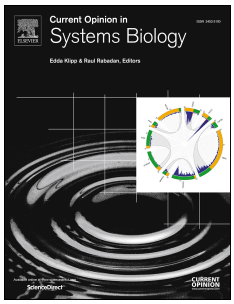
Journal Pre-proof



Advances in constraint-based modelling of microbial communities

Almut Heinken, Arianna Basile, Ines Thiele

PII: S2452-3100(21)00031-7

DOI: https://doi.org/10.1016/j.coisb.2021.05.007

Reference: COISB 346

To appear in: *Current Opinion in Systems Biology*

Received Date: 15 February 2021

Revised Date: 30 April 2021

Accepted Date: 16 May 2021

Please cite this article as: Heinken A, Basile A, Thiele I, Advances in constraint-based modelling of microbial communities, *Current Opinion in Systems Biology*, https://doi.org/10.1016/j.coisb.2021.05.007.

Advances in constraint-based modelling of microbial communities

Almut Heinken[1,2], Arianna Basile [1,3], Ines Thiele[1,2,4,5#]

[1] School of Medicine, National University of Ireland, University Road, Galway, Ireland
[2] Ryan Institute, National University of Ireland, University Road, Galway, Ireland
[3] Department of Biology, University of Padova, Via U. Bassi 58/b, 35121, Padova, Italy
[4] Division of Microbiology, National University of Galway, University Road, Galway, Ireland
[5] APC Microbiome Ireland, Cork, Ireland

[#]Correspondence: ines.thiele@nuigalway.ie

Abstract

Microbial communities are near universally present in nature. A wealth of meta-omics data has been gathered from numerous ecosystems, such as the human gut, ocean, or soil. Constraint-based Reconstruction and Analysis (COBRA) is a valuable tool for the contextualisation of meta-omics data and allows for the mechanistic prediction of metabolic fluxes. Advances in genome-scale reconstruction and multispecies modelling tools have enabled the construction and interrogation of constraint-based multispecies models on the microbiome scale spanning hundreds of organisms. Here, we give a comprehensive overview of the areas of application for these multiscale, strain-and molecule-resolved multispecies models, and discuss key works, in which computational modelling yielded novel biological knowledge. We show that constraint-based microbiome modelling can complement experimental approaches and has valuable applications spanning from ecology, human health, industry to environmental conservation.

1. Introduction

Microbiomes are present in diverse ecosystems, such as soil, oceans, plants, food, and in the bodies of humans and animals, and play an important role in processes from the global carbon cycle, in biotechnology and food production, to human health and wellbeing [1-4]. When microbes encounter each other, they may engage in mutualistic, commensal, competitive, or predatory interactions [5]. Understanding these complex interactions requires predictive computational models that can integrate different types of data and capture the principles of microbe-microbe crosstalk [6,7]. High-throughput techniques, such as 16S rRNA sequencing, as well as meta-omics (i.e., metagenomics, metatranscriptomics, metaproteomics, and metabolomics) have generated a wealth of publicly available data on the composition and activity of microbiomes in different environments [8-11], yet the interpretation of this data is lacking behind. To gain novel insight by cross-linking different data types, computational systems biology methods are needed [12,13]. A commonly used systems biology approach is Constraint-Based Reconstruction and Analysis (COBRA) [14]. As COBRA modelling can complement *in vivo* and *in vitro* models by integrating different types of omics data and provide mechanistic insight into microbiome structure and function [15,16], it has been applied to a wide variety of studies simulating microbe-microbe, host-microbe, and microbiome interactions. Here, we comprehensively review applications of COBRA-based modelling of microbial communities.

Principles of Constraint-based Reconstruction and Analysis

The constraint-based reconstruction and analysis approach relies on genome-scale metabolic reconstructions that have been traditionally built manually in a bottom-up manner [17]. Such genome-scale reconstructions can readily be converted into a variety of condition-specific, mathematical models. By implementing physicochemical and condition-specific constraints, the computation of the solution space, which contains all flux distributions feasible under the given constraints, becomes possible [18]. The starting point for a genome-scale reconstruction is the sequenced and annotated genome of the target organism, from which a draft reconstruction, consisting of metabolites and the reactions that link them, is built [19]. Draft reconstructions can also be generated automatically by online platforms, such as Pathway Tools [20], KBase [21], and ModelSEED [22]. However, these draft reconstructions have typically gaps, as genome annotations are incomplete and misannotations may be present [23], and hence, they require a manual curation effort [24,25] to ensure that the *in silico* organism represents as good as possible the organism's known biology. To obtain a high-quality genome-scale reconstruction, the draft reconstruction is extensively curated based on refinement of genome annotations, which is often done manually [26,27], and published biochemical and physiological traits of the target organism [19]. Previously, such curation efforts could take up months to years for well-studied organisms [19]. Recently, tools, which facilitate the curation process of reconstructions have been developed, such as CarveMe [28], gapseq [29], and DEMETER [30]. A protocol for multi-strain reconstructions from a single strain template reconstruction is also available [31]. While these pipelines require the genome sequence (and annotation) as in inputs, pipelines have become available to assemble genome-scale reconstruction directly from metagenomic samples [32,33]. However, all these pipelines do not fully replace manual curation and the integration of organism-specific experimental data. To address this challenge to some extent, DEMETER provides a large-scale collection of experimental data, e.g., on carbon sources and fermentation products, which is incorporated during the semi-automatic reconstruction process [30].

Constraint-based modelling of multispecies interactions

While the first decade of constraint-based modelling mainly focussed on the reconstruction and analysis of single strains [34], in the last decade, an increasing number of studies has demonstrated that COBRA modelling can successfully be used for predicting interspecies interactions (Figure 1a) [35]. An advantage of constraint-based modelling is that at its core are the strain-and molecule-resolved metabolic networks resulting into mechanism-based predictions. Moreover, constraint-based modelling does not require enzyme kinetics as an input parameter [17]. These advantages of COBRA, as well as the increasing availability of genome-scale reconstructions, have motivated a variety of studies that used COBRA modelling to interrogate multispecies interactions (Figure 1a). We performed an extensive literature review of studies that used constraint-based modelling to integrate at least two organisms through COBRA methods as either the main focus of the paper or to complement experimental results. Based on the resulting list of 122 studies (Table S1), which, to the best of our knowledge, represents a comprehensive overview of the field, we identified eight main areas of application (Figure 1b).

2. Current applications of constraint-based modelling of multispecies interactions

Development of multispecies modelling tools

A multitude of efforts have been made to develop constraint-based modelling tools that enable two or more reconstructed organisms to communicate *in silico* in a biologically plausible manner. One way to accomplish this is by using a compartmentalised approach, in which the individual genome-scale reconstructions are integrated by placing them into a shared compartment representing the environment, allowing for tractable metabolic exchange and uptake of available nutrients by the joined organisms [36]. Examples include optCom [37], cFBA [38], SteadyCom [39], mgPipe [40], KBase [21], and MICOM [41]. Typically, compartmentalised modelling frameworks rely on the formulation of a community biomass reaction summarising the growth of all joined organisms. This approach also enables the contextualisation of multi-species models with 16S rRNA or metagenomic sequencing data to enforce growth at experimentally observed ratios [40]. Another strategy to simulate multi-species interactions relies on dynamic flux balance analysis, which simulates flux changes over time [42,43]. Here, a steady-state flux solution is computed at a given time point. The joint *in silico* environment (or medium) and biomass reaction(s) are then updated based on this flux solution by setting the constraints for the next time point to be solved. Advantages of dynamic community models include that they allow for the prediction of growth rates, of dynamic changes in the community over time, and of metabolite concentrations rather than fluxes [44]. Examples include DMMM, [43], dOptCom [45], and FLYCOP [46]. Finally, spatiotemporal modelling capture both spatial and temporal dynamics by placing organisms into a simulated lattice of grids and then simulate the temporal dynamics of microbial communities. Examples include COMETS [47], and its successor, COMETS 2 [48], BacArena [49], ACBM [50], and IndiMeSH [51]. For a more detailed review of strategies and assumptions used by the different constraint-based multispecies modelling tools, the reader is directed to [44,52-54].

Interactions in synthetic communities

When microbes encounter each other, they may engage in mutualistic, commensal, neutral, or competitive interactions [5]. As real microbial communities found in nature are complex, synthetic communities consisting of a limited set of defined species are a useful tool for studying basic principles of microbial ecology [5]. Constraint-based modelling is a valuable complementary approach to *in vitro* models as it can predict metabolic interactions in a molecule-resolved manner, while maintaining inter-organism borders and the strain-level origin of metabolites [55].

A variety of COBRA studies have simulated synthetic microbial communities to predict interspecies interactions. For instance, the outcome of the co-growth of two- and three-species consortia, which had been engineered to be obligately mutualistic, was correctly predicted *in silico* [47]. In a follow-up study, the behaviour of a microbe-microbe pair when introducing a third species to the community has been analysed, revealing that adding another mutualist reduced selection for mutualism while adding an exploiter increased selection for mutualism in the original pair [56]. The evolution of a two-species community has been modelled by simulating random gene losses [57]. Over 16,000 independent evolutionary trajectories emerged that resulted in different metabolic interdependencies in the pair [57]. A different approach combined constraint-based modelling with evolutionary game theory to uncover evolutionary paths towards the development of obligate mutualism mediated by amino acid exchanges [58]. In another study, over 2 million pairwise growth

simulations on different nutrient environments have been performed and the outcomes (e.g., mutualism, competition) as well as secreted metabolites were determined [59]. In agreement with previous studies [60,61], anoxic conditions promoted mutualistic interactions, which could be explained by an increase in exchange of products that microbes secreted at no metabolic cost [59]. Multispecies models for thousands of co-occurring communities with up to 40 organisms have been computed and revealed two distinct types of communities [62]. Cooperative and competitive communities have been characterised by multiple auxotrophies and overlapping nutrient requirements, respectively [62]. Taken together, these studies demonstrate that COBRA modelling can be used to explore key mechanisms of microbial evolution and ecology.

Human microbiome

The human microbiome plays an important role for human health and wellbeing, and has been implicated in a growing number of disease states [63]. Recently, the paradigm of personalised medicine, or targeted interventions, which take host genetics, lifestyle, and the microbiome into account, has been proposed [64,65]. As constraint-based models are mechanistic and can readily integrate meta-omics data [13] as well as condition-specific constraints (e.g., diet) [66], they can serve as a computational framework for such personalised predictions [15,67]. Ultimately, novel dietary or therapeutic interventions could be proposed [64,68]. Previously, simulations on the human microbiome level have been hampered by the lack of genome-scale reconstructions representing the diversity of the human microbiome [36]. To fill this gap, a resource of 773 gut microbial reconstructions, Assembly of Gut Organisms through Reconstruction and Analysis (AGORA) has been assembled through an iterative, semi-automated refinement pipeline [61], which incorporated extensive experimental data and manually curated genome annotations. An update of AGORA in both size and scope, following the same reconstruction protocol and standards, has been recently compiled [69].

Fuelled by the continuing high interest in the field, and enabled by the release of AGORA, modelling of the human gut microbiome is the largest area of applications of COBRA-based multispecies modelling studies (Figure 1a, b). While early works focussed on interrogating the interactions in small model communities [70-72], more recent studies have modelled the gut microbiome on the microbiome scale with application to several diseases linked to the gut microbiome. For instance, inflammatory bowel disease (IBD) is associated with dysbiosis of the gut microbiome and changes in gut microbial metabolites have been implicated in IBD [73,74]. Several studies interrogated personalised models for IBD patients and controls, and they found that the structure and function of dysbiotic microbiomes have been disrupted compared to controls [26,75-79]. Colorectal cancer (CRC) is another disease that has been linked to the gut microbiome. Certain taxa, such as *Fusobacterium* sp., have been shown to be associated with CRC [80]. Personalised modelling using entire gut microbiome-level models, consisting of an average of 70 species, predicted that the presence of *Fusobacterium* sp. was linked in CRC to lower butyrate production potential [81]. Personalised community models of the CRC tumour microbiome have also been constructed and the production potential for detrimental hydrogen sulphide was predicted [82,83]. Metabolic modelling has also revealed that metabolites increased in CRC confer a growth advantage to CRC-associated microbes [84]. Changes in the gut microbiome in

Parkinson's disease (PD) have also been repeatedly reported [85]. COBRA modelling combined with metabolomics proposed a role of the transsulphuration pathway in PD [86], which was further supported in a follow-up study [87].

Recent studies have expanded the scope of host-microbe modelling beyond gut prokaryotes. For instance, the lung microbiome in cystic fibrosis patients was simulated [88]. Another study modelled the interactions between the human fungal pathogen *Candida albicans* and commensal gut microbes, predicting and experimentally confirming species that could promote or inhibit *C. albicans* growth [89]. A constraint-based model of the interactions between human alveolar macrophages and SARS-CoV-2 has predicted guanylate kinase as a potential antiviral drug target [90]. To further expand the scope of human microbiome modelling, an organ-resolved whole-body model of human metabolism, which can be contextualised with physiological data and dietary regimes, and integrated with a personalised microbiome has been made available enabling novel *in silico* studies focusing on host-microbiome crosstalk [91].

Animal microbiome

Besides the human microbiome, COBRA modelling has yielded insight into the interactions between animals and their commensal microbes. For instance, the metabolic interactions between mouse and a gut symbiont have been modelled, revealing that the microbe could rescue lethal gene deletions in the host [92]. Other works have used constraint-based modelling to understand the metabolic dependencies between insects and their obligate endosymbionts for whiteflies [93], xylem-feeding insects [94], and pea aphids [95]. The interactions between the parasitic worm *Brugia malayi* and its obligate bacterial endosymbiont has been modelled leading to propose novel drug targets, some of which have been experimentally confirmed [96]. COBRA modelling could also complement gnotobiotic animal models to drive discovery of the mechanisms underlying host-microbe interactions. One study combined-high throughput screening, personalised *in silico* modelling, and validation in the model nematode *Caenorhabditis elegans* to elucidate how diet and the microbiome affect the efficacy of the Type 2 diabetes drug metformin [97].

Bioremediation

Bioremediation is the application of microorganisms to degrade or remove environmental contaminants and pollutants. In a first effort to *in silico* design communities optimal for bioremediation efficiency, dynamic FBA has been used to simulate a community of two Fe(III) reducers engaging in uranium removal from groundwater [43]. A naphthalene-degrading microbial community from contaminated soil has also been reconstructed and modelled based on metaproteomic data [98]. Another study modelled the degradation of the herbicide and pollutant atrazine by a soil community [99]. The performance of the atrazine degrader *Arthrobacter* was better in the presence of non-degrading soil bacteria than alone [99]. Similarly, co-cultures of human microbes have been shown to be more efficient in converting food waste to commodity chemicals compared with each microbe alone [100].

Biotechnology and food microbiology

Microbial consortia are commonly used in biotechnology to produce metabolites of industrial interest. Constraint-based modelling is useful for cost-efficient engineering of

such consortia and proposing optimal growth conditions *in silico* [101]. For example, Ye and colleagues have built an artificial metabolic ecosystem consisting of *Ketogulonicigenium vulgare* and *Bacillus megaterium* to produce vitamin C [102]. Furthermore, optimal media have been developed *in silico* to improve the purity of biogas produced from anaerobic digestion [103]. A COBRA model of medium-chain fatty acid production from syngas by a co-culture of *Clostridium autoethanogenum* and *Clostridium kluyveri* has proposed several strategies to optimise yield [104]. Another study modelled interactions between >800 metabolic models from metagenome-assembled genomes to understand the mechanisms underlying methanogenesis and acetogenesis in different feedstocks [105]. This analysis proposed that anaerobic digestion consortia are shaped by a highly cooperative behaviour as postulated by the red queen hypothesis [105]. In another application, the impact of ammonia addition in four reactors fuelled by different carbon sources has been investigated [106]. Microbial communities also play an important role in the food industry [107]. Here, constraint-based modelling can provide insight into the growth conditions of key organisms, such as lactic acid bacteria (LAB) [107], and could especially elucidate the properties of co-cultures [4]. For example, pairwise cultures of different species of LAB were simulated to identify pairs resulting in optimal lactate yield [108].

Environmental microbiome

Interactions in environmental microbiomes, such as in freshwater, sediments, and soil, play an important role in the global carbon cycle with implications for global warming [2]. In one study, the interactions between two methanotrophic bacteria, which are an important methane sink, have been modelled [109]. Such approaches could serve to tailor mitigation approaches for freshwater methane emission [109]. The direct interspecies electron transfer (DIET) in a subsurface community has been modelled for two *Geobacter* species [110]. Moreover, a soil microbial community model has been generated from metagenomic sequence data to investigate *in silico* the influence of agricultural activities on soil metabolic profiles [111]. Modelling of marine microbiomes has never been performed to our knowledge, but has valuable applications [112].

Plant microbiome

Plant microbiomes play a key role for the survival of their hosts, the quintessential example being the symbiosis between legumes and endosymbiotic rhizobial bacteria [113]. Two studies have thus far modelled these cross-kingdom interactions. A genome-scale reconstruction of the clover *Medicago truncatula* has been integrated with a reconstruction of its rhizobial symbiont, *Sinorhizobium meliloti* [114], and has subsequently been used to derive a zone-specific, compartmentalised model of the nodulated legume and its symbiont deemed Virtual Nodule Environment' (ViNE) [113].

3. Outlook

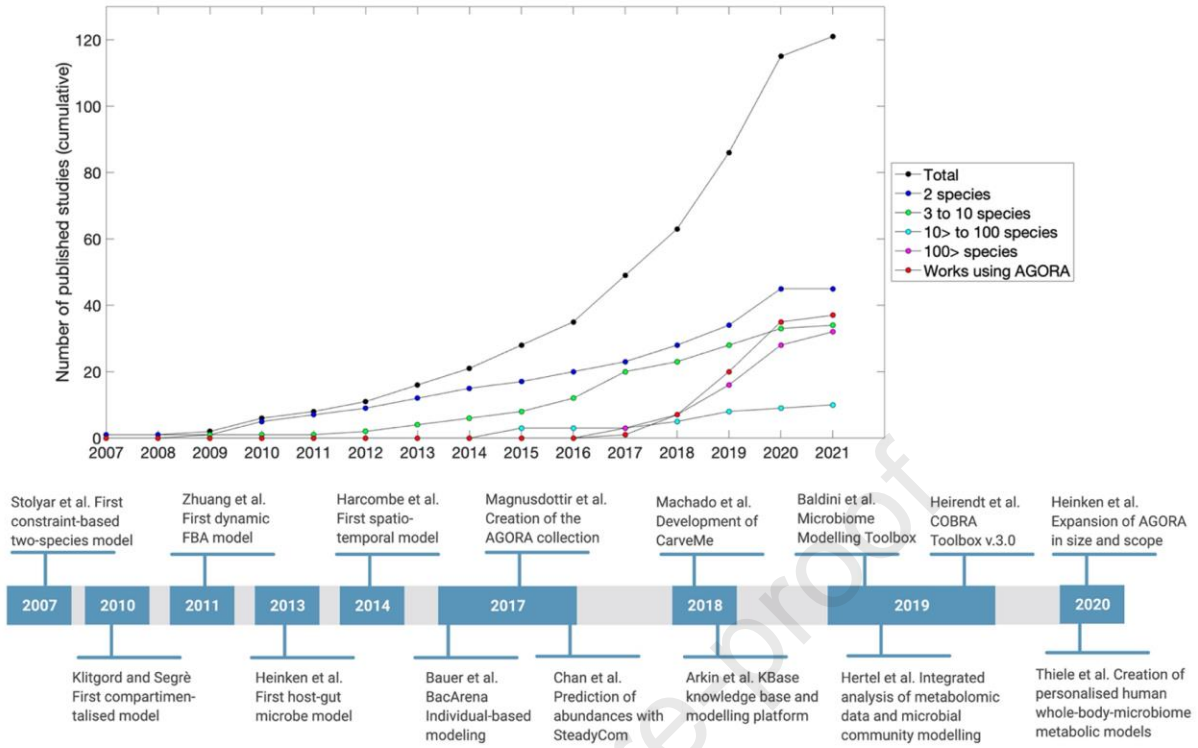
Recent years have seen a steep increase of COBRA-based multispecies modelling applications in both size and scope with more than 120 published studies (Figure 1a, Table S1). While the first years of community modelling focussed on small communities of ten or less species, an increasing number of works modelled communities on a microbiome scale (Figure 1a). These applications were enabled by a growing number of curated genome-scale reconstructions [28,61,105,115], which are freely available to the research community on websites, such as Virtual Metabolic Human (www.vmh.life) [116] and BiGG Models

(bigg.ucsd.edu) [115]. Moreover, to facilitate multiscale modelling, a variety of scalable, tractable COBRA-based methods and tools have been developed for the simulation of metabolic interactions, many of which provide easy to use interfaces and/or tutorials on platforms, such as the community-maintained COBRA Toolbox [117] and cobrapy [118]. Having these platforms allows researchers of different levels and fields to apply this powerful modelling approach to their research question.

Areas of application for COBRA multispecies modelling are manifold and include, as discussed in the respective sections, ecology in synthetic microbial communities, the human microbiome, animal-microbe interactions, plant-microbe interactions, bioremediation and biotechnology, and finally the microbiomes of environments, such as soil or ocean. The majority of constraint-based modelling studies to date focussed on the human gut microbiome (Figure 1b), while other important ecosystems, such as rumen, ocean, or soil, have been less commonly investigated through constraint-based multispecies modelling. This is in line with a generally observed research focus on the human microbiome, while other ecosystems, such as animal microbiomes, have received less attention so far [119]. In future efforts, as more metagenomic sequencing data from less studied microbiomes becomes available, microbial communities from these ecosystems could be distinguished by their context-specific flux profiles analogous to the methods for the stratification of patients and controls discussed in this review. While COBA multispecies modelling was previously hampered by the availability of curated genome-scale reconstructions, this limitation has been largely overcome by the availability of automated or semi-automated genome-scale reconstruction tools (see above). However, this is only the case for prokaryotes as fewer high-quality reconstructions of eukaryotes, such as non-human host organisms, are available [120]. Other yet existing limitations of the COBRA approach include the steady-state assumption and the prediction of fluxes rather than concentrations, which can be partially overcome by using dynamic flux balance analysis methods [44].

