Review Article

Application of emerging technologies for gut microbiome research

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Abstract

Microbiome is associated with a wide range of diseases. The gut microbiome is also a dynamic reflection of health status, which can be modified, thus representing great potential to exploit the mechanisms that influence human physiology. Recent years have seen a dramatic rise in gut microbiome studies, which has been enabled by the rapidly evolving high-throughput sequencing methods (i.e. 16S rRNA sequencing and shotgun sequencing). As the emerging technologies for microbiome research continue to evolve (i.e. metatranscriptomics, metabolomics, culturomics, synthetic biology), microbiome research has moved beyond phylogenetic descriptions and towards mechanistic analyses. In this review, we highlight different approaches to study the microbiome, in particular, the current limitations and future promise of these techniques. This review aims to provide clinicians with a framework for studying the microbiome, as well as to accelerate the adoption of these techniques in clinical practice.

Keywords: Culturomics, faecal microbiota transplant, metagenomics, microbiome, multi-omics

INTRODUCTION

The gut microbiome is associated with a wide range of diseases as well as a dynamic reflection of well-being. Interest in the gut microbiome by clinicians and the general public is at an all-time high. The gut microbiome, being greatly influenced by environmental exposures, such as diet and medications, represents a vast area of great promise to identify novel medical treatment.

Historically, studying the microbiome dated back to the 17th century; the first microscopes were developed, facilitating the discovery and identification of microorganisms that were previously invisible to the naked eye. Koch's concept of pathogenicity provided the framework to explain disease as a consequence of microbial infection.[1] Then, medical microbiology focused on the role of disease-forming microorganisms that needed to be eliminated. However, over the past century, it has been highlighted that only a small proportion of microbes are associated with pathogenicity; currently, only 11 organisms have been formally recognised as distinct causes of cancer in humans.[2] With the introduction of microbial ecology (environmental microbiome research), the interest in microbiome research has shifted to commensal microbes, which are the majority of our body's microbes, with beneficial interactions with the human host, and thereby are essential for host–microbial coexistence. This concept of the holobiont (or meta-organism) highlights that microbes occur within complex communities,[3] in which their inter-microbial interactions and host–microbial signalling are critical to host–microbial homeostasis. An imbalance to this system, either by introduction of pathogens (i.e. infection) or by extreme environmental changes (e.g. antibiotics), would result in an altered composition of the microbial community, often

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reflected by a loss in microbial diversity (i.e. dysbiosis), and would have a downstream impact on the human physiology, thereby impacting health and propagating disease.

Currently, the exponential wealth of microbiome research is enabled by advances in genomic sequencing coupled with established computational biology pipelines.[4] The microbiome impacts every organ and aspect of human physiology. However, the mechanism of microbial pathways modulating host biology remains to be fully elucidated. Although more recent high-throughput sequencing technologies provide important information about both composition and functionality of the gut microbiome, other microbial research methodologies, from multiple branches of biology and engineering, are still required to better appreciate mechanistic host–microbial interactions. This review will discuss the different approaches in microbiome studies, in particular, the current limitations and future promise of these techniques. This review also aims to provide clinicians with a framework for studying the microbiome with hope to accelerate the adoption of these techniques in clinical practice.

Defining the microbiome

This review adopted the Microbiome Support 2019 workshop consensus[5] definition of *microbiota* and *microbiome*. The term microbiota refers to all the living microorganisms (i.e. bacteria, archaea, fungi, algae and small protists), whereas the microbiome includes not only the community of living microorganisms, but also the spectrum of molecules produced by these microorganisms, including their structural elements, metabolites and molecules produced by the coexisting host and structured by the surrounding environmental conditions. By this definition, all mobile genetic elements such as phages, viruses and extracellular DNA included in the microbiome are not part of the microbiota. It is worth noting that microbiota is often confused with the term *metagenome* in many published papers. The term metagenome refers to the collection of genomes and genes from members of the microbiota.

CULTUROMICS — TRADITIONAL METHODS AND CURRENT ADVANCES

Accurate identification of microbes was previously challenging, as it was heavily dependent on traditional culture methods. Before advancements were made in sequencing technology, it was estimated that ~80% of the bacteria within the human gut were unculturable at that time, and thus unknown.[6] However, with recent high-throughput automated innovations, hundreds of new bacterial species have been isolated in the last few years.[4] With advances in automated sample handling, a single stool sample can be aliquoted into many micro-chambers, each with its own unique culture conditions and incubation setting. This approach developed by researchers to culture bacteria in a high-throughput setting, which cannot be cultured using conventional techniques, is termed *culturomics*.

Initial culturomics efforts[7] were labour-intensive, experimenting with 212 culture conditions to generate >30,000 colonies from 341 unique bacterial species, of which more than half were identified from the human gut for the first time. Culturomics methods continue to develop, such as the addition of ethanol to faecal samples to enrich the growth of sporulated bacteria, resulting in isolation of 69 novel bacterial species.[8] While this high-throughput method has dramatically contributed to broaden the knowledge of gut bacteria, this approach remains relatively labour-intensive, in comparison to other microbiome study methodologies such as metagenomics. Thus, some groups have investigated the most profitable conditions for optimising culturomics by determining a methodological minimal number of conditions, while not losing significant bacterial diversity.[9]

METAGENOMICS — AMPLICON, AMPLICON-PLUS, SHOTGUN, LONG READS

The majority of microbiome research charts community-wide ecological maps using next-generation sequencing metagenomics to provide insight into the microbial determinants of health and disease. This culture-free, high-throughput technology typically encompasses two particular sequencing strategies: amplicon sequencing, using unique variable regions of the bacterial 16S rRNA/internal transcribed spacer (ITS) as a phylogenetic marker, and shotgun sequencing, which captures the complete breadth of DNA within a sample and involves breaking all the genomes present in the sample into small DNA fragments, which are sequenced individually and subsequently pieced back together using bioinformatic tools. A brief comparison of the technical considerations between the two methods is presented in Table 1.

