB110 plasmid	0.04
SA0029	*pre*	plasmid recombination enzyme	0.04
SA0577		hypothetical protein, similar to FimE recombinase	0.23
SA1189	*parC*	topoisomerase IV subunit A	2.06
SA1489	*tag*	DNA-3-methyladenine glycosidase	2.44
SA1806		probable ATP-dependent helicase [Bacteriophage phiN315]	0.09
RNA synthesis and modification			
SA0017	*vicR*	response regulator	0.11
SA0097		hypothetical protein, similar to transcription regulator AraC/XylS family	0.07
SA0104		hypothetical protein, similar to transcription regulator GntR family	0.21
SA0108	*sarH1*	staphylococcal accessory regulator A homologue	0.11
SA0142		hypothetical protein, similar to DNA-binding protein	7.00
SA0322		hypothetical protein, similar to transcription regulator	2.69
SA1041	*pyrR*	pyrimidine operon repressor chainA	3.49
SA1465	*tgt*	tRNA-guanine transglycosylase	0.49
SA1583	*rot*	repressor of toxins Rot	4.32
SA1591		arsenical resistance operon repressor homologue	3.57
SA1678		transcription regulator Fur family homologue	2.25
SA1805		repressor homologue [Bacteriophage phiN315]	0.28
SA1869	*sigB*	sigma factor B	0.30
SA1870	*rsbW*	anti-sigmaB factor	0.26
SA1961		hypothetical protein, similar to transcription antiterminator BglG family	0.25
SA1999		hypothetical protein, similar to regulatory protein, SIR2 family	0.37
SA2089	*sarR*	staphylococcal accessory regulator A homologue	3.28
SA2147	*tcaR*	TcaR transcription regulator	0.43
SA2384	*ermA*	rRNA methylase Erm(A)	0.02
SA2424		hypothetical protein, similar to transcription regulator Crp/Fnr family protein	2.91
Protein synthesis, modification and folding			
SA0009	*serS*	seryl-tRNA synthetase	2.28
SA0330		hypothetical protein, similar to ribosomal-protein-serine N-acetyltransferase	0.40
SA0815		peptidyl-prolyl cis-trans isomerase homologue	2.63
SA0855	*trpS*	tryptophanyl-tRNA synthetase	0.36
SA1039	*lsp*	lipoprotein signal peptidase	2.04
SA1116	*rpsO*	30S ribosomal protein S15	2.51
SA1697		hypothetical protein, similar to protein-tyrosine phosphatase	0.49
SA1717		glutamyl-tRNAGln amidotransferase subunit C	0.44
SA1725		Staphopain, Cysteine Proteinase	2.27
SA1856		hypothetical protein, similar to glycoprotein endopeptidase	0.48
SA1917		hypothetical protein, similar to phosphatase	2.39
Adaption to atypical conditions			
SA0144	*capA*	capsular polysaccharide synthesis enzyme Cap5A	0.26

