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Modeling metabolism of the human gut microbiome

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The human gut microbiome plays an important part in human health. The complexity of the microbiome makes it difficult to determine the detailed metabolic functions and cross-talk occurs between the individual species. *In silico* systems biology studies of the microbiome can help to identify metabolite exchanges among gut microbes. Constraint-based reconstruction and analysis methods use biochemically accurate genome-scale metabolic networks of microorganisms to simulate metabolism between species in a given microbiome and help generate novel hypotheses on microbial interactions. Here, we review metabolic modeling studies that have investigated metabolic functions of the gut microbiome.

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Introduction

The gut microbiome is beneficial to human metabolism as the gut microbes break down large indigestible compounds from the diet (e.g. fibers) and secrete essential nutrients [1–3], including short-chain fatty acids, which can be taken up by the host and used as energy precursors [4]. Moreover, gut microbes produce essential amino acids and vitamins that the intestinal cells can take up [5]. In addition to the benefits of intestinal microbes, studies have also reported connections between an imbalance in the microbiome ('dysbiosis') [6] and several diseases [7–11]. However, most of these studies investigated correlations between the microbiome and disease status, meaning that the concrete metabolic pathways underlying these observations remain largely unknown. Using current experimental methods, it is difficult and time-consuming to elucidate detailed mechanisms underlying metabolic exchanges among hundreds of microbes and how these exchanges may affect the human metabolism.

Several factors can affect the gut microbiome composition. Claesson *et al.* [12] observed that microbiomes among elderly individuals varied more than the microbiomes among their young control subjects and that the composition correlated with both diet and health. The same year, Yatsunenko *et al.* [13] also observed age-related changes in the gut microbiomes of their subjects, as well as between the geographical locations of their cohorts. Several studies have shown that modifications to the host's diet can shift the microbiome composition, for example [14–16]. However, David *et al.* [17] showed that, while lifestyle changes led to short-term alterations in an individual's microbiome, the overall community structure was stable long-term. In addition, despite the high variability of the microbiome among individuals and the diverse influences to the microbiome composition, functional annotations (see Glossary) seem to be stable among diverse microbiomes in healthy individuals [18].

Despite our steadily increasing knowledge about the microbiome, much remains to be discovered concerning how microbes interact metabolically, both among each other and with the human host. Computational methods can help to guide the exploration of the microbiome by forming hypotheses based on genome-scale metabolic models and testing them in the laboratory. One method that could facilitate elucidating such mechanisms is the constraint-based reconstruction and analysis (COBRA) approach [19], which has been successfully applied in studies on metabolic pathways, individual species metabolism, and inter-species metabolic interactions [20,21]. COBRA studies of the gut microbiome are based on genome-scale metabolic reconstructions (GENREs), which represent the full known set of metabolic pathways that occur in that organism based on genomic and experimental knowledge. Here, we review COBRA studies that have investigated microbiome metabolic interactions, with special focus on human gut microbial studies (Figure 1).

Genome-scale metabolic reconstructions of human gut microbes

GENREs are based on a collection of metabolic functions, which can be inferred from the list of genes identified in an organism [22]. First, the genome sequence is annotated and the resulting set of genes encoding for metabolic enzymes is extracted. From the set of metabolic enzymes and experimental data, we can infer the list of metabolic reactions that can occur in the organism.

Glossary
Biomass reaction: A theoretical reaction that takes up metabolites representing the building blocks of a cell, for example, amino acids, lipids, nucleotides, vitamins. The biomass reaction is often used to represent cell growth.
Functional annotation: Describes the functional role of a gene's product, that is, protein or RNA. Genes linked to metabolic pathways generally have annotations that describe enzymatic reactions.
Gap-filling: Refining a metabolic reconstruction by adding metabolic reactions that were not identified through genome annotation. Gap-filled reactions connect metabolic pathways that are known to occur in the organism of interest.
Objective function: A reaction or combination of reactions whose flux is either maximized or minimized in a simulation of a metabolic model. Typically in microbial metabolic models, the biomass reaction is set as the objective function and its flux maximized.

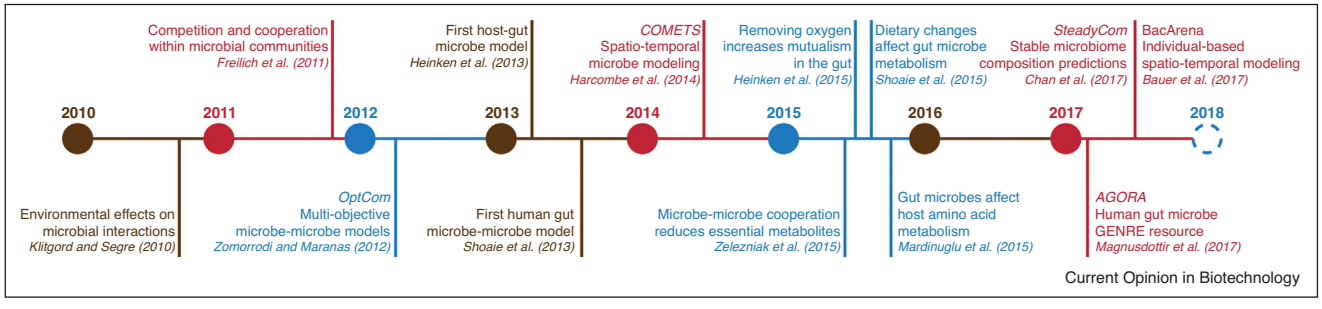
Today, GENREs can be created automatically through several different platforms (e.g. Model SEED [23], KBase [24], Pathway Tools [25]) within minutes to hours, instead of months [22]. However, these automatically created reconstructions require further manual refinement to resolve various issues [26] including stoichiometric consistency [27], reaction directionality [28,29], gene annotations [30], and biological functions of the organism based on experimental knowledge [22]. Numerous algorithms have been developed to accelerate and facilitate the gap-filling (see Glossary) process, for example, SMILEY [26], GapFind [31], fastGapFill [32], SONEC [33], and EnsemblFBA [34]. While each of these algorithms have used different approaches and thus result in potential alternate gap filling solutions, biochemical and phenotyping data is needed to manually evaluate the biological relevance of the computed results and validate the gap filling solutions [35].

Until 2017, manually curated genome-scale metabolic reconstructions had been published for fewer than 20 human gut microbial species [36]. Even though many aspects of microbial and host–microbial metabolism could be studied using these reconstructions, having such a limited set available hindered simulations of complex microbiomes. Nonetheless, previous studies have successfully modeled gut microbial interactions using small sets of microbial GENREs as representatives of the respective taxonomic group [37••,38,39••]. Since microbial composition and diversity are important in many diseases, it is important to model such microbiomes on a large scale. In 2017, we published AGORA, the first comprehensive resource of 773 strain-specific human gut microbial GENREs [40••], which have been manually curated using literature and genomic evidence with specific focus on microbial metabolism of human dietary components, that is, the breakdown simple sugars and of complex carbohydrates and fermentative capabilities of the respective microbes.

COBRA modeling of microbial communities

In the first microbial community COBRA model, Stolyar *et al.* [41] structured their pairwise community model based on the organelle compartmentalization of eukaryotic GENREs. While this community model structure has largely been maintained throughout multispecies modeling to date, several other community modeling approaches exist [42]. In 2012, Zomorrodi and Maranas [43] presented OptCom, a COBRA microbial community modeling framework that could model more than one objective function (see Glossary) at the same time. OptCom uses a nonlinear multi-objective optimization approach to model a system where both the overall community biomass and each individual microbe's biomass are optimized at the same time. Khandelwal *et al.* [44] introduced a nonlinear optimization approach where all microbes in a community grow at the same rate while finding the set of relative biomass abundances that give rise to the highest community growth rate. Klitgord and Segre [45] were the first to systematically investigate the effects of different environmental metabolites on the metabolic interactions between microbial metabolic models. Using a compartmentalized approach, for every pair of seven microbial GENREs, they could identify growth media that could induce syntrophic interactions among the microbes. They hypothesized that environmental changes can more readily drive beneficial interactions between microbes than genomic changes. In turn, Chubiz

Figure 1



Timeline illustrating some of the highlights of COBRA microbiome modeling studies in the last decade. Vertical lines represent the approximate time of year that the study was published.

et al. [46] performed gene knock-out studies using *in silico* microbial pairs and found that cooperating species were less affected by gene deletions than species that competed for resources. Freilich *et al.* [47] first investigated competition between two microbial GENREs. They performed a large-scale pairwise interaction study using 118 automatically generated GENREs and found that most their model pairs would compete for resources and impact each other negatively rather than cooperate. They found that cooperating microbial pairs usually had few growth-requiring metabolites in common. Also, using a large set of automatically generated microbial reconstructions, Zelezniak *et al.* [48•] found that community models of microbes that could cooperate required fewer metabolites in their growth medium than microbial communities with few cooperating species.

Dynamic COBRA modeling of microbiomes

Microbiome communities exist in dynamic environments and shape their environments constantly through the production and the removal of metabolites. Traditional COBRA methods cannot recapitulate such dynamics of microbial communities. Therefore, efforts have been made to include temporal and spatial dynamics in the COBRA approach. In 2014, Harcombe *et al.* [49] presented a modeling framework, named COMETS, enabling the dynamic modeling of metabolic exchanges between multi-species bacterial colonies. The same year, Zomorrodi *et al.* [50] presented an extension to the OptCom framework [43], d-OptCom, which models dynamic changes in the extracellular metabolites and individual microbe biomass concentrations. In 2015, Louca and Doebeli [51] published a dynamic model where experimental metabolic and growth data was integrated with the COBRA model to assess changes in metabolic phenotypes within the microbiome over time. In 2017, Henson and Phalak [52] examined the cross-feeding of fermentation products between three gut microbes in a biofilm model composed of COBRA modeling and kinetic equations for metabolite and biomass diffusion across the biofilm. The same year, Bauer *et al.* [53•] published BacArena, which combines COBRA with agent-based modeling to simulate spatiotemporal dynamics of metabolic interactions in microbial communities. Additionally, since multiple copies of individual strains can be simulated simultaneously in BacArena, it can be used to explore different metabolic phenotypes of a single strain at different times or locations in a microbial community [54]. Finally, van Hoek and Merks [55] published a spatial dynamic flux balance analysis modeling framework to study microbe population dynamics and evolution in the human gut microbiome.

COBRA studies of the gut microbiome

In the decade since the first microbial community COBRA model was published, very few community models have been used to study gut microbial interactions. For instance, Shoaie *et al.* [38] compared *in silico* predicted metabolic interactions between three different bacteria that were contextualized with experimentally measured carbohydrate uptake rates and fermentation product secretion rates from germ-free mice colonized with the same microbes. The *in silico* predicted short-chain fatty acid production of the microbial communities matched their *in vivo* observations. In 2014, El-Semman *et al.* [56] applied the OptCom [43] framework to model the interactions between the gut microbes *Bifidobacterium adolescentis* and *Faecalibacterium prausnitzii*. They found that the *F. prausnitzii* model could secrete more butyrate in the presence of *B. adolescentis* model through its acetate secretion. Later, Mardinoglu *et al.* [57•] investigated how the gut microbiome could affect host amino acid metabolism by simulating the *in silico* co-culture of two microbes, *Bacteroides thetaiotamicron* and *Eubacterium rectale*, and confirmed the *in silico* predicted amino acid production with metabolic measurements from germ-free and conventionally raised mice. That same year, Shoaie *et al.* [37••] validated *in silico* predictions of the metabolic interactions between four different microbes using fecal metabolomics data and metabolite serum levels in germ-free mice colonized with the same microbial communities. The largest microbial community model was constructed by Heinken and Thiele [39••], which consisted of 11 human gut microbes as well as a small intestinal cell model [58], and found that the presence of a host cell drove the microbial pairs to competitive interactions due to host-derived carbohydrates. The same authors showed in another *in silico* study that removing oxygen from the environment promoted mutualism in pairwise interaction metabolic models of 11 human gut microbes [59]. Steinway *et al.* [60] combined genus-level and species-level GENREs with a Boolean dynamic model to identify interactions within a microbiome consisting of 12 taxonomic groups. Using *in vitro* experiments, they validated the *in silico* model prediction that *Barnesiella intestinihominis* can diminish the growth of Clostridium difficile. In 2016, Granger *et al.* [61] used gut microbial reconstructions to present an update of VisANT, a visualization tool that facilitates the analysis of metabolic exchanges and flux distributions within COBRA community models. In 2017, Budinich *et al.* [62] presented a multi-objective approach to model microbial communities, in which every individual microbe in the community optimizes its own objective function. Similarly, Chan *et al.* [63••] developed the scalable SteadyCom framework, which predicts relative abundances and flux distributions of individual microbes in a microbial community. The authors applied SteadyCom to a community of nine human gut microbes and predicted relative abundances in the *in silico* community that resembled experimentally observed microbiome compositions. In 2017, we simulated all possible pairwise interactions between the 773 microbes from the AGORA resource [40••]. We found that removing oxygen and

increasing dietary fibers in the growth media decreased the number of negative interactions among the microbial pairs.

Host–microbiome metabolic models

The first study of a metabolic model between a mammalian host and a microbe was published by Bordbar *et al.* in 2010 [64]. To date, only two studies have been published on *in silico* modeling of a gut microbiome using host metabolic models. In the first study in 2013, Heinken *et al.* [65••] combined a mouse metabolic reconstruction with a reconstruction of the human gut microbe *Bacteroidetes thetaiotaomicron*. They found that the presence of the microbe could rescue the phenotype of the mouse model simulated with inborn errors of metabolism. Two years later, Heinken and Thiele [39••] published a study on the effects of different microbial communities on the metabolism of a human small intestinal cell [58]. The authors could show that the presence of pathogens in the community would lead to the loss of important metabolic functions of the host cell as well as that the microbiome is able to synthesize numerous precursors of hormone metabolism and thus support *in silico* the hypothesis that the gut microbiome can serve as an endocrine organ.

Metabolic modeling of the gut microbiome and integration with omics data

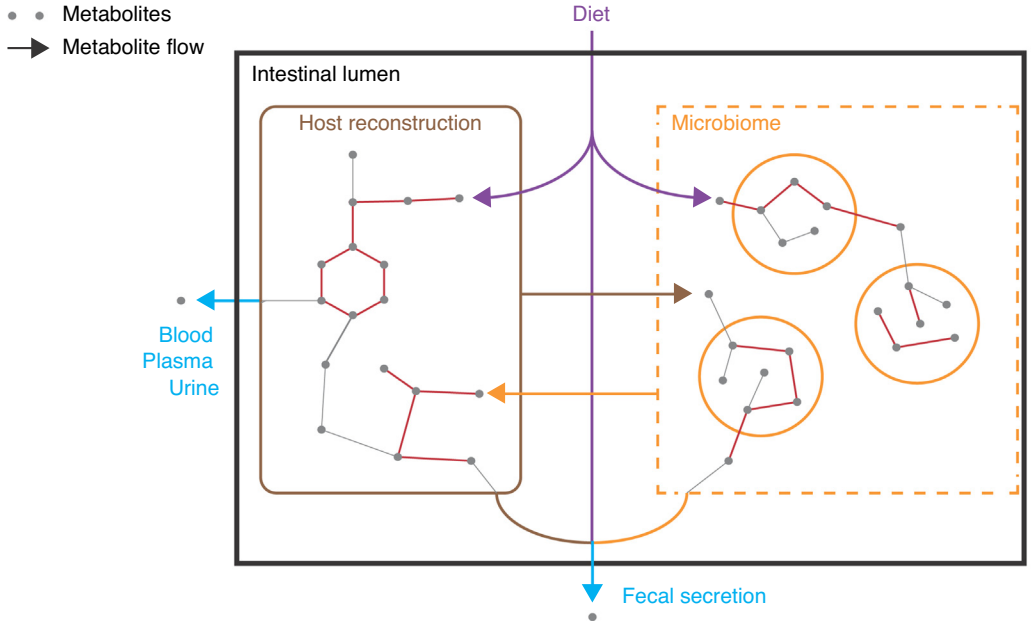
Omics data are crucial during the reconstruction process of GENREs, most notably genomic [22] but also proteomic [66] and metabolomics [67,68] data. Importantly, omics data have been used to convert a generic GENRE into condition-specific metabolic models, by applying

Figure 2

(a)

Data type	(Meta)genomics	(Meta)proteomics (Meta)transcriptomics	Metabolomics	Nutrition
Input data	16S rRNA data. Metagenomic reads. Host genetics.	Gene expression levels. Protein levels.	Media metabolites. Stool metabolites. Blood metabolites. Urine metabolites.	Food frequency questionnaires. Standardized diets. Growth media.
Affected constraints	Microbiome composition. and relative abundances. Host phenotype.	Active metabolic pathways. in individual microbes and host reconstruction.	Output metabolites. Metabolite secretion into body fluids.	Input metabolites.

(b)



Schematic overview of a compartmentalized host–microbiome metabolic model setup. Various data types can be integrated with the model to simulate personalized microbiomes, nutrition, metabolites detected in body fluids, or gene expression data. Colors illustrate the parts of the model that can be contextualized with the different data types.

those omics data as constraints on extracellular and/or intracellular reactions. Numerous corresponding methods have been developed by the COBRA community, many of which are accessible through the COBRA toolbox v3 [69], and which have been reviewed and compared elsewhere, for example [70,71]. Here, we describe how omics data could be used for modeling the human gut microbiota (Figure 2). For instance, metagenomics data can be used to determine the microbiome community structure, for example, by mapping metagenomic reads from stool samples to a set of gut microbial reference genomes, for example, provided by [18], using the sequence alignment tools, such as SAMtools [72]. However, the presence of a microbe in a microbial sample does not imply that all its genomically encoded metabolic functions are active, hence, additional data will be required, such as metatranscriptomics, metaproteomics, and metametabolomics data (e.g. [73,74]). In principle, the COBRA methods developed for contextualizing single organism GENREs could be also applied for large-scale microbial community models. Despite the increasing availability of 'omics' data and integration platforms, only few studies [37••,38,54,75] have used 'omics' data to constrain gut microbial community GENREs, leaving many open opportunities for future *in silico* microbiome studies.

Conclusions

COBRA methods have been applied successfully in the analysis of small microbial communities consisting of up to eleven microbes [39••]. Metabolic models of microbial communities matched metabolomics data from representative *in vitro* studies [37••,76,77], showing that COBRA methods are suitable for studying microbial communities. Additionally, the COBRA community has generated necessary tools to construct and simulate with large-scale microbial community models [69,78,79]. COBRA studies of the human gut microbiome will also require realistic intake fluxes to represent the human diet. Additionally, it will be necessary to formulate biologically feasible objective functions, for example, ones that represent microbial community growth. Chan *et al.* [63••] used a multi-objective biomass function that aims to optimize the growth of every individual microbe, as well as the collective growth of the community. Other approaches include setting a lower bound on every microbe's growth rate while maximizing the collective community growth, for example, Heinken and Thiele [39••]. We envision that future COBRA studies of the human gut microbiome will explore the use of personalized gut microbiome metabolic models, for example, based on metagenomics data from stool samples. To-date, only one such study has been performed to our knowledge, in which metagenomics data from healthy and diseased individuals were used to generate personalized gut microbial community models, consisting hundreds of microbial species-resolved or strain-resolved GENREs, to gain functional insight into the emergent metabolic capabilities of the human gut microbiota [75]. Such models could be further personalized by coupling them with human metabolic reconstructions (e.g. Recon 3 [67,80]) and constrained based on personal dietary reports and omics data sets, such as metatranscriptomics data from stool samples or metabolomics data from stool, blood, or urine (Figure 2). Personalized metabolic models could be applied in personalized medicine, for example, in order to propose personalized treatments of metabolic diseases.

Conflicts of interest

None declared.

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