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COMMENTARY - NATURE METHODS

EcoFAB: Advancing microbiome science through standardized fabricated microbiomes

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Abstract |

There is immense interest in advancing microbiome science. However, current efforts are largely focused on disparate and often irreproducible experimental systems. Here we present the results of a one-and-a-half-day workshop that brought together a small group (~60) of thought leaders in microbial genomics, systems biology, microbial ecology, and bioinformatics to discuss approaches for the development of standardized model microbiomes. In all, we describe a community strategy to advance next-generation integrated efforts around model microbial communities that will help to standardize screening and systems biology-based prediction of microbial community activitiesecological frameworks.

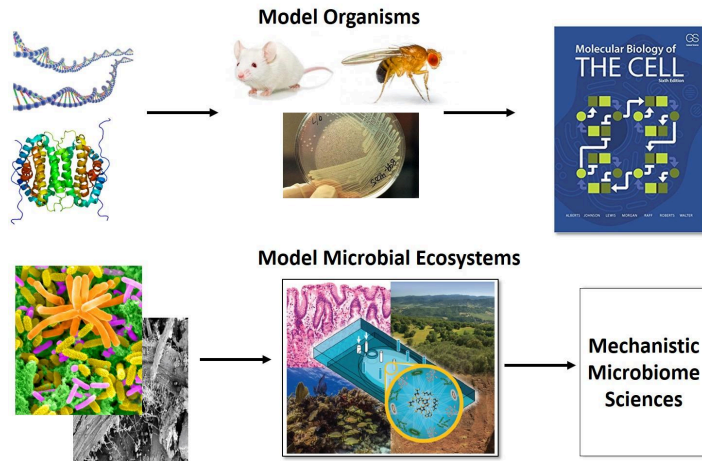


Figure 1. Need for model microbial ecosystems to advance mechanistic microbiome science just as model organisms enabled the field of molecular biology.

Introduction

Microorganisms are ubiquitous on our planet and inhabit every ecosystem we know. Most In addition, mecosystems are extremely complex, consisting of hundreds to hundred of thousands of different species and strains. The sheer diversity of these microbiomes in addition to their temporal and spatial dynamics renders reproducible studies impossible. For example, when we analyze the diversity of several environmental samples from the same location using amplicon sequencing, we find that even careful replicates have different microbial compositions. While we still can describe these samples and infer diversity, the function and dynamics of these specific microbial systems cannot be identified and extended to other soils because of the heterogeneity and temporal dynamics of natural ecosystems. As a result our data cannot be reproduced by other researchers, which has stifled progress in microbiome sciences and rendered many studies to be mostly to descriptive studies in nature that have little ability to test “stand alone incidents”. Thus, the fundamental essence of science, that is to build upon prior results and data, is currently challenging to fulfill in microbiome studies. While other research fields have developed and adapted models to gain reproducible insights into complex systems, such as *Escherichia coli*, yeast, fruit fly, zebra fish, mouse and Arabidopsis plants, standardized model systems are largely non-existent for microbiome sciences. This lack of controlled, reproducible, and standardized model systems with which to perturb and study microbiomes greatly limits progress in microbiome science. We thus advocate for the establishment of defined model systems for microbiome sciences. We argue that advancing integrated efforts of scientists around model microbial communities is urgently needed to enable standardized screening and systems biology-based prediction of microbial community activities within ecological frameworks.

It is noteworthy that there are attempts by individual labs and small research consortia to established well-defined model systems, but these systems are often highly specialized to represent a defined ecosystem and for most parts are not easily accessible by others. There is an opportunity to build on the great tradition of model organisms, where communities of scientists agreed upon particular organisms, which lead to rapid advances in our understanding of molecular biology and cellular life. We argue that advancing integrated efforts of scientists around model microbial communities is urgently needed to enable standardized screening and systems biology-based prediction of microbial community activities within ecological frameworks. Model system are compliments, not replacements, to field and clinical studies. Importantly, as the success of defined mouse models for the study of human medicine shows, these models enable mechanistic insights and allow concerted efforts of the entire scientific community making them highly cost-effective and able to speed up research and development.

Current challenges in microbiome science to be addressed by laboratory ecosystems

Standardized laboratory ecosystems, EcoFABs, could rapidly overcome current challenges in microbiome science. We have made little progress over the last decade to tackle some of the most fundamental questions in microbiome science including: Defining ecological design principles for microbiome assembly, structure, interactions, and integrated activities; understanding of the ecological function of genes, microbes and metabolites; predicting the health and trajectories of microbiomes. We argue that the development of a few widely accepted EcoFABs (Box1) would greatly accelerate microbiome science by enabling reductionistic studies focused on microbiome assembly, structure, interactions, and integrated activities among microbes, their environment, and potentially their host. Comparisons across EcoFABs would facilitate abstraction of generalizable ecological principles. These systems should be designed to enable investigations of microbiome functions and interactions at different temporal and spatial scales to advance a foundational understanding of the ecological function of genes, gene products, metabolites, and microbes. Experimental standardization will enable cross-experiment computational comparisons to develop generalizable principles and models that accurately predict the state and trajectory of microbiomes, ultimately, to enable the engineering, design, and management microbiome activities.

Recommendations to advance community development and acceptance of model laboratory microbiomes:

EcoFAB development must balance relevance with reproducibility, which is a significant challenge. They

Overarching design principles for laboratory microbiomes

- Favor simplicity but embrace necessary complexity in recapitulating natural environments.
- Design to integrate diverse technologies that enable mechanistic studies.
- Enable experiments across spatial and temporal scales relevant to microbiomes.
- Built-in sensors to capture minimum reporting information.
- Enable extensibility of designs and data—create the core platform and data portal and let the larger community

must be experimentally tractable, broadly applicable to address diverse questions, and ideally be composed of organisms known to interact in natural settings. Given these diametrically opposed design criteria, development of a few foundational EcoFABs will be a major effort for the scientific community. Most microbial communities will not constitute useful laboratory models, just as most individual species are not suitable model organisms. At our workshop (see: eco-fab.org) we determined that intelligently selecting useful systems should be guided by the overarching design principles (Box 1).

Establishing field and clinical relevance of EcoFABs will require demonstration that important microbiome activities are recapitulated in these standardized laboratory systems. Hence, developing model microbiomes should leverage existing studies to benchmark that field/clinical results are recapitulated in EcoFABs such that mechanisms identified will be translational to natural systems.

The goal of achieving reproducibility across labs should guide the construction of basal culture conditions, including minimal microbiomes. At a minimum, systems must display reproducible dynamics and genotype-environment interactions as validated through multi-lab comparisons. Because much of the diversity within microbiomes is likely neutral, resulting in stochastic assembly and dynamics and irreproducibility, it will be necessary to either use highly simplified communities or a “functional group paradigm”, where microbes are defined in particular ecological or functional niches, where the same functional dynamics are observed, despite differences in strains of the same functional groups.

Advancing the development of laboratory microbiomes will require organization and community engagement. Historically, development and establishment of model organisms has taken decades. Typically, they result when the larger community recognizes the value of extensive resources developed over the career of a few individuals [ref]. To rapidly advance model microbiomes, we must learn from successful model organism communities to rapidly accelerate the development and acceptance of model microbiomes. This requires consideration of the attributes and resources that are required to meet the needs of large research communities and their ability to meet the goals of funding agencies. Our vision is that the community should develop the scientific basis and necessary resources for a few broadly applicable EcoFABs that can be adapted and extended by individual groups of scientists—analogous to the smart phone and its diverse apps.

Effective advancement of the few broadly applicable laboratory microbiomes will require a group responsible for developing and implementing data standards, data sharing, and

EcoFAB dissemination and adoption. To help organize these efforts we formed a Steering Committee with three main responsibilities: 1) defining criteria for design, dissemination, and data standards, 2) broadly promoting the development of EcoFABs, 3) organizing multi-laboratory ring-trials to evaluate reproducibility, and 4) preparing annual white paper(s) identifying key challenges. Complementing this effort will be topical working groups spanning the major microbiomes (Animal, Plant, Soil, and Aquatic).

Developing broadly applicable EcoFABs requires fabricating the environment, defining the organisms, and developing the protocols for 'booting-up', monitoring, and maintaining the EcoFAB. The necessity of robust and detailed protocols cannot be overemphasized. They should define the inoculum and assessment of viability, environmental fabrication and setup, culture conditions, and acceptable dynamic ranges of experimental parameters (e.g. genetic, epigenetic, metabolic, community stability, etc). Initially, communities of scientists can rely on common portals for design and protocol sharing coupled with exchange of model biota. Longer-term, engagement of industry to produce laboratory environments and culture collections to host standardized microbiomes will be important steps in standardization and dissemination.

One of the most exciting opportunities for standardized microbiomes is the ability to compare data across experiments. This is only possible if groups follow the same protocols, collect the minimal data, and embrace an open science culture.

While there are innumerable advanced technologies that may be applied and shared to characterize the EcoFAB, standard measures should favor widely available and low-cost methods that are accessible to the greatest number of scientists. Incorporation of standardized sensors into EcoFABs is a great opportunity to collect data that can be standardized across the community. The resulting data must be available in common data portals to enable large-scale meta-analysis.

Verifying that the designs, protocols, and data types collected are sufficient to enable multiple labs to generate the same results will result from multi-lab 'ring-trial' experiments. Here, carefully designed experiments will be performed across labs to assess EcoFAB reproducibly. If successful, these experiments will define the reference set, enabling other researchers to benchmark their work. Adoption of EcoFABs by the wider scientific community will not only depend on these validation studies, but also funding opportunities and ease of integration into existing research programs. The latter will be greatly enhanced through availability of chambers, media, microbes, etc. with sufficient quality control standards. We advocate that the community first develop

minimal systems that are broadly applicable with defined scientific and technical parameters. Adoption of a few, robust EcoFABS as model systems should be the immediate goal and will enable the larger community to develop increasingly complex systems to address specific research goals. Reproducible laboratory systems will also be invaluable tools in training future generations of microbiome scientists.

Conclusions

Development of reproducible standardized laboratory ecosystems along with common protocols and data standards represent important steps in advancing microbiome science. They will enable researchers to build on each other's work, test predictions, identify governing mechanisms, and ultimately build predictive models. Moving microbiome science from largely observational and correlative studies to reproducible mechanistic investigations identifying causal mechanisms. However, rapid development of these EcoFABS will require organization of the broader scientific community, engagement of funding agencies, and potentially foundations and commercial partners. Ultimately, standardization across systems will enable the development of general principles that are applicable across microbiomes, and ultimately validated in studies of natural systems.

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Competing interests statement

The authors declare no competing interests. TRN is listed as an inventor on a related provisional patent application.

References

EcoFAB design principles:

1. Keep it simple: Base EcoFABs and minimal analyses should be as simple and inexpensive as possible to enable widespread adoption, large numbers of replicates, and integrated studies across labs, recognizing that some studies will require specialized EcoFABs
2. Design for extensibility: Enable extensions to address the needs of large groups of researchers from diverse fields
3. Standardize system dynamics: EcoFABs should be standardized to 'boot-up' and display reproducible dynamics
4. Accurately recapitulate important aspects of natural systems: EcoFABs should capture key natural processes
5. Enable mechanistic studies: Genetically tractable organisms should be used when possible to enable mechanistic insight at the molecular level
6. Embrace complexity: Allow experimentation from minimal to complex microbiomes and habitats
7. Build in simple analytics and sensors: Enable debugging, comparison, and standardization by including inexpensive sensors and analytical measures
8. Space and time are important: Move towards manipulation, imaging, and analysis spanning relevant scales from individual microbes to whole communities over time
9. Future proof data: Data standards, analytical approaches, system tolerances, and modeling approaches must be considered upfront to enable future cross-EcoFAB meta-analyses. When possible, data should also be preserved in its original, raw form to enable future analyses.
10. Give it away. To maximize impact and usefulness, the EcoFAB resources should be freely accessible to the greater scientific community. This means that the base-level capabilities will need to be common to most research groups and that we will embrace a culture of sharing and inter-lab cooperation. Open access experimental design and standardized reporting methods will also aid the overall inclusiveness of the EcoFAB movement.

Box 1 |

Figures |