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Table S2 - *Continued*

N315 ORF	Gene	Gene product function	Fold change SG511-Berlin/ SA137/93A [1]
SA0145	*capB*	capsular polysaccharide synthesis enzyme Cap5B	0.18
SA0146	*capC*	capsular polysaccharide synthesis enzyme Cap8C	0.22
SA0147	*capD*	capsular polysaccharide synthesis enzyme Cap5D	0.19
SA0148	*capE*	capsular polysaccharide synthesis enzyme Cap8E	0.25
SA0149	*capF*	capsular polysaccharide synthesis enzyme Cap5F	0.30
SA0150	*capG*	capsular polysaccharide synthesis enzyme Cap5G	0.35
SA0151	*capH*	capsular polysaccharide synthesis enzyme O-acetyl transferase Cap5H	0.49
SA0152	*capI*	capsular polysaccharide synthesis enzyme Cap5I	0.30
SA0153	*capJ*	capsular polysaccharide synthesis enzyme Cap5J	0.26
SA0154	*capK*	capsular polysaccharide synthesis enzyme Cap5K	0.22
SA0155	*capL*	capsular polysaccharide synthesis enzyme Cap5L	0.44
SA0156	*capM*	capsular polysaccharide synthesis enzyme Cap5M	0.37
SA0157	*capN*	capsular polysaccharide synthesis enzyme Cap5N	0.44
SA0158	*capO*	capsular polysaccharide synthesis enzyme Cap8O	0.39
SA0659		hypothetical protein, similar to CsbB stress response protein	0.36
SA0723	*clpP*	ATP-dependent Clp protease proteolyticsubunit homologue	2.02
SA0755		hypothetical protein, similar to general stress protein 170	0.35
SA1941	*dps*	general stress protein 20U	2.54
SA1984	*asp23*	alkaline shock protein 23, ASP23	0.15
SA2175		hypothetical protein, similar to small heat shock protein	0.36
SA2336	*clpL*	ATP-dependent Clp proteinase chain clpL	0.16
Detoxification			
SA0033	*aadD*	kanamycin nucleotidyltransferase	0.02
SA0132		hypothetical protein, similar to tetracyclin resistance protein	0.43
SA0551		mercuric reductase homologue	0.43
SA0650	*norA*	quinolone resistance protein	0.12
SA0681		hypothetical protein, similar to multidrug resistance protein	0.19
SA2385	*ant(9)*	O-nucleotidylltransferase	0.10
Phage-related functions			
SA1759		lytic enzyme [Bacteriophage phiN315]	0.37
SA1765		hypothetical protein [Bacteriophage phiN315]	0.29
SA1787		hypothetical protein [Bacteriophage phiN315]	0.43
SA1810	*int*	integrase [Bacteriophage phiN315]	2.05
Transposon and IS elements			
SA0034		transposase for IS-like element	0.05
SA0762	*tnpA*	transposase A for Tn*554*	0.45
SA1483	*tnpB*	transposase B for Tn*554*	0.31
SA1603		truncated transposase	2.17
SA1623		truncated transposase [Pathogenicity island SaPIn3]	0.34
SA2289	*tnp*	transposase	0.23
SA2383		hypothetical protein	0.21
SA2386	*tnpC*	transposition regulatory protein tnpC transposon Tn*554*	0.23
SA2387	*tnpB*	transposition regulatory protein tnpB transposon Tn*554*	0.18
SA2388	*tnpA*	transposition regulatory protein tnpA transposon Tn*554*	0.15
SAS069		hypothetical protein, similar to transposase for IS*232*	2.48
Pathogenic factors (toxins and colonization factors)			
SA0270		hypothetical protein, similar to secretory antigen precursor SsaA	5.72
SA0276		conserved hypothetical protein, similar to diarrheal toxin	0.45
SA0309	*geh*	glycerol ester hydrolase	0.44
SA0357		hypothetical protein, similar to exotoxin 2	0.17
SA0519	*sdrC*	Ser-Asp rich fibrinogen-binding, bone sialoprotein-binding protein	9.39

