Studying on the protein stress response in *Staphylococcus aureus*

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Contents

Summary

Being one of the most important pathogenic bacteria and due to the widespread emergence of antibiotic resistance, *Staphylococcus aureus* became a topic of several studies mainly addressing its pathophysiology and virulence. During infection,*S.aureus* is subjected to various environmental changes. Understanding of the regulatory mechanisms controlling stress gene expression of *S.aureus* in response to environmental stress is very essential in studying its fitness and virulence.

In this work, the changes in protein expression profiles as well as the gene transcription of *S.aureus* after heat exposure, osmotic stress and in response to the antibiotic puromycin were studied in order to provide detailed insights into the response of *S.aureus* to various kinds of environmental stress under in vitro conditions, namely:
(1) to investigate the global response of *S.aureus* to heat stress conditions using transcriptomic and proteomic analyses.
(2) to study the transcriptome and proteome of *S.aureus* in response to antibiotic substance puromycin.
(3) to define the proteome signatures of *S.aureus* under NaCl stress condition.
(4) to complete the proteome map of cytoplasmic proteins of *S.aureus* by identifying proteins exclusively synthesized during the exposure to stress.

Firstly, the high resolution 2-D protein gel electrophoresis technique combined with MALDI-TOF-MS and a DNA array approach were used to investigate the cellular response of *S.aureus* to heat stress. A switch from normal growth temperature to high temperature condition revealed complex changes in the protein expression pattern as well as the genes expression profile. In particular, both

transcriptome and proteome analysis revealed the induction of main cellular chaperone machineries GroE and DnaK, the ATP-dependent proteases ClpB, ClpP . *S.aureus* cells showed that the heat shock repressor protein CtsR was highly up-regulated after heat exposure . In contrast to *B. subtilis* ,the *groES/L* and *dnaK*-operon are regulated by both heat shock regulators, HrcA and CtsR. They act together synergistically to maintain low basal levels of expression of these operons in the absence of stress. Surprisingly, the members of the σ^B-dependent response, strongly heat-inducible in *B. subtilis* are not induced in heat-stressed cells of *S.aureus* grown in synthetic medium. Other proteins involved in protein folding, refolding, and degradation, as well as DNA repair systems, and intermediary metabolism were also found to be up-regulated. Heat stress treatment also resulted in a strong synthesis level of genes belonging to the SOS response such as *uvrA* and *urvB* indicated the relevance of heat stress response and SOS response. According to the microarrays analysis data, among the heat-induced transcripts were several bacteriophage replication/packaging genes such as intergrase SACOL0318, transcriptional regulator of the RinA family SACOL0364 as well as the small and large subunit of the terminase SACOL0366 and SACOL0367.

The effect of puromycin stress on *S.aureus* cells was analyzed, using a gel-based proteomic approach and transcriptomic analyses with DNA microarrays. We compared the protein synthesis pattern as well as the transcription data of *S.aureus* in response to puromycin stress with that in response to heat shock. The results demonstrated that both stress conditions induced specific, overlapping and general responses. We recognized overlapping responses between heat and puromycin stress. The proteomic and transcriptomic results did show induction of DnaK, GroEL, GrpE as well as ClpB and ClpP. Exposure to puromycin stress mediated a significant increase of DNA repair enzymes, as well as affected proteins involved in protein repair and degradation.

Finally, the protein expression profile of *S.aureus* in response to NaCl stress was analyzed with 2D gel based proteomic approach. The synthesis of some enzymes belonging to amino acid synthesis pathways was elevated after stress, for example, enzymes of the metabolism of glutamate, an amino acid which was probably required in higher concentration after salt stress. Our result also suggested that

osmotic stress induced the expression of Asp23, which encodes an alkaline shock protein. Our proteome analyses also revealed the repression of the synthesis of many enzymes belong to different metabolism pathways such as DNA metabolism, purin/pyrimidin metabolism or energy metabolism. Interestingly, in *S.aureus*, the northern blot analysis revealed no significant induction of *opuA, B* or *C* of the osmoprotectant transport system which play an essential role in salt adaptation of the model Gram-positive bacteria *B. subtilis*. In this study, the level of their transcripts remained almost unaltered.

In summary, the signatures for stress or starvation stimuli can be used as diagnostic tools for the prediction of the mode of action of new antibiotics or for studying the physiological state of cells grown. Expression of the respective genes under in vivo conditions could provide some ideas on the environmental signals that specifically influence the survival of *S.aureus* within and outside the host.

Abbreviation

Abbreviation	**Fullname**
2D	two-dimensional
2DE	two-dimensional electrophoresis
2D-PAGE	two-dimensional polyacrylamide gel electrophoresis
ATP	adenosine-5-triphosphate
B.subtilis	*Bacillus subtilis*
BMM	Belitsky minimal medium
CBB	Coomassie Brilliant Blue
cDNA	complementary deoxyribonucleic acid
CFU	colony forming unit
CHAPS	3-[(3-cholamidopropyl)dimethyl ammonio]-1-propane sulfonate
DNA	deoxyribonucleic acid
DTT	dithiolthreitol
E. coli	*Escherichia coli*
EDTA	ethylenediamine tetra acetic acid
GTP	guanosine triphosphate
IEF	isoelectric focusing
IPG	non-linear immobilized pH gradients
kb	kilo bases
kDa	kilo Daltons
L.monocytogenes	*Listeria monocytogenes*
LB	Luria Bertani broth medium
MIC	minimal inhibitory concentration
Mr	molecular weight
MALDI-TOF	matrix assisted laser desorption/ ionisation time of flight
MS	mass spectrometry

nm	nanometer
OD	optical density
ORF	open reading frame
PCR	polymerase chain reaction
pI	isoelectric point
PMSF	phenylmethylsulphonylfluoride
RNA	ribonucleic acid
rpm	rounds per minute
S.typhimurium	*Salmonella typhimurium*
S.aureus	*Staphylococcus aureus*
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
U	unit
V	voltage
v/v	volume per volume
w/v	weight per volume

Chapter 1

Introduction

1.1 An overview of *Staphylococcus aureus* and its proteomics

1.1.1 *S.aureus*-one of the most important pathogenic bacteria

S.aureus is a spherical Gram-positive, catalase positive bacterium which appears in pairs, short chains or bunched, grape like yellow cluster when viewed in the microscope (Figure 1.1). Most isolated *S.aureus* strains are covered by polysaccharide capsule. Beneath the capsule, *S. aureus* harbours a cell wall which lacks the outer membrane with a thick and highly crosslinked peptidoglycan layer (Anthony *et al.*, 1988; Beveridge *et al.*, 1999 ; Popescu *et al.*,1996 ; Van

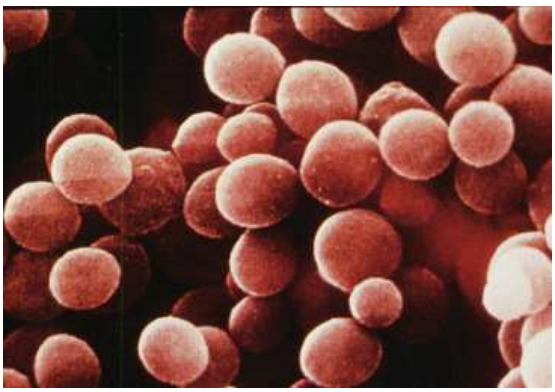

Figure 1.1: Electromicroscopic view of *S.aureus*

Wely *et al.*, 2001) (Figure 1.2).

S.aureus causes a wide variety of diseases, from mild skin infections to severe life threatening systemic infections. *S.aureus* is commonly associated with wound infections, catheter-related infections, toxic shock syndrome (TSS) and food poisoning (Bennett *et al.*, 2001; Todar, 2004).

Being one of the most important pathogenic bacteria, *S.aureus* has an increasing importance as a result of the spread of antibiotic resistance. Because of its adaptability and resistance to environmental stresses, *S. aureus* can survive extremely well outside the host and is one of the major causes of community acquired infections. The pathogenesis of *S.aureus* is very complex and involves the highly coordinated synthesis of cell wall- associated proteins and extracellular toxins.

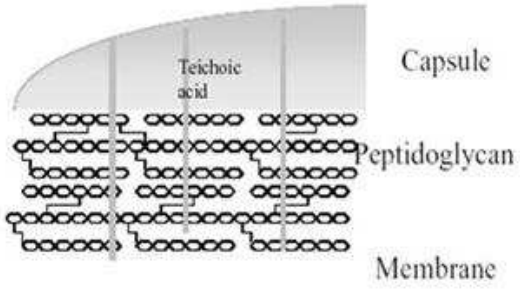

Figure 1.2: Schematic presentation of *S.aureus* cell wall

1.1.2 Proteomics of *S.aureus*

With the publication of the first complete genome sequence of a living organism in 1995, new fields in molecular genetics such as phylogenomics or comparative genomics have emerged. However, the genome sequence of an organism predicts the number of coding sequences and represents only the "blue-print of life", not "life itself".The post genomic era of *S. aureus* began with the publication of the genome sequence of two reference strains (Kuroda *et al.*, 2001). This provided the experimental basis for bringing the "blue print into real life", namely "the genome sequence of *S. aureus* to life" by an application of experimental tools encompassed under the term "functional genomics". Transcriptomic and proteomic approaches are the major tools for functional genomics. Transcriptomics, relying on DNA arrays that may cover the complete genome, provides

information on the global gene expression pattern of a cell while proteomics provides additional information on the expression levels, stabilization, localization, interaction and post-translational modifications of the proteins (e.g. phosphorylation, glycosylation). To obtain this global view of the synthesis and distribution of many *S.aureus* proteins in the cell, the highly sensitive two dimensional gel electrophoresis (2-DE) separation technique is a well established technique. It has to be complemented with protein identification, relying on tryptic peptide mass mapping via matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS) (Hecker *et al.*, 2003). With the creation of cytoplasmic and extracellular protein maps, an experimental tool is available for more sophisticated physiological studies. Such studies can be addressed by proteomics, thus generating new information on regulatory systems and enzymatic pathways involved in particular cellular responses. Two major classes of proteins synthesized at different growth conditions can be distinguished:
- Vegetative proteins synthesized during growth and cell cycle with many house -keeping functions
- Proteins synthesized only in response to environmental stimuli with mainly adaptive function against stress or starvation.
Many proteins of these different groups are involved in the fitness of *S.aureus*. In addition to proteins having the function of damaging the host, these proteins are essential for survival outside and within the host that may contribute indirectly to pathogenicity (Hecker *et al.*, 2003).

1.2 Environmental stimuli and proteomic signatures

Environmental stimuli and starvation are typical conditions in most natural ecosystems, including many different micro habitats in the host that limit bacterial growth. The adaptation to stress or starvation is crucial for survival in nature. As a result of this longstanding interaction of bacteria with a continuously changing set of environmental stimuli, a very complex adaptational network has evolved. Analysing this network forms the basis for understanding the cell

physiology in natural ecosystem (Hecker and Völker, 2001). The stress and starvation genes are more or less silent in growing cells, but are strongly activated by defined environmental stimuli. To define the genes induced by a single stimulus, to identify the corresponding proteins and to understand their adaptive function are the most important steps in exploring adaptional networks. As a good tool for elucidating these networks and their modules, proteomics can be used:
- To define single stimulons, i.e. the entire set of proteins/ genes induced or repressed by one stimulus. For this "stimulon approach" the protein pattern of stressed cells has to be analysed and compared to that of untreated cells to identify the induced or repressed proteins.
- To dissect each stimulon into single regulons, i.e. the basic modules of global gene expression. For this "regulon approach" the protein pattern of the wild type has to be compared with that of the respective regulatory mutant under conditions that activate the regulators
- To analyse overlapping regions between single regulons because these do not exist independently but are tightly connected and form a complicated adaptational network
- To analyse the kinetics of gene expression programmes (Hecker *et al.*, 2003).
In *S.aureus*, a comprehensive exploration of the adaptational network will not only provide basic knowledge on *S.aureus* physiology, but will also give many clues on the function of still unknown proteins indicated by the induction profile of genes by environmental stimuli. Relying on the fact that environmental stimuli such as heat, oxidative or anaerobic stress might be essential cellular signals in the host environment which controll the expression of virulence genes, proteomics has been used as an excellent experimental tool to visualize changes in the protein synthesis pattern of living cells and to define the structure and function of the single stimulons or regulons in response to different stresses (Fuchs *et al.*, 2007, Wolf *et al.*, 2008, Hochgräfe *et al.*, 2008).

1.3 Function and regulation of stress response

1.3.1 Heat stress response

Heat stress response is a rapid reaction of bacteria to temperature up-shift. It is presumed that damaged proteins, such as unfolded and denatured proteins are detected by cellular systems which induce a large set of so called heat shock proteins (HSP). (Boorstein *et al.*, 1994; Gupta,1995). The heat shock response is found in all living cells studied so far (Craig, 1985). Many of the HSPs are molecular chaperones (e.g., GroEL, GroES. DnaK, DnaJ) and ATP-dependent proteases (e.g.,ClpP, ClpC) (Sherman and Goldberg, 1992, 1996, Kandror *et al.*, 1996) play a critical role in the refolding of denatured proteins and in protein degradation under normal and stress conditions. Reports studying heat shock proteins in the Gram-positive bacterium *Bacillus subtilis* and in the Gram-negative bacterium *Escherischia coli* indicated that heat shock proteins are also important for the protection against other environmental stresses such as high salt concentration or heavy metal stress (VanBogelen and Neidhardt, 1987; Inbar and Ron, 1993; Hecker and Völker, 1998).

1.3.1.1 Regulation of heat stress response in Gram-positive bacteria

The model organism for studying the heat shock response in Gram-positive bacteria was *B. subtilis*. The heat shock response of this bacterium includes the induction of proteins from several regulatory groups (Zuber and Schumann, 1994; Hecker and Völker, 1998).

Class I heat shock genes encode classical chaperones (DnaK, GroES, GroEL). The *dnaK* and *groESL* operons controlled by the HrcA repressor recognizing the highly conserved CIRCE operator (Zuber and Schumann, 1994) were identified at elevated levels in *B. subtilis* on 2D gel after heat shock (Hecker and Völker., 1990). The HrcA regulon consists of the *dnaK* and *groE* operons that are preceded by a σ^A type promoter and a CIRCE element (controlling IR of chaperone expression) (TTAGCACTC-N9-GAGTGCTAA) which was shown to be involved in the regulation of the heat shock response (Zuber and Schumann, 1993). The

dnaK operon includes the genes *hrcA, grpE, dnaK* and *dnaJ* of which *hrcA* gene encodes a transcriptional repressor of the *dnaK* and *groE* operons (Wetzstein *et al.*, 1992; Schulz and Schumann, 1996). In the absence of heat shock, the binding of activated HrcA to the CIRCE element is modulated by the GroE molecular chaperone amount which allows a basal level of transcription of the downstream operon (Mogk *et al.*, 1997). After exposure to heat stress, HrcA is inactivated and not able to bind to the CIRCE sequence and GroE is titrated by nonnative proteins arising as a result of the heat shock. The more nonnative proteins are removed from the cytoplasm, the more the GroE chaperonins will be free to convert inactive HrcA into its active form, resulting in a turn-off of the HrcA regulon until the default state has been reached (Schumann, 2003).

Class II genes encode general stress proteins (GSPs) whose transcription is regulated by the alternative sigma factor σ^B and strongly induced by heat, ethanol, acid or salt stress, as well as by starvation for glucose, phosphate, and oxygen. (Bernhardt *et al.*,1997; Hecker and Völker, 1998, 2001; Hecker, 2003). In unstressed cells, the anti-sigma factor RsbW sequesters σ^B in an inactivate complex, preventing its association with the RNA polymerase core enzyme. In stressed cells, the regulation to activate σ^B depends on two classes of stress. The first class consists of energy-stress signals caused by carbon, phosphorus, oxygen starvation or the addition of uncouplers. The second class includes environmental-stress signals such as acid, ethanol, heat or salt stress (Price, 2002)

Class III heat shock genes are repressed by CtsR (Class three stress gene Repressor). In *B. subtilis*, a cis element that contains a heptameric tandem consensus sequence is found upstream of the *clpC* operon as well as of *clpE* and *clpP* and is shown to be the binding site of the CtsR repressor (Krüger *et al.*, 1998). The class III heat shock proteins include proteins involved in protein renaturation, protein repair, or ATP-dependent proteolysis such as the ATP-dependent Clp proteases (ClpC, E, P, X) that are controlled by the CtsR repressor. The ClpC ATPase of *B. subtilis* is shown to be involved in enzymes synthesis, sporulation, cell division, and survival under stress conditions. Both the *clpC* operon (*ctsR-mcsA-mcsB-clpC*) and the *clpP* gene are preceeded by overlapping σ^B and σ^A-dependent promoters (Schumann *et al.*, 2002). ClpP protein is essential for

growth at high temperature and for stress tolerance (Gerth *et al.*, 1998; Msadek *et al.*, 1998).

Class IV heat shock genes: Only one class IV heat shock gene, the *htpG* gene, has been identified so far in *B. subtilis* (Schumann, 2003). The regulatory site for *htpG* (GAAAGG) has been identified immediately downstream of its σ^A-dependent promoter (Versteeg *et al.*, 2003). This gene, which is assumed to code for a molecular chaperone, is induced about ten-fold after a heat shock of 48° C, both at the level of transcription and translation (Schulz *et al.*, 1997).

Class V heat shock genes: This group has been described quite recently and consist of two members, *htrA* and *htrB* (Darmon *et al.*, 2002), encoding putative membrane-anchored proteases. Both genes are preceded by a -10 region of σ^A-type promoters but lack an obvious -35 region. Instead, the control regions have a 4-fold-repeated octameric consensus sequence (TTTTCATA) positioned close to the -35 regions (Noone *et al.*, 2001). Both genes are under the positive control of the CssRS two-component signal transduction system (for control of secretion stress regulator and sensor), which responds not only to heat but also to secretion stress (Darmon *et al.*, 2002).

Class VI heat shock gene: Class VI summarizes genes whose expression is also responsive to heat stress, but the mechanism of induction is not affected by any one of the previously mentioned regulators (Schumann, 2003). Ten class VI heat shock genes have been reported previously in *B. subtilis*, where four are arranged in monocistronic transcriptional units (*ftsH, clpX, ykoZ, and sacB*) (Gerth *et al.*, 1996; Deuerling *et al.*, 1997; Schumann *et al.*, 2002; Zuber *et al.*, 2001) and six in bicistronic units (*lonA-orfX, ahpC-ahpF*, and *nfrA-ywcH*) (Riethdorf *et al.*, 1994; Antelmann *et al.*, 1996; Moch *et al.*, 2000).

1.3.1.2 Heat stress response in *S.aureus*

The ubiquitous nature of *S.aureus* derives mostly from its ability to survive in a great variety of environmental extremes, such as nutrient starvation, a wide range of pH and growth temperatures, restriction of metal ions, high salt concentration or desiccation. An increasing amount of data indicates that the capacity to

survive stress conditions is highly correlated with virulence in *S.aureus* (Clements and Foster, 1999). Analyses of the complete *S.aureus* genome sequence (Kuroda *et al.*, 2001) indicate that, at least four different types of heat shock response regulatory mechanisms may co-exist (Clements and Foster, 1999, review):

Class I, which includes the *dnaK* operon, are regulated by the HrcA repressor, which recognizes CIRCE operator (Ohta *et al.*, 1994)

Class II genes encode general stress proteins whose expression requires the stress sigma factor (Gertz *et al.*, 2000).

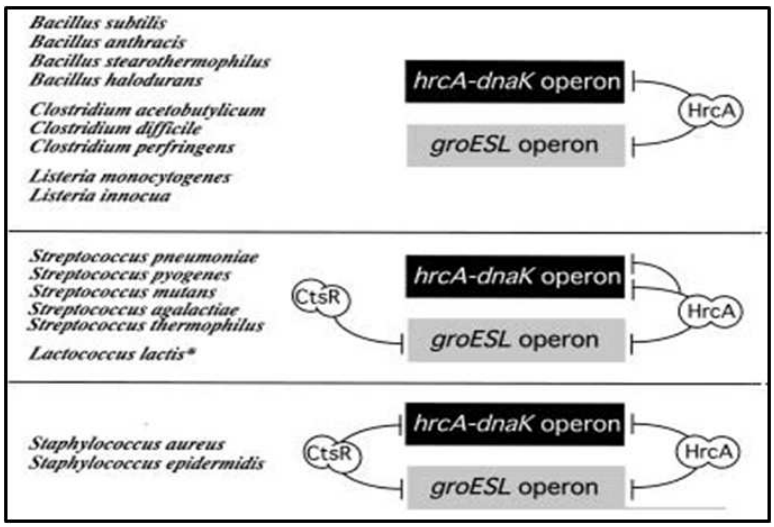

Figure 1.3: Dual regulation by CtsR and HrcA of the dnaK and groESL operon is present in several Gram positive bacteria

Class III heat shock genes, are negatively regulated by CtsR, which recognizes a directly repeated heptanucleotide operator sequence (A /GGTCAAA NAN A/GGTCAAA) (Derre *et al.*, 1999). While in *B. subtilis* and in other bacteria of the Bacillus group, the *dnaK* and *groESL* operons are only controlled by HrcA, in the Staphylococcus group both operons are dually regulated by CtsR and HrcA . These two repressor control the expression of *dnaK* and *groESL* operon by binding directly and specifically to their promoter regions (Chastanet *et al.*, 2003) (Figure 1.3).

The entire HcrA regulon is embedded within the CtsR regulon, with the syn-

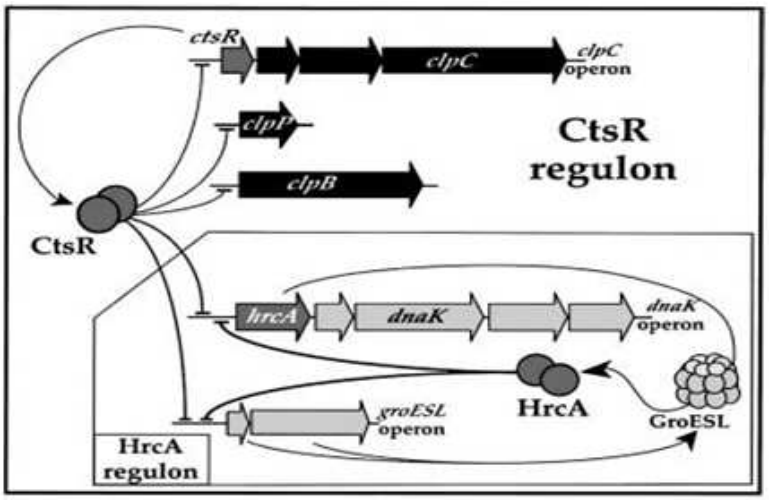

Figure 1.4: The HrcA regulon of S. aureus is entirely embedded within the CtsR regulon,with the synthesis of HrcA itself being repressed by CtsR.

thesis of HrcA itself repressed by CtsR, as HrcA is the first gene of the dnaK operon. Many Clp genes of *S.aureus* (*clpB, clpP, clpC*) are also controlled by CtsR (Chastenet *et al.*, 2003) (Figure 1.4)

Besides, according to the genome sequence of *S.aureus*, there are also orthologues of many genes belonging to class VI heat shock gene of *B. subtilis* which are not controlled by HrcA, or CtsR .

1.3.2 Puromycin stress response

1.3.2.1 General

Antibiotics are natural or synthetic compounds that inhibit the growth or kill the bacteria (bacteriostatic or bacteriolytic antibiotics). Even though antibiotics are successfully applied in the therapy of many otherwise lethal infectious diseases, bacteria are able to adapt to several antibiotics by the development of specific resistance mechanisms. Thus, the study of the bacterial response to antibiotics by transcriptomic and proteomic approaches is required to understand those mechanism them. Puromycin is a cinnamamido adenosine antibiotic substance found

in *Streptomyces alboniger* which inhibits protein synthesis by binding to RNA. It is an antineoplastic and antitrypanosomal agent and is used in research as an inhibitor of protein synthesis. Puromycin is structural analog of the aminoacyl-tRNAs. One of the most striking differences between the two molecules is the adenine moiety carrying two methyl groups on its amino nitrogen, and the tyrosyl residue, which forms the amino acid residue, is also methylated in its phenolic oxygen group (Ingram *et al.*, 1972). Another important difference is that instead of an ester linkage attaching the amino acyl groups, puromycin uses an amide grouping. Puromycin inhibits protein synthesis by prematurely terminating a peptide chain. In simple terms, the part of puromycin that resembles an aminoacyl end of tRNA can bind to the A site of a ribosome (forming a peptide bond), but the end product will not participate in translocation to the P site (Fig.1.5). This lack of participation in translocation results in the dissociation of unfinished polypeptide chains from the ribosome and early peptide termination. This early termination results in an accumulation of truncated, nonfunctional polypeptides in the cell.

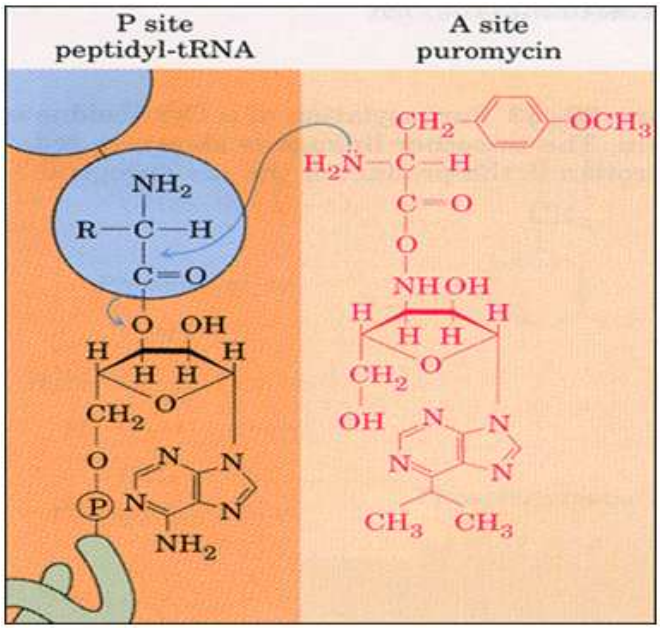

Figure 1.5: Antibiotic action of puromycin. (Garret, R.; Grisham, C. Biochemistry. Austin: Saunders College Publishing; 1995)

1.3.2.2 Puromycin stress response in Gram-positive bacteria

Treatment with puromycin have been reported to induce the heat shock response in *B. subtilis* (Hecker *et al.*, 1996, Bandow *et al.*,2003), although no class II general stress protein were induced after puromycin stress in *B. subtilis* (Mogk *et al.*, 1998; Movahedi and Waites, 2002) An enhanced transcription of htrA was also seen in Lactobacillus cells as well as in *Listeria monocytogenes* cells after exposure to puromycin (Smeds *et al.*, 1998; Wilson *et al.*, 2006). It was also indicated that the same stress proteins induced after heat shock were also induced after puromycin stress in in *Streptococcus pneumonia* (Wai-Leung Ng *et al.*, 2002).

1.3.3 Salt stress response

1.3.3.1 General

The exposure of organisms to conditions of high salinity results in a decrease of their cytoplasmic water activities. This phenomenon consequently brings about a reduction in the turgor pressure and a shrinkage of the cytoplasmic volume. called plasmolysis. Sudden plasmolysis results in the inhibition of a variety of physiological processes, ranging from nutrient uptake to an increase in the adenosine triphosphate (ATP) levels of the cells (Ohwada and Sagisaka, 1988). Generally, two basic schemes of bacterial adaptation to high osmolality have been identified:

1. The accumulation of intracellular ions, a strategy followed frequently by extreme halophilic archaea and by halotolerant bacteria whose entire physiology has been adapted to a high-saline environment (Galinski and Trüper, 1994; Ventosa *et al.*, 1998).

2. The intracellular amassing of osmotically active compounds that are highly appropriate with cellular functions, the so-called compatible solutes since their molecules that are accumulated during conditions of osmotic stress are not greatly inhibitory to cellular processes (Brown 1972, 1980). These organic solutes are accumulated by many microorganisms through synthesis or uptake from the environment to counteract the outflow of water under hypertonic growth conditions

(Galinski and Trüper, 1994; Csonka and Epstein, 1996; Miller and Wood, 1996). Osmoprotectants are operationally defined as exogenously provided organic solutes that enhance bacterial growth in media of high osmolarity. These substances may be compatible solutes themselves, or they may act as precursor molecules that can be enzymatically converted into these compounds. The intracellular amassing of compatible solutes is not restricted to the prokaryotic world but is also widely used as an adaptive strategy in fungal, plant, animal, and even human cells to offset the deleterious effects of high osmolarity and high ionic strength (Rhodes and Hanson, 1993; Burg *et al.*, 1997; Hohmann, 1997).

The prominent compatible solutes found in bacteria are K^+ ions, the amino acids glutamate, glutamine, proline, choline, alanine and γ- aminobutyrate, the quaternary amines glycine betaine and other fully N-methylated amino acid derivatives, as well as the sugars sucrose, trehalose (α-D-glucopyranosyl- α-D-glucopyranoside), and glucosylglycerol (Flowers *et al.*, 1977; Reed *et al.*,1986; Yancey *et al.*, 1982). Among them, proline, choline and glycine betaine were considered as important osmoprotective compounds (Reed *et al.*, 1985; Landfald and Strom, 1986; Christian,1955 ; Strom *et al.*, 1986).

Studies with *E. coli* and *Salmonella typhimurium* revealed that initially large amounts of K^+ are rapidly taken up from the environment via turgor-responsive transport systems . Concomitantly, glutamate synthesis is increased to provide counterions for the strong increase in positive charges. Glutamate synthesis is dependent on the prior uptake of K^+, and glutamate is required to maintain the steady-state K^+ pool (McLaggan *et al.*, 1994; Yan *et al.*,1996). High intracellular concentrations of K^+, however, have negative effects on protein function and DNA-protein interactions in non-halophilic bacteria; thus, the massive accumulation of K^+ is an inadequate strategy for coping with prolonged high osmolarity. Instead, the initial increase in cellular K^+ content is followed by the accumulation of compatible solutes, which allows the cell to discharge large amounts of K^+ through specific and nonspecific efflux systems (Csonka and Epstein, 1996; Stumpe *et al.*, 1996). Two transporters are primarily responsible for the uptake of osmoprotectants across the cytoplasmic membrane of these enteric bacteria: ProP and ProU. These transporters were originally identified as osmotically stimulated

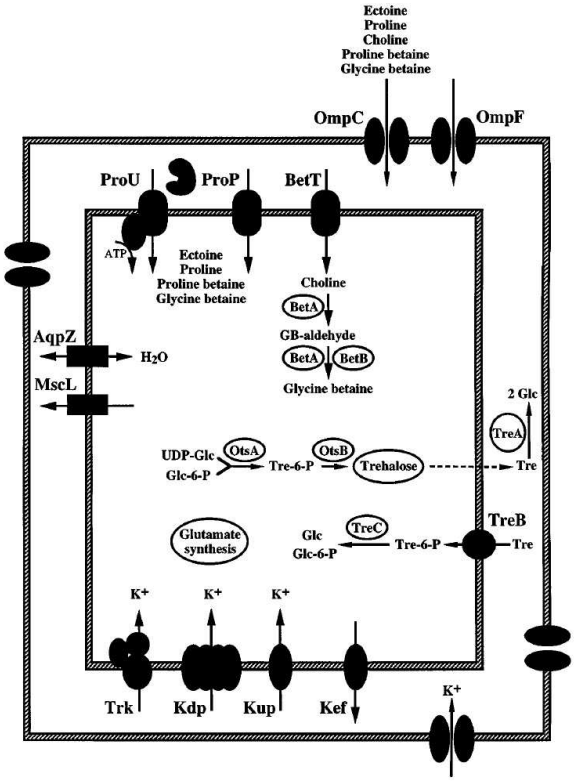

Figure 1.6: Osmostress response systems of *E. coli*. GB : glycine betaine, Glc : glucose, Glc-6-P: glucose-6-phosphate, Tre : trehalose, Tre-6-P: trehalose-6-phosphate UDP-Glc: uridine disphosphate-glucose

uptake systems for proline, but subsequent studies established their pivotal role in the uptake of a wide spectrum of osmoprotectants, of which glycine betaine and proline betaine are transported with high affinity (Csonka and Epstein, 1996). Permeation of osmoprotectants across the *E. coli* and *S. typhimurium* outer membrane is accomplished by passive diffusion through the nonspecific porins OmpC and OmpF.

