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Journal of Chromatography B, 787 (2003) 179–195
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Review

Proteomics of *Staphylococcus aureus*—current state and future challenges

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Abstract

This paper presents a short review of the proteome of *Staphylococcus aureus*, a gram-positive human pathogen of increasing importance for human health as a result of the increasing antibiotic resistance. A proteome reference map is shown which can be used for future studies and is followed by a demonstration of how proteomics could be applied to obtain new information on *S. aureus* physiology. The proteomic approach can provide new data on the regulation of metabolism as well as of the stress or starvation responses. Proteomic signatures encompassing specific stress or starvation proteins are excellent tools to predict the physiological state of a cell population. Furthermore proteomics is very useful for analysing the size and function of known and unknown regulons and will open a new dimension in the comprehensive understanding of regulatory networks in pathogenicity. Finally, some fields of application of *S. aureus* proteomics are discussed, including proteomics and strain evaluation, the role of proteomics for analysis of antibiotic resistance or for discovering new targets and diagnostics tools. The review also shows that the post-genome era of *S. aureus* which began in 2001 with the publication of the genome sequence is still in a preliminary stage, however, the consequent application of proteomics in combination with DNA array techniques and supported by bioinformatics will provide a comprehensive picture on cell physiology and pathogenicity in the near future.

Keywords: Reviews; Proteomics; *Staphylococcus aureus*

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1570-0232/02/$ – see front matter
PII: S1570-0232(02)00907-8

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1. Introduction

1.1. Functional genomics, proteomics and life

The publication of the first complete genome sequence of a living organism in 1995 opened a new era in biology [1]. The exponential increase in genome sequence information that followed after this hallmark has posed a challenge for bioinformatics and for researchers to design new ''global'' experiments to attempt to understand the functions of all of these new genes. Many prokaryotic and later eukaryotic genomes had to be annotated, and the huge amount of sequence information was the reason that new fields in molecular genetics such as phylogenomics or comparative genomics emerged. A large number of genes coding for proteins with unknown functions have been derived from genome sequencing, among them new global regulators such as alternative sigma factors, response regulators or other activators. It appears that even for model organisms such as *Escherichia coli* or *Bacillus subtilis* many chapters of their cell physiology or molecular biology remain empty.

The genome sequence, however, represents only the ''blue-print of life'', functional genomics is required to bring the blue-print into real life. 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 concentration, modification and sub-cellular localization of those molecules that act as cellular effectors, the proteins. If one assembles all the data available from sequence information, mRNA profiling, proteomics and metabolomics to a cell model supported by bioinformatic tools, a new and almost complete picture of a living cell is no longer a vision but may become reality in the near future (such an emphatic overview of living cells is now being termed ''systems biology''). However, it should not be ignored that for these biotechnological tools to be utilized to their undoubted capacity, a substantial commitment to excellent experimental design is necessary.

1.2. Staphylococcus aureus—one of the most important pathogenic bacteria

S. aureus is a gram-positive human pathogen of 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 and nosocomial infections. The pathogenesis of *S. aureus* is very complex and involves the strongly coordinated synthesis of cell wall-associated proteins and extracellular toxins. Due to its great variety of virulence factors, *S. aureus* causes a broad spectrum of infections ranging from superficial abscesses, osteomyelitis, endocarditis, and toxic shock syndrome.

The post-genomic era of *S. aureus* began in 2001 with the publication of the genome sequence of two reference strains [2]. This provided the experimental basis for bringing ''the genome sequence of *S. aureus* to life'', by a consequent application of the experimental tools encompassed under the term ''functional genomics'', including proteomics. The *S. aureus* genome codes for approximately 2600 proteins. At least 40% of the identified proteins show no similarity to proteins of known function, and a further-one-third of these proteins seem to be unique to *S. aureus* and might be good candidates for

possible novel virulence factors. Almost all known *S. aureus* virulence factors could be identified in this study, as well as 70 new candidates. The expression of virulence genes must be strongly coordinated and is realised by an arsenal of regulatory proteins. The genome analysis identified 124 open reading frames (ORFs) encoding putative transcription regulators. Among them are 63 regulators with helix-turn-helix motifs, 17 two-component systems, five *sarA* homologs and two sigma factors [2].

The present review will show that we are just beginning the post-genome era of *S. aureus,* and this will undoubtedly contribute to an essential understanding of the biology of this species. Whereas ''classical studies'' have focused on the elucidation of single components of cellular life of *S. aureus*, the new approaches provide an opportunity to look at cells as an entity. These studies will provide an increasingly complete picture on the cell physiology of growing and non-growing *S. aureus* cells, as well as a comprehensive understanding of its pathogenicity which may be regarded as part of its cell physiology in the host environment. A comprehensive picture of the biology of this bacterial species is not only interesting from a scientific point of view but may also open new fields for diagnostics and therapy.

2. Proteomics of *S. aureus*—a reference map

Global regulation of gene expression provides living cells only with those proteins that are necessary for growth or survival. These proteins are produced in a required and tightly adjusted amount and transported to their specific locations inside or outside the cell. Therefore, only a part of the genome is active under certain physiological circumstances. Proteomics is an excellent experimental tool to visualize changes in the protein synthesis pattern of living cells. 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 and has to be complemented with the identification of proteins, relying on tryptic peptide mass mapping via matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) or often more sophisticated MS techniques [3] to characterize post-translationally modified peptides or proteins that were poorly amenable to peptide mapping. Both approaches provide data that can immediately link a separated protein with its respective *S. aureus* genome sequence.

The ''theoretical proteome map'' comprising the predicted p*I* and M_r of all ORFs derived from the genome sequence shows two major peaks for bacteria, a neutral peak and a more alkaline one [4,5]. Therefore, the proteomic pattern relying on a pH gradient from pH 4 to 7 only provides the main window for cytoplasmic proteins. Alkaline gel systems (up to pH 12) are required to cover the more alkaline proteins, however these are technically challenging and usually only resolve proteins with isoelectric point (p*I*) values up to approximately 9.5–10 [6–9]. Furthermore, many proteins are located at the cell surface, or even secreted into the extracellular space. If one combines these four subproteomic fractions—neutral and alkaline cellular proteins, surface-bound and extracellular proteins—the majority of proteins synthesized in a cell under defined physiological conditions can be visualized by this powerful approach. On this way towards the entire proteome of an organism, some proteins have, however, not been captured by these technologies. Above all, intrinsic membrane proteins with multiple membrane-spanning domains often escape detection by 2-DE because of their hydrophobic nature. In *Pseudomonas aeruginosa*, over 250 outer membrane proteins (OMPs) have been characterized [10], however, this data set is still limited to those proteins that are hydrophilic and does not necessarily appear to depend on the number of transmembrane spanning regions. This methodology becomes generally more difficult in gram-positive bacteria due to the presence of peptidoglycan, and membrane fractions from such organisms are commonly contaminated with abundant cellular constituents. Furthermore, very large or very small proteins or proteins of low abundance are frequently not included in proteomic studies utilizing 2-DE. Different techniques such as application of zoom gels, low- or high-percentage gels or chromatographic prefractionation techniques have been used to increase the overall proteome coverage [11–13].

Even by application of all these approaches the physiology of the cell does not allow the coverage of an entire proteome in one single experiment. The expression of several genes is controlled by environmental stimuli with the consequence that many genes are more or less silent when the specific environmental stimuli are not imposed on the cell. The combined application of an analytical with a physiological approach is necessary for visualizing the entire proteome.

