Tạp chí Công nghệ Sinh học 9(3): 373-390, 2011

GENE EXPRESSION ANALYSIS OF *STAPHYLOCOCCUS AUREUS* UNDER HEAT STRESS

Le Thi Nguyen Binh[1, 2], Susanne Engelmann[1], Nong Van Hai[2], Michael Hecker[1]

[1]*University of Greifswald, Germany*
[2]*Institute of Biotechnology, Vietnam*

SUMMARY

Staphylococcus aureus is a very important human pathogen. Studies on the mechanisms of the response of *S. aureus* to environmental stresses have become one center of attention during the last few years because of their resistance to environmental stress. 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 transcriptional profiles of *S. aureus* after heat exposure were studied in order to provide detailed insights into the response of *S. aureus* to environmental stress under *in vitro* conditions. A DNA array approach was 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 gene expression profile. In particular, the transcriptome analysis revealed the induction of main cellular chaperone machineries GroE and DnaK, the ATP-dependent proteases ClpB, ClpP.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 genes 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 *uvrB* indicated the relevance of heat stress response and SOS response. According to the microarrays analysis data, among the heat-induced transcripts there 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. In summary, the signatures for stress 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.

Keywords: *Staphylococcus aureus, heat shock, transcriptomics, Nothern Blot, DNA array*

INTRODUCTION

Staphylococcus aureus is one of the major causes of community acquired infections. Because of its adaptability and resistance to environmental stresses, *S. aureus* can survive extremely well outside the host. Being one of the most important pathogenic bacteria and due to its widespread emergence of antibiotic resistance, *S. aureus* became a topic of many studies mainly addressing its pathophysiology and virulence. But there were only a few studies focusing on the cellular physiology of *S. aureus*. During infection, *S. aureus* is subjected to various environmental changes. Therefore, understanding of the regulatory mechanisms controlling stress gene expression and moreover, of cellular physiology of *S. aureus* in response to environmental stress is very essential in studying its pathogenicity.

The post genomic era of *S. aureus* began with the publication of the genome sequence of two reference strains (Kuroda *et al.*, 2001) and provided the experimental basis for bringing the genome sequence of *S. aureus* to life by an application of experimental tools encompassed under the term "functional genomics". Functional genomics enables the understanding of the cell physiology and infection biology of this bacterium.

Transcriptomic approaches in response to changes in the environment including mutants in central regulatory genes 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.

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 *et al.*, 2003). The stress genes are more or less silent in growing cells, but are strongly activated by environmental stimuli.

In *S. aureus*, a comprehensive exploration of the adaptational network will not only provide basic knowledge on *S. aureus* physiology, but also give many clues on the function of still unknown proteins indicated by the induction profile of genes by environmental stimuli. Rely on the fact that environmental stimuli such as heat, oxidative or anaerobic stress might be essential cellular signals in the host environment controlling the expression of virulence genes.

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 or restriction of metal ions. An increasing amount of data indicates that the capacity to survive stress conditions is highly correlated with virulence in *S. aureus* (Clements, Foster; 1999).

MATERIALS AND METHODS

Bacterial strains and growth condition

S. aureus COL (Shafer, Iandolo, 1979) was grown aerobically with shaking at 120 rpm and 37°C in the synthetic medium (Gerzt *et al.*, 1999). The condition of heat stress was then achieved by shifting cell culture at OD_{500} of 0.5 to 48°C.

RNA preparation

Total RNA from *S. aureus* was isolated using the acid-phenol method (Fuchs *et al.*, 2008) with some modifications. The quality of RNA was ensualized by gel electrophoresis and by analysis with a Bioanalyzer (Agilent Technologies, Palo Alto, CA).

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 manufacturer's 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 sizes of the transcripts. The hybridization signals were detected using a Lumi-Imager (Roche Diagnostics, Mannheim, Germany) and analyzed using the software package Lumi-Analyst (Roche Diagnostics, Mannheim, Germany).