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Table S2 - *Continued*

N315 ORF	Gene	Gene product function	Fold change SG511-Berlin/ SA137/93A [1]
SA0521	*sdrE*	Ser-Asp rich fibrinogen-binding, bone sialoprotein-binding protein	12.15
SA0587		lipoprotein, streptococcal adhesin PsaA homologue	13.65
SA0744	*ssp*	extracellular ECM and plasma binding protein	0.42
SA0841		hypothetical protein, similar to cell surface protein Map-w	2.02
SA1004		hypothetical protein, similar to fibrinogen-binding protein	0.48
SA1430		hypothetical protein, similar to enterotoxin A precursor	3.54
SA1629	*splC*	serine protease SplC [Pathogenicity island SaPIn3]	0.50
SA1642	*seg*	extracellular enterotoxin type G precursor [Pathogenicity island SaPIn3]	4.55
SA1643	*sen*	enterotoxin SeN [Pathogenicity island SaPIn3]	3.39
SA1644	*yent2*	enterotoxin YENT2 [Pathogenicity island SaPIn3]	2.25
SA1645	*yent1*	enterotoxin Yent1 [Pathogenicity island SaPIn3]	3.27
SA1648	*seo*	enterotoxin SeO [Pathogenicity island SaPIn3]	2.40
SA1812		hypothetical protein, similar to synergohymenotropic toxin precursor	0.48
SA1898		hypothetical protein, similar to SceD precursor	0.16
SA1973		hypothetical protein, similar to hemolysin III	0.42
SA2093	*ssaA*	secretory antigen precursor SsaA homologue	2.04
SA2097		hypothetical protein, similar to secretory antigen precursor SsaA	0.34
SA2290	*fnbB*	fibronectin-binding protein homologue	0.10
SA2291	*fnb*	fibronectin-binding protein homologue	0.16
SA2423	*clfB*	clumping factor B	2.41
SA2430	*aur*	zinc metalloproteinase aureolysin	2.53
Miscellaneous			
SA0083		conserved hypothetical protein	0.35
SA0231		hypothetical protein, similar to flavohemoprotein	3.16
SA1193	*mprF*	oxacillin resistance-related MprF (FmtC) protein	0.40
SA1559		hypothetical protein, similar to smooth muscle caldesmon	0.32
SA1617		hyp. protein, similar to nuclear antigen, Kaposi's sarcoma-associated herpesvirus	3.32
SA1709		hypothetical protein, similar to ferritin	2.88
Hypothetical proteins			
SA0019		conserved hypothetical protein	0.14
SA0020		conserved hypothetical protein	0.47
SA0024		hypothetical protein	0.08
SA0025		hypothetical protein	0.10
SA0037		conserved hypothetical protein	0.11
SA0043		conserved hypothetical protein	0.45
SA0078		hypothetical protein	0.17
SA0079		conserved hypothetical protein	0.18
SA0080		conserved hypothetical protein	0.16
SA0084		hypothetical protein, similar to homo sapiens CGI-44 protein, PRO1975 protein	0.17
SA0085		conserved hypothetical protein	0.04
SA0088		hypothetical protein	0.19
SA0089		hypothetical protein, similar to DNA helicase	0.21
SA0092		hypothetical protein	0.02
SA0093		hypothetical protein	0.07
SA0094		hypothetical protein	0.21
SA0095		hypothetical protein	0.02
SA0096		hypothetical protein	0.05
SA0100		conserved hypothetical protein	0.22
SA0101		hypothetical protein	0.49
SA0129		hypothetical protein	7.68
SA0141		hypothetical protein	2.58

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Table S2 - *Continued*

N315 ORF	Gene	Gene product function	Fold change SG511-Berlin/ SA137/93A [1]
SA0203		hypothetical protein	0.32
SA0221		hypothetical protein	3.94
SA0228		hypothetical protein	4.27
SA0269		hypothetical protein	13.63
SA0271		conserved hypothetical protein	18.12
SA0278		hypothetical protein	0.49
SA0279		hypothetical protein	0.41
SA0280		hypothetical protein	0.41
SA0281		conserved hypothetical protein	0.10
SA0282		conserved hypothetical protein	0.10
SA0283		hypothetical protein	0.12
SA0284		hypothetical protein	0.07
SA0286		conserved hypothetical protein	0.23
SA0287		conserved hypothetical protein	0.08
SA0288		conserved hypothetical protein	0.11
SA0289		conserved hypothetical protein	0.22
SA0290		conserved hypothetical protein	0.22
SA0291		hypothetical protein	2.89
SA0292		hypothetical protein	5.58
SA0308		conserved hypothetical protein	0.39
SA0326		conserved hypothetical protein	0.31
SA0327		conserved hypothetical protein	0.27
SA0345		conserved hypothetical protein	0.33
SA0355		hypothetical protein, similar to hypothetical protein virulence plasmid pXO1-38	0.34
SA0359		conserved hypothetical protein	0.28
SA0360		conserved hypothetical protein	0.37
SA0370		conserved hypothetical protein	4.49
SA0372		hypothetical protein	3.83
SA0395		hypothetical protein [Pathogenicity island SaPIn2]	0.36
SA0401	*lpl5*	hypothetical protein [Pathogenicity island SaPIn2]	3.04
SA0402	*lpl6*	hypothetical protein [Pathogenicity island SaPIn2]	0.46
SA0478		conserved hypothetical protein	2.45
SA0509		conserved hypothetical protein	0.05
SA0518		conserved hypothetical protein	2.15
SA0529		conserved hypothetical protein	0.23
SA0552		hypothetical protein	2.05
SA0554		conserved hypothetical protein	0.37
SA0555		conserved hypothetical protein	0.48
SA0556		conserved hypothetical protein	2.99
SA0570		hypothetical protein	2.39
SA0588		conserved hypothetical protein	13.07
SA0609		conserved hypothetical protein	0.42
SA0636		conserved hypothetical protein	0.49
SA0637		conserved hypothetical protein	0.39
SA0651		hypothetical protein	0.31
SA0673		conserved hypothetical protein	0.46
SA0701		conserved hypothetical protein	2.68
SA0704		conserved hypothetical protein	2.65
SA0707		conserved hypothetical protein	2.04
SA0710		conserved hypothetical protein	4.37
SA0725		conserved hypothetical protein	0.43