Nevertheless, the establishment of a protein reference map is an essential starting point for all physiological studies that may follow. This reference map is usually established from cells grown under ''standard conditions''. From a physiological point of view essential environmental stimuli can be imposed in later experiments, not only to cover new parts of the proteome repressed under standard conditions, but at the same time to obtain new information on cell physiology under stress or starvation conditions. Some proteomic studies on *S. aureus* have recently become available in the literature, providing new information towards master two-dimensional (2D) gels [14–18], and thus allowing further study on how proteins respond to different genetic and environmental stimuli. The *S. aureus* reference map of cytoplasmic proteins has been recently published by Cordwell et al. [14] (Fig. 1). *S. aureus* strains COL and 8325 were grown to mid-exponential phase in tryptone soy broth at 37 °C. Neutral or weakly acid

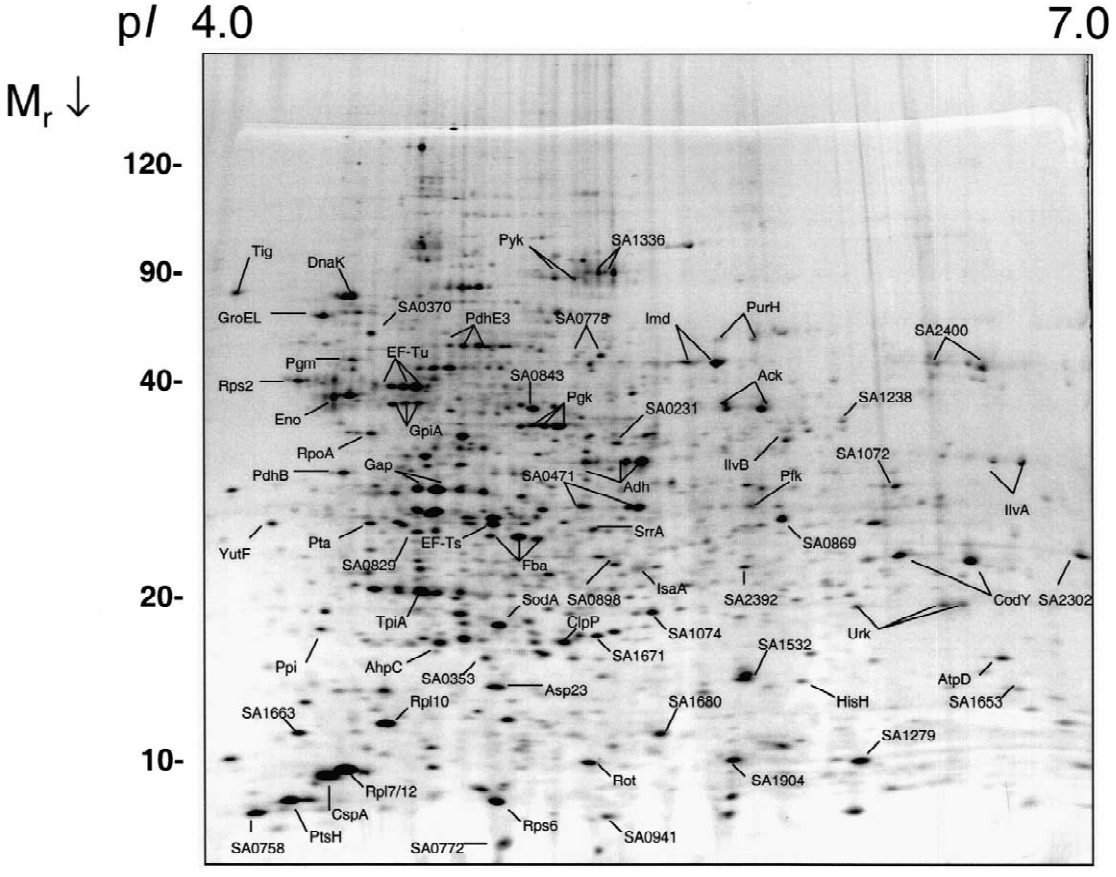

Fig. 1. Silver-stained two-dimensional electrophoresis gel of *S. aureus* 8325 whole cell proteins. Identifications of landmark proteins are shown [14]. Proteins were separated using a pH 4–7 immobilized pH gradient and proteins characterized using tryptic-digest MALDI-TOF-MS.

proteins (pH 4–7; Fig. 1) were separated, as were more alkaline proteins (pH 6–11). Three hundred and seventy-seven proteins from cells grown under these ''standard conditions'' were analyzed using MALDI-TOF-MS, corresponding to 266 expressed ORFs, or approximately 12% of the *S. aureus* proteome. Each gene, on average, was present at ''1.42 spots'' on 2D gels, indicating some post-transcriptional processing. In total, 36 proteins were identified in more than one 2D gel spot, in most cases as a result of p*I* variation. The protein pattern obtained is typical of growing cells with the glycolytic enzymes, the GroEL and DnaK machines or some translational proteins (e.g., elongation factors Tu and Ts) among the most abundant proteins. Because alkaline proteins were also covered, 22 ribosomal proteins could be detected. Because a complex medium was used for the ''standard growth conditions'' most of the amino acid biosynthesis enzymes were probably not synthesized by the organism. Nevertheless, this proteome picture with many reference spots can be regarded as a marker gel for further physiological studies.

A systematic proteome study of cell-surface associated proteins in *S. aureus* is still lacking. Surface proteins of *S. aureus* are bound to the cell wall envelope by a mechanism requiring a C-terminal sorting signal with an LPXTG motif [19,20]. A second targeting mechanism for surface proteins involves the binding of polypetides to cell wall structures.

Because of their key role in pathogenicity extracellular proteins have been analyzed in several studies [17,18,21–25]. As found in most bacteria, the synthesis of extracellular proteins is strongly regulated at the transcriptional level and mainly occurs in the stationary phase of growth at high cell densities (Fig. 2, Table 1). In the later stages of the stationary phase the majority of extracellular proteins disappear again, probably because of the action of extracellular proteases [26–28].

The data on the proteome of *S. aureus* are being integrated in a comprehensive proteome database Staph 2D (http://microbio2.biologie.uni-greifswald.de:8880/staph2d). The structure of this database follows that of Sub-2D database [29]. Reference gels of cytoplasmic and extracellular proteins of various *S. aureus* strains are available [15,18].

3. Physiological proteomics of *S. aureus*—cell physiology and pathogenicity

With the production of reference maps for cytoplasmic and extracellular proteins an experimental tool is available for more sophisticated physiological studies. Many such questions can be addressed by proteomics, thus generating new information on regulatory systems and enzymatic pathways involved in particular cellular responses.

Firstly, two major classes of proteins produced at different growth-phases can be distinguished: vegetative proteins synthesized during growth and cell cycle with mainly house-keeping functions (growth physiology); and proteins synthesized only in response to an environmental stimulus with mainly adaptive functions against stress or starvation (stress physiology). An alternative classification approach looks for enzymes integrated into metabolic networks, for structural proteins forming the cellular architecture, for signal transduction proteins integrated into various signalling pathways, or for proteins with adaptive function against stress and starvation stimuli [5]. Obviously there are many overlapping areas between these groups forming the protein interaction network of the cell. Many proteins of these different groups are involved in the pathogenicity of *S. aureus*. This pathogenicity protein group is rather heterogenous. In addition to proteins having the function of damaging the host there are also proteins that are essential for survival outside and within the host that may contribute indirectly to pathogenicity.

3.1. Metabolism and proteomics

The identification of many, if not all, proteins of single metabolic pathways offers the chance to analyse the regulation of entire metabolic pathways and not only of selected enzymatic steps. This approach, for example, was used for the analysis of the regulation of main carbon catabolic pathways such as glycolysis or the TCA (trichloroacetic acid) cycle in *B. subtilis* [30]. A similar approach can be used for *S. aureus* grown in the presence or absence of glucose because almost all glycolytic enzymes have been detected on 2D gels [14], offering the chance to analyse the regulation of glycolysis as an

