

2024 Annual Meeting of the Livestock Research Group

# RUMEN GATEWAY

GLOBAL RESEARCH ALLIANCE FLAGSHIP PROJECT ON  
AGRICULTURAL GREENHOUSE GASES



**QUEEN'S  
UNIVERSITY  
BELFAST**



Dr. Fernanda Godoy Santos

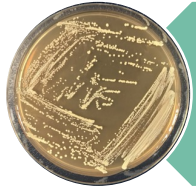
BSc Animal Science, MSc, PhD Agricultural Microbiology



# BACKGROUND AND AIMS



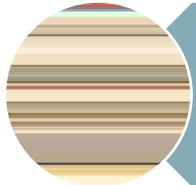
Hungate 1000 collection (2018) – Sequencing of 410/501 ruminal microorganisms.



Isolate, phenotype and genotype novel rumen microbes.



“Global culture Hubs” across the world (diversity and representativity).



Protocol development, sharing and training.



Comprehensive database and open-access biobank.



# PROSPECTIVE PROJECT OUTCOMES IMPACTS



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**Rumen function:** Expand our understanding on the rumen microbiome.

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**Hinder methanogenesis:** Provision of potential direct-fed microbials to re-direct hydrogen away from methanogenesis (hydrogen sinks).

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**Feed interaction:** Mechanistic understanding of the action of feed interventions to mitigate methane emissions.

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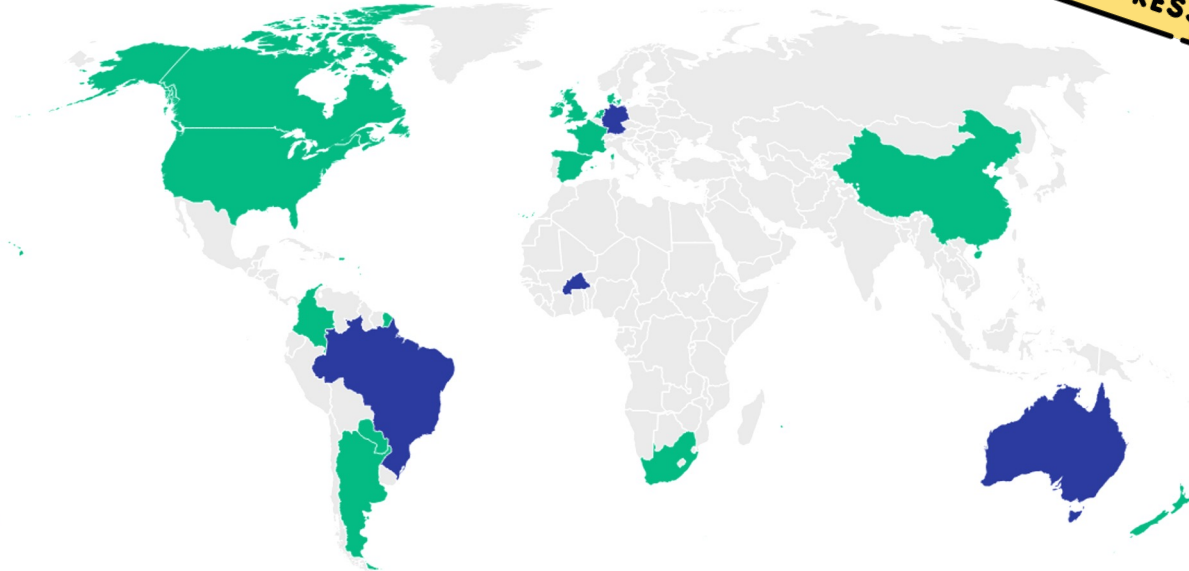
**Discovery:** Microbial resource availability for bioactive compound discovery.