DNA-microarray analyses

The isolated 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).

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 *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 (http://cybert.microarray.ics.uci.edu) (Baldi, Long, 2001). The parameters for the Bayesian standard deviation estimation applied in

the Cyber-T analysis were: sliding window size was 101 and confidence value for the Bayesian variance estimate was 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 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 co-hybridized with equivalent amounts of Cy5-labelled genomic DNA pooled from N315, Mu50, and COL (Charbonnier *et al.*, 2005).

RESULTS

The effect of high temperature on gene transcription of *S. aureus*

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. Additionally, to establish appropriate time points for cell sampling for DNA array analyses, total RNA was extracted from exponentially growing and heat treated 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 1, 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 which was absent in exponentially growing cells and increased strongly 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.

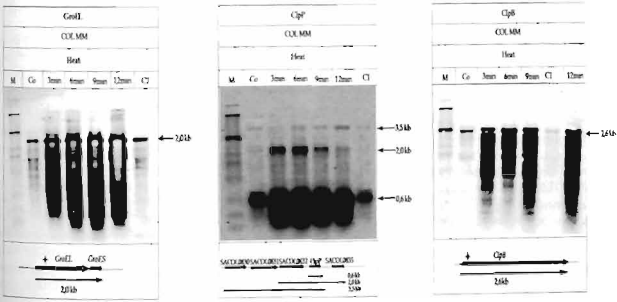

Figure 1. Northern blot analyses of genes whose transcription was induced by heat stress. For RNA preparation, cells were grown in synthetic medium to an OD_{500} of 0.5 and shifted to heat stress conditions. RNA was isolated before (co) and 3, 6, 9, 12 minutes after shift to 48°C

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 twofold 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 1 and 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.

After heat exposure, we could not detect any induction of sigmaB-dependent genes. The alternative sigma factor σ^B has been found in some pathogenic gram-positive bacteria, including *S. aureus* (Wu *et al.*, 1996; Kullik, 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, Völker, 1998). In *S. aureus*, σ^B-dependent induction of sigB by heat stress in complex medium has been described previously (Kullik, 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 σ^B-dependent proteins.

The transcriptome 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^D (Derre *et al.*, 1999).

Among the genes induced by heat shock there 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 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 encodong a purine nucleoside phosphorylase was also induced.

The transcriptome experiments also suggested the induction of genes to the function of which in heat resistance was still unknown. For example, *Frp* gene encoding NAD(P)H-flavin-dependent oxidoreductase (4.5 fold) 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 up-regulated, for instant, genes encoding a repressor protein (SACOL0321), and an antirepressor protein (SACOL0325), replicative DNA helicase (SACOL0343), N-6-adenine-methyltransferase (SACOL0346), 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 1).

Table 1. Genes whose transcription was induced under heat stress conditions.

ID	COL Locus	Function	Average ratio
Amino acid biosynthesis			
SACOL0502	SACOL0502	cysteine synthase	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	coproporphynnogen III oxidase	2.31
nbrI	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	SACOL1388	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
lmrE	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	2.45
nbBA	SACOL1818	3,4-dihydroxy-2-butanone-4-phosphate synthase	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	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 sub	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	antirepressor, Putative	13.83
dut	SACOL0357	deoxyuridine 5'-triphosphate nucleotidohydrolase	8.07
int	SACOL0318	Integrase	8.06
SACOL0346	SACOL0346	N-6-adenine-methyltransferase	22.81
SACOL0368	SACOL0368	portal protein, HK97 family	2.51
SACOL0343	SACOL0343	replicative DNA helicase.	23.75
SACOL0321	SACOL0321	repressor protein, Putative	14.10
ssb1	SACOL0339	single-stranded DNA binding protein	8.07
SACOL0367	SACOL0367	Terminase,large subunit	3.95
SACOL0366	SACOL0366	Terminase, small subunit.	6.72
SACOL0364	SACOL0364	transcriptional regulator, RinA family	11.90
Purines, pyrimidines, nucleosides, and nucleotides			
pyrB	SACOL1212	aspartate carbamoyltransferase catalytic sub	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	2.09
SACOL0998	SACOL0998	oligopeptide ABC transporter, ATP-binding	2.20
SACOL0996	SACOL0996	oligopeptide ABC transporter	2.08
SACOL1000	SACOL1000	oligopeptide ABC transporter, permease	2.61
SACOL0999	SACOL0999	oligopeptide ABC transporter permease	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		Total 113 genes have been identified to be upregulated from 2.05 to 30.65	

