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Metabolic engineering of human gut microbiome: Recent developments and future perspectives

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ABSTRACT

Many studies have demonstrated that the gut microbiota is associated with human health and disease. Manipulation of the gut microbiota, e.g. supplementation of probiotics, has been suggested to be feasible, but subject to limited therapeutic efficacy. To develop efficient microbiota-targeted diagnostic and therapeutic strategies, metabolic engineering has been applied to construct genetically modified probiotics and synthetic microbial consortia. This review mainly discusses commonly adopted strategies for metabolic engineering in the human gut microbiome, including the use of *in silico*, *in vitro*, or *in vivo* approaches for iterative design and construction of engineered probiotics or microbial consortia. Especially, we highlight how genome-scale metabolic models can be applied to advance our understanding of the gut microbiota. Also, we review the recent applications of metabolic engineering in gut microbiome studies as well as discuss important challenges and opportunities.

1. Introduction

Dysbiosis of the human gut microbiota has been indicated to be associated with various human diseases, such as metabolic diseases (diabetes, obesity)(Ridaura et al., 2013; Wang et al., 2012), inflammatory bowel disease (Lloyd-Price et al., 2019), and cancer (Yu et al., 2017). To treat diseases, many studies have attempted to reverse the dysbiosis of the gut microbiota in patients through fecal microbiota transplantation (FMT) (Suez et al., 2018) or oral supplementation with probiotics (Li et al., 2022a). However, the mechanisms underlying the efficacy of FMT in treating human diseases are unclear (Segal et al., 2020). Particularly, a number of side effects of FMT, such as abdominal pain, bloating, diarrhea, stuffy nose, fever, and vomiting, have been shown in previous studies (De Leon et al., 2013; Suskind et al., 2015). In addition, traditional probiotics, such as lactic acid bacteria and bifidobacteria, often resulted in conflicting clinical results (Dickson, 2019; Feizizadeh et al., 2014), had limited therapeutic efficacy for human diseases (Li et al., 2022a), and even led to side effects (Suez et al., 2019).

To address these problems, metabolic engineering has recently been applied to develop gut microbiota-targeted diagnostics and therapeutics of human diseases, including genetically modified probiotics (i.e. next-generation probiotics) (Kurtz et al., 2019; O'Toole et al., 2017) and synthetic microbial consortia with desired characteristics and functions (Shen et al., 2015; Tanoue et al., 2019). Metabolic engineering is a field that uses engineering strategies, including computational models and genetic tools, to investigate and manipulate microorganisms (Nielsen, 2001; Stephanopoulos et al., 1998). Interestingly, traditional probiotics could be genetically engineered as live microorganism-based delivery systems of drugs (Chen et al., 2020) or biosensors (Daeffler et al., 2017; Riglar and al., 2017), which have the potential for the detection and treatment of diseases.

In this review, we focus on the useful strategies, i.e. an adapted iterative cycle of Learn-Design-Build-Test (LDBT), for metabolic engineering in the human gut microbiome. First, we introduce how we understand the roles of gut microbiota in determining human health. Then, we introduce different metabolic modeling approaches for microbial communities and compare their advantages and disadvantages. Next, we review the recent advances in metabolic engineering for constructing genetically modified probiotics and synthetic microbial consortia. Finally, we discuss the challenges and opportunities for applications of metabolic engineering in the gut microbiome field.

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2. Strategies for metabolic engineering of the gut microbiota

Despite many advances in our understanding of how the human gut microbiota functions in the past few years, the enormous complexity of the gut microbiome makes it difficult to directly prove its mechanisms and causal relationships (Li et al., 2022b). In addition, many factors could influence the gut microbiota, such as age (Kumar et al., 2016), body mass index (Yun et al., 2017), drug (Mardinoglu et al., 2016), diet (Wu et al., 2011), lifestyle (Olm et al., 2022), genetic factor (Gaulke and Sharpton, 2018) and environmental condition (Yatsunenko et al., 2012). In short, more studies are still needed to deeply understand the causal relationship between the gut microbiota and human health. Therefore, here we introduce an adapted iterative cycle of Learn-Design-Build-Test (LDBT) for engineering the gut microbiome for development of the gut microbe-targeted diagnostics and therapeutics of human diseases, which is slightly different from the typical cycle of Design-Build-Test-Learn (DBTL) applied in metabolic engineering. In the LDBT cycle, the learning process is placed in the first step before the design phase,

