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Current Opinion in Microbiology

From complex gut communities to minimal microbiomes via cultivation

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The mammalian gut microbiota is dominated by populations of bacteria, mostly strict anaerobes. Because these bacteria can influence the health of their host, it is important to investigate their diversity and functions, which can be done via culture-based or molecular approaches. In recent years, microbiologists have very often preferred the use of molecular techniques, as they do not limit the analysis to the fraction of communities that can be grown in the laboratory. In reality, cultivation and molecular methods are complementary, and we are now witnessing a period of unification. Obtaining strains that can be grown *in vitro* is currently indispensable for the description of novel diversity and eventually the improvement of taxonomic and sequence databases. Moreover, cultivation allows using host-specific minimal consortia of microbes that are helpful for detailed and standardized studies of gut microbial communities and microbe-host interactions. Molecular techniques are helpful because they can provide insights into strain-level diversity and the functional potential of organisms. Furthermore, genomic and metagenomic data allow inferring growth conditions for uncultured bacteria and also enable detailed genetic studies. In the present manuscript, we highlight recent work on culture-based investigation of mammalian gut bacteria and microbe-host interactions and give our opinions on challenges and perspectives in the field.

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Current Opinion in Microbiology 2017, **38**:148–155

This review comes from a themed issue on **Microbiota**

Edited by **François Leulier** and **Maria Elena**

http://dx.doi.org/10.1016/j.mib.2017.05.013

Introduction

The intestinal microbiota is a complex and dynamic ecosystem that influences mammalian physiology and the susceptibility to chronic diseases. Our gut symbionts can preclude infection by enteropathogens and participate in immune system maturation and metabolic health, as reviewed in Refs. [1–3]. These examples justify the importance of studying the mammalian gut microbiota and especially its bacteria, which are dominant members of this ecosystem [4].

The use of molecular techniques has revolutionized the investigation of microbial ecosystems as they increased throughput of analysis and brought light to previously unexplored phylogenetic diversity [5,6]. However, due to the popularization of molecular tools, culture-based approaches have been neglected by many in recent years despite several advantages [7,8]. For instance, interpretation of the gigantic amount of data accumulated by high-throughput sequencing requires key isolates for precise taxonomic and functional classification. Culture-based investigations of bacterial communities complements very well molecular studies via characterization of novel bacterial functions [9••], description of yet unexplored diversity [10], and hypothesis-driven functional studies in animal models [11].

In the following sections, we present recent work pertaining to the investigation of mammalian gut microbiota by cultivation and discuss important notions in the field.

What have we learnt?

Since 2005, molecular studies of the gut microbiome have generated major hopes pertaining to its role in health and diseases. These hopes have found a great echo in the media, but the reality looks somewhat different: the metagenomics era fell short of its target to provide an unbiased and comprehensive view of gut microbial ecosystems and microbe–host interactions. The field still suffers from a lack in standardization [12–15], and more mechanistic studies dissecting the interplay between gut bacteria and their host are needed to counteract the exponential accumulation of descriptive data on shifts in microbiota composition associated with certain host phenotypes. Past and future development of metagenomic tools have and will be of great value to the field, but this should go hand in hand with the use of culture-based approaches in order to shed light on causal relationships. It is important to further promote the isolation of gut bacteria to enrich our knowledge of prokaryotic

diversity. Although there is currently no clear consensus in the field, it is safer to say that approximately half of human gut prokaryotes can be cultured, rather than a minority [16•].

Culturing efforts of the last few years have shown that it is worthwhile to isolate bacteria [17•,18•,19•]. The odds of finding new taxa are substantial: approximately 1 of each 100 colonies analyzed represents a new taxon, which can lead to the discovery of dominant and important commensals [20,21]. Browne *et al.* [17•] recently isolated 137 bacterial species from the human gut, including 67 novel taxa. They proposed that approximately half of the bacterial diversity in human feces has the genetic potential to form spores, with consequences for host-to-host transmission of strains. The term 'culturomics', which refers to large-scale cultivation of gut bacteria on agar plates followed by high-throughput mass spectrometry-based identification and genome sequencing, was coined by French researchers in 2012 [22]. Their efforts in cultivating bacterial strains from the human gut has already brought hundreds of novel taxa to light [18•].

The projects aforementioned are unique as per their scale (number of isolates described and genomes generated), but nonetheless relied on classical anaerobic cultivation on solid media. Isolation campaigns in the 1970s were already prolific in obtaining gut bacteria, but capacities to describe and archive isolates were limited at the time [23]. Several innovative approaches for comprehensive and high-throughput cultivation of gut bacteria have been published in recent years [24–27]. Because exact media requirements are yet unknown for many bacteria and cross-feeding between strains is important in native communities, the major challenge is to efficiently separate the multitude of different taxa existing in native ecosystems while, in the best case, still allowing exchange of metabolites, under strict anaerobic conditions. Thereafter, handling and identification of thousands of isolates, including slow growers, is an additional difficulty. This non-trivial logistics combined with the need for specific equipment may explain why new approaches have not yet spread widely into culture labs despite their elegance. Innovative co-culture techniques have also been developed [28,29]. These systems open new ways for studying mechanisms underlying the impact of bacteria-derived factors on the host, and vice versa. However, their usefulness is dependent on the pool of cultured bacteria and cell lines available for testing interactions, and obviously, co-culture adds another layer of complexity.

Future culture projects should be based on additional implementation of classical approaches, on the one hand. On the other hand, we now have the chance to integrate molecular approaches and culturing efforts even further. For instance, inferring metabolic functions and nutritional requirements from genome information to isolate unknown bacteria has successfully been used in the past [9••], but could be intensified thanks to progress made in binning shotgun sequences into so-called 'metagenomic species' [30,31]. Moreover, targeted sorting of strains with specific metabolic functions by single-cell, non-invasive molecular methods combined with cultivation is a promising avenue to explore [32••] (Figure 1).

Of man and animals

Are gut ecosystems so specific that one can confidently determine their host species of origin by looking at their community structure? How functionally different are various strains of a given bacterial species, or distinct yet closely related species originating from different hosts?

Comprehensive answers to these questions will require additional research, but it is clear that gut ecosystems have been shaped over thousands of years of co-evolution with their hosts [33,34]. Mammals and other animals such as birds are characterized by various dietary modes and by different digestive physiologies that markedly impact microbial communities in their intestine [35,36]. Readers are re-directed to other papers for more detailed information on factors that may contribute to the establishment of a host-specific gut microbiota [37,38]. We discuss only a few aspects here.

The host genome and associated physiological traits such as immune responses and metabolites/substrates production (*e.g.*, mucus, bile acids, anti-microbial peptides) are major selective factors for the gut microbiome [39–42]. From a microbial perspective, the expression of strain-specific signals (*e.g.*, cell surface structures), which may emerge from co-evolution or selection by the host upon initial colonization, can favor persistence in specific niches in the gut of a given host [43]. Several studies have reported that certain taxa preferentially colonize a particular host species [19•,44,45], and entire bacterial families such as *Muribaculaceae*, *Deferribacteraceae*, or *Coriobacteriaceae* are rather unique in some gut environments [19•,46–48,49••].

We now have tools to test the ecological distribution of bacteria via sequence-based approaches in a large-scale manner [50••]. Molecular studies have also shown that the gut microbiome of different mammals (human, mouse, pig) is characterized by limited overlap in terms of gene diversity [45,51]. Although the gut microbiota is characterized by functional redundancy and it is unclear to what extent ecosystems with different phylogenetic makeups may be functionally equal [52], microbiota transfer experiments have revealed host-specific traits of colonization [53], either due to the presence/absence of specific taxa well adapted to the recipient host or to failure in colonization by taxa from a different host [54•].