Table 2. Genes whose transcription was repressed under heat stress conditions

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	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	galactosyltransferase	0.29
cap5A	SACOL0136	capsular polysaccharide biosynthesis protein	0.18
cap5B	SACOL0137	capsular polysaccharide biosynthesis protein	0.17
cap5C	SACOL0138	capsular polysaccharide biosynthesis protein	0.12
cap5D	SACOL0139	capsular polysaccharide biosynthesis protein	0.23
cap5E	SACOL0140	capsular polysaccharide biosynthesis protein	0.24
cap5F	SACOL0141	capsular polysaccharide biosynthesis protein	0.16
cap5H	SACOL0143	capsular polysaccharide biosynthesis protein	0.17
cap5I	SACOL0144	capsular polysaccharide biosynthesis protein	0.17
cap5J	SACOL0145	capsular polysaccharide biosynthesis protein	0.22
cap5K	SACOL0146	capsular polysaccharide biosynthesis protein	0.29
cap5L	SACOL0147	capsular polysaccharide biosynthesis protein	0.46
cap5N	SACOL0149	capsular polysaccharide biosynthesis protein	0.41
cap5O	SACOL0150	capsular polysaccharide biosynthesis protein	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
agrC2	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	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 sub	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	SACOL2578	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	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,	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	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 sub.	0.36
nrdF	SACOL0793	ribonucleotide-diphosphate reductase beta sub.	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	0.27
SACOL1916	SACOL1916	amino acid ABC transporter, permease	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, lucA/lucC	0.34
oppF	SACOL0994	oligopeptide ABC transporter, ATP-binding	0.20
oppD	SACOL0993	oligopeptide ABC transporter, ATP-binding	0.22
SACOL0995	SACOL0995	oligopeptide ABC transporter,	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	0.36
SACOL2474	SACOL2474	peptide ABC transporter, permease protein	0.37
SACOL2475	SACOL2475	peptide ABC transporter, permease protein,	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	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		Total 60 proteins have been down regulated from 0.09 to 0.49 folds	

DISCUSSION

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 (Schumann *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 analysed 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.*, 2001) in *S. aureus*. Moreover, an orthologue of the CtsR class III stress regulator was defined as well as several potential target genes (Derré *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.

The obtained transcriptomic analysis data indicate that under heat stress conditions, *S. aureus* cells showed the induction of three classes of heat inducible genes. Among them were proteins such as chaperones DnaK, GroEL, GroES, and GrpE.

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 *B. subtilis*, GroEL and DnaK are regulated by the HrcA repressor and transcribed during heat shock by the vegetative sigma factor A (Schumann *et al.*, 1996; Yuan *et al.*, 1995). In this work, it was shown that in *S. aureus*, genes belonging to class I heat shock response were strongly induced after heat exposure. Transcriptome analysis data show that the gene encoding the chaperones DnaK, GroEL, GroES and GrpE was highly up regulated 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 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 a strong induction of gene encoding the chaperones as well as proteases which are typical for protect function. For example, among the heat induced genes are also those belonging to the Clp machinery: *clpP* encodes the proteolytic component ClpP and *clpB* of 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 *et al.*, 1996; Derre *et al.*, 2000; Schumann *et al.*, 1996). Frees and colleagues demonstrated that the action of a ClpP proteolytic complex is essential for staphylococci to grow under heat-shock conditions. 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).

