The UK Crop Microbiome Cryobank
Biobanking the Phytobiome to ask biological questions

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1. A unique resource

Microbiome biobanks are required to underpin Phytobiomes research. Integral to this is the need to preserve soil & plant microbiota and associated metadata. This is required for protection of intellectual property, compliance with legislation to validate the stringency of research and to provide resources for future reference and commercial use.

The UK Crop Microbiome Cryobank is a newly constructed biological resource of 36,000 microbial isolates and 4,800 soil rhizoplane samples representing 6 economically important crops: wheat, oil seed rape (canola), barley, fava bean, oats, and sugar beet. Well characterised bulk soils with known cropping history were sourced from the UK BBSRC ASSIST network or from Hutton Institute and Rothamsted farms (Fig 1)

Fig 1. Soil sites from the BBSRC Assist Programme JHI and Rothamsted Research sites. Soil texture data was clustered using complete linkage producing three major groups (Texture clusters 1, 2, and 3). Texture cluster 1 is formed by sandier soils (sandy loam); cluster 2 is formed by clay soils (clay loam and clay), while texture cluster 3 is formed by silt soils (clay loam and silt clay loam).

2. Experimental Approach

Approx. 50 kg of soil was passed through a 5 mm sieve, then homogenised and used to culture plants (without added fertiliser) from surface sterilised seeds in 20cm pots under controlled glasshouse conditions for each crop, with 3 plants per pot. In total, five replicate pots for each of 6 crops (+unplanted bulk soil) and 9 soil sites were prepared, equating to 315 pot systems on which the core resource is based. Genomic DNA was prepared for culture-free descriptive assessments of all 315 samples. A strain library was prepared by culturing bacteria on 1/10th Tryptic Soy Agar (TSA) from the rhizosphere and bulk soil. Inoculant dilutions allowed up to 20-40 colony forming units per cultured plate, avoiding nutrient competition effects on isolate development. This resulted in up to 96 microbial isolates per crop per pot system, and a unique collection of approximately 36,000 isolates was generated.

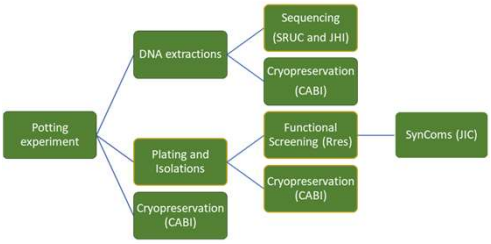

Fig. 2. Project workflow, the success of the project workplan is dependent on sample processing and culturing, each partner is responsible for a specific work-package:

3. Cryopreservation

All samples were cryopreserved at ultra-low temperature between -175 and -190ºC, utilising liquid nitrogen as a cryo-refrigerant. For the bulk soil and soil rhizoplane samples, a careful approach was required to ensure that sample integrity was not compromised during cooling. This was achieved through the application of optimised techniques involving controlled rate cooling utilising a ViaFreeze™ Duo Controlled rate cooler with a cooling regime of -1ºC per minute and an encapsulation dehydration method. For the microbial isolates a 'next generation, high throughput' approach, developed for use in biomedicine, was utilised, employing a specially designed manifold plate holder for the Via Freeze cooler, where glycerol (10% w/v) was used as a cryoprotectant, and samples preserved using a cooling rate of -1ºC minute.

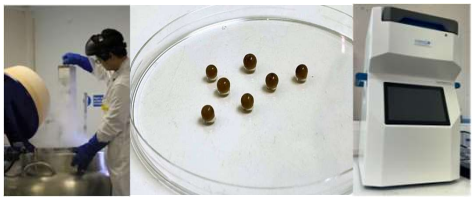

Fig 5. (L to R) Cryotank, Encapsulated soil in calcium alginate beads before storage in liquid nitrogen & the ViaFreeze Duo Controlled rate cooer

4. Characterization, Sequencing and Data handling

All soils have been characterised and the culture collection of 35,000 isolates is being screened for phenotypic traits. The community composition of the rhizosphere has been determined by ribosomal RNA gene amplicon sequencing for bacterial (16S) and fungal (ITS2) taxonomy. This informed sample selection for higher resolution analysis using a shotgun metagenomic sequencing approach.

Selection of samples for shotgun metagenomics sequencing reduces redundancy and contributes to cost efficiency. Selected culturable microbial isolates are being whole genome sequenced to provide understanding of functional diversity as well as their phylogeny. This also permits potential further integration of the different sequence-based datasets. Sequencing technology and data analysis approaches rapidly evolve, so any dataset is representative of a technological 'snapshot'.

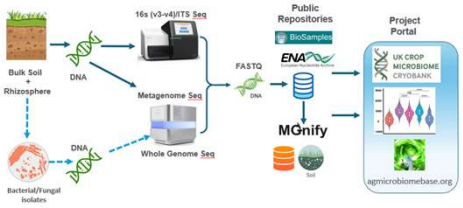

Fig 4. Cryobank Sequencing Strategy, All metadata is publicly availble through www.agmicrobiomebase.org/data with links to the European Nucleotide Archive

5. Demonstrating the utility of the resource

The UK Crop Microbiome Cryobank demonstrates the utility of a biobanking approach designed around experimental hypothesis. A work-package led by the John Innes Centre is now generating crop-associated synthetic microbial communities (SynComs) with plant growth promoting traits.

Initial analysis of data resources generated through the project is allowing us to answer 'biological questions', such as do some crops host a larger bacterial diversity than others? Do crops consistently recruit a preferred consortium of microbes from different soils and what are the trends within recruitment patterns? Would this knowledge affect crop rotation choice? Can we use the data to construct SynComs using an evidenced based approach? Further, we have evidence that shows that plants select on the basis of phenotype rather than their taxonomic status. As a publicly funded resource, we are keen to collaborate, so please do reach out to us!

- Data
- Microbial Isolates
- Soil Samples
- Deposit
- Collaboration
- Methods and Protocols
- Newsletter

Please Scan Here!

Reference:
Ryan MJ, Mauchline TH, Malone JG et al. The UK Crop Microbiome Cryobank: a utility and model for supporting Phytobiomes research. *CABI Agric Biosci* 2023;4:53.

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