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ANALYSIS OF PROTEIN RESPONSE OF *STAPHYLOCOCCUS AUREUS* UNDER HEAT STRESS

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SUMMARY

Being one of the most important pathogenic bacteria, *Staphylococcus aureus* became a topic of several studies mainly addressing its pathophysiology and virulence. 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 of *S. aureus* after heat exposure were studied in order to provide detailed insights into the response of *S. aureus* to various kinds of environmental stress under in vitro conditions. The high resolution 2-D protein gel electrophoresis technique combined with MALDI-TOF-MS was used to investigate the cellular response of *S. aureus* to heat stress. The 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. 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. In summary, the protein response signatures for stress can provide some ideas on the environmental signals that specifically influence the survival of *S. aureus* within and outside the host.

Keywords: *heat shock, MALDI-TOF-MS, proteomics, Staphylococcus aureus, transcriptomics*

INTRODUCTION

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. 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 adaptational networks.

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. 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, proteomics has been used as an excellent experimental tool to visualize changes in the protein synthesis pattern of living cells and define the structure and function of genes in response to different stresses (Fuchs *et al.*, 2007; Wolf *et al.*, 2008; Hochgrafer *et al.*, 2008). Proteomics, based on the highly sensitive two dimensional gel electrophoresis (2-DE) which is a well established technique to obtain the global view of the synthesis and distribution of the proteins in the cell will confirm our findings at the transcriptional level. It was 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).

Heat stress response is a rapid response of bacteria to temperature up-shift. It is presumed that damaged proteins, such as unfolded and denatured proteins are detected by cellular systems that induce a large set of so called heat shock proteins (HSP). (Boorstein *et al.*, 1994; Gupta, 1995). Many of the HSPs are molecular chaperones (e.g., GroEL, GroES. DnaK, DnaJ) and ATP-dependent proteases (e.g.,ClpP, ClpC) (Sherman , Goldberg, 1992, 1996, Kandror *et al.*, 1996) which 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 , Neidhardt, 1987; Inbar , Ron, 1993; Hecker , Völker, 1998).

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).

In this paper, changes in the synthesis of cytoplasmic proteins before and at different times after shifting the cells to high temperature condition was studied. To observe the synthesis rate of each protein, two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) (Eymann *et al.*, 1996) of radioactively labelled protein extracts was performed, following with protein identification by means of MALDI-TOF-MS.

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 synthetic medium (Gerzt *et al.*, 1999) in 500-ml Erlenmeyer flasks under vigorous agitation at 37°C to an optical density at 500 nm (OD_{500}) of 0.5.

Condition of heat stress was then achieved by shifting cell culture at OD_{500} of 0.5 to 48°C.

L-[35S] methionine-labeling and preparation of cytoplasmic protein fractions

To label newly synthesized proteins, the pulse labeling experiment was carried out as previously described (Wolf *et al.*, 2008).

2-D PAGE and gel imaging

Protein extracts (100 mg of radioactively labeled proteins, 250 mg of fluorescence-labeled proteins, and 250 mg of unlabeled proteins for Coomassie staining) were loaded onto commercially available IPG strips (pH 4–7, GE Healthcare, Uppsala, Sweden). 2-D PAGE was performed as described previously (Büttner *et al.*, 2001).

Gels containing radioactively labeled proteins were stained with silver nitrate and scanned with a light scanner with integrated transparency unit (Quatographic, Braunschweig, Germany). All protein gels containing nonradioactively labeled proteins were stained with colloidal CBB G-250 and scanned with the light scanner.

Data analysis

For each sampling time point in the experiments gel images generated from at least two independent cell cultures were analyzed with the Delta2D software (Decodon, Greifswald, Germany). All autoradiograms in a stress experiment were overlaid and fused to one single image using the union image fusion algorithm of Delta2D (Luhn *et al.*, 2003).

Protein identification

For protein identification by MS, preparative 2-D protein gels were used. Protein spots that could not be definitely assigned to a spot on the *S. aureus* reference 2-D map (Kohler *et al.*, 2006) were identified by MALDI-TOF-MS.

RESULT

Heat stress response

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 were examined.

When shifting the cells from 37°C to 48°C the growth rate of the cells was drastically decreased. At 50°C and 52°C the cells seemed to stop growing after the temperature upshift. Therefore, we chose 48°C as the appropriate temperature for heat stress experiments.

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 labelled protein extracts was used (Eymann *et al.*, 1996).

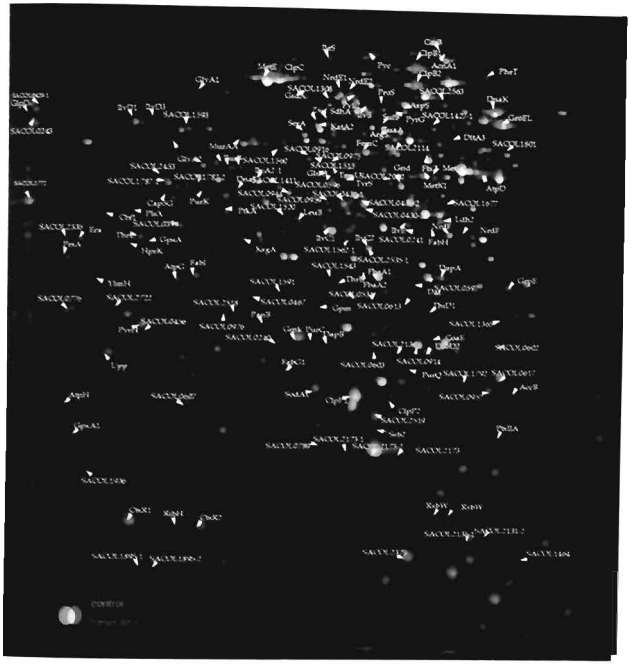

Figure 1. The dual-channel image of the protein synthesis pattern of *S. aureus* COL before (green image) and 10 min after the exposure to 48°C (red image). Cytoplasmic proteins were labelled with L-^{35} S]methionine and separated by 2D-PAGE as described in Section 2. Image analysis of the autoradiograms 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 1 and table 2.

The 2D gels were silver stained, dried, and exposed to Phosphor Imager screens. The resulting autoradiograms represent the synthesized proteins at the respective time points. Proteins synthesised only upon heat treatment appeared in red, whereas green labelled spots represent proteins the synthesis of which was switched off under these conditions. Interesting protein spots were identified by MALDI-TOF/MS and MASCOT.

Proteins whose synthesis was significantly induced in at least 3 experiments were shown in table 1 and proteins whose synthesis was repressed by heat stress were shown in table 2. 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.

Figure 2. 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).

Proteome analysis data show 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, Schumann, 1994; Hecker , Volker,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.

Table 1. Proteins whose synthesis was induced under heat stress conditions.

ID			Ratio (min)			
	Symbol	Function	5	10	30	60
Amino acid biosynthesis						
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
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	7.70	7.64	8.75	4.90
SACOL21312	**SACOL2131**	Cellular processes: Dps family protein	19.58	17.97	14.62	10.68
DNA metabolism						
SACOL0006	**GyrA**	DNA metabolism	8.00	6.11	10.91	10.03
Hypothetical protein						
SACOL2379	**SACOL2379**	Hypothetical protein	7.16	15.19	1.23	0.67
SACOL1204	**YlmH**	Hypothetical protein	90.54	128.50	97.64	104.60
SACOL0455	**SACOL0455**	Hypothetical proteins	6.52	7.74	6.31	3.14
SACOL0776	**SACOL0776**	Hypothetical proteins	32.60	22.15	18.82	9.01
SACOL1992	**SACOL1992**	Hypothetical proteins	3.41	5.91	3.16	11.30
Protein fate						
SACOL0979	**ClpB**	Degradation of proteins, peptides, and glycopeptides	3.60	5.83	5.21	8.40
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	1.85	1.69	1.18	8.63
SACOL2016	**GroEL**	Protein fate, Protein folding and stabilization	4.67	22.98	13.71	0.22
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	3.81	3.18	2.83
Protein synthesis						
SACOL0536	**KsgA**	Protein synthesis, tRNA and rRNA base modification	0.76	0.69	0.76	0.25
Purines, pyrimidines						
SACOL0018	**PurA**	Purines, pyrimidines, nucleosides, and nucleotides	0.60	3.62	25.15	27.80
Transport and binding proteins						
SACOL2148	**PtsIIA**	ABC transporter	1.88	2.70	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
Biosynthesis of cofactors						
SACOL1817	**RibH**	Biosynthesis of cofactors, prosthetic groups	2.39	1.63	3.28	1.50

