IPD Project Details

Project ID: IPD1913

Project Title: Proteomics and metabolic burden analysis to understand the impact of recombinant protein production on host cells

Description: Recombinant protein production (RPP) using Escherichia coli as an expression host is highly efficient, however, it also inÂ¬ leads to strong host cell metabolic burden. Energy and biomass precursors are withdrawn from the hostâ€™s metabolism as they are required for plasmid replication, heterologous gene expression and protein production. In this study, we aim to investigate the effect of early and growth phase induction point on RPP. We have analysed and compared the proteomics data for growth-and protein production-phase to understand the cellular dynamics and recombinant protein-dependent product formation in process with respect to the two different hosts (M15 and DH5â•º) and medium (minimal and complex) cultures. Differential gene analysis shows that significant changes in carbohydrate metabolism, transport metabolism, nucleotide metabolism, amino acid metabolism and energy metabolism. Comprehension of the obtain results suggest that growth inhibition does not showing effect on recombinant protein and product yield but there is an changes in intracellular nucleobases, translation, transcription and cellular metabolism which leads to the degradation and instability during product formation. Overall, deeper knowledge of the cellular behaviour during recombinant protein production can be used to better exploit omics data with the goal of rational strain development.

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Sample Preparation: To prepare the samples for proteomic studies, total protein was identified by BCA Method and 50 ug of total protein were used. 50 ug of total protein was reconstituted in 20 ?L of resuspension buffer containing 7 M urea and 2 M thiourea, add 2 ul of 100mM DTT to the solution to make final DTT concentration 10 mM and gently vortex and incubated at 37 °C for 30 min. This was followed by the addition of 6 ?L of 100 mM iodoacetamide prepared in 30 mM and incubated at 25 °C (Room temperature) in the dark for 30 min.

Peptide Separation: Adjusted the pH 7-8 of the solution by addition of 25 mM ammonium bicarbonate, Samples were then digested using sequencing grade trypsin (1

?g per 50 ?g of total protein; Pierce Biotechnology, USA) overnight at 37 °C. The enzymatic digestion was terminated by the addition of formic acid to pH 3.0 to 4.0. The tryptic peptides were desalted using C18 Spin column (Thermo Scientific, USA) Eluted samples were vacuum- dried and reconstituted in 0.1% (v/v) formic acid before being subjected to LC-MS/MS.

Protein Characterization: LC-MS/MS analysis was performed using Orbitrap Fusion Lumos Tribrid Mass Spectrometer equipped with nano-LC Easy nLC 1200 (Thermo Fischer Scientific, Singapore). Liquid chromatography separation was performed at a flow rate of 300 nl/ml on a C18 pre-column (Acclaim PepMapTM 100, 75um X 2 cm, nanoViper, P/N 164946, Thermofisher Scientific Incorporation) followed by analytical column (Acclaim PepMapTM RSLC C18, 75um X 50 cm, 2um, 100 Å, P/N ES803). The peptides were separated using a gradient of 2% solvent B to 10% in 5 min followed by gradient increase to 45% and sharp increase to 95%, then retention of 95% for 10 min. Solvent A was aqueous solution in 0.1% formic acid, and solvent B was 95% acetonitrile in 0.1% formic acid. The eluted peptides were injected into the mass spectrometer and the MS1 data were acquired in full scan mode at 120000 orbitrap resolution with mass range from 375-2000 Da. Data were acquired using the Thermo Xcalibur software setup version 4.3.73.11 (Thermo Fischer Scientific, Inc 2019). Precursors were allowed to fragment using Higher-energy C-trap dissociation (HCD) in ion trap (IT) detector with collision energy of 28 in a data dependent MSn Scan acquisition. Charge state screening of precursor ions and monoisotopic precursor selection was enabled. The parent ions once fragmented were excluded for 40 sec with exclusion mass width of +/- 10ppm. The lock mass option (polydimethylcyclosiloxane; m/z 445.120025) enabled accurate mass measurement in the MS Mode. For analysis, raw LC-MS/MS data files obtained from the mass spectrometer were processed with Proteome DiscovererTM (Version 2.4.1.15, Thermo FisherTM Scientific Inc). The Proteome Discoverer processing workflow was employed in the label free quantitation (LFQ) of relative protein abundance across the samples and controls. For the search process, Sequest HT tools were used. Peak lists obtained from MS/MS spectra were identified using the MSF files. Protein identification was conducted against a concatenated target/decoy version of the uniport database (E coli +DH5?+ K12) and AAR peptide sequence along with Proteome Discoverer contaminant database. The identification settings were as follows: trypsin digestion with maximum of 2 missed cleavages; minimum peptide length 6; precursor mass tolerance 10 ppm; fragment mass tolerance 0.6 Da; fixed modifications; carbamidomethyl c (+57.021464 Da), variable modifications; oxidation of m (+15.994915 Da), acetylation of protein n-term (+42.010565 Da). Peptides and proteins inferred from the spectrum results using Uniprot database (Escherichia+coli+DH5?+K12) and AAR peptide sequence). Peptide Spectrum Matches (PSM's), peptides and proteins were validated at a target False Discovery Rate (FDR) strict to 0.01 and relaxed to 0.05.

Experiment Type: Top-down

Species: Escherichia coli-562 No Data

Tissue: Unknown No Data

Cell Type: Unknown No Data

Disease: Unknown No Data

Instrument Details: Orbitrap Fusion Lumos (MS:1002732) Data in instrument_details

Protein Modifications: No PTMs

PubMed ID: