RuminOmics E-Newsletter

Connecting the animal genome, gastrointestinal microbiomes and nutrition to improve digestion efficiency and the environmental impacts of ruminant livestock production

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Welcome to the winter 2014 newsletter

Momentum continues to build in the RuminOmics project. The collection of samples involving 1000 cows is nearly complete (see later for details). The last 12 months of the project will be challenging to try to put together this huge and enormously valuable dataset in order to join the triangle of host genome, ruminal microbiome and emissions. Most of the other experiments in the project are now complete or nearly so but await full analysis and reporting and the writing of Christmas Cards!.

John Wallace with his wife in Australia.

The aim of one key experiment was to determine if samples from the mouth, from the regurgitated food bolus or from faeces reflected the microbial community of the rumen. If they did, it was thought possible that the proxy samples, each of which is much easier to obtain than ruminal digesta, could be used as indicators of rumen microbial community composition. The experiments were carried out at MTT in Finland and analysed by MTT and other partners. What has emerged has surprised us. None of the samples contains the same community as the rumen. The patterns are remarkably consistent over the five dietary treatments tested. Faeces is especially different from the other samples, which was expected, but perhaps not to the extent that was found. Archaea are as abundant in faeces as in ruminal digesta, while protozoa are absent, but there are major differences in the bacterial communities as well. The other samples are much more rumen-like, but they are not identical, with common features across diets. This does not mean that the proxy samples cannot be useful, far from it. Correlations with emissions may enable large-scale sampling that is useful for host genetic analysis.

We wish all our friends and collaborators across the globe a Happy Christmas and prosperous and exciting 2015.

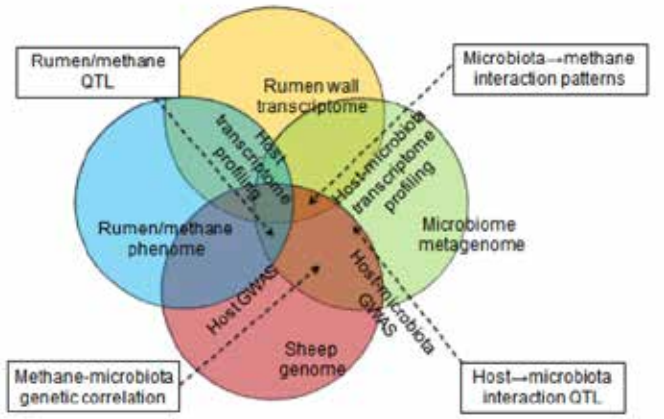
John Wallace
RuminOmics Project Coordinator

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement n° 289319

RuminOmics and Pangenome

John Wallace (Ruminomics Project Coordinator) travelled to Australia at the invitation of the Pangenome project, a project that has many parallels with RuminOmics. The name, Pangenome, encapsulates what the project is about: investigating how all the genes in the organism, both host and microbial, influence methane emissions and related feed efficiency. John was accompanied on his travels by Noelle Cockett of Utah State University, who leads the US sheep genome project, and Phil Vercoe, leader of the Pangenome project.

The concept of the Pangenome project is similar to, but rather more complicated than, RuminOmics, because it encompasses transcriptomics as well. A Venn-type diagram shows the aims of the project.



The visit began with a trip to the CSIRO facilities in Townsville, east Queensland, where Ed Charmley and Nigel Tomkins lead the research. Respiration chambers are located out of town at Lansdowne, the experimental farm where respiration chambers are located. The primary aim of the experiments here are to investigate the consequences of inhibiting methanogenesis.

Next stop was CSIRO, Brisbane, to meet Chris McSweeney and Stu Denman, who carry out ruminal community analysis on the respiration-chamber cattle, and scientists working with Brian Dalrymple who are following the transcriptome of the rumen epithelium throughout foetal development and after birth.

The whistlestop tour continued with a visit to Armidale, NSW, to see Roger Hegarty, Robert Herd, Hutton Oddy and Jude Bond from the University of New England. Their field system for measuring methane in sheep is a simple gas-accumulation chamber affectionately known as the 'butter-box' system. Hutton Oddy has made remarkable progress in understanding the heritability of methane emission and its relationship with rumen volume and the properties of rumen contents (particle size, density) using CT scanner technology. John Nolan, famed for his 15N tracer analyses in sheep, joined the discussion to explore the possibilities of nitrate as an alternative nitrogen sink to methane.

A 5-h flight to Perth, WA, completed the air travel. Once on the ground, visits were made to the UWA farm at Ridgefield, Pingelly, and the laboratory/animal facilities at Floreat.

Sheep in the 'butter-box' respiration system.

The farm at Pingelly, where antimethanogenic shrubs are strip-cultivated alongside grazing forage.

The outcomes of the visit were a much improved understanding between the projects about what research is being carried out, an agreement to pool resources where practicable, and a decision to incorporate the Pangenome project in the workshop planned for EAAP, Warsaw 2015.

One Thousand Very Special European Cows

Under Work Package 3 of the RuminOmics Project, researchers are busy generating a set of phenotypic data on a scale unprecedented for dairy cows. Previous projects have recorded either a limited number of measurements on a large number of cows or detailed measurements on relatively few cows. For RuminOmics we are recording detailed measurements on 1,000 cows distributed across four countries (United Kingdom, Italy, Sweden and Finland). Each cow is very special to us, but collectively the sampled population is representative of the EU dairy industry and will comprise 800 Holstein-Friesian cows (UK and Italy) and 200 Scandinavian Red and White cows (Sweden and Finland). All cows are managed under commercial conditions, so results will be directly applicable to the industry.

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From measurements of milk yield, milk composition, live weight and feed intake we will calculate feed and energetic efficiency. Analysis of faecal samples will add digestibility coefficients for major nutrients, which will be related to parameters of rumen fermentation determined from samples of rumen fluid. In turn, these will be related to direct measurements of methane emissions and nitrogen excretion. Thus, we will be able to examine relationships between digestive efficiency and environmental impacts. More importantly, we will be able to identify cows that have both superior efficiency and lower impact.

Samples collected from these cows will be used in other work packages to determine if the cow's genome is linked to her rumen microbiome, and also to confirm how accurately methane emissions and feed digestibility can be predicted from milk fatty acid profile. Overall, this project will provide unique information to link genetics, rumen microorganisms, metabolism, feed efficiency, milk composition, and methane emissions in dairy cows.

Data warehouse strategy for RuminOmics

The Ruminomics project is generating large datasets, ranging from genomics data, to proteomics profiles and phenotypic information. One of the project tasks is to develop a data warehouse system to store all the information generated by the project and to make it accessible to the community.

In the last couple of years, European Bioinformatics Institute (EBI) has progressively released a number of public databases to store new kind of data and to allow the community to submit different information to EBI. These databases, specifically the BioSamples (http://www.ebi.ac.uk/biosamples/) and Metagenomics (https://www.ebi.ac.uk/metagenomics/) databases, are extremely flexible and well matched for the type of data the Ruminomics project is generating.

The project has then decided to progressively submit, during the next year, the project data to the EBI databases, specifically the raw sequencing data and the collected sample information. This decision was also driven by the recent advances and development of the EBI RDF platform (https://www.ebi.ac.uk/rdf/), where data present in specific EBI databases is made accessible using new semantic web technologies.

The combination of long term storage, standardisation and availability offered by EBI resources and the improved accessibility of these databases and datasets were the main factors that led to this decision. With this strategy, the Ruminomics project will also avoid replicating existing databases and creating its own resources to store the main project data and information. In the past these strategies have indubitably led to issues with long term data availability, accessibility and have made it more difficult to integrate new data with existing public databases. Instead, the Ruminomics project will focus on producing high quality information and will concentrate on making the data submitted to different EBI databases, accessible for the researchers community from one single convenient online resource.

