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Reconstructing functional networks in the human intestinal tract using synthetic microbiomes

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The human intestinal tract harbors one of the most densely populated and open microbial ecosystems. The application of multi-omics approaches has provided insight into a wide array of complex interactions between the various groups of mainly anaerobic colonic microbes as well as the host–microbe dialogue. Integration of multi-omics techniques in cultivation based experiments that vary in complexity from monocultures to synthetic microbial communities identified key metabolic players in the trophic interactions as well as their ecological dynamics. A synergy between these approaches will be of utmost importance to reconstruct the functional interaction networks at the ecosystem level within the human intestinal microbiome. The improved understanding of microbiome functioning at ecosystem level will further aid in developing better predictive models and design of effective microbiome modulation strategies for health benefits.

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Introduction

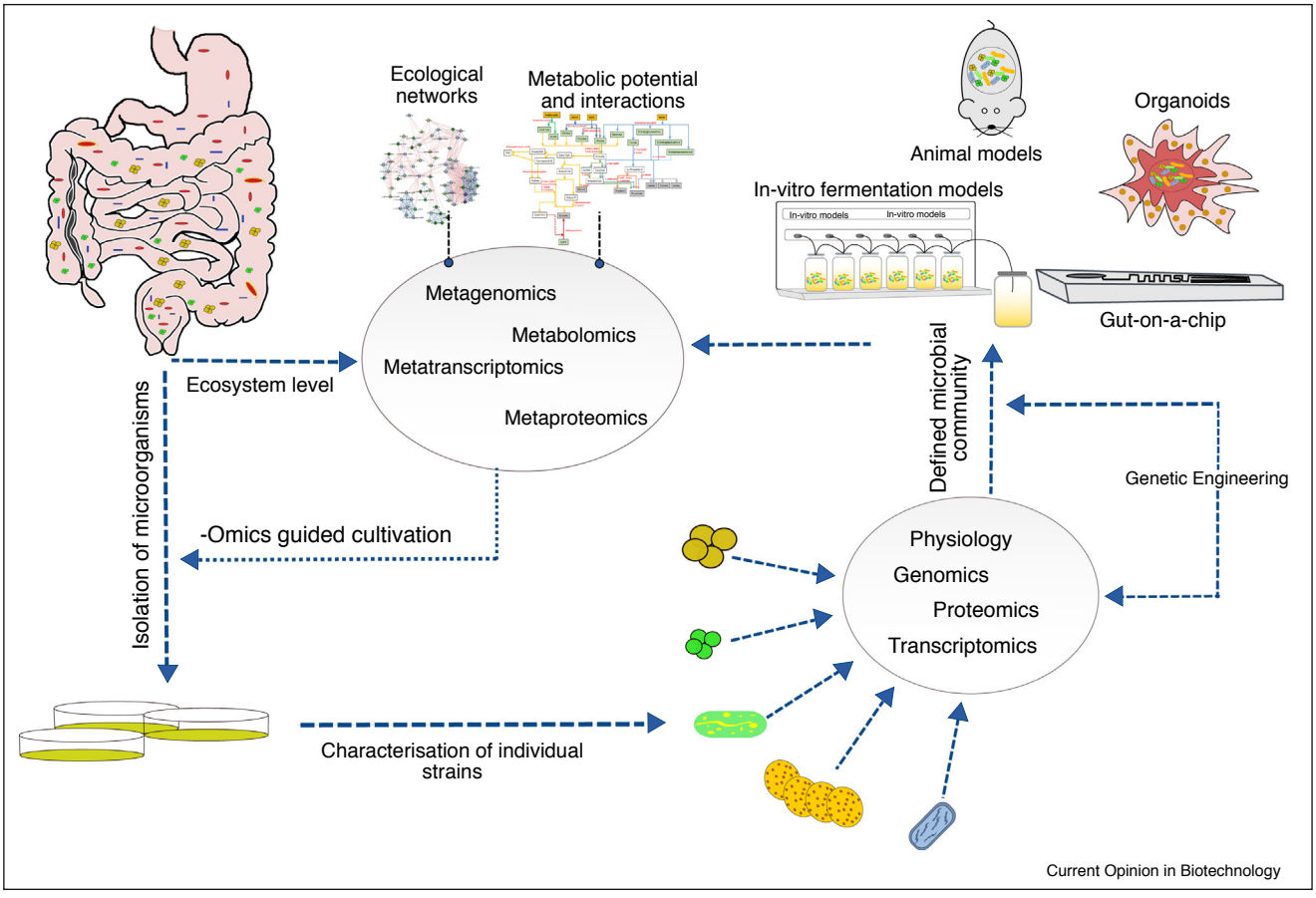
The microbes in the colon are in a continuous state of dynamic interactions with the host as well as other microbes. Consequently, microbes play a major role in balancing human health while the human host also has an impact on the survival of microbes [1–3]. The trophic interactions in the intestinal tract facilitate co-existence of complementary species that share the resources derived from consumed food and products generated by the host [4•]. Studying the metabolic interactions as well as identifying emergent biosynthetic pathways resulting from multiple interacting species is challenging due to the complexity of the intestinal microbiome that includes over 1000 species of mainly anaerobic bacteria, archaea, and fungi [5].