The transcriptome analysis also revealed that the heat shock repressor CtsR was highly up-regulated after heat exposure. Frees and colleagues observed this same phenomenon and suggested that CtsR may need a cofactor for repressor function (Frees *et al.*, 2004; Anderson *et al.*, 2006).

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.

Heat stress treatment also resulted in a strong synthesis 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.

According to the microarrays analysis data, among

the heat-induced transcripts were several bacteriophage replication/packaging genes such as intergrase SACOL0318, replicative DNA helicase SACOL0343, N-6-adenine-methyltransferase SACOL0346, deoxyuridine 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 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.

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).

Acknowledgements: *We are grateful to Jan Pané-Farré and Stephan Fuchs for excellent support and lengthy, fruitful discussions on DNA microarray data analyses. We thank Thomas Meier and Anita Harang for excellent technical assistance This work was supported by grants of the BMBF and the Land MV.*

REFERENCES

Anderson KL, Roberts C, Disz T, Vonstein V, Hwang K, Overbeek R, Olson PD, Projan SJ, Dunman PM (2006) Characterization of the *Staphylococcus aureus* heat shock, cold shock, stringent, and SOS responses and their effects on log-phase. *J. Bacteriol* 188(19): 6739-6756.

Baldi P, Long AD (2001) A Bayesian framework for the analysis of microarray expression data: regularized *t*-test and statistical inferences of gene changes. *Bioinformatics* 17(6): 509-519

Bernhardt J, Völker U, Völker A, Antelmann H, Schmid R, Mach H, Hecker M (1997) Specific and general stress proteins in *Bacillus subtilis* - a two-dimensional protein electrophoresis study. *Microbiology* 143: 999-1017.

Charbonnier Y, Gettler B, François P, Bento M, Renzoni A, Vaudaux P, Schlegel W, Schrenzel J (2005) A generic approach for the design of whole-genome oligoarrays validated for genomotyping, deletion mapping and gene expression analysis on *Staphylococcus aureus*. *BMC Genomics* 1471-2164-6-95

Chastanet A, Ferrè T, Msadek T (2003) Comparative genomics reveal novel heat shock regulatory mechanisms in *Staphylococcus aureus* and other Gram-positive bacteria. *Mol Microbiol* 47: 1061-1073.

Clements M, Foster S (1999) Stress resistance in *Staphylococcus aureus*. *Trends Microbiol* 7: 458-462.

Derré I, Rapoport G, Msadek T (1999) CtsR, a novel regulator of stress and heat shock response, controls *clp* and molecular chaperone gene expression in Gram-positive bacteria. *Mol Microbiol* 31: 117-131.

Derré I, Rapoport G, Msadek T (2000) The CtsR regulator of stress response is active as a dimer and specifically degraded *in vivo* at 37°C *Mol Microbiol* 38: 335-347

Frees D, Chastanet A, Qazi S, Sorensen K, Hill P, Msadek T, Ingmer H (2004) Clp ATPases are required for stress tolerance, intracellular replication and biofilm formation in *Staphylococcus aureus*. *Mol Microbiol* 54: 1445-1462

Fuchs S, Jan Pané-Farré J, Kohler C, Hecker M, Engelmann S (2007) Anaerobic gene expression in *Staphylococcus aureus*. *J Bacteriology* 189: 4275-4289.

Gertz S, Engelmann S, Schmid R, Ohlsen K, Hacker J, Hecker M (1999) Regulation of sigma B-dependent transcription of sigB and asp23 in two different Staphylococcus aureus strains. *Mol Gen Genet* 261: 558-566.

Gertz S, Engelmann S, Schmid R, Ziebandt AK, Tischer K, Scharf C, Hacker J, Hecker M (2000) Characterization of the sigma B Regulon in *Staphylococcus aureus*. *J. Bacteriol* 182(24): 6983-6991.

Hecker M, Schumann W, Völker U (1996) Heat-shock and general stress response in *Bacillus subtilis*. *Mol Microbiol* 19(3): 417-28.

Hecker M, Volker U (1998) Non-specific, general, and multiple stress resistance of growth-restricted *Bacillus subtilis* cells by the expression of the sigmaB regulon. *Mol Microbiol* 29: 1129-1136.

Hecker M, Engelmann S, Cordwell SJ (2003) Proteomics of *Staphylococcus aureus* -current state and future challenges. *J. Chromatograph B* 787: 179-195.

Kullik I, Giachino P(1997) The alternative sigma factor s in *Staphylococcus aureus*: regulation of the sigB operon in

sponse to growth phase and heat shock. *Arch Microbiol* 67: 351-159.

uroda M, Ohta T, Uchiyama I, Baba T, Yuzawa H, obayashi I, Cui L, Oguchi A, Aoki K, Nagai Y, Lian J, o T, Kanamori M, Matsumaru H, Maruyama A, furakami H, Hosoyama A, Mizutani-Ui Y, Takahashi IK, Sawano T, Inoue R, Kaito C, Sekimizu K, Hirakawa L Kuhara S, Goto S, Yabuzaki J, Kanehisa M, Yamashita , Oshima K, Furuya K, Yoshino C, Shiba T, Hattori M, agasawara N, Hayashi H, Hiramatsu K (2001) Whole enome sequencing of meticillin-resistant *Staphylococcus ureus*. *Lancet* 357(9264): 1225-1240.

rüger E, Msadek T, and Hecker M (1996) Alternate romoters direct stress induced transcription of the acillus subtilis clpC operon. *Mol Microbiol*, 20: 713-723

laiques E, Ubeda C, Campoy S, Salvador N, Lasa I, ovick RP, Barbe J, Penades JR (2006) β-Lactam ntibiotics induce the SOS response and horizontal transfer f virulence factors in *Staphylococcus aureus*. *J Bacteriol* 88: 2726-2729.

lazmanian SK, Liu G, Ton-That H, Schneewind O (1999) *taphylococcus aureus* sortase, an enzyme that anchors rface proteins to the cell wall. *Science* 285: 760-763.

lowahedi S, Waites W (2000) Cold Shock Response in orulating Bacillus subtilis and Its Effect on Spore Heat esistance *J. Bacteriol*. 184: 5275-5281

oenfleh MW, Weraarchakul W, Wilkinson BJ (1998) ntibodies to a range of *Staphylococcus aureus* and *scherichia coli* heat shock proteins in sera from patients ith *S. aureus* endocarditis. *Infect Immun* 66: 3024-3027.

Schumann W, Schulz A (1996) hrcA, the first gene of the *Bacillus subtilis* dnaK operon encodes a negative regulator of class I heat shock genes. *J Bacteriol* 178(4): 1088-1093.

Schumann W (2003) The *Bacillus subtilis* heat shock stimulon *Cell Stress Chaperon* 8(3): 207-217.

Shafer WM, Iandolo JJ (1979) Genetics of staphylococcal enterotoxin B in methicillin-resistant isolates of *Staphylococcus aureus*. *Infect Immun* 25(3): 902-911.

Weitzstein M, Voelker U, Dedio J, Loenau S, Zuber U, Schiesswohl M, Herget C, Hecker M, Schumann W (1992) Cloning, sequencing and molecular analysis of the dnaK locus from *Bacillus subtilis*. *J Bacteriol* 174: 3300-3310.

Wolf C, Hochgräfe F, Kusch H, Albrecht D, Hecker M, Engelmann S (2008) Proteomic analysis of antioxidant strategies of *Staphylococcus aureus*: diverse responses to different oxidants. *Proteomics* 8(15): 3139-3153.

Wu S, de Lencastre H, Tomasz A (1996) A Sigma-B, a putative operon encoding alternate sigma factor of *Staphylococcus aureus* RNA polymerase: molecular cloning and DNA sequencing. *J Bacteriol* 178, 6036-6042

Yuan G, Wong SL (1995) Isolation and characterization of *Bacillus subtilis* groE regulatory mutants: evidence for orf39 in the dnaK operon as a repressor gene in regulating the expression of both groE and dnaK. *J Bacteriol* 177, 6462-6468

Zhang JK, Aronson A (1994) A *Bacillus subtilis* bglA gene encoding phospho-glucosidase is inducible and closely linked to a NADH dehydrogenaseencoding gene *Gene* 140: 85-90.

PHÂN TÍCH SỰ BIỂU HIỆN GEN CỦA *STAPHYLOCOCCUS AUREUS* SAU XỬ LÝ SỐC NHIỆT

Lê Thị Nguyên Bình[1, 2, *], Susanne Engelmann[2], Nông Văn Hải[1], Michael Hecker[2]

[2]Trường Đại học Tổng hợp Greifswald, Đức
[1]Viện Công nghệ sinh học, Việt Nam

TÓM TẮT

Staphylococcus aureus là một trong những vi khuẩn gây bệnh ở người rất nguy hiểm vì thế mà trở thành một đối tượng nghiên cứu rất quan trọng. Trong những năm gần đây, nghiên cứu về cơ chế phản ứng của *S. aureus* với các kích thích từ ngoại cảnh đã và đang rất được chú ý bởi vì loài vi khuẩn này có tính đề kháng mạnh với các điều kiện môi trường bất lợi. Bài báo này trình bày kết quả nghiên cứu về những thay đổi của biểu hiện gen và protein của *S. aureus* dưới tác động của sốc nhiệt, qua đó trình bày một cái nhìn chi tiết về phản ứng của *S. aureus* ở điều kiện *in vitro* đối với tác động của ngoại cảnh bất lợi. Trước hết, việc phân tích hệ protein và mức độ biểu hiện mRNA của *S. aureus* dưới tác dụng của sốc nhiệt được thực hiện bằng kỹ thuật điện di hai chiều kết hợp với phân tích bằng máy khối phổ và phương pháp DNA array. Sự thay đổi từ nhiệt độ môi trường sống thông thường lên nhiệt độ cao đã gây ra ở *S. aureus* một loạt những thay đổi trong hệ protein cũng như hệ gen của vi khuẩn này. Cụ thể là các protein nội bào có chức năng bảo vệ như GroEL, DnaK và các

*Author for correspondence: Tel: 84-4-37562934, E-mail: lenguyenbinh@ibt.ac.vn

protease phụ thuộc ATP như ClpB và ClpP đã cảm ứng gia tăng rõ rệt. Thêm vào đó sự biểu hiện của protein ức chế CtsR cũng tăng mạnh. Một điều đáng ngạc nhiên là các protein chịu sự điều khiển của nhân tố sigma B, thường cảm ứng rất mạnh sau sốc nhiệt ở vi khuẩn *Bacillus subtilis* lại không hề tăng mức biểu hiện ở *S. aureus* trong trường hợp này. Ngoài ra, các phân tích ở mức độ phiên mã gen cũng chỉ ra sự tăng mức phiên mã của các gen thuộc nhóm SOS, các gen chịu trách nhiệm phiên mã, đóng gói σ thể thực khuẩn thể, hoặc các gen điều hòa quá trình dịch mã thuộc nhóm RinA. Tóm lại, những biểu hiện đặc trưng của *S. aureus* đối với các điều kiện không có lợi có thể trở thành công cụ hữu hiệu trong việc chẩn đoán phản ứng của vi khuẩn này đối với một loại kháng sinh mới hoặc việc nghiên cứu các trạng thái sinh lý của tế bào.

Từ khóa: *DNA array, lai Northern, phân tích transcriptomic, sốc nhiệt, Staphylococcus aureus*