

The HUNGATE1000.

A catalogue of reference genomes from the rumen microbiome.

Bill Kelly

AgResearch, New Zealand

RuminOmics/Rumen Microbial Genomics Network Workshop

Dublin, Ireland

Project history.

- A workshop was held in Palmerston North in February 2011 to discuss establishing a Rumen Microbial Genomics (RMG) Network.
 - The need for reference ruminant microbial genomes was identified as a key area for RMG Network collaboration.
 - Discussion resulted in a proposal to sequence 1000 genomes including anaerobic fungi and ciliate protozoa.
- Proposals successfully submitted to:
- New Zealand Government to support the objectives of the Livestock Research Group of the Global Research Alliance on Agricultural Greenhouse Gases.
- US Department of Energy Joint Genome Institute (JGI), Community Sequencing Program. Letters of support from 23 rumen microbiology research groups.



Dick Clarke and Bob Hungate, DSIR Applied Biochemistry Division, Palmerston North, New Zealand, circa 1971.



HUNGATE1000

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RMG NETWORK

RUMEN MICROBIAL GENOMICS NETWORK

Aim.

- To produce a reference set of rumen microbial genome sequences from cultivated rumen bacteria and methanogenic archaea, together with representative cultures of rumen anaerobic fungi and ciliate protozoa.

Purpose.

- To support international efforts to develop methane mitigation and rumen adaptation technologies.
- To initiate genome-enabled research aimed at understanding rumen function in order to find a balance between food production and greenhouse gas emissions.
- To underpin the analysis and comprehension of metagenomic sequence datasets.

Scope of the project.

Bacteria and Archaea:

Important criteria for selection of cultures for inclusion in this project include:

- Novel isolates
- Coverage of the phylogenetic diversity found in the rumen
- Known species that are not yet sequenced
- Multiple strains from genera that show high phenotypic diversity
- Well-studied strains with known properties
- Numerically dominant organisms identified from analysis of metagenome data,
- Different host animals and diets

Anaerobic Fungi and Ciliate Protozoa:

- There is little information available on the genomic make-up of rumen anaerobic fungi and ciliate protozoa.
- As part of the Hungate1000 project representative cultures will be selected for sequencing by members of the RMG network who have specific expertise in working with these organisms.

Rumen microbial genomes.

