International Journal of Medical Microbiology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Medical Microbiology

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

Mini Review

Global analysis of bacterial membrane proteins and their modifications

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ARTICLE INFO

Keywords:
Shotgun proteomics
Membrane proteins
Posttranslational modifications
Bacteria
Virulence

ABSTRACT

Membrane proteins are situated at the interface of bacterial cell and its environment, and are therefore involved in vital physiological processes such as nutrient exchange, signal transduction and virulence. Due to their distinct biophysical properties, especially hydrophobicity, they are difficult subjects to study. Classical proteomics technologies have relied on multidimensional separation of proteins on gels, which largely limited the choice of detergents and made the development of specialized enrichment protocols for membrane proteins necessary. Shotgun proteomic approaches, based on the digestion of whole proteomes and subsequent analysis of peptides by LC–MS, has largely circumvented these problems due to its compatibility with potent detergents. Here we briefly present and discuss the major developments in bacterial membrane proteomics and argue that recent developments in biochemical sample preparation and high resolution mass spectrometry have the potential to comprehensively identify and quantify membrane proteins without the need for specific enrichment procedures prior to LC–MS analysis.

Introduction

Bacterial membrane proteins play an essential role in many biological areas such as in signal transduction and pathogenicity. Membrane proteins represent approximately 20 to 30% of the entire genetic complement of a bacterial cell. Despite their importance, there is still a general lack of knowledge of many bacterial proteins due to the fact that many are of low abundance, insoluble and of a hydrophobic nature hence making them difficult to identify and characterize (Poetsch and Wolters, 2008). Proteomics has established itself as a critical tool in many areas in biology. This is largely due to the advances made in mass spectrometry (MS)-based technologies, which provides high accuracy, sensitivity and resolution in a highly efficient and high throughput manner. MS-based proteomics is revolutionizing biomedical research, significantly impacting the microbiology field. Bacteria are amenable for global quantitative gene expression analyses, due to their relatively simple proteomes compared to their more complex eukaryotic counterparts. The successful development of MS-based quantitative proteomics has allowed researchers to produce numerous studies that identify and characterize membrane proteins in bacteria (Table 1).

Historically, proteomic studies of bacterial proteins have been performed using a technique known as two-dimensional polyacrylamide gel electrophoresis (2D-PAGE), followed by MS analysis in order to identify the proteins present (Santoni et al., 2000). In this approach proteins are successively separated based on their isoelectric points and molecular weights and then visualized by specific stains in the form of protein spots on the gel. This technique has the capability to resolve thousands of proteins, and is very advantageous when studying certain protein features like isoforms. Numerous 2D-PAGE studies have assisted in advancing the identification and knowledge of bacterial membrane proteins as well as the proteomes of many bacterial species in general (Curreem et al., 2012; Li et al., 2007; Peng et al., 2005; Yun et al., 2008); However, this technique faces inherent problems: it is relatively time consuming and it is difficult to identify certain protein classes (e.g. proteins of extreme sizes or PI values). Utilizing 2D-PAGE to identify bacterial membrane proteins poses additional problems. The intrinsic hydrophobic and low abundant nature of membrane proteins presents an extreme challenge with respect to solubilizing them in order to be compatible with isoelectric focusing techniques used in 2D-PAGE. This ultimately results in the reduction of the transfer efficiency of the membrane proteins to the second dimension gel. Moreover, 2-D PAGE is not suitable when studying certain classes of membrane proteins such as integral membrane proteins

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http://dx.doi.org/10.1016/j.ijmm.2014.12.017
1438-4221/

Table 1
Selected proteomics based studies that have focused on bacterial membrane proteins within the last decade.

Topic	Method	Year	Reference
Vibrio cholera OMVs	Shotgun	2014	Altindis et al. (2014)
Helicobacter pylori outer membranes	2D-PAGE	2004	Baik et al. (2004)
Bacillus subtilis global membrane profiling	Shotgun	2004	Eymann et al. (2004)
Neisseria meningitides OMVs	2D-PAGE	2006	Ferrari et al. (2006)
Bacillus subtilis stress conditions	Shotgun	2010	Hahne et al. (2010)
Synechocystis sp. membrane complexes	2D-PAGE	2004	Herranen et al. (2004)
Escherichia coli membrane proteins	2D-PAGE	2004	Lai et al. (2004)
Escherichia coli OMVs	Shotgun	2007	Lee et al. (2007)
Escherichia coli envelope proteins	2D-PAGE	2006	Lok et al. (2006)
Staphylococcus aureus global profiling	Both	2005	Scherl et al. (2005)
Streptococcus pyogenes surface proteins	Shotgun	2007	Severin et al. (2007)
Mycobacterium tuberculosis membrane	Shotgun	2005	Sinha et al. (2005)
Escherichia coli membrane methods	Shotgun	2013	Tanca et al. (2013)
Meningococcal OMVs	2D-PAGE	2006	Vipond et al. (2006)
Bacteria general membrane proteins	2D-PAGE	2004	Wilmes and Bond (2004)
Vibrio alginolyticus outer membranes	2D-PAGE	2005	Xu et al. (2005)
Shigella flexneri outer membranes	2D-PAGE	2005	Ying et al. (2005)
Neisseria gonorrhoeae envelope proteins	Shotgun	2014	Zielke et al. (2014)

(Santoni et al., 2000). Several improvements have been made in an attempt to alleviate these issues with the 2D-PAGE approach as well as improving the coverage of membrane proteins in general. For example, using various detergents such as zwitterionic or anionic detergents have been shown to improve the solubility of membrane proteins (Vuckovic et al., 2013). Other sample preparation methods, such as cell surface and membrane shaving (Solis and Cordwell, 2011), biotinylation (Macher and Yen, 2007), use of various organic solvents such as methanol (Blonder et al., 2006), and synthetic membrane systems known as nanodiscs (Yan et al., 2011) can be utilized to increase the efficiency and purification of bacterial membrane proteins prior to MS analysis. These and other membrane protein extraction techniques are extensively covered in a recent review (Vuckovic et al., 2013).

Despite several improvements made to the 2D-PAGE MS proteomics approach, the overall limitations with respect to analysis of membrane proteins lead to the recent application of gel-free MS based approaches, better known as "shotgun" proteomics. It is very well suited to identify proteins in complex mixtures in a very robust and high throughput manner. This mini-review will focus on these gel-free MS based approaches that have provided new information about bacterial membrane proteins especially within the context of quantitative proteomics, post translational modifications (PTMs) and pathogenicity.

MS-based shotgun proteomics

A typical shotgun MS-based proteomics workflow is explained in detail in Fig. 1. One critical advantage of this approach is that the entire protein content of a cell can be extracted utilizing a variety of methods depending on the type of sample and analysis that is required. For example, in the context of bacterial membrane proteins, detergent based extraction methods have been widely employed as they serve as an efficient means to isolate many insoluble and low abundant targets (Vuckovic et al., 2013). One such detergent known as sodium dodecyl sulfate (SDS) is very popular in large scale global proteomics studies. Specific techniques that are compatible with the shotgun proteomics platform, such as filter-aided sample preparation (FASP) (Wisniewski et al., 2009), have been developed in order to utilize SDS and subsequently remove it prior to protein digestion, as the presence of even minor amounts of SDS can cause problems with chromatography as well as the inhibition of many proteases such as trypsin.

These workflows can be universally employed to virtually any type of biological organism or material, and have been utilized for identification of many bacterial membrane proteins. For example, this approach identified a large number of insoluble ABC transporters (protein family of integral membrane proteins) in *Geobacillus thermoleovorans* and *Oceanobacillus iheyensis* (Graham et al., 2006, 2007).

Importantly, these workflows have the possibility to produce a comprehensive coverage of bacterial membrane proteins even without specialized enrichment procedures. To demonstrate this, for the purpose of this review we re-analyzed our recently published proteogenomics study in *Escherichia coli*, which with 2626 identified proteins represents one of the largest proteome datasets of this organism to date (Krug et al., 2013). We predicted how many membrane proteins were identified in this study, using a Hidden Markov Model approach as previously described (Krogh et al., 2001). The criterion was that each protein must have been predicted with at least one transmembrane domain, and that at least 18 amino acids or more are predicted to be located inside the membrane. This resulted in 503 hits out of a potential 898 total hits to be predicted as membrane proteins in the *E. coli* proteome, corresponding to nearly a 60% coverage of membrane proteins without any specialized enrichment. It is noteworthy to mention that other similar proteomics approaches previously performed could potentially show this same trend of membrane protein coverage without specialized membrane protein extraction protocols (Macek et al., 2007, 2008; Ravikumar et al., 2014; Soares et al., 2013; Soufi et al., 2008, 2010; Wolff et al., 2007).

Quantitative proteomics

One important advantage of the Shotgun MS-based proteomics approaches is that they have the ability to adapt quantitative proteomics strategies that can compare two or more perturbations or conditions in a biological system in a proteome-wide global approach. Several methodologies exist each with their respective advantages as well as limitations. One common approach is to utilize stable (non-radioactive) isotope labelling to quantify the relative abundance of a protein from different samples directly from the acquired mass spectral data. Two major strategies for introducing a stable isotope label into the proteome exist: chemical and metabolic labelling. One strategy is known as stable isotope labelling by amino acids in cell culture (SILAC) (Ong et al., 2002), which has been successfully applied to many bacterial systems (Ravikumar et al., 2014; Soufi et al., 2010). SILAC metabolically (in vivo) incorporates the label, which is typically introduced in form of labelled lysine, arginine or another amino acid. Protein samples from two or three states are labelled with either unlabeled ("light") or labelled ("heavy") version of the amino acid, and can

Please cite this article in press as: Soufi, B., Macek, B., Global analysis of bacterial membrane proteins and their modifications. Int. J. Med. Microbiol. (2015), http://dx.doi.org/10.1016/j.ijmm.2014.12.017