# Synthetic ecology of the human gut microbiota

*Gino Vrancken[1,2,3], Ann C. Gregory[1,2,3], Geert R. B. Huys[1,2], Karoline Faust[1] and Jeroen Raes[1,2]**

Abstract | Despite recent advances in sequencing and culturing, a deep knowledge of the wiring and functioning of the human gut ecosystem and its microbiota as a community is still missing. A holistic mechanistic understanding will require study of the gut microbiota as an interactive and spatially organized biological system, which is difficult to do in complex natural communities. Synthetic gut microbial ecosystems can function as model systems to further current understanding of the composition, stability and functional activities of the microbiota. In this Review, we provide an overview of the current synthetic ecology strategies that can be used towards a more comprehensive understanding of the human gut ecosystem. Such approaches that integrate in vitro experiments using cultured isolates with mathematical modelling will enable the ultimate goal: translating mechanistic and ecological knowledge into novel and effective therapies.

Dysbiosis
Qualitative and quantitative changes in the intestinal microbiota that alter their metabolic activities and local distributions.

Cross-feeding
The process by which one organism uses the metabolic products of another organism.

Syntrophy
A type of cooperative bidirectional cross-feeding whereby two organisms gain through the metabolic reactions of each other.

[1]*Department of Microbiology and Immunology, Rega Institute, KU Leuven, Leuven, Belgium.*

[2]*VIB-KU Leuven Center for Microbiology, Leuven, Belgium.*

[3]*These authors contributed equally: Gino Vrancken, Ann C. Gregory.*

**e-mail: jeroen.raes@kuleuven.vib.be*

https://doi.org/10.1038/s41579-019-0264-8

The human gut microbiota is a complex and dynamic microbial community that is integral to the maintenance of health[1,2] and the regulation of the host immune system[3,4]. Recent breakthroughs in culture-independent, high-throughput profiling have enabled the rapid, large-scale quantification of the composition of the gut microbial community in health and disease (FIG. 1). Alterations of the gut microbiota (that is, dysbiosis) have been linked to many diseases and conditions, such as obesity[5–8] and diabetes mellitus[9–12]. As a result, modulating the gut microbiota has been viewed as a potential source of novel therapeutics for treating diseases that are associated with dysbiosis[13,14]. Nonetheless, the clinical translation of microbiota-based therapies has been slow, with one of the main obstacles being lack of a mechanistic understanding of the metabolic and ecological interactions between microorganisms and with the host.

Although a relatively complete atlas of the taxa that make up the human gut microbiota has been compiled in the past 10 years, much more limited progress has been made in elucidating the wiring of the human gut ecosystem — that is, the distinct characteristics of individual community members and the complex web of interactions among them[2,8]. Sequencing data alone have been shown to be inadequate to fully predict microbial phenotypes and functions in a community. For example, a recent study revealed that 75% of metabolic models of gut bacterial species, based on genomics and literature information on growth requirements alone, failed to predict the growth of the same bacterial species in different media[15]. Analyses pairing complementary 'omics' data (that is, metatranscriptomics, metaproteomics and metabolomics) with shotgun data arguably have more predictive power and are able to assess active species, pathways and genes in an ecosystem. However, it is not yet possible to (fully) assess the complex ecological interactions using 'omics' data alone[16]. Here, we argue that a synthetic ecology approach — that is, the study of complex microbial systems using synthetic communities — could form the solution to this problem.

Synthetic microbial communities are systems of known and reduced complexity that are amenable to experimental intervention and modelling, enabling a systems-level understanding[17–19]. From a bottom-up ecological perspective (going from components to communities), synthetic communities are a way to study how microbial community structure emerges (for example, through competition and cooperation) and to identify the conditions necessary to generate specific interaction patterns (for example, cross-feeding, syntrophy and auxotrophy). From a top-down ecological perspective (starting with the system), they can answer questions about the overall function and the resistance and resilience of microbial systems[18]. The study of synthetic microbial communities requires (i) controlled in vitro environments, (ii) biologically relevant bacterial strains and (iii) mathematical models of the ecological interactions to simulate and test.

Work on creating human gut-specific, controlled in vitro environments, isolating intestinal bacterial strains and mathematically modelling gut bacterial communities is currently under way. Still, the use of a

Auxotrophy
A type of cross-feeding whereby the metabolic reactions of one organism are required for the growth of another organism.

Resistance
The ability of a system to withstand disturbance.

synthetic-ecology approach is still relatively new for the gut microbiota field. It was once thought that as few as ~20% of the human gut microorganisms were culturable[20], indicating that the design of synthetic gut microbial communities would always be limited. Now, however, new methods such as culturomics[21] are overturning this paradigm and have inspired a culturing renaissance[21–24] (FIG. 1). Furthermore, recent efforts to sequence the genomes of these cultured gut microorganisms[21,24] (see also the Human Microbiome Project)[25] and to assemble complete or nearly complete metagenome-assembled genomes (MAGs) from shotgun metagenomes[26–28] are improving our ability to identify bacterial taxa of interest, down to the strain level. The combination of these advances allows us to begin designing meaningful, synthetic gut microbial communities and study those communities in vitro.

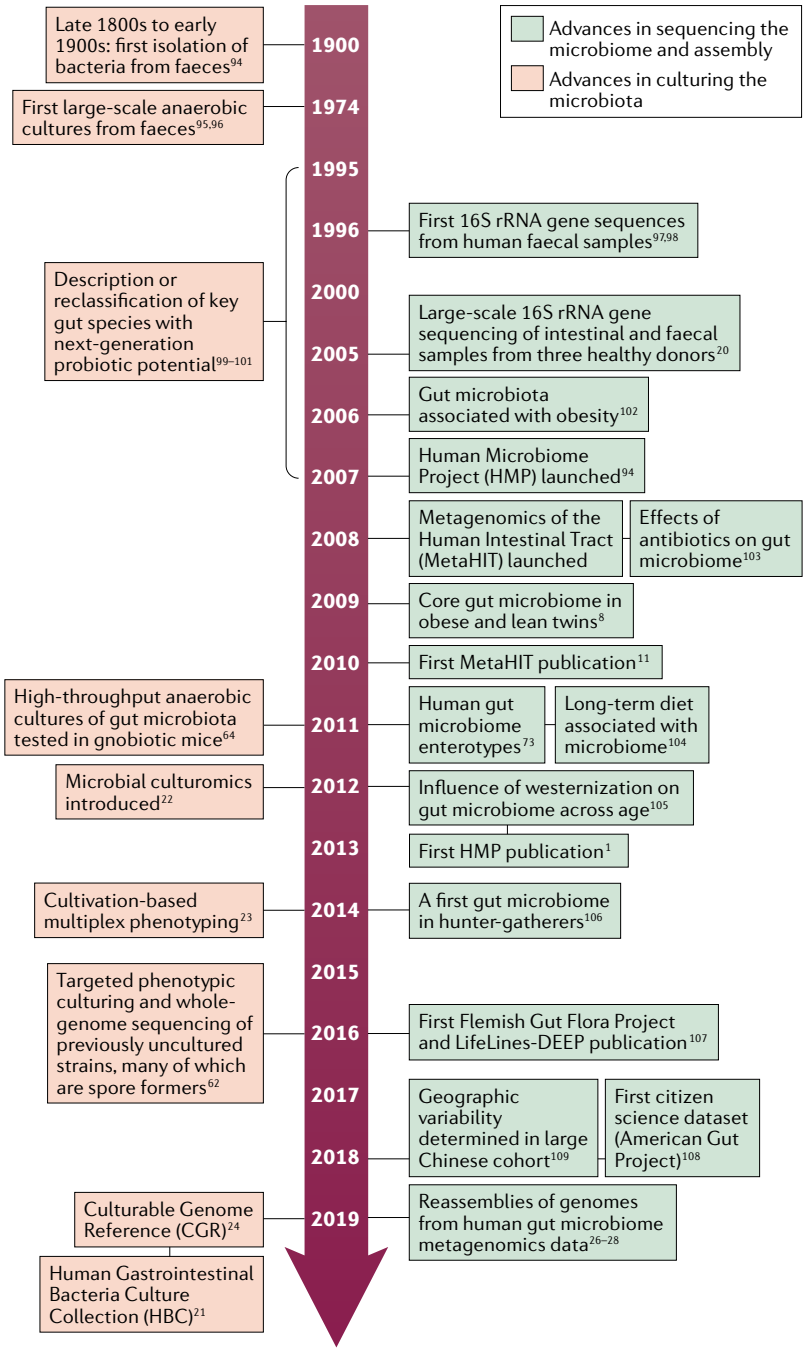


Fig. 1 | **Timeline of select highlights in the sequencing and culturing of the human gut microbiota.** Sequencing has provided important insights into the diversity of the human gut microbiome and its associations with health and disease (right). In parallel, advances in culturing have increased the number of cultured isolates from the gut microbiota (left)[94–109].