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Table S2 - *Continued*

N315 ORF	Gene	Gene product function	Fold change SG511-Berlin/ SA137/93A [1]
SA0739		conserved hypothetical protein	4.96
SA0752		hypothetical protein	0.44
SA0768		conserved hypothetical protein	0.42
SA0770		conserved hypothetical protein	0.31
SA0771		conserved hypothetical protein	0.34
SA0772		conserved hypothetical protein	0.36
SA0773		conserved hypothetical protein	0.42
SA0779		hypothetical protein	2.66
SA0789		conserved hypothetical protein	0.43
SA0800		conserved hypothetical protein	2.11
SA0801		conserved hypothetical protein	2.57
SA0804		conserved hypothetical protein	3.82
SA0805		conserved hypothetical protein	2.12
SA0833		conserved hypothetical protein	0.29
SA0872		conserved hypothetical protein	2.62
SA0873		conserved hypothetical protein	3.36
SA0903		conserved hypothetical protein	0.41
SA0906		conserved hypothetical protein	0.48
SA0908		conserved hypothetical protein	0.39
SA0919		conserved hypothetical protein	0.32
SA0929		conserved hypothetical protein	18.84
SA0933		hypothetical protein	0.39
SA0955		hypothetical protein	0.50
SA0999		conserved hypothetical protein	0.45
SA1002		hypothetical protein	2.80
SA1040		conserved hypothetical protein	2.32
SA1049		hypothetical protein	11.12
SA1133		conserved hypothetical protein	0.35
SA1154		conserved hypothetical protein	0.45
SA1168		hypothetical protein	2.51
SA1250		conserved hypothetical protein	0.26
SA1252		conserved hypothetical protein	0.50
SA1265		conserved hypothetical protein	0.39
SA1295		conserved hypothetical protein	0.36
SA1320		hypothetical protein	0.15
SA1321		hypothetical protein	0.18
SA1331		conserved hypothetical protein	0.47
SA1335		conserved hypothetical protein	2.29
SA1419		conserved hypothetical protein	0.49
SA1432		conserved hypothetical protein	0.28
SA1433		conserved hypothetical protein	0.21
SA1436		conserved hypothetical protein	0.19
SA1437		conserved hypothetical protein	0.20
SA1497		conserved hypothetical protein	2.01
SA1544		hypothetical protein, similar to soluble hydrogenase 42 kD subunit	0.21
SA1573		hypothetical protein	0.40
SA1582		conserved hypothetical protein	0.46
SA1590		hypothetical protein	0.47
SA1595		hypothetical protein	0.23
SA1596		hypothetical protein	0.13
SA1597		hypothetical protein	0.10

