Identification of a novel LysR-type transcriptional regulator in *Staphylococcus aureus*

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Contents

I. Abstract

Staphylococcus aureus is a facultative pathogen which causes a variety of infections. The treatment of staphylococcal infections is complicated because the bacteria is resistant to multiple common antibiotics. *S. aureus* is also known to express a variety of virulence factors which modulate the host's immune response in order to colonize and invade certain host cells, leading to the host cell's death. Among the virulence factors is a LysR-type transcriptional regulator (*lttr*) which is required for efficient colonization of secondary organs. In a recent report, which used transposon screening on *S. aureus*-infected mice, it was found that the amount of a novel *lttr*852 mutant bacteria recovered from the kidneys was significantly lower compared to the wildtype strains.

This doctoral thesis therefore focused on phenotypical and molecular characterization of *lttr*852. An assessment of the *S. aureus* biofilm formation and the hemolysis revealed that *lttr*852 was not involved in the regulation of these virulence processes. RNA-sequencing for potential target genes of *lttr*852 identified differentially expressed genes that are involved in branched chain amino-acid biosynthesis, methionine sulfoxide reductase and copper transport, as well as a reduced transcription of genes encoding urease and of components of pyrimidine nucleotides. Promoter fusion with GFP reporters as as well as OmniLog were used to identify conditions under which the *lttr852* was active. The promoter studies showed that glucose and high temperatures diminish the *lttr*852 promoter activity in a time-dependent manner, while micro-aerobic conditions enhanced the promoter activity. Copper was found to be a limiting factor. In addition, the impact on promoter activity of the *lttr*852 was tested in the presence of various regulators, but no central link to the genes involved in virulence was identified.

The present work, thus, showed that *lttr*852, a new member of the class of LysR-type transcriptional regulators in *S. aureus*, has an important role in the rapid adaptation of *S. aureus* to the changing microenvironment of the host.

II. Zusammenfassung

Staphylococcus aureus ist ein fakultativer Erreger, der eine Vielzahl von Infektionen verursacht. Die Behandlung von Staphylokokken-Infektionen ist aufgrund des Auftretens einer Resistenz gegen mehrere gängige Antibiotika kompliziert. Es ist bekannt, dass *S. aureus* eine Vielzahl von Virulenzfaktoren exprimiert, um die Immunantwort des Wirts zu umgehen, und so in bestimmte Wirtszellen einzudringen und diese zu kolonisieren, was zum Tod von Wirtszellen führen kann. Unter den Virulenzfaktoren befindet sich ein Transkriptions-regulator vom LysR-Typ (*lttr*), der für eine effiziente Besiedlung von Sekundärorganen erforderlich ist. In einem kürzlich durchgeführten Transposon-Screen, bei dem Mäuse mit *S. aureus* infiziert wurden, wurde ein neuartiger *lttr*, der *lttr*852 identifiziert, bei dem aus den Nieren gewonnenen Bakterien signifikant dezimiert waren.

Diese Doktorarbeit befasste sich mit der phänotypischen und molekularen Charakterisierung von *lttr*852. Die Auswertung der Biofilmbildung und der Hämolyse von *S. aureus* ergab, dass *lttr*852 nicht an der Regulation dieser Virulenzprozesse beteiligt war. Die RNA-Sequenzierung für potenzielle Zielgene von *lttr852* identifizierte sowohl eine erhöhte Expression von Genen, die in der Aminosäuren-Biosynthese, Methionin-Sulfoxid-Reduktase und dem Kupfertransport involviert sind, als auch eine verringerte Transkription von codierenden Genen der Urease und Komponenten der Pyrimidin Nukleotide. Die Promotorfusion mit dem GFP-Reporter sowie das OmniLog System wurden verwendet, um Bedingungen zu identifizieren unter denen das *lttr852* aktiv ist. Die Promotorstudien ergaben, dass die Anwesenheit von Glucose und eine erhöhte Temperatur die Promotoraktivität von *lttr*852 zeitabhängig herabsetzt, wobei die Aktivität durch mikroaerobe Bedingungen begünstigt wird. Kupfer wurde als limitierender Faktor identifiziert. Außerdem wurde der Einfluss diverser Regulatoren auf die transkriptionelle Regulation von *lttr*852 kontrolliert, jedoch keine zentrale Rolle in der Regulation von Virulenzgenen zugewiesen.

Damit konnte innerhalb der vorliegenden Arbeit gezeigt werden, dass *lttr*852*,* ein neues Mitglied der Klasse der LysR-Typ Transkriptionsregulatoren in *S. aureus*, eine wichtige Rolle in der schnellen Adaption von *S. aureus* an die wechselnde Mikroumgebungen des Wirts hat.

1. Introduction

1.1 *Staphylococcus aureus*

Staphylococcus aureus was first identified by Sir Alexander Ogston in 1880, while he was investigating pus from an abscess of a human patient (Ogston, 1882). Four years later, the German researcher Friedrich Julius Rosenbach established the name for these bacteria, which appear under the microscope to be arranged in grape-like clusters leading to the name *Staphylococcus aureus* - derived from the Greek words ´staphyle´ and ´kokkos´ and the Latin word ´aureus´ meaning golden, as the colonies are often yellow-pigmented. The organisms are Gram positive cocci with a diameter of 0.8 - 1.2 µm, facultative anaerobic, and grow at temperatures between 16°C and 42°C (Fig. 1.1). *S. aureus* produces catalase and coagulase, ferments mannitol and is novobiocin-sensitive. Since its first description, the taxonomic classification of staphylococci was changed several times. Today, staphylococci are placed within the family of *Staphylococcaceae* comprising 36 different species (Flügge, 1886; Götz et al., 2006). Since *S. aureus* contains a circular genome of approximately 2.8 Mbp with a GC-content of 33 mol% it taxonomically belongs to the bacilli class (Kuroda et al., 2001).

Figure 1.1: Macroscopic and microscopic images of *Staphylococcus aureus*.
Left: *S. aureus* colonies on horse blood agar (source: www.wikimedia.org). Right: Electron-microscopic image of *S. aureus* (gold) and white blood cell (blue) (source: www.wikimedia.org).

Staphylococcal infections became a major problem as *S. aureus* developed antibiotic resistances, thereby limiting treatment options. Before the 1950s, *S. aureus* infections routinely were treated with the β-lactam antibiotic benzylpenicillin, but shortly after introduction of the therapy, benzylpenicillin-resistant strains developed (Eriksen, 1961; Sutherland and Rolinson, 1964). Thus, in 1959, the β-lactam derivate methicillin was synthesized and immediately upon the introduction, first methicillin-resistant *S. aureus* (MRSA) strains were isolated (Barber, 1961; Chambers, 1997). MRSA strains carry a *mec* gene on the staphylococcal chromosomal cassette (SCCmec), which encodes the penicillin-binding protein 2a (PBP-2a). PBP-2a has a lower affinity to β-lactams when compared to other penicillin-binding proteins (PBP) (Ubukata et al., 1990). Therefore, *S. aureus* can grow in the presence of many antibiotics and hence is resistant to methicillin, nafcillin and cephalosporins (Georgopapadakou et al., 1982; Hartman and Tomasz, 1984; Utsui and Yokota, 1985).

1.2 *S. aureus* host colonization and diseases

S. aureus is an opportunistic pathogen which can asymptomatically colonize about 20-30% of the healthy population as a human commensal (Kluytmans et al., 1997). Aside from humans, *S. aureus* is able to colonize animals such as pigs and horses, but also bats, parrots and chinchillas (Voss et al., 2005; Morgan, 2008). Natural asymptomatic reservoirs of *S. aureus* in healthy human beings are primarily the epithelium of the anterior nasal vestibule, but also skin lesions, skin glands, mucosal surfaces, throat, gastro-intestinal tract, rectum and vagina (Wertheim et al., 2005; Senn et al., 2012). If a person is a persistent carrier of *S. aureus,* there is an increased risk of developing an infection with this organism (Williams, 1963). In addition, miscellaneous studies showed that nasal carriage are associated with influencing host factors such as gender, age, diet, smoking and drug addiction and pre-existing diseases, particularly skin diseases as well as rheumatoid arthritis. However, host determinants influencing colonization are not fully understood (Tuazon et al., 1975; Berman et al., 1987; Miller et al., 2003; Choi et al., 2006; Laudien et al., 2010; Johannessen et al., 2012; Olsen et al., 2012).

The attachment of *S. aureus* to the host surface is an important step in the colonization and depends on bacterial surface structures and adhesins (Speziale et al., 2009; Weidenmaier et al., 2012). *S. aureus* is a prominent pathogen in hospital-acquired infections. One way for *S. aureus* to enter the human body system is via medical intervention, as *S. aureus* is located on the skin and thereby can enter the body via open wounds or by way of contaminated instruments, leading to 37% of all surgical-site infections (SSI) (Pal et al., 2019). In this context, the worldwide epidemic of community associated methicillin-resistant *S. aureus* (CA-MRSA) has been a big problem over the last decade (DeLeo et al., 2010; Otto, 2013). As soon as the bacterium has entered the human body it can be disseminated by blood stream to single organs like liver and kidneys, leading to pyogenic abscesses. The infection of some organs with *S. aureus* can lead to specific diseases such as endocarditis, which starts with the damage of cardiac endothelium by inflammation or direct trauma. An infection can also lead to osteomyelitis, native joint septic arthritis and prosthetic joint infection in bones (Torda et al., 1995; Gupta et al., 2001; Byren et al., 2009) where *S. aureus* is able to form biofilms (Clauss et al., 2013). Isolates from acute and chronic osteomyelitis form more biofilm than strains isolated from nasal colonization or sepsis and have been shown, to be more cell-invasive for osteoblast, whereby isolates from chronic osteomyelitis developed small colony variants (SCVs) at higher rates. It has been speculated that SCV formation may cause persistence in the host (Proctor et al., 2006; Shi and Zhang, 2012; Kalinka et al., 2014).

S. aureus is a major human pathogen that causes a wide range of clinical infections. The pathophysiology of *S. aureus* can be categorized in local tissue infection including skin and soft tissue infections (SSTIs), pneumonia, endocarditis, urinary tract infections, meningitis, epidural abscess, osteomyelitis, septic arthritis, SSI, which can lead to a whole body system infection including toxic-shock syndrome (TSS), bacteraemia and sepsis (reviewed in Tong et al., 2015).

S*. aureus* bacteraemia (SAB) has a world-wide population incidence ranging from 10 to 30 per 100 000 persons and year. Moreover, in 38% to 44% cases bacteraemia marks the beginning of sepsis and TSS leading to morbidity and mortality (Guilarde et al.,

2006; Ammerlaan et al., 2009). SAB often results from SSTIs, pleuropulmonary infection, infection of implants and infective endocarditis (Engemann et al., 2003). SAB can activate a cascade of innate immune response, involving pro-inflammatory cytokines such as IL-6, IL-8, MCP-1, TNF-α and IL-1β, leading to a cytokine storm causing septic shock and cell death up to multiple organ failure (Laster et al., 1988; Bone et al., 1992; Bozza et al., 2007; Harrison, 2010; Sridharan and Upton, 2014; Kell and Pretorius, 2018).

1.3 Host-pathogen interactions during *Staphylococcus aureus* infections

During infection with bacterial pathogens, the host possesses different kinds of defense mechanisms ranging from the production of antimicrobial peptides, coagulation of blood, or complement activation. Pathogenic bacteria have developed strategies to overcome these host responses in order to establish an infection. *S. aureus* expresses a variety of virulence factors, which generally are classified into two groups: surface-associated and secreted virulence factors.

1.3.1 Surface-associated virulence factors

On one side *S. aureus* can be phagocytosed by immune cells, on the other side surface adhesins can cause internalization by non-professional phagocytic cells (NPPC) such as epithelial and endothelial cells, keratinocytes, osteoblasts and fibroblasts (Tompkins et al., 1990; Beekhuizen et al., 1997; Jevon et al., 1999; Lammers et al., 1999; Peacock et al., 1999; Kintarak et al., 2004; Fraunholz and Sinha, 2012; Strobel et al., 2016). The first step in this process is the adhesion of *S. aureus* to the cell surface, which is necessary in order to form a biofilm or to cause internalization by NPPC. *S. aureus* expresses a broad range of surface proteins involved in their adhesion to extracellular matrix, plasma proteins or directly to host cells. Surface associated virulence factors involved in adhesion and being produced by *S. aureus* are teichoic acids and microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) (reviewed in Heilmann, 2011).

1.3.1.1 Teichoic acids

S. aureus possesses two types of teichoic acids, which are either covalently linked to peptidoglycan, named wall teichoic acid (WTA), or to the cytoplasmic membrane, known as lipoteichoic acid (LTA) (Xia et al., 2010). The structure of WTAs is highly conserved and consists of two main units, the cell wall linkage unit and the main polymer chain. Studies showed that WTAs extend well beyond the peptidoglycan layer, therefore being able to bind extracellular metal cations (Matias and Beveridge, 2006; Wickham et al., 2009; Brown et al., 2013). In several organisms they modulate susceptibility to cationic antibiotics. Moreover, they are required for β-lactam resistance in MRSA (Campbell et al., 2011). LTA is an amphiphilic, negatively charged glycolipid with the simple structure of a single unbranched 1,3-linked poly glycerophosphate chain. That chain is covalently linked to a glycolipid which also occurs among free membrane lipids (Duckworth et al., 1975; Fischer et al., 1990). It elicits antibody formation, activates the activity of the complement system in an antibody independent manner and stimulates the secretion of cytokines and radicals from monocytes and macrophages (Hummell et al., 1985; Bhakdi et al., 1991; Keller et al., 1992; Fischer, 1994).

1.3.1.2 Microbial surface component recognizing adhesive matrix molecules (MSCRAMM)

The family of MSCRAMMs belongs to the largest group of cell wall anchor in *S. aureus* which initiate adherence by binding to components of the extracellular matrix like collagen, fibrinogen and fibronectin (Foster and Hook, 1998; Marraffini et al., 2006; Foster et al., 2014; Josse et al., 2017). They possess immunoglobulin G (IgG) like folded domains, they are typically covalently anchored to the cell wall peptidoglycan via a LPCTG-motif and they can bind to their ligands by a ´dock, lock and latch´ mechanism (Marraffini et al., 2006; Foster et al., 2014). The most known MSCRAMMs with a major role for *S. aureus* virulence are protein A (SpA), fibronectin-binding proteins (FnBPs) and clumping factors (Clf) (Foster and Hook, 1998).

The SpA exists of five immunoglobulin binding domains, it confers the interaction of *S. aureus* to the host protein von Willebrand factor and it attaches to the Fc domain of

human IgG and the Fab domain of IgG and immunoglobulin M (IgM) (Forsgren and Nordstrom, 1974; Cedergren et al., 1993; Cary et al., 1999; Hartleib et al., 2000). It is released during staphylococcal growth in order to escape from the protective immune responses, for example from opsonophagocytic killing (Forsgren and Quie, 1974; Ton-That et al., 1999). Moreover, if SpA binds to IgM, it triggers the cross linking to B cell receptors, therefore resulting in proliferative supraclonal expansion as well as apoptotic of the activated B cells (Goodyear and Silverman, 2003). Studies showed that SpA also plays a role in the formation of renal abscesses, septic arthritis and lethal sepsis. In addition, it mediates the invasion via a zipper like mechanism across the epithelia cells of the airway, through the activation of RhoA GTPase and proteolytic activity (Palmqvist et al., 2002; Cheng et al., 2009; Kim et al., 2011; Soong et al., 2011).

S. aureus expresses two fibronectin-binding (Fnb) protein homologues FnbA and FnbB, which are not only essential for the adhesion to cells but also for the internalization by cells. The N-terminal domain of the FnBPs can bind to several ligands like fibronectin, fibrinogen, elastin, plasminogen and histones (Wann et al., 2000; Schwarz-Linek et al., 2003; Roche et al., 2004; Meenan et al., 2007; Pietrocola et al., 2016; Pietrocola et al., 2019; Speziale and Pietrocola, 2020). The ligand fibronectin is a binding bridge between the FnBPs and the $\alpha_5\beta_1$ integrin on the host cell surface, which is sufficient to induce the uptake of *S. aureus* via zipper-type mechanism (Sinha et al., 1999). Beside the internalization of *S. aureus*, FnBPs are also important for the biofilm formation (O'Neill et al., 2008). The mechanism is based on a multiple zinc ion dependent hemophilic bond, with low affinity bonds between the A domains of the FnBPs of neighboring cells, leading to cell detachment during the dispersal phase, allowing biofilm formation (Herman-Bausier et al., 2015).

S. aureus has two clumping factors - ClfA and ClfB - and as the same supplies, both mediates direct binding to human platelets which can lead to the formation of platelet clumps. Their structure is very similar and only differs in the binding domain. As a consequence, ClfA binds to fibrinogen and fibrin, whereas ClfB binds to cytokeratin 10, loricrin and the α-chain of fibrinogen (McDevitt et al., 1997; Ni Eidhin et al., 1998; O'Brien et al., 2002; Walsh et al., 2004; Walsh et al., 2008; McAdow et al., 2011;

Mulcahy et al., 2012). Since the ligands of ClfA are widespread in the human body, they are involved in various types of infections, including sepsis, endocarditis, kidney abscesses, arthritis and skin infections (Moreillon et al., 1995; Josefsson et al., 2001; Josefsson et al., 2008; Cheng et al., 2009; Kwiecinski et al., 2014). With loricrin and keratin 10, both being components of the nasal squamous cells, whereas ClfB promotes the nasal colonization (Mulcahy et al., 2012).

1.3.2 Secreted virulence factors

To establish an infection, *S. aureus* secretes a wide array of small peptides, exotoxins and proteins. Secretable expanded repertoire adhesive molecules (SERAMs) are as the name indicates involved in adhesion of *S. aureus*. Other cofactors or secreted enzymes (exoenzymes) which are playing part in the virulence of *S. aureus* are for example lipases, including phospolipases (β-toxin, PI-PLC), glycerol ester hydrolases (SAL1 & 2) and fatty acid modifying enzymes (FAMES) or proteases such as serine protease (SspA, SplA-F, exfoliative toxins), metalloprotease (aureolysin) or cysteine protease (staphopain) (Tam and Torres, 2019). A large proportion of secreting virulence factors are extracellular toxins, like cytolysins, including α, β, γ, δ-toxin, Panton-Valentine leukocidine (PVL) and PSM or the superantigenes, including *sea*, *seb*, *sec* and *tsst-1* (Reviewed in Tam, 2019). Only a few secreted virulence factors are explained more in detail below.

1.3.2.1 Secretable expanded repertoire adhesive molecules (SERAM)

S. aureus also owns secretory factors that can bind to host proteins and ligands including extracellular fibrinogen binding protein (Efb), extracellular matrix binding protein (Emp), extracellular adhesive protein (Eap), coagulase (Coa) and von Willebrand factor binding protein (vWbp) which are all part of the SERAMs. The anchorless proteins both interact with the host immune system and other defense mechanisms (Chavakis et al., 2005).

The extracellular fibrinogen binding protein Efb consists of two structurally and functionally distinct domains and interacts with the α chain of fibrinogen, whereas Efb stimulates the binding of fibrinogen to platelets (Boden and Flock, 1994; Palma et al., 1998; Heilmann et al., 2002). Due to the binding of Efb to platelets it inhibits the platelet activation and thrombus formation, leading to *S. aureus* survival within the blood and therefore stronger *S. aureus* infection (Zhang et al., 2011).

The Emp interacts with fibronectin, fibrinogen, and vitronectin, and associates with the cell surface (Hussain et al., 2001). The binding domain is located in the C-terminus, whereas the N-terminus is essential for polymerization as Emp seems to have a fibrous structure composed of Emp polymers (Geraci et al., 2017). It binds preferentially to skin and cartilage extracellular matrix but also to host tissue components, leading to the assumption that Emp plays a role in wound infections, biofilm and abscesses formation (Johnson et al., 2008; Cheng et al., 2009; Bur et al., 2013).

Eap is expressed in a growth dependent manner, secreted in a large amount into the extracellular milieu where it binds to extracellular matrix host ligands like fibrinogen, fibronectin and prothrombin, in order to simplify the inflammation and adhesion of *S. aureus* (Palma et al., 1999; Hansen et al., 2006; Joost et al., 2009; Bur et al., 2013; Eisenbeis et al., 2017).

Both Coa and vWbp promote coagulation; they are sharing the same D1-D1 domain over which they activate prothrombin nonproteolytically (Bjerketorp et al., 2002; Friedrich et al., 2003; Kroh et al., 2009). The complex of these SERAMs with prothrombin converts soluble fibrinogen into insoluble fibrin (Friedrich et al., 2003; Kroh et al., 2009). The two coagulases differ in the amino acid sequence level (Kroh and Bock, 2012). Coa has a linker region at the C-terminus and a tandemly repeated fibrinogen binding motif. This fibrinogen binding motif is not present in vWbp, but instead it has a specific motif which binds to von Willebrand factor allowing *S. aureus* to adhere to vessel walls of activated endothelial cells (Bjerketorp et al., 2002; Claes et al., 2014; Ko and Flick, 2016).

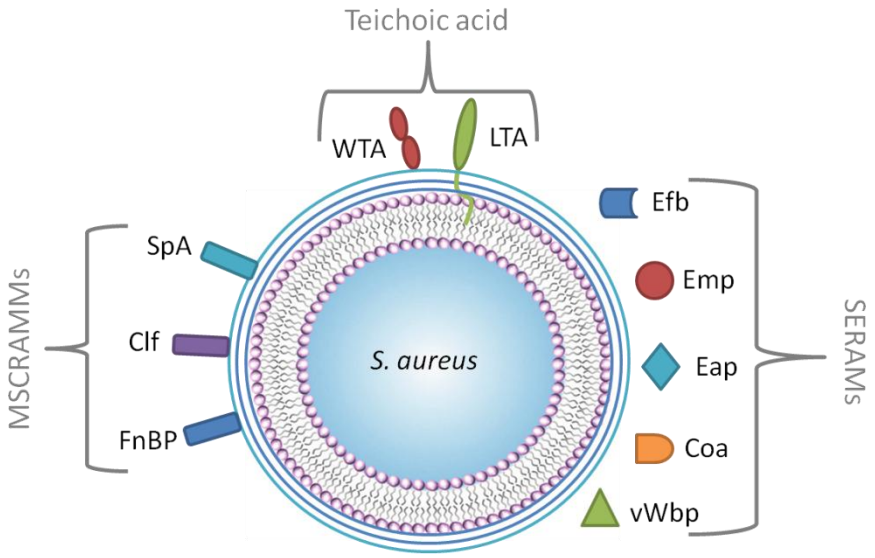


Figure 1.2: Graphical overview of *S. aureus* virulence factors involved in adhesion.
S. aureus possesses a broad range of surface proteins involved in their adhesion to extracellular matrix, plasma proteins or directly to host cells. Surface associated virulence factors expressed by *S. aureus* are teichoic acids, microbial surface component recognizing adhesive matrix molecules (MSCRAMMs) and secretable expanded repertoire adhesive molecules (SERAMs). For details on individual components refer to section 1.3.1 (Figure modified after Lowy, 1998; Choi et al., 2014).

1.3.2.2 Pore-forming toxins in *S. aureus*

Many of the diseases caused by *S. aureus* have been linked to the expression of a variety of secreted virulence factors such as α-toxin, leukocidins and PSMs, which belong to pore-forming toxins. These factors belong to a specific class of lipid binding proteins, which form membranous pores to disrupt the cellular integrity. A variety of such pore-forming toxins are secreted by *S. aureus*, targeting several types of host cells.

α-toxin is encoded by the single genetic locus *hla* and secreted as a water-soluble monomer. It forms a pre pore by oligomerizing into a heptamer on the plasma membrane, which is stabilized by caveolin 1, followed by a β-barrel transmembrane channel which is formed as the stem domain is inserted into the membrane (Gouaux et al., 1994; Song et al., 1996; Seilie and Bubeck Wardenburg, 2017). The expression is primarily controlled by the quorum sensing accessory gene regulator (*agr*) system. As

agr is active at the late log-phase and stationary phase of growth, the *hla* transcription is also active at that period of time (Novick et al., 1993; Novick et al., 1995). Another system which coordinates the environmental signals and monitors the expression of *hla* is the two-component system SaeR/S (Novick and Jiang, 2003; Voyich et al., 2009). The α-toxin is able to lyse endothelial cells, epithelial cell, leukocytes and platelets (Berube and Bubeck Wardenburg, 2013).

Leukocidins are bi-component pore-forming toxins. Until now, seven leukocidins have been identified in *S. aureus*: HlgAB, HlgCB, LukAB/HG (Ventura et al., 2010), LukED (Gravet et al., 1998), Panton-Valentine leukocidine (LukSF-PV/PVL) (Panton and Valentine, 1932), LukMF (Barrio et al., 2006) and LukPQ (Koop et al., 2017), whereby the last two are associated with zoonotic infections (Koop et al., 2017). The receptors of leukocidins vary in sequence and structure on the surface of different mammalian species, which are required for leukocidin toxicity (Spaan et al., 2017). It is still unclear how the transition to a pore formation function, after the receptor has bound (Spaan et al., 2017).

There are three different PSMs encoded in *S. aureus:* PSMα, β and γ (Wang et al., 2007). All PSMs have an α-helix amphipathic structure, whereby PSMα consists of four peptides and is positively charged, the PSMβ is negatively charged and has two peptides, and the PSMγ is encoded within the coding sequence of RNAIII and appears neutral (Peschel and Otto, 2013). The PSMs are regulated by *agr* quorum sensing system, whereby *psmα* and *psmβ* are both directly regulated by *agr*A (Cheung et al., 2011). When PSMs are released into the host system, they spread on surfaces, cause the formation of biofilm and can lead to cytolysis. On that regard, PSMα3 has the strongest cytolytic activity, whereas PSMβ peptides are not cytolytic at all (Wang et al., 2007; Cheung et al., 2012).

1.3.3 Internalization of *S. aureus* by non-professional phagocytes

In 1986, *S. aureus* was first reported to invade non phagocytic cells and is nowadays known to internalize and persist inside phagocytes or endothelial cells (Fig. 1.3) (Hamill et al., 1986; Fraunholz and Sinha, 2012). For the internalization of *S. aureus* into NPPCs exist different mechanisms with specific receptors (reviewed in Alva-Murillo et al., 2014).

The best-known internalization mechanism is via α5β1 integrin host receptor. The transmembrane receptor is a cation dependent glycoprotein, containing non-covalently associated α and β subunits, and can bind to the Arg-Gly-Asp or LDV domain of ligands such as fibrinonectin, fibrinogen, vitronectin and laminin (Kumar, 1998; Takada et al., 2007). The β1 integrin mediates the adhesion and endocytosis of *S. aureus* (Sinha et al., 1999; Hoffmann et al., 2011). As α5β1 integrin gets activated, it triggers particular signaling pathways and several focal adhesions. Examples are proteins such as actinin, paxillin, zyxin and tensin that accumulate in the host cell around the area of the pathogen (Agerer et al., 2003; Agerer et al., 2005; Schroder et al., 2006). Subsequently, focal adhesion kinase and Src kinase associate with Arp2/3 complex via coractin and actin binding protein, leading to an actin polymerization which binds to the endocytosis regulator dynamin-2 (McNiven et al., 2000; DeMali et al., 2003; Agerer et al., 2005; Selbach and Backert, 2005; Hauck and Ohlsen, 2006). This mechanism is named the zipper-type mediated internalization.

Heat shock proteins (Hsp) or Hsp-related proteins of the host cell are also playing a role in the internalization into NPPCs (Dziewanowska et al., 2000). *S. aureus* adhesion proteins such as FnBPs can bind directly to Hsp60 of the host, or Hsp60 binds in combination with integrin over a Fn bridge. Hsc70 can bind directly to autolysin, which mediates the internalization of *S. aureus*, with the mechanisms of endocytosis remaining unknown (Dziewanowska et al., 2000; Hirschhausen et al., 2010).

Another mechanism is activated via in the host cell membrane anchored Toll like receptors (TLRs) which mediate an intrinsic signaling pathway, leading to a certain response against the pathogen. Of several TLRs, TLR2 is most likely to be involved in

the invasion, since it recognizes different pathogen associated molecular patterns (PAMP) such as peptidoglycan, lipoproteins or lipoteichoic acid. In this context, TLR2 is building heterodimer with TLR1 or TLR6 (Ozinsky et al., 2000; Takeuchi et al., 2002). It is still unclear whether the receptor heterodimers are triggering a signal activity that results in internalization or if the binding of PAMPs mediates endocytosis (Fournier, 2012; Alva-Murillo et al., 2014).

Iron-regulated surface determinant B (IsdB) was found to support the invasion of *S. aureus* into HeLa cells (Mazmanian et al., 2003; Zapotoczna et al., 2013; Pietrocola et al., 2016). IsdB primarily served in iron-acquisition of *S. aureus* utilizing iron from hemoglobin of erythrocytes. During adhesion, IsdB interacts with integrins and stabilizes their conformation, therefore leading to interaction with ligands containing a RGD motif, followed by the internalization via an integrin dependent pathway. However, the endocytic pathway has not been determined yet (Zapotoczna et al., 2013).

1.3.4 Intracellular survival of *S. aureus*

Once *S. aureus* is within the host cell, there are different opportunities for the pathogen destiny, depending on virulence determinants based on the staphylococcal strain and on the host cell type and their sensitivity regarding the pathogen (Krut et al., 2003; Strobel et al., 2016). Some NPPCs have the ability to implement an endocytic degradation of included *S. aureus* even before the pathogen has the ability to survive within the host cell for an extended period (Melly et al., 1960; Hamill et al., 1986; Lowy et al., 1988; Amano et al., 2006; Tuchscherr et al., 2011). Right after the internalization, *S. aureus* is located in a membrane bound vacuole also known as phagosome. It matures from an early phagosome to a late phagosome, and after a while, fuses with a lysosome to rebuild a phagolysosome (Pitt et al., 1992; Desjardins et al., 1994). With growing maturation, the phagosomal protein compositions are changing and antimicrobial effectors are generated in order to create an inhospitable environment for most microorganisms. These include acidification, concentration of ROS, generation of nitrogen-based radicals and depletion of nutrients (Hackam et al.,

1997; Haas, 2007; Fairn and Grinstein, 2012; Uribe-Querol and Rosales, 2017). *S. aureus* possesses strategies to survive, grow or even escape from the environment in the vesicle (Fig. 1.3). A specific phenotype named small colony variants (SCVs) allows *S. aureus* to withstand the niche given milieu. SCVs have a thick cell wall, grow slow and have no pigment, they are not hemolytic, more resistant to a variety of antibiotics and they were shown to survive and grow within host cell phagosomes (Fraunholz and Sinha, 2012). However, the process of intracellular persistence of *S. aureus* is still poorly characterized.

1.3.5 Phagosomal escape and host cell death induced by intracellular *S. aureus*

In 1998, the escape of *S. aureus* from phagosomes of bovine mammary epithelial cells was described for the first time for bovine mastitis isolates. Meanwhile, phagosomal escape has been observed in epithelial and endothelial cells derived from different host species (Fig. 1.3) (Bayles et al., 1998; Giese et al., 2011; Chi et al., 2014; Grosz et al., 2014; Strobel et al., 2016). In these NPPCs, phagosomal escape of *S. aureus* is followed by replication within the host cytoplasm and subsequent host cell death. In order to escape from the phagosomes as well as from the host cell, *S. aureus* not only secrets specific proteins which have been identified as PSM (Grosz et al., 2014; Giese et al., 2011), but also pore forming toxins or Panton-Valentine leukocidins have been described to participate in the process (Jarry & Cheung, 2006).

As already mentioned, there are three different PSMs encoded in *S. aureus,* PSMα, β and γ, with the α-type having been identified as a key mediator of phagosomal escape (Wang et al., 2007; Grosz et al., 2014). The *psmα* operon and the δ-toxin (*hld*) are both encoded by RNAIII, which is an agr effector (Balaban and Novick, 1995; Novick and Jiang, 2003). The PSMs of *S. aureus* are secreted by an essential transporter named phenol-soluble modulin transporter, as in absence the PSM peptides are going to accumulate in the cytosol leading to cell death (Chatterjee et al., 2013). For the phagosomal escape, additional factors are necessary, since an overexpression of PSMs did not lead to the process (Giese et al., 2011). However, the escape of internalized *S. aureus* is possible if either PSMβ or δ-toxin are co-expressed with β-toxin (Giese et

al., 2011). Nevertheless, the mode of action of PSM during phagosomal escape is still not resolved (Moldovan and Fraunholz, 2019).

In combination, β- and δ-toxins play an important role for phagosomal escape of *S. aureus*. β -toxin is known as a sphingomyelinase, encoded by *hlb* with the activity being responsible for hemolysis, but not hemolytic towards erythrocytes in rabbits (Doery et al., 1963; Dinges et al., 2000; Huseby et al., 2007). However, this toxin is able to induce phagosomal escape in the presence of δ-toxin (Giese et al., 2011). The amphipathic peptide δ-toxin is encoded by RNAIII which in turn is induced by the agr-system. The toxin was shown to possess cytolytic properties, lyses lysosomes, mitochondria and erythrocytes (Bernheimer and Schwartz, 1964; Verdon et al., 2009).

The pore-forming toxin Panton-Valentine leukocidin (PVL) is composed of two components, LukS-PV and LukF-PV, which are encoded by several bacteriophages and reveal cytolytic activity (Kaneko et al., 1997; Tomita and Kamio, 1997; Kaneko et al., 1998; Goerke et al., 2009). The LukSF-PV were identified to be involved in the lysis of neutrophils as they bind to specific chemokine receptors C5aR and C5L2. Also, PVL facilitates the escape of *S. aureus* from human keratinocyte endosomes (Spaan et al., 2013; Chi et al., 2014). It was shown that PVL interacts with an Rab5-decorated endosome and permeabilizes the membrane after 2 hours of infection, so the bacteria can be released via a pore formation into the cytosol where PVL apparently promotes the induction of apoptosis by caspase dependent pathways (Chi et al., 2014).

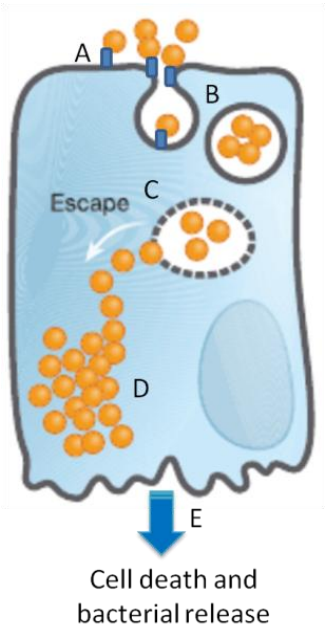


Figure 1.3: Schematic representation of *S. aureus* infection within the host cell.
(**A**) Adherence of *S. aureus* to host cell by MSCRAMMs, which triggers the (**B**) internalization into the host cell via a zipper-like mechanism, resulting in the formation of phagosomes implied with *S. aureus*. The pathogen is able to survive within the phagosome or (**C**) escapes from them into the cytoplasm where (**D**) *S. aureus* is able to replicate and (**E**) subsequently induces host cell death. (Figure modified after Horn et al., 2018; Moldovan and Fraunholz, 2019).

1.4 Regulation of virulence in *S. aureus*

S. aureus is a pathogen, which causes many illnesses by invading host tissue. In order to establish an infection within the host, the bacterium owns a diverse arsenal of virulence factors. The virulence factors can be classified into two groups, namely surface associated factors and secreted proteins, whose expression have to be coordinated during an infection. In *S. aureus* 36 regulatory families are activating or repressing genes in response to environmental and physiological conditions (Ibarra et al., 2013). Regulators involved in the expression of virulence factors are based on a broad range, including two-component systems (TCS), multiple antibiotic resistance regulator (MarR), LysR-type transcriptional regulator (LTTR) and many more (Fig. 1.4).

1.4.1 Two-component systems (TCS)

The two-component signal transduction system is known to be a widely distributed mechanism in *S. aureus* that perceives environmental changes and coordinates the regulatory response. As the name supplies, the system consists of two proteins - a sensor histidine kinase and the a-response regulator - that interact with one another. In a typical TCS, the membrane-associated histidine kinase gets activated by an external signal, leading to its autophosphorylation and followed by the phosphorylation of the response regulator. On the basis of phosphorylation of the response regulator it comes to an conformational change, which triggers to bind at a specific DNA sequence motif, resulting in the activation or repression of the target gene (Casino et al., 2010). Sixteen different TCS are encoded in most of *S. aureus* strains (Barakat et al., 2009; Galperin et al., 2010; Ulrich and Zhulin, 2010; Villanueva et al., 2018). There are two major regulators of virulence gene expression that are generally referred to as global regulators (AgrCA, SaeRS). Others are involved in antibiotic resistance, bacterial respiration and nutrient sensing or cell wall metabolism (Fig. 1.4) (Brunskill and Bayles, 1996; Giraudo et al., 1999; Martin et al., 1999; Fournier et al., 2000; Fedtke et al., 2002; Novick and Jiang, 2003; Kamps et al., 2004; Pragman et

al., 2004; Gardete et al., 2006; Meehl et al., 2007; Stauff et al., 2007; Hiron et al., 2011; Sun et al., 2012; Haag and Bagnoli, 2017).

1.4.1.1 The agr quorum-sensing system (QS)

One of the main staphylococcal virulence regulators is the accessory gene regulator. The *agr* system is one of the best-studied models of quorum sensing and regulates the expression level of more than 100 genes (Dunman et al., 2001; Queck et al., 2008). The *agr* locus encodes two divergent RNA transcripts, RNAII and the regulatory RNAIII (Novick et al., 1993; Morfeldt et al., 1995). *agr*BDCA of the quorum-sensing is part of RNAII, whereby AgrB is a transmembrane endopeptidase that evolves the AgrD propeptide into four different types of active pheromone named autoinducing peptides (Novick et al., 1993; Ji et al., 1995; Novick et al., 1995; Ji et al., 1997; Saenz et al., 2000; Qiu et al., 2005). Once the threshold concentration of autoinducing peptides is reached, AgrC and AgrA are stimulated as they form the sensor kinase and response regulator of TCS (Ji et al., 1995). Auto-inducing peptides are binding to the extracellular sensor domain of the dimeric, membrane bound protein AgrC which than undergoes transient autophosphorylation (George Cisar et al., 2009). The phosphate is transferred to the response regulator AgrA, which leads to a phosphorylation that triggers a conformational change to the protein, resulting in the formation of dimers (Srivastava et al., 2014). The phosphorylated AgrA is the main regulator of the agr auto-induction cycle and binds within the P2 and P3 promoters of agr with different affinities to drive the expression of RNAII and RNAIII (Cheung et al., 2004; Koenig et al., 2004; Queck et al., 2008; Wang and Muir, 2016). The expression of RNAII leads to a positive feedback loop which is a hallmark of quorum-sensing system enabling *S. aureus* to produce exoproteins (Novick et al., 1995). *Agr* upregulates the expression of secreted proteins and toxins such as PSMs, lipases, proteases, hemolysins, leukocidins, TSS toxins and exfoliative toxins, and represses the expression of surface proteins, including protein A and fibronectin-binding proteins (Fig. 1.4) (Recsei et al., 1986; Vandenesch et al., 1991; Dassy et al., 1993; Novick et al., 1993; Ji et al., 1995; Morfeldt et al., 1995; Novick et al., 1995; Wolz et al., 2000; Dunman et al., 2001; Kato et al., 2011; Haag and Bagnoli, 2017). Several *S. aureus* regulators modulate *agr* activity in a positive (CcpA, MgrA,

SarA) or negative way (CodY, SarR, SrrAB, sigmaβ) (Cheung and Projan, 1994; Yarwood et al., 2001; Horsburgh et al., 2002; Pragman et al., 2004; Ingavale et al., 2005; Seidl et al., 2006; Lauderdale et al., 2009; Majerczyk et al., 2010; Reyes et al., 2011; Roux et al., 2014; Jenul and Horswill, 2019).

1.4.1.2 *S. aureus* accessory element (Sae)

The staphylococcal accessory element is a TCS and consists of two transmembrane domains. It is important for the pathogenesis as it regulates the secretion of toxins, exoenzymes and immune evasion factors (Fig. 1.4) (Liu et al., 2016). The *sae* locus encodes a two-component signal transduction system, which contains four genes *sae*RSPQ. The genes encoding *sae*RS are predicted to be histidine kinase, whereas *sae*PQ are response regulator, whereby their proteins form a protein complex which regulates the sensor kinase phosphorylation, thereby affecting the expression level of *sar*R induced genes (Giraudo et al., 1999; Jeong et al., 2012). The P1 and P3 promoter impel the expression of the genes located in the *sae* locus. Many genes that are involved in the immune evasion and adhesion to the host are positively modulated by *sae*RS such as efb, eap, sspA, fnbA and fnbB and many more (Giraudo et al., 1994; Giraudo et al., 1997; Harraghy et al., 2005; Kuroda et al., 2007). External stimuli that result membrane perturbation such as low pH and high NaCl concentration were shown to repress the *sae*RS activation, whereas the presence of H_2O_2 and α-defensins induces the activity (Novick and Jiang, 2003; Weinrick et al., 2004; Geiger et al., 2008; Geiger et al., 2012). Like for *agr*, there are several other global regulatory mechanisms influencing the *sae* regulation such as *agr* and *fur*, which is a ferric iron uptake regulator (Johnson et al., 2011).

1.4.1.3 Other TCS regulating respiration, fermentation and nitrate metabolism

Pathogens are able to colonize various niches that exhibit different environmental conditions (Chan and Foster, 1998). During an infection, *S. aureus* passes a huge diversity of environments beginning with aerobic respiration, which switches to anaerobic respiration due to oxygen limitation in abscesses. *S. aureus* is a facultative anaerobe pathogen that allows to grow without oxygen as it uses either an anaerobic

respiration with nitrate as the terminal electron acceptor, or it ferments carbohydrates (Strasters and Winkler, 1963; Burke and Lascelles, 1975; Fuchs et al., 2007; Haag and Bagnoli, 2017). To deal with environmental oxygen levels in order to regulate respiratory activity and divert energy fluxes into different metabolic pathways, *S. aureus* possesses three TCS named SrrAB, NreCBA and AirRS.

The TCS of the staphylococcus respirator response (SrrAB) controls the induction of genes required for anaerobic growth in this organism, whereas *srr*A is the cytoplasmic response regulator and *srr*B the signal transduction system of the membrane bound histidine kinase (Fig. 1.4) (Pragman et al., 2004). Under low oxygen conditions, *srr*A binds to the *agr*, *srr*, *spa* and other promoters, and represses their transcription. Under aerobic conditions, it enhances the level of *spa* (Yarwood et al., 2001; Pragman et al., 2004; Pragman et al., 2007). Furthermore, *srr*AB is a major regulator for *ica* operon under low oxygen conditions, which encodes the polysaccharide intercellular adhesin that may protect *S. aureus* against non-oxidative killing by neutrophils (Ulrich et al., 2007). *srr*AB was also shown to regulate fermentative enzymes such as alcohol dehydrogenase and lactate dehydrogenase, and to negatively regulate some enzymes of the tricarboxylic acid cycle (TCA) (Throup et al., 2001). Besides the regulation under hypoxia, the TCA plays a role in the resistance to nitrosative stress (Kinkel et al., 2013).

The TCS for nitrogen regulation (NreCBA) is involved in metabolism and transport of nitrate and nitrite (Fedtke et al., 2002). Unlike the other mentioned TCS, NreB is not membrane bound but is found in the cytosol. An activation of *nre*B could be found by incubation with cysteine desulfurase, ferrous iron and cysteine (Kamps et al., 2004). The *nre*B is predominant under aerobic conditions, as the autokinase is active, therefore leading to the transfer of the phosphorylgroup to the response regulator *nre*C (Kamps et al., 2004; Reinhart et al., 2010). The phosphorylation of *nre*B can be decreased by *nre*A in a nitrate dependent manner, by direct inhibition (Nilkens et al., 2014).

1.4.2 Network of metabolism and virulence regulation

Beside the regulation of virulence, *S. aureus* owns a set of transcriptional factors involved in metabolic and nutrient response regulation. These in turn can be divided into catabolite responsive regulators (*ccp*A, *cod*Y, *rpi*Rc), nitrogen dependent regulators (*nre*C, *cod*Y), oxygen dependent regulators (*srr*A/B, *mgr*A, *per*R) and metal dependent regulators (*fur*, *mnt*R, *zur*, *cop*). Many of these regulators can directly and indirectly influence the function of virulence regulators and vice versa, in consequence of close interconnection (Fig. 1.4).

The glucose-mediated regulation was one of the first described catabolite regulators, with the catabolite control protein A (CcpA) being the major regulator for the carbon catabolite repression which requires histidine containing protein kinase (Magasanik, 1961; Henkin et al., 1991; Jankovic et al., 2001). Beside the metabolization of glucose, *ccp*A has an impact on growth, biofilm formation, the transcription of selected virulence determinants and antibiotic resistance in *S. aureus* (Seidl et al., 2006; Seidl et al., 2008). It was shown that CcpA down regulates the expression of *RNA*III which in turn is an effector molecule of *agr,* with an impact on α-toxin and spa encoding protein A, and therefore CcpA is directly linked to their decrease. In addition, CcpA abolishes the capsule formation in the presence of glucose as it decreases the *cap* operon transcription (Seidl et al., 2006). In addition, it upregulates the expression of *cidA*, *icaA* and polysaccharide intercellular adhesin (PIA) production, presumably via the downregulation of the tricarboxylic acid cycle genes *citB* and *citZ,* which all are factors influencing the biofilm formation in *S. aureus* (Seidl et al., 2008).

Beside CcpA, the highly conserved global repressor *cod*Y regulates the carbon metabolism, and additionally the nitrogen metabolism, responding to intracellular concentrations of branched-chain amino acids (BCAA) and GTP (Slack et al., 1995; Shivers and Sonenshein, 2004; Tojo et al., 2005; Shivers et al., 2006). It was shown that CodY negatively regulates virulence gene expression of the *agr* locus, the hemolytic α-toxin and the operon responsible for the production of PIA (Majerczyk et al., 2010). Furthermore, it has an repressive impact on the expression of cysteine protease, serine protease, V8 protease, peptide, amino acid transporters and amino acid catabolic gene

(Somerville and Proctor, 2009). A RNA-Seq transcriptional profile revealed that the availability of isoleucine, leucine, valine and GTP mediates a metabolic reorganization, therefore controlling virulence factor production necessary for invasion of host cell (Waters et al., 2016).

The activity of the TCA cycle is dependent on the stress induction and can be regulated by virulence factors, whereby the pentose phosphate pathway is regulated by RpiRc (Zhu et al., 2011). This RpiRc has furthermore an impact on the transcription of *RNAIII*, which is an effector molecule of the quorum sensing system and therefore plays a role in virulence determinant synthesis next to the regulation of the central metabolism (Zhu et al., 2011). Data showed that RpiRc affects the transcription of *RNAIII* in concert with the regulators *sar*A and sigma β (Gaupp et al., 2016). Moreover, RpiRc is a repressor for lukED and lukSF, therefore regulating the expression of leukocidins which are known to prevent bacterial killing and biofilm clearance by neutrophils (Balasubramanian et al., 2016; Bhattacharya et al., 2018).

It is well established that humane immune system uses copper to kill invading microorganisms. In order to escape from this environmental stress, *S. aureus* possesses strategies to control the intracellular level of copper to avoid toxicity, including membrane copper exporters CopA and copper chaperon CopZ (Sitthisak et al., 2007a). The *cop*AZ operon gets derepressed by the Cu-senstitive operon repressor (*cso*R) due to intracellular binding of copper (Grossoehme et al., 2011). CopB and CopL proteins are additional factors to suppress cooper toxicity, whereas *cop*B encodes a copper exporter and *cop*L a membrane bound, surface exposed copper binding lipoprotein (Rosario-Cruz et al., 2019).

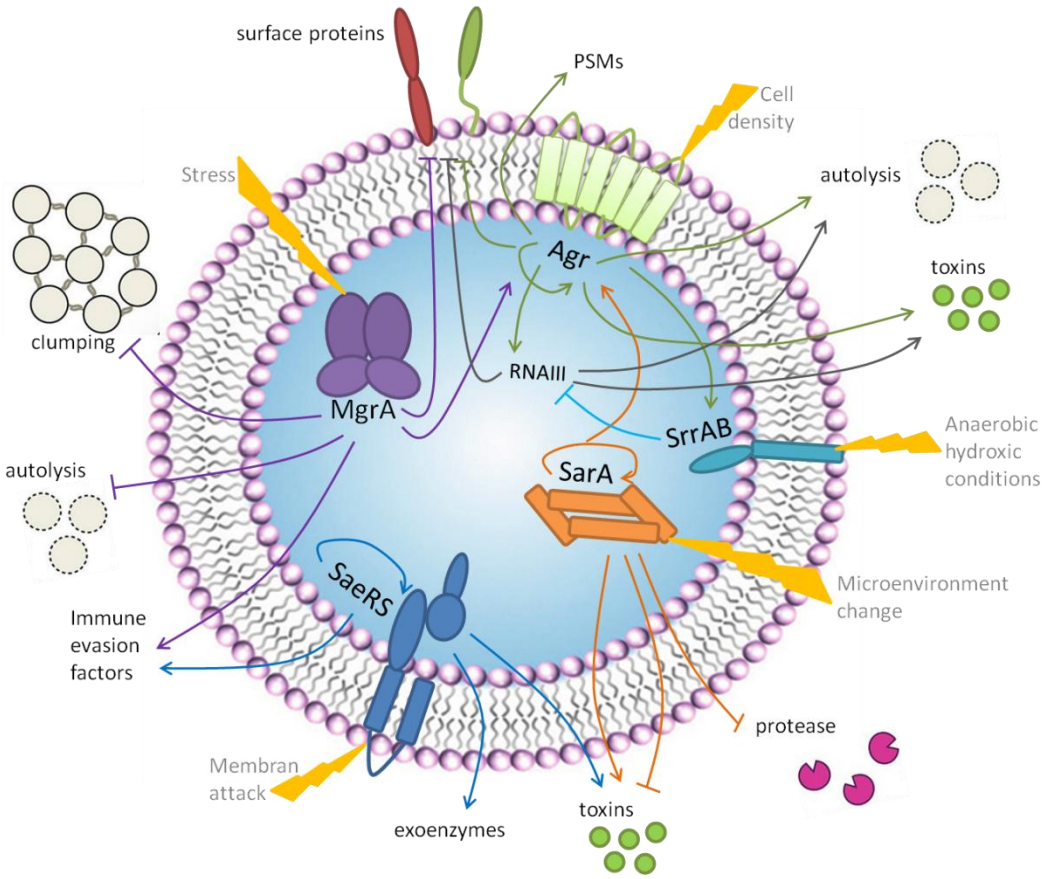


Figure 1.4: Graphical illustration of metabolism and virulence network regulation.
S. aureus perceives various signals from the environment, here marked with yellow flashes. Dependent on the signal the pathogen possesses several regulators that can response in a specific way by expressing or repressing different virulence factors. Virulence regulators are interconnected and can regulate the same virulence factor. The different factors are valuable for *S. aureus* in order to survive. For details on individual components refer to section 1.4 (Figure modified after Fisher et al., 2018; Jenul and Horswill, 2019).

