International Journal of Medical Microbiology 304 (2014) 110–120

Contents lists available at ScienceDirect

International Journal of Medical Microbiology

journal homepage: www.elsevier.com/locate/ijmm

Mini Review

The *Staphylococcus aureus* proteome

Andreas Otto[a], Jan Maarten van Dijl[b], Michael Hecker[a], Dörte Becher[a,*]

[a] *Institut für Mikrobiologie, Ernst-Moritz-Arndt-Universität Greifswald, Friedrich-Ludwig-Jahn-Str. 15, D-17489 Greifswald, Germany*
[b] *Department of Medical Microbiology, University of Groningen, University Medical Center Groningen, Hanzeplein 1, P.O. Box 30001, 9700 RB Groningen, The Netherlands*

ARTICLE INFO

Keywords:
Physiological proteomics
Entire proteome
2D gel electrophoresis
MS-based proteomics
Stress/starvation responses
Virulence factors
Surfacome
Membrane vesicles
Biofilms

ABSTRACT

Staphylococcus aureus is a Gram-positive commensal bacterium that is regarded as a major threat for modern health care systems. This relates both to the ability of *S. aureus* to overcome antibiotic therapy by developing high-level resistance against multiple antibiotics and this bacterium's extensive arsenal of virulence factors. Understanding the mechanisms of resistance and functional studies on stress and starvation responses are the main goals of proteomics in staphylococcal research. This review high-lights recent advances in gel-based and gel-free proteomics analyses of *S. aureus* and pinpoints the importance of location-specific proteomics studies targeting the cytosol, the membrane, the cell surface and the extracellular milieu in combination with integrated global proteome studies. Emerging hot topics in staphylococcal proteomics are discussed with special focus on *in vivo* proteomics, membrane vesicles, biofilm formation and the acquisition of absolute proteome data for systems biological modeling approaches.

Introduction

In recent years the rapid development of multiple antibiotic resistances in pathogenic bacteria has gained much attention from both specialists and the broader public, because infections with resistant bacteria lead to increased morbidity and mortality of patients as well as to increased healthcare costs. Especially the threat of nosocomial and community-acquired infections by multi-resistant *Staphylococcus aureus* leading to life-threatening diseases is seen as a major challenge for the future of modern health care (CDC, 1999; Klevens et al., 2007).

S. aureus is a Gram-positive commensal bacterium that colonizes about one third of the human population without affecting its host (Wertheim et al., 2005). Nevertheless, *S. aureus* is a notorious causative agent of many different diseases, ranging from mild skin infections to severe systemic infections, including endocarditis, osteomyelitis, pneumonia and sepsis (Lowy, 1998). Whilst it is largely unknown which determinants inside the human host trigger the switch from a commensal lifestyle toward pathogenicity (Otto, 2010, 2012b), the molecular effectors of pathogenicity are better understood due to intensive research (Sibbald et al., 2006; Dreisbach et al., 2011b). These effectors (i.e. virulence factors) belong to distinct groups characterized by their subcellular localization or their function. First, adhesins are located at the bacterial cell surface where they function in cell to cell adherence for example within biofilms or in host–pathogen interactions thereby facilitating colonization and invasion of the host (Foster and Hook, 1998). Second, toxins are actively involved in the degradation of host cells to break the host's defenses and acquire nutrients (Dinges et al., 2000). Third, factors helping in evading the host's immune responses and proteins needed for survival inside the host contribute to the virulence of *S. aureus* (Engelmann and Hecker, 2009; Bestebroer et al., 2010; Spaan et al., 2013).

The fast adaptation of *S. aureus* to the host defenses or new antimicrobial therapies through evolution of existing genes or horizontal acquisition of new virulence genes makes it necessary to develop novel therapeutic strategies to combat staphylococcal infections. An important strategy is the development of new antibiotics, which has received insufficient attention especially since the early 1980s until present (Bandow and Metzler-Nolte, 2009). Furthermore, the development of vaccines or protective antibodies would be most welcome for fighting infections caused by *S. aureus* (Ziebandt et al., 2010; van den Berg et al., 2011). Both approaches require a thorough understanding of the staphylococcal physiology and pathophysiology in general, and the function of the effectors of virulence in particular. To achieve this important objective, global and well-integrated functional studies are needed including genomics, transcriptomics, proteomics and metabolomics. Here, proteomics is unique in the information that is provided on proteins – the main workhorses of life – thereby reflecting the actual condition of the cell. Proteomics becomes even more powerful when combined with transcriptomics as the profiling of transcripts

* Corresponding author.
E-mail address: dbecher@uni-greifswald.de (D. Becher).

1438-4221/$ – see front matter © 2013 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.ijmm.2013.11.007

gives deep and early-stage insights in the global control of gene expression, which closely reflects the physiological needs of the cell (Becher et al., 2009). Proteomics nestled in between other powerful 'omics' techniques has become pivotal in the analysis of complex biological systems at a global scale. In general, proteomics studies aim at the understanding of complex biological systems at the level of basic physiology or more complex regulations due to imposed stress and starvation. Consequently, based on to the importance of research covering basic physiology, virulence and finding possible new targets in antibiotic therapy against *S. aureus*, it is of increased importance that robust proteomics workflows are readily available for the staphylococcal research community (Hecker et al., 2009, 2010; Francois et al., 2010).

