Task 8.2 Rumen Shotgun Metaproteomics

Shotgun metaproteomics has become an established method to analyse the total proteins found in environmental samples. The approach takes the extracted protein, digests with trypsin before separation and analysis of the resulting peptides with high resolution mass spectroscopy. The proteins are then identified by mapping the peptides to a reference protein database. The shotgun approach offers significant advantages with regards to throughput and avoids the problems of contaminant carry over from ruminal digesta samples that inhibit SDS PAGE gel approaches.

As part of WP8, shotgun metaproteomics was carried out to compare the proteins in digesta samples from different ruminant species, five cows and five reindeer. A further 20 cows were also selected by rank for their methane emission profiles with 10 high and 10 low phenotypes respectively.

Different sampling techniques were used in each case with digesta taken via cannula in the case of the reindeer and cattle whereas the high and low methane emitters' samples were taken by gastric intubation. Proteins were extracted using bead-beating in a buffer designed to maximise solubility of the proteins. The extracts were run on 1D SDS PAGE, which served to separate humic contaminants before digestion of the entire sample in trypsin.

The peptides were analysed using an Ultimate 3000 RS LC nano system coupled to a LTQ OrbiTrap Velos Pro and were identified using MASCOT. They were then mapped to proteins from the NCBInr reference database using Proteome Discoverer software. The list of proteins were summarised by mapping the GI reference numbers to UniProtKB database. Taxonomic and functional summaries using gene ontology (GO) terms were produced (Fig 1, Fig 2). Proteins were also mapped, where possible, to the KEGG orthology (KO) global pathways to produce a 'metapathways' map for each of the sample categories (Fig 3).

A selection of the most abundant and important proteins was produced including structural components of eukaryotes from the host and ciliate protozoa, plant proteins from the diet and key metabolism enzymes from the bacteria that carry out the degradation of dietary plant fibre. The amino acid sequence identity of the proteins was strongly linked with taxonomy. Moreover, the metazoa, plant and microbial origins of the proteins were closely related to species expected to be found in ruminal digesta samples. Some enzymes involved in methane metabolism were also detected especially from the samples taken by ruminal cannula. These were from the ruminal Archaeal genera *Methanobrevibacter* and *Methanosphaera* and were key enzymes directly involved in the conversion of CO_2 and H_2 into methane.

In all samples, shotgun metaproteomics was an effective tool to identify enzymes and other proteins that were involved in important functions of the rumen microbiota. Moreover, the GO and KEGG summaries provided a way to compare the functional activity between the different sample groups. Recent developments improving on the depth of metaproteome characterisation along with quantitative techniques offer even more scope for future research.

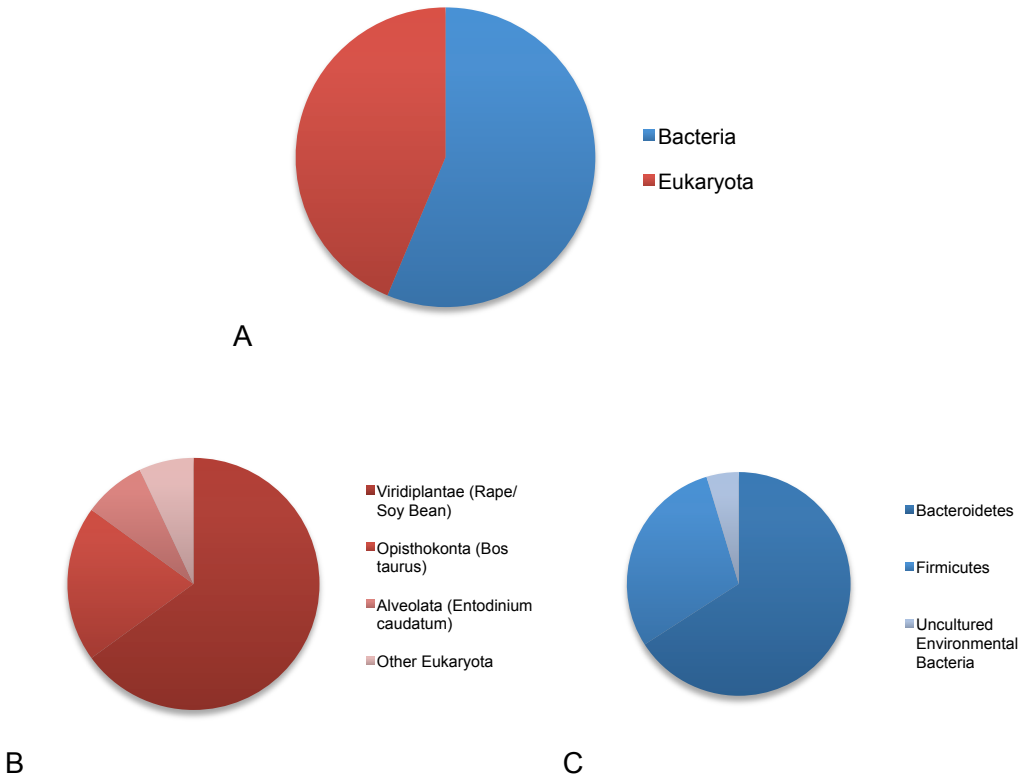

Figure 1 Taxonomy summary of shotgun metaproteome of rumen fluid from dairy cows. (A. Distribution of domains. B. Eukaryotic phylum summary. C. Bacterial phylum summary)

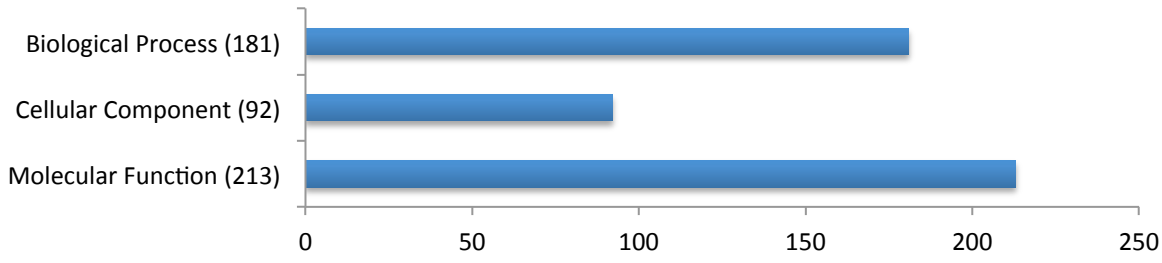

Figure 2. Function summary of unique proteins identified in rumen digesta of dairy cows. Relative abundance of gene ontology (GO) categories.

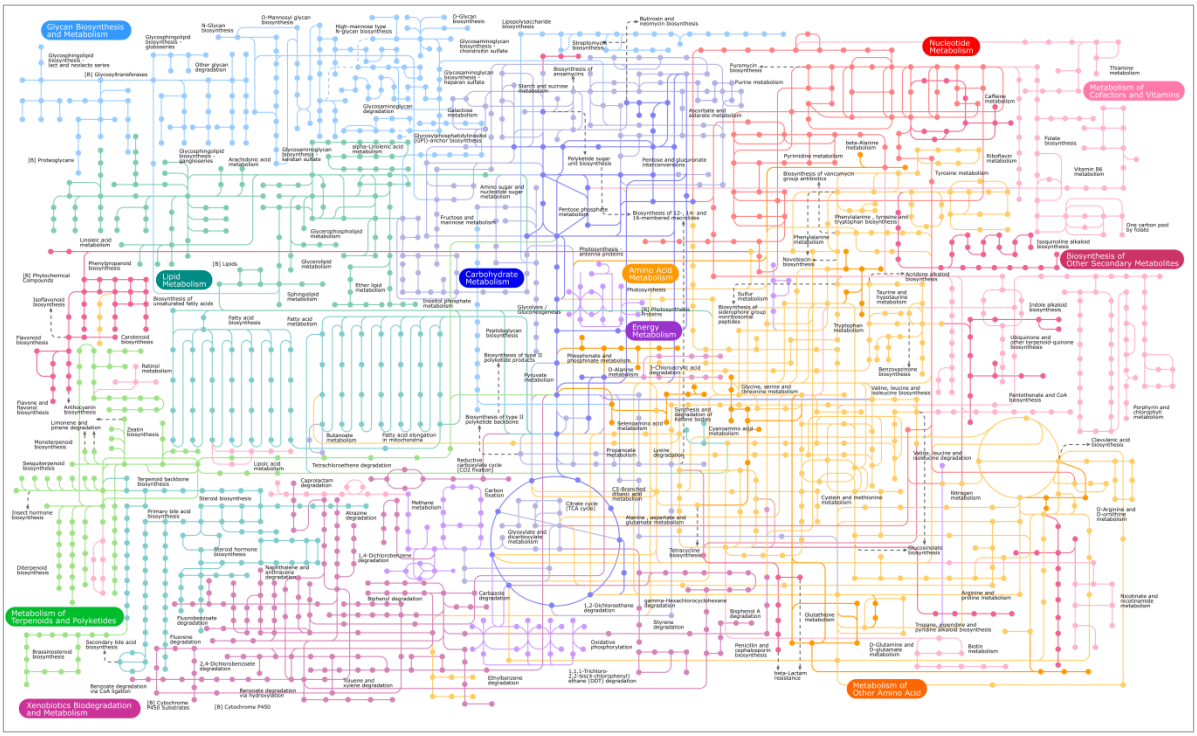

Figure 3. KEGG Global metabolism pathways map