## GLOBAL CULTURE HUBS

■ Collaboration agreement ■ Accession agreement



Created with Datawrapper



**JGI - DNA extraction protocol for  
genome sequencing.**

## GLOBAL COLLABORATIONS



- ✓ **Collaboration agreement:**
  - ✓ 20 Institutions (14 countries).
- ✓ **Accession agreement:**
  - ✓ Potential new collaborators – in progress
    - EMBRAPA-Brazil;
    - CIRAD/UAC-Burkina Faso;
    - Department of Agriculture and Fisheries-Queensland-Australia;
    - Bavarian State Research Center for Agriculture-Germany.
- ✓ **Different forms of collaboration.**

Rumen Gateway Project:  
New collaborator form



<https://forms.office.com/e/zwjuvJi2i3>





# DELIVERABLES

+Officially launched 12/10/2023



Implementation  
of a mutual  
agreement across  
different  
countries.

| Deliverable | Description  | Project month | Dates         |
|-------------|--|---------------|---------------|
| 1           | Inventory of unsequenced rumen microbes in storage globally                  | 6             | June 2023     |
| 2           | Development of standard operating procedures for culturing across the 'hubs' | 6             | June 2023     |
| 3           | 150 existing unsequenced rumen microbes sequenced by the JGI                 | 12            | December 2023 |
| 4           | 450 existing unsequenced rumen microbes sequenced by the JGI                 | 24            | December 2024 |
| 5           | Culture and phenotyping of a minimum of 1,000 novel rumen microbes           | 36            | June 2025     |
| 6           | Sequencing of the microbes isolate in deliverable 5 by the JGI               | 42            | June 2025     |
| 7           | Deposition of all isolates in culture collections                            | 42            | June 2025     |
| 8           | Submission of the publication to a high impact journal                       | 42            | June 2025     |

## Rumen Gateway Management Board



WP1  
Bacteria

WP2  
Archaea

WP3  
Fungi

WP4  
Virus/Protozoa

- ✓ Routine WP meeting.
- ✓ Recording meetings (Time zone).
- ✓ Development and sharing of comprehensive protocols.



Principal Investigator: Professor Sharon Huws  
s.huws@qub.ac.uk



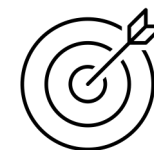
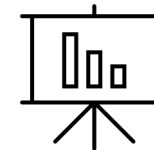
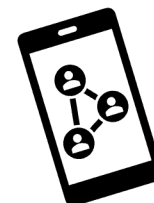


# PROJECT LOGO:



## Workshop demands results:

- ✓ Cultivation techniques for anaerobic microorganisms
- ✓ Sharing standard protocols and practical laboratory experiences across labs, including open operational discussions
- ✓ Biobank system and operational logistics
- ✓ Metadata organization
- ✓ Anaerobic medium preparation
- ✓ Culture stock procedures
- ✓ Isolation and taxonomic identification of rumen microbiota
- ✓ Molecular protocols (DNA extraction, PCR, etc.)
- ✓ Genomic and metagenomic analysis
- ✓ Analytical tools (e.g., HPLC, GC-MS, NMR) for metabolic studies



## Information Collection for Project Dissemination

Rumen Gateway Project:  
Information collection for  
dissemination



## Workshop demand and hub contribution survey

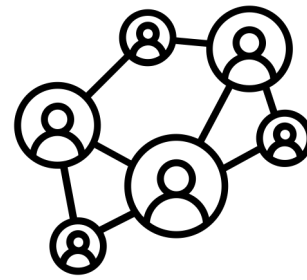
Rumen Gateway Project:  
Workshop demand and hub  
contribution survey



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<https://forms.office.com/e/UyEaV51F1c>



# METADATA – COMPREHENSIVE DATABASE/PROJECT INTEGRATION



- ✓ Extended JGI isolate metadata template (shared Excel file).
- ✓ Project integration:
  - ✓ **HoloRuminant project** (Isolation and phenotyping);
  - ✓ **Global Methane Genetics - Breed4Food** (Database integration);
  - ✓ **Targeting Rumen Hydrogen Production & Utilization – FFAR**  
(Hydrogen sink phenotyping for direct feed microorganism project)
  - ✓ **AMR** (phenotyping)



| Main parameters  |
|--|
| • Partner ID   |
| • Rumen Gateway ID   |
| • Isolate partner collection ID                                |
| • Preliminary taxonomic classification                         |
| • Preliminary taxonomic classification method (16S, MALDI-TOF) |
| • Isolation method   |
| • Geographical location  |
| • Collection date  |
| • Sample source  |
| • Contact name   |





# DILUTION TO EXTINCTION AND DIRECT PLATE

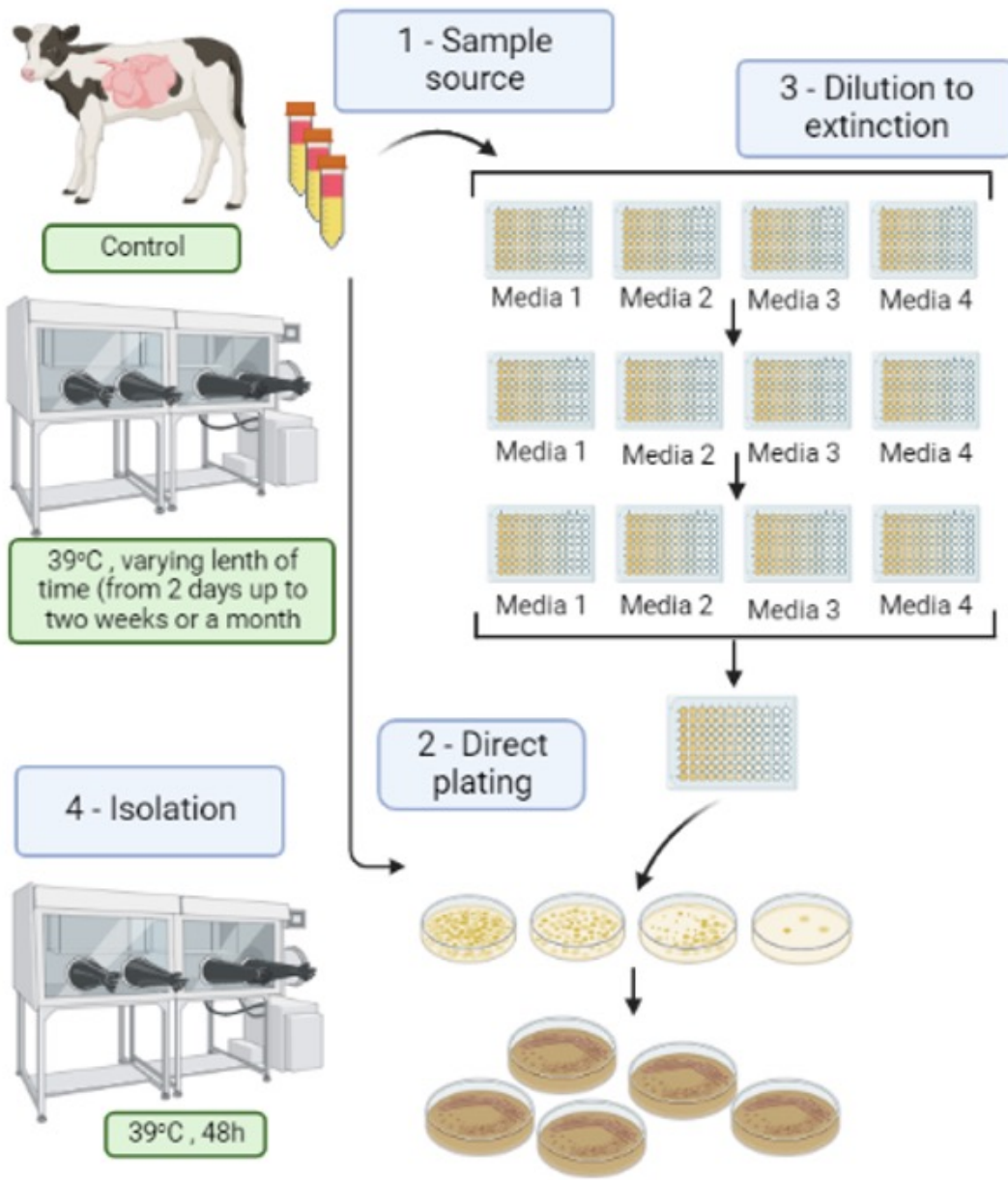
AARHUS UNIVERSITY (AU) → 38

QUEENS UNIVERSITY OF BELFAST (QUB) → 673

THE UNIVERSITY COURT OF THE UNIVERSITY OF  
ABERDEEN (The Rowett, UoA) → 15

Distinct medium, host (species, age, diet)

## Bacteria – Batch-1 726 -16S sequences

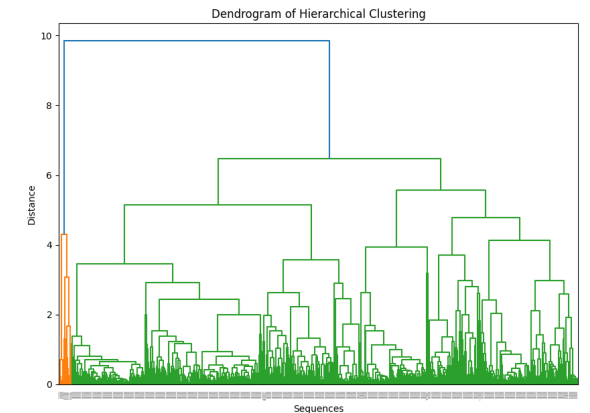
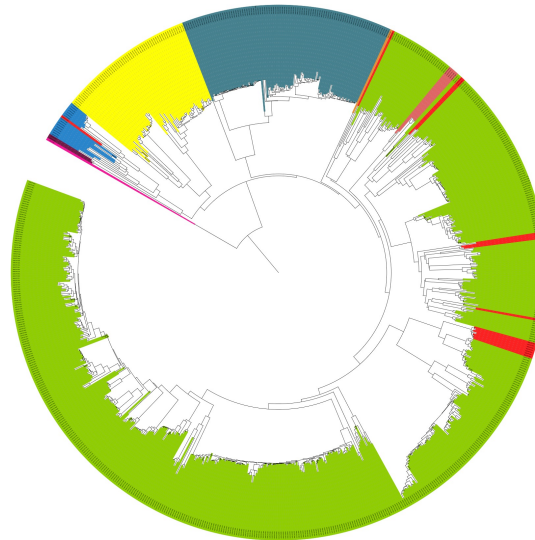
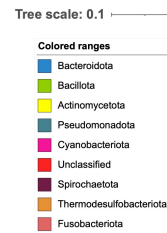




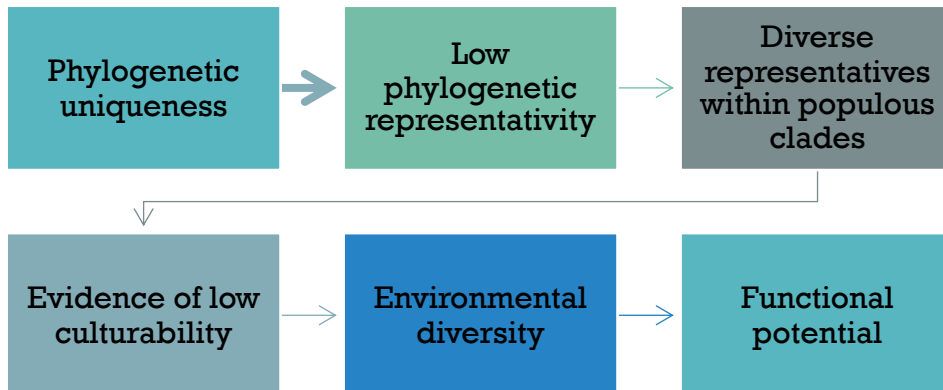
# SYSTEMATIC SELECTION OF ISOLATES FOR GENOME SEQUENCING AT JGI



- ✓ Comparison against Hungate sequences and public databases (SILVA; GTDB; Greengenes2...)
- ✓ Hierarchical clustering;
- ✓ Management board discussions.



Clustering based on a 97% similarity threshold using hierarchical clustering - **88 clusters.**

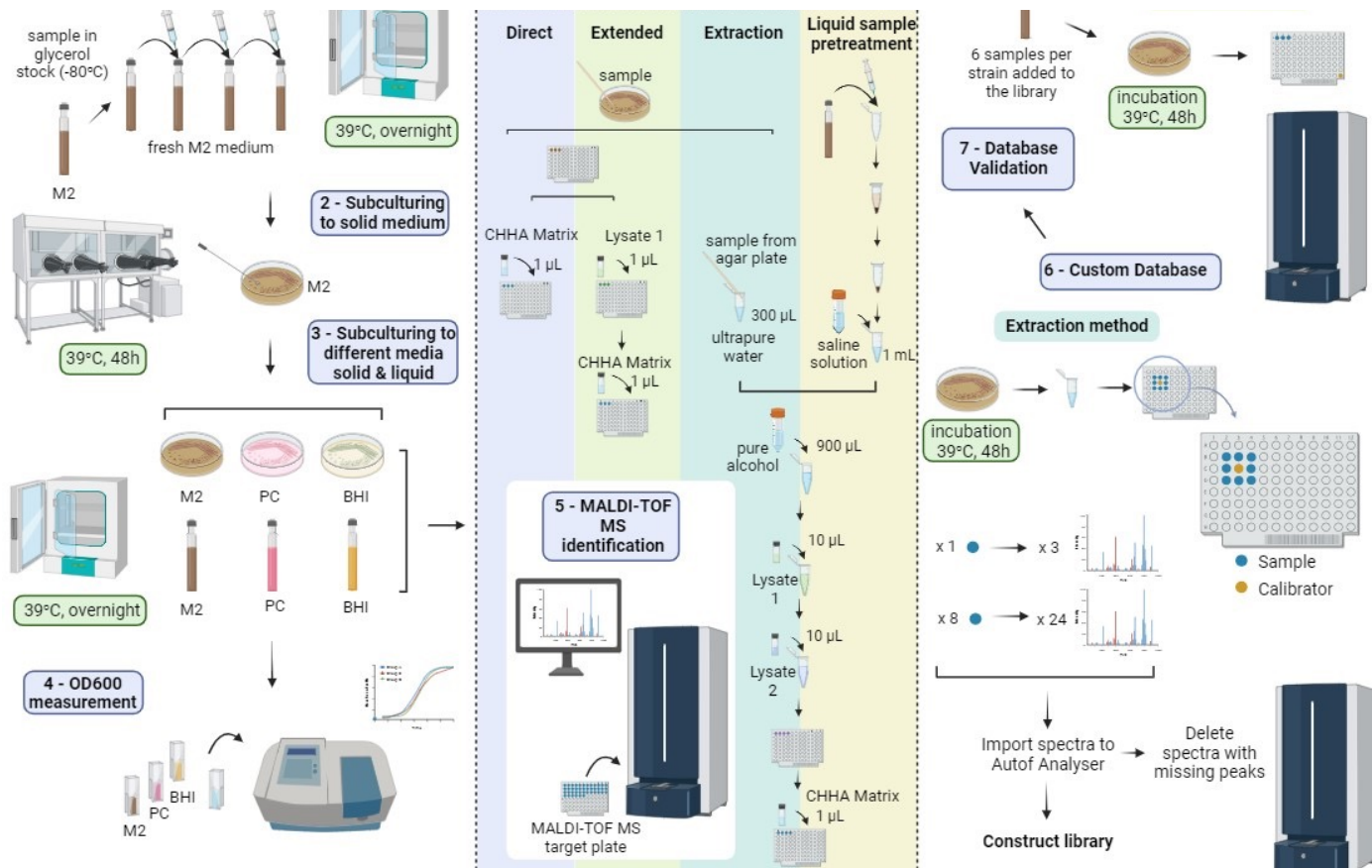


| Range of % of identity | Hungate 16S genome assemblies | Hungate 16S PCR | Greengenes2* |
|------------------------|-------------------------------|-----------------|--------------|
| <80%                   | 4                             | 3               | 0            |
| <80% and >85%          | 19                            | 2               | 0            |
| <85% and >90%          | 76                            | 59              | 1            |
| <90% and >95%          | 167                           | 138             | 41           |
| <95% and >97%          | 44                            | 25              | 8            |
| <97% and >100%         | 416                           | 499             | 676          |

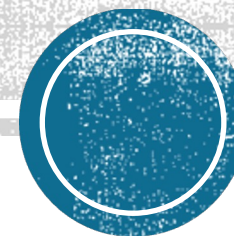
**Greengenes2\*=23,467,470 sequences**

| 726          | SILVAdb | GTdb |
|--------------|---------|------|
| Domain       | 711     | 134  |
| Unclassified | 15      | 592  |
| Phylum       | 9       | 9    |
| Unclassified | -       | -    |
| Class        | 12      | 10   |
| Unclassified | -       | -    |
| Order        | 24      | 15   |
| Unclassified | -       | -    |
| Family       | 35      | 20   |
