Label-free Quantitative Proteomic Analysis of Proteins Essential for Bacterial Growth on Biomass

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Introduction

Plants and crops present a huge and relatively untapped source of biomass which can be exploited in the process of sustainably producing biofuels. The breakdown of complex plant carbohydrates is seen commonly through a variety of soil bacteria that require simple sugars for metabolism. A vast array of biocatalysts are produced by countless bacteria to target and degrade the biomass. We aim to understand the bacterial mechanisms and processes the enzymes are utilised and see how they can be applied to industrial process of enzymatically producing biofuels.

The biocatalysts that are responsible for the release of sugars from complex biomass substrates can be exploited in the large scale degradation of biomass. The sugars that are released from hydrolysis are then mixed with yeasts and allowed to ferment. The subsequent ethanol that is produced is able to be refined and used as a fuel produced from a sustainable and reliable source (Saha, 2003; Herve *et al.,* 2009).

Label free proteomics allows us to closely study a complex biological system for relatively little cost, whilst maximising the potential of the LC-MS system. The soil bacterium *Cellvibrio japonicus* is the initial candidate for analysis due to the plethora of carbohydrate active enzymes in its genome. We aim to quantitate the abundance and regulation of essential proteins when bacteria are cultivated in the presence of varying forms of biomass. Up-regulated potential candidates will be confirmed through cross-lab studies.

Methods

- *Cellvibrio japonicus* has been selected as initial candidate to study growth on biomass.

*C. japonicus g*rown with following biomass as sole carbon and energy source in M9 minimal media:

- Sugarcane Bagasse
- Miscanthus gigantus

- System that we are running is a Dionex Ultimate 3000 LC coupled with Bruker HCT Ultra MS.
  - 145min run, flow rate 0.4µl/min
  - 120min gradient 0-40% Buffer B (95% ACN, 5% H_2O, 0.1% FA)
  - 10min wash with 90% Buffer B
  - 15min equilibration
- Digested proteins are separated on a Dionex 15cm C18 column, with 3µm beads, ran at 60°C

- Test system using simple and complex protein mixtures:
  - Simple; pure protein digests
  - Complex; *E. coli* proteins

-*E. coli* proteins prepared through HPLC protein fractionation on Dionex Ultimate 3000 with RP-1S monolyth column or through crude preparation of digesting freeze dried culture

- Data has been analysed using the Nonlinear Dynamics software Progenesis LC-MS and Mascot database searches. (figure 1)
  - Progenesis LC-MS has also been excellent in analysing chromatography, giving quick indication of good, sharp peptide elution.

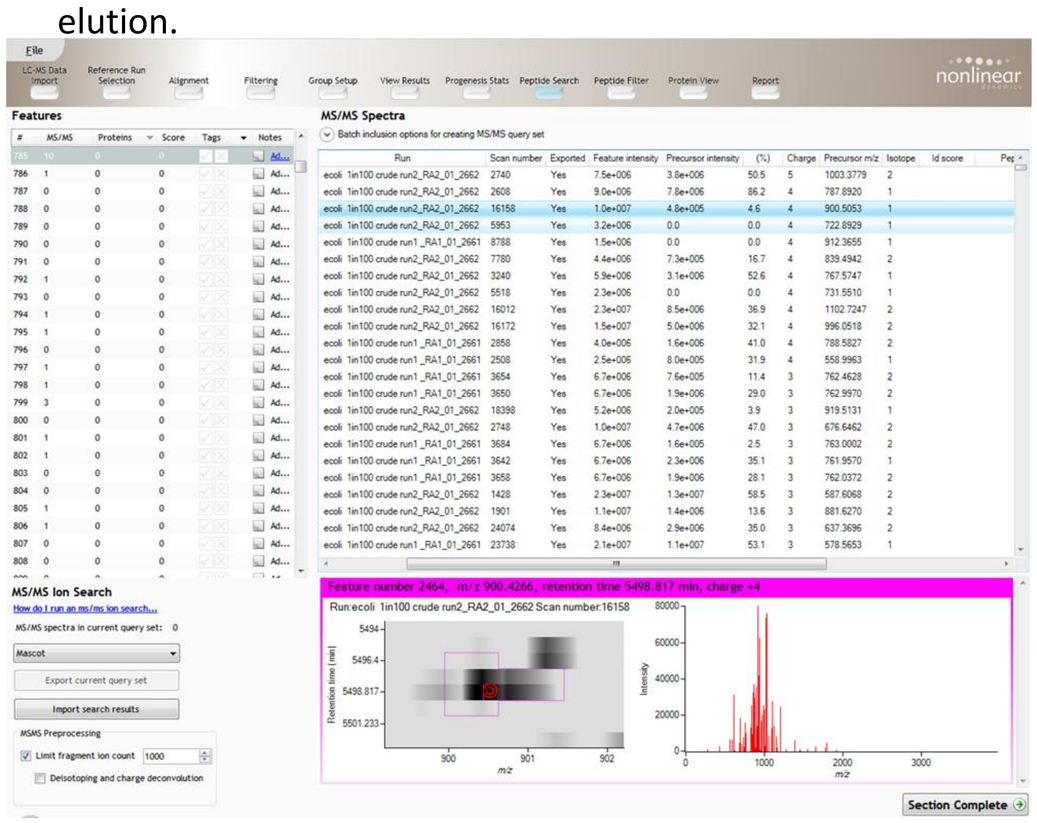

Figure 1: Progenesis LC-MS analysis of *E.coli* peptides. Selecting any of the features in the table will show details of the peptide retention time and subsequent MS mass:charge data that is collected

Results

- *C. japonicus* was successfully grown on glucose and both types of complex biomass

- Positive identification of 4 pure protein digests using LC-MS
  - Cytochrome C
  - Alcohol dehydrogenase
  - Carbonic Anhydrase
  - Myoglobin

- Positive identification of *E. coli* proteins through LC-MS analysis.

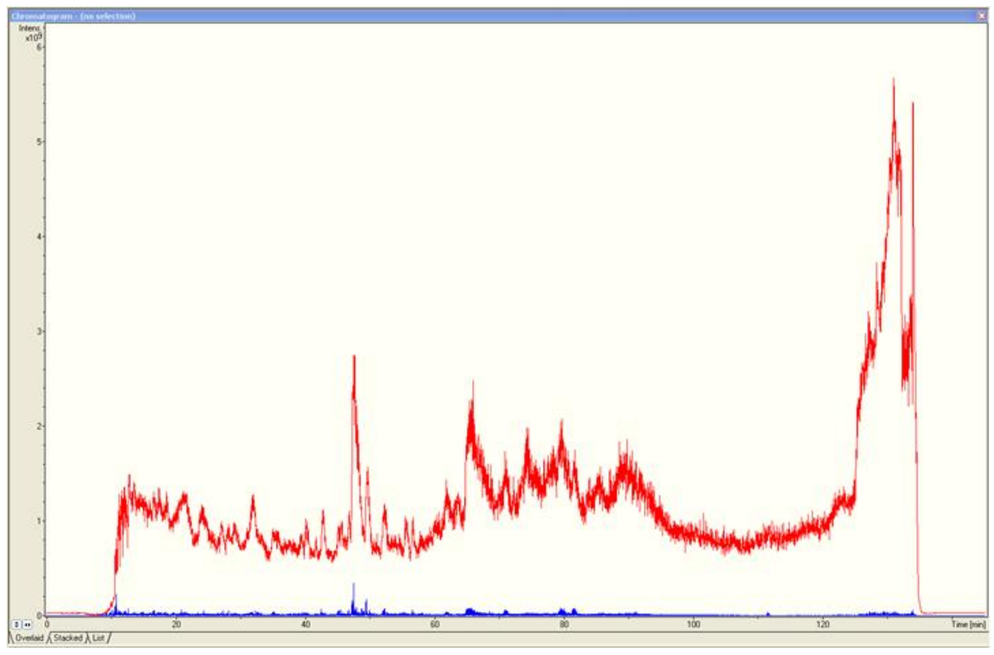

Figure 2: Mass Spectra of total protein digest of *E. coli.* MS 1 (red) and MS 2 (blue) allowed for protein identification through Progenesis LC-MS and Mascot

Discussion

There has been successful identification of 4 pure proteins when run individually at varying concentrations. Similarly there has been positive identification of *E. coli* proteins from both crude and HPLC preparations. This shows that the system is separating peptides effectively. More work needs to be carried out to refine each of the *E. coli* protein preparation methods so that more proteins are detected with a higher sequence coverage through LC-MS and Mascot analysis.

Future work

- Continue to analyse complex mixture of *E. coli* proteins through LC-MS. Analyse *E. coli* spiked with pure protein to detect proteins in a complex mixture.
- Apply *C. japonicus* to LC-MS and detect up-regulated proteins.
- 2-D gel analysis of *C. japonicus*, compare with LC-MS results

References

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