GLOBAL RESEARCH ALLIANCE
ON AGRICULTURAL GREENHOUSE GASES

GRA FLAGSHIP PROJECT TEMPLATE

Title: Hungate Collection: Second Coming
Leader: Professor Sharon Huws, School of Biological Sciences/Institute for Global Food Security, Queen's University Belfast, UK.
GRA Council Champions: *list the names of at least 5 GRA Members and Partners (including at least 3 Member countries)*

1. *Dr Hugo Jiminez, Agrosavia, Columbia*
2. *Professor Itzik Mizrahi, Ben-Gurion University, Israel*
3. *Professor Wei-Yun and Professor Yanfen Cheng, Nanjing Agricultural College, China*
4. *Dr. Shengguo Zhao, Chinese Academy for Agriculture, China*
5. *Professor Sinead Waters, Teagasc, Ireland*
6. *Professor Richard Dewhurst, SRUC, UK*
7. *Dr David Ruiz Yanez, CSIC, Spain*
8. *Dr Diego Morgavi, INRAE, France*
9. *Dr Rodolpho Prado, Universidade de Maringa, Brazil*
10. *Dr Rob Gruninger, D Renee Petri and Dr Tim McAllister, AAFC Canada*
11. *Professor Leluo Guan, University of Alberta, Canada*
12. *Dr Matthias Hess, UcDavis, California, USA.*
13. *Dr Garrett Suen and Dr Hilario Montovani, Madison University, USA.*
14. *Professor Hauke Smidt, Wageningen University and Research, Netherlands*
15. *Dr Graeme Attwood and Nik Palevich, AgResearch, New Zealand*

Countries involved:
As hubs and co-hubs of culturomics:
Brazil
Canada
China
Columbia
France
Ireland
Israel
Netherlands
New Zealand
Spain
UK
USA

As partners sending samples to the nearest hub:
Open to all and hope that LMIC countries without infrastructure to become hubs can participate through sending samples and being named as a co-author on a publication.

Start date and project length:
01/06/21 for 2 years with some flexibility

Brief description of project:

The rumen is a complex, dynamic ecosystem composed of mainly anaerobic bacteria, protozoa, anaerobic fungi, methanogenic archaea and phages. These microbes interact with each other and have a symbiotic relationship with the host, providing energy from the breakdown of plant cell wall carbohydrates that are largely inedible by human. The rumen bacteria far outnumber the other microbial groups (approx. 10^{10}-10^{11} cells/mL), with rumen protozoa, fungi and archaea being reasonably similar in densities (10^{3}-10^{5} cells/mL), but the number and diversity of the rumen viruses remains largely unknown. Our understanding of most microbiomes, including the rumen, has been hindered by poor culture collections and a lack of effort placed on culturing in an era that has seen an explosion in metataxonomic and metagenomic sequencing. This has led to an assumption that many microbes are unculturable, when it is well recognised that with some effort they could be cultured. Nonetheless, the emphasis on culture, termed culturomics when combined with genome sequencing, is returning as these culture collections are hugely valuable to our fundamental understanding of microbiomes, use as probiotics/direct-fed microbials and a source of bioactive compounds for biotechnological applications.

In terms of the rumen microbiome, the seminal work of Robert Hungate in the 1960s resulted in many of the culture technologies for anaerobic bacteria, that are still widely used throughout the world. The recent Hungate collection Joint Genome Initiative (previous GRA flagship) represents a major step change in our understanding by providing 501 rumen bacterial and archaeal genomes, resulting largely from the pioneering work of Robert Hungate, which allowed culture of these microbes in the laboratory. This is an immense achievement, which has greatly enhanced our ability to understand the rumen microbiome and resulted in much scientific impact in terms of follow on publications. Nonetheless, the project has now ended, due to funding rather than reaching the end of the rumen microbial cultures available. As such, many genomes for rumen microbes remain unavailable. The Hungate collection is also underrepresented by the Phylum Bacteroidetes and family Bacteroidales compared with their representation within the global rumen census dataset (previous GRA flagship project) and genomes from so called unulturable rumen bacteria are also hugely underrepresented. Some genomes from so called unculturable rumen bacteria have recently been assembled from metagenomics sequences (called metagenomically assembled genomes (MAGs)), nonetheless, there is a need to get these microbes in culture to test key hypothesis arising from the genomic information in the laboratory are somewhat hindered. Undoubtedly, there is a crucial need to develop methods for isolation and culture of 'missing' rumen microbes and subsequently obtain 'omic level information. Likewise, many cultures remain in our culture collections, which were not sequenced as part of the initial Hungate collection; the phenotypes and genomes of which are crucial to drive further innovation in sustainable livestock production.

In terms of advances in rumen culturomics, our ability to culture rumen bacteria has notably improved in recent years, through the development of new culture media and use of dilution to extinction. For example, development of the enhanced rumen bacterial media RMO2, and use of the dilution to extinction technique allowed the isolation of 54 novel bacteria. Indeed, partners in QUB and Ben-Gurion university have also recently placed substantial emphasis on rumen bacterial culturomics, with over 100 novel isolates (according to 16S rDNA) being in pure culture (as yet unpublished), including those which are most closely related to so-called unculturable bacteria, illustrating the potential power of such applications. Whilst such efforts will undoubtedly form the basis of further understanding and biotechnological exploitation of rumen microbiome, the potential to use some of these bacteria as direct-fed microbials in the diets of ruminants in order to re-direct hydrogen away from the methane biochemical pathways are immense. In particular in combination with additives, which reduce methane emissions, these potential direct-fed microbials could not only further reduce methane output but provide a production enhancement in a sustainable manner. Consequently, given the impetus, the aims of this GRA flagship are to isolate, phenotype and genotype novel rumen

bacteria (or the latter 2 only if novel cultures are already available) from cultures or samples sent from as many GRA member countries to QUB, which will form the flagship hub (with the option given to other partners with resources available to also be a hub of activity for the flagship, whilst utilising aligned protocols), in order to drive future innovations for enhanced sustainability of livestock production. We will instruct member countries appropriately as to how to send DNA, cultures or samples and protocols involving minimal input from countries with limited resources have already been developed. We also recognise the governmental challenges that sometimes can be associated with member countries sending samples and we will work closely with the Northern Ireland government to overcome obstacles and also we will aim via the CLIFF-GRAD program to train staff/students from a variety of countries, particularly those who experience difficulties in sending samples, such that they can get involved and that they will be trained to carry out the research in their own country and get maximal benefit from the flagship. In essence, every attempt will be made to ensure this flagship is open to all member countries, ensuring it's global impact in enhancing sustainable livestock production.

Key partners and existing resources:
See above list of partners and country of residence. Partners who will act as the hub and co-hubs have substantial infrastructure for anaerobic microbiology. Most of these partners are also partners in the following substantial projects:

EU Horizon 2020 (Acronym: MASTER) https://www.master-h2020.eu/
FACCE ERA-Net Gas (Acronym: RumenPredict) https://www.eragas.eu/en/eragas/research-projects/rumenpredict.htm
FACCE ERA-Net Gas (Acronym: SeaSolutions) https://seasolutions.ie/
FACCE ERA-Net Gas (Acronym: INTEGRITY) https://www.eragas.eu/en/eragas/Research-projects/INTEGRITY.htm
DAFM/DAERA (Acronym: MethAbatehttps://www.agriland.ie/farming-news/cross-border-research-project-to-cut-methane-emissions-on-the-cards/
EU Horizon 2020 (Acronym: Holoruminant) https://cordis.europa.eu/project/id/101000213

Our aim is to add value to the existing infrastructure and funding and providing facilities allowing partners from countries lacking infrastructure to become involved and shape the flagship. Involvement of LMIC countries is particularly required as the samples will no doubt represent unique biodiversity given the differences in diet.

Benefits and outcome from Flagship project:
Without the need to culture from samples received globally, we already have over 200 pure cultures available within QUB, Ben-Gurion University, AAFC Canada and many others partners. Therefore at a minimum, we will phenotype these 200 using the Omnilog system and classical Gram staining etc. Following 16S rDNA sequencing we will choose those which are of most interest to genome sequence alongside partners and in line with funding availability for the sequencing effort. This GRA flagship will undoubtedly lead to a very impactful publication, involving all partners which have participated in some way. It will also help to assemble and annotate metagenomic and metatranscriptomic sequences, leading to a deeper understanding of the rumen microbiome. The follow on publications utilising these datasets are expected to be large, as are those resulting from the Hungate collection, therefore ensuring major legacy and impact. These sequences will also be used to further enhance the CowPI 16S rDNA based functional inference tool, thus enhancing the understanding within studies that have been unable to find funding to go beyond metataxonomy as is the case for many of the studies coming from LMIC countries. As well as providing resources for biotechnological exploitation of novel bioactives, the most crucial expected outcome of this flagship is to provide biological

resources to investigate the possibility of using some of these microbes as direct-fed microbials to re-direct hydrogen within the rumen for further reducing methane emissions and enhancing productivity in a sustainable manner. These cultures will be kept within -80 and -20°C freezers within each hub, ensuring that each culture is at least duplicated. We expect many follow on projects will ensue as a consequence of this flagship and the intention is to ensure that the isolates are available to all either through the hubs or through deposition in cheap and accessible culture collections, such as DSMZ (subject to IP discussions).

The benefits to the GRA from this flagship are immense. We will be utilising the skill sets of the most knowledgeable anaerobic microbiologists globally to form the most comprehensive culture collection, alongside phenotype and genotypic biological and bioinformatic resources. The impact of this project will be immense in terms of 1. Global visibility and engagement with the GRA, 2. Brining global GRA partners together and maximising country value from being members of the GRA, 3.Publication impact, 3. Potential for global novel innovations from the project. Prof Huws has led the Rumen Microbial Genomics network for 8 years and within this time has been an advocate of the GRA, organised global workshops and communicated to all members from developed and non-developed countries with respect to opportunities etc. and will continue to do so whilst marketing the potential to become involved in this project and disseminating outcomes.

Further Resourcing needs:
We require a post-doctoral scientist for a period of 2 years (at a minimum) at a cost of approximately £170,000 and would value the appointment of a scientist from a low or middle income country supported by CLIFF-Grad scholarships to increase the impact of the flagship and the training opportunity in rumen microbiology for all GRA partner countries. This person will communicate to all partners to coordinate sample delivery from GRA members, intellectual property discussions (alongside internal experts within QUB with consultancy with New Zealand partners who conducted the Hungate collection research using similar governance) and lead the culturing and storage aspects of the project. In return we will use existing funds for an affordable level of consumables and will apply for appropriate funding to enhance capabilities, whilst also asking those with resources to become co-hubs. Resourcing mechanisms We will use a small proportion of budgets within existing projects (EU FACCE JPI RumenPredict, EU Horizon 2020 MASTER and EU Horizon 2020 Holoruminant) to purchase consumables to culture and phenotype bacterial isolates and sequence a proportion of those which are of interest, initially using 16S rDNA sequencing for identification and thereafter a further refined list for genome sequencing (£250/bacterium via MicrobesNG including illumina Hi-seq and oxford nanopore sequencing, assembly and annotation of closed genomes). We will also seek further funding to increase our capacity to phenotype and genotype isolates, from funders such as the EU (Cost Action possibly), UKRI and especially the JGI CSP calls.

Resourcing mechanisms:
Thirty % funding will be covered from funding within the projects named above.

Linkages: As noted we will also uses resources within EU FACCE JPI RumenPredict and Horizon 2020 projects MASTER and Holoruminant. Between ourselves, Ben-Gurion University and AAFC Canada we have over 200 isolates to work with already and intent from the other countries noted that they will be involved get involved in various capacities (sending DNA, pure cultures or samples or through exchange/training). The partners are world leaders in this area and have extensive networks of stakeholders, including academics, industry, farmers, policy makers, which will be used to disseminate data and maximise potential impact from this project beyond academic resources and publications.