The colon is the most densely populated site in the human intestinal tract, and an anaerobic fermentative lifestyle is the major physiological characteristic of the high numbers of bacteria and archaea that reside there. These convert the substrates originating from host-associated glycans or dietary fibers and proteins that have not been taken up by the host. Fermentation end products such as short-chain fatty acids (SCFAs), including acetate, propionate and butyrate, as well as medium chain fatty acids (MCFAs), like caproate, and branched chain fatty acids (BCFAs), such as iso-butyrate and isovalerate, play a crucial role in normal host physiology [1,6–9]. This central metabolism in the colon results in a thriving ecosystem giving rise to highly complex and dynamic interactions between the microbes themselves and between the host and microbes. Consequently, diet is considered as a promising avenue for modulating the microbiome for achieving health benefits by supporting the growth of known beneficial microbes [10,11]. However, our understanding of the complex metabolic interactions resulting from different dietary fibers is limited. Finally, understanding the ecological principles governing the assembly, structure, and function of the microbiome under the influence of diet and consequent metabolic interactions have not been studied in detail. Therefore, integrating the ecological information obtained through population level microbiome studies and the physiological information obtained through *in-vitro* and *in-vivo* studies is vital for reconstructing the functional interaction networks at the community level to design better microbiome modulation strategies.

Reconstructing functional networks using fecal samples

It is important to acknowledge that intestinal microorganisms are not independently growing free-living entities. Information obtained from investigation of a given bacterium in isolation may not represent its natural lifestyle. Therefore, it will be crucial to study bacterial populations as communities by growing multiple bacterial species together in well-controlled settings that mimic the natural ecosystem. Integration of multi-disciplinary approaches will be crucial for improving our knowledge regarding the physiology, interaction networks and role of intestinal microorganisms in human health (Figure 1).

Figure 1



Synergistic approach to understanding individual to ecosystem level microbial interactions and their impact on the host.

