IPD Project Details

Project ID: IPD2983

Project Title: Comparative quantitative proteomic analysis of Mycobacterium tuberculosis H37Rv cultured under metal-limited (Chelated Sauton's medium) and metal-rich (7H9) conditions

Description: Wild-type Mycobacterium tuberculosis H37Rv was cultured under two nutritional conditions representing metal limitation and metal sufficiency. Cells grown in chelated Sauton's medium (metal-limited) and Middlebrook 7H9 medium (metal-rich) were harvested during logarithmic growth, and total cellular proteins were extracted for label-free quantitative proteomic analysis. LC-MS/MS was performed to identify and quantify changes in protein abundance associated with adaptation to metal availability. The dataset provides a comprehensive comparison of the intracellular proteome of M. tuberculosis under metal-limited and metal-rich growth conditions and identifies proteins involved in metal acquisition, transport, metabolism, and stress adaptation

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Sample Preparation: Briefly described as Wt Mtb, bacteria were cultured in Chelated Sauton’s media and 7H9 media. Protein lysate was prepared by bead beating for 7 cycles (1 min ON/2 min OFF), and the samples were run on a 10% SDS-PAGE gel. Protein gel bands were excised, destained, and dehydrated. In-gel tryptic digestion was performed using trypsin solution (1 µl trypsin reconstituted + 19 µl 25 mM ammonium bicarbonate solution (ABC) (Trypsin gold, Promega, Catalogue No. V5280) at 37°C. 20 µl of extraction buffer (1:1 ratio of 1% TFA and 100% acetonitrile (ACN) Solution) was added to each sample, and the above step was repeated thrice or until elution of all the digested peptides. Peptides were cleaned up by loading them onto C18 zip tips (Merck, ZTC 18S960). After one wash with 0.1% formic acid in LC-MS grade water (Sigma), digested peptides were eluted using 50% and 100% acetonitrile (with 0.1% formic acid). Finally, extracted peptides were vacuum-dried and processed for LC-MS measurements.

Peptide Separation: Dried peptides were reconstituted in 2% acetonitrile, 0.1% TFA and analysed on an Orbitrap Exploris 240 mass spectrometer coupled to an easy nLC Vanquish Neo (ThermoFisher Scientific) using a 25 cm long, 75 µm inner diameter fused-silica column packed in-house with 3µm C18 particles (ThermoFisher Scientific, 89870).

Peptides were eluted using a nonlinear gradient from 1 to 35 % acetonitrile (80% ACN) over 110 min and directly sprayed into the mass spectrometer equipped with a nanospray-Flex ion source (ThermoFisher Scientific).

Protein Characterization: Full scan MS spectra (375-1200 m/z) were acquired in profile mode at a resolution of 60,000 at m/z 200, auto mode for maximum injection time and an automatic gain control target value of 3 × 10^6 charges. Up to 15 most intense peptides per full scan were isolated using a 1.6 Th window and fragmented using higher-energy collisional dissociation (normalised collision energy of 30). MS/MS spectra were acquired in centroid mode with a resolution of 15000, in auto mode with maximum injection time, and an automatic gain control target value of 1 × 10^5. Single-charged ions, ions with a charge state above 5, and ions with unassigned charge states were not considered for fragmentation, and dynamic exclusion was set to 25s to minimise the acquisition of fragment spectra of already acquired precursors. MS raw data were processed using Proteome Discoverer 3.2, applying default parameters. Acquired spectra were searched against the Mtb ‘one sequence per gene’ database (taxonomy identifier 83332) downloaded from UniProt (accessed 18th September 2025; 3568 sequences) and using the Sequest HT search engine integrated in Proteome Discoverer 3.2. Protein quantification and data normalisation relied on the INFERYS Rescoring and CHIMERYS search algorithm (MASID) implemented in Proteome Discoverer 3.2. Identifications were filtered to obtain false discovery rates (FDRs) below 1% for both peptide spectrum matches (minimum length of 7 amino acids) and proteins using a high-confidence target-decoy strategy. For protein assignment, spectra were correlated with the UniProt TB database, including a list of common contaminants. Searches were performed with tryptic specifications and default mass tolerance settings for MS and MS/MS spectra. Carbamidomethyl at cysteine residues, oxidations at methionine, and acetylation at the N-terminus were defined as fixed modifications. The minimal peptide length was set to 7 amino acids, and the FDR for proteins and peptide-spectrum matches was set to 1%. The match-between-run feature with a 1 min time window was used. To compare Wt in 7H9 vs CSM, total spectral counts for each interactor were first normalised and then expressed relative to Wt/controls in fold-change (log2).

Experiment Type: Bottom-up

Species: Data in species_details No Data

Tissue: Data in tissue_details No Data

Cell Type: Data in cell_details No Data

Disease: Data in disease_details No Data

Instrument Details: Data in instrument_details Data in instrument_details

Protein Modifications: No PTMs are included in the dataset

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