Please find attached the slide deck from the Microbiome Outreach event in September and we very much thank you for your participation. We hope you enjoyed the opportunity to meet face to face and we thank you for your participation. The main outcomes of the breakout session are summarised below.

Since the meeting, it has been very busy: the main experiment has been progressing well, with samples being transferred to CABI for long term cryopreservation. Additionally, we have also held our annual review which is a requirement of the funder.

We would like to take the opportunity to remind you of the 3rd International Plant Microbiome Symposium, taking place in Dundee, Scotland between 24 – 26th May 2022.

Please do contact us should you have any questions, and we look forward to further engagement and discussions in the new year.

With Best Wishes,

Matthew Ryan, Tim Mauchline, Nic Holden, Jake Malone & Ian Clark on behalf of the UK Crop Microbiome Cryobank Team

www.agmicrobiomebase.org

Twitter: @pmicrobiome

Domains covered:

The Cryobank covers a range of UK Crops. This will help establish a baseline of holdings, while allowing for method development. However, it was acknowledged that there are many crops of other UK and international importance, especially related to horticulture where the establishment of biobanking capacity should be targeted in future. These include (but are not limited to) Strawberry, Hops, Apple, Rice, Beans, ornamentals, other perennials and commodity crops such as coffee, cotton and cocoa.

Targeted approaches

The initial focus of the project is the cryobanking of DNA, bulk soil and rhizosphere material. While other material will be stored, they are not a key focus of the project. However, future projects should look to other niches including the endosphere as well as the phyllosphere and the seed associated microbiome.

There were a number of questions asked in plenary. These included the need to consider environmental contamination including heavy metals, where in the field

samples were collected from (addressed by Tim Mauchline), the requirement to not forget fungi, the importance of mycorrhizae and the need to consider the broader holobiont relationship.

Cryobanking as a service:

The methods developed during the project could have much wider application. This includes the protection of IP through deposits related to the Budapest treaty, storage (safe deposit) of inoculum for i) environmental regulation, ii) as a permanent record of organisms released into the environment for iii) business continuity & academic research

There is an urgent need to develop standards both for industry and academic research science but also for the operation of microbiome cryobanks. It is important that standards are coordinated internationally. The provision of inoculum for baseline studies was also highlighted, and the cryobank is a good example of this.

Industry outreach

Participants stressed the importance to reach out to and engage with industry. CHAP is involved on the project steering group and is well placed to provide links. An event with CHAP was suggested. In addition to the elements included under “Cryobanking as a service”, it was also pointed out that the mechanism that we are using to cryopreserve our samples is a fantastic delivery mechanism. The outputs of most potential value for industry will come out of the work package managed by Jake Malone. However, this is not due to begin until late 2022. This gives an opportunity for industry to help mould the development of synthetic communities for potential agricultural application related to the development of biocontrol solutions, biofertilisers to enhance nutrient availability to plants and adjuvants.

The need to engage with industry was highlighted on several occasions, and the importance of a clear, unified intellectual property strategy was agreed.

The supporting science

The science supporting both cryobanking and soil health / crop microbiome is developing at a rapid pace. This is aided by the continuing evolution of genomics and bioinformatics. Specific areas for development include:

1. The application of advanced cryobiological techniques – CABI is involved in proposals led by Kew (Prof Hugh Pritchard) in this area.
2. The need to assess the impacts of cryopreservation on the stability of samples. To include both functional potential, genomic integrity and the maintenance of

community structure, but also to ensure that cryopreserved samples do not represent a subset of freeze tolerant microbiota.

3. Development of Synthetic Communities and the incorporation of gnotobiotic approaches.
4. Culturomics and Phenomics – development of microbial culture collections, supported with strong metadata. Collections should also relate microbial species identification to function, especially in terms of beneficial functions related to plant growth promotion.
5. Bioinformatics: functional data, RNA based technologies, eDNA approaches - see below.

AgmicrobiomeBase and Bioinformatics

The importance of metadata capture was a recurring theme. AgMicrobiomeBase will capture complete datasets related to the physico-chemical characteristics of the soil, sample provenance, the physical isolate but also the digital sequence information through EMBL and links to EBI's MGnify.

We were asked whether AgMicrobiomeBase could be used to see if microbes are linked to certain characteristics and environments. The answer is yes, and links to MGnify will also help with this.

Discussions centred on the ability of the bioinformatics approaches to provide functional data. We discussed the feasibility of different technological platforms and the need to rationalise functional assessment over taxonomic community assessment. However, the nature of technological developments in microbiome research can also be seen as an opportunity. While we will use contemporary approaches to best address our specific questions, an advantage of such a rapidly developing field is that the datasets can easily be reanalysed in response to technological advancements. There is a need to define which functional traits will form the focus of analysis in metagenome datasets: there are a number of different routes that can be taken and it will be important to prioritise based on agritech & microbiome research community needs. A good point of inclusion was for detrimental, e.g. pathogenic traits (effectors/ microbial secondary metabolites) that can be difficult for pathogen control (e.g. DAPG), in addition to the beneficial ones, such as plant-growth promotion / anti-pathogenic etc. Relating culture-independent functional analysis to isolate / community functional assays is potentially possible, although identification of the genetic basis to *in vitro* / *in planta* functional traits in the metagenomes requires consideration that a given isolate may not necessarily be present in sufficient abundance to be detectable in metagenome sequences. We did identify limitations related to experimental selection criteria: but we have to standardise the approach, otherwise it will be impossible to account for microbial physiological responses and ecological interactions with the microbiota communities between samples. Another point of limitation is that the microbiome analyses are based on

DNA, as opposed to RNA, so encompasses the entire microbiome, rather than functionally, metabolically active or viable microbes. However due to budget limitations, it is not currently feasible to pursue an RNA based approach. It can also be argued that a DNA approach offers the more comprehensive description of microbiome potential, from which future targeted RNA studies can be derived.

Future Research Priorities -The UK Plant Microbiome Initiative and broader networks

The cryobank serves as an excellent avenue for collaboration on a national and international scale to coordinate and expand this type of resource. The future research priorities should be related to both industry needs. A key area of focus should be Biodiversity Conservation which should not be ignored, this is related to climate change and its mitigation and presents a unique opportunity for cryobanking.

The crop microbiome project could easily form the core of a network of crop/plant & soil microbiome UK community, to share findings and technical approaches, coordinate research efforts, and provide a hub for ECR. There is significant support for developing the community under the auspices of the UK Plant Microbiome Initiative. This could also form a natural network for the KTN microbiome outputs. Links to the KTN are already in place and the team contributed to the strategic roadmap for microbiome research, that aims to link up efforts, and has produced a 'landscape' map of all UKRI-funded microbiome projects. Further, there is an effort to coordinate biobanking effort across domains utilising a ‘One Health’ approach and ensure that the wider research community and UK innovation is supported.

Opportunities also link to the possibility of a Big Data-focused doctoral training school DTP that could be pursued with appropriate university partners.

Finally, the need for collaboration and to link in with international biobanking initiatives, and other resources centres such as DSMZ (German Collection of Microorganisms and Cell Cultures) was emphasised - this is crucial to avoid duplication and reach the goal of common standards.

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