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Defining a healthy human gut microbiome: a systems biology approach

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Thesis

DEFINING A HEALTHY HUMAN GUT MICROBIOME:

A SYSTEMS BIOLOGY APPROACH

by

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DEDICATION

I dedicate this work to my grandmother, whose love of knowledge led me on this path, and my mother, whose constant encouragement ensured I stay on it.

ACKNOWLEDGMENTS

Thank you to Dr. Zomorrodi, for providing tireless guidance throughout this project.

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NANEH ROZA VARTAN

ABSTRACT

Despite the association of the human gut microbiome and various diseases, a systematic definition of what constitutes a healthy human gut microbiome has not been established. This is crucial for microbiome research as it provides a basis for evaluating whether a given microbiome sample may deviate from the homeostasis state and is thus prone to the development of chronic diseases. This work aims to propose one such definition by using species/strain-resolved Genome-scale (GEM) models of metabolism. More specifically, we have constructed sample-specific GEMs from 30 healthy subjects using the taxonomic profiling of fecal metagenomic samples. We then computationally simulated these GEMs under a relevant diet (a supplemented typical Western diet) to determine which microbes in each sample contribute to the production of 17 key metabolites curated from literature and reported to be produced and secreted by the gut microbiota of healthy subjects. Beyond this pilot study, we plan to expand our analyses by creating samples-specific GEMs for a large-scale database of all publicly available metagenomic data from healthy subjects (~2,500 samples so far). We will additionally identify a core set of microbial species/strains that are necessary to perform all essential functions of a healthy microbiome. Taken together, this project offers a new paradigm to

establish a healthy baseline microbiome definition by identifying generalized and personalized microbial blueprints that could serve as viable markers of health.

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LIST OF ABBREVIATIONS

AGORA……………. Assembly of Gut Organisms through Reconstruction and Analysis

FBA ………………………………….……………………….Flux Balance Analysis

GEM...Genome Scale Model

IBD………………………………...……...……………Inflammatory Bowel Disease

SCFA…………………………………………………...…… Short Chain Fatty Acid

VMH……………………………...………………………..Virtual Metabolic Human

INTRODUCTION

The human microbiome encompases the microscopic organisms living in and on the human body. The symbiotic relationship between the microbiota and the human host have long been acknowledged. The gut microbiota is the most dense and complex human microbiota that resides in the digestive tract (see Figure 1 for a general overview of the human microbiome). Alterations in the taxonomic composition (i.e., changes in the identity and abundance of microbial taxa) of the gut microbiota, alson known as gut dysbiosis, has been linked to a variety of chronic human conditions such as inflammatory bowel disease, obesity, diabetes, cardiovascular disease, and autism, among others (Gupta et al., 2020; Madhogaria et al., 2022).

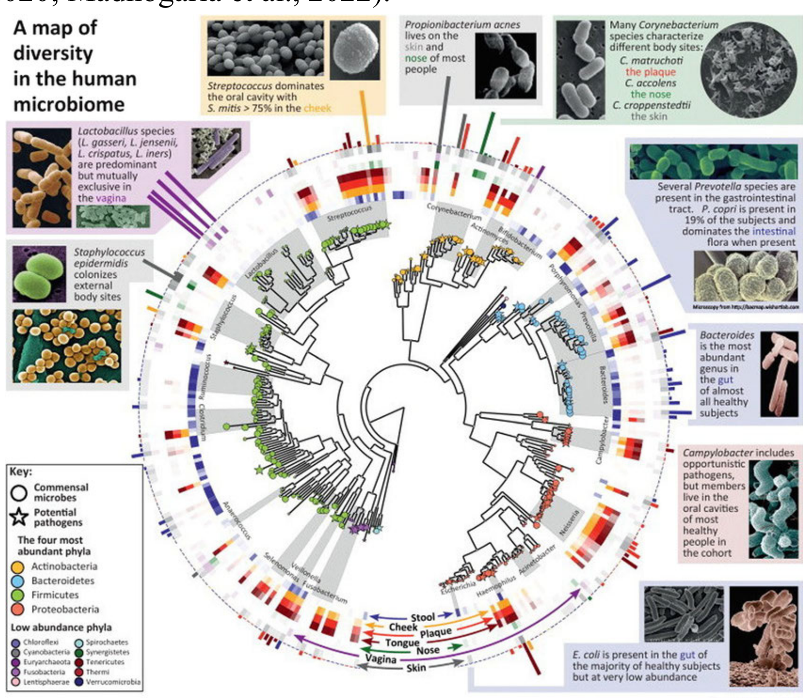

Figure 1: presentation of various phylogenies discovered as part of the human microbiome as a demonstration of the great diversity present therein

(Figure from: Al Khador & Shatat, *Pediatric Nephrology*, 2017, 32(6), 921–931.)