Table 2. Proteins whose synthesis was repressed under heat stress conditions

ID	Symbol	Function	Ratio (min)			
			5	10	30	60
Amino acid biosynthesis						
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 poly saccharides&lipopolysacchandes	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	0.16
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,	0.20	0.10	0.10	0 07

		Glutamate				
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'-Deoxynbonucleotide 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 D-isomer specific 2-hydroxyacid dehydrogenase family protein, putative	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						
20 Hypothetical protein						

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 sigB dependent stress response in *S aureus* cultivated in synthetic medium after heat stress.

Table 1 shows the changes in the synthesis level of some Clp proteases encoded by CtsR-regulated genes: ATPase subunit ClpB and proteolytic subunit ClpP (Derré *et al.*, 2000; Krüger *et al.*, 1996; 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). 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 1 , the synthesis rate of some enzymes belonging to amino acid metabolism pathways such as serine hydroxymethyltransferase GlyA, trans-sulfuration enzyme family proteins

SACOL0430 and SACOL0431 as well as enzymes involved in the purine ribonucleotide biosynthesis pathwaysuch as adenylosuccinate synthetase PurA was induced in heat treated cells.

DISCUSSION

In *B. subtilis* , a model Gram positive bacteria, at least four classes of heat shock genes encoding cytoplasmic proteins have been identified (Hecker *et al.*, 1996; Schumann, 2003).

Unlike *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 σB (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.*, 2000). 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 this study, effects of heat stress on *S. aureus* cells was analysed, using a gel-based proteomic approach which offers the opportunity to find proteins of *S. aureus* whose synthesis was increased or decreased after stress such as heat stress.

Two groups of interesting proteins have been identified: Those whose synthesis was induced after heat stress and those whose synthesis 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.

In this work, it was shown that in *S. aureus*, proteins belonging to class I heat shock protein were strongly induced after heat exposure. Proteome analysis data show that the synthesis of the chaperones DnaK, GroEL, GroES and GrpE was up regulated with an induction factor of at least three fold under stress condition.

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* (Derre *et al.*, 2000; Schumann, 2003). In our work, ClpP and ClpB were also found to be induced by the temperature up shift by proteomics analyses.

The proteome analysis also revealed that the heat shock repressor protein 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).

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 *et al.*,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 , 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).

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.

Collectively, the overview of induction after heat stress obtained by transcriptomics and proteomics reveals that members of protein fate processes are the largest group which can be detected by 2D gel approach suggesting protein damage is a priority under heat stress.

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PHÂN TÍCH SỰ BIỂU HIỆN PROTEIN CỦA *STAPHYLOCOCCUS AUREUS* TRONG ĐIỀU KIỆN SỐC NHIỆT

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TÓM TẮT

Trong những năm gần đây, *Staphylococcus aureus* đã và đang là một trong những đối tượng nghiên cứu trọng tâm về bệnh học của vi khuẩn. Hiểu biết về cơ chế điều hòa sự biểu hiện protein cũng như gen của *S. aureus* dưới các tác động của điều kiện bất lợi cũng đóng vai trò quan trọng trong việc tìm hiểu độc tính của vi khuẩn này. Bài báo này trình bày kết quả nghiên cứu về những thay đổi của biểu hiện protein của *S. aureus* dưới tác động của nhiệt độ cao, 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. Ở đây, việc phân tích hệ protein của *S. aureus* dưới tác dụng của sốc nhiệt đã được thực hiện bằng kỹ thuật điện di hai chiều kết hợp với phân tích bằng máy khối phổ. 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 của vi khuẩn này. Cụ thể là các protein nội bào có chức năng bảo vệ như GroEL, DnaK và các 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, việc nhận biết các protein mới được tổng hợp dưới tác động của các điều kiện bất lợi cũng góp phần để hoàn thành bản đồ protein tế bào chất của *S. aureus*. 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 nghiên cứu các trạng thái sinh lý tế bào của vi khuẩn này.

Từ khóa: *MALDI-TOF-MS, phân tích, proteomic, sốc nhiệt, Staphylococcus aureus*