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Development of Microbiome Biobanks – Challenges and Opportunities

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The microbiome research field is rapidly evolving, but the required biobanking infrastructure is currently fragmented and not prepared for the biobanking of microbiomes. The rapid advancement of technologies requires an urgent assessment of how biobanks can underpin research by preserving microbiome samples and their functional potential.

Introduction

Traditionally, microbiology has relied on culture collections and their associated services to underpin and ensure the quality and reproducibility of microbiological research [1]. Microbiome science signals a paradigm shift in the scientific approach from preservation of axenic samples in culture collections towards preservation of complex communities, which requires the supporting infrastructure to be developed. 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 mouse microbiomes [5]. Culture collections typically operate a business model where organisms are propagated for sale and distribution. However, for microbiome samples with only a finite supply source, this is not an option, although collections can provide 'mock', 'synthetic', or 'constructed' microbial consortia for quality control (QC) and product supply.

Conversely, 'biobanks' may contain tissues or samples that are 'frozen' or 'fixed' as a measure or 'snapshot' in time. Various institutes biobank patients' stool samples for future medical use, for example, AdvancingBio (USA), OpenBiome (USA), Stool Bank East (The Netherlands), Metagenopolis (France), or HMGU Biobank (Germany). Stool banks are leading the way through development of sample handling processes and quality protocols. This experience will not only improve the quality 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].

Preservation and Storage

The challenges of preserving microbiome samples optimally are immense. Researchers should be aware of unintentionally and fundamentally altering the functionality and integrity of the microbiome, which is a dynamic system that changes in response to environmental influences and biotic factors. At the functional level, the removal of a single critical microbial component due to the application of a non-optimized storage approach could irreversibly affect the integrity of the system. Bell [7] quoted Adams [8] 'If you try and take a cat apart to see how it works, the first thing you have on your hands is a nonworking cat', and this is an important issue when endeavoring to conserve a microbiome sample. How would you distinguish a 'whisker' from a 'heart' and assess what a component does in the

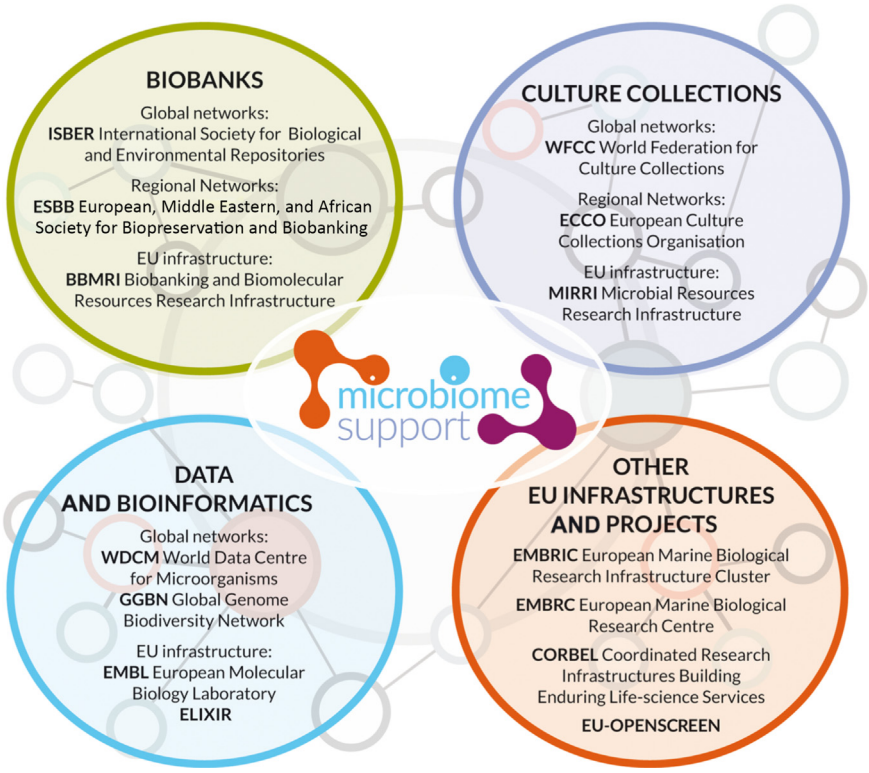

Figure 1. The Current European and International Landscape Underpinning Microbiome Research Is Fragmented.

microbiome and its relationship with the total functionality of the whole system? For example, the very nature of a crop-related microbiome changes when one changes the crop variety, the management practices, or adds or eliminates microbes.

When considering microbiome preservation there are two essential questions that need to be answered: (i) what should be preserved, and (ii) what is the best way of preserving it?

The question about why and what to preserve is a controversial one, and ultimately serves not only to underpin research quality and the generation of new microbiome-sourced microbial products (which may also have commercial value) but also to allow for preservation during a time of altered agricultural and medical practices and climate change. Similarly, there is a need to ensure that products such as probiotics remain stable over time.

Sometimes it makes scientific sense to preserve the whole community, such as symbionts with their host, rhizobacteria with soil/roots, gut bacteria in stool samples, or even enrichment cultures from natural complex communities [9]. Preserving genomic DNA may allow for studies to be repeated for confirmation of results and ensure the integrity and repeatability of research but it will only provide information about a 'snapshot in time' and will only deliver evidence for an organism being present in the system and will not be an indication of whether the organism is (or was) viable or active at the time of sampling. Similarly, storing total RNA will allow for transcriptomic assessments of what microbes were viable and potentially functional. Therefore, with respect to the current state of the art, an approach where nucleic acids, the 'intact' microbiome sample, or even protein extracts or metabolic fractions are stored is called for.

Capacity is one significant challenge to this approach. It is not practical to store large amounts of soil, hence the question of how much sample is required to be representative of the microbiome in question becomes critical. For example, in agriculture a single field encompasses thousands of localized microbiomes. Indeed, how many site-specific and temporal samples can provide a true snapshot of the field and its microbiomes? In precision agriculture, we are moving to smaller and smaller grids and not relying on a few samples over a large field. We need to calculate what can provide us the best, most accurate example.

Further, over time, there might be genomic drift in the microbiome as a result of processing and storage. Genomic shift was something considered with eukaryotic primary cell lines [10]. With microbiomes, the genetic shift and impact on species abundance could happen faster. Therefore, there is a fundamental requirement to assess and optimize the preservation techniques for microbiome samples and investigate cryobiological and alternative approaches that may be applied. The question of how requirements can be delivered and the technologies and advances that are required to conserve DNA and microbiome samples optimally needs to be addressed, preferably through a targeted and coordinated research program.

Historically, cryopreservation and freeze drying have been the methods of choice for the storage of fungi and bacteria [1] in pure form because they conserve the genomic integrity of the organism, maintaining it as close to the original

'unpreserved' wild type as possible. However, even in these systems, pathogenicity and other key functional traits may be compromised if suboptimal preservation approaches are applied [1].

Cryopreservation has been the 'gold standard' for microbial storage since the 1960s [1], and there are few reports of cryopreservation for microbiome samples. Kerckhof *et al.* [11] evaluated a cryopreservation protocol for a methanotrophic coculture, an oxygen-limited autotrophic nitrification/denitrification biofilm, and fecal material from a human donor, and succeeded in preserving both community structure (composition and abundance of taxa) and functionality of microbiomes. Vekeman and Heylen [12] described methods for the cryopreservation of mixed communities but only at –80°C and not at ultra-low temperatures.

It is widely accepted that, when samples are cryopreserved, only the freeze-tolerant cells will survive. This translates to a microbiome system of multiple components in which cryopreservation, if not applied optimally, will result in unintended selective pressures on the community. This represents the primary challenge when preserving microbiome samples, with the aim of maintaining composition and functional potential of the microbiome in as close a state as possible to that originally isolated from the field or host.

Assessing Success and Quality

A variety of approaches from metagenomics [13] to transcriptomics [14] have been used to assess the microbiome with respect to both its construction and functionality. These approaches could be used to assess the success of preservation and the storage regime but each has its limitations. However, a combination of approaches, such as that proposed by Easterly *et al*. [15] – who used an integrated, quantitative metaproteomics approach 'the metaQuantome' to reveal connections between taxonomy and protein function in complex microbiomes such as the human oral microbiome – may be the way forward. At the very least, tests should be undertaken before preservation/storage in order to characterize the microbiome and then postpreservation to ensure the compositional and functional integrity.

Summary Recommendations and the Way Forward

The question of why and what should be conserved has to be addressed in detail considering scientific, economic, social, and environmental perspectives. Taking into consideration diversity and complexity of microbiomes across environments, a prioritized list should be agreed upon to focus the efforts and achieve advancements.

The biggest technological bottleneck is the development of optimized methodologies for the preservation of microbiomes and for the assessment of preservations' success in terms of maintaining the composition and functionality of microbiomes. The clear complementarity between culture collections and biobanks necessitates an approach to enable that both work together to ensure that this critical microbiome research field has effective support. This will require the identification of infrastructural overlaps to gauge what is required and what is available/missing within the EU and beyond.

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