SOP_iTRAQ_proteome

Protein extraction and iTRAQ labeling

Proteins were extracted from frozen cell pellets (see sample processing). Cells were washed with cold water before being re-suspended in 1 M tetraethylammonium bromide (TEAB) pH 8.0 containing 0.05 % SDS. Proteins were extracted using glass beads (size 425-600 µm, Sigma, UK) in a disruptor (Disruptor Genie, USA) for 8 cycles (alternatively 45 s vortexing and 45 s incubation on ice). Samples were centrifuged at 13,000 rpm for 15 min and the supernatants were transferred into new reaction tubes. Total protein concentrations were quantified using the Bradford assay (Sigma, UK).

100 µg protein of each sample was reduced, alkylated, digested and labeled with iTRAQ 8-plex reagents as described in detail by Zaparty *et al* (2010). Biological triplicates of each growth condition were used and these samples were labeled with iTRAQ 8-plex reagents as follows (6 reagents used): 113, 114 and 115 were used for cells grown on L-fucose whilst 116, 117 and 118 were used for cells grown on D-glucose (and used as control). Samples were combined before drying in a vacuum concentrator (Eppendorf Concentrator 5301, Germany).

Peptides separation, mass spectrometry and data analysis

Dried labeled iTRAQ peptides were fractionated using a HILIC technique on an uHPLC 3000 system (Dionex, UK) operated at a flow rate of 0.4 ml/min and a wavelength of 280 nm (Ow *et al*, 2010). Samples were re-supended in buffer A (10 mM ammonium formate in 80 % acetonitrile pH 3) before injection into a 4.6 x 200 mm Poly HYDROXYETHYL-A column (Hichrom Limited, UK). Peptides were separated using a gradient as follows: 5 min of 3 % buffer B (10 mM ammonium formate in 5 % acetonitrile pH 5) followed by a ramp to 25 % buffer B for 85 min, then up to 95 % buffer B for 35 min and finally maintained at 95 % for 10 min before ramping back to 3 % buffer B for 10 min. Fractionated peptides were collected every minute and then dried in a vacuum concentrator (Eppendorf Concentrator 5301, Germany).

Selected fractions were cleaned using micro spin C_{18} column (Nest Group, USA). Samples were resuspended in buffer A containing 0.1 % formic acid and 3 % acetonitrile prior to submission to a QStar XL Hybrid ESI Quadrupole time-of-flight tandem mass spectrometer, ESI-qQ-TOF–MS/MS (Applied Biosystems / MDS Sciex (now ABSciex), Canada), coupled with a nano-LC system (LC Packings Ultimate 3000, Dionex, UK). Mass

spectrometry parameters were applied as described by Zaparty *et al* (2010). Briefly, peptides were separated on a PepMap C-18 revered phase l/min with aμcapillary column (LC Packings) at a flow rate of 3 gradient generated by increment of buffer B containing 0.1 % formic acid and 97 % acetonitrile. The electrospray ionization voltage was set at 270 V whilst mass detector range was set to 350 to 1600 m/z and operated in the positive ion mode. Peptides with +2, +3, and +4 charge states were selected for fragmentation.

Raw mass spectrometry data were directly submitted to Mascot Daemon V2.2.0 with the iTRAQ 8-plex option. The search parameters were set up as follows with MS and MS/MS tolerances: 0.8 and 0.4 Da respectively, ion charges +2, +3 and +4, trypsin used with up to two missed cleavages. Variable modification of methionine and fixed modification of MMTS, iTRAQ 8-plex N-terminal and K were used. Data were searched against the *S. solfataricus* P2 database (2972 entries) downloaded from NCBI (Aug 2014). Furthermore, the false positive rates were also performed by searching the data with a reversed database of *S. solfataricus* P2. Results from the Mascot Daemon were exported into an excel file before submission to our in-house proteomic pipeline (Pham *et al*, 2010 and Bewley *et al*, 2011) for quantitation and assessment of regulated proteins. The protein identification was obtained from Mascot results while peak areas obtained from Mascot results were used for quantitation. Subsequently a rigorous statistical method was used to determine regulated proteins (Pham *et al*, 2010).