We hope that other European projects will consider the potential benefits of the new EBI RDF platform and will follow the same approach, since we believe this will lead to an overall improvement of data availability and accessibility for the whole scientific community.

Rumen microbial metaproteomics: 2D PAGE vs Shotgun

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Nearly 40 years ago, Patrick O'Farrell of the University of Colorado (USA) developed a method of separating the proteins extracted from a culture of Escherichia coli. It was called two dimensional polyacrylamide gel electrophoresis or 2D PAGE for short. Ten years ago, Paul Wilmes and Philip Bond of the University of East Anglia (UK) applied this technique to an even more complex microbial sample from an activated sludge wastewater system. They proposed the term 'metaproteomics' for the large-scale characterization of the entire protein complement of environmental microbiota.

2D PAGE begins with solubilisation of the extracted proteins in a buffer designed to break down the bonds and molecular interactions that give them their complex three dimensional structures (Rabilloud et al., 1997). This solution is added to a pH gradient strip and 'focussed' in the first dimension to the point where the proteins have a neutral charge. A current is applied and the proteins then migrate in the second dimension through a polyacrylamide gel, separating according to size. After staining, the result is a pattern of spots that reveals all the proteins and their

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isoforms, like a jigsaw puzzle of the proteome of a single organism or the metaproteome of a microbial community (**Figure 1**). Individual proteins can be identified by a precise mass measurement using a mass spectrometer revealing the picture of the structure and functions of the microbial community.

So much for the theory. Unfortunately, the rumen is a hostile environmental for biological molecules. Protein degrading enzymes are abundant and tannins and humic compounds from the dietary plant fibre can obscure protein spots on a gel. This means that rumen microbial metaproteomes are more likely to appear as a dark smear with the proteins impossible to identify.

The latest generation of mass spectrometers is now providing an alternative to gels by sequencing the proteins almost directly from the original extract. This process, know as shotgun proteomics, starts with the digestion of the entire extract, breaking up the proteins into short peptides before mass spectrometer analysis. If 2D PAGE can be likened to a jigsaw puzzle, then Shotgun metaproteomics takes the pieces and cuts them up even smaller before the picture can be put together. This would be an impossible task if it were not for the sensitivity and accuracy of the mass sprectrometer analysis and the software and computing power that maps the peptide fragments to the 87 Million or so non-redundant protein references available in the online repositories (UniProt).

The result is a list of the different proteins detected in the sample. As the technology, software and databases improves, these lists will get longer and longer. Methods to determine the relative and absolute abundance of proteins are also under development either inferred from the number of spectra or by using stable isotope labelling.

The challenge ahead is to process and present the data to summarise and compare the biological functions of metaproteomes. This requires an effective way of classifying and categorising proteins to make comparisons between samples. The metaproteomics option at UniPept http://unipept.ugent.be/ is an excellent example (**Figure 2**). It uses the lowest common ancestor (LCA) approach to generate a taxonomic summary from the tryptic peptides and an interactive diagram to explore the constituents of the metaproteome.

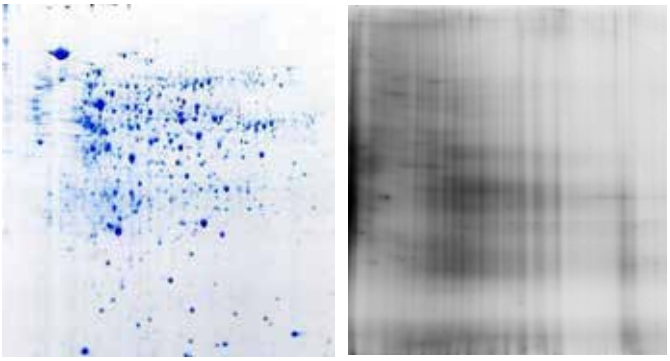

Figure 1. 2D SDS PAGE metaproteomics, in theory and in practice.

Figure 2. UniPept ‘Sunburst’ diagram of a shotgun metaproteome of ruminal digesta.

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