Bacterial proteomic workflow

CURRENT STATUS: POSTED

Hanna Sidjabat
Hanna Sidjabat Group
h.sidjabat@uq.edu.au*Corresponding Author*

DOI:
10.1038/protex.2018.021

SUBJECT AREAS
Microbiology *Computational biology and bioinformatics*

KEYWORDS
Proteome, bacteria, protein expression, probiotics, pathogens

Abstract

This protocol desribes the bacterial proteomic workflow used in the article: "The use of SWATH to analyse the dynamic changes of bacterial proteome of carbapenemase-producing Escherichia coli under antibiotic pressure" published in Scientific Reports.

The workflow begins with growing the bacterial strains, isolating the bacterial proteins, processing the samples for submission to LCMS (protein quantitation, digestion, clean up, desalting). Then, once the samples are being processed on LCMS, the data analysis requires high performance computers.

Introduction

The workflow begins with growing the bacterial strains, isolating the bacterial proteins, processing the samples for submission to LCMS (protein quantitation, digestion, clean up, desalting). Then, once the samples are being processed on LCMS, the data analysis requires high performance computers.

Reagents

PBS, 50mM ammonium bicarbonate, Trypsin Gold, AII, DTT, acetonitrile, bacterial media.

Equipment

Tissue lyser, stainless steel beads, SCIEX5600, ESI, softwares: protein pilot, peakview, markerview.

Procedure

The isolates were cultured on Luria Bertani agar overnight. Subsequently, a single colony from each isolate was cultured in Mueller-Hinton broth. An optimization step to establish optimum bacterial growth condition to obtain the highest number of identifiable proteins was also performed. The number of identified proteins from an overnight bacterial culture and from bacteria harvested at 0.5 of OD600 were compared. In the overnight culture, outer membrane proteins were abundant, reducing the number of other identified proteins. A bacterial culture harvested at OD600 ~0.5 thus provided a better sample for protein analysis (data not shown). Therefore, the culture of E. coli in Mueller-Hinton broth without or with appropriate antibiotics for 4 hours with shaking at 250 rpm at 37oC to reach the exponential growth phase at OD600 of 0.5 was used.

Protein quantitation:

Proteins were quantified in triplicates using the BCA assay (Sigma-Aldrich) and bovine serum albumin

(BSA) as standard. After incubation at 37oC for 30 min, a microplate reader (BMG Labtech, Ortenberg/Germany) was used to determine the protein concentration at 562 nm.

Reduction and alkylation:

Aliquots of 1 µg protein/µL were mixed with ammonium bicarbonate (100 µl of 50 mM) for 10 s. Ten microliters of 20 mM DTT/bicarbonate were added to each sample and incubated for 1 h at 60oC. Iodoacetamide (10 µl of 1 M in 100 mM bicarbonate) was added to each tube and incubated for another hour at 37oC, protected from light.

Trypsin digestion and desalting:

Samples were digested with Trypsin Gold (Promega, USA) to cleave proteins into peptides on the carboxyl side of amino acid residues lysine and arginine. Following digestion, 0.1% formic acid (100 µL) was added and samples were centrifuged (15,000 rpm for 15 min) using a 10 kDa size exclusion membrane (PALL, Nanosep Cheltenham Vic, Australia). The flow-through was retained for a desalting step using a 3 mm piece of an Empore C18 (Octadecyl) SPE Extraction Disk. The disk was excised and placed in a gel loader tip and 5 µL of a POROS R3 slurry were added to form a micro-column. This column was washed with trifluoroacetic acid (20 µL, 0.1% in water). Peptides were eluted from the micro-column by three washes of acetonitrile (20 µL, 0.1% formic acid). Elutes were pooled and samples were dried at room temperature in a vacuum evaporator for 45 min. Subsequently, the samples were reconstituted with 100 µl of 0.1% formic acid in H_2O and centrifuged for 2 min at 10,000g to remove particulates.

Sample analysis by LC-MS/MS

LC-MS/MS analysis of digested E. coli lysates was performed on a Tandem Quadrupole Time-of-Flight

mass spectrometer Sciex TripleTOF 5600 (Sciex) coupled to an Eksigent 1D+ Nano LC system and a nanoFlex cHiPLC system (Eksigent) with a Nanospray III Ion Source (Sciex). Peptides were separated using a linear gradient (60 min for 5 to 80% B at 500 nL/min) of 0.1% formic acid in water and 0.1% formic acid in acetonitrile, and were delivered by a nanospray III electrospray interface (105 mm stainless steel emitter, Thermo Fisher THIES528).

Data acquisition of peptide separation using LC-MS/MS was performed using two different methods: the information-dependent acquisition (IDA) method and the data-independent (SWATH) acquisition method. Technical duplicates were performed for the IDA method.

Following the LC-MS/MS analysis, the mass spectral data generated from the IDA method were processed through two different search algorithms, Mascot (Matrix Science, v. 2.4.0) and Paragon (ABSciex, ProteinPilot Software v. 4.5.0.0, 1654). To generate an ion library, LC-MS/MS mass spectral data were firstly analysed using Mascot and the Eubacteria database from SwissProt for a qualitative analysis to identify and detect the presence or absence of the proteins of interest. Secondly, the analysis using Paragon involved a search against a suitable FASTA-formatted E. coli protein database from UniProt for the identification of peptides from the mass spectral data54. The data from Paragon were loaded onto PeakView (Sciex, v.1.2.0.3) to interrogate the SWATH data bank using the ion library generated in ProteinPilot. PeakView performs targeted and non-targeted data processing and generates extracted ion chromatograms (XIC). The data were then transferred to Markerview (Sciex, v. 1.2.1.1) for result interpretation and quantitative analysis. Markerview allows for a rapid review of the data to determine up and down-regulation of protein expression26. The data were processed using principal component analysis (PCA), which is an unbiased multivariate statistical analysis method that compares data across multiple samples, revealing groupings among data sets and graphically present the groupings in a Scores plot.

SWATH Analysis:

Specific to analyse the data from the SWATH method, three different software programs were used.

Firstly, Paragon was used to streamline protein identification and quantitation by identifying hundreds of peptide modifications and non-tryptic cleavages simultaneously to build an ion library. Then, using PeakView (Sciex, v. 1.2.0.3), the SWATH data were interrogated against the ion library. PeakView allows mass spectral data to be explored and interpreted for processing accurate mass data, structural interpretation and batch analysis. MarkerView was then used to review the data to determine the up or down regulation of protein expression in the bacterial samples through the use of Principle Components Analysis (PCA), a statistical tool that produces a visual representation of patterns in a dataset. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE55 partner repository with the dataset identifier PXD008019.

Timing

All together from culturing the bacteria until the completion of the data analysis is approximately 2-4 weeks, depending the availability of the LC-MS to run the samples.

Troubleshooting

We had performed optimisation of sample lysis method to obtain good protein yield.

Anticipated Results

PCA analysis of the detected proteins which indicate the upregulation or down regulation of certain proteins.

References

Liu, Y. et al. Quantitative measurements of N-linked glycoproteins in human plasma by SWATH-MS. Proteomics 13, 1247-1256, doi:10.1002/pmic.201200417 (2013).

Gillet, L. C. et al. Targeted data extraction of the MS/MS spectra generated by data-independent acquisition: a new concept for consistent and accurate proteome analysis. Mol Cell Proteomics 11, O111 016717, doi:10.1074/mcp.O111.016717 (2012).

Collins, BC, ... Aebersold, R. 2017. Multi-laboratory assessment of reproducibility, qualitative and quantitative performance of SWATH-mass spectrometry. Nat Com, 8(1):291.

Acknowledgements

Jolene Gien and Amanda Bordin.