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Method Article

Gel-free sample preparation techniques and bioinformatic enrichment analysis to in depth characterise the cell wall proteome of mycobacteria

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A B S T R A C T

The comprehensive characterisation of the cell wall proteome of mycobacteria is of considerable relevance to both the discovery of new drug targets as well as to the design of new vaccines against *Mycobacterium tuberculosis*. However, due to its extremely hydrophobic nature, the coverage of proteomic studies of this subcellular compartment is still far from complete. Here, we report novel gel-free cell wall sample preparation procedures and quantitative LC–MS/MS measurements on a Q Exactive mass spectrometer. We combine these with a novel post-measurement bioinformatic analysis to filter out likely cytosolic contaminants. This reveals a subset of proteins that are highly enriched for cell wall proteins. The success of this approach is verified by peptide-centric measurement of the abundance of known subcellular markers, as well as analysis of the percentage of predicted membrane proteins within the purified fraction. While *M. smegmatis* was used during this study to establish and optimise the sample preparation procedures, these can easily be applied to other mycobacterial species, such as *M. bovis* BCG or *M. tuberculosis*.

- Improved gel-free cell wall sample preparation gives higher yields of tryptic peptides for LC–MS/MS measurement.
- Higher yields of tryptic peptides provide better quantitation and coverage of cell wall proteome.
- Post-measurement enrichment analysis filters out high abundance cytosolic contaminants that have carried through the experimental analysis.

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A R T I C L E I N F O

Method name: Cell wall enrichment and bioinformatic enrichment analysis

Keywords: Cell wall proteomics, Mycobacteria, Proteomics, Bioinformatic enrichment analysis

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Specifications Table

Subject area	Immunology and Microbiology
More specific subject area	Cell wall proteomics
Method name	Cell wall enrichment and bioinformatic enrichment analysis
Name and reference of original method	Partly based on previous methods [1–3] This new cell wall proteomics approach was applied to a biological question in our recent study [4]
Resource availability	The dataset used in this publication is part of a larger dataset freely accessibly on PRIDE (Accession Number: PXD008075). http://www.biochem.mpg.de/5111795/maxquant http://www.biochem.mpg.de/5111810/perseus

Method details

Introductory remarks

The study of the cell wall proteome of mycobacteria is an active field of research, given its attractiveness for drug and vaccine targets, with a number of studies and protocols being published for *M. tuberculosis* laboratory strains H37Rv [5–7] and H37Ra [8], clinical strains of *M. tuberculosis* [9], *M. bovis* BCG [3,5], *M. marinum* [10] and *M. smegmatis* [11,12]. While these mycobacterial species differ in their pathogenicity and environmental habitat, which may uniquely shape the composition of the cell wall proteome, this protocol – although optimised using the fast-growing mycobacterial species *M. smegmatis* as a surrogate – can be directly applied to all mycobacterial species.

A major hurdle to obtaining complete coverage of the cell wall proteome of mycobacteria is the high hydrophobicity of the cell wall itself. This poses a major challenge for the complete extraction of cell wall proteins from the cell wall, as well as for the efficient generation of tryptic peptides from hydrophobic proteins. Historically, different experimental approaches have been used to characterise the cell wall proteome of mycobacteria. These include differential centrifugation [1,2], phase separation using Triton X114 [1,3], cell surface protein biotinylation followed by enrichment with magnetic streptavidin beads [13], trypsin shaving [11] as well as detergent extraction of outer membrane proteins [10]. Most of these approaches rely on gel-based separation prior to tryptic digestion and analysis by mass spectrometry; all of them suffer from the presence of high-abundance cytosolic contaminants in the cell wall fraction. To overcome this challenge, we have developed sample preparation procedures and a novel downstream data analysis method that together enable the comprehensive identification and quantitation of the cell wall proteome of mycobacteria. Our method comprises three steps: differential centrifugation, gel-free sample preparation and *in silico* enrichment analysis (Fig. 1). We have successfully applied this method to quantify the changes in the cell wall proteome of *M. smegmatis* after exposure to rifampicin [4].

Growth and harvest of M. smegmatis

Only MilliQ water should be used for any buffers and aqueous solutions during the protocol. Likewise, all plastic tubes should be mass spectrometry compatible. Growth condition will vary according to the experimental question being asked for each individual study. We used standard growth condition for optimisation of the current procedure. The *M. smegmatis* strain mc^2 155 was grown in 7H9 Middlebrook broth (BD, Maryland, USA) supplemented with 0.1% Tween-20, 0.2% glycerol (v/v) and 10% OADC (Becton Dickinson) at 37 °C with constant agitation at 120 rpm. Cells were grown until mid-log phase (OD_{600} ~1.2), harvested by centrifugation at 3,500 × *g* for 10 min at 4 °C and cell pellets were subsequently washed three times with phosphate-buffered saline pH 7.4 (PBS).

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