Within.a microbiome, the various microbes present interact with each other and with the human host in a number of ways. These interactions are what give rise to the community level functions which surpass the functions available to any single species on its own. Most of these interactions are metabolic in nature, creating cooperation and competition among the present species and establishing kind of symbiosis which ultimately leads to the production and consumption of metaboltes available in their environment. These interactions can be beneficial, where one species uses metabolites produced by another, or detrimental, where several species compete for the same metabolites possibly leading to growth inhibition. Microbial interactions canhave a physical nature, as well, though this aspect cannot be computationally modeled at this time. The interactions between a microbial community and their host organism is also largely metabolite based. In the case of humans, the community recieves metabolites from the host's diet, which can then be used by the various species present to produce byproducts which go on to alter the host's physiology via immune modulation and influence of metabolic systems (Zomorrodi & Segrè, 2016).

Maintaining the microbiome homeostasis is thus crucially associated with the human health. Therefore, understanding what a healthy human gut microbiota may entail is critical for assessing deviations from the homeostasis state and to evaluate the clinical status of the human host. Previous studies suggested that a healthy human gut microbiome can be defined based on measures of diversity and resilience. Diversity refers to the number of species present which are able to maintain similar levels of metabolic production and toxin clearance with redundancy. Resilience refers to the microbiome's ability to reestablish its baseline metabolic production and response to toxic metabolites or antibiotics after a dysruption which may alter the composition of the overall microbiome (Lloyd-Price et al., 2016; Wilmanski et al., 2021).

Nevertheless, currently, there is no systematic definition of a healthy human gut microbiome. In this work, we aimed to tackle this gap by using a systems biology-based analysis of healthy human microbiomes based on Genome-scale Models (GEMs) of metabolism. For a single species, GEMs describe the list of all metabolic reactions occurring in an organism as encoded by its genome, as well as the genes producing enzymes which go on to catalyze these reactions and the reversibility of these reactions themselves. GEMs also describe a biomass reaction, which denotes the combination of the fractional contribution of each component of the biomass, as seen in one gram of dry cell mass. This ideally reflects the molecular composition of the microbe in question

To simulate the microbiome as a whole, GEMs for microbes present within a microbiome sample can be integrated into a community scale model (see Methods). This accounts for the presence of and interactions between the various species present in the community.

Here, we use GEMs of metabolism to computationally assess whether a given microbiome sample is capable of producing key metabolites that are known to be differentially produced by the gut microbiota in healthy and nonhealthy subjects.

Microbiota GEMs have been used previously to investigate the role of the gut microbiota in human diseases such as inflammatory bowel disease (IBD) (Agus et al., 2021) and Parkinson's disease (Bicknell et al., 2022). This will enable us to assess whether a microbiome sample is capable of performing core metabolic functionalities needed to maintain the health of the human host.

SPECIFIC AIMS

Despite mounting evidence of the involvement of dysbiosis in the microbiome – especially that of the gut – in various diseases, a systematic method for the definition of what encompasses a healthy state is as of yet undefined. This work aims to introduce one such method using the adherence of metabolic models to a set of proposed healthy parameters as obtained via flux balance analysis (FBA) focused on a set of marker metabolites shown through metabolomic studies in the past to have marked differences between healthy and nonhealthy cohorts. The work herein is presented as a proof of concept for the viability of this method.

METHODS

Taxonomic profiling of metagenomic samples

MetaPhlAn 3.0 pipeline (Truong et al., 2015) was used to conduct the taxonomic profiling of the metagenomic samples.

Community GEM reconstruction

Individual GEMs for species/strains present in the metagenomic samples studied here were obtained from the Virtual Metabolic Human (VMH) database (Heinken et al., 2020; Magnúsdóttir et al., 2017). These models were integrated into community models for each sample using a customized pipeline implementing the MGPipe procedure from the Microbiome Modeling Toolbox (Baldini et al., 2019) in Python 3. This was accomplished by integrating all individual species/strains GEMs, which can exchange metabolites through a shared intestinal lumen compartment.

The community model additionally contains a diet and fecal compartment representing the intake of dietary nutrients and metabolites secretions into feces, respectively. A simplified schema of such a model can be seen in Figure 1. Furthermore, we added a community biomass reaction to the community model encompassing all species found within each sample with an abundance above 0.001. The reactants of this community biomass reaction are the biomasses of taxa present in each sample and their stoichiometric coefficients are their relative abundances.

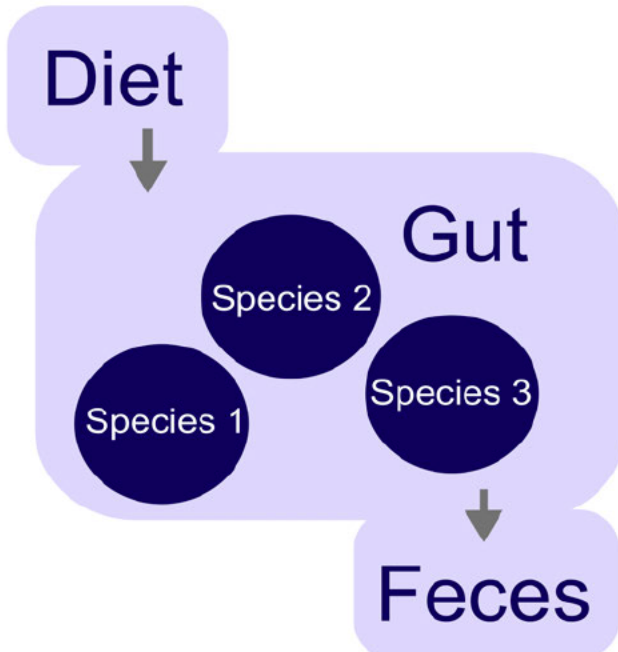

Figure 2: Simplified overview of the composition of a community GEM

Flux Balance Analysis: An Overview

In the case of a single species, flux balance analysis (FBA) requires the construction of a genome scale metabolic network model of present species via metagenomic analysis, which allows the stipulation of ongoing biochemical reactions within the specific microbe depending on the presence of coding enzymatic genes and prior experimental knowledge of ongoing processes. To obtain such a model, the genome of interest is sequenced and annotated in order to extract enzymes and determine their function. While a kinetic approach to this optimization is possible, a stoichiometric analysis yields much more relevant information thanks to the wealth of experimental data which can be taken into consideration therewith (Maranas & Zomorrodi, 2016) This stoichiometric analysis requires several elements.

From the information presented in a model, a biomass reaction – which simulates a microbe's growth by the production of amino acids, lipids, nucleotides, and any cofactors necessary – can be constructed, which is then optimized under environmentally specific conditions, accounting for the presence of all metabolites available in the system as well as any limiting reagents to known processes.

In order to eliminate the need for kinetic data, a steady state is imposed on the model prior to optimization. This eliminates time as a variable being taken into consideration when ensuring the conservation of each metabolite within the model throughout the various reactions it is involved in.

This steady state is simulated for each metabolite as follows:

$$\sum_{j \in J} S_{ij} v_j = 0, \quad \forall\, i \in I$$

Where v_j is the flux of reaction j and S_{ij} is the stoichiometric coefficient of reaction j associated with metabolite i.

This construction can also be represented as:

$$\boldsymbol{S} \cdot \boldsymbol{v} = 0$$

Where $\boldsymbol{S}$ refers to the stoichiometric matrix and $\boldsymbol{v}$ refers to the flux vector. This can be expanded into the following matrix:

$$\begin{bmatrix} S_{11} & S_{12} & \cdots & S_{1N} \\ S_{21} & S_{22} & \cdots & S_{2N} \\ \vdots & \vdots & \ddots & \vdots \\ S_{M1} & S_{M2} & \cdots & S_{MN} \end{bmatrix} \begin{bmatrix} v_1 \\ v_2 \\ \vdots \\ v_N \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \\ \vdots \\ 0 \end{bmatrix}$$

Each metabolite is a participant in multiple reactions, thus, the number of metabolites, M, is smaller than the number of reactions in a metabolic network, N. Because of this, the system of equations denoting the steady state of a metabolite is underdetermined which implies that there exist an infinite number of flux vectors which can satisfy this state. This means that the above equation captures all possible metabolic phenotypes of the system regardless of their physiologic relevance. Constraint based methods, like FBA, attempt to constrain this set of possibilities such that physiologically irrelevant metabolic phenotypes are eliminated and only biologically feasible solutions remain.

Lower and upper bounds are imposed on each metabolic reaction to account for thermodynamic and flux capacity constraints. This also accounts for the reversibility or lack thereof for a given reaction. This can be represented as follows:

$$LB_j \leq v_j \leq UB_j$$

Irreversible reactions which only occur in the forward direction are represented by a lower bound of 0 and an upper bound of M, where M is either the maximum possible flux for the reaction as previously found experimentally or a large number such as 1000 relative to the transport flux of the limiting metabolite within the reaction. Irreversible reactions occurring only in the backward direction are represented by a lower bound of -M and an upper bound of 0. Reversible reactions, as defined by a possible change in sign of Gibbs free energy under various physiologically relevant concentrations of involved metabolites, are represented by a lower bound of -M and an upper bound of M. These constraints further limit the possible fluxes for each reaction upon optimization.

As an example, the biomass of a given microbe under specific conditions can be examined by the following formulation:

$$\text{maximize } \ v_{biomass}$$

$$\text{subject to}$$

$$\sum_{j \in J} S_{ij} v_j = 0, \quad \forall\, i \ \in I \tag{1}$$

$$LB_j \leq v_j \leq UB_j, \quad \forall \text{ j } \in \text{J} \tag{2}$$

$$v_j \ \in \ \mathbb{R} \tag{3}$$

Here, a solution flux of 0 would denote that the organism cannot grow under the simulated conditions whereas a positive solution would imply growth.

Because the microbiome as a whole is not populated by a single specie but rather a diverse host of possible microbes, this analysis must be conducted with a network approach where the genomes of all present microbes are taken into consideration in the model as an attempt to aggregate the ongoing processes of all present species and reach one, optimized solution of reaction fluxes representative of the whole network (Kuang et al., 2020). A simplified summary of this composition can be seen in Figure 2. The above conditions can be extended to this more complex model, and new constraints can be imposed to assess the behavior of each included species as an individual member of the community.

While this presents additional challenges and requires more careful handling of the various genome scale metabolic network models involved, it also intends to form a more cohesive understanding of the biochemical processes within this ecosystem as a whole by presenting the environment of the microbiome in a single model.

