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Current Opinion in Microbiology

Advancing gut microbiome research using cultivation

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Culture-independent approaches have driven the field of microbiome research and illuminated intricate relationships between the gut microbiota and human health. However, definitively associating phenotypes to specific strains or elucidating physiological interactions is challenging for metagenomic approaches. Recently a number of new approaches to gut microbiota cultivation have emerged through the integration of high-throughput phylogenetic mapping and new simplified cultivation methods. These methodologies are described along with their potential use within microbiome research. Deployment of novel cultivation approaches should enable improved studies of xenobiotic tolerance and modification phenotypes and allow a drastic expansion of the gut microbiota reference genome catalogues. Furthermore, the new cultivation methods should facilitate systematic studies of the causal relationship between constituents of the microbiota and human health accelerating new probiotic development.

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Current Opinion in Microbiology 2015, **27**:127–132

This review comes from a themed issue on **Microbial systems biology**

Edited by **Eric Brown** and **Nassos Typas**

http://dx.doi.org/10.1016/j.mib.2015.08.004

Introduction

For centuries microbiologists relied on cultivation to study microorganisms, including the microorganisms that colonize the human body. These efforts provided the foundation for microbiology and the identification of pathogens responsible for a wide range of infectious disease. However, comparison of microscopic cell counts with the number of colony forming units growing on nutrient agar plates from the same sample highlighted that a large number of microorganisms are recalcitrant to culture. With the advent of molecular taxonomy it became clear that this 'plate count anomaly' resulted from vast amounts of unknown organisms. Potential reasons for the limited ability of researchers to cultivate all microorganisms from a given environment include complex cross feeding relationships, differential nutrient requirements or very slow growth rates. This finding spurred interest in studying this potentially significant new biology, which so far had gone by unnoticed. A new era of microbiology began with metagenomics focused on the enumeration and characterization of this previously unknown microbial diversity. Since then complex communities have been characterized in virtually every environmental niche [1,2].

Over the past decade evidence has been accumulating regarding the influence of the gut microbiome on human health [3]. Studies have identified correlations between gut microbiome composition and several disease states [4]. The causal relationship between the gut microbiota as a whole and various diseases has been supported by germ-free animal studies in which fecal/cecal transplantations from different hosts lead to distinct phenotypes in recipient animals [5,6]. Recently, some findings of these animal studies have been verified in humans through landmark clinical studies [7,8]. However, in spite of the progress made by culture-independent approaches and fecal transplantation studies, definitive and causal links between specific strains or microbial communities and disease states remain limited. Furthermore, the underlying interactions between specific strains in the microbiome cannot be definitively mapped using culture-independent approaches.

To map microbial interactions and to narrow down on specific constituents of the microbiome that have a causal relationship to disease states, individual strains or communities must be cultivated and interrogated experimentally. Accordingly, there is a renewed and growing interest in cultivation methods to study the gut microbiota [9]. This interest has resulted in several new approaches to mine for new bacteria and these approaches have the potential to propel our understanding of the gut microbiome and its causal relationship to human health states. Furthermore, these new cultivation methods have also revealed that culture-independent approaches have their biases and that polyphasic approaches to study the gut microbiome are needed to further our knowledge of the incredible diversity living on and within the human body [10••,11••,12••]. Cultivation-based approaches are likely to dramatically expand our knowledge of the gut microbiome and open new avenues for the development of next-generation probiotics.

Novel approaches for cultivation of the gut microbiota

A number of advanced cultivation methods have been developed in order to study difficult to culture organisms

over the past decades, these include encapsulation of bacteria into microdroplets or gel particles [13,14], diffusion chambers simulating the natural environment of the samples [15], microfabricated cultivation chips [16]. These techniques enable cultivation of novel species; however, they require access to complex microfluidic or microfabrication technology. In this article focus is on recent simple cultivation methodologies that can be readily implemented in most biology laboratories.

Most simple cultivation procedures have been developed to cultivate specific anaerobic microorganisms from the gut microbiota [17]. However, a couple of pioneering studies compared the species recovered using culture-independent and culture-dependent methods from human fecal samples [18–20]. These studies showed that 30–60% of live cells identified by microscope counts could in fact be cultured as determined by colony counts on solid growth media. Yet, due to the limited sequencing capability available at the time, accurate comparisons of the microbial diversity covered by each method was challenging. With the advent of next-generation sequencing and mass spectrometry based phylotyping it became possible to phylogenetically characterize thousands of isolates with a reasonable effort [21]. The novelty of the recent gut microbiota cultivation approaches arises in large part through the integration of such phylogenetic profiling methods with new cultivation conditions (Figure 1).

Personalized culture collections

In a remarkable study Goodman *et al.* sparked renewed interest in cultivation-based approaches for studying the gut microbiome [10••]. The authors developed a new gut microbiota medium (GMM) and cultivated individual strains of the gut microbiota by diluting fecal samples such that only single cells are added to each culture well (Figure 1a). Individual cells are cultured in liquid media separately in microtiter plates and cultures are collected after growth. The resulting culture collections are comprised of isolated strains that were all derived from the original sample. Notably, >50% of the species identified from a sample using culture-independent methods were covered by the isolates in the culture collections. Furthermore, personalized culture collections were used to inoculate germ-free animals along with uncultured fecal samples and the authors showed that the microbiome response to dietary changes were similar between the two groups. These results challenged the hypothesis that uncultivated microorganisms played a key role in the dynamic responses of the gut microbiome to perturbations.

Culturomics

A next major step was the development of a parallel cultivation setup coupled to rapid taxonomic identification termed culturomics (Figure 1b). In a key study Lagier *et al.* used 212 different cultivation conditions chosen to mimic biological niches relevant to the gut environment to isolate bacteria from human fecal samples [11••]. The authors isolated over 30,000 colonies comprising over 300 different species. Notably, over half of the species identified in this study had not previously been identified in the human gut, including a number of entirely new species and genera. The authors compared the taxonomy of the cultured isolates to that resulting from 16S rDNA sequencing of uncultured samples and found that only 51 out of a total of 571 species identified overlapped between the two approaches highlighting the need for using both cultivation-based and cultivation-independent approaches to study the gut microbiome. Culturomics has since been applied to diverse samples as well as to cultivate eukaryotic organisms [22–24].

Cultivation-based multiplex phenotyping

Rettedal *et al.* tested a variety of individual growth media with the goal of identifying a specific cultivation medium that yielded the best representation of the human gut microbiota as characterized by culture-independent methods. It was found that a modified version of the Gifu anaerobic media introduced a minimal bias in the phylogenetic representation of the isolates cultured from human fecal samples compared to the culture-independent characterization. Furthermore, the media allowed the cultivation of over 30% of the viable cells identified through microscopic observation in the diluted fecal samples. Notably, the taxa making up more than 80% of the gut microbiota as determined by culture-independent methods were in fact cultured on the optimized growth medium. Supplementation of this medium with a variety of antibiotics allowed a rapid assessment of the phylogenetic distribution of antibiotic tolerance phenotypes and offered a direct coupling of tolerance phenotypes to specific taxa (Figure 1c). Furthermore, this information was used to tailor selective media in order to culture previously uncultivated bacteria through the rational combination of specific antibiotics.

It should be noted that the recent cultivation studies differed in their approaches to map bacterial phylogeny. While it remains challenging to arrive at a generally accepted species definition [25], the relative consistency of findings between the recent studies in spite of applying different phylogenetic criteria supports the robustness of the overall conclusion; that a majority of the gut microbiome is amiable to culture. However, since bacterial genomes with identical 16S rDNA sequences can vary significantly in their genomic content and accordingly, the identification of a 16S rDNA tag in a culture condition does not necessarily imply that all strains with this 16S rDNA tag can be cultured. Further studies mapping the genomic content of cultured and uncultured samples are required in order to investigate such potential biases.