As a model of Gram-positive bacteria, *B.subtilis* initiates a two-step adaptation response to cope with the unfavourable osmotic conditions (Bremer and Kramer, 2000; Kempf and Bremer, 1998). Initially, K^+ is rapidly taken up (Whatmore and Reed, 1990) and subsequently replaced in part by proline (Whatmore *et al.*, 1990). These osmolytes can be accumulated to high levels through either de novo synthesis or uptake of osmoprotectants from the environment without interfering

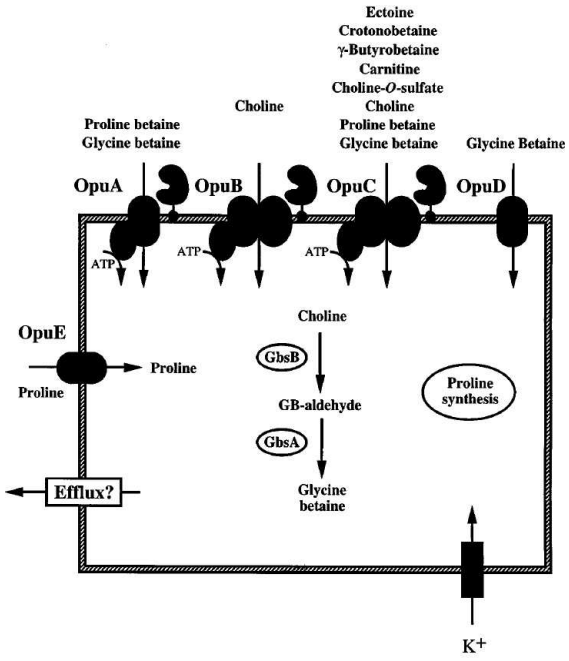

Figure 1.7: Osmostress response systems of *B. subtilis*

with central cellular functions.

In addition, *B. subtilis* can efficiently scavenge a wide variety of compatible solutes such as choline, proline or glycine betaine from environmental sources by means of five osmoregulated transport systems (OpuA to OpuE) (Kappes *et al.*, 1996; Kappes *et al.*, 1999; Kempf and Bremer, 1995; von Blohn *et al.*, 1997) and can aquire choline for the production of the osmoprotectant glycine betaine (Kappes *et al.*, 1999) In *B.subtilis*, the accumulation of compatible solutes offsets the detrimental effects of high osmolarity on cell physiology and permits growth over a wide range of osmotic conditions (Boch *et al.*, 1994). Proteomic and transcriptomic approaches have shown that in *B. subtilis* salt stress triggers generally the induction of the SigB regulon (Petersohn *et al.*, 2001; Steil *et al.*, 2003; Höper *et al.*, 2006). This non-specific stress protection also includes protection against osmotic stress. Membrane proteins such as OpuD and OpuE are involved in the uptake of osmoprotectants. The immediate response to salt stress is followed by the induction of the sigmaW regulon (Höper *et al.*, 2006). The σ^W-dependent proteins seem to be involved in the maintenance of cell integrity during cell sur-

face or alkaline stress (Cao *et al.*, 2002). Furthermore, salt stress seems to cause oxidative stress in the cells as indicated by the induction of the PerR-dependent catalase KatA, the alkyl hydroperoxide reductase subunits AhpC and AhpF, the glutamyl tRNA reductase HemA as well as enzymes involved in cysteine biosynthesis and the formation of [4Fe-4S] clusters (CysC, YurU) (Höper *et al.*, 2006) and those induced also by iron limitation (Hoffmann *et al.*, 2002). Transcriptomic analysis revealed a strong induction of the *proJH* genes which are involved in the regulation of proline synthesis in response to osmotic shock (Belitsky *et al.*, 2001).

1.3.3.2 Salt stress response in *S.aureus*

S.aureus is one of the most osmotolerant bacteria of the nonhalophilic eubacteria and is able to grow at low water activities (equivalent to hight salt concentration). Although there are some studies on osmoregulation in *S.aureus* (Christian and Waltho, 1964) very little is known about mechanisms conferring the high salt tolerance of this bacterium. Osmoregulation in this organism is therefore inherently interesting for several reasons. Unlike many other organisms which grow in environments of elevated osmolarity, *S.aureus* can also perfectly grow at low osmolarities, suggesting an efficient means of regulating cytoplasmic osmolarity (Graham *et al.*, 1992)

In contrast to enteric bacteria, cytoplasmic levels of K^+ do not change strikingly in *S.aureus* grown in media of increasing osmolarity (Graham and Wilkinson, 1992; Kunin and Rudy, 1991. Choline, glycine betaine, proline, and taurine act as osmoprotectants for *S.aureus* (Miller *et al.*, 1991; Graham and Wilkinson, 1992). Transport systems for glycine betaine (Pourkomailian and Booth 1992, 1994; Bae *et al.*,1993; Stimeling *et al.*, 1994), choline (Kaenjak *et al.*,1993) and an osmotically activated, low-affinity transport system for proline have been already described in *S.aureus* (Bae and Miller, 1991; Townsend and Wilkinson, 1992).

However, some of the mechanisms *S.aureus* utilizes to survive under osmotic stress are similar to those of Gram-negative enteric bacteria such as *E.coli* and *S.typhimurium*, both of which accumulate compatible solutes including proline and glycine betaine via transport (Stewart *et al.*, 2005.). There are, however, several major differences, as enterics have a two-phase process in which they adapt

to osmotic stress, this process first allows the co-transport and rapid accumulation of potassium and glutamate ions, followed by the activation of transport systems that lead to the accumulation of compatible solutes (Booth, 1998). In contrast under osmotic stress conditions, *S.aureus* cells increase the compatible solute pools in the cytoplasm and inhibit the transport of potassium and glutamate (Pourkomailian and Booth, 1992).

Among a series of compatible solutes, glycine betaine and proline are the principal ones of *S.aureus*, with glycine betaine being the most potent osmoprotectant (Bae and Miller, 1991). Transport of these osmolytes is mediated by three basic systems: an osmotically sensitive, low affinity glycine betaine/proline transport system (BPII) and two osmotically insensitive, high affinity transport systems; one specific for proline (PPI) and the other specific for glycine betaine (BPI) (Bae and Miller, 1992; Pourkomailian and Booth, 1992, 1994; Townsend and Wilkinson, 1992; Pourkomailian, 1998).

The active transport of glycine betaine and proline in *S.aureus* is achieved by sodium-dependent transport, and thus is energized by sodium motive force. To date, regulation of gene expression in *S.aureus* in response to salt stress has not yet been well characterized.

Aim of thesis

Our approach was to analyse protein/genes of *S.aureus* whose expression is highly stimulated under different environmental stress conditions as well as antibiotic stress , in order to identify specific proteins for these conditions.
In this work, the changes in protein expression profiles as well as the gene transcription of *S.aureus* after heat exposure, osmotic stress and in response to the antibiotic puromycin were studied in order to provide detailed insights into the response of *S.aureus* to various kinds of environmental stress under in vitro conditions, namely:
(1) to investigate the global response of *S.aureus* to heat stress conditions using transcriptomic and proteomic analyses.
(2) to study the transcriptome and proteome of *S.aureus* in response to antibiotic substance puromycin.
(3) to define the proteome signatures of *S.aureus* under NaCl stress condition.

(4) to complete the proteome map of cytoplasmic proteins of *S.aureus* by identifying proteins exclusively synthesized during the exposure to stress.

Chapter 2

Materials and Methods

2.1 Cell culture

2.1.1 Strains used in this study

The *S.aureus* strain used in this study was the clinical isolate *Staphylococcus aureus* COL, which is highly resistant against methicillin (MRSA) (Shafer and Iandolo, 1979)

2.1.2 Glycerol culture

S.aureus COL was spreaded on LB-Agar plates. Single colonies were picked up, then inoculated and grown in LB medium. This culture was incubated and shaken at 37° C for about 16-18 hours. 1.8 ml steril glycerol was added to 4.2 ml overnight culture and kept at -80° C.

2.1.3 Cultivation

The synthetic medium was inoculated with exponentially growing cells of *S.aureus* COL grown over night in the same medium to an initial OD_{500} of 0.07 to 0.1. Cells were cultivated with vigorous agitation at 37° C.

2.1.4 Media for cell cultivation

Name	Components	Concentration
LB (Broth medium)	Casein peptone	10 g/l
	Yeast extract	5 g/l
	NaCl	5 g/l
	Glucose	1 g/l
Synthetic medium contains: Basic medium, amino acids, vitamins and metal elements		
Basic medium	Glucose	7.5 mM
	Citric acid	0.142 mM
	Na_2HPO_4	10 mM
	KaH_2PO_4	10 mM
	$MgSO_4$ x 7 H_2O	0.81 mM
	NH_4Cl	9.34 mM
	MOPS pH 7.0	40 mM
Amino acid	Alanine,valine,leucine,isoleucine	8g/l
	Aspartate, glutamate	8g/l
	Serine, threonine, cysteine	8g/l
	Phenylalanine,tryptophane,histidine	8g/l
	Arginine, lysine, proline	8g/l
	Glycine	80g/l
Vitamins	Cyanocobalamin	0.036μM
	4-Aminobenzoat	0.29μM
	Biotin	0.04μM
	Nicotin acid	0.81μM
	Ca-Panthotenat	0.21μM
	Pyrioxamindihydrochloride	0.62μM
	Thiamindichloride	0.29μM
	Riboflavine	0.26μM
Metal elements	Cyanocobalamin	0.036μM
	$ZnCl_2$	0.51μM
	$MnCl_2$	0.5μM
	Boric acid	0.097μM
	$CoCl_2$	1.46μM
	$CuCl_2$	0.015μM
	$NiCl_2$	0.1μM
	Na_2MoO_4	0.148μM

All components of the basic medium (except glucose and MOPS) were autoclaved for 20 minutes at 120° C. Glucose and MOPS were separately autoclaved only for 10 minutes at 120° C. Amino acids, vitamins and metal elements were filtrated for sterilization.

2.1.5 Stress experiment

For stress kinetic experiments, 10 to 50 ml of cell culture at an optical density at 500 nm of 0.5 were transferred to new preheated Erlenmeyer flasks and exposed to stress conditions. Cells were harvested at fixed time intervals after imposition of stress. As a control 20 to 50 ml of the untreated culture were harvested immediately before and at the end of the stress experiment (Engelmann and Hecker, 2008).

2.2 Protein preparation

2.2.1 Preparation of cytoplasmic proteins

For preparation of cell extracts, at an optical densities OD_{500}=0.5 of different time points after exposure to stress, cells of 50 ml culture were separated from the supernatant by centrifugation (7,000 x g) for 10 min at 4° C. Cell pellets were washed twice with 1 ml ice cold TE buffer, resuspended in 1 ml TE buffer and transferred into screw top tubes containing 500 μl glass beads. Cells were disrupted by homogenization with glass beads using the Ribolyser (Thermo Electron Corporation) for 30 seconds at 6.5 m/s. The lysate was centrifuged for 25 min at 21,000 x g at 4° C so that cell debris is removed and the supernatant is transferred to a new tube. In order to remove insoluble and aggregated proteins which disturbed the isoelectric focusing of the proteins, the supernatant was centrifuged once again for 45 min at 21,000 x g at 4° C. Protein concentration was determined using Roti-Nanoquant (Roth, Karlsruhe, Germany).The protein solution was stored frozen at -20° C.

2.2.2 Preparation of cytoplasmic protein extracts labeled with [^{35}S]-L-methionine (pulse-labelling reaction)

Cells were grown in synthetic medium at 37° C to an optical density of 0.5. Then 10 ml of the culture volume were transferred to a new Erlenmeyer flask and 10 μl (100 Ci) L-[^{35}S]-methionine were added to the culture. The labeling reaction was stopped after 5 min by adding 1 ml stop solution and by transferring the flask to ice. Afterwards, cells were pelleted by centrifugation (8,000 x g) for 5 min at 4° C. Cell pellets were washed twice with 1 ml ice cold TE buffer and were resuspended in 400 μl TE buffer. For cell lysis 10 μl lysostaphin solution (10 mg/ml) were added to the cell suspension. After incubation on ice for 10 min, cells were disrupted by sonication. For this step a breaker of ice water was placed around the sample tube to keep it cold. The samples were sonicated for 1 min (0.5 $^{s-1}$, low) followed by a 1 min cooling break. This process was repeated three times. Sonication was complete when the solution appeared noticeably less cloudy than the starting solution. After sonication, the sample was centrifuged at 21,000 x g for 10 min at 4° C. The supernatant was removed to a new tube and the centrifugation process was repeated for 30 min. Protein concentration was determined using ROTI-Nanoquant (Roth, Karlsruhe Germany). The protein solution was stored frozen at -20° C.

2.2.3 Determination of the labelling efficiency of radioactively labelled protein extract

10 μl of the protein extract were dropped on filter paper plates. The paper plates were dried and incubated in 10% (v/v) ice cold TCA for 10 minutes, then incubated in 5% (v/v) ice cold TCA for 10 minutes and finally the plates were washed two times in 96% (v/v) ethanol at room temperature. The filter papers were dried under red light and then were transferred into Scintillation cuvette, covered with 1ml Toluene.

2.2.4 Determination of protein concentration

10 μl of each protein solution was used. Otherwise an appropriate volume of the protein extract was diluted prior to measurement. The mixture of protein solution together with ROTI-Nanoquant was measured at 590 nm and 450 nm (Ultraspec 3000, Pharmacia Biotech). The protein amount (m Protein) was calculated using the following formula:

$$m_{Protein}[\mu g] = \frac{\frac{E590}{E450} - 0.3846}{0.054945}$$

$$C_{Protein}[\mu g/\mu l] = \frac{m_{Protein} \cdot D}{SV}$$

D : Dilution range of samples. SV : Sample volume.

The ratio E_{590nm}/E_{450nm} must always be the same for each protein extract. Protein extracts in TE or water should be frozen at -20° C before measuring the protein concentration.

2.3 Two dimensional protein gel electrophoresis

2.3.1 Isoelectric focussing (IEF)

1. Protein samples (80-100 μg of radioactively labeled proteins, 350-600 μg of unlabeled proteins for Colloidal Coomassie staining) were made up to 360 μl with 8 M urea/2 M thiourea. If the volume containing the desired amount of cytoplasmic proteins exceeds 40 μl the volume should be reduced by using a speed vac.

2. Subsequently 40 μl 10x rehydration buffer were added and the solution was mixed by shaking at room temperature for 30 min. The rehydration mix was centrifuged for 5 min at 21,000 x g (-20° C) to remove insoluble proteins.

3. The supernatant was equally dispensed in one slot of the rehydration chamber. The IPG strips were positioned with the gel side down. Rehydration occured over

night for at least 15 h and no longer than 24 h.

4. The isoelectric focusing was performed with the Multiphor II unit at -20° C. The strips were positioned in the DryStrip Aligner in adjacent grooves and all strips were aligned so that the anodic gel edges were lined up. The electrode strips which already soaked with distilled water were placed across the cathodic and anodic ends of the aligned IPG strips. The electrode strips must at least partially contact the gel surface of each IPG strip. The IPG strips were covered with mineral oil.

5. The isoelectric focusing was performed by using the following voltage profile: Step 1: 500V (gradient), 2mA, 5W, 2Vh. Step 2: 3500 V (gradient), 2mA, 5W, 3kVh. Step 3: 3500 V, 2mA, 5W, 23.5 kVh.

6. After IEF the second-dimension was performed immediately or the IPG strips could be stored at -20° C .

Rehydration solution

2,4 g	Urea
760 mg	Thiourea
50 mg	Chaps
15 mg	DTT
52 μl	Pharmalyte 3-10
	ad. 5 ml distilled water

2.3.2 SDS PolyAcrylamide Gel Electrophoresis (PAGE)

1. The separation of the focused proteins according to their molecular weight was done in 12.5% acrylamide and 2.6% bis-acrylamide polyacrylamide gels using the Tris-glycine system

2. For preparing 12 slab gels 335.3 ml of 40% acrylamide, 179 ml 2% bis-acrylamide, 272.54 ml 1.5 M Tris-HCL (pH 8.8), 11.48 ml 10% SDS, and 300 ml deionized water were mixed while stirring on a magnetic stirrer. 2.8 ml 10% APS and 0.55 ml TEMED were added to this solution and mixed by stirring. The gels were poured immediately by filling the gel cassette about 1 cm below

the top of the glass plates. The gels were overlayed immediately after pouring with a thin layer (1 ml) of water-saturated n-butanol or water immediately after pouring the gels to minimize the gel exposure time to oxygen and to create a flat gel surface. Polymerization takes at least 3 h. Each gel should be inspected and the top of surface of each gel should be straight and flat. The butanol was completely removed and the gel surface was rinsed with deionized water.

3. The stacking gel was prepared by mixing 10.8 ml 40% acrylamide, 3.4 ml 2% bisacrylamide, 30 ml upper buffer (4x), and 74 ml deionized water. Afterwards 0.3 ml 10% APS and 0.05 ml TEMED were added to the solution while stirring. An appropriate amount of the stacking gel solution was used to quickly rinse the top of each gel and was overlaid with a thin layer (1 ml) of water. The stacking gel should polymerize within 1 hour.

4. Equilibration buffer A and B were prepared. Each IPG strips was placed with the gel side up in one slot of an equilibration chamber and 4-5 ml Equilibration buffer A were added to each slot. The IPGs were equilibrated for at least 15 min with gently shaking. The equilibration solution A was decanted and 4-5 ml equilibration solution B were added to each slot. The IPGs were equilibrated again for at least 15 min while shaking. Equilibration solution B was decanted and the IPG strips were placed on filter paper so that they rest on an edge to help drain the equilibration solution.

5. Running buffer (1x) was added to the gel system. Gels were put in the gel system filled with running buffer (1x).

6. The IPG strips were placed between the plates on the surface of the stacking gel by gently pushing the IPG strip down so that the entire lower edge of the IPG strip was in contact with the top surface of the stacking gel. No air bubbles should be trapped between the IPG strips and the stacking gel.

7. Electrophoresis was performed at constant power (2 W per gel) at 12° C. A constant temperature during electrophoresis is very important for gel to gel reproducibility. When starting electrophoresis the buffer temperature should be at 12° C .

8. After electrophoresis, gels were removed from their glass plates in preparation

for staining. Each gel should be marked to identify the acidic end of the first dimension preparation.

Equilibration solution

Main solution	36 g Urea 30ml Glycerol 4 g SDS 10 ml Tris /HCl pH 6,8 adjust 100 ml distilled water
Solution A	add 3,5 mg/ml DTT
Solution B	add 45 mg/ml Iodoacetamid

Separating gel (4%)

Amount	**100 ml**
A.bidest	60 ml
40 % (w/v)Acrylamid	9 ml
2 %(w/v)Bisacrylamid	4,5 ml
0,5 M Tris-HCl, pH 6,8	25 ml
10 % (w/v) SDS	1 ml
10% (w/v) APS (Ammoniumpersulfat)	380 μl
TEMED	62,5 μl

Stacking gel (12,5%)

Amount	**1000 ml**
A. bidest	266 ml
40 % (w/v)Acrylamid	304 ml
2 %(w/v)Bisacrylamid	170 ml
1,5 M Tris-HCl, pH 8,8	250 ml
10 % (w/v) SDS	10 ml
10 % (w/v) APS (Ammoniumpersulfat)	2,5 ml
TEMED	500 μl

2.3.3 Protein detection

2.3.3.1 Colloidal Coomassie staining

250 ml Coomassie staining solution were added to each gel and the gels were incubated for 24 h on a stirrer.

1. After removing Coomassie staining solution the gels were rinsed with deionized water for several times.

2. After staining, the gels were sealed in foil.

3. Gels were scanned with a Scanner X finity ultra (Quato Graphic) in transmission mode at a resolution of 200 dpi.

4. Gels could be stored at 4 ° C.

2.3.3.2 Silver Staining

The resulting 2D gels were treated with fixing solution for silver staining (200-250 ml per gel) for 1 to 2 h.

1. Gels were washed three times with 200 to 250 ml 50% (v/v) ethanol for 20 min and pretreated with 200 to 250 ml sodium thiosulfate solution for 1 min.

2. The gels were rinsed three times with deionized water for 20 seconds.

3. Gels were incubated with 200 to 250 ml silver nitrate solution for 20 min.

4. After removing the silver nitrate solution the gels were rinsed twice with deionized water for 20 s.

5. For developing, gels were incubated in 200 to 250 ml potassium carbonate solution for 2 to 10 min. The developing reaction was stopped by incubating the gels with 200 to 250 ml 1% (w/v) glycine for 20 s, rinsing with deionized water for 20 s and stopping again with 200 to 250 ml 1% (w/v) glycine for 20 min.

6. Gels were washed two times with deionized water for 20 min and sealed in foil.

7. Gels were scanned with a Scanner X finity ultra (Quato Graphic) in transmission mode at a resolution of 200 dpi.

8. Gels could be stored at 4° C.

2.3.3.3 Detection of L-[^{35}S]-methionine labelled proteins

1. After silver staining and scanning, gels were placed on Whattman paper, covered with cellophane sheets and dried on a vacuum dryer at 75° C for at least 2 to 4 hours

2. The dried gels were exposed to "Storage Phosphor Screens" for 2 h to several days (depending on signal intensity). Storage Phosphor Screens were scanned with a Storm 840 Phosphor Imager at a resolution of 200 m and a color depth of 16 bit (65536 gray scay levels)

2.3.4 Protein identification by mass spectrometry

For identification of proteins by MALDI-TOF-MS, Coomassie stained protein spots were cut from gels using a spot cutter (Proteome Work^{TM}) with a picker head of 2 mm and transferred into 96-well microtiter plates.

1. Digestion with trypsin and subsequent spotting of peptide solutions onto the MALDI targets were performed automatically in the Ettan Spot Handling Workstation (GE Healthcare, Little Chalfont, United Kingdom) using the following standard procedure: The gel pieces were washed twice with 100 μl 50 mM ammonium bicarbonate/ 50% (v/v) methanol for 30 min and once with 100 μl 75% (v/v) acetonitrile for 10 min. After 17 min drying, 10 μl trypsin solution containing 20 ng/μl trypsin were added and the mixture was incubated at 37° C for 120 min. For peptide extraction gel pieces were covered with 60 μl 50% (v/v) acetonitrile / 0.1% (w/v) TFA and incubated for 30 min at 37° C. The supernatant containing the peptides was transferred into a new microtiter plate and the extraction was repeated with 40 μl of the same solution. The supernatants were dried completely at 40° C for 220 min completely and the peptides were dissolved in 2.2 μl of 0.5% (w/v) TFA / 50% (v/v) acetonitrile.

2. 0.7 μl of this solution was directly spotted onto the MALDI target. Then, 0.4 μl of matrix solution was added and mixed with the sample solution by aspirating

the mixture five times. Prior to the measurement in the MALDI-TOF instrument the samples were dried on the target for 10 to 15 min.

3. MALDI-TOF-MS analyses of spotted peptide solutions was carried out on a Proteome-Analyzer 4700 (Applied Biosystems, Foster City, CA, USA). The spectra were recorded in a reflector mode in a mass range from 900 to 3700 Da. For one main spectrum 25 subspectra with 100 spots per sub spectrum were accumulated using a random search pattern. If the autolytical fragment of trypsin with the monoisotopic (M+H)+ m/z at 2211.104 reaches a signal to noise ratio (S/N) of at least 10, an internal calibration was automatically performed using the peak for one point calibration. The peptide search tolerance was 50 ppm but the actual standard deviation was between 10 and 20 ppm.

4. Calibration was performed manually for the less than 1% samples for which automatic calibration fails. After calibration the peak lists were created using the "peak to mascot" script of the 4700 ExplorerTM software with the following settings: mass range from 900 to 3700 Da; peak density of 50 peaked per range of 200 Da, minimal area of 100 and maximal 200 peaks per protein spot and minimal S/N ratio of 6. The resulting peak lists were compared with organism specific sequence databases by using the mascot search engine (Matrix Science, London, UK). Peptide mixtures that yielded at least twice a Mowse score of at least 64 (depending on the size and quality of the database) and a sequence coverage of at least 30% were regarded as positive identifications.

5. Proteins that fail to exceed the 30% sequence coverage cut-off were subjected to MALDI-MS/MS. MALDI-TOF-TOF analysis was performed for the three strongest peaks of the TOF spectrum. For one main spectrum 20 sub-spectra with125 shots per sub-spectrum were accumulated using a random search pattern. The internal calibration was automatically performed as one-point calibration if the mono-isotopic arginine (M+H)+ m/z at 175.119 or lysine (M+H)+ m/z at 147.107 reaches an S/N of at least 5. The peak lists were created using the "peak to mascot" script of the 4700 ExplorerTM software with the following settings: mass range from 60 Da to a mass that was 20 Da lower than the precursor mass; peak density of 5 peaks per 200 Da; minimal area of 100 and maximal 20 peaks per precursor and a minimal S/N ratio of 5. Database searches were performed using

the GPS explorer software with the organism specific databases. Proteins with a Mowes score of at least 64 in the reflector mode that was confirmed by subsequent peptide/fragment identifications of the strongest peaks (MS/MS) were regarded as identified. MS/MS analysis was particularly useful for the identification of spots containing more than one protein.

Materials and Chemicals for MALDI-TOF MS

Washing solution 1	50 mM ammoniumbicarbonate 50% (v/v)methanol
Washing solution 2	75% (v/v) Acetonnitril (ACN)
Trypsine solution	20 ng/mL trypsin 20 mMammoniumbicarbonate
Gel digestion solution	50% (v/v) ACN 0.1% (w/v) TFA (Trifloroacetic acid)
Diffusion solution	0.5% (w/v) TFA 50% (v/v) ACN
Matrix solution	50% (v/v) ACN 0.5% (w/v) TFA cyano-4-hydroxycinnamic acid

2.3.5 Quantitation and bioinformatic approaches

Evaluating high resolution 2-D gels by manual comparison of two gels was not always possible, consequently, an image evaluation software "Delta 2D" (Decodon GmbH, Greifswald, Germany) was applied. By means of this program, it was possible to display the dual channel imaging of the 2-D gel pair in red and green color, and to warp the protein spots as well. At least two different data sets of each experiment had to be analyzed in order to screen for differences in the amount or synthesis of the proteins identified on 2D gels. The dual channel imaging technique is an excellent tool for identifying all proteins induced or repressed by growth-restricting stimuli. In this technique, two digitized images of 2D gels were generated and combined in alternate additive dual-color channels. The first one (densitogram), showing protein levels visualized by various staining techniques, was false-colored green. The second image (autoradiograph), representing proteins synthesized and radioactively labeled during a 5-min pulse-labeling with

L-[^{35}S]-methionine, was false-colored red. When the two images were combined, proteins accumulated and synthesized in growing cells were colored yellow. However, proteins not previously accumulated in the cell but newly synthesized after the imposition of a stress or starvation stimulus were colored red. Looking for such red-colored proteins was a simple approach for visualizing all proteins induced by a single stimulus, thereby defining the entire stimulon. Proteins repressed by the stimulus can also be visualized by this powerful technique. Green-colored proteins that were no longer synthesized (no longer red) but still present in the cell were the candidates for repression by the stimulus.

2.3.6 RNA preparation

Total RNA from *S.aureus* was isolated using the acid-phenol method (Fuchs *et al.*, 2007) with some modifications. Samples (20 ml) from exponentially growing cultures (OD_{500} of 0.5) and stressed culures at different times after the shift were treated with 10 ml of ice-cold killing buffer (20 mM Tris-HCl [pH 7.5], 5 mM $MgCl_2$, 20 mM NaN_3). The cells were immediately separated from the supernatant by centrifugation (for 5 min at 7,155 g at 4° C, washed with ice-cold killing buffer, and resuspended in lysis buffer (3 mM EDTA, 200 mM NaCl). For mechanical disruption, the cell suspension was transferred into screw-top tubes containing glass beads (reach the 500 μl line on the screw top) (diameter of glass beads 0.1 to 0.11 mm; Sartorius, Goettingen, Germany) and 500 μl of water-saturated phenol-chloroform-isoamyl alcohol (25:24:1, v/v/v). Cells were then disrupted by homogenisation using a Ribolyser (Thermo Electron Corporation) for 30 s at 6.5 m/s. Afterwards, the resulting RNA solution was extracted once with water-saturated phenol-chloroform-isoamylalcohol (25:24:1,v/v/v), twice with chloroform-isoamyl alcohol (24:1, v/v), and once with water-saturated ether. RNA was precipitated by using 70% ethanol and resuspended in deionised water. The quality of RNA was ensured by gel electrophoresis and by analysis with a Bioanalyzer (Agilent Technologies, Palo Alto, CA).

2.3.7 Northern Blot analysis

Digoxigenin-labeled RNA probes were prepared by in vitro transcription with T7 RNA polymerase by using PCR fragments as templates (Gertz *et al.*,1999) The PCR fragments were generated by using chromosomal DNA of *S.aureus* COL isolated with a chromosomal DNA isolation kit (Promega, Madison, WI), according to the manufacturers recommendations, and the respective oligonucleotides. Northern blot analyses were carried out as previously described (Wetzstein *et al.*,1992). The digoxigenin-labeled RNA marker I (Roche, Indianapolis, IN) was used to calculate the sizes of the transcripts. The hybridisation signals were detected using a Lumi-Imager (Roche Diagnostics, Mannheim, Germany) and analyzed using the software package Lumi-Analyst (Roche Diagnostics, Mannheim, Germany).

2.3.8 DNA-microarray analyses

After isolation , RNA for the DNA-microarray experiments was further purified in order to eliminate traces of contaminating DNA. Briefly, the RNA was incubated with 7 U RNase-free DNase (Qiagen) for 10 minutes at room temperature followed by two phenol-chloroform-isoamyl alcohol and two chloroform-isoamyl alcohol extraction steps in order to remove the DNase.

The integrity of the RNA was checked with the Bioanalyzer (Agilent Technologies, Palo Alto, CA) and the concentration and purity were assessed using a NanoDrop ND-1000 spectrophotometer(NanoDrop Technologies, Inc.,Rockland, DE). Synthesis of Cy5-dCTP or Cy3-dCTP (Perkin-Elmer) labeled cDNA was done with 10 μg of total RNA as template by direct reverse-transcription using Superscript II (Invitrogen) and random hexamers (Promega) according to the manufacturer's instructions. To degrade the RNA after cDNA synthesis the reaction mix was incubated for 30 minutes at room temperature with *E. coli* RNase H (Invitrogen). Labeled cDNA was then purified with the QIAquick PCR Purification Kit (Qiagen). The Cy-dye incorporation was analysed with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Inc., Rockland, DE). A sample volume corresponding to 30 pmol incorporated dye of the labeled cDNA was used

for two-colour competitive hybridization experiments. In total four independent hybridization experiments with each representing a biological replicate including a control and a treated sample were carried out. To account for the dye bias two of the four replicates were dye swapped.