The use of the 16S ribosomal RNA gene as a phylogenetic marker has proven to be an efficient and cost-effective strategy for microbiome analysis. In a landmark inflammatory bowel diseases (IBD) microbiome study which characterised stool, ileum and rectal mucosal microbiome of paediatric Crohn's disease patients, Gevers *et al.*[10] were able to accurately predict IBD disease activity using a simple microbial dysbiosis index from 16s readouts. This study also highlighted the capability of using 16S rRNA sequencing to characterise microbially low-biomass samples such as ileum and rectal biopsies. However, it is important to note some technical considerations with amplicon sequencing, as these differences in the methodology would generate different results. Bacterial 16s rRNA genes consist of nine hypervariable regions (V1–V9). So, it would be important to pick the common primers to enable other investigators to compare the results. The Human Microbiome Project,[11] which characterised 300 healthy individuals across several different sites of the human body (nasal passages, oral cavity, skin, gastrointestinal tract and urogenital tract), used both V1–3 and V3–5 primers. For gut microbiome sequencing, most research projects would pick

Table 1. Comparison of different sequencing platforms for microbiome metagenomic sequencing.

Types of microbiome sequencing	Short read		Long read
	Amplicon sequencing 'Partial genomes'	Shotgun sequencing 'Complete genomes'	
Microorganism identified	Bacteria, archaea, eukaryotes, fungi (no viruses)	Bacteria, fungi, protists, archaea, viruses	Bacteria, archaea, fungi, protists
Region of amplification	16S, 18S, ITS	Whole genome	16S rRNA, 18S rRNA, ITS rRNA, whole genome
Taxonomic composition readout	Up to genus level	Up to species level and functional genes	Up to strain level
Commonly used sequencing platform	Illumina, Ion Torrent, MGI	Illumina, Ion Torrent, MGI	PacBio, ONT
Amount of DNA required	10–20 ng	100 ng–1 μg	100 ng–1 μg
Sequencing data output	Megabytes	Gigabytes	Gigabytes
Estimated cost	$	$$$	$$–$$$
Availability of open-source analytical packages	+++	++	+

ITS: internal transcribed spacer, MGI: MGI Technologies, ONT: Oxford Nanopore Technologies

the V4 or V3–4 primers, which has the advantage of identifying both archaea as well as the majority of the bacterial species. As amplicon sequencing only provides a partial genome representation, it is unable to annotate all amplicons with bacterial species-level resolution. Genus-level resolution for most bacterial taxa with 16S amplicon sequencing, however, is possible. Recent advances in amplicon sequencing, coupled with long-read sequencing technologies, have now enabled full-length sequencing of the entire V1–V9 16S gene, thereby accurately annotating all amplified sequences to the species level.[12] Further enhancements in this platform by including other target regions such as ITS and 23S, paired with long-read sequencing technologies, bring promise that amplicon sequencing methods may achieve strain-level resolution, which would then be particularly useful for clinical applications.

Given the limitations of amplicon sequencing described above, increasing numbers of researchers are now reliant on shotgun sequencing, which reads all genomic DNA in a sample, rather than just one specific region of DNA. This provides subspecies strain-level resolution and functional insights. In the context of IBD, shotgun sequencing enabled Hall *et al.*[13] to conduct a pan-genome analysis using 266 stool samples from 20 IBD patients and 16 controls sampled longitudinally, on subspecies strains of *Ruminococcus gnavus,* whereby a distinct clade of *R. gnavus* strains was identified specifically encoding for 199 IBD-specific microbial genes involved in oxidative stress responses, adhesions, iron acquisition and mucous utilisation. This study illustrated that even among pathognomic bacterial species associated with IBD,[14] such as *R. gnavus*, there is still a need for strain-level resolution to better study host–microbial interactions.

Shotgun sequencing also enables the profiling of fungi, viruses and many other types of microorganisms. Metagenomic analysis of faecal samples from patients with and without colorectal cancer demonstrated unique virome[15] and fungal[16] signatures, when compared to cancer-free controls. Thus, the microbiome research field is expected to increasingly rely on this more sophisticated methodology when studying microbiome–host interactions in health and disease. However, the relatively high costs of shotgun metagenomics and more demanding bioinformatic requirements limit this methodology to be applied on large population cohorts. Another key limitation of shotgun sequencing is its applicability for low-biomass samples, whereby human DNA is rampantly abundant, and therefore, having such samples undergo shotgun sequencing results in expensive sequencing at high-depth reads with subsequently low traces of microbial reads.[17] However, technologies to dehost human DNA are an active area of method development, and when such methods are able to efficiently deplete host DNA before shotgun sequencing, we thereby expect shotgun sequencing to truly displace amplicon sequencing for microbiome research.

Both amplicon and shotgun sequencing are short-read sequencing technologies. There is emerging data demonstrating that long-read sequencing improves identification of a wider range of species and better differentiates between strains within a species.[18,19] Two of the dominant technologies providing long-read sequencing include Pacific Biosciences' (PacBio) single-molecule real-time sequencing and Oxford Nanopore Technologies' (ONT) nanopore sequencing. Application of long-read sequencing has been useful for the assembly of small bacterial genomes.[20] Although long reads uncover the genomic regions that are inaccessible to short-read sequences, there are still concerns that long-read sequencing is less accurate and less cost-effective compared to short-read sequencing,[21] thereby limiting its applicability for wide-scale adoption. As the sequencing platforms and downstream bioinformatic pipelines continue to advance, we anticipate wider adoption of long-read sequencing to study rare microbial genomes in low-diversity microbial communities.

MULTI-OMICS MICROBIOME INTEGRATED ANALYSIS

The microbiome impacts every organ system and aspect of physiology. As an increasing number of researchers characterise

sparsely populated low-biomass microbiome communities in seemingly 'sterile' organs, such as skin, lungs, reproductive organs and bile ducts, their findings are often questioned. Often, the findings are challenged by high false-positive signals from potential contamination and sequencing artefacts. Therefore, common to microbiome research, there are often internal validations of the microbial readouts, particularly in such studies, when characterising patients' samples with low biomass, using experimental validation methods (e.g. electron microscopy) and microbiome *multi-omics* (e.g. culturomics, metatranscriptomics, metabolomics). In a recent finding by Mishra *et al.*[22] who profiled microbial communities in human foetal tissues in the second trimester of gestation using 16s rRNA sequencing, they provided additional microbial readouts to demonstrate the presence of these microbes of interest, such as culturomics, electron microscopy, as well as *in vitro* experimental validation experiments, affirming the function of these bacterial strains. This landmark study advocates the important role of microbial exposure for early-life immune priming, whereby gut microbes present in the second trimester of gestation to activate memory T cells. This display of microbiome multi-omics further provides better understanding of the microbiome communities uncovered.

Multi-omics also enables microbiome researchers to understand the function of microbial communities in the gut microbiome, validating from the metagenomic readouts, in which genes are expressed and which are translated into proteins [Figure 1]. In Schirmer *et al.*'s[23] IBD metatranscriptomics study, it has been further shown that meta-transcriptional profiles, whereby RNA extracted from a faecal sample is reverse transcribed into cDNA and sequenced, provided important insight into gut microbial community dynamics, including IBD-specific transcriptional activity. Furthermore, it is suggested that microbial transcriptional programmes are more rapidly responsive to environmental cues such as changes in inflammation and oxygen levels, which may not be reflected at the DNA level. However, limitations to interpretation of faecal metatranscriptomics readouts include variation due to subject-specific transit times and selection bias where stool samples only capture extractable, non-degraded RNA restricted to organisms present in stool.

The influence of the microbiome extends beyond the local environment. One way it does so is through *microbiome metabolites*, classes of small molecules produced or modified by the gut microbiome, which are important regulators of host–microbial interactions. In Franzosa *et al.*'s IBD microbiome metabolomics study, IBD-specific microbial metabolites were identified by correlating faecal metabolomics and faecal metagenomics; patients with IBD were found to have increased levels of bile acids and sphingolipids and depletion of triacylglycerols and tetrapyrroles.[24] Such microbial metabolites serve as promising novel biomarkers for personalised medical therapy. It has been suggested that IBD patients with microbial communities capable of converting primary bile acids to secondary bile acids would more likely respond to anti-cytokine biologic therapy,[25] and likewise, Crohn's disease patients with increased abundance of microbes capable of butyrate production are more likely to achieve clinical remission when treated with anti-integrin therapy.[26]

As alluded to above, one of the key groups of microbial metabolites is formed of short-chain fatty acids (SCFAs), such as acetate, propionate and butyrate, which are the products of fermentation of carbohydrates by the gut microbiota in the colon.[27] SCFAs are important gut molecules that play diverse immune-modulatory roles, such as regulating histone acetylation[28] and regulating T cells in the gut.[29] Given their potential anti-inflammatory effects, coupled with the reduction of SCFAs and their corresponding SCFA-producing bacteria

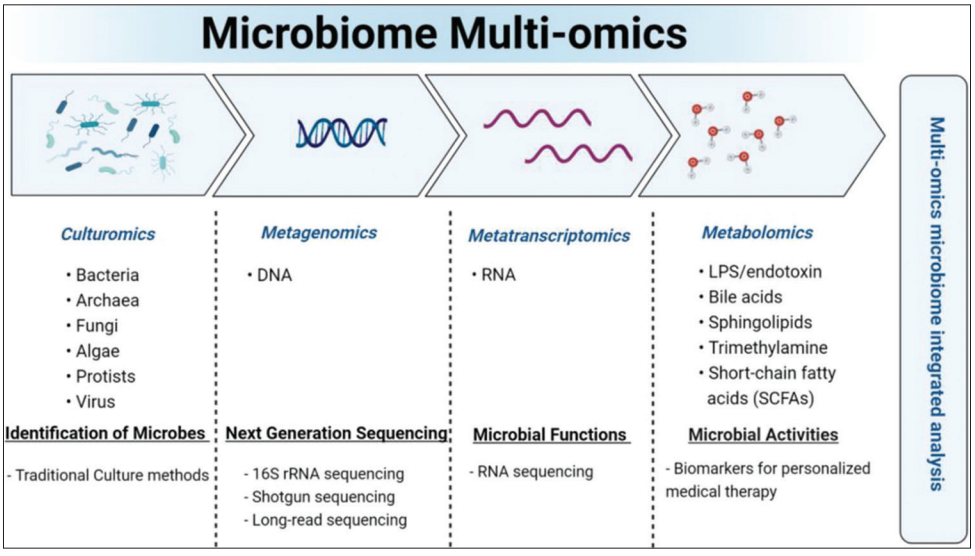

Figure 1: Diagram shows microbiome multi-omics and related applications. [Created with BioRender.com] LPS: lipopolysaccharide, SCFAs: short-chain fatty acids