With this model, the overall reaction to alterations in the nature of species present, metabolite availability, or changes in available metabolites – given that the reactions by which they are processed can be determined – will be able to be simulated *in silico*. This development can allow more thorough analysis of conditions known to have a connection to the gut microbiome as a contributing factor, including the simulation of the effect of various diets and the addition or removal of species from this environment.

In silico diet composition

The average European diet provided in the VMH database was used with supplementation of taurocholate, glycocholate, taurochenodeoxycholate, and glycochenodeoxycholate (Heinken et al., 2019b). This supplemented diet was used with no further modifications.

Community GEM simulations

We computationally simulated the community GEMs using constraint-based methods as follows. We maximized the secretion flux of each metabolite into the fecal compartment while requiring the community biomass reaction flux to be bound within 0.4 to 1.0 (Heinken et al., 2019a) in order to represent a realistic turnover of microbial biomass as described before. The in silico diet composition was used to constrain the intake of nutrients into the community model. An uptake flux of -10 was permitted through all dietary exchange reactions corresponding to metabolites present in the diet.

The following formulation was used for these simulations:

Maximize/Minimize flux of metabolite secretion into feces

Subject to

$$\sum_{j \in J} S_{i,j} v_j = 0 \text{ , } \forall\, i \in \{metabolites\} \quad (1)$$

$$LB_j \le v_j \le UB_j \text{ , } \forall\, j \in \{reactions\} \quad (2)$$

$$v_j \in \mathbb{R} \quad (3)$$

Here, i and j are indices for metabolites and reactions, respectively. S_{ij} is the stoichiometric coefficient of metabolite i in reaction j. v_j is the flux of reaction j, and LB_j and UB_j denote lower and upper bounds on v_j. Constraint (1) represents the steady-state mass balance for each metabolite. Constraint (2) requires the reaction fluxes to be within the imposed lower and upper bounds. The objective function of this problem is to either minimize or maximize the fecal exchange reaction of a metabolite m as noted to be the trend in a healthy community from literature, seen in Table 1.

Table 1: Target metabolites used in this study as indicators of a healthy human microbiome.

Metabolite	Optimization Direction
Acetate	Maximize
Butyrate	Maximize
Propionate	Maximize
P-Cresol	Minimize
1- Oleoylglycerol	Maximize
2-Arachidonoylglycerol	Maximize
Indole	Maximize
Indole-3-propionate	Maximize
Indole-3-acetate	Maximize
L-Phenylalanine	Minimize
Coprostanol	Maximize
L-Glutamate	Minimize
L-Tryptophan	Minimize
Deoxycholic acid	Maximize
Allodeoxycholic acid	Maximize
Allolithocholic acid	Maximize
Taurodeoxycholic acid	Maximize

RESULTS

We chose 30 healthy subjects and 30 non-healthy controls from the publicly available metagenomic datasets. These subjects were drawn from multiple studies; the unhealthy samples comprised subjects with colorectal cancer, ulcerative colitis, and Crohn's disease drawn at random from multiple studies (MetaHIT Consortium et al., 2014; Schirmer et al., 2016; Tanca et al., 2017b; Thomas et al., 2019; Zeller et al., 2014). Healthy subjects were those that served as healthy controls in previously published studies of human microbiome and disease (Schirmer et al., 2016; Tanca et al., 2017a; Zeller et al., 2014).

We next conducted a complete survey of existing literature to extract a list of metabolites that a healthy microbiome is expected to be able to product. We put together 17 metabolites that have been reported to be produced by the microbiome of healthy subjects at either higher or lower levels compared to unhealthy samples (see Table 1). Metabolites that are produced at higher levels in healthy samples include short chain fatty acids (acetate, butyrate, propionate), bile acids (deoxycholic acid, taurodeoxycholic acid, allolithocholic acid, and allodeoxycholic acid), as well as other metabolites including indole and p-cresol. These metabolites have been reported to have protective effects to the host. In contrast, that are produced at lower levels in healthy subjects – namely p-cresol, l-glutamate, l-tryptophan, and l-phenylalanine – have determinantal effects on the host or are broken down to be used for the building of other essential compounds.

Next, we constructed species-resolved community GEMs for each sample by integrating the GEMs of individual species present in each sample. We extracted the GEMs of individual species/strains present in each sample (informed by taxonomic profiling of metagenomic samples) and then integrated them into a community model. These model captures the entire metabolism of a given microbiome sample at metagenome scale and at species/strain-level resolution. Next, we computationally simulated each microbiota GEM under a typical Western diet by using multi-cell FBA (see Methods) in order to assess the capability of each microbiome sample to produce these 17 target metabolites.

Our simulation of community models for these samples reveled several differences in the ability of the microbiomes to produce the target metabolites between the healthy and nonhealthy samples. For example, we found some metabolites that were present in the models for nonhealthy samples but did not exist in any of the models for the healthy samples or vice versa. The absence of a metabolite in the community GEM for a given sample implies that the sample is not able produce that metabolite under any conditions because it lacks the enzymatic machinery in any of its constituent microbes to produce that metabolite. For instance, coprostanol is a metabolite that is present only in the unhealthy models but not in any of the healthy models. On the other hand, allodeoxycholic acid and allolitholitic acid were absent in the majority of unhealthy models but were present in all the healthy models. In the following, we will present our results for each specific metabolite examined in our analyses.

Short Chain Fatty Acids

Short chain fatty acids (acetate, butyrate, and propionate) are among the most important microbiota-derived metabolites that modulate host's phonotypes and the ability to produce them at sufficient levels is an indicative of a healthy microbiome. In our simulations, for acetate, butyrate and propionate, we found that the production levels in healthy samples is overall higher than those in the nonhealthy samples (see Figures 3,4,5), which is consistent with previous reports (Wilmanski et al., 2021).

Short chain fatty acids, and especially the three metabolites investigated here, play a vital role in maintaining a healthy state (Nogal et al., 2021). They are known to play a regulatory role in glucose, cholesterol, and lipid metabolism, as well as having a role in immune and anti-inflammatory response. They are also involved in maintaining the gut barrier integrity. They are produced by bacteria through fermentation processes as well as by the breakdown of amino acids.

Acetate

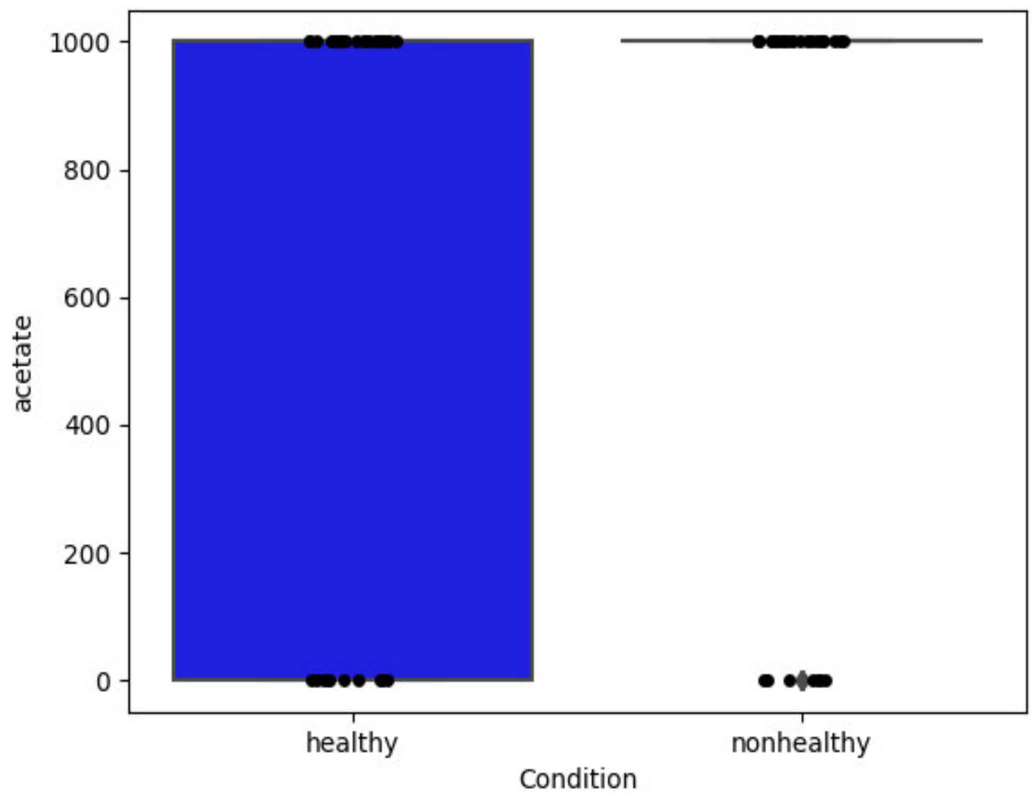

Figure 3: Acetate production in healthy vs. nonhealthy samples

Acetate production in healthy samples was found to be higher than in nonhealthy samples as seen in Figure 3. This is expected, as acetate has a beneficial role in human physiology. Previous findings also support this result (Nogal et al., 2021).

Butyrate

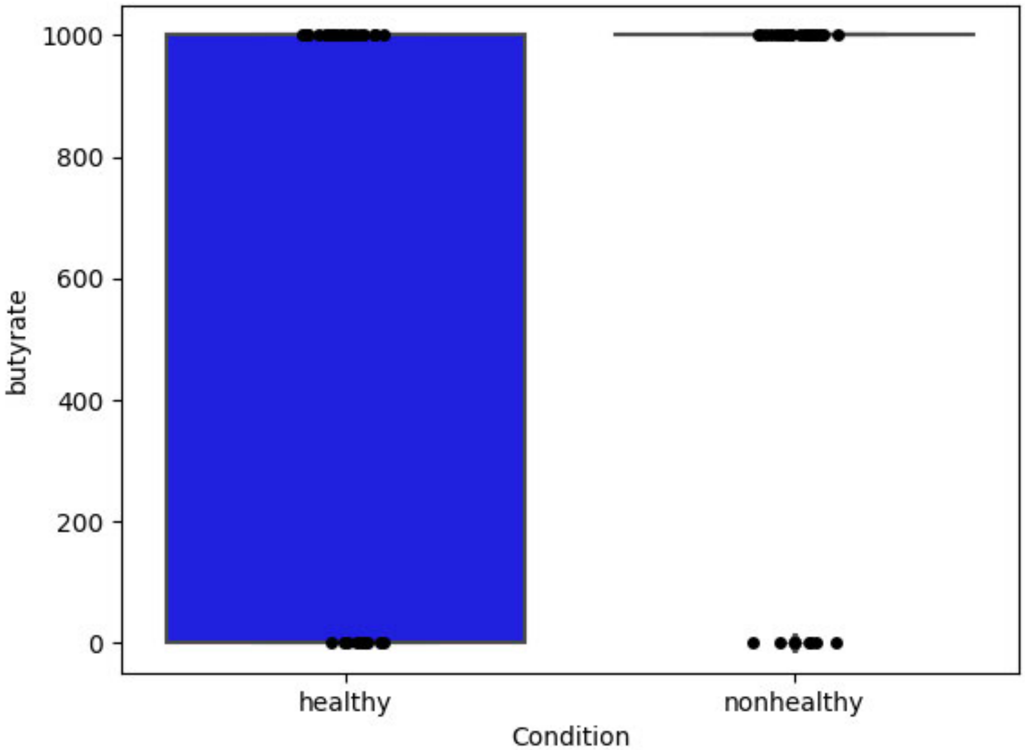

Figure 4: Butyrate production in healthy vs. nonhealthy samples

Butyrate was produced at an overall higher flux in healthy samples than among in samples (see Figure 4). This result also matches expectation and previous findings as butyrate is known to have a beneficial effect for the host (Nogal et al., 2021).

Propionate

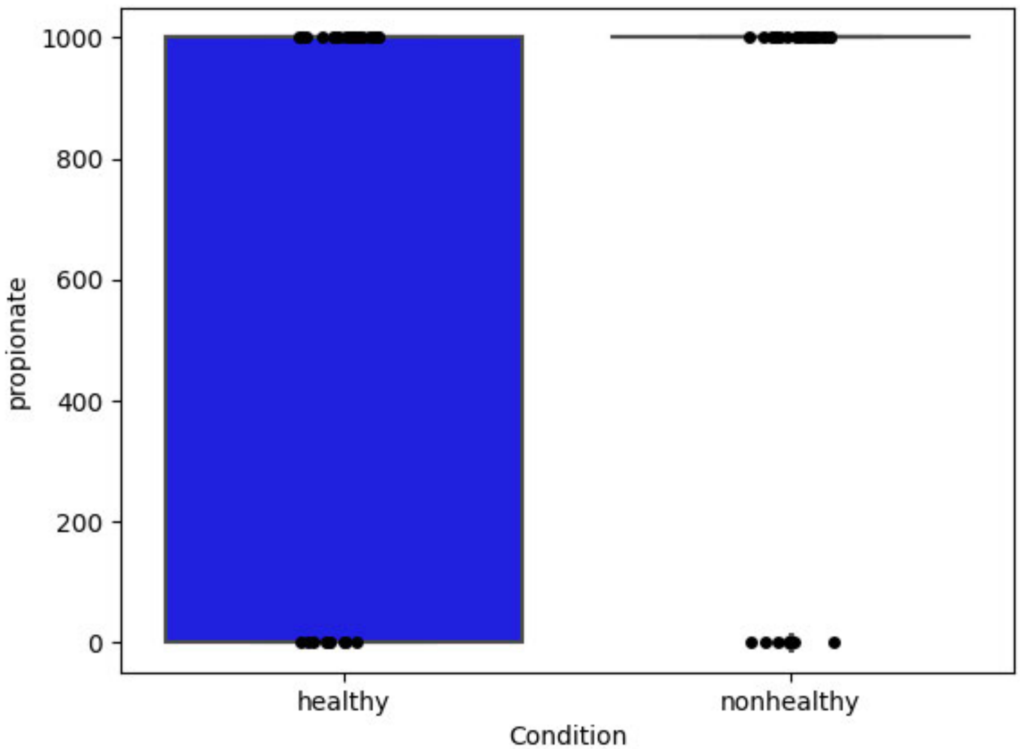

Figure 5: Propionate production in healthy vs. nonhealthy samples

Propionate production was found to be higher in healthy samples than in nonhealthy samples (see Figure 5). With consideration of the beneficial effects of propionate on the human host, this result is expect and agrees with previous findings (Nogal et al., 2021).

Bile Acids

The flux of various bile acids is known to vary depending on health status and specific conditions (Guzior & Quinn, 2021b). Several of the metabolites considered belonging to this category were seen to have potential use as indicators for the healthy status of a sample if used in conjunction with other parameters to verify their results, as they were either predominantly present and carrying a nonzero flux in healthy samples.

Bile acids are known to act as regulators of the gut microbiota (Ridlon et al., 2014). They have direct and indirect antimicrobial effects on the gut microbiome and a clear decline in their production is seen in various disease states. Despite this, an accumulation of secondary bile acids in particular is also associated with a nonhealthy state. As such, bile acids are expected to reside in a clear range in healthy individuals.

In our studies, we investigated four different secondary bile acids that have been reported to have higher production in healthy subjects, namely, deoxycholic acid, taurodeoxycholic acid, allodeoxycholic acid, and allolithocholic acid than in nonhealthy subjects. Reduced levels are associated with inflammation and bacterial overgrowth (Ridlon et al., 2014).

Deoxycholic Acid

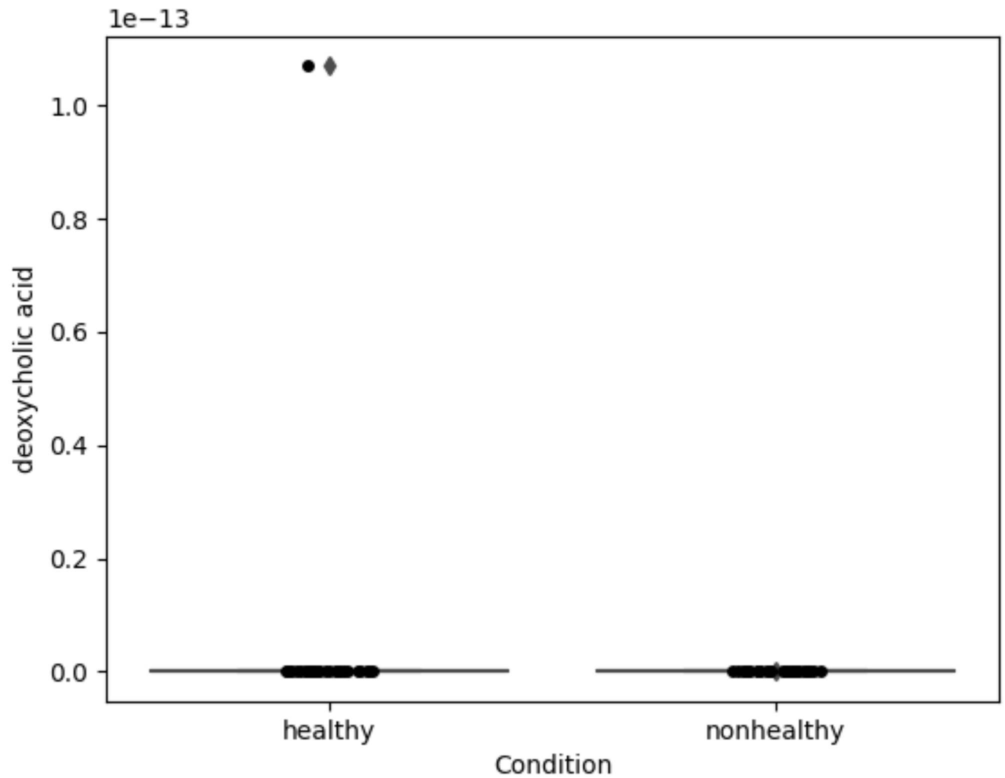

Figure 6: Deoxycholic acid production in healthy vs. nonhealthy samples

Deoxycholic acid was produced only by samples belonging to the healthy group examined, as seen in Figure 6. This agrees with expectation as a moderate production of this metabolite is known to be associated with a healthy state (Ridlon et al., 2014).

Taurodeoxycholic Acid

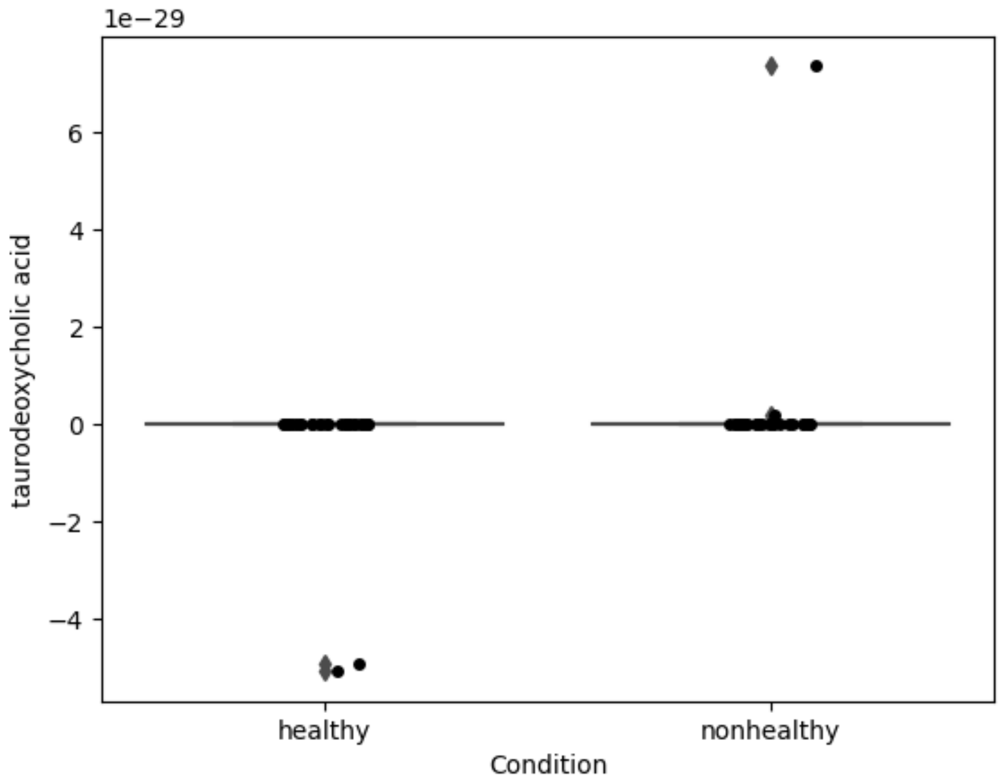

Figure 7: Taurodeoxycholic acid production in healthy vs. nonhealthy samples

Taurodeoxycholic acid was found to be used by healthy models but produced by nonhealthy models as seen in Figure 7. This clear distinction supports its use as a viable marker, as it presents a clearly defined pattern among the analyzed samples. These results are, however, surprising, as despite the known beneficial effects of this metabolite in improving gut barrier function (Wang et al., 2018), nonhealthy samples showed higher production than healthy samples, which actually showed the metabolite being used rather than produced.

Allodeoxycholic Acid

Allodeoxycholic acid was not detected in the models constructed using normalized abundance data. This is possibly caused by the limitation of only adding species with an abundance above 0.001 to the community composition and thus having eliminated species which originally interacted with this metabolite.

Allolithocholic Acid

Allolithocholic acid was not detected in the models constructed using renormalized abundance data. This is possibly caused by the limitation of only adding species with an abundance above 0.001 to the community composition and thus having eliminated species which originally interacted with this metabolite.

Amino Acids

Various amino acids are known to be used by a healthy gut microbiome in the production and modification of various byproducts (Wilmanski et al., 2021). Thus, their use in healthy samples are shown, overall, to be higher than in nonhealthy samples. Some amino acids are also known to be produced *de novo* by the gut microbiome (Lin et al., 2017). In either case, healthy samples are expected to show a higher flux of the fecal exchange reaction for amino acids than nonhealthy samples. A minimization of the fecal exchange fluxes of l-glutamate, l-tryptophan, and l-phenylalanine within the renormalized models showed the same pattern, wherein some healthy samples showed indication of use of these metabolites (see Figures 8, 9, 10).

Some amino acids, including l-glutamate and l-tryptophan examined in this study alongside l-phenylalanine, are known to have a beneficial effect on host immunity and gut barrier integrity maintenance (Ma & Ma, 2019). L-Phenylalanine and l-tryptophan are also used in the production of other, immunomodulatory metabolites (Dodd et al., 2017).

L-Glutamate

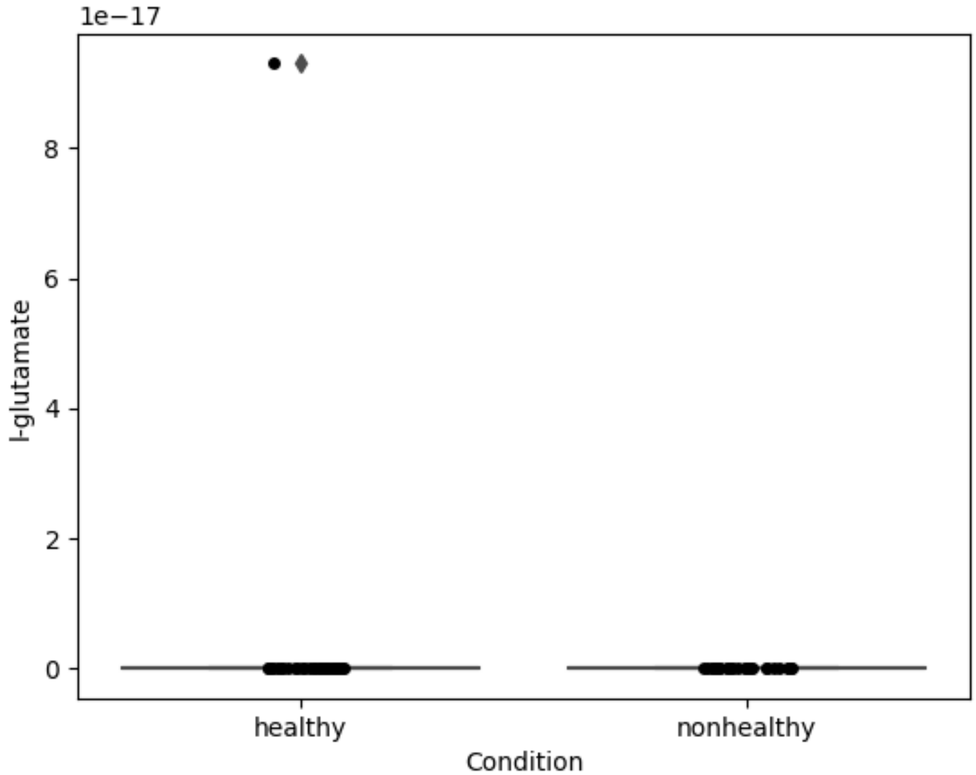

Figure 8: L-glutamate production in healthy vs. nonhealthy samples

L-Glutamate was only found to be produced in a subset of healthy samