Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Microbiology

Designer microbiomes for environmental, energy and health biotechnology

Marc Strous and Christine Sharp

Biotechnology conventionally uses pure strains of microorganisms to realize a desired conversion. The design of functional microbiomes is becoming a powerful alternative for when an aseptic environment is not an option, either for economic reasons or if the environment is intrinsically open. Rapid technological developments in combined -omics approaches is enabling the engineering and optimization of highly complex microbiomes. This review outlines emerging principles of design and provides examples of successful approaches and interventions in wastewater treatment, bioenergy production and the human intestinal microbiome.

Address
Department of Geoscience, University of Calgary, Calgary, Alberta, Canada

Corresponding author: Strous, Marc (mstrous@ucalgary.ca)

Current Opinion in Microbiology 2018, **43**:117–123

This review comes from a themed issue on **Environmental microbiology**

Edited by **Alberto Scoma** and **Julia Vorholt**

https://doi.org/10.1016/j.mib.2017.12.007

Introduction

The term 'Microbiome' was originally coined to mean a microbial community associated with a host, such as a protist, plant or animal [1]. Currently, it is often used more broadly, to mean any microbial community that is characterized using data from next generation sequencing technology. The data might consist of 16S rRNA gene amplicons [2], shotgun sequences (metagenomics) [3,4•,5•], or even metaproteomes [6,7•] and metabolomes [8].

Natural microbiomes are complex, often comprised of hundreds of different and poorly studied populations that interact in unknown ways [9,10]. A microbiome typically exhibits unpredictable responses to poorly constrained environmental dynamics. For that reason, the use of microbiomes is not a natural fit with biotechnology. Biotechnology applications typically aim to produce products for food, fuel or pharmaceutical markets that must meet certain specifications, see for example [11]. Meeting such specifications usually depends on the aseptic cultivation of a single strain of bacteria or fungi, excluding all other biological 'contaminants' [12]. In addition, single strains can be genetically engineered, by knocking out genes associated with an unfavorable phenotype or by introducing entirely new properties [13••].

As long as the product has sufficient value, the extra costs of maintaining an aseptic production environment are not a problem. However, some products have little economical value, such as clean water in wastewater treatment, clean soil in bioremediation and fuel in bioenergy production. In those cases, maintaining an aseptic environment is too costly, and use of a microbial community, or microbiome, becomes an essential aspect of the biotechnology application. Environments might also be intrinsically open, as is the case for the human intestinal microbiome.

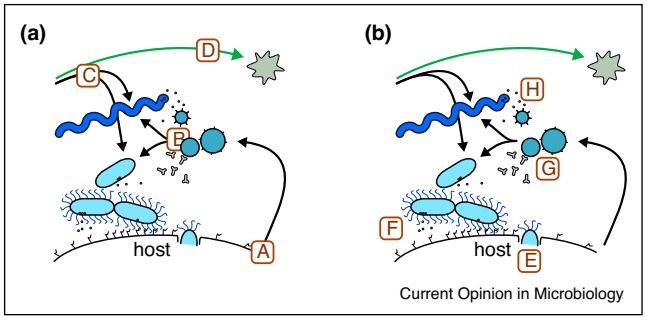
Compared to a pure culture, a microbiome can convert a much more complex mixture of substrates, has limited capacity in terms of its product portfolio, and displays much better robustness, both because of its adaptable community structure and because it displays evolution. Here we review key concepts and examples of the engineering of designer microbiomes that robustly perform a desired function for applications with little added value, where aseptic operation is not an option.

Fundamentals of microbiome engineering

At the current state of knowledge, a microbiome cannot be engineered by 'micromanagement' at the level of the gene, as is possible for well studied model organisms or simple consortia [14]. Some investigators engineer microbial communities at the level of the species, by creating a defined model community that consists of a few isolated strains [15,16]. Although the study of such communities might lead to valuable new insights in microbial ecology, maintenance of such a community in a biotechnological application would still require aseptic conditions. In an open system, invasion of wild populations would quickly overturn the designer community with a natural microbiome, leading to unpredictable outcomes [17].

A microbiome in an open system has the potential to perform any possible biological conversion, perhaps even including conversions that have not yet been discovered, as shown by discoveries such as anaerobic ammonium oxidation [18]. Each of these possible conversions is

Figure 1



Examples of primary **(a)** and secondary selection **(b)**. Energy metabolism drives primary selection (a) for those populations that rapidly effectively the available resources. The host excretes a substrate A that is fermented by the first microbial population. This population produces two products B, which are each respired by a different microbial population that use an electron acceptor C (e.g. oxygen) provided by a liquid flow. The liquid flow removes cells of population D from this microbiome, because it is unable to metabolize the substrates to sustain itself. Ecological interactions define forces of secondary selection. (b) The host gains energy by phagocytosis E of one of the bacterial populations. This population uses quorum sensing F to decide whether to produce a compound that is toxic to the host. A second microbial population displays strain heterogeneity G as a defense against attack by a phage. This population is also resistant against a toxin H excreted by a third microbial population.

performed by a different ecological guild. For example, nitrifiers perform aerobic ammonium oxidation. Successful engineering of a microbiome comes down to the engineering of an environment that favors those guilds that together predictably and robustly perform the desired conversions (Figure 1). In other words, we need to engineer environments with suitable selective forces, that provide ecological niches for the right ecological guilds. This requires a solid understanding of microbial ecophysiology and transport processes, and can be informed by -omics data.

In microbial ecophysiology, the microbial redox tower defines which specific ecological guilds are selected in a given environment. Aerobic respiration provides the highest fitness in the presence of oxygen, followed by respiration of oxidized forms of nitrogen (e.g. nitrate, nitrite), respiration of oxidized manganese and iron, fermentation, sulfate reduction and finally methanogenesis. Thus, the bioreactor should provide the correct redox environment to select for the desired processes. This can be challenging because potential rates of bioconversions generally exceed transport rates (by passive diffusion or active mixing) leading to gradients in space and time, and variations in redox conditions, which may yield unexpected outcomes [19••,20] (Figure 2).

Each microbial guild also has a characteristic maximum growth rate, defined by thermodynamics (growth yield) and kinetics (activation energy). This provides an additional handle to select for desired or exclude undesired ecological guilds. The growth rate can also be used to select for a microbiome that forms biofilms or aggregates. This is often desirable because (a) it enables cost-effective separation of dissolved products and biomass, (b) it enables a high biomass concentration leading to higher conversion rates, (c) it leads to formation of gradients, enabling the co-occurrence of different microbial conversions in a single compartment, (d) aggregation of biomass leads to a more robust bioprocess. Biofilms and aggregates are selected for by maintaining a hydraulic residence time smaller than the doubling time of the bacteria performing the desired conversion. This way, suspended cells will be 'washed out' of the bioreactor and only aggregated or attached cells will remain. In addition, surface area or settling needs to be provided, yielding biofilms or aggregates, respectively.

These 'primary' selective forces act directly on the metabolism of individual populations and are fairly well understood. However, microbiomes also display emergent properties in the form of ecological interactions between populations and, if present, the host. Well known examples of such interactions are viral, bacterial or eukaryotic predation [21•], excretion of secondary metabolites including antimicrobials [22], quorum sensing [23•] and specific cell to cell interactions [24•]. Understanding the outcome of such ecological interactions, or 'secondary' selective forces, is currently the main challenge of microbiome research (Figure 1).

Finally, it is important to realize that our primary and secondary selective forces operate on a 'founder' community, the inoculum. To create a robust designer microbiome, the founder community needs to contain a mix of specific populations necessary for a successful selection outcome [25•,26]. Interestingly, in natural ecosystems, some populations display a vast amount of microdiversity, or strain heterogeneity, whereas others can be almost clonal [27••]. Such microdiversity likely results from adaptive evolution in response to dynamic primary [28•] and secondary [29•] selective forces, and needs to be preserved during selection. From a practical point of view, the inoculum needs to come from an environment that hosts a microbiome as similar as possible to the desired microbiome.

Examples of microbiome engineering

Anaerobic ammonium oxidation (anammox) has become a classic case in which a then unknown process turned out to be important [18]. A bioreactor was designed to perform sulfide oxidation coupled to denitrification. To improve conversion rates, the bioreactor was designed as a fluidized bed reactor with biomass retention. This enabled growth of a slowly growing microbiome and led to the serendipitous enrichment of anaerobic ammonium oxidizers. This way the investigators got more than they