in patients with IBD, SCFAs have been often considered a promising therapeutic option for the clinical management of IBD patients. Thus, there have been small, but promising results[30,31] in animal models as well as a subset of IBD patients who respond to augmentation of SCFA-producing bacteria. However, more robust clinical studies are still required to test the safety and efficacy of such approaches.

IgA-Seq, which pairs 16S amplicon sequencing with bacterial fluorescence-activated cell sorting for bacterial taxa coated with host secretory immunoglobulin IgA, has been shown to identify more accurately pathogenic members of the gut microbiome driving inflammatory response in IBD. This approach, which ingeniously integrates existing sequencing methods with previous knowledge of human immunology, now allows investigators to focus on microbes which have interaction with the host mucosal immune system (i.e. microbe members that would tag with IgA). Shapiro *et al.*[32] thereby used IgA-SEQ in the Ocean State Crohn's and Colitis Area Registry to identify multiple potential bacterial contributors to IBD, exclusively based on IgA coating. A low IgA coating of *Oscillospira* was predictive for poor prognosis, whereby a significant proportion of patients with low IgA coating of *Oscillospira* required subsequent surgical resection. Although IgA coating in IBD patients marks bacteria associated with increased inflammation, it is important to note that IgA coating does not only represent pathogenesis, but is also important for physiological host defence systems. Therefore, when interpreting putative IgA-targeted microbial strains, the results should be interpreted with caution as these microbes of interest may not only regulate pathogenesis of the disease, but may also be critical players in the normal immunoregulatory responses and, in fact, are proactive against intestinal inflammation. Nonetheless, IgA-SEQ represents a novel approach to incorporate microbial sequencing with other experimental biological research methods to gain better insight into host–microbiome interactions in health and disease.

IN VITRO HOLOBIONT SYSTEMS — SHIME®, HuMIx, RapidAIM

Another approach to study host–microbiome interactions is the use of high-throughput *in vitro* models, mostly for validation experimentation of *in silico* results obtained from metagenomics or multi-omics approaches described above. For this review, we will highlight three such established models used for microbiome research — Simulator of the Human Intestinal Microbial Ecosystem (SHIME®), Human Microbial X (cross) talk (HuMix) and Rapid Assay of Individual Microbiome (RapidAIM).

SHIME[33] is an *in vitro* gut model that recapitulates the physiological conditions of the human gastrointestinal tract. It comprises five dynamic simulating compartments connected in series — stomach, small intestine, ascending colon, transverse colon and descending colon. The simulated stomach and small intestine compartments re-enact the 'fill and draw' principle, while allowing the investigator to determine the concentration of nutrients, pancreatic enzymes and bile within. The distal three compartments which run under constant volume and controlled pH are stirred continuously to modulate retention times in the large intestines [Appendix, Supplementary Figure 1a]. The SHIME system has been a useful tool for the study of probiotics, prebiotics and oral therapeutics metabolism. To investigate the colonisation capacity of various probiotics, Abbeele *et al.*[34] added mucin to SHIME, creating a more representative dynamic gut model, which helped to better discover the organisms that would benefit from mucosal adhesion. They were also able to study the efficacy of the colonisation effect by adding mucin to the system to study the microbiome stability over a long timeframe, as well as to monitor microbial adaptation. In brief, the advantages of SHIME[35] include the ability to study microbes through integrating the entire gastrointestinal tract, facilitate colon region-specific research, as well as enable mechanistic research by controlling for multiple parametric options. However, it is important to note that SHIME is still dependent on stirrers for mixing, rather than a peristalsis-like mode of mixing, and there is still an absence of host cells in the conventional SHIME model to allow for holistic host–microbe interaction interrogation. To further improve on this, Marzorati *et al.* introduced the Host–Microbiota Interaction (HMI™), whereby an additional module is attached to the end of the SHIME system, which provides a mucosal area for bacteria to adhere, while allowing bilateral transport of metabolites with variable permeation coefficients. HMI also allows for microaerophilic settings that enhance the formation of biofilms.[36]

In view of the need to better represent host–microbiome interactions, scientists from the University of Luxembourg introduced a three-dimensional organotypic model of human colonic epithelium using microfluidics-based principles – the HuMix model.[37,38] The HuMix comprises three microchambers (microbial, epithelial cell and perfusion) m whereby intestinal cells and microbes are co-cultured but separated by a nanoporous membrane [Appendix, Supplementary Figure 1b]. The HuMix allows for real-time monitoring of oxygen concentrations, which is important as the oxygen gradient varies across different topographic locations of the gastrointestinal tract, as well as in various inflammatory diseases (e.g. IBD). This module also allows easy access to each of the individual cell contingents for end-point microscopic assays, facilitating high-resolution microbiome multi-omics analyses. As a proof of concept, Shah *et al.*[37] demonstrated the utility of HuMix to study host–microbiome interactions in co-culturing *Lactobacillus rhamnosus* GG and Caco-2 cell cultures with transcriptomics, metabolomics and immunological readouts. The HuMix model promises to perform systematic investigation of host–microbe interactions to enable translational microbiome for novel therapeutic discovery.

To further drive drug discovery while studying its association with the microbiota, Li *et al.*[39] developed a rapid and scalable assay to comprehensively assess microbiome responses to drugs – the RapidAIM. Using the RapidAIM, they developed a pipeline to co-culture individual stool samples in uniform non-selective media together with a wide range of pharmaceutical compounds, which would then undergo high-throughput metaproteomics to measure microbial biomass levels as well as drug concentrations. This gives us insights into microbiome–drug responses. This approach brings promise of the potential of personalised medicine in the near future through principles of microbial pharmacogenomics, whereby one may prescribe medications based on microbiome profiles. As the current platform is still limited by the time-consuming mass spectrometry analysis, the developers of this module are working to consider a fast pass screening process, perhaps using tandem mass tags, to enable future clinical adaptation.

MICROBIOME-DIRECTED INTERVENTIONS

The exponential growth of microbiome knowledge obtained through the methodologies described above has further cemented the importance of microbiome in health and disease. Furthermore, the microbiome can be potentially modified, and presents untapped opportunities to exploit mechanisms that influence human physiology, such as to achieve health and prevent disease. Microbiome-directed interventions can be broadly classified as either untargeted (with general improvement in the microbial composition and functions) or targeted (with specific modification in metabolism-related gut microbiota) [Figure 2].

Common examples of untargeted microbiome-based therapeutics include faecal microbiota transplant (FMT), antibiotics, prebiotics, probiotics and postbiotics, as well as dietary changes. FMT, which takes faecal samples from healthy donors void of detectable pathogens and implants that faecal material into the gut of recipients with missing healthy gut microbes, is currently accepted as an effective treatment for recurrent *Clostridioides difficile* infections, with over 90% efficacy.[40] Despite the non-specific nature of the treatment, there has been an increasing number of FMT clinical trials conducted for other indications with considerable efficacy, such as for ulcerative colitis,[41] anticancer response to immune checkpoint inhibitors[42,43] as well as for type 2 diabetes mellitus.[44] Although FMT has thus far had a remarkably low rate of serious adverse events, it is important to note that the capacity to safely deliver FMT depends heavily on standardised, highly specialised laboratories for stool preparation.[45] These laboratories will require expertise in appropriate sample collection, preparation and storage, as well as thorough health screening of donors. Well-conducted clinical FMT trials may better identify specific bacteria or metabolites responsible for microbiota-based therapeutic effect, which will further guide the development of targeted bacteriotherapy as a more sustainable and safer approach.

One of the most promising targeted microbial-based interventions is bioengineered therapeutics as demonstrated by Ho *et al.*,[46] who engineered *Escherichia coli (E. coli)* Nissle *(E.* Nissle*)* into a probiotic that would attach to surfaces of colorectal cancer cells to secrete myrosinases, which transform glucosinolates found in cruciferous vegetables (such as broccoli) into sulforaphane, an organic small molecule with known anticancer activity. This bioengineered *E.* Nissle strain when taken with broccoli extract resulted in >95% inhibition of colorectal cancer cell lines grown *in vitro.* Another promising emerging targeted microbial-based intervention is phage therapy. Bacteriophages are viruses that infect and kill bacteria without negative effects on human cells and have been in the limelight for their potential to treat patients with life-threatening antibiotic-resistant infections, as evidenced by its use to treat a patient with disseminated resistant *Acinetobacter baumannii* infection.[47] The limitations

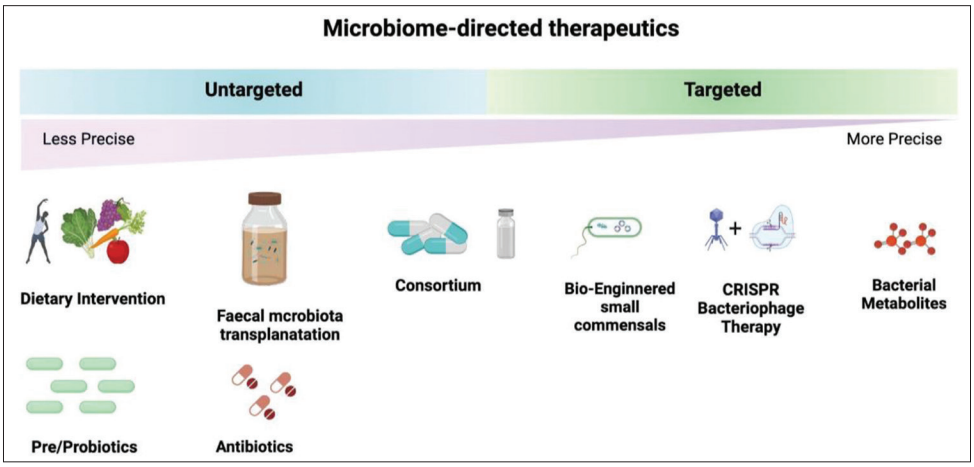

Figure 2: Diagram shows the current pipeline for microbiome-directed therapeutics ranging from untargeted interventions (i.e. faecal microbiota transplantation, pre/probiotic and consortium) to targeted interventions (i.e. bioengineered small molecules and bacteriophage). [Created with BioRender.com]

associated with phage therapy include possible emergence of bacterial resistance against such phages, reduced activity due to immune response to phages, as well as the laborious effort to isolate and formulate specific phages for clinical adoption.[48]

CONCLUSION

Recent years have seen a dramatic rise in gut microbiome studies, enabled by the rapidly evolving high-throughput sequencing methods (i.e. 16S rRNA sequencing and shotgun sequencing). Human cohort studies using metagenomics will continue to be integral for translating the microbiome in health and disease. Multi-omics (i.e. metatranscriptomics, metabolomics, culturomics, synthetic biology) will uncover the 'microbial dark matter' within the microbiome by providing a complete annotation of microbes, gene, proteins and metabolites within the human microbiome, as well as aid in the holistic interpretation of microbiome–host interactions. With this, more microbiome research would then be hypothesis-driven functional studies, rather than hypothesis generating. As our understanding of microbiome–host interactions improves, so will our targeted microbiome-based interventions for microbiome-based precision medicine.

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Conflicts of interest

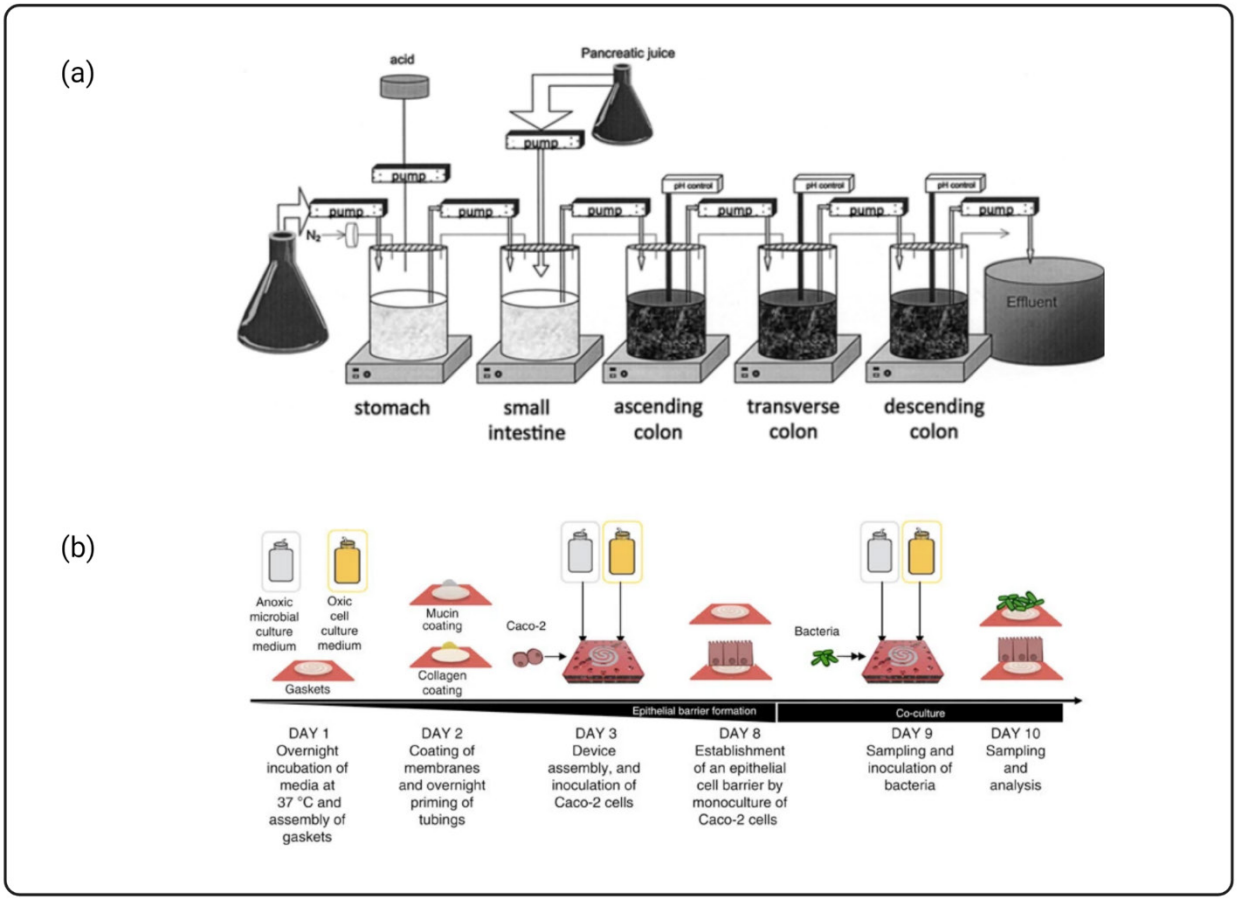
Lee J is a co-founder of AMILI and serves as a member of the scientific advisory board. Sundarajoo S and Toh KY are employees of AMILI.

