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Bringing the gut microbiota into focus through microbial culture: recent progress and future perspective

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Abstract

Ever-more powerful ‘omics’ –based technologies are allowing us to pry deeper and more clearly into the workings of the human gut microbiota. Culture of the component microbes has fallen somewhat behind these efforts for a number of reasons, not least of which being the perceived difficulty in growing microbial species that have previously eluded all efforts to tame them. However, recent advances in the field are beginning to bring success in this area, allowing holistic study of microbes and microbial communities in defined systems. Innovative approaches to the culture and study of the human microbiota will ultimately guide medical practice, as the importance of a robust gut microbial ecosystem in the maintenance of health is increasingly realized.

Introduction

Ninety percent of the cells of the human body are microbes, and the importance of this microbial majority to human health has only recently begun to be fully realized [1]. Along with this realization has come recognition that the discipline of microbiology requires an overhaul; even though many great advances have been made in the field since pioneers such as Pasteur and Koch began to deliberately grow microbial species *in vitro* using prepared media over 130 years ago, for the most part microbiology culture from human and animal-derived specimens is still carried out by cultivating microbial isolates using empirically-devised nutrient formulations [2]. Whilst this traditional approach to culture still has value, it is limited by many factors, not least of which is the enormous range and combinations of possible growth substrates that microbes are able to utilize and the time taken for some species to grow under sub-optimal conditions. As our appreciation of the role of the microbiota in human health increases, and our efforts to catalogue the diversity of the microbial communities across various body sites have come of age [1, 3–6], there is a growing need to understand the function and dynamics of human-associated microbial ecosystems. This short review will focus on contemporary approaches to modeling the human gut microbial ecosystem using culture-based techniques.

Why culture matters

It is unfortunate that, following early molecular surveys of the diversity of the human gastrointestinal tract and the concomitant realization that only a small fraction of the range

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of microbes found in this niche had been hitherto cultured [7, 8], the rest was often labeled ‘unculturable’. As a result, there followed a general lack of investment in attempts to culture microbes from the human gut, a situation that was exacerbated by the generally fastidious, anaerobic growth requirements of most of the strains that had successfully been grown from e.g. fecal samples [9, 10], and the emerging availability of ever more powerful, sequence-based methods of ecosystem assessment with the promise of deeper insight with less effort. Recognizing that culturability is a function of the determination and innovation of microbiologists, ‘as-yet uncultured’ is now considered to be the more correct terminology for microbial phylotypes known only by their sequenced signatures [11].

So, why should microbiologists expend their energy to culture the as-yet uncultured microbes of the human gut? There is a limit to the amount of information that can be gleaned from purely molecular-based approaches to microbial ecology, and thus one first, obvious benefit of the availability of cultured microbes is the concomitant ability to obtain completely sequenced genomes from these species. A clear example of the value of culture to metagenomics can be seen as a result of the availability of the National Institutes of Health supported Human Microbiome Project (HMP)’s reference genome collection (http://www.hmpdacc.org/reference_genomes/reference_genomes.php) [12] which serves as a rich database of information that offers some context to metagenomic surveys of the human microbiome [3], and has been used as a key resource in many studies, for example: [13–16].

Beyond this practical use of sequence information from isolated representative microorganisms, cultivation of microbes in groups also provides information about the ecological role of communities associated with health or disease, and allows for greater understanding of the stochastic interactions that lead to extant traits such as an ability to break down dietary substrates. A purely sequence-based description of a given microbial ecosystem gives valuable information about community content and metabolic potential, but little else. When datasets from 16S rRNA- or metagenomics-based studies are considered, it is tempting to assume importance of given species or functions based on the relative read abundances in a given sample. However, it is becoming increasingly clear that microbial ecosystems contain species that punch above their weight in terms of the relative function they provide to the community as a whole; for these so-called “keystone” species, numerical abundance is less important than the ability to serve a key metabolic function upon which the larger community structure may rely [17]. An excellent example of this concept has recently been reported by Ze and colleagues [18], who elegantly demonstrated the role of a *Firmicutes* species of the human gut, *Ruminococcus bromii*, in the primary stages of breakdown of dietary resistant starch. Under carefully controlled growth conditions within simple defined communities, *R.bromii* efficiently initiated resistant starch degradation, which stimulated the growth of the co-cultured species without conferring a growth advantage on *R.bromii* itself [18].

Metagenomic applications to microbial community analysis are undoubtedly powerful, but suffer from some key limitations; for example depending on the depth of sequencing used, microbial species may only be detected above a certain threshold, below which species may still be present in clinically relevant concentrations, but not reliably detectable using molecular methods [19]. Because such low-abundance species may have an overall important role in their home community as indicated by the example of *R.bromii* above [18], it is critical that these rare phylotypes be sampled and studied effectively. Culture-based approaches offer the route through which this may be accomplished.

Culturing gut isolates *in vitro*

Culturing isolates of the gut microbial ecosystem can be approached in 2 main ways: The first of these, a targeted approach, may be taken to selectively culture a specific species or group of strains based on a particular trait, such as ability to exclusively use a given growth substrate. For example, the *Verrucomicrobia* organism, *Akkermansia muciniphila*, was first isolated from feces through its unusual ability to use mucin as a sole carbon and nitrogen source [20]. In this case, culture enrichments of a fecal sample on a specialized mucin-containing medium were analyzed using a molecular fingerprinting technique (Denaturing Gradient Gel Electrophoresis), finally zeroing in on the microbe giving a consistent pattern through each passage [20]. An even more targeted variation on this theme would be to utilize the genome of a given uncultured species to infer its necessary growth requirements. This was best demonstrated by Renesto *et al.*, who tailored a growth medium specific to *Tropheryma whippelii*, the causative agent of Whipple's disease, based on analysis of genome sequence derived from minimal culture of the microbe [21]. This analysis revealed an absolute requirement for exogenous L-glutamine and several amino acids for *T. whippelii* culturability, a growth requirement that was subsequently demonstrated at the bench with bespoke media [21]. While this latter approach depended on the intracellular culturability of *T. whippelii* in tissue culture cells, since this work was published there have been significant strides taken in the ability to sequence partial bacterial genomes from DNA isolated from single cells and accomplished using multiple displacement amplification [14]. McLean and colleagues have used a single-cell sequencing approach combined with single cell sorting by FACS, to isolate, amplify and sequence a portion of the genome of a novel TM6 bacterium from a hospital water pipe biofilm [22]. By doing so, the authors demonstrated that this bacterium had a small genome seemingly lacking in biosynthesis pathways for most amino acids and vitamins [22]. The further use of metatranscriptomics can more directly point to the metabolic pathways used by an as-yet-uncultured organism in a given environment; Bomar and colleagues used this tactic to investigate the physiology of uncultured microbes from leech gut contents, concentrating on genes encoding hydrolytic enzymes, binding proteins and transporters, and as a result were able to determine the nutritional requirements for two previously uncultured bacterial species from this niche [15]. As this groundbreaking work continues, it may soon become possible to routinely custom-make growth medium for targeted growth of microbes that have thus far evaded cultivation, opening them up to detailed physiological study for the first time.

The second, more shotgun approach to culture of as-yet uncultivated microbes has been developed by Lagier and colleagues, and named 'culturomics' [19]. In their landmark work, the authors demonstrated the culture of 340 different bacterial species (32 of which were novel) as well as 5 fungal species and a giant virus, using a process whereby first the more predominant microbial population from 3 separate stool samples was eliminated using cocktails of lysogenic phage, or by filtration methods [19]. This was followed by growth enrichment steps with blood, rumen fluid or fecal water, and finally 212 separate culture conditions were applied to the resulting pool of microbes that had been diluted to give single cells [19]. The perceived bottleneck of the culturomic method was the identification step, which would normally be facilitated through time-consuming sequencing (for example, of 16S rRNA amplicons); however Lagier *et al.* were able to demonstrate that a mass spectroscopy approach (matrix-assisted laser desorption-ionization time-of-flight, MALDI-TOF) [23] was faster and cheaper, greatly facilitating the work [19]. Combined with automated colony picking technology and high throughput growth arrays, culturomics represents a powerful technique that should revolutionize microbial culture.

Culturing gut microbial communities *in vitro*

Although the pure culture approaches of gut microbial species thus far outlined have enormous value, it is important to remember that microbes in nature rarely grow under axenic conditions. As well, microbes grown in isolation of their originating communities are unlikely to behave in an identical way as they would when part of a consortium population [24, 25]. To address this disparity, microbial ecologists have developed several technical innovations to allow culture of whole gut microbial communities *in vitro* under physiologically relevant conditions using continuous culture or ‘chemostat’ approaches [26, 27]. Chemostats lend themselves well to the study of the human gut because the gut itself can be approximated to a continuous culture vessel, where nutrients are constantly added as food intake, and waste products periodically removed as food is digested and transits through the intestine [26]. Using chemostat apparatus at the bench, it is possible to create conditions that mimic the environment of specific sections of the gut, and to then seed the culture vessels with gut content (for practical purposes, fecal samples are often used) [26]. The technology required to run chemostats has improved significantly since Gibson and MacFarlane first described a system to model the human gut microbiota [28]; semi and fully automated systems now allow tight control with the result that technical – and to some extent biological – replicates can be easily obtained [29, 30].

Although useful, chemostats that mimic the environment of the gut lumen only cannot accurately model the gut microbiota present at the mucosal surface, which is known to be distinct from the luminal community [31]. To tackle this discrepancy, mucin is usually added to culture media to at least maintain the sub-populations of the gut microbiota that depend on this substrate [27, 30, 32]. In order to further develop this concept, Van den Abbeele *et al.* have simulated the geography of the mucosal environment using mucin-covered microcosms [33]. Importantly, the microbial ecosystems that developed within this adapted, microcosm-containing system more closely mimicked those found *in vivo*, and particularly were enriched in *Firmicutes* belonging to *Clostridium* clusters IV and XIVa [33], recognized as important components of the core human gut microbiota [34]. A further improvement of *in vitro* gut models would be the incorporation of host cell feedback to the system. Several groups are currently developing systems that combine human intestinal tissue culture with continuous flow of chemostat material to better mimic the *in vivo* situation, reviewed in [27]. The further addition of immune cells to these systems will add even greater clinical relevance.

Concluding remarks

Much progress has been made over the past decade, leading to an enhanced ability to isolate and culture microbes that were previously dismissed as ‘unculturable’. Progress has also been made in developing *in vitro* models that closely mimic the gut microbial environment. The time has now come to combine these efforts in order to rationally explore the microbe-microbe and host-microbe interactions in engineered ecosystems using defined experimental communities created from well characterized isolates. Without the considerable encumbrances of *in vivo* experimentation, next generation *in vitro* models can offer extraordinary insight into gut microbial function. Using ever-more-powerful ‘omics’ technologies, we are at the point of being able observe gut ecosystem dynamics in response to external manipulation in unprecedented depth under tightly controlled conditions. Such work is of extraordinary importance to medicine, as we increasingly realize the critical role that the gut microbiota plays in the maintenance of health [35]. In particular, such approaches could be applied to the rational design of microbial ecosystem therapeutics [36] suited to the treatment of selected diseases whilst also being tailored to a given patient’s lifestyle and gut microbiota individuality.

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Highlights

Efforts to culture microbes have fallen behind ‘omics’ approaches to study the gut microbiota

Cultivating microbes is important to fully enable understanding of microbial physiology and cross-talk

Novel approaches to growing and studying gut microbial communities *in vitro* are under development

Understanding gut microbial physiology is an essential part of human medicine

Figure 1.

Schematic outlining the general approaches to culturing microbial diversity. In the empirical approach, media components are used based on laboratory observations; in this case the diversity yield is low because the enormous range of microbial growth rates and conditions that unknown targets may use makes the approach time-consuming and inefficient. In the bioinformatic approach, the molecular signatures of a microbial target are studied before culture is attempted to infer culture conditions. Diversity yield is intentionally low, and target is efficiently recovered. In the culturomics approach, basic knowledge about ecosystem targets is known, and high throughput techniques such as flow cytometry and robotic media preparation allow multiple conditions to be tested for growth support simultaneously. As a result, the diversity yield is very high.