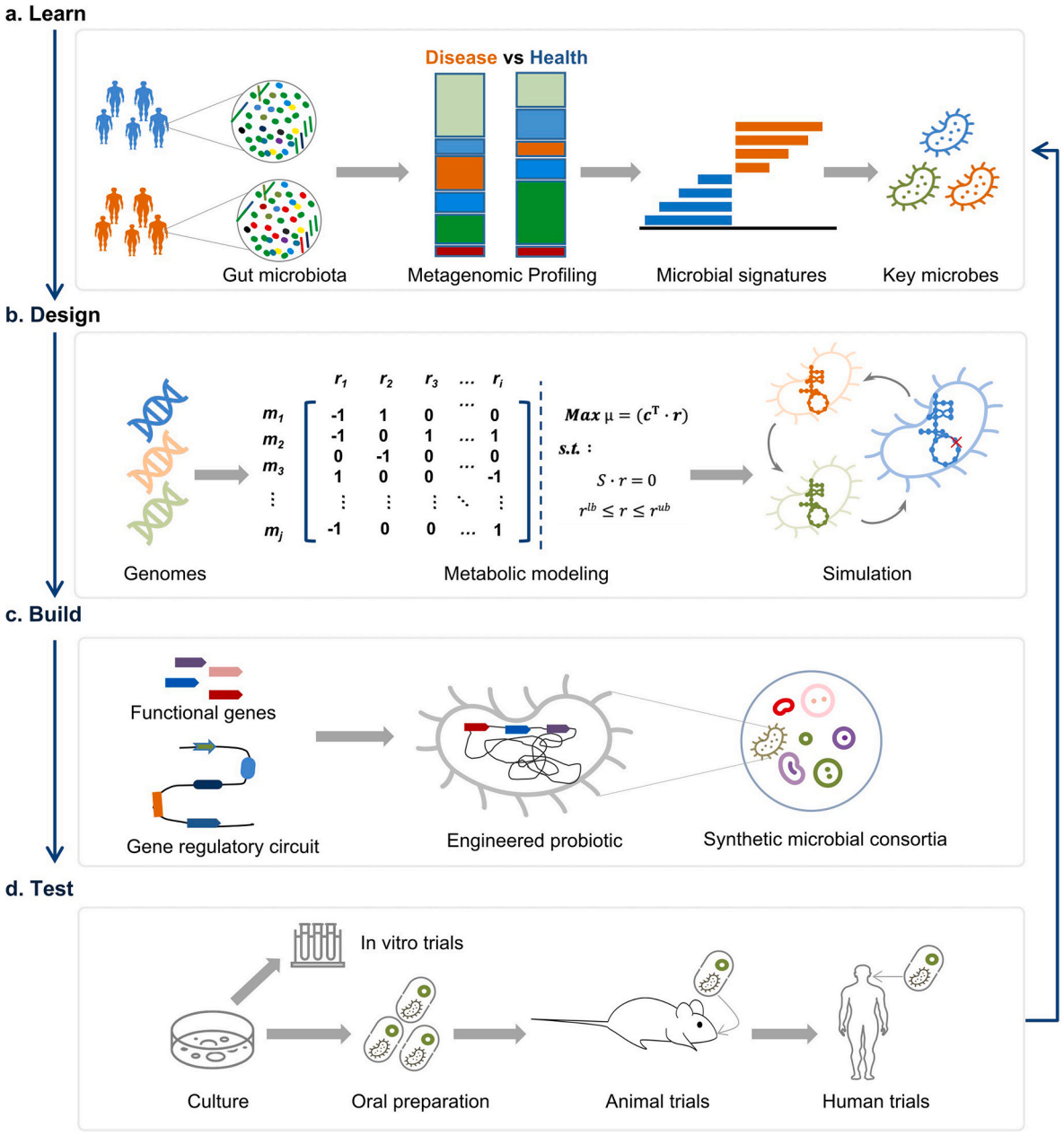

Fig. 1. Iterative strategies of Learn-Design-Build-Test (LDBT) in metabolic engineering of the gut microbiota for diagnostics and therapeutics of human diseases. **a.** Key species contributing to human health can be identified by using next-generation sequencing techniques, such as amplicon sequencing, metagenome shotgun sequencing. **b.** Genome-scale metabolic models (GEMs) and community metabolic models (CMMs) can be applied not only to study the detailed metabolic capabilities of key species or synthetic microbial consortia, but also to investigate interspecies interactions. For model simulations, the metabolic network is defined as a stoichiometric coefficient matrix S, where rows represent metabolites and columns represent reactions. Flux balance analysis (FBA) is widely used to simulate reaction fluxes at a steady state when maximizing an objective function under given condition; c is a vector with coefficients for all reactions that specify a linear combination of reaction fluxes to be maximized; r is a vector with fluxes of all reactions; r^{lb} and r^{ub} denote a vector with lower and upper bounds for all reaction fluxes, respectively; μ indicates objective function that is maximized to simulate metabolic fluxes. **c.** Genetically engineered probiotics and synthetic microbial consortia can be further developed using exogenous genes or gene regulatory circuits. **d.** The constructed microbes or synthetic microbial consortia can be finally tested *in vitro* trials, animal models and human clinic trials.

whereas the DBTL cycle starts from the design phase. The new cycle highlights the importance of determining the causality between human diseases and gut microbiota, which could provide knowledges and insights for the design process. The LDBT strategies mainly consist of characterization and understanding of the gut microbiota, *in silico* design using metabolic modeling, engineering probiotics, and synthetic microbial consortia as well as testing their efficacy for treating human diseases (Fig. 1). First, we will introduce the characterization of human gut microbiota that can help us to understand association or causality between the key microbes, human health and environment.

2.1. Characterizing the gut microbiota using next-generation sequencing technique

To investigate links between the human gut microbiota and diseases, next-generation sequencing techniques, including amplicon-based and metagenome shotgun sequencing, have been widely applied for rapid and large-scale quantifications of the composition and function of microbial communities (Bowers et al., 2017) (Fig. 1a). Therefore, unprecedented metagenomics data have been accumulated and a large number of uncultured species have been discovered from international projects, such as the metagenomics of the human intestinal tract (MetaHIT) consortium (Qin et al., 2010), the human microbiome project (Lloyd-Price et al., 2019), the TEDDY study (Stewart et al., 2018). This provides us with more insights into the mechanisms underlying the impact of gut microbiota on human physiology. Furthermore, comprehensive gene and genome resources from the human gut microbiome have been mined. Here, we summarize the currently constructed gene or genome catalogs in Table 1. Early studies have constructed gene catalogs consisting of millions of prokaryotic genes, including the integrated gene catalog (IGC) (Qin et al., 2010) and MetaHIT (Qin et al., 2010). Moreover, several studies have recovered over 90,000 prokaryotic draft genomes of human gut microbiota from shotgun metagenomic data by assembling sequencing reads into contigs and then performing contig binning (Almeida, 2019; S Nayfach, 2019). The unique children gut genome catalog (ELGG) was also built and included 32,277 genomes, encoding over 80 million protein sequences (Zeng et al., 2022). Recently, the number of prokaryotic genomes has been expanded to 204,938 in the Unified Human Gastrointestinal Genome (UHGG) catalogs (Almeida et al., 2021), which facilitates us to understand the mechanisms of dysbiosis of the gut microbiota in human diseases.

In addition to bacteria, the gut microbiota consists of other communities, including fungi and viruses, which play key roles in the gut ecosystem and diseases (Flint et al., 2012). Therefore, researchers have also constructed the related gene and genome catalogs, such as the unified archaeal protein catalog (UAPC) for human gut archaeome (Chibani et al., 2022), the reference database for sequence-based identification of fungi (UNITE) for human gut mycobiome (Nilsson et al., 2019), the human gut virome database (GVD) (Gregory et al., 2020). Compared to bacteria that account for a large majority of the gut microbial members, the population of fungi in the healthy human is small with a limited number of species (Hallen-Adams and Suhr, 2017). With the development of other high-throughput technologies, increasing studies have attempted to profile the gut microbiota using longitudinal multi-omics data, such as metatranscriptomics, metabolomics, and metaproteomics (Lloyd-Price et al., 2019; Zhong et al., 2019; Zhou et al., 2019). This also has given us a more complete picture of gut microbial metabolism that might influence human health.

Table 1
The existed gene and genome catalogs of human gut microbiome.

Databases	Gene num. (million)	MAG num.	Sample num.	Citation
MetaHIT	~3.3	–	124	Qin et al. (2010)
IGC	~9.9	–	1267	Li et al. (2014)
ELGG	>80	32,277	6122	Zeng et al. (2022)
HGM	–	60,664	3,810	(S Nayfach, 2019)
MGS	–	92,143	11,850	Almeida (2019)
UHGG	>170	204,938	Public databases	Almeida et al. (2021)
UAPC	~1.8	1,167	691	Chibani et al. (2022)
GVD	–	33,242	2,697	Gregory et al. (2020)

Note: MAG: metagenome-assembled genome; MetaHIT: metagenomics of the human intestinal tract; IGC: the integrated gene catalog; ELGG: early-life gut genomes catalog; HGM: the global human gut MAG; MGS: the metagenome species; UHGG: the unified human gastrointestinal genome collection; UAPC: the unified archaeal protein catalogue; GVD: the human gut virome database.

2.2. Metabolic modeling of the human gut microbiota

As introduced above, increasing studies have accumulated tons of metagenomics data that help us to understand the gut microbiota related to human diseases (Almeida et al., 2021; Qin et al., 2010). After identifying key gut microbes, genome-scale metabolic models (GEMs) can be applied not only to study their detailed metabolic capabilities, but also to investigate interactions between gut microbes, such as cross-feeding, and competition (Karlsson et al., 2011) (Fig. 1b). This has advanced our understanding of the gut microbiota, and has enabled rationally engineering of probiotics or design the optimal synthetic microbial consortia for disease diagnostics and therapeutics (Fig. 1c). In the following, we will discuss the general workflow of how to reconstruct GEM and community metabolic model (CMM), respectively.

2.2.1. A general workflow of GEM reconstruction

GEMs comprise a set of biochemical reactions catalyzed by the corresponding enzymes in an organism. To reconstruct the GEM of an organism of interest, the gene-protein-reaction (GPR) associations need to be extracted first, based on its genome as well as the mapped gene annotation from a number of biochemical reaction databases such as MetaCyc (Caspi et al., 2018), KEGG (Kanehisa et al., 2016) and BIGG (King et al., 2016) (Fig. 2a). There are several widely used tools for model reconstruction and analyses, such as COBRA (Becker et al., 2007), RAVEN (Agren et al., 2013), Model SEED (Henry et al., 2010), KBase (Arkin et al., 2018), and CarveMe (Machado et al., 2018), which could automatize many steps of the reconstruction and generate an initial draft model with the collected GPRs associations. A detailed protocol for model reconstruction was also summarized in the previous review (Thiele and Palsson, 2010).

Due to missing annotations, incorrect biomass synthesis reaction or thermodynamically infeasible reactions, these generated draft models always have inaccurate phenotypic predictions and need to be manually curated and refined, such as parameter optimizations for biomass reaction, addition of exchange or transport reactions and gap filling (Fig. 2a). Once the reconstruction of a GEM is completed, it can be used for simulation using the concept of flux balance analysis (FBA) (Orth et al., 2010), where all biochemical reactions in the GEM are formulated as a stoichiometric coefficient matrix S with rows representing metabolites and columns representing reactions (Fig. 1b). Through FBA, reaction fluxes at the steady state can be predicted when maximizing an objective function under a certain number of constraints, which has been formulated mathematically as shown in Fig. 1b. Hereby the metabolic capability of species can be investigated, such as growth rate, targeted product or by-product synthesis. For instance, pan/core GEMs of probiotic *Limosilactobacillus reuteri* were reconstructed and used to analyze the metabolic characteristics and phenotypic diversity among different strains (Luo et al., 2021).

2.2.2. Community metabolic modeling in human gut microbiota

Community metabolic modeling mainly consists of three steps: 1, identification of relevant species by metagenomic data analysis; 2,

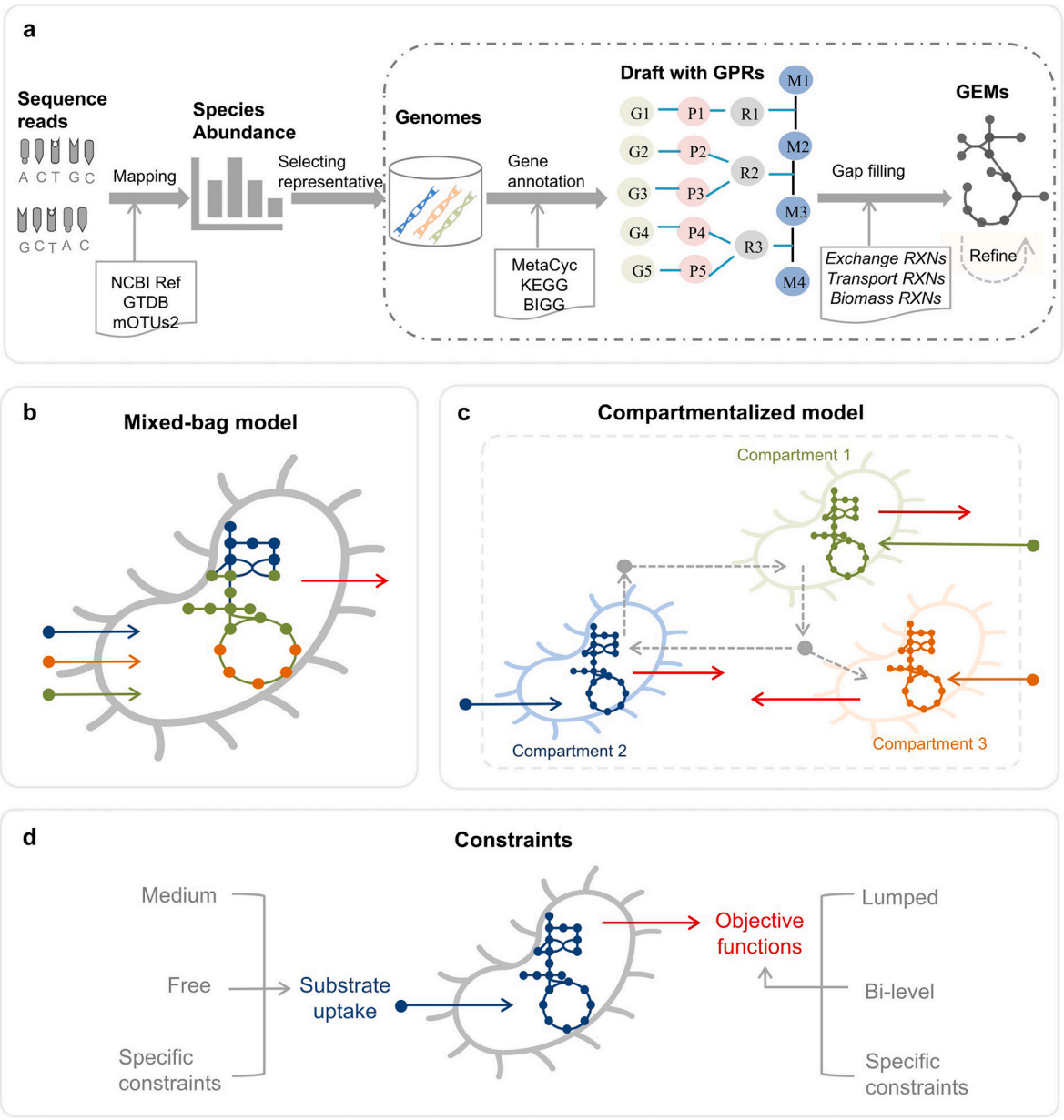

Fig. 2. Reconstruction of GEM and CMM in the human gut microbiota. **a**. The general workflow for metabolic reconstruction. The dashed box illustrates the pipeline for a GEM reconstruction, mainly consisting of the steps: I, the gene-protein-reaction (GPR) associations are collected based on the genomic contents and the public databases, such as MetaCyc, KEGG, generating an initial draft model; II, the draft model needs to be manually curated, such as biomass reaction adjustment, addition of exchange or transport reactions and gap filling; III, after a series of refinements, a finalized GEM is achieved with a complete metabolic network that could convert substrates into biomass components. For CMM, model reconstruction mainly consists of three steps: I, identification of relevant species by metagenomic data analysis; II, model reconstruction of individual species within a microbial community; III, integration of individual species models into a CMM of the microbial consortia. **b.** Mixed-bag model integrates all metabolic reactions from individual species within a microbial community into one cytosolic compartment and one extracellular compartment. The model ignores species boundaries and can be regarded as the typical GEM of a single superorganism. The blue, orange and green lines and dots indicate substrate uptakes. The red line indicates object function, such as biomass synthesis, target compound production. **c.** Compartmentalized model separates metabolic reactions belonging to each species by creating a cytosolic compartment specific to that species. Also, it creates a common extracellular environment compartment for the entire microbial community as shown in dashed box. In this way, species can interact or exchange metabolites with each other through the shared space. **d.** The commonly used constraints in CMMs for substrate uptakes and objective functions. The specific constraints are usually according to the taxonomic abundance profiles or experimental data. In a compartmentalized model, one lumped objective function is usually optimized, while bi-level objective functions have been applied in some cases.

model reconstruction of individual species within a microbial community; 3, integration of individual species models into a CMM of the microbial consortia. Firstly, by mapping sequence reads to reference genomes from the National Center for Biotechnology Information (NCBI) Reference Sequence (RefSeq) database (Pruitt et al., 2012), or taxonomic marker genes, species and their abundances can be determined using metagenomic analysis tools, such as Kraken2 (Wood et al., 2019), MetaPhlAn2 (Truong et al., 2015), mOTUs2 (Milanese et al., 2019) (Bolyen et al., 2019; Truong et al., 2015)(Figs. 1a and 2a). Then the representative genome of individual species within a microbial community can be selected from public databases, e.g. NCBI RefSeq (Pruitt et al., 2012). Also, we can obtain high-quality draft genomes by assembling sequence reads into contigs and then performing contig binning (Li et al., 2015). Further, we can reconstruct draft GEMs for

individual species with complete genomes by following the general workflow of metabolic modeling as introduced above (Fig. 2a).

After metabolic models of individual species are obtained, they can be converted into one of two types of steady-state CMMs: (i) mixed-bag models (Fig. 2b) or (ii) compartmentalized models (Fig. 2c). With the increasing availability of high-quality genomes and automatic modeling tools, a large number of gut microbial GEMs have been reconstructed. For example, the AGORA models, a set of GEMs for 773 human gut microbes, were semi-automatically generated using the Model SEED pipeline (Magnúsdóttir et al., 2017), but the quality of these models was found to be poor (Babaei et al., 2018). In another study, over 14,000 metagenomic GEMs were reconstructed directly from metagenomes rather than reference genomes using the metaGEM pipeline (Zorrilla et al., 2021), which suggested that the intraspecies metabolic diversity could be captured by these strain-level GEMs. Further, these model resources can be applied to predict metabolic capabilities or growth medium for individual gut microbes or to investigate interspecies interactions (Fig. 1b). For instance, GEMs can be used to build a CMM of multi-species by integrating with gut microbial composition data from metagenomic sequencing, which was suggested to be feasible for evaluating the overall metabolic potential of the community (Kumar et al., 2018). An overview of some reconstructed CMMs is provided in Table 2.

2.2.3. Mixed-bag model

Mixed-bag community models can be constructed by merging all metabolic reactions from individual species comprising a microbial community into one cytosolic compartment and one extracellular compartment like in a typical bacterial GEM. This approach ignores species boundaries and can be regarded as a single superorganism (Fig. 2b). Usually, these models are built by first reconstructing GEMs of individual species using their high-quality genomes and then building a mixed-bag model for the microbial community under study. For example, GEMs of multiple cultivable and isolated species, including *Bacteroides thetaiotaomicro*, *Faecalibacterium prausnitzii*, *Thermosynechococcus elongatus,* and *Meiothermus ruber*, have been first reconstructed and then combined into mixed-bag models, which have been used to explore the metabolic capabilities of microbial community or interactions between external environment (e.g., nutrient condition) and the community (Faria et al., 2016; Henry et al., 2016) (Table 2). Moreover, mixed-bag community models with larger sizes have been reconstructed, using 58 children gut bacterial GEMs (Kumar et al., 2018) and AGREDA models consisting of 818 GEMs of human gut bacterial species (Blasco et al., 2021). The AGREDA models were reconstructed for studying diet metabolism of the human gut microbiota, based on the AGORA models and 16S rRNA sequencing data. In these mixed-bag models, an overall biomass synthesis reaction is always created for the microbial community. The new biomass reaction is a reaction combining the individual species' biomasses. In addition, a mixed-bag model for the microbial community can be reconstructed by directly annotating all gene content from the metagenome. For example, the HMP unified metabolic analysis network (HUMAnN) tool was developed to directly perform the metabolic reconstruction of an entire microbial community from metagenomic data (Abubucker et al., 2012). Overall, it is relatively simple to reconstruct mixed-bag community models and these models therefore easily scale with the number of species to be considered. Also, it is computationally efficient to use the model to perform different simulation analyses, including the prediction of growth conditions and synthesis of a target product or by-product for the microbial community.

2.2.4. Compartmentalized model

Although the mixed-bag model is adequate to predict the overall metabolic capability of a microbial community, it does not enable the investigation of interspecies interactions or metabolic exchanges, which can be achieved by using a compartmentalized CMM instead. Unlike the mixed-bag model, the compartmentalized model contains all metabolic reactions belonging to each species by creating a cytosolic compartment specific to that species (Fig. 2c). In addition, the compartmentalized CMM creates a common extracellular environment compartment, which enables species to share external metabolites and medium. In this way, species can interact or exchange metabolites with others through the shared extracellular environment. Moreover, the compartmentalized CMM not only maintains the biomass composition reaction of each species, but also creates a new lumped biomass reaction consisting of individual species' biomasses for the entire microbial community.

After completing the reconstruction of compartmentalized models, simulation analysis is done by applying FBA and optimizing a lumped biomass synthesis reaction as the objective function. The lumped reaction accounts for all species' growths and thus includes all biomass composition reactions of individual species comprising the microbial community. The relative abundance of species in the microbial community is usually used as a constraint, which formulates a weighted linear combination of the biomass composition reactions of individual

Table 2
The community metabolic models (CMMs) of gut microbes.

