Identifying the Proteome of Different Mycobacterial Species Using Orbitrap™ Mass Spectrometry

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Introduction

Advances in next generation sequencing, of whole bacterial genomes, have led to a plethora of useful genetic data. However, these data often lack correlation with expressed protein profiles. It has been well demonstrated that even very closely related genomes, such as in mycobacteria, express drastically different phenotypes. These phenotypes often have major roles in pathogenicity. Therefore, it is just as important to have a method for examining the proteome of a bacterium as it is its genome. These studies are further complicated in mycobacteria due to the mycolic acid and extraction techniques needed to obtain a complete protein profile. We setout to develop a method for the extraction, purification and identification of the mycobacterial proteome in various species. This method was developed using reverse phase liquid chromatography and a Q Exactive ™ Plus Orbitrap™ mass spectrometer for peptide and protein identification. Both Top-down and Bottom-up approaches were employed for characterization of the proteins.

Methods

Mycobacterium abscessus, *M. chelonae*, *M. fortuitum*, *M. peregrinum*, and *M. scrofulaceum* bacteria were grown for 4 days on 7H11 agar plates (Hardy Diagnostics). Cells were lysed with the two lysing methods (bead beating and sonication) and various buffer compositions were tested for each method. Sonication was performed with different size beads as well as with a different number of beads. SPE was performed using TopTip POROS R2 (Glygen Corporation). Numerous clean-up methods were used for cleaning lysates before loading on SPE cartridges. For bottom-up experiments, tryptic peptides were generated from in-gel digestion of SDS-PAGE gels (Life Technologies). Bottom-up and Top-down LC-ESI-MS/MS was performed on nanoLC 1000 chromatograph (Thermo Scientific) and a Q Exactive ™ Plus mass spectrometer (Thermo Scientific).

Preliminary Data

The preferred method of protein extraction is sonication in 6M Gu-HCl utilizing glass beads. This method consistently extracted more proteins than the other solvents and bead beating methods. The size of the bead and the number of beads had no significant effect on protein concentration. However, beads were shown to be necessary for lysing cells during sonication. SPE recoveries of intact proteins were less than expected due to complexity of mycobacterium cell wall. Different strategies (liquid-liquid extraction, Cleanascite lipid removal reagents, DNase I treatment) were tested to increase recovery of protein following SPE. All of these strategies were less effective than desalting the cell lysate with PD10 desalting columns (Sephadex G-25, GE Healthcare). Additionally, the use of TopTip POROS R2 (10-200 uL, Glygen Corporation)) SPE tips significantly increased protein recovery. All protein and peptide lysates were then analyzed on a Q Exactive ™ Plus mass spectrometer (Thermo Scientific). Proteins were identified using Sequest searches in the Proteome Discoverer™ (PD) Software (version 1.4, Thermo Scientific). PD Searches resulted in identification of greater than 3500 proteins, including all of the ribosomal proteins from tested species. These proteins include cytoplasmic, intracellular and membrane proteins. Despite top-down proteomics complexity, we were able to identify ~100 unique proteoforms. These top-down experiments are on the cutting edge of this field and will only get better.

Novel Aspect

A novel strategy for identification and characterization of mycobacterial species proteome using bottom-up and top-down LC-MS/MS approach.