The design and evaluation of the customized StaphChip oligoarray manufactured by Agilent Technologies (Palo Alto, CA) used in this study has been described below (Charbonnier *et al.*, 2005). The StaphChip used in this study was based on the whole genome sequences of *S.aureus* strains: COL, MRSA252, MSSA476, Mu50, MW2, N315, USA300, and 8325.

Hybridization and washing of slides was carried out as described by Charbonnier and colleagues (Charbonnier *et al.*, 2005). The slides were scanned with an Agilent scanner. Spot intensity values were extracted with the "Feature Extraction" software provided by Agilent (Palo Alto, CA). Visualization and analyses of expression data were done in GeneSpringGX 7.3.1 (Agilent) and with the Cyber-T program for the analysis of paired expression data (Baldi and Long, 2001) (http://www.cybert.microarray. ics.uci.edu) . The parameters for the Bayesian Standard Deviation Estimation applied in the Cyber-T analysis were: sliding window size = 101 and confidence value for the Bayesian variance estimate = 12. Genes with a p-value $p<0.001$ associated with the Bayesian t-statistic and a mean fold change of two in the four hybridization experiments were considered as biologically significant expression changes.

Hybridization and scanning parameters.

Unless specified, equivalent amounts of cDNA (or genomic DNA) labelled with Cyanine-3 or Cyanine-5, were diluted in 250 μl Agilent hybridization buffer, and hybridized at a temperature of 60° C for 17 hours in a dedicated hybridization oven (Robbins Scientific). For comparative genome hybridization, genomic DNA from each individual *S.aureus* strain was labelled with Cy3 and cohybridized with equivalent amounts of Cy5-labelled genomic DNA pooled from N315, Mu50 and COL (Charbonnier *et al.*, 2005). Slides were washed, dried under Nitrogen flow and scanned (Agilent) using 100% PMT power for both wavelengths. Data were extracted and processed using Feature Extraction software (version 5.0, Agilent). For gene expression analysis, saturated spots were excluded from sub-

sequent analysis. Local background subtracted signals were corrected for unequal dye incorporation or unequal load of labelled product. The algorithm consisted of a rank consistency filter and a curve fit using the default LOWESS (locally weighted linear regression) method. Spots showing a reference signal lower than background plus two standard deviations were also excluded from subsequent analyses. For comparative genome hybridization, local background-subtracted data were expressed as Log ratios and 10 analyzed by two-way clustering using GeneSpring 6.1 (SiliconGenetics).

Chapter 3

Results

3.1 Heat stress response

3.1.1 Growth under heat stress conditions in synthetic medium

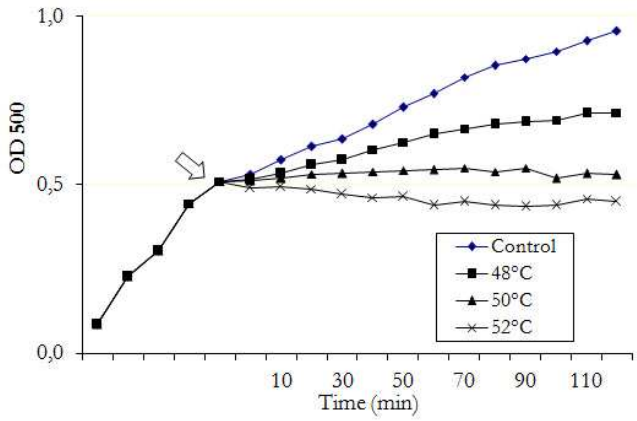

Figure 3.1: Growth curve of *S. aureus* COL *S.aureus* was grown in synthetic medium to an OD500 of 0.5 and shifted to 48° C, 50° C and 52° C. The time point of the shift was set to zero which indicated by arrow.

In order to select an optimal temperature for the heat shock experiments, the growth rates of *S.aureus* COL grown at different temperatures in synthetic medium

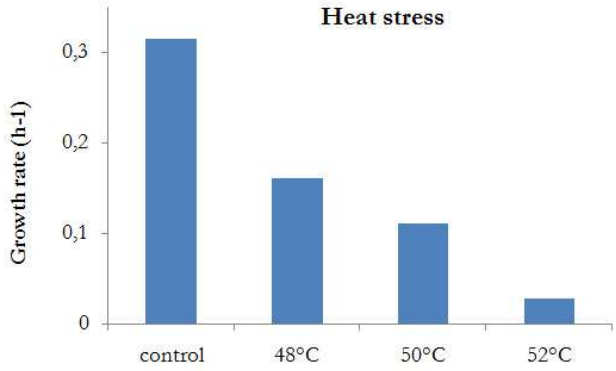

Figure 3.2: Growth rates of *S.aureus* COL grown at different temperatures

were examined. 37° C was chosen as the standard growth temperature. The cells were grown in synthetic medium to an optical density of 0.5 at OD_{500} and heat stress was performed by shifting the cells to 48° C, 50° C, and 52° C for two hours. Meanwhile, the cell density was measured every 10 minutes. When shifting the cells to 48° C the growth rate of the cells decreased from 0.359 to 0.151 (Figure 3.2). At 52° C the cells seemed to stop growing after the temperature upshift. To obtain a less severe depression of the physiology, we wanted to apply a stress level which would result in a reduction of approximately 50% of the growth rate. Therefore, we chose 48° C as the appropriate temperature for heat stress experiments.

3.1.2 Global gene expression analyses of *S.aureus* during heat stress

In order to get a global view on the influence of high temperature on gene regulation, transcriptomic studies were carried out by using full-genome DNA microarrays. In order to guarantee high RNA quality for DNA microarray, total RNA was checked for induction of marker genes by Northern blot analysis first. Additionaly, to establish appropriate time points for cell sampling for DNA array analyses, total RNA was extracted from exponentially growing and heat treated

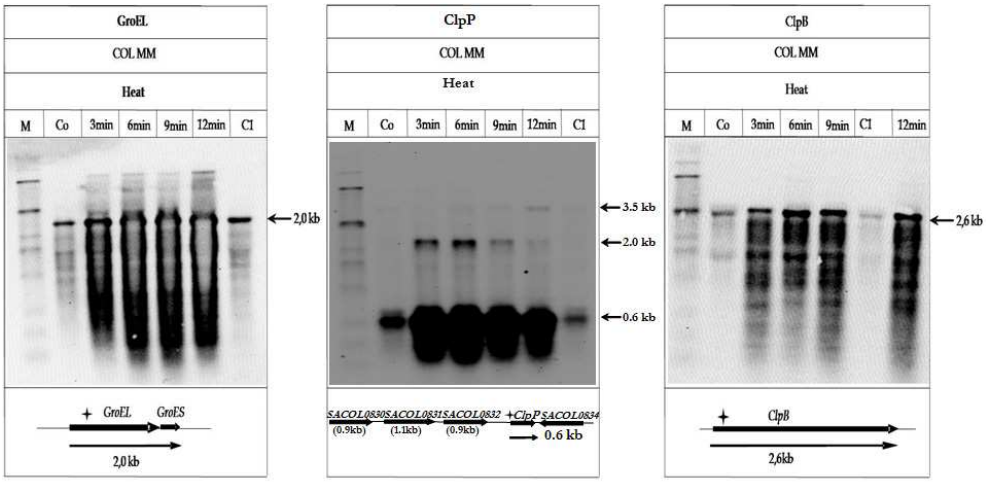

Figure 3.3: Northern blot analyses of genes whose transcription was induced by heat stress. For RNA preparation, cells were grown in synthetic medium to an OD_{500} of 0.5 and shifted to heat stress conditions. RNA was isolated before and 3, 6, 9, 12 minutes after shift to 48° C.

cells at different time points (3, 6, 9 and 12 minutes). Transcription of *groEL* was analyzed as marker for HrcA-dependently regulated genes, and *clpB* and *clpP* as markers for CtsR-regulated genes. Using a *groEL* probe, a transcript was detected whose transcription was increased after heat stress. As shown in Fig 3.3, this major signal with a size of about 2.0 kb appeared only slightly in the control and was strongly induced after stress and corresponds to the bicistronic operon *groEL-groES.* In the case of *clpB,* a transcript of approximately 2.6 kb was detected to be strongly increased after different time of exposure to heat stress. The transcription of *clpP* was also increased 3, 6, 9, and 12 minutes after heat stress. In this experiment, besides the transcript of about 0.6 kb corresponding to *clpP*, we also observed other transcripts of about 2.0 kb and 3.5 kb which were induced by heat stress as well. According to the region view of *clpP*, it can be suggested that SACOL0830, SACOL0831 and SACOL0832 might transcribed together and might be induced by heat shock as well. These results indicated that *groEL-groES, clpB* and *clpP* were expressed at increased levels after heat stress. According to these data, we chose 10 minute shift to 48° C as an appropriate time point for DNA microarrays experiments.

By comparing gene transcription under control and stress conditions, we were able to identify differently expressed genes. For each gene the ratios of the transcript levels were calculated. All genes showing an at least two fold induction or repression of transcription in both experiments were considered to be differentially expressed under heat stress.

Accordingly, the transcription of 310 genes was increased while the transcription of 266 genes was decreased under heat shock conditions. The expression profiles of up and down regulated genes are shown in Table 3.1 and 3.2. The transcriptomics analyses have shown the induction of many HrcA-CIRCE controlled genes. The transcription of seven genes possibly regulated by HrcA was strongly increased after heat stress: *dnaK-dnaJ- grpE- hrcA* with an induction factor that varied from 15.11 fold to 20.67 fold and *groEL -groES* with an induction ratio of 7.7 to 8 fold.

Moreover, the transcription genes belonging to the CtsR regulon was also induced by high temperature e.g.s *clpB* and *clpP* encoding proteins involved in repair and degradation of damaged proteins were strongly induced at the transcriptome level (135 fold and 10 fold, respectively). Transcriptome analysis also showed the induction of the transcription factor CtsR at a very high level of 61,6 fold after 10 minute heat exposure. Besides, the DNA arrays data revealed a light induction of the genes SACOL0830, SACOL0831 and SACOL0832(as shown in fig.3.3) at an average ratio of 2.1, 1.8 and 1.7 respectively, indicating that these genes might by induced by heat stress as well.

After heat exposure, we could not detect any induction of sigmaB-dependent genes. The alternative sigma factor σ^B B has been found in some pathogenic gram-positive bacteria, including *S.aureus* (Wu *et al.*, 1996; Kullik and Giachino, 1997). In *B. subtilis*, sigma B-dependent genes have been described to be strongly induced by heat and other environmental stresses as well as starvation condition (Hecker and Völker, 1998). In *S.aureus*, σ^B-dependent induction of sigma B by heat stress in complex medium has been described previously(Kullik and Giachiano., 1997). In this study, we looked for sigB-dependent induction after heat stress in cells grown in synthetic medium. Surprisingly, after heat exposure, we could not detect any response of sigma B-dependent proteins. The transcrip-

tome analysis revealed the induction of alkyl hydroperoxide reductase, subunit C (*ahpC*) and alkyl hydroperoxide reductase, subunit F (*ahpF*) at 3.1 and 3.16 fold, respectively. These two genes were defined as such that controlled neither by HrcA, nor by CtsR and σ^B(Derre *et al.*, 1999).

Among the genes induced by heat shock were some putative virulence factors including five pathogenicity island genes SACOL0893, SACOL0898, SACOL0903, SACOL0904, SACOL0905 with an induction rate that varied from 2.2 to 8.4 fold and members of the urease system *ureA-ureG* which were upregulated 5.24, 4.58, 3.68, 3.48, 3.66 and 3,26 fold, respectively.

As shown in Table 3.1, the heat treatment also resulted in the induction of enzymes involved in purine and pyrimidine biosynthesis. Among them were *pyrB, pyrC, pyrE,* and *pyrF* forming an operon as well as the regulator *pyrR* with the induction factors of 5.6, 4.2, 3.4, 3,2 and 6.1 fold , respectively. The *deoD1* gene encoding a purine nucleoside phosphorylase was also induced.

The transcriptome experiments also suggested the induction of genes whose function in heat resistance was still unknown. For example, *frp* gene encoding NAD(P)H-flavin-dependent oxidoreductase (4.5 fold) or the SACOL0453 gene encoding a NAD(P)H flavinoxidoreductase (2.96 fold) which could contribute to specific degradation are induced by heat treatment. Furthermore, the array data also showed that genes involved in the metabolism of alternative carbon sources such as 6-phospho-beta-glucosidase (*bglA*) (Zhang *et al.*,1994), glycerate kinase (SACOL0805) and glycosyl transferase (SACOL1498) were highly transcribed under heat shock conditions.

Surprisingly, the transcription of genes localized on prophage L54a was also upregulated, for instant, genes encoding a repressor protein (SACOL0321), an antirepressor protein (SACOL0325), replicative DNA helicase (SACOL0343), N-6-adenine-methyltransferase (SACOL0346), deoxyuridine 5'-triphosphate nucleotidohydrolase (SACOL0357), and transcriptional regulator of the RinA family SACOL0364 as well as the small and large subunit of the terminase (SACOL0366 and SACOL0367) (Table 3.1).

Table 3.1: Genes whose transcription was induced under heat stress condition

ID	COL Locus	Function	Average Ratio
Amino acid biosynthesis			
SACOL0502	SACOL0502	cysteine synthase/cystathionine beta-synthase family protein	3.55
vraS	SACOL1943	ornithine–oxo-acid transaminase	3.10
cysE	SACOL0575	sensor histidine kinase VraS	6.70
rocD	SACOL0960	serine acetyltransferase	2.22
SACOL0503	SACOL0503	trans-sulfuration enzyme family protein	3.09
Biosynthesis of cofactors, prosthetic groups and carriers			
entB	SACOL0172	isochorismatase	2.83
SACOL1051	SACOL1051	isochorismate synthase family protein	2.53
SACOL1640	SACOL1640	coproporphyrinogen III oxidase	2.31
ribH	SACOL1817	riboflavin synthase, beta subunit	3.00
coaBC	SACOL1223	phosphopantothenoylcysteine decarboxylase/phosphopantothenate–cysteine ligase	2.76
moaA	SACOL2261	molybdenum cofactor biosynthesis protein A	2.38
hutH	SACOL0008	histidine ammonia-lyase	2.92
Cellular processes			
ahpC	SACOL0452	alkyl hydroperoxide reductase, C subunit	3.16
ahpF	SACOL0451	alkyl hydroperoxide reductase, subunit F	3.10
SACOL1645	SACOL1645	comE operon protein 2	2.35
SACOL0813	SACOL0813	comf operon protein 1, putative	3.91
betB	SACOL2628	betaine aldehyde dehydrogenase	2.76
SACOL1644	SACOL1644	competence protein ComEC/Rec2, putative	2.79
SACOL0814	SACOL0814	competence protein F	3.98
SACOL1004	SACOL1004	competence protein, putative	3.31
SACOL1003	SACOL1003	adaptor protein	2.74
kataA	SACOL1368	catalase	2.88
ebh	SACOL1472	Cell wall associated fibronectin-binding protein	2.22
lytN	SACOL1264	cell wall hydrolase	7.10
SACOL1657	SACOL1657	enterotoxin type A, putative	3.86
sspA	SACOL1057	V8 Protease	2.44
trmE	SACOL2738	tRNA modification GTPase	2.90
SACOL2131	SACOL2131	Dps family protein	10.89
SACOL1781	SACOL1781	cell wall surface anchor family protein	2.73
Cell envelope			
fnbB	SACOL2509	fibronectin binding protein B	14.18
SACOL1762	SACOL1762	thiol peroxidase, putative	2.52
SACOL0985	SACOL0985	surface protein, putative	3.66
mreC	SACOL1704	rod shape-determining protein MreC	2.89
SACOL0414	SACOL0414	lipoprotein, putative	2.37
SACOL2439	SACOL2439	lipoprotein, putative	6.13
SACOL1220	SACOL1220	fibronectin/fibrinogen binding-related protein	3.29
DNA metabolism			
gyrA	SACOL0006	DNA gyrase, A subunit	2.31
gyrB	SACOL0005	DNA gyrase, B subunit	2.97
polA	SACOL1737	DNA polymerase I	2.41
dnaG	SACOL1619	DNA primase	2.26
radA	SACOL0572	DNA repair protein RadA	12.75
SACOL1493	SACOL1493	DNA replication protein DnaD, putative	4.66
vraR	SACOL1942	DNA-binding response regulator VraR	2.95
dinP	SACOL1955	DNA-damage-inducible protein P	2.74
recG	SACOL1241	ATP-dependent DNA helicase RecG	2.18
nth	SACOL1492	endonuclease III	4.35
uvrB	SACOL0823	excinuclease ABC subunit B	3.64
uvrA	SACOL0824	excinuclease ABC, A subunit	2.81
SACOL1954	SACOL1954	exonuclease	6.27
SACOL0004	SACOL0004	recombination protein F	3.00
SACOL1154	SACOL1154	recombination and DNA strand exchange inhibitor protein	2.15
rpoB	SACOL0588	DNA-directed RNA polymerase beta subunit	2.22
Energy metabolism			
ipdC	SACOL0173	indole-3-pyruvate decarboxylase	2.99
SACOL0981	SACOL0981	isopropylmalate synthase-related protein	44.87
zwf	SACOL1549	glucose-6-phosphate 1-dehydrogenase	2.48
gidA	SACOL2737	glucose-inhibited division protein A	2.40
murI	SACOL1161	glutamate racemase	4.75
gltX	SACOL0574	glutamyl-tRNA synthetase	9.43

SACOL0805	SACOL0805	glycerate kinase family protein	2.30
SACOL1498	SACOL1498	glycosyl transferase, group 1 family protein	2.49
SACOL1207	SACOL1207	glyoxalase family protein	4.31
menD	SACOL1052	2-succinyl-6-hydroxy-2,4-cyclohexadiene-1-carboxylic acid synthase/2-oxoglutarate decarboxylase	2.45
ribBA	SACOL1818	3,4-dihydroxy-2-butanone-4-phosphate synthase/GTP cyclohydrolase II	2.25
bglA	SACOL0251	6-phospho-beta-glucosidase	5.66
SACOL2278	SACOL2278	acyl-CoA dehydrogenase-related protein	2.59
SACOL0111	SACOL0111	acetoin reductase	2.45
acuA	SACOL1784	acetoin utilization protein AcuA	2.58
SACOL2178	SACOL2178	alcohol dehydrogenase, zinc-containing	2.66
SACOL2177	SACOL2177	alcohol dehydrogenase, zinc-containing	3.06
cydA	SACOL1094	cytochrome d ubiquinol oxidase, subunit I	4.02
cydB	SACOL1095	cytochrome d ubiquinol oxidase, subunit II	3.16
frp	SACOL2534	NAD(P)H-flavin oxidoreductase	4.28
SACOL0453	SACOL0453	NAD(P)H-flavin oxidoreductase, putative	2.96
SACOL0959	SACOL0959	NADH-dependent flavin oxidoreductase, Oye family	5.43
trxB	SACOL0829	thioredoxin-disulfide reductase	2.81
fdhD	SACOL2273	formate dehydrogenase accessory protein	6.82
SACOL1952	SACOL1952	ferritins family protein	12.87
acs	SACOL1783	acetyl-coenzyme A synthetase	3.05
Fatty acid and phospholipid metabolism			
SACOL1814	SACOL1814	lysophospholipase, putative	2.24
Pathogenicity island protein			
SACOL0898	SACOL0898	pathogenicity island protein	2.16
SACOL0893	SACOL0893	pathogenicity island protein	2.52
SACOL0903	SACOL0903	pathogenicity island protein	3.79
SACOL0904	SACOL0904	pathogenicity island protein	5.70
SACOL0905	SACOL0905	pathogenicity island protein	8.38
Protein fate			
groES	SACOL2017	chaperonin, 10 kDa	7.74
groEL	SACOL2016	chaperonin, 60 kDa	8.06
dnaJ	SACOL1636	dnaJ protein	18.91
dnaK	SACOL1637	dnaK protein	15.46
clpB	SACOL0979	ATP-dependent Clp protease, ATP-binding subunit ClpB	135.49
grpE	SACOL1638	heat shock protein GrpE	15.11
hrcA	SACOL1639	heat-inducible transcription repressor HrcA	20.67
clpP	SACOL0833	ATP-dependent Clp protease, proteolytic subunit ClpP	10.09
SACOL1419	SACOL1419	oligoendopeptidase F, putative	2.48
def	SACOL1100	peptide deformylase	2.09
SACOL1555	SACOL1555	peptidase, M20/M25/M40 family	2.47
secA	SACOL0816	translocase	2.41
Protein synthesis			
SACOL0578	SACOL0578	RNA methyltransferase, TrmH family	4.60
cysS	SACOL0576	cysteinyl-tRNA synthetase	7.70
Prophage L54a			
SACOL0325	SACOL0325	prophage L54a, antirepressor, putative	13.83
dut	SACOL0357	prophage L54a, deoxyuridine 5'-triphosphate nucleotidohydrolase	8.07
int	SACOL0318	prophage L54a, integrase	8.06
SACOL0346	SACOL0346	prophage L54a, N-6-adenine-methyltransferase	22.81
SACOL0368	SACOL0368	prophage L54a, portal protein, HK97 family	2.51
SACOL0343	SACOL0343	prophage L54a, replicative DNA helicase, putative	23.75
SACOL0321	SACOL0321	prophage L54a, repressor protein, putative	14.10
ssb1	SACOL0339	prophage L54a, single-stranded DNA binding protein	8.07
SACOL0367	SACOL0367	prophage L54a, terminase, large subunit, putative	3.95
SACOL0366	SACOL0366	prophage L54a, terminase, small subunit, putative	6.72
SACOL0364	SACOL0364	prophage L54a, transcriptional regulator, RinA family	11.90
Purines, pyrimidines, nucleosides, and nucleotides			
pyrB	SACOL1212	aspartate carbamoyltransferase catalytic subunit	5.67
pyrC	SACOL1213	dihydroorotase	4.20
deoD1	SACOL0121	purine nucleoside phosphorylase	4.26
SACOL0569	SACOL0569	putative ATP:guanido phosphotransferase	56.14
SACOL1520	SACOL1520	pyridine nucleotide-disulfide oxidoreductase	2.52
SACOL0640	SACOL0640	pyridine nucleotide-disulfide oxidoreductase	8.64
pyrR	SACOL1210	pyrimidine regulatory protein PyrR	6.15
pdp	SACOL2128	pyrimidine-nucleoside phosphorylase	2.11
carB	SACOL1215	carbamoyl-phosphate synthase large subunit	2.99

carA	SACOL1214	carbamoyl-phosphate synthase small subunit	3.55
pyrE	SACOL1217	orotate phosphoribosyltransferase	3.43
pyrF	SACOL1216	orotidine 5'-phosphate decarboxylase	3.21
thiD2	SACOL2085	phosphomethylpyrimidine kinase	2.77
Putative virulence factor			
ureD	SACOL2286	urease accessory protein UreD	3.48
ureE	SACOL2283	urease accessory protein UreE	3.66
ureF	SACOL2284	urease accessory protein UreF	3.84
ureG	SACOL2285	urease accessory protein UreG	3.26
ureC	SACOL2282	urease, alpha subunit	3.68
ureB	SACOL2281	urease, beta subunit	4.58
ureA	SACOL2280	urease, gamma subunit	5.24
Regulatory functions			
ccpA	SACOL1786	catabolite control protein A	4.67
SACOL1393	SACOL1393	transcriptional antiterminator LicT, putative	2.78
ctsR	SACOL0567	transcriptional regulator CtsR	61.64
czrA	SACOL2137	transcriptional regulator CzrA	9.21
SACOL1550	SACOL1550	transcriptional regulator, AraC family	2.38
SACOL0890	SACOL0890	transcriptional regulator, Cro/CI family	2.21
SACOL0420	SACOL0420	transcriptional regulator, Cro/CI family	2.91
SACOL0563	SACOL0563	transcriptional regulator, GntR family	8.59
SACOL2256	SACOL2256	transcriptional regulator, MarR family	2.84
SACOL2531	SACOL2531	transcriptional regulator, MarR family	4.29
SACOL2189	SACOL2189	transcriptional regulator, Sir2 family	2.89
SACOL2086	SACOL2086	transcriptional regulator, TenA family	2.25
SACOL2593	SACOL2593	transcriptional regulator, TetR family	2.78
SACOL2374	SACOL2374	transcriptional regulator, TetR family. putative	2.95
sarV	SACOL2258	staphylococcal accessory regulator V	2.45
SACOL2035	SACOL2035	redox-sensing transcriptional repressor Rex	2.37
rnpA	SACOL2739	ribonuclease P	3.03
gltC	SACOL0513	transcriptional regulatory protein GltC	4.06
Ribosomal protein			
rpsN1	SACOL1370	30S ribosomal protein S14	2.65
prmA	SACOL1635	ribosomal protein L11 methyltransferase	18.88
rpmG1	SACOL1369	ribosomal protein L33	2.30
Transport and binding proteins			
SACOL2138	SACOL2138	cation efflux family protein	7.58
SACOL0997	SACOL0997	oligopeptide ABC transporter, ATP-binding protein	2.09
SACOL0998	SACOL0998	oligopeptide ABC transporter, ATP-binding protein	2.20
SACOL0996	SACOL0996	oligopeptide ABC transporter, oligopeptide-binding protein	2.08
SACOL1000	SACOL1000	oligopeptide ABC transporter, permease protein	2.61
SACOL0999	SACOL0999	oligopeptide ABC transporter, permease protein	2.69
SACOL2375	SACOL2375	transporter, CorA family	2.61
SACOL2279	SACOL2279	transporter, putative	3.02
uraA	SACOL1211	uracil permease	7,16
SACOL1096	SACOL1096	TrkA potassium uptake family protein	2.48
SACOL0122	SACOL0122	tetracycline resistance protein, putative	4.17
SACOL0250	SACOL0250	PTS system, IIA component	7.30
SACOL0781	SACOL0781	osmoprotectant ABC transporter, ATP-binding protein, putative	2.24
SACOL2257	SACOL2257	drug transporter, putative	3.26
SACOL2523	SACOL2523	drug transporter, putative	3.27
SACOL2356	SACOL2356	ABC transporter, ATP-binding protein	2.31
Unknown function			
SACOL0220	SACOL0220	flavohemoprotein, putative	2.55
SACOL0976	SACOL0976	hydrolase, haloacid dehalogenase-like family	2.39
SACOL1162	SACOL1162	HAM1 protein	3.27
SACOL1683	SACOL1683	HesA/MoeB/ThiF family protein	2.78
SACOL1400	SACOL1400	ImpB/MucB/SamB family protein	4.34
SACOL0820	SACOL0820	LysM domain protein	2.61
SACOL0191	SACOL0191	M23/M37 peptidase domain protein	3.77
SACOL0064	SACOL0064	metallo-beta-lactamase family protein	2.50
SACOL0418	SACOL0418	mttA/Hcf106 family protein	6.36
SACOL0417	SACOL0417	MttB family protein	6.47
SACOL2722	SACOL2722	N-acetyltransferase family protein	2.73
SACOL1669	SACOL1669	O-methyltransferase family protein	2.19
SACOL1669	SACOL1669	O-methyltransferase family protein	2.19
SACOL1771	SACOL1771	OsmC/Ohr family protein	2.53
SACOL2594	SACOL2594	oxidoreductase, short chain dehydrogenase/reductase family	3.01

SACOL0498	SACOL0498	PAP2 family protein	2.32
SACOL1967	SACOL1967	PcrB-like protein	4.11
SACOL2529	SACOL2529	phospholipase/carboxylesterase family protein	2.08
SACOL0573	SACOL0573	PIN/TRAM domain protein	11.27
rarD	SACOL2719	rarD protein	2.74
SACOL0921	SACOL0921	CBS domain protein	2.72
SACOL2245	SACOL2245	acetyltransferase, GNAT family	2.66
SACOL2366	SACOL2366	acetyltransferase, GNAT family	3.00
SACOL2012	SACOL2012	acetyltransferase, GNAT family	4.41
SACOL1678	SACOL1678	bacterial luciferase family protein	3,53
SACOL1833	SACOL1833	crcB family protein	3.43
crcB	SACOL1832	crcB protein	3.63
SACOL0982	SACOL0982	Sua5/YciO/YrdC/YwlC family protein	28.27
ypfP	SACOL1022	ypfP protein	2.69
SACOL0714	SACOL0714	acetyltransferase, GNAT family	4.46
Hypothetical protein			
SACOL0613	SACOL0613	hypothetical protein	2.05
SACOL2040	SACOL2040	hypothetical protein	2.07
SACOL2041	SACOL2041	hypothetical protein	2.12
SACOL1616	SACOL1616	hypothetical protein	2.15
SACOL1539	SACOL1539	hypothetical protein	2.16
SACOL2127	SACOL2127	hypothetical protein	2.16
SACOL0710	SACOL0710	hypothetical protein	2.21
SACOL0924	SACOL0924	hypothetical protein	2.23
SACOL0496	SACOL0496	hypothetical protein	2.24
SACOL1099	SACOL1099	hypothetical protein	2.25
SACOL0614	SACOL0614	hypothetical protein	2.29
SACOL0466	SACOL0466	hypothetical protein	2.34
SACOL0354	SACOL0354	hypothetical protein	2.34
SACOL1953	SACOL1953	hypothetical protein	2.36
SACOL0715	SACOL0715	hypothetical protein	2.36
SACOL0129	SACOL0129	hypothetical protein	2.36
SACOL1643	SACOL1643	hypothetical protein	2.40
SACOL0324	SACOL0324	hypothetical protein	2.42
SACOL0897	SACOL0897	hypothetical protein	2.42
SACOL0922	SACOL0922	hypothetical protein	2.44
SACOL0822	SACOL0822	hypothetical protein	2.50
SACOL2503	SACOL2503	hypothetical protein	2.51
SACOL1009	SACOL1009	hypothetical protein	2.51
SACOL2502	SACOL2502	hypothetical protein	2.52
SACOL2547	SACOL2547	hypothetical protein	2.54
SACOL2440	SACOL2440	hypothetical protein	2.57
SACOL1050	SACOL1050	hypothetical protein	2.58
SACOL0974	SACOL0974	hypothetical protein	2.60
SACOL0615	SACOL0615	hypothetical protein	2.66
SACOL2354	SACOL2354	hypothetical protein	2.67
SACOL0465	SACOL0465	hypothetical protein	2.69
SACOL0463	SACOL0463	hypothetical protein	2.73
SACOL0848	SACOL0848	hypothetical protein	2.75
SACOL2596	SACOL2596	hypothetical protein	2.77
SACOL0512	SACOL0512	hypothetical protein	2.80
SACOL1761	SACOL1761	hypothetical protein	2.80
SACOL0467	SACOL0467	hypothetical protein	2.82
SACOL1153	SACOL1153	hypothetical protein	2.89
SACOL2520	SACOL2520	hypothetical protein	2.93
SACOL1190	SACOL1190	hypothetical protein	2.97
SACOL0063	SACOL0063	hypothetical protein	2.98
SACOL1658	SACOL1658	hypothetical protein	3.00
SACOL1163	SACOL1163	hypothetical protein	3.00
SACOL1499	SACOL1499	hypothetical protein	3.00
SACOL0978	SACOL0978	hypothetical protein	3.05
SACOL0074	SACOL0074	hypothetical protein	3.06
SACOL1656	SACOL1656	hypothetical protein	3.11
SACOL2033	SACOL2033	hypothetical protein	3.24
SACOL0219	SACOL0219	hypothetical protein	3.27
SACOL1944	SACOL1944	hypothetical protein	3.37
SACOL1945	SACOL1945	hypothetical protein	3.38
SACOL1438	SACOL1438	hypothetical protein	3.50

SACOL2034	SACOL2034	hypothetical protein	3.51
SACOL0769	SACOL0769	hypothetical protein	3.51
SACOL2550	SACOL2550	hypothetical protein	3.54
SACOL0942	SACOL0942	hypothetical protein	3.56
SACOL1218	SACOL1218	hypothetical protein	3.58
SACOL0218	SACOL0218	hypothetical protein	3.64
SACOL0003	SACOL0003	hypothetical protein	3.74
SACOL1526	SACOL1526	hypothetical protein	3.84
SACOL1815	SACOL1815	hypothetical protein	3.89
SACOL0965	SACOL0965	hypothetical protein	3.96
SACOL2715	SACOL2715	hypothetical protein	3.99
SACOL1059	SACOL1059	hypothetical protein	4.08
SACOL1491	SACOL1491	hypothetical protein	4.14
SACOL2551	SACOL2551	hypothetical protein	4.14
SACOL2179	SACOL2179	hypothetical protein	4.44
SACOL0316	SACOL0316	hypothetical protein	4.55
SACOL0579	SACOL0579	hypothetical protein	4.59
SACOL0072	SACOL0072	hypothetical protein	4.66
SACOL0580	SACOL0580	hypothetical protein	4.70
SACOL1705	SACOL1705	hypothetical protein	5.30
SACOL0419	SACOL0419	hypothetical protein	5.51
SACOL0577	SACOL0577	hypothetical protein	5.57
SACOL2013	SACOL2013	hypothetical protein	5.66
SACOL1633	SACOL1633	hypothetical protein	5.88
SACOL0984	SACOL0984	hypothetical protein	6.52
SACOL1634	SACOL1634	hypothetical protein	7.24
SACOL0323	SACOL0323	hypothetical protein	7.68
SACOL0329	SACOL0329	hypothetical protein	8.24
SACOL0356	SACOL0356	hypothetical protein	9.76
SACOL0641	SACOL0641	hypothetical protein	10.02
SACOL0319	SACOL0319	hypothetical protein	10.03
SACOL0341	SACOL0341	hypothetical protein	10.09
SACOL0811	SACOL0811	hypothetical protein	10.32
SACOL0983	SACOL0983	hypothetical protein	10.50
SACOL2571	SACOL2571	hypothetical protein	10.60
SACOL0320	SACOL0320	hypothetical protein	11.00
SACOL0348	SACOL0348	hypothetical protein	11.41
SACOL0327	SACOL0327	hypothetical protein	11.68
SACOL0568	SACOL0568	hypothetical protein	11.74
SACOL0355	SACOL0355	hypothetical protein	12.64
SACOL0326	SACOL0326	hypothetical protein	12.69
SACOL0361	SACOL0361	hypothetical protein	12.95
SACOL0342	SACOL0342	hypothetical protein	13.48
SACOL0333	SACOL0333	hypothetical protein	14.49
SACOL0331	SACOL0331	hypothetical protein	14.75
SACOL0347	SACOL0347	hypothetical protein	16.11
SACOL0335	SACOL0335	hypothetical protein	17.99
SACOL0340	SACOL0340	hypothetical protein	18.73
SACOL0350	SACOL0350	hypothetical protein	20.20
SACOL0337	SACOL0337	hypothetical protein	20.39
SACOL0338	SACOL0338	hypothetical protein	21.71
SACOL0334	SACOL0334	hypothetical protein	22.52
SACOL0362	SACOL0362	hypothetical protein	23.30
SACOL0336	SACOL0336	hypothetical protein	24.77
SACOL0358	SACOL0358	hypothetical protein	25.92
SACOL0359	SACOL0359	hypothetical protein	26.59
SACOL0352	SACOL0352	hypothetical protein	28.90
SACOL0344	SACOL0344	hypothetical protein	28.92
SACOL0363	SACOL0363	hypothetical protein	29.02
SACOL0353	SACOL0353	hypothetical protein	29.54
SACOL0345	SACOL0345	hypothetical protein	30.65
SACOL0351	SACOL0351	hypothetical protein	32.63

Table 3.2: Genes whose transcription was repressed under heat stress condition

ID	COL Locus	Function	Average Ratio
Amino acid biosynthesis			
SACOL1360	SACOL1360	aspartate kinase	0.39
metE	SACOL0428	5-methyltetrahydropteroyltriglutamate–homocysteine methyltransferase	0.39
argG	SACOL0964	argininosuccinate synthase	0.34
pheS	SACOL1148	phenylalanyl-tRNA synthetase, alpha subunit	0.43
pheT	SACOL1149	phenylalanyl-tRNA synthetase, beta subunit	0.30
serA	SACOL1773	D-3-phosphoglycerate dehydrogenase	0.28
SACOL0430	SACOL0430	trans-sulfuration enzyme family protein	0.34
SACOL0431	SACOL0431	trans-sulfuration enzyme family protein	0.41
trpB	SACOL1408	tryptophan synthase subunit beta	0.48
thrC	SACOL1363	threonine synthase	0.36
hisI	SACOL2696	phosphoribosyl-ATP pyrophosphatase/phosphoribosyl-AMP cyclohydrolase	0.48
hisA	SACOL2698	phosphoribosylformimino-5-aminoimidazole carboxamide ribotide isomerase	0.36
hisF	SACOL2697	hisF protein (cyclase)	0.41
hisD	SACOL2702	histidinol dehydrogenase	0.42
hom	SACOL1362	homoserine dehydrogenase	0.41
thrB	SACOL1364	homoserine kinase	0.32
hisH	SACOL2699	imidazole glycerol phosphate synthase, glutamine amidotransferase subunit	0.40
hisB	SACOL2700	imidazoleglycerol-phosphate dehydratase	0.42
putA	SACOL1816	proline dehydrogenase	0.30
SACOL0429	SACOL0429	bifunctional homocysteine S-methyltransferase/5,10-methylenetetrahydrofolate reductase protein	0.30
Biosynthesis of cofactors			
SACOL0564	SACOL0564	pyridoxine biosynthesis protein	0.35
ctaB	SACOL1125	protoheme IX farnesyltransferase	0.40
SACOL2579	SACOL2579	phytoene dehydrogenase	0.13
Cell envelope			
cap5M	SACOL0148	capsular polysaccharide biosynthesis galactosyltransferase Cap5M	0.29
cap5A	SACOL0136	capsular polysaccharide biosynthesis protein Cap5A	0.18
cap5B	SACOL0137	capsular polysaccharide biosynthesis protein Cap5B	0.17
cap5C	SACOL0138	capsular polysaccharide biosynthesis protein Cap5C	0.12
cap5D	SACOL0139	capsular polysaccharide biosynthesis protein Cap5D	0.23
cap5E	SACOL0140	capsular polysaccharide biosynthesis protein Cap5E	0.24
cap5F	SACOL0141	capsular polysaccharide biosynthesis protein Cap5F	0.16
cap5H	SACOL0143	capsular polysaccharide biosynthesis protein Cap5H	0.17
cap5I	SACOL0144	capsular polysaccharide biosynthesis protein Cap5I	0.17
cap5J	SACOL0145	capsular polysaccharide biosynthesis protein Cap5J	0.22
cap5K	SACOL0146	capsular polysaccharide biosynthesis protein Cap5K	0.29
cap5L	SACOL0147	capsular polysaccharide biosynthesis protein Cap5L	0.46
cap5N	SACOL0149	capsular polysaccharide biosynthesis protein Cap5N	0.41
cap5O	SACOL0150	capsular polysaccharide biosynthesis protein Cap5O	0.47
SACOL0119	SACOL0119	cell wall surface anchor family protein	0.38
SACOL1043	SACOL1043	glycosyl transferase, group 1 family protein	0.33
SACOL2578	SACOL2578	glycosyl transferase, group 2 family protein	0.07
lrgB	SACOL0248	antiholin-like protein LrgB	0.34
cap5G	SACOL0142	UDP-N-acetylglucosamine 2-epimerase Cap5G	0.13
lrgA	SACOL0247	murein hydrolase regulator LrgA	0.29
atl	SACOL1062	bifunctional autolysin	0.25
dltD	SACOL0938	DltD protein	0.48
Cellular processes			
agrA	SACOL2026	accessory gene regulator protein A	0.21
agrB	SACOL2023	accessory gene regulator protein B	0.18
argC2	SACOL2025	accessory gene regulator protein C	0.18
sarS	SACOL0096	staphylococcal accessory regulator S	0.29
sarY	SACOL2289	staphylococcal accessory regulator Y	0.29
arlS	SACOL1450	sensor histidine kinase ArlS	0.48
kdpD	SACOL2070	sensor histidine kinase KdpD	0.45
rsbW	SACOL2055	serine-protein kinase RsbW	0.35
rpoF	SACOL2054	sigma factor B	0.31
SACOL2581	SACOL2581	staphyloxanthin biosynthesis protein	0.39
sdrE	SACOL0610	sdrE protein	0.39

epiG	SACOL1871	epidermin immunity protein F	0.41
epiE	SACOL1872	epidermin immunity protein F	0.44
mscL	SACOL1383	large conductance mechanosensitive channel protein	0.34
clfB	SACOL2652	clumping factor B	0.29
SACOL0861	SACOL0861	cold shock protein, CSD family	0.30
DNA metabolism			
hup	SACOL1513	DNA-binding protein HU	0.36
kdpE	SACOL2071	DNA-binding response regulator KdpE	0.42
SACOL0201	SACOL0201	DNA-binding response regulator, AraC family	0.44
SACOL0678	SACOL0678	integrase/recombinase, phage integrase family	0.34
Energy metabolism/Central metabolism			
malA	SACOL1551	alpha-glucosidase	0.43
SACOL2484	SACOL2484	alkylhydroperoxidase, AhpD family	0.21
SACOL0517	SACOL0517	alpha-amylase family protein	0.40
atpH	SACOL2098	ATP synthase F1, delta subunit	0.50
atpC	SACOL2094	ATP synthase F1, epsilon subunit	0.40
atpG	SACOL2096	ATP synthase F1, gamma subunit	0.46
atpA	SACOL2097	ATP synthase subunit A	0.50
atpD	SACOL2095	ATP synthase subunit B	0.41
folC	SACOL1709	folylpolyglutamate synthase/dihydrofolate synthase	0.42
pflB	SACOL0204	formate acetyltransferase	0.25
SACOL2301	SACOL2301	formate dehydrogenase, alpha subunit, putative	0.29
hutG	SACOL2327	formiminoglutamase	0.31
fumC	SACOL1908	fumarate hydratase, class II	0.37
glk	SACOL1604	glucokinase	0.38
gntP	SACOL2514	gluconate transporter, permease protein	0.30
gntK	SACOL2515	gluconokinase	0.15
SACOL1912	SACOL1912	glucosamine-6-phosphate isomerase, putative	0.35
gltB	SACOL0514	glutamate synthase, large subunit	0.28
gltD	SACOL0515	glutamate synthase, small subunit	0.24
femC	SACOL1329	glutamine synthetase FemC	0.38
glnR	SACOL1328	glutamine synthetase repressor	0.48
sdhA	SACOL1159	succinate dehydrogenase	0.36
sdhC	SACOL1158	succinate dehydrogenase, cytochrome b558 subunit	0.41
qoxA	SACOL1069	quinol oxidase, subunit I	0.34
qoxB	SACOL1070	quinol oxidase, subunit II	0.40
qoxC	SACOL1068	quinol oxidase, subunit III	0.38
qoxD	SACOL1067	quinol oxidase, subunit IV	0.25
crtN	SACOL2576	dehydrosqualene desaturase	0.10
crtM	SACOL2577	dehydrosqualene synthase	0.25
glmS	SACOL2145	D-fructose-6-phosphate amidotransferase	0.33
pckA	SACOL1838	phosphoenolpyruvate carboxykinase	0.45
mtlD	SACOL2149	mannitol-1-phosphate 5-dehydrogenase	0.47
manA1	SACOL2135	mannose-6-phosphate isomerase, class I	0.32
SACOL1602	SACOL1602	metallo-beta-lactamase family protein	0.38
SACOL0638	SACOL0638	phosphomevalonate kinase	0.41
SACOL2293	SACOL2293	NAD/NADP octopine/nopaline dehydrogenase family protein	0.27
metK	SACOL1837	S-adenosylmethionine synthetase	0.21
pflA	SACOL0205	pyruvate formate-lyase-activating enzyme	0.42
sucA	SACOL1449	2-oxoglutarate dehydrogenase, E1 component	0.44
SACOL0607	SACOL0607	azoreductase	0.42
Fatty acid metabolism			
accC	SACOL1571	acetyl-CoA carboxylase	0.49
SACOL1662	SACOL1662	acetyl-CoA carboxylase, biotin carboxyl carrier protein, putative	0.36
SACOL1661	SACOL1661	acetyl-CoA carboxylase, biotin carboxylase, putative	0.40
SACOL0213	SACOL0213	acyl-CoA dehydrogenase family protein	0.41
glpF	SACOL1319	glycerol uptake facilitator protein	0.27
SACOL0962	SACOL0962	glycerophosphoryl diester phosphodiesterase GlpQ, putative	0.45
geh	SACOL2694	lipase	0.44
Purines, pyrimidines, nucleosides, and nucleotides			
purD	SACOL1083	phosphoribosylamine–glycine ligase	0.24
purK	SACOL1074	phosphoribosylaminoimidazole carboxylase, ATPase subunit	0.20
purE	SACOL1073	phosphoribosylaminoimidazole carboxylase, catalytic subunit	0.28
purM	SACOL1080	phosphoribosylaminoimidazole synthetase	0.20
purH	SACOL1082	phosphoribosylaminoimidazolecarboxamide formyltransferase/IMP cyclohydrolase	0.21
purQ	SACOL1077	phosphoribosylformylglycinamidine synthase I	0.16
purL	SACOL1078	phosphoribosylformylglycinamidine synthase II	0.22

purS	SACOL1076	phosphoribosylformylglycinamidine synthase, PurS protein	0.19
purN	SACOL1081	phosphoribosylglycinamide formyltransferase	0.20
purB	SACOL1969	adenylosuccinate lyase	0.42
purF	SACOL1079	amidophosphoribosyltransferase	0.17
xpt	SACOL0458	xanthine phosphoribosyltransferase	0.26
nrdE	SACOL0792	ribonucleotide-diphosphate reductase alpha subunit	0.36
nrdF	SACOL0793	ribonucleotide-diphosphate reductase beta subunit	0.26
guaA	SACOL0461	bifunctional GMP synthase/glutamine amidotransferase protein	0.34
guaB	SACOL0460	inosine-5'-monophosphate dehydrogenase	0.31
Protein metabolism			
valS	SACOL1710	valyl-tRNA synthetase	0.50
SACOL1803	SACOL1803	pseudouridine synthase, family 1	0.51
serS	SACOL0009	seryl-tRNA synthetase	0.39
glyS	SACOL1622	glycyl-tRNA synthetase	0.39
Regulatory functions			
rsbV	SACOL2056	anti-anti-sigma factor RsbV	0.42
SACOL2147	SACOL2147	transcriptional antiterminator, BglG family/DNA-binding protein	0.41
SACOL2290	SACOL2290	transcriptional regulator, AraC family	0.21
norR	SACOL0746	transcriptional regulator, MarR family	0.45
Ribosomal protein			
rplM	SACOL2207	50S ribosomal protein L13	0.39
rplJ	SACOL0585	ribosomal protein L10	0.45
rpsR	SACOL0439	ribosomal protein S18	0.47
rpsI	SACOL2206	ribosomal protein S9	0.36
Transport and binding proteins			
SACOL0882	SACOL0882	ABC transporter, ATP-binding protein	0.09
SACOL0306	SACOL0306	ABC transporter, ATP-binding protein	0.22
SACOL1427	SACOL1427	ABC transporter, ATP-binding protein	0.30
SACOL0690	SACOL0690	ABC transporter, ATP-binding protein	0.42
SACOL0883	SACOL0883	ABC transporter, permease protein	0.08
SACOL0305	SACOL0305	ABC transporter, permease protein	0.27
SACOL0689	SACOL0689	ABC transporter, permease protein	0.40
SACOL0884	SACOL0884	ABC transporter, substrate-binding protein	0.06
SACOL0688	SACOL0688	ABC transporter, substrate-binding protein	0.37
SACOL0506	SACOL0506	ABC transporter, substrate-binding protein	0.39
SACOL1915	SACOL1915	amino acid ABC transporter, ATP-binding protein	0.27
SACOL1916	SACOL1916	amino acid ABC transporter, permease/substrate-binding protein	0.35
SACOL0630	SACOL0630	amino acid permease	0.06
SACOL1743	SACOL1743	amino acid permease	0.28
SACOL1728	SACOL1728	amino acid permease	0.37
SACOL1367	SACOL1367	amino acid permease	0.42
SACOL2169	SACOL2169	aerobactin biosynthesis protein, IucA/IucC family	0.34
oppF	SACOL0994	oligopeptide ABC transporter, ATP-binding protein	0.20
oppD	SACOL0993	oligopeptide ABC transporter, ATP-binding protein	0.22
SACOL0995	SACOL0995	oligopeptide ABC transporter, oligopeptide-binding protein	0.20
oppC	SACOL0992	oligopeptide ABC transporter, permease protein	0.27
opuD2	SACOL2176	osmoprotectant transporter, BCCT family	0.06
oppB	SACOL0991	oligopeptide ABC transporter, permease protein	0.33
SACOL2473	SACOL2473	peptide ABC transporter, ATP-binding protein	0.45
SACOL2472	SACOL2472	peptide ABC transporter, ATP-binding protein	0.47
SACOL2476	SACOL2476	peptide ABC transporter, peptide-binding protein	0.36
SACOL2474	SACOL2474	peptide ABC transporter, permease protein	0.37
SACOL2475	SACOL2475	peptide ABC transporter, permease protein, putative	0.38
SACOL2663	SACOL2663	PTS system, fructose-specific IIABC components	0.30
SACOL2552	SACOL2552	PTS system, IIABC components	0.08
SACOL0175	SACOL0175	PTS system, IIABC components	0.13
SACOL0516	SACOL0516	PTS system, IIBC components	0.23
SACOL2148	SACOL2148	PTS system, mannitol-specific IIA component	0.15
SACOL2319	SACOL2319	Na+/H+ antiporter family protein	0.46
nhaC	SACOL2292	Na+/H+ antiporter NhaC	0.34
SACOL0682	SACOL0682	Na+/H+ antiporter, MnhD component, putative	0.43
SACOL0685	SACOL0685	Na+/H+ antiporter, MnhF component, putative	0.44
SACOL0686	SACOL0686	Na+/H+ antiporter, MnhG component, putative	0.46
nupC	SACOL0566	nucleoside permease NupC	0.39
pbuX	SACOL0459	xanthine permease	0.21
SACOL2242	SACOL2242	xanthine/uracil permease family protein	0.46
SACOL2483	SACOL2483	transporter, putative	0.31
SACOL1114	SACOL1114	Mn2+/Fe2+ transporter, NRAMP family	0.39

SACOL0088	SACOL0088	Na/Pi cotransporter family protein	0.38
SACOL2314	SACOL2314	sodium/bile acid symporter family protein	0.42
SACOL1979	SACOL1979	sodium-dependent transporter	0.30
SACOL0788	SACOL0788	proton-dependent oligopeptide transporter family protein	0.42
SACOL0093	SACOL0093	L-lactate permease	0.36
SACOL2707	SACOL2707	cobalt transport family protein	0.45
Unknown function/Others			
SACOL1847	SACOL1847	conserved domain protein, putative	0.24
SACOL1365	SACOL1365	hydrolase, haloacid dehalogenase-like family	0.33
isaA	SACOL2584	immunodominant antigen A	0.28
SACOL0388	SACOL0388	prophage L54a, holin, SPP1 family	0.46
SACOL0770	SACOL0770	radical activating enzyme family protein	0.47
SACOL1606	SACOL1606	rhomboid family protein	0.50
SACOL1941	SACOL1941	ribonuclease BN, putative	0.29
SACOL1607	SACOL1607	5-formyltetrahydrofolate cyclo-ligase family protein	0.47
SACOL1187	SACOL1187	antibacterial protein (phenol soluble modulin)	0.32
SACOL0740	SACOL0740	decarboxylase family protein	0.36
SACOL0777	SACOL0777	urea amidolyase-related protein	0.38
spoVG	SACOL0541	spoVG protein	0.38
SACOL1663	SACOL1663	urea amidolyase-related protein	0.38
SACOL0399	SACOL0399	oxidoreductase, putative	0.28
SACOL2321	SACOL2321	oxidoreductase, short chain dehydrogenase/reductase family	0.29
SACOL2053	SACOL2053	S1 RNA binding domain protein	0.46
SACOL0723	SACOL0723	LysM domain protein	0.42
Hypothetical protein			
nrdI	SACOL0791	hypothetical protein	0.41
SACOL2175	SACOL2175	hypothetical protein	0.09
SACOL2174	SACOL2174	hypothetical protein	0.10
SACOL0599	SACOL0599	hypothetical protein	0.13
SACOL0427	SACOL0427	hypothetical protein	0.18
SACOL2580	SACOL2580	hypothetical protein	0.19
SACOL2621	SACOL2621	hypothetical protein	0.19
SACOL2720	SACOL2720	hypothetical protein	0.20
SACOL1090	SACOL1090	hypothetical protein	0.20
SACOL2168	SACOL2168	hypothetical protein	0.22
SACOL2164	SACOL2164	hypothetical protein	0.23
SACOL0673	SACOL0673	hypothetical protein	0.24
SACOL1848	SACOL1848	hypothetical protein	0.26
SACOL0741	SACOL0741	hypothetical protein	0.27
SACOL1850	SACOL1850	hypothetical protein	0.28
SACOL0866	SACOL0866	hypothetical protein	0.28
SACOL2461	SACOL2461	hypothetical protein	0.28
SACOL2379	SACOL2379	hypothetical protein	0.30
SACOL1802	SACOL1802	hypothetical protein	0.30
SACOL2681	SACOL2681	hypothetical protein	0.30
SACOL1846	SACOL1846	hypothetical protein	0.31
SACOL2300	SACOL2300	hypothetical protein	0.31
SACOL0092	SACOL0092	hypothetical protein	0.33
SACOL1849	SACOL1849	hypothetical protein	0.33
SACOL1978	SACOL1978	hypothetical protein	0.34
SACOL0565	SACOL0565	hypothetical protein	0.34
SACOL0787	SACOL0787	hypothetical protein	0.34
SACOL1836	SACOL1836	hypothetical protein	0.34
SACOL0862	SACOL0862	hypothetical protein	0.35
SACOL0863	SACOL0863	hypothetical protein	0.36
SACOL2093	SACOL2093	hypothetical protein	0.36
SACOL0272	SACOL0272	hypothetical protein	0.36
SACOL2705	SACOL2705	hypothetical protein	0.38
SACOL2625	SACOL2625	hypothetical protein	0.41
SACOL0444	SACOL0444	hypothetical protein	0.41
SACOL0864	SACOL0864	hypothetical protein	0.42
SACOL0274	SACOL0274	hypothetical protein	0.42
SACOL1664	SACOL1664	hypothetical protein	0.43
SACOL0273	SACOL0273	hypothetical protein	0.43
SACOL1020	SACOL1020	hypothetical protein	0.43
SACOL1605	SACOL1605	hypothetical protein	0.43
SACOL2704	SACOL2704	hypothetical protein	0.43
SACOL1126	SACOL1126	hypothetical protein	0.43

SACOL0372	SACOL0372	hypothetical protein	0.43
SACOL0275	SACOL0275	hypothetical protein	0.44
SACOL1042	SACOL1042	hypothetical protein	0.44
SACOL0849	SACOL0849	hypothetical protein	0.44
SACOL1017	SACOL1017	hypothetical protein	0.45
SACOL1603	SACOL1603	hypothetical protein	0.45
SACOL2479	SACOL2479	hypothetical protein	0.45
SACOL1789	SACOL1789	hypothetical protein	0.46
SACOL0381	SACOL0381	hypothetical protein	0.46
SACOL0865	SACOL0865	hypothetical protein	0.46
SACOL2706	SACOL2706	hypothetical protein	0.46
SACOL1361	SACOL1361	hypothetical protein	0.46
SACOL0628	SACOL0628	hypothetical protein	0.47
SACOL1660	SACOL1660	hypothetical protein	0.48
SACOL2052	SACOL2052	hypothetical protein	0.49
SACOL1899	SACOL1899	hypothetical protein	0.49
SACOL0380	SACOL0380	hypothetical protein	0.49

3.1.3 Protein synthesis profiles in response to heat stress

In the present approach, quantification of the synthesis of cytoplasmic proteins before and at different times after shifting the cells to high temperature condition was performed. To study changes in the synthesis rate of each protein, two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) of radioactively labeled protein extracts was used (O'Farrell, 1975 , Eymann *et al* ., 1996).

The 2D gels were stained, dried, and exposed to PhosphorImager screens. The resulting autoradiograms represent the synthesized proteins at the respective time points. Proteins synthesized only upon heat treatment appeared in red, whereas spots which appeared in green represent proteins the synthesis of which was switched off under these conditions.

Interesting protein spots were identified by MALDI-TOF/MS and Mascot software. Proteins whose synthesis was significantly induced in at least 3 experiments were shown in table 3.3 and proteins whose synthesis was repressed by heat stress were shown in table 3.4. As a result, the synthesis rate of at least 29 protein spots was induced, whereas at the same time the synthesis rate of at least 116 protein spots seemed to be repressed.

Table 3.3: Proteins whose synthesis was induced under heat stress condition

COL Locus	ID	Function	Ratio			
Amino acid biosynthesis						
			5min	**10min**	**30min**	**60min**
SACOL2105	GlyA	Amino acid biosynthesis, Glycine, serine threonine	33.47	56.96	46.26	56.26
SACOL0430	SACOL0430	Amino acid biosynthesis: Aspartate family	4.39	7.25	12.83	15.58
SACOL0431	SACOL0431	Amino acid biosynthesis: Aspartate family	29.79	34.53	18.98	16.69
Biosynthesis of cofactors						
SACOL1817	RibH	Biosynthesis of cofactors, prosthetic groups	2.39	1.63	3.28	1.5
Cellular processes						
SACOL0567	CtsR	Cellular processes, Adaptations to atypical conditions	14.31	15.96	20.57	16.59
SACOL2131	SACOL2131	Cellular processes: Dps family protein	7.7	7.64	8.75	4.9
DNA metabolism						
SACOL0006	GyrA	DNA metabolism	8	6.11	10.91	10.03
Protein fate						
SACOL0979	ClpB	Degradation of proteins, peptides, and glycopeptides	3.6	5.83	5.21	8.4
SACOL0833	ClpP	Degradation of proteins, peptides, and glycopeptides	2.85	2.92	2.62	3.17
SACOL0570	ClpC/or MetE	Degradation of proteins, peptides, and glycopeptides	12.33	12.55	13.15	12.25
SACOL1637	DnaK	Protein fate, Protein folding and stabilization	8.85	11.69	11.18	8.63
SACOL2016	GroEL	Protein fate, Protein folding and stabilization	7.67	22.98	13.71	6.22
SACOL1638	GrpE	Protein fate, Protein folding and stabilization	2.39	2.96	7.12	9.19
SACOL2054	RpoF	Protein fate	14.73	21.59	22.01	13.58
SACOL1636	DnaJ	Protein fate	4.33	3.91	3.18	2.83
Purines, pyrimidines metabolism						
SACOL0018	PurA	Purines, pyrimidines, nucleosides, and nucleotides	0.6	3.62	25.15	27.8
Transport and binding proteins						
SACOL2148	PtsIIA	ABC transporter	1.88	2.7	87.34	237.75
SACOL2335	SACOL2335	ABC transporter, ATP-binding protein	3.58	2.92	0.98	0.17
SACOL1952	SACOL1952	Cations and iron carrying compounds, ferritins family	13.43	10.73	8.97	4.98
SACOL2708	SACOL2708	ABC transporter, ATP-binding protein, putative	3.72	1.66	0.29	1.13
Unknown function						
SACOL2597	SACOL2597	hydrolase, alpha/beta hydrolase fold family putative	3.17	0.72	0.06	0.04
SACOL2722	SACOL2722	N-acetyltransferase family protein putative	1.57	2.75	0.18	0.46
Hypothetical protein						
SACOL2379	SACOL2379	Hypothetical protein	7.16	15.19	1.23	0.67
SACOL1204	YlmH	Hypothetical protein	90.54	128.5	97.64	104.6
SACOL0455	SACOL0455	Hypothetical proteins	6.52	7.74	6.31	3.14
SACOL0776	SACOL0776	Hypothetical proteins	32.6	22.15	18.82	9.01
SACOL1992	SACOL1992	Hypothetical proteins	3.41	5.91	3.16	11.3

Table 3.4: Proteins whose synthesis was repressed under heat stress condition

COL Locus	ID	Function	Ratio			
Amino acid biosynthesis						
			5min	**10min**	**30min**	**60min**
SACOL1787	SACOL1787	Aromatic amino acid family	0.19	0.16	0.11	0.09
SACOL1431	DapB	Aspartate family	0.14	0.15	0.17	0.18
SACOL1435	LysA	Aspartate family	0.09	0.03	0.13	0.07
SACOL1364	ThrB	Aspartate family	0.26	0.14	0.14	0.16
SACOL1363	ThrC	Aspartate family	0.39	0.18	0.18	0.24
SACOL1329	FemC	Glutamate family	0.33	0.27	0.21	0.12
SACOL1773	SerA	Serine family	0.30	0.09	0.10	0.17
SACOL2050	IlvA2	Valine, leucine and isoleucine family	1.30	0.41	0.89	0.14
SACOL2043	IlvB	Valine, leucine and isoleucine family	0.32	0.29	0.17	0.18

SACOL2045	IlvC	Valine, leucine and isoleucine family	0.47	0.18	0.20	0.61
SACOL0600	IlvE	Valine, leucine and isoleucine family	0.10	0.05	0.04	0.03
SACOL2047	LeuB	Valine, leucine and isoleucine family	0.19	0.17	0.12	0.44
Biosynthesis of cofactors, prosthetic groups, and carriers						
SACOL0918	SufB	FeS assembly protein	0.35	0.17	0.15	0.19
SACOL0915	SufD	FeS assembly protein	0.39	0.17	0.20	0.29
SACOL0914	SufC	Biosynthesis of cofactors, prosthetic groups	0.28	0.26	0.19	0.75
SACOL0916	SACOL0916	Biosynthesis of cofactors, prosthetic groups	0.25	0.20	0.19	0.23
SACOL1735	CoaE	Pantothenate and coenzyme A	0.07	0.04	0.05	0.09
SACOL2615	PanB	Biosynthesis of cofactors, prosthetic groups,	0.14	0.20	0.21	0.18
SACOL0626	ThiD1	Biosynthesis of cofactors, prosthetic groups	0.29	0.18	0.18	0.13
Cell envelope						
SACOL2092	MmurAA	Biosynthesis and degradation of murein sacculus and peptidoglycan	0.13	0.12	0.16	0.11
SACOL1411	SACOL1411	Biosynthesis and degradation of murein sacculus and peptidoglycan	0.49	0.20	0.18	0.16
SACOL0142	Cap5G	Biosynthesis and degradation of surface polysaccharides	0.14	0.14	0.21	0.13
Cellular processes						
SACOL1624	Era	Adaptations to atypical conditions	0.40	0.21	0.14	0.27
SACOL2173	SACOL2173	Adaptations to atypical conditions	0.09	0.08	0.10	0.09
SACOL1199	FtsZ	Cellular processes, Cell division	0.28	0.22	0.22	0.17
SACOL1368	KatA	Cellular processes, Detoxification	0.33	0.26	0.12	0.23
SACOL0118	SodA1	Cellular processes, Detoxification	0.12	0.07	0.13	0.10
DNA metabolism						
SACOL2737	GidA	DNA replication, recombination, and repair	0.26	0.19	0.21	0.19
SACOL0534	SACOL0534	DNA replication, recombination, and repair	0.18	0.22	0.19	1.20
SACOL0438	Ssb2	DNA replication, recombination, and repair	0.25	0.18	0.25	0.20
Energy metabolism						
SACOL1800	Dat	Energy metabolism, Amino acids and amines	0.30	0.19	0.23	0.15
SACOL1430	DapA	Energy metabolism, Amino acids and amines. Aspartate family	0.23	0.27	0.23	0.35
SACOL0961	GluD	Energy metabolism, Amino acids and amines, Glutamate	0.20	0.10	0.10	0.07
SACOL1593	SACOL1593	Energy metabolism, Amino acids and amines, Glycine, serine and threonine	0.25	0.25	0.30	0.25
SACOL1562	SACOL1562	Energy metabolism, Amino acids and amines, Valine, leucine and isoleucine	0.06	0.10	0.09	0.09
SACOL2095	AtpD	Energy metabolism, ATP-proton motive force interconversion	0.71	0.40	0.20	0.24
SACOL2096	AtpG	ATP-proton motive force interconversion	0.39	0.44	0.26	0.12
SACOL2098	AtpH	ATP-proton motive force interconversion	0.18	0.13	0.08	0.06
SACOL0944	SACOL0944	Electron transport, respiration chain	0.10	0.08	0.05	0.04
SACOL2618	Ldh2	Energy metabolism, Fermentation, lactate	0.07	0.06	0.03	0.09
SACOL2535	SACOL2535	Energy metabolism, Fermentation, lactate	0.27	0.23	0.20	0.12
SACOL1321	GlpD	Energy metabolism, Glycerol metabolism	0.04	0.33	0.41	0.39
SACOL1514	GpsA	Energy metabolism, Glycerol metabolism	1.60	1.64	0.73	0.09
SACOL2117	FbaA	Glycolysis/gluconeogenesis	0.15	0.19	0.18	0.36
SACOL2415	Gpm	Glycolysis/gluconeogenesis	0.27	0.22	0.23	0.46
SACOL1746	PfkA	Glycolysis/gluconeogenesis	0.11	0.07	0.08	0.11
SACOL1123	Pyc	Glycolysis/gluconeogenesis	0.20	0.11	0.11	0.08
SACOL1745	Pyk	Glycolysis/gluconeogenesis	0.07	0.08	0.21	0.06
SACOL1554	Gnd	Pentose phosphate pathway	0.05	0.05	0.04	0.05
SACOL1549	Zwf	Pentose phosphate pathway	0.12	0.15	0.14	0.29
SACOL0617	SACOL0617	Energy metabolism, Sugars	0.11	0.09	0.21	0.14
SACOL1385	AcnA	Energy metabolism, TCA cycle	0.10	0.12	0.10	0.07
SACOL1308	SACOL1308	Energy metabolism, TCA cycle	0.26	0.14	0.16	0.11
SACOL1159	SdhA	Energy metabolism, TCA cycle	0.16	0.27	0.21	0.08
SACOL0975	SACOL0975	Energy metabolism: Electron transport	0.59	0.36	0.23	0.32
Fatty acid and phospholipid metabolism						
SACOL1245	FabG1	Fatty acid and phospholipid metabolism	0.14	0.12	0.12	0.22
SACOL0987	FabH	Fatty acid and phospholipid metabolism	0.24	0.34	0.12	0.23
SACOL1016	FabI	Fatty acid and phospholipid metabolism	2.27	0.40	0.38	0.35
SACOL1243	PlsX	Fatty acid and phospholipid metabolism	0.28	0.18	0.14	0.20
Protein fate						
SACOL1801	SACOL1801	Degradation of peptides	0.55	0.23	0.20	0.42
SACOL0957	SACOL0957	Protein folding and stabilization	0.10	0.05	0.10	0.07
SACOL1591	SACOL1591	Protein modification and repair	0.13	0.10	0.12	0.17

Protein synthesis						
SACOL0663	ArgS	Protein synthesis, tRNA aminoacylation	0.11	0.14	0.20	0.13
SACOL1685	AspS	Protein synthesis, tRNA aminoacylation	0.39	0.28	0.45	1.74
SACOL1206	IleS	Protein synthesis, tRNA aminoacylation	0.17	0.14	0.29	0.27
SACOL0562	LysS	Protein synthesis, tRNA aminoacylation	0.08	0.11	0.09	0.22
SACOL1149	PheT	Protein synthesis, tRNA aminoacylation	0.14	0.16	0.15	0.12
SACOL1282	ProS	Protein synthesis, tRNA aminoacylation	0.26	0.16	0.30	0.56
SACOL1778	TyrS	Protein synthesis, tRNA aminoacylation	0.14	0.10	0.06	0.13
SACOL1676	TrmU	Protein synthesis, tRNA and rRNA base modification	0.28	0.23	0.11	0.13
Purines, pyrimidines, nucleosides, and nucleotides						
SACOL0792	NrdE	2'-Deoxyribonucleotide metabolism	0.33	0.27	0.21	0.40
SACOL0793	NrdF	2'-Deoxyribonucleotide metabolism	0.11	0.10	0.17	0.20
SACOL1277	PyrH	Nucleotide and nucleoside interconversions	0.11	0.19	0.29	0.31
SACOL0603	SACOL0603	Nucleotide and nucleoside interconversions	0.36	0.31	0.75	2.74
SACOL1221	Gmk	Purine ribonucleotide biosynthesis	0.25	0.28	0.22	0.42
SACOL0461	GuaA	Purine ribonucleotide biosynthesis	0.18	0.23	0.49	0.72
SACOL0460	GuaB	Purine ribonucleotide biosynthesis	0.16	0.15	0.48	0.36
SACOL0544	PrsA	Purine ribonucleotide biosynthesis	0.27	0.30	0.24	0.16
SACOL1969	PurB	Purine ribonucleotide biosynthesis	0.15	0.22	0.18	0.15
SACOL1075	PurC	Purine ribonucleotide biosynthesis	0.14	0.10	0.05	0.13
SACOL1074	PurK	Purine ribonucleotide biosynthesis	0.42	0.24	0.12	0.13
SACOL1077	PurQ	Purine ribonucleotide biosynthesis	0.24	0.20	0.28	0.36
SACOL1215	CarB	Pyrimidine ribonucleotide biosynthesis	0.28	0.16	0.14	0.13
SACOL2119	PyrG	Pyrimidine ribonucleotide biosynthesis	0.20	0.18	0.14	0.23
SACOL2104	Upp	Salvage of nucleosides and nucleotides	0.07	0.11	0.05	0.07
Other						
SACOL1898	Cbf1	Mobile and extrachromosomal element functions, Plasmid functions	0.45	0.30	0.21	0.14
SACOL2055	RsbW	Regulatory functions, Protein interactions	0.58	0.27	0.27	0.26
SACOL0731	SACOL0731	Regulatory functions, Protein interactions	0.33	0.31	0.16	0.10
SACOL0825	HprK	Signal transduction, PTS	0.43	0.12	0.15	0.13
			0.47	0.08	0.05	0.05
SACOL1427	SACOL1427	ABC transporter	0.11	0.13	0.11	0.10
SACOL2519	SACOL2519	Hypothetical proteins: Conserved	0.14	0.10	0.11	0.06
SACOL2535	SACOL2535	Central intermediary metabolism: Other	0.20	0.15	0.17	0.20
SACOL0976	SACOL0976	Hydrolase, haloacid dehalogenase-like family	0.03	0.04	0.09	0.05
SACOL1560	SACOL1560	Amino acids and amines	0.32	0.29	0.27	0.19
SACOL1677	SACOL1677	Aminotransferase, class V, putative	0.23	0.19	0.14	0.08
SACOL2000	SACOL2000	aminotransferase, putative	0.19	0.10	0.34	0.17
Hypothetical protein						
SACOL0467	SACOL0467	hypothetical proteins	0.23	0.14	0.13	0.25
SACOL0456	SACOL0456	hypothetical protein	0.14	0.22	0.29	0.43
SACOL0597	SACOL0597	hypothetical protein	0.30	0.30	0.21	0.21
SACOL0613	SACOL0613	hypothetical protein	0.08	0.05	0.06	0.05
SACOL0789	SACOL0789	hypothetical protein	0.12	0.11	0.11	0.09
SACOL1365	SACOL1365	hypothetical protein	0.26	0.13	0.12	0.35
SACOL1772	SACOL1772	hypothetical protein	0.12	0.07	0.23	0.10
SACOL1792	SACOL1792	hypothetical protein	0.06	0.04	0.05	0.03
SACOL2133	SACOL2133	hypothetical protein	0.12	0.08	0.05	0.14
SACOL2519	SACOL2519	hypothetical protein	0.17	0.21	0.21	0.44
SACOL0918	SufB	hypothetical protein	0.21	0.18	0.27	0.23
SACOL0915	SufD	hypothetical protein	0.37	0.41	0.13	0.09
SACOL1936	SACOL1936	hypothetical protein	0.24	0.18	0.23	0.17
SACOL2518	SACOL2518	hypothetical protein	0.36	0.35	0.25	0.52
SACOL1543	SACOL1543	hypothetical protein, aldo/keto reductase	0.11	0.41	0.27	0.18
SACOL0241	SACOL0241	hypothetical protein, Fermentation	0.05	0.02	0.04	0.06
SACOL2114	SACOL2114	hypothetical protein, Fermentation	0.44	0.48	0.26	0.37
SACOL0596	SACOL0596	hypothetical protein, Glycine. serine threonine metabolism	0.24	0.21	0.26	0.18
SACOL0602	SACOL0602	hypothetical protein, haloacid dehalogenase-like hydrolase	0.10	0.07	0.07	0.06
SACOL0607	SACOL0607	NADPH-dependent FMN reductase	0.18	0.24	0.20	0.17

Proteome analysis data shows that the major chaperones regulated by HrcA-CIRCE element were highly induced after heat stress.

This important group of heat-shock-induced proteins includes the chaperones DnaK, GroES, and GroEL which are controlled by the HrcA repression system in *B. subtilis* and dually regulated by CtsR and HrcA in *S.aureus* (Zuber and Schumann, 1994; Hecker and Völker, 1998; Chastanet *et al.*, 2003). Quantitation of relative synthesis rates at the next time points demonstrated that the synthesis of these proteins continued to be induced 10 minutes and 30 minutes after stress. Later on, the induction rate slowed down, manifested in the synthesis profile of proteins after 60 minutes heat treatment.

Dual channel imaging of protein gels did not show any up-regulation in the synthesis of proteins whose expression requires the σ^B stress sigma factor. This result was in good agreement with the data obtained by transcriptome analysis. Apparently, there was no sigma B-dependent stress response in *S.aureus* cultivated in synthetic medium after heat stress.

Table 3.4 shows the changes in the synthesis level of some Clp proteases encoded by CtsR-regulated genes: ATPase subunit ClpB and proteolytic subunit ClpP (Derre *et al.*, 2000; Krüger *et al.*,1997; Msadek *et al.*,1998). Within the first 10 minutes exposure to heat stress, both ClpB and ClpP were highly induced.

Another candidate belonging to Clp-proteases group is the ATPase subunit ClpC that was suggested to be induced by heat stress in a CtsR dependent manner as well (Krüger *et al.*, 1996, 1997). Unfortunately, on the gel, ClpC is located right under MetE so ClpC has not yet been surely identified since ClpC might be present at very low levels. The synthesis of many other proteins was observed to be up-regulated at a high rate instantly after shift from 37° C to 48° C.

As shown in Table 3.3 , the synthesis rate of some enzymes which belong to amino acid metabolism pathways such as serine hydroxymethyltransferase GlyA, trans-sulfuration enzyme family proteins SACOL0430 and SACOL0431 as well as enzymes involve in the purine ribonucleotide biosynthesis pathwaysuch as adenylosuccinate synthetase PurA was induced in heat treated cells.

Collectively, the overview of induction profiles after heat stress obtained by tran-

scriptomics and proteomics reveals that members of protein fate processes are the largest group which can be detected by both DNA arrays and 2D gel approach suggesting protein damage is a priority under heat stress(Fig 3.4, 3.5 and 3.6).

3.1.4 Detailed transcriptional analysis of heat shock genes

The influence of high temperatures on the transcription of selected genes was subsequently confirmed by Northern blotting experiments to validate the data obtained from proteome and DNA microarray analyses.

We selected *codY*, which acts as a global regulator involved in the adaptation of bacterium to starvation. The expression of CodY was suggested to be induced by heat stress (Frees *et al.*, 2005).

According to the gene region view (Fig 3.7), we predicted that the genes *clpY, clpQ* and *xerC* might form an operon together with *codY*, therefore Northern blot analysis using probes corresponding to *codY, clpY, clpQ* and *xerC*, respectively, were performed as well. From the proximity of the *xerC, clpQ, clpY* and codY genes, we expected that they form an operon with a size of approximately 3,7 kb, and this notion was confirmed by the size of the transcript detected with the *codY* as well as *clpY, clpQ* and *xer C* probes. This transcript was present in cells grown at normal condition and showed a slightly increasing amount in heat shocked cells (Fig 3.7).

Besides, we also detected two other transcripts of 2.5kb and 1.4kb respectively. But these transcripts might be the products of RNA degradation.

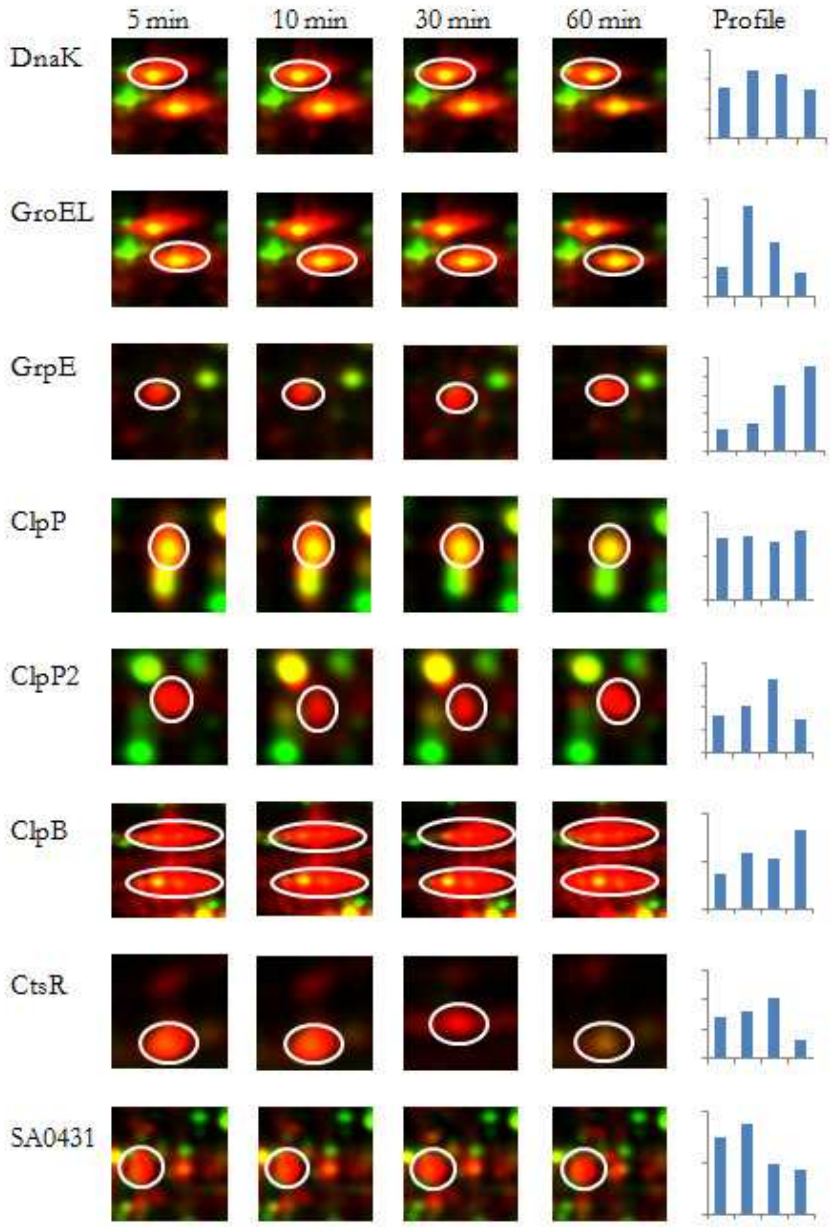

Figure 3.4: Details of dual channel images of selected marker proteins induced in response to heat stress. Protein synthesis pattern of unstressed *S. aureus* cells was compared with protein synthesis patterns at different time points after exposure to heat shock . The bar graphs on the right displayed relative synthesis rates of the individual proteins at the different time points

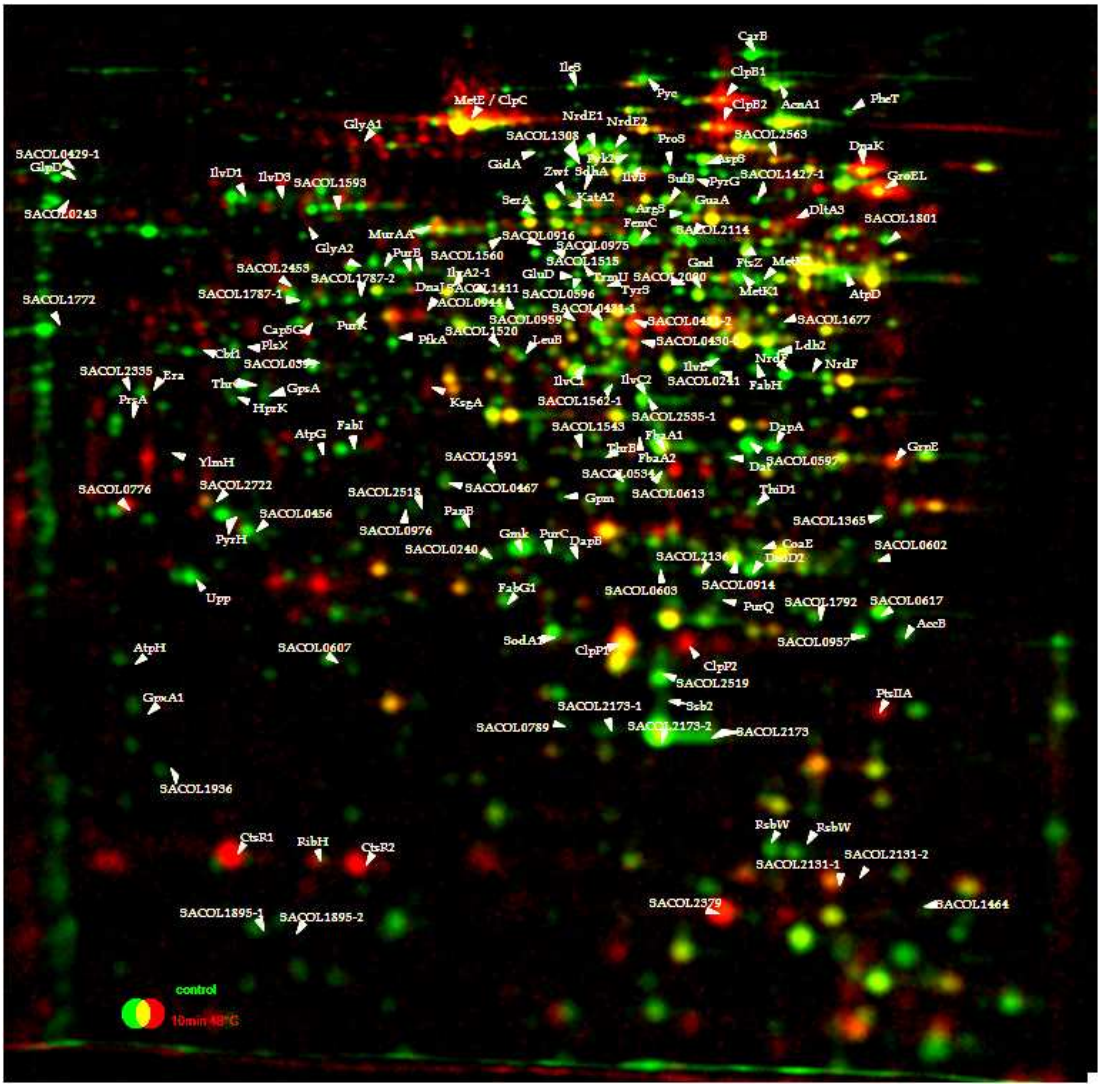

Figure 3.5: The dual-channel image of the protein synthesis pattern of *S.aureus* COL before (green image) and 10 min after the exposure to 48° C (red image). Cytoplasmic proteins were labeled with L-^{35}[S]methionine and separated by 2D-PAGE as described in Section 2. Image analysis of the autoradiograms was performed using the Decodon Delta 2D software. Proteins that were synthesized at increased/decreased levels in response to heat stress were identified and listed in Table 3.3 and Table 3.4.

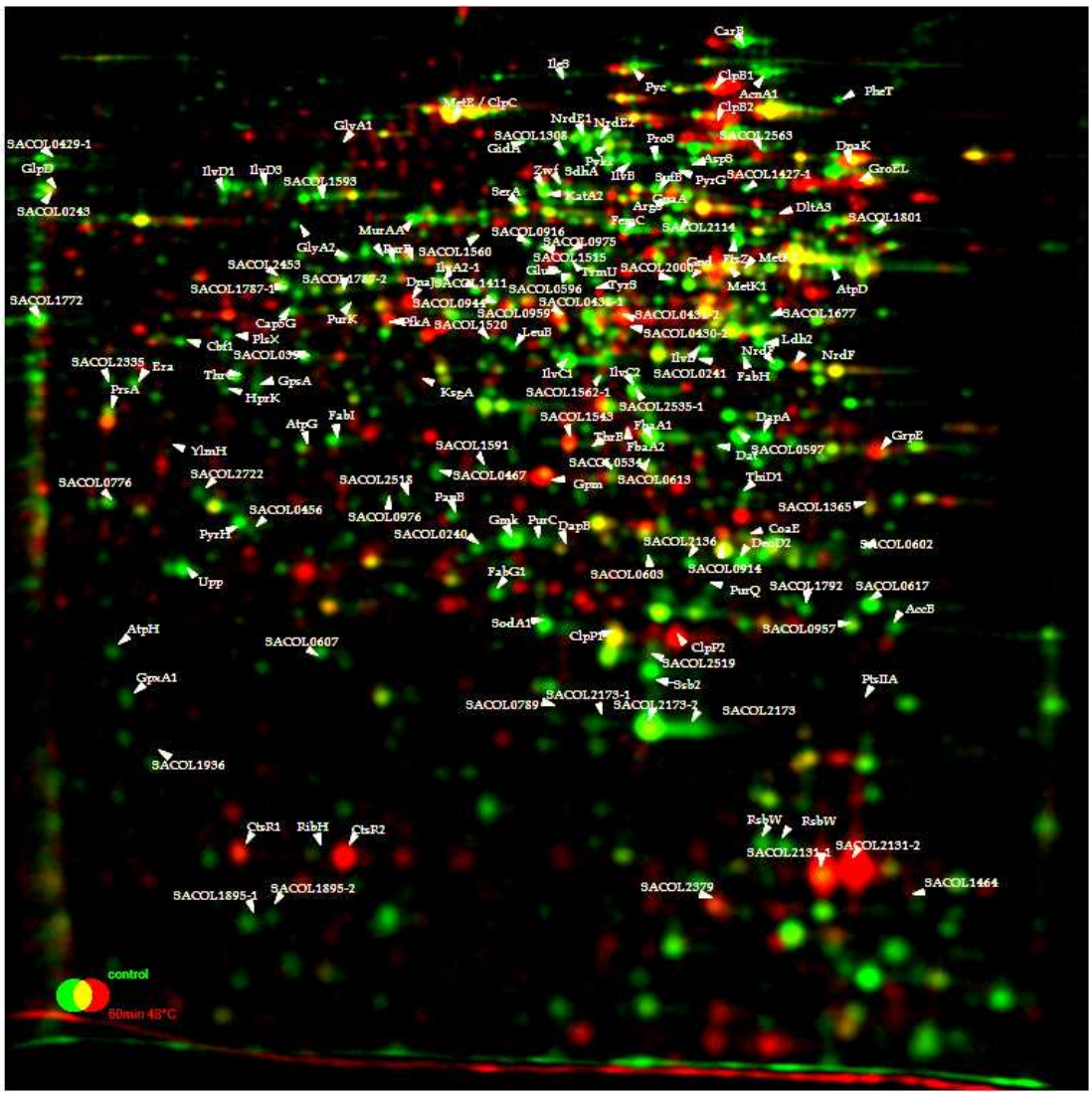

Figure 3.6: The dual-channel image of the protein synthesis pattern of *S.aureus* COL before (green image) and 60 min after the exposure to 48° C (red image).

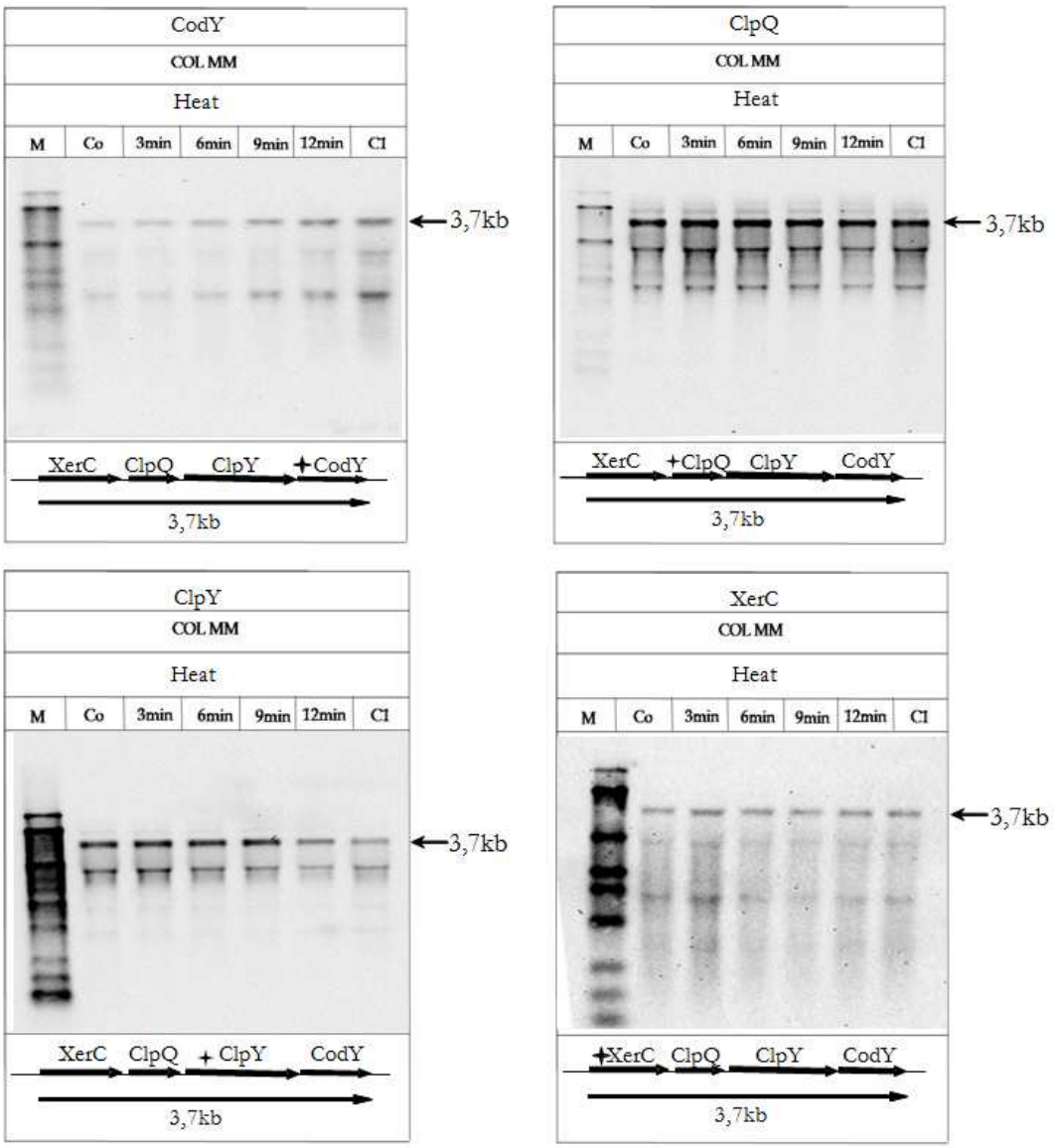

Figure 3.7: Transcript analyses of *codY, clpQ, clpY* and *xerC* in response to heat shock. Relevant probes which had been used in each Northern blot experiments were indicated by star. 10 μg RNA each were applied isolated from *S.aureus* COL before and at different times (3, 6, 9, 12 min) after the exposure to heat stress.

3.2 Puromycin stress response

3.2.1 Growth behavior of *S.aureus* in response to puromycin

Firstly, the minimal inhibitory concentration (MIC) was determined for the antimicrobial substance puromycin. The MIC of the antimicrobial substance were defined as the lowest concentration resulting visibly inhibited bacterial growth.In this case the MIC of puromycin corresponded to 2 μg/ml.

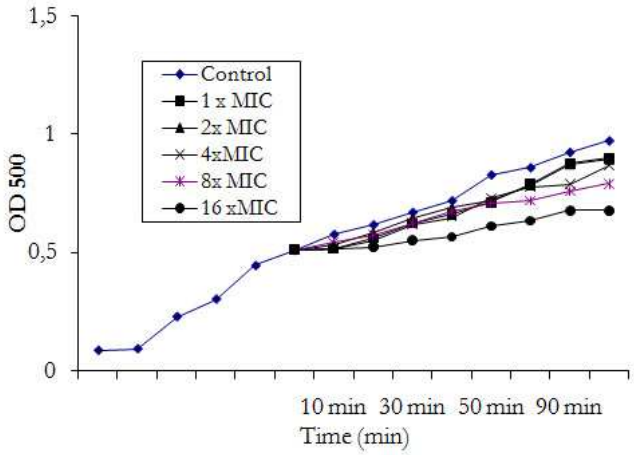

Figure 3.8: Growth of *S.aureus* COL in the presence of different concentrations of puromycin. S. aureus was grown in synthetic medium to an OD500 of 0.5 and treated with different concentrations of puromycin.

To investigate global changes in gene expression of *S.aureus* in response to puromycin, cells were treated with sublethal but growth-inhibitory concentrations. For anaylsing the growth behaviour of *S. aureus* COL in response to puromycin, different concentrations of the antibiotic (1xMIC; 2xMIC; 4xMIC; 8xMIC; 16xMIC) were added to exponentially growing cultures (OD_{500} of 0.5). As reflected in the growth curves, 16 μg/ml of puromycin (8xMIC) are required to inhibit the growth rate, results in a reduction of approximately 50% (Fig.3.9).

For further experiments, cells were treated with 8xMIC (16 μg/ml)puromycin. After exposure to puromycin, samples were harvest at 3, 6, 9, 12 minutes for RNA preparation. For proteome analysis experiments, samples were labelled with L-[

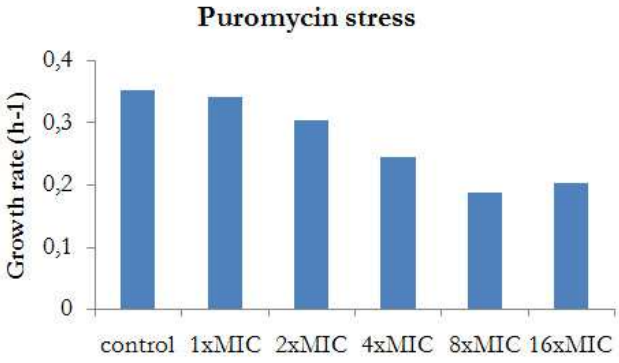

Figure 3.9: Growth rate of *S.aureus* under puromycin stress.

^{35}S]-methionine at the same time points.

3.2.2 The influence of puromycin on gene transcription of *S.aureus.*

Transcriptome analysis data demonstrated that after puromycin stress, genes belonging to class I and III heat-shock response were strongly induced. Genes encoding both HrcA and CtsR-dependent chaperones and proteases are strongly transcribed after exposure to puromycin. As for HrcA-dependent genes, *dnaK, dnaJ* and *grpE* were detected as strongly up-regulated after puromycin stress with an induction rate of 4.5, 4.6 and 6.3 fold, respectively. The *groEL-groES* operon also showed a high induction rate of 4.49 and 6.1 fold after stress exposure (Table 3.5). Besides, *hrcA* itself was also detected to be highly transcribed (11.59 fold). In the case of CtsR-dependent proteases, transcriptome analysis pointed out that the transcription of *clpP* and *clpB* was induced by puromycin at a rate of 3.47 and 12.2 fold, respectively. Moreover, transcription of *ctsR* encoding the transcriptional regulator CtsR was increased by puromycin (8.0 fold). As expected, similarly to the heat stress response of *S.aureus* grown in synthetic medium, we detected no induction of the sigma-B-dependent general stress genes after puromycin stress. During growth under puromycin stress conditions, the mRNA levels of several genes of unknown function were found to be up-regulated

compared to cells grown under control conditions. For example, *prmA* encoding the ribosomal protein L11 which was also induced by heat stress or SACOL0569 encoded guanido phosphotransferase,and SACOL0981 encoded isopropylmalate synthase-related protein.

Table 3.5: Genes whose transcription was induced after puromycin stress

COL Locus	ID	Function	Average Ratio
Protein fate			
SACOL1636	dnaJ	dnaJ protein	4.95
SACOL1637	dnaK	dnaK protein	4.54
SACOL1638	grpE	heat shock protein GrpE	6.3
SACOL1639	hrcA	heat-inducible transcription repressor HrcA	11.59
SACOL2016	groEL	chaperonin, 60 kDa	4.49
SACOL2017	groES	chaperonin, 10 kDa	6.01
SACOL0833	clpP	ATP-dependent Clp protease, proteolytic	3.47
SACOL0979	clpB	ATP-dependent Clp protease, ATP-binding	12.22
SACOL0567	ctsR	transcriptional regulator CtsR	8
Protein synthesis			
SACOL1635	prmA	ribosomal protein L11 methyltransferase	5.18
Others			
SACOL0569	SACOL0569	putative ATP:guanido phosphotransferase	7.69
SACOL0811	SACOL0811	hypothetical protein	4.04
SACOL0981	SACOL0981	isopropylmalate synthase-related protein	2.76
SACOL1634	SACOL1634	hypothetical protein	2.16
SACOL0568	SACOL0568	hypothetical protein	2.78

Table 3.6: Genes whose transcription was repressed after puromycin stress

COL Locus	ID	Function	Average Ratio
Amino acid metabolism			
SACOL0505	gltD	Glutamate synthase, subunit	0.48
Protein fate			
SACOL0165	SACOL0165	4-phosphopantetheinyl transferase	0.46

3.2.3 Protein expression profile of *S.aureus* in response to puromycin

Proteome analyses were performed after exposure to puromycin. Proteins synthesis with induction ratios of at least two fold in two biological replicates were considered as significantly up-regulated.

Accordingly, in response to puromycin stress, approximately the synthesis of 20 proteins was up-regulated and the synthesis of 35 proteins was down-regulated (Table 3.7 and 3.8).

The strongest induction of these 20 proteins was found after 10 min of puromycin stress. The most strongly induced proteins belong to the class I heat-specific chaperones which are regulated by HrcA and CtsR, and and class III proteins

which are only regulated by CtsR (Chastanet *et al.*, 2003).

Thereby, the protein synthesis rate of DnaK and GrpE of class I heat shock proteins was highly increased during 60 minutes after exposure to stress at a rate varying from three to nine fold.

Similarly, GroEL and GroES were also strongly induced with the highest induction rate at 30 and 60 minutes after stress. In the case of Clp proteases, on 2D protein gels, ClpP and ClpB were detected to be induced strongly indicating that cells suffer from damaged proteins in to the presence of puromycin (Fig 3.10, 3.11 and 3.12).

The proteome data revealed no significant induction of the sigmaB-dependent general stress response after puromycin treatment. This result has consolidated the data obtained by proteome analysis of *S.aureus* grown in synthetic medium under heat stress condition.

Table 3.7: Proteins whose synthesis was induced after puromycin stress

COL Locus	**ID**	**Function**	**Ratio**			
Amino acid biosynthesis						
			5min	**10min**	**30min**	**60min**
SACOL1363	ThrC	threonine synthase	1.90	2.36	2.10	1.78
SACOL1506	AroC	chorismate synthase	1.10	2.35	1.91	2.81
DNA metabolism						
SACOL0016	DnaB	replicative DNA helicase	1.50	1.66	2.13	2.89
Energy metabolism						
SACOL0842	Eno	enolase	1.68	2.20	1.81	2.49
SACOL2151	GlmM	phosphoglucosamine mutase	0.99	1.78	2.71	3.57
SACOL1104	PdhC	pyruvate dehydrogenase complex E2 component, dihydrolipoamide acetyltransferase	1.32	1.91	1.90	4.23
SACOL1277	PyrH	uridylate kinase	1.56	1.55	2.78	3.72
Others						
SACOL0932	SACOL0932	D-isomer specific 2-hydroxyacid dehydrogenase family protein	0.93	1.46	1.89	2.16
SACOL1297	SACOL1297	conserved hypothetical protein	0.65	0.80	0.66	0.45
SACOL1902	SACOL1902	conserved hypothetical protein	0.63	1.82	2.37	2.32
SACOL2486	SACOL2486	conserved hypothetical protein	0.63	0.66	0.49	0.34
SACOL0594	Tuf	translation elongation factor Tu	1.46	1.71	1.98	2.26
Protein fate						
SACOL0833	ClpP	ATP-dependent Clp protease, proteolytic subunit	2.01	2.60	4.61	7.97
SACOL1637	DnaK	dnaK protein	2.36	4.88	8.72	8.68
SACOL2016	GroEL	chaperonin, 60 kDa	2.19	3.86	6.80	6.55
SACOL2017	GroES	chaperonin, 10 kDa	3.45	4.41	5.02	6.19
SACOL1638	GrpE	heat shock protein GrpE	3.81	5.28	6.65	6.98
SACOL0979	ClpB	ATP-dependent Clp protease	1.72	3.53	3.57	3.09

Table 3.8: Proteins whose synthesis was repressed after puromycin stress

COL Locus	ID	Function	Ratio			
Amino acid biosynthesis						
			5min	**10min**	**30min**	**60min**
SACOL2105	GlyA	serine hydroxymethyltransferase	0.69	1.02	0.64	0.50
SACOL2048	LeuC	3-isopropylmalate dehydratase, large subunit	0.56	0.54	0.47	0.53
SACOL0428	MetE	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase	0.55	0.29	0.34	0.41
SACOL0430	SACOL0430	trans-sulfuration enzyme family protein	0.61	0.93	0.48	0.42
GcvP	GcvP	glycine dehydrogenase	0.57	0.87	0.54	0.50
Biosynthesis of cofactors, prosthetic groups, and carriers						
SACOL2266	MoeA	molybdopterin biosynthesis MoeA protein	1.28	1.00	1.14	0.41
SACOL0918	SufB	FeS assembly protein SufB	0.69	0.57	0.48	0.42
SACOL0915	SufD	FeS assembly protein SufD	0.75	0.74	0.53	0.44
Energy metabolism						
SACOL2273	Fdh	formate dehydrogenase accessory protein	0.24	0.10	0.19	0.08
SACOL1838	PckA	phosphoenolpyruvate carboxykinase (ATP)	0.64	0.52	0.46	0.46
SACOL0841	Pgm	phosphoglycerate mutase, 2,3-bisphosphoglycerate-independent	0.71	0.55	0.32	0.18
SACOL1123	PyC	pyruvate carboxylase	0.39	0.25	0.27	0.11
SACOL1745	Pyk	pyruvate kinase	0.53	0.50	0.37	0.28
SACOL1449	SucA	2-oxoglutarate dehydrogenase, E1 component	0.22	0.16	0.21	0.12
SACOL1263	SucD	succinyl-CoA synthase, alpha subunit	0.88	0.83	0.66	0.49
Protein synthesis						
SACOL1288	InfB	translation initiation factor IF-2	0.40	0.44	0.60	0.47
SACOL0533	MetS	methionyl-tRNA synthetase	0.49	0.39	0.35	0.26
SACOL0816	SecA	preprotein translocase, SecA subunit	0.39	0.47	0.57	0.36
SACOL0009	SerS	seryl-tRNA synthetase	0.68	0.40	0.69	0.41
SACOL1729	ThrS	threonyl-tRNA synthetase	0.55	0.75	0.54	0.34
Purine, pyrimydine metabolism						
SSACOL1214	CarA	carbamoyl-phosphate synthase, small subunit	0.77	0.68	0.50	0.58
SACOL0461	GuaA	GMP synthase	0.67	0.70	0.55	0.47
SA0686	NrdE	ribonuceloside diphosphate reductase major subunit	0.50	0.24	0.28	0.21
SACOL1083	PurD	phosphoribosylamine-glycine ligase	0.57	0.56	0.47	0.45
SACOL1082	PurH	phosphoribosylaminoimidazolecarboxamide formyltransferase/IMP cyclohydrolase	0.51	0.68	0.51	0.40
Others						
SACOL1571	AccC	Acetyl-CoA carboxylase, biotin carboxylase	0.59	0.84	0.44	0.34
SACOL1411	FemB	femB protein	0.72	0.44	0.51	0.47
SACOL1198	FtsA	cell division protein FtsA	0.69	0.29	0.78	1.41
SACOL2737	GidA	glucose inhibited division protein A	0.51	0.52	0.33	0.34
SACOL1206	IleS	isoleucyl-tRNA synthetase	0.47	0.12	0.38	0.12
SACOL0761	NagA	N-acetylglucosamine-6-phosphate deacetylase	0.85	0.49	0.59	0.65
SACOL1737	PolA	DNA polymerase I	0.40	0.15	0.77	0.23
SACOL0588	RpoB	DNA-directed RNA polymerase. beta subunit	0.30	0.08	0.18	0.13
SACOL1361	SACOL1361	hypothetical protein	0.20	0.11	0.24	0.08
SACOL0438	Ssb2	single-stranded DNA-binding protein	0.68	0.74	0.49	0.58
SACOL0597	SACOL0597	conserved hypothetical protein	0.52	0.33	0.25	0.45
SACOL0934	SACOL0934	conserved hypothetical protein	0.93	0.46	0.39	0.35
SACOL1011	SACOL1011	conserved hypothetical protein	0.62	0.90	0.47	0.65

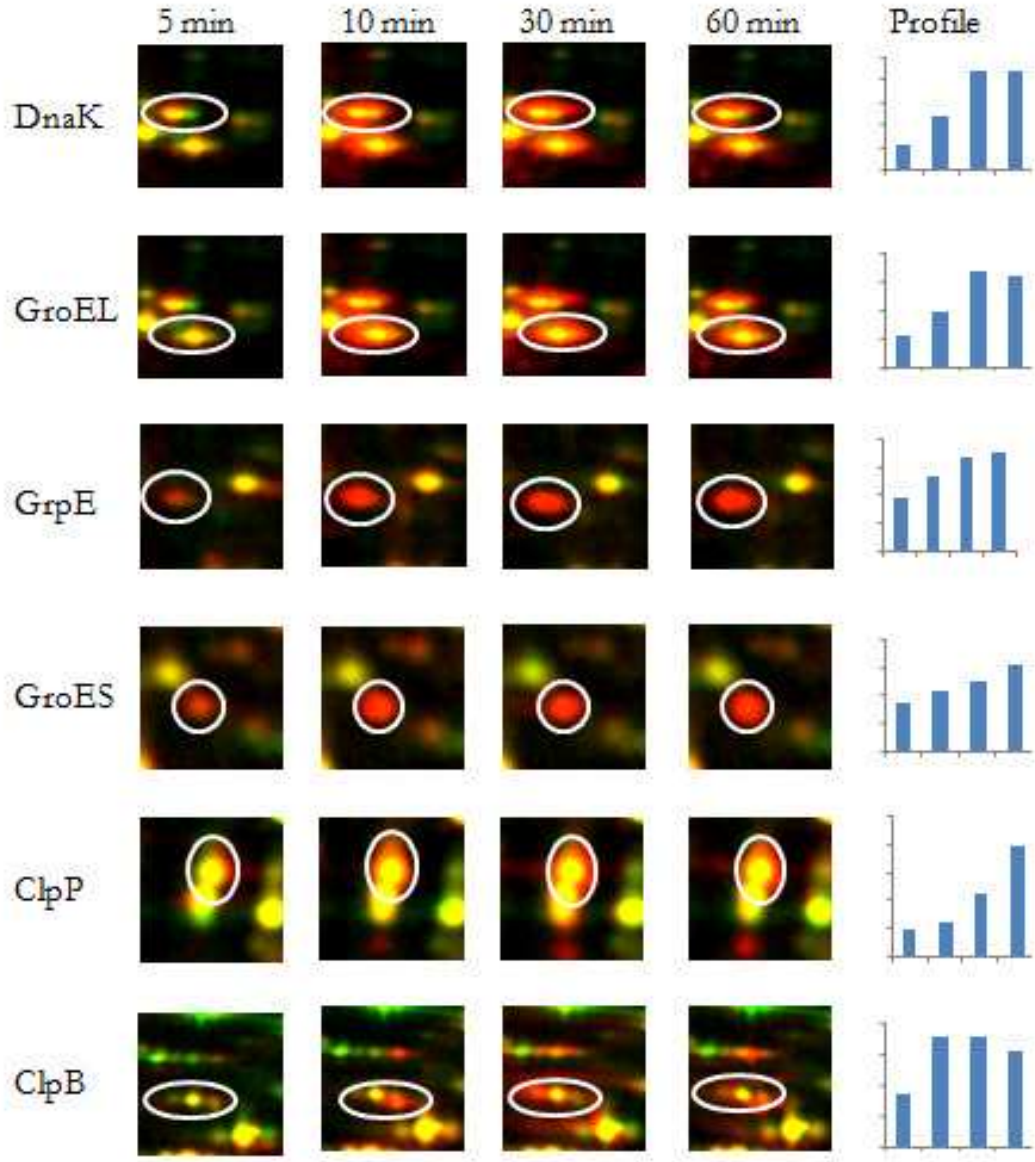

Figure 3.10: Details of dual channel images of selected marker proteins induced in response to puromycin stress. Protein synthesis pattern of unstressed *S. aureus* cells was compared with protein synthesis patterns at different time points after adding puromycin . The bar graphs on the right displayed relative synthesis rates of the individual proteins at the different time points .

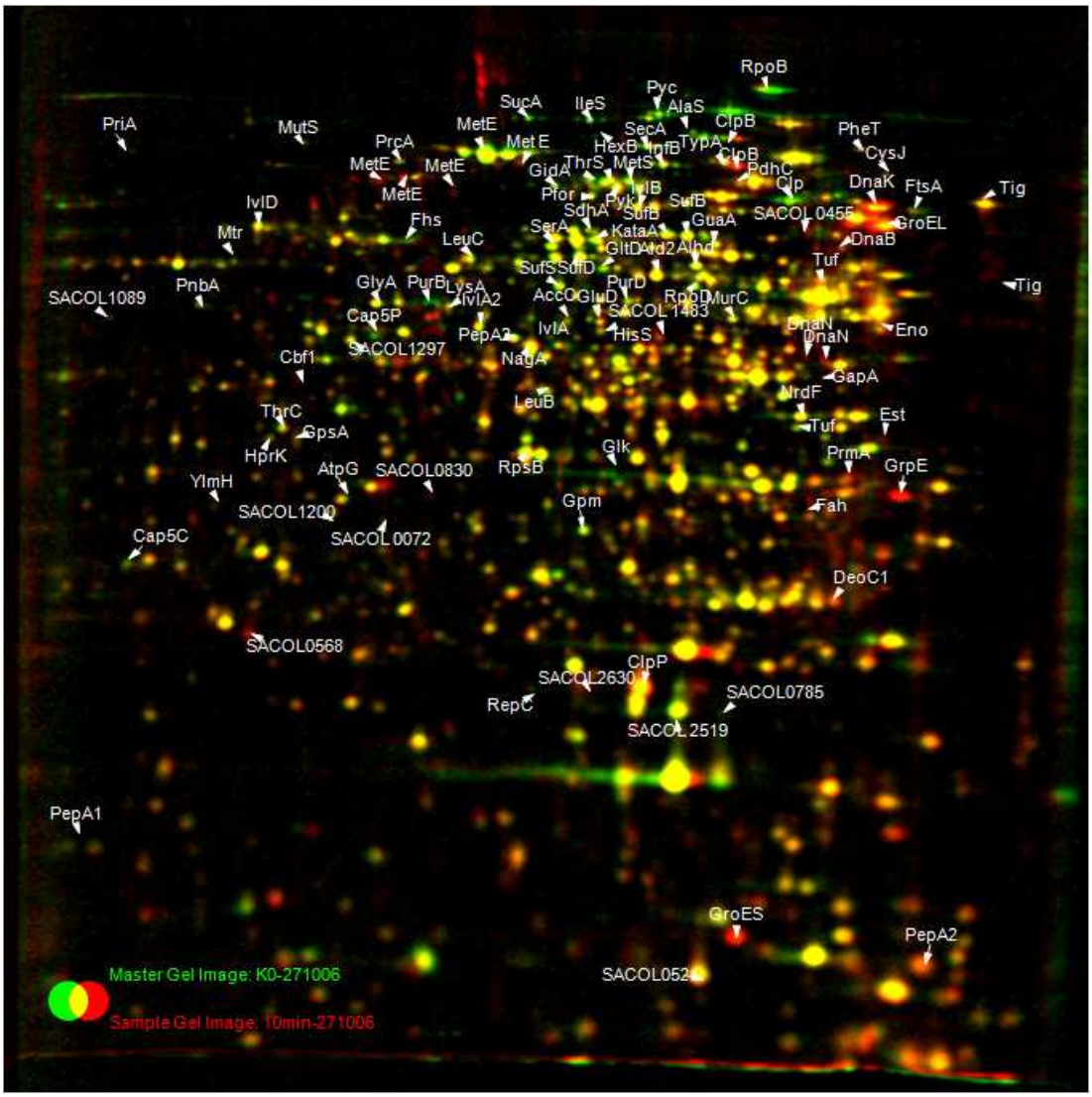

Figure 3.11: Dual-channel images of the protein synthesis patterns of *S.aureus* COL during exponential growth (green image) and 10 minutes after puromycin stress (16μg/ml)(red image).

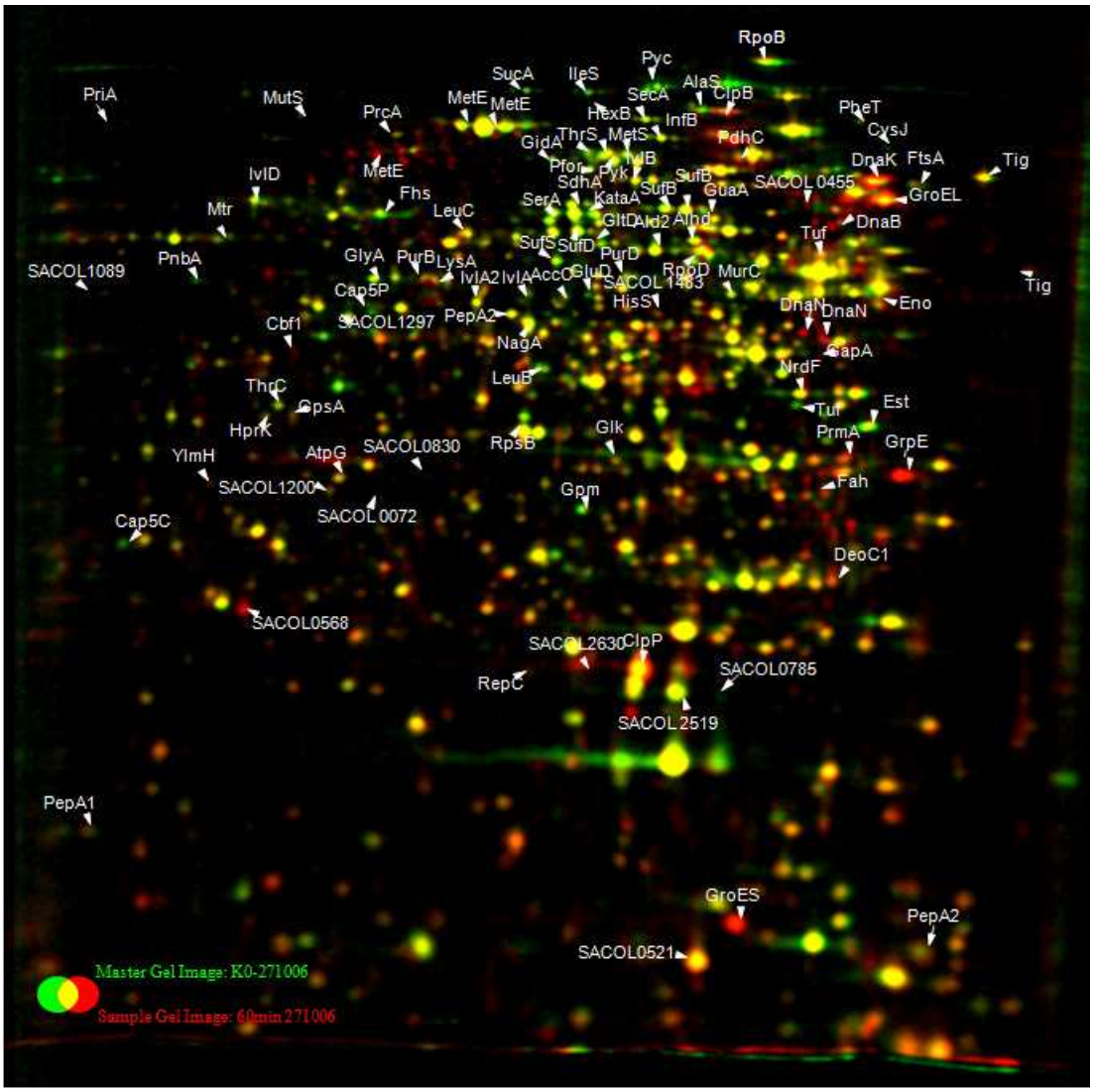

Figure 3.12: Dual-channel images of the protein synthesis patterns of *S.aureus* COL during exponential growth (green image) and 60 minutes after puromycin stress (16μg/ml)(red image).

It could be speculated that both heat and puromycin stress cause an accumulation of denatured proteins within the cytoplasm which is indicated by an increased synthesis of protease and chaperones such as the HrcA-and CtsR-regulated proteins such as DnaK, GrpE, GroEL, GroES, ClpP, ClpB by both heat and puromycin stress.

The results of the transcriptome and proteome analyses after puromycin stress revealed a large overlap with the results obtained by heat stress. There are 11 out of 20 puromycin induced proteins which are indicative for a heat-shock signature.

The repression profile of proteins after puromycin stress also has a similarity with heat stress. After puromycin stress, 14 proteins whose expression was decreased was also detected to be repressed by heat stress.

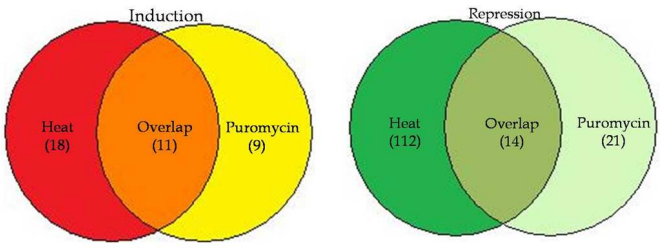

Figure 3.13: Overview of the number of commonly shared (generally induced/repressed) and unique (specifically induced/repressed) proteins in the proteome of *S.aureus* after heat and puromycin stress.

The proteome analysis also showed the induction of proteins involved in glycolysis such as pyruvate dehydrogenase PdhC and enolase Eno, with the highest induction after 60 minutes exposed to puromycine stress. Besides, some enzymes belonging to the amino acid synthesis pathways such as threonine synthase ThrC and chorismate synthase AroC were also induced by puromycin stress. In addition, some proteins required for the protein synthesis, for example glycyl-tRNA synthetase GlyS or translation elongation factor Tuf were up-regulated after adding puromycin to the medium. The proteome experiments also suggested the induction of many genes whose functions that might contribute to puromycin resistance are still unknown. These are the D-isomer specific 2-hydroxyacid dehydrogenase family protein SACOL0932 or the uridylate kinase PyrH of the nu-

cleotide and nucleoside interconversions pathway.

As shown in Table 3.8, the protein synthesis of four enzymes taking part in glycolysis was down regulated- PfkA, Pgm, Pyc as well as Pyk and two enzymes of the TCA cycle SucA and Suc were also repressed after puromycin stress. Data shown in table 3.8 also demonstrated that many enzymes involve in purine, pyrimidine synthesis were repressed by puromycin. The synthesis of GuaA, NrdE, PurD and PurH was switched off when puromycin was added to the medium. The decrease of some other metabolic proteins might be linked to the slower growth rate in response to puromycin stress.

In addition, other proteins involve in growth-phase-related functions such as fatty acid and phospholipid metabolism; amino acid metabolism; biosynthesis of cofactors, prosthetic groups, and carriers, cell envelope as well as cell division were among the repressed proteins.

3.2.4 Detailed transcriptional analyses of some genes under puromycin stress condition

The regulatory effect of puromycin on the transcription of selected genes was subsequently confirmed by Northern blotting experiments to complement the data obtained by DNA microarrays analyses.

We selected the transcriptional regulator encoding gene *codY* which was already analyzed after heat stress, in order to examine what happened in the case of puromycin stress. Northern blot analyses using *clpQ, clpX,* and *xerC* probes were performed as well since these genes were demonstrated to be transcribed together as a four-cistronic operon (Frees *et al.*, 2005). We were interested if there is also an induction of those genes under puromycin stress condition.

But unfortunately after stress exposure, no detectable induction could be observed. The transcripts were present in mRNA extracted from normal grown cells as the same level as in mRNA extracted from cells grown under stress condition.

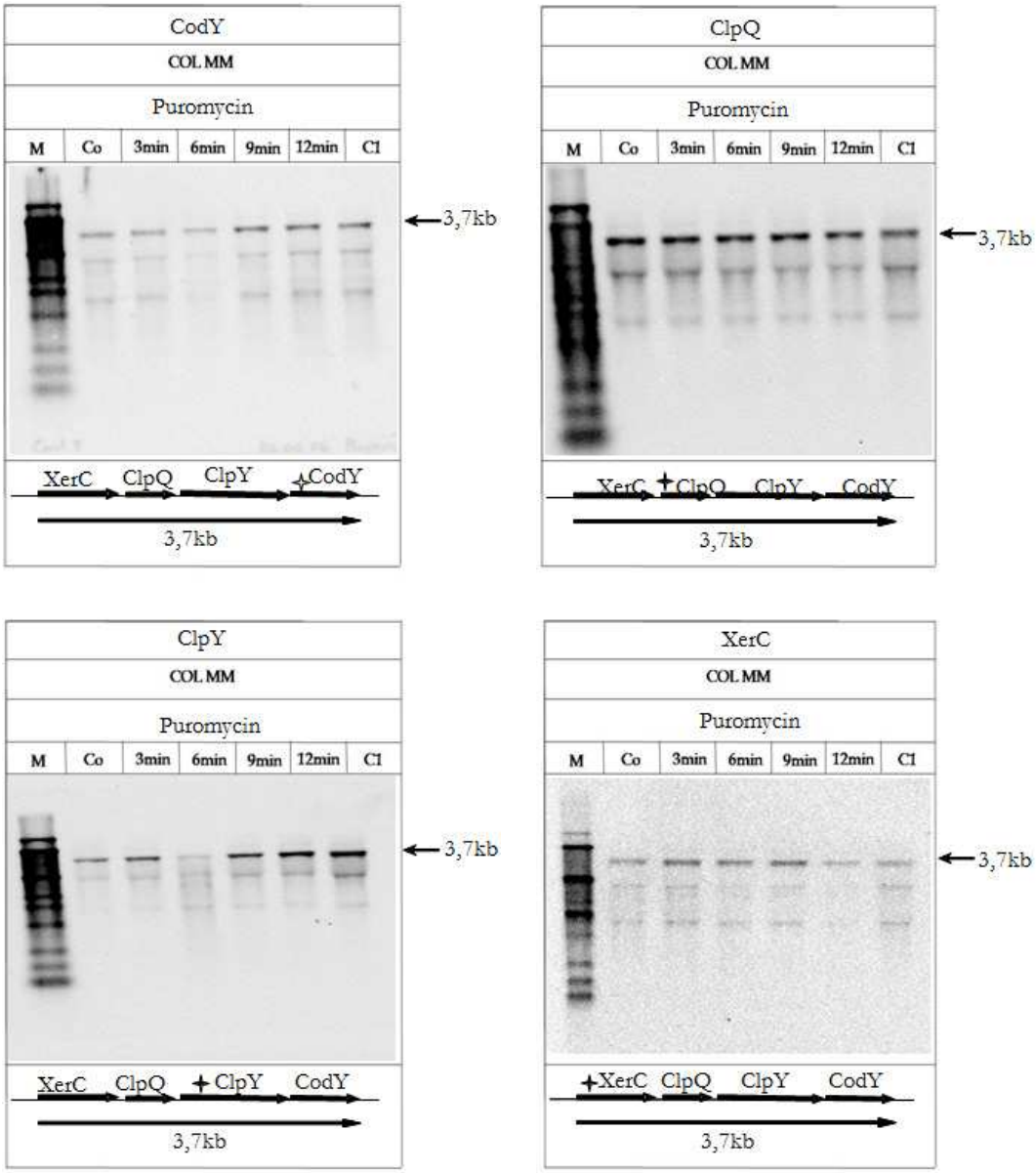

Figure 3.14: Northern blot analyses of *codY, clpY, clpQ, xerC* under puromycin stress conditions. For RNA preparation, cells were grown in synthetic medium to an OD_{500} of 0.5 and were treated with puromycin. RNA was isolated before (Co) and 3, 6, 9, 12 min after adding puromycin. To ensure that the mRNA levels of the respective genes remained unchanged during the course of the experiment in an aerobically grown culture, RNA was also prepared from a non treated culture 12 min after the stress (C1). Relevant probes which had been used in each Northern blot experiment were indicated by star. Relevant transcripts were indicated by arrows .

3.3 Salt stress response

3.3.1 Bacterial growth

Firstly, the ability of *S.aureus* to grow in the presence of salt was tested. *S.aureus* was grown in synthetic medium to an OD_{500} of 0.5 and then different amount of NaCl were added to the medium to determine the appropriate concentration of salt for stress experiment. Growth experiments were carried out with synthetic medium and synthetic medium supplemented with 2%, 4%, 6%, and 8% w/v NaCl.

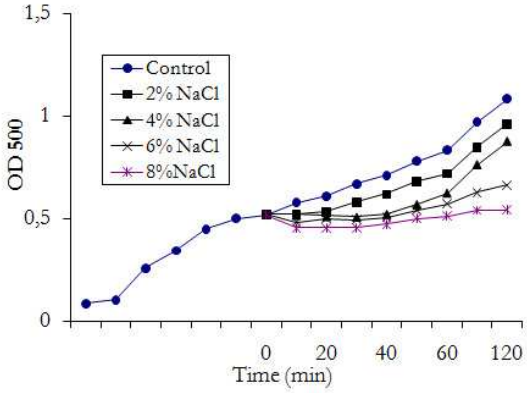

Figure 3.15: Growth curves of *S.aureus* in the presence of NaCl S. aureus COL was grown in synthetic medium to an $OD_{500}$of 0.5 and exposed to different concentrations of NaCl (2, 4, 6, and 8%(w/v) NaCl).

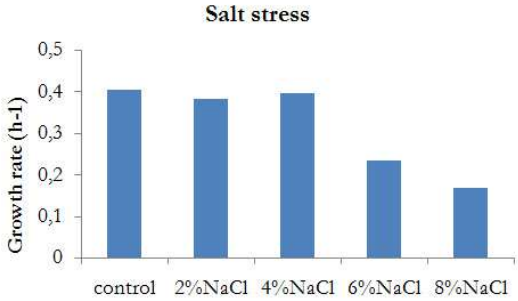

Figure 3.16: Growth rate of *S. aureus* COL during NaCl stress.

The growth curves and growth rate (Fig.3.15 and 3.16) indicated that, growth rate of cells after adding 2%, 4% and 6% NaCl to synthetic medium was slightly reduced during stress. In this growth experiments, we determined that a reduction in the growth rate of approximately 50% required the addition of 6% w/v NaCl to the growth medium. To obtain a less severe stress to the cell but strong enough to enhance the protein synthesis, 6% w/v salts were considered as the appropriate concentrations in the present study.

3.3.2 Protein synthesis profile

Our study focused on the cytosolic proteins in a pI range of 4 to 7. Protein expression was determined using protein extract of cells grown in the absence of salt under standard conditions and was compared with the synthesis pattern of cells grown in the presence of 6 % NaCl. In this study, we identified only protein spots which were considered to be up- or down-regulated by a factor of greater than two in the presence of salts. Figure 3.17, 3.18 and 3.19 shows 2D gels of protein extracts of *S.aureus* grown either in the absence or the presence of 6% salt. Comparison of synthesis patterns in the presence and absence of NaCl revealed that 101 spots displayed different synthesis rates. Among these proteins, 35 proteins were identified to be significantly up-regulated after salts stress and 76 of them to be significantly down regulated at under salt stress conditions and could be identified on 2D gel.

3.3.2.1 The induction of proteins of some metabolic pathways after addition of NaCl

Figure 3.17 showed the changes in synthesis pattern of enzymes involved in the purine/pyrimidine metabolism. The regulatory protein PyrR was down regulated during the first 10 minutes after addition of NaCl. Afterwards the protein synthesis was slightly increased.

Whereas dihydroorotase PyrC were induced immediately after salt stress at and 2.9 fold. The protein synthesis rate was then decreased after 30 minutes stress.

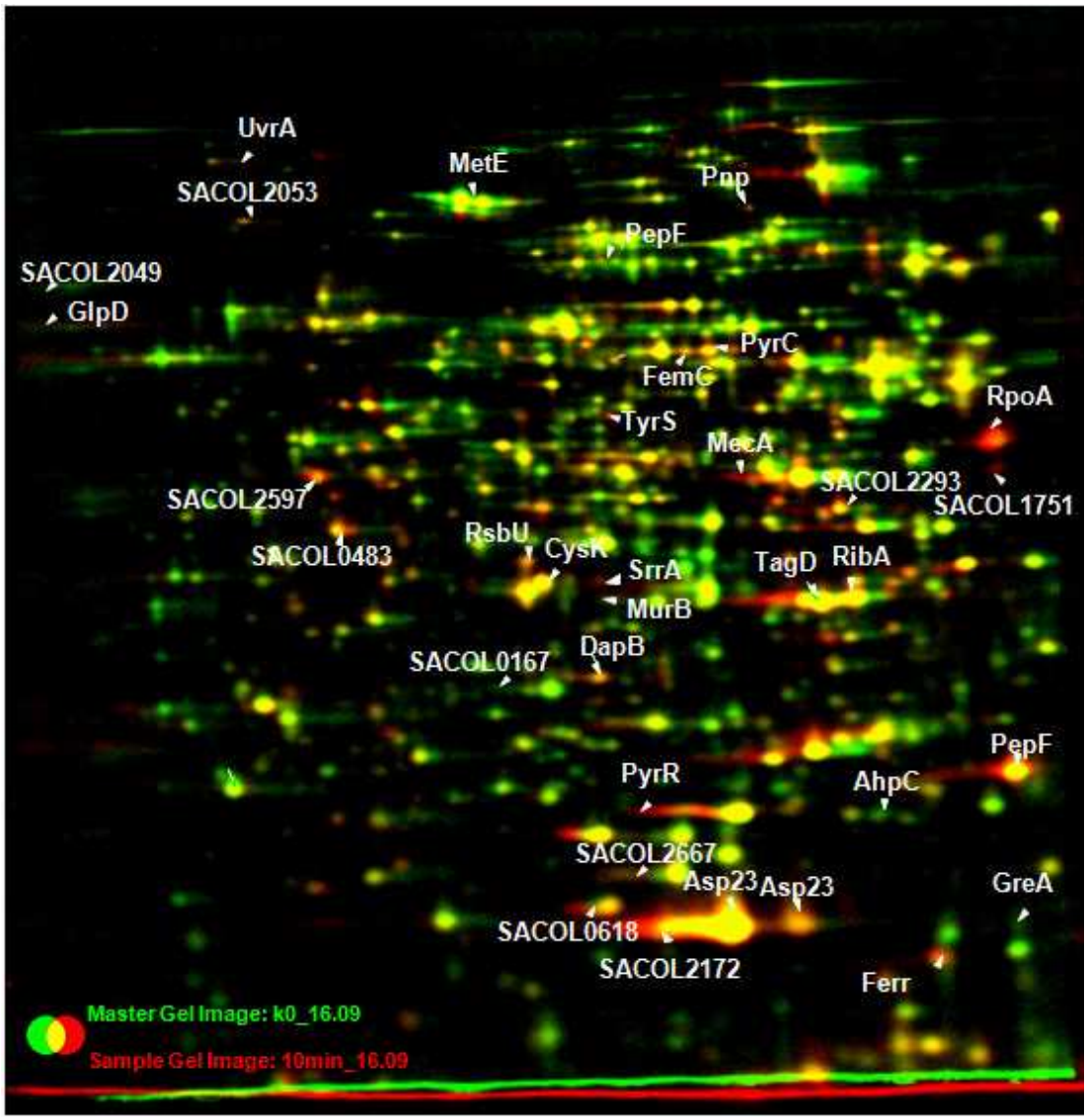

Figure 3.17: The dual-channel image of the protein synthesis pattern of *S.aureus* COL before (green image) and 10 min after adding 6% w/v NaCl (red image). Cytoplasmic proteins were labeled with L-^{35}[S]methionine and separated by 2D PAGE. Image analysis of the autoradiograms was performed using the Decodon Delta 2D software. Proteins that are synthesized at increased levels (induction rate of at least 2 fold) in response to NaCl stress are indicated by white labels.

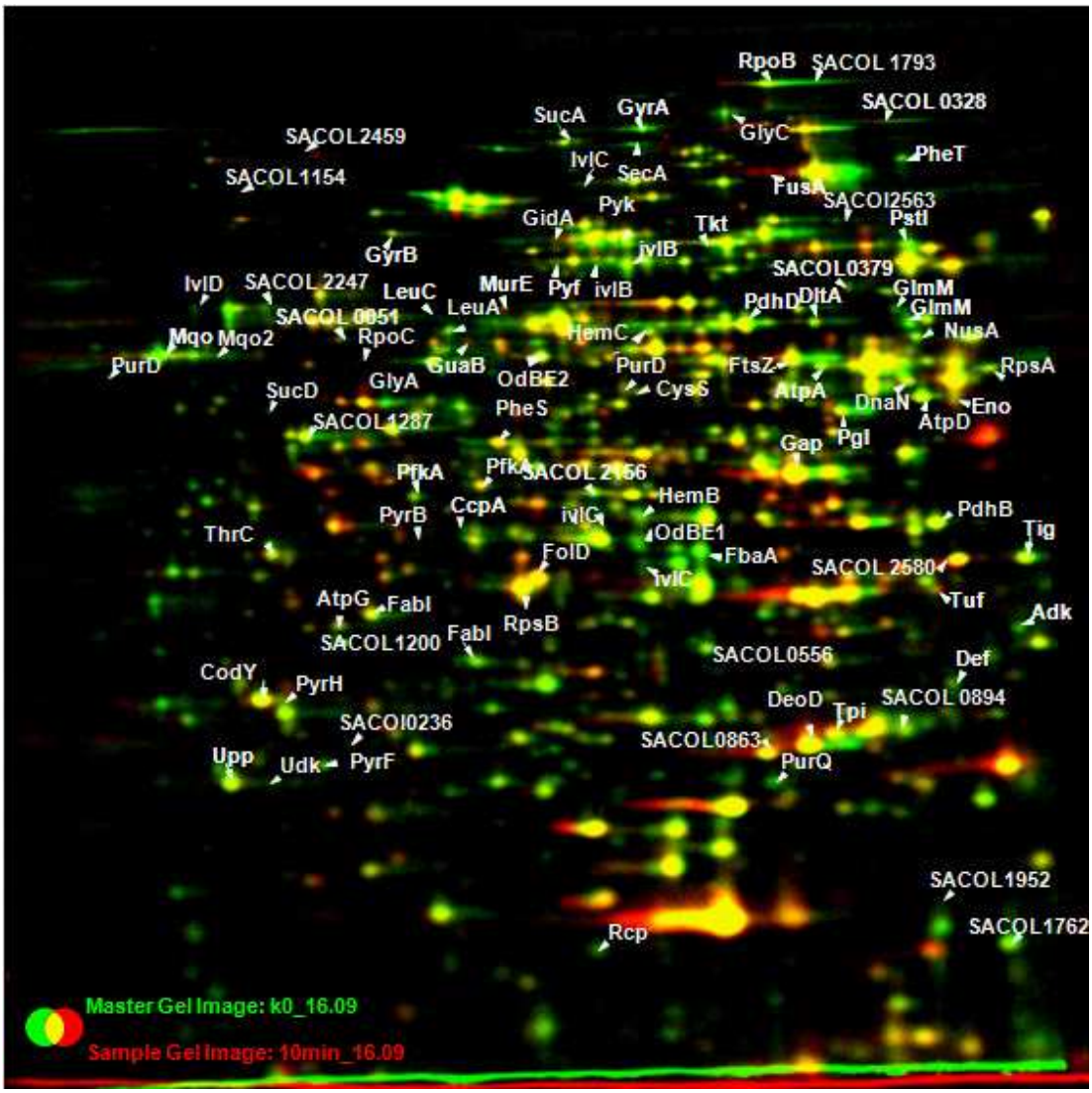

Figure 3.18: The dual-channel image of the protein synthesis pattern of *S. aureus* COL before (green image) and 10 min after adding 6% w/v NaCl (red image).Proteins that are synthesized at decreased levels (synthesis rate of ≤ 0.5) in response to NaCl stress are indicated by white labels.

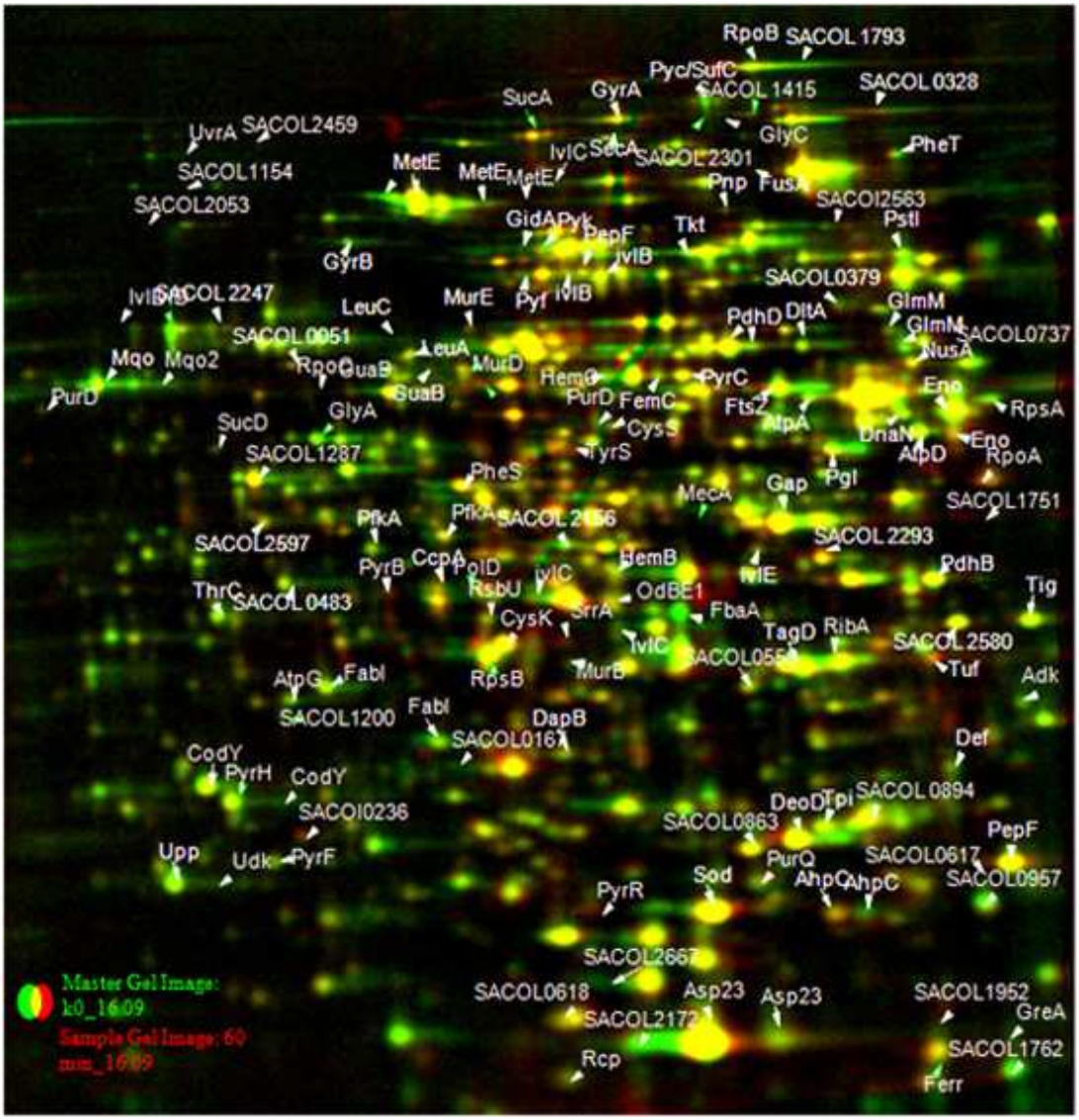

Figure 3.19: The dual-channel image of the protein synthesis pattern of *S. aureus* COL before (green image) and 60 min after adding 6% w/v NaCl (red image). Cytoplasmic proteins were labeled with L-^{35}[S]methionine and separated by 2D PAGE Image analysis of the autoradiograms was performed using the Decodon Delta 2D software.

The synthesis of some enzymes belonging to amino acid synthesis pathways was elevated after stress. For example enzymes of the metabolism of glutamate, an amino acid which was probably required in higher concentration after salt stress (Höper*et al.*,2005) were slightly induced. The synthesis rate of glutamine synthetase FemC and acetylglutamate kinase SACOL0167 was also increased after 30 minutes and 60 minutes salt stress at 2.2 and 2.1 fold, respectively.

In case of other amino acid metabolism pathways, some enzymes which belong to these pathways were also induced. Cysteine synthase CysK catalyzing the synthesis of cysteine was immediately induced after 5 and 10 minutes stress at 2.9 and 2.6 fold but after 30 and 60 minutes the induction rate reduced and reached the original level. Dihydrodipicolinate reductase DapB which belong to the aspartate metabolism also followed the same pattern. The induction peaked at 5 and 10 minutes after stress and then decreased at 30 and 60 minutes.

As for the proteins involved in the energy metabolism, SACOL0617, a putative hexulose-6-phosphate synthase was strongly induced right after salt stress. However, the synthesis rate of SACOL0617 was declined at 30 minutes and reached the normal level at 60 minutes.

Another enzyme of the energy metabolism pathway, the aerobic glycerol-3-phosphate dehydrogenase GlpD showed a temporal induction at 30 minutes after stress. It might be considered that GlpD was enhancedly synthesized to serve *S.aureus* growth on glycerol.

Table 3.9: Proteins whose synthesis was induced under salt stress condition

COL Locus	**ID**	**Function**	**Ratio**			
Amino acid biosynthesis						
			5min	**10min**	**30min**	**60min**
SACOL0167	SACOL0167	acetylglutamate kinase	0.09	0.42	1.81	2.03
SACOL0428	MetE	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase	3.38	1.19	2.86	1.04
SACOL0557	CysK	cysteine synthase	2.96	2.64	1.47	1.42
SACOL2049	SACOL2049	3-isopropylmalate dehydratase, small subunit	1.55	1.32	4.59	0.56
SACOL1329	FemC	glutamine synthetase FemC	1.64	2.23	1.65	1.53
SACOL1431	DapB	dihydrodipicolinate reductase	2.75	2.54	1.28	1.00
Energy metabolism						
SACOL1321	GlpD	aerobic glycerol-3-phosphate dehydrogenase	1.61	1.88	3.51	1.04
SACOL0617	SACOL0617	hexulose-6-phosphate synthase, putative	3.75	2.81	1.59	0.80
Cell envelope						
SACOL0698	TagD	glycerol-3-phosphate cytidylyltransferase	2.38	1.71	1.15	0.99
SACOL0801	MurB	UDP-N-acetylenolpyruvoylglucosamine reductase	0.52	0.90	2.45	1.23
Regulatory function						
SACOL1210	PyrR	pyrimidine operon regulatory protein	0.62	1.69	2.53	1.64
SACOL1535	SrrA	DNA-binding response regulator SrrA	1.70	1.72	2.17	2.68

SACOL2057	RsbU	sigma factor B regulator protein	2.00	2.01	0.94	1.01
Transcription						
SACOL1665	GreA	Transcription elongation factor	0.89	0.48	2.13	1.24
SACOL1293	Pnp	polyribonucleotide nucleotidyltransferase	3.04	2.19	1.19	1.36
SACOL2213	RpoA	DNA-directed RNA polymerase alpha subunit	6.01	6.27	2.43	2.59
Others						
SACOL0452	AhpC	alkyl hydroperoxide reductase	1.16	0.84	2.08	2.40
SACOL1984	Asp23	alkaline shock protein 23	3.25	2.34	1.53	1.36
SA2457	Ferr	ferritins family protein	0.55	0.48	2.18	2.60
SACOL1213	PyrC	dihydroorotase	2.90	2.43	1.08	1.06
SA1587	RibA	riboflavin biosynthesis protein	2.08	2.13	1.66	1.05
SACOL0033	MecA	penicillin-binding protein 2	0.10	1.04	2.01	1.72
SACOL1005	PepF	oligoendopeptidase F	3.69	3.07	3.14	2.37
SACOL1952	SACOL1952	ferritins family protein	1.18	0.75	2.84	2.49
SACOL2172	SACOL2172	IS1272-related, transposase, degenerate	0.93	2.88	1.86	1.07
SACOL0778	TyrS	tyrosyl-tRNA synthetase	2.34	2.42	1.17	1.44
SACOL0824	UvrA	excinuclease ABC, A subunit	0.88	1.07	2.38	0.65
SACOL0618	SACOL0618	SIS domain protein	2.08	1.85	1.48	1.52
SACOL1751	SACOL1751	DHH subfamily 1 protein	4.72	3.63	3.42	3.10
SACOL2053	SACOL2053	S1 RNA binding domain protein	1.27	1.67	3.30	1.07
SACOL2293	SACOL2293	NAD/NADP octopine/nopaline dehydrogenase family protein	2.56	2.08	1.87	1.75
SACOL2459	PnbA	para-nitrobenzyl esterase	3.44	2.25	4.35	1.17
SACOL2597	SACOL2597	hydrolase, alpha/beta hydrolase fold family	2.06	2.32	0.97	1.18
SACOL2667	SACOL2667	isochorismatase family protein	0.03	1.36	2.25	0.68

3.3.2.2 The induction of σ^B- dependent general stress protein

σ^B which has been shown to be important for survival under extreme conditions in *B. subtilis* has also been identified in *S. aureus* (Chan *et al.*, 1998, Kullik *et al.*, 1998, Nicolas *et al.*, 1999.). In response to salt tress, some σ^B-dependent proteins were detected to be induced. For instance, the alkaline shock protein Asp23 which was identified as a σ^B-dependent protein (Gertz *et al.*,1999) was up regulated during the first 5 and 10 minutes after stress at 3.2 and 2.3 fold, respectively (Table 3.9). The 2D gel quantitation data also showed the induction of some other proteins which were classified to be σ^B dependent (Pané-Farré *et al.*, 2005) such as the putative hexulose-6-phosphate synthase SACOL0617 or the SIS domain protein SACOL0618. In the case of NAD/NADP octopine/nopaline dehydrogenase SACOL2293 and hydrolase SACOL2597, their synthesis was increased, but it was an undurable induction that only took place in the first 10 minutes after stress. As for para-nitrobenzyl esterase PnbA, the protein synthesis induction was virtually stable during 30 minutes of stress experiment.

3.3.2.3 The synthesis of many proteins is down regulated by salt stress

The presence of 6% NaCl in the growth medium not only induced but also repressed the the synthesis of proteins. In this experiment, we could identified 76 proteins whose synthesis was decreased after NaCl stress.

Table 3.10: Proteins whose synthesis was repressed under salt stress condition

COL Locus	ID	Function	Ratio			
Amino acid biosynthesis						
			5min	**10min**	**30min**	**60min**
SACOL2046	LeuA	2-isopropylmalate synthase, Pyruvate family	0.45	0.50	0.86	1.09
SACOL2048	LeuC	3-isopropylmalate dehydratase, Pyruvate family	0.31	0.36	1.58	0.15
SACOL1148	PheS	phenylalanyl-tRNA synthetase, alpha subunit	0.30	0.48	0.68	0.91
SACOL1149	PheT	phenylalanyl-tRNA synthetase, beta subunit	0.40	0.30	0.42	0.47
SACOL1123	Pyc	pyruvate carboxylase, catalyzes the ATP dependent carboxylation of pyruvat to form oxaloacetat (aspartate synthesis)	0.88	0.45	0.62	0.57
SACOL2043	ivlB	valine, leucine and isoleucine biosynthesis	0,48	0.64	0.63	0.93
SACOL2045	ivlC	acetolactate synthase, large subunit, biosynthetic type , Valine, leucine and isoleucine biosynthesis	0.26	0.30	0.50	0.81
SACOL2042	ivlD	ketol-acid reductoisomerase, Valine, leucine and isoleucine biosynthesis	0.42	0.51	0.97	0.80
SACOL0167	SACOL0167	acetylglutamate kinase	0.09	0.42	1.81	2.03
Cell envelope						
SAO126	GlyC	glycocardiolipin , Cell envelope: Biosynthesis and degradation of surface polysaccharides and lipopolysaccharides(S.a MU50)	0.48	1.94	1.28	0.88
SACOL1762	SACOL1762	t hiol peroxidase, putative	0.53	0.40	1.82	1.11
SACOL1199	FtsZ	cell division protein, Cell division	0.58	0.46	0.64	0.64
SACOL0935	DltA	D-alanine-activating enzyme/D-alanine-D-alanyl carrier protein ligase, Cellular processes Toxin production and resistance	0.39	0.42	0.34	0.47
Cofactor biosynthesis						
SACOl0236	SACOL0236	diphosphocytidyl-2C-methyl-D-erythritol synthase, putative	0.48	0.45	1.18	1.63
SACOL1715	HemB	delta-aminolevulinic acid dehydratase, porphyrin biosynthesis	0.47	0.64	0.66	1.20
SACOL1717	HemC	Biosynthesis of cofactors, prosthetic groups, and carrier	0.45	0.95	0.93	0.99
DNA metabolism, DNA replication, recombination, and repair						
SACOL2737	GidA	glucose inhibited division protein A	0.20	0.49	0.82	0.85
SACOL0002	DnaN	DNA polymerase III, beta subunit, DNA replication	0.45	0.61	0.81	0.72
SACOL0006	GyrA	DNA gyrase, A subunit , Tpe II of Topiisomerase	0.35	0.41	0.55	0.63
SACOL0005	GyrB	DNA gyrase, B subunit, DNA replication	0.53	0.58	0.56	0.49
SACOL1154	SACOL1154	DNA mismatch repair MutS2 family protein	0.48	0.64	1.57	0.46
Energy metabolism						
SACOL2097	AtpA	ATP synthase F1, alpha subunit, ATP synthase	0.37	0.58	1.13	0.48
SACOL2095	AtpD	ATP synthase F1, beta subunit, ATP synthase	0.47	0.38	0.60	0.59
SACOL2096	AtpG	ATP synthase F1, gamma subunit, ATP synthase	0.26	0.50	0.54	0.68
SACOL0842	Eno	enolase, Glycolysis	0.65	0.55	0.49	0.96
SACOL1016	FabI	fructose-bisphosphate aldolase, class II , Glycolysis	0.46	0.58	0.64	0.81
SACOL2117	FbaA	fructose-bisphosphate aldolase, class II , Glycolysis	0.92	0.56	0.56	0.47
SA2457	Ferr	ferritins family protein , Iron storage protein, possible glutamate synthase(S.a Mu50)	0.55	0.48	2.18	2.60

SACOL2151	GlmM	phosphoglucosamine mutase, Aminosugars metabolism	0.30	0.31	0.46	0.43
SACOL2623	Mqo2	malate:quinone oxidoreductase, TCA	0.53	0.57	0.43	1.15
SACOL1103	PdhB	pyruvate dehydrogenase complex E1 component, beta subunit , Transform pyruvat in to acetyl coA	0.56	0.37	0.69	0.88
SACOL1105	PdhD	pyruvate dehydrogenase complex E3 component, lipoamide dehydrogenase, Glycolysis	1.00	0.68	0.47	0.83
SACOL1149	PfkA	6-phosphofructokinase , Glycolysis and gluconeogenesis	0.81	0.98	0.46	0.64
SACOL1149	PfkA	6-phosphofructokinase , Glycolysis and gluconeogenesis	0.58	0.66	0.39	0.77
SACOL0966	PgI	Pentose phosphate pathway	0,52	0.44	0.51	0.57
SACOL1902	PstI	phosphoenolpyruvate-protein phosphotransferase , pyruvat metabolism	0.39	0.47	0.65	0.56
SACOL1745	Pyk	pyruvate kinase, Convert phosphoenol pyruvate to pyruvate in the glycolysis	0.48	0.85	1.38	0.99
SACOL1263	SucD	succinyl-CoA synthase, alpha subunit, TCA	0.37	0.59	1.02	1.01
SACOL1666	Udk	uridine kinase, Purines, pyrimidines, nucleosides, and nucleotides: Salvage of nucleosides and nucleotides	0.46	0.58	1.10	1.02
SACOL2301	SACOL2301	formate dehydrogenase, alpha subunit, putative	0.45	0.83	1.16	0.64
Protein fate: Degradation of proteins, peptides, and glycopeptides						
SACOL1005	PepF	oligoendopeptidase F	0.38	0.56	0.62	0.99
SACOl2563	SACOL2563	ATP-dependent Clp protease, putative	0.25	0.32	0.72	0.50
SACOL0816	SecA	preprotein translocase, SecA subunit , Translocase	0.52	0.26	0.84	0.42
SACOL0556	SACOL0556	chaperonin,	0.23	0.80	1.11	1.08
SACOL0957	SACOL0957	peptidyl-prolyl cis-trans isomerase, cyclophilin-type	0.73	0.46	0.79	0.77
SACOL1722	Tig	trigger factor, stringent control	0.60	0.38	1.15	1.30
Protein synthesis: Ribosomal proteins: synthesis and modification						
SACOL1277	RpsA	ribosomal protein S1	0.72	0.38	0.66	0.82
SACOL1287	SACOL1287	30S ribosomal protein L7 Ae	0.41	0.61	0.79	1.24
SACOL0593	FusA	translation elongation factor G , Translation elongation factor	0.26	1.84	1.85	0.53
SACOL0594	Tuf	translation elongation factor Tu, Translation elongation factor	0.58	0.57	0.45	0.91
SACOL0576	CysS	cysteinyl-tRNA synthetase, Protein synthesis: tRNA aminoacylation	0.74	0.55	0.50	0.71
Purine/ Pyrimidin metabolism						
SACOL1083	PurD	phosphoribosylamine-glycine ligase, Purine/ Pyrimidin metabolism, Purine ribonucleotide biosynthesis	0.49	0.51	0.77	0.51
SACOL1077	PurQ	phosphoribosylformylglycinamidine synthase I. Purine/ Pyrimidin metabolism, Purine ribonucleotide biosynthesis	0.78	0.49	1.31	1.13
SACOL1212	PyrB	aspartate carbamoyltransferase, Purine/ Pyrimidin metabolism, Pyrimidine ribonucleotide biosynthesis	0.25	0.29	0.88	0.62
SACOL1216	PyrF	orotidine 5-phosphate decarboxylase, Purine/ Pyrimidin metabolism, Pyrimidine ribonucleotide biosynthesis	0.36	0.39	0.81	1.00
SACOL2218	Adk	adenylate kinase, Phosphotranferase that catalyse the reversible reaction convert ATP to ADP	0.47	0.40	0.89	0.81
SACOL1277	PyrH	uridylate kinase, Nucleotide and nucleoside interconversions	0.47	0.56	0.71	0.95
Regulatory functions: DNA interactions						
SACOL1786	CcpA	catabolite control protein A , Signal transduction pathway	0.47	0.62	0.81	1.06
SACOL1272	CodY	transcriptional regulator CodY, Regulatory functions: DNA interactions	0.47	0.33	0.81	1.01
Transcription: DNA-dependent RNA polymerase						
SACOL0588	RpoB	DNA-directed RNA polymerase, beta subunit	0.70	0.37	0.52	0.85

SACOL0589	RpoC	DNA-directed RNA polymerase, beta subunit	0.42	0.32	1.71	0.40
SACOL1665	GreA	transcription elonggation factor	0.89	0.48	2.13	1.24
SA1206	NusA	N utilization substance protein A, Transcription termination process (S.a Mu50)	0.46	0.53	0.85	1.10
Other						
SACOL1415	SACOL1415	Transport and binding proteins: Amino acids, peptides and amines	0.29	1.76	1.15	1.10
SACOL2667	SACOL2667	isochorismatase family protein	0.03	1.36	2.25	0.68
SACOL2156	SACOL2156	ATP-binding protein, Mrp/Nbp35 family	0.49	0.74	0.48	0.68
SACOL0379	SACOL0379	prophage L54a, tail tape measure protein, TP901 family	0.49	0.57	0.55	0.66
Hypothetical protein						
SACOL0051	SACOL0051	Hypothetical protein	0.36	0.84	0.48	1.27
SACOL0328	SACOL0328	peptide ABC transporter, ATP-binding protein	0.69	0.47	0.58	1.23
SACOL1793	SACOL1793	Hypothetical protein	0.53	0.31	0.51	0.49
SACOL2247	SACOL2247	Hypothetical protein	0.35	0.50	0.92	0.92
SACOL0737	SACOL0737	Hypothetical protein	0.46	0.42	0.49	0.65
SACOL1200	SACOL1200	Hypothetical protein	0.29	0.49	0.77	0.82

The synthesis of the enzymes that catalyse almost all steps of the glycolytic pathway or tricarboxylic cycle such as Eno, FbaA, PdhB, PdhD, PfkA, PgI, Pyc and Pyk was down regulated (Fig. 3.15,3.16, Table 3.10) indicating a strong repression of the gene in the non growing-cell in response to NaCl stress. However, as show in figure 3.20, those enzymes are present in green color, indicated that they are still present in the cell but their synthesis has been switched off. Figure 3.20 also showed that after NaCl stress, many proteins are strongly synthesized but not yet accumulated (present in red color).

The synthesis of several proteins of amino acid metabolism pathways was down regulated by salt stress. For examples, IvlC, IvlD, IvlS of valine, leucine and isoleucine biosynthesis pathways or PheT and PheS of phenylalanine, tyrosine and tryptophan biosynthesis pathways were repressed under NaCl stress condition.

Except some transient induction of several enzymes catalysing the purine, pyrimidine metabolism mentioned above, the synthesis of PurD and PurQ enzymes of the purine ribonucleotide biosynthesis as well as the synthesis of PyrD, PyrF, PyrH enzymes of the pyrimidine ribonucleotide biosynthesis were switched off in response to salt stress.

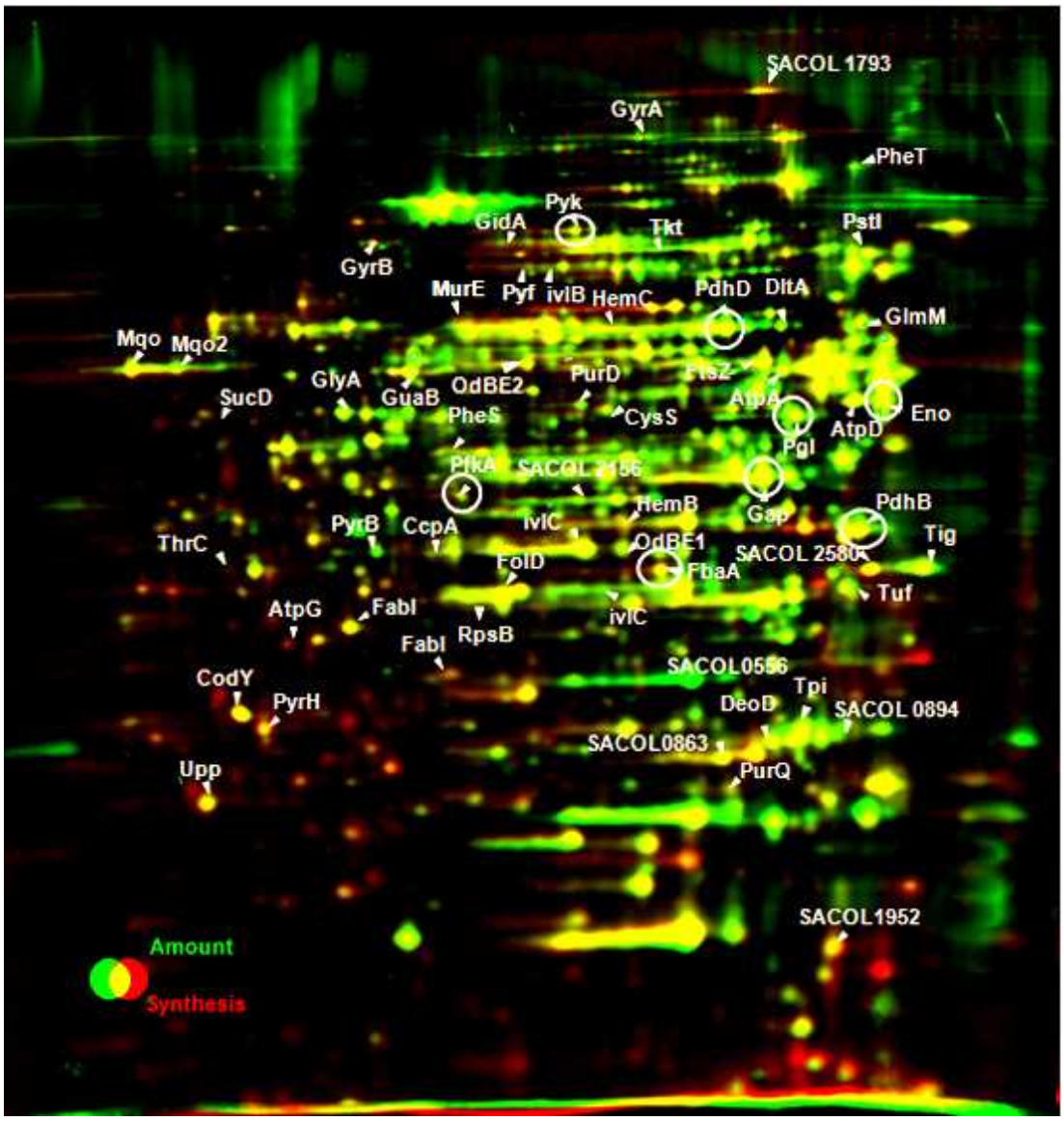

Figure 3.20: Overview of the intracellular proteome of *S.aureus* before and after challenge with 6% w/v NaCl. Figure displays false colored dual channel images of the accumulation of proteins as revealed by silver staining (colored green) and synthesis of proteins as revealed by radioactive labeling (colored red). Yellow spots are synthesized and accumulated at the same time, green spots are still present but no longer synthesized, red spots are only synthesized and not yet accumulated. Enzymes belong to TCA cycle and glycolysis were indicated by white circle.

3.3.3 Detailed transcriptional analyses of genes under NaCl stress

Northern blotting experiments for selected genes were performed to validate the data obtained from proteome analyses. RNA were isolated from cell grown at normal condition and 3, 6, 9 minutes after shift to high salt concentration conditions.

We selected especially gene encodes protein which present to be induced under high salt concentration conditions but for some reason could not be identified on 2D gels, for instance,*sodA* gene encodes superoxide dismutase.

Besides, we also selected *opuA, opuB, opuC* of the osmoprocetant transport system which play an essential role in salt adaptation in *B. subtilis* (Kappes *et al.*, 1999; Steil *et al.*, 2003) but were not covered in 2D gel analysis because of their nature as membrane proteins.

Northern Blots performed using a *sodA* specific probe revealed a slight induction of *sodA* transcript under salt stress. A transcript of 0.6 kb already present under control conditions was slightly increased after exposure to salt stress (3, 6, 9 minutes, figure 3.21). In the case of *opuA*, a large transcript of 3.4 kb and a small transcript of 1.6kb were detected (Fig.3.21).

The same two transcripts were detected using *opuB* and *opuC* probes indicating that *opuA, opuB* and *opuC* are co-transcribed.

Unfortunately, no significant induction of *opuA, B* or *C* could be demonstrated after salt stress. The level of transcripts remained almost unaltered in mRNA extracted from cell grown under control conditions as well as from cells after exposure to salt stress.

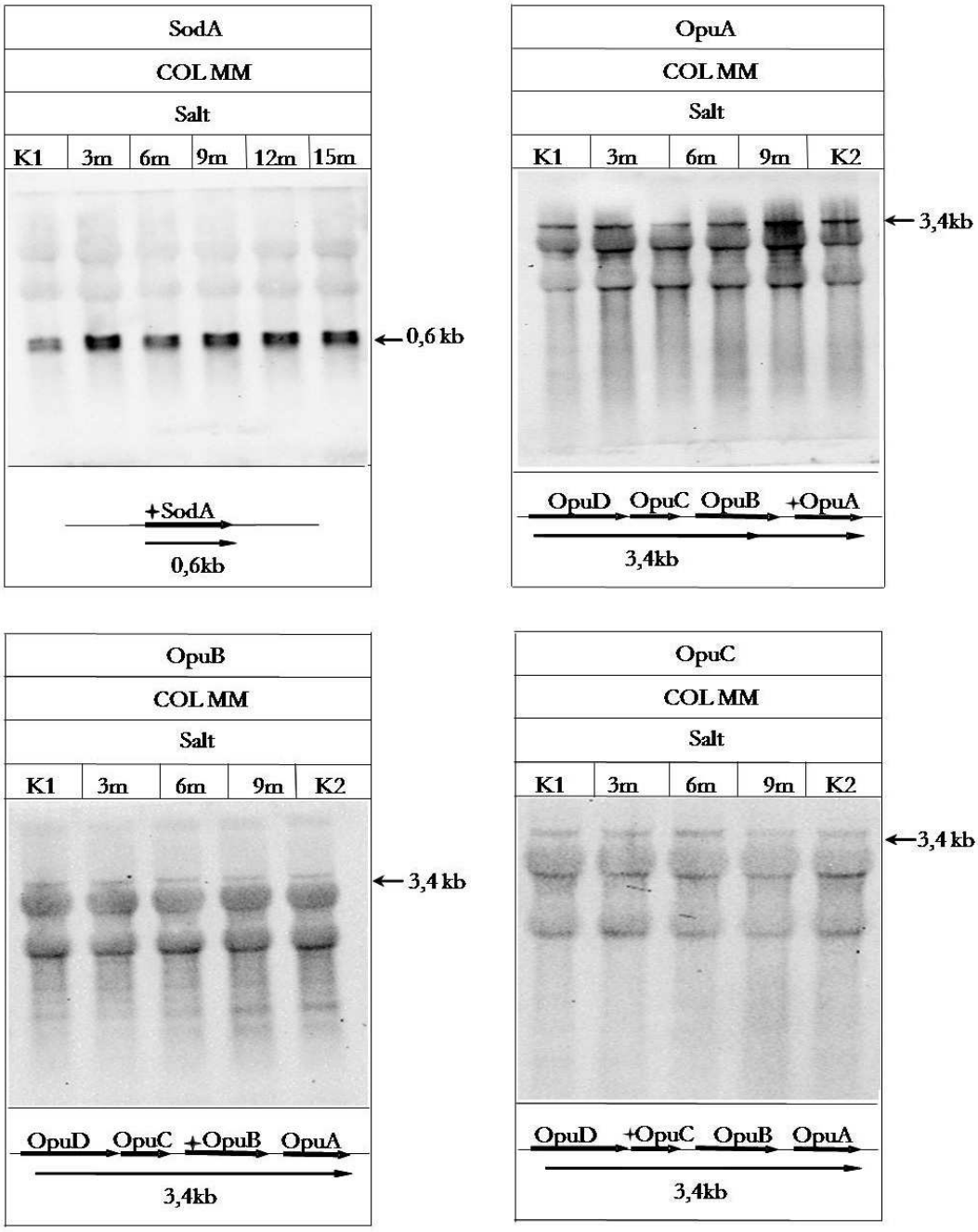

Figure 3.21: Transcript analysis of *sodA* and the *opuABC* operon in response to salt stress.Relevant probes which have been used in each Northern blot experiment were indicated by star. Relevant transcripts are indicated by arrows.

Chapter 4

Discussion

Studies on the mechanisms of the response of *S.aureus* to environmental stimuli have become one center of attention during the last few years because of the resistance to environmental stress of *S.aureus* .

4.1 Heat stress response

As a model organism for studying the heat shock response in Gram-positive bacteria, differences in the proteome of heat shocked *B.subtilis* cells and the mechanisms of heat shock response of this organism have been examined quite throughout (Schulz *et al.*, 1996; Bernhardt *et al.*, 1997; Movadehi *et al.*, 2000). In *B.subtilis*, at least four classes of heat shock genes encoding cytoplasmic proteins have been identified (Hecker *et al.*, 1996; Schumann *et al.*, 1996 ; Schumann, 2003).

In the case of pathogenic bacteria, heat-shock proteins might play an important role, as sera from infected patients contain antibodies to several heat-shock proteins and they are induced early in infection of human epithelial cells with *S.aureus* (Qoronfleh *et al.*,1998).

In this study, effects of heat stress on *S.aureus* cells was analyzed, using a gel-based proteomic approach and transcriptomic analyses with DNA microarrays .

Unlike the model organisms *B.subtilis*, little was known until recently about the regulation of stress response in *S.aureus*. Analysis of the complete genome sequence (Kuroda *et al.*, 2001) and several recent reports indicate the existence of the σ^B (Gertz *et al.*, 2000) and HrcA (Kuroda *et al.*, 1999) in *S.aureus*. Moreover, an orthologue of the CtsR stress regulator was defined as well as several potential target genes (Derr *et al.*, 1999). In *S.aureus*, there seems to be a regulatory overlap between class I and class III genes, with dual heat shock regulation by CtsR and HrcA.

In our work, the combination of proteomics with transcriptomics approach offers the opportunity to find genes/proteins of *S.aureus* whose transcription/synthesis was increased or decreased after stress such as heat stress. Two groups of interesting proteins/gene have been identified: Those whose synthesis/transcription was induced after heat stress and those whose synthesis/transcription was repressed at certain time points after heat stress.

In general, our result suggested that there is a high degree of similarity between the response of *S.aureus* and *B.subtilis* to heat stress. The obtained proteomic and transcriptomic analysis data indicated that under heat stress conditions, *S.aureus* cells showed the induction of three classes of heat inducible genes. Among them were genes which encode the chaperones DnaK, GroEL, GroES, and GrpE. In *B.subtilis*, *groEL* and *dnaK* are regulated by the HrcA repressor and transcribed during heat shock by the vegetative sigma factor σ^A (Schulz *et al.*, 1996; Yuan and Wong, 1995). In *S.aureus*, the expression of *dnaK* and *groEL/groES* is controlled by both HrcA and CtsR repressor, which act together synergistically to maintain low levels of expression in the absence of stress (Chastanet *et al.*, 2003). In this work, transcriptome and proteome analysis data showed that in *S.aureus*, the transcription of genes belong to *dnaK* and *groESL* operon as well as the synthesis of the chaperones DnaK, GroEL, GroES and GrpE were up regulated with an induction factor of at least three fold under stress condition. Moreover, the transcription profile showed the high induction of HrcA , one of the main regulators of the heat shock response, which could not be identified on the 2D gels. The transcription profile also showed that the gene encodes ribosomal protein L11 was up regulated by heat exposure. The ribosomal protein

L11 exhibits significant homology to the deduced amino acid sequence of *orf35* in *B.subtilis*, which is one part of the heptacistronic *dnaK* operon (Schumann, 2003).

The exposure to heat stress condition leads to the accumulation of misfolded proteins, resulting in an increased demand for not only the chaperones but also for the proteases which are typically induced under heat shock conditions. For example, among the heat induced proteins are also proteins belonging to the Clp machinery: the proteolytic component ClpP and the chaperone ClpB. Clp-proteases belong to class III heat shock protein that regulated by CtsR repressor which were showed to be highly induced by heat stress in *B. subtillis* (Kruger and Hecker, 1998; Derre *et al.*, 2000; Schumann *et al.*, 2002). In our work, ClpP and ClpB were also found to be induced by the temperature up shift by proteomics and transcriptomic analyses. Frees and colleagues demonstrated that the action of a ClpP proteolytic complex is essential for staphylococci to grow under heat-shock conditions (Frees *et al.*, 2004). Our Northern blot result figured out that the transcription of ClpB was highly induced, in support of this notion ClpB was recently shown to be required for growth at very high temperatures (Frees *et al.*, 2004). In the case of SACOL0568 and SACOL0569 which were suggested to be co-transcribed and formed a tetracistronic with *ctsR* and *clpC* in *S.aureus* (Wang *et al.*, 2004), trancriptomic analysis data also revealed a highly induction rate of both gene after heat stress at 11.46 and 54.07 fold, respectively.

Both transcriptome and proteome analysis revealed that the heat shock repressor protein CtsR was highly up-regulated after heat exposure. Frees and colleagues observed the same phenomenon and suggested that CtsR may need a cofactor for repressor function (Frees *et al.*, 2004; Anderson *et al.*, 2006). The proteomic and transcriptomic analysis data showed no induction of ClpC protease, which is also regulated by CtsR repressor and is observed to be required for *S.aureus* to grow at high temperature condition (Frees *et al.* 2004, Anderson *et al.*, 2006). This result is caused by the fact that *clpC* probe did not exist in the *S.aureus* COL arrays which were used in this study. As for the proteome analysis, ClpC protein might be present at very low levels and thus could not be surely identified on 2D-gels.

Interestingly, in this work, we have detected the induction of *ahpC-ahpF* in *S.aureus* after exposing to heat shock. In *B.subtilis*, these genes belong to a group of genes whose expression is also responsive to stress, but the mechanism of induction is not affected by any of the previously mentioned regulators (Schumann, 2003). Therefore, *ahpC-ahpF* are controlled by one or more unknown mechanisms.

In the present study, under heat shock condition, no induction of proteins which are controlled by sigma B factor (Gerzt *et al.*, 1999) could be detected. Sigma B was indicated to control a stress/ starvation regulon that comprises a very large set of general stress genes in *B.subtilis* (Hecker and Völker,1996). In *B.subtilis*, this σ^B dependent genes are strongly induced by heat, ethanol, acid or salt stress, as well as by starvation for a carbon source, phosphate, and oxygen (Bernhardt *et al.*, 1997, 1999; Hecker and Volker, 1998; Büttner *et al.*, 2001). In our study, *S.aureus* cells were inoculated in synthetic medium, however the sigma B activity was increased by heat stress only when the cells were grown in a complex medium (Kullik *et al.*, 1997; Gertz *et al.*, 2000). In synthetic medium, sigma B was highly active but could not be further activated by heat stress (Gertz *et al.*, 2000).

Heat stress treatment also resulted in a strong expression level of genes belonging to the SOS response such as *uvrA* and *urvB*, which were also induced by the antibiotic mitomycine (Anderson *et al.*, 2006). That indicated the relevance of heat stress response and SOS response .

We also observed number of proteins with high synthesis rate which are involved in the biosynthesis of purine/ pyrimidine implicating that the cell needed more nucleoside precursor for DNA metabolism.

According to the microarrays analysis data, among the heat-induced transcripts were several bacteriophage replication/ packaging genes such as intergrase SACOL 0318, replicative DNA helicase SACOL0343, N-6-adenine- methyltransferase SAC OL0346, deoxy-uridine 5'-triphosphate nucleotido-hydrolase SACOL0357, transcriptional regulator of the RinA family SACOL0364 as well as the small and large subunit of the terminase SACOL0366 and SACOL0367 (Table 3.1). Anderson and colleagues have shown that SOS induction also activates the expression of numerous bacteriophage genes in *S.aureus*. The concerted functions of these

factors may account, in part, for the observation that SOS induction results in phage-mediated pathogenicity island dissemination among staphylococci (Mazmanian *et al.*, 1999; Maiques *et al.*, 2006). In the current work, as heat shock cause DNA damage and consequently bring about an induction of DNA repair and DNA synthesis, heat shock might lead the phages into the lysis cycle but the mechanism of that process has not been well examined.

The transcriptomic data also showed the induction of genes encoding urease enzyme (*urea, B, C, D, E, G*), which were strongly upregulated in *S.aureus* biofilms (Beenken *et al.*, 2004). The thermo dependent protein denaturating properties of urea have been well documented (Bennion *et al.*, 2003)

Taken together, heat shock lead to an up-regulation of enzymes involved in DNA metabolism, protection, and repair. Consequently, it can be concluded that under heat shock conditions, the DNA damage occur in *S.aureus*. Another indication for this conclusion is the induction of phage genes. Under oxidative stress conditions, similar phenomena have been observed in *S.aureus*(Wolf *et al.*, 2008)

4.2 Puromycin stress

The ribosome is the target of many antibiotics, that interfere with translation via different molecular mechanisms of action. Aminoglycosides interfere with the ribosomal proofreading activity and cause an increase in mistranslation. The resulting accumulation of mistranslated and, therefore, misfolded proteins leads, just as the increase in growth temperature, to the induction of chaperons and proteases. Similarly, puromycin- a protein synthesis inhibitor that causes abortive translation and leads to the accumulation of truncated and misfolded proteins, thereby also induces the heat-shock signature (Brötz-Oesterhelt *et al.*, 2004). We compared the protein synthesis pattern as well as the transcription data of *S.aureus* in response to heat shock with that in response to puromycin stress.

In general, our results suggest that, there is an overlap of the induced /repressed proteins profiles under heat stress and puromycin stress in *S.aureus*. According to the proteome analyses, the protein synthesis level of GroEL/GroES increased

not only after heat shock but also after exposure to puromycin stress, similar induction pattern was also observed for DnaK and GrpE. In *B.subtilis*, it was also shown that puromycin stress stimulated heat shock protein production, the response was rapid, treatment with puromycin most probably produced nonnative proteins and thereby titrated the GroE chaperonin system, this in turn may lead to an accumulation of inactive HrcA repressor and induction of the CIRCE regulon (Hecker *et al.*,1996, Mogk *et al.*, 1997).

The microarray analysis also suggested the induction of the *groESL* operon as well as the *dnaK-dnaJ-grpE* operon. Under heat shock conditions, these chaperones were also highly induced, indicating that similar signalling pathways played their roles in adaptation of *S.aureus* to heat and puromycin stress. This data were in good agreement with the result gathered in *Streptococcus pneumonia* by Wai-Leung and colleagues which showed that *hrcA-grpE-dnaK-dnaJ*, and *groEL-groES* operons was highly induced by puromycin (Wai-Leung Ng *et al.*, 2002)

Similar to heat stress, after puromycin stress, the proteomic and transcriptomic results did not show any induction of genes which is belong to the σ^B-dependent stress response. Beside the fact that synthetic medium was used in this study, it has also been proposed that because puromycin inhibits the de novo synthesis of σ^B, which in turn reduces the induction of σ^B-dependent genes(Moch *et al.*, 2000).

As expected, genes belonging to the CtsR regulon such as *clpB, clpP* were highly induced. The CtsR repressor which was supposed to be inactivated and removed during several stresses such as heat shock, puromycin in *B.subtilis* (Krüger *et al.*, 1998, Gerth *et al.*, 1998) was also up regulated after *S.aureus* cells were exposed to puromycin stress .The same phenomenon was observed in heat stress response.

Puromycin stress might lead to an accumulation of misfolded proteins therefore might cause an increase in the synthesis of proteases and chaperones (Azzam and Algranati, 1973) which is similar to heat stress response. However, treatment with this antibiotic also brought about an additional response that is not observed during the shift to high growth temperature such as the induction of replicative DNA helicase DnaB. Puromycin, which is also a strong inhibitor of protein synthesis (Azzam and Algranati, 1973), therefore leads to a decreased

synthesis of enzymes belonging to amino acid synthesis pathway such as SerS, ThrS.

4.3 Salt stress

Although osmoadaptation of *S.aureus* has been studied fairly extensively in the past, it remains unclear why this organism is considerably more osmotolerant than other bacteria (Vilhelmsson and Miller, 2002). From studies in *E. coli*, *Lactococcus lactis* or *L.monocytogenes*, it was supposed that salt stress might induce a set of proteins related to heat stress (Kilstrup *et al.*, 1997, Duch *et al.*, 2002). In *B.subtilis*, it was indicated that a diverse range of environmental stresses such as heat shock, salt stress, or starvation stress such as oxygen limitation induced the same set of proteins, called general stress proteins (Hecker *et al.*, 1996).

In this work, we compared the protein synthesis pattern in response to salt stress and heat stress in order to examine what happened in the case of *S.aureus*, and furthermore to get some insights to high salt tolerance of *S.aureus*.

Surprisingly, there is only a small overlap of proteins induced by heat and salt stress implying that different signalling pathways did play a role in adaptation to these various kinds of environmental stress. The proteomic data also showed that osmotic stress induces the expression of AhpC which was shown to be unspecifically induced by heat stress in *B.subtilis* (Schumann, 2003). In this work, AhpC has also been detected to be up-regulated by heat stress in *S.aureus* . Our results are in good agreement with Höper and colleagues who reported that AhpC was induced after salt stress in *B.subtilis* as well (Höper *et al.*, 2006).

In *B.subtilis*, proline is the most prominent compatible solute synthesized in osmotically stressed cells when no compatible solutes are provided exogenously (Whatmore *et al.*,1990 ; Kuhlmann *et al.*, 2002). Therefore, in *B.subtilis*, glutamate as the precursor for proline, is probably required in higher concentrations after salt stress when extracellular compatible solutes are not available in the growth medium (Whatmore *et al.*,1990, Kuhlmann *et al.*, 2002). In the case of

S.aureus, the cells accumulate the compatible solutes glycine, proline and betaine within the cytoplasm, without inhibiting cellular processes (Townsend and Wilkinson, 1992). Osmotic stress does not affect glutamate levels in *S.aureus* cells, but rather results in dramatic increases in glutamine (and/or alanine) (Anderson and Witter, 1982; Killham and Firestone, 1984; Miller and Leschine, 1984). Therefore, as show in table 3.9, it was reasonable that the synthesis rate of glutamine synthetase FemC was induced after salt stress.

Our result suggested that osmotic stress induced the expression of Asp23, which encodes an alkaline shock protein, and the gene was shown to be preceded by a σ^B-dependent promoter (Gertz *et al.*,1999). We additionaly observed that the expression of some other proteins belonging to the σ^B-dependent proteins was induced by high osmolarity such as hexulose-6-phosphate synthase SACOL0617, the SIS domain protein SACOL0618 and the hydrolase SACOL2597, indicating that could be part of a general environmental-response mechanism.

The observed results agreed well with the fact that acclimation to NaCl influences several metabolic pathways. For instance, the induction of CysK, which catalyzes the last step in cysteine biosynthesis. Duché and colleagues also found the same result in *L. monocytogenes* after exposure to high salinity (Duché *et al.*, 2002). CysK was shown to be induced after oxidative stress in *B.subtilis* as well as in *S.aureus* (Antelmann *et al.*, 1997, Wolf *et al.*, 2008). Besides, in *B.subtilis*, salt stress seems to cause oxidative stress response in the cells as well, indicated by the induction of the PerR regulon (Hörper *et al.*, 2005). In *S.aureus*, the mechanism that caused the induction of CysK during oxidative stress might be a decreased availability of free cysteine, an sulfur containing amino acid which is low molecular weight thiol and is suggested to play a crucial role as antioxidants (Lithgow *et al.*, 2004). But the regulation and function that CysK might play in *S.aureus* under NaCl stress as well as the relation between salt stress and oxidative stress is still unknown.

We additionaly detected the induction of some enzymes involved in the purine/pyrimidine biosynthesis such as PyrR and PyrC, reflecting a particular need for DNA precursor. The gene *pyrR* was also suggested to be induced by salt stress in *B.subtilis* in a transcriptional profiling analysis by Steil and colleagues (Steil *et*

al., 2003).

Our proteome analyses revealed that NaCl caused the significant repression of 76 proteins that could be identified on 2D gels. Among them are the enzymes of the sugar metabolism pathways such as GapA, Eno, FbaA, PdhB, PdhD, PfkA, PgI, Pyc and Pyk or IvlC, IvlD, IvlS , PheT, and PheS which belong to amino acid metabolism pathways their synthesis rate was decreased in response to salt stress and increased when growth processes resume again. This results were similar to those previously obtained by Höper *et al.*, 2006 and Steil *et al.*, 2003 in a series of proteomic and transcriptomic experiments in *B.subtilis*.

The 2D gel- based proteomics allows a rapid visual overview of the most essential physiological responses of the cell but does not cover intrinsic membrane proteins that might play an essential role in salt adaptation. For instance, the ABC transporter systems for the uptake of compatible solutes such as membrane proteins OpuA, OpuB, OpuC, OpuD, and OpuE which play a crucial function in the adaptation to osmotic stress in *B.subtilis* (Bremer *et al.*, 2003), but in our gel-based proteome analysis, those membrane proteins were not covered on the proteomics main window pI 4-7.

Northern blot analysis with total mRNA from NaCl stressed *S.aureus* cells and *opuA, opuB,* and *opuC* probes was carried out in order to get some insights into the regulator mechanism of those ABC transporter system in *S.aureus*. Our result suggested, that as in *B.subtilis* (Kempf and Bremer , 1995) *opuA, opuB*, and *opuC* form a four cistronic operon together with *opuD* in *S.aureus*. Surprisingly, no significant induction of *opuA, B* or *C* could be demonstrated after salt stress. The level of transcripts remained almost unaltered. This might be due to the fact that our stress condition was not severe enough to activate the ABC transporter systems for the uptake of the compatible solutes or the genes were probably constitutively expressed , which could be an explanation for the high salt tolerance of *S.aureus*.

Taken together, the transcriptome and proteome analyses of *S.aureus* under heat, puromycin, and NaCl shock might help us to understand the physiology of *S.aureus* under different stress conditions and could provide some ideas about the environmental signals that could specifically influence protein synthesis in dif-

ferent tissues. A combination of proteomics with transcriptomics is probably one of the best choice to come to a comprehensive understanding of stress adaptation.

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Erklärung

Hiermit erkläre ich, dass diese Arbeit bisher von mir wieder an der Mathematisch-Naturwissenschaftlichen Fakultät der Ernst-Moritz-Arndt-Universitt Greifswald noch einer anderen wissenschaftlichen Einrichtung zum Zwecke der Promotion eingereicht wurde.

Ferner erkläre ich, dass ich diese Arbeit selbständig verfasst und keine anderen als die darin angegebenen Hilfsmittel benutzt habe.