Model for species	Model type	Simulation method	Growth condition	Citation
Desulfovibrio vulgaris and *Methanococcus maripaludis*	Compartmentation	FBA	Specific medium	Stolyar et al. (2007)
Bacteroides thetaiotamicron, Eubacterium rectale and *Methanobrevibacter smithii*	Compartmentation	FBA	Specific medium	Shoaie et al. (2013)
Desulfovibrio vulgaris and *Methanococcus meripaludis*	Compartmentation	OptCom	Specific medium	Zomorrodi and Maranas (2012)
E. coli	Compartmentation	cFBA	Specific medium	Khandelwal et al. (2013)
Bifidobacterium adolescentis and *Faecalibacterium prausnitzii*	Compartmentation	FBA, OptCom	Rich media	El-Semman et al. (2014)
EBBR and FBBR	Compartmentation	CASINO	Specific medium	Shoaie et al. (2015)
AGORA	Compartmentation	MICOM	Minimal medium	Diener et al. (2020)
Over 14,000 MAGs	Compartmentation	SMETANA	Complete media	(Zelezniak et al., 2015; Zorrilla et al., 2021)
Bacteroides thetaiotaomicron and *Faecalibacterium prausnitzii*	Mixed-bag and compartmentation	FBA, FVA	Minimal medium	Faria et al. (2016)
AGREDA	Mixed-bag and compartmentation	FBA	Minimal medium and 20 growth media (diet)	Blasco et al. (2021)
Entire microbial community	Mixed-bag	HUMAnN	–	Abubucker et al. (2012)
Thermosynechococcus elongatus and *Meiothermus ruber*	Mixed-bag	FBA	LB or minimal medium	Henry et al. (2016)

Note: FBA: flux balance analysis; FVA: flux variability analysis; HUMAnN: the HMP unified metabolic analysis network; MICOM: microbial community; CASINO: community and systems-level interactive Optimization toolbox; cFBA: community flux balance analysis. EBBR: *E. rectale*, *B. adolescentis*, *B. thetaiotaomicron*, and *R. bromii*; FBBR: *F. prausnitzii*, *B. adolescentis*, *B. thetaiotaomicron*, and *R. bromii*; LB: Lysogeny broth; AGORA: assembly of gut organisms through reconstruction and analysis, including 773 human gut bacteria; MAGs: metagenome assembled genomes; AGREDA: AGORA-based reconstruction for diet analysis, including 818 human gut bacteria. Specific medium indicates the available media components from experiments.

species or limits the substrate uptake rates of the community members (Shoaie et al., 2013; Shoaie and Nielsen, 2014; Stolyar et al., 2007) (Fig. 2d). In addition to using the simplest FBA with a lumped objective function, a number of other modeling algorithms have been developed for compartmentalized CMM, such as OptCom (Zomorrodi and Maranas, 2012), CASINO (Shoaie et al., 2015), cFBA (Khandelwal et al., 2013). The OptCom algorithm was performed with a bi-level objective function, which attempts not only to maximize growth rates of individual species (i.e. inner objective function), but also to optimize community growth (i.e. outer objective function) (Fig. 2d). The bi-level optimization algorithm to large extent simplifies the microbial community, where the metabolic capacity of individual species is dependent on each other. Thus, cFBA was proposed to consider the balanced growths between species comprising the microbial community. Nevertheless, the cFBA and OptCom algorithms are computationally limited to be applied for a small size of the microbial community.

By integrating network properties into the community objective function, the CASINO algorithm iteratively optimizes the CMM at both the species level and community level, which enables simulation for a larger size of the microbial community, compared to the cFBA and OptCom algorithms. Recently, the MICOM framework was developed to reconstruct CMMs using AGORA models (Magnúsdóttir et al., 2017), which efficiently expands metabolic modeling to the whole microbial community (Diener et al., 2020). As introduced above, the metaGEM pipeline first reconstructed strain-level GEMs directly from metagenomes (Zorrilla et al., 2021). Then these metagenomic GEMs were integrated into a compartmentalized CMM for analyzing intraspecies or interspecies metabolic interactions, using the SMETANA framework (Zelezniak et al., 2015), which can evaluate flux-balanced metabolic exchanges in the co-occurring microbial species.

Compared to the mixed-bag model, compartmentalized models require much more manual curations of individual species GEM, greater complexity, and more intensive computational cost, which result in more difficulties to scale up to larger microbial communities. However, the integration of species compartmentation in the CMMs not only enables the investigation of interspecies interactions in detail, but also gains a more accurate prediction of the overall metabolic capabilities of the entire community. Based on the deep understanding of the key gut microbes related to human health through the simulation design using GEM or CMM, metabolic engineering approaches can be applied to genetically modify the related species or build synthetic microbial consortia (Fig. 1c), such as probiotic *L. reuteri* (Namrak et al., 2022) and the consortium of *Ketogulonicigenium vulgare* and *Bacillus megaterium* (Ye et al., 2014). Although there are a small number of cases successfully applying metabolic models for engineering of the gut microbes, these suggest great potential towards the development of gut microbe-targeted diagnostic and treatment strategies, which will be discussed as below.

3. Metabolic engineering for the microbiota-based diagnostics and therapeutics of human diseases

Metabolic engineering has mainly been applied to develop genetically engineered probiotics and synthetic microbial consortia (Fig. 1c), summarized in Table 3. Oral supplementation with the engineered microbes as drug delivery systems has the potential to detect and treat diseases (Öhnstedt et al., 2022; Yuvaraj et al., 2006), mainly in three ways: 1) producing beneficial proteins; 2) enabling biosensing in the gut; and 3) designing defined microbial consortia.

3.1. Engineered microbes producing beneficial proteins for disease therapeutics

Metabolic engineering has been widely applied to manipulate probiotics to produce human-derived proteins that can benefit host health (Fig. 3a). By investigating the metabolic capability of probiotic strain

Table 3
List of the engineered probiotics and synthetic microbial consortia.

Species	Disease	Engineering	Testing	Citations
Escherichia coli	*Pseudomonas aeruginosa* infection	Building the synthetic circuit for both detection and treatment	*In vitro* model	(Gupta et al., 2013; Saeidi et al., 2011)
Escherichia coli	Colon cancer	Producing BMP-2 protein for therapeutics	*In vitro* model system (DLD-1)	Yuvaraj et al. (2012)
Escherichia coli	Intestinal inflammation	Constructing the synthetic circuit in response to nitric oxide for diagnostics	*In vitro* model	Archer et al. (2012)
Escherichia coli	Liver metastasis	Expressing β-galactosidase for diagnostics	Mouse model	Danino et al. (2015)
Escherichia coli	Intestinal inflammation	Building bacterial thiosulfate and tetrathionate sensors for diagnostics	Mouse model	(Daeffler et al., 2017; Riglar and al., 2017)
Escherichia coli	Phenylketonuria	Expressing phenylalanine-degrading enzymes for therapeutics	Mouse and monkey models	Isabella et al. (2018)
Escherichia coli	Hyperammonemia	Upregulating arginine biosynthesis for therapeutics	Human clinical trial	Kurtz et al. (2019)
Lactobacillus reuteri	Nonalcoholic fatty liver disease	Secreting IL-22	Mouse model	Oh et al. (2020)
Lactobacillus reuteri	Colitis	Expressing chemokine CXCL12	Mouse model	Öhnstedt et al. (2022)
Lactococcus lactis	Crohn's disease	Producing IL-10 protein for therapeutics	Human clinical trial	Braat et al. (2006)
Lactococcus lactis	Food allergy	Producing murine IL-10 protein for therapeutics	Mouse model	Frossard et al. (2007)
Lactococcus lactis	Colon cancer	Producing scFv SIgA antibody for therapeutics	Jurkat and Ramos cells	Yuvaraj et al. (2008)
Lactococcus lactis	Inflammatory bowel disease	Expressing elafin protein for therapeutics	Mouse model	Motta et al. (2012)
Lactococcus lactis	Type 1 diabetes	Secreting proinsulin autoantigen and IL-10 for therapeutics	Mouse model	Takiishi et al. (2012)
Lactococcus lactis	Type 1 diabetes	Secreting GAD-65 and IL-10 for therapeutics	Mouse model	Robert et al. (2014)
Lactococcus lactis	Type-2 Diabetes	Producing glucagon like peptide-1 for therapeutics	Mouse model	Agarwal et al. (2014)
Saccharomyces boulardii	*Clostridioides difficile* infection	Producing a tetra-specific antibody for therapeutics	Mouse model	Chen et al. (2020)
Bacteroides ovatus	Colitis	Building the synthetic circuit for both detection and treatment	Mouse model	Hamady et al. (2010)

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Table 3 (*continued*)

Species	Disease	Engineering	Testing	Citations
Bacteroides ovatus	Colitis	Building the synthetic circuit for both detection and treatment	Mouse model	Hamady et al. (2011)
Bacteroides ovatus	Intestinal disorder	Building the synthetic circuit for both detection and treatment	*In vitro* model	Farrar et al. (2005)
33 gut commensals	*Clostridioides difficile* infection	Developing the microbial consortia for therapeutics	Human clinical trial	Petrof et al. (2013)
8 gut commensals	Hyperammonemia	Developing consortia with minimal urease gene content for therapeutics	Mouse model	Shen et al. (2015)
11 gut commensals	Cancer	Inducing interferon-γ-producing CD8 T cells for therapeutics	Mouse model	Tanoue et al. (2019)
7 gut commensals	Intestinal disorder	Engineering propionate-producing microbial consortia for therapeutics	*In vitro* model	El Hage et al. (2019)
100 gut commensals	Aging	Controlling composition and function of gut microbiota for therapeutics	*In vitro* colon model	Perez et al. (2021)

Note: BMP: bone morphogenetic protein 2; DLD-1: human colon cancer cell line; scFv: single chain variable fragments; SIgA, secretory IgA; IL-22 (10): the cytokine interleukin-22 (10); GAD: glutamic acid decarboxylase.

L. reuteri KUB-AC5 under various carbon sources using its GEM, a recent study identified essential and preferable nutrients as well as key metabolic pathway including enzymes galactohydrolase and sucrose phosphorylase, which were suggested to be crucial for optimizing the probiotic growth and metabolism (Namrak et al., 2022). This indicates GEMs offer a powerful platform for deciphering cell metabolisms, thus enabling probiotic strain optimization and engineering for the overproduction of biomass or beneficial proteins.

A number of early studies genetically engineered *Lactococcus lactis* by expressing human-derived proteins in order to treat intestinal diseases, such as Crohn's disease (Braat et al., 2006), colon cancer (Yuvaraj et al., 2008), inflammatory bowel disease (IBD) (Motta et al., 2012) as well as to reverse diabetes (Agarwal et al., 2014; Robert et al., 2014; Takiishi et al., 2012). Also, *Escherichia coli* has been genetically modified with the delivery of bone morphogenetic protein 2, which can induce apoptosis of DLD-1 cells, and this was tested in an *in vitro* model of human colon cancer (Yuvaraj et al., 2012). Moreover, the probiotic *Saccharomyces boulardii* was engineered to produce a tetra-specific antibody that could neutralize toxins related to *Clostridioides difficile* infection (CDI) in mouse models (Chen et al., 2020), indicating the potential of yeast immunotherapy. These studies suggest that metabolic engineering has the potential to modify probiotics as drug delivery systems for non-invasive disease therapeutics.

In addition, metabolic engineering has been applied to genetically modify probiotics to reduce metabolites that are detrimental to human health, by expressing related enzymes. For example, to treat phenylketonuria, *E. coli* was engineered to express phenylalanine-metabolizing enzymes in the mammalian gut, which efficiently degraded phenylalanine that is toxic for patients with this disease (Isabella et al., 2018). To improve hyperammonemia, another study also genetically modified *E. coli* to reduce systemic ammonia by converting it into arginine (Kurtz et al., 2019). These studies provide novel and feasible strategies to eliminate toxic metabolites by expressing the relevant enzymes and therefore treat toxicity or other diseases.

3.2. Engineered microbes with biosensors for disease detection and treatment

Genetically engineered microbes can also be applied for diagnostic purposes (Fig. 3b). In an early study, *E. coli* was built to sense and respond to the presence of the mammalian inflammatory signal nitric oxide by integration of a synthetic gene regulatory circuit in an *in vitro* model (Archer et al., 2012). Moreover, probiotic *E. coli* was incorporated with a thiosulfate or tetrathionate sensor that can respond to colitis in mice (Daeffler et al., 2017; Riglar and al., 2017). Interestingly, an engineered *E. coli* expressing β-galactosidase was applied to detect liver metastasis (Danino et al., 2015), by producing easily detectable signal luciferin in urine. Here, luciferin was degraded from the combined molecule of luciferin and galactose, which was fed to mice and catalyzed by β-galactosidase.

In addition, probiotics can be engineered with a synthetic genetic system for both detection and treatment of diseases. For example, several studies have genetically engineered *E. coli* to first sense *Pseudomonas aeruginosa* infection through its quorum-sensing molecule N-3-oxododecanoyl homoserine lactone (3OC12-HSL) (Gupta et al., 2013; Saeidi et al., 2011). The engineered *E. coli* then responds to the signal molecule by producing an engineered chimeric bacteriocin or pyocin that can specifically kill *P. aeruginosa* for therapeutic purposes. Moreover, several studies have genetically modified the gut bacterium *Bacteroides ovatus* to sense the presence of xylan, and then secrete the relevant biologically active molecules, such as human keratinocyte growth factor-2, transforming growth factor-β1, murine interleukin-2, for treatment of intestinal disorders (Farrar et al., 2005; Hamady et al., 2010, 2011). These reports suggest that metabolic engineering of probiotics with sensors enables us to develop novel disease diagnostics and therapeutics in a metabolite-inducible manner.

3.3. Synthetic microbial consortia

The fast development of metabolic engineering also enabled the construction of synthetic microbial consortia as reviewed early (Vázquez-Castellanos et al., 2019), which extends genetic modifications from single probiotics to the multi-species microbial community. Through simulation analysis of interspecies interactions using community metabolic modeling, researchers have designed and constructed synthetic microbial consortia, mainly applied in industrial biotechnology. For example, a previous study used a CMM to investigate the co-culture of *Ketogulonicigenium vulgare* and *Bacillus megaterium* for producing vitamin C (Ye et al., 2014). The metabolic simulation suggests that *B. megaterium* possibly boosts *K. vulgare* growth and biosynthesis of vitamin C precursor 2-keto-l-gulonic acid (2-KLG) via supplying essential growth factors and nutrients. This was consistently observed in the further experiment where the *K. vulgare* growth rate and 2-KLG production were both increased in the co-culture compared to the mono-culture. This study confirms utility of the metabolic model for designing synthetic microbial consortia.

A recent study designed artificial microbial consortia consisting of 100 commensals, which could regulate the composition and function in an *in vitro* colon model of the gut microbiota of the elderly (Perez et al., 2021). The alterations in gut microbiota were related to the increased level of branched-chain amino acids that can benefit the elderly. Moreover, increasing studies have designed and constructed various synthetic microbial consortia of human gut commensals, which have been shown to be safe and efficient for the treatment of patients with CDI in a human clinical trial up to 6 months (Petrof et al., 2013), for cancer immunotherapy by inducing interferon-γ-producing CD8 T cells in mice

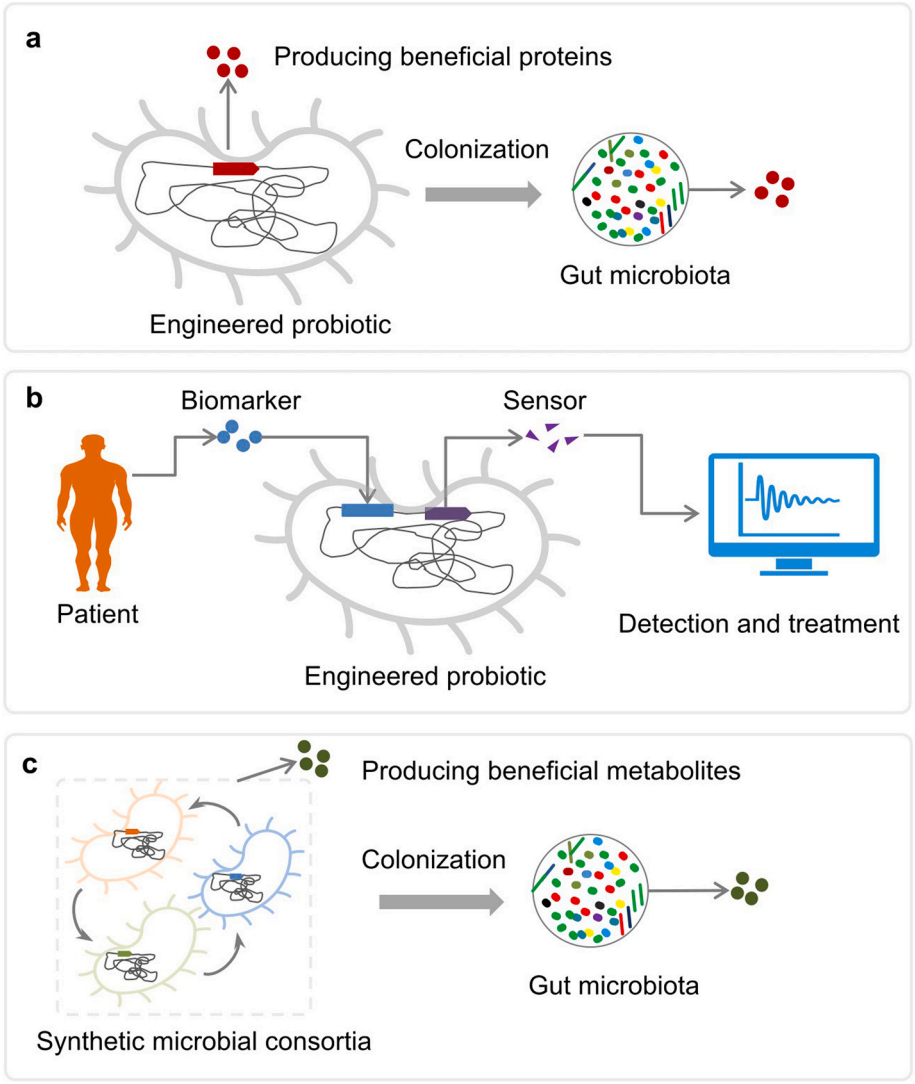

Fig. 3. Engineered probiotics and synthetic microbial consortia for disease diagnostics and therapeutics. **a.** Engineered probiotics that can produce proteins beneficial for human health. The red dots indicate excreted proteins. **b.** Engineered probiotics that can respond to disease biomarker and then produce sensor for disease detection and treatment. **c.** Synthetic microbial consortia that can produce beneficial metabolites for reversing dysbiosis of the gut microbiota. The green dots indicate excreted metabolites. The engineered probiotics or synthetic microbial consortia first colonize the gut and then confer the defined function on the microbiota.

tumor models (Tanoue et al., 2019), for restoration of antibiotic-induced dysbiosis by engineering of the consortia with producing propionate (El Hage et al., 2019) (Fig. 3c). To treat hyperammonemia, researchers also constructed synthetic microbial consortia consisting of 8 bacteria with minimal urease gene content (Shen et al., 2015), which showed that transplantation of the defined microbial consortia caused a decrease in fecal urease activity and ammonia production. These studies suggest that metabolic engineering has the potential to develop synthetic multi-species microbial communities feasible and efficient for reversing dysbiosis of the gut microbiota and disease therapeutics.

Last but not least, these engineered probiotics or synthetic microbial consortia could be further tested *in vitro* models (Gupta et al., 2013; Saeidi et al., 2011), animal models (Daeffler et al., 2017; Riglar and al., 2017) and even human clinic trials (Braat et al., 2006; Kurtz et al., 2019) for their performance evaluation (Fig. 1d). Based on the results obtained from testing, researchers could learn from the data and make further modifications to the design process. This iterative LDBT cycle is repeated until the desired function is achieved with high efficiency.

4. Challenges and future perspectives for metabolic engineering of the gut microbiota

Although metabolic engineering has been increasingly applied in the gut microbiota related to human health, there are still a number of challenges that need to be addressed. One of the challenges is to first identify the unambiguous causality between the gut microbiota composition and function and human diseases. Associations between the gut microbiota and a unique disease have often been shown to be inconsistent across different studies(Karlsson et al., 2013; Wang et al., 2012), which could be caused by various factors, such as drug intake (Mardinoglu et al., 2016), diet (Wu et al., 2011), ethnicity (Gaulke and Sharpton, 2018), geography (Yatsunenko et al., 2012), age (Kumar et al., 2016), and lifestyle (Olm et al., 2022). Therefore, when conducting experiment design and analyses, researchers should take these factors into account, and perform integrative analysis of complex data including multi-omics, dietary composition, and clinic data. Another challenge could be that the vast majority of studies are based on fecal samples, whereas many important microbial metabolisms and interactions have taken place in the proximal colon or ileum (Kastl et al., 2020). Addressing these issues will help to elaborate on the underlying links between human diseases and gut microbiota.

GEM is a powerful tool for studying the metabolisms of microorganisms. Recently, there is an increasing interest to reconstruct enzyme-constrained metabolic models (Domenzain et al., 2022; Sánchez et al., 2017), which have incorporated enzyme's abundance, turnover number or proteomics data, and improved the phenotypic predictions. These GEMs can be further used to construct a CMM of the gut microbiota for simulating its metabolic potential or interspecies interactions (Faria

et al., 2016; Karlsson et al., 2011). However, it needs to address a number of challenges for community metabolic modeling. A compartmentalized CMM is usually constructed using GEMs of hundreds of species comprising the gut microbiota (Magnúsdóttir et al., 2017). Thus, the CMM needs considerable manual curation of many draft models, which is time-consuming and difficult to accelerate. To overcome this, a human gut microbiota-specific model database, including complete metabolites, reactions and genes with unified and standardized ID, should be established, like the BIGG platform (King et al., 2016). Meanwhile, the database should include various detailed medium or dietary components like growth media defined in the MetaCyc, which will expand phenotypic simulations at different conditions, and thus accelerate model curation and refinement. As several GEM reservoirs of human gut microbes, such as AGORA (n = 773) (Magnúsdóttir et al., 2017), AGORA2 (n = 7,302) (Heinken et al., 2023), metaGEM collection (n > 14,000) (Zorrilla et al., 2021), have been reconstructed, these models could be regarded as knowledge base for conveniently constructing CMMs. However, the quality of these models needs to be further evaluated and improved as discussed in an earlier report (Babaei et al., 2018).

Moreover, CMMs often include a part of microbial species with little physiological knowledge, incomplete gene annotations or few experimental data, which are required to reconstruct a high-quality model and can lead to limited predictive performance. To address this issue, high-throughput microbial culturomics technique could be used to isolate more strains for their detailed mechanistic studies. For example, using automation technology and machine learning, a recent study developed a culturomics framework for efficiently isolating individual bacteria from microbial ecosystems, by integrating imaging-based phenotypic data with high-resolution genomics data (Huang et al., 2023). The technique would help collect more experimental and mechanistic data for individual species, thus accelerating the reconstruction of a high-quality metabolic model.

An additional challenge is to integrate the gut microbial relative abundances into a CMM for designing an appropriate objective function for the entire microbial community. To overcome this, researchers first need to determine the overall goal of the microbial community, such as maximizing biomass growth or optimizing metabolite production. Next process is to identify relevant species, i.e., determine which species in the community play a critical role in achieving the goal, involved in considering the metabolic capabilities of each species and how they interact with each other. In many cases, there will be tradeoffs between different species or between individual species and the microbial community. Several studies have discussed the tradeoffs as well as proposed different methods to incorporate them into the objective functions, such as cFBA (Khandelwal et al., 2013), CASINO (Shoaie et al., 2015), MICOM (Diener et al., 2020), which nevertheless need to be further validated with more experimental data. These processes are particularly important for the accurate predictions of microbial metabolic capabilities or interspecies interactions within the community.

The steady-state metabolic model of microbial community provides us insights into interspecies interactions at a snapshot of one environmental condition. However, the microbial community suffers the compositional or functional disturbance caused by interspecies interactions or varied environments. Therefore, dynamic metabolic modeling algorithms have been proposed (Dukovski et al., 2021; Geng et al., 2021; Henson and Hanly, 2014; Luo et al., 2022), which nevertheless have been limited to small-sized microbial communities, due to the required model parameters and computational cost. Thus, before performing the dynamic metabolic modeling, it is essential to identify and validate a small number of key species, driving key metabolisms and interactions in contribution to the gut microbiota-related diseases. Additionally, it is crucial to measure several factors, such as biomass growth, secreted metabolites, and consumed substrates. Then the dynamic model could accurately fit these measurements, providing valuable insights into the systems being studied.

Metabolic modeling of the microbial community has helped researchers to design and develop next-generation probiotics and synthetic microbial consortia by *in silico* analysis of metabolic capacities and interspecies interactions. There are still some hurdles to overcome during metabolic engineering of gut microbes. Over the last decades, there has been a great improvement in development of the genetic tools, e.g., CRISPR/Cas-based genome editing tool for *in vivo* and *in situ* gene editing (Deltcheva et al., 2011). However, currently only a few model microorganisms have efficient tools for gene editing (Zheng et al., 2022), and there are still needs to develop more efficient tools for many types of gut microbes. In addition, genetically engineered probiotics or synthetic microbial consortia as drug delivery systems could bring unexpected results and even cause biosafety problems after getting into the human body (Kleter et al., 2005; Wegmann et al., 2017), which have thus raised controversies and concerns about their uses in clinic. Particularly, one challenge is the regulatory policies on genetically modified probiotics, which will likely be classified as drugs, and not dietary supplements (Venugopalan et al., 2010). One positive aspect of this is that there might be a higher acceptance for side effects in proportion to the clinical effects, while one negative side is that there might be an increasing costs and time to market. Another limitation is the personal difference in response to probiotics or synthetic microbial consortia (Li et al., 2022a). Therefore, further studies using metabolic engineering strategies need to be performed for the identification of key factors that contribute to the differential responses, which might help to develop a personalized intervention.

Despite the challenges, the iterative strategies of LDBT for metabolic engineering of the human gut microbiome hold great promise for the development of the gut microbe-targeted diagnostics and therapeutics of human diseases, which could reverse dysbiosis of the gut microbiota, and ultimately achieve personalized and precision medicine.

Author contributions

JN and PL conceived and designed the review. PL and HL drew all graphs. PL wrote the first draft. PL, BJ, SR and JN revised the manuscript. All authors critically reviewed and approved the manuscript.

Declaration of competing interest

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Data availability

No data was used for the research described in the article.

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