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Table S2 - *Continued*

N315 ORF	Gene	Gene product function	Fold change SG511-Berlin/ SA137/93A [1]
SA1618		conserved hypothetical protein	3.29
SA1619		hypothetical protein	2.94
SA1639		hypothetical protein [Pathogenicity island SaPIn3]	0.36
SA1641		hypothetical protein [Pathogenicity island SaPIn3]	3.83
SA1692		conserved hypothetical protein	0.30
SA1768		hypothetical protein [Bacteriophage phiN315]	2.37
SA1770		hypothetical protein [Bacteriophage phiN315]	2.15
SA1773		hypothetical protein [Bacteriophage phiN315]	2.19
SA1774		hypothetical protein [Bacteriophage phiN315]	2.80
SA1802		hypothetical protein [Bacteriophage phiN315]	0.45
SA1803		hypothetical protein [Bacteriophage phiN315]	0.34
SA1823		hypothetical protein [Pathogenicity island SaPIn1]	4.58
SA1824		hypothetical protein [Pathogenicity island SaPIn1]	3.25
SA1838		conserved hypothetical protein	0.24
SA1839		hypothetical protein, similar to SdrH	0.48
SA1849		conserved hypothetical protein	2.11
SA1850		conserved hypothetical protein	2.83
SA1918		conserved hypothetical protein	2.19
SA1932		hypothetical protein, similar to hypothetical protein T13D8.31 - *Arabidopsis thaliana*	2.12
SA1934		hypothetical protein	0.46
SA1946		conserved hypothetical protein	0.09
SA1971		hypothetical protein	0.19
SA1976		conserved hypothetical protein	3.70
SA1985		hypothetical protein	0.09
SA1986		hypothetical protein	0.07
SA2101		conserved hypothetical protein	0.18
SA2106		hypothetical protein, similar to protein of pXO2-46	0.15
SA2113		hypothetical protein	0.35
SA2133		conserved hypothetical protein	2.18
SA2143		conserved hypothetical protein	2.51
SA2195		conserved hypothetical protein	0.35
SA2196		conserved hypothetical protein	0.23
SA2218		hypothetical protein	3.70
SA2262		conserved hypothetical protein	0.09
SA2265		hypothetical protein	0.28
SA2271		hypothetical protein	0.36
SA2272		hypothetical protein	0.25
SA2315		conserved hypothetical protein	0.37
SA2329		conserved hypothetical protein	3.29
SA2350		conserved hypothetical protein	0.45
SA2355		conserved hypothetical protein	0.12
SA2359		hypothetical protein	0.33
SA2366		conserved hypothetical protein	0.27
SA2367		conserved hypothetical protein	0.18
SA2371		conserved hypothetical protein	2.68
SA2372		hypothetical protein	2.76
SA2373		hypothetical protein	3.31
SA2374		conserved hypothetical protein	0.28
SA2377		conserved hypothetical protein	2.63
SA2407		conserved hypothetical protein	2.15
SA2432		conserved hypothetical protein	0.25

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Table S2 - *Continued*

N315 ORF	Gene	Gene product function	Fold change SG511-Berlin/ SA137/93A [1]
SA2449		hypothetical protein	0.46
SA2450		hypothetical protein	0.41
SA2451		hypothetical protein	0.40
SA2452		conserved hypothetical protein	0.48
SA2479		conserved hypothetical protein	0.48
SA2480	*drp35*	Drp35	2.45
SA2483		hypothetical protein	0.31
SA2488		hypothetical protein	0.34
SA2491		conserved hypothetical protein	0.46
SAS001		conserved hypothetical protein	0.48
SAS058		hypothetical protein	2.02

[1] Fold change in transcript level of at least 2-fold is indicated as mean of the "median of ratios".

8 Publications

Journal articles

Sass, P., and G.Bierbaum. 2008. "Native *graS* mutation supports the susceptibility of *Staphylococcus aureus* strain SG511 to antimicrobial peptides." IJMM, accepted for publication.

Sass, P., A. Jansen, C. Szekat, V. Sass, H.-G. Sahl, and G. Bierbaum. 2008. „The lantibiotic mersacidin is a strong inducer of the cell wall stress response of *Staphylococcus aureus.*" BMC microbiology, **8**:186.