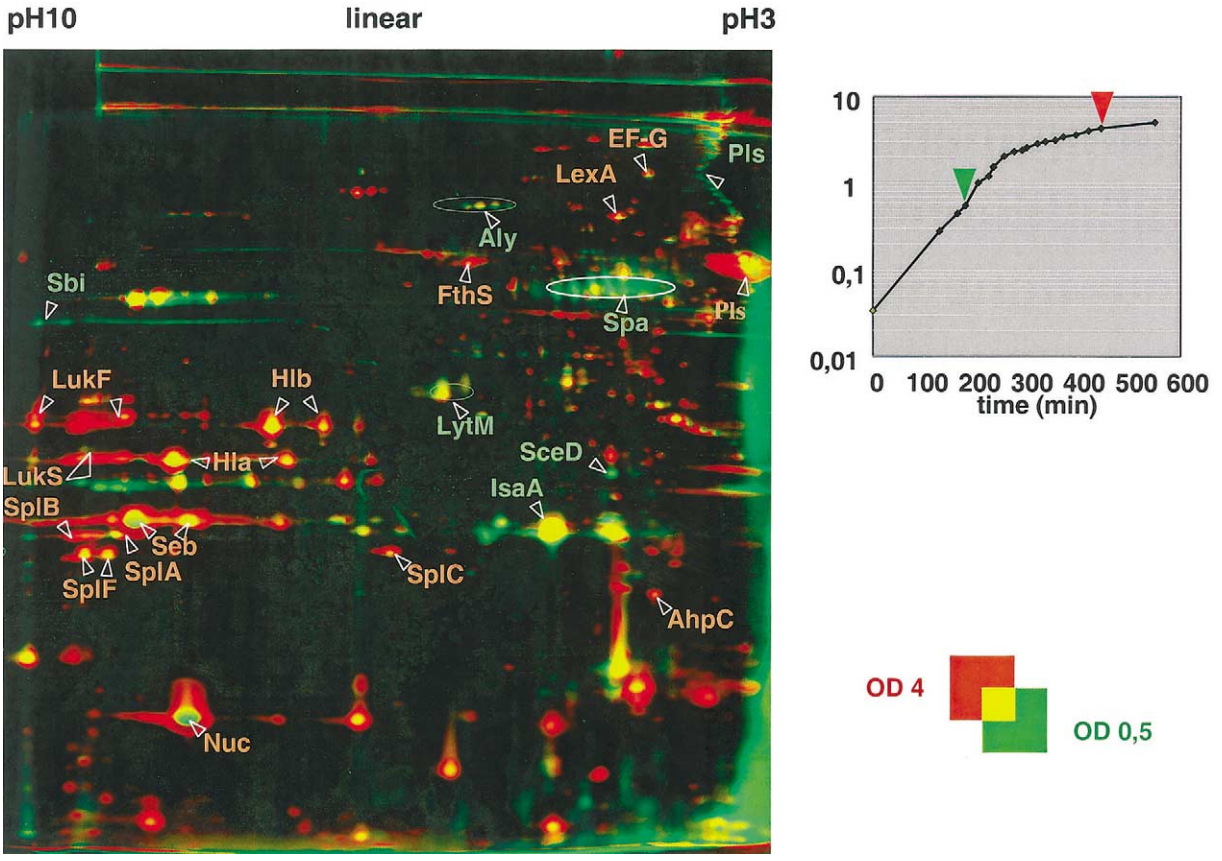

Fig. 2. The extracellular proteome of *S. aureus* COL under low (green image) and high (red image) cell densities. Proteins in the supernatant of cells grown in LB-medium at A_{540}=0.5 or 4, respectively, were precipitated with 10% TCA and the resulting protein pellets were dissolved in urea/thiourea solution. Proteins were separated by two-dimensional gel electrophoresis technique and stained with silver. Extracellular proteins whose amount is increased at high cell densities are labelled red and those proteins only present at low cell densities are labelled green.

entity, rather than examine the effects on each individual enzyme constituent. First results show that glucose excess strongly activates glycolysis but represses the TCA cycle of *S. aureus* as in *B. subtilis*, triggering an overflow metabolism even in the presence of oxygen (Engelmann et al., in preparation). Similar and extended studies are required for a better understanding of the regulation of cell metabolism in *S. aureus*, which is also crucial for understanding its behaviour in the host cell (pathophysiology). For many branches of growth physiology, including cell cycle control or stringent control the proteomic approach has not yet been applied in a systematic manner that would allow a more comprehensive picture of the relationship between the regulation of gene expression and these parts of growth physiology. Using the new opportunities of the proteomics approach new information on cell metabolism and growth physiology of *S. aureus* should be expected soon.

3.2. Stress and starvation responses

Stress and starvation are typical conditions in most natural ecosystems, including many different microhabitats 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 adapta-

Table 1
Extracellular proteins of *S. aureus* COL

Protein	Function/similarity
AhpC	Alkyl hydroperoxide reductase subunit C
Aly	Hypothetical protein, autolysin homologue
EF-G	Elongation factor G=85 kDa, vitronectin-binding protein
FthS	Formate-tetrahydrofolate ligase *Streptococcus mutans*
Hla	α-Haemolysin
Hlb	β-Haemolysin
IsaA	Immunodominant antigen A
LexA	SOS regulatory LexA protein
LukF	Leukocidin F subunit=γ-haemolysin subunit B
LukS	Leukocidin S subunit=γ-haemolysin subunit C
LytM	Peptidoglycan hydrolase
Nuc	Thermonuclease
Pls	Methicillin-resistance surface protein precursor
SceD	Hypothetical protein, similar to SceD precursor
Seb	Enterotoxin B
Spa	IgG binding protein A
Sbi	IgG-binding protein Spi
SplA	Serine protease SplA
SplB	Serine protease SplB
SplC	Serine protease SplC
SplF	Serine protease SplF

tional network has evolved. Analysing this network forms the basis for understanding the cell physiology in natural ecosystems [31]. The stress and starvation 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 functions as an entity are the most important steps in exploring adaptational networks. Proteomics is a good tool for elucidating this network and its single modules: proteomics can be used:

(i) To define a single stimulon, i.e., the entire set of proteins/genes induced or repressed by one stimulus. The definition of the stimulon structure will give an overview on stress adaptation because all proteins induced together by the stimulus manage stress adaptation. Following this ''stimulon approach'' the pattern of induced proteins has to be analysed and compared in untreated and treated cells to identify the newly induced or repressed proteins.

(ii) To dissect each stimulon into single regulons, i.e., the basic modules of global gene expression. The genes of a regulon are distributed on the genome but are controlled by one global regulator. For this ''regulon approach'' the protein pattern of the wild-type has to be compared with that of a global regulator mutant under conditions that activate the global regulator [32].

(iii) To analyse overlapping regions between single regulons because these do not exist independently, but are tightly connected and form the complicated adaptational network (Fig. 3).

(iv) To analyse the kinetics of gene expression programmes.

The allocation of proteins to functional groups (stimulons, regulons) is a promising strategy for a first prediction of the function of unknown proteins. Because the genome of *S. aureus* encodes more than 1000 proteins with unknown function, the elucidation of these functions is necessary for a complete understanding of *S. aureus* biology, including its pathogenicity. 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. This approach, however, only provides preliminary information: a protein strongly induced by oxidative stress will somehow be involved in oxidative stress adaptation, but de-

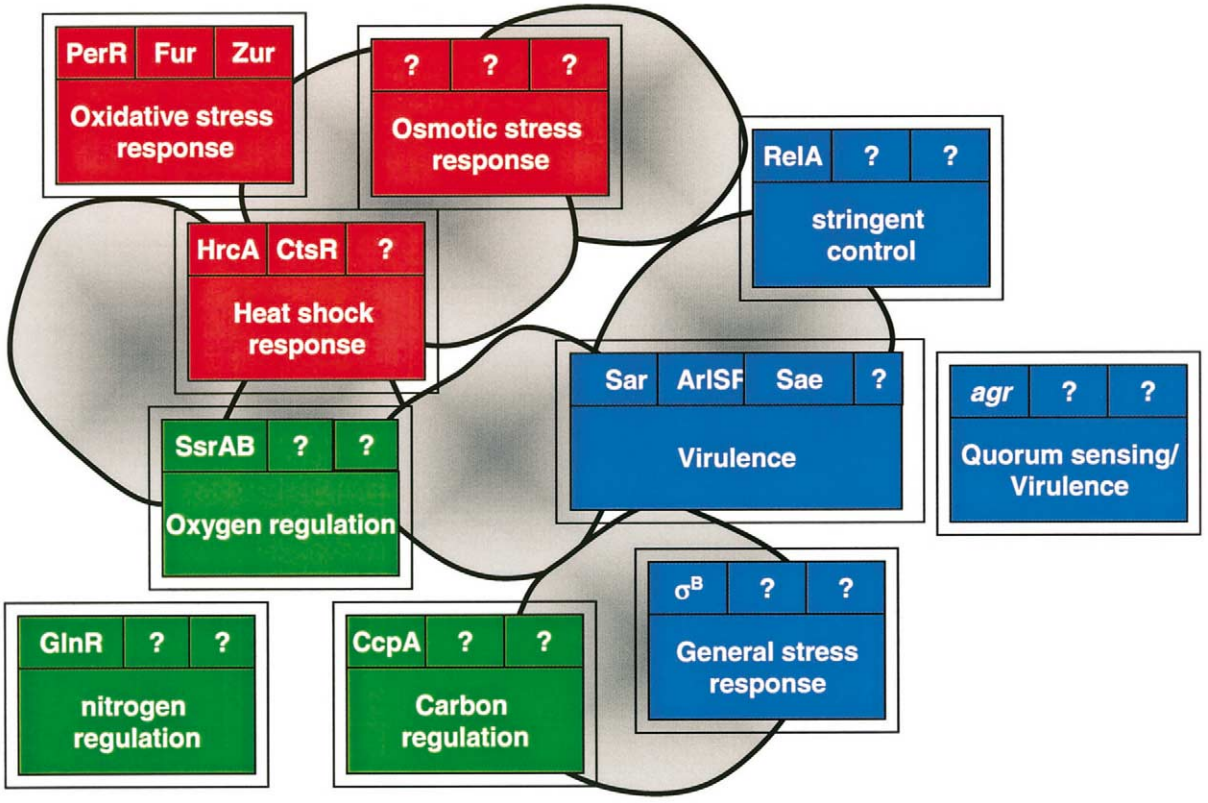

Fig. 3. Proteomics and transcriptomics will be used more and more to analyse this adaptational network of *S. aureus*. Only very few stimulons and regulons (wild type/mutant comparison) have been already studied in this way (e.g., Gertz et al. [15] for the σ^B-regulon, Throup et al. [63] for the SrhSR-regulon).

tailed and in most cases time-consuming studies are required to explore the exact role of the single proteins in question.

Despite the fact that environmental stimuli such as oxygen or nutrient limitation, oxidative, alkaline or acid stress and many other stimuli might be the essential cellular signals in the host environment controlling the expression of virulence genes, proteomics has so far only been used in rare cases to define the structure and function of the single stimulons or regulons. Since cells are usually grown in complex media, pulse-labelling experiments with ^{35}S-L-methionine to follow the immediate changes in protein induction or repression cannot be carried out. Such labelling experiments are, however, necessary to visualize the rapid changes in the gene expression pattern after imposition of the stress stimulus. To solve this problem *S. aureus* cells have to be cultivated in synthetic media, which allows ^{35}S-L-methionine incorporation studies. Alternatively, proteomics could be replaced by mRNA profiling studies that can be performed in complex media as well. All these studies aim to explore the complex adaptational network that will offer many different proteomic signatures for various stress or starvation conditions.

3.3. Stress and starvation stimulons and regulons—proteomic signatures

Only a few proteomic signatures have been analysed for *S. aureus*. Proteomic signatures [33] indicating the growth-limiting condition of a cell population are a promising tool for the evaluation or prediction of the physiological situation of cells grown in a bioreactor or in a natural ecosystem (e.g.,

biofilm). Cell wall-active antibiotics, such as oxacillin, induced a complex proteomic signature [16] indicating oxidative damage (methionine sulfoxide reductase) or protein stress (GroES). Only a few proteins, however, have been identified so far and are not sufficient to obtain a complete proteomic signature for cell wall stress. In the study of Cordwell et al. [14], the response of *S. aureus* strains to submicellar concentrations of Triton X-100 (TX-100) was investigated. TX-100 appears to reduce methicillin resistance in previously resistant *S. aureus* strains to levels seen in methicillin sensitive strains [34,35]. This resulted in a proteomic signature containing many elements of the *sigB* (encoding sigma factor B, σ^B;) [15] and *sarA* regulons [36], further implicating these two global regulators in *S. aureus* antibiotic resistance. Intriguingly, the proteomic signature was slightly different for previously resistant strains when compared to sensitive strains, and these differences included enzymes encoding proteins involved in cell wall and peptidoglycan biosynthesis, including phosphoglucosamine mutase (GlmM).

3.3.1. Heat stress response

Preliminary proteome data have been published on the heat stress response of *S. aureus*. A temperature shift from 37 to 46 °C induced the production of at least eight proteins. Two of these were GroEL and GroES [37]. Besides these proteins, Dnak, ClpP and AhpC belong to the heat inducible protein stimulon in *S. aureus* growing in complex medium (Gertz et al., unpublished). The heat stress response of *S. aureus* has been fairly well elucidated, relying more on general methods of molecular genetics than on proteomics. The global repressors HrcA and CtsR might control the heat induction of the GroESL/DnaK machine and of the Clp proteases/ATPases [15,38,39].

3.3.2. General stress response

In *B. subtilis* the general stress regulon controlled by σ^B also belongs to the heat stress stimulon because σ^B-dependent proteins are among other stress and starvation stimuli strongly induced by heat stress [31,40]. The regulation of σ^B-dependent stress response in *S. aureus*, however, differs remarkably from that of *B. subtilis* (see below). There is a relatively high level of constitutive σ^B-expression in cells grown in synthetic medium which is not increased further by heat stress. In cells cultivated in a complex medium (LB), a transient heat induction of *sigB* was found which is, however, too low to be reflected in a distinct increase of the protein abundance [41]. As a consequence, the level of σ^B-dependent stress proteins in cells grown at 37 or 48 °C is similar. This is the reason why the ''stimulon approach'' failed to detect any general stress proteins. Because of the low or even lacking heat inducibility of the proteins, the question arises whether the σ^B-dependent general stress proteins belong to the heat stress stimulon in *S. aureus* at all.

To analyse the σ^B-dependent stress regulon a ''regulon approach'' was used, relying on a comparison of the wild type with a *sigB* mutant strain. By this approach about 25 proteins were either absent or present in lower amounts in the *sigB* mutant (Fig. 4, Table 2). Because the experiments had been done before the genome sequence became available, MALDI-TOF-MS techniques could not be used for protein identification. Therefore, N-terminal sequencing of 18 of these proteins allowed the identification of their genes (Table 2) and the analysis of the promoter structure in front of these genes. In all cases σ^B-dependent promoters were found but only a few genes seemed to be controlled solely by σ^B [15].

It is interesting to note that the signal transduction pathway from stress to σ^B activity is remarkably different from that of *B. subtilis*. The *sigB* operon encodes, in addition to σ^B, only the anti sigma factor RsbW, the anti-anti-sigma factor RsbV and the RsbV~P-phosphatase, RsbU [42,43]. The starvation induction module of *B. subtilis* (RsbP, Q) was not found in the genome of *S. aureus*, which may explain why energy limitation does not induce σ^B (Engelmann et al., unpublished). Furthermore, the proteins that stimulate the RsbU~P-phosphatase activity in response to stress are not present either, probably explaining the low and only transient heat induction of σ^B. The high expression of σ^B-dependent stress proteins during slow growth in synthetic medium and the lack of a strong regulation by environmental stimuli may indicate that the proteins are required under all life circumstances. This unusual induction profile also renders the classification of the regulon as a general stress regulon dubious,

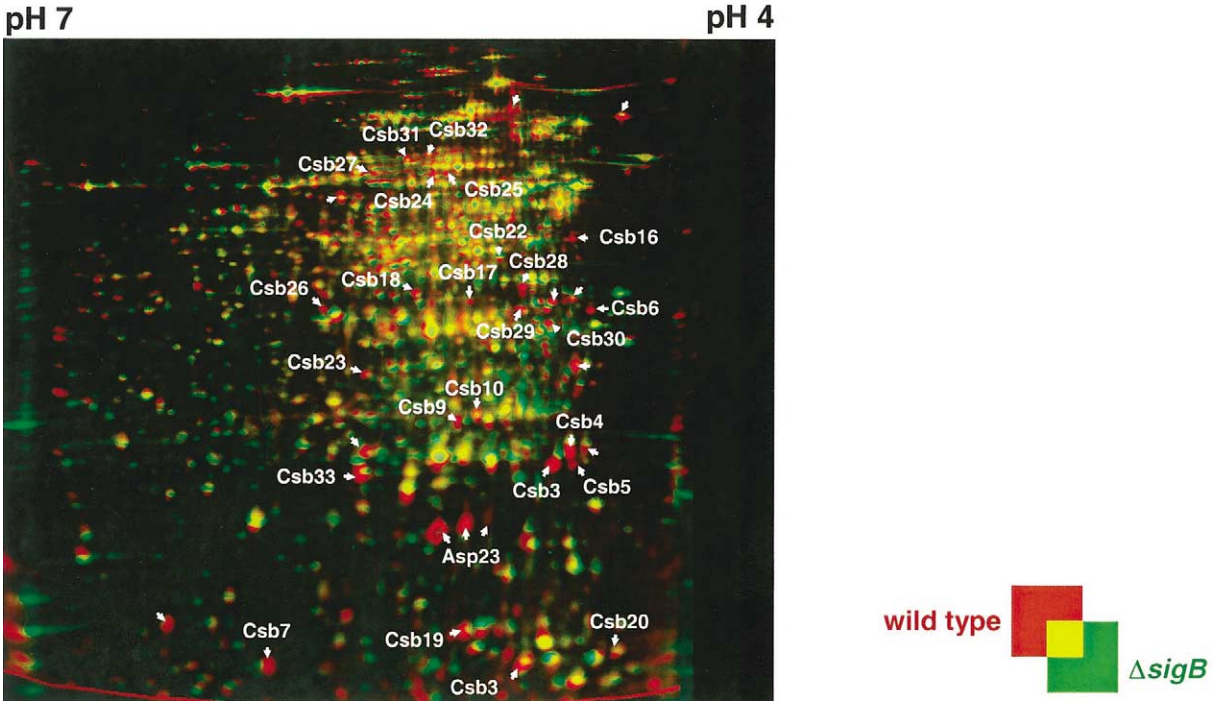

Fig. 4. Silver-stained two-dimensional electrophoresis gel of cytoplasmic protein extracts of *S. aureus* COL (wild type) and its isogenic *sigB* mutant (Δ*sigB*). Cells were grown in synthetic medium described earlier [15] and protein extracts were prepared from cells at an absorbance (500 nm) of 1.

because this regulon is characterized by a complex induction profile by a range of different stress and/or environmental stimuli [31]. It is tempting to speculate that by a long-standing interaction with its host, *S. aureus* has lost the stress induction pattern, possibly because of a permanent need for a high level of stress proteins.