Fecal samples have been widely used in batch and continuous fermentation systems to investigate the fate of dietary fibers and resulting microbial interaction networks. Resistant carbohydrates, which include resistant starch, non-starch polysaccharides (NSP) and oligosaccharides (including pre-biotics, e.g. fructo-ologosaccharides, galacto-oligosaccharides), are important determinants of microbial composition and function [6,10,12,13]. Mucus-derived glycans are another important growth and energy source, and their utilization has major implications for host health as mucus acts as a barrier against pathogen invasion [14•,15,16]. Most of our understanding of the microbial metabolic interactions has been derived from investigating faecal samples by metagenomic and to some extent by metatranscriptomic and metaproteomic approaches. These have been used for both *in-vivo* and *in-vitro* anaerobic fermentation systems. Inoculation of *in vitro* anaerobic fermentation systems containing different carbohydrates has revealed a predominance of *Bacteroides* species [17]. Several *Bacteroides* species are capable of utilizing diverse carbohydrates and thus are considered to be one of the most metabolically versatile groups in the human intestinal tract. Dietary interventions in humans and subsequent molecular analysis of fecal samples have revealed phylotypes related to *Ruminococcaceae* as dominant groups in resistant starch utilization, whereas phylotypes related to *Lachnospiraceae* were dominant in NSP degradation [18]. A recent dietary intervention study investigating the effect of resistant starch 2 (RS2) in human subjects included metagenomics and observed that *Ruminococcus bromii* contributed the majority of the key genes for RS2 degradation, further validating its role as a key degrader of resistant starch [19•,20]. A major challenge in reconstructing microbial interaction networks using fecal samples is the presence of a large number of unknown functions that have not been annotated well. In natural samples, the unknown contribution of bacteriophages, and the high variability across different inocula pose major challenges in deciphering the microbial interactions. Moreover, the role of uncultured microorganisms in governing ecological outcomes *via* hitherto unknown interactions makes predictive modelling a challenging activity. Finally, most currently employed sequencing-based molecular

techniques are incapable of species/strain level identification and annotation with high confidence, while the design and application of qPCR primers to allow discrimination at strain or species level is often technically challenging and expensive [21,22]. Strain-level resolution can be obtained from shotgun metagenome sequencing data, albeit limited to the top 0.1% of the microbes in the total community and at a higher cost [23,24]. For a better understanding of complex systems, such as the human intestinal microbiome, a pragmatic approach would be to study the ecosystem in parts under well-controlled conditions. Studying defined microbial communities could provide a promising avenue where major properties such as known species composition and their genetic potential (sequenced genome), as well as known general physiological characteristics can be leveraged to better understand the metabolic roles and interaction networks and to develop predictive models for the microbiome.

Reconstructing functional networks using cultured microorganisms from the human intestinal tract

Specialist bacteria capable of degrading complex dietary fibers and mucus are key players in the community as they provide simple carbohydrates for other microbes in the community. Known examples of such bacteria are *R. bromii*, *Eubacterium rectale* and *Bacteroides thetaiotaomicron* capable of degrading complex polysaccharides, and *Akkermansia muciniphila*, *Barnesiella intestinihominis* and *Bacteroides caccae* that are capable of degrading mucus [20,25,26••,27,28]. An experimentally verified metabolic interaction network is the one between *A. muciniphila* and butyrate producers *Anaerostipes caccae, Eubacterium hallii,* and *Faecalibacterium prausnitzii* [14•]. The butyrate producers benefitted from simple sugars released from mucus by *A. muciniphila*, and in return *A. muciniphila* benefitted from the *E. hallii*-mediated production of vitamin B12, an important co-factor in the propionate biosynthesis pathway.

In-vitro growth assays have identified polysaccharide-degrading bacteria that utilize the dietary carbohydrates reaching the colon undigested. For example, resistant starch can be utilized by *R. bromii* and *E. rectale*, xylan can be utilized by *Bacteroides intestinalis*, *Bacteroides ovatus*, *Bacteroides dorei*, *Bacteroides cellulosilyticus*, *Bacteroides xylanisolvens* and *Roseburia intestinalis*, whereas pectin can be used by *B. ovatus*, *B. thetaiotaomicron*, some strains of *F. prausnitzii*, *Eubacterium eligens* and *Lachnospira pectinoschiza* [6,20,29–31]. Co-culture experiments combining degraders and non-degraders have revealed interesting cross-feeding pathways, such as utilization of lactate to produce butyrate or propionate [32]. This has allowed reconstructing the dominant metabolic pathway starting from degradation of dietary carbohydrates to production of dominant SCFAs detected in feces *viz.* acetate, butyrate, and propionate. Formate and lactate are known intermediates of microbial fermentation but are detected in low amounts in feces. Conversion of formate produced by amylolytic bacteria (*R. bromii*) to acetate by an acetogen (*Blautia hydrogenotrophica*) has been recently shown to be a contributing factor for high amounts of acetate [33]. Potential emergent properties that are related to biosynthetic pathways for amino acid, vitamin and co-factor metabolism and other non-central metabolic pathways have been identified using RNA-sequencing in both *in-vivo* and *in-vitro* co-culture experiments [33–37]. However, the influence of regulation of secondary biosynthesis pathways in the presence of interacting partners and subsequent impact on the overall community level functional interaction network is largely unknown. Therefore, there is a need to incorporate high complexity in terms of phylogenetic and functional diversity in experiments aimed at reconstructing the functional interaction network in the human intestinal tract.