Sass, P., and G. Bierbaum. 2007. "Lytic activity of recombinant bacteriophage ϕ11 and ϕ12 endolysins on whole cells and biofilms of *Staphylococcus aureus*." Appl. Env. Microbiol. **73(1)**:347-352.

Oral presentations

Sass, P., A. Jansen, C. Szekat, and G. Bierbaum. 2008. "Extensive induction of the cell wall stress response of *Staphylococcus aureus* by subinhibitory concentrations of the lantibiotic mersacidin." Joint Annual Conference of the Association of General and Applied Microbiology (VAAM) and the German Society for Biochemistry and Molecular Biology (GBM), Frankfurt am Main, Germany, March 2008.

Sass, P., V. Sass, H.-G. Sahl and G. Bierbaum. 2007. "Transcriptional profiles of *Staphylococcus aureus* strains in the presence of the cell wall biosynthesis inhibitor mersacidin." SFB/TRR34-Meeting on the "Pathophysiology of Staphylococci in the Post-Genome-Era", Isle of Vilm, Rügen, Germany, September 2007.

Poster presentations

Sass, P., and G.Bierbaum. 2008. "High susceptibility of *Staphylococcus aureus* strain SG511 to antimicrobial peptides is based on a mutation in the histidine kinase gene *graS*." Workshop of the Transregional Collaborative Research Center 34 (Greifswald, Tübingen, Würzburg) at the Kloster Banz, Bad Staffelstein, Germany. 2008.

Dischinger, J., C. Szekat, **P. Sass** and G. Bierbaum. 2008. „Analysis of a new two-component lantibiotic lichenicidin". VAAM Workshop, 2008

Dischinger, J., C. Szekat, **P. Sass** and G. Bierbaum. 2008. „Analysis of a new two-component lantibiotic gene cluster in *Bacillus licheniformis* DSM13". DGHM, Dresden, 2008.

Dischinger, J., C. Szekat, **P. Sass** and G. Bierbaum. 2008. „ Lichenicidin: A novel two-component lantibiotic produced by *Bacillus licheniformis* DSM13". VAAM, Frankfurt am Main, 2008.

Sass, P., V. Sass, H.-G. Sahl and G. Bierbaum. 2007. "Effects of the cell wall biosynthesis inhibitor mersacidin on the transcriptional profile of Staphylococcus aureus." ProkaGen 2007 3rd European Conference on Prokaryotic Genomes, Göttingen, 2007.

Sass, P., V. Sass, H.-G. Sahl and G. Bierbaum. 2007. "Transcriptional profiles of *Staphylococcus aureus* strains in the presence of the lantibiotic mersacidin." Gordon Research Conference on «Staphylococcal Diseases». Les Diablerets, Switzerland, 2007.

Sass, P., V. Sass, H.-G. Sahl and G. Bierbaum. 2007. "Transcriptional profile of a *Staphylococcus aureus* strain with reduced sensitivity towards mersacidin." VAAM, Osnabrück, 2007.

Sass, P., and G. Bierbaum. 2006. "Lytic activity of bacteriophage ϕ11 endolysin modules to lyse staphylococcal cells and biofilms." Int. Symp. on Staphylococci and Staphylococcal Infections, Maastricht, Netherlands, 2006.

Sass, P. and G. Bierbaum. 2006. "Recombinant overexpression of His-tagged phage ϕ11 and ϕ12 endolysins and their influence on planktonic and sessile *Staphylococcus aureus* cells." VAAM, Jena, 2006.

Jansen, A., M. Türck, C. Szekat, I. Rumpel, **P. Sass**, and G. Bierbaum. 2004. „Untersuchungen zur Evolution von Antibiotika-Resistenzen bei *Staphylococcus aureus*." BMBF Pathogenomics Network Meeting, Würzburg, 2004.

Declaration (Eidesstattliche Erklärung)

Hiermit erkläre ich an Eides statt, dass ich für meine Promotion keine anderen als die angegebenen Hilfsmittel benutzt habe, und dass die inhaltlich und wörtlich aus anderen Werken entnommenen Stellen und Zitate als solche gekennzeichnet sind.
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Bonn, November 2008

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Peter Saß