To date and to the best of our knowledge, there have been only a handful of studies of in vitro synthetic gut microbial communities (with at least two bacterial strains), with most of the early studies exploring the inhibitory effect of bifidobacterial strains on other gut commensals or pathogens[29–31]. Many of these in vitro studies provided some of the first insights on nutritional interactions[32] between certain gut bacterial taxa. However, we still know very little. If all the strains in the different in vitro studies mentioned above are counted independently, the interactions of <100 different gut microbial strains have been studied experimentally (FIG. 2). This number is only a very small fraction of the number of cultured gut microorganisms (~5%), and it pales in comparison to the number of gut microorganisms that have been detected in amplicon-based metagenomic studies (~0.002%). Currently, we are poised to begin filling the gaps between the numbers of sequenced, of cultured and of in vitro-tested members of the gut microbiota, but the challenge is substantial.

In this Review, we provide an overview of (i) the available in vitro experimental systems, (ii) current isolation methods and (iii) mathematical modelling options to study synthetic gut microbial communities. With a synthetic ecology approach, we can gain a deeper understanding of the black box of microbial interactions in the gut ecosystem. First, we can identify the functional niche and capacity of every isolated gut inhabitant, in a wide range of environmental conditions. Next, we can begin to disentangle the interactions between major gut inhabitants. Lastly, we can begin to study the bottom-up and top-down ecology of these synthetic gut microbial ecosystems through targeted perturbations of nutrients, small molecules, specific antibiotics, organisms (including genetically modified microorganisms) and/or metabolite tracking using, for example, stable isotope probing[33], to identify ecological tipping points[34]. Such knowledge could then be used to improve our understanding of the complex gut ecosystem and how we can modulate it in order to prevent and treat different diseases and conditions associated with dysbiosis.

## In vitro systems

In vitro systems are defined and/or engineered environments outside of a living organism that provide a simple, controlled system to study ecological interactions. Although in vitro experimentation has many disadvantages, in particular the lack of host interactions, the ability to automate and miniaturize environments for high-throughput analyses makes in vitro studies an excellent starting point prior to in vivo experimentation[35]. For the human gut, several in vitro environments have been defined and engineered to study synthetic microbial communities (FIG. 3). The most relevant

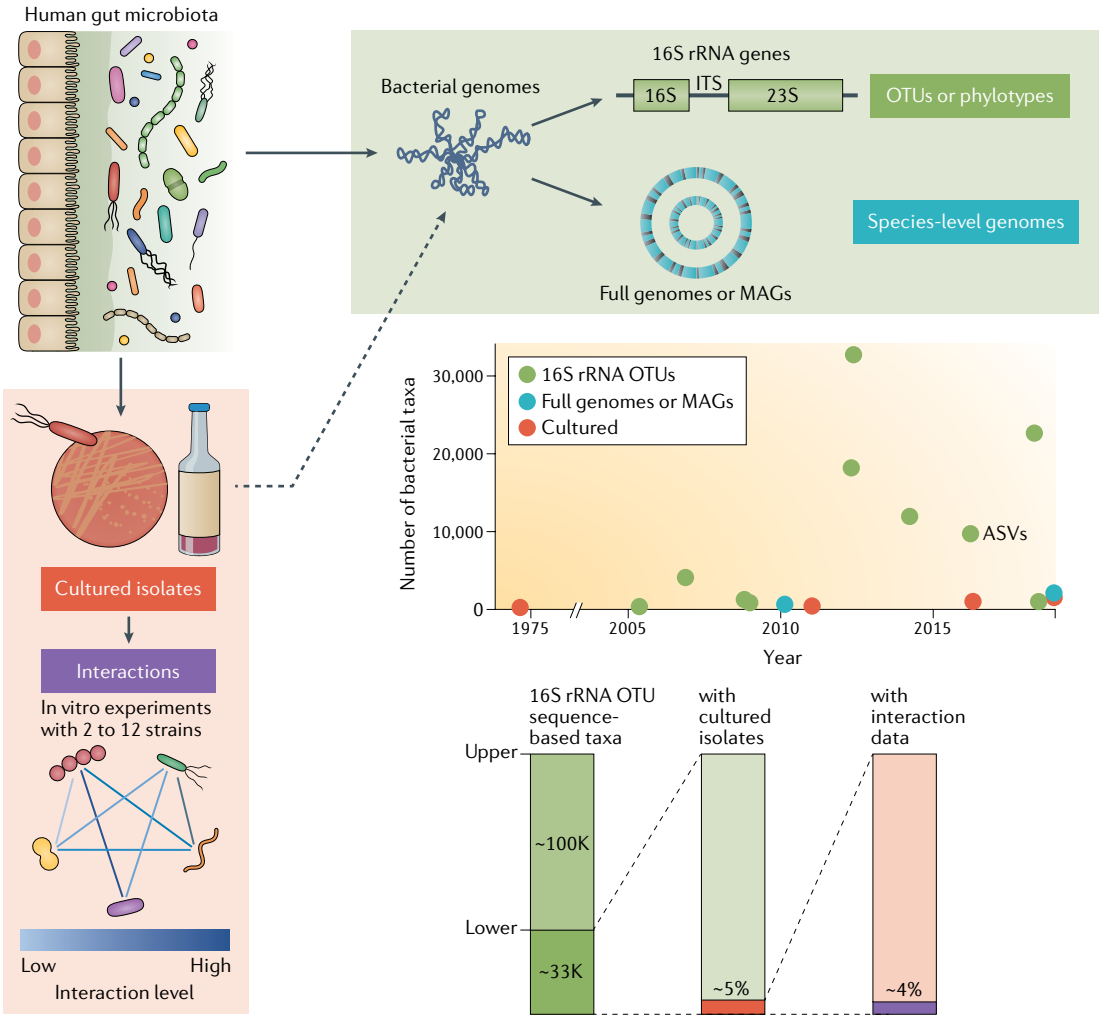


Fig. 2 | **Gaps between sequenced, cultured and experimentally verified interacting bacterial species in the human gut microbiota.** Many more members of the gut microbiota have been sequenced than cultured, and even fewer of the cultured species have then been used to study interactions in vitro. The scatterplot shows the published number of 16S ribosomal RNA (rRNA) 97% operational taxonomic units (OTUs) or phylotypes, species-level metagenome-assembled genomes (MAGs) or full genomes, and cultured bacterial species in selected studies reported in FIG. 1 (REFS[1,8,11,20,24,26,62,64,96,102,103,105,106,108,109,114]). A study that used a different 16S rRNA classification level (amplicon sequence variants (ASVs)) is identified in the plot. Furthermore, studies that combined sequencing efforts across multiple human body sites were excluded from the plot. The bar plots show the number of 16S rRNA OTU sequence-based species, the percentage of the sequence-based species with cultured isolates, and the percentage of bacterial species with known species–species in vitro interaction experiments[29,38,43,110–117]. The sequence-based species stacked bar plot shows the lower range at 33,888 bacterial species, which is the largest number of deduplicated OTUs found in REF.[1]; the upper range is the combined, not-deduplicated number of all OTUs across all studies included in the figure. The percentage bar plots show the percentage of the lower-range sequence-based species with cultured isolates and the percentage of cultured isolates with interaction data, respectively. ITS, internal transcribed spacer.

Resilience
The ability of a system to recover from disturbance.

Metagenome-assembled genomes
(MAGs). Genomes assembled using sequencing data from environmental samples.

Tipping points
Thresholds at which, if conditions are changed beyond that level, the system suddenly switches to a different state.

Operational taxonomic units
(OTUs). Operational definitions used to classify and group closely related organisms.

Phylotypes
Groups of phylogenetically related organisms.

systems range from the classical batch fermenter with various modifications, all the way to multistaged continuous fermenters with or without the addition of components to simulate interactions with the human host (for example, through the addition of human cells to otherwise standard microbial cultivation systems)[36].

***Batch fermentation.*** Batch fermentations are the simplest and most commonly used in vitro culture models. Batch fermentation experiments are closed systems, with all components necessary to initiate microbial growth included in a controlled environment at the beginning of an experiment. Two commonly used deviations from this closed setup, however, include the possibility of pH correction and the gradual addition of a key nutrient (also known as fed-batch). After addition of the bacterial strain or mixture of strains, the system can be monitored for overall growth and growth by strain. Overall growth is often monitored by conventional methods, such as plate counts, optical density measurements or flow cytometry. In mixed cultures, individual strain growth has also been followed using fluorescent markers, but the number of fluorescent markers previously greatly limited the number of strains that could be

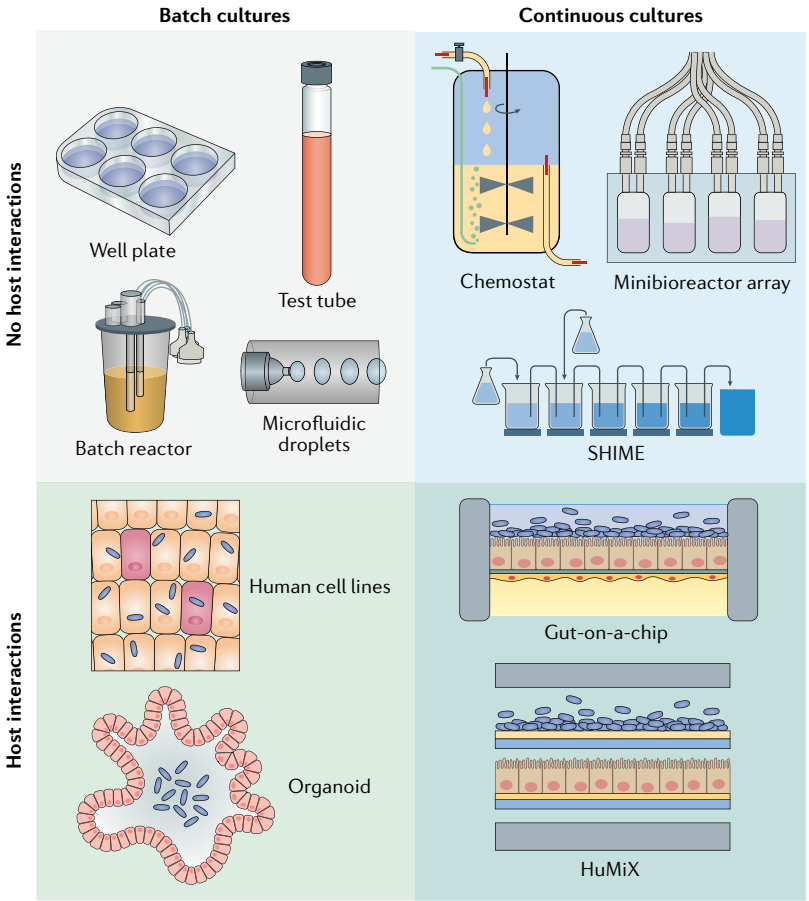


Fig. 3 | **In vitro culture systems for batch and continuous culture of gut microbiota.** The current in vitro systems are either batch cultures, without or with host interactions, or continuous cultures, also without or with host interactions. The top two panels show the seven most common systems lacking host interactions that have been used to study the gut microbiota. The bottom panels display the four current systems that have been developed to simulate gut human–microbiota interactions. HuMiX, human–microbial crosstalk; SHIME, Simulator of the Human Intestinal Microbial Ecosystem.

grown together[37–40]. More recently, periodic sampling of the system and high-throughput amplicon or shotgun sequencing has greatly increased the number of strains and the complexity of mixed cultures that can be used, as a limitation is no longer imposed by the number of fluorescent markers[37–40]. The simplicity of set-up of batch fermentations makes them the easiest in vitro culture systems to parallelize, enabling high-throughput analyses. Nonetheless, a major limitation is that, because batch fermentations are closed systems, it is impossible to quantify stable states. Examples of standard batch fermentation systems include batch reactors, test tubes, well plates and microfluidic droplets (FIG. 3, top left).

***Continuous culture systems.*** Continuous culture systems, unlike batch fermentations, enable counterbalancing of the inflow and outflow of a medium and, thus, the maintenance of cultures in a specific growth rate and physiological state. By physically coupling several continuous reactors in sequence through overflow weirs, several different environmental circumstances can be imposed sequentially. For example, alterations to pH or residence time in the medium, as well as the addition of particular components (such as enzymes and bile salts), can be used to simulate conditions in different compartments of the intestinal tract[36]. However, continuous-culture models inoculated with human faecal samples have not been able to reproduce the exact in vivo community compositions or microbial community states observed in the human gut[36], although progress is being made by immobilizing faecal microbiota on gel beads[41]. A limitation is that, although many of the kinetic aspects (that is, transit time characteristics) of the human gastrointestinal tract are incorporated (with the notable exception of microbial metabolite absorption by the human gut), the presence of the human host is largely ignored, with certain partial exceptions such as including human cell lines or mucus-coated beads[42]. Nonetheless, continuous-culture models are a rapid screening tool for xenobiotic transformations and the impact of nutraceuticals[36]. Examples of standard continuous-culture systems include chemostats and minibioreactor arrays (FIG. 3, top right).

***Microtiter well plate cultures.*** A microtiter well plate format is a specific type of batch fermentation system that offers a cheap, high-throughput way to study multiple different microbial interactions in parallel. Using this format for mixed cultures has previously been shown to be a valuable tool when predicting community dynamics in the gut from pairwise interactions[43]. However, due to the small volume used in most microtiter well-plate experimental culture models, it is more difficult to control environmental conditions (such as pH and nutrient addition) than in a full-scale fermenter. Furthermore, there are limitations on the amount of sampling possible with such systems. Microtiter well plates can potentially be used as continuous-culture systems by performing serial transfers[44], but it is unclear whether the community dynamics monitored in such a setting would be entirely free of the influence of fluctuating nutrient concentrations. These problems might be mitigated in more elaborate systems in the future (and potentially in a high-throughput fashion, thanks to advances in microfluidics).

***Advances and challenges.*** Importantly, across all types of in vitro culture systems, no truly representative system exactly mimicking the in vivo microbiota has yet been developed. Steps in this direction have been achieved by the gut-on-a-chip model[44,45], the HuMiX system[46], the mini-bioreactor system for growth screening[47] and the combinatorial growth of strains in microtiter plates, although mostly with the aim of following growth through optical density and 16S ribosomal RNA (rRNA) sequencing, with the purpose of mathematical model parameterization[43]. Although efforts are ongoing[46], an important challenge still lies in further miniaturizing these systems and increasing their high-throughput analyses capacity. Another challenge is the integration of spatial structure. The microbial compositions of communities in the gut lumen and mucosa are known to differ[48]. The latter, which is in closer contact with host cells, may have a particularly important role in disease development[49]. The incorporation of human cell lines in high-throughput fermentation systems — for example, through a layer of differentiated

Xenobiotic transformations
Biological transformation of the chemical structure of a molecule normally absent from the microbial ecosystem, such as pharmaceuticals, pesticides and so forth.

Nutraceuticals
Nutrients that, besides macronutrient components, also have pharmaceutical properties.

Caco-2 cells, such as in the HuMiX system[46] — enables the investigation of host cellular responses to particular gut microbial strains or their mixtures, as well as the reverse interactions (FIG. 3, bottom).

Overall, given the large number of gut microbiota members that still need to be assessed, currently the most viable option to start uncovering this complexity is controlled and repeatable in vitro systems. Still, it is worth mentioning that the use of living systems as the 'container' (that is, in vivo experiments) for microbial trials is also needed, and most likely would be the next step following in vitro experimentation. In vivo experiments are crucial for studying many aspects of ecological interactions, ranging from exploring the role of the microbiota in host development and immune responses to understanding the complex interactions between host dietary or nutritional choices and the gut ecosystem. Most of the current in vivo gut microbiota studies have used mice[50,51], but many host-specific differences between mice and humans exist and should not be ignored. These differences include immunological differences (for example, type of antimicrobial peptides), which select a different microbiota in mice than in humans[52,53]; transit time; type of food and propensity for coprophagy; and macroscopic and microscopic structure of the gut (for example, length and relative transit time, or the presence of a functional caecum)[54]. Thus, current in vivo experiments are also still limited in their capacity to directly predict microbial behaviour in the human gastrointestinal tract. However, given the obvious unavailability of gnotobiotic humans, the use of mice or functionally more equivalent model animals (for example, the porcine model) remains our best available in vivo option for certain research lines. This said, work on incorporating human cell lines in model systems[55] and organoids[56] may fill this gap in the near future, but currently all these systems are in the developmental phase.

## Isolating the human gut microbiota

After the field almost completely abandoned microbial isolation in favour of the depth and throughput offered by DNA-based approaches, one of the ironic conclusions of evaluating one and a half decades of metagenomics-based research is that microbiologists need cultures after all. For most of the challenges as well as for the synthetic ecology solutions outlined above, the availability of well-documented strain collections is a crucial prerequisite. In addition to the anaerobic reference cultures preserved and catalogued by public microbial resource centres such as the German Collection of Microorganisms and Cell Cultures (DSMZ) and the Culture Collection University of Gothenburg (CCUG), recent years have also witnessed host-specific culture repository initiatives such as the Mouse Intestinal Bacterial Collection (miBC)[57], the Human Gastrointestinal Bacteria Culture Collection (HBC)[21] and the Culturable Genome Reference (CGR)[44] catalogue from human faecal samples (FIG. 2). Still, coverage of well-characterized gut species in these culture collections is low compared to what is predicted from metagenomic inventories. The recently reported reconstruction of >150,000 microbial genomes from >9,400 geographically widespread human metagenomes, encompassing intestinal, oral, skin and vaginal body sites, indicated that 77% of the resulting species-level genome bins have never been described before[28]. This highlights the continuing need for improving existing isolation methods or for repurposing cell-capturing technologies such as droplet microfluidics from other fields.

Increased insight into the reasons why a large fraction of gut microorganisms resist cultivation under standard laboratory conditions has triggered a series of simple but effective modifications to conventional isolation strategies[58]. The most obvious of these modifications stems from the observation that the high phylogenetic diversity of the human gut microbiota is mirrored by myriad metabolic functions and nutritional preferences. By multiplexing bacterial isolation conditions through the serial addition of specific growth promoters and/or inhibitors, coupled to high-throughput identification with MALDI-TOF MS, the so-called culturomics approach has broadened the scope of conventional single-medium strategies[12]. According to a recent report[59], culturomics has resulted in the discovery of 232 novel human gut species since 2015. It is worth noting, however, that only a small minority (~1–2%) of these newly proposed names have so far been validated and thus have official standing in prokaryotic nomenclature[60], which may compromise their future inclusion in strictly curated taxonomic reference frameworks and prevent effective progress in the annotation of metagenomic reads. Surprisingly, recent work has challenged the need for a wide range of media; a survey[15] on the growth preferences of 96 phylogenetically and metabolically diverse human gut strains representing core microbiome species, species used as probiotics and enteropathogens demonstrated that 76 strains (representing 79.2% of all tested strains) were able to grow on a single, defined medium. Although this observation does not guarantee comparable recovery rates during isolation from faecal samples, it suggests that laboratories that are not able to roll out a dedicated culturomics pipeline but that do have basic anaerobic incubation infrastructure can also contribute to the discovery of new gut bacteria, although less efficiently. Next to multiplexing nutritional resources, the supplementation of isolation media with (combinations of) antimicrobials has been successfully used as a simple modification of traditional culturing approaches for the targeted selection of previously uncultured bacteria[23]. In this study, antibiotics capable of inhibiting or suppressing the growth of abundant taxa could select for less abundant populations, provided that these populations can grow in the presence of the antibiotic. Likely, this particular type of modification could be extended to non-antibiotic drugs, given the recent observation that some classes of human drugs such as antipsychotics may also exhibit anticommensal activities and thus selectively inhibit the growth of specific members of the gut microbiota[61]. Even prior to the actual isolation stage, simple modifications such as the treatment of faecal samples with ethanol, which kills ethanol-sensitive vegetative cells but does not affect spores, have been shown to unlock novel bacterial diversity, including potentially new families[62].

An additional opportunity to scale up the isolation and culture collection building of human gut

Gnotobiotic
Without microbial commensals.

Organoids
Tissue-culture-generated structures from certain human cell types, in this paper to be understood as differentiated gut tissue.

Core microbiome
The collection of gut organisms common to the majority of subjects in a given population.

Dilution-to-extinction
Serial dilution of a microbial sample to extremely low densities that allow the isolation of single cells.

microorganisms comes from the implementation of miniaturized, versatile and high-throughput technologies, most of which were initially developed for other purposes in molecular and cell biology laboratories. Especially, single-cell-based approaches in which physical isolation or sorting precedes the actual growth stage seem promising[63]. As such, a personalized gut microbiota culture collection study[64] demonstrated that the principle of dilution-to-extinction is an interesting approach to capture slow-growing and low-abundance gut microorganisms that might escape isolation during traditional plating. However, to reach the scale and depth of metagenomics, future isolation efforts will require integration in automated platforms and dedicated pipelines. Recent advances in the analysis and separation of free microbial cells by Raman microspectroscopy[65] as well as in the physical stochastic confinement of individual cells in nanofabricated and microfabricated compartments and microfluidic devices[66] are two prime examples that may pave the way towards the isolation of previously uncultured species and the discovery of novel microbial taxa[63], as well as being a high-throughput method for inferring interactions[67]. Still, the often-challenging integration of such single-cell tools in strictly anaerobic laboratory systems has strongly limited the number of successful culture-based applications in the human microbiota field.

## Mathematical modelling

Next to experimental in vitro work, mathematical modelling can provide further system-level understanding and generate hypotheses. Models are mathematical representations of our knowledge of a system. A mathematical model can describe the behaviour of the gut community in altered conditions, and can therefore be applied to predicting the effects of perturbations such as antibiotic treatment[68] or to optimizing therapeutic microbiota for functions such as pathogen suppression[69] or immune system modulation[70]. Models also pinpoint knowledge gaps: a deviation of experimental observations from model expectations implies a lack of understanding, necessitating further experiments and adjustments. An example is the genome-scale metabolic model of a microorganism. If the model fails to describe an observed physiology (for example, growth on formate), this may point to incomplete or erroneous gene annotations[71]. In addition, models enable exploring ecosystem properties that are hard to gauge experimentally. For example, a mathematical analysis of interaction networks has linked community stability to the proportion of negative interactions such as competition[72]. Finally, models can suggest the mechanisms underlying observations. Alternative gut community types[73,74] are a good case in point: model simulations have suggested multistability[75,76], priority effects (the exclusion of competitors by species arriving first)[77] or differences in water uptake and nutrient influx in the colon[78] as possible drivers of different community compositions. Another example is a spatial colon model that explains the high diversity in the colon through a combination of cross-feeding and spatial stratification[79].

Microbial communities can be modelled at different levels of resolution, ranging from metabolic models that take the internal metabolism of every community member into account, to topological models (that is, networks) that represent community members as nodes and their interactions as directed or undirected edges[80–82] (see the overview in TABLE 1). Modelling approaches differ in their ability to account for the factors that shape community dynamics; for example, the generalized Lotka–Volterra (gLV) model, which describes community dynamics as a function of growth rates and species interactions, cannot predict the impact of spatial structure on the dynamics. Modelling approaches also differ in their treatment of causality: metabolic and kinetic models describe the mechanism underlying metabolite-mediated interactions such as cross-feeding, whereas the gLV model relies on generic interaction coefficients[83,84]. In vitro experiments are required in order to benchmark and compare different modelling approaches, to find an optimal compromise between prediction accuracy and the number of parameters.

Fitting the neutral model, which neglects species interactions, and the gLV model to faecal sequencing data led to the conclusion that gut microbial community dynamics are not neutral, but can be described by the gLV model to some extent[68,69,85]. Parameterizing the gLV model enables the inference of ecological interactions directly from sequencing data, but this technique faces substantial challenges. For example, interaction inference requires densely sampled time series data and is, for the moment, limited to the most abundant species[86]. Alternatively, ecological interactions can be obtained from metabolic models by comparing growth rates in monocultures and co-cultures in silico[87]; however, the accuracy of these predicted interactions has not yet been assessed systematically. Following this approach, the anoxic gut environment was predicted to induce mutualism[88,89]. Metabolic models require metabolic reconstructions, which can be obtained automatically from the genome within minutes[90,91], but take several months of curation effort to refine[71]. The AGORA database offers semi-curated metabolic reconstructions for 773 gut bacteria[92]. Given the challenges of obtaining an accurate metabolic reconstruction, experimental validation with growth data collected for a range of substrates is essential.

When applying community models to the human gut microbiota in vivo, a number of challenges have to be overcome. Hundreds of species live in the human gut[28], each of which has a specific pH optimum, particular consumption and production kinetics for a range of metabolites, and specific interactions with other gut microorganisms and the human host. Spatial structure, such as the mucus layer and food particles as well as gradients, influences community dynamics. In addition, the gut ecosystem is not static, but varies over time as conditions change and strains immigrate, are lost or evolve. However, a model has to reduce system complexity in order to be useful. In vitro experiments help pinpoint the most relevant processes while controlling confounding factors and enable systematically measuring model parameters. For example, measured growth rates of gut bacteria were used to test and subsequently to refine metabolic reconstructions[15]. The large species number in the human gut is challenging

Table 1 | **Selected mathematical modelling approaches applied to the human gut microbiota**

| Modelling approach[a] | Summary | Requirements | Assumptions and limitations | Strengths | Remarks and references |
|---|---|---|---|---|---|
| Metabolic models | The flow of metabolites through each reaction in each species is determined such that an objective function (for example, biomass production) is optimized | Metabolic reconstruction for each community member | Metabolism is assumed to be at steady state. The objective function is difficult to define. High-quality metabolic reconstruction requires a large percentage of annotated genes and labour-intensive curation | Metabolite-mediated interaction mechanisms are modelled at the enzymatic level. Medium composition is taken into account. Meta-omics data can be integrated | By default the model is static, but dynamic extensions exist[118] |
| Kinetic models | The change of species abundances and concentrations of key metabolites over time is described | Production and consumption rates for key metabolites, maximal specific growth rates, Monod (half-saturation) constants | The model assumes that growth is limited only by the substrates considered in the growth function and that model parameters do not change | Metabolite-mediated interaction mechanisms are taken into account | Model can be extended to work with groups of species[83] |
| Generalized Lotka–Volterra model | The change of species abundances over time is described at the level of populations | Growth rates and interaction matrix | The model assumes that higher-order interactions (involving more than two species) do not matter. The effect of interaction partners is assumed to scale linearly with their abundance (no saturation) and to be additive. Interaction strengths are assumed to be constant | This model requires comparatively few parameters and no curation | Interaction mechanism is not modelled explicitly. Model can be extended to integrate environmental effects[119] |
| Neutral model | The change of species abundances over time is described at the level of individuals | Number of individuals in the local and in the metacommunity, immigration rate, death rate, speciation rate | Growth rates do not differ between species. Interactions do not affect community dynamics | The neutral model can be used as a null model to test the importance of interactions | Model can be extended to cover different spatial structures[120] |
| Network (topological model) | Species are represented as nodes, and their interactions as directed or undirected edges | Ecological interactions between species, and optionally their strengths | The model is static. Interactions and their strengths are assumed not to change | The model is parameter-free | More validation will be required in order to learn the extent to which ecological networks capture the properties of real ecosystems[121,122] |

[a]Overview of microbial community modelling approaches mentioned in the main text. The list is not comprehensive; for a more systematic and detailed review of microbial community modelling approaches, see REF.[81].

not only because of the amount of work to characterize their physiology, but also because the number of all possible interactions scales exponentially with the species number. Many models assume that community dynamics can be described in terms of pair-wise interactions (which scale only quadratically with species number), and thus ignore interactions involving more than two species. Two different studies tested whether this simplifying assumption holds, by modelling observed community dynamics using parameters obtained from monocultures and co-cultures[38,43]. The studies both revealed that the dynamics of the community could be predicted from co-cultures, thereby ruling out a strong effect of higher-order interactions. Another study reduced species number further by grouping strains into guilds based on their functional role (for example, non-butyrate-forming fibre degraders) and was able to correctly predict the response of these guilds to pH change in continuous bioreactors seeded with faecal samples[83]. In vitro experiments and modelling strategies such as these are an important step towards predictive modelling in vivo.

## Future strategies

Synthetic gut microbial communities will improve our understanding of emergent properties, from bottom-up intercellular processes (such as cross-feeding, syntrophy and auxotrophy) to top-down community processes (such as community structure and resilience). So far, in vitro batch trial experiments with defined gut cultures (such as monocultures and co-cultures) have elucidated potential intercellular interactions and in vitro systems, allowing partial simulation of the conditions in the human gastrointestinal tract and yielding first insights into the full-ecosystem processes. Key to gaining a mechanistic understanding of the human gut ecosystem will be extensive, well-documented culture collections of human microbiota members used in in vitro ecology experiments feeding into and driven by mathematical models.

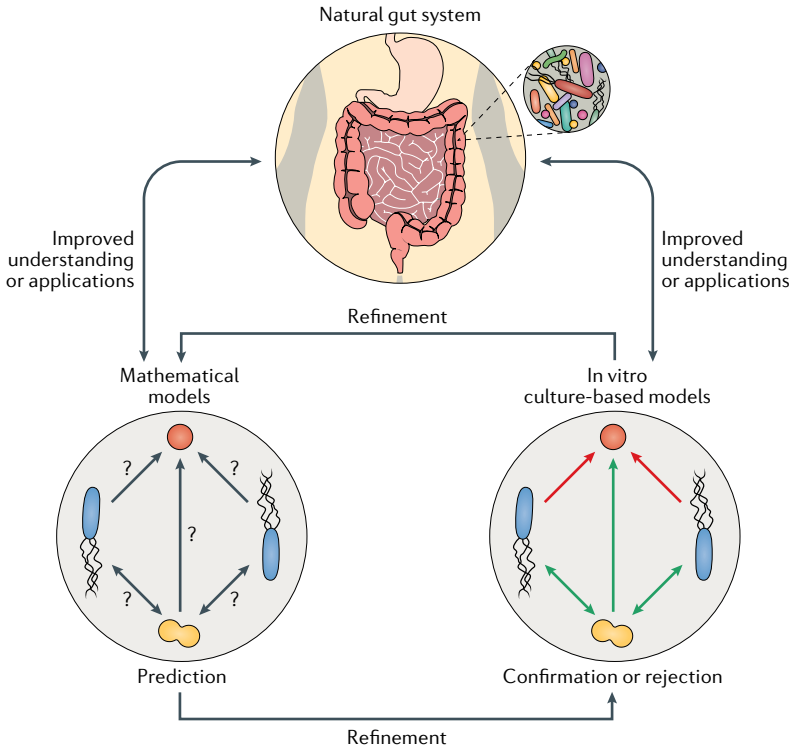


Fig. 4 | **Connections between different types of human gut microbiota models.** Through refinement, a continuous feedback loop between mathematic models and in vitro culture systems will enable both a better understanding of the human gut system and the development of applications.

Specifically, for the gut ecosystem, a priority will be to repurpose existing technologies to enable parallel anaerobic fermentation and allow high-throughput analyses. Many parallel bioreactors are coming on the market to meet this demand, but few have the option to add gut-specific functions, such as the addition of human cells. In this respect, co-development between biotechnology companies and academia, could be an option. Lastly, microfluidics also holds promise as a high-throughput method, but it is in very early development[67]. Given the potentially wide applications of anaerobic microbiota-oriented high-throughput technologies, ranging from health therapeutics to industrial bioreactors, innovation interest is likely to increase substantially in the coming years.

Once sufficient high-throughput capacity has been developed, we envision a consistent feedback loop between mathematical modelling and culturing, in which mathematical models guide culturing efforts through hypothesis generation, and culturing results feed back to generate new models (FIG. 4). The current mathematical models are grounded by current culture collections, which are biased by the source of the microbial isolates. Efforts to study the gut microbiota have mostly focused on faecal samples collected from Western individuals. To ensure that the global human microbiota diversity is properly captured and framed, however, worldwide sampling and quantitative profiling[93] of populations should be performed and coupled to metadata collection (for example, covering dietary records, immunological parameters and xenobiotic use), so that we can use these samples and sequencing data, respectively, to extend the known taxa and culture collections and to refine mathematical models. In addition to the impact of growing culture collections, mathematical models will continuously be refined through studies of ecological interactions, growth parameters, nutritional dependencies and other properties, and will be complemented by human and animal intervention and perturbation studies[14] that will further inform the models and enable better incorporation of the host context.

Cycling through this loop will lead to a deeper functional and systemic understanding of the gut ecosystem and guide the development of novel modulation strategies through, for example, synthetic probiotics and small molecules. Once modulation strategies have been properly evaluated and tested in vivo in animal models, human clinical trials will be the ultimate stepping stone towards pharmaceutical use.

In summary, the human gut is a complex ecosystem for which modulation strategies can be envisaged, but a mechanistic understanding is needed of the intricate interactions that drive its ecology. Given the vast array of metabolic activities and multiple points of interaction of the gut microbiota with the human host, the opportunities for health-promoting interventions are bountiful. The key steps towards the development of a functional model of the colon ecosystem that can be used for the design of therapeutic solutions appear all to be in place, but they have yet to be aligned and combined. Synthetic ecology holds promise as the road to a renaissance in human microbiota-based therapeutics for health and well-being.

Published online 2 October 2019


1. Huttenhower, C. et al. Structure, function and diversity of the healthy human microbiome. *Nature* **486**, 207–214 (2012).
2. Lloyd-Price, J., Abu-Ali, G. & Huttenhower, C. The healthy human microbiome. *Genome Med.* **8**, 51–62 (2016).
3. Gensollen, T., Iyer, S. S., Kasper, D. L. & Blumberg, R. S. How colonization by microbiota in early life shapes the immune system. *Science* **352**, 539–544 (2016).
4. Honda, K. & Littman, D. R. The microbiota in adaptive immune homeostasis and disease. *Nature* **535**, 75–84 (2016).
5. Bäckhed, F. et al. The gut microbiota as an environmental factor that regulates fat storage. *Proc. Natl Acad. Sci. USA* **101**, 15718–15723 (2004).
6. Ley, R. E. et al. Obesity alters gut microbial ecology. *Proc. Natl Acad. Sci. USA* **102**, 11070–11075 (2005).
7. Ridaura, V. K. et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science* **341**, 1241214 (2013).
8. Turnbaugh, P. J. et al. A core gut microbiome in obese and lean twins. *Nature* **457**, 480–484 (2009).
9. Karlsson, F. H. et al. Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature* **498**, 99–103 (2013).
10. Larsen, N. et al. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLOS ONE* **5**, e9085 (2010).
11. Qin, J. et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* **464**, 59–65 (2010).
12. Tilg, H. & Moschen, A. R. Microbiota and diabetes: an evolving relationship. *Gut* **63**, 1513–1521 (2014).
13. Cani, P. D. Human gut microbiome: hopes, threats and promises. *Gut* **67**, 1716–1725 (2018).
14. Schmidt, T. S. B., Raes, J. & Bork, P. The human gut microbiome: from association to modulation. *Cell* **172**, 1198–1215 (2018).
15. Tramontano, M. et al. Nutritional preferences of human gut bacteria reveal their metabolic idiosyncrasies. *Nat. Microbiol.* **3**, 514–522 (2018). **This study applied high-throughput techniques to study the growth characteristics in 96 gut species.**
16. Lynch, M. D. J. & Neufeld, J. D. Ecology and exploration of the rare biosphere. *Nat. Rev. Microbiol.* **13**, 217–229 (2015).
17. De Roy, K., Marzorati, M., Van den Abbeele, P., Van de Wiele, T. & Boon, N. Synthetic microbial ecosystems: an exciting tool to understand and apply microbial communities. *Environ. Microbiol.* **16**, 1472–1481 (2014).
18. Großkopf, T. & Soyer, O. S. Synthetic microbial communities. *Curr. Opin. Microbiol.* **18**, 72–77 (2014).

19. Johns, N. I., Blazejewski, T., Gomes, A. L. C. & Wang, H. H. Principles for designing synthetic microbial communities. *Curr. Opin. Microbiol.* **31**, 146–153 (2016).
20. Eckburg, P. B. et al. Diversity of the human intestinal microbial flora. *Science* **308**, 1635–1638 (2005).
**This is the first paper to quantitatively predict the number of gut microbial species missing from culture collections**.
21. Forster, S. C. et al. A human gut bacterial genome and culture collection for improved metagenomic analyses. *Nat. Biotechnol.* **37**, 186–192 (2019).
**This report provides an introduction to the science, practice and applications of phenotypically multiplexed isolation of human gut bacteria**.
22. Lagier, J. C. et al. Microbial culturomics: paradigm shift in the human gut microbiome study. *Clin. Microbiol. Infect.* **18**, 1185–1193 (2012).
**This paper provides an introduction to the science, practice and applications of high-throughput isolation of human gut bacteria**.
23. Rettedal, E. A., Gumpert, H. & Sommer, M. O. A. Cultivation-based multiplex phenotyping of human gut microbiota allows targeted recovery of previously uncultured bacteria. *Nat. Commun.* **5**, 4714 (2014).
24. Zou, Y. et al. 1,520 reference genomes from cultivated human gut bacteria enable functional microbiome analyses. *Nat. Biotechnol.* **37**, 179–185 (2019).
25. NIH Human Microbiome Project. View dataset. *HMPDACC* https://www.hmpdacc.org/hmp/catalog/grid.php?dataset=genomic (2019).
26. Almeida, A. et al. A new genomic blueprint of the human gut microbiota. *Nature* **568**, 499–504 (2019).
27. Nayfach, S., Shi, Z. J., Seshadri, R., Pollard, K. S. & Kyrpides, N. C. New insights from uncultivated genomes of the global human gut microbiome. *Nature* **568**, 505–510 (2019).
28. Pasolli, E. et al. Extensive unexplored human microbiome diversity revealed by over 150,000 genomes from metagenomes spanning age, geography, and lifestyle. *Cell* **176**, 649–662.e20 (2019).
**Pasolli et al. contribute an impressive addition to our genome-level knowledge of human gut inhabitants based on innovative analysis of existing data**.
29. Gibson, G. R. & Wang, X. Regulatory effects of bifidobacteria on the growth of other colonic bacteria. *J. Appl. Bacteriol.* **77**, 412–420 (1994).
30. Lievin, V. et al. Bifidobacterium strains from resident infant human gastrointestinal microflora exert antimicrobial activity. *Gut* **47**, 646–652 (2000).
31. Touré, R., Kheadr, E., Lacroix, C., Moroni, O. & Fliss, I. Production of antibacterial substances by bifidobacterial isolates from infant stool active against *Listeria monocytogenes*. *J. Appl. Microbiol.* **95**, 1058–1069 (2003).
32. Louis, P., Hold, G. L. & Flint, H. J. The gut microbiota, bacterial metabolites and colorectal cancer. *Nat. Rev. Microbiol.* **12**, 661–672 (2014).
33. Berry, D. & Loy, A. Stable-isotope probing of human and animal microbiome function. *Trends Microbiol.* **26**, 999–1007 (2018).
34. Lahti, L., Salojärvi, J., Salonen, A., Scheffer, M. & De Vos, W. M. Tipping elements in the human intestinal ecosystem. *Nat. Commun.* **5**, 4344 (2014).
35. Saeidnia, S., Manayi, A. & Abdollahi, M. From in vitro E experiments to in vivo and clinical studies; pros and cons. *Curr. Drug Discov. Technol.* **12**, 218–224 (2016).
36. Wilmes, P., Marta, C. & Tom, Vd. W. Resolving host–microbe interactions in the gut: the promise of in vitro models to complement in vivo research. *Curr. Opin. Microbiol.* **24**, 28–33 (2018).
37. Crost, E. H. et al. Mechanistic insights into the cross-feeding of *Ruminococcus gnavus* and *Ruminococcus bromii* on host and dietary carbohydrates. *Front. Microbiol.* **9**, 2558 (2018).
38. D'Hoe, K. et al. Integrated culturing, modeling and transcriptomics uncovers complex interactions and emergent behavior in a three-species synthetic gut community. *eLife* **7**, e37090 (2018).
39. Falony, G. et al. In vitro kinetic analysis of fermentation of prebiotic inulin-type fructans by *Bifidobacterium* species reveals four different phenotypes. *Appl. Environ. Microbiol.* **75**, 454–461 (2009).
40. Rivière, A., Selak, M., Geirnaert, A., Van den Abbeele, P. & De Vuyst, L. Complementary mechanisms for degradation of inulin-type fructans and arabinoxylan oligosaccharides among bifidobacterial strains suggest bacterial cooperation. *Appl. Environ. Microbiol.* **84**, e02893-17 (2018).
41. Berner, A. Z. et al. Novel polyfermentor intestinal model (PolyFermS) for controlled ecological studies: validation and effect of pH. *PLOS ONE* **8**, e77772 (2013).
42. Van den Abbeele, P. et al. Incorporating a mucosal environment in a dynamic gut model results in a more representative colonization by lactobacilli. *Microb. Biotechnol.* **5**, 106–115 (2012).
43. Venturelli, O. S. et al. Deciphering microbial interactions in synthetic human gut microbiome communities. *Mol. Syst. Biol.* **14**, e8157–e8157 (2018).
**The authors successfully predict the in vitro dynamics of a 12-species gut community from mono-cultures and co-cultures, thereby illustrating that higher-order interactions do not dominate the dynamics**.
44. Kim, H. J., Li, H., Collins, J. J. & Ingber, D. E. Contributions of microbiome and mechanical deformation to intestinal bacterial overgrowth and inflammation in a human gut-on-a-chip. *Proc. Natl Acad. Sci. USA* **113**, E7–E15 (2016).
45. Kim, H. J., Huh, D., Hamilton, G. & Ingber, D. E. Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow. *Lab Chip* **12**, 2165–2174 (2012).
46. Shah, P. et al. A microfluidics-based in vitro model of the gastrointestinal human–microbe interface. *Nat. Commun.* **7**, 11535 (2016).
47. Marzorati, M. & Van De Wiele, T. An advanced in vitro technology platform to study the mechanism of action of prebiotics and probiotics in the gastrointestinal tract. *J. Clin. Gastroenterol.* **2015**, S124–S125 (2016).
48. Zoetendal, E. G. et al. Mucosa-associated bacteria in the human gastrointestinal tract are uniformly distributed along the colon and differ from the community recovered from feces. *Appl. Environ. Microbiol.* **68**, 3401–3407 (2002).
49. Tytgat, H. L. P., Nobrega, F. L., van der Oost, J. & de Vos, W. M. Bowel biofilms: tipping points between a healthy and compromised gut? *Trends Microbiol.* **27**, 17–25 (2019).
**This study gives an excellent introduction to gut biofilms and their role in pathology**.
50. Elzinga, J., van der Oost, J., de Vos, W. M. & Smidt, H. The use of defined microbial communities to model host–microbe interactions in the human gut. *Microbiol. Mol. Biol. Rev.* **83**, e00054-18 (2019).
51. Schaedler, R. W., Dubos, R. & Costello, R. The development of the bacterial flora in the gastrointestinal tract of mice. *J. Exp. Med.* **122**, 59–66 (1965).
**This classic study describes one of the first model artificial communities**.
52. Byndloss, M. X., Pernitzsch, S. R. & Bäumler, A. J. Healthy hosts rule within: ecological forces shaping the gut microbiota. *Mucosal Immunol.* **11**, 1299–1305 (2018).
**This report provides a clear overview of the role of the human host in controlling the human gut microbiota; especially of interest is the role of anaerobiosis and its failure in a variety of gut afflictions**.
53. Salzman, N. H. et al. Enteric defensins are essential regulators of intestinal microbial ecology. *Nat. Immunol.* **11**, 76–83 (2010).
54. Nguyen, T. L. A., Vieira-Silva, S., Liston, A. & Raes, J. How informative is the mouse for human gut microbiota research? *Dis. Model. Mech.* **8**, 1–16 (2015).
55. von Martels, J. Z. H. et al. The role of gut microbiota in health and disease: in vitro modeling of host–microbe interactions at the aerobe–anaerobe interphase of the human gut. *Anaerobe* **44**, 3–12 (2017).
56. Fair, K. L., Colquhoun, J. & Hannan, N. R. F. Intestinal organoids for modelling intestinal development and disease. *Phil. Trans. R. Soc. B* **373**, 20170217 (2018).
57. Lagkouvardos, I. et al. The Mouse Intestinal Bacterial Collection (miBC) provides host-specific insight into cultured diversity and functional potential of the gut microbiota. *Nat. Microbiol.* **1**, 16131 (2016).
58. Sommer, M. O. A. Advancing gut microbiome research using cultivation. *Curr. Opin. Microbiol.* **27**, 127–132 (2015).
59. Bilen, M. et al. The contribution of culturomics to the repertoire of isolated human bacterial and archaeal species. *Microbiome* **6**, 94 (2018).
60. Oren, A., Garrity, G. M. & Parte, A. C. Why are so many effectively published names of prokaryotic taxa never validated? *Int. J. Syst. Evol. Microbiol.* **68**, 2125–2129 (2018).
61. Maier, L. et al. Extensive impact of non-antibiotic drugs on human gut bacteria. *Nature* **555**, 623–628 (2018).
62. Browne, H. P. et al. Culturing of 'unculturable' human microbiota reveals novel taxa and extensive sporulation. *Nature* **533**, 543–546 (2016).
63. Huys, G. R. B. & Raes, J. Go with the flow or solitary confinement: a look inside the single-cell toolbox for isolation of rare and uncultured microbes. *Curr. Opin. Microbiol.* **44**, 1–8 (2018).
64. Goodman, A. L. et al. Extensive personal human gut microbiota culture collections characterized and manipulated in gnotobiotic mice. *Proc. Natl Acad. Sci. USA* **108**, 6252–6257 (2011).
**This study paves the way for in vivo synthetic ecology in model organisms**.
65. Wang, Y., Huang, W. E., Cui, L. & Wagner, M. Single cell stable isotope probing in microbiology using Raman microspectroscopy. *Curr. Opin. Biotechnol.* **41**, 34–42 (2016).
66. Wu, F. & Dekker, C. Nanofabricated structures and microfluidic devices for bacteria: from techniques to biology. *Chem. Soc. Rev.* **45**, 268–280 (2016).
67. Hsu, R. H. et al. Microbial interaction network inference in microfluidic droplets. *Cell Syst.* https://doi.org/10.1016/j.cels.2019.06.008 (2019).
68. Stein, R. R. et al. Ecological modeling from time-series inference: insight into dynamics and stability of intestinal microbiota. *PLOS Comput. Biol.* **9**, e1003388 (2013).
69. Buffie, C. G. et al. Precision microbiome reconstitution restores bile acid mediated resistance to *Clostridium difficile*. *Nature* **517**, 205–208 (2015).
**In this work, the authors parameterize the gLV model to identify gut bacteria inhibiting *C. difficile*, which were subsequently shown to confer infection resistance in mice**.
70. Stein, R. R. et al. Computer-guided design of optimal microbial consortia for immune system modulation. *eLife* **7**, e30916 (2018).
71. Thiele, I. & Palsson, B. Ø. A protocol for generating a high-quality genome-scale metabolic reconstruction. *Nat. Protoc.* **5**, 93–121 (2010).
72. Coyte, K. Z., Schluter, J. & Foster, K. R. The ecology of the microbiome: networks, competition, and stability. *Science* **350**, 663–666 (2015).
73. Arumugam, M. et al. Enterotypes of the human gut microbiome. *Nature* **473**, 174–180 (2011).
74. Costea, P. I. et al. Enterotypes in the landscape of gut microbial community composition. *Nat. Microbiol.* **3**, 8–16 (2018).
75. Gonze, D., Lahti, L., Raes, J. & Faust, K. Multi-stability and the origin of microbial community types. *ISME J.* **11**, 2159–2166 (2017).
76. Goyal, A., Dubinkina, V. & Maslov, S. Multiple stable states in microbial communities explained by the stable marriage problem. *ISME J.* **12**, 2823–2834 (2018).
77. Gibson, T. E., Bashan, A., Cao, H.-T., Weiss, S. T. & Liu, Y.-Y. On the origins and control of community types in the human microbiome. *PLOS Comput. Biol.* **12**, e1004688 (2016).
78. Cremer, J., Arnoldini, M. & Hwa, T. Effect of water flow and chemical environment on microbiota growth and composition in the human colon. *Proc. Natl Acad. Sci. USA* **114**, 6438–6443 (2017).
79. van Hoek, M. J. A. & Merks, R. M. H. Emergence of microbial diversity due to cross-feeding interactions in a spatial model of gut microbial metabolism. *BMC Syst. Biol.* **11**, 56–56 (2017).
80. Faust, K. & Raes, J. Microbial interactions: from networks to models. *Nat. Rev. Microbiol.* **10**, 538–550 (2012).
81. Song, H.-S., Cannon, W., Beliaev, A. & Konopka, A. Mathematical modeling of microbial community dynamics: a methodological review. *Processes* **2**, 711–752 (2014).
82. Widder, S. et al. Challenges in microbial ecology: building predictive understanding of community function and dynamics. *ISME J.* **10**, 2557–2568 (2016).
83. Kettle, H., Louis, P., Holtrop, G., Duncan, S. H. & Flint, H. J. Modelling the emergent dynamics and major metabolites of the human colonic microbiota. *Environ. Microbiol.* **17**, 1615–1630 (2015).
84. Momeni, B., Xie, L. & Shou, W. Lotka–Volterra pairwise modeling fails to capture diverse pairwise microbial interactions. *eLife* **6**, e25051 (2017).
85. Marino, S., Baxter, N. T., Huffnagle, G. B., Petrosino, J. F. & Schloss, P. D. Mathematical modeling of primary succession of murine intestinal microbiota. *Proc. Natl Acad. Sci. USA* **111**, 439–444 (2014).

86. Cao, H.-T., Gibson, T. E., Bashan, A. & Liu, Y.-Y. Pitfalls in inferring human microbial dynamics from temporal metagenomics data. *Bioessays* **39**, 1600188 (2017).
87. Freilich, S. et al. Competitive and cooperative metabolic interactions in bacterial communities. *Nat. Commun.* **2**, 589–589 (2011).
88. Heinken, A. & Thiele, I. Anoxic conditions promote species-specific mutualism between gut microbes in silico. *Appl. Environ. Microbiol.* **81**, 4049–4061 (2015).
89. Pacheco, A. R., Moel, M. & Segrè, D. Costless metabolic secretions as drivers of interspecies interactions in microbial ecosystems. *Nat. Commun.* **10**, 103 (2019).
90. Devoid, S. et al. Automated genome annotation and metabolic model reconstruction in the SEED and Model SEED. *Methods Mol. Biol.* **985**, 17–45 (2013).
91. Machado, D., Andrejev, S., Tramontano, M. & Patil, K. R. Fast automated reconstruction of genome-scale metabolic models for microbial species and communities. *Nucleic Acids Res.* **46**, 7542–7553 (2018).
92. Magnúsdóttir, S. et al. Generation of genome-scale metabolic reconstructions for 773 members of the human gut microbiota. *Nat. Biotechnol.* **35**, 81–89 (2017).
93. Vandeputte, D. et al. Quantitative microbiome profiling links gut community variation to microbial load. *Nature* **551**, 507–511 (2017).
94. Tissier, H. *Recherches sur la flore intestinale des nourissons: état normal et pathologique* (G. Carré et C. Naud, 1900).
95. Finegold, S. M., Attebery, H. R. & Sutter, V. L. Effect of diet on human fecal flora: comparison of Japanese and American diets. *Am. J. Clin. Nutr.* **27**, 1456–1469 (1974).
96. Moore, W. E. & Holdeman, L. V. Human fecal flora: the normal flora of 20 Japanese-Hawaiians. *Appl. Microbiol.* **27**, 961–979 (1974).
97. Millar, M. R. et al. Application of 16S rRNA gene PCR to study bowel flora of preterm infants with and without necrotizing enterocolitis. *J. Clin. Microbiol.* **34**, 2506–2510 (1996).
98. Wilson, K. H. & Blitchington, R. B. Human colonic biota studied by ribosomal DNA sequence analysis. *Appl. Environ. Microbiol.* **62**, 2273–2278 (1996). **This pioneering gut 16S rRNA gene study lays the foundations for the microbiome field**.
99. Chassard, C., Delmas, E., Lawson, P. A. & Bernalier-Donadille, A. *Bacteroides xylanisolvens* sp. nov., a xylan-degrading bacterium isolated from human faeces. *Int. J. Syst. Evol. Microbiol.* **58**, 1008–1013 (2008).
100. Derrien, M., Vaughan, E. E., Plugge, C. M. & de Vos, W. M. *Akkermansia municiphila* gen. nov., sp. nov., a human intestinal mucin-degrading bacterium. *Int. J. Syst. Evol. Microbiol.* **54**, 1469–1476 (2004).
101. Duncan, S. H., Louis, P. & Flint, H. J. Lactate-utilizing bacteria, isolated from human feces, that produce butyrate as a major fermentation product. *Appl. Environ. Microbiol.* **70**, 5810–5817 (2004).
102. Ley, R. E., Turnbaugh, P. J., Klein, S. & Gordon, J. I. Microbial ecology: human gut microbes associated with obesity. *Nature* **444**, 1022–1023 (2006).
103. Dethlefsen, L., Huse, S., Sogin, M. L. & Relman, D. A. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16s rRNA sequencing. *PLOS Biol.* **6**, e280 (2008).
104. Wu, G. D. et al. Linking long-term dietary patterns with gut microbial enterotypes. *Science* **334**, 105–108 (2011).
105. Yatsunenko, T. et al. Human gut microbiome viewed across age and geography. *Nature* **486**, 222–227 (2012).
106. Schnorr, S. L. et al. Gut microbiome of the Hadza hunter-gatherers. *Nat. Commun.* **5**, 3654 (2014).
107. Falony, G. et al. Population-level analysis of gut microbiome variation. *Science* **352**, 560–564 (2016).
108. McDonald, D. et al. American gut: an open platform for citizen science microbiome research. *mSystems* **3**, e00031-18 (2018).
109. He, Y. et al. Regional variation limits applications of healthy gut microbiome reference ranges and disease models. *Nat. Med.* **24**, 1532–1538 (2018).
110. Belenguer, A. et al. Two routes of metabolic cross-feeding between *Bifidobacterium adolescentis* and butyrate-producing anaerobes from the human gut. *Appl. Environ. Microbiol.* **72**, 3593–3599 (2006).
111. Bunesova, V., Lacroix, C. & Schwab, C. Mucin cross-feeding of infant bifidobacteria and *Eubacterium hallii*. *Microb. Ecol.* **75**, 228–238 (2018).
112. Das, P., Ji, B., Kovatcheva-Datchary, P., Bäckhed, F. & Nielsen, J. In vitro co-cultures of human gut bacterial species as predicted from co-occurrence network analysis. *PLOS ONE* **13**, e0195161 (2018).
113. Egan, M. et al. Cross-feeding by *Bifidobacterium breve* UCC2003 during co-cultivation with *Bifidobacterium bifidum* PRL2010 in a mucin-based medium. *BMC Microbiol.* **14**, 282 (2014).
114. Falony, G., Calmeyn, T., Leroy, F. & De Vuyst, L. Coculture fermentations of bifidobacterium species and *Bacteroides thetaiotaomicron* reveal a mechanistic insight into the prebiotic effect of inulin-type fructans. *Appl. Environ. Microbiol.* **75**, 2312–2319 (2009).
115. Newton, D. F., MacFarlane, S. & MacFarlane, G. T. Effects of antibiotics on bacterial species composition and metabolic activities in chemostats containing defined populations of human gut microorganisms. *Antimicrob. Agents Chemoth.* **57**, 2016–2025 (2013).
116. Pinto, F., Medina, D. A., Pérez-Correa, J. R. & Garrido, D. Modeling metabolic interactions in a consortium of the infant gut microbiome. *Front. Microbiol.* **8**, 2507 (2017).
117. Ze, X., Duncan, S. H., Louis, P. & Flint, H. J. *Ruminococcus bromii* is a keystone species for the degradation of resistant starch in the human colon. *ISME J.* **8**, 2507 (2012).
118. Gottstein, W., Olivier, B. G., Bruggeman, F. J. & Teusink, B. Constraint-based stoichiometric modelling from single organisms to microbial communities. *J. R. Soc. Interface* **13**, 20160627 (2016).
119. Gonze, D., Coyte, K. Z., Lahti, L. & Faust, K. Microbial communities as dynamical systems. *Curr. Opin. Microbiol.* **44**, 41–49 (2018).
120. Rosindell, J., Hubbell, S. P. & Etienne, R. S. The unified neutral theory of biodiversity and biogeography at age ten. *Trends Ecol. Evol.* **26**, 340–348 (2011).
121. Delmas, E. et al. Analysing ecological networks of species interactions. *Biol. Rev*. https://doi.org/10.1111/brv.12433 (2018).
122. Röttjers, L. & Faust, K. From hairballs to hypotheses — biological insights from microbial networks. *FEMS Microbiol. Rev.* **42**, 761–780 (2018).



## Acknowledgements

The authors thank all members of the Raes laboratory, as well as D. Gonze, for the many discussions that contributed to this work. G.H. is supported by the European Union's Horizon 2020 Research and Innovation Programme (EU H2020) AD-GUT project, grant agreement no. 686271. The Raes laboratory is supported by the VIB Grand Challenges Programme, the Rega Institute for Medical Research, KU Leuven, the FWO EOS Programme (30770923), FP7 METACARDIS (305312) and the H2020 initiatives SYSCID (733100), PIBD-SET (668023) and IMMUNAID (779295); K.F. has received funding from the European Research Council (ERC) under EU H2020 grant agreement no. 801747.


## Author contributions

G.V. provided scoping and coordination. G.V. and A.C.G. wrote the introduction and the sections on in vitro systems and future strategies; A.C.G. made draft figures; G.R.B.H. contributed the section on isolation; and K.F., the section on mathematical modelling as well as the table. J.R. provided overall guidance. All authors polished and approved the text.

## Competing interests

The authors declare no competing interests.

## Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

## Peer review information

*Nature Reviews Microbiology* thanks Samuel Forster and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

### RELATED LINKS

**Human Microbiome Project:** https://www.hmpdacc.org/hmp/overview/
**German Collection of Microorganisms and Cell Cultures (DSMZ):** https://www.dsmz.de/
**Culture Collection University of Gothenburg (CCUG):** www.ccug.se/