Preserving the microbiome and curating meta-data for Phytobiomes research

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Summary

What is CABI and what is a Phytobiome?

Understanding the History and context

EU Microbiome Support – biobanking- why is it needed?

The UK Crop Microbiome Cryobank – In practice

The EU Microbiome Biobanking Enabler (EU Microbe)

A cryobiological approach

Opportunities
CABI in Brief

- **Not-for-profit** intergovernmental organisation established in **1910** by a UN treaty
- Provides scientific expertise and information about **agriculture** and the **environment**
- Expertise in: scientific publishing and international development
- Owned by **48 member countries**
- **400 staff worldwide** in 21 locations
- Annual turn-over **£28m** (2013)
- Compliant with requirements for **Joint Management** of strategic EC programmes, following successful 4-pillar audit in 2011
our mission
CABI is a not-for-profit international organization that improves people’s lives by providing information and applying scientific expertise to solve problems in agriculture and the environment
Phytobiomes: Complex Systems of Plant-based Agriculture

Plants

Microbiomes and macroorganisms/macrofauna

Soil

Arthropods, other animals and plants

All influenced by management practices
A straw poll at two 2022 conferences

Have you ever obtained a reference strain from a public culture collection? Approx. 70%

Have you ever deposited a strain in a public culture collection or biobank? Approx. 10%

Have you uploaded sequence data to ENA, GENBANK or similar? >40%

Having uploaded the sequence, have you then deposited the archive material in a collection / DNA bank / biobank < 1%!!
“State of the Ark”

- Existing Culture Collections: Only axenic cultures are ‘generally’ stored
- Cultures are rarely stored as consortia or as ‘microbiome’
- Human and animal microbes are often prioritised over environmental and plant associated organism

**Culture collections** (generally) preserve living organisms and their derivatives, often propagatable.

**Biobanks** are either reference collections of dead/fixed material such as tissue banks & museum collections or ‘viable’ seed banks or medical related e.g. blood banks, ivf clinics
An uncomfortable reality – fungi as a case example

The World Data Centre for Microorganisms provides a global view of microorganisms held, and there are almost 3.2 million strains available for reference and research, of which 849,724 are fungal strains.

More than 75% of the currently described fungi are not available as living samples for study.

The data are extracted from 793 culture collections in 77 countries and regions. However, these collections represent existing fungal diversity rather poorly.

The collections and strains are distributed disproportionately in the ‘Global North”, with most collections in Europe (250) and North America (197)

Africa is one of the ‘megadiverse’ regions of the world, yet it has only 18 WDCM registered collections.

Historical ‘culture collection’ data sets are often fragmented (no GPS, lack of provenance, isolation details etc.)

Collections supporting ‘traditional’ microbiology – an example

CABI-IMI collection

Founded in its current form by Major Dade – National Status (1947)

The collection retains the historical ‘IMI’ acronym for strain accessions

A culture collection of 30,000 living microorganisms, DNA and associated data representing 6,000 species

Strains deposited by scientists from across the world, mainly from CABI projects

Culture maintained using state-of-the-art (incl. Stirling Cycling) preservation techniques to keep the cultures in optimal condition

IDA under the Budapest Treaty for Patent Deposits

Incorporates UK, BAS & NCWRF collections

Focus on agriculture and the environment

Over 55% plant associated

Global Distribution
Moving towards the future – The challenge!

The genomics revolution has changed the ‘status quo’. The collections and biobanking community has to evolve to meet the needs of researchers to provide the tools and solutions needed to underpin R&D

Seth Goldenberg!

Save the microbes to save the planet.
A call to action of the International Union of the Microbiological Societies (IUMS)

Rino Rappuoli¹,²* Paul Young³, Eliora Ron⁴, Simone Pecetta⁵,⁶ and Mariagrazia Pizza⁵,⁷
Microbes are everywhere in the food system

Diverse microbial communities consisting of fungi, bacteria, protozoa and other micro-organisms occur in all parts of our food system and are essential in its functioning and health for food security and climate change mitigation.

Individual microbes can be harmful to plant, animal and human health. If environmental conditions are in their favour, these microbes are often a natural part of microbial communities in low numbers.

**Soil environment**

Soil-environment communities live deeper underground and the special plants that grow there have adaptations to survive in low light and nutrient-poor soils. Microbes in this environment contribute to pollination and disease control, which benefit crops and other non-harvested plants and crops.