This brings us to an essential question; what is the function of this large regulon. To cope with this problem, the function of all genes under σ^B control

Table 2
σ^B-Dependent cytoplasmic proteins of *S. aureus* COL

Protein	Function/similarity
Csb3	*S. aureus*: Yly1; hypothetical 18.6 kDa protein
Csb4	*B. subtilis*: YckG; hexulose-6-phosphate-synthase
Csb7	*B. subtilis*: YdfG; unknown
Csb8	*B. subtilis*: YwmG; unknown
Csb9	*S. aureus*: hypothetical protein
Csb10	*B. subtilis*: YurI; unknown, V296 vegetative protein, similar to ABC transporter protein
Csb12	*B. subtilis*: YtrI; unknown
Csb16	*L. monocytogenes*: DapE; succinyl-diaminopimelate-desuccinylase
Csb19	*B. subtilis*: YdaG; (GS26), unknown
Csb22	*Arthrobacter* sp.: ODH; opine dehydrogenase, norvalin dehydrogenase
Csb24	*Streptomyces coelicolor*: putative aldehyde dehydrogenase
Csb28	*B. subtilis*: YhxD; unknown, similar to ribitol-dehydrogenase
Csb33	*B. subtilis*: NagB; *N*-acetylglucosamine-6-phosphate-isomerase

should be completely understood. For this purpose a functional genomic assay search for the entire set of σ^B-dependent genes is required. A few years ago, some homologs of σ^B-dependent genes of *B. subtilis* were found to be under RpoS-control in *E. coli* [44–47]. Because RpoS is involved in the expression control of virulence genes in gram-negative [48–52] it was tempting to speculate that σ^B might be the equivalent of RpoS in gram-positive bacteria, also controlling the expression of virulence genes [15,18,41,53]. The function of this regulon in *S. aureus*, however, is still a matter of debate. Because *sigB* mutants of *S. aureus* are characterized by an impaired stress tolerance [53] the general stress proteins should provide the cells with multiple stress resistance, as in *B. subtilis* [31,40]. If this statement is true, a general prediction of the function of many still unknown proteins belonging to the σ^B-regulon will be feasible. This multiple stress resistance will probably be essential for its survival in various ecosystems, including the host, but the data on a putative involvement of σ^B in pathogenicity are still contradictory [53–55]. However, at least one of the global regulators of virulence genes, SarA, is partly under σ^B-control [15,56–58]. Furthermore, σ^B might be involved in the formation of biofilms of *S. aureus* and *S. epidermidis* [59,60]. Finally, σ^B might somehow be involved in the resistance control against the antibiotics methicillin and teicoplanin, since *sigB* mutants are more susceptible to these antibiotics, while strains over-producing σ^B become hyper-resistant [43,61,62]. Further experiments are, however, required to present more clear data on the involvement of σ^B-dependent genes in pathogenicity.

3.3.3. Oxygen availability and limitation

Oxygen availability is probably a crucial environmental stimulus for *S. aureus*. Within surface lesions, oxygen concentration will therefore not restrain bacterial growth, but because *S. aureus* has the ability to invade different tissues, oxygen might become one of the most crucial growth limiting signals forcing adaptational processes against oxygen limitation. *S. aureus* as a facultative anaerobe is able to cope with oxygen limitation by anaerobic respiration or even by fermentation. Recently, a two-component signal transduction system (*srhSR* and *srrAB*, respectively) sharing strong similarity with the ResDE regulon of *B. subtilis* was described [63,64]. ResDE of *B. subtilis* is a main global regulator controlling aerobic or anaerobic respiration [65–67]. Throup et al. [63] used the proteomics (regulon) approach to analyse the size and function of this two-component system, which might play a similarly essential role in the adaptation of *S. aureus* to changing oxygen concentrations. This was shown by a strong growth defect of a *srhS* mutant under anaerobic conditions. The differential display proteome approach of the wild type with the *srhS* mutant indicated that the synthesis of many proteins seems to be regulated by the response regulator SrhR in dependence of oxygen. The data suggest that among other things SrhR controls the up-regulation of glycolytic and fermentative enzymes as well as the down-regulation of aerobic TCA cycle enzymes that are no longer required in the absence of a terminal electron acceptor. This crucial type of regulation probably no longer exists in the mutant, leading to a total imbalance of energy metabolism and finally to a severe growth defect [63]. A similar situation may be typical for small colony variants that may have a defect in aerobic respiration [68–70] (Engelmann et al., unpublished). It is tempting to speculate that the SrhSR two-component system is required to maintain the energy metabolism of those cells even in the presence of oxygen.

3.3.4. Growth-phase dependent gene expression patterns—the role of sar and agr

SarA and *agr* are key regulators involved in the expression of many virulence genes whose products are required for host cell adhesion, for evasion of host defence mechanisms, tissue damage or tissue invasion. The expression of these genes is regulated in a coordinated manner during the growth cycle that ensures expression of cell surface-associated proteins during growth and that of extracellular virulence factors during the post-exponential phase of growth after reaching a critical cell density [71–76]. SarA is a DNA-binding protein that activates the transcription of several target genes including *spa*, coding for protein A, *fnb* coding for the fibronectin-binding protein or even *agr* itself [77–79]. The *agr* locus encodes a two-component signal transduction system that responds to a quorum sensing signal [80,81].

After reaching a critical cell density indicated by the quorum sensing octapeptides, the sensorkinase AgrC becomes phosphorylated and activates the response regulator AgrA by phosphorylation. Phosphorylated AgrA stimulates the transcription of RNAIII, which activates the synthesis of extracellular proteins and represses the production of cell wall-associated proteins [80–82].

The sequential activity of SarA and *agr* during growth and stationary phase might be one of the reasons for this sequential expression of SarA-dependent cell wall-associated, and *agr*-dependent extracellular proteins. Since SarA and *agr* seem to be the major factors in regulating virulence gene expression, many laboratories have focused on the analysis of *sar*/*agr*-dependent gene expression [18,36]. Nevertheless, a proteomic view of both regulons should reveal new members of the regulon that have escaped classical studies. Therefore the extracellular proteomes of a wild type strain and of a *sarA* mutant strain were compared. These studies revealed three proteins (β-haemolysin, aureolysin and V8 protease) as already described in the literature (see Ziebandt et al. [18]), as well as four proteins newly described as SarA-induced. A further seven proteins were shown to be SarA-repressed [18].

In a similar approach with an *agr* mutant, 10 proteins were found to be positively regulated by RNAIII. In contrast two proteins seem to be repressed in the presence of RNAIII (Ziebandt et al., in preparation). It was presumed that RNAIII mainly up-regulates extracellular virulence factors but down-regulates cell surface virulence factors. After reaching a high cell density, virulence factor expression switches to a more invasive system including extracellular proteins capable of destroying host cells (proteases, lipases, toxins) or of damaging the immune response system (superantigens) are induced. Staphylococcal proteases can cleave and degrade a number of host proteins, including the heavy chains of all human immunoglobulin classes, plasma-proteinase inhibitor and elastin. Very recently it was shown that proteases also play a role in the transition of *S. aureus* cells from adhesive to an invasive phenotype by degrading bacterial cell surface proteins such as fibronectin-binding protein and protein A [77,83,84].