Leveraging the concept of minimal microbiomes for reconstructing functional networks of the human intestinal microbiome

One approach to better understand the microbial interaction networks and develop predictive modelling tools is to grow microorganisms in combinations as co-, tri- or even more complex cultures, building up to create a consortium of microorganisms that could be representative of a functioning minimal microbial community of the human intestinal tract. The first attempt at developing a defined microbial community in a host was done in 1965 by Russell W. Schaedler *et al.*, who composed the 'Schaedler flora' comprising five dominant bacterial isolates in mice [38]. The 'Schaedler flora' was further modified to include three more isolates. This Altered Schaedler flora (ASF) has been widely used to study the relationship between the murine host and intestinal microbiota [39,40]. A proof of concept study showed the applicability of the ASF in therapeutically modulating the murine host metabolism as to decrease intestinal ammonia levels as the eight bacteria that make up the ASF have a minimal urease gene content [41••].

A number of other defined microbial communities have been designed to investigate microbial interactions, develop predictive models and study specific hypotheses such as conferring colonization resistance (CR) against pathogens in a host (Table 1). The complexity of these defined microbial communities ranges from 2 to 33 bacterial isolates while the selection is often based on characteristics such as dominance and prevalence. These defined microbial communities can be considered as a minimal microbiome, a term coined previously to describe the smallest set of microbes and/or microbial functions needed to develop a stable community [42]. These minimal microbiomes allow researchers to gain mechanistic insights regarding several aspects of host-microbiome and within microbiome interactions [26••,43••,44,45]. The recently developed Minimal

Table 1

Defined intestinal microbial communities and their application. This summary only lists those that include three or more strains