**Aquatic environment**

The fish we eat have microbiomes, sometimes to protect us from food-borne infection.

**Shellfish and disease**

Shellfish are extensively and massively eaten in seafood in Europe, causing food poisoning.

**Single celled algae**

Phytoplankton algae are the main primary producer for fish and shellfish.

**Agricultural soil**

Nitrogen fixing and other plant-growing nutrients are produced by soil microbes. They can convert atmospheric nitrogen into a form that is useful to plants and act as protectors against pathogens.

**Plants and disease**

Indigenous soil microbes can naturally control plant disease and help crops. If environmental conditions are right.

**Animal environment**

Animals have their own gut microbes. Skin and mucosal immune systems are under microbial control. At the same time, microbes help control pathogens and supporting immune functioning.

**Animals and disease**

Pathogens make the association that causes disease, which is often linked closely to the immune system and the immune response.

**Microbes on food**

In the human gut, food microbiomes are associated with probiotics and antithetists, which help promote health.

**Human environment**

Humans have their own gut microbes. The gut microbiome is strongly influenced by the food we eat.

**Humans and disease**

Microbes can impact on human disease regulation and human immune system. Inflammation.

**Food waste**

Food waste is a significant source of microorganisms. It can impact on food spoilage and food-borne pathogens in the environment.

**Microbiome support**

Microbiome support can help with nutrient absorption and disease prevention.
Scientific Life

Development of Microbiome Biobanks – Challenges and Opportunities


The EU project MicrobiomeSupport assessed resource infrastructure needs in this important area of research (Figure 1). In this paper we consider why and what we need to preserve, and how it should underpin microbiome research.

Microbiomes in the Context of Biobanks and Culture Collections

Microbiomes are dynamic and complex systems consisting of bacteria, archaea, fungi, algae, protists, and viruses, and the principles of microbiome formation/functioning are the same regardless of host organism or environment. A recent revisit of microbiome definition proposes that it is the theatre of activities of microorganisms living in a given ecosystem [2].

Whilst every ‘culture collection’ has microorganisms isolated from microbiomes, these represent the culturable components preserved in an axenic state. The German DSMZ collection is one of the few collections with broader, collective deposits of culturable microbiome samples, including strains isolated from Arabidopsis [3], human intestinal microbiomes [4], and of the product available for subsequent use but will be translatable to scientists working in other domains such as food and agriculture. In the agricultural domain, the Rothamsted Sample Archive (UK) consists of wheat grain, straw, soil, and herbage together with fertilizers. Seed banks, for example, the Kew Millennium Seed Bank (UK), contain seeds and associated microbial endophytes. Whilst a culture collection will ensure that their microbes are preserved optimally [1] around a sustainability model of ‘growth and supply’, a biobank will generally store the sample not necessarily focusing on the viability or stability of all the constituent microbial components. This represents a clear demarcation of a living ‘culture collection’ and a ‘biobank’ archive repository, although there are occasional exceptions.

The Microbiota Vault (www.microbiotavault.org) represents the first major step towards a comprehensive microbiome resource. This initiative is a proposal for a vault for microbes important to humans and calls for an international microbiome preservation effort [6].
Fragmentation of infrastructure is common in Europe and beyond

BIOBANKS
Global networks:
ISBER International Society for Biological and Environmental Repositories
Regional Networks:
ESBB European, Middle Eastern, and African Society for Biopreservation and Biobanking
EU infrastructure:
BBMRI Biobanking and Biomolecular Resources Research Infrastructure

CULTURE COLLECTIONS
Global networks:
WFCC World Federation for Culture Collections
Regional Networks:
ECCO European Culture Collections Organisation
EU infrastructure:
MIRRI Microbial Resources Research Infrastructure

DATA AND BIOINFORMATICS
Global networks:
WDCM World Data Centre for Microorganisms
GGBN Global Genome Biodiversity Network
EU infrastructure:
EMBL European Molecular Biology Laboratory
ELIXIR

OTHER EU INFRASTRUCTURES AND PROJECTS
EMBRIC European Marine Biological Research Infrastructure Cluster
EMBRC European Marine Biological Research Centre
CORBEL Coordinated Research Infrastructures Building Enduring Life-sciences Services
EU-OPENScreen

Horizon2020 European Union Funding for Research & Innovation

The need for a data infrastructure to support European and global microbiome research

High-quality microbiome research relies on the integrity, management and quality of supporting data.

Currently biobanks and culture collections have different formats and approaches to data management.

A need for

I) a standard data format to underpin research, particularly in line with the FAIR data standards of findability, accessibility, interoperability and reusability.

II) a unified, coordinated approach that ensures compatibility of data & to ensure linkage between bioinformatic databases and the wider research community

Historical and provenance data + Physical Organism or Material + Genomic Metadata

→ Links to Nucleotide archives & MGNify

Why do we need ‘microbiome’ biobanks?

To aid the development of standards
To allow deposits to ensure compliance with legislation including IP, Nagoya etc.
As a source of new potential products for industry, medical and environmental applications
To protect IP e.g. stable storage of SynComs, LBP’s, outputs from academia and industry
For biodiversity conservation
To provide resources to underpin research, furthering our scientific knowledge but also to ensure the reproducibility and stringency of research
To ensure the link between provenance, sample and bioinformatic meta data
Translating to the Microbiome

The UK Crop Microbiome Cryobank (UKCMB) – to establish a cryopreserved and characterised crop microbiome resource (including baseline sample characterisation and metagenomic analysis thereof) to underpin UK and international crop research, building on the UK Agritech capability provided through the Centre for Crop Health and Protection (CHAP).

The UKCMCB will provide a comprehensive platform to facilitate research towards optimising plant yield in an integrated crop management framework. This provides an infrastructural baseline for other projects and science-based activities.

Focussing on key UK crops including wheat, OSR, barley, oats, sugar beet & beans (further details in Nic’s talk)

see www.agmicrobiomebase...
Summary of the key projected outputs of UK Crop Microbiome CryoBank

- A cryopreserved resource of characterised material from crop microbiomes with a prioritised collection strategy. Frozen samples will be made **publicly available** to the user community through the Agmicrobiomebase.org database and will be dynamically linked to genomic data.

- Robust methodologies for collection and storage of **intact microbial communities in environmental samples and extracts of total DNA**, which will be available to researchers.

- Enhanced capability to sustainably maintain the resource in a **genotypically and phenotypically stable** state.

- Genomic characterisation of the samples for assessing microbial diversity (including symbionts, endophytes, pathogens), from whole community taxonomies (bacteria, fungi, viruses) to individual isolate genomes.

- An added value demonstration of the utility of the UK-CMCB to the user community through PGPR isolation and **synthetic community construction**.

- A validated sequence resources database, ‘**AgMicrobiome Base**’ linked to EBI, available to Agritech sector and researchers, including model organisms and novel product outputs.
1. Collection, Production and Culture (Rothamsted)

2. Genomics and Bioinformatics (SRUC & Hutton)

3. Cryopreservation - Optimisation and Application (CABI)

4. SynComs for Agritech (JIC & UEA)

UK Crop Microbiome Cryobank

barley, oats, oil seed rape, potato, sugar beet & wheat
3 x soil types (x3)

AgMicrobiome BASE
www.agmicrobiomebase.org

EBI MGnify

Sequence data

Provenance data and strain information
Where do we start – Cryopreservation?

Storage at ultra low temperature in a cryogenic gas or liquid

Used widely since the 1960’s and the ‘Rolls Royce” of methods

Needs a careful approach to ensure sample integrity is not compromised

Optimal cryopreservation regimes are required for preserving functional potential and maintaining population structure

Huge resource and potential: Cryo-Storage of microbiome samples – ‘a snap shot in time’
What can we learn: Factors affecting survival in cryopreservation

• Growth conditions
• Cell type
• Cryoprotection
• Cooling rate
• Storage temperature
• Thawing
Cryoinjury & cryo-recalcitrance
Cryomicroscopy: 
*Aspergillus repens*
How can we predict what will survive?

Most Bacteria – flagellate species more vulnerable?
Any organism producing a resting stage
Sporulating fungi e.g. those producing ascospores or conidia

*Problematical*

- Algae
- Basidiomycetous fungi
- Protozoa
- Nematodes unless in an anhydrobiotic state
- Chromists including inc. Phytophthora

Caution – we traditionally preserve populations of cells. **Not** lines derived from single cells / spores

By utilising different methods we hope to capture a broader representative proportion of the ‘living’ microbiota in each sample
Cryopreservation - utilising two methods

Controlled rate cooling using a Stirling Cryocooler
Will capture most freeze tolerant organisms

An encapsulation vitrification approach
Will capture more delicate and freeze recalcitrant organisms

We chose not to select freeze drying (as proved later!) or sub-optimal storage at -80°C
Progress to date – Construction of the resource is almost complete!