3.4. A pathogenicity network model

The single regulons activated in response to environmental stimuli do not function independently from each other but are tightly connected forming a complex adaptational network. Because of the crucial role of SarA and *agr* in the control of pathogenicity, the interaction of both regulons and their integration into the network may form a core region of pathogenicity. Proteomics and DNA array technologies have already been used and will continue to be used to provide a more comprehensive knowledge, not only on the single members of both regulons, but also on their kinetics of expression in a temporal manner [18,36]. While SarA and its regulon may be active at early infection stages ensuring adhesion on the host and possibly biofilm formation, this ''settlement phase'' might be replaced in later infection stages by the cell density-dependent and *agr*-controlled induction of extracellular virulence genes. Recent data suggest that σ^B might interfere with this temporal activation of SarA followed by *agr*. In a *sigB* mutant, many *agr*-dependent extracellular proteins are overproduced during exponential growth while the same genes remained repressed in the wild type [18]. These data suggest that σ^B prevents a premature activation of the *agr*-dependent production of extracellular virulence proteins. These still preliminary data suggest that pathogenicity exists through a network of interacting regulons (σ^B is involved in SarA expression, SarA controls *agr*, etc.) and is characterized by tightly controlled temporal gene expression patterns, ensuring the activity of the regulons and their proteins exactly at that moment when they are absolutely required.

Recent studies indicate that this pathogenicity network is not confined to the interactions between SigB, SarA and *agr*. Newly described global regulators and their regulons, such as ArlSR, SsrAB, Sae, and various Sar-like proteins, will probably show that the network is much more complex [63,64,85–88]. A new two-component system, ArlSR, for instance, seems to be involved in adhesion and autolysis. An *arlS* mutant of *S. aureus* 8325-4 showed an increased adhesion to polystyrene followed by biofilm formation and a dramatic decrease of extracellular proteolytic activation [86].

The consequent usage of proteomics will not only offer the chance to characterize all members and functions of these new regulons in a comprehensive manner, but will also elucidate their interaction within the pathogenicity network.

Up to now, however, we are far removed from this aim. In addition to proteomics, other highly sophisticated techniques such as signature tagged mutagenesis that informs us on gene activity in the host [89,90] or DNA array techniques [91] are required to elucidate the network. This last approach, which will be used more and more in future studies, does not only allow a more complete characterization of the genes belonging to stimulons and regulons compared to proteomics (because—as outlined above—many proteins still escape proteomic detection), but also because of its high sensitivity offers the chance to analyse the gene expression pattern of *S. aureus* cells in infection models. This combined application of proteomics and DNA techniques supplemented by bioinformatics will provide a continuously growing picture on that what is really going on during the growth cycle or during infection and disease.

4. Application of *S. aureus* proteomics

There are several fields that may use the proteomic information summarized in this article including: strain evaluation, mechanisms of antibiotic resistance and new targets and serological proteomics—new antigens, new vaccines and protection, new diagnostic tools.

4.1. Strain evaluation

Comparisons of cytosolic proteins from *S. aureus* strains using proteomics [14] showed they were remarkably conserved in two areas—spot co-ordinates and abundance, and protein expression. This means that not only do phenotypically different strains express typically the same set of proteins under basal conditions, but that these proteins are highly conserved at the amino acid sequence level, and hence migrate identically on 2D gels across strain boundaries. This is quite dissimilar to results found in other bacteria, for example, *Helicobacter pylori*, where different strains produce completely different 2D gel patterns [92]. Comparing the protein pattern of extracellular proteins from different *S. aureus* strains, however, showed a very different composition. There is a set of proteins, including the haemolysins, lipases and proteases, that are secreted in almost all strains, however, most of the strains produce at least one or more unique exoproteins (enterotoxins, exotoxins, leukocidin) that are not seen in other strains and that might be responsible for the different pathogenic potential of each [18,21]. The combination of one-dimensional protein gel electrophoresis and MALDI-TOF-MS could be a good tool for a rapid analysis of pathogenic factors in clinical isolates [21].

4.2. Mechanisms of antibiotic resistance and new targets

S. aureus is a leading cause of infection in immunocompromised patients and is responsible for the majority of nosocomially-acquired infections in the Western world. However, antibiotic resistance has become an increasing problem in its treatment with the rapid proliferation of not only methicillin resistant strains, but also strains resistant to the latest generation of glycopeptide antibiotics, including vancomycin and teicoplanin. The emergence of multi-drug resistant *S. aureus* strains urgently demands new antibiotics. Functional and comparative genomics offers a big chance for finding new antibiotics with completely new action mechanisms. Surprisingly, however, antibiotic resistant and sensitive strains appear to be highly conserved at the proteome level, at least in initial reports [14]. It seems likely that cell wall constituents, which are poorly amenable to the proteomics approach, may hold some of the answers to resistance in those strains. Hence development of new methodology for the micro-characterization of highly hydrophobic proteins in proteomics continues to be a priority. Furthermore, the discovery that approximately one-third of the genome codes for proteins with still unknown function is one of the general conclusions from genome sequencing. Among these genes are a few that code for essential proteins. These new essential genes can be found by a systematic muta-

tion approach of all unknown genes. Those still unknown, but essential genes that occur only in prokaryotic, but not in eukaryotic genomes, might be excellent candidates for new drug targets. Although conclusive results are still lacking one can expect that many research programmes are already running in several laboratories to use this new opportunity for target identification.

4.3. Serological proteomics—new antigens, new diagnostic tools, new vaccines and protection

A comprehensive knowledge on the surface and extracellular proteins produced by a set of different *S. aureus* strains is a good basis for the development of new diagnostic tools and new vaccines. Bacterial vaccine candidate antigens can be found by screening of sera from patients suffering from *S. aureus* infections. This serological proteomic approach was followed by Vytvytska et al. [17] who identified 15 potential vaccine candidates including proteins thought to be confined to the bacterial cytosol. Similar work reported by Lorenz et al. [93], and Vytvytska et al. [17] also confirmed the presence of a highly abundant ''immunodominant staphylococcal antigen'' (IsaA), with no sequence similarity to proteins of known function. This protein has been detected in the *S. aureus* cytosol [14] and extracellular matrix [18], and appears to be σ^B-regulated. Cordwell et al. [14] also showed significant differences in IsaA abundance levels, and possibly in post-translational modification in the antibiotic resistant COL strain when compared to antibiotic sensitive strains. Therefore, IsaA is an obvious choice for further study, with a focus at elucidating its biological function and ability to interact with other *S. aureus* proteins, as well as those derived from the host [93]. An alternative approach has been taken by Etz et al. [94] who utilized fusion peptide display to *E. coli* outer membrane proteins to screen against patient sera. At least 60 potential vaccine candidates were characterized using this approach. The apparently highly enriched selection of both surface-exposed and predicted secreted proteins, in comparison to traditional Western blotting methods constitutes an important finding. Etz et al. [94] were also able to report on which regions of each protein were capable of binding antibodies, and hence some information on surface-accessible epitopes. These new staphylococcal antigens are not only good candidates for the development of diagnostic protein chips but may also provide a reasonable approach for rational vaccine design.

5. Conclusion

The sequencing of the genome of *S. aureus* marks the beginning of a new era in *S. aureus* biology that will substantially influence future *S. aureus* research over the next 10 years or far beyond. The sequencing of the *S. aureus* genome, however, yields only a first, more introductory chapter in the ''Book of *S. aureus* Life''. A long way has to be followed to bring this blue-print of life to ''real'' life. This does not only mean an understanding of the physiology of the growth or stationary phase of cells cultivated in vitro, but also of the physiology of cells grown in different habitats in the host including the complex interaction of cells with the host system. To provide this new and comprehensive information constitutes the big challenge for the single fields of functional genomics are such as transcriptomics, proteomics or bioinformatics. Despite the big role that *S. aureus* plays in human life its functional genomics is still at a very preliminary state. Transcriptomics will be used more and more to assign the 2600 genes to various regulation groups and to uncover regulation of global genome expression, and proteomics will be applied to explore the complex protein interaction networks of cells, including information on protein modification, protein damage, repair or degradation, protein localization and targeting. This review shows that proteomics of *S. aureus* is still in a preliminary state. An essential goal for future studies will be to make full use of the big potential of proteomics to observe events in the cell never seen before by looking at the majority of almost all cell proteins. This new and wider view visualized by proteomics, however, will need techniques of molecular genetics to elucidate and analyse the observed phenomena at the molecular level. Proteomics in combination with molecular genetics, biochemistry or biophysics are the tools required to lead to a new understanding of *Staphylococcus* life.

