IPD Project Details

Project ID: IPD1450

Project Title: Total proteome analysis of engineered/adapted E.coli strains with glucose/xylose co-utilization ability

Description: This project was about developing a bacterial strain that could consume a mixture of glucose and xylose simultaneously with higher ethanol productivity. In this study, an ethanologenic strain (SSK42) was made deficient in Carbon Catabolite Repression (CCR) by deleting the ptsG gene encoding EIIBCGlc component of PTS transport system. This strain (SCD00) was then evolved for several generations on xylose containing minimal media. A strain (SCD78) was finally obtained that, unlike its parent strain could consume glucose and xylose simultaneously. Then we performed proteomics analysis of evolved and un-evolved strain. The strain SSK42 was also considered for proteome analysis as a reference for analysis. The starting strain â€“ SSK42, is the derivative of E.coli B.

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Sample Preparation: All the three strains were grown in bioreactor until early to mid-log phase in AM1 minimal media containing 10 g/L glucose and xylose each, under micro-aerobic conditions at pH- 6.5. Samples from bioreactor were immediately centrifuged and pellet collected at 4?C. The pellet was washed twice with cold MQ water and re-suspended in sonication buffer. Sonication buffer included Triton X-100 (1% v/v), sodium chloride (150mM), Tris-HCl pH-7.4 (50mM), EDTA (1mM), PMSF(1mm), Lysozyme (1mg/ml), 1,4-dithiothreitol (1mM) and Phos-stop tablets (anti phosphatases) from Pierce. Cell suspension equivalent to an OD.600nm of 75 to 100 was taken in a pyrex tube and volume was increased to 5 ml with cold sonication buffer. Culture was maintained on ice all time. Sonication was performed for 3 min with 3 sec on – 5 sec off cycle at 20% amplitude. After effective lysis, lysate was centrifuged at 12000g for 10 min at 4?C in a table top centrifuge and supernatant collected in Eppendorf tubes. Protein content was determined using BCA kit (G-Biosciences) following the manufacturer's instruction. A total 50µg protein was taken per reaction to proceed further for reduction, alkylation and digestion. Appropriate volume of protein sample (lysate supernatant) was diluted with urea (10M in 50mM ammonium bicarbonate solution) to a final volume of

150 µl. DTT was added to a final concentration of 10mM and gently mixed and incubated at 56?C for 45 min. Then, iodoacetamide (200mM stock in 50mM ammonium bicarbonate) was added to 50 mM final concentration, gently mixed and incubated at room temperature in dark for 45 min. DTT (30 mM final concentration) was added again and incubated at room temperature in dark for 45 min to consume any unreacted iodoacetamide. Calcium chloride (1mM in 50mM ammonium bicarbonate solution) was added to the reaction to reduce the urea concentration to <0.5M.

Peptide Separation: Protein solution was then subjected to trypsin digestion. Trypsin from Pierce was used for the purpose, in a 1:50 (w/w trypsin: protein) ratio as suggested by the manufacturer and reaction content was incubated at 37?C overnight. Two drops of concentrated formic acid were added to lower the reaction pH and stop the trypsin reaction. C18 columns from Pierce were used for sample cleanup before being analyzed on mass spectrometer (Orbitrap lumos).

Protein Characterization: Analysis of raw data was performed using Proteome DiscovererTM supplied by Thermo ScientificTM. Further analysis including normalization (Quantile normalization) and differential expression analysis was performed using R package – NormalyzerDE.

Experiment Type: Shotgun proteomics

Species: Data in species_details No Data

Tissue: Unknown No Data

Cell Type: Data in cell_details No Data

Disease: Unknown No Data

Instrument Details: Data in instrument_details Data in instrument_details

Protein Modifications: No PTMs

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