Original host of bacterial isolates	Defined intestinal microbial communities	No. of isolates	Selection approach	Application(s)	Ref (s)
Human	Microbial Ecosystem Therapeutic (MET)	33	Cultivation of bacteria from donor feces. Screened for antibiotic resistance. Susceptible isolates chosen representing *Bacteroidetes*, *Firmicutes*, *Actinobacteria* and *Proteobacteria*.	Proposed alternative to fecal transplant by repopulating the intestinal tract with defined bacterial communities representative of the normal microbiota.	[52••]
	Synthetic Gut Community (SGC-1)	3	Isolated from human feces, abundant with genome sequence available. *Faecalibacterium prausnitzii* and *Roseburia intestinalis* were chosen for ability to produce butyrate, while *Blautia hydrogenotrophica* was chosen for its ability to utilize CO_2 and H_2 apart from its ability to produce acetate. All belong to *Firmicutes*.	A minimal model to investigate interactions between the intestinal bacteria as well to develop predictive models for community dynamics.	[50••]
	Synthetic Human Gut Microbiome Communities	12	Isolated from human feces and chosen to cover major functions and phylogenetic diversity present in the human intestinal tract. The community has representatives from phyla *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, and *Proteobacteria*.	Useful for developing predictive models for microbial community dynamics as well as investigate microbial interactions involved in community assembly.	[51••]
	Model 15-member human gut microbiota	15	Isolated from human feces, representatives from phyla *Bacteroidetes*, *Firmicutes*, and *Actinobacteria*.	Used for investigating the spatial organization of the key intestinal tract bacteria at different scales.	[53]
	Synthetic Microbiota (SM)	14	Genome sequenced human intestinal isolates representing the five dominant phyla *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, *Verrucomicrobia*, and *Proteobacteria* and ability to carry out important core metabolic functions such as mucus and dietary fiber degradation as well as short chain fatty acid production.	Effect of dietary fiber deprivation was investigated along with its effect on mucus layer.	[26••]
Mice	Oligo-Mouse-Microbiota (Oligo-MM^{12}) plus Facultative anaerobes (FA^3)	15	Bacterial isolates cultivated from the specified pathogen-free (SPF) mice. Isolates representative of most prevalent and abundant phyla *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, *Verrucomicrobia*, and *Proteobacteria*.	The Oligo-MM^{12} was tested for its ability to confer colonization resistance against *Salmonella enterica* serovar *Typhimurium*. Incorporating three isolates of FA^3 provided colonization resistance similar to the conventional complex mice microbiota	[43••]
	Schaedler flora (SF)	5	Dominant bacteria isolated from mice. Aerobic and aerotolerant anaerobic bacteria	Initially used to create gnotobiotic mouse.	[38]
	Altered Schaedler flora (ASF) [39,54••,55]	8	Modified version which included ASF356 (*Clostridium* sp.), ASF360 (*Lactobacillus intestinalis*), ASF361 (*Lactobacillus murinus*), ASF457 (*Mucispirillum schaedleri*), ASF492 (*Eubacterium plexicaudatum*), ASF500 (*Pseudoflavonifractor* sp.), ASF502 (*Clostridium* sp.) and ASF519 (*Parabacteroides goldsteinii*)	Widely used for investigating mechanisms host-microbiota relationship as well as microbe–microbe interactions.	
	Altered Schaedler flora (ASF), Shen *et al.*, 2015	7 out of 8 original strains	*Parabacteroides goldsteinii* (ASF519) ASF356 (*Clostridium* sp.), ASF361 (*Lactobacillus murinus*), ASF457 (*Mucispirillum schaedleri*), ASF492 (*Eubacterium plexicaudatum*), ASF500 (*Pseudoflavonifractor* sp.), ASF502 (*Clostridium* sp.). Missing strain was ASF360	Original ASF strains were maintained in was laboratory mice. Proportional abundances varied in the host and had minimal urease activity. This ASF was demonstrated to treat hyperammonemiain mice model	[41••]
Minimal	Bacteriome (MIBAC-1)	18	*Bacteroidetes*, *Firmicutes*, *Verrucomicrobia*, *Proteobacteria*, *Actinobacteria* representative of strains enriched in mouse.	Update to the Altered Schaedler flora. Highly representative of mouse gut microbiome and can be used for studying microbe–microbe and host–microbe interactions	[46••]

Bacteriome (MIBAC-1) consisting of 18 mouse derived bacterial strains is an example of one such a minimal microbiome and may replace the ASF in future studies of the mouse microbiome. From the extensive culture collection of mouse intestinal microorganisms, a minimal bacterial consortium (Oligo-MM^{12}) was designed to investigate CR against *Salmonella enterica* serovar *Typhimurium* [43••,46••]. *In-vivo* experiments with Oligo-MM^{12} revealed the importance of facultative anaerobes in improving CR. These two studies demonstrated the importance of combining large scale culturing approaches and multi-omics to investigate mechanisms of host–microbe interactions.

When designing a minimal microbiome, it is important to consider the major factors influencing the intestinal microbiome. For example, diet is a major source of carbon and energy and other nutrients required for growth for intestinal bacteria, alongside host-derived compounds such as mucus. Diet, especially components that cannot be digested by the host, influence the composition and metabolic (fermentation) activity of the microbiome [2,18,47]. For instance breakdown of starch and fructo-oligosaccharides results in cross-feeding not only *via* the partial breakdown products of complex substrates but also due to the fermentation end products (lactate and acetate) produced by primary degraders and consumed by butyrate-producing bacteria [48]. High functional redundancy, especially with regards to the butyrate producers using monosaccharides leads to competition for resources in the intestinal microbiome [49]. Recently, two studies used synthetic communities to model community dynamics and metabolic interactions between dominant and prevalent human intestinal bacteria [50••,51••]. Investigation of pair-wise interactions and community dynamics of a consortium of 12 human intestinal bacterial strains was used to build predictive models of community assembly and co-existence [51••]. Using a combination of mathematical modelling, culturing, metabolite measurements, and transcriptomics of a three species synthetic community, an emergent metabolic behavior was identified in *F. prausnitzii*, which downregulated the B12 production pathway due to its availability from partners in the tri-culture [50••]. The design of the three species synthetic community incorporated both potential cross-feeding as well as competitive interactions thereby allowing the investigation and predictive modelling of metabolic interactions driving such ecological interactions [50••].

Conceptual understanding for the design of minimal microbiomes

Complex ecological processes determine the successful assembly of microbial communities, and thermodynamic constraints, metabolic pathways, and regulatory circuits play a major role in successful survival and propagation at the level of individual microbial cells [56,57]. Therefore, integrating these features in top-down and bottom-up approaches for the design of minimal microbiomes is essential. The latter approach would involve understanding the metabolic roles played by each of the bacteria identified in the human intestinal microbiome. The size and complexity of a minimal microbiome can be tuned to address two main broadly defined aims, that is, 1) unravelling metabolic interactions, 2) investigating key ecological concepts. For example, lactate and acetate are produced as a result of fermentation and breakdown of polysaccharides by bacteria such as *E. rectale*, *R. bromii* or *Bifidobacterium* spp. and they can be subsequently used by *E. hallii* and related species to produce butyrate [58,59•]. Two-species systems have been used in order to understand trophic metabolic interactions addressing polysaccharide degradation and butyrate production [60,61•]. Similarly, trophic metabolic interactions between mucus degraders and butyrate producers have been studied using two-species systems [14•]. The two-species systems can be upgraded to incorporate ecosystem processes of competition, by including two competing polysaccharide degraders, and two butyrate producers that compete for polysaccharide breakdown products. Such four-species cultures can be used to investigate pairwise species competition and complementarity as well as metabolic inter-dependencies. To address specific ecological concepts, the design should aim at higher complexity to more comprehensively mimic the human intestinal microbiome. For example, to investigate the effect of functional redundancy on community assembly, selection of bacterial species that have functional overlap at different trophic levels will be crucial. Ecophysiology guided approaches that incorporate the knowledge of physiology, metabolic potential of each species with their ecological roles, and properties such as prevalence, dominance and rarity will be important in the rational design of minimal microbiomes that mimic natural ecosystems.

Challenges, opportunities, and future prospects

Several bacteria remain uncharacterized

There exists a major lacuna in our understanding of the metabolic roles of individual species, especially of some core species such as *Subdoligranulum variable*, *Coprococcus eutactus*, *Lachnospira pectinoschiza* and members of *Dialister* and *Collinsella*, to just name a few. In addition to these, bacteria related to the genus *Oscillibacter*, uncharacterized *Lachnospiraceae* and uncharacterized *Ruminococcoceae* have been cultured and sequenced as part of the human microbiome project, MetaHIT reference genomes, Culturable Genome Reference (CGR) and Human Gastrointestinal Bacteria Culture Collection (HBC) and are consistently identified in molecular profiling studies of the microbiome [62–65]. However, due to a lack of metabolic characterization, their roles in the community remain elusive. In addition to the cultured bacterial species, there remain a few key bacterial groups that

have not yet been grown as pure cultures, one example being *Oscillospira* and related bacteria [66,67]. While high-throughput cultivation strategies, also termed culturomics, have achieved success in cultivating a claimed >70% of the human intestinal bacteria, isolation of some key species will require more targeted approaches [68]. These approaches will require integrating the knowledge of their ecology and predicted nutrient requirements based on metagenome-assembled genomes.

Using *in-silico* approaches to model and predict microbial community level interactions and dynamics has received considerable interest [69–71,72••]. However, a major challenge with the currently available bioinformatics tools is the accuracy of functional annotations for genomes and metagenomes. Improvements in the accuracy of genome annotation tools will be crucial for metabolic modelling approaches that are used to simulate and predict microbial interactions in defined as well as natural communities. Recently, a large number of semi-curated constraint-based metabolic models of human intestinal bacteria were created [73•]. These models are now being used to investigate microbial interactions in communities as well as in pairs of microorganisms [74•]. A graph theory-based approach employing metabolic networks to identify species complementarity and competition is also available [75]. Results and observation of both constraint-based metabolic models and graph theory-based metabolic networks are only as good as the functional gene annotation that the current bioinformatics tools provide. A major challenge is to annotate transporter genes, which encode key functions that influence the accuracy of *in-silico* prediction of microbial interactions [73•,76]. By integrating multi-omics data and physiological studies, metabolic models have been developed for *A. muciniphila* (iAkkMuc_588), *F. prausnitzii* (*iFpraus_v1.0*), *Bacteroides thetaiotamicron* (iBth1201), *Eubacterium rectale* (iEre400), and the methanogen *Methanobrevibacter smithii* (iMsi385) [77–79]. Focus on developing improved metabolic models for these and other core microorganisms will be crucial for improving the accuracy of our understanding of the metabolic interaction networks and predictive modelling, involving the designing of minimal microbiomes with known ecophysiological properties.

Minimal microbiome(s) to understand the intricacies of the intestinal microbiome

Minimal microbiomes will be crucial for unravelling active metabolic networks and potential interactions which may be hidden due to the extensive technical noise and several unknowns in the studies based on natural communities (for e.g. feces). Minimal microbiomes allow for studying emergent metabolic behaviors that could explain the evolution of co-operation and competition between the microbial members [26••,45,50••,51••]. There still remains a wide-open field for similar studies investigating several combinations of core and non-core species to address diverse research questions. Multi-species interactions, which incorporate competition for mucus or dietary fiber breakdown products and other nutrients, and potential emergent properties of these interactions have not been investigated. Importantly, the effect of diet and mucus degrading key stone species on the overall community dynamics remains understudied. We propose that future development of minimal microbiomes should address these questions by designing-specific minimal microbiomes. For instance, to investigate the ecological and metabolic interaction dynamics in the mucus layer, a mucus-based minimal microbiome which could include mucin degraders and other co-occurring bacteria can be designed.

The understanding of ecophysiological features of natural microbiomes using minimal microbiomes can have far reaching implications in the design and development of therapeutics. More than two decades ago, a mixture of ten different facultative aerobic and anaerobic bacterial strains was shown to inhibit *Clostridioides difficile* in five patients suffering from chronic relapsing diarrhoea [80••]. Years later, a defined consortium of 33 bacterial strains (MET-1) has shown potential in treatment of *C. difficile* infections [52••]. However, the mechanism of action of these live therapeutics is unknown. Therefore, investigation of host–microbe interaction dynamics will be crucial for unravelling the mechanism of action of such minimal microbiomes. Development of predictive models for *in-situ* behavior of minimal microbiomes will be necessary for achieving effective therapeutic success in humans. Designing minimal microbiomes with defined functional outputs, such as the production of butyrate, sequestering of ammonia, or synthesis of vitamin B12, holds a promise for targeted intervention strategies. In addition to these live microbial therapeutics, cell-free supernatants with bioactive metabolites can be produced in industrial scale fermenters using minimal microbiomes to mimic the natural extracellular components in the human intestinal tract.

Conclusions/outlook

The last few years have seen a rise in studies that move forward from mere associations to identifying mechanisms of how microbes influence host health. One of the major focus areas has been the understanding of metabolic interactions and ecological dynamics. Moving forward from co-cultures and tri-cultures, the studies employing minimal microbiomes are expected to provide insights that are relevant at the ecosystem level. Synergy between culture independent and dependent experimental approaches driven by specific hypotheses is expected to play a crucial role in advancing our knowledge of microbial communities associated with human and animal hosts and for developing effective microbiome modulation strategies.

Conflict of interest statement

Nothing declared.

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