Taken together, we expect that COBRA microbiome modelling will continue to be a valuable tool for elucidating how microbiome community structure translates into function. Future studies could see the use of COBRA microbiome models combined with experimental approaches and meta-omics techniques to answer key questions on the structure and function of microbial ecosystems, with potential applications spanning from theoretical ecology, biomedicine, and industry, to bioremediation and conservation of the environment.

a) Growing number and milestones of COBRA multispecies modelling



b) Areas of application of COBRA multispecies modelling

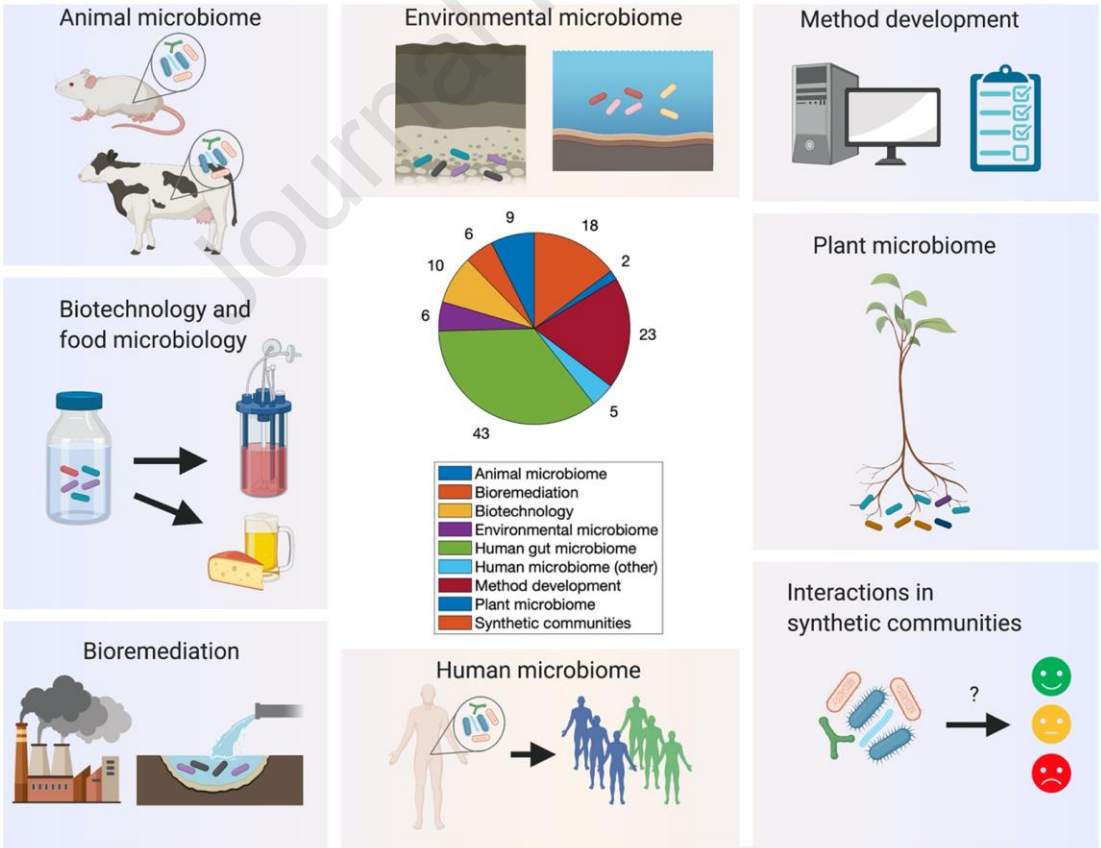


Figure 1: Increasing number, size, and scope of multi-species constraint-based modelling studies. a) Published studies (cumulative) that used constraint-based modelling to predict the interactions between and emergent properties of two or more species. A timeline of key milestones in the development of models and tools for multi-species modelling is also shown. b) Areas of application identified for the 122 studies that used constraint-based modelling to simulate multispecies interactions. Shown is the main focus of the modelling section in the study. Detail on the 122 studies can be found in Table S1. Created with BioRender.com.

Table S1: List of published applications of COBRA multi-species modelling.

Acknowledgements

This study was funded by grants from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 757922) to IT, by the National Institute on Aging grants (1RF1AG058942-01 and 1U19AG063744-01), and by the EMBO short-term fellowship 8720 to A.B.

Reference annotations

*Of special interest

Chng et al, Nat Ecol Evol 2020, 4:1256-1267.

Microbial taxa associated with robust recovery of diversity after antibiotic depletion have been recovered from 500 metagenomes. The food webs between these recovery-associated bacterial species (RABs) have been explored through metabolic modelling and revealed an enrichment in degradation capabilities of mucins and plant- and animal-associated carbohydrates.

Curran et al, Elife 2020, 9

The interactions between the parasitic worm *Brugia malayi*, the agent of lymphatic filariasis, and its bacterial obligate endosymbiont *Wolbachia* have been modelled and potential drug targets have been predicted. Three predicted targets have been experimentally validated and confirmed to have anti-filarial activity.

diCenzo et al, Nat Commun 2020, 11:2574.

A compartmentalised whole-organism reconstruction of the legume *Medicago truncatula* was joined with a reconstruction of its rhizobial endosymbiont *Sinorhizobium meliloti*. The combined model, called 'Virtual Nodule Environment' (ViNE), enables simulating the symbiotic nitrogen fixation by the bacterium and its interactions with the host plant.

Effenberger et al, J Crohns Colitis 2020.

Through constraint-based modelling, the butyrate production capacity of the microbiomes from IBD patients undergoing either azathioprine or anti-tumor-necrosis-factor antibodies therapy was predicted. The predicted butyrate production capacity of patients in remission was 1.7 higher compared to patients without remission and thus indicative of treatment success.

Heinken et al, bioRxiv 2020

AGORA2 is introduced, a resource of over 7,000 curated genome-scale-reconstructions of human microbes. AGORA2 also accounts for strain-specific reconstructions of microbial drug metabolism for over 100 prescription drugs.

Hertel et al Cell Rep 2019, 29:1767-1777 e1768

Personalised microbiome models of Parkinson's disease patients and age-matched controls were analysed in the context of metabolomic data from a separate cohort of patients and controls, revealing a potential role for the transsulphuration pathway in PD.

Mirhakkak et al, ISME J 2020

The interactions between intestinal fungi and prokaryotes was modelled by joining a reconstruction of the pathogenic fungi Candida albicans pairwise with over 900 reconstructions of gut bacteria and archaea. A negative effect of *Alistipes putredinis* on the growth of *C. albicans* has been predicted and confirmed through metagenomic sequencing of stool samples from 24 human subjects and *in vitro* experiments.

**Of outstanding interest

Machado et al Nat Ecol Evol 2021, 5:195-203.

Metabolic modelling of ecological dynamics was performed by simulating cooperation and competition in thousands of microbial communities of up to 40 members from diverse environments. Host-associated habitats were driven by cooperative interactions as in the red queen hypothesis while free-living habitats were dominated by resource competition.

Pryor et al, Cell 2019, 178:1299-1312 e1229.

Personalised microbiome modelling combined with *in vitro* screening revealed a role for bacterial agmatine production in the efficacy of the Type 2 diabetes drug metformin. Validation in the model nematode *C. elegans* confirmed model predictions and demonstrated an effect of metformin on the lifespan of the host.

Thiele et al, Mol Syst Biol 2020, 16:e8982.

An organ-resolved, sex-specific, anatomically corrected whole-body reconstruction of human metabolism is presented that can be personalised by integrating physiological parameters, diet, drug use, and context-specific microbiome models in several body sites.

