Establishing a Baseline for a Healthy Gut Microbiome

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Abstract. The human gut microbiome, a complex and dynamic ecosystem, plays a critical role in host health. Genome-scale metabolic modeling enables mechanistic exploration of microbial metabolism and interspecies interactions within this environment. In this study, we aim to integrate curated taxonomic profiles from GutFeelingKB with genome-scale metabolic models from the BiGG database to simulate the metabolic behavior of health-associated gut microbes. Using SMETANA, MICOM, and SteadyCom, we will explore metabolic cooperation, resource competition, and community stability. The resulting metabolic profiles will be analyzed using dimensionality reduction and clustering techniques to identify microbial ecotypes and metabolic subnetworks characteristic of a healthy gut. The main goal of this work is to contribute to the functional stratification of the gut microbiome and support the development of simulation-driven approaches to microbiome-based diagnostics and therapeutic design.

Keywords: Gut Microbiome Health, Genome-scale metabolic models, Dysbiosis, Machine Learning, Metagenomics, Microbial community modeling

1 Contextualization and Motivation

1.1 Gut Microbiome in Health and Disease

The gut microbiome is a diverse and metabolically active microbial community that contributes to essential physiological processes, including digestion, nutrient absorption, immune regulation, and the production of bioactive compounds [1,2]. It helps maintain homeostasis, protects against pathogens, and modulates inflammatory responses [3]. Its composition is influenced by various factors that modulate microbial diversity and function, such as genetics [4], diet [5], age [6], geography [6], and medications [7], all of which contribute to differences in microbiome resilience and susceptibility to microbial imbalance often associated with disease, more commonly known as dysbiosis [8]. Dysbiosis has been linked to a wide range of diseases, including inflammatory bowel diseases [9,10], metabolic disorders like obesity and type 2 diabetes[11,12], and neurological conditions such as depression and neurodegeneration [13,14].

Despite considerable inter-individual variability, studies identified a possible "healthy core" microbiome, comprising taxa and metabolic functions that are consistently associated with health across individuals and populations[15].

Given the microbiome's influence on host health [16], there has been a surge of interest in microbiome-based interventions aimed at restoring microbial balance and reducing disease risk. Strategies such as pre and probiotics [17], fecal microbiota transplantation [18], and dietary modifications have been explored to modulate microbial communities and improve clinical outcomes [19]. In parallel to these therapeutic approaches, advances in high-throughput sequencing and computational biology have driven a broader integration of microbiome research into precision medicine and systems biology. These developments have enabled the identification of microbiome-derived biomarkers and the modeling of complex host–microbe interactions[20,21]. Yet, the high degree of inter-individual variability and the functional complexity of microbial ecosystems remain major challenges. Addressing these requires integrative analytical frameworks that combine multi-omics data, metabolic modeling, and Machine Learning (ML) to better characterize microbiome in health and disease [20].

1.2 Aims

This project aims to characterize the metabolic landscape of the healthy gut microbiome by combining community-level metabolic simulations and unsupervised ML. Starting from taxonomic profiles curated in the Gut Feeling Knowledge Base (GutFeelingKB), we retrieve Genome-scale Metabolic Models (GEMs) for representative microbial strains from the BiGG Models database. These models are used to simulate microbial interactions and metabolic behavior using Species METabolic interaction ANAlysis (SMETANA), Microbial Community Modeling (MICOM), and Steady-state Community modeling (SteadyCom). The resulting simulation outputs are analyzed using dimensionality reduction techniques coupled with clustering algorithms to identify microbial ecotypes and health-associated metabolic subnetworks. The aim is to produce a functional, simulation-based stratification of healthy microbial communities, forming the basis for understanding metabolic organization in the healthy gut.

2 State-of-the-Art

2.1 Metagenomics in Microbiome Research

Metagenomics has transformed microbiome research by enabling the cultivation-independent analysis of microbial communities directly from environmental or host-associated samples[22,23]. Through high-throughput shotgun sequencing, metagenomics provides detailed insights into the taxonomic composition and functional potential of complex microbial ecosystems, including those inhabiting the human gut. Unlike targeted approaches such as 16S ribosome RNA gene sequencing, shotgun metagenomics captures information at the species and even

strain level, allowing for more precise profiling of microbiome structure, which is essential for studying health-associated microbial patterns and for linking specific taxa to metabolic functions relevant to host physiology.

While Metagenome-Assembled Genomes (MAGs) have expanded the known diversity of unculturable gut microbes, reference-based profiling remains essential for comparative studies and clinical applications. In this context, GutFeelingKB [24] provides a manually curated reference collection of microbial organisms consistently identified in the healthy human gut. GutFeelingKB is derived from metagenomics samples spanning the National Institute of Health (NIH) Human Microbiome Project [25], the George Washington University cohort, and other publicly available datasets, with a pipeline that integrates taxonomic profiling using CensuScope [26] and alignment against the Filtered-nt database, followed by extensive manual validation to ensure high-confidence taxonomic assignments.

While metagenomics provides insights into taxonomic composition and genetic potential, it cannot directly resolve dynamic metabolic activity, nutrient exchange, or pathway usage. The presence of a gene does not guarantee its expression, limiting functional predictions. To address these gaps, researchers integrate metagenomics with GEMs and multi-omics approaches [20]. By linking taxonomic profiles to GEMs, community-level metabolism can be simulated, enabling predictions of metabolic outputs and the functional roles of microbes in health and disease. This systems-level perspective bridges microbial composition with biological function.

2.2 Metabolic Modeling of the Gut Microbiome

GEMs address these limitations by representing microbial metabolism as stoichiometric networks, allowing simulations of biochemical fluxes under defined conditions. Their construction follows the principles of Constraint-Based Reconstruction and Analysis (COBRA) [27], a widely adopted framework for modeling metabolic networks. These models rely on the pseudo-steady-state assumption, which posits that the concentration of internal metabolites remains constant over time. Under this assumption, the system is typically analyzed using Flux Balance Analysis (FBA) [28], a linear programming approach that estimates flux distributions that satisfy the mass balance constraints while optimizing a predefined objective function, such as biomass production:

$$\max_{\mathbf{v}} \; c^T \cdot \mathbf{v} \quad \text{subject to} \quad \mathbf{S} \cdot \mathbf{v} = 0, \quad \mathbf{v}_{\min} \leq \mathbf{v} \leq \mathbf{v}_{\max} \tag{1}$$

In this formulation (Eq. 1), $\mathbf{c}$ is the objective coefficient vector that defines the biological goal of the simulation, such as maximizing biomass production. The steady-state assumption is encoded by the constraint $\mathbf{S} \cdot \mathbf{v} = 0$, where $\mathbf{S}$ is the stoichiometric matrix and $\mathbf{v}$ is the flux vector representing the rate of each reaction. The bounds $\mathbf{v}_{\min} \leq \mathbf{v} \leq \mathbf{v}_{\max}$ impose physiological or environmental constraints on each reaction, such as thermodynamic directionality,

enzyme capacities, or nutrient availability. Solving this linear programming problem yields a feasible steady-state flux distribution that satisfies all constraints while optimizing the defined objective, thereby enabling quantitative predictions of metabolic behavior under specific conditions.

Several tools have been developed to automate or semi-automate the reconstruction process using genome annotations and biochemical databases. Notable examples include AGORA [29] and AGORA2 [30], curated collections of gut microbial models; ModelSEED [31] and merlin [32], which facilitate draft reconstructions from genomic data; and CarveMe [33], a fast, template-based tool for generating GEMs at scale. Additionally, tools like gapSeq [34] support gap-filling and refinement of metabolic networks to ensure model functionality. While such pipelines enable the generation of new models, curated repositories such as the BiGG Models [35] database provide a standardized and quality-controlled collection of GEMs for a wide range of microbial species. These resources support the integration of metagenomics data with predictive metabolic modeling in microbiome studies.

Beyond individual species modeling, community-level metabolic modeling allows exploration of interactions within complex microbial ecosystems such as the gut microbiome[20,36]. These approaches simulate metabolite exchange, resource competition, and cooperation, which shape community structure and function and generally follow one of two frameworks: steady-state or dynamic. Steady-state models assume constant internal metabolite concentrations and employ constraint-based formulations such as FBA and its community extensions. In contrast, dynamic approaches use ordinary differential equations to model temporal changes in biomass and metabolite levels, enabling simulations of ecological succession. Additionally, alternative methods have been developed that rely on different modeling strategies, providing complementary insights into microbial community behavior. Each approach offers a distinct perspective, enriching our understanding of these complex ecosystems. This study focuses on three representative steady-state modeling tools—SteadyCom, MICOM, and SMETANA—each of which offers a distinct perspective on simulating microbial communities.

SteadyCom [37] is a constraint-based algorithm designed for modeling microbial communities under ecological steady-state conditions. It represents the community as a multicompartment system in which each organism has its own compartment, and all species interact via a shared extracellular compartment that defines the environmental medium. The key assumption in SteadyCom is that all species in a stable community grow at a uniform, time-averaged growth rate—reflecting ecological equilibrium. Unlike classical FBA, fluxes in SteadyCom are not normalized per unit biomass but are instead scaled by species abundance.

A central feature of the method is the coupling of biomass flux and species abundance: an organism can only carry flux if it has nonzero biomass and growth rate, realistically excluding inactive or extinct species. The framework solves

a convex optimization problem to compute the maximum feasible community growth rate while satisfying species-specific and community-level mass balance constraints. It is particularly well-suited for modeling long-term microbial coexistence in environments like the human gut or industrial bioreactors. SteadyCom is also computationally efficient, requiring a fixed number of linear programs independent of the number of species—an advantage over dynamic models such as cFBA.

MICOM [38] extends constraint-based community modeling by incorporating species-specific relative abundances into a multi-objective optimization framework. Like SteadyCom, it assumes a shared extracellular environment and individual taxon compartments but does not impose the assumption of equal growth rates. Instead, MICOM introduces a cooperative trade-off strategy that balances the growth of individual taxa with the global community objective.

MICOM's two-stage optimization begins by maximizing the weighted sum of taxon-specific growth rates, where the weights correspond to observed abundances. A second stage then adjusts fluxes to maintain near-optimal growth while optimizing community-level functions. The framework allows for simulation of dietary conditions, prediction of taxon-specific growth rates, and evaluation of metabolic interactions under defined environmental constraints. However, it requires reliable abundance data as input, and low-abundance taxa may be excluded if coverage is insufficient. MICOM does not natively support flux variability analysis and focuses on steady-state growth predictions rather than dynamic behaviors.

SMETANA [39] introduces SMETANA, an alternative framework for modeling microbial communities that infers metabolic interactions without relying on biomass optimization or predefined growth rates. While it assumes steady-state mass balance, it diverges from FBA-based approaches like SteadyCom and MICOM by estimating cross-feeding and metabolic dependencies through pairwise metabolite exchange simulations in minimal media.

The method constructs growth-supporting media for each organism and evaluates whether other species can complement missing metabolic functions via extracellular metabolite exchange. This generates directional interaction networks and species-specific support scores that reflect obligate or facultative dependencies. SMETANA does not require abundance or growth rate data and scales well to large communities, making it well-suited for exploring metabolic complementarity in natural or synthetic consortia.

2.3 Machine Learning in Microbiome Analysis

Analyzing microbiome metabolic models poses unique computational challenges due to the high dimensionality and complexity of microbial community interactions. While supervised machine learning methods—such as random forests and support vector machines—have demonstrated value in phenotype classification

[40], this study emphasizes unsupervised approaches for exploratory discovery of metabolic patterns.

Dimensionality reduction techniques are particularly effective for this purpose [41]. These techniques project high-dimensional flux distributions into low-dimensional spaces, enabling the visualization and interpretation of complex metabolic profiles. When coupled with clustering algorithms, they uncover intrinsic groupings that may correspond to functional ecotypes or transitions between health and disease states.

This unsupervised framework offers three key advantages in the context of metabolic modeling: (1) it operates without requiring labeled training data, making it well-suited for exploratory analysis; (2) it reveals dominant patterns in community-level metabolic behavior that emerge from simulation data; and (3) it enables the stratification of microbial communities into functional ecotypes, supporting biologically meaningful subgrouping beyond taxonomic classification. Crucially, the biological interpretability of these methods ensures that the identified patterns retain mechanistic relevance to host–microbiome interactions.

2.4 Pipeline Overview and Tool Integration

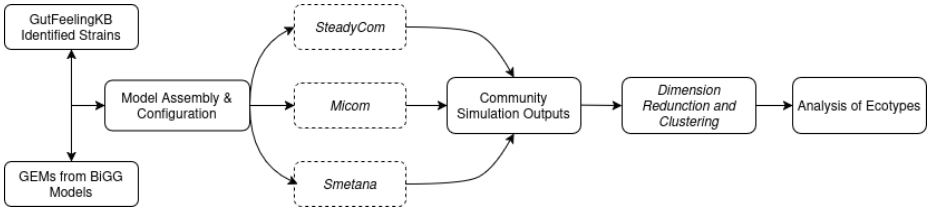

Fig. 1. Overview of the microbiome analysis pipeline.

Each tool mentioned can contribute to a modular yet integrated framework for characterizing the metabolic structure of the gut microbiome. GutFeelingKB and BiGG Models will be combined to generate a curated set of microbial strains and high-quality GEMs, providing a reliable foundation for community-level simulations. These models can then be assembled into microbial communities that reflect healthy gut conditions. SteadyCom, MICOM, and SMETANA will be applied in parallel to capture complementary aspects of microbial interaction: SteadyCom will estimate stable coexistence under shared growth rates; MICOM will simulate flux distributions informed by relative abundances; and SMETANA will identify metabolic dependencies and cross-feeding potentials without requiring abundance data. The outputs of these simulations will then be processed using unsupervised machine learning techniques—including dimensionality reduction and clustering—to identify functionally coherent microbial ecotypes that differentiate healthy from dysbiotic microbiome states.

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