

Proteomic Analysis of *Cellvibrio japonicus* UEDA 107 using Gas Phase Fractionation and 2D Gel Electrophoresis



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Introduction

Plants and crops contain a large proportion of lignocellulose. Lignocellulosic biomass represents a huge and relatively untapped source of organic carbon (1). Current processing methods for this crystalline substrate involve extreme conditions for hydrolysis; however the use of carbohydrate degrading enzymes could increase the viability and reduce the cost of the process. There are a plethora of bacteria that have evolved a catalogue of carbohydrate degrading enzymes in order to survive in and around complex carbohydrate carbon sources. The analysis of the enzymes associated with carbohydrate degradation and metabolism are of significance to food, textile and **biorefineries** industries. The target bacterium for this research is *Cellvibrio japonicus* UEDA 107, which encodes for more than 130 enzymes associated with carbohydrate degradation (2,3). The analysis of the proteome of *C. japonicus* when it is grown on a variety of complex carbon sources facilitates the quantification of protein expression. Two proteomics methods of LC-MS Gas Phase Fractionation (GPF) (4,5) and 2-D Gel Electrophoresis (2DE) have been employed in order to validate results.

Methods

- **Cell Growth.** *Cellvibrio japonicus* grown on liquid M9 minimal media supplemented with 0.5% (w/v) glucose or xylan until OD_{600 nm} 0.8 (mid log).
- **GPF.** Dionex Ultimate 3000 coupled with Bruker HCT Ultra. 180 min gradient 0-40% Buffer B (95% ACN, 5% H₂O, 0.1% FA) 0.3 µl/min. Separations carried out using Dionex 50 cm, C18 column, 3 µm particle size at 45°C. MS1 was collected and 3 replicates analysed in Progenesis LC-MS to deduce mass ranges for 6 bins containing equal number of features (Figure 1). Then MS2 was collected for each bin and 3 replicates analysed in Progenesis LC-MS.
- **2DE.** 75 µg protein stained with 300 pmol IC5 dye as per manufacturers instructions (Dojindo Laboratories). Isoelectric focussing was carried out on 18 cm pH 4-7 IPG strips (GE) using a IPGPhor3 (GE). SDS-PAGE was carried out on Dodeca XL (Bio-Rad). Gels were visualised using a Pharos FX imager (Bio-Rad) with a PMT voltage of 60-70%.
- **Data Analysis.** Progenesis LC-MS and SameSpots (Nonlinear Dynamics) was coupled with Mascot database searches. Gel features were filtered by normalised spot volume (>100,000), p value (<0.05) and by fold change (>2). LC-MS features were filtered by charge state (+2 and +3) and p value (<0.05).

Results

GPF. Pilot data has shown 6414 features after alignment from all 6 bins (Figure 1). The number of protein IDs has risen by more than 300 proteins compared to MS without GPF, an increase of ~25% in proteome coverage. An increase due to the selection of more precursors. The carbohydrate degrading proteins identified from LC-MS database searches with the best MASCOT score were β 1-4 glucosidase and xylanase enzymes.

2DE. Glucose was compared to xylan as sole carbon source for growth. 6 replicates of each have been analysed. There was shown to be 533 features in glucose media and 367 in xylan media post filtering (Figure 2). A total of 49 spots were highlighted as differentially expressed when two sets of six replicates were compared.

Comparing 2DE and GPF. The top 24 MASCOT scores of GPF and 24 most abundant spots in 2DE were compared (Table 1). There were 7 common ID between the two techniques, even though approaches are orthologous.