2D gel-based proteomics in *S. aureus* research

Historically, the success of proteomics as a research field is closely linked to the introduction of two-dimensional gel electrophoresis (2DE). Despite new, gel-free and mass spectrometry-based workflows that have been introduced in proteomics-based research since then, 2DE as a *top-down* proteomics approach is still indispensable in life science research. Since the first application in 1975 by O'Farrell (O'Farrell, 1975), 2D-gel-based proteomics has remained unparalleled in providing panorama views of the main constituents of cellular life, the proteins (Hecker et al., 2010). This can mainly be attributed to the matching of the analytical window of 2D gels with the physico-chemical properties of the mostly soluble, cytosolic proteins. For *S. aureus* strain COL, in the p*I* range of 4–7 (main window) and in the p*I* range of 6–11 (alkaline region), approximately 700 proteins can be simultaneously distinguished and traced throughout different physiological situations (Becher et al., 2009). This allows for a clear visualization of the main biochemical pathways at a glance. Moreover, the abundance of individual proteins on a 2D gel can be assessed by staining with fluorescent dyes, which allows for a quantitative and sensitive detection of proteins over a wide dynamic range (Maass et al., 2011). By taking advantage of suitable software for image analysis, proteomics samples can thus be differentially analyzed at the level of individual proteins (Berth et al., 2007). The features of a global view on different protein species, including post-translational modifications (e.g. phosphorylation, limited proteolysis) and unambiguous information on gradual or absolute changes in the presence of specific proteins depending on the biological setting can be integrated into comprehensive comparisons of physiological processes, which is needed for understanding the behavior of the human pathogen *S. aureus*. One crucial application that is unique to gel-based proteomics, especially in prokaryotes, and that cannot yet be replaced by gel-free techniques is the visualization of newly synthesized (e.g. radioactively labeled) proteins at short time scales. This way, it is possible to distinguish proteins already present in the cell from those emerging in the context of growth and starvation (Bernhardt et al., 2003; Hecker et al., 2008).

Mass spectrometry-based proteomics – deeper insights through technological improvements

With the advent of modern mass spectrometry (MS) techniques combining high resolution and high mass accuracy, and the availability of novel gel-free sample preparation methods, an even further analytical depth has been achieved by proteomics (Cox and Mann, 2011). The nowadays predominantly gel-free MS-based proteomics approaches differ markedly in terms of comprehensiveness of the data acquired, versatility of the accessible samples and sensitivity. Thus, it is even possible to detect changes in proteins of very low abundance. This development came along with a shift in the perspective from protein-centered studies as is the case in 2D gel-based studies toward shotgun proteomics relying on digested protein extracts with peptides as the analytical targets. Today the newer techniques allow for the identification of thousands of peptides within a single liquid chromatography (LC) MS run providing substantially more information within a significantly shorter time than ever before (Kocher et al., 2011; Thakur et al., 2011). Furthermore, the latest proteomics techniques are geared toward reduced sample consumption: whilst for 2D gel-based proteomics μg quantities of sample proteins are needed, gel-free *in vivo* proteomic approaches manage to analyze samples in the *ng* range (Schmidt et al., 2010). For quantitative proteomics workflows an impressive diversity of approaches has been implemented so far. These range from relative to absolute quantification approaches based on the implementation of *in vivo* or *in vitro* isotopic labeling or even label-free analyses (Otto et al., 2012a).

A pathogen dissected: subcellular proteomics of *S. aureus*

Classical proteomics workflows for panorama views on basic cell physiology and determinants of virulence in S. aureus

For *S. aureus*, 2D gel-based physiological studies have been published comprising starvation experiments (e.g. glucose limitation and starvation (Kohler et al., 2003, 2005; Seidl et al., 2009)), the influence of an anaerobic life style on staphylococcal physiology (Fuchs et al., 2007), and the impact of oxidative stress on *S. aureus* (Hochgräfe et al., 2008; Wolf et al., 2008). It becomes clear that the classical proteomics techniques are perfectly suited to reflect changes of the entire metabolism by visualization of the main metabolic pathways in a single protein-centered assay. Consequently, 2D gel-based studies were instrumental in the comparison of the main Gram-positive bacterial model organisms, *Bacillus subtilis* and *S. aureus*, coping with oxidative stress affecting both protein expression and post-translational modifications readily visible on 2D gels (Hecker et al., 2009; Pöther et al., 2009, 2013).

Over the last years, a shift from single stress and starvation experiments toward the establishment of stress/signature libraries for *S. aureus* could be observed. Whilst a thorough understanding of the basic physiology under different stress conditions is most valuable for understanding the basic physiological adaptations of this pathogen, proteomics signature libraries have a high potential to assist in finding key enzymes and therefore key potential targets for antimicrobial therapies over a wide range of stimuli. Prerequisites for such libraries are sophisticated 2D analysis software packages relying on the powerful dual-channel imaging technique together with the possibility of high-throughput protein identification by Matrix-assisted laser desorption/ionization time-of-flight (MALDI ToF) MS (Bernhardt et al., 1999; Domon and Aebersold, 2006). Following this concept, Fuchs and coworkers have stored and processed results from 50 experimental data sets for *S. aureus* grown without oxygen as terminal electron acceptor in the database "Protecs" (Fuchs et al., 2010). By integration of altogether four different growth experiments related to anaerobiosis, a deeper understanding of the reliance of *S. aureus* on a terminal electron acceptor other than oxygen was achieved (Pagels et al., 2010). Additionally, these data libraries can be used to predict the physiological state and life style of cells grown under infection-related settings, such as biofilm formation/growth and to pinpoint the differences between planctonically growing *S. aureus* cells and biofilm-forming *S. aureus* cells (Resch et al., 2006). Another important aspect of 2D gel-based data libraries is the possibility to predict the mode of action of new antibiotics through the determination of particular stress signatures (Brötz-Oesterhelt et al., 2005). After this