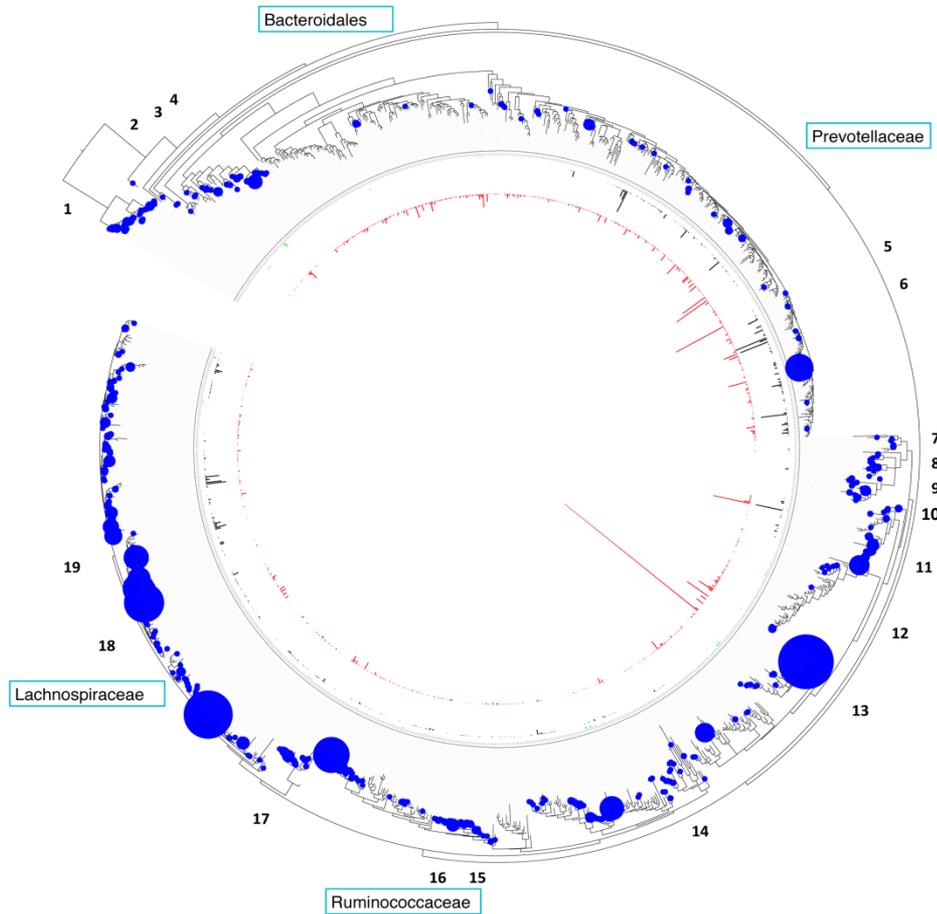
2011

- Genome sequence information was available for 14 bacteria and 1 methanogen.
- Some of the first rumen bacterial genomes to be sequenced were those of cultivated bacteria considered to have key roles in breakdown of the cellulose (*Fibrobacter* and *Ruminococcus*) and hemicellulose (*Butyrivibrio* and *Prevotella*) components of plant cell walls.
- As expected, these fibrolytic bacteria have a particularly wide spectrum of degradative abilities. Between them these four bacteria have 78 different families of polysaccharide degrading enzymes and 22 different families of carbohydrate binding modules.

2013

- Genome sequence information is available for 25 bacteria, 4 methanogens and 1 anaerobic fungus.
- The NIH human microbiome project currently lists >1100 reference bacterial genomes.

Do cultured isolates capture the phylogenetic diversity found in the rumen?



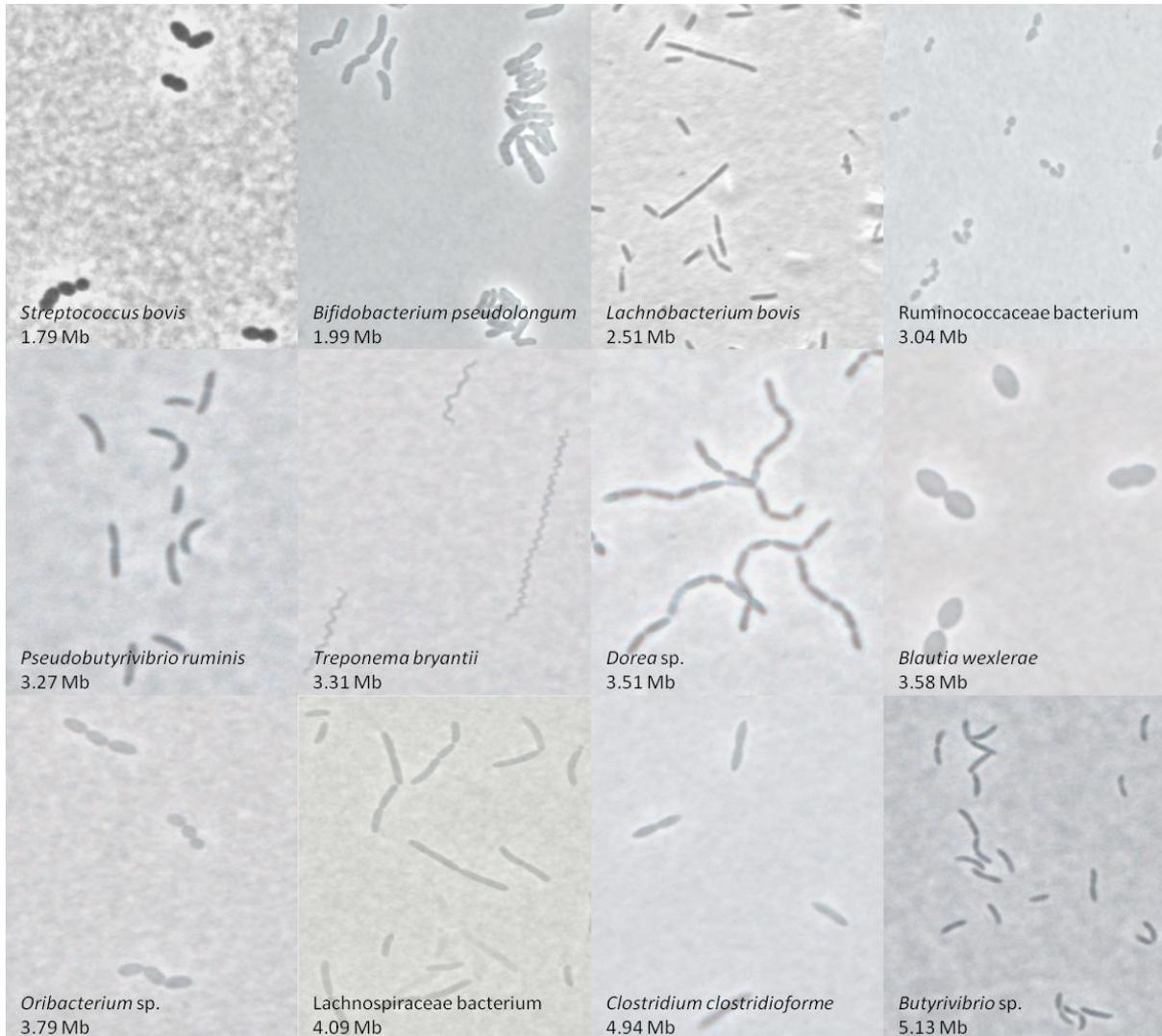
Publically available genome sequences of rumen microbes.		
	Organism	Family
1	<i>Methanobrevibacter ruminantium</i>	Methanobacteriaceae
2	<i>Wolinella succinogenes</i>	Helicobacteriaceae
3	<i>Actinobacillus succinogenes</i>	Pasteurellaceae
4	<i>Bastia succiniciproducens</i>	Pasteurellaceae
5	<i>Prevotella bryantii</i>	Prevotellaceae
6	<i>Prevotella ruminicola</i>	Prevotellaceae
7	<i>Desulfovibrio desulfuricans</i>	Desulfovibrionaceae
8	<i>Slackia heliotrinireducens</i>	Coriobacteriaceae
9	<i>Desulfotomaculum ruminis</i>	Peptococcaceae
10	<i>Megasphaera elsdenii</i>	Veillonellaceae
11	<i>Selenomonas ruminantium</i>	Veillonellaceae
12	<i>Treponema saccharophilum</i>	Spirochaetaceae
13	<i>Lactobacillus ruminis</i>	Lactobacillaceae
14	<i>Synergistes jonesii</i>	Synergistaceae
15	<i>Ruminococcus albus</i>	Ruminococcaceae
16	<i>Ruminococcus flavefaciens</i>	Ruminococcaceae
17	<i>Fibrobacter succinogenes</i>	Fibrobacteraceae
18	<i>Butyrivibrio proteoclasticus</i>	Lachnospiraceae
19	<i>Eubacterium cellulosolvens</i>	Lachnospiraceae

16S rRNA gene sequence data from rumen metagenome datasets were analysed to show the different bacterial taxa present in the rumen. This was then overlaid with known rumen bacterial isolates and indicates that there are cultured representatives for most bacterial groups present in the rumen. Bacteria related to *Bacteroides* and *Prevotella* are relatively poorly represented.

Hungate1000 sequencing.

- Genomic DNA has been isolated from 106 bacterial strains and sent to JGI.
- Genomic DNA is being prepared from additional cultures.
- Strains are listed in the GOLD (Genomes On Line) database and on the Hungate1000 website (www.hungate1000.org.nz)
- Sequencing originally used the Illumina HiSeq2000 platform but has now switched to PacBio.
- Draft genome sequence information is available for 52 bacteria (as of June 21st).
- The 52 genomes have 193 Mb of sequence and encode 175,083 genes.
- They range from the 1.79 Mb *Streptococcus bovis* to the 5.13 Mb *Butyrivibrio* sp.
- Includes 27 strains of *Butyrivibrio* belonging to 6 possible species.
- Includes 4 unnamed strains identified as belonging to novel genera.
- Data available at <https://img.jgi.doe.gov> Integrated Microbial Genomes (IMG).

Novel rumen bacterial genomes.



Future

- DSMZ type strains
- Bacterial cultures from international participants
- Import permits
- Most wanted list
- Culturing

We invite researchers who are interested in this project, or who have cultures they would like to include, to contact us at hungate1000@agresearch.co.nz or www.hungate1000.org.nz.

Acknowledgements.

The Hungate1000 project is a team effort.

- Rechelle Perry, Kenneth Teh, Nick Palevich, Adrian Cookson and Suzanne Lambie have grown the cultures, confirmed their identity and done the DNA isolation for all the strains.
- Nicki Kenters, Sam Noel and Laura Olthof isolated new bacterial strains.
- Chris Creevey did the meta-analysis.
- Lynne Goodwin and Nicole Shapiro are the JGI project managers.
- Gemma Henderson, Graeme Attwood and Sinead Leahy.

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New Zealand Government