Acknowledgements

We are very grateful to Anne-Kathrin Ziebandt, Christian Kohler, Silke Gertz, Harald Weber, Sandra Lövenich, and Jan Pané-Farré who contribute to this article with excellent results on proteomics of *S. aureus*. Helga Korthase is acknowledged for her assistance in preparing the manuscript and Volker Brözel for critical comments on the manuscript. Furthermore, we thank Renate Gloger for excellent technical assistance. We are especially grateful to Jörg Hacker, Wilma Ziebuhr, and Knut Ohlsen for a longstanding and fruitful cooperation and Decodon GmbH for providing Delta2D Software. This work was supported by grants of the BMBF (031U107A/031U207A, 03U213B), the Bayerische Forschungsstiftung and the Fonds der Chemischen Industrie to M.H. and by the access to the Australian Proteome Analysis Facility funded under the Australian government Major National Research Facility (MNRF) program.

References

[1] R.D. Fleischmann, M.D. Adams, O. White, R.A. Clayton, E.F. Kirkness, A.R. Kerlavage, C.J. Bult, J.-F. Tomb, B.A. Dougherty, J.M. Merrick, K. McKenney, G.G. Sutton, W. FitzHugh, C.A. Fields, J.D. Gocayne, J.D. Scott, R. Shirley, L.I. Liu, A. Glodek, J.M. Kelley, J.F. Weidman, C.A. Phillips, T. Spriggs, E. Hedblom, M.D. Cotton, T. Utterback, M.C. Hanna, D.T. Nguyen, D.M. Saudek, R.C. Brandon, L.D. Fine, J.L. Fritchman, J.L. Fuhrmann, N.S. Geoghagen, C.L. Gnehm, L.A. McDonald, K.V. Small, C.M. Fraser, H.O. Smith, J.C. Venter, Science 269 (1995) 496.

[2] M. Kuroda, T. Ohta, I. Uchiyama, T. Baba, H. Yuzawa, I. Kobayashi, L. Cui, A. Oguchi, K. Aoki, Y. Nagai, J. Lian, T. Ito, M. Kanamori, H. Matsumaru, A. Maruyama, H. Murakami, A. Hosoyama, Y. Mizutani-Ui, N.K. Takahashi, T. Sawano, R. Inoue, C. Kaito, K. Sekimizu, H. Hirakawa, S. Kuhara, S. Goto, J. Yabuzaki, M. Kanehisa, A. Yamashita, K. Oshima, K. Furuya, C. Yoshino, T. Shiba, M. Hattori, N. Ogasawara, H. Hayashi, K. Hiramatsu, Lancet 357 (2001) 1225.

[3] M. Wilm, A. Shevchenko, T. Houthaeve, S. Breit, L. Schweigerer, T. Fotsis, M. Mann, Nature 379 (1996) 466.

[4] K. Büttner, J. Bernhardt, C. Scharf, R. Schmid, U. Mäder, C. Eymann, H. Antelmann, A. Völker, U. Völker, M. Hecker, Electrophoresis 22 (2001) 2908.

[5] R.A. VanBogelen, K.Z. Abshire, B. Moldover, E.R. Olson, F.C. Neidhardt, Electrophoresis 18 (1997) 1243.

[6] S.J. Cordwell, D.J. Basseal, B. Bjellqvist, D.C. Shaw, I. Humphery-Smith, Electrophoresis 18 (1997) 1393.

[7] A. Görg, C. Obermaier, G. Boguth, A. Harder, B. Scheibe, R. Wildgruber, W. Weiss, Electrophoresis 21 (2000) 1037.

[8] M.P. Molloy, N.D. Phadke, H. Chen, R. Tyldesley, D.E. Garfin, J.R. Maddock, P.C. Andrews, Proteomics 2 (2002) 899.

[9] S. Ohlmeier, C. Scharf, M. Hecker, Electrophoresis 21 (2000) 3701.

[10] A.S. Nouwens, S.J. Cordwell, M.R. Larsen, M.P. Molloy, M. Gillings, M.D. Willcox, B.J. Walsh, Electrophoresis 21 (2000) 3797.

[11] S.J. Cordwell, A.S. Nouwens, N.M. Verrills, D.J. Basseal, B.J. Walsh, Electrophoresis 21 (2000) 1094.

[12] S.P. Gygi, B. Rist, S.A. Gerber, F. Turecek, M.H. Gelb, R. Aebersold, Nat. Biotechnol. 17 (1999) 994.

[13] M.P. Washburn, D. Wolters, J.R. Yates III, Nat. Biotechnol. 19 (2001) 242.

[14] S.J. Cordwell, M.R. Larsen, R.T. Cole, B.J. Walsh, Microbiology 148 (2002) 2765.

[15] S. Gertz, S. Engelmann, R. Schmid, A.-K. Ziebandt, K. Tischer, C. Scharf, J. Hacker, M. Hecker, J. Bacteriol. 182 (2000) 6983.

[16] V.K. Singh, R.K. Jayaswal, B.J. Wilkinson, FEMS Microbiol. Lett. 199 (2001) 79.

[17] O. Vytvytska, E. Nagy, M. Bluggel, H.E. Meyer, R. Kurzbauer, L.A. Huber, C.S. Klade, Proteomics 2 (2002) 580.

[18] A.-K. Ziebandt, H. Weber, J. Rudolph, R. Schmid, D. Höper, S. Engelmann, M. Hecker, Proteomics 1 (2001) 480.

[19] O. Schneewind, P. Model, V.A. Fischetti, Cell 24 (1992) 267.

[20] O. Schneewind, D. Mihaylova-Petkov, P. Model, EMBO J. 12 (1993) 4803.

[21] K. Bernardo, S. Fleer, N. Pakulat, O. Krut, F. Hunger, M. Krönke, Proteomics 2 (2002) 740.

[22] S. Herbert, P. Barry, R.P. Novick, Infect. Immun. 69 (2001) 2996.

[23] Y. Kawano, Y. Ito, Y. Yamakawa, T. Yamashino, T. Horii, T. Hasegawa, M. Ohta, FEMS Microbiol. Lett. 189 (2000) 103.

[24] Y. Kawano, M. Kawagishi, M. Nakano, K. Mase, T. Yamashino, T. Hasegawa, M. Ohta, Microbiol. Immunol. 45 (2001) 285.

[25] M. Nakano, Y. Kawano, M. Kawagish, T. Hasegawa, Y. Iinuma, M. Oht, Microbiol. Immunol. 46 (2002) 11.

[26] G.R. Drapeau, J. Bacteriol. 136 (1978) 607.

[27] K. Rice, R. Peralta, D. Bast, J. de Azavedo, M.J. McGavin, Infect. Immun. 69 (2001) 159.

[28] A. Sabat, K. Kosowska, K. Poulsen, A. Kasprowicz, A. Sekowska, B. van Den Burg, J. Travis, J. Potempa, Infect. Immun. 68 (2000) 973.

[29] J. Bernhardt, K. Büttner, J.Y. Coppée, C. Lelong, N. Ogasawara, C. Scharf, V. Vagner, R. Schmid, U. Völker, M. Hecker, in: W. Schumann, S.D. Ehrlich, N. Ogasawara (Eds.), Functional Analysis of Bacterial Genes: A Practical Manual, Wiley, Weinheim, 2001, p. 63.

[30] S. Tobisch, D. Zühlke, J. Bernhardt, J. Stülke, M. Hecker, J. Bacteriol. 181 (1999) 6996.

[31] M. Hecker, U. Völker, Adv. Microb. Physiol. 44 (2001) 35.

[32] M. Hecker, S. Engelmann, Int. J. Med. Microbiol. 290 (2000) 123.

[33] R.A. VanBogelen, E.E. Schiller, J.D. Thomas, F.C. Neidhardt, Electrophoresis 20 (1999) 2149.
[34] H. Komatsuzawa, J. Suzuki, M. Sugai, Y. Miyake, H. Suginaka, J. Antimicrob. Chemother. 34 (1994) 885.
[35] H. Komatsuzawa, M. Sugai, C. Shirai, J. Suzuki, K. Hiramatsu, H. Suginaka, FEMS Microbiol. Lett. 134 (1995) 209.
[36] P.M. Dunman, E. Murphy, S. Haney, D. Palacios, G. Tucker-Kellogg, S. Wu, E.L. Brown, R.J. Zagursky, D. Shlaes, S.J. Projan, J. Bacteriol. 183 (2001) 7341.
[37] T. Ohta, K. Honda, M. Kuroda, K. Saito, H. Hayashi, Biochem. Biophys. Res. Commun. 193 (1993) 730.
[38] I. Derré, G. Rapoport, T. Msadek, Mol. Microbiol. 31 (1999) 117.
[39] A. Schulz, W. Schumann, J. Bacteriol. 178 (1996) 1088.
[40] C.W. Price, in: G. Storz, R. Hengge-Aronis (Eds.), Bacterial Stress Responses, American Society for Microbiology, Washington, DC, 2000, p. 179.
[41] S. Gertz, S. Engelmann, R. Schmid, K. Ohlsen, J. Hacker, M. Hecker, Mol. Gen. Genet. 261 (1999) 558.
[42] I. Kullik, P. Giachino, Arch. Microbiol. 167 (1997) 151.
[43] S. Wu, H. de Lencastre, A. Tomasz, J. Bacteriol. 178 (1996) 6036.
[44] H. Antelmann, S. Engelmann, R. Schmid, A. Sorokin, A. Lapidus, M. Hecker, J. Bacteriol. 179 (1998) 7251.
[45] S. Engelmann, C. Lindner, M. Hecker, J. Bacteriol. 177 (1995) 5595.
[46] F. Spiegelhalter, E. Bremer, Mol. Microbiol. 29 (1998) 285.
[47] U. Völker, K.K. Andersen, H. Antelmann, K.M. Devine, M. Hecker, J. Bacteriol. 180 (1998) 4212.
[48] C.Y. Chen, N.A. Buchmeier, S. Libby, F.C. Fang, M. Krause, D.G. Guiney, J. Bacteriol. 177 (1995) 5303.
[49] F.C. Fang, S.J. Libby, N.A. Buchmeier, P.C. Loewen, J. Switala, J. Harwood, D.G. Guiney, Proc. Natl. Acad. Sci. USA 89 (1992) 11978.
[50] C.A. Nickerson, R. Curtiss III, Infect. Immun. 65 (1997) 1814.
[51] S.J. Suh, L. Silo-Suh, D.E. Woods, D.J. Hassett, S.E. West, D.E. Ohman, J. Bacteriol. 181 (1999) 3890.
[52] F.H. Yildiz, G.K. Schoolnik, J. Bacteriol. 180 (1998) 773.
[53] I. Kullik, P. Giachino, T. Fuchs, J. Bacteriol. 180 (1998) 4814.
[54] P.F. Chan, S.J. Foster, E. Ingham, M.O. Clements, J. Bacteriol. 180 (1998) 6082.
[55] R.O. Nicholas, T. Li, D. McDevitt, A. Marra, S. Sucoloski, P.L. Demarsh, D.R. Gentry, Infect. Immun. 67 (1999) 3667.
[56] A.L. Cheung, Y.T. Chien, A.S. Bayer, Infect. Immun. 67 (1999) 1331.
[57] R. Deora, T. Tseng, T.K. Misra, J. Bacteriol. 179 (1997) 6355.
[58] A.C. Manna, M.G. Bayer, A.L. Cheung, J. Bacteriol. 180 (1998) 3828.
[59] J.K. Knobloch, K. Bartscht, A. Sabottke, H. Rohde, H.H. Feucht, D. Mack, J. Bacteriol. 183 (2001) 2624.
[60] S. Rachid, S. Cho, K. Ohlsen, J. Hacker, W. Ziebuhr, Adv. Exp. Med. Biol. 485 (2000) 159.
[61] M. Bischoff, M. Roos, J. Putnik, A. Wada, P. Glanzmann, P. Giachino, P. Vaudaux, B. Berger-Bächi, FEMS Microbiol. Lett. 194 (2001) 77.
[62] K. Morikawa, A. Maruyama, Y. Inose, M. Higashide, H. Hayashi, T. Ohta, Biochem. Biophys. Res. Commun. 288 (2001) 385.
[63] J.P. Throup, F. Zappacosta, R.D. Lunsford, R.S. Annan, S.A. Carr, J.T. Lonsdale, A.P. Bryant, D. McDevitt, M. Rosenberg, M.K. Burnham, Biochemistry 40 (2001) 10392.
[64] J.M. Yarwood, J.K. McCormick, P.M. Schlievert, J. Bacteriol. 183 (2001) 1113.
[65] M.M. Nakano, Y. Zhu, M. Lacelle, X. Zhang, F.M. Hulett, Mol. Microbiol. 37 (2000) 1198.
[66] M.M. Nakano, Y. Zhu, J. Bacteriol. 183 (2001) 1938.
[67] M.M. Nakano, J. Bacteriol. 184 (2002) 1783.
[68] R.A. Proctor, J.M. Balwit, O. Vesga, Infect. Agents Dis. 3 (1994) 302.
[69] R.A. Proctor, P. van Langevelde, M. Kristjansson, J.N. Maslow, R.D. Arbeit, Clin. Infect. Dis. 20 (1995) 95.
[70] C. von Eiff, C. Heilmann, R.A. Proctor, C. Woltz, G. Peters, F. Götz, J. Bacteriol. 179 (1997) 4706.
[71] A.L. Cheung, J.M. Koomey, C.A. Butler, S.J. Projan, V.A. Fischetti, Proc. Natl. Acad. Sci. USA 89 (1992) 6462.
[72] A.L. Cheung, P. Ying, J. Bacteriol. 176 (1994) 580.
[73] E. Morfeldt, L. Janzon, S. Arvidson, S. Lofdahl, Mol. Gen. Genet. 211 (1988) 435.
[74] H.L. Peng, R.P. Novick, B. Kreiswirth, J. Kornblum, P. Schlievert, J. Bacteriol. 170 (1988) 4365.
[75] S.J. Projan, R.P. Novick, in: A.B. Crossley, G.L. Archer (Eds.), The Staphylococci in Human Disease, Churchill Livingstone, 1997, p. 55.
[76] P. Recsei, B. Kreiswirth, M. O'Reilly, P. Schlievert, A. Gruss, R.P. Novick, Mol. Gen. Genet. 202 (1986) 58.
[77] A.L. Cheung, K. Eberhardt, J.H. Heinrichs, Infect. Immun. 65 (1997) 2243.
[78] Y. Chien, A.C. Manna, S.J. Projan, A.L. Cheung, J. Biol. Chem. 274 (1999) 37169.
[79] J.H. Heinrichs, M.G. Bayer, A.L. Cheung, J. Bacteriol. 178 (1996) 418.
[80] G. Ji, R.C. Beavis, R.P. Novick, Proc. Natl. Acad. Sci. USA 92 (1995) 12055.
[81] R.P. Novick, S.J. Projan, J. Kornblum, H.F. Ross, G. Ji, B. Kreiswirth, F. Vandenesch, S. Moghazeh, Mol. Gen. Genet. 248 (1995) 446.
[82] R.P. Novick, H.F. Ross, S.J. Projan, J. Kornblum, B. Kreiswirth, S. Moghazeh, EMBO J. 12 (1993) 3967.
[83] A. Karlsson, P. Saravia-Otten, K. Tegmark, E. Morfeldt, S. Arvidson, Infect. Immun. 69 (2001) 4742.
[84] M.J. McGavin, C. Zahradka, K. Rice, J.E. Scott, Infect. Immun. 65 (1997) 2621.
[85] A.L. Cheung, G. Zhang, Front Biosci. 1 (2002) 1825.
[86] B. Fournier, D.C. Hooper, J. Bacteriol. 182 (2000) 3955.
[87] B. Fournier, A. Klier, G. Rapoport, Mol. Microbiol. 41 (2001) 247.
[88] A.T. Giraudo, A. Calzolari, A.A. Cataldi, C. Bogni, R. Nagel, FEMS Microbiol. Lett. 177 (1999) 15.

[89] J. Mecsas, Curr. Opin. Microbiol. 5 (2002) 33.
[90] J.E. Shea, J.D. Santangelo, R.G. Feldman, Curr. Opin. Microbiol. 3 (2000) 451.
[91] G.K. Schoolnik, Adv. Microb. Physiol. 46 (2002) 1.
[92] P.R. Jungblut, D. Bumann, G. Haas, U. Zimny-Arndt, P. Holland, S. Lamer, F. Siejak, A. Aebischer, T.F. Meyer, Mol. Microbiol. 36 (2000) 710.
[93] U. Lorenz, K. Ohlsen, H. Karch, M. Hecker, A. Thiede, J. Hacker, FEMS Immunol. Med. Microbiol. 29 (2000) 145.
[94] H. Etz, D.B. Minh, T. Henics, A. Dryla, B. Winkler, C. Triska, A.P. Boyd, J. Sollner, W. Schmidt, U. von Ahsen, M. Buschle, S.R. Gill, J. Kolonay, H. Khalak, C.M. Fraser, A. von Gabain, E. Nagy, A. Meinke, Proc. Natl. Acad. Sci. USA 99 (2002) 6573.