| Unclassified | -       | -    |
| Genus        | 65      | 34   |
| Unclassified | 7       | -    |
| Species      | -       | 40   |
| Unclassified | -       | -    |





# MALDI TOF DATABASE





- QUEENS UNIVERSITY OF BELFAST (QUB)
- UNIVERSITY OF BRITISH COLUMBIA (UBC)
- NANJING AGRICULTURAL UNIVERSITY (NAU)
- INSTITUTE OF ANIMAL SCIENCES, CHINESE ACADEMY OF AGRICULTURAL SCIENCES (IAS-CAAS)
- CORPORACION COLOMBIANA DE INVESTIGACION AGROPECUARIA (AGROSAVIA)
- INSTITUT NATIONAL DE RECHERCHE POUR L'AGRICULTURE, L'ALIMENTATION ET L'ENVIRONNEMENT (INRAE)
- THE UNIVERSITY OF GALWAY (UOG)
- AGENCIA ESTATAL CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS (CSIC)
- UNIVERSIDAD NACIONAL DE ASCUNCION (UNA)
- UNIVERSITY OF KWAZULU-NATAL (UKZN)
- AGRICULTURAL RESEARCH COUNCIL (ARC)
- UNIVERSITY OF PRETORIA (UP)
- WAGENINGEN UNIVERSITY , DEPARTMENT AGROTECHNOLOGY AND FOOD SCIENCES (WU)
- THE REGENTS OF THE UNIVERSITY OF CALIFORNIA ON BEHALF OF IT'S DAVIS CAMPUS (UCDAVIS)
- THE UNIVERSITY COURT OF THE UNIVERSITY OF ABERDEEN (The Rowett, UoA)
- AARHUS UNIVERSITY (AU)
- INSTITUTO NACIONAL DE TECNOLOGIA AGROPECUARIA (INTA)
- BIOMEDIT, LLC (BiomEdit)
- AGRESEARCH LIMITED (AGRESEARCH)
- THE BOARD OF TRUSTEES OF THE UNIVERSITY OF ILLINOIS, (UIUC)



**RUMEN GATEWAY TEAM  
AT SOBS - IGFS  
THE QUEEN'S UNIVERSITY BELFAST**

