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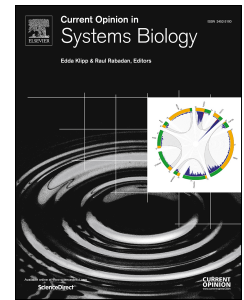
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Advances in constraint-based modelling of microbial communities

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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 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

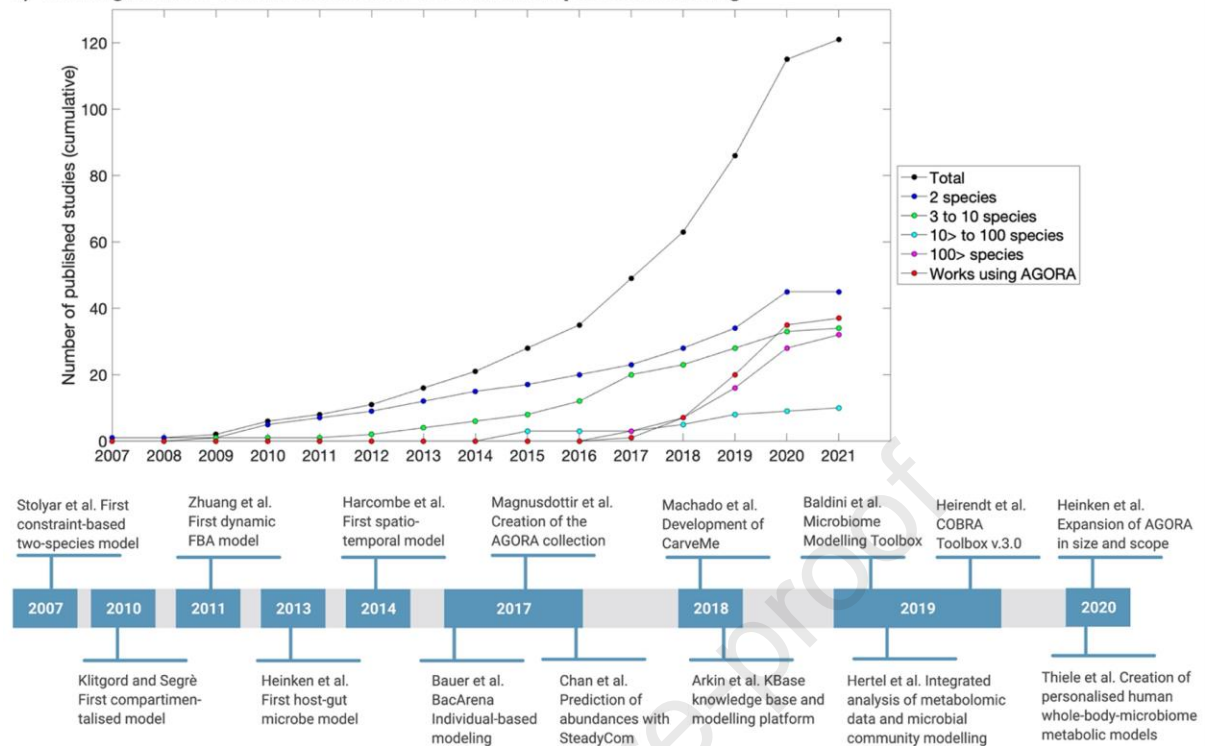
Recent years have seen a steep increase of COBRA-based multispecies modelling applications in both size and scope with more than 120 published studies (Figure 1a, Table S1). While the first years of community modelling focussed on small communities of ten or less species, an increasing number of works 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.