1.5 LysR-type transcriptional regulators

The LysR-type transcriptional regulator (LTTR) family was initially described in 1988 (Henikoff et al., 1988). Today, it is a well-characterized group of bacterial transcriptional regulators which could be clustered on the basis of sequence similarity and conservation of DNA-binding-domain. Based on their amino acid sequence, 800 members were identified, due to which they count as the largest known family of prokaryotic DNA binding proteins (Schell, 1993). LTTRs are highly conserved in bacteria, but functional orthologues are found in archaea and eukaryotic organisms (Perez-Rueda and Collado-Vides, 2001; Sun and Klein, 2004; Stec et al., 2006;

Maddocks and Oyston, 2008). Due to evolutionary pressure and genetic divergence, multiple paralogues of LTTRs can be present within a given genome, which are engendered by gene duplication. Although the structure and functions might be similar, sometimes the control of various regulons differs in limited or no cross-talk (Maddocks and Oyston, 2008). The protein family is involved in the expression of an extremely diverse set of genes. Hence, LTTR are implicated in regulating numerous cellular functions including environmental recognition, motility, cell division, oxidative stress responses, attachment, secretion, virulence, quorum sensing, metabolism, efflux pumps and nitrogen fixation (Schlaman et al., 1992; Kovacikova and Skorupski, 1999; Deghmane et al., 2000; Cao et al., 2001; Deghmane et al., 2002; Kim et al., 2004; Russell et al., 2004; Byrne et al., 2007; Lu et al., 2007; Sperandio et al., 2007; Maddocks and Oyston, 2008; Habdas et al., 2010; O'Grady et al., 2011; Toledo et al., 2011; Shimada et al., 2013; Matthias and Rest, 2014; Takao et al., 2014; Santiago et al., 2015). These global transcriptional regulators can be auto-regulators, activators and or repressors of a single or operonic genes which can be located somewhere on the bacterial chromosome and are most of the time divergently transcribed (Lindquist et al., 1989; Schell, 1993; Parsek et al., 1994; Heroven and Dersch, 2006; Hernandez-Lucas et al., 2008). In general, a co-inducer is necessary for the transcriptional activation or repression of the target gene, but sometimes the protein has a function on *lttr* via feedback loop (Celis, 1999; van Keulen et al., 2003; Picossi et al., 2007; Maddocks and Oyston, 2008) (Fig. 1.5). The co-inducers have structural diversity and can be aromatics, various aliphatics, ions and amino acid derivates, to stimulate various LTTRs (Schell, 1993).

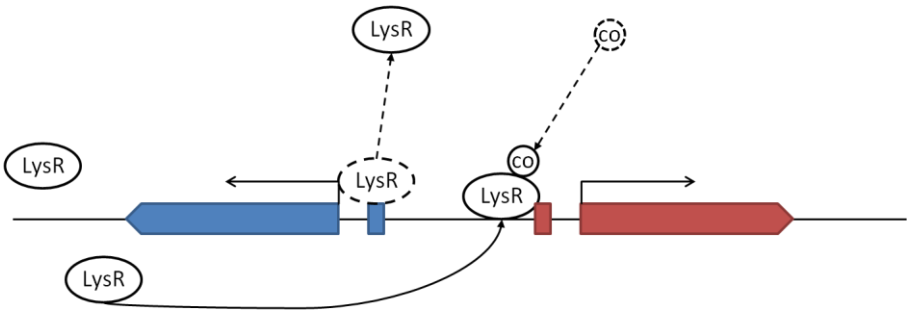


Figure 1.5: A schematic model of a LTTR dependent transcriptional regulation.
First, the *lysR* gene is transcripted if the LysR protein dissociates from its promoter. In the depicted case, the transcribed LsyR protein binds upstream at the promoter of a divergently transcribed target gene. Only if a co-inducer gets in contact with the LysR protein, the target gene gets transcribed (Figure modified after Maddocks and Oyston, 2008).

1.5.1 Structure and transcriptional regulation of LTTRs

The family of LysR-type transcriptional regulators is defined by its high degree of sequence conservation within important structural domains. *lttr*s consist of a length of approximately 330 amino acids, with a characteristically high G + C content, which have been acquired within the genome of *S. aureus* and many other bacteria by horizontal transfer (Viale et al., 1991; Schell, 1993; Maddocks and Oyston, 2008). Present in all *lttr*s is a helix-turn-helix (HTH) motif, which can bind to DNA and a co-factor binding domain (Fig. 1.6) (Henikoff et al., 1988; Schell, 1993; Perez-Rueda and Collado-Vides, 2001; Maddocks and Oyston, 2008).

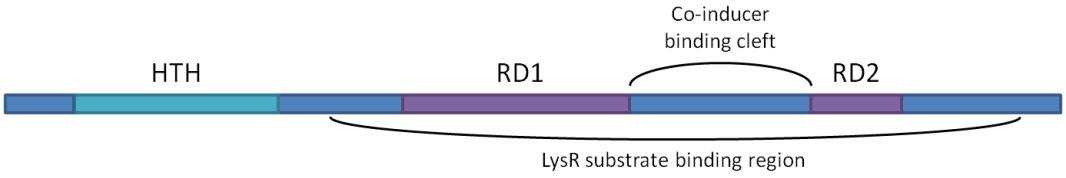


Figure 1.6: Schematic representation of *lttr* gene structure by the example of *E. coli* LysR.
HTH domain on the N-terminal side and RD1 as well as RD2 on the C-terminal side. The C-terminal is also called LysR substrate binding region as there is the co-inducer cleft within RD1 and RD2 (Figure modified after Maddocks and Oyston, 2008).

The HTH motif can be located at the N-terminus whereby the transcriptional regulator acts as a repressor, whereas it behaves as an activator when being located at the C-terminus. *lttr*s can also act as dual regulators, whereby the HTH is located 20-90 amino acids from the N-terminus (Perez-Rueda and Collado-Vides, 2000; Maddocks and Oyston, 2008). The HTH motif of the LysR family is a member of a common three helical bundle HTH DNA binding motif in an open conformation, including a winged-helix variety. LysR family members own a fourth helical structure. In addition, they form a single anti-parallel β-plated sheet hairpin between the second and third helices, resulting in a bundle, which interacts with the DNA, the tetra-helical structure and the ribbon helix-helix structure (Pabo and Sauer, 1984; Brennan and Matthews, 1989; Huffman and Brennan, 2002; Aravind et al., 2005; Maddocks and Oyston, 2008). However, conformational changes can occur in order to allow various communication between the ligand and DNA binding regions. Therefore, besides LTTRs with tetrameric crystal forms like AgrP, AphB, CbnR and TsaR, there exist LTTRs with octameric crystal forms with compact subunits such as CrgA and BenM (Muraoka et al., 2003; Sainsbury et al., 2009; Monferrer et al., 2010; Ruangprasert et al., 2010; Zhou et al., 2010; Taylor et al., 2012). There are even further differences within tetrameric LTTRs, classified into a closed form, which have an additional head-to-head interaction between the regulatory domain subunits at the contact helix and an open form (Muraoka et al., 2003; Monferrer et al., 2010; Zhou et al., 2010; Taylor et al., 2012).

The C-terminus of *lttr*s is better known as co-factor binding domain, connected via a hinge region to the HTH domain (Maddocks and Oyston, 2008). This region includes two distinct sub-domains of α and β-strands, named regulatory domain 1 and 2 (RD1 and RD2) (Neidle et al., 1989). The domains are connected by a hinge-like region between residues 95 and 210 (Burn et al., 1989; Cebolla et al., 1997; Jorgensen and Dandanell, 1999). The connection of the two crossover regions forms a cleft at which the co-inducer binds (Stec et al., 2006; Maddocks and Oyston, 2008). Studies showed that this causes conformational changes at the co-inducer binding site which can be related to the different binding ability of LTTRs and due to that having a relatively low conservation of the C-terminus (Maddocks and Oyston, 2008). Co-inducer binding

clefts were found in C-terminal region of lower than 80 amino acids as well as between 225 and 290 amino acids, which have an effect on the binding capability of the proteins, leading to a co-inducer independent phenotype (Burn et al., 1989; Storz et al., 1990; Maddocks and Oyston, 2008). It is possible that two different co-inducers bind to the same LTTR, but also that the same co-inducer binds to different LTTRs (Collier et al., 1998; Maddocks and Oyston, 2008; Ruangprasert et al., 2010).

Regulated genes can possess several binding sites for LTTR. By DNA-footprinting, DNase I-protection studies and mutagenesis, multiple binding sites for LTTR protein were identified such as GltC in *Bacillus subtilis*, GcvA and CysB in *E. coli*, AtzR in *Pseudomonas sp*. or LadA in *Myxococcus xanthus*, ranging from -218 bp up to an internal binding site of +350 bp, with the majority binding around -35 to +20 bp of the so-called regulatory binding site (RBS) and -40 to -20 bp of the activation binding site (ABS) (Belitsky et al., 1995; Wilson et al., 1995; Lochowska et al., 2004; Porrua et al., 2007; Viswanathan et al., 2007; Maddocks and Oyston, 2008). One of the various mechanisms by which LTTRs operate involves four subunits, of which two LTTR subunits bind to the RBS, whereas the two other two bind to the ABS. Due to oligomerization this leads to a tetrameric protein which causes the DNA to bend between 50° and 100°, also known as apo-form. The angle of the DNA bending can be lowered for 9° to 50° if a co-inducer binds to the LTTR tetrameric protein, leading to a higher binding affinity of RNA polymerase, thus initiating transcriptional activation or repression (Fig. 1.7) (van Keulen et al., 2003; Tropel and van der Meer, 2004; Maddocks and Oyston, 2008).

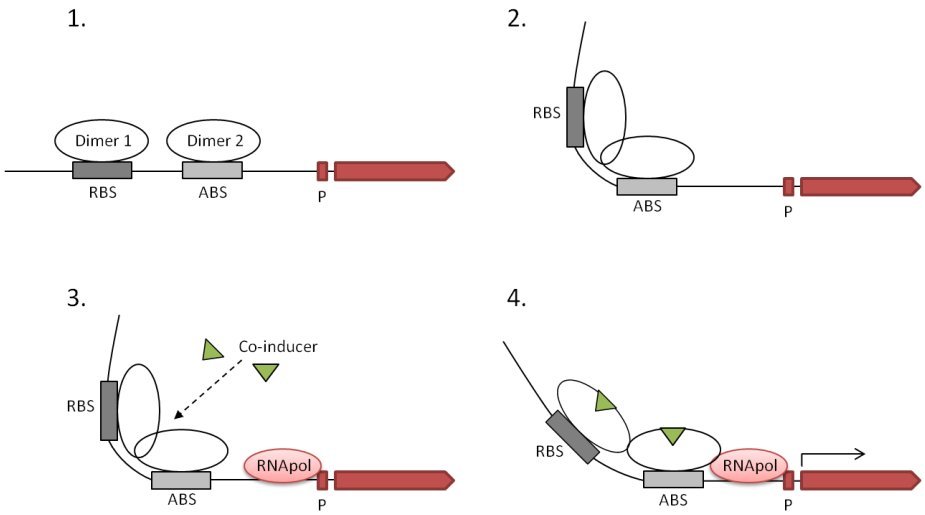


Figure 1.7: A more particularized schematic representation of LTTR transcriptional activation. (**1**) LTTR dimer 1 binds at RBS and LTTR dimer 2 at ABS. (**2**) DNA bending results from protein-protein oligomerization of both LTTR dimers, which leads to the formation of a tetrameric protein. (**3**) Binding of RNA polymerase at the promoter of the target gene, but no activation of the transcription. (**4**) After the co-inducer binds to the LTTR tetramer the DNA bending relaxes and the LTTR tetramer is coming into contact with the RNA polymerase, leading to the transcription of the target gene (Figure modified after Maddocks and Oyston, 2008).

There are different approaches of LTTR regulation, the classical ones are the transcriptional activation and negative autoregulation. It is commonly observed that this type of regulation can be coupled via a feedback loop. For example, IlvY of *E. coli* and *Salmonella spp.,* which is classically regulated, whereby expression of *ilvY* is negatively auto regulated by IlvY and the expression of *ilvC* is activated by IlvY (Blazey and Burns, 1980; Rhee et al., 1999; Maddocks and Oyston, 2008). LTTRs are also able to repress the transcription. An LTTR which only acts as a global repressor is the LTTR RovM, which is the regulator of the virulence activator gene *rov*A, found in *Yersinia pseudotuberculosis* and controls the cell invasion, virulence and motility (Heroven and Dersch, 2006). Since now, no co-inducer is known. The difference between transcriptional repressor and activator is very complex. But it is possible that LTTRs act as both, which depends on both the DNA binding site and the nature of the co-inducer (Picossi et al., 2007; Maddocks and Oyston, 2008). Both transcriptional options were found for the LTTR contact regulated gene A (*crg*A) and *leu*O (Maddocks and Oyston,

2008). CrgA was found to be necessary in *Neisseria meningitidis* for the adhesion to epithelial cells. The co-factor is α-methylene-γ-butyrolactone and the gene itself is involved in the synthesis of a capsule for adhesion. However, it can also repress the expression of *pilC1* after the initial phase of induction, which is necessary for the bacterial adhesion (Deghmane et al., 2000). LeuO has been implicated in the bacterial stringent response and in the virulence of *Salmonella,* whereby the co-inducer is still unknown (Hernandez-Lucas et al., 2008). An LTTR can also auto-regulate the activation or repression of a target gene in a positive way. Members of this subgroup are HexA and PecT of *Erwinia spp.* and LrhA of *E. coli* (Gibson and Silhavy, 1999; Maddocks and Oyston, 2008), which regulate genes required for flagellation, motility and chemotaxis. Transcriptional regulators involved in global, complex regulatory networks are negatively regulated by these LTTRs. So far, no co-inducer or environmental stimuli have been found for this group.

1.5.2 *S. aureus* LTTRs

So far, there have been just five LTTRs identified in *S. aureus* including CidR, HutR, GltC, CcpE and RpvA (Yang et al., 2005; Hartmann et al., 2013; Ibarra et al., 2013; Han et al., 2019).

CidR activates the expression of two operons including *cidABC* and *alsSD* that play pro- and anti-death death mechanisms simultaneously (Yang et al., 2006; Chaudhari et al., 2016). It is regulated in a classical way by a combination of transcriptional activation and negative auto-regulation via a feedback loop (Yang et al., 2005). During the metabolism of glucose, an upregulation of *cidBC* expression promotes the generation of acetate, which in turn potentiates cell death in the stationary phase by cytoplasmic acidification. In order to decrease the cell death, the expression of *cidABC* and *alsSD* is upregulated via *cidR*. CidC itself is able to produce acetate once it is localized in the membrane. AlsSD consumes protons and competes against CidC for pyruvate, while the activity of CidA inhibits the cytoplasmic abundance of CidC (Yang et al., 2005; Yang et al., 2006; Chaudhari et al., 2016). Due to that, CidR has an effect on the stationary-

phase survival of S*. aureus.* Additionally, it was found to have an effect on the antibiotic tolerance (Yang et al., 2005).

The least investigated LTTR in *Staphylococcaceae* is HutR, a putative repressor of the histidine utilization operon, which is located upstream of the *hutU* operon in the divergent direction and therefore shares the same regulatory region with the target operon (Ravcheev et al., 2011; Ibarra et al., 2013). In a global analysis of transcriptional regulators of *S. aureus*, HutR was identified to be present in all *S. arueus* strains and is involved in the metabolic regulation (Ibarra et al., 2013). Until today, no co-inducer is known.

Involved in the synthesis of glutamate is the classical LysR-type transcriptional regulator GltC. It can act as a transcriptional activator and transcriptional repressor, depending on the binding site at the promoter region and the nature of the co-incuder (Picossi et al., 2007; Maddocks and Oyston, 2008; Dormeyer et al., 2019). The mechanism of this LTTR was described within *B. subtilis* and identified in *S. aureus* via sequence similarity. If *B. subtilis* is grown with the carbon source glucose, GltC activates the expression of the glutamate synthase genes *gltAB*, but if intracellular glutamate is surplus, the *gltAB* genes are not transcribed as the glutamate-degrading glutamate dehydrogenases inhibits GltC (Dormeyer et al., 2019). It was also shown that this *lttr* is repressed by CodY, which leads to the suggestion that *gltB* and *gltD* are repressed by CodY and indirectly influenced via *gltC* (Pohl et al., 2009).

The LTTR catabolite control protein (CcpE) was identified due to homology to the *Bacillus subtilis* CcpC regulator (Hartmann et al., 2013). It is a major positive regulator of the tricarboxylic acid (TCA) cycle activity. Most of the genes the regulon has an effect on are involved in the pathogenesis of *S. aureus* (Hartmann et al., 2013; Ding et al., 2014). It activates the expression of *citB* in a direct manner, as sodium citrate binds directly to the oligomeric state of CcpE protein, leading to an active form of LTTR (Ding et al., 2014). This LTTR does not bind to the promoter of *citZ* (Hartmann et al., 2013). It is believed that CcpE positively regulates a citrate consuming enzyme or alters a pathway that diverts acetyl-CoA from citrate production or negatively regulates a citrate-producing enzyme. In addition, some studies showed that a downregulation of

ccpE seems to protect the bacterium from the human whole blood mediated killing and facilitates the bacterial survival within the host (Ding et al., 2014).

Just recently the LTTR RpvA was described, it represents a regulator of persistence and virulence, whose mutation leads to higher susceptibility to antibiotics norfloxacin and ampicillin and various stresses including oxidative stress, heat and starvation in late exponential and early stationary phase (Han et al., 2019). Possible target genes of RpvA are *hla*, *hlgB* and *crtM* which were detected via gel shift assay. Beside the carotenoid biosynthesis genes crtI, crtM and crtN, also the expression of virulence genes are upregulated for *hla*, *hlgA*, *hlgB*, *hlgC*, *lukF*, *lukS*, *lukD*, *sea* and *coa* which increase the statement that RpvA is important for *S. aureus* virulence. Additionally, RvpA could repress multiple genes including *gapR*, *gapA*, *tpi*, *pgm*, *eno*, *glpD*, and *acs* expression. Within the cell, this LTTR has a positive effect on the production of the following metabolites: dihydroxyacetone phosphate, 2-phosphoglycerate, acetyl-CoA, glycerol 3-phosphate, L-glutamate.

Recently, *lttr852* was identified by Tn-Seq to be critical for bacterial colonization of secondary organs in a murine model of metastatic bloodstream infection. However, the regulatory role of the *lttr852* has remained largely elusive.

1.6 Aim of the study

The aim of this study was the functional characterization of LTTR852 by investigating the effects of the deletion of this *lttr* on invasion and escape from HEK 293T cells. The role of the *lttr852* in biofilm formation and hemolysis was also assessed under chemical induction with AHT. Furthermore, RNA-Seq was performed in order to gain insight into the expression profile of the regulon controlled by *lttr852*. Promoter activity studies was applied to study the activity of the *lttr852* under different growth conditions to identify factors under which the promoter is active. These promoter activity studies were further applied in different *S. aureus* regulators to determine the role of the *lttr852* in the virulence regulatory network of *S. aureus*. This study also aimed to identify a potential co-inducer of the *lttr852* by using a transcription factor

domain swap to identify possible target genes. Lastly, the ability of the *S. aureus lttr852* mutant strain to metabolize different compounds was also assessed.

2. Results

2.1 The LysR-type transcriptional regulator *lttr*852 is essential for the colonization of mice secondary organs

In a previous study Das et al., (2016) used deep sequencing of transposon insertion sites (Tn-Seq) of mice which where intravenously infected with *S. aureus* strain 6850 transposon library. Five genes were identified, RSAU_000958 [*pur*M], RSAU_000901, RSAU_000571 [*lta*A], RSAU_002542, a putative DNA-binding protein and a novel RSAU_000852, whose mutants were significantly depleted in homogenates of liver, kidneys and tibia. Except for RSAU_000852, the other four genes have been characterized and shown to be important in the physiology of *S. aureus.* The RSAU_000852 was termed a LysR-type transcriptional regulator since it is homologous to genes encoding LysR-type transcriptional regulator. Interestingly, the Tn-Seq data revealed that the number of bacteria with a mutation in *lttr*852 recovered significantly lower from the kidneys compared to the wildtype strains (Sup. Fig. 7.1). To validate the observations from the transposon mutant screen, an isogenic knockout mutant within the *S. aureus* strain 6850 which is deficient in the expression of *lttr*852 (Δ852) was generated. Mice were intravenously infected with Δ852 and wildtype strains and the kidneys, livers and tibias were assessed in order to determine the capacity of the *S. aureus* Δ852 to establish infections in secondary organs. As shown in Figure 2.1, while CFU of wildtype (WT) and Δ852 strains recovered from the liver did not differ significantly (Fig. 2.1 A), the CFU of Δ852 strain recovered from the kidneys (Fig. 2.1 B) and bones (Fig. 2.1 C) were significantly less compared to wildtype strains. Serum levels of the inflammatory cytokine interleukin 6 were also significantly lower in mice infected with the Δ852 mutant strain compared to mice infected with the wildtype strain. These results corroborate the requirement of *lttr*852 for successful *S. aureus* infection of secondary organs during blood stream infection. Sequence alignments showed that the sequence variants shared 96% identity at the amino acid level which indicated that this *lttr* is highly conserved in *S. aureus.* Since this *lttr* is highly conserved in *S. aureus,* efforts were made in this thesis to characterize the function of this novel

regulator, which demonstrates to be essential for colonization of secondary organs by *S. aureus.* However, due to the complexity associated with mouse experiment, efforts were replicate conditions present in the kidneys *in vitro* in order to characterize the *lttr*852.

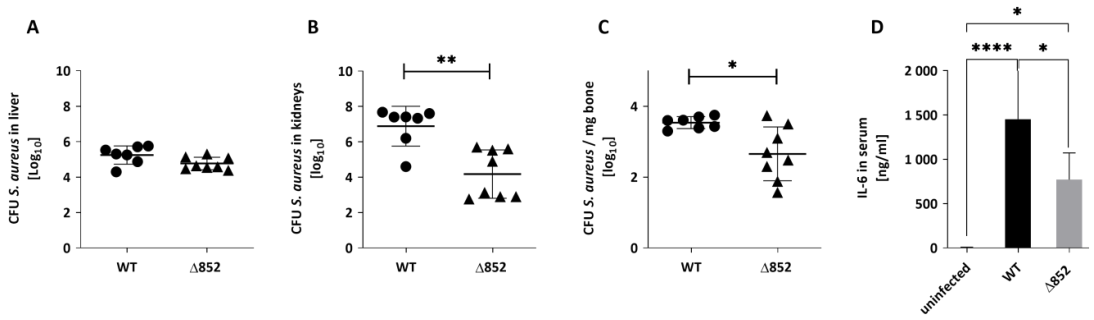


Figure 2.1: *lttr*852 is necessary for successful infection of secondary organs by *S. aureus*. Mice were intravenously infected with WT (●) and Δ852 (▲) and at 24 h of infection CFU were determined in liver (**A**), kidneys (**B**) and tibiae (**C**). Each symbol represents the data of an individual animal. Data are pooled from three independent experiments. (**D**) Serum levels of IL-6 in uninfected or infected mice with wildtype or Δ852 mutant strain at 24 h of infection. Each column represents the mean values ± standard deviations (SDs) from three independent experiments. *, $p < 0.05$; **, $p < 0.01$; ****, $p < 0.001$ (Figure modified after Groma et al., 2020)

*2.2 lttr*852 of *S. aureus* is not important during cell invasion and phagosomal escape *in vitro*

S. aureus strains possess the capacity to invade different cell types (Strobel et al., 2016). Since there was a significant variation in the CFUs of wildtype and Δ852 recovered from the infected mice, the role of the *lttr*852 in cellular invasion by *S. aureus* was assessed using human empryonic kidney (HEK) 293T cells infected *in vitro.* The HEK 239T cells were growth to 80% confluence and then infected with *S. aureus* 6850 wildtype and *lttr*852 mutant at a MOI of 5. The gentamycin protection assay was used to assessed invasion after 1.5, 2, 3 and 4 hours post inoculation. Serial 10-fold dilutions were then plated on TSB pates and the CFU were determined the next day. As shown in Figure 2.2 A, the CFUs did not significantly differ between

HEK 293T cells infected with WT and ∆852 strains. However, the invasiveness of both strains showed to be dependent on the duration of the inoculation.

S. aureus strains have previously been found to be able to mature and to escape from phagosomes of different host cell types (Fraunholz and Sinha, 2012; Grosz et al., 2014; Moldovan and Fraunholz, 2019). The *lttr*852 impact on the phagosomal escape capability of *S. aureus* was investigated by infecting HEK 293T YFP-CWT (**y**ellow **f**luorescent **p**roteins - **c**ell **w**all **t**argeting domain), engineered to tag escaped bacterial cells with yellow fluorescent proteins, with *S. aureus* strains carrying a red fluorescent protein plasmid SarAP1-mRFP. The HEK 293T YFP-CWT cells were infected with the wildtype and ∆852 strains as described above. The relative escape rates from HEK 239T YFP-CWT cells were calculated from the ratio of YFP to RFP bacteria. As shown in the Figure 2.2 B, escape of both WT and mutant strains demonstrate to be temporal as the lowest relative escape of 5 was observed 1.5 h post inoculation (p.i.) and the highest 4 h p.i.. However, the relative escape rate did not significantly differ between WT and Δ852 strains. Taken together, the data suggests that *lttr852* may not be involved in *S. aureus* invasion and phagosomal escape.

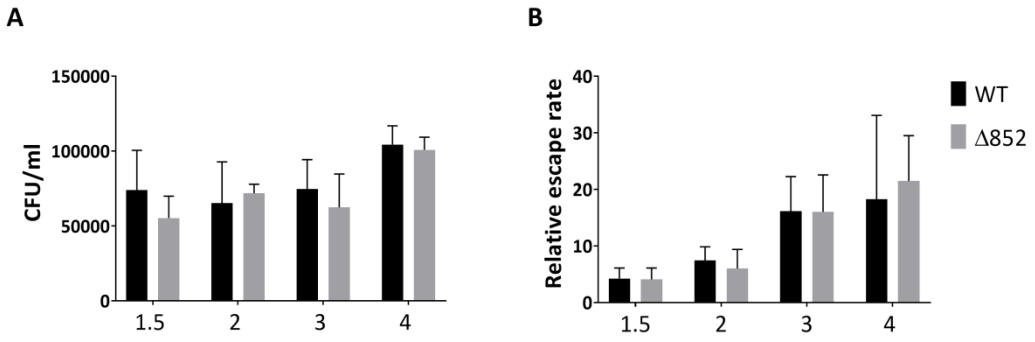


Figure 2.2: *lttr*852 is not involved in *S. aureus* invasion and phagosomal escape.
HEK 293T YFP-CWT cells were infected with *S. aureus* WT, Δ852 at a MOI of 5 and assessed for invasion and phagosomal escape every hour for 4 hours p. i. (**A**) Colony forming units per ml of HEK 239T YFP-CWT cells infected with *S. aureus* wildtype (black) and Δ852 (grey) strains after 1.5, 2, 3 and 4 h p.i.. (**B**) Relative escape rate of HEK 239T YFP-CWT cells infected with *S. aureus* wildtype SarAP1-mRFP (black) and Δ852 SarAP1-mRFP (grey) strains after 1.5, 2, 3 and 4 h p.i.. The graph represents the mean values ± SDs from three independent experiments performed in triplicates.

2.3 Induced expression of *lttr*852

*lttr*852 was not found to be expressed in over 100 conditions, including infection-simulating conditions, in a study by Mader et al. (2016). It was therefore of interest to inducibly express the unknown *lttr* to test for transcriptional changes. For this purpose, the plasmid p2085_852-AHT was transformed into *S. aureus* 6850 Δ852 as well as into the wildtype strain. The plasmid p2085_852-AHT is based on the pALC2084 and thereby puts the open reading frame (ORF) of the unknown *lttr* under control of an anhydrous tetracycline (AHT) inducible promoter $P_{xyl/tet}$ (Bateman et al., 2001). Cultures of *S. aureus* wildtype, *lttr*852 mutant as well as of both strains carrying the inducible plasmid were pulsed with 200 µg/ml of AHT at 4 h and 24 h to induce the expression of the p2085_852-AHT plasmid. The induction of the *lttr852* was assessed by performing RT-PCR with the primers RT-852-fw and RT-852-rev.

As shown in Figure 2.3 A, a significant transcription of the *lttr*852 was observed in the WT and Δ852 strains carrying the inducible plasmid compared to both strains without the inducible plasmid, upon AHT induction already after 4 h. In contrast, induction with AHT after 24 h resulted in a vigorous expression of *lttr*852 in the wildtype ($\Delta\Delta^{CT}$=57.84) and Δ852 strain ($\Delta\Delta^{CT}$=70.88), which carried the plasmid p2085_852-AHT (Fig. 2.3 B). The RT-PCR data therefore showed that the AHT system works best when the induction was implemented in the stationary-phase (24 h), as the *lttr*852 expression level was around 60 times higher in the strains carrying the inducible plasmid under induced conditions compared to the strains without any plasmid under the same conditions.

The translation of *lttr*852 under AHT induction was further confirmed with a Coomassie stain after 24 h. WT and Δ852 strains as well as the WT and Δ852 strains carrying the p2085_852-AHT plasmid were cultured for 24 h and either induced for 1 h with 200 µg/ml AHT or uninduced. The bacteria were then pelleted, lysed to isolated total proteins and the total protein was quantified using the Bradford assay. Equal amounts were loaded and electrophorased on SDS-PAGE. Since the AHT system is based on a tet promoter, which has a protein size of 23.3 kDa (red arrow), this size was

used as a control to check if the system got activated via AHT or not. The protein size of LTTR852 is 33.8 kDa (green arrow). As shown in Figure 2.3 D, only strains carrying the plasmid under AHT induction showed two extra bands on the SDS-PAGE (4, 8; green, red arrow). This confirmed that the AHT inducible promoter system was able to stimulate the over-expression of the *lttr*852 and that AHT causes no side effects.

To confirm the observation from the colloidal Coomassie staining, a His-Tag which was cloned immediately after the *lttr*852 in the AHT inducible plasmid was used. To ensure that the His-Tag had no impact on the expression of the *lttr*852, RT-PCR was repeated with *S. aureus* wildtype and Δ852 carrying p2085_852-His grown for 24 h in TSB and induced with 200 µg/ml AHT for 1 h. The over-expression with AHT functioned also in both His-tagged samples as shown in Figure 2.3 C. As the detection of the His tagged samples were not explicitly possible with the Coomasie blue staining, a Western blot analysis with a His-Tag antibody was performed on bacteria cultures induced at the log-phase and stationery-phase. As shown in Figure 2.3 E, AHT supply resulted in a strong induction in both wildtype and Δ852 strains compared to the uninduced condition. However, there was no significant difference between the samples of the two tested phases. As a specific antibody was used for the His-Tag it could be consequently said that the whole AHT-system for the over-expression of the unknown *lttr*852 gene works on the relative *lttr*852 expression as well as on the protein level. This construct was used in further experiments to analyze the phenotypical and molecular characterization of *lttr*852.

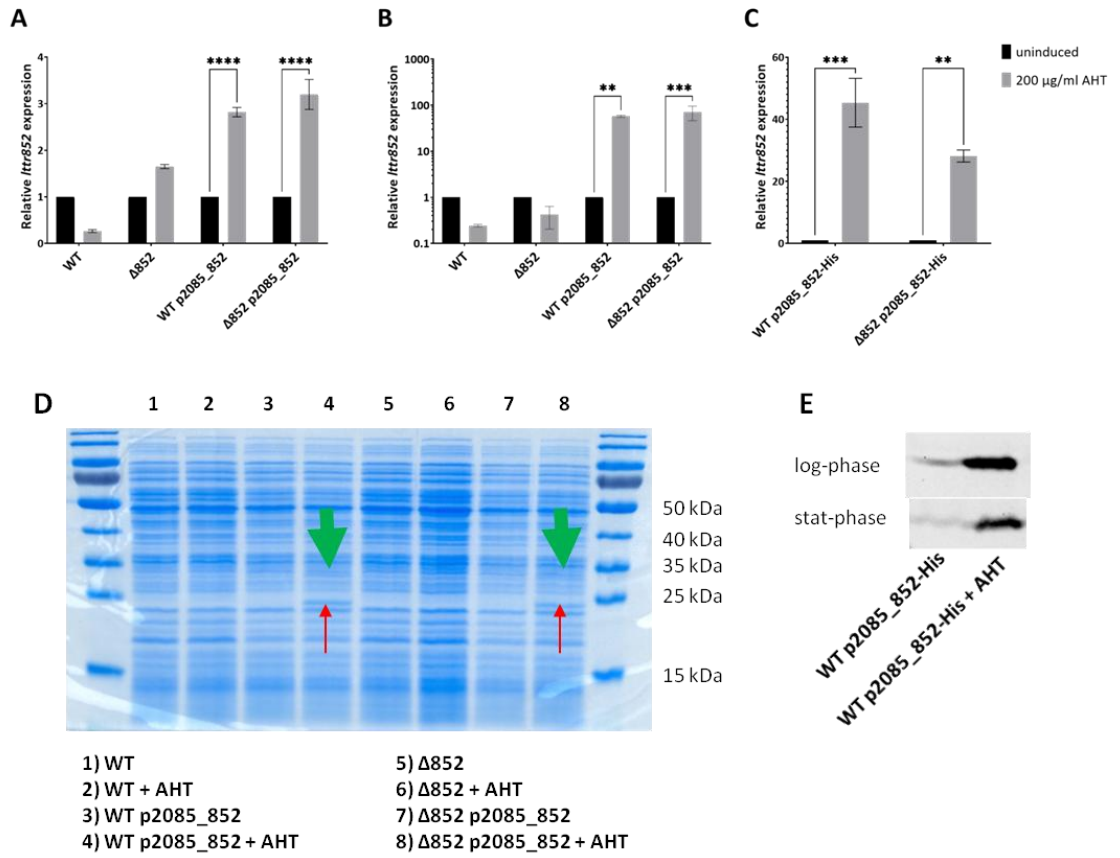


Figure 2.3: *lttr*852 is inducible over-expressed by transgenic *S. aureus*.
Relative *lttr852* expression in S. *aureus* wildtype, Δ852 and S. *aureus* wildtype, Δ852 carrying the plasmid p2085_852-AHT under AHT induction (grey) and uninduction (black) after 4h (**A**) and 24 h (**B**). (**C**) Relative *lttr*852 expression in *S. aureus* wildtype and Δ852 carrying the plasmid p2085_852-His under no induction (black) and induction with 200 µg/ml AHT (grey) after 24 h. (**D**) SDS-PAGE of wildtype ± AHT (1, 2), WT p2085_852-AHT ± AHT (3, 4), Δ852 ± AHT (5, 6) and Δ852 p2085_852-AHT ± AHT (7, 8). Green arrow at 33.8 kDa marking LTTR852 and red arrow at 23.3 kDa marking the Tet-promoter. (**E**) Western-blot of *S. aureus* wildtype carrying p2085_852-His, not induced or induced with 200 µg/ml AHT at the log-phase and stat-phase, detecting LTTR852 tagged with a His via His antibody. Each column represents the mean values ± SDs from three independent experiments. Statistical analysis was performed using two-way ANOVA. **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

2.4 Potential role of *lttr*852 for virulence

Virulence mechanisms are important for bacteria in order to invade the host, evade host defense and cause disease. LTTRs have been found to play a role in virulence in other bacterial spp. The PA2121 in *P. aeruginosa* is an LTTR which functions as biofilm formation repressor, whereas the LcrX promotes biofilm formation in *Xanthomonas axonopodis* pv. *glycines* (Park et al., 2019a; Yang et al., 2019). Since the over-expression of the unknown *lttr* was successful as shown above, the impact of the *lttr*852 deletion on hemolysis and biofilm formation was assessed.

2.4.1 Hemolysis is not affected by *lttr*852 deletion

Lysis of erythrocytes can be caused by the expression of several hemolysins of *S. aureus* (Vandenesch et al., 2012). To determine if *lttr*852 plays a role in hemolysis, a quantitative erythrocyte lysis assay was performed. The hemolytic activity of the Δ852, complementation, as well as in WT and Δ852 carrying the p2085_852-AHT plasmid were compared to hemolytic activity of the wildtype strain. Additionally, the hemolytic activity was also similarly tested in the presence or absence of AHT. As shown in Figure 2.4, although there was a slight decrease in the relative hemolytic activity due to the Δ852 strain (97.7%), it was not statistically significant (p=0.0905) compared to the WT strain. Neither the presence of the plasmid nor the induction with AHT resulted in a statistically significant difference of the hemolysis in the WT strains. However, the presence of the p2085_852-AHT plasmid in the Δ852 strain resulted in statistically, significant induction of hemolysis in the absence (p=0.0275) and presence (p=0.0481) of AHT. Complementation did not result in a significant difference in the hemolytic activity compared to WT and Δ852 strains. Taken together, the novel *lttr* showed a minor tendency towards playing a role in the hemolytic activity of *S. aureus*.

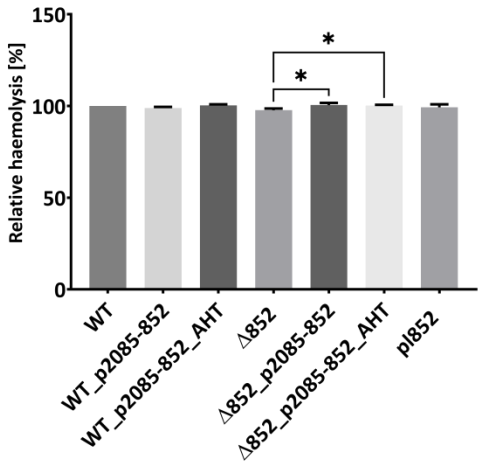


Figure 2.4: Deletion of *lttr*852 does not affect lysis of sheep erythrocytes.
Relative hemolysis of *S. aureus* WT carrying inducible plasmid ± AHT, Δ852, Δ852 carrying inducible plasmid ± AHT and complementation in percent, compared to *S. aureus* WT. Each column represents the mean values ± SDs from three independent experiments. Statistical analysis was performed using two-way ANOVA. *, $p < 0.05$.

2.4.2 *lttr852* deletion does not alter biofilm formation

S. aureus is able to form biofilm which is an organized structure of bacteria that leads to survival strategy. The biofilm formation in *S. aureus* is tightly regulated by complex genetic factors. As *lttr*852 might impact biofilm formation, the ability to form a biofilm was analyzed by measuring the optical density of crystal violet stained biofilm formation. Besides the analysis of wildtype, *lttr*852 mutant and complementation, the biofilm formation was also tested in wildtype and Δ852 carrying the plasmid for *lttr*852 over-expression. The biofilm formation of the strain *S. epidermidis* RP62A was used as a positive control as it is a known biofilm forming bacteria and *S. carnosus* TM300 was used as a negative control. Since biofilm formation is influenced by bacteria growth rate, the optical density of each bacteria was measured prior to fixing and staining with crystal violet. The biofilm formation was calculated by generating the ratio of the biofilm formed (OD_{550}) and the growth rate at OD_{600}, to eliminate bias due to growth rate and additionally described as a percentage of the positive control. Since AHT was dissolved in ethanol, an additional control of all strains treated with ethanol was setup, as ethanol is known to enhance *S. aureus* biofilm formation (Redelman et al., 2012). All *S. aureus* strains showed reduced biofilm formation in Figure 2.5, independent on the

treatment. However, independent of the *S. aureus* strain, the ratio of the biofilm formation was higher after the treatment with AHT or EtOH irrespective of *lttr*852. In conclusion it demonstrates that this *lttr* also had no impact on the biofilm formation of *S. aureus*.

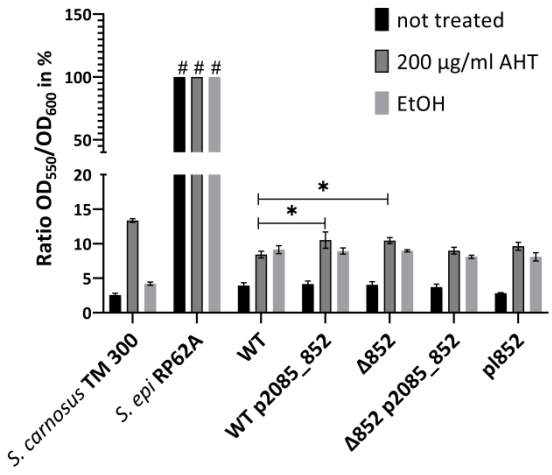


Figure 2.5: Biofilm formation is not affected by *lttr*852 even after induction with AHT.
Ratio of growth rate of an OD_{600} to an OD_{550} for biofilm formation, for negative control *S. carnosus* TM300, *S. aureus* WT, WT carrying inducible plasmid, Δ852, Δ852 carrying inducible plasmid and complementation, compared to as a percentage of the positive control *S. epidermidis* RP62A for not induced (black), induced with 200 µg/ml AHT (dark grey) or treated with EtOH (light grey). Each column represents the mean values ± SDs from three independent experiments. Statistical analysis was performed using two-way ANOVA. *, $p < 0.05$.

2.5 The regulon of *lttr*852 includes inter alia *msr*A, *cop*A, *pyr*B

LysR-type transcriptional regulated genes are known to often regulate genes which are located immediately upstream or downstream of these genes (Maddocks and Oyston, 2008). Downstream of this novel *lttr* lies the chaperon protein *clp*B which is involved in the recovery of cells from heat induced damage and therefore part of a stress-induced multi-chaperone system (Lee et al., 2004; Doyle and Wickner, 2009). The gene for *leu*A1 is responsible for the 2-isopropylmatate synthesis and lies upstream of *lttr*852. Both genes are divergently transcribed to this unknown *lttr*. The impact of *lttr*852 on the expression of these neighboring genes was analyzed in *S. aureus* wildtype, Δ852 and the *lttr*852 mutant carrying the inducible plasmid. For this purpose, total RNA was

isolated from overnight cultures of the strains and the expression of *clp*B and *leu*A1 were analyzed under inducing and non-inducing conditions. The expression of these two genes in the different strains was normalized to expression in the wildtype under non-inducing conditions. As in figure 2.6 A, deletion of the *lttr*852 resulted in a statistically not significant 2-fold increase in expression of the *leu*A1 under non-induced conditions. The insertion of p2085_852-AHT plasmid into the Δ852 strain did not result in the down regulating expression of the *leu*A1, as to that seen in the wildtype. Although the addition of 200 µg/ml AHT to the bacteria culture of Δ852 and Δ852 p2085_852-AHT resulted in 3-fold upregulation of the *leu*A1 expression, compared to the wildtype, the observed difference in expression was not statistically significant. In summary, deletion of the *lttr*852 from *S. aureus* indicated a trend towards the upregulation of the *leu*A1 (Fig. 2.6 A). The expression of the *clp*B, however shows to be downregulated in the Δ852, although not significantly under inducing and non-inducing conditions with AHT. The insertion of the p2085_852-AHT plasmid to the Δ852 strain however restored the expression of *clp*B to the level observed in the *S. aureus* WT strain (Fig. 2.6 B). The evidence from this data indicates that the *lttr*852 may not influence the expression of the two genes, *leu*A1 and *clpB*, flanking it.

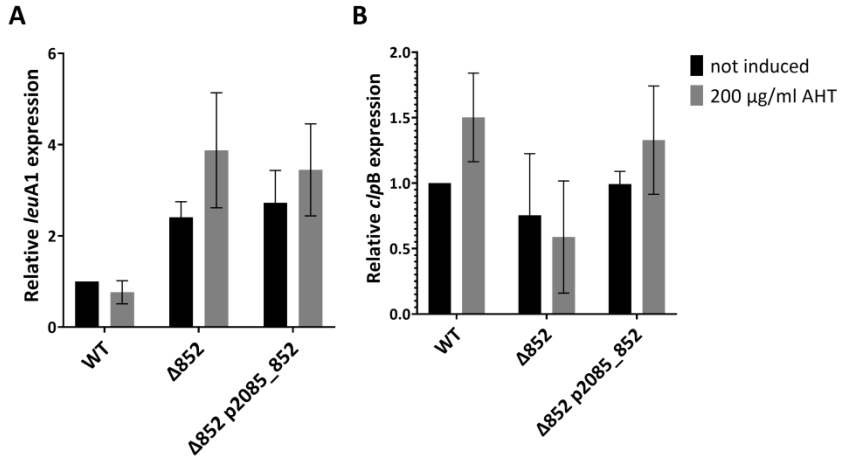
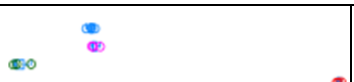
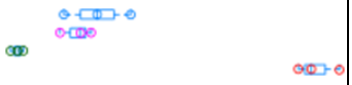
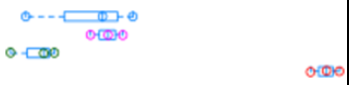
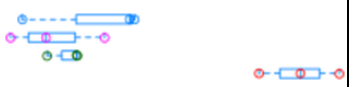


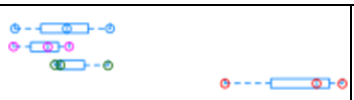
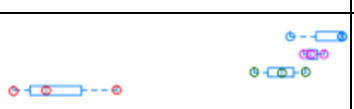
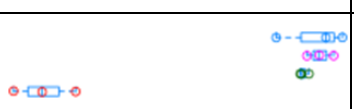
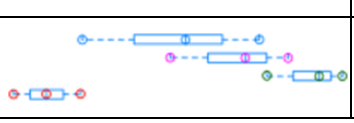
Figure 2.6: Up- and downstream genes are no potential targets of the transcriptional regulator.
(**A**) Relative *leu*A1 expression in *S. aureus* 6850 WT, Δ852 and Δ852 p2085_852-AHT with and without 200 µg/ml AHT treatment, which lies upstream of *lttr*852. (**B**) Relative *clp*B expression, which lies downstream of *lttr*852*,* of *S. aureus* 6850 WT, Δ852 and Δ852 p2085_852-AHT with and without 200 µg/ml AHT treatment*.* Each column represents the mean values ± SDs from three independent experiments.

To analyze the expression profile of the regulon controlled by the unknown *lttr*, RNA-Seq was performed and assessed by DEseq2 analysis. The following strains were used, *S. aureus* wildtype, WT induced with 200 µg/ml AHT to exclude any side effects of AHT, WT carrying the inducible plasmid and being induced with AHT to generate the strongest induction of *lttr*852 and the *lttr*852 mutant. After the RNA isolation, the transcription level of *lttr*852 for the strains was again validated by RT-PCR to be sure that the induction worked (Sup. Tab. 7.1). *S. aureus* wildtype carrying the inducible plasmid under AHT induction showed a significantly higher relative expression of the *lttr*852 compared to WT and Δ852 strains. The samples were then sent for RNA-Seq after controlling for RNA integrity. The variance of the three samples of wildtype and wildtype induced with AHT are still within the limits, the variance between the other two samples is very low (Sup. Fig. 7.1). Several targets were found specifically down- and upregulated upon differential expression analysis of the RNA-Seq data after induction of *lttr*852 with AHT as showed in Table 2.1. Genes that were observed to be downregulated when *lttr*852 expression was induced via AHT included members of pyrimidine biosynthesis pathway (*car*B*, pyrB* and *pyrC*), the urease operon (*ure*B, *ure*C, *ure*E and *ure*F), tagatose-6-phosphate kinase (*lac*C) and PTS system lactose-specific transporter subunit IIBC (*lac*E). Upregulated genes after *lttr*852 induction include the holin-like murein hydrolase regulator (*lrg*A), the peptide methionine sulfoxide reductase (*msr*A2), the components of a copper-translocation machinery (*cop*A and *cop*Z) as well as the genes of branched chain amino acid biosynthesis (*ilv*D, *ilv*B, *leu*A2).

Table 2.1: Regulated genes upon induced expression of *lttr*852

Reading locus ID, gene, annotation, expression pattern, log_2 fold change (FC) and adjusted p-value. Above can be read the upregulated genes by *lttr*852, followed by the downregulated genes. The expression pattern is illustrated from low to high expression level and has following color code: *S. aureus* wildtype: blue; wildtype treated with 200 ng/ml AHT: magenta; *S. aureus* Δ852: green; *S. aureus* p2085_p852-AHT, *lttr*852 expression induced with 200 ng/ml AHT: red (Table modified after Groma et al., 2020).

Locus ID	Gene	Annotation	Expression pattern low expr. level → high expr. level	log_2FC	Adj. p-value
UP-REGULATED BY LTTR					
RSAU_000852	*lttR*	LysR type regulator		12.7000	0.00e+00
RSAU_001242	*msrA*	Peptide methionine sulfoxide reductase MsrA		1.4700	4.36e-58
RSAU_002398	*copA*	Copper-translocating P-type ATPase		1.2400	4.86e-43
RSAU_002532	*yceI*	YceI-like domain protein / polyisoprenoid-binding protein		1.1700	1.12e-30
RSAU_000210	*lrgB*	Antiholin-like protein LrgB		1.4600	1.68e-22
RSAU_002399	*copZ*	Copper chaperone copZ-ion-binding		1.2600	6.90e-22
RSAU_001866	*rnaIII*	RNAIII		1.0900	3.87e-20
RSAU_001886	*ilvD*	Dihydroxy-acid dehydratase		1.7000	2.28e-15
RSAU_002445	*gabT*	4-aminobutyrate aminotransferase		1.4900	7.66e-14
RSAU_001515	*cadA*	Cadmium-transporting ATPase		1.0500	1.25e-13
RSAU_001887	*ilvB*	Acetolactate synthase large subunit		1.5500	2.02e-13
RSAU_000265	*sirA*	Nucleoside recognition domain protein		1.2100	2.92e-13
RSAU_000209	*lrgA*	Holin-like murein hydrolase regulator LrgA		1.2100	1.55e-12

RSAU_002444	*bcaP*	Branched-chain amino acid permease BcaP		1.1500	2.36e-12
RSAU_000394	*metN2*	Methionine ABC transporter ATP-binding protein		1.2200	7.18e-12
RSAU_000396	*metQ2*	Methionine ABC transporter, substrate-binding protein		1.1900	1.01e-10
RSAU_000010	*azlC*	predicted Branched-chain amino acid protein (azaleucine resistance)		1.1200	9.22e-10
RSAU_001890	*leuA2*	2-isopropylmalate synthase		1.3200	2.61e-08
RSAU_001892	*leuC*	3-isopropylmalate dehydratase large subunit		1.1600	2.45e-07
RSAU_000395	*metP2*	methionine uptake ABC transporter, permease		1.0000	9.67e-07
RSAU_001894	*ilvA*	Threonine dehydratase biosynthetic		1.0700	1.03e-06
RSAU_001889	*ilvC*	Ketol-acid reductoisomerase		1.1700	1.42e-06
DOWN-REGULATED BY RSAU_000852					
RSAU_002123	*ureC*	Urease subunit alpha		-1.2800	1.57e-25
RSAU_001086	*carB*	Carbamoyl-phosphate synthase large chain		-1.1900	1.76e-16
RSAU_002125	*ureF*	Urease accessory protein UreF		-1.1100	2.62e-13
RSAU_001083	*pyrB*	Aspartate carbamoyltransferase catalytic		-1.2600	1.82e-09
RSAU_002124	*ureE*	Urease accessory protein UreE		-1.1000	3.67e-08
RSAU_001084	*pyrC*	Dihydroorotase		-1.0200	6.00e-08
RSAU_000955	*purQ*	Phosphoribosylformylglycinamidine synthase I		-1.0600	8.92e-08

RSAU_002122	*ureB*	Urease subunit beta		-1.0500	1.47e-06
RSAU_002037	*lacC*	Tagatose-6-phosphate kinase		-1.1500	1.66e-06
RSAU_002034	*lacE*	PTS system lactose-specific transporter subunit IIBC		-1.0100	1.67e-06

Some of the observed differentially expressed genes from the RNA-Seq were confirmed using RT-PCR (Fig. 2.7). The expression of WT p2085_852-AHT after *lttr*852 induction with AHT, versus Δ852 showed that, *lrg*A ($\Delta\Delta^{CT}$=2.124; p-value=0.1646) and *RNA*III ($\Delta\Delta^{CT}$=1.464; p-value=0.1643) were upregulated, whereas *msr*A ($\Delta\Delta^{CT}$=2.033) and *cop*A ($\Delta\Delta^{CT}$=1.835) were significantly upregulated. Significantly downregulated genes included *lac*C ($\Delta\Delta^{CT}$=0.5195), *leu*A2 ($\Delta\Delta^{CT}$=0.4431), *ure*C ($\Delta\Delta^{CT}$=0.4484), *car*B ($\Delta\Delta^{CT}$=0.4838), and *pyr*B ($\Delta\Delta^{CT}$=0.2641).

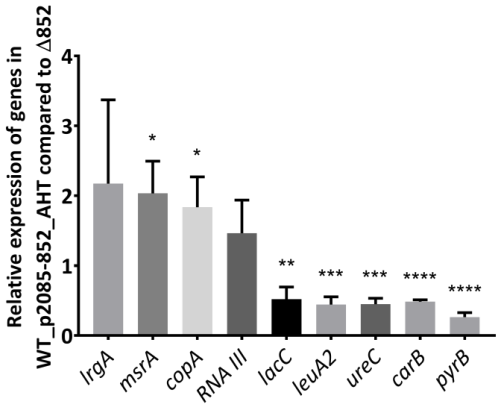


Figure 2.7: *lttr*852 targets identified by RNA-Seq were validated using RT-PCR.
Relative expression level of *lgr*A, *msr*A, *cop*A, *RNA*III, *lac*C, *leu*A2, *ure*C, *car*B and pyrB in WT p2085-852-AHt induced via 200 µg/ml AHT compared to the expression in Δ852 set on 1. Statistical analysis was performed using Student´s t-test. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$. (Figure modified after Groma et al., 2020).

2.6 *lttr852* is controled by glucose, micro-aerobic condition, copper and oxidative stress

Since the conditions under which the novel *lttr* is expressed in *S. aureus in vivo* and *in vitro* has so far not been characterized, the highly conserved promoter region of the *lttr*852 was taken from strain 6850 and fused with a codon-adapted green fluorescent protein (GFP) to generate the p2085_Pr852_GFP plasmid (Fig. 2.8 A). This construct is designed such that an increased transcription of the *lttr*852 corresponded to an increased GFP emission. The plasmid was introduced into *S. aureus* wildtype as well as into the Δ852 strain in order to assess whether the activation of the promoter was based on a feedback loop or not. The activation of the *lttr*852 promoter was analyzed in different media and under stress inducing conditions typically found in the kidneys *in vivo*.

2.6.1 *lttr852* promoter is most strongly expressed in glucose free medium

To determine if commonly used media for the culture of *S. aureus* influence the activity of the *lttr*852 promoter, *S. aureus* WT carrying p2085_Pr852_GFP were cultivated overnight in BHI, LB, TSB, CDM or RPMI. The next day these bacteria cultures were diluted to an OD_{600} of 0.1 with fresh media and 400 µl were plated into a 48-well plate. Growth rate of the bacteria was measured at OD_{600} and the GFP emission as relative fluorescence units (excitation 488 nm, emission 515 nm) was taken every 10 min for 24 h using a TECAN microplate reader. As shown in Figure 2.8**:** A, *S. aureus* WT with the GFP plasmid showed best growth in BHI medium while the least growth was observed in the RPMI. There is a slight delay of GFP emission when compared to growth rate, which is most likely caused by the maturation time of GFP fluorophore (Fig. 2.8 B). However, *S. aureus* wildtype p2085_Pr852_GFP growing in TSB, CDM and RPMI had the lowest GFP emission level, whereas in BHI it reached almost a level of around 3 000 units and the GFP emission in LB is even 1 000 units higher. As the relative fluorescence is dependent on the number of viable bacteria with induced promoter activity in the well, Figure 2.8 C showing the ratio indicated the highest *lttr*852 promoter activity in *S. aureus* grown in LB. Similarly, *S. aureus* Δ852 strains

carrying the GFP-plasmid showed the same dynamic, indicating that the *lttr*852 promoter dependent phenotype may be based on the media composition (Fig. 2.8 D).

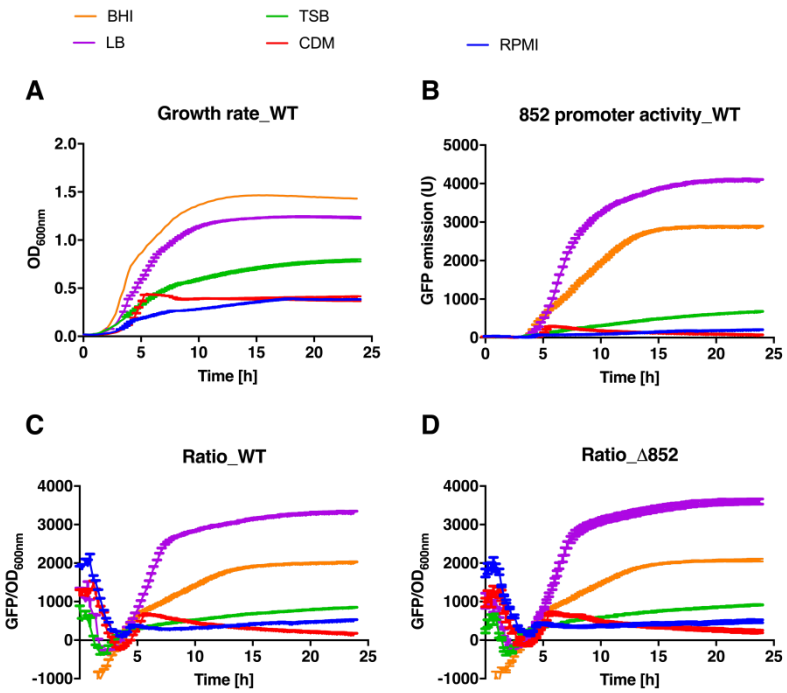


Figure 2.8: Culture media has an influence on the strength of *lttr*852 promoter activity.
S. aureus WT p2085_Pr852_GFP analysis of growth rate by OD_{600} (**A**) and GFP emission (**B**). Ratio of GFP emission to growth rate with an OD of 600 of *S. aureus* WT p2085_Pr852_GFP (**C**) and Δ852 p2085_Pr852_GFP (**D**), grown in BHI (orange), LB (purple), TSB (green), CDM (red) and RPMI (blue).

2.6.2 Glucose enhances the transcription of the *lttr*852 in the log-phase

Media composition were demonstrated to affect the *lttr*852 promoter activity. A media dependent influence could also be observed during the transduction of p2085_Pr852_GFP. After generating the GFP promoter plasmid, it was transduced into *E. coli* which were plated on LB before it was transduced via several intermediate steps into the strain of interest which were plated on TSB. During the process it could be observed that bacteria carrying p2085_Pr852_GFP plated on LB agar without glucose displayed a sharper GFP fluorescence, than when grown on TSB agar containing 2.5 g/L glucose (Fig. 2.9 A). This led to the assumption that glucose might repress the promoter activity of *lttr*852.

To verify if glucose could repress the *lttr*852 promoter activity, bacterial growth rate as well as GFP emission were recorded for *S. aureus* wildtype harboring the reporter plasmid p2085_Pr852_GFP in chemically defined medium containing different glucose concentrations from 0 to 75 mM. S*. aureus* was still able to grow in the absence of glucose although not optimally, whereas the addition of 1 mM glucose already improved the growth of the bacteria to the same OD_{600} as the addition of 75 mM (Fig. 2.9 B). The GFP emission of *S. aureus* p2085_Pr852_GFP growing in the absence or the presence of 1 mM glucose is very low, but already the addition of 5 mM glucose increased the GFP emission remarkably. However, glucose concentrations of 10 mM and above resulted in peak GFP emission already after 4 h and a reduction in the emission with time (Fig. 2.9 C). The correlation between OD_{600} and GFP emission revealed that a glucose concentration above 10 mM reproducible increases the *lttr*852 promoter activity to a certain point, which afterwards decreases (Fig. 2.9 D). The data indicate that the presence of glucose may be essential for the induction of the *lttr*852 promoter activity with optimal induction at 5 mM glucose.

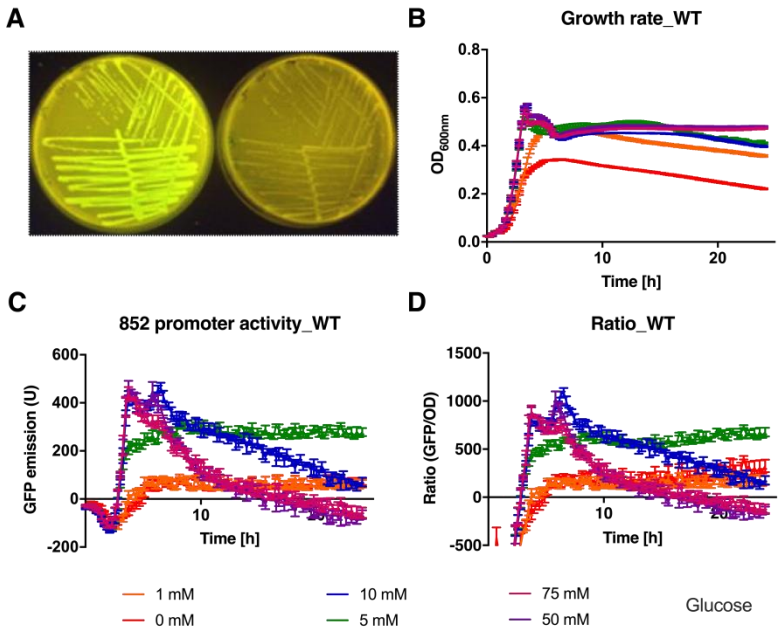


Figure 2.9: Promoter activity of *lttr*852 with an optimal induction at 5 mM glucose.
(**A**) Picture showing the green fluorescence intensity of *S. aureus* WT p2085_Pr852_GFP on LB-agar (left panel) or on TSB-agar (right panel) (Figure modified after Groma et al., 2020). (**B**) Growth rate of *S. aureus* wildtype p2085_Pr852_GFP in CDM supplemented with different glucose concentrations from 0-75 mM. 0 mM = red; 1 mM = orange; 5 mM = green, 10 mM =

blue; 50 mM = purple; 75 mM = pink. (**C**) *lttr852* promoter activity detected via GFP emission. (**D**) Ratio of promoter activity to bacterial density in CDM with different glucose concentrations

2.6.3 Transcription of *lttr*852 is enhanced under micro-aerobic conditions

The kidneys are known to be involved in glucose homeostasis, thus establishing a glucose gradient in the organ (Mather and Pollock, 2011), moreover the oxygen level in kidneys vary as well (Zhang et al., 2014). To analyse the impact of oxygen on *lttr*852 bacterial growth rate and relative fluorescence units of GFP were recorded under micro-aerobic conditions in BHI, LB, TSB, CDM and RPMI, as it was already shown in 2.8, that there might be medium dependent effects as well. The experiment was implemented as described above, the only modification was that the 48 well-plates were covered with an adhesive foil, thereby limiting oxygen availability during the growth.

The micro-aerobic condition did not have any effect on the growth rate of *S. aureus* wildtype p2085_Pr852_GFP in any of the used media, if compared to Figure 2.8 C (Fig. 2.10 A, left). The GFP emission under micro-aerobic condition was very low for bacteria grown in TSB, CDM and RPMI. However, in BHI and LB, the GFP emission were notably higher than under aerobic condition, reaching a relative fluorescence unit of 5 000 for BHI and 12 500 for LB (Fig. 2.10. A, middle). The calculated ratio for RPMI, CDM and TSB under micro-aerobic condition were also very low, but in BHI it reached a level of 4 000 and in LB even a level of almost 10 000 which is approximately three times higher than under aerobic condition (Fig. 2.10 A, right). Growth rate, GFP emission and their ratio in *S. aureus* Δ852 p2085_Pr852_GFP showed the same dynamic as in wildtype (Fig. 2.10 B), suggesting that the LTTR852 promoter activity may be enhanced under micro-aerobic condition but not based on a feedback loop.

To reinforce this statement, relative *lttr*852 expression was analyzed for *S. aureus* wildtype, growing under micro-aerobic and aerobic condition in LB, as the oxygen effect was strongest in this medium. *lttr*852 mutant was not tested as *lttr*852 expression was not detectable. The samples were prepared as for the promoter activity assay and harvested after 24 h. The relative *lttr*852 expression was found to be

significantly higher under micro-aerobic condition compared to aerobic conditions in both strains (Fig. 2.10 C).

To confirm the micro-aerobic conditions, the formate acetyltransferase gene *pfl*B, which is only expressed under low oxygen conditions was tested via RT-PCR (Fuchs et al. 2014). Relative *pfl*B expression was induced 44-fold in *S. aureus* wildtype cultured in foil-sealed wells when compared to *S. aureus* wildtype from wells not covered with a foil and therefore having access to the environmental atmosphere (Fig. 2.10 D). The relative *pfl*B expression was also significant in *S. aureus lttr*852 mutant under micro-aerobic condition, proving that bacteria grown in foil-sealed wells have a lower available oxygen. The observations from these experiments indicate that the promoter activity of *lttr*852 is enhanced under micro-aerobic conditions.

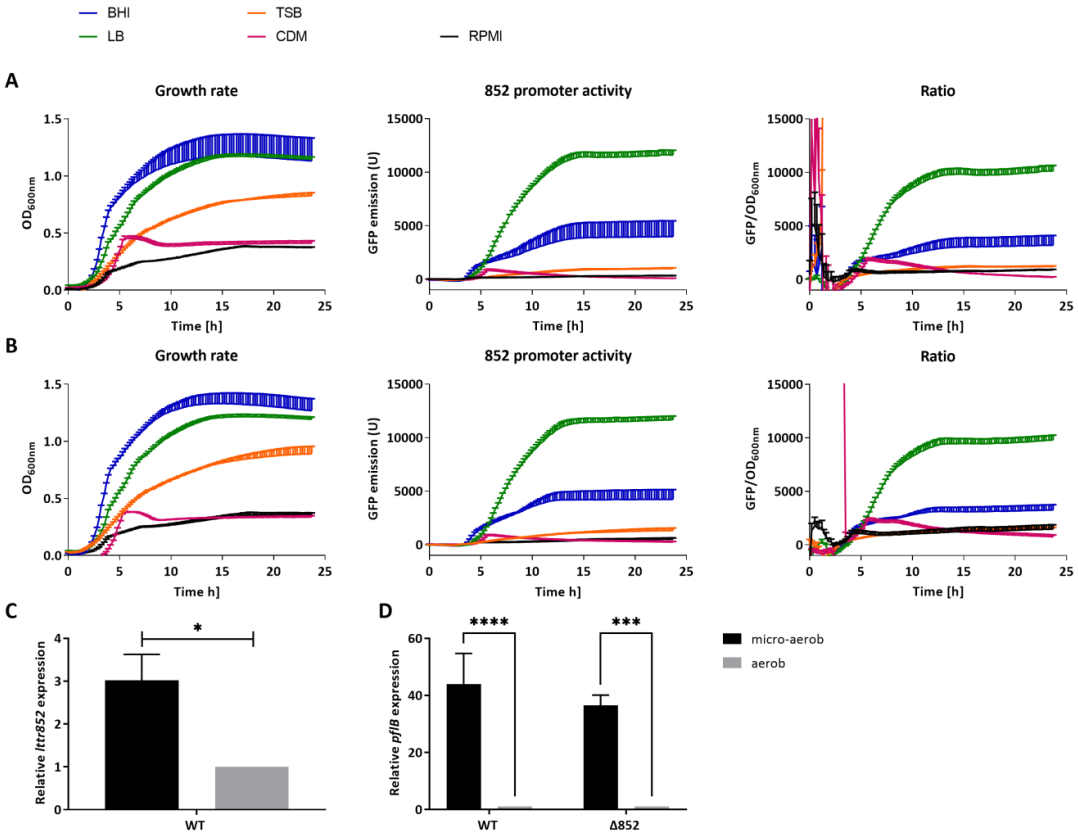


Figure 2.10: Enhanced promoter activity of *lttr*852 under micro-aerobic conditions.
Detection of growth rate OD_{600} (left), GFP emission (middle) and its ratio (right) in BHI (orange), LB (purple), TSB (green), CDM (red) and RPMI (blue) in (**A**) *S. aureus* wildtype p2085_Pr852_GFP and (**B**) Δ852 p2085_Pr852_GFP. (**C**) Relative *lttr*852 expression of *S. aureus* wildtype grown under micro-aerobic (black) compared to aerobic condition (grey). Each

column represents the mean values ± SDs from three independent experiments. Statistical analysis was performed using Student´s t-test. *, $p < 0.05$. (**D**) Relative *pfl*B expression of *S. aureus* wildtype and Δ852 grown under micro-aerobic (black) and aerobic condition (grey). Each column represents the mean values ± SDs from three independent experiments. Statistical analysis was performed using two-way ANOVA. ***, $p < 0.001$; ****, $p < 0.0001$.

2.6.4 *lttr*852 promoter activity is critical at 42°C exposure

The optimum growth temperature for *S. aureus* is 37°C although the bacteria can grow in temperature ranges of 15 to 45°C (Missiakas and Schneewind, 2013). The optimum growth temperature of *S. aureus* corresponds to the core temperature of most mammals (Refinetti, 2010). However, during infections, there are physiological changes within the host, which include altered temperature relation (Small et al., 1986; Granger et al., 2013). Temperature changes during infection may therefore trigger the unknown *lttr* activity. In order to determine the impact of temperature on *lttr*852 promoter activity, *S. aureus* wildtype and *lttr*852 mutant carrying p2085_Pr852_GFP were grown in CDM without glucose under micro-aerobic conditions and in addition the temperature was changed from 37°C to 42°C after 2, 4 and 6 h or not changed at all. Under all conditions, almost every sample reached the same OD_{6oo} of around 0.4 after 5 h. The OD_{600} at 37°C however, flattened a bit to an OD_{600} of 0.3, but a temperature shift to 42°C independent on the time point resulted in a light decrease in the OD_{600} to 0.25 (Fig. 2.11 A left). Interestingly the promoter activity showed to be influenced by the timing of the exposure of the bacteria to 42^{o}C temperature. As shown in Figure 2.11, culturing the bacteria at 42^{o}C from the start as well as an increase of the temperature to 42^{o}C after 2 h showed to repress promoter activity while exposure at later time points did not affect the LTTR promoter activity. A similar trend was observed in both WT and Δ852 strains. It can therefore be deduced from the observations that the time of exposure of the bacteria to 42^{o}C may be critical for the induction of the *lttr*852 promoter activity although it does not enable a self-regulation.

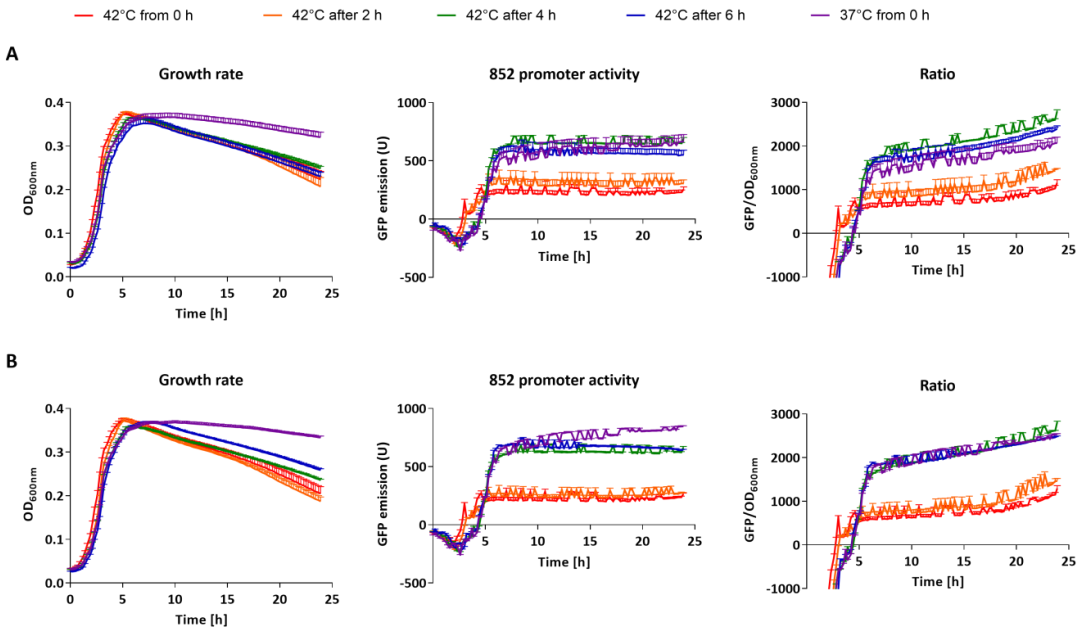


Figure 2.11: Exposure of *S. aureus* to 42°C within the first 2 hours of culture impacted *lttr*852 promoter activity.
Analysis of growth rate (left), *lttr*852 promoter activity profile (middle) and ratio of GFP emission to bacterial growth rate (right) in (**A**) *S. aureus* wildtype p2085_Pr852_GFP and (**B**) *lttr*852 mutant p2085_Pr852_GFP under different temperature settings; control temperature at 37°C throughout the whole measurement (purple); same behavior of *lttr*852 promoter activity found if temperature switch from 37°C to 24°C was implemented after 4 h (green) or 6 h (blue); significantly lower *lttr*852 promoter activity due to switch from 37°C to 24°C implemented after 2 h (orange) or 42°C throughout the whole measurement (red)

2.6.5 A mix of trace elements evoke *lttr*852 based phenotype in *S. aureus*

Since the media compositions are critical for the *lttr*852 promoter activity, the effect of the stock of components CDM is made of were assessed. Therefore seven diverse CDM media were crafted, whereas one main component was always omitted. But glucose was one of the CDM components additionally omitted, since it was already shown to have an impact on *lttr*852 promoter activity. As a control, CDM and CDM without glucose were also tested (Fig. 2.9). It is known that bacteria will not grow without amino acids, therefore this omission was left out.

S. aureus wildtype and Δ852 carrying the GFP plasmid were grown overnight in CDM without glucose to avoid the influence of glucose residues. The bacteria were then

washed once with PBS and diluted to an OD_{600} of 0.1 in one of the eight CDMs. Growth rate and GFP emission were tracked using a TECAN reader under aerobic and micro-aerobic conditions. Only the ratio of GFP emission and growth rate are shown in Figure 2.12. The ratio in standard CDM already showed an increase in the first 5 h, followed by a flattening of the curve. Additionally, the mean was higher under micro-aerobic conditions compared to aerobic conditions (Fig. 2.12 A). In the other tested media, the relative promoter activity was also observed to be higher under micro-aerobic conditions compared to aerobic conditions, however the addition of vitamins to the glucose deficient CDM abolished this difference (Fig. 2.12 D). The relative promoter activity was similar in 7 media combinations as shown in Figure 2.12 for both WT and Δ852 strains, however, only a repeatedly observed difference between wildtype and *lttr*852 mutant was observed in CDM without glucose and trace elements. In *S. aureus* Δ852, the *lttr* promoter activity demonstrate to peak at about 5 h under both micro-aerobic and aerobic conditions, whereas no such peaks were observed in *S. aureus* wildtype (Fig. 2.12 F). Additionally the *lttr*852 promoter activity for both strains grown in CDM without glucose and trace elements was very low compared to all the other used media in this experiment. The data from this experiment brought a *lttr*852 based *in vitro* phenotype, for the *lttr*852 promoter activity in the absence of the stock of trace elements of CDM.

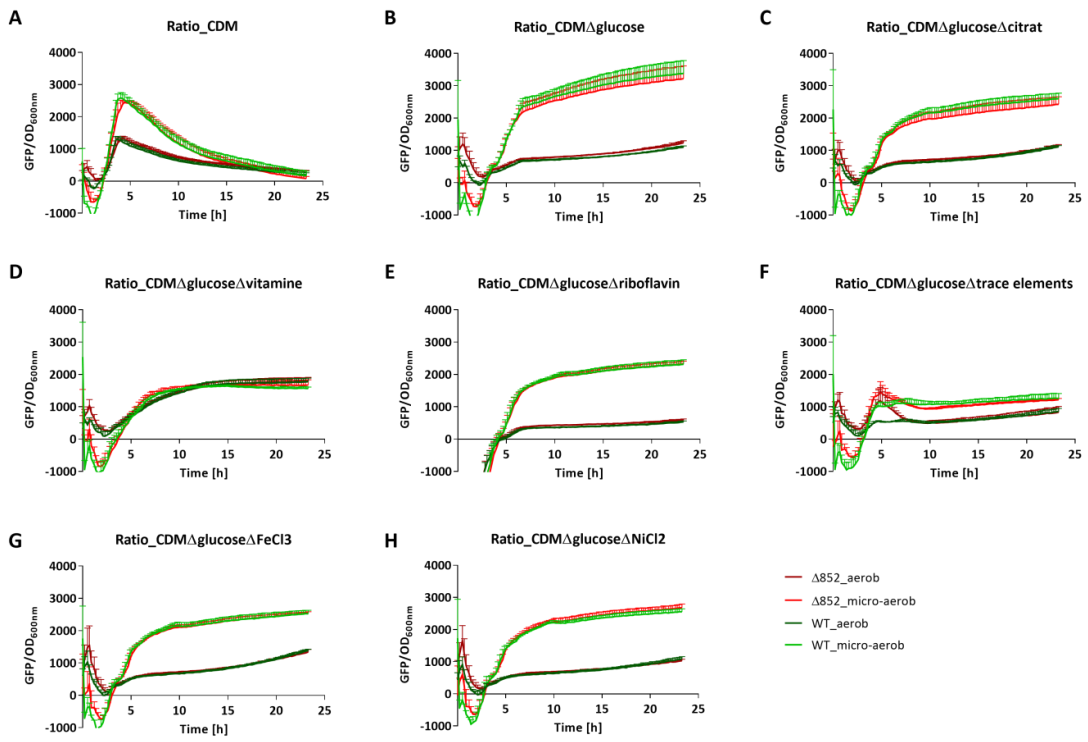


Figure 2.12: Verifying the impact of individual substances of the chemical defined medium. Monitoring ratio of GFP emission to growth rate OD_{600} in *S. aureus* wildtype p2085_Pr852_GFP (green) and Δ852 p2085_Pr852_GFP (red) under aerobic (dark) or micro-aerobic (light) conditions grown in (**A**) CDM, (**B**) CDMΔglucose, (**C**) CDMΔglucoseΔcitrat, (**D**) CDMΔglucoseΔvitamin, (**E**) CDMΔglucoseΔriboflavin, (**F**) CDMΔglucoseΔtrace elements, (**G**) CDMΔglucoseΔ$FeCl_3$ or (**H**) CDMΔglucoseΔ$NiCl_2$.

2.6.6 *lttr*852 promoter activity shows a strain and time dependent requirement for copper ions

Some LysR-type transcriptional regulators were found to use trace elements as their co-inducer (Maddocks and Oyston, 2008; Braz et al., 2010) and due to the result of 2.6.5 it was hypothesized that substances composing the trace element stock could be responsible for the different *lttr*852 promoter activity profile of *S. aureus* wildtype and *lttr*852 mutant.

To identify the cause of the observed differential adjustment of *lttr*852 promoter activity between *S. aureus* WT and *lttr*852 mutant grown in CDM without glucose and trace elements, the ratio of GFP emission to OD_{600} was analyzed in *S. aureus* wildtype

and Δ852 carrying p2085_Pr852_GFP. Bacteria were grown overnight in CDM without glucose to avoid influence of glucose residues, next day washed once with PBS and diluted with an OD_{600} of 0.1 in CDM lacking glucose and one of the six substances the trace element stock is made of. Growth rate and GFP emission were tracked with a TECAN reader under micro-aerobic and aerobic conditions, but only ratio is shown in Figure 2.13. There was no difference in the ratio regarding both strains. However, a very low GFP emission was observed in bacteria cultured in CDM lacking glucose and copper sulfate, which suggested that copper sulfate is required for the *lttr*852 promoter activity.

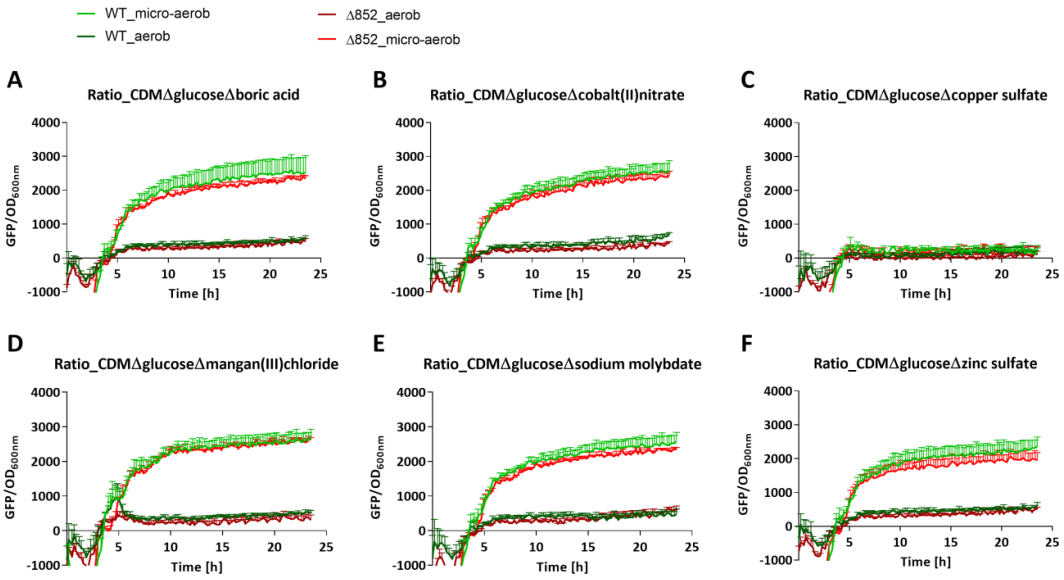


Figure 2.13: Copper sulfate has a massive impact on *lttr*852 promoter activity.
Monitoring ratio of GFP emission to growth rate OD_{600} in *S. aureus* wildtype p2085_Pr852_GFP (green) and Δ852 p2085_Pr852_GFP (red) under aerobic (dark) or micro-aerobic (light) conditions, grown in (**A**) CDMΔglucoseΔboric acid, (**B**) CDMΔglucoseΔcobalt(II)nitrate, (**C**) CDMΔglucoseΔcopper sulfate, (**D**) CDMΔglucoseΔmangan(III)chloride, (**E**) CDMΔglucoseΔsodium molybdate or (**F**) CDMΔglucoseΔzinc sulfate.

As the verification of the trace elements showed an abolished *lttr*852 promoter activity only when copper sulfate was omitted, it was of interest to determine if copper sulfate was necessary for the activation of the *lttr*852 promoter. Therefore, growth rate, GFP emission and ratio of *S. aureus* wildtype and Δ852 carrying the GFP plasmid were checked, in different concentrations of copper sulfate in CDM without glucose was

assessed under micro-aerobic conditions. The OD_{600} of stationary growth phase of *S. aureus* WT p2085_Pr852_GFP grown in medium containing 200 nM or less copper sulfate was under 0.4, whereas bacteria grown in media containing 400 nM copper sulfate or more reached an OD_{600} of around 0.4 (Fig. 2.14 A, left). Although OD_{600} dynamics did not differ crucially between the strains, a remarkably lower GFP signal was detected when *S. aureus* WT p2085_Pr852_GFP was grown in CDM with a concentration of 200 nM or less copper sulfate (Fig. 2.14 A, middle). The GFP/OD ratio confirmed that there was a reproducible dose-dependent increase in *lttr*852 promoter activity upon addition of copper sulfate, which was fully recovered by the addition of at least 400 nM copper sulfate (Fig. 2.14 A, right). The experiment was also performed in the knock-out mutant *S. aureus* Δ852 p2085_Pr852_GFP, but this strain behaved as *S. aureus* p2085_Pr852_GFP, thus indicating the absence of a LTTR852 feedback-loop (Fig. 2.14 B).

Next, the relative *lttr*852 expression was assessed by RT-PCR in *S. aureus* wildtype and complementation strains. Strains were grown for 24 h under micro-aerobic conditions in CDM lacking glucose either with or without copper sulfate. The result showed that copper sulfate was necessary for the relative transcription of *lttr*852, as the expression of *lttr*852 was consistently 2.5-fold higher in the presence of copper sulfate (Fig. 2.14 C).

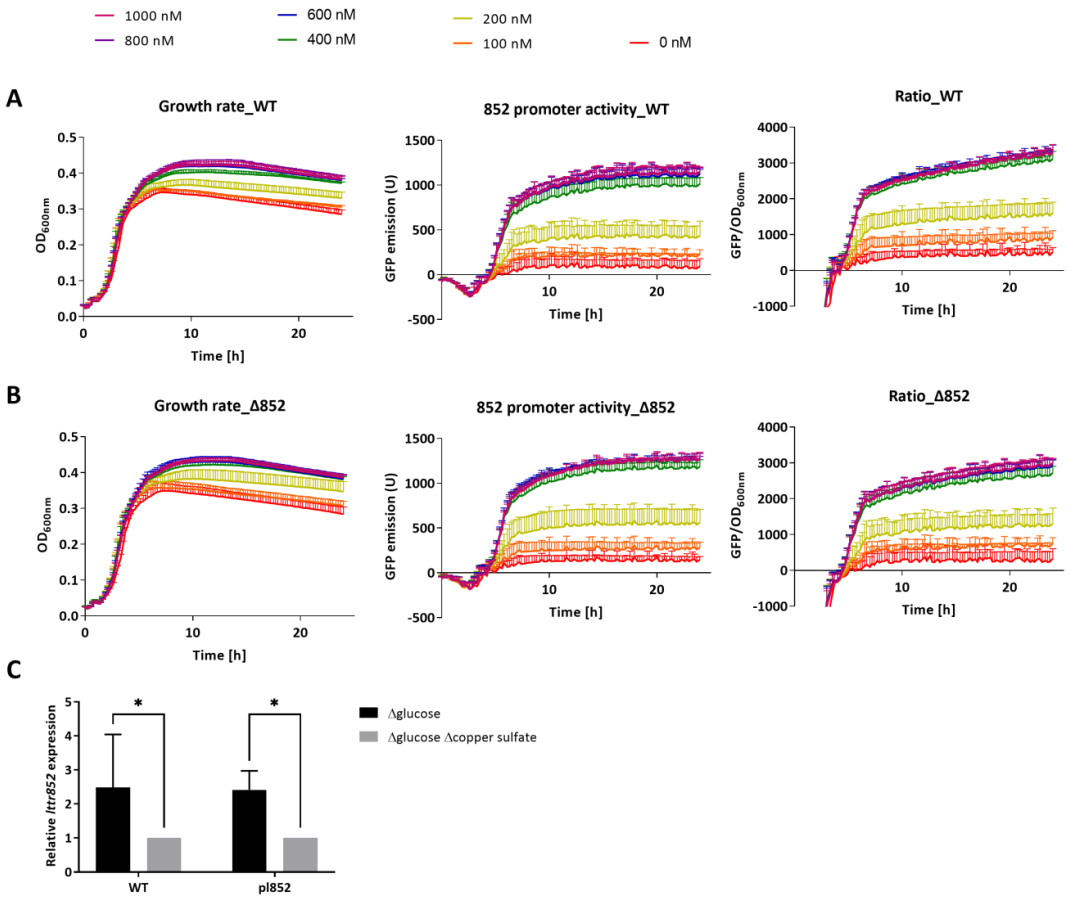


Figure 2.14: *lttr*852 promoter activity is depending on copper.
Monitoring of growth rate (left), GFP emission (middle) and ratio (right) for *S. aureus* WT p2085_Pr852_GFP (**A**) and *lttr*852 mutant p2085_Pr852_GFP (**B**) grown in CDM omitting glucose and a copper sulfate concentration of 1000 nM (magenta), 800 nM (purple), 600 nM (blue), 400 nM (green), 200 nM (yellow), 100 nM (orange) or no copper sulfate addition (red). (**C**) Relative *lttr*852 expression in *S. aureus* WT and complementation grown in CDM lacking glucose and copper sulfate, which is based as one, compared to growth in CDM lacking glucose. Each column represents the mean values ± SDs from three independent experiments. Statistical analysis was performed using two-way ANOVA. *, $p < 0.05$.

As observed in the experiments on the influence of elevated temperatures (chapter 2.6.4), *lttr*852 promoter activity during bacterial growth was dependent on the timing of temperature shift to 42°C. Similarly, here 1 mM copper sulfate was added at specified timepoints to micro-aerobic cultures of *S. aureus* wildtype and Δ852 strain carrying the GFP promoter reporter plasmid. The copper concentration was chosen, as it previously had been shown to be sufficient in activating the *lttr*852 promoter (Chapter 2.6.6). OD_{600} and GFP emission were recorded in a TECAN plate reader and

the GFO/OD ratio was calculated. The highest OD_{600} level was reached when copper sulfate was added after 2 h. A slight reduction of OD_{600} levels were recorded when copper sulfate was added at the start of the experiment compared to when copper sulfate was added after 4 h (Fig. 2.15 A, left). The addition of copper sulfate after 6 h or its complete omission resulted in reduced growth with the stationary growth phase commencing at an OD_{600} of 0.35. *lttr*852 promoter activity was reduced when copper sulfate was added only after 4 h or later, when compared to a culture in which copper sulfate was present at the start of the culture (Fig. 2.15 A, middle). The observed differences in the relative promoter activity reproducibly were dependent on the time point when the copper sulfate was added during the bacteria culture (Fig. 2.15 A, right). Again, growth rate and GFP expression were similar in both strains, wild type and its isogenic Δ852 mutant, excluding a direct involvement of *lttr*852 in copper dependency (Fig. 2.15 B).

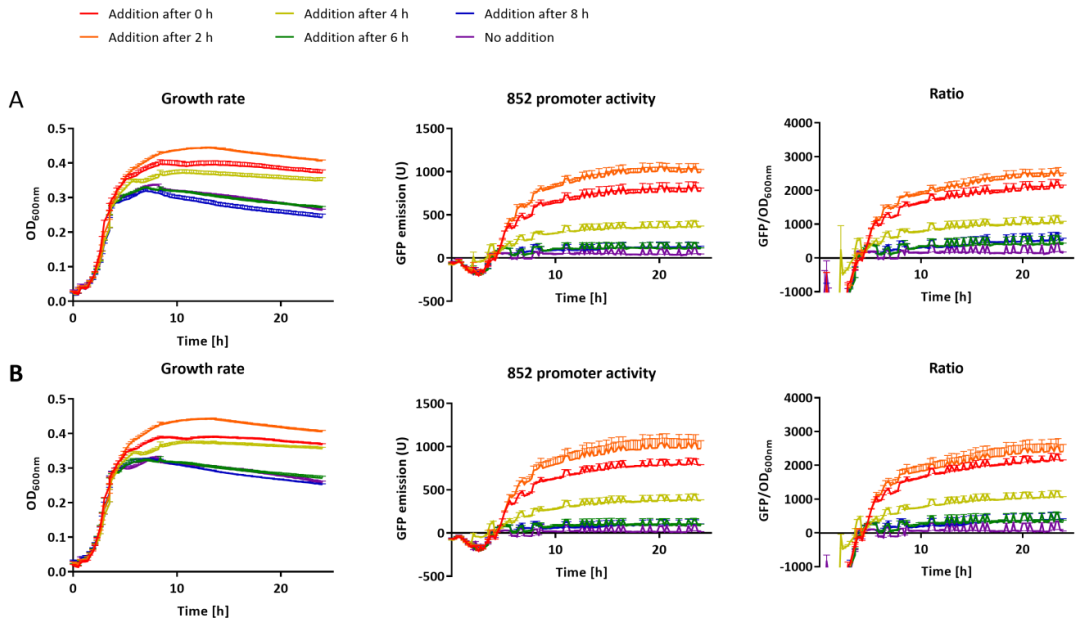


Figure 2.15: Time dependent impact of copper on *lttr*852 promoter activity.
Monitoring of growth rate, *lttr*852 promoter activity profile and ratio of GFP emission to bacterial growth rate in (**A**) *S. aureus* wildtype p2085_Pr852_GFP and (**B**) *lttr*852 mutant growing under micro-aerobic condition in CDM lacking glucose and adding 1 mM copper sulfate after 0 h (red), 2 h (orange), 4 h (yellow), 6 h (green) and 8 h (blue) or not at all (purple).

Since copper influenced the activity of the *lttr*852 promoter, it was therefore of interest to verify the correlation between some copper dependent systems of *S. aureus* and the novel LysR-type transcriptional regulator. CsoR is a copper sensing transcriptional repressor, and it mediates the copper regulation of gene expression in *S. aureus*. Downstream of the copper induced *cso*R lies *cso*Z, a putative copper chaperone and a hypothetical gene (Baker et al., 2011). To determine whether the *lttr*852 promoter activity is linked to the CsoR repressor or the putative copper chaperone CsoZ, the transposon insertion mutant *S. aureus* USA 300 JE2 NE1750 (*cso*R) and JE2 NE1322 (*cso*Z) were used. These two strains from the Nebraska library were transduced with the p2085_Pr852_GFP plasmid. The effect of glucose and copper depletion under micro-aerobic condition on the *lttr*852 promoter was assessed as previously described. No effect was observed on the growth rate of either strain in either media (Fig. 2.16 A-C, left). As in the absence of copper the *lttr*852 promoter activity reached the same level as in the presence of copper in NE1759 and NE1322, the promoter activity showed to be repressed by *cso*R and *cso*Z (Fig. 2.16 A-C, middle). Taken together, copper demonstrated to be a limiting factor for *lttr*852 promoter activity which is abrogated in NE1750 and NE1322.

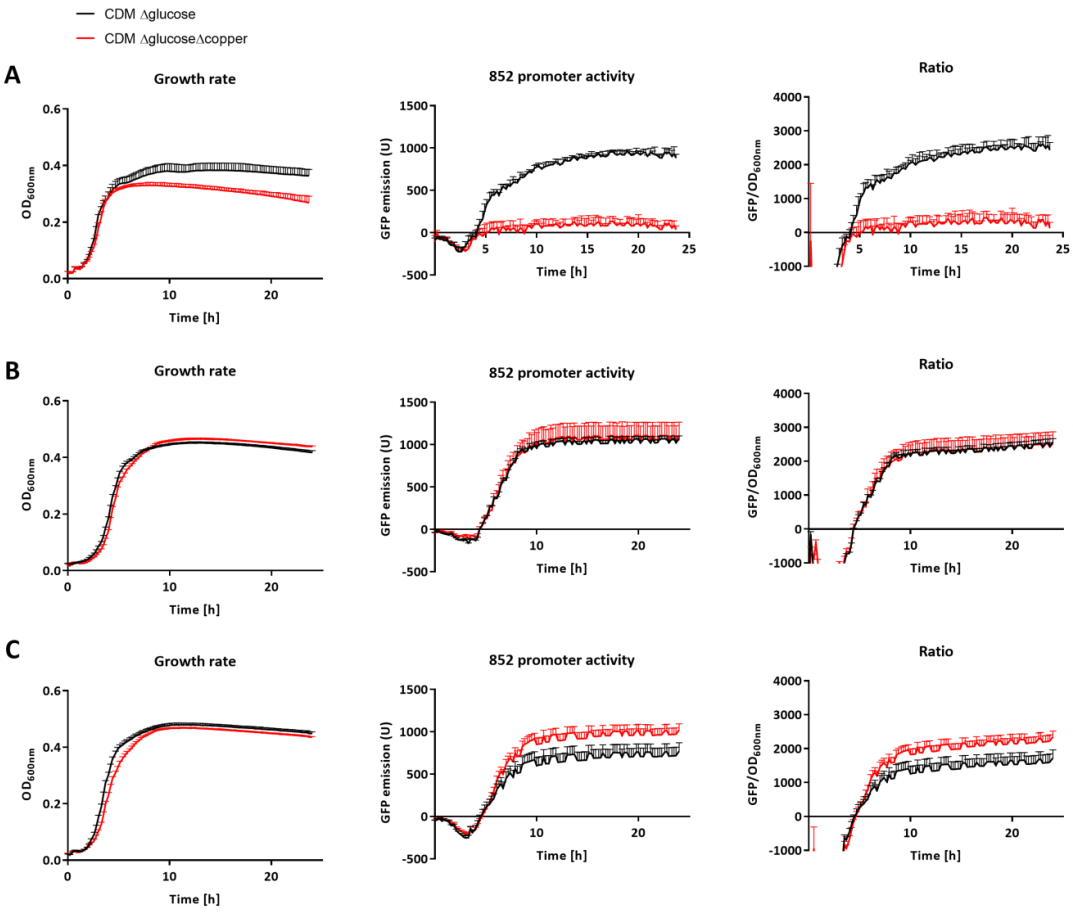


Figure 2.16: Copper impact on promoter activity is abrogated in the transposon insertion mutant of *cso*R and *cso*Z.
Monitoring of growth rate (left), promoter activity via GFP emission (middle) and ratio (right) of (**A**) *S. aureus* 6850 WT p2085_Pr852_GFP, (**B**) *S. aureus* USA300 JE2 NE p2085_Pr852_GFP or (**C**) *S. aureus* USA300 JE2 NE p2085_Pr852_GFP grown in CDM omitting glucose (black) or CDM omitting glucose and copper sulfate (red) under micro-aerobic condition.

As the copper effect on the *lttr*852 promoter was absent in strains with an insertional disruption of *S. aureus* USA300 background, the promoter activity of *lttr*852 was additionally assessed in *S. aureus* Cowan, RN4220, and JE2, as well as in 6850 Δ852 and JE2 Tn_852, which carried transposon insertions within the structural gene for *lttr*852. All strains were transduced with the reporter plasmid p2085_Pr852_GFP and were grown as illustrated above.

The promoter activity relative to the growth rate of *S. aureus* 6850 Δ852 and Cowan were similar to those of *S. aureus* 6850 WT in the absence of glucose alone and

glucose and copper (Fig. 2.17 A, B, E). In *S. aureus* USA300 JE2, the promoter activity of *lttr*852 grown in CDM without glucose reached a level of 3 500, whereas the ratio level for *S. aureus* 6850 WT is around 1 000 higher (Fig. 2.17 C). However, if USA300 JE2 was grown in CDM without glucose and copper sulfate, the growth rate was very similar to the observed growth rate in CDM with copper sulfate. Similarly was the behaviour between the two growth conditions for the relative *lttr*852 promoter activity of *S. aureus* USA300 JE2 Tn_852, whereby the ratio just reached a level of 1 500 (Fig. 2.17 D). The ratio of *lttr*852 promoter activity to the growth rate of the RN4220 strain declined rapidly in the first 6 h in both media but recovered to reach a maximum ratio of about 3 000 in the CDM without glucose alone 14 hours. However, in the absence of glucose and copper, the relative *lttr*852 promoter activity in RN4220 strain reached a maximum of 1 200, which was reproducible different from the repressed activity that was observed in the *S. aureus* 6850 WT strain. Taken together, the data suggests that each strain regulates the *lttr*852 promoter activity differently in the presence or absence of copper sulfate.

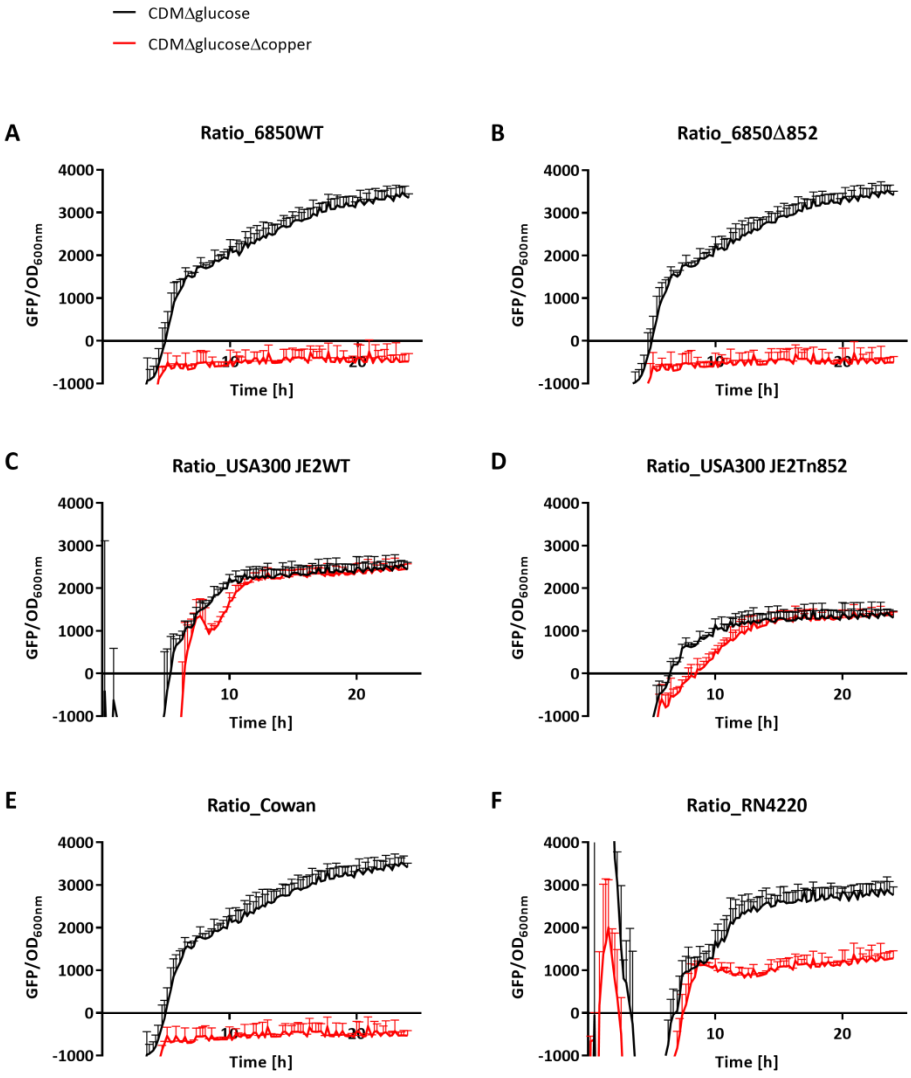


Figure 2.17: *lttr*852 promoter activity is strain dependent.
Monitoring ratio of GFP emission and OD_{600} of (**A**) *S. aureus* 6850 WT p2085_Pr852_GFP, (**B**) 6850 Δ852 p2085_Pr852_GFP, (**C**) USA300 JE2 p2085_Pr852_GFP, (**D**) USA300 JE2 Tn_852 p2085_Pr852, (**E**) Cowan p2085_Pr852_GFP or (**F**) RN4220 p2085_Pr852_GFP grown in CDM omitting glucose (black) or CDM omitting glucose and copper sulfate (red).

2.6.7 *lttr*852 is induced in response to oxidative stress

An infection with *S. aureus* can lead to an oxidative stress induced damage of the kidneys. The oxidative stress response gene, *msr*A2, was one of the genes identified as a possible target of *lttr*852*,* therefore hydrogen peroxide was used to assess the role of the *lttr852* in the resistance of the bacteria to reactive oxidative species released by this compound. To that effect, the relative *lttr*852 expression of *S. aureus* WT, Δ852 and complementation were assessed. Strains were growing in CDM for 6 h and oxidative stress induced by a pulsing of 25 mM hydrogen peroxide for 1 h into the bacteria culture. As shown in Figure 2.18 A upregulation of the *lttr*852 due to the H_2O_2 oxidative stress was very significant in the complemented strain compared to the wildtype and Δ852. The response to oxidative stress was further assessed analyzing the expression of the general stress *dps* gene and one of the superoxide dismutase genes, *sodA*. The expression of *dps* was significantly upregulated in all three strains after exposure to H_2O_2 stress. No differences in the expression of the *dps* was observed between WT and Δ852 after H_2O_2 incubation. However, the complemented strain had significantly higher ($p < 0.001$) upreguation of the *dps* compared to the WT and Δ852 strains pulsed with H_2O_2 (Fig. 2.18 B). Similar, the induction with H_2O_2 resulted in a statistically significant upregulation of *sod*A in all the three strains. The *sodA* gene again was significantly upregulated in the complemented strain compared to wildtype and Δ852 (Fig. 2.18 C).

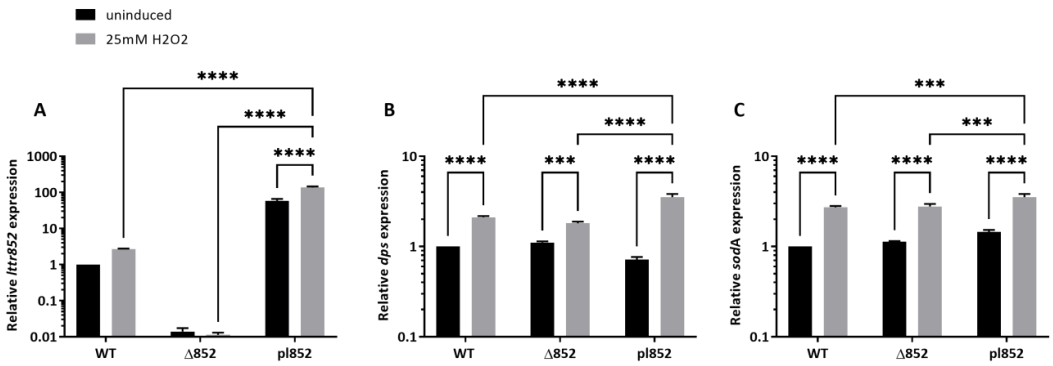


Figure 2.18: Peroxide stress induces transcription of *lttr*852.
Relative expression of (**A**) *lttr*852, (**B**) *dps* or (**C**) *sod*A in *S. aureus* WT, *lttr*852 mutant and pl852 under induction with 25 mM H_2O_2 (grey) or no induction (black). Each column represents the mean values ± SDs from three independent experiments. Statistical analysis was performed using two-way ANOVA. ***, $p < 0.001$; ****, $p < 0.0001$.

Additionally, the correlation of the novel uncharacterized LTTR and the response of potential target genes to oxidative stress were also tested. The induction with hydrogen peroxide did not show any effect on *msrA* expression in *S. aureus* wildtype, however *msr*A was significantly upregulated in *S. aureus lttr*852 mutant and its complementation (Fig. 2.19 A). Furthermore, the expression of *msr*A was statistically significant higher in *S. aureus lttr*852 mutant compared to WT and complementation. The relative expression of *pyr*B was significantly higher in *S. aureus* wildtype and Δ852 after the induction of oxidative stress, but a stronger upregulation was observed in *S. aureus* wildtype compared to *lttr*852 mutant strain (Fig. 2.19 B). The induction with H_2O_2 caused a statistically significant lower expression of *leu*A2 in *S. aureus* wildtype compared to *lttr*852 mutant and its complementation (Fig. 2.19 C). For *car*B the induction was not significant for the complemented strain, however under induction *car*B was strongly expressed in *S. aureus* wildtype than in the complemented strain (Fig. 2.19 D). Oxidative stress induces a significant upregulation of *RNA*III in *S. aureus* wildtype and complemented strains and compared to each other the expression is significantly higher in the wildtype (Fig. 2.19 E). There is hydrogen peroxide dependent regulation of *ure*B, however under this condition the expression of *ure*B is significantly lower in *S. aureus* wildtype compared to Δ852 and its complementation (Fig. 2.19 F).

A significant upregulation due to H_2O_2 treatment in all strains could be found for *cop*A (Fig. 2.19 G). *lrg*A is only upregulated after induction with hydrogen peroxide in *S. aureus* wildtype (Fig. 2.19 H). Oxidative stress has no impact on the expression of *lac*C (Fig. 2.19 I). Taken together, the *lttr*852 is induced in response to oxidative stress and that under oxidative stress *msr*A was significantly upregulated in Δ852 compared to *S. aureus* wildtype and pl852, leading to the suggestion that LTTR852 represses methionine sulfoxide reductase.

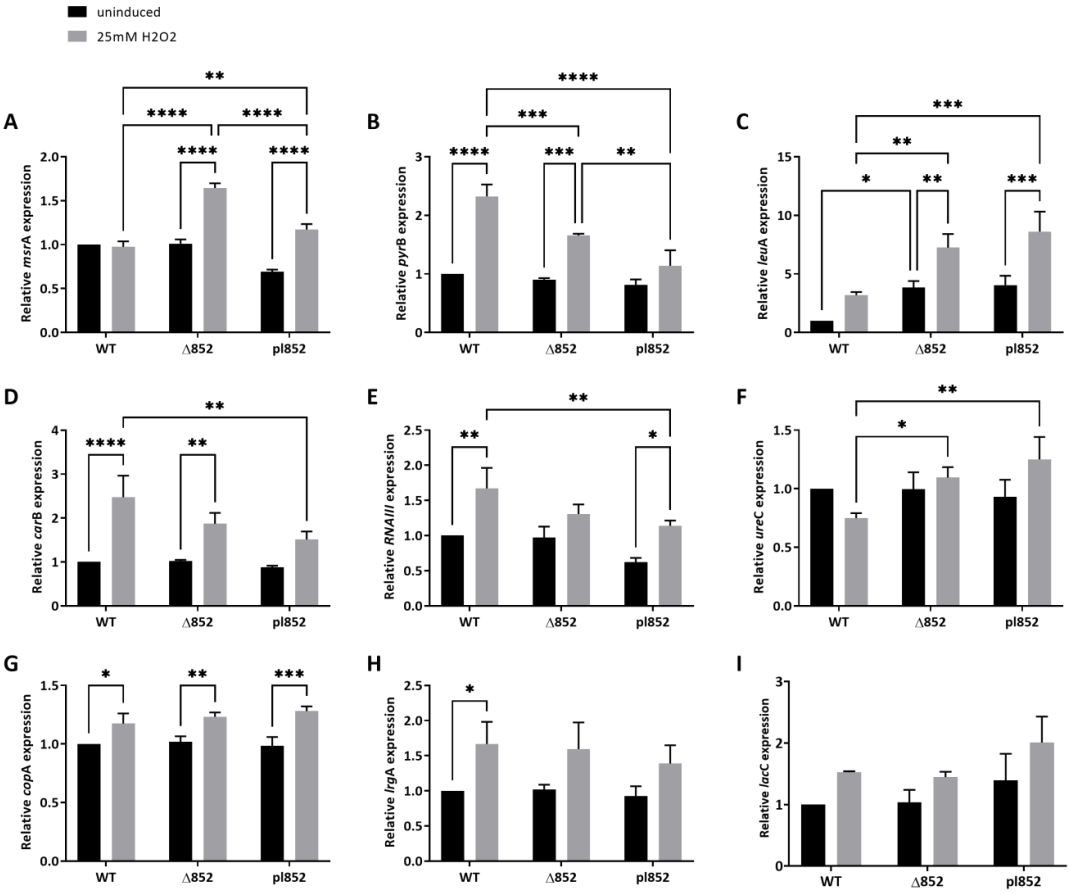


Figure 2.19: Transcription of potential *lttr*852 target genes under oxidative stress.
Relative expression level of (**A**) *msr*A, (**B**) *pyr*B, (**C**) *leu*A2, (**D**) *car*B, (**E**) *RNA*III, (**F**) *ure*C, (**G**) *cop*A, (**H**) *lrg*A, and (**I**) *lac*C in *S. aureus* WT, *lttr*852 mutant and pl852 under induction with 25 mM H_2O_2 (grey) or no induction (black). Each column represents the mean values ± SDs from three independent experiments. Statistical analysis was performed using two-way ANOVA. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

2.7 *lttr*852 is not regulated by major global regulators in *S. aureus*

*lttr*852 is part of the LysR-transcriptional regulator family, whereby it is known that the transcriptional regulator can operate over feedback loop, therefore it was of interest to check if promoter activity of *lttr*852 is influenced in a positive or negative manner by some major regulators. To decipher the regulation of the novel uncharacterized LTTR, some promoter activity assays were performed in various mutants from the Nebraska library to investigate a conjunction with the *lttr*852 gene. The p2085_Pr852_GFP plasmid was transduced into the relevant mutants from the library and the impact of various regulatory factors on *lttr*852 promoter activity. For 24 h the growth rate and promoter activity of *lttr*852 were recorded and afterwards the ratio was calculated. The tested strains were grown in CDM with and without glucose under micro-aerobic condition, as under this condition a strong impact on the *lttr*852 promoter activity was already detected.

2.7.1 Weak regulation of *lttr*852 promoter activity by two component systems

There are several two-component systems which play a major role in transcriptional regulation in *S. aureus*. The two-component systems that have an impact in bacterial stress response during host infection include the Agr, SrrAB and NreBC system.

The accessory gen regulator, *agr,* is a quorum-sensing system and was chosen as the importance of this system matters for virulence regulation (Gong et al., 2014). Here in particular the insertional disruption of ***agrA*** (NE1532) was used, which is required for high-level post-exponential phase expression of a series of secreted proteins, to create NE1532 p2085_Pr852_GFP. Compared to *S. aureus* wildtype p2085_Pr852_GFP grown in CDM without glucose, growth rate was slightly delayed for NE1532 p2085_Pr852_GFP, however the expression of the *lttr*852 promoter was also delayed. Under this condition the ratio of the promoter activity to the growth rate was repeatedly observed lower when the *agr*A was disrupted (Fig. 2.20 A).

The two component system, ***srr*AB,** regulates genes required for nitric oxide, resistance and anaerobic metabolism in *S. aureus* (Kinkel et al., 2013). *lttr*852

promoter activity in the insertional mutants of *srr*A (NE1309) and *srr*B (NE588) were analyzed with the NE1309 p2085_Pr852_GFP and NE588 p2085_Pr852_GFP strains. Both strains showed the same affect on growth rate and GFP emission compared to wildtype in both media (Fig. 2.20 B, C).

The *lttr*852promoter activity was tested in relation to ***nre*C,** a member of the NreBC two-component regulatory system that is involved in the control of dissimilatory reduction of nitrate and nitrite in response of oxygen (Fedtke et al., 2002). The variance of growth rate of NE1669 p2085_Pr852_GFP was broad-based in the log-phase. However, the *lttr*852 promoter activity in this mutant was stronger compared to that of wildtype in the CDM without glucose (Fig. 2.20 D).

A comprehensive valuation reveals that in the insertional mutant of *agr*A and *nre*C the promoter activity of the *lttr*852 is weakly divergently regulated, whereas the two-component system of SrrA/B does not have an impact on the promoter activity of *lttr*852.

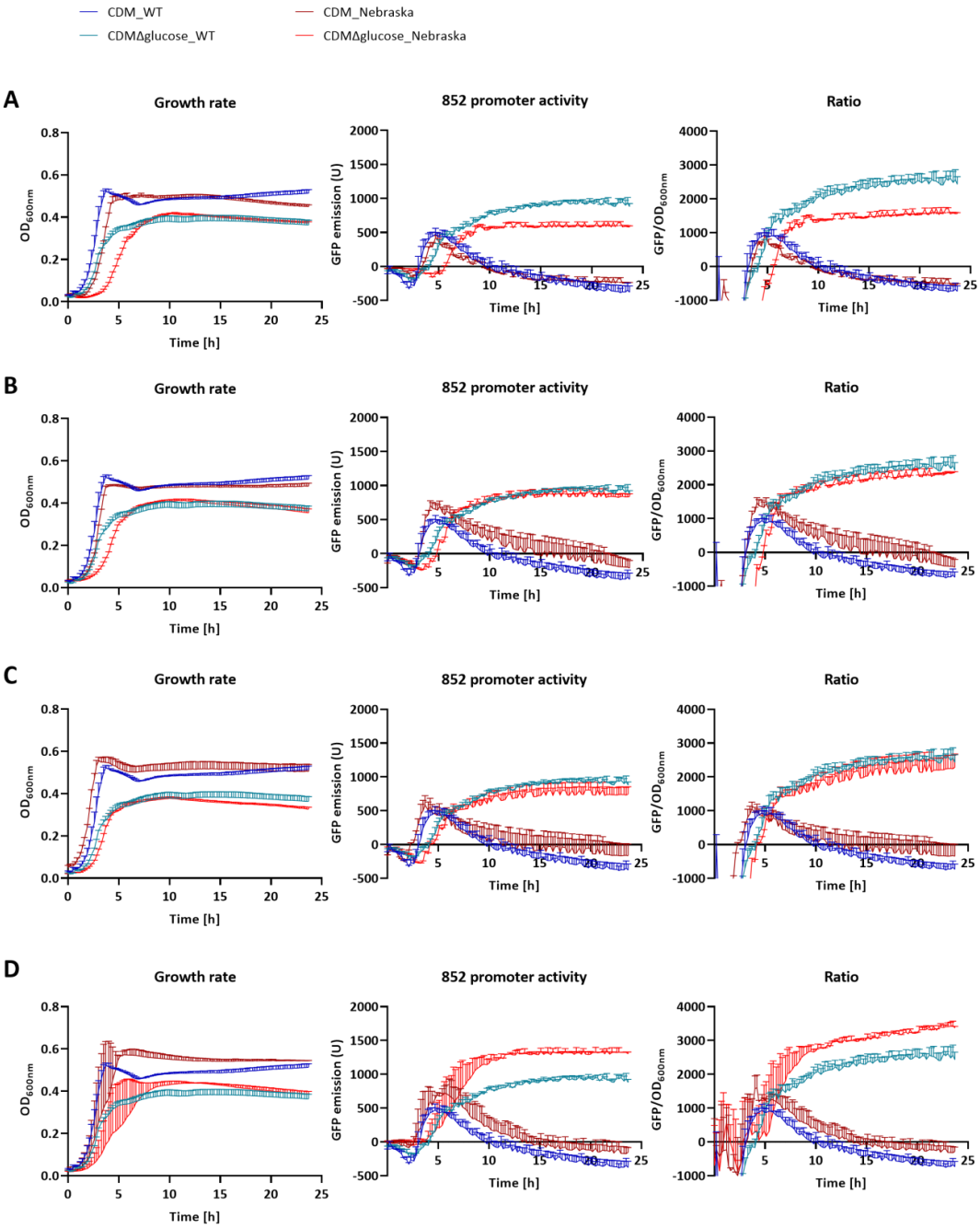


Figure 2.20: Divergently regulation of *lttr*852 promoter activity by the absence of *agr*A and *nre*C.

Monitoring of growth rate, GFP emission and their calculated ratio of strains from the Nebraska library (red) with insertional mutant in two component system of (**A**) *agr*A, (**B**) *srr*A, (**C**) *srr*B and (**D**) *nre*C carrying the plasmid p2085_Pr852_GFP, compared to *S. aureus* 6850 wildtype p2085_Pr852_GFP (blue), each strain grown in either CDM (dark) or CDM omitting glucose (light) under micro-aerobic condition.

2.7.2 Slight regulation of *lttr852* promoter activity by *cym*R, *ccp*E and *cod*Y

S. aureus is able to adapt to different environments and therefore it is necessary that the regulation between metabolism and virulence gene expression are tightly intertwined. There are several regulatory factors linking metabolism and virulence regulation in *S. aureus* such as the transcriptional regulator RpiR and CymR, the catabolite control protein A (CcpA) and E (CcpE) and the global repressor CodY. Thus, the impact of those regulators on the *lttr*852 promoter activity were analyzed.

There are three representatives of the RpiR family of transcriptional regulators. **RpiRc** was selected due to its potential as a regulator of the pentose phosphate pathway and RNAIII levels (Gaupp et al., 2016). The dynamic for growth rate and GFP emission of the insertional disruption of NE1142 p2085_Pr852_GFP were almost the same as for *S. aureus* wildtype p2085_Pr852_GFP, therefore having no impact on the *lttr*852 promoter activity (Fig. 2.21 A).

Involved in the metabolism of biofilm formation is ***cym*R**, which is the main regulator of the cysteine metabolism (Soutourina et al., 2009). The promoter activity of *lttr*852 in Nebraska NE1293 p2085_Pr852_GFP showed a delay of growth in both media with a even more deference in growth in the absence of glucose (Fig. 2.21 B, left). There was also a delay in GFP emission. Additionally, the promoter activity was upregulated in CDM whereas it was downregulated when glucose was omitted in comparison to *S. aureus* wildtype grown in the same media (Fig. 2.21 B, middle).

Another link between metabolism and virulence gene expression are **CcpA** and **CcpE**. CcpE is one of the described LTTRs in *S. aureus* and a major regulator of the tricarboxylic acid cycle, which in the presence of glucose represses virulence associated genes (Ding et al., 2014; Hartmann et al., 2014). The insertional disruption of *ccpA* (NE1670) and *ccpE* (NE1560) both interfere with the development of a catabolite-controlled protein. Although the growth rate of wildtype and both mutants were quite similar (Fig. 2.21 C, D, left), the experiment demonstrated that the *lttr*852 promoter activity behaves differently in both Nebraska mutants, but only if glucose was omitted. The range of GFP emission in the NE1670 p2085_Pr852_GFP was big, but

it reached a higher level compared to wildtype (Fig. 2.21 C, middle). The range for GFP emission in NE1560 p2085_Pr852_GFP was very low, however there was a consistent increase between eight and twelve hours, which later on increased with the same consistence as for *S. aureus* wildtype p2085_Pr852_GFP (Fig. 2.21 D).

CodY is another representative of a major regulatory protein, with a linkage between metabolism and virulence regulation which serves mainly as a repressor of virulence genes (Pohl et al., 2009; Majerczyk et al., 2010). A delayed of growth and GFP emission was initially observed for NE1555 p2085_Pr852_GFP. Additionally, the growth rate of the insertional mutant of *cod*Y grown in CDM reached an OD_{600} of 0.8 (Fig. 2.21 E, left) with a corresponding increase in GFP emission. Consequently, the ratio showed an increase for the promoter activity in NE1555 p2085_Pr852_GFP compared to *S. aureus* wildtype p2085_Pr852_GFP (Fig. 2.21 E, middle, right).

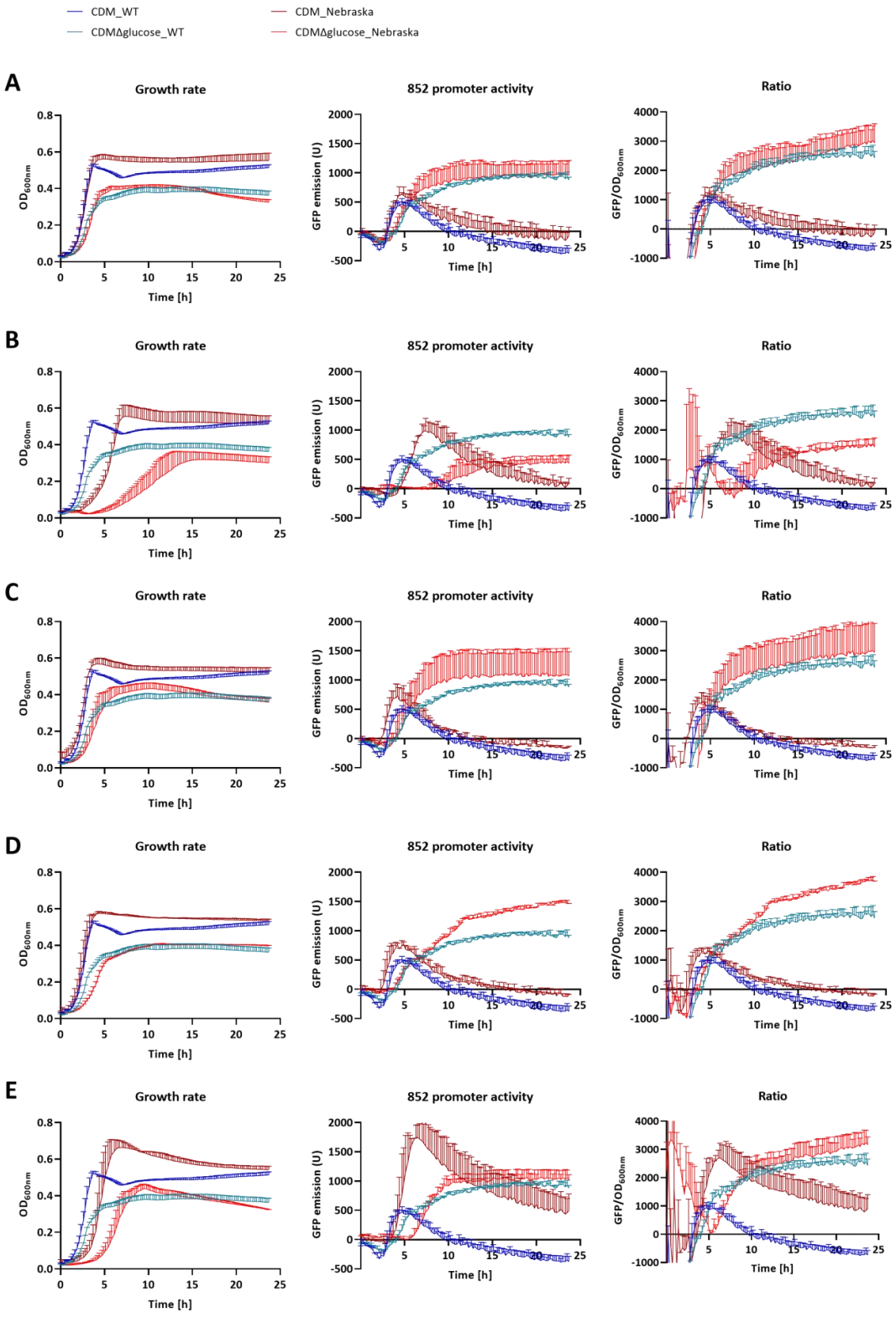


Figure 2.21: Weak regulation of *lttr*852 promoter activity by miscellaneous regulators.
Monitoring of growth rate, GFP emission and their calculated ratio of strains from the Nebraska library (red) with insertional mutant in two component system of (**A**) *rpir*C, (**B**) *cym*R,

(**C**) *ccp*A, (**C**) *ccp*E and (**E**) *cod*Y carrying the plasmid p2085_Pr852_GFP, compared to *S. aureus* 6850 wildtype p2085_Pr852_GFP (blue), each strain grown in either CDM (dark) or CDM omitting glucose (light) under micro-aerobic condition.

2.8 Identification of the enigmatic co-inducer by transcription factor domain swapping

Previous results suggested potential target genes of *lttr*852 (Chapter 2.5). However, the log2 FC of these potential target genes were low after induced expression of the transcriptional regulator (Table 2.1). This implied the lack of a small-molecule co-inducer which is usually required for LTTRs activity (Maddocks and Oyston, 2008). Since LTTRs are comprised of two domains, a HTH-domain that binds to the promoter sequences and a co-inducer binding domain, both domains of *lttr*852 were "re-wired" with the more thoroughly characterized domains of the LTTR *cid*R in order to verify the regulon identified by RNA-Seq (Fig. 4.1 A) or screen for a potential co-inducer (Fig. 4.1 B). Genetic engineering resulted in two AHT-inducible so-called "domain swap" (DS) plasmid constructs: I) inducibly expressing a transgene with the HTH domain of *lttr*852 and the SBD of *cid*R (p2085_852cidR, DS-I), and II) a construct comprising the HTH of *cid*R and SBD of *lttr*852 (p2085_cidR852, DS-II). As controls, plasmids were constructed that allowed AHT-inducible expression of each original transcriptional regulator, *lttr*852 and *cid*R, respectively.

Successful cloning of the constructs was established by PCR and DNA sequencing and the resulting plasmids were transferred to *S. aureus* by electroporation into RN4220 and subsequently transduced into the target strain *S. aureu*s 6850. Next, growth rates of the different strains upon induction with AHT were assessed. For that strains were grown overnight in TSB containing 10 µg/ml chloramphenicol (TSB_{Cm10}) and were freshly diluted to an OD_{600} of 0.1. 400 µl per sample were transferred into wells of a 48-well plate. 200 ng/ ml AHT was added after 5 h of incubation. Bacterial growth was monitored with a TECAN microplate reader for a total of 24 h at 37°C (Fig. 2.22 A).

No consistent growth differences were observed between the used strains. After 24 h, samples were harvested, and the relative expression of the constructs were checked by RT-PCR. For detection, specific primers based on the HTH domain of the tested construct were used (Table 5.4). The induction via AHT was statistically significant for each tested plasmid, whereas the highest induction was detected for the plasmid of the DS II with a 110-fold expression (Fig. 2.22 B).

The translation of the domain-swaps was confirmed by the detection of His-tag which was directly cloned after the domain-swaps. Strains were grown overnight in TSB at 37°C and adjusted to the lowest measured OD_{600} the following day. Afterwards, the strains were grown for one more hour before they were either induced with 200 µg/ml AHT or the same concentration of EtOH, which was used as solvent control. Total protein was isolated from the strains and quantified with a Bradford assay. 5 µg of each sample was denatured and loaded onto SDS-PAGE gels, electrophoretically separated and transferred onto nitrocellulose. Subsequently, Western blot analysis with a His-Tag antibody was performed (Fig. 2.22 C). AHT induction caused a strong expression of all constructs when compared to the respective uninduced condition. Thus, inducible expression of all constructs was detected on RNA as well as on protein level.

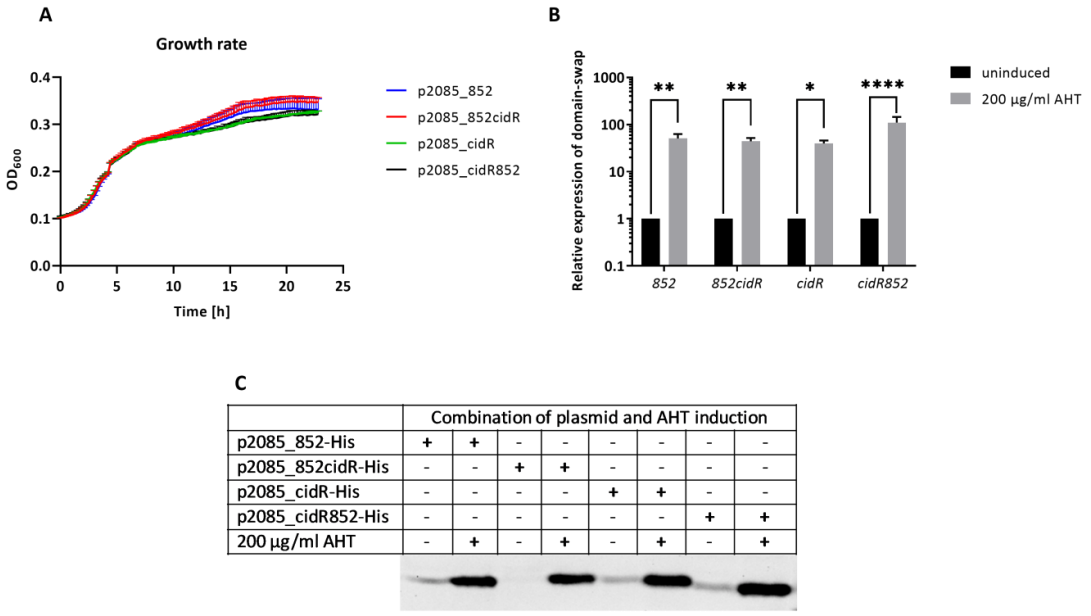


Figure 2.22: Engineering of DS I and II resulted in inducible expression of the constructs.
(**A**) Growth rate of *S. aureus* 6850 carrying either p2085_852 (blue), p2085_852cidR (red), p2085_cidR (green) or p2085_cidR852 (black) with induction of 200 ng/ml AHT after 5 h. (**B**) Relative expression of *852*, *852cidR, cidR* or *cidR852*, with and without induction of 200 ng/ml AHT. (**C**) Detection of His-tagged after the plasmids via His-tag antibody and Western blot.

Next, was tested if the natural substrate of CidR could be used to induce transcriptional changes. Therefore, the control plasmid p2085_cidR was transduced into *S. aureus* Δ852 mutant to exclude interference of the endogenous LTTR852. Relative expression levels of *cid*R and *cid*A were also tested, since the *cid*ABC operon is activated by endogenous CidR in the presence of acetic acid. *S. aureus* Δ852 p2085_cidR was cultivated in TSB and after 24 h, 200 ng/ml AHT was added and additionally 26 mM acetic acid for 5 min, 10 min or 60 min. The samples were harvested and analyzed by RT-PCR.

Under no induction, the relative expression of *cid*A was lower than the relative expression of *cid*R and the addition of 26 mM acetic acid did not increase the expression of *cid*A independent on the induction time (Fig. 2.23 A-C). As expected, induction with AHT resulted in a statistically significant increase in expression of *cid*R, whereas the expression of *cid*A was still low (Fig. 2.23 A-C). However, this did not change in bacteria induced with 200 µg/ml AHT and 26 mM acetic acid for 10 or

60 min (Fig. 2.23 B, C). The longer *S. aureus lttr852* mutant p2085_cidR was induced with the components, the more significant the differences in the expression of targeted genes (Fig. 2.23 A-C). This suggested that the workflow for this strategy needs to be optimized.

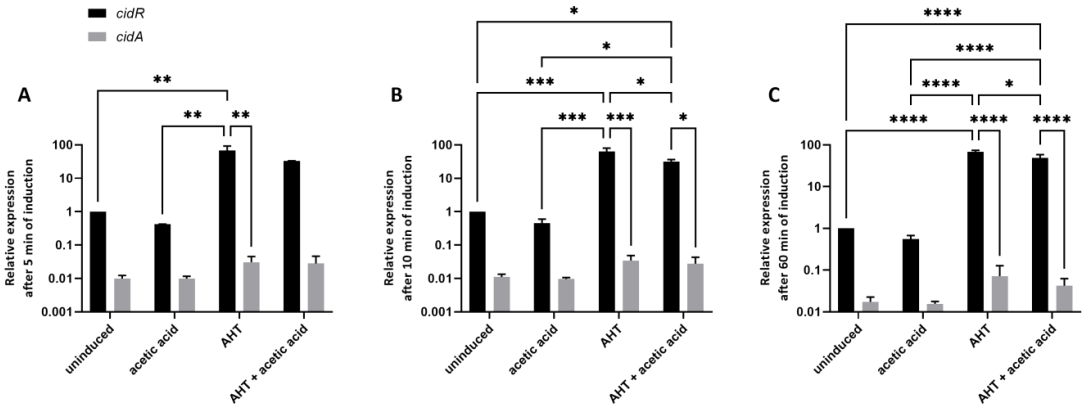


Figure 2.23: Addition of acetic acid upon induced *cid*R expression does not lead to transcription of the *cid*R target gene *cid*A.
Relative expression of *cid*R (black) and *cid*A (grey) of domain-swap control *S. aureus* p2085_cidR under no induction, induction with 2.5 mM acetic acid or200 ng/ml AHT or both for (**A**) 5 min, (**B**) 10 min or (**C**) or 60 min. Each column represents the mean values ± SDs from two independent experiments. Statistical analysis was performed using two-way ANOVA. *, p < 0.05; **, *p* < 0.01; ***, *p* < 0.001; ****, *p* < 0.0001.

2.9 Metabolic differences between *S. aureus* wildtype and its isogenic *lttr*852 mutant

Using BioLog phenotype assay physiological differences between *S. aureus* 6850 WT and its isogenic Δ852 mutant in response to various metabolites were tested. Nine different 96-well panels were used to test differential utilization of 190 carbon sources (panel PM1 and PM2), 95 nitrogen sources (PM3), 59 phosphorus and 35 sulphur sources (PM4), 284 nitrogen-related metabolites (PM6, PM7, PM8) and panels to test for effects of osmolytes (PM9) or pH and ions (PM10). The individual plates were prepared as described in the manufacturer's manual and the subsequent measurements were performed with an OmniLog® instrument. Kinetic data analysis was done by the OmniLog PM software.

Thereby differences in the dynamics and amounts of metabolized substances were detected (Table 2.2., Sup. Tab. 7.2, 7.3). The most prominent hits included butyric acid (difference 5018 units) or di-aminoacid glutamine-asparagine (difference 5117 units) with highest resistance, meaning cell respiration is stronger in *S. aureus* wildtype compared to *lttr*852 mutant. By contrast, substances such as the di-aminoacid valine-glycine (difference -5367 units) demonstrated largest sensitivity indicating that the substances were less metabolized by *S. aureus* wildtype (Tab. 2.2).

Where available, substances demonstrating highest sensitivity or resistance between WT and mutant were mapped to the Kyoto Encyclopedia of Genes and Genomes (KEGG) and were tested for selective enrichment of specific pathways (Tab. 2.3). For *S. aureus* wildtype, around 50 substances were affecting the metabolic pathways, whereas 83 substances had an impact on the metabolic growth of *lttr*852 mutant. Pathways which only show up in wildtype were involved in the biosynthesis of alkaloids derived from histidine, purine, ornithine, lysine and nicotinic acid (each case 9 substances involved), galactose metabolism (9 substances involved), amino and nucleotide sugar metabolism (each case 8 substances involved) and metabolism of the amino-acids alanine, aspartate and glutamate (each case 8 substances involved). Whereas for *lttr*852 mutant, the following pathways came out; pyrimidine metabolism (each case 14 substances involved), 2-oxocarboxylic acid metabolism (14 substances involved), amino-acid metabolism of cysteine, methionine (12 substances involved), glycine, serine, threonine (each case 11 substances involved), arginine and proline (each case 11 substances involved).

Table 2.2: Substances with resistance (green) or sensitivity (red) effect on *S. aureus* 6850 wildtype when compared to Δ852 using the OmniLog system. Shown are ten best hits for resistance as well as sensitivity. Full tables are given in supplemental tables 7.2 and 7.3.

Plate	Source	Well number	Substance	p-value difference
PM08	N-Source	A12	Gly-Asn	5117,00
PM02	C-Source	D12	Butyric Acid	5018,00
PM08	N-Source	A10	Asp-Gly	4673,00
PM04	S-Source	F11	Cysteamine	3952,00
PM04	S-Source	F09	L-Cysteinyl-Glycine	3871,00
PM04	S-Source	G09	Glycyl-L-Methionine	3780,00
PM08	N-Source	D12	Thr-Gln	3690,00
PM04	P-Source	A12	Adenosine- 3,5-Cyclic Monophosphate	3486,00
PM04	S-Source	F08	D-Cysteine	3445,00
PM03	N-Source	A12	L-Glutamic Acid	3429,00
PM01	C-Source	H05	D-Psicose	-1878,00
PM01	C-Source	G03	L-Serine	-2108,00
PM01	C-Source	E01	L-Glutamine	-2135,00
PM02	C-Source	H03	L-Pyroglutamic Acid	-2164,00
PM02	C-Source	H01	L-Ornithine	-2298,00
PM01	C-Source	F01	Glycyl-L-Aspartic Acid	-2495,00
PM07	N-Source	H12	g-Glu-Gly	-2743,00
PM01	C-Source	H06	L-Lyxose	-3105,00
PM01	C-Source	H01	Glycyl-L-Proline	-3118,00
PM07	N-Source	H06	Val-Gly	-5367,00

Table 2.3: KEGG metabolic pathways classes differentially affected by *S. aureus* 6850 wildtype and Δ852 as obtained with the OmniLog system.

# metabolites *S. aureus* WT	Pathway class	# metabolites *S. aureus* Δ852
50	Metabolic pathways	83
24	Biosynthesis of secondary metabolites	36
22	Microbial metabolism in diverse environments	37
19	ABC transporters	29
19	Biosynthesis of plant secondary metabolites	26
17	Biosynthesis of antibiotics	31
14	Biosynthesis of amino acids	26
14	Protein digestion and absorption	26
13	Central carbon metabolism in cancer	21
11	Carbon metabolism	18
9	Aminoacyl-tRNA biosynthesis	21
9	Glyoxylate and dicarboxylate metabolism	13
9	Purine metabolism	11
7	Mineral absorption	14
7	Methane metabolism	10
Metabolic pathways specifically affected in *lttr*852 mutant		
	2-Oxocarboxylic acid metabolism	14
	Pyrimidine metabolism	14
	Cysteine and methionine metabolism	12
	Arginine and proline metabolism	11
	Glycine, serine and threonine metabolism	11
Metabolic pathways specifically affected in WT		
9	Biosynthesis of alkaloids derived from histidine and purine	
9	Biosynthesis of alkaloids derived from ornithine, lysine and nicotinic acid	
9	Galactose metabolism	
8	Alanine, aspartate and glutamate metabolism	
8	Amino sugar and nucleotide sugar metabolism	

3. Discussion

S. aureus is a widespread pathogen capable of infecting various niches within the host. For adaptation to a wide variety of microenvironments within these niches *S. aureus* relies on a network of transcriptional regulators, which therefore are crucial for its virulence. In a previous study of a complex transposon mutant library, the AraC-type transcriptional regulator Rsp was identified to be crucial for dissemination in the host (Das et al., 2016). In a follow-up study, Dr. Sudip Das and Prof. Dr. Eva Medina, intravenously infected experimental animals with the mutant library and enumerated bacterial mutants recovered from different host tissues by TnSeq. Thereby they identified a LysR-type transcriptional regulator, here termed LTTR852, whose mutants were recovered at significantly reduced CFU from kidneys of the animals, whereas CFU from livers were unaffected (Supplemental Fig. 1). This was corroborated with a clean KO mutant which demonstrated that together with the kidneys, also tibiae of hosts had lower numbers of *S. aureus* suggesting that *lttr*852 is involved in establishment of secondary site infection (Groma et al.2020). The present study therefore sought to characterize this novel LysR-type transcriptional regulator in *Staphylococcus aureus* and assess its role in the establishment of an infection niche in secondary infection sites.

3.1 *lttr*852 does neither influence adherence to and invasion into host cells nor phagosomal escape and intracellular replication of *S. aureus*

While *S. aureus* spreads within mice, it invades a variety of non-professional phagocytic cells like the epithelia, endothelial cells and osteoblast (Strobel et al., 2016; Horn et al., 2018). *S. aureus* has been shown to escape from HeLa cells, HEK 239T cells and upper airway epithelia (Giese et al., 2011; Grosz et al., 2014). Due to the observed significant impact of *lttr*852 mutation on the bacteria recovered from the kidney, its role in *S. aureus* adherence, internalization and replication within the host was

investigated. Several factors such as fibronectin-binding proteins and secondary adhesins such as the bi-functional autolysin wall teichoic acid or extracellular adherence protein are important for *S. aureus* adhesion and invasion of host cells (Sinha et al., 1999; Sinha et al., 2000; Weidenmaier et al., 2004; Hussain et al., 2008; Hirschhausen et al., 2010). Transcription of *atl* was reported to show a $\log_2$-fold change of 3.74 upon enrichment or depletion of the respective transposon mutant in murine kidneys compared to intravenously inoculated transposon mutant pool (Groma et al., 2020). However, none of the adhesins were found to be regulated by *lttr*852 within the RNA-seq data (Sup. Tab. 7.1). Furthermore, if HEK 293T cells were infected a time-dependent increase could be detected for the number of recovered bacteria in both wildtype and Δ852, however no *lttr*852 dependent impact on adhesion and internalization could be determined (Fig. 2.2). This is a strong indication that the novel uncharacterized LTTR and its regulon may not be involved in the invasion of non-phagocytic host cells.

After invasion by neutrophils and macrophages, *S. aureus* has been shown to evolve mechanisms to replicate and survive in phagosomes (Gresham et al., 2000; Moldovan and Fraunholz, 2019). The phagosome maturation is a dynamic and complex process based on multiple interactions between the phagosome and various intracellular compartments (Fairn and Grinstein, 2012; Flannagan et al., 2012; Gutierrez, 2013). Via Rab GTPases it is possible to generate an intracellular vesicle trafficking pathway as they belong to GTPases that regulate intracellular transport (Stenmark, 2009; Mizuno-Yamasaki et al., 2012). A student generated during his master-thesis HeLa fluorescent reporter cell lines, expressing caveolin-YFP, YFP-Rab3a, -clathrin and -cwt and additional phagosomal escape markers for *S. aureus,* as an array for phenotype profiling in endovascular trafficking. Although, an infection with *S. aureus* 6850 wildtype and *lttr*852 mutant showed super species of co-localization to phagosomal vesicles expressing Rab3a and LC3II, no obvious differences between the infected cells could be observed, suggesting that *lttr*852 may not be linked to phagosome maturation (Master-Thesis, Haubenthal, T. 2019).

The pathogen is reported to escape phagoendosomes in non-professional phagocytes by replicating within the host cell cytoplasm and subsequently lysing the host cell. Since deficiency in these processes would lead to a failure to spread within the host organism, the relative escape rate of *S. aureus* wildtype and its isogenic Δ852 mutant were investigated in HEK 239T escape reporter cells. Although consistently higher escape rates were observed with longer incubation periods, there was no significant difference between the wildtype and Δ852 strains (Fig. 2.2), suggesting that *lttr*852 may not be involved in escape from non-phagocytic host cells.

As several hemolysins of *S. aureus* can cause lysis of erythrocytes, the dependency of LTTR852 on hemolysis was checked, but the deletion of *lttr852* does not affect the lysis of sheep erythrocytes (Fig. 2.4). The quorum sensing system agr is crucial for virulence of *S. aureus* and subsequent escape of bacteria from host cells (Qazi et al., 2001; Shompole et al., 2003). The *agr* operon and the *agr*-regulated pore-forming α-toxin, which mediates beside hemolysis the escape of *S. aureus* into the cytosol in cystic fibrosis cells (Jarry and Cheung, 2006) were not significantly regulated in the differential RNA-seq results obtained in this thesis (Tab. 2.1). Other important mediators for phagosomal escape of *S. aureus* are the α-type phenol-soluble modulins, which are also directly regulated by *agr* (Wang et al., 2007; Queck et al., 2008; Giese et al., 2011; Grosz et al., 2014) and the PSM δ-toxin which are partly encoded within the direct *agr* effector *RNA*III (Balaban and Novick, 1995; Novick, 2003). Interestingly, in this work *RNA*III was upregulated upon AHT-induced expression of *lttr852* (Tab. 2.1). Yet, no connection to phagosomal escape based on *lttr*852 was found.

S. aureus possesses stress response pathways in order to refold or degradate damaged proteins via molecular chaperons or proteases (Gottesman et al., 1997; Kuroda et al., 2001; Fleury et al., 2009). *Clp*B which is located downstream of *lttr*852 is part of the stress induced multi-chaperon system and involved amongst others in biofilm formation, endocytosis and cell wall autolysis (Frees et al., 2004; Chatterjee et al., 2005). The gene *clp*B was identified under AHT induction, but even than there was no regulation seen according to *lttr*852 (Fig. 2.6 B). The biofilm formation according to *lttr*852 was also tested, but here as well as under over-expression of *lttr852* an impact

on the biofilm formation dependent on *lttr*852 could not be detected (Fig. 2.5), suggesting that the novel uncharacterized LTTR may not be involved in biofilm formation.

3.2 Impact of the microenvironment on *lttr*852 in *S. aureus*

Successful invasion and subsequent establishment of an infection of host cells by *S. aureus* involves a complex interplay of the pathogen with the host microenvironment. Thus, the pathogen needs to adapt to various environmental stresses and additionally overcome host immune responses. The pathogen successfully establishes an infection niche by adjusting its gene expression and virulence factors to overcome host barriers that result in the so-called infection bottlenecks (Fig. 6.1). Due to the fact that the CFUs of the *lttr*852 mutant were significantly lower in the secondary infection sites, it raises the question whether the number of bacteria entering the secondary infection site are already reduced or if there is a deficiency to establish an infection at secondary infection sites, leading to a niche specificity for *S. aureus* harboring the *lttr*852.

At the beginning of this thesis a phenotype based on *lttr*852 was only observed *in vivo* and as experiments in mice are limited due to animal protection laws, it was of interest to identify an *in vitro* phenotype, in order to characterize this novel LTTR. In order to determine an *in vitro* phenotype for *lttr*852, attempts were made to mimic the micro-environmental pressure the pathogen has to go through while circulating in the mice. It is known that LysR-type transcriptional regulators can impact the bacterial response to various stressors, such as temperature, osmotic or oxidative stress (Farr and Kogoma, 1991; Banos et al., 2011). Therefore, the growth habit dependent on LTTR852 was investigated under different growth media conditions (2.6.1), as well as under osmotic (2.6.2), oxidative (2.6.7) and temperature stresses (2.6.4).

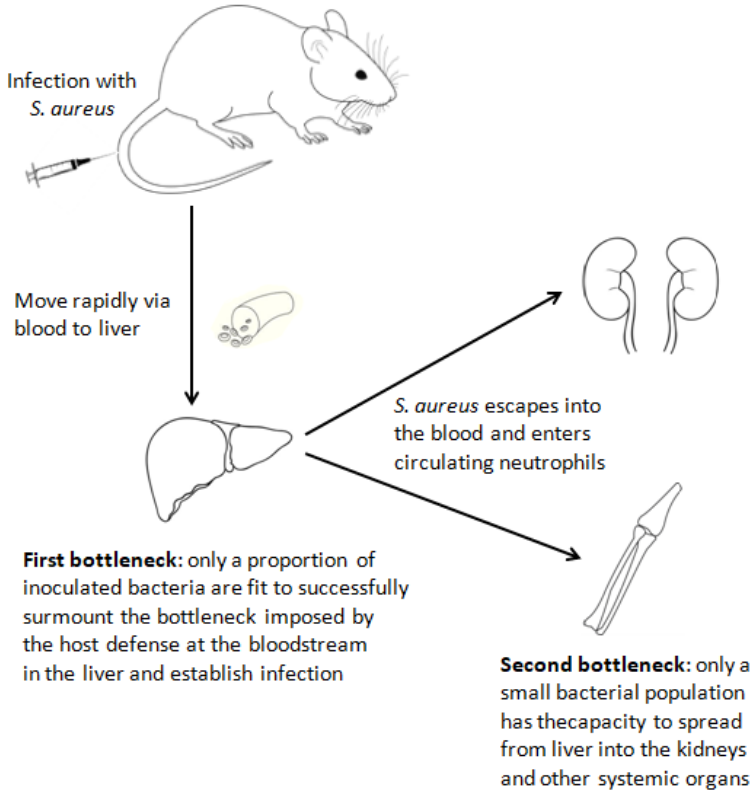


Figure 3.1: Host bottleneck effect during infection with *S. aureus*.
After the injection with *S. aureus*, bacteria move through the vasculature and enters the liver as the first bottleneck. Most of the pathogens are phagocytosed by Kupffer cells. Some *S. aureus* survive by building an abscess or by being taken up by neutrophils or they are even able to escape from the organ via blood vessels. Therefore, only a few *S. aureus* succeed to enter organs of the secondary bottleneck, such as kidneys and tibia, where they can lead to form abscesses.

Additionally, promoters must process different metabolic and physicochemical signals in order to determine their output. In extremely competitive niches it helps the pathogen to achieve the optimal fitness. Promoters react to distinct signals with inclusive reactions to more general environmental changes, in consequence their regulation is in a more precise way (Cases and de Lorenzo, 2005). Therefore, the transcription initiation of the *lttr*852 promoter was also checked under the above-mentioned environmental conditions and additionally under the induction of essential components. The subsequent chapters will give a summary and an assessment of the impact of the microenvironment on *lttr*852 in *S. aureus*.

3.2.1 High temperature represses *lttr*852 promoter activity in a time dependent manner

An Infection by pathogens can cause fever, mainly to activate the immune system of the host, but also to affect DNA, RNA or protein and many virulence factors of the pathogen, however, bacteria developed miscellaneous strategies to adapt to temperature changes (Small et al., 1986; Lam et al., 2014; Evans et al., 2015). *S. aureus* grows optimally at 37°C, which correlates to the core temperature of its mammalian host, but the pathogen is also able to grow sub-optimally between 42°C and 16°C. Due to an infection with *S. aureus*, mice core temperature might change and therefore the growth ability and *lttr*852 promoter activity of *S. aureus* were analyzed dependent on the LysR-Type transcriptional regulator, under changing temperatures from 37°C to 42°C.

The OD_{600} for *S. aureus* under 37°C showed an optimal growth rate, whereas the OD_{600} under 42°C, independent on the time point the temperature was changed, showed a marginally different growth kinetic as already observed by Fleury (Fleury et al., 2009). Nevertheless, *lttr*852 promoter activity shows to exhibit a temperature dependent repression, in a time-dependent activation. A growth temperature of 42°C from the beginning reduced the *lttr*852 promoter activity but independent on a feedback loop of LTTR852, as the *lttr*852 activity in wildtype and *lttr*852 mutant were similar (Fig. 2.11 A, B). It is known that most purine- and pyrimidine-synthesis pathway components and arginine deiminase are downregulated if *S. aureus* was exposed at 48°C (Fleury et al., 2009). However, the KEGG analysis showed a better metabolization of purine, pyrimidine and agrinine by the Δ852 strain (Tab. 2.3). Additionally stress exposed *S. aureus* exhibited an impact on some TCA cycle components like *thi*E, *thi*M, *thi*D, being increased transcripted at 48°C (Fleury et al., 2009), whereas RNA-seq which was performed under 37°C showed a downregulation of *thi*M and *thi*D in the overexpression of *lttr*852 (Sup. Tab. 7.1). As promoter activity of *lttr*852 was repressed under high temperature, there might be a correlation between *lttr*852 and high temperature which has to be further investigated. Beside temperature, it seems that time might also play a role in the *lttr*852 promoter activity. A temperature switch from

37°C to 42°C in the first two hours resulted in the reduction of bacterial growth rate, but when the temperature was shifted from 37°C to 42°C after 4 h hours, the promoter activity was similar to growth under standard 37°C (Fig. 2.11). A high temperature of 42°C in the log-phase showed an impact on the *lttr*852 promoter activity, however the promoter activity was not affected when treated with high temperature at the stationary phase. Also, the addition of copper at certain time-points diminished the *lttr*852 promoter activity the later copper was added to the culture (Fig. 2.15). Almost no *lttr*852 promoter activity was detected when copper was added after six hours, therefore also leading to the suggestion that time might be important. It is known that time-dependent genetic effects have an impact on gene expression (Bryois et al., 2017), but how exactly time plays a role for the novel uncharacterized LTTR needs to be examined.

3.2.2 *lttr*852 transcription is repressed by glucose in the stationary phase

Culture medium are important for *in vitro* experiments, as growth rate and transcriptional regulation can be affected by the use of different media. For example, the gram-negative bacterium *Xanthomonas axonopodis* pv. *glycines* (*Xag*) is a LysR-type carbohydrate related transcriptional regulator, which was seen more abundant in the minimal medium XVM2 compared to TSB, although bacteria were grown significantly less in XCM2 (Park et al., 2019b). In this study following media were tested BHI, TSB, LB, CDM and RPMI (Fig. 2.8). Although, as suspected the best growth condition of *S. aureus* was found in the rich BHI medium, whereas the lowest detection was found if grown in RPMI medium, the GFP emission which correlated to the level of *lttr*852 promoter activity, was strongest in bacteria grown in LB (Fig. 2.8). This suggested that chemical components of LB might activate the *lttr*852 promoter activity or media such as TSB, CDM or RPMI contain substances which repress the activity of the *lttr*852 promoter.

However, during the *lttr*852 promoter cloning process, it was observed that bacteria carrying p2085_Pr852_GFP plated on LB agar without glucose displayed a sharper GFP fluorescence, than when grown on TSB agar containing 2.5 g/L glucose (Fig. 2.9).

Glucose therefore demonstrates to inhibit the transcription of the *lttr*852. As an activation of *lttr*852 promoter was already observed upon plating *S. aureus* carrying the promoter activity reporter plasmid p2085_Pr852_GFP on agar without glucose (Fig. 2.9), differenct concentrations of glucose were analyzed to determine the effect of glucose inclusion in the culture medium on the *lttr*852 promoter activity. Two different kinetic GFP emission were detected. The absence of glucose as well as the addition of 1mM amounts resulted in low GFP emission, whereas in presence of 5 mM glucose it reached an unit of 300. On the other hand, the addition of 10 mM glucose or more to the medium resulted in rapid increase in GFP emission to 450 unit in the first 7 hours and a constant drop from 8 to 24 hours (Fig. 2.9, C). It seems that the metabolism of glucose has an impact on the activation or respression of the *lttr*852 promoter. The glycolytic and pentose phosphate pathways primarily catabolize carbohydrates, whereas the TCA cycle activity is mainly repressed in the abundance of nutrients in the culture media (Collins and Lascelles, 1962; Somerville and Proctor, 2009). When the concentration of essential nutrients such as glucose decreases, or growth inhibitory molecules like lactic acid or acetic acid accumulate, an exit from the exponential phase occurs. This suggests that the *lttr*852 may be activated in the presence of glucose and repressed if glucose is no longer available. However, the postexponential catabolism of nonpreferred carbon sources such as acetate requires TCA cycle activity (Somerville et al., 2002). The increased enzymatic activity of the TCA cycle (Somerville et al., 2003) may therefore contribute to the repression of the *lttr*852 promoter activity during the postexponential phase. With the aid of the Biolog assay, the metabolism of a variety of substances in *S. aureus* wildtype and Δ852 were analyzed, however after mapping the outcome via KEGG, several components of the TCA cycle were either better metabolized by *S. aureus* wildtype or Δ852 (Sub. 7.2, 7.3). Kidneys are involved in glucose homeostasis through processes of gluconeogenesis, glucose filtration, re-absorption and consumption (Mather and Pollock, 2011), therefore this might explain the nescessity of *lttr*852 in *S. aureus* to adapt to the environment found in the kidneys, as lower CFU level of Δ852 were found in the kidneys.

3.2.3 *lttr*852 mutants may be more sensitive to oxygen content

It is known that *S. aureus* is a facultative anaerobic bacterium and therefore able to adapt to an environment with different oxygen levels. For example, the limited blood flow in the kidneys generates oxygen gradients and low tissue oxygen tension, thereby creating conditions that require anaerobic glucose metabolism in the inner medulla of the kidneys (Chen et al., 2016). Due to that, the *lttr*852 promoter activity was analyzed under low oxygenation, whereas the *lttr*852 promoter activity in bacteria grown under micro-aerobic conditions reached an GFP emission of over 10 000 units and therefore being consistently stronger when compared to aerobic conditions (Fig. 2.8, 2.10). Additionally, the relative *lttr*852 expression was significantly higher under micro-aerobic condition compared to aerobic condition (Fig. 2.10 C). Both experiments led to the assumption that the promoter may be enhanced under micro-aerobic conditions. As the availability of oxygen is important for the catabolic fate of pyruvate, there might be a link to *lttr*852. It is known that under aerobic growth condition, pyruvate is enzymatically oxidized to acetyl coenzyme A (acetyl-Coa), whereas under anaerobic condition, pyruvate is reduced primarily to lactic acid, allowing continuation of glycolysis due to concomitant reoxidation of NADH (Krebs, 1937; Gardner and Lascelles, 1962). Additionally, *S. aureus* wildtype was more sensitive for the metabolization of L-lactic acid via Biolog assay (Sup. Tab. 7.2), therefore also giving a hint that *lttr*852 is somehow involved in the catabolization of carbohydrates.

As the implementation of the method to generate micro-aerobic condition could not easily be verified during culture, the lower oxygen content was confirmed by the RT-PCR measurements with an RNA abundance of the formate acetyltransferase gene *pfl*B, which is only expressed under low oxygen conditions (Fig. 2.10 D) (Fuchs et al., 2007). The enhanced transcriptional activity of RSAU_000852 may explain the observed lack of fitness of Δ852 mutant in secondary host tissues such as kidneys, as the renal physiology is influenced by a distinctive inhomogeneity of oxygen availability (Aukland and Krog, 1960; Leichtweiss et al., 1969; Baumgartl et al., 1972; Brezis et al., 1984; Brezis et al., 1989; Brezis and Rosen, 1995).

3.2.4 Potential involvement of *lttr*852 in response to oxidized methionine

Usually, oxidative stress results from either mitochondrial dysfunction, impaired antioxidant system, excessive reactive oxygen species (ROS) production, or a combination of these factors (Cui et al., 2012; Nita and Grzybowski, 2016). ROS are part of the unspecified defense system of the organisms, however excessive ROS level can cause tissue damage by mechanisms including lipid peroxidation, DNA damage and protein modification and thus can have a negative effect on tissue function and structure (Cabiscol et al., 2000; Dobashi et al., 2000; Cai and Yan, 2013; Nita and Grzybowski, 2016; Su et al., 2019). Well known ROS are superoxide (O_2·-), hydrogen peroxide (H_2O_2) and the peroxy radical (OH·) (Vatansever et al., 2013). However, oxidizing agents can oxidize the sulfur atom of methionine residues, resulting in methione sulfoxide, but these can be reconverted to methionine by the methionine sulfoxide reductase (MSR) that restore normal protein functions (Brot et al., 1981; Moskovitz et al., 1997; Singh et al., 2018).

Via RNA-seq, *msr*A was identified as a potential target of *lttr*852 (Tab. 2.1) as it was found to be upregulated 1.74-fold in the wildtype carrying the plasmid p2085_852-AHT and induced with AHT, compared to the Δ852 p2085_852-AHT strain. These data were corroborated by RT-PCR data (Fig. 2.7). Although methionine sulfoxide reductases msrA and msrB enzymes have the same function of methionine sulfoxide reduction, they differ not only in substrate specificity, but also in active site composition, protein folding, sub-cellular localization and evolution in bacteria as well as plants or mammals. Four methionine sulfoxide reductases are encoded by *S. aureus,* three of which possess MsrA-functionality, and MsrB is encoded by a single gene (Singh et al., 2018). Comparison of the transcriptome of *S. aureus* grown in medium and *S. aureus* recovered from experimental animals during acute and chronic infection showed an upregulation of the operon encoding *msr*A1 and *msr*B (Szafranska et al., 2014). As kidneys are highly vulnerable to damage caused by reactive ROS (Ozbek, 2012) and as CFU differences between wildtype and Δ852 were significant in the kidneys, it could be that *msr*A is stronger regulated by *S. aureus* wildtype in order to overcome ROS.

Furthermore, with the OmniLog system it was possible to analyze which metabolic pathways classes are differentially affected by *S. aureus* 6850 wildtype and Δ852, however the methionine metabolism was specifically affected in *lttr*852 mutant (Tab. 2.3). As the metabolization of substances of the methionine metabolism were detected as resistance, meaning that the cell respiration for those substanzes is stronger in *S. aureus* wildtype and therefore leading to the suggestion of potential involvement of *lttr*852 in methionine metabolism.

A well-known LTTR which is responsible for the oxidative stress response against H_2O_2 in *Escherichia coli*, *Salmonella enterica* servoar Typhimurium is OxyR (Farr and Kogoma, 1991). The innate immune system is known to produce peroxide as antibacterial agents *in vivo*. This also reinforces the important role of LTTRs when *S. aureus* is presented with oxidative stress in the host. Based on the ability of *S. aureus* to survive oxidative stress and additionally the upregulated expression of the oxidative stress response gene *msr*A, it was of interest to analyze if *lttr*852 may be important for *S. aureus* upon encounter of oxidative stress in the host. Therefore *S. aureus* wildtype, Δ852 and complementation were exposed to 25 mM H_2O_2, but the *lttr*852 expression was only found to be significantly upregulated in the complementation (Fig. 2.18 A). But as the expression of general stress protein *dps* was significantly upregulated for all three strains in the presence of peroxide (Fig. 2.18 B), it confirmed that the amount and time of induction with H_2O_2 was enough to generate oxidative stress. *S. aureus* possesses two superoxide dismutases, encoded by *sod*A and *sod*M, which are encountered during host infection or generated from aerobic metabolism and which inactivate harmful superoxide radicals (Clements et al., 1999; Valderas and Hart, 2001; Karavolos et al., 2003). Therefore, *sod*A was checked under oxidative stress, however there was an upregulation of *sod*A under H_2O_2 induction in all tested strains, but there was no dependency on *lttr*852 (Fig. 2.18 B), leading to the suggestion that *lttr*852 is not correlated to SODs. However, the expression of the possible target genes were also tested under the induction with hydrogen peroxide. Only in Δ852 and pl852 the induction showed a significant upregulated expression of *msr*A due to oxidative stress (Fig. 2.19 A). Although peroxide has been shown to lead

to methionine oxidation (Levine et al., 1996; Sjoberg et al., 2018), it is known that *msr*A1 is not transcribed by the induction with peroxide (Singh et al., 2001b; Singh and Moskovitz, 2003), explaining the same level of *msr*A in wildtype under H_2O_2 treatment or uninduction. Therefore an induction with the cell wall antibiotic oxacillin would be better, as the most strongly induced protein under this treatment is MsrA1 (Singh et al., 2001a). However, under oxidative stress *msr*A was significantly upregulated in Δ852 compared to *S. aureus* wildtype and pl852, leading to the suggestion that LTTR852 represses methionine sulfoxide reductase.

3.2.5 Copper is a limiting factor for *lttr*852 transcription

In the present thesis, RNA-seq suggests that the unknown LTTR is involved in *cop*AZ expression, as they were found upregulated after the over-expression of *lttr852*, thereby modulating the copper response of *S. aureus* (Tab. 2.1). *S. aureus* survives high copper stress by expressing transmembrane copper export proteins CopA and CopB. The *cop*AZ and *cop*BL operons are linked to the *cso*R transcriptional regulator, which binds intracellular copper and leads to their de-repression (Rosario-Cruz et al., 2019). CopZ acts as a cytosolic buffer and is a chaperone protein. Bound to the membrane and being exposed to the surface is CopL. It is a copper-binding lipoprotein, which prevents copper uptake by binding extracellular copper to prevent it from entering or re-entering the cell. However, *lttr*852 promoter activity was checked in the presence and absence of copper sulfate in *S. aureus* 6850 wildtype p2085_Pr852_GFP and *S. aureus* USA 300 JE2 NE1750 (*cso*R) and JE2 NE1322 (*cso*Z) which were also transduced with the p2085_Pr852_GFP plasmid. As the promoter activity of *lttr*852 was not repressed in both mutants if copper was omitted, it demonstrates that *cso*R and *cso*Z are having a repressing effect on *lttr*852 in the absence of copper (Fig. 2.16 B, C). But as the used mutants had a different background, the experiment was repeated in multiple genetic backgrounds. The repression of *lttr*852 promoter activity in the absence of copper could be attributed in *S. aureus* of multiple genetic backgrounds (Fig. 2.17). However, in USA 300 JE2 the promoter activity showed the same kinetic ratio as in the *cso*R and *cso*Z mutant, therefore leading to the result that CsoR and

CsoZ do not have an impact on *lttr*852 promoter activity in this background. Therefore, the experiment should be repeated in *S. aureus* 6850 ΔcsoR and ΔcsoZ. However, the different *lttr*852 promoter activity between the *S. aureus* strains can be explained, as the USA 300 JE2 strain carries a copper resistance locus (*cop*XL), which is uniquely associated with the SCCmec element and not found in other *S. aureus* lineages (Purves et al., 2018). CopL in USA 300 JE2 may supply copper to the bacterium when it is transferred to a copper-depleted growth media. Interestingly *cop*A mutants were predominantly isolated from bacteria recovered from liver, suggesting that *cop*A may not be required for bacterial survival in this organ (Groma et al., 2020).

Further analysis demonstrated availability of copper ions as a limiting factor for the activation of *lttr*852 transcription. The *lttr*852 promoter exhibited a dose dependent increase in activity upon addition of copper sulfate. The *lttr*852 promoter activity was fully restored by addition of at least 400 nM copper sulfate (Fig. 2.14). It is tempting to speculate that *lttr*852 is important in host microenvironments identified by the presence of copper ions. Copper is involved in processes such as cellular respiration, iron transport and free radical scavenging in both, the host and the pathogen (Jimenez and Speisky, 2000; Collins et al., 2010; Festa and Thiele, 2011). The copper balance in the host is mainly regulated by two copper activated P-type ATPase transporters ATP7A and ATP7B (Lutsenko et al., 2007). In macrophages, copper transport into the secretory pathway is additionally regulated by oxygen (White et al., 2009). Limiting cellular oxygen stimulates copper transport from the Golgi by increasing copper binding to the ATP7A protein. Hypoxia increases the expression of ATP7A in RAW264.7 macrophages (White et al., 2009). During tissue infection or injury, tissue-specific macrophage subpopulations can change their penotype and function (Murray and Wynn, 2011). In the kidneys, copper is normally detected in the proximal tubules, distal tubules and glomeruli and there are five types of tissue-resident macrophages (Kodama et al., 1993; Kirby et al., 1998; Kawakami et al., 2013). Copper also serves as an antibacterial agent and is pumped into phagosomes of macrophages in order to kill bacteria. The increased levels of phagosome associated Cu may be due to elevation of the steady-state levels of the plasma membrane Ctr1, high-affinity copper ion importer

and ATP7A (White et al., 2009). RNA interference depletion of ATP7A in RAW264.7 cells reduced microbicidal activity against bacteria consistent with the notion that ATP7A pumps Cu into the phagosome. Therefore, the bactericidal activity of copper added to RAW264.7 is enhanced by H_2O_2 (White et al., 2009). A college with very good knowledge in working with macrophages checked the growth effect in M1 macrophages dependent on *lttr*852, by adding CuSO4 exogenously to the macrophages to increase the possibility of having higher intracellular concentration of copper, according to literatures protocols. Although, in the first experimental procedure Δ852 exhibits a mild growth defect in M1 macrophages which were copper (II) sulfate stimulated, the experiment has to be repeated to check whether there is indeed a phenotype of the *lttr*852 mutant under these conditions (Data not shown).

All in all, the data point to the function of the transcriptional regulator LTTR encoded by RSAU_000852 to establish an efficient infection in host specific niches. In conclusion one can maintain with certainty that the promoter of *lttr*852 is activated by the presence of glucose in the log-phase, whereas after metabolization it is repressed as well as under high temperatur treatment in the log-phase. Additionally the promoter activity is enhanced under micro-aerobic conditions and copper beeing a limiting factor for the promoter activity (Fig. 3.2).

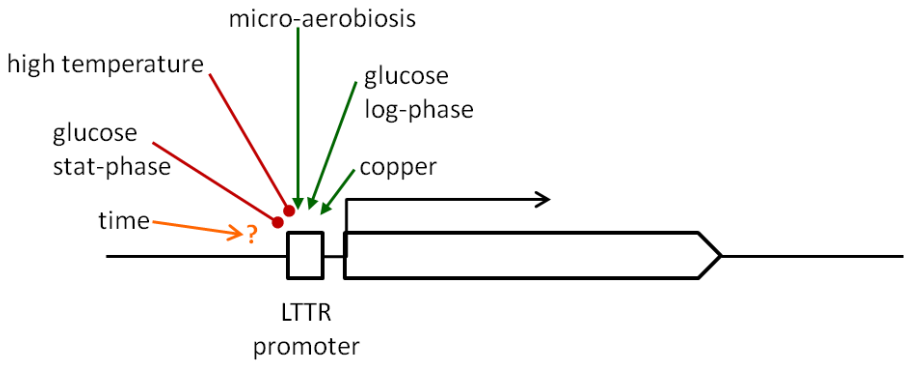


Figure 3.2: Factors regulating the *lttr852* promoter activity in an enhanced or repressed manner.
The regulation of *lttr*852 promoter showed a higher activity under micro-aerobic conditions, in presence of glucose in the log-phase and copper is essential for the activation. The promoter is inhibited via glucose in the stat-phase and under high temperature. *lttr*852 promoter activity seems to be regulated in a time dependent manner, which needs to be examined.

3.3 Potential target genes of *lttr*852 include *msr*A, *cop*A, *pyr*B

LTTRs belong to the largest known family of prokaryotic DNA-binding proteins with 800 members identified on the basis of their amino acid sequence (Schell, 1993). They can either act as activators, repressors or even both, of single and operonic genes. They are divergently transcribed most of the time, but the location can be elsewhere on the bacterial chromosome (Heroven and Dersch, 2006; Hernandez-Lucas et al., 2008). Numerous genes regulated by LTTRs have products that are involved in multiple bacterial functions such as metabolism, motility, cell division, quorum-sensing, virulence, oxidative stress response, to name a few (Kovacikova and Skorupski, 1999; Deghmane et al., 2000; Cao et al., 2001; Deghmane et al., 2002; Kim et al., 2004; Russell et al., 2004; Byrne et al., 2007; Lu et al., 2007; Sperandio et al., 2007). Only five LTTRs have so far been identified in *S. aureus* including CidR, HutR, GltC, CcpE and RpvA (Yang et al., 2005; Hartmann et al., 2013; Ibarra et al., 2013).

In this study, a sixth LTTR of *S. aureus* 6850 encoded by RSAU_000852 (NCTC8325 Locus ID SAOUHSC_0913) was identified with unknown function and whose expression was rarely reported. Mader et al. (2016) identified a network of differentially regulated genes in *S. aureus* strain HG001 under 106 different conditions. However, the NCTC82325 *lttr*852 homolog, SAOUHSC_0913 was never found to be expressed in any of the tested conditions (Mader et al., 2016). Indeed, only few studies demonstrate expression or differential expression of the novel uncharacterized LTTR. One of such studies by Kenny et al. (2009) found a 2.94-fold downregulation in the presence of 0.01 µM linoleic acid, while a 3.75-fold induction was reported in strain COL when challenged with 1 mM diamide (Posada et al., 2014). To identify potential targets of the transcriptional regulator, AHT-inducible expression of *lttr852* in *S. aureus* 6850 and subsequent RNA-seq was attempted (Tab. 2.1). Induction of *lttr852* correlated with the upregulation of genes responsible for the expression of the peptide methionine sulfoxide reductase *msr*A2 as well as components of a copper-translocation machinery, *cop*A and the copper chaperone *cop*Z (Sitthisak et al., 2007b), which are involved in copper efflux. These three genes were already mentioned and their correlation with *lttr*852 explained in chapters 3.2.4 and 3.2.5.

Other genes that were upregulated are involved in the branched chain amino acid biosynthesis *ilv*D (Dihydroxy-acid dehydratase), *ilv*B, *leu*A2 as well as the holin-like murein hydrolase regulator *lrg*A and the antiholin-like protein *lrg*B. Involved in the first step of the L-leucine biosynthesis pathway is isopropylmalate synthase 2 (*leu*A2) which has been identified as a target of LTTR852 in RNA-seq, beside other genes of the BCCA biosynthesis machinery (*leu*C, *leu*D, *leu*B and *ilv*E) and a BCAA transporter named *bca*P (Kaiser et al., 2018). This suggests, that BCAA biosynthesis is induced upon activation of the transcriptional regulator BrnQ2 (RSAU_000252). Another virulence-associated BCAA transporter upregulated in *S. aureus* during chronic infections, suggesting CodY de-repression and emphasizing the importance of branched chain amino-acid metabolism for *S. aureus* survival within the host (Szafranska et al., 2014; Groma et al., 2020). In the RNA-seq neither *brn*Qs nor *Cod*Y targets were significantly regulated, suggesting that a *cod*Y independent stimulation of BCAA biosynthesis may exist in *S. aureus*. CodY was found to be highly enriched in a chromatin precipitation analyses (Majerczyk et al., 2010) for the promoter region of *lttr852*, within the intergenic region of the upstream gene *clp*B and RSAU_000852. Therefore, the relative transcription level of *clp*B and *leu*A1 (2-isopropylmalate synthase-like protein) were checked, as they directly bound the structural gene for the LTTR RSAU_000852. There was no significance between wildtype and *lttr*852 mutant for *leu*A1 and *clp*B under induction, however a trend can be spotted (Fig. 2.6). Since the expression construct was encoded by a plasmid, *lttr852* hence may act in trans and lead to *leu*A1 transcription, which was not previously identified by RNA-seq. Similarly, RT-PCR found *leu*A2 to be downregulated. However, this downregulation was in contrast to the data obtained by RNA-seq. This discrepancy needs further investigation.

Continuing with genes that had a reduced expression after *lttr852* induction, including members of the urease operon *ure*B, *ure*C, *ure*E, and *ure*F. Urease is an enzyme that generates ammonia and CO_2 from urea and is essential for pH homeostasis and viability in urea-rich environments under weak acid stress (Zhou et al., 2019). As urease was shown to be required for bacterial fitness in murine kidneys with low pH, the infection niche of *S. aureus* wildtype in the kidney needs to be assessed (Zhou et

al., 2019). Changing the metabolic flux is based on a complex network, whereby the urease transcription is regulated by *ccp*A, *agr* and *cod*Y. The promoter activity of *lttr*852 was examined in the insertional mutant of CcpA. Compared to the activity in wildtype the ratio of GFP emission to growth rate had a wide range and therefore a *ccp*A dependent regulation for *lttr852* could not be described (Fig. 2.12 C), this leads to the suggestion that *ccp*A and *lttr852* are not standing in correlation for urease transcription.

Reduced expression after *lttr*852 induction was also detected for genes of pyrimidine biosynthesis *pyr*B, *pyr*C, and *car*B. The Biolog assay data revealed that L-glutamine, which is metabolized via carB was better metabolized by *lttr*852 mutant (Tab. 2.1) and also the pyrimidine pathway was listed as having a higher impact in the *lttr*852 mutant. The data point out that the *de novo* UMP biosynthesis pathway is repressed by the novel uncharacterized LTTR. The Tagatose-6-phosphate kinase *lac*C as well as the lactose-specific IIBC compound *lac*E, were also found to be repressed by the LTTR852 (Breidt et al., 1987). LacC is involved in the first step of the sub pathway that synthesizes D-glyceraldehyde 3-phosphate and glycerone phosphate from D-tagatose 6-phosphate and LacE is a major carbohydrate active transport system and part of the phosphoenolpyruvate-dependent sugar phosphotransferase system. It catalyzes the phosphorylation of incoming sugar substrates concomitantly with their translocation across the cell membrane. The enzyme II LacEF PTS system is involved in lactose transport. D-lactose as well as D-tagatose were strongly metabolized by the *lttr*852 mutant thus emphasizing the repression of lactose metabolism by LTTR852 (Tab. 2.1). It has been hypothesized that the increased PTS expression enables the pathogenesis of *S. aureus* in mastitis and may also facilitate *S. aureus* adaptation to secondary organs during bloodstream infection (Sharer et al., 2003; Groma et al., 2020).

3.4 Potential interaction of regulators with *lttr852* promoter not found

The regulation of virulence in *S. aureus* is based on different regulators which interact with each other in a complex manner. LTTRs are part of the LysR-transcriptional regulator family which are known to operate in feedback loops (Maddocks and Oyston, 2008), in order to estimate the function of *lttr*852 in the regulatory network, the activity of *lttr*852 promoter was analyzed in a variety of mutants. Mutation in *agr*A, *cym*R, *ccp*E and *cod*Y affected *lttr*852 promoter activity less drastic, while mutation in *ssr*A/B, *nre*C, *cop*A/B, *rpir*C and *ccp*A did not affect the promoter activity of *lttr*852.

AgrA belongs to the agr system which is one of the main global regulator systems and prominent to mediate quorum-sensing in *S. aureus* (Novick and Jiang, 2003). The *lttr*852 promoter activity in the *agr*A mutant showed a reduced activation when glucose was omitted (Fig. 2.22 A), leading to the suggestion that in presence of *agr*A the *lttr*852 promoter might be enhanced but only in the absence of glucose as promoter activity in both strains was similar in CDM. As the effect appears only under one condition it cannot be assumed that *agrA* has a direct impact on the promoter, but maybe indirect as other regulators can modulate the *agr* activity in a positive or negative way (Jenul and Horswill, 2019).

However, the promoter activity was also tested under the control of the two-component regulatory system of NreC, SrrA/B. The two component system of SrrA and B regulate genes required for nitric oxide, resistance and anaerobic metabolism in *S. aureus* (Kinkel et al., 2013). However, in the insertional mutant of *srr*A/B no difference in *lttr*852 promoter activity could be detected (Fig. 2.20 B, C), leading to the suggestion that under the tested conditions *lttr852* is not regulated by SrrA/B. NreC is involved in the control of dissimilatory reduction of nitrate and nitrite in response to oxygen (Fedtke et al., 2002). The promoter activity was upregulated in the insertional mutation of NE1669 compared to *S. aureus* 6850 wildtype, under both growth conditions (Fig. 2.20 D), therefore assuming that *lttr*852 might be repressed in the presence of *Nre*C. However, the metabolization of nitrate/nitrite analyzed via Biolog for *S. aureus* wildtype and Δ852, no significant difference between the strains could be detected (Sup. Tab. 7.4), therefore the correlation between NreC and *lttr*852 should be

further examined. NreC is also known to repress genes being important for biofilm growth and/or maturation (Beenken et al., 2004; Resch et al., 2005), although *lttr852* shows to be repressed by NreC it was already verified that *lttr*852 is not involved in biofilm formation.

CymR is the main regulator of the cysteine metabolism and it plays a role in biofilm formation (Soutourina et al., 2009). The insertional mutation of *cym*R showed a delay in growth in both media conditions, however the *lttr*852 promoter activity was higher in CDM, whereas it was lower in CDM omitting glucose in the absence of the gene *cym*R (Fig. 2.21 B), therefore leading to the suggestion that CymR represses *lttr*852 promoter activity in the presence of glucose, whereas in the absence of glucose CymR activates it. Cystein is a core player in the celullar metabolism as it is involved in glutathione synthesis, carbon and energy metabolism and contributes for sulfur and energy production (Serpa, 2020). Although the metabolism of many cysteine-based substances was lower in *S. aureus* wildtype compared to Δ852, no definite statement on the interaction of CymR and *lttr*852 promoter activity can be done at that time.

The promoter activity of the novel uncharacterized LTTR in the insertional disruption of *ccp*E showed an increased activation after 10 h in CDM omitting glucose (Fig. 2.21 D), therefore suggesting that *lttr852* is repressed by CcpE after 10 h of growth. CcpE is involved in the regulation of TCA-cycle activity especially having an impact on CitB an aconitase, which is responsible for the interconversion of citrate to isocitrate (Ding et al., 2014). As TCA-cycle is mainly active when the concentration of essential nutrients decreases, therefore also *ccp*E being more active at the stationary phase, it could explain why the repression of the promoter activity of *lttr*852 directly or indirectly by CcpE appears after 10 h.

CodY is a major regulatory protein with a linkage between metabolism and virulence regulation and serves mainly as a repressor of virulence genes (Pohl et al., 2009; Majerczyk et al., 2010). In the insertional mutant of *cod*Y (NE1555), the GFP emission for the *lttr*852 promoter was repeatedly observed higher compared to wildtype if grown in CDM, although already the growth rate reached a higher OD_{600} level (Fig. 2.21 E), therefore it seems that CodY might repress the activity of the novel

uncharacterized LTTR promoter. However, the transcription of *lttr852* was not de-repressed in a *cod*Y mutant (Majerczyk et al., 2010) and additional *cod*Y was not seen to be regulated by LTTR852 in this thesis (Sup. Tab. 7.1). Therefore, the precise correlation between *lttr*852 and *cod*Y needs to be explored further.

In summary, there are hints that the novel uncharacterized LTTR is regulated by important global regulators in a network. But as two different *S. aureus* backgrounds were compared to each other, there might be a background specific effect as seen for *cso*R and *cso*Z, descibed above (3.2.5). Therefore, the experiment should be repeated for the hints in *S. aureus* 6850 background. Moreover, the regulation of *lttr*852 by other global regulators remain elusive as only a few regulators were checked.

3.5 Overcoming the lack of co-inducers

It seems that in this study the concentration of the hitherto unknown co-inducer of LTTR852 was limiting under the tested conditions, since LTTRs might require a co-inducer for DNA-binding or multimerization. LTTRs comprise approximately 330 amino acids consisting of a helix–turn–helix motif at the N-terminus. The C-terminus harbors the LysR-substrate binding region, which lies between RD1 and RD2 (Schell, 1993). To stimulate various LTTRs to activate transcription, there are different co-inducers with structural diversity necessary (e.g. amino acid derivates, aromatics, ions, various aliphatics) (Schell, 1993). There are some well-studied LTTRs, including global activators as OxyR which is responsible for the oxidative stress response of H_2O_2 in *Escherichia coli*, *Salmonella enterica* servoar Typhimurium (Farr and Kogoma, 1991), or ToxR which locally activates the quorum sensing via toxoflavin in *Burkholderia glumae* (Kim et al., 2004). It is also possible that co-inducer can operate as an activator as well as a repressor such as α-Methylene-γ-butyrolactone, needed during pili and capsule synthesis in *Neisseria meningitidis* (Reviewed in Maddocks and Oyston, 2008). However, till now not all co-inducers are known, for example for YofA, which is involved in the cell division of *Bacillus subtilis* (Lu et al.,

2007), or in *Yersinia pestis* the unknown metabolite is acting as a co-repressor (Heroven and Dersch, 2006) to name some.

3.5.1 Rewiring *lttr*852 regulons by domain swapping

The transcription factor domain-swaps can be used to verify potential LTTR852 target genes or co-inducer. LTTRs are comprised of two domains, a HTH-domain that binds to the promoter sequence and a co-inducer binding domain. The concept was to rewire both domains of the novel uncharacterized LTTR with the more thoroughly characterized LTTR *cid*R, of which the target gene as well as the co-inducer is known. The successful generation of the domain swap was confirmed by the detection of the relative expression of the specific domain-swap (Fig. 2.22 B) and additionally by the detection of *His*-tags cloned behind the domain-swap, via Western blot (Fig. 2.22 D). Acetic acid is the co-inducer of the LTTR system of *cid*A/*cid*R. CidR binds at the area in front of the *cidA* promoter and it gets only activated if acetic acid binds to CidR and due to that, the DNA strand relaxes and CidR gets in contact with the *cid*A promoter and therefore leading to its transcription. The relative expression of *cid*A was supposed to be upregulated in the presents of acetic acid, however the induction of *S. aureus lttr*852 mutant p2085_cidR with 26 mM acetic acid did not lead to an upregulation of *cid*A. An explanation for that could be that cidR in *S. aureus* is responsible for the acetoin metabolism in cell death and lysis (Yang et al., 2006), suggesting that the chosen time point was too late and therefore the induction in the log-phase might be a better option. Although the concentration of acetic acid was selected according to literature (Yang et al., 2005), 26 mM acetic acid might be too low. Therefore, further studies should be done to find the right conditions under which the control for the domain-swap works out, to subsequently continue the identification of potential target genes and co-inducer of LTTR852.

3.5.2 Biolog screen give rise to a co-inducer metabolite

Further experiments conducted to identify the co-inducer and possible target genes of LTTR852, another way was to examine the metabolic differences between *S. aureus* wildtype and *lttr*852 mutant via a Biolog screen. With this method a lot of chemical substances were tested, and several of them had either resistant or sensitive for their metabolization in *S. aureus* wildtype compared to Δ852 (Sup. Tab. 7.2, 7.3). If the substances were mapped according to their sensitivity of metabolization in *S. aureus* wildtype and *lttr*852 mutant and additionally to their occurrence of their related pathways via KEGG pathway database (not shown), no unambiguous statement on the impact on a certain pathway, dependent on the novel uncharacterized LTTR could be done (Tab. 2.2). For both strains, most of the metabolized substances are found to be part of the metabolic pathways or the biosynthesis of metabolites. However, alanine, aspartate and glutamate were better metabolized in the wildtype, whereby the metabolism of arginine and proline were higher in the *lttr*852 mutant (Tab. 2.3). The metabolism of all these substances are immediately or indirect interconnected with the tricarboxylic acid cycle, which acts as a signal transduction pathway to translate external environmental signals into intracellular metabolic signals that adjust the activity of transcriptional regulators (Sadykov et al., 2008). This suggests again that environmental signals are having an impact on the control of *lttr*852, but a statement on how exactly cannot be done yet. Furthermore, the screen was just done once, therefore a second and third repetition would be necessary to conceivably get a clear impression of the impact of LTTR852 on certain pathways. Additionally, the screen could be repeated with the reporter plasmid p2085_Pr852_GFP, so that more components could be identified under which the promoter of *lttr*852 is enhanced or repressed. Furthermore, with the Biolog assay the domain-swap II could be used to screen for possible co-inducer.

3.6 Perspectives

The scientific question of this thesis was based on a Tn-seq screen of *S. aureus* mutant pools recovered from liver and kidneys of intravenously infected animals, whereby the LysR-type transcriptional regulator LTTR of RSAU_000852 was found to be important for the colonization of secondary organs like kidneys and bones (Das et al., 2016). Since the infected tissues were harvested after 24 h of infection, which is quite an early time point during infection, a potential role of *lttr*852 for bacterial survival in the primary infection site at later time points could not be assessed. It is known that *S. aureus* can survive up to 28 days in a host, by changing its phenotype and virulence factor expression in order to escape host immune response and establish a chronic infection (Tuchscherr et al., 2011). To better understand the *lttr*852 function within the host, it would be of benefit if intravenous infection and organ isolation could be repeated at later time points to determine the importance of the *lttr*852 in the colonization of secondary organs, as well as in primary infection niches at later time points. In order to generate and/or confirm available information about the live cycle of *S. aureus* dependent on *lttr*852, a stable plasmid linked with GFP has to be generated and the mice has to be processed in order to clear their body, so that the infection can be tracked during the systemic infection. It is conceivable to clear various organs or tissues by different tissue clearing techniques which enables visualization by turning the tissue transparent (Chung et al., 2013; Du et al., 2018). Nowadays, with the polyethylene glycol (PEG)-associated solvent system (PEGASOS) it is even possible to turn the whole adult mouse body transparent (Jing et al., 2018). By using CLARITY-based tissue clearing and imaging in combination with infection, it is possible to detect *lttr*852 dependent differences within a mouse, but this will require a plasmid that remains stable after the clarification process. Animal protection laws may make this implementation a bit more complicated. Another possibility to check the impact of *lttr*852 within the liver would be to infect Kupffer cells of the liver and to analyze the *lttr*852 dependent behavior of *S. aureus* during infection and escape.

In this thesis, RNA-Seq data of potential LTTR852 regulated genes under the induction of AHT showed only moderate fold-change levels in the expression analysis, although

they were significant (Sup. Tab. 7.1). Although conditions were found under which the *lttr*852 promoter was more or less active, under oxidative stress however, the expression of potential target genes could not be confirmed (Fig. 2.19). This could be attributed to the requirement of a yet unidentified co-inducer molecule of LysR-type transcriptional regulator RSAU_000852 which may be present in the mice body. Even though RNA-Seq study identified transcribed LTTR852 target genes after AHT induction of the *lttr*852 in vitro, the conditions prevailing within the mouse under which the lttr852 is naturally induced cannot be predicted with the identification of the co-inducer.

4. Material

4.1 Bacterial strains

Table 4.1: Bacterial strains used in this study

Strain	Description	Source
Escherichia coli		
DH5α	*fhu*A2 *lac(del)U*169 *pho*A *glnV*44ϕ80´*lacZ*(del)M15 *gyr*A96 *rec*A1 *rel*A1 *end*A1 *thi*-1 *hsd*R17; used for cloning	(Hanahan, 1983) BRL Life Technology
Top10F´	Non-pathogenic laboratory strain of *E. coli* NC_000913, F'[*lac*I^{q}Tn10(*tet*^{R})] *mcr*Aδ(*mrr-hsd*RMS-*mcr*BC) *ϕ*80*lacZδ*M15δ *lac*X74 *deo*R *nup*G *rec*A1 *ara*D139 *δ*(*ara-leu*)7697 *gal*U *gal*K *rps*L (Str^{R}) *end*A1 λ^{-}; used for cloning	(Gravet et al., 1998) (Durfee et al., 2008) Invitrogen
Staphylococcus aureus		
RN 4220	Non-hemolytic derivative of NCTC 8325-4, *sau*1^{-}, *hsdR*$^{-}$, developed for cloning and laboratory studies. Genome: NCTC 8325, NCBI Accession: NC_007795	(Kreiswirth et al., 1983)
RN 4220 Pr852_GFP	RN 4220 expressing GFP under control of the promoter of 852; used for analysis of 852 promoter activity	This study
6850	Methicillin-sensitive *S. aureus* (MSSA), isolated from a patient with a skin abscess, progressed to bacteraemia, osteomyelitis, septic arthritis and multiple systemic abscesses; *β*-lactam resistant	(Vann and Proctor, 1987)
6850 SarAP1-mRFP	6850 expressing *monomeric red fluorescent protein* (mRFP) under control of *SarAP*1 promoter	K. Paprotka

6850 p2085_852-AHT	6850 expressing 852 under control of an AHT inducible promoter	This study
6850 p2085_852-His	6850 expressing 852 under control of an AHT inducible promoter; with hexahistidin (His)-tag	This study
6850 p2085_852cidR	6850 expressing transcription factor domain swap *852cidR* under control of an AHT inducible promoter	This study
6850 p2085_852cidR-His	6850 expressing transcription factor domain swap *852cidR* under control of an AHT inducible promoter; with His-tag	This study
6850 p2085_cidR852	6850 expressing transcription factor domain swap *cidR852* under control of an AHT inducible promoter	This study
6850 p2085_cidR852-His	6850 expressing transcription factor domain swap *cidR852* under control of an AHT inducible promoter; with His-tag	This study
6850 p2085_cidR	6850 expressing *cid*R under control of an AHT inducible promoter	This study
6850 p2085_cidR-His	6850 expressing *cid*R under control of an AHT inducible promoter; with His-tag	This study
6850 Pr852_GFP	6850 expressing GFP under control of the promoter of 852; used for analysis of 852 promoter activity	This study
6850 Δ852	6850 with deletion of the ORF RSAU_000821	Raupach not published
6850 Δ852 SarAP1-mRFP	6850 Δ852 expressing mRFP under control of *SarAP*1 promoter	This study
6850 Δ852 p2085_852-AHT	6850 Δ852 expressing 852 under control of an AHT inducible promoter; with His-tag	This study

6850 Δ852 p2085_852-His	6850 Δ852 expressing 852 under control of an AHT inducible promoter; with His-tag	This study
6850 Δ852 Pr852_GFP	6850 Δ852 expressing GFP under control of the promoter of 852; used for analysis of 852 promoter activity	This study
6850 pl852	Δ821 complemented with pL821, cam^R	Raupach not published
USA 300 JE2	USA300 LAC MRSA, plasmid-cured; erm^S	(Fey et al., 2013)
USA 300 JE2 Pr852_GFP	USA 300 JE2 expressing GFP under control of the promoter of 852; used for analysis of 852 promoter activity	This study
USA 300 JE2 Tn852	JE2, (SAUSA300_000852)	(Fey et al., 2013)
USA 300 JE2 Tn852 Pr852_GFP	USA 300 JE2 Tn852 expressing GFP under control of the promoter of 852; used for analysis of 852 promoter activity	This study
Cowan	No hla and hlb production, unable to escape from phagosomes	ATCC 12598
Cowan Pr852_GFP	Cowan expressing GFP under control of the promoter of 852; used for analysis of 852 promoter activity	This study
NE1532	JE2, *agrA*::Bursa (SAUSA300_1992)	(Fey et al., 2013)
NE1532 Pr852_GFP	NE1532 expressing GFP under control of the promoter of 852; used for analysis of 852 promoter activity	This study
NE1309	JE2, *srr*A::Bursa (SAUSA300_1442)	(Fey et al., 2013)
NE1309 Pr852_GFP	NE1309 expressing GFP under control of the promoter of 852; used for analysis of 852 promoter activity	This study
NE588	JE2, *srrB*::Bursa (SAUSA300_1441)	(Fey et al., 2013)
NE588 Pr852_GFP	NE588 expressing GFP under	This study

	control of the promoter of 852; used for analysis of 852 promoter activity	
NE1669	JE2, *nreC*::Bursa (SAUSA300_2337)	(Fey et al., 2013)
NE1669 Pr852_GFP	NE1669 expressing GFP under control of the promoter of 852; used for analysis of 852 promoter activity	This study
NE590	JE2, *copA*::Bursa (SAUSA300_0078)	(Fey et al., 2013)
NE590 Pr852_GFP	NE590 expressing GFP under control of the promoter of 852; used for analysis of 852 promoter activity	This study
NE1660	JE2, *copZ*::Bursa (SAUSA300_2495)	(Fey et al., 2013)
NE1660 Pr852_GFP	NE1660 expressing GFP under control of the promoter of 852; used for analysis of 852 promoter activity	This study
NE1142	JE2, *rpiRc*::Bursa (SAUSA300_2264)	(Fey et al., 2013)
NE1142 Pr852_GFP	NE1142 expressing GFP under control of the promoter of 852; used for analysis of 852 promoter activity	This study
NE1293	JE2, *cymR*::Bursa (SAUSA300_1583)	(Fey et al., 2013)
NE1293 Pr852_GFP	NE1293 expressing GFP under control of the promoter of 852; used for analysis of 852 promoter activity	This study
NE1670	JE2, *ccpA*::Bursa (SAUSA300_1682)	(Fey et al., 2013)
NE1670 Pr852_GFP	NE1670 expressing GFP under control of the promoter of 852; used for analysis of 852 promoter activity	This study
NE1560	JE2, *ccpE*::Bursa (SAUSA300_0658)	(Fey et al., 2013)
NE1560 Pr852_GFP	NE1560 expressing GFP under control of the promoter of 852; used for analysis of 852 promoter activity	This study

NE1555	JE2, *codY*::Bursa (SAUSA300_1148)	(Fey et al., 2013)
NE1555 Pr852_GFP	NE1555 expressing GFP under control of the promoter of 852; used for analysis of 852 promoter activity	This study
NE1750	JE2, *csoR*::Bursa (SAUSA300_2043)	(Fey et al., 2013)
NE1750 Pr852_GFP	NE1750 expressing GFP under control of the promoter of 852; used for analysis of 852 promoter activity	This study
NE1322	JE2, *csoZ*::Bursa (SAUSA300_2042)	(Fey et al., 2013)
NE1322 Pr852_GFP	NE1322 expressing GFP under control of the promoter of 852; used for analysis of 852 promoter activity	This study
Staphylococcus epidermidis		
ATCC12228	Non-biofilm forming, non-infection associated strain; used as negative control	(Zhang et al., 2003)
RP62A	Biofilm-producing methicillin-resistant strain; used as positive control	(Gill et al., 2005)

4.2 Plasmids

Table 4.2: Plasmids used in this study

Name	Description / Purpose	Source
p2085	Derivative of pALC2084 (Bateman et al., 2001), shuttle vector with pC194 and pUC19 backbones, containing *tetR* and $P_{xyl/tet}$ promoter driving GFPuvr expression, Cmp	(Giese et al., 2009)
p2085_pl852	Expression plasmid for RSAU_000852 under control of its native promoter	Raupach not published
p2085_852-AHT	Complementation of LTTR852 under control of AHT-inducible promoter	This study
pAHT-SSR42	Complementation of SSR42 under control of AHT-inducible promotor	Horn

p2085_852-His	Complementation of LTTR852 under control of AHT-inducible promoter, with His-tag	This study
SarAP1-mRFP	mRFP under control of *SarAP*1 promoter	Paprotka
p2085_Pr852_GFP	GFP transcription under control of *lttr852* promoter; used for promoter activity studies	This study
p2085_Prhla_GFP	GFP transcription under control of *hla* promoter; used as template plasmid	Horn
p2085_852cidR	Transcription factor domain swapping; substrate binding domain of *lttr852*; Helix-turn-helix binding side of *cidR*; used for analysis of potential co-inducer; AHT-inducible	This study
p2085_852cidR-His	Transcription factor domain swapping; AHT-inducible; with His-tag	This study
p2085_cidR852	Transcription factor domain swapping; substrate binding domain of *cidR*; Helix-turn-helix binding side of *LTTR852*; used for analysis of potential target genes; AHT-inducible	This study
p2085_cidR852-His	Transcription factor domain swapping; AHT-inducible; with His-tag	This study
p2085_cidR	Control for transcription factor domain swapping; substrate binding domain and Helix-turn-helix binding side of *cidR*; AHT-inducible	This study
p2085_cidR-His	Transcription factor domain swapping; AHT-inducible; with His-tag	This study
pLVTHM	2nd generation lentiviral expressing shRNA from H1 promoter and GFP, pLVTHM was a gift from Didier Trono (Addgene plasmid #12247)	(Wiznerowicz and Trono, 2003)
pVSVG	2nd generation Envelope plasmid, pMD2.G was a gift from Didier Trono (Addgene plasmid #12259)	(Wiznerowicz and Trono, 2003)
psPAX	2nd generation lentiviral packaging vector, psPAX2 was a gift from Didier Trono (Addgene plasmid #12260)	(Wiznerowicz and Trono, 2003)

pLVTHM YFP-CWT	pLVTHM with YFP-CWT for detection of phagosomal escape of *S. aureus* in host cells	(Grosz et al., 2014)

4.3 Cell lines

Table 4.3: Cell lines used in this study

Name	Description	Source
HeLa 229	Adherent tumor epithelial cells derived from human cervix adeno carcinoma containing human papilloma virus (HPV-18)	ATCC
HeLa 229 YFP-CWT	HeLa 229 expressing YFP-CWT for detection of phagosomal escape of *S. aureus* in host cells	(Grosz et al., 2014)
HEK 293T	Adherent epithelial cells derived from human embryonic kidney	ATCC
HEK 293T YFP-CWT	HEK 293T expressing YFP-CWT for detection of phagosomal escape of *S. aureus* in host cells	This study

4.4 Oligonucleotides

Table 4.4: Oligonucleotides used for cloning

Name	Sequence 5´-3´	Purpose
852- PmeI-fw	GATGTTTAAACATGAATTTGGATTGGTACTATAC	Cloning of p2085_852-AHT
852- PstI-rev	GAATTCTGCAGTTATTATAATTGTTCATTGGC	Cloning of p2085_852-AHT
pGFP-Inf-PromF	GAATTCTTAGGAGGATGATTATTTATGAGTAAAGGAGAAGAAC	Cloning of p2085_Pr852GFP
pGFP vec R	GCATGCAAGCTTTTAAAAAGCAAATATGAGCCAAATAAA	Cloning of p2085_Pr852GFP
Pr852-GFP_fw	TAAAAGCTTGCATGCGATATTTTGAAATAATTTC	Cloning of p2085_Pr852GFP

Pr852-GFP_rev	TCCTCCTAAGAATTCTTCAAAAAAGTTAATAATTC	Cloning of p2085_Pr852GFP
D-Swap_p2085_852-HTH_fw	CGTCGCCTAGGGAGGGTTTAAACATGAATTTGGATTGGTACTATAC	Fragment_ HTH-852 domain swap I
D-Swap_852-HTH_cidR-SBD_rev	GCCCATGTTTATATGCCCTTGAAACATCGTTTTTTTAAG	Fragment_ HTH-852 domain swap I
D-Swap_852-HTH_cidR-SBD_fw	CTTAAAAAAACGATGTTTCAAGGGCATATAAACATGGG	Fragment_ SBD-cidR domain swap I
D-Swap_cidR-SBD_p2085_rev	CGACGGCCAGTGAATTCTGCAGTTAGCCTAAACGATCTTTC	Fragment_ SBD-cidR domain swap I
D-Swap_p2085_cidR-HTH_fw	CGTCGCCTAGGGAGGGTTTAAACATGGATATCAAACATATGAAATATTT	Fragment_ HTH-cidR domain swap II
D_Swap_cidR-HTH_852-SBD_rev	CGACAACATTTAATTTCGATGTTTCCAGTCCATTCAAG	Fragment_ HTH-cidR domain swap II
D_Swap_cidR-HTH_852-SBD_fw	CTTGAATGGACTGGAAACATCGAAATTAAATGTTGTCGTG	Fragment_ SBD-852 domain swap II
D-Swap_852-SBD_p2085_rev	CGACGGCCAGTGAATTCTGCAGTTATAATTGTTCATTGGCAATATAC	Fragment_ SBD-852 domain swap II
852_His_rev	GACGGCCAGTGAATTCTGCAGTTAATGATGGTGGTGATGATGTAATTGTTCATTGGCAATATAC	Cloning His-Tag
cidR_His_rev	GACGGCCAGTGAATTCTGCAGTTAATGATGGTGGTGATGATGGCCTAAACGATCTTTCAAAAATTC	Cloning His-Tag
MF39 Seq2085s	CTAATGCGCTGTTAATCACTTTAC	Cloning His-Tag

Table 4.5: Oligonucleotides used for quantitative real time PCR

Name	Sequence 5´-3´	Target
RT-852-F	TGAACAATATGAAAGTGGCA	RSAU_000852
RT-852-R	GGATGCTCGTTAAAGAATG	RSAU_000852
RT-852-R_JE2	GGATGCTCGTTAAAGAAAG	USA300 JE2_0878
RT-pyrAB-F	GAGCAACCTGACGCTTTAC	carB
RT-pyrAB-R	GTTCTAAACATTTCACGGTCTTC	carB
RT-clpB-F	CAACAAGCAAACCAATTG	clpB
RT-clpB-R	GATGTCACGTGATTTCCC	clpB
RT-copA-F	CGCTGTAGAAACTGTCGAATTAG	copA
RT-copA-R	GGATAATAGTCAACTTTAGCTTGC	copA
RT-dps-F	AATCAACAAGTAGCAAACTGG	dps
RT-dps-R	TTAATGTACCTACAGGGTTTCC	dps
RT-gyrB-F	CGACTTTGATCTAGCGAAAG	gyrB
RT-gyrB-R	ATAGCCTGCTTCAATTAACG	gyrB
RT-lacC-F	GATCATGCCGGCATCAAG	lacC
RT-lacC-R	CCTGAAATAGCAACTGCTTC	lacC
RT-lacC-F_JE2	GATCATGCCGACATCAAG	USA300 JE2_*lac*C
RT-leuA1-F	CAAATTGAAAATTCATAATTTAGTG	leuA1
RT-leuA1-R	GAAGTCCTTGTTGCATCTTC	leuA1
RT-leuA2-F	GGGATGGTATGCAACAAAGTAATGT	leuA2
RT-leuA2-R_JE2	GGACTTAAAATGTCTCTGAATTGATG	USA300 JE2_*leu*A2
RT-lrgA-F	CTGGTGCTGTTAAGTTAGGC	lrgA
RT-lrgA-R	GTATTGTTGAGACGATTATTAGTCC	lrgA
RT-msrA-F	GGACCACGGTCTTGATATTG	msrA
RT-msrA-R	GGCGGACATATTGAAAATCC	msrA
RT-pyrB-F	CGAATATTAACATCCCAATTGCG	pyrB
RT-pyrB-R	GCACCTAATGCTTTTAAACTATGG	pyrB
RT-RNAIII-F	ACATAGCACTGAGTCCAAGG	RNAIII
RT-RNAIII-R	TCGACACAGTGAACAAATTC	RNAIII
RT-sodA-F	ACGACAAACATCACAATACG	sodA
RT-sodA-R	ATTTCCCAGAATAATGAATGG	sodA

RT-ureC-F	GCAGACCTTGTTATTTCTAATGC	ureC
RT-ureC-R	CAATACCACCAGCAGTGA	ureC
RT-ureC-F_JE2	GCAGACCTTGTCATTTCTAATGC	USA300 JE2_*ure*C

4.5 Enzymes

Table 4.6: Enzymes used in this study

Name	Manufacturer
EcoRI (10 U/µl)	Thermo Scientific
FastAP (Thermosensitive alkaline phosphatase)	Thermo Scientific
MssI (PmeI) (5 U/µl)	Thermo Scientific
Phusion High-Fidelity DNA polymerase	Thermo Scientific
PstI (10 U/µl)	Thermo Scientific
Restriction enzymes	Thermo Scientific
RNase A	Thermo Scientific
Taq Polymerase	Thermo Scientific
TURBO^{TM} DNase	Genaxxon Bioscience
T4 DNA ligase	Thermo Scientific

4.6 Antibodies

Table 4.7: Antibodies used in this study

Name	Origin	Dilution	Manufacturer
Anti-6-His antibody	Rabbit	1:1000	Sigma-Aldrich
Rabbit IgG	Goat	1:2000	Santa Cruz

4.7 Antibiotics

Table 4.8: Antibiotics used in this study

Name	Concentration	Manufacturer
Ampicillin	100 µg/ml in H_2O	Carl-Roth
Chloramphenicol	10 µg/ml in EtOH	Sigma-Aldrich
Erythromycin	5 µg/ml in EtOH	Carl-Roth
Lysostaphin	20 µg/ml in H_2O	Sigma-Aldrich
Penicillin- Streptomycin	1000 U/ml	GIBCO

4.8 Size standards

Table 4.9: Size standards used in this study

Size standard / loading dye	Manufacturer
Gene Ruler 50 bp DNA ladder	Thermo Scientific
Gene Ruler 1kb DNA ladder	Thermo Scientific
Page Ruler Prestained Protein Ladder	Thermo Scientific
Loading dye 6x	Thermo Scientific

4.9 Kits

Table 4.10: Kits used in this study

Name	Purpose	Manufacturer
In-Fusion HD Cloning Kit	Cloning	Takara
NucleoSpin Gel and PCR Clean up	PCR purification	Machery-Nagel
NucleoSpin Plasmid QuickPure	Plasmid isolation *E. coli*	Machery-Nagel
QIAmp DNA Mini Kit	Genomic DNA isolation	Qiagen
QIAprep Spin Miniprep Kit	Plasmid isolation *S. aureus*	Qiagen
RevertAid First Strand cDNA Synthesis Kit	Reverse transcriptase	Thermo Scientific
TOPO® TA Cloning® kit	TOPO TA cloning	Invitrogen
TURBO DNA free Kit	Digestion of DNA	Thermo Scientific

4.10 Media

Table 4.11: Cell culture media used in this study

Media and solutions	Ingredients	Quantity
Dulbecco's Modified Eagle's Medium (DMEM) full medium	DMEM D6429 Fetal calf serum (FCS) Sodium Pyruvate Penicillin-Streptomycin	500 ml 10% 1 mM 1000 U/ml
DMEM infection medium	DMEM D6429 FCS Sodium Pyruvate	500 ml 10% 1 mM
Roswell Park Memorial Institute (RPMI) full medium 1640	RPMI 1640 Glutamax™ FCS Sodium Pyruvate Penicillin-Streptomycin	500 ml 10% 1 mM 1000 U/ml
RPMI infection medium	RPMI 1640 Glutamax™ FCS Sodium Pyruvate	500 ml 10% 1 mM
Cryopreservative medium	FCS Dimethyl sulfoxide (DMSO)	90% 10%

Table 4.12: Bacterial culture liquid media used in this study

Bacterial culture broth	Ingredients	Quantity
Luria-Bertani (LB) broth	Tryptone Sodium chloride Yeast extract	10 g/l 10 g/l 5 g/l
Tryptic Soy Broth (TSB)	Tryptic Soy Broth	30 g/l
Tryptic Soy Broth without glucose	Tryptic Soy Broth without glucose	27.5 g/l
Tryptic Soy Broth with 5% sheep blood	Tryptic Soy Broth Columbia blood agar base	30 g/l 5%
RPMI	RPMI 1640 Glutamax™	Ready for use
BHI (Brain Heart Infusion)	BHI	37 g/l
CDM (Chemical Defined Medium)	Citrate Glucose Amino acids Vitamins (10.000 stock) Riboflavin (1.000 stock) Trace Metal Mix A5 with Co (1.000 stock) Iron(III)chloride (10.000 stock) Nickel(II)chloride (1.000 stock) Basic medium (2x stock) d H_2O	0.1245 mM 0.75 mM 1 mM (Tyrosin 0.1 mM) 3x 3x 1x 1.5x 1.5x 1x Rest volume

Table 4.13: Ingredient mix of CDM used in this study

CDM mix	Ingredients	Quantity
Basic medium (2x stock)	Sodium phosphate Potassium dihydrogen phosphate Magnesium sulfate Ammonium chloride Sodium chloride	4.44975 g/l 2.7218 g/l 0.397221 g/l 0.989565 g/l 0.99348 g/l
Vitamins (10,000x stock)	4-Aminobenzoic acid Thiamine hydrochloride Ca-D-Pathothenic acid Cyanocobalamin D-Biotin Nicotinamid Pyridoxine hydrochloride	2.9 mM 2.9 mM 2.1 mM 0.36 mM 0.4 mM 8.1 mM 6.2 mM
Trace metal mix A5 with Co (1,000x stock)	Boric acid Mangan(II)chloride Zinc sulfate Sodium molybdate Copper(II)sulfate Cobalt(II)nitrate	2860 mg/l 1810 mg/l 222 mg/l 390 mg/l 79 mg/l 49 mg/l

Table 4.14: Bacterial culture agar media used in this study

Bacterial culture agar	Components	Quantity
Luria-Bertani agar	Tryptone Sodium chloride Yeast extract Agar	10 g/l 10 g/l 5 g/l 15 g/l
Luria-Bertani softagar	Tryptone Sodium chloride Yeast extract Agar	10 g/l 10 g/l 5 g/l 6 g/l
Tryptic Soy Agar (TSA)	Tryptic Soy Broth Agar	30 g/l 15 g/l
Phage top agar	Tryptone Sodium chloride Yeast extract Agar Sodium citrate	10 g/l 10 g/l 5 g/l 6 g/l 20 mM

4.11 Buffers

Table 4.15: Buffer for working with *S. aureus* in this study

Buffer	Components	Quantity
Annealing buffer	Sodium chloride Tris-HCl pH 7.9 Magnesium chloride Dithiothreitol (DTT)	100 mM 50 mM 10 mM 1 mM
Buffer A	Glucose Tris pH 7.6 Ethylendiamintetraacetat (EDTA)	10% 12.5mM 10mM
Electroporation buffer	Saccharose Glycerol	0.5 M 10% (v/v)
Genomic DNA lysis buffer	Lysostaphin Tris-HCl pH 8.0 EDTA Triton X-100 RNase A	200 µg/ml 20 mM 2 mM 1.2% (v/v) 160 µg/ml
Phage buffer	LB media Calcium chloride	1 ml 0.5 mM
Protein lysis buffer	Tris-HCl pH 8.0 Potassium chloride Magnesium chloride DTT	20 mM 150 mM 1 mM 1 mM

Table 4.16: Buffer for agarose gel electrophoresis, SDS-PAGE, staining, Western blot

Buffer	Components	Quantity
1x TRIS-Acetate-EDTA buffer (TAE buffer)	Tris-HCL (pH 8.5) EDTA Acetic acid	40 mM 1 mM 20 mM
1x Tris buffered saline (TBS buffer)	Tris-HCL (pH 7.6) Sodium chloride	20 mM 137 mM
1x TBS-T	Tris-HCL (pH 7.6) Sodium chloride Tween-20 (w/v)	20 mM 137 mM 0.05%

2.5 x Laemmli buffer	Tris-HCl pH8 Glycine (w/v) Sodium dodecyl sulfate (SDS) (w/v) 2-mercaptoethanol (w/v) Bromphenol blue (w/v)	62.5 mM 10% 2% 5% 0.05%
SDS-PAGE running buffer	Tris Glycine SDS (w/v)	25 mM 0.25 M 0.1%
Polyacrylamide (PAA) 7.5% gel	d H_2O Acrylamide Tris HCl pH 8.8 Ammonium persulphate (APS) Tetramethylethylenediamine (TEMED)	10 ml 5 ml 5 ml 200 µl 20 µl
PAA 3.5% stacking gel	d H_2O Acrylamide Tris HCl pH 6.8 Ammonium persulfate (APS) Tetramethylethylenediamine (TEMED)	6.24 ml 1.25 ml 2.5 ml 200 µl 20 µl
Colloidal Coomassie fixation solution	Acetic acid Methanol	7% 40%
Colloidal Coomassie staining solution A	Phosphoric acid Ammonium persulfate (w/v)	2.375% 10%
Colloidal Coomassie staining solution B	Coomassie G-250 (w/v)	5%
Colloidal Coomassie neutralization solution	Tris pH 6.5 (w/v)	1.2%
Colloidal Coomassie washing solution	Methanol	25%
Semi-Dry blotting buffer	Tris Glycine SDS Methanol (v/v)	44 mM 40 mM 1.3 mM 20%
Western blot blocking buffer	1x TBS-T Nonfat dried milk powder	1x TBS-T 5%
Enhanced Chemoluminescence (ECL) 1	d H_2O Luminol p-coumaric acid Tris HCl pH 8.5	up to 25 ml 250 µl 110 µl 2.5 ml
ECL 2	d H_2O Tris-HCl pH 8.5 Hydrogen peroxide (H_2O_2) (30%)	up to 25 ml 16 µl 2.5 ml

1% agarose RNA gel	Agarose Diethylpyrocarbonat (DEPC)-treated H_2O 10x 3-(N-morpholino)propanesulfonic acid (MOPS) Formaldehyde 37%	0.8 g 68 ml 8 ml 4 ml

4.12 Chemicals

Table 4.17: Chemicals and products used in this study

Chemicals and products	Manufacturer
Acetic acid	Carl-Roth
Acrylamide Rotiphorese Gel 30	Carl-Roth
Adhesive PCR plate foil	Thermo Scientific
4-Aminobenzoic acid	Sigma-Aldrich
Ammonium chloride	Carl-Roth
Ammonium persulphate	Merck
Anhydrous tetracycline	Sigma-Aldrich
Aqua-Phenol	Carl-Roth
D-Biotin	Sigma-Aldrich
Boric acid	Sigma-Aldrich
Brain Heart Infusion	Sigma-Aldrich
Bromphenol blue	Carl-Roth
Calcium chloride	Carl-Roth
Ca-D-Pathothenic acid	Sigma-Aldrich
Citrate	Carl-Roth
Cobalt (II) nitrate	Sigma-aldrich
Columbia blood agar base	Sigma-Aldrich
Coomassie G-250	Carl-Roth
Copper(II)sulfate	Sigma-Aldrich
p-coumaric acid	Sigma-Aldrich
Cyanocobalamin	Sigma-Aldrich

Diethylpyrocarbonat	Carl-Roth
Dimethyl sulfoxide	Intas
Dithiothreitol	Carl-Roth
dNTPS	Genaxxon Bioscience
Dulbecco's Modified Eagle's Medium	Corning
Dulbecco's Phosphate Buffered Saline	Sigma-Aldrich
Ethylendiamintetraacetate	Applichem
European agar	Oxoid
Fetal calf serum	GIBCO
Formaldehyde	Sigma-Aldrich
Glucose	Carl-Roth
Glycerol	Carl-Roth
Glycine	Sigma-Aldrich
Hoechst 34580	Thermo Fisher
Hydrogen peroxide (30%)	Carl-Roth
Intas HD Green	Quanta Biosciences
Iron(III)chloride	Sigma-Aldrich
Luminol	Carl-Roth
Magnesium chloride	Sigma-Aldrich
Magnesium sulfate	Sigma-Aldrich
Mangan(II)chloride	Sigma-Aldrich
2-mercaptoethanol	Sigma-Aldrich
Methanol	Carl-Roth
3-(N-morpholino)propanesulfonic acid	Sigma-Aldrich
Nickel(II)chloride	Sigma-Aldrich
Nicotinamid	Sigma-Aldrich
Non-fat dried milk powder	Applichem
Perfluoralkoxy-Polymere (PFA)	Morphisto
Phosphate buffered saline (PBS)	Croning
Polyvinylidene fluoride (PVDF)	Roche
Potassium chloride	Sigma-Aldrich

Potassium dihydrogen phosphate	Carl-Roth
Pyridoxine hydrochloride	Sigma-Aldrich
Quanta Biosciences Perfecta SYBR Green FastMix	Quanta Biosciences
Riboflavin	Sigma-Aldrich
RNase A	Carl-Roth
RPMI 1640 Glutamax™	GIBCO
Saccharose	Carl-Roth
Sodium chloride	VWR
Sodium citrate	Sigma-Aldrich
Sodium dodecyl sulfate	Carl-Roth
Sodium molybdate	Sigma-Aldrich
Sodium phosphate	Sigma-Aldrich
Sodium pyruvate	PAA
Tetramethylethylenediamine	Fluka Analytics
Thiamine hydrochloride	Sigma-Aldrich
Trace metal mix A5	Sigma-Aldrich
TRI Reagent™ Solution	Thermo Scientific
Tris(hydroxymethyl)aminomethan	Sigma-Aldrich
Tris(hydroxymethyl)aminomethan - hydrochloride	Sigma-Aldrich
Triton X-100	Carl-Roth
Tryptone	BD
Tryptone Soy Broth	Sigma-Aldrich
Tryptone Soy Broth without glucose	Sigma-Aldrich
Tween-20	Carl-Roth
Yeast extract	Carl-Roth
Zinc sulfate	Sigma-Aldrich

4.13 Technical equipment

Table 4.18: Technical equipment used in this study

Equipment	Manufacturer
Autoclave	WEBECO, Sytec VX 150
Chemiluminescence camera system (Intas LabImage Chemostar)	Intas
Electrophoresis power supplies (Peqlab EV202)	Peqlab Biotechnology
FastPrep FP120	MP Biomedicals
Gel imager (BiostepDark hood DH-40/50)	Biostep GmbH
Megafuge 1.0R	Heraeus
MicroPulser™	Bio-Rad
Microcentrifuge (Hettich MIKRO 200)	Hettich Lab tech
NanoDrop 1000 spectrophotometer	Peqlab Biotechnology
OmniLog®	Biolog
PCR Thermocycler (PEQLAB peqSTAR)	VWR International
PerfectBlue™ Gel system	Peqlab Biotechnology
PerfectBlueTM Semi-Dry Electroblotter	Peqlab Biotechnology
pH electrode SenTix	WTW series inolab
Photometer Ultraspec 3100 Pro	Amersham Biosciences
Plate reader (TECAN MPlex)	TECAN
Refrigerated Microcentrifuge (Hitachi himac CT15RE)	Hitachi-Koki
StepOne Plus real-time PCR system	Applied Biosystems
Thermo mixer comfort	Eppendorf
Vortex REAX 2000	Heidolph

4.14 Applied Software

Table 4.19: Applied Software and web tools used in this study

Software	Manufacturer / Homepage
ApE A plasmid Editor 8.5.2.0	M. Wayne Davis (biologylabs.utah.eu)
Argus x1 version 7.6.17	Biostep GmbH
CodonCode Aligner 4.0.4	CodonCode Corporation
EndNoteX9	Thomson Reuters
GraphPad Prism7	GraphPad
Harmony®	PerkinElmer Operetta®
ND-100 V3.7.1	NanoDrop Technologies, Inc. Wilmington
Office 2007	Microsoft
Omnilog PM Software	Biolog
StepOne Software v.2.3	Life Technologies
TECAN i-Control	TECAN

5. Methods

5.1 Cultivation of bacteria

5.1.1 Bacterial culture conditions

Escherichia coli were grown in LB agar or broth supplemented with the required antibiotics if needed. If not specifically mentioned, all *Staphylococcus aureus* strains were grown in Tryptic soy broth (TSB) or on Tryptic soy agar (TSA) with glucose (0.25%, w/v) and the corresponding antibiotics when required. The trains were cultivated overnight aerobically at 37°C with agitation on an orbital shaker at 180 rpm.

5.1.2 Bacterial cryo-stocks for preservation

For preservation bacterial cryo-stocks were prepared by growing bacteria in liquid culture and mixed with 50% sterile glycerol to an end concentration of 12.5% (v/v) and stored at -80°C until use.

5.1.3 Bacterial growth curves

To determine differences in bacterial growth, it was recorded via a TECAN MPlex plate reader. *S. aureus* strains were grown overnight at 37°C in the according medium. Next day bacterial culture was diluted to an OD_{600} of 0.1 in fresh medium. A 48 micro-well plate was used, filled in a technical triplicate of three with 400 µl per well. As a reference a sterile medium was used. If needed, half of the well plate was covered with an adhesive strip to generate micro-aerobic condition. The plate reader was preheated to 37°C before starting the measurement. The optical density was measured at 600 nm every ten minutes for 24 h and the plate was shaken with an amplitude of 6 mm between each measurement. At every time point the growth rate was determined from the mean of three biological replicates.

5.1.4 BIOLOG Phenotype Microarray characterization of *S. aureus*

The BIOLOG Phenotype Microarray™ (BIOLOG, Hayward, CA, USA) was performed, according to the manufacturer's recommendations. Phenotype MicroArrays are preconfigured sets of phenotypic tests deployed on microplate panels. Each well of the array is designed to test a different phenotype after inoculation with a standardized cell suspension, allowing simultaneous testing of thousands of phenotypes in a single experiment. The technology is based on the measurement of bacterial respiration, which produces NADH as a universal reporter (Bochner et al., 2001). If bacteria are able to metabolize a specific substrate, electrons from NADH reduce a tetrazolium dye in an irreversible reaction generating and forming a strong purple color in the panel wells. If it is weakly positive or negative, respiration is slowed or stopped, and the result is less color or no color. The redox assay provides for both amplification and precise quantification of phenotypes. Bacterial cultures were platted on TSB-Agar and re-suspended using a sterile stick in tubes containing 10 ml of GN/GP-IF-0a (BIOLOG inoculating fluid), 120 µl of 100× BIOLOG Redox Dye Mix G and 1 ml of the appropriate additive until 85% transmittance was reached as measured using the turbidimeter provided by BIOLOG. A volume of 100 μl of this final suspension was added to each of the 96 wells of the PM plates. The PM plates were then sealed to avoid drying and incubated at 37 °C in the OmniLog® (BIOLOG, Hayward, CA, USA) incubator reader for 1 day. Incubation and recording of phenotypic data is performed automatically by the OmniLog™, generating a kinetic response curve for each well (Bochner, 2003; 2009). The kinetic data analysis was done by the OmniLog PM software, which contains a suite of algorithms that work in conjunction with the OmniLog PM system and Phenotype MicroArray panels to automate incubation at a fixed user-controlled temperature with complete collection of colorimetric assay data over time (Fig. 4.1).

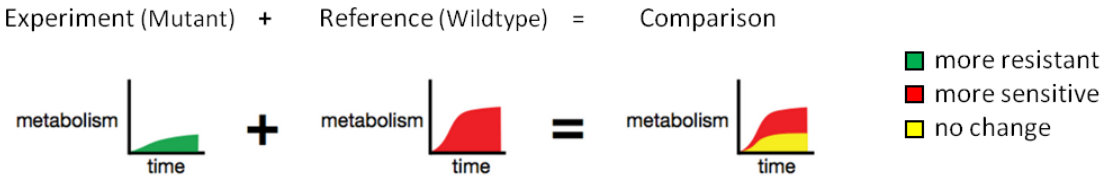


Figure 5.1: Pattern of kinetic data from PM panels recorded by the OmniLog PM system. Schematic presentation of the evaluation from the outcome of the kinetic data, whereas metabolism for mutant is presented in red and metabolism for wildtyp is marked in green, after lying on top of each other yellow area shows the equality of both strains. Red patch means wildtyp is more sensitive to the substance than the mutant, if patch turns green wildtype would be more resistant. Figure adapted from Biolog.

5.2 Genetic manipulation of bacteria

5.2.1 Preparation of competent *E. coli*

E. coli DH5α were grown overnight in LB at 37°C. Next day 1 ml of the overnight culture was inoculated with 100 ml of fresh LB and grown until the logarithmic growth phase of OD_{600} 0.4-0.7 nm was reached. Bacteria were centrifuged for 10 min at 4.000 and resuspended in 20 ml 0.1 M calcium chloride solution and incubated for 30 min on ice. These Bacteria were resuspended in 10 ml 0.1 M calcium chloride containing 20% glycerol. Aliquot of 100 µl were stored at -80°C.

5.2.2 Transformation of *E. coli*

To transform *E. coli*, 100 µl of chemical competent *E. coli* were thawed on ice and incubated with the plasmid of interest for 30 min on ice. Next, Bacteria got heat-shocked for 90 sec at 42°C and cooled off for 2 min on ice. Afterwards, 1 ml LB was added to the bacteria and incubated at 180 rpm for 1 h at 37°C. Bacteria were platted on LB agar with the corresponding antibiotics.

5.2.3 Preparation of electro-competent *S. aureus*

S. aureus strains were grown overnight at 37°C and next day they were diluted to OD_{600} 0.5 nm in 100 ml of fresh TSB. Bacterial were grown at 180 rpm and 37°C until they reached OD_{600} of 0.6-0.8 nm. Before harvested at 3.000 x g for 10 min at 4°C, bacteria were incubated for 15 min on ice. The Bacterial pellet was washed with 30, 25 and than 10 ml of sterile ice-cold water. Afterwards the pellet was washed twice with 10 and 5 ml of ice-cold sterile 10% glycerol and centrifuged at 3.500 x g for 15 min at 4°C. At least the pellet was resuspended in 1-2.5 ml ice-cold 10% glycerol and splitted into aliquots of 50 µl which were kept at -80°C.

5.2.4 Electroporation of *S. aureus*

After electro-competent *S. aureus* were thawed for 5 min on ice, they were incubated for 15 min at room temperature and 120 µl of electroporation buffer and 5 µg of plasmid DNA of interest were added. The mixture was incubated at room temperature for 20 min before it got electroporated. A Bio-Rad MicroPulser was used at 1.8 kV and a 2.5 milli-second pulse for 2 mm thick electroporation cuvettes. Immediately after the electroporation pulse, 1 ml of TSB was added, collected in a falcon and incubated for 1.5 h at 200 rpm at 37°C. Bacteria were platted on TSB agar with the corresponding antibiotic.

5.2.5 Transduction of DNA via phage transduction

Bacteriophage 11 or 80 was used to transduce genetic material into *S. aureus*. With this method it is possible to transduce plasmid DNA from one strain into another.

5.2.6 Amplifying of phages

S. aureus strain RN4220 was grown in phage buffer overnight. 300 µl of bacteria culture were incubated for 2 min at 53°C and afterwards 100 µl bacteriophage 11 or 80 was added to the culture and incubated at room temperature for 2h. The mixture was added to 55°C warm LB-soft agar containing 0.5 mM calcium chloride and plated on TSB at 37°C overnight. The soft agar was scraped off and incubated with 3 ml phage

buffer for 3 h. Afterwards the bacteriophages were centrifugated at 2.000 rpm for 10 min and the supernatant was stored at 4°C.

To prepare the transducing phages, donor strain harbouring the respective plasmid was grown overnight in 5 ml phage buffer. Next day culture was diluted 100-fold in fresh phage buffer and grown to an OD_{600} of 0.1. After the level got reached 100 µl donor bacteria was diluted again with fresh phage buffer and additionally with 100 µl of bacteriophage 11 or 80 solution and incubated at 30°C until lysis was visible. By centrifugation at 14.000 rpm for 1 min the cellular soil was separated from the transducing phages and could be stored at 4°C. Recipient strain was grown in phage puffer overnight and next day 300 µl of this culture was heat shocked at 53°C for 2 min, before 100 µl of the transducing phage solution was added. This solution incubated for 2.5 h at RT, afterwards 0.6% of LB phage top agar containing sodium citrate was added, before it was platted on TSA with the respective antibiotic. To avoid secondary site mutation, *S. aureus* insertional transposon mutant USA300 JE2_Tn852 obtained from the Nebraska library was transduced via phage 80 into the genetic background of wildtype *S. aureus* JE2.

5.3 Desoxyribonucleic acid

5.3.1 Isolation of Plasmid DNA from *E. coli*

For isolation of plasmid DNA from *E. coli* strains NucleoSpin Plasmid Kit was used according to manufacturer´s protocol, with some modifications. *E. coli* was grown in 5 ml LB overnight at 37°C, with corresponding antibiotics. Bacteria were harvested and plasmid was isolated by passing though spin columns. Elution was performed using 20 µl of pre-warmed sterile water. Plasmid DNA was stored at -20°C.

5.3.2 Isolation of Plasmid DNA from *S. aureus*

For isolation of plasmid DNA from *S. aureus* strains QIAprep Spin Miniprep Kit was used according to manufacturer´s instructions, with some modifications. Briefly, *S. aureus* was grown overnight at 37°C in TSB. Bacteria were harvested and resuspended in buffer A1. To lyse bacteria 50 µg of lysostaphin was added and bacteria were incubated at 37°C and 1400 rpm until the mixture becomes clear, indicating cell wall lysis. Afterwards the plasmid DNA was isolated according to the manufacturer´s instructions. DNA was eluted from columns using 20 µl of pre-warmed sterile water and stored at -20°C.

5.3.3 Isolation of genomic DNA from *S. aureus*

For isolation of genomic DNA form *S. aureus* DNA mini kit (Qiagen) was used according to manufacturer´s instructions, with some modifications. *S. aureus* was grown overnight at 37°C in TSB. Bacteria were harvested and resuspended in 180 µl of freshly made lysisbuffer and incubated for 30 min at 37°C at 1400 rpm. To the visible lysed bacteria cells 200 µl of buffer AL and 20 µl of proteinase K solution was added and incubated for 30 min at 56°C and 1400 rpm. The lysate was processed as instucted and eluted with 20 µl of prewarmed sterile water and stored at -20°C.

5.3.4 Polymerase-Chain-Reaction (PCR)

Polymerase-Chain-Reaction were performed either with Phusion polymerase with 3´-5´exonuclease activity or with Taq polymerase without any proofreading ability. PCR were performed in a volume of 25-50 µl using a template of 50-100 ng, or 1 µl of heat-lysed bacteria suspensions if PCR was used to screen for a transformed plasmid in the bacteria. Also, 1x polymerase buffer, 0.2 pmol of forward and reverse primer, 0.2 µM dNTPs and 1U of the thermostable polymerase were added for the PCR. The reaction starts with an initial step at 95°C for 5 min and continuous with 30 cycles of denaturation at 95°C for 30 sec, annealing at 50-60 °C depending on the primer´s melting temperature for 30 sec and elongation depending on the product length and the used polymerase at 72°C. The duration of the elongation for Phusion is 30 sec/kb

and 1 min/kb for Taq. The reaction ends with a final elongation step for 10 min at 72°C and the product could be stored at 4°C for further use. Depending on the DNA size a 1% or 2% TAE agarose gel was used and 0.0005% HD Green Plus was added to visualize it under UV-light. If necessary, PCR product were purified using Nucleospin Gel and PCR Clean-up kit according to manufacturers protocol.

5.3.5 Annealing of oligonucleotides

Oligonucleotides were annealed by mixing 20 µM of each oligonucleotide to a tenth with annealing puffer and heated for 5 min at 95°C. Annealing occurred by slowly cooling the heated onligonucleotides to RT.

5.3.6 Phosphorylation of DNA

DNA obtained via PCR amplification was phosphorylated using 1 µl of PNK in a tenth of ligase buffer at 37°C for 20 min.

5.3.7 Cloning of DNA into a vector

Cloning vectors and purified PCR fragments were digested using appropriate restriction enzymes and buffers. In case of incompatible restriction enzyme buffers, a serial digestion was performed. After restriction the vector was dephosphorylated using FastAP for 15 min at 37°C. Ligation was performed in a total volume of 10 µl by using an insert vector ratio of 3:1 and an incubation with T4 DNA ligase at 16°C overnight. The ligation mixture was transformed into electro competent *E. coli* DH5α.

5.3.8 In-Fusion cloning

The In-Fusion HD Cloning kit (Takara) was used to clone plasmids. According to the manufacurer´s recommendation fragments contained 15-19 bp overlaps to each other or the vector. Depending on the molar ratio of the fragments and vector, they were mixed together with 5x In-Fusion HD Enzyme Premix and deionized water and incubated for 15 min at 50°C and afterwards stored on ice. The resulting plasmids

were transformed into chemo-competent *E. coli* DH5α and sequenced via Sanger sequencing.

5.3.9 Construction of plasmids used in this study

5.3.9.1 Construction of AHT induced transcription of LTTR852

For inducible complementation plasmid p2085_852-AHT was constructed. Using enzymes PmeI and PstI to linearize the vector pAHT-SSR42 containing an AHT-inducible promoter which was amplified from plasmid p2085. Sequence of LTTR852 was amplified from genomic DNA of *S. aureus* 6850 using oligonucleotides 852-PmeI-fw and 852-PstI-rev and trimmed with the enzymes PmeI and PstI. The ligament was cloned via T4 Ligase into the prepared vector. The resulting sequence was initially cloned into TOPO TA according to the cloning kit. Digestion with PmeI and PstI resulted in the final plasmid p2085_852-AHT, which was cloned into *S. aureus* 6850 wildtype and Δ852. 200 ng/ml AHT was added to *S. aureus* in liquid culture, for induction of LTTR852 transcription.

5.3.9.2 Construction of His-Tag construct

In order to detect proteins via Western blot, the proteins were tagged with an His after SBD in the plasmid sequence. The oligonucleotides were designed reverse complementary harbouring the base exchange in the middle. Fragments carrying the His-tag were amplified from plasmid p2085_852-AHT of *E. coli* DH5α with the oligonucleotides MF39 Seq 2085 s and either 852_His_rev or cidR_His_rev depending on the SBD and afterwards the fragments were trimmed with the enzymes PmeI and EcoRI. The isolated plasmid p2085_852-AHT of *E. coli* DH5α was trimmed with the same enzymes and afterwards the fragment was cloned via T4 Ligase into the prepared vector. At the end the new plasmid was transformed into *E. coli* DH5α (4.2.2), electroporated into RN4220 (4.2.4) and finally transducted via phages into the target strain (4.2.5.2).

5.3.9.3 Construction of GFP promoter reporter construct

For construction of p2085_Pr852_GFP (Fig. 5.2) the plasmid p2085_Prhla_GFP from Horn was used as a template. The vector p2085_Prhla_GFP was amplified with the oligonucleotides pGFP-Inf-Prom F and pGFP vec R. The promoter sequence was amplified using the primers Pr852-GFP_fw and Pr852-GFP_rev. The GFP coupled promoter sequence of LTTR852 was cloned in the prepared vector using the In-Fusion system (4.3.8).

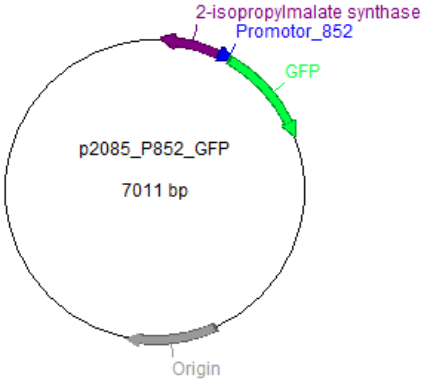


Figure 5.2: Schematic illustration of the plasmid p2085_Pr852_GFP.
Designing plasmid p2085_Pr852_GFP, using the backbone of p2085 with a fragment of the divergently transcribed gene *leu*A1 (magenta) lying downstream of *lttr852*; the promoter fraction of *lttr852* is marked blue with the attached GFP sector (green); the plasmid has a total size of 7011 bp.

5.3.9.4 Construction of transcription factor domain swapping

Domains of LTTR852 were re-wire with the more thoroughly characterized domains of LTTR CidR, leading to the inducible plasmid p2085_852cidR (domain-swap I; Fig. 4.2 A), p2085_cidR852 (domain-swap II; Fig. 4.2 B) and the control p2085_cidR. Using enzymes PmeI and EcoRI to linearize the vector p2085_852-AHT. Fragments of domain swap and its control were amplified from oligonucleotides, whereby D-Swap_p2085_852-HTH_fw and D-Swap_852-HTH_cidR-SBD_rev for fragment one with 313 bp of p2085_852cidR and fragment two with 658 bp with oligonucleotides D-Swap_cidR-SBD_p2085_fw and D-Swap_852-HTH_cidR-SBD_rev. For p2085_cidR852 the first fragment was amplified by D-Swap_p2085_cidR-HTH_fw and D-Swap_cidR-HTH_852-SBD_rev for fragment one with 312 bp and D-Swap_852-SBD_p2085_fw and D-Swap_cidR-HTH_852-SBD_rev for fragment two with 647 bp. And for the control plasmid p2085_cidR the oligonucleotides D-Swap_p2085_cidR-HTH_fw and D-Swap_cidR-SBD_p2085_rev were used to amplify one fragment. The fragments were cloned via In-Fusion into the prepared vector

(4.3.8). The resulting plasmid were transformed into *E. coli* DH5α (4.2.2). For induction of domain-swap transcription 200 ng/ml AHT was added to liquid culture of *S. aureus*.

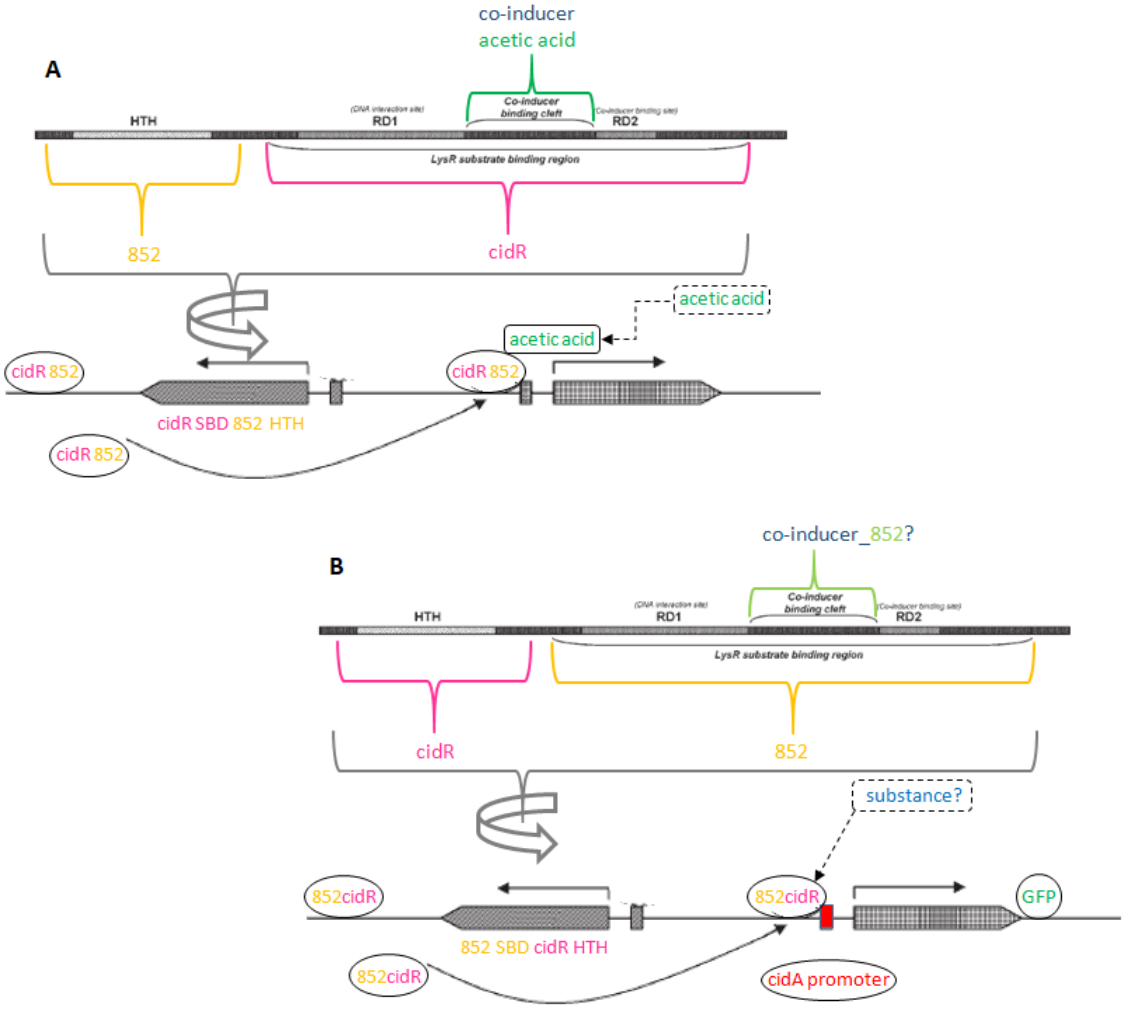


Figure 5.3: Molecular cloning model of schematic domain swapping.
(**A**) Domain-swap I to find the specific target gene for LTTR852. Transcription of domain-swap protein I based on helix-turn-helix from LTTR852 (yellow) fused with co-factor binding side of *cid*R (pink), binding downstream at promoter of LTTR852 target gene, where acetic acid as a co-inducer (green) of *cid*R binds. (**B**) Domain-swap II to find the unknown co-inducer for LTTR852. Transcription of domain-swap protein II based on helix-turn-helix from *cid*R (pink) fused with co-factor binding side of LTTR852 (yellow), binding downstream at promoter of target gene *cid*A (red). Unknown substance (blue) binds at domain-swap II and activates transcription of *cid*A, whereby GFP is integrated behind the target gene *cid*A.

5.4 Ribonucleic acid techniques

5.4.1 RNA isolation

Bacterial RNA was extracted using the TRIzol method (Lasa et al., 2011). At the desired optical densities, bacteria were harvested, flash-frozen in liquid nitrogen and stored at -80°C. The Bacteria pellets were thawed on ice, following by addition of 0.5 mM EDTA and resuspended in 400 µl Buffer A. The mixture was transfered to a Lysin matrix B tube (MP Biomedicals) containing 500 µl of acid-phenol to lyse the bacteria cells by using a Fast Prep FP120 at 6 m/s for 45 seconds at 4°C. If not mentioned otherwise, every step was performed on ice or a cooling centrifuge. Phases were separated at 10.000 x g for 10 min. The upper aqueous layer was mixed with 1 ml TRI reagent and incubated for 5 min at room temperature. Afterwards 100 µl chloroform was added, the samples were shaked and incubated at room temperature for 3 min. Phases were separated by centrifugation at 17.900 x g for 10 min. The subsequent aqueous phase was re-incubated with 200 µl chloroform and incubated for 5 min at room temperature. After this separation step the RNA in the aqueous phase was precipitated by using absolute isopropanol for 15 min at room temperature following centrifugation for 30 min at 17.900 x g. The RNA-pellet was washed once with 75% EtOH, air-dried in a fume hood and dissolved in DEPC-treated water and stored at -20°C.

5.4.2 RNA digestion by DNaseI

Purifying total RNA residing DNA contamination after isolation, TURBODNA-free™ DNase kit was used according to manufaturer´s instructions. Briefly, 10 µg of total RNA were digested mixed with TURBO DNase buffer and 1,5 µl DNaseI for 30 min at 37°C. The digestion reaction was stopped by addition of 5 µl DNase I-inactivation reagent, proximately incubated for 5 min at room temperature. By centrifugation at 10.000 x g for 2 min, RNA was separated from the inactivation reagent and the upper aqueous layer was collected and frozen at -20°C.

5.4.3 Generation of cDNA via reverse transcription

Generation of complementary DNA was generated by reverse transcription messenger RNA using RevertAid Kit according to manufacturer´s instructions. Briefly, 1 µg RNA was mixed with a genomic DNA clean-up reagent and incubated for 2 min at 42°C. Afterwards, primer mix, reaction buffer and reverse transcriptase enzyme was added and incubated for 30 min at 42°C. The resulting cDNA was diluted 5-fold and stored at -20 °C.

5.4.4 Quantitative real time PCR (qRT-PCR)

Quantitative real time PCR was performed in a 96 well format, on a StepOnePlus^{TM} real time PCR system using Quantita Biosciences Perfecta SYBR Green FastMix. The reaction composition and cycle settings were chosen according to manufacturer´s recommendation. The SYBR Green master mix was diluted to 1x in dH_2O and mixed with 0.9 µM of forward and reverse primers and 10 ng cDNA template to a total volume of 20 µl per well. All reactions were performed in technical triplicates. After an initial hold step at 95°C for 10 min, each cycle of total 40 cycles were run for 15 seconds at 95°C followed by 60°C for 1 min. The expression of housekeeping gene *gyrB* (gyrase subunit B) was used to normalize the relative transcription levels to the expression of the target genes. The $2^{-\Delta\Delta CT}$ method was used to analyse the relative transcription (Livak and Schmittgen, 2001).

5.4.5 RNA-Sequencing (RNA-seq)

Illumina sequencing was done by Max-Planck Genome Centre Cologne. DNase I-treated RNA samples were rRNA depleted and converted into cDNA libraries for sequencing. On an illumina HiSeq2000 machine a paired end read sequencing was performed, with a total of 6 million reads for sequencing of each cDNA library. Cutadapt was used to remove adapters and only reads exceeding a mean base quality 5 within all sliding windows of 5 bp were mapped to the *S. aureus* 6850 genome. Read mapping was conducted using Bowtie2 (Langmead et al., 2009). To identify differentially regulated transcripts, DESeq2 was used (Love et al., 2014). RNA-seq was

carry out in biological triplicates. Statistical analysis was performed by Maximilian Klepsch.

5.5 Investigation of promoter activities

The promoter activity was determined by measuring the GFP fluorescence (excitation: 488±9 nm, emission: 518±20 nm) and put it into proportion with the measurement of the optical density (600 nm) of growing bacteria during a certain time slot. A TECAN MPlex plate reader was used to measure GFP and optical density. Bacteria were grown overnight at 37°C under shaking. Next day bacteria were washed once with PBS and inoculated in fresh media, if the medium ingredients varied for the measurement. Bacteria culture were diluted to an OD_{600} of 0.1 and triplicates of 400 µl were added in wells of a 48 micro-well plate. To generate micro-aerobic conditions, the part of the micro-well plate was pasted over with an adhesive PCR plate foil that provides an excellent bond that eliminates evaporation at high temperatures. The micro-well plate was shaked at an amplitude of 6 mm and every ten minutes the optical density as well as the GFP fluorescence were measured for a certain time course.

5.6 Investigation of virulence in *S. aureus*

5.6.1 Quantitative haemolysis assay

S. aureus can secret haemolysins and cause disruption of erythrocytes which leads to the release of haemoglobin. The release of haemoglobin over a certain time can be measured in order to determine the haemolytic potential of *S. aureus*. Briefly, sheep erythrocytes were washed twice with 0.9% NaCl and diluted afterwards with the same amount of fresh 0.9% NaCl to get a final concentration of 50% erythrocytes. Bacteria were grown overnight in TSB at 37°C, normalized to their optical density and the supernatants were collected. The supernatants were sterile filtered with a diameter of 0.45 µm. A mixture of 5% (v/v) of the sterile-filtered supernatant with 1 ml of the 50% erythrocytes dilution was incubated for 1 h at 37°C at 550 rpm. For a positive control

20 µl of 50% erythrocytes were mixed with 980 µl H_2O_2 and for the negative control 1 ml 1% erythrocytes were mixed with 50 µl TSB. After that the suspension was centrifuged and the supernatant was analyzed. The released haemoglobin was measured at an absorbance of 405±9 nm using a TECAN reader. Haemoglobin release was compared to a negative control where erythrocytes were incubated with sterile water. The percentage of *S. aureus* wildtype haemolysis potential defined the relative haemolysis.

5.6.2 Quantitative biofilm assay

Molecular components of *S. aureus* are involved in biofilm formation. For the quantitative biofilm assay trains were grown overnight in TSB medium at 37°C. Next day the cultures were diluted in fresh TSB medium to an OD_{600} of 0.05 and 200 µl of each sample were added at least four times into a 96 well plate. If necessary 200 ng/µl AHT was added per well before an incubation overnight with no shaking at 37°C. Following day, the supernatant was discarded, and each well was washed two times with 200 µl PBS. To fixate the biofilm the plate was incubated at 65°C for one hour. Afterwards 50 µl of 0.1% crystal violet (w/v) was added per well to stain the biofilm for 2 min at room temperature. Then the staining was removed, and the plate was washed three times with 200 µl of destilled water. After the plate dry at room temperature it was analysed in an TECAN Infinite M200 plate reader at a wavelength of 550 nm. As a positive control the strain *S. epidermidis* RP62A was used.

5.7 Protein techniques

5.7.1 Isolation of bacteria proteins

Cytosolic and secreted bacterial proteins were isolated from *S. aureus*. Therefore, overnight culture of *S. aureus* was either centrifuged or inoculated in fresh TSB to an OD_{600} of 0.1. They grow as long as certain time points were reached, then normalized and centrifuged at 6.000 rpm for 20 min at 4°C.

5.7.2 Isolation of secreted bacterial proteins

For the isolation of secreted bacterial proteins, the supernatant were blended in a fresh falcon with four time of ice-cold acetone to precipitate secreted proteins. The samples were stored overnight at -20°C and next day they got centrifuged for 1 h at 6.000 rpm at 4°C. After that the precipitated proteins were washed once with 1 ml ice-cold acetone for 10 min at 6.000 rpm and 4°C. The protein pellets were air-dried and resuspended in an appropirate amount of PBS and stored at -20°C.

To isolate cytosolic bacterial proteins the pellets were resuspended in 200 µl lysisbuffer, transferred to a lysing matrix B tube and lysed for 45 seconds at 6 m/s using a FasPrep FP120. After centrifugation for 10 min at 4°C the protrusion was harvested and stored at -20 °C. A Bradford assay using Roti Quant was done to measure the protein amount of the supernatant and the pellet at different time points. The amount of the cytosolic and secreted bacterial proteins was calculated by using 1 mg/ml BSA solution to calibrate a standard line according to the manufacturer protocol.

5.7.3 SDS-PAGE

Electrophoresis SDS-PAGE was running to separate proteins according to their masses. The samples were mixed with Laemmli buffer and got denatured by heating up to 95°C for 5 min. The gel was made of a 3,5% PAA stacking and a 7,5% PAA resolving gel. A Page Ruler^{TM} prestrained protein ladder was used as a standard size. The proteins were separated for 15 min at 80 V and afterwards at 120 V until the front line got out.

5.7.4 Colloidal coomassie staining

The colloidal coomassie staining was used to visualize proteins which were separated by SDS-PAGE. Proteins in the gel were fixed for 1 h in 500 ml fixing solution and stained overnight in freshly set up coomassie staining solution. Next day the gel was incubated for 5 min in neutralization solution and washed afterwards for 15 min in 500 ml washing solution until excess colour pigments were removed. At the end the gel was washed in dH_2O until the water was sufficient destained and got scanned.

5.7.5 Western blotting

A semi-dry blotting was used to transfer the proteins from the SDS-PAGE on a PVDF membrane. After a transfer of 1h at 0,8 mA/cm^2, the membrane was washed once with TBS-T and afterwards blocked in TBS-T for 1h. Incubation with the first antibody was done at 4°C overnight under tumbling. Next day the membrane was washed three times for 5 min in TBS-T before it was incubated in the secondary antibody for 1h at room temperature. Then the membrane was washed three times for 10 min in TBS-T. The development of the Western blot was generated by mixing ECL-1 and ECL-2 solution in equivalent amounts and adding it to the membrane. The signal was detected using an Intas LabImage Chemostar system.

5.8 Cell culture

5.8.1 Thawing of cell lines

To revitalize, frozen cells were washed in 10 ml of according pre-warmed cell culture medium for 5 min at 1.500 rpm. Cells were resuspended in 20 ml of fresh medium and transferred into a cell culture flask. Cells were than incubated at water-vapor saturated atmosphere with 5% CO_2 at 37°C.

5.8.2 Cultivation of cell lines

Cells were cultivated in the according cell culture medium supplemented with 10% FCS, 5% sodium-pyruvate and 5% penicillin/streptomycin in 75 cm^2 cell culture flasks at 37°C and 5% CO_2 saturation. When cells reached a confluency of approximately 80-90% they were passaged. For this, cells were washed twice with 10 ml PBS before 1 ml trypsin was added, followed by an incubation at 37°C for about 5 min until cells become detached. To stop the reaction 10 ml pre-warmed cell culture medium was added. Desired number of cells where diluted with the required amount of fresh culture medium.

5.8.3 Cell line cryo-stocks for preservation

Cryo-stocks of cell lines were made for preservation. For that cells were grown until reaching confluency, using trypsin to detach, washed once with PBS and centrifuged for 10 min at 1.500 rpm. The cell pellet was resuspended in a freezing medium containing FCS as well as 10% DMSO and 1 ml was transferred to a cryo tube. For short term storage the cryo tubes were kept at -80°C in an isopropanol chamber and for long term storage cells were transferred to a liquid nitrogen container.

5.8.4 Generation of YFP-CWT cell lines

To generate a stable integration of YFP-CWT escape marker (Giese et al., 2011) in Human Embryonic Kidney (HEK) cell lines the lentiviral vector pLVTHM (Wiznerowicz et al., 2003) was used. HEK 293T cells were grown on a 15 cm dish and blended with 20 µg of the pLVTHM YFP-CWT vector, 10 µg psPAx and 10 µg pVSVG for four to eight hours. After the incubation growth medium was exchanged and two days later the supernatant was harvested and sterile filtered with a 0.45 µm Filter. HEK 293T YFP-CWT cells were infected in presence of 10 mg/ml polybrene and then sorted on a fluorescence activated cell sorter.

5.8.5 Infection of cells with *S. aureus*

On the first day a 12 well micro-well plate was pre-coated for two hours with 500 µl FBS and washed afterwards once with PBS before cells were seeded at a density of $1x10^5$ per well in cell culture media. *S. aureus* strains were grown overnight in TSB at 37°C. Next day, cells were washed once with PBS and the cell culture medium was replaced by 500 µl infection media. For the infection the bacterial culture was inoculated in fresh TSB at an OD_{600} of 0.4 and grown at 37°C with agitation for 45 min until the logarithmic growth phase was reached. Bacteria were harvested, washed once with PBS and once with infection media and subsequently resuspended in infection media. Bacteria number was counted of a dilution using a Thoma chamber. Cells were infected with an MOI of 5 and to set it on the time point zero the microwell plate was centrifuged at 1 000 rpm for 10 min to sediment the bacteria. After 1 h post infection the medium was replaced by 500 µl infection media containing 10 µg/ml lysostaphin to digest extracellular bacteria. 1.5 h post infection media was replaced by 500 µl infection medium containing 2 µg/ml lysostaphin until the desired time. After the corresponding time points the cells were washed twice with PBS and went on depending on the method.

5.8.6 Numeration of colony forming unit

Enumeration of colony forming units (CFU) of *S. aureus* were done from infected cells at certain time points. Infected cells (4.8.5) were washed twice with PBS and afterwards incubated for approximately 20 min with 500 µl dH_2O at 37°C until the cells were lysed. Furthermore, the cells were cracked mechanically and the whole solution was collected. The bacteria were plated with various dilutions on TSA and incubated overnight at 37°C. Next day CFU were counted and calculated.

5.8.7 Detection of phagosomal escape of *S. aureus*

After certain infection time points the cells were washed twice with PBS and incubated afterwards with 300 µl 4% PFA for 30 min in the dark. All the next steps were implemented in the dark. Following the cells were washed again with 500 µl PBS and

covered with 300 µl of 0.1% Triton for 5 min. Subsequently the samples were coated with 500 µl 50 mM ammonium chloride. After 5 min the cells were incubated with an Höchst staining solution for 30 min. After the dye the wells were washed three times with PBS and stored with 4% PFA at 4°C in the darkness until the plates were measured at the Operetta.

5.9 Statistics

Statistical analysis was performed using GraphPad Prism7. Significance of differences from at least three independent replicates were determined by a two-way ANOVA test for comparison of more than two independent data sets, or Student's *t* test for comparison of two independent data sets. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

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Figures:

Figure 1.1:
left: Nathan Reading on Flickr Nathan R. on twitter; 9. Februar 2011; *Staphylococcus aureus* growing on Columbia horse blood agar; www.wikimedia. org; 07.03.2021

right: National Institute of Allergy and Infectious Diseases (NIAID); 26. August 2010; Scanning electron micrograph of Staphylococcus aureus bacteria (gold) outside a white blood cell (blue). www.wikimedia.org; 07.03.2021

7. Appendix

7.1 Abbreviations

agr	**A**ccessory **g**ene **r**egulator	nm	**N**ano**m**eter
AHT	**A**n**h**ydrous **t**etracycline	NPPC	**N**on **p**rofessional **p**hagocytic **c**ell
BHI	**B**rain **h**eart **i**nfusion	Nre	**N**itrogen **re**gulation
bp	**B**ase **p**airs	OD	**O**ptical **d**ensity
Ccp	**C**atabolite **c**ontrol **p**rotein	p	**P**-value
CDM	**C**hemical **d**efined **m**edium	p/pl	**Pl**asmid
cDNA	**C**omplementary **DNA**	PAA	**P**oly**a**cryl**a**mide
CFUs	**C**olony-**f**orming **u**nits	PBP	**P**enicillin-**b**inding **p**rotein
Clf	**Cl**umping **f**actors	PBS	**P**hosphate **b**uffered **s**aline
Cm	**C**enti**m**eter	PCR	**P**olymerase **c**hain **r**eaction
CO_2	**C**arbon di**o**xide	PFA	**Perfluoral**koxy-Polymere
Coa	**Coa**gulase	pmol	**P**ico**mol**ar
CWT	**C**ell **w**all-***t**argeting domain*	Pr	**Pr**omoter
DEPC	**Die**thyl**p**yro**c**arbonat	PSM	**P**henol-**s**oluble **m**odulins
d H_2O	**D**estilled Water	PVDF	**P**oly**v**inyli**d**ene **f**luoride
dNTP	**D**eoxyribo**n**ucleosid **t**risphos**p**hate	PVL	**P**antone-**v**alentine **l**eukocidin
DMEM	**D**ulbecco's **m**odified **e**agle's **m**edium	qRT-PCR	**Q**uantitative **r**eal **t**ime **PCR**
DMSO	**Dim**ethyl **s**ulf**o**xid	RD	**R**egulatory **d**omain
DNA	**D**esoxyribo**n**ucleic **a**cid	R/rev	**Rev**erse
DNase	**D**esoxyribo**n**ukle**ase**	RNA	**R**ibo**n**ucleic **a**cid
DTT	**Di**thio**t**hreitol	RNase	**R**ibo**n**ucle**ase**
Eap	**e**xtracellular **a**dhesive **p**rotein	ROS	**R**eactive **o**xygen **s**pecies
ECL	**E**nhanced **C**hemo**l**uminescence	rpm	**R**evolutions **p**er **m**inute
EDTA	**E**thylen**d**iamin**t**etra**a**cetat	RPMI	**R**oswell **p**ark **m**emorial **i**nstitute
Efb	**E**xtracellular **f**ibrinogen **b**inding protein	rRNA	**R**ibosomal **r**ibo**n**ucleic **a**cid
Emp	**E**xtracellular **m**atrix binding **p**rotein	RT	**R**oom **t**emperature
EtOH	**Et**hanol	SAB	***S. a**ureus* **b**acteremia
expr.	**Expr**ession	S.	***S**taphylococcus*
FCS	**F**etal **c**alf **s**erum	SCCmec	**S**taphylococcal **c**assette **c**hromosome **mec**
Fig	**Fig**ure	SDS	**S**odium **d**odecyl **s**ulfate
Fnb	**F**ibro**n**ectin-**b**inding	sec	**Sec**onds
F/fw	**F**or**w**ard	seq	**Seq**uencing

g	**G**ram	SERAM	**S**ecretable **e**xpanded **r**epertoire **a**dhesive **m**olecules
g	**G**ravitational acceleration	SpA	***S**. arueus* **p**rotein **A**
GFP	**G**reen **f**luorescent **p**rotein	Srr	***S**taphylococcus* **r**espirator **r**esponse
h	**H**ours	SSTIs	**S**kin and **s**oft **t**issue **i**nfection**s**
HCl	**H**ydro**ch**l**o**ric acid	stat	**Stat**ionary growth phase
HEK	**H**uman **e**mbryonic **k**idney	Sup	**Sup**plement
HeLa	Cervical cancer cells taken from **He**nrietta **La**cks	t	**T**ime
His	Hexa**his**tidin	Tab	**Tab**le
H_2O	Water	TAE	**T**RIS-**A**cetat-**E**DTA
H_2O_2	Hydrogen peroxide	Tag	DNA-Polymerase from **T**hermus **aq**uaticus
Hsp	**H**eat **s**hock **p**rotein	TCA	**T**ri**c**arboxylic **a**cid cycle
HTH	**H**elix **t**urn **h**elix	TBS	**T**ris **b**uffered **s**aline
ID	**Id**entificator	TBS-T	**TBS**-**T**ween
Ig	**I**mmuno**g**lobulin	TCS	**T**wo-**c**omponent **s**ystems
IL	**I**nter**l**eucin	TEMED	**Te**tra**m**ethyl**e**thylene**d**iamine
IsdB	**I**ron regulated **s**urface **d**eterminant **B**	Tn	**T**ransposon
kb	**K**ilo **b**ase	Tris	**Tris**(hydroxymethyl)aminometha n
kDA	**K**ilo **Da**lton	TSA	**T**ryptic **s**oy **a**gar
kV	**K**ilo**v**olt	TSB	**T**ryptic **s**oy **b**roth
l	**L**iter	TSS	**T**oxic-**s**hock **s**yndrome
LB	**L**uria-**B**ertani	U	**U**nits
log	**Log**arithmic growth phase	UV	**U**ltra**v**iolet
LTA	**l**ipo**t**eichoic **a**cid	V	**V**olt
LTTR	**L**ysR-**t**ype **t**ranscriptional **r**egulator	v/v	**V**olume per **v**olume
M	**M**olar	vWbp	**V**on **W**illebrand **b**inding **p**rotein
mA/cm^2	**M**ili**a**mper per quadrat **c**enti**m**eter	WT	**W**ild-**t**ype
mg	**M**ili**g**ram	WTA	**W**all **t**eichoic **a**cid
min	**Min**utes	w/v	**W**eight per **v**olume
ml	**M**ili**l**iter	X	X Times
mM	**M**ili**m**olar	YFP	**Y**ellow **f**luorescent **p**rotein
mm	**M**ili**m**eter	µg	Micro**g**ram
MOI	**M**ultiplicity **o**f **i**nfection	µl	Micro**l**iter
MOPS	N-**Mo**rpholino **p**ropane**s**ulfonic acid	µM	Micro**m**olar
mRFP	**M**onomeric **r**ed **f**luorescent **p**rotein	µm	Micro**m**eter
mRNA	**M**essenger RNA	%	Percentage
m/s	**M**eter per **s**econds	°C	Grad celsius
MSCRAMM	**M**icrobial **s**urface **c**omponent **r**ecognizing **a**dhesive **m**atrix **m**olecules	°	Grad
MRSA	**M**ethicillin-**r**esistant ***S**. **a**ureus*	Δ	Delta
MSSA	**M**ethicillin-**s**ensitive ***S**. **a**ureus*	$\Delta\Delta^{CT}$	Delta delta **t**hreshold **c**ycles
mut	**Mut**ant	®	**R**egistered
NaCl	Sodium **ch**l**o**ride (**Na**trium**ch**l**o**rid)	™	**T**rade**m**ark
ng	**N**ano**g**ram		

7.2 List of Figures

7.3 List of Tables

7.4 Supplementary information

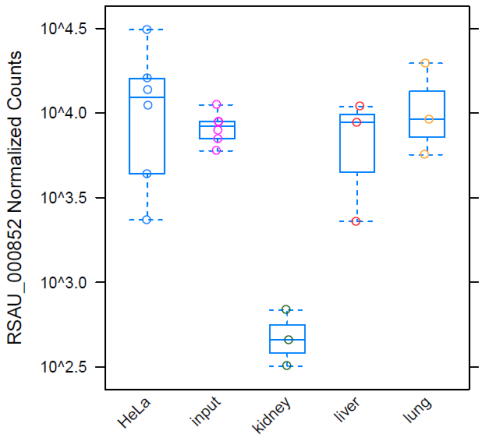

Figure 7.1: Tn-Seq data revealed that mutants in *lttr852* were significantly depleted from the bacteria recovered from the kidneys.
Tn-seq data from mice infected with mutation in *lttr*852, recovered from kidney, liver and lung. Control infection of HeLa cells and number of inputs.

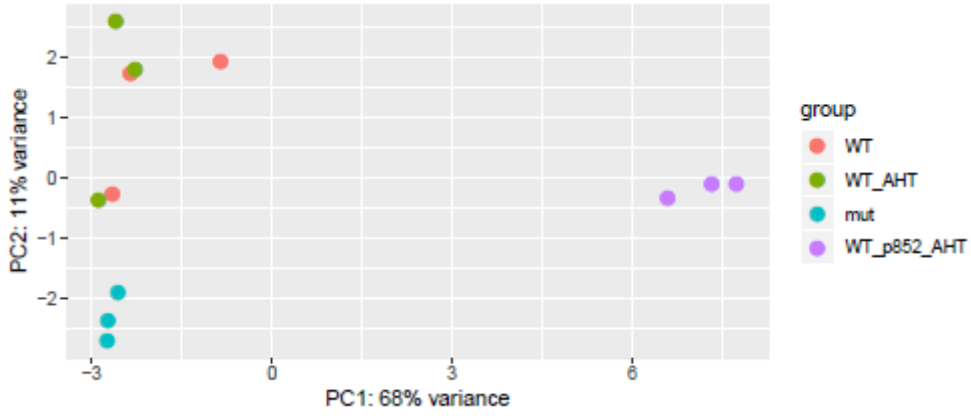

Figure 7.2: Low variance whitin the strains sendet for RNA-Seq.
Measurement of variance between *S. aureus* WT p2085_852 either treated with 200 µg/ml AHT (green) or not (red) and, Δ852 p2085_852 either treated with 200 µg/ml AHT (purple) or not (blue).

Table 7.1: RNA-Seq data of regulated genes upon induced expression of *lttr*852.

S. aureus 6850	Gene	Product	log2FC	p-value
RSAU_002123	ureC	Urease subunit alpha	-1,280811711	3,23884E-28
RSAU_001083	pyrB	Aspartate carbamoyltransferase catalytic	-1,261718567	4,37335E-11
RSAU_001086	carB	Carbamoyl-phosphate synthase large chain	-1,19108872	8,00316E-19
RSAU_002037	lacC	Tagatose-6-phosphate kinase	-1,154477101	9,60133E-08
RSAU_002125	ureF	Urease accessory protein UreF	-1,10935421	3,02249E-15
RSAU_002124	ureE	Urease accessory protein UreE	-1,097385848	1,15717E-09
RSAU_000955	purQ	Phosphoribosylformylglycinamidine synthase I	-1,060211486	3,31278E-09
RSAU_002122	ureB	Urease subunit beta	-1,049115426	8,38139E-08
RSAU_001084	pyrC	Dihydroorotase	-1,020758042	2,12742E-09
RSAU_002034	lacE	PTS system lactose-specific transporter subunit IIBC	-1,012646715	9,687E-08
RSAU_001087	pyrF	Orotidine 5'-phosphate decarboxylase	-0,99478419	1,68102E-09
RSAU_001161	0	Similar to processing proteinase-like protein	-0,969517374	2,4678E-17
RSAU_002074	rplE	50S ribosomal protein L5	-0,935437126	1,97065E-10
RSAU_000069	sirA	Iron-regulated ABC transporter siderophore-binding protein SirA	-0,925484465	7,9243E-06
RSAU_001082	pyrP	Uracil permease	-0,921811459	5,43924E-07
RSAU_001219	guaC	Guanosine monophosphate reductase	-0,910866185	5,23783E-08
RSAU_002077	rpsQ	30S ribosomal protein S17	-0,909500624	1,47027E-08
RSAU_002036	lacD	Tagatose 1,6-diphosphate aldolase	-0,894973483	3,62899E-05
RSAU_001163	phbB	Putative 3-oxoacyl-acyl carrier protein reductase	-0,893707301	2,95586E-17
RSAU_001162	0	Zinc peptidase, M16 family	-0,893220456	1,72753E-20
RSAU_002126	ureG	Urease accessory protein UreG	-0,878416381	1,3143E-12
RSAU_000958	purM	Phosphoribosylformylglycinamidine cyclo-ligase	-0,877178292	1,91117E-05
RSAU_002073	rpsN	30S ribosomal protein S14	-0,851745212	1,58954E-07
RSAU_002078	rpmC	50S ribosomal protein L29	-0,850922956	1,446E-07
RSAU_001064	murD	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate synthetase	-0,844833903	2,1702E-10
RSAU_002127	ureD	Urease accessory protein UreD	-0,840992398	5,84141E-18
RSAU_002079	rplP	50S ribosomal protein L16	-0,838431287	1,11538E-08
RSAU_000115	capM	Capsular polysaccharide synthesis enzyme Cap5M	-0,836806353	7,40096E-08
RSAU_000959	purN	Phosphoribosylglycinamide formyltransferase	-0,826760693	0,000121137
RSAU_001063	mraY	Phospho-N-acetylmuramoyl-pentapeptide-transferase	-0,821862997	2,85529E-08
RSAU_001088	pyrE	Orotate phosphoribosyltransferase	-0,804002823	9,83695E-07
RSAU_002070	rplR	50S ribosomal protein L18	-0,803794789	1,2844E-07
RSAU_001179	mutL	DNA mismatch repair protein mutL	-0,797966612	3,55129E-15
RSAU_002096	pycA	RND multidrug transporter; Acriflavin resistance protein	-0,796793505	1,13878E-09
RSAU_002076	rplN	50S ribosomal protein L14	-0,783499586	7,31594E-07

RSAU_000960	purH	Bifunctional purine biosynthesis protein: phosphoribosylaminoimidazolecarboxamide formyltransferase IMP	-0,781920749	0,000129341
RSAU_001085	pyrAA	Carbamoyl-phosphate synthase small chain	-0,774410914	1,08943E-06
RSAU_002403	crtM	Squalene/phytoene synthase	-0,773748269	7,39441E-07
RSAU_002071	rplF	50S ribosomal protein L6	-0,770008538	7,6645E-09
RSAU_002080	rpsC	30S ribosomal protein S3	-0,753983434	1,81335E-07
RSAU_002081	rplV	50S ribosomal protein L22	-0,742681727	6,20927E-07
RSAU_000657	fadA	UPF0178 protein ECTR2_633	-0,732782432	8,47368E-08
RSAU_000952	purK	Phosphoribosylaminoimidazole carboxylase ATPase subunit	-0,719928242	0,000105573
RSAU_000017	purA	Adenylosuccinate synthetase	-0,707993158	1,85593E-05
RSAU_001117	ftsY	Signal recognition particle-docking protein FtsY	-0,703757125	2,65017E-07
RSAU_002063	rpmJ	50S ribosomal protein L36	-0,700752482	3,45326E-05
RSAU_002064	infA	Translation initiation factor IF-1	-0,698118758	2,81112E-08
RSAU_002409	0	Acetyltransferase, GNAT family protein	-0,696034651	2,11914E-05
RSAU_001598	ftsK	FtsK/SpoIIIE family protein	-0,695865383	8,00141E-09
RSAU_001140	pyrH	Uridylate kinase	-0,692598217	1,51268E-10
RSAU_001237	parC	DNA topoisomerase IV subunit A	-0,687016933	1,09612E-14
RSAU_000944	fmt	Autolysis and methicillin resistant-related protein FmtA	-0,684136794	5,28911E-10
RSAU_000491	rplJ	50S ribosomal protein L10	-0,6807613	4,92261E-06
RSAU_002068	rpmD	50S ribosomal protein L30	-0,680319059	2,94238E-06
RSAU_001062	pbpA	Penicillin-binding protein 1	-0,68008017	5,14255E-09
RSAU_002257	sbi	IgG-binding protein SBI	-0,674576886	0,00011978
RSAU_002402	crtN	Squalene/phytoene synthase	-0,674168092	1,7244E-05
RSAU_002061	rpsK	30S ribosomal protein S11	-0,66504899	1,43357E-06
RSAU_002069	rpsE	30S ribosomal protein S5	-0,662815505	2,26478E-06
RSAU_002065	adk	Adenylate kinase	-0,660650375	8,35662E-09
RSAU_001385	lpdA	Dihydrolipoamide dehydrogenase	-0,6590378	3,66781E-10
RSAU_001116	smc	Chromosome segregation protein SMC	-0,657672571	1,23055E-06
RSAU_002062	rpsM	30S ribosomal protein S13	-0,64599334	3,20484E-07
RSAU_000907	htrA	Serine protease htrA-like protein	-0,638990334	3,76141E-07
RSAU_002151	murI	Similar to membrane-bound protease, CAAX family	-0,630658455	9,61322E-09
RSAU_001546	coaE	Dephospho-CoA kinase	-0,624300274	1,7204E-15
RSAU_000375	lpl5	Uncharacterized lipoprotein SAV0441	-0,616811614	9,11455E-05
RSAU_001383	bfmBAB	Branched-chain alpha-keto acid dehydrogenase, E1 component, beta subunit	-0,605532453	1,58159E-07
RSAU_001795	purB	Adenylosuccinate lyase	-0,60474435	1,36311E-08
RSAU_001081	pyrR	Bifunctional protein pyrimidine operon regulatory protein/uracil phosphoribosyltransferase, PyrR	-0,588665521	3,75126E-12
RSAU_002558	gidB	RRNA small subunit methyltransferase division protein GidB	-0,587903632	1,405E-06

RSAU_002066	secY	Preprotein translocase subunit SecY	-0,586612782	1,42642E-09
RSAU_001791	ligA	DNA ligase, NAD-dependent	-0,585565193	4,2952E-07
RSAU_000493	lukE	Methyltransferase small domain protein	-0,581894627	1,04683E-06
RSAU_000980	pdhC	Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex	-0,581535495	1,06439E-07
RSAU_001132	topA	DNA topoisomerase I	-0,569731918	2,88958E-07
RSAU_001028	mutS2	Recombination and DNA strand exchange inhibitor protein	-0,569650908	1,02478E-06
RSAU_001384	bfmBAA	Branched-chain alpha-keto acid dehydrogenase, E1, alpha subunit	-0,569496915	3,01707E-07
RSAU_001112	fabD	Malonyl CoA-acyl carrier protein transacylase	-0,562298157	1,79054E-08
RSAU_001094	priA	Primosomal protein n'	-0,557166027	2,78879E-09
RSAU_001180	glpP	Glycerol uptake operon antiterminator regulatory protein	-0,555301088	6,46978E-07
RSAU_000430	ksgA	Ribosomal RNA small subunit methyltransferase A	-0,554899696	1,22039E-05
RSAU_001547	mutM	Formamidopyrimidine-DNA glycosylase	-0,554370229	8,37471E-09
RSAU_002067	rplO	50S ribosomal protein L15	-0,550459889	5,36356E-05
RSAU_001931	thiM	Hydroxyethylthiazole kinase	-0,54343153	1,26943E-08
RSAU_001794	0	DNA-binding protein, putative	-0,536327939	1,36592E-06
RSAU_000376	0	Putative membrane protein	-0,529992814	2,21324E-05
RSAU_001065	div1b	Cell division protein FtsQ	-0,528143664	3,66184E-05
RSAU_001827	pmtB	Membrane protein	-0,526117676	7,10862E-05
RSAU_001523	valS	Valyl-tRNA synthetase	-0,525776757	6,2033E-08
RSAU_002113	modA	Molybdenum ABC transporter molybdate-binding protein	-0,523983284	0,000142607
RSAU_002408	oatA	O-acetyltransferase OatA	-0,523450096	6,84775E-12
RSAU_001111	plsX	Putative glycerol-3-phosphate acyltransferase PlsX	-0,523291002	2,19663E-06
RSAU_001518	#NV	#NV	-0,522191798	2,91242E-07
RSAU_001599	pheT2	Phenylalanyl-tRNA synthetase domain protein	-0,518108494	7,88605E-06
RSAU_002060	rpoA	DNA-directed RNA polymerase subunit alpha	-0,516912063	3,18278E-06
RSAU_000117	capO	Capsular polysaccharide synthesis UDP-N-acetyl-D dehydrogenase	-0,516420618	3,28955E-05
RSAU_001314	0	Putative dynamin family protein	-0,516054394	3,86417E-08
RSAU_000752	tpiA	Triosephosphate isomerase	-0,512440961	5,17005E-05
RSAU_001942	atpG	F0F1 ATP synthase subunit gamma	-0,511330484	3,13938E-05
RSAU_000812	dltD	D-lipoteichoic acid biosynthesis protein DltD	-0,510748329	5,80606E-06
RSAU_000676	nagA	N-acetylglucosamine-6-phosphate deacetylase	-0,510330167	2,23086E-08
RSAU_000753	pgm	2,3-bisphosphoglycerate-independent phosphoglycerate mutase	-0,506368328	5,97033E-06
RSAU_000544	mvaK2	Phosphomevalonate kinase	-0,504908907	9,62143E-09
RSAU_001178	mutS	DNA mismatch repair protein MutS	-0,504382108	1,4279E-08

RSAU_000810	dltB	D-alanine transfer protein DltB	-0,500484523	4,22747E-08
RSAU_001965	pyrG	CTP synthase	-0,496535648	3,82644E-06
RSAU_001388	ispA	Geranyltranstransferase	-0,49130815	1,005E-06
RSAU_001702	0	DNA double-strand break repair Rad50 ATPase	-0,48914827	4,77549E-07
RSAU_001256	femB	Aminoacyltransferase FemB methicillin resistance	-0,487852891	1,17315E-12
RSAU_002059	rplQ	50S ribosomal protein L17	-0,467730287	1,77741E-06
RSAU_001933	tenA	Putative thiaminase-2	-0,465944782	3,73619E-05
RSAU_001787	gatA	Aspartyl/glutamyl-tRNA amidotransferase subunit A	-0,462991041	5,25277E-08
RSAU_000818	kdpE	Putative pyridine nucleotide-disulfide oxidoreductase	-0,461812763	3,17839E-11
RSAU_000697	opuBB	Glycine/betaine ABC transporter permease	-0,461253619	3,75108E-07
RSAU_001522	folC	Dihydrofolate synthase / Folylpolyglutamate synthase	-0,461179899	9,85671E-06
RSAU_000443	0	Polysaccharide biosynthesis family protein	-0,460669527	0,000125219
RSAU_002559	gidA	TRNA uridine 5-carboxymethylaminomethyl modification enzyme GidA	-0,46016947	3,34564E-05
RSAU_001141	frr	Ribosome recycling factor	-0,460154126	7,2612E-06
RSAU_002032	0	Alpha/beta cell surface hydrolase	-0,459272964	3,15699E-05
RSAU_001480	alaS	Alanyl-tRNA synthetase	-0,454475582	7,13087E-06
RSAU_001313	ypcP	5'-3' exonuclease	-0,454118652	2,38124E-08
RSAU_001432	era	GTP-binding protein Era	-0,454016039	0,000102953
RSAU_001402	gcvPA	Glycine dehydrogenase, subunit 1 cleavage system	-0,45368956	1,05819E-06
RSAU_000118	capP	Capsular polysaccharide synthesis UDP-N-acetylglucosamine 2-epimerase	-0,45163854	1,00501E-05
RSAU_002097	fmhB	Peptidoglycan pentaglycine interpeptide biosynthesis protein FmhB	-0,450004308	4,03929E-09
RSAU_001790	camS	Putative pheromone cAM373 lipoprotein CamS	-0,446616497	6,71552E-06
RSAU_001457	cysE	Similar to hydrolase (HAD superfamily)	-0,445222739	0,000106046
RSAU_001701	cbf1	3'-5' exoribonuclease yhaM	-0,444784529	4,84523E-07
RSAU_001386	recN	DNA repair protein RecN	-0,443681542	1,34024E-06
RSAU_001903	tex	S1 RNA binding domain protein	-0,443249666	3,86394E-06
RSAU_000808	gyaR	D-isomer specific 2-hydroxyacid dehydrogenase protein	-0,440853761	1,52641E-06
RSAU_001548	polA	DNA polymerase I	-0,434536102	1,1527E-08
RSAU_001535	0	Predicted in CGM	-0,433987306	3,52457E-05
RSAU_001133	gid	Methylenetetrahydrofolate--tRNA-(uracil-5-)- methyltransferase TrmFO	-0,429398578	2,91661E-07
RSAU_000793	sufD	FeS assembly protein SufD	-0,428382494	2,74137E-05
RSAU_000188	rihA	Inosine-uridine preferring nucleoside hydrolase	-0,425515267	3,68621E-05
RSAU_001932	thiD	Phosphomethylpyrimidine kinase	-0,423137017	3,76096E-05
RSAU_000794	sufS	Cysteine desulfurase, SufS subfamily	-0,422036192	4,5563E-05
RSAU_000839	pgi	Glucose-6-phosphate isomerase	-0,420517251	1,51873E-08

RSAU_000627	dhaL	Dihydroxyacetone kinase subunit DhaL	-0,417790547	5,24877E-05
RSAU_001164	0	ACT domain-containing protein	-0,416581674	7,45645E-07
RSAU_000308	ychF	GTP-dependent nucleic acid-binding protein engD	-0,415361459	4,24082E-05
RSAU_000979	pdhB	Pyruvate dehydrogenase E1 component beta subunit	-0,414003613	7,56829E-05
RSAU_000807	nagD	Haloacid dehalogenase-like hydrolase	-0,4126895	0,000106475
RSAU_001615	leuS	Leucyl-tRNA synthetase	-0,411367031	6,6467E-05
RSAU_000905	prfC	Peptide chain release factor 3	-0,411030144	1,02449E-05
RSAU_001159	ftsK	DNA translocase FtsK/SpoIIIE family protein	-0,410770984	7,79015E-05
RSAU_001597	murC	UDP-N-acetylmuramate--L-alanine ligase	-0,405909737	1,24724E-09
RSAU_001220	narI	SCP-like extracellular family protein	-0,405029694	4,51268E-05
RSAU_001692	hemH	Ferrochelatase	-0,400732695	9,88192E-05
RSAU_001584	htrA1	Putative serine protease do-like htrA	-0,39642832	3,79073E-09
RSAU_001706	0	Cro family transcriptional regulator	-0,394464747	0,000151131
RSAU_001534	tig	Trigger factor	-0,39443859	5,86949E-07
RSAU_001481	recD	Helicase RecD/TraA family protein	-0,388453621	3,31756E-06
RSAU_000795	iscU	SUF system FeS assembly protein, NifU family	-0,383533929	5,1498E-05
RSAU_001074	ileS	Isoleucyl-tRNA synthetase	-0,383300646	2,57332E-08
RSAU_001119	ffh	Signal recognition particle protein	-0,3743121	9,06149E-06
RSAU_000976	def	Peptide deformylase 2	-0,371957975	7,9414E-05
RSAU_001344	gpsA	NAD(P)H-dependent glycerol-3-phosphate dehydrogenase	-0,368227075	1,58669E-05
RSAU_000943	nanE	Cell envelope-related transcriptional attenuator domain protein	-0,367581704	3,61678E-05
RSAU_001786	gatB	Aspartyl/glutamyl-tRNA amidotransferase subunit B	-0,367280422	1,04497E-06
RSAU_001959	rho	Transcription termination factor Rho	-0,364748839	2,13831E-05
RSAU_001559	maeB	NADP-dependent malic enzyme, malate dehydrogenase	-0,358143687	7,40238E-05
RSAU_001941	atpD	F0F1 ATP synthase subunit beta	-0,356271711	8,37648E-05
RSAU_000754	eno	Phosphopyruvate hydratase	-0,354422133	5,26416E-05
RSAU_001393	accC	Acetyl-CoA carboxylase biotin carboxylase subunit	-0,35341953	9,64312E-06
RSAU_002557	yyaA	DNA-binding protein Spo0j-like protein	-0,349081752	4,09045E-05
RSAU_000693	ltaS	Glycerol phosphate lipoteichoic acid synthase	-0,336987166	7,66257E-06
RSAU_000942	0	Transcriptional regulator, LytR family protein	-0,322032767	1,56244E-05
RSAU_000588	mntB	ABC transporter permease protein	-0,322031139	4,28958E-06
RSAU_000796	sufB	FeS assembly protein SufB	-0,31788269	9,34565E-06
RSAU_001255	femA	Aminoacyltransferase FemA essential for expression of methicillin resistance	-0,298604224	1,23087E-05
RSAU_000427	metG	Methionyl-tRNA synthetase	-0,29424675	3,11693E-06
RSAU_000728	secA	Preprotein translocase subunit SecA	-0,266652923	2,81782E-05
RSAU_002357	fbp	Fructose-1,6-bisphosphatase class 3	0,246521096	4,58221E-05

RSAU_000829	mnhA	Monovalent cation/H+ antiporter subunit A	0,278190112	2,42101E-05
RSAU_001494	dtd	D-tyrosyl-tRNA(Tyr) deacylase	0,282147832	7,75961E-05
RSAU_000494	rpoB	DNA-directed RNA polymerase subunit beta	0,29149538	4,2046E-05
RSAU_000599	abcA	ABC ATP binding cassette transporter, ABC protein	0,301706538	9,2412E-05
RSAU_000948	qoxA	Quinol oxidase polypeptide II	0,312167128	4,87405E-05
RSAU_000974	rnjA	Ribonuclease J 1	0,315907888	1,743E-06
RSAU_001240	dsbA	Putative membrane protein	0,319831663	0,000120302
RSAU_000847	cdr	Coenzyme A disulfide reductase	0,324818248	2,20353E-05
RSAU_001032	sdhA	Succinate dehydrogenase flavoprotein subunit	0,333307446	2,06041E-07
RSAU_000501	0	Putative N-acyl-L-amino acid amidohydrolase	0,336861756	0,000108586
RSAU_001963	fba	Fructose-bisphosphate aldolase	0,343188684	5,83387E-06
RSAU_001589	acsA	Acetyl-coenzyme A synthetase-CoA	0,347982599	5,34018E-06
RSAU_001592	ccpA	Catabolite control protein A	0,353277138	2,86382E-05
RSAU_000520	folE2	GTP cyclohydrolase folE2	0,360889208	3,9528E-07
RSAU_001478	yrrK	Holliday junction resolvase-like protein	0,362868463	1,98081E-06
RSAU_000333	pbuX	Xanthine permease	0,365580429	2,9635E-06
RSAU_001756	yfhP	Membrane-bound metal-dependent hydrolase, putative	0,365622759	1,48661E-05
RSAU_001543	dnaB	Chromosome replication initiation and membrane attachment protein	0,371532873	9,4654E-05
RSAU_000803	lipA	Lipoyl synthase	0,372333241	1,49351E-09
RSAU_001035	rdgB	Non-canonical purine NTP pyrophosphatase	0,379728717	2,74701E-07
RSAU_000825	mnhE	Monovalent cation/H+ antiporter subunit E	0,380322868	0,000140304
RSAU_001591	acuC	Acetoin utilization protein AcuC	0,382330621	8,85596E-06
RSAU_000874	mecA	Negative regulator of genetic competence MecA	0,383993225	1,94458E-05
RSAU_002358	0	Membrane protein, putative	0,384222146	2,18247E-05
RSAU_000061	nptA	Na+/Pi-cotransporter family protein	0,384875273	2,33633E-06
RSAU_001606	vraG	Phosphotransferase enzyme family protein	0,386231275	1,78421E-06
RSAU_001617	0	Putative Fe-S oxidoreductase	0,3866793	2,5562E-05
RSAU_001002	lacR	Putative Heme biosynthesis related protein	0,388078284	0,000122265
RSAU_001637	tal	Transaldolase, putative	0,390241772	1,16893E-05
RSAU_000945	qoxD	Quinol oxidase polypeptide IV	0,391119491	4,11402E-05
RSAU_001489	rarA	ATPase, AAA family protein	0,393439001	2,19087E-06
RSAU_002170	lyrA	Lysostaphin resistance protein A	0,39458346	7,23448E-11
RSAU_000600	nupC2	Na+ dependent nucleoside transporter protein	0,396141291	2,65018E-05
RSAU_001418	sodA	Superoxide dismutase	0,397325189	1,93973E-05
RSAU_001636	#NV	#NV	0,398494528	2,45676E-05
RSAU_002276	0	Peptidase C39 like family protein	0,399672324	8,39151E-05
RSAU_002225	hsp20	Hsp20 small heat shock protein, putative	0,401316734	0,00010958
RSAU_002349	0	Major facilitator transporter, glucarate	0,40530678	3,49814E-05
RSAU_001036	rpmJ	Phosphoesterase family protein	0,412159273	2,11544E-08

RSAU_001332	0	Neutral zinc metallopeptidase family protein	0,413230924	2,90906E-05
RSAU_000671	0	Putative exported protein	0,414259998	7,21984E-05
RSAU_000335	guaA	GMP synthase	0,416755513	9,89626E-08
RSAU_000149	oppF	Oligopeptide ABC transporter ATP-binding protein	0,416770978	6,99227E-07
RSAU_002150	0	DeoR helix-turn-helix domain protein	0,420880637	2,80651E-05
RSAU_001056	rpsQ	Haloacid dehalogenase-like hydrolase	0,426550853	9,95981E-05
RSAU_001698	gvpP	Gas vesicle protein	0,428648411	4,52128E-06
RSAU_000521	bshB2	Putative GlcNAc-PI de-N-acetylase family protein	0,453972439	3,47329E-10
RSAU_001748	perR	Peroxide regulator PerR, FUR family	0,455424587	4,22537E-05
RSAU_001354	fer	Ferredoxin	0,455948837	0,000100232
RSAU_002137	fda	D-isomer specific 2-hydroxyacid dehydrogenase	0,45834739	7,10894E-08
RSAU_001616	0	Multidrug resistance transporter protein B	0,458546433	4,12449E-05
RSAU_000895	0	2',5' RNA ligase protein	0,458672086	4,41088E-07
RSAU_001810	blaR1	Beta-lactamase regulatory protein BlaR1	0,458862105	3,61405E-06
RSAU_001089	moaD	3-demethylubiquinone-9 3-methyltransferase protein	0,463270344	8,14308E-07
RSAU_002484	manP	PTS system, fructose-specific IIABC component	0,463544526	4,9745E-05
RSAU_001477	0	YrzB like protein	0,465073085	5,37223E-10
RSAU_002108	moaC	Molybdenum cofactor biosynthesis protein C	0,466093919	3,27385E-05
RSAU_000130	ausA	Non-ribosomal peptide synthetase	0,466489224	5,9437E-06
RSAU_000503	hchA	Chaperone protein HchA	0,466690591	3,38452E-07
RSAU_000804	rsbU	Hypothetical DUF1027 domain protein	0,469004187	5,68794E-06
RSAU_002384	amiD2	Secretory antigen SsaA protein	0,469107481	7,75374E-07
RSAU_002541	rarD	RarD protein	0,470787209	0,000105825
RSAU_001277	hipO	Hippurate hydrolase	0,473523915	3,84187E-05
RSAU_001593	aroA	Bifunctional 3-deoxy-7-phosphoheptulonate synthase/chorismate mutase	0,473684825	3,05289E-08
RSAU_001001	ctaB	Protoheme IX farnesyltransferase	0,475803548	3,54185E-05
RSAU_000629	0	FIG01107983: hypothetical protein	0,480623008	1,08454E-06
RSAU_000300	0	Cyclase family protein	0,487131391	0,00015284
RSAU_000817	sufA	Iron-sulfur cluster assembly accessory family protein	0,487460339	6,62917E-07
RSAU_000585	gatB	Sodium/hydrogen exchanger family protein	0,491793894	4,42707E-11
RSAU_001215	lysP2	Amino acid permease	0,495891762	2,90856E-09
RSAU_001380	0	Disulfide isomerase, putative	0,496260299	3,81813E-05
RSAU_001525	amoA	Membrane AbrB duplication domain protein	0,498843134	1,87218E-05
RSAU_000252	brnQ	Branched-chain amino acid transport system II carrier protein	0,505962447	5,48597E-08
RSAU_001935	ssb	Single-stranded DNA-binding protein	0,507144834	1,26864E-05

RSAU_000081	butA	Dehydrogenase-acetoin / Acetoin reductase	0,509165308	1,82027E-07
RSAU_000222	lytM	Peptidoglycan hydrolase	0,510737433	9,39133E-07
RSAU_000154	0	Staphylococcal tandem lipoprotein	0,51330342	3,78522E-05
RSAU_001400	0	Rhodanese-like domain-containing protein	0,515280119	0,00012732
RSAU_000756	secG	Preprotein translocase subunit SecG	0,51589058	2,453E-07
RSAU_001986	#NV	#NV	0,521503924	1,99031E-06
RSAU_001580	serA	D-3-phosphoglycerate dehydrogenase	0,522403631	9,65344E-06
RSAU_001567	uspA_1	Universal stress protein family	0,523756428	2,11805E-06
RSAU_001780	ftnA	Ferritin protein	0,528672687	1,78499E-05
RSAU_000131	acpS	4'-phosphopantetheinyl transferase protein	0,52931929	2,59323E-06
RSAU_001057	0	Acetyltransferase family protein	0,539118139	9,23557E-06
RSAU_001718	pfoS/R	Phosphotransferase system, EIIC protein	0,558557476	3,91534E-07
RSAU_001376	cysM	Glyoxalase/bleomycin resistance protein/dioxygenase superfamily protein	0,559606943	5,53024E-06
RSAU_001230	mscL	Large conductance mechanosensitive channel protein	0,562072372	3,16012E-06
RSAU_002167	hutU	Urocanate hydratase	0,562284397	4,48213E-09
RSAU_000739	trxB	Thioredoxin reductase	0,564467617	8,86058E-10
RSAU_002245	gmk	Addiction module toxin Txe/YoeB family protein	0,566899794	6,24797E-06
RSAU_002172	0	MOSC domain-containing protein	0,568211676	5,01291E-06
RSAU_001544	nrdR	Transcriptional regulator NrdR	0,568626141	2,31796E-08
RSAU_000776	bglA	LysE type translocator family protein	0,569937601	2,14605E-05
RSAU_001789	putP	High affinity proline permease	0,572538271	2,17583E-09
RSAU_001628	arsB	Arsenical pump membrane protein efflux pump	0,575639333	1,13945E-06
RSAU_001865	amiE	Carbon-nitrogen hydrolase family protein	0,581417015	5,50888E-09
RSAU_001404	aroK	Shikimate kinase	0,582620038	3,45636E-06
RSAU_000775	gpmA1	Phosphoglycerate mutase family protein	0,582932479	3,34877E-07
RSAU_002274	bcr	Drug resistance transporter protein	0,584340023	1,12657E-07
RSAU_000381	psmA4	Phenol-soluble modulin alpha 4	0,585022631	6,05948E-09
RSAU_000662	mgrA	HTH-type transcriptional regulator mgrA	0,585690944	1,60279E-09
RSAU_002540	0	2-oxoglutarate/malate translocator protein	0,587944635	2,29862E-06
RSAU_001579	0	Aminotransferase class-V protein	0,591565849	4,17257E-06
RSAU_000405	gltB	Glutamate synthase large subunit	0,593171359	9,03928E-05
RSAU_002419	0	Short chain dehydrogenase oxidoreductase	0,594987432	5,65364E-06
RSAU_000891	agcS	Sodium/alanine symporter family protein	0,597827167	1,1077E-05
RSAU_000988	0	Putative membrane protein	0,606335421	3,4523E-14
RSAU_000727	yfiA	Modulation protein/ribosomal protein S30EA	0,614817596	1,35462E-08
RSAU_000140	ptsG	Pts system, glucose-specific iiabc component	0,622896748	2,68668E-17
RSAU_001361	rluB	Ribosomal large subunit pseudouridine synthase B	0,630648395	3,51333E-11
RSAU_001221	lexA	Repressor LexA	0,633718444	2,89242E-11
RSAU_002166	hutI	Imidazolonepropionase	0,653926927	4,50444E-14

RSAU_002165	hipO2	N-L-amino acid amidohydrolase	0,655584274	6,66182E-05
RSAU_001055	psmB2	Antibacterial protein (phenol soluble modulin)	0,656118583	2,30102E-06
RSAU_001983	czrB	Cation-efflux system membrane protein	0,658662103	2,27246E-14
RSAU_000431	veg	Veg protein	0,661544136	4,97223E-06
RSAU_000129	glyS	Major facilitator transporter	0,662554044	2,0285E-15
RSAU_001987	#NV	#NV	0,665236679	0,000101721
RSAU_001276	dapD	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-acetyltransferase	0,667899333	1,50134E-06
RSAU_000641	amiD7	Secretory antigen SsaA-like protein	0,678213181	5,27273E-12
RSAU_000698	hisC	Histidinol-phosphate aminotransferase	0,678353564	1,73003E-09
RSAU_001811	blaZ	Beta-lactamase	0,678809398	2,85755E-08
RSAU_001282	cspA	Cold shock protein CspA	0,682027359	3,98511E-07
RSAU_001783	gapR	Hypothetical protein SAV1896	0,683290361	2,47286E-15
RSAU_001054	psmB1	Antibacterial protein (phenol soluble modulin)	0,688621906	7,31182E-06
RSAU_000699	0	Putative 5'(3')-deoxyribonucleotidase	0,691361821	5,35291E-10
RSAU_000012	metX	Homoserine O-acetyltransferase, putative	0,6923267	6,52499E-05
RSAU_002224	sarZ	HTH-type transcriptional regulator sarZ	0,698588347	8,94363E-13
RSAU_000861	oppC	Oligopeptide transport system permease protein oppC	0,70233318	6,88277E-06
RSAU_001362	scpB	Segregation and condensation protein B	0,703510126	3,43731E-09
RSAU_002363	sasA	Glyoxalase/bleomycin resistance protein/dioxygenase superfamily protein	0,707878384	4,58666E-13
RSAU_000366	argC	Putative exported protein	0,714745349	1,14336E-05
RSAU_000937	aspC	Putative aminotransferase	0,720433044	8,82644E-12
RSAU_000470	pdxS	Pyridoxal biosynthesis lyase PdxS	0,729722909	1,16651E-09
RSAU_000363	#NV	#NV	0,734709233	1,42558E-14
RSAU_000577	0	Phage integrase family integrase/recombinase	0,739170147	2,55607E-05
RSAU_001274	dapA	Dihydrodipicolinate synthase	0,740084894	4,79518E-08
RSAU_000787	metN2	Methionine ABC transporter ATP-binding protein	0,742384682	2,43352E-06
RSAU_000406	gltD	Glutamate synthase small subunit	0,745328805	9,39112E-06
RSAU_000873	spxA	Spx family transcriptional regulator	0,7517309	2,31373E-14
RSAU_000724	0	DegV family domain-containing protein	0,756798807	2,64467E-19
RSAU_002498	arlS	Flavin reductase family, FMN-binding protein	0,761296514	2,83858E-13
RSAU_002454	cudC	Transcriptional regulator, putative	0,775051542	2,37164E-08
RSAU_000770	cspC	Cold-shock protein	0,77554205	7,95309E-06
RSAU_002418	acrR	Transcriptional regulator TetR family protein	0,776152667	2,99041E-05
RSAU_000011	azlD	Branched-chain amino acid transport protein, AzlD	0,776387643	8,00013E-06
RSAU_001819	0	Exported protein	0,77873492	3,3137E-10
RSAU_002411	pfoS/R	Membrane regulatory protein	0,782306994	5,5472E-05
RSAU_000060	norC	Major facilitator transporter	0,783785298	4,77834E-06

RSAU_001275	dapB	Dihydrodipicolinate reductase	0,792850311	3,21873E-08
RSAU_001581	tagD	Hydrolase, haloacid dehalogenase-like family protein	0,798409087	3,13942E-10
RSAU_000155	acpD	FMN-dependent NADH-azoreductase	0,799199933	1,31542E-17
RSAU_000546	merA	Putative; Mercuric reductase pyridine nucleotide-disulfide oxidoreductase	0,813952833	1,23795E-09
RSAU_000769	nuc	Thermonuclease precursor	0,828980986	2,3215E-05
RSAU_001363	scpA	Segregation and condensation protein A	0,831165712	1,90368E-15
RSAU_000857	eap	Putative cell surface protein, Map-w protein	0,832861343	3,44328E-12
RSAU_002311	ydaH	Putative aminobenzoyl-glutamate transport protein	0,841753207	9,43102E-17
RSAU_001273	asd	Aspartate-semialdehyde dehydrogenase	0,844624492	7,32483E-10
RSAU_001181	#NV	#NV	0,851094015	1,30265E-05
RSAU_002456	cudT	Transporter, betaine/carnitine/choline transporter family	0,851334804	9,76509E-05
RSAU_001891	leuB	3-isopropylmalate dehydrogenase	0,86005464	8,30936E-05
RSAU_000788	metI	ABC transporter permease protein	0,864726205	6,05269E-08
RSAU_000860	oppB	Oligopeptide transport system permease protein	0,86916917	1,68616E-10
RSAU_001893	leuD	3-isopropylmalate dehydratase small subunit	0,878756194	4,7631E-05
RSAU_001864	0	Nitroreductase family protein	0,880239329	9,65637E-19
RSAU_000287	engB	Ribosomal-protein-serine N-acetyltransferase	0,890765691	3,35449E-12
RSAU_000816	kdpA	Putative DUF1450-containing protein	0,902353661	3,35955E-07
RSAU_000471	pdxT	Glutamine amidotransferase subunit PdxT	0,902417489	6,15882E-18
RSAU_000362	0	Hypothetical protein	0,918983608	3,28318E-13
RSAU_001961	0	HxlR family transcriptional regulator	0,920885937	1,33528E-14
rsaD	#NV	#NV	0,944756708	1,59655E-06
RSAU_002531	0	S-adenosyl-l-methionine hydroxide adenosyltransferase family protein	0,961778104	7,93208E-06
RSAU_002177	0	Membrane protein	0,966044543	1,75408E-09
RSAU_000395	metI	Binding-protein-dependent transport system inner membrane protein	1,001876704	5,10668E-08
RSAU_001515	cadA	Cadmium-transporting ATPase	1,051831873	1,13353E-15
RSAU_001894	ilvA	Threonine dehydratase biosynthetic	1,06517764	5,58623E-08
RSAU_001866	#NV	#NV	1,092955056	1,27561E-22
RSAU_000010	azlC	Branched-chain amino acid protein azlC	1,120017868	2,05499E-11
RSAU_002444	0	Amino acid permease family protein	1,149853015	3,69535E-14
RSAU_001892	leuC	3-isopropylmalate dehydratase large subunit	1,160854039	1,05117E-08
RSAU_002532	yceI	YceI-like domain protein	1,167873034	1,84398E-33
RSAU_001889	ilvC	Ketol-acid reductoisomerase	1,171703427	8,00433E-08
RSAU_000396	0	Methionine ABC transporter, substrate-binding protein, putative	1,189463046	1,95343E-12
RSAU_000209	lrgA	Holin-like murein hydrolase regulator LrgA	1,208557342	2,17377E-14
RSAU_000265	sirA	Nucleoside recognition domain protein	1,213503145	3,60927E-15

RSAU_000394	metN	Methionine ABC transporter ATP-binding protein	1,216942296	1,18527E-13
RSAU_002398	copA	Copper-translocating P-type ATPase	1,235378921	6,01421E-46
RSAU_002399	copZ	Copper chaperone copZ-ion-binding	1,258851983	1,9926E-24
RSAU_001890	leuA	2-isopropylmalate synthase	1,319798638	7,86675E-10
RSAU_000210	lrgB	Antiholin-like protein LrgB	1,456590003	4,158E-25
RSAU_001242	msrA	Peptide methionine sulfoxide reductase MsrA	1,4663958	3,60108E-61
RSAU_002445	gabT	4-aminobutyrate aminotransferase	1,491176812	6,63775E-16
RSAU_001887	ilvB	Acetolactate synthase large subunit	1,55117472	2,16427E-15
RSAU_001886	ilvD	Dihydroxy-acid dehydratase	1,699098939	1,50666E-17
RSAU_000852		LysR family regulatory protein	12,71327807	0

Table 7.2: Evaluation of Biolog plates of substances which are more sensitive metabolized by *S. aureus* 6850 wild-type.

Plate	Source	Well number	Substance	p-value
PM08	N-Source	A12	Gly-Asn	5117,00
PM02	C-Source	D12	Butyric Acid	5018,00
PM08	N-Source	A10	Asp-Gly	4673,00
PM04	S-Source	F11	Cysteamine	3952,00
PM04	S-Source	F09	L-Cysteinyl-Glycine	3871,00
PM04	S-Source	G09	Glycyl-L-Methionine	3780,00
PM08	N-Source	D12	Thr-Gln	3690,00
PM04	P-Source	A12	Adenosine- 3,5-Cyclic Monophosphate	3486,00
PM04	S-Source	F08	D-Cysteine	3445,00
PM03	N-Source	A12	L-Glutamic Acid	3429,00
PM06	N-Source	A07	Ala-Gly	3412,00
PM04	S-Source	G12	L-Methionine Sulfone	3407,00
PM08	N-Source	A11	Glu-Ala	3157,00
PM08	N-Source	C12	Pro-Asn	3097,00
PM04	S-Source	H12	Tetramethylene Sulfone	2975,00
PM06	N-Source	D12	Glu-Tyr	2919,00
PM04	S-Source	F07	L-Cysteine	2826,00
PM02	C-Source	A10	Laminarin	2778,00
PM08	N-Source	F11	D-Leu-Gly	2720,00
PM01	C-Source	A12	Dulcitol	2608,00
PM08	N-Source	D11	Thr-Asp	2501,00
PM06	N-Source	E09	Gly-Met	2465,00
PM06	N-Source	E12	Gly-Ser	2396,00
PM08	N-Source	F08	D-Ala-Gly	2379,00
PM01	C-Source	A10	D-Trehalose	2341,00
PM04	S-Source	F12	L-Cysteine Sulfinic Acid	2324,00
PM04	S-Source	F03	Thiosulfate	2281,00
PM04	S-Source	G02	S-Methyl-L-Cysteine	2255,00

PM08	N-Source	C06	Phe-Glu	2220,00
PM06	N-Source	D09	Glu-Gly	2196,00
PM06	N-Source	D06	Gln-Gly	2146,00
PM06	N-Source	A12	Ala-Pro	2118,00
PM01	C-Source	B08	D-Xylose	2044,00
PM07	N-Source	A12	Lys-Phe	2038,00
PM04	S-Source	G01	N-Acetyl-L-Cysteine	2038,00
PM04	P-Source	A11	Adenosine- 2,3-Cyclic Monophosphate	2036,00
PM07	N-Source	C10	Phe-Gly	1989,00
PM08	N-Source	A09	Asp-Gln	1985,00
PM07	N-Source	E11	Thr-Ala	1974,00
PM02	C-Source	A09	Inulin	1957,00
PM02	C-Source	E12	5-Keto-D-Gluconic Acid	1949,00
PM04	S-Source	H11	Methane Sulfonic Acid	1880,00
PM04	S-Source	F05	Thiophosphate	1872,00
PM06	N-Source	D07	Glu-Asp	1860,00
PM07	N-Source	D12	Pro-Tyr	1829,00
PM06	N-Source	D11	Glu-Trp	1762,00
PM04	P-Source	B12	Guanosine- 3,5-Cyclic Monophosphate	1742,00
PM06	N-Source	E10	Gly-Phe	1730,00
PM01	C-Source	A07	L-Aspartic Acid	1702,00
PM02	C-Source	C08	3-Methyl Glucose	1691,00
PM01	C-Source	E09	Adonitol /Xylitol/ Ribitol	1691,00
PM07	N-Source	D07	Pro-Gly	1656,00
PM02	C-Source	A07	Gelatin	1622,00
PM04	S-Source	F06	Dithiophosphate	1597,00
PM02	C-Source	A08	Glycogen	1586,00
PM06	N-Source	D08	Glu-Glu	1567,00
PM06	N-Source	E07	Gly-Leu	1560,00
PM08	N-Source	A02	L-Glutamine	1527,00
PM07	N-Source	B11	Met-Gly	1527,00
PM02	C-Source	C12	Palatinose	1499,00
PM07	N-Source	F10	Trp-Gly	1492,00
PM03	N-Source	B11	L-Threonine	1468,00
PM08	N-Source	D10	Ser-Glu	1468,00
PM04	P-Source	B10	Guanosine- 5-Monophosphate	1448,00
PM01	C-Source	D08	a-Methyl-D-Galactoside	1441,00
PM08	N-Source	G12	D-Ala-Gly-Gly	1426,00
PM01	C-Source	B10	Formic Acid	1406,00
PM02	C-Source	F10	Succinamic Acid	1392,00
PM04	S-Source	G04	Lanthionine	1385,00
PM02	C-Source	E10	b-Hydroxy-Pyruvic Acid	1371,00
PM02	C-Source	D06	D-Tagatose	1360,00

PM04	P-Source	A10	Adenosine- 5-Monophosphate	1357,00
PM02	C-Source	A11	Mannan	1346,00
PM01	C-Source	A08	L-Proline	1328,00
PM02	C-Source	A06	Dextrin	1318,00
PM04	P-Source	A03	Pyrophosphate	1302,00
PM01	C-Source	B09	L-Lactic Acid	1290,00
PM01	C-Source	D09	a-D-Lactose	1269,00
PM06	N-Source	E08	Gly-Lys	1262,00
PM04	S-Source	G03	Cystathionine	1259,00
PM07	N-Source	B12	Met-His	1257,00
PM01	C-Source	C06	L-Rhamnose	1254,00
PM07	N-Source	A02	L-Glutamine	1239,00
PM04	P-Source	C10	Cytidine- 5- Monophosphate	1236,00
PM04	P-Source	E09	Thymidine- 3-Monophosphate	1235,00
PM04	S-Source	H07	Hypotaurine	1227,00
PM04	S-Source	H04	D,L-Lipoamide	1220,00
PM04	P-Source	E12	Thymidine 3 ,5 - Cyclic Monophosphate	1216,00
PM06	N-Source	B12	Arg-Lys	1195,00
PM02	C-Source	B09	2-Deoxy-D-Ribose	1193,00
PM02	C-Source	B06	D-Arabitol	1193,00
PM04	S-Source	G11	L-Methionine Sulfoxide	1188,00
PM02	C-Source	A12	Pectin	1185,00
PM04	S-Source	H10	2-Hydroxyethane Sulfonic Acid	1176,00
PM01	C-Source	A09	D-Alanine	1152,00
PM04	P-Source	D10	Uridine- 5 - Monophosphate	1147,00
PM08	N-Source	H11	Phe-Gly-Gly	1136,00
PM02	C-Source	E09	g-Hydroxy-Butyric Acid	1135,00
PM04	P-Source	D07	O-Phospho-L-Threonine	1134,00
PM01	C-Source	C12	Thymidine	1115,00
PM01	C-Source	F08	Mucic Acid	1111,00
PM07	N-Source	B03	Lys-Thr	1108,00
PM07	N-Source	D06	Pro-Gln	1107,00
PM03	N-Source	B12	L-Tryptophan	1098,00
PM08	N-Source	B12	Lys-Asp	1094,00
PM01	C-Source	B06	D-Gluconic Acid	1090,00
PM07	N-Source	F04	Thr-Met	1077,00
PM02	C-Source	E08	b-Hydroxy-Butyric Acid	1075,00
PM07	N-Source	F05	Thr-Pro	1061,00
PM06	N-Source	C12	Asp-Lys	1059,00
PM01	C-Source	F11	D-Cellobiose	1055,00
PM01	C-Source	E12	Adenosine	1047,00
PM04	P-Source	B07	D-3-Phospho-Glyceric Acid	1035,00
PM06	N-Source	E06	Gly-His	1035,00

PM04	P-Source	B06	D-2-Phospho-Glyceric Acid	1014,00
PM07	N-Source	A11	Lys-Lys	1010,00
PM04	P-Source	E10	Thymidine- 5 -Monophosphate	1001,00
PM08	N-Source	E01	Thr-Phe	971,00
PM06	N-Source	E05	Gly-Gly	968,00
PM08	N-Source	E08	Val-Glu	966,00
PM06	N-Source	F12	His-Trp	961,00
PM07	N-Source	C12	Phe-Phe	955,00
PM01	C-Source	D12	Uridine	951,00
PM04	P-Source	C06	D-Glucosamine-6-Phosphate	950,00
PM03	N-Source	A11	L-Cysteine	945,00
PM02	C-Source	C09	b-Methyl-D-Glucuronic Acid	922,00
PM01	C-Source	F09	Glycolic Acid	916,00
PM03	N-Source	E12	N-Acetyl-D-Galactosamine	916,00
PM04	S-Source	G05	Glutathione	910,00
PM04	S-Source	H02	Thiourea	903,00
PM04	P-Source	D08	Uridine- 2 - Monophosphate	899,00
PM03	N-Source	G10	D,L-a-Amino-Caprylic Acid	874,00
PM01	C-Source	F07	Propionic Acid	870,00
PM03	N-Source	F12	Inosine	866,00
PM02	C-Source	G12	L-Methionine	862,00
PM06	N-Source	G12	Ile-Ser	859,00
PM01	C-Source	B07	D,L-a-Glycerol- Phosphate	846,00
PM04	P-Source	D12	Uridine- 3 ,5 - Cyclic Monophosphate	845,00
PM04	P-Source	C07	6-Phospho-Gluconic Acid	842,00
PM03	N-Source	C12	L-Ornithine	832,00
PM04	P-Source	C09	Cytidine- 3 - Monophosphate	824,00
PM08	N-Source	C07	Gln-Glu	823,00
PM04	P-Source	C12	Cytidine- 3 ,5 -Cyclic Monophosphate	808,00
PM04	P-Source	D11	Uridine- 2 ,3 - Cyclic Monophosphate	791,00
PM04	S-Source	F10	L-Cysteic Acid	789,00
PM06	N-Source	A02	L-Glutamine	785,00
PM08	N-Source	E12	Val-Pro	783,00
PM06	N-Source	A11	Ala-Phe	773,00
PM03	N-Source	G12	a-Amino-N-Valeric Acid	770,00
PM04	P-Source	D09	Uridine- 3 - Monophosphate	770,00
PM03	N-Source	D12	Agmatine	769,00
PM02	C-Source	B05	D-Arabinose	769,00
PM02	C-Source	D09	L-Xylose	767,00
PM02	C-Source	B10	i-Erythritol	763,00
PM08	N-Source	C03	Met-Thr	760,00
PM08	N-Source	F12	D-Leu-Tyr	757,00
PM06	N-Source	F02	Gly-Trp	745,00

PM03	N-Source	F07	Guanosine	744,00
PM02	C-Source	D08	Xylitol	741,00
PM01	C-Source	B12	L-Glutamic Acid	731,00
PM04	P-Source	B02	Dithiophosphate	730,00
PM08	N-Source	H08	Gly-Phe-Phe	718,00
PM07	N-Source	F09	Trp-Glu	717,00
PM07	N-Source	F12	Trp-Lys	712,00
PM01	C-Source	C08	Acetic Acid	698,00
PM01	C-Source	A11	D-Mannose	684,00
PM06	N-Source	H12	Leu-Phe	674,00
PM07	N-Source	F03	Thr-Leu	654,00
PM04	P-Source	C05	2-Deoxy-D-Glucose 6-Phosphate	653,00
PM01	C-Source	E10	Maltotriose	645,00
PM06	N-Source	B02	Ala-Thr	637,00
PM08	N-Source	H06	Gly-Gly-Phe	628,00
PM02	C-Source	B11	D-Fucose	627,00
PM02	C-Source	B08	Arbutin	623,00
PM07	N-Source	G07	Tyr-Glu	618,00
PM03	N-Source	E10	D-Mannosamine	613,00
PM02	C-Source	B07	L-Arabitol	609,00
PM02	C-Source	B12	3-0-b-D-Galacto-pyranosyl-D-Arabinose	573,00
PM01	C-Source	A06	D-Galactose	573,00
PM04	P-Source	D05	O-Phospho-D-Serine	569,00
PM04	P-Source	E11	Inositol Hexaphosphate	559,00
PM02	C-Source	C07	b-Methyl-D-Galactoside	547,00
PM07	N-Source	D10	Pro-Phe	535,00
PM04	S-Source	F04	Tetrathionate	530,00
PM04	S-Source	H03	1-Thio-b-D-Glucose	529,00
PM04	S-Source	H01	L-Djenkolic Acid	529,00
PM01	C-Source	E06	a-Hydroxy Glutaric Acid-g-Lactone	523,00
PM01	C-Source	E08	b-Methyl-D-Glucoside	517,00
PM01	C-Source	D04	1,2-Propanediol	514,00
PM07	N-Source	A10	Lys-Leu	510,00
PM04	S-Source	H09	Butane Sulfonic Acid	507,00
PM01	C-Source	C05	Tween 20	499,00
PM04	P-Source	C11	Cytidine- 2 ,3 -Cyclic Monophosphate	497,00
PM01	C-Source	D10	Lactulose	492,00
PM02	C-Source	G10	L-Leucine	490,00
PM01	C-Source	D06	a-Keto-Glutaric Acid	485,00
PM01	C-Source	E05	Tween 80	482,00
PM06	N-Source	F06	His-Gly	465,00
PM06	N-Source	E02	Gly-Ala	461,00
PM07	N-Source	G06	Tyr-Gln	456,00

PM08	N-Source	H09	Leu-Gly-Gly	450,00
PM07	N-Source	B10	Met-Glu	447,00
PM06	N-Source	C07	Asn-Glu	440,00
PM08	N-Source	B11	Leu-Tyr	427,00
PM03	N-Source	H12	Met-Ala	425,00
PM07	N-Source	G12	Tyr-Phe	421,00
PM02	C-Source	F12	L-Tartaric Acid	418,00
PM04	S-Source	G10	N-Acetyl-D,L-Methionine	418,00
PM07	N-Source	E12	Thr-Arg	413,00
PM02	C-Source	C05	Maltitol	396,00
PM04	P-Source	D06	O-Phospho-L-Serine	395,00
PM08	N-Source	B04	His-Glu	394,00
PM04	P-Source	B05	L-a -Phosphatidyl-D,L-Glycerol	394,00
PM07	N-Source	F11	Trp-Leu	394,00
PM04	S-Source	G07	L-Methionine	391,00
PM08	N-Source	B01	Gly-Asp	383,00
PM04	S-Source	H08	p-Amino Benzene Sulfonic Acid	369,00
PM02	C-Source	C10	a-Methyl-D-Mannoside	368,00
PM04	P-Source	E08	Methylene Diphosphonic Acid	368,00
PM07	N-Source	F02	Thr-Gly	362,00
PM01	C-Source	C11	D-Melibiose	355,00
PM06	N-Source	H11	Leu-Met	335,00
PM07	N-Source	G08	Tyr-Gly	335,00
PM04	S-Source	G08	D-Methionine	325,00
PM04	P-Source	B09	Guanosine- 3 -Monophosphate	324,00
PM04	P-Source	A05	Tripolyphosphate	317,00
PM06	N-Source	E03	Gly-Arg	315,00
PM06	N-Source	B11	Arg-Leu	309,00
PM04	P-Source	B11	Guanosine- 2 ,3 -Cyclic Monophosphate	305,00
PM02	C-Source	E05	Dihydroxy-Fumaric Acid	303,00
PM07	N-Source	C11	Phe-Ile	300,00
PM08	N-Source	C01	Lys-Gly	292,00
PM04	S-Source	F02	Sulfate	291,00
PM07	N-Source	D11	Pro-Pro	285,00
PM03	N-Source	E09	D-Galactosamine	280,00
PM08	N-Source	B10	Leu-Pro	274,00
PM06	N-Source	D10	Glu-Ser	267,00
PM04	P-Source	E02	O-Phospho-L-Tyrosine	264,00
PM07	N-Source	A09	Lys-Ile	258,00
PM08	N-Source	G03	Gly-D-Ala	252,00
PM03	N-Source	F11	Uridine	249,00
PM06	N-Source	A10	Ala-Lys	245,00
PM02	C-Source	D05	Stachyose	244,00

PM08	N-Source	F03	b-Ala-Gly	239,00
PM06	N-Source	B10	Arg-Ile	236,00
PM04	P-Source	A02	Phosphate	232,00
PM04	P-Source	A09	Adenosine- 3 -Monophosphate	228,00
PM01	C-Source	B05	D-Glucuronic Acid	223,00
PM04	P-Source	C01	Phosphoenol Pyruvate	218,00
PM06	N-Source	F11	His-Ser	217,00
PM06	N-Source	F04	Gly-Val	208,00
PM03	N-Source	A10	L-Aspartic Acid	208,00
PM06	N-Source	E04	Gly-Cys	206,00
PM07	N-Source	A08	Lys-Glu	203,00
PM07	N-Source	E09	Ser-Tyr	201,00
PM07	N-Source	E10	Ser-Val	198,00
PM01	C-Source	A04	D-Saccharic Acid	196,00
PM01	C-Source	A05	Succinic Acid	192,00
PM06	N-Source	H08	Leu-Gly	191,00
PM07	N-Source	D09	Pro-Leu	188,00
PM03	N-Source	B10	L-Serine	174,00
PM01	C-Source	E07	a-Hydroxy-Butyric Acid	171,00
PM02	C-Source	D07	Turanose	170,00
PM08	N-Source	E11	Val-Phe	170,00
PM04	P-Source	C08	Cytidine- 2 - Monophosphate	167,00
PM08	N-Source	H05	Gly-Gly-Leu	163,00
PM02	C-Source	F06	Quinic Acid	158,00
PM08	N-Source	C11	Pro-Arg	155,00
PM02	C-Source	H11	2,3-Butanone	150,00
PM06	N-Source	D04	Cys-Gly	147,00
PM03	N-Source	C11	L-Homoserine	147,00
PM08	N-Source	A08	Asp-Ala	144,00
PM04	P-Source	B04	b-Glycerol Phosphate	144,00
PM03	N-Source	G11	d-Amino-N-Valeric Acid	144,00
PM04	S-Source	H05	Taurocholic Acid	124,00
PM07	N-Source	A07	Lys-Arg	120,00
PM08	N-Source	H12	Tyr-Gly-Gly	117,00
PM01	C-Source	C07	D-Fructose	113,00
PM04	P-Source	B08	Guanosine- 2 -Monophosphate	105,00
PM08	N-Source	B02	Gly-Ile	103,00
PM06	N-Source	G11	Ile-Pro	103,00
PM08	N-Source	C10	Phe-Val	103,00
PM07	N-Source	C09	Phe-Ala	99,00
PM06	N-Source	G06	Ile-Gly	96,00
PM01	C-Source	G09	Mono Methyl Succinate	88,00
PM03	N-Source	D11	Putrescine	87,00

PM01	C-Source	H11	b-Phenylethylamine	86,00
PM08	N-Source	E10	Val-Met	84,00
PM08	N-Source	E09	Val-Lys	69,00
PM06	N-Source	E11	Gly-Pro	68,00
PM01	C-Source	D07	a-Keto-Butyric Acid	67,00
PM02	C-Source	C11	b-Methyl-D-Xyloside	63,00
PM03	N-Source	A09	L-Asparagine	62,00
PM03	N-Source	C01	L-Tyrosine	61,00
PM03	N-Source	E11	N-Acetyl-D-Glucosamine	59,00
PM03	N-Source	D10	Ethylenediamine	58,00
PM08	N-Source	A07	Ala-Val	52,00
PM02	C-Source	F11	D-Tartaric Acid	52,00
PM07	N-Source	E08	Ser-Ser	48,00
PM08	N-Source	F10	D-Leu-D-Leu	46,00
PM08	N-Source	H04	Gly-Gly-lle	45,00
PM04	P-Source	A08	Adenosine- 2 -Monophosphate	44,00
PM06	N-Source	C10	Asp-Glu	42,00
PM03	N-Source	E08	D-Glucosamine	42,00
PM04	P-Source	C03	D-Glucose-1-Phosphate	38,00
PM01	C-Source	G11	D-Malic Acid	36,00
PM08	N-Source	D09	Ser-Gln	35,00
PM06	N-Source	C11	Asp-Leu	27,00
PM02	C-Source	D10	g-Amino-Butyric Acid	25,00
PM03	N-Source	C10	L-Citrulline	24,00
PM02	C-Source	G11	L-Lysine	24,00
PM08	N-Source	F09	D-Ala-Leu	23,00
PM04	S-Source	G06	D,L-Ethionine	19,00
PM07	N-Source	F08	Trp-Asp	19,00
PM02	C-Source	D11	d-Amino-Valeric Acid	18,00
PM07	N-Source	B09	Met-Gln	18,00
PM08	N-Source	C09	Phe-Tyr	18,00
PM03	N-Source	B02	Glycine	16,00
PM02	C-Source	D04	L-Sorbose	16,00
PM02	C-Source	F09	Sorbic Acid	14,00
PM07	N-Source	F07	Trp-Arg	12,00
PM02	C-Source	E11	Itaconic Acid	11,00
PM02	C-Source	G09	L-Isoleucine	10,00
PM06	N-Source	A09	Ala-Leu	9,00
PM01	C-Source	F10	Glyoxylic Acid	7,00
PM07	N-Source	G05	Tyr-Ala	6,00
PM03	N-Source	A08	L-Arginine	5,00
PM06	N-Source	H10	Leu-Leu	5,00
PM07	N-Source	G11	Tyr-Lys	5,00

PM06	N-Source	A08	Ala-His	4,00
PM04	P-Source	D04	Phospho-L-Arginine	4,00
PM03	N-Source	F10	Uracil	4,00
PM03	N-Source	A07	L-Alanine	3,00
PM07	N-Source	A04	Leu-Trp	3,00
PM08	N-Source	A04	Ala-Gln	2,00
PM06	N-Source	B09	Arg-Glu	2,00
PM08	N-Source	C08	Phe-Met	1,00

Table 7.3: Evaluation of Biolog plates of substances which are more resitant for the metabolization by *S. aureus* 6850 wild-type.

Plate	Source	Well number	Substance	p-value
PM07	N-Source	H06	Val-Gly	-5367,00
PM01	C-Source	H01	Glycyl-L-Proline	-3118,00
PM01	C-Source	H06	L-Lyxose	-3105,00
PM07	N-Source	H12	g-Glu-Gly	-2743,00
PM01	C-Source	F01	Glycyl-L-Aspartic Acid	-2495,00
PM02	C-Source	H01	L-Ornithine	-2298,00
PM02	C-Source	H03	L-Pyroglutamic Acid	-2164,00
PM01	C-Source	E01	L-Glutamine	-2135,00
PM01	C-Source	G03	L-Serine	-2108,00
PM01	C-Source	H05	D-Psicose	-1878,00
PM01	C-Source	G02	Tricarballylic Acid	-1855,00
PM08	N-Source	H02	Gly-Gly-D-Leu	-1767,00
PM02	C-Source	C01	Gentiobiose	-1762,00
PM08	N-Source	H01	Gly-Gly-Ala	-1750,00
PM02	C-Source	C02	L-Glucose	-1746,00
PM01	C-Source	C01	D-Glucose-6-Phosphate	-1745,00
PM07	N-Source	H01	Tyr-Trp	-1720,00
PM01	C-Source	C03	D,L-Malic Acid	-1639,00
PM08	N-Source	G01	g-Glu-Gly	-1587,00
PM01	C-Source	G05	L-Alanine	-1565,00
PM02	C-Source	G02	L-Alaninamide	-1510,00
PM01	C-Source	F03	m-Inositol	-1505,00
PM01	C-Source	G04	L-Threonine	-1501,00
PM01	C-Source	G01	Glycyl-L-Glutamic Acid	-1433,00
PM04	P-Source	D03	Cysteamine-S-Phosphate	-1381,00
PM01	C-Source	D02	D-Aspartic Acid	-1365,00
PM02	C-Source	D01	D-Raffinose	-1334,00
PM01	C-Source	G06	L-Alanyl-Glycine	-1317,00
PM01	C-Source	F02	Citric Acid	-1303,00
PM01	C-Source	B03	Glycerol	-1299,00
PM02	C-Source	G05	Glycine	-1293,00

PM04	P-Source	E01	O-Phospho-D-Tyrosine	-1286,00
PM01	C-Source	G07	Acetoacetic Acid	-1227,00
PM01	C-Source	C02	D-Galactonic Acid-g-Lactone	-1187,00
PM01	C-Source	B02	D-Sorbitol	-1173,00
PM02	C-Source	H12	3-Hydroxy-2-Butanone	-1166,00
PM02	C-Source	D03	Sedoheptulosan	-1136,00
PM04	P-Source	C02	Phospho-Glycolic Acid	-1134,00
PM03	N-Source	H01	Ala-Asp	-1113,00
PM01	C-Source	C04	D-Ribose	-1106,00
PM01	C-Source	G12	L-Malic Acid	-1080,00
PM03	N-Source	H04	Ala-Gly	-1071,00
PM06	N-Source	H01	Ile-Trp	-1041,00
PM01	C-Source	D03	D-Glucosaminic Acid	-1026,00
PM02	C-Source	B02	N-Acetyl-Neuraminic Acid	-1021,00
PM01	C-Source	D01	L-Asparagine	-1015,00
PM01	C-Source	H04	Tyramine	-1008,00
PM01	C-Source	F04	D-Threonine	-987,00
PM07	N-Source	H02	Tyr-Tyr	-981,00
PM02	C-Source	G04	L-Arginine	-958,00
PM07	N-Source	G01	Trp-Phe	-940,00
PM03	N-Source	G01	Xanthine	-911,00
PM02	C-Source	C03	D-Lactitol	-850,00
PM03	N-Source	H10	Gly-Glu	-815,00
PM08	N-Source	G02	g-D-Glu-Gly	-807,00
PM02	C-Source	E02	Caproic Acid	-803,00
PM02	C-Source	C04	D-Lyxose	-759,00
PM01	C-Source	H02	p-Hydroxy-Phenylacetic Acid	-744,00
PM07	N-Source	F01	Thr-Glu	-721,00
PM01	C-Source	E03	D-Glucose-1-Phosphate	-715,00
PM01	C-Source	B04	L-Fucose	-708,00
PM01	C-Source	F12	Inosine	-687,00
PM01	C-Source	E04	D-Fructose-6-Phosphate	-679,00
PM07	N-Source	H03	Val-Arg	-678,00
PM03	N-Source	B01	L-Glutamine	-651,00
PM01	C-Source	B01	D-Serine	-609,00
PM02	C-Source	F01	D-Lactic Acid Methyl Ester	-601,00
PM02	C-Source	B04	Amygdalin	-594,00
PM01	C-Source	H08	Pyruvic Acid	-588,00
PM04	P-Source	D01	D-Mannose-1-Phosphate	-583,00
PM07	N-Source	H10	Val-Tyr	-582,00
PM01	C-Source	H12	Ethanolamine	-566,00
PM02	C-Source	F04	Oxalic Acid	-559,00
PM02	C-Source	H09	Dihydroxy-Acetone	-552,00

PM06	N-Source	H02	Ile-Tyr	-550,00
PM08	N-Source	F01	Val-Ser	-548,00
PM03	N-Source	H02	Ala-Gln	-547,00
PM07	N-Source	H09	Val-Leu	-537,00
PM01	C-Source	F05	Fumaric Acid	-536,00
PM04	P-Source	E03	Phosphocreatine	-536,00
PM01	C-Source	B11	D-Mannitol	-533,00
PM04	P-Source	C04	D-Glucose-6-Phosphate	-525,00
PM02	C-Source	A02	Chondroitin Sulfate C	-523,00
PM02	C-Source	F03	Melibionic Acid	-512,00
PM01	C-Source	D11	Sucrose	-509,00
PM01	C-Source	H09	L-Galactonic Acid-g-Lactone	-506,00
PM08	N-Source	H10	Leu-Leu-Leu	-503,00
PM01	C-Source	H07	Glucuronamide	-494,00
PM08	N-Source	H03	Gly-Gly-Gly	-487,00
PM01	C-Source	G08	N-Acetyl-b-D-Mannosamine	-483,00
PM07	N-Source	E01	Ser-Ala	-423,00
PM01	C-Source	F06	Bromo-Succinic Acid	-422,00
PM06	N-Source	G01	His-Tyr	-402,00
PM07	N-Source	H11	Val-Val	-401,00
PM02	C-Source	H08	Putrescine	-397,00
PM01	C-Source	A03	N-Acetyl-D-Glucosamine	-393,00
PM02	C-Source	G08	4-Hydroxy-L-Proline (trans)	-384,00
PM07	N-Source	H04	Val-Asn	-376,00
PM07	N-Source	H05	Val-Asp	-371,00
PM02	C-Source	G06	L-Histidine	-349,00
PM07	N-Source	H08	Val-Ile	-343,00
PM02	C-Source	H10	2,3-Butanediol	-306,00
PM02	C-Source	A03	a-Cyclodextrin	-296,00
PM01	C-Source	A02	L-Arabinose	-280,00
PM02	C-Source	E01	Capric Acid	-271,00
PM03	N-Source	H03	Ala-Glu	-269,00
PM01	C-Source	G10	Methyl Pyruvate	-263,00
PM02	C-Source	H02	L-Phenylalanine	-257,00
PM03	N-Source	F01	N-Acetyl-D-Mannosamine	-257,00
PM06	N-Source	F01	Gly-Thr	-253,00
PM06	N-Source	F03	Gly-Tyr	-240,00
PM06	N-Source	H07	Leu-Glu	-239,00
PM03	N-Source	H09	Gly-Gln	-237,00
PM02	C-Source	G01	Acetamide	-231,00
PM07	N-Source	G10	Tyr-Leu	-229,00
PM02	C-Source	F05	Oxalomalic Acid	-225,00
PM04	P-Source	E04	Phosphoryl Choline	-206,00

PM07	N-Source	H07	Val-His	-198,00
PM08	N-Source	G10	Phe-b-Ala	-197,00
PM01	C-Source	H03	m-Hydroxy-Phenylacetic Acid	-186,00
PM08	N-Source	D01	Pro-Glu	-186,00
PM07	N-Source	D01	Phe-Pro	-181,00
PM07	N-Source	G02	Trp-Ser	-176,00
PM08	N-Source	H07	Val-Tyr-Val	-176,00
PM04	P-Source	B03	D,L-a-Glycerol Phosphate	-160,00
PM03	N-Source	H07	Ala-Thr	-158,00
PM06	N-Source	H03	Ile-Val	-158,00
PM01	C-Source	H10	D-Galacturonic Acid	-148,00
PM03	N-Source	E01	Histamine	-131,00
PM04	P-Source	E05	O-Phosphoryl-Ethanolamine	-128,00
PM01	C-Source	D05	Tween 40	-116,00
PM06	N-Source	D01	Asp-Phe	-113,00
PM07	N-Source	C01	Met-Ile	-110,00
PM06	N-Source	E01	Glu-Val	-105,00
PM03	N-Source	H11	Gly-Met	-103,00
PM04	S-Source	H06	Taurine	-94,00
PM04	P-Source	B01	Thiophosphate	-84,00
PM01	C-Source	C10	Maltose	-77,00
PM07	N-Source	G03	Trp-Trp	-76,00
PM03	N-Source	G03	Uric Acid	-71,00
PM08	N-Source	G09	Leu-D-Leu	-62,00
PM07	N-Source	E02	Ser-Gly	-62,00
PM04	P-Source	D02	D-Mannose-6-Phosphate	-53,00
PM07	N-Source	B01	Lys-Pro	-50,00
PM08	N-Source	G06	Gly-D-Thr	-48,00
PM07	N-Source	G09	Tyr-His	-40,00
PM08	N-Source	G11	Ala-Ala-Ala	-38,00
PM02	C-Source	H06	Sec-Butylamine	-32,00
PM03	N-Source	H08	Gly-Asn	-27,00
PM01	C-Source	C09	a-D-Glucose	-26,00
PM08	N-Source	E02	Thr-Ser	-22,00
PM08	N-Source	G08	Leu-b-Ala	-15,00
PM08	N-Source	G04	Gly-D-Asp	-14,00
PM03	N-Source	G05	Allantoin	-13,00
PM08	N-Source	G05	Gly-D-Ser	-13,00
PM03	N-Source	H06	Ala-Leu	-12,00
PM03	N-Source	A02	Ammonia	-12,00
PM01	C-Source	E11	2`-Deoxy-Adenosine	-9,00
PM03	N-Source	H05	Ala-His	-7,00
PM03	N-Source	F06	Guanine	-5,00

PM08	N-Source	G07	Gly-D-Val	-4,00
PM06	N-Source	H04	Leu-Ala	-4,00
PM02	C-Source	H07	D,L-Octopamine	-3,00
PM03	N-Source	G02	Xanthosine	-2,00
PM03	N-Source	G04	Alloxan	-1,00
PM08	N-Source	F02	b-Ala-Ala	-1,00
PM06	N-Source	H09	Leu-Ile	-1,00
PM03	N-Source	D01	N-Acetyl-D,L-Glutamic Acid	-1,00

Table 7.4: Evaluation of Biolog plates of substances which are metabolized in the similar in *S. aureus* wildtype and Δ852.

Plate	Source	Well number	Substance	p-value
PM04	P-Source	E07	2-Aminoethyl Phosphonic Acid	0,00
PM02	C-Source	E06	2-Hydroxy-Benzoic Acid	0,00
PM02	C-Source	E07	4-Hydroxy-Benzoic Acid	0,00
PM03	N-Source	E04	Acetamide	0,00
PM03	N-Source	F02	Adenine	0,00
PM03	N-Source	F03	Adenosine	0,00
PM06	N-Source	A03	Ala-Ala	0,00
PM06	N-Source	A04	Ala-Arg	0,00
PM06	N-Source	A05	Ala-Asn	0,00
PM08	N-Source	A03	Ala-Asp	0,00
PM06	N-Source	A06	Ala-Glu	0,00
PM08	N-Source	A05	Ala-Ile	0,00
PM08	N-Source	A06	Ala-Met	0,00
PM06	N-Source	B01	Ala-Ser	0,00
PM06	N-Source	B03	Ala-Trp	0,00
PM06	N-Source	B04	Ala-Tyr	0,00
PM02	C-Source	C06	a-Methyl-D-Galactoside	0,00
PM06	N-Source	B05	Arg-Ala	0,00
PM06	N-Source	B06	Arg-Arg	0,00
PM06	N-Source	B07	Arg-Asp	0,00
PM06	N-Source	B08	Arg-Gln	0,00
PM06	N-Source	C01	Arg-Met	0,00
PM06	N-Source	C02	Arg-Phe	0,00
PM06	N-Source	C03	Arg-Ser	0,00
PM06	N-Source	C04	Arg-Trp	0,00
PM06	N-Source	C05	Arg-Tyr	0,00
PM06	N-Source	C06	Arg-Val	0,00
PM06	N-Source	C08	Asn-Val	0,00
PM06	N-Source	C09	Asp-Asp	0,00
PM06	N-Source	D02	Asp-Trp	0,00
PM06	N-Source	D03	Asp-Val	0,00

PM08	N-Source	F04	b-Ala-His	0,00
PM08	N-Source	F06	b-Ala-Phe	0,00
PM02	C-Source	A04	b-Cyclodextrin	0,00
PM02	C-Source	B03	b-D-Allose	0,00
PM03	N-Source	A06	Biuret	0,00
PM03	N-Source	E02	b-Phenylethylamine	0,00
PM02	C-Source	E03	Citraconic Acid	0,00
PM03	N-Source	F04	Cytidine	0,00
PM03	N-Source	F05	Cytosine	0,00
PM03	N-Source	G07	D,L-a-Amino-N-Butyric Acid	0,00
PM02	C-Source	H05	D,L-Carnitine	0,00
PM02	C-Source	E04	D,L-Citramalic Acid	0,00
PM03	N-Source	E07	D,L-Lactamide	0,00
PM08	N-Source	F07	D-Ala-D-Ala	0,00
PM03	N-Source	C03	D-Alanine	0,00
PM03	N-Source	C04	D-Asparagine	0,00
PM03	N-Source	C05	D-Aspartic Acid	0,00
PM03	N-Source	C06	D-Glutamic Acid	0,00
PM03	N-Source	C07	D-Lysine	0,00
PM02	C-Source	F07	D-Ribono-1,4-Lactone	0,00
PM03	N-Source	C08	D-Serine	0,00
PM03	N-Source	C09	D-Valine	0,00
PM03	N-Source	G09	e-Amino-N-Caproic Acid	0,00
PM03	N-Source	D09	Ethanolamine	0,00
PM03	N-Source	D08	Ethylamine	0,00
PM03	N-Source	E05	Formamide	0,00
PM03	N-Source	G08	g-Amino-N-Butyric Acid	0,00
PM02	C-Source	A05	g-Cyclodextrin	0,00
PM06	N-Source	D05	Gln-Gln	0,00
PM03	N-Source	E06	Glucuronamide	0,00
PM08	N-Source	B03	His-Ala	0,00
PM06	N-Source	F05	His-Asp	0,00
PM08	N-Source	B05	His-His	0,00
PM06	N-Source	F07	His-Leu	0,00
PM06	N-Source	F08	His-Lys	0,00
PM06	N-Source	F09	His-Met	0,00
PM06	N-Source	F10	His-Pro	0,00
PM06	N-Source	G02	His-Val	0,00
PM03	N-Source	D04	Hydroxylamine	0,00
PM04	P-Source	A07	Hypophosphite	0,00
PM06	N-Source	G03	Ile-Ala	0,00
PM06	N-Source	G04	Ile-Arg	0,00
PM08	N-Source	B06	Ile-Asn	0,00

PM06	N-Source	G05	Ile-Gln	0,00
PM06	N-Source	G07	Ile-His	0,00
PM06	N-Source	G08	Ile-Ile	0,00
PM08	N-Source	B07	Ile-Leu	0,00
PM06	N-Source	G09	Ile-Met	0,00
PM06	N-Source	G10	Ile-Phe	0,00
PM06	N-Source	H05	Leu-Arg	0,00
PM08	N-Source	B08	Leu-Asn	0,00
PM06	N-Source	H06	Leu-Asp	0,00
PM08	N-Source	B09	Leu-His	0,00
PM07	N-Source	A03	Leu-Ser	0,00
PM07	N-Source	A05	Leu-Val	0,00
PM03	N-Source	B03	L-Histidine	0,00
PM02	C-Source	G07	L-Homoserine	0,00
PM03	N-Source	B04	L-Isoleucine	0,00
PM03	N-Source	B05	L-Leucine	0,00
PM03	N-Source	B06	L-Lysine	0,00
PM03	N-Source	B07	L-Methionine	0,00
PM03	N-Source	B08	L-Phenylalanine	0,00
PM03	N-Source	B09	L-Proline	0,00
PM03	N-Source	D03	L-Pyroglutamic Acid	0,00
PM02	C-Source	H04	L-Valine	0,00
PM03	N-Source	C02	L-Valine	0,00
PM07	N-Source	A06	Lys-Ala	0,00
PM08	N-Source	C02	Lys-Met	0,00
PM07	N-Source	B02	Lys-Ser	0,00
PM07	N-Source	B04	Lys-Trp	0,00
PM07	N-Source	B05	Lys-Tyr	0,00
PM07	N-Source	B06	Lys-Val	0,00
PM02	C-Source	F02	Malonic Acid	0,00
PM07	N-Source	B07	Met-Arg	0,00
PM07	N-Source	B08	Met-Asp	0,00
PM08	N-Source	F05	Met-b-Ala	0,00
PM03	N-Source	D05	Methylamine	0,00
PM07	N-Source	C02	Met-Leu	0,00
PM07	N-Source	C03	Met-Lys	0,00
PM07	N-Source	C04	Met-Met	0,00
PM07	N-Source	C05	Met-Phe	0,00
PM07	N-Source	C06	Met-Pro	0,00
PM07	N-Source	C07	Met-Trp	0,00
PM08	N-Source	C04	Met-Tyr	0,00
PM07	N-Source	C08	Met-Val	0,00
PM01	C-Source	E02	m-Tartaric Acid	0,00

PM02	C-Source	B01	N-Acetyl-D-Galactosamine	0,00
PM02	C-Source	G03	N-Acetyl-L-Glutamic Acid	0,00
PM03	N-Source	D06	N-Amylamine	0,00
PM03	N-Source	D07	N-Butylamine	0,00
PM03	N-Source	A04	Nitrate	0,00
PM03	N-Source	A03	Nitrite	0,00
PM03	N-Source	D02	N-Phthaloyl-L-Glutamic Acid	0,00
PM03	N-Source	G06	Parabanic Acid	0,00
PM08	N-Source	C05	Phe-Asp	0,00
PM07	N-Source	D02	Phe-Ser	0,00
PM07	N-Source	D03	Phe-Trp	0,00
PM04	P-Source	E06	Phosphono Acetic Acid	0,00
PM07	N-Source	D04	Pro-Ala	0,00
PM07	N-Source	D05	Pro-Asp	0,00
PM07	N-Source	D08	Pro-Hyp	0,00
PM08	N-Source	D02	Pro-lle	0,00
PM08	N-Source	D03	Pro-Lys	0,00
PM08	N-Source	D04	Pro-Ser	0,00
PM08	N-Source	D05	Pro-Trp	0,00
PM8	N-Source	D06	Pro-Val	0,00
PM02	C-Source	D02	Salicin	0,00
PM02	C-Source	F08	Sebacic Acid	0,00
PM08	N-Source	D07	Ser-Asn	0,00
PM08	N-Source	D08	Ser-Asp	0,00
PM07	N-Source	E03	Ser-His	0,00
PM07	N-Source	E04	Ser-Leu	0,00
PM07	N-Source	E05	Ser-Met	0,00
PM07	N-Source	E06	Ser-Phe	0,00
PM07	N-Source	E07	Ser-Pro	0,00
PM03	N-Source	F09	Thymidine	0,00
PM03	N-Source	F08	Thymine	0,00
PM04	P-Source	A06	Triethyl Phosphate	0,00
PM04	P-Source	A04	Trimetaphosphate	0,00
PM07	N-Source	F06	Trp-Ala	0,00
PM07	N-Source	G04	Trp-Tyr	0,00
PM08	N-Source	E03	Trp-Val	0,00
PM03	N-Source	E03	Tyramine	0,00
PM08	N-Source	E04	Tyr-lle	0,00
PM08	N-Source	E05	Tyr-Val	0,00
PM03	N-Source	A05	Urea	0,00
PM08	N-Source	E06	Val-Ala	0,00
PM08	N-Source	E07	Val-Gln	0,00

7.5 Publications and presentations

7.5.1 Publications

Groma, M., Horst, S. A., Das, S., Huettel, B., Klepsch, M., Rudel, T., Fraunholz, M. (2020). Identification of a Novel LysR-Type Transcriptional Regulator in Staphylococcus aureus That Is Crucial for Secondary Tissue Colonization during Metastatic Bloodstream Infection. *mBio, 11*(4). doi:10.1128/mBio.01646-20

7.5.2 Oral presentation

Groma, M. *S. aureus* 6850 Δ852 a LysR-Type Transcritpional Regulator. PhD meeting, DFG Transregional Collaborative Research Centre 34 (2017, Greifswald, Germany).

Groma, M. *S. aureus* 6850 Δ852 a LysR-Type Transcritpional Regulator. Retreat, the Department of Microbiology at the University of Würzburg (2017, Rothenburg ob der Tauber, Germany).

Groma, M. Characterization of a noval LysR-type transcriptional regulator in *S. aureus* 6850. Retreat, the Department of Microbiology at the University of Würzburg (2019, Kloster Bronnbach, Germany).

7.6 Contribution by others

This work was conducted under the supervision of Prof. Dr. Thomas Rudel, Chair of Microbiology (University of Würzburg), Dr. Martin Fraunholz (University of Würzburg) and Prof. Dr. Eva Medina, group leader of Infection Immunology (Helmholz Centre for Infection Research, Braunschweig). Several parts of this doctoral thesis have been conducted in collaboration with others and their contributions are indicated below:

- *S. aureus* Δ852 was generated by Dr. Sudip Das (Department of Microbiology, University of Würzburg, Germany)
- Cloning of the complementation plasmid pl852 was performed by Linda Raupach at the Department of Microbiology (University of Würzburg, Germany)
- Mouse experiments were performed by Dr. Sarah Horst and Prof. Dr. Eva Medina (Helmholz Centre for Infection Research, Braunschweig, Germany)
- RNA-seq was performed in collaboration with Dr. Bruno Hüttel (Max PlanckGenome Centre, Cologne, Germany)
- Processing and statistical analysis of RNA-seq was performed by Dr. Maximilian Klepsch (Department of Microbiology, Univesity of Würzburg, Germany)
- Providing OmniLog® by Dr. Meina Neumann-Schaal (Leibniz Institute DSMZ, Braunschweig, Germany)

7.7 Danksagung

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7.8 Affidavit

Eidesstattliche Erklärungen nach §7 Abs. 2 Satz 3, 4, 5 der Promotionsordnung der Fakultät für Biologie

Affidavit

I hereby declare that my thesis entitled: „**Identification of a novel LysR-type transcriptional regulator in *Staphylococcus aureus***" is the result of my own work. I did not receive any help or support from commercial consultants. All sources and / or materials applied are listed and specified in the thesis.

Furthermore I verify that the thesis has not been submitted as part of another examination process neither in identical nor in similar form.

Besides I declare that if I do not hold the copyright for figures and paragraphs, I obtained it from the rights holder and that paragraphs and figures have been marked according to law or for figures taken from the internet the hyperlink has been added accordingly.

Eidesstattliche Erklärung

Hiermit erkläre ich an Eides statt, die Dissertation: „**Identification of a novel LysR-type transcriptional regulator in *Staphylococcus aureus***", eigenständig, d. h. insbesondere selbständig und ohne Hilfe eines kommerziellen Promotionsberaters, angefertigt und keine anderen, als die von mir angegebenen Quellen und Hilfsmittel verwendet zu haben.

Ich erkläre außerdem, dass die Dissertation weder in gleicher noch in ähnlicher Form bereits in einem anderen Prüfungsverfahren vorgelegen hat.

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