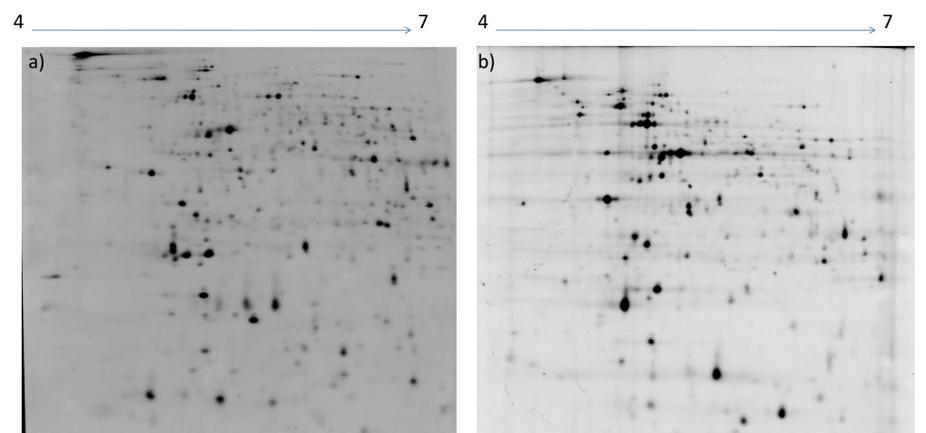
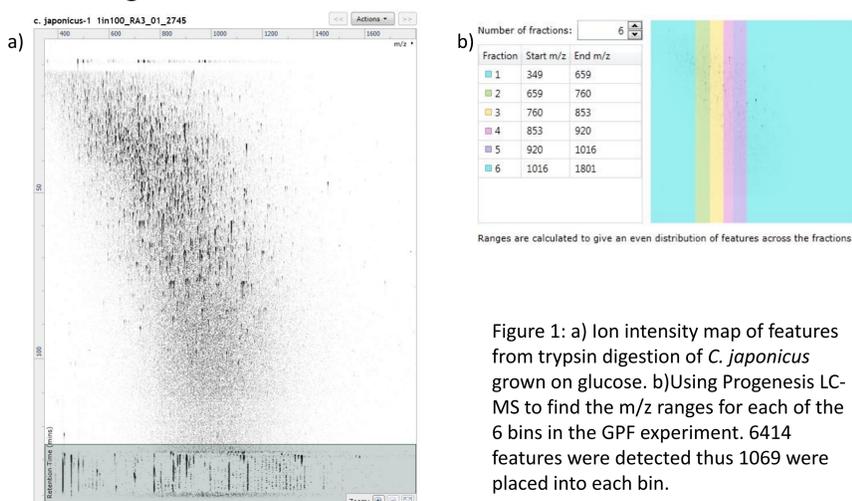


Figure 2: 2DE gels from *C. japonicus* grown on (a) glucose and (b) xylan

Prot_acc	2DE		GPF	
	Spot No	Protein	Prot_hit_num	prot_desc
gi 157164833	5	phosphopyruvate hydratase	2	phosphopyruvate hydratase [<i>Cellvibrio japonicus</i> Ueda107]
gi 192359288	18	putative peroxidase	3	thiol peroxidase [<i>Cellvibrio japonicus</i> Ueda107]
gi 192360445	6	elongation factor Tu	9	elongation factor Tu [<i>Cellvibrio japonicus</i> Ueda107]
gi 192361283	15	hypothetical protein CJA_0471	13	hypothetical protein CIA_0147 [<i>Cellvibrio japonicus</i> Ueda107]
	17	hypothetical protein CJA_0471		
gi 192360427	14	elongation factor P	21	elongation factor P [<i>Cellvibrio japonicus</i> Ueda107]
gi 192359320	2	chaperonin GroEL	23	chaperonin GroEL [<i>Cellvibrio japonicus</i> Ueda107]
gi 4512117	10	succinyl-CoA synthetase beta chain	24	succinyl-CoA synthetase beta subunit [<i>Cellvibrio japonicus</i> Ueda107]

Table 1: Comparison of the identities of Top 24 most abundant spots and LC-MS features when *C. japonicus* was grown on xylan. Only features that were common are shown. 7/24 were commonly identified by the two techniques.

Discussion

The utilisation of different carbon sources in minimal media has demonstrated how *C. japonicus* is able to adapt to different environments by changes in protein expression. The use of 2DE and GPF in parallel has provided an interesting platform from which to analyse changes to the *C. japonicus* proteome. The differences in methodology provided insights into varying aspects of the proteome and displayed some concordance between the methods. Further work is required to identify those protein spots that have been shown to be differentially expressed shown using 2DE. In addition, further replicates, using GPF, are needed to highlight quantifiable changes to the proteome.

References

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