We have preserved 4,800 individual cryovials consisting of:

2,400 preserved by Stirling Cycle Controlled Rate Cooling

2,400 preserved by encapsulation dehydration

Representing all bulk soil and rhizoplane samples from the Rothamsted pot experiments

Data is being uploaded to AgMicrobiomeBase & EBI platforms (Nic’s talk)
A question of functional potential

Assaying the functionality of *Brassica napus* rhizosphere microbiomes to identify a preferred method for cryogenic preservation.

Looked at effects of freeze drying, controlled rate cooling and plunge cooling against control samples

Biolog Eco Plates were used for community-level physiological profiling. BacTiter-Glo™ assessed ATP levels. Fluorescently marked substrates assayed exoenzyme activity and MicroResp™ was used to compare respiration
Enzyme and ATP assays along with Biolog plates showed freeze-drying to be the least effective method for preservation. Freeze-drying had too greater impact on the microbiome.

Plunge cooling in liquid nitrogen and controlled rate cooling were repeatedly found to conserve microbial functionality equally.

Biolog assays suggested both preservation methods caused slight drops in functional diversity. On all other measures (ATP, enzyme activity and respiration) there were no statistically significant differences compared to the control soils.

Controlled rate cooling is however safer and more convenient compared to plunge cooling.

Once Cryopreserved at ultra-low temperature, samples will not change over time.
The EU Microbiome (RI) Biobanking Enabler (EU Microbe)

Partners

AIT (Lead)
CABI
DSMZ
EMBL
HMGU
INRAe
MUG
RTD services
Sorbonne
(Roscoff)
Project Outcomes:

- Validated protocols for preservation, isolation and co-cultivation of complex microbiome samples
- Novel isolates representative of key microbiome members from different domains
- Novel synthetic consortia reproducing the key functionalities of selected ecosystems
- Customised data infrastructure tools for microbiome biobanking
- Guidelines for implementation of standardised microbiome biobanking workflows in selected fields
- Guidelines for establishment of ethical and legal framework conditions that enable microbiome biobanking
- Business models for the implementation and exploitation of novel microbiome-based technologies and resources
- Portfolio of training and educational resources

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WP: Validated protocols for preservation and propagation of complex microbiome samples

The aim is to develop:

- new preservation approaches for integrated preservation of microbiomes and synthetic consortia;
- new sampling procedures and stabilization reagents not requiring cooling chains for microbiome samples used for nucleic acid-based analysis;
- novel propagation technologies for defined complex samples (i.e., synthetic consortia);
- approaches for optimized assessment of microbiome functionalities.

Working with EMBL to ensure data is handled appropriately and uploaded through biosamples and represented in MGNify.

Work is focussed on Soil, Seeds and Marine systems. First stage involved testing existing preservation protocols. Initial results are very encouraging. Methods conserve biodiversity – genomic approaches will provide further insights into the success of preservation approaches and will provide the baseline from which future progress can be measured.
Priorities Identified in the Strategic Roadmap

1. Foster a “Microbiome Centres of Excellence” approach
2. Create Microbiome Research & Innovation Collaboration Networks
3. Encourage Microbiome entrepreneurship, seed funding, regulatory and intellectual property rights support
4. Ensure support for and access to emerging, enabling technology
5. Establish Microbiome research standards
6. Develop “Next Generation” bio-banking
7. Harness the potential for new and rapid diagnostics
8. Invest in Microbiome process development and pilot-scale manufacturing
9. Promote a supportive regulatory environment
10. Improve Microbiome education, skills, and talent pipeline
11. Prioritise support for specific opportunities where the UK has a distinct advantage
12. Increase strategic funding for Microbiome Research and Innovation
Established a group to directly address the requirements for microbiome biobanking in a ‘one health context’

Report due end 2023!

Timely with UK’s reaffirmed participation in Horizon Europe!
Summary: the next steps.

Need for broader UK, European and International Infrastructure for Microbiome Biobanking

UK Crop Microbiome provides is a model of an ‘agri-focussed’ microbiome resource

Key areas for future scientific research retention of functional potential & stability of ‘SynComs’

Facilities for deposits to ensure regulatory compliance (Environmental, Patents, Nagoya etc.)

Development of standard protocols and reference standards to ensure reproducibility and quality

Encourage all plant microbiome scientists to be responsible!!

Ensure engagement of industry – we share common challenges
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