REFERENCES

1. Falkow S. Molecular Koch's postulates applied to microbial pathogenicity. Rev Infect Dis 1988;10(Suppl 2):S274-6.
2. Cullin N, Azevedo Antunes C, Straussman R, Stein-Thoeringer CK, Elinav E. Microbiome and cancer. Cancer Cell 2021;39:P1317-41.
3. Bassler BL. Small talk. Cell-to-cell communication in bacteria. Cell 2002;109:421-4.
4. Lagier J-C, Dubourg G, Million M, Cadoret F, Bilen M, Fenollar F, *et al*. Culturing the human microbiota and culturomics. Nat Rev Microbiol 2018;16:540-50.
5. Berg G, Rybakova D, Fischer D, Cernava T, Vergès MC, Charles T, *et al*. Microbiome definition re-visited: Old concepts and new challenges. Microbiome 2020;8:103.
6. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, *et al*. Diversity of the human intestinal microbial flora. Science 2005;308:1635-8.
7. Lagier J-C, Armougom F, Million M, Hugon P, Pagnier I, Robert C, *et al*. Microbial culturomics: Paradigm shift in the human gut microbiome study. Clin Microbiol Infect 2012;18:1185-93.
8. Browne HP, Forster SC, Anonye BO, Kumar N, Neville BA, Stares MD, *et al*. Culturing of "unculturable" human microbiota reveals novel taxa and extensive sporulation. Nature 2016;533:543-6.
9. Diakite A, Dubourg G, Dione N, Afouda P, Bellali S, Ngom II, *et al*. Optimization and standardization of the culturomics technique for human microbiome exploration. Sci Rep 2020;10:9674.
10. Gevers D, Kugathasan S, Denson LA, Vázquez-Baeza Y, Van Treuren W, Ren B, *et al*. The treatment-naive microbiome in new-onset Crohn's disease. Cell Host Microbe 2014;15:382-92.
11. Zeevi D, Korem T, Godneva A, Bar N, Kurilshikov A, Lotan-Pompan M, *et al*. Structural variation in the gut microbiome associates with host health. Nature 2019;568:43-8.
12. Singer E, Bushnell B, Coleman-Derr D, Bowman B, Bowers RM, Levy A, *et al*. High-resolution phylogenetic microbial community profiling. ISME J 2016;10:2020-32.
13. Hall AB, Yassour M, Sauk J, Garner A, Jiang X, Arthur T, *et al*. A novel Ruminococcus gnavus clade enriched in inflammatory bowel disease patients. Genome Med 2017;9:103.
14. Lloyd-Price J, Arze C, Ananthakrishnan AN, Schirmer M, Avila-Pacheco J, Poon TW, *et al*. Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. Nature 2019;569:655-62.
15. Nakatsu G, Zhou H, Wu WKK, Wong SH, Coker OO, Dai Z, *et al*. Alterations in enteric virome are associated with colorectal cancer and survival outcomes. Gastroenterology 2018;155:529-41.
16. Coker OO, Nakatsu G, Dai RZ, Wu WKK, Wong SH, Ng SC, *et al*. Enteric fungal microbiota dysbiosis and ecological alterations in colorectal cancer. Gut 2019;68:654-62.
17. Allaband C, McDonald D, Vázquez-Baeza Y, Minich JJ, Tripathi A, Brenner DA, *et al*. Microbiome 101: Studying, analyzing, and interpreting gut microbiome data for clinicians. Clin Gastroenterol Hepatol 2019;17:218-30.
18. Bertrand D, Shaw J, Kalathiyappan M, Ng AHQ, Kumar MS, Li C, *et al*. Hybrid metagenomic assembly enables high-resolution analysis of resistance determinants and mobile elements in human microbiomes. Nat Biotechnol 2019; 37:937-44.
19. Xie H, Yang C, Sun Y, Igarashi Y, Jin T, Luo F, *et al*. PacBio long reads improve metagenomic assemblies, gene catalogs, and genome binning. Front Genet 2020;11:516269.
20. Moss EL, Maghini DG, Bhatt AS. Complete, closed bacterial genomes from microbiomes using nanopore sequencing. Nat Biotechnol 2020;38:701-7.
21. Bharti R, Grimm DG. Current challenges and best-practice protocols for microbiome analysis. Brief Bioinform 2019;22:178-93.
22. Mishra A, Lai GC, Yao LJ, Aung TT, Shental N, Maskowitz RA, *et al.* Microbial exposure during early human development primes fetal immune cells. Cell 2021;184:3394-409.e20.
23. Schirmer M, Franzosa EA, Lloyd-Price J, McIver LJ, Schwager R, Poon TW, *et al*. Dynamics of metatranscription in the inflammatory bowel disease gut microbiome. Nat Microbiol 2018;3:337-46.
24. Franzosa EA, Sirota-Madi A, Avila-Pacheco J, Fornelos N, Haiser HJ, Reinker S, *et al*. Gut microbiome structure and metabolic activity in inflammatory bowel disease. Nat Microbiol 2019;4:293-305.
25. Lee JWJ, Plichta D, Hogstrom L, Borren NZ, Lau H, Gregory SM, *et al.* Multi-omics reveal microbial determinants impacting responses to biologic therapies in inflammatory bowel disease. Cell Host Microbe 2021;29:1294-304.e4.
26. Ananthakrishnan AN, Luo C, Yajnik V, Khalili H, Garber JJ, Stevens BW, *et al*. Gut microbiome function predicts response to anti-integrin biologic therapy in inflammatory bowel diseases. Cell Host Microbe 2017;21:603-10.e3.
27. Wong JM, de Souza R, Kendall CW, Emam A, Jenkins DJ. Colonic health: Fermentation and short chain fatty acids. J Clin Gastroenterol 2006;40:235-43.
28. Davie JR. Inhibition of histone deacetylase activity by butyrate. J Nutr 2003;133 (7 Suppl):2485S-93S.
29. Arpaia N, Campbell C, Fan X, Dikiy S, van der Veeken J, deRoos P, *et al.* Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. Nature 2013;504:451-55.
30. Deleu S, Machiels K, Raes J, Verbeke K, Vermeire S. Short chain fatty acids and its producing organisms: An overlooked therapy for IBD? EBioMedicine 2021;66:103293.
31. Parada Venegas D, De la Fuente MK, Landskron G, González MJ, Quera R, Dijkstra G*, et al*. Short Chain Fatty Acids (SCFAs)-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases. Front Immunol 2019;10:277.
32. Shapiro JM, de Zoete MR, Palm NW, Laenen Y, Bright R, Mallette M, *et al.* Immunoglobulin a targets a unique subset of the microbiota in

inflammatory bowel disease. Cell Host Microbe 2021;29:83-93.e3.
33. Molly K, Vande Woestyne M, Verstraete W. Development of a 5-step multi-chamber reactor as a simulation of the human intestinal microbial ecosystem. Appl Microbiol Biotechnol 1993;39:254-8.
34. Van den Abbeele P, Roos S, Eeckhaut V, MacKenzie DA, Derde M, Verstraete W, *et al.* Incorporating a mucosal environment in a dynamic gut model results in a more representative colonization by lactobacilli. Microb Biotechnol 2012;5:106-15.
35. Van de Wiele T, Van den Abbeele P, Ossieur W, Possemiers S, Marzorati M. The Simulator of the Human Intestinal Microbial Ecosystem (SHIME®). In: Verhoeckx K, Cotter P, López-Expósito I, *et al.*, editors. The Impact of Food Bioactives on Health: *In Vitro* and *Ex Vivo* Models. Cham: Springer International Publishing; 2015. p. 305-17. Available from: https://doi.org/10.1007/978-3-319-16104-4_27.
36. Marzorati M, Vanhoecke B, De Ryck T, Sadaghian Sadabad M, Pinheiro I, Possemiers S, *et al.* The HMI™ module: A new tool to study theHost-Microbiota Interaction in the human gastrointestinal tract *in vitro*. BMC Microbiol 2014;14:133.
37. Shah P, Fritz JV, Glaab E, Desai MS, Greenhalgh K, Frachet A*, et al.* A microfluidics-based *in vitro* model of the gastrointestinal human–microbe interface. Nat Commun 2016;7:1-15.
38. Wilmes P. HuMiX, an *in vitro* model to study the human gut microbiome and immune system. 2016. Available from: https://orbilu.uni.lu/handle/10993/37976.
39. Li L, Ning Z, Zhang X, Mayne J, Cheng K, Stintzi A, *et al*. RapidAIM: A culture- and metaproteomics-based rapid assay of individual microbiome responses to drugs. Microbiome 2020;8:33.
40. Lai CY, Sung J, Cheng F, Tang W, Wong SH, Chan PKS*, et al*. Systematic review with meta-analysis: Review of donor features, procedures and outcomes in 168 clinical studies of faecal microbiota transplantation. Aliment Pharmacol Ther 2019;49:354-63.
41. Narula N, Kassam Z, Yuan Y Colombel JF, Ponsioen C, Reinisch W, *et al.* Systematic review and meta-analysis: Fecal microbiota transplantation for treatment of active ulcerative colitis. Inflamm Bowel Dis 2017;23:1702-9.
42. Baruch EN, Youngster I, Ben-Betzalel G, Ortenberg R, Lahat A, Katz L, *et al.* Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients. Science 2021;371:602-9.
43. Davar D, Dzutsev AK, McCulloch JA, Rodrigues RR, Chauvin JM, Morrison RM, *et al*. Fecal microbiota transplant overcomes resistance to anti-PD-1 therapy in melanoma patients. Science 2021;371:595-602.
44. Ng SC, Xu Z, Mak JWY, Yang K, Liu Q, Zuo T, *et al.* Microbiota engraftment after faecal microbiota transplantation in obese subjects with type 2 diabetes: A 24-week, double-blind, randomised controlled trial. Gut 2022;71:716-23.
45. Cammarota G, Ianiro G, Tilg H, Rajilić-Stojanović M, Kump P, Satokari R, *et al*. European consensus conference on faecal microbiota transplantation in clinical practice. Gut 2017;66:569-80.
46. Ho CL, Tan HQ, Chua KJ, Kang A, Lim KH, Ling KL, *et al*. Engineered commensal microbes for diet-mediated colorectal-cancer chemoprevention. Nat Biomed Eng 2018;2:27-37.
47. Schooley RT, Biswas B, Gill JJ, Hernandez-Morales A, Lancaster J, Lessor L, *et al*. Development and use of personalized bacteriophage-based therapeutic cocktails to treat a patient with a disseminated resistant acinetobacter baumannii infection. Antimicrob Agents Chemother 2017;61:e00954-17. doi: 10.1128/AAC.00954-17.
48. Principi N, Silvestri E, Esposito S. Advantages and limitations of bacteriophages for the treatment of bacterial infections. Front Pharmacol 2019;10:513.

APPENDIX



Supplementary Figure 1: Schematic representation of the *in vitro* holobiont systems: (a) SHIME® (Simulator of the Human Intestinal Microbial Ecosystem); (b) HuMix (Human Microbial X (cross) talk) model. Figures are adapted from Van de Wiele T *et al* (2015) and Shah P, *et al* (2016).