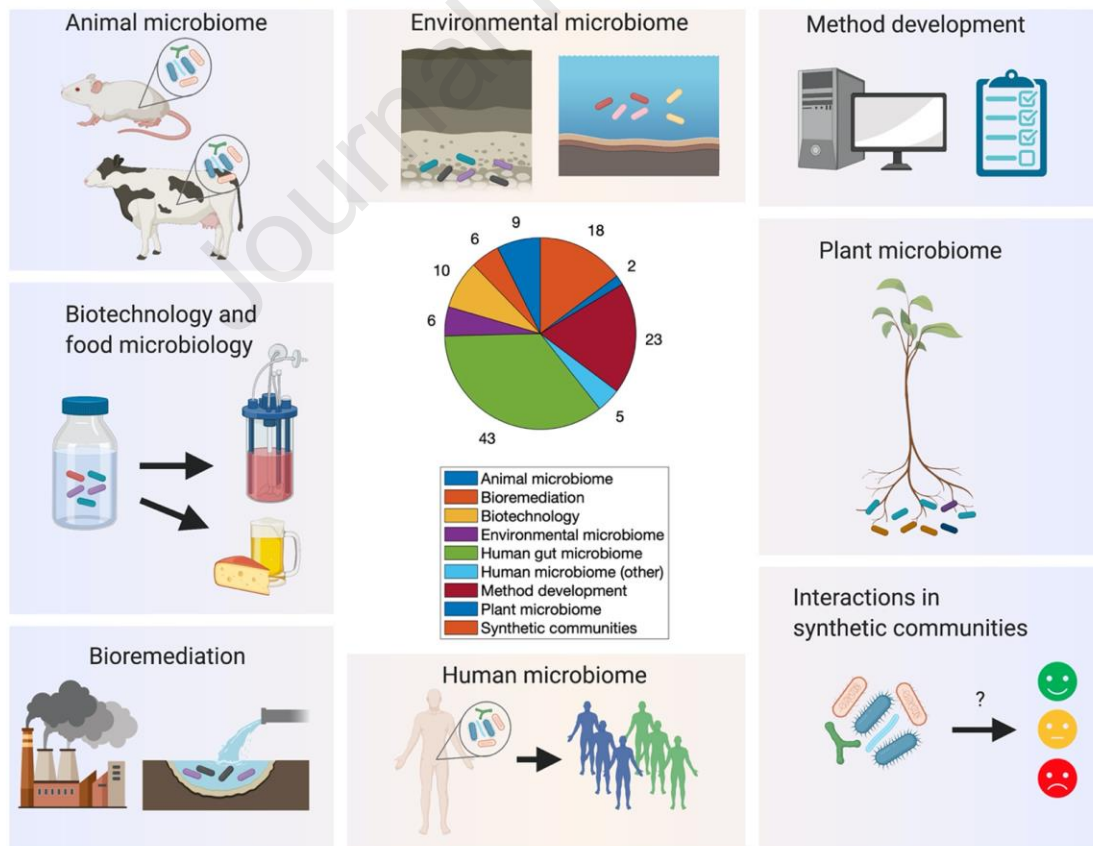
Areas of application for COBRA multispecies modelling are manifold and include, as discussed in the respective sections, ecology in synthetic microbial communities, the human microbiome, animal-microbe interactions, plant-microbe interactions, bioremediation and biotechnology, and finally the microbiomes 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 COBRA 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



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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.

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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.

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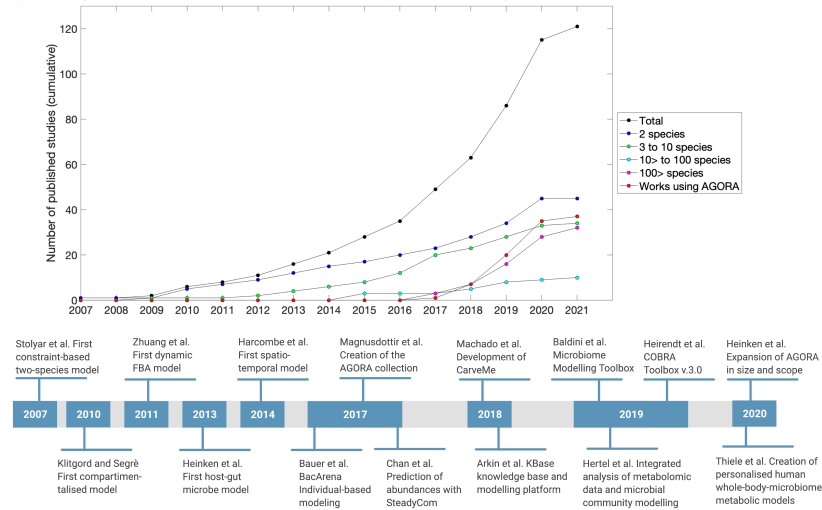
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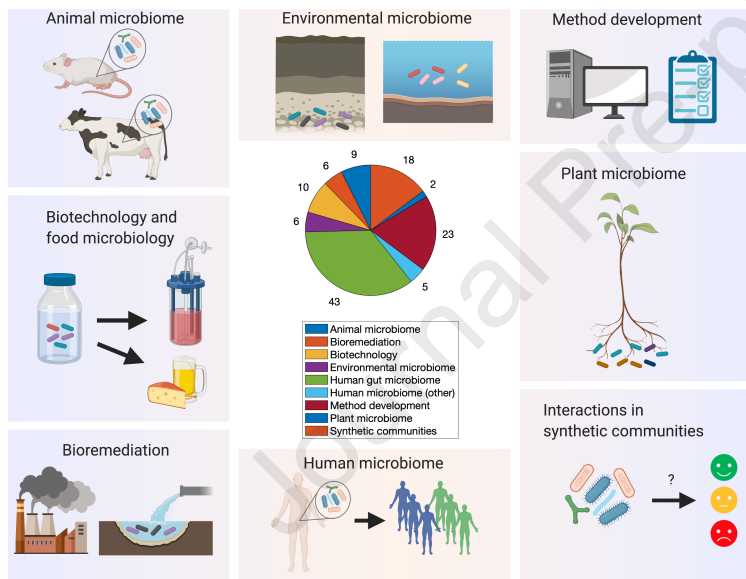
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Journal Pre-proof

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:

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