References

1. Qian X, Chen L, Sui Y, Chen C, Zhang W, Zhou J, Dong W, Jiang M, Xin F, Ochsenreither K: **Biotechnological potential and applications of microbial consortia**. *Biotechnol Adv* 2020, **40**:107500.
2. Hutchins DA, Jansson JK, Remais JV, Rich VI, Singh BK, Trivedi P: **Climate change microbiology - problems and perspectives**. *Nat Rev Microbiol* 2019, **17**:391-396.
3. Lynch SV, Pedersen O: **The Human Intestinal Microbiome in Health and Disease**. *N Engl J Med* 2016, **375**:2369-2379.
4. Rau MH, Zeidan AA: **Constraint-based modeling in microbial food biotechnology**. *Biochem Soc Trans* 2018, **46**:249-260.
5. Grosskopf T, Soyer OS: **Synthetic microbial communities**. *Curr Opin Microbiol* 2014, **18**:72-77.
6. Widder S, Allen RJ, Pfeiffer T, Curtis TP, Wiuf C, Sloan WT, Cordero OX, Brown SP, Momeni B, Shou W, et al.: **Challenges in microbial ecology: building predictive understanding of community function and dynamics**. *ISME J* 2016, **10**:2557-2568.
7. Bengtsson-Palme J: **Microbial model communities: To understand complexity, harness the power of simplicity**. *Comput Struct Biotechnol J* 2020, **18**:3987-4001.
8. Campanaro S, Treu L, Rodriguez RL, Kovalovszki A, Ziels RM, Maus I, Zhu X, Kougias PG, Basile A, Luo G, et al.: **New insights from the biogas microbiome by comprehensive genome-resolved metagenomics of nearly 1600 species originating from multiple anaerobic digesters**. *Biotechnol Biofuels* 2020, **13**:25.
9. Creasy HH, Felix V, Aluvathingal J, Crabtree J, Ifeonu O, Matsumura J, McCracken C, Nickel L, Orvis J, Schor M, et al.: **HMPDACC: a Human Microbiome Project Multi-omic data resource**. *Nucleic Acids Res* 2021, **49**:D734-D742.
10. Fierer N: **Embracing the unknown: disentangling the complexities of the soil microbiome**. *Nat Rev Microbiol* 2017, **15**:579-590.
11. Sunagawa S, Coelho LP, Chaffron S, Kultima JR, Labadie K, Salazar G, Djahanschiri B, Zeller G, Mende DR, Alberti A, et al.: **Ocean plankton. Structure and function of the global ocean microbiome**. *Science* 2015, **348**:1261359.
12. Tavassoly I, Goldfarb J, Iyengar R: **Systems biology primer: the basic methods and approaches**. *Essays Biochem* 2018, **62**:487-500.
13. Dahal S, Yurkovich JT, Xu H, Palsson BO, Yang L: **Synthesizing Systems Biology Knowledge from Omics Using Genome-Scale Models**. *Proteomics* 2020, **20**:e1900282.
14. Palsson B: *Systems biology : properties of reconstructed networks*. Cambridge ; New York: Cambridge University Press; 2006.
15. Sen P, Oresic M: **Metabolic Modeling of Human Gut Microbiota on a Genome Scale: An Overview**. *Metabolites* 2019, **9**.
16. Zhang X, Li L, Butcher J, Stintzi A, Figeys D: **Advancing functional and translational microbiome research using meta-omics approaches**. *Microbiome* 2019, **7**:154.
17. O'Brien EJ, Monk JM, Palsson BO: **Using Genome-scale Models to Predict Biological Capabilities**. *Cell* 2015, **161**:971-987.
18. Orth JD, Thiele I, Palsson BO: **What is flux balance analysis?** *Nat Biotechnol* 2010, **28**:245-248.
19. Thiele I, Palsson BO: **A protocol for generating a high-quality genome-scale metabolic reconstruction**. *Nat Protoc* 2010, **5**:93-121.

20. Karp PD, Midford PE, Billington R, Kothari A, Krummenacker M, Latendresse M, Ong WK, Subhraveti P, Caspi R, Fulcher C, et al.: **Pathway Tools version 23.0 update: software for pathway/genome informatics and systems biology**. *Brief Bioinform* 2021, **22**:109-126.
21. Arkin AP, Cottingham RW, Henry CS, Harris NL, Stevens RL, Maslov S, Dehal P, Ware D, Perez F, Canon S, et al.: **KBase: The United States Department of Energy Systems Biology Knowledgebase**. *Nat Biotechnol* 2018, **36**:566-569.
22. Henry CS, DeJongh M, Best AA, Frybarger PM, Linsay B, Stevens RL: **High-throughput generation, optimization and analysis of genome-scale metabolic models**. *Nat Biotechnol* 2010, **28**:977-982.
23. Liberal R, Pinney JW: **Simple topological properties predict functional misannotations in a metabolic network**. *Bioinformatics* 2013, **29**:i154-161.
24. Hamilton JJ, Reed JL: **Software platforms to facilitate reconstructing genome-scale metabolic networks**. *Environ Microbiol* 2014, **16**:49-59.
25. Mendoza SN, Olivier BG, Molenaar D, Teusink B: **A systematic assessment of current genome-scale metabolic reconstruction tools**. *Genome Biol* 2019, **20**:158.
26. Heinken A, Ravcheev DA, Baldini F, Heirendt L, Fleming RMT, Thiele I: **Systematic assessment of secondary bile acid metabolism in gut microbes reveals distinct metabolic capabilities in inflammatory bowel disease**. *Microbiome* 2019, **7**:75.
27. Magnusdottir S, Ravcheev D, de Crecy-Lagard V, Thiele I: **Systematic genome assessment of B-vitamin biosynthesis suggests co-operation among gut microbes**. *Front Genet* 2015, **6**:148.
28. Machado D, Andrejev S, Tramontano M, Patil KR: **Fast automated reconstruction of genome-scale metabolic models for microbial species and communities**. *Nucleic Acids Res* 2018, **46**:7542-7553.
29. Zimmermann J, Kaleta C, Waschina S: **gapseq: informed prediction of bacterial metabolic pathways and reconstruction of accurate metabolic models**. *Genome Biol* 2021, **22**:81.
30. Heinken A, Magnusdottir S, Thiele I: **DEMETER: Data-driven refinement of genome-scale reconstructions**. *submitted* 2021, **https://github.com/opencobra/cobratoolbox/tree/master/src/reconstruction/demeter**.
31. Norsigian CJ, Fang X, Seif Y, Monk JM, Palsson BO: **A workflow for generating multi-strain genome-scale metabolic models of prokaryotes**. *Nat Protoc* 2020, **15**:1-14.
32. Zorrilla F, Patil KR, Zelezniak A: **metaGEM: reconstruction of genome scale metabolic models directly from metagenomes**. *bioRxiv* 2021:2020.2012.2031.424982.
33. Bidkhori G, Lee S, Edwards LA, Chatelier EL, Almeida M, Ezzamouri B, Onate FP, Ponte N, Shawcross DL, Proctor G, et al.: **The Reactobiome Unravels a New Paradigm in Human Gut Microbiome Metabolism**. *bioRxiv* 2021:2021.2002.2001.428114.
34. Oberhardt MA, Palsson BO, Papin JA: **Applications of genome-scale metabolic reconstructions**. *Mol Syst Biol* 2009, **5**:320.
35. Zengler K, Palsson BO: **A road map for the development of community systems (CoSy) biology**. *Nat Rev Microbiol* 2012, **10**:366-372.
36. Thiele I, Heinken A, Fleming RM: **A systems biology approach to studying the role of microbes in human health**. *Curr Opin Biotechnol* 2013, **24**:4-12.

37. Zomorrodi AR, Maranas CD: **OptCom: a multi-level optimization framework for the metabolic modeling and analysis of microbial communities**. *PLoS Comput Biol* 2012, **8**:e1002363.
38. Khandelwal RA, Olivier BG, Roling WF, Teusink B, Bruggeman FJ: **Community flux balance analysis for microbial consortia at balanced growth**. *PLoS One* 2013, **8**:e64567.
39. Chan SHJ, Simons MN, Maranas CD: **SteadyCom: Predicting microbial abundances while ensuring community stability**. *PLoS Comput Biol* 2017, **13**:e1005539.
40. Baldini F, Heinken A, Heirendt L, Magnusdottir S, Fleming RMT, Thiele I: **The Microbiome Modeling Toolbox: from microbial interactions to personalized microbial communities**. *Bioinformatics* 2018.
41. Diener C, Gibbons SM, Resendis-Antonio O: **MICOM: Metagenome-Scale Modeling To Infer Metabolic Interactions in the Gut Microbiota**. *mSystems* 2020, **5**.
42. Mahadevan R, Edwards JS, Doyle FJ, 3rd: **Dynamic flux balance analysis of diauxic growth in Escherichia coli**. *Biophys J* 2002, **83**:1331-1340.
43. Zhuang K, Izallalen M, Mouser P, Richter H, Risso C, Mahadevan R, Lovley DR: **Genome-scale dynamic modeling of the competition between Rhodoferax and Geobacter in anoxic subsurface environments**. *ISME J* 2011, **5**:305-316.
44. Gottstein W, Olivier BG, Bruggeman FJ, Teusink B: **Constraint-based stoichiometric modelling from single organisms to microbial communities**. *J R Soc Interface* 2016, **13**.
45. Zomorrodi AR, Islam MM, Maranas CD: **d-OptCom: Dynamic multi-level and multi-objective metabolic modeling of microbial communities**. *ACS Synth Biol* 2014, **3**:247-257.
46. Garcia-Jimenez B, Garcia JL, Nogales J: **FLYCOP: metabolic modeling-based analysis and engineering microbial communities**. *Bioinformatics* 2018, **34**:i954-i963.
47. Harcombe WR, Riehl WJ, Dukovski I, Granger BR, Betts A, Lang AH, Bonilla G, Kar A, Leiby N, Mehta P, et al.: **Metabolic resource allocation in individual microbes determines ecosystem interactions and spatial dynamics**. *Cell Rep* 2014, **7**:1104-1115.
48. Dukovski I, Bajić D, Chacón JM, Quintin M, ila JCC, Sulheim S, Pacheco AR, Bernstein DB, Rieh WJ, Korolev KS, et al.: **Computation Of Microbial Ecosystems in Time and Space (COMETS): An open source collaborative platform for modeling ecosystems metabolism**. *arXiv* 2020.
49. Bauer E, Zimmermann J, Baldini F, Thiele I, Kaleta C: **BacArena: Individual-based metabolic modeling of heterogeneous microbes in complex communities**. *PLoS Comput Biol* 2017, **13**:e1005544.
50. Karimian E, Motamedian E: **ACBM: An Integrated Agent and Constraint Based Modeling Framework for Simulation of Microbial Communities**. *Sci Rep* 2020, **10**:8695.
51. Borer B, Ataman M, Hatzimanikatis V, Or D: **Modeling metabolic networks of individual bacterial agents in heterogeneous and dynamic soil habitats (IndiMeSH)**. *PLoS Comput Biol* 2019, **15**:e1007127.
52. Bauer E, Thiele I: **From Network Analysis to Functional Metabolic Modeling of the Human Gut Microbiota**. *mSystems* 2018, **3**.
53. Biggs MB, Medlock GL, Kolling GL, Papin JA: **Metabolic network modeling of microbial communities**. *Wiley Interdiscip Rev Syst Biol Med* 2015, **7**:317-334.
54. Succurro A, Ebenhoh O: **Review and perspective on mathematical modeling of microbial ecosystems**. *Biochem Soc Trans* 2018, **46**:403-412.

55. Zomorrodi AR, Segre D: **Synthetic Ecology of Microbes: Mathematical Models and Applications**. *J Mol Biol* 2016, **428**:837-861.
56. Harcombe WR, Betts A, Shapiro JW, Marx CJ: **Adding biotic complexity alters the metabolic benefits of mutualism**. *Evolution* 2016, **70**:1871-1881.
57. McNally CP, Borenstein E: **Metabolic model-based analysis of the emergence of bacterial cross-feeding via extensive gene loss**. *BMC Syst Biol* 2018, **12**:69.
58. Zomorrodi AR, Segre D: **Genome-driven evolutionary game theory helps understand the rise of metabolic interdependencies in microbial communities**. *Nat Commun* 2017, **8**:1563.
59. Pacheco AR, Moel M, Segre D: **Costless metabolic secretions as drivers of interspecies interactions in microbial ecosystems**. *Nat Commun* 2019, **10**:103.
60. Heinken A, Thiele I: **Anoxic Conditions Promote Species-Specific Mutualism between Gut Microbes In Silico**. *Appl Environ Microbiol* 2015, **81**:4049-4061.
61. Magnusdottir S, Heinken A, Kutt L, Ravcheev DA, Bauer E, Noronha A, Greenhalgh K, Jager C, Baginska J, Wilmes P, et al.: **Generation of genome-scale metabolic reconstructions for 773 members of the human gut microbiota**. *Nat Biotechnol* 2017, **35**:81-89.
62. Machado D, Maistrenko OM, Andrejev S, Kim Y, Bork P, Patil KR, Patil KR: **Polarization of microbial communities between competitive and cooperative metabolism**. *Nat Ecol Evol* 2021, **5**:195-203.
63. Fan Y, Pedersen O: **Gut microbiota in human metabolic health and disease**. *Nat Rev Microbiol* 2021, **19**:55-71.
64. Nielsen J: **Systems Biology of Metabolism: A Driver for Developing Personalized and Precision Medicine**. *Cell Metab* 2017, **25**:572-579.
65. Kashyap PC, Chia N, Nelson H, Segal E, Elinav E: **Microbiome at the Frontier of Personalized Medicine**. *Mayo Clin Proc* 2017, **92**:1855-1864.
66. Thiele I, Clancy CM, Heinken A, Fleming RMT: **Quantitative systems pharmacology and the personalized drug–microbiota–diet axis**. *Current Opinion in Systems Biology* 2017, **4**:43-52.
67. van der Ark KCH, van Heck RGA, Martins Dos Santos VAP, Belzer C, de Vos WM: **More than just a gut feeling: constraint-based genome-scale metabolic models for predicting functions of human intestinal microbes**. *Microbiome* 2017, **5**:78.
68. Thiele I, Clancy CM, Heinken A, Fleming RMT: **Quantitative systems pharmacology and the personalized drug-microbiota-diet axis**. *Curr Opin Syst Biol* 2017, **4**:43-52.
69. Heinken A, Acharya G, Ravcheev DA, Hertel J, Nyga M, Okpala OE, Hogan M, Magnúsdóttir S, Martinelli F, Preciat G, et al.: **AGORA2: Large scale reconstruction of the microbiome highlights wide-spread drug-metabolising capacities**. *bioRxiv* 2020:2020.2011.2009.375451.
70. Shoaie S, Ghaffari P, Kovatcheva-Datchary P, Mardinoglu A, Sen P, Pujos-Guillot E, de Wouters T, Juste C, Rizkalla S, Chilloux J, et al.: **Quantifying Diet-Induced Metabolic Changes of the Human Gut Microbiome**. *Cell Metab* 2015, **22**:320-331.
71. Shoaie S, Karlsson F, Mardinoglu A, Nookaew I, Bordel S, Nielsen J: **Understanding the interactions between bacteria in the human gut through metabolic modeling**. *Sci Rep* 2013, **3**:2532.
72. Heinken A, Thiele I: **Systematic prediction of health-relevant human-microbial co-metabolism through a computational framework**. *Gut Microbes* 2015, **6**:120-130.

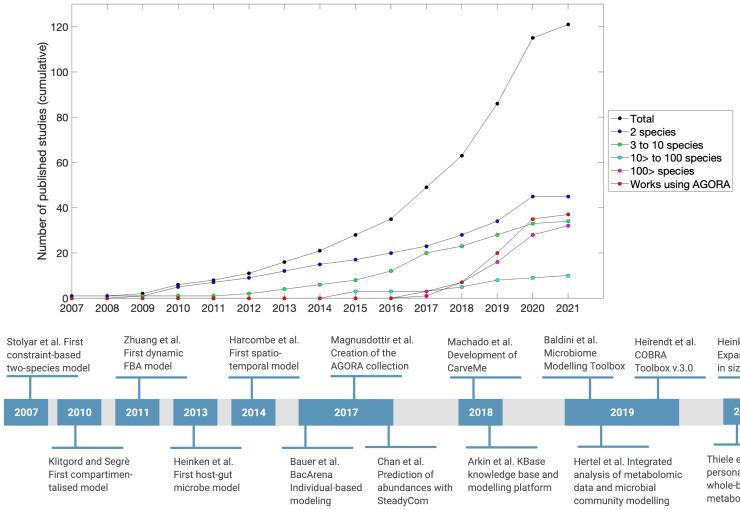
73. Lavelle A, Sokol H: **Gut microbiota-derived metabolites as key actors in inflammatory bowel disease**. *Nat Rev Gastroenterol Hepatol* 2020, **17**:223-237.
74. Schirmer M, Garner A, Vlamakis H, Xavier RJ: **Microbial genes and pathways in inflammatory bowel disease**. *Nat Rev Microbiol* 2019, **17**:497-511.
75. Heinken A, Thiele I: **Systematic interrogation of the distinct metabolic potential in gut microbiomes of inflammatory bowel disease patients with dysbiosis**. *bioRxiv* 2019:640649.
76. Effenberger M, Reider S, Waschina S, Bronowski C, Enrich B, Adolph TE, Koch R, Moschen AR, Rosenstiel P, Aden K, et al.: **Microbial butyrate synthesis indicates therapeutic efficacy of azathioprine in IBD patients**. *J Crohns Colitis* 2020.
77. Aden K, Rehman A, Waschina S, Pan WH, Walker A, Lucio M, Nunez AM, Bharti R, Zimmerman J, Bethge J, et al.: **Metabolic Functions of Gut Microbes Associate With Efficacy of Tumor Necrosis Factor Antagonists in Patients With Inflammatory Bowel Diseases**. *Gastroenterology* 2019, **157**:1279-1292 e1211.
78. Yilmaz B, Juillerat P, Oyas O, Ramon C, Bravo FD, Franc Y, Fournier N, Michetti P, Mueller C, Geuking M, et al.: **Microbial network disturbances in relapsing refractory Crohn's disease**. *Nat Med* 2019, **25**:323-336.
79. Bauer E, Thiele I: **From metagenomic data to personalized in silico microbiotas: predicting dietary supplements for Crohn's disease**. *NPJ Syst Biol Appl* 2018, **4**:27.
80. Wirbel J, Pyl PT, Kartal E, Zych K, Kashani A, Milanese A, Fleck JS, Voigt AY, Palleja A, Ponnudurai R, et al.: **Meta-analysis of fecal metagenomes reveals global microbial signatures that are specific for colorectal cancer**. *Nat Med* 2019, **25**:679-689.
81. Hertel J, Heinken A, Martinelli F, Thiele I: **Integration of constraint-based modelling with faecal metabolomics reveals large deleterious effects of Fusobacteria species on community butyrate production**. *bioRxiv* 2020:2020.2009.2009.290494.
82. Hale VL, Jeraldo P, Chen J, Mundy M, Yao J, Priya S, Keeney G, Lyke K, Ridlon J, White BA, et al.: **Distinct microbes, metabolites, and ecologies define the microbiome in deficient and proficient mismatch repair colorectal cancers**. *Genome Med* 2018, **10**:78.
83. Hale VL, Jeraldo P, Mundy M, Yao J, Keeney G, Scott N, Cheek EH, Davidson J, Greene M, Martinez C, et al.: **Synthesis of multi-omic data and community metabolic models reveals insights into the role of hydrogen sulfide in colon cancer**. *Methods* 2018, **149**:59-68.
84. Garza DR, Taddese R, Wirbel J, Zeller G, Boleij A, Huynen MA, Dutilh BE: **Metabolic models predict bacterial passengers in colorectal cancer**. *Cancer Metab* 2020, **8**:3.
85. Nishiwaki H, Ito M, Ishida T, Hamaguchi T, Maeda T, Kashihara K, Tsuboi Y, Ueyama J, Shimamura T, Mori H, et al.: **Meta-Analysis of Gut Dysbiosis in Parkinson's Disease**. *Mov Disord* 2020, **35**:1626-1635.
86. Hertel J, Harms AC, Heinken A, Baldini F, Thinnes CC, Glaab E, Vasco DA, Pietzner M, Stewart ID, Wareham NJ, et al.: **Integrated Analyses of Microbiome and Longitudinal Metabolome Data Reveal Microbial-Host Interactions on Sulfur Metabolism in Parkinson's Disease**. *Cell Rep* 2019, **29**:1767-1777 e1768.
87. Baldini F, Hertel J, Sandt E, Thinnes CC, Neuberger-Castillo L, Pavelka L, Betsou F, Krüger R, Thiele I: **Parkinson's disease-associated alterations of the gut microbiome can invoke disease-relevant metabolic changes**. *bioRxiv* 2019:691030.

88. Henson MA, Orazi G, Phalak P, O'Toole GA: **Metabolic Modeling of Cystic Fibrosis Airway Communities Predicts Mechanisms of Pathogen Dominance**. *mSystems* 2019, **4**.
89. Mirhakkak MH, Schauble S, Klassert TE, Brunke S, Brandt P, Loos D, Uribe RV, Senne de Oliveira Lino F, Ni Y, Vylkova S, et al.: **Metabolic modeling predicts specific gut bacteria as key determinants for Candida albicans colonization levels**. *ISME J* 2020.
90. Renz A, Widerspick L, Drager A: **FBA reveals guanylate kinase as a potential target for antiviral therapies against SARS-CoV-2**. *Bioinformatics* 2020, **36**:i813-i821.
91. Thiele I, Sahoo S, Heinken A, Hertel J, Heirendt L, Aurich MK, Fleming RM: **Personalized whole-body models integrate metabolism, physiology, and the gut microbiome**. *Mol Syst Biol* 2020, **16**:e8982.
92. Heinken A, Sahoo S, Fleming RM, Thiele I: **Systems-level characterization of a host-microbe metabolic symbiosis in the mammalian gut**. *Gut Microbes* 2013, **4**:28-40.
93. Ankrah NYD, Luan J, Douglas AE: **Cooperative Metabolism in a Three-Partner Insect-Bacterial Symbiosis Revealed by Metabolic Modeling**. *J Bacteriol* 2017, **199**.
94. Ankrah NYD, Wilkes RA, Zhang FQ, Aristilde L, Douglas AE: **The Metabolome of Associations between Xylem-Feeding Insects and their Bacterial Symbionts**. *J Chem Ecol* 2020, **46**:735-744.
95. Blow F, Ankrah NYD, Clark N, Koo I, Allman EL, Liu Q, Anitha M, Patterson AD, Douglas AE: **Impact of Facultative Bacteria on the Metabolic Function of an Obligate Insect-Bacterial Symbiosis**. *mBio* 2020, **11**.
96. Curran DM, Grote A, Nursimulu N, Geber A, Voronin D, Jones DR, Ghedin E, Parkinson J: **Modeling the metabolic interplay between a parasitic worm and its bacterial endosymbiont allows the identification of novel drug targets**. *Elife* 2020, **9**.
97. Pryor R, Norvaisas P, Marinos G, Best L, Thingholm LB, Quintaneiro LM, De Haes W, Esser D, Waschina S, Lujan C, et al.: **Host-Microbe-Drug-Nutrient Screen Identifies Bacterial Effectors of Metformin Therapy**. *Cell* 2019, **178**:1299-1312 e1229.
98. Tobalina L, Bargiela R, Pey J, Herbst FA, Lores I, Rojo D, Barbas C, Pelaez AI, Sanchez J, von Bergen M, et al.: **Context-specific metabolic network reconstruction of a naphthalene-degrading bacterial community guided by metaproteomic data**. *Bioinformatics* 2015, **31**:1771-1779.
99. Xu X, Zarecki R, Medina S, Ofaim S, Liu X, Chen C, Hu S, Brom D, Gat D, Porob S, et al.: **Modeling microbial communities from atrazine contaminated soils promotes the development of biostimulation solutions**. *ISME J* 2019, **13**:494-508.
100. Perisin MA, Sund CJ: **Human gut microbe co-cultures have greater potential than monocultures for food waste remediation to commodity chemicals**. *Sci Rep* 2018, **8**:15594.
101. Garcia-Jimenez B, Torres-Bacete J, Nogales J: **Metabolic modelling approaches for describing and engineering microbial communities**. *Comput Struct Biotechnol J* 2021, **19**:226-246.
102. Ye C, Zou W, Xu N, Liu L: **Metabolic model reconstruction and analysis of an artificial microbial ecosystem for vitamin C production**. *J Biotechnol* 2014, **182-183**:61-67.
103. Koch S, Benndorf D, Fronk K, Reichl U, Klamt S: **Predicting compositions of microbial communities from stoichiometric models with applications for the biogas process**. *Biotechnol Biofuels* 2016, **9**:17.
104. Benito-Vaquerizo S, Diender M, Parera Olm I, Martins Dos Santos VAP, Schaap PJ, Sousa DZ, Suarez-Diez M: **Modeling a co-culture of Clostridium autoethanogenum**

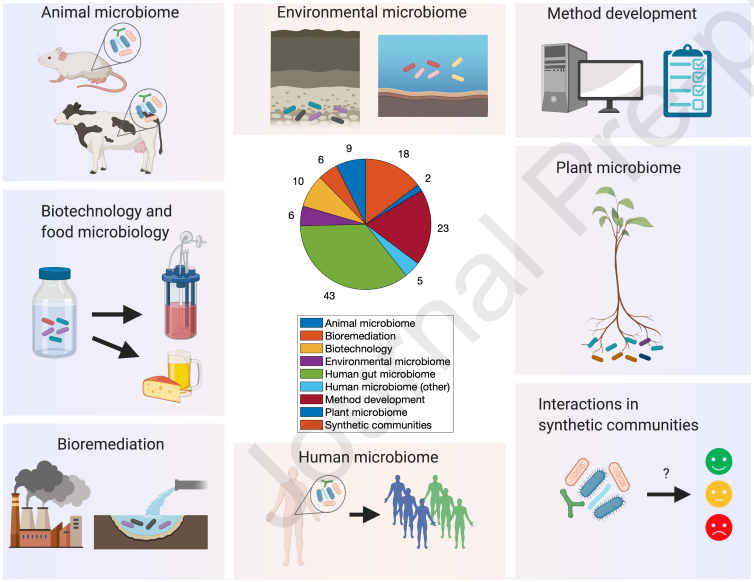
and Clostridium kluyveri to increase syngas conversion to medium-chain fatty-acids. *Comput Struct Biotechnol J* 2020, **18**:3255-3266.
105. Basile A, Campanaro S, Kovalovszki A, Zampieri G, Rossi A, Angelidaki I, Valle G, Treu L: **Revealing metabolic mechanisms of interaction in the anaerobic digestion microbiome by flux balance analysis**. *Metab Eng* 2020, **62**:138-149.
106. Yan M, Treu L, Zhu X, Tian H, Basile A, Fotidis IA, Campanaro S, Angelidaki I: **Insights into Ammonia Adaptation and Methanogenic Precursor Oxidation by Genome-Centric Analysis**. *Environ Sci Technol* 2020, **54**:12568-12582.
107. Teusink B, Molenaar D: **Systems biology of lactic acid bacteria: For food and thought**. *Curr Opin Syst Biol* 2017, **6**:7-13.
108. Ibrahim M, Raman K: **Two-species community design of Lactic Acid Bacteria for optimal production of Lactate**. *bioRxiv* 2020:2020.2010.2024.353805.
109. Islam MM, Le T, Daggumati SR, Saha R: **Investigation of microbial community interactions between Lake Washington methanotrophs using -------genome-scale metabolic modeling**. *PeerJ* 2020, **8**:e9464.
110. Nagarajan H, Embree M, Rotaru AE, Shrestha PM, Feist AM, Palsson BO, Lovley DR, Zengler K: **Characterization and modelling of interspecies electron transfer mechanisms and microbial community dynamics of a syntrophic association**. *Nat Commun* 2013, **4**:2809.
111. Alvarez-Silva MC, Alvarez-Yela AC, Gomez-Cano F, Zambrano MM, Husserl J, Danies G, Restrepo S, Gonzalez-Barrios AF: **Compartmentalized metabolic network reconstruction of microbial communities to determine the effect of agricultural intervention on soils**. *PLoS One* 2017, **12**:e0181826.
112. Fondi M, Fani R: **Constraint-based metabolic modelling of marine microbes and communities**. *Mar Genomics* 2017, **34**:1-10.
113. diCenzo GC, Tesi M, Pfau T, Mengoni A, Fondi M: **Genome-scale metabolic reconstruction of the symbiosis between a leguminous plant and a nitrogen-fixing bacterium**. *Nat Commun* 2020, **11**:2574.
114. Pfau T, Christian N, Masakapalli SK, Sweetlove LJ, Poolman MG, Ebenhoh O: **The intertwined metabolism during symbiotic nitrogen fixation elucidated by metabolic modelling**. *Sci Rep* 2018, **8**:12504.
115. Norsigian CJ, Pusarla N, McConn JL, Yurkovich JT, Drager A, Palsson BO, King Z: **BiGG Models 2020: multi-strain genome-scale models and expansion across the phylogenetic tree**. *Nucleic Acids Res* 2020, **48**:D402-D406.
116. Noronha A, Modamio J, Jarosz Y, Guerard E, Sompairac N, Preciat G, Danielsdottir AD, Krecke M, Merten D, Haraldsdottir HS, et al.: **The Virtual Metabolic Human database: integrating human and gut microbiome metabolism with nutrition and disease**. *Nucleic Acids Res* 2019, **47**:D614-D624.
117. Heirendt L, Arreckx S, Pfau T, Mendoza SN, Richelle A, Heinken A, Haraldsdottir HS, Wachowiak J, Keating SM, Vlasov V, et al.: **Creation and analysis of biochemical constraint-based models using the COBRA Toolbox v.3.0**. *Nat Protoc* 2019, **14**:639-702.
118. Ebrahim A, Lerman JA, Palsson BO, Hyduke DR: **COBRApy: COnstraints-Based Reconstruction and Analysis for Python**. *BMC Syst Biol* 2013, **7**:74.
119. Levin D, Raab N, Pinto Y, Rothschild D, Zanir G, Godneva A, Mellul N, Futorian D, Gal D, Leviatan S, et al.: **Diversity and functional landscapes in the microbiota of animals in the wild**. *Science* 2021, **372**.

744 120. Gu C, Kim GB, Kim WJ, Kim HU, Lee SY: **Current status and applications of genome-**
745 **scale metabolic models**. *Genome Biol* 2019, **20**:121.
746

a) Growing number and milestones of COBRA multispecies modelling



b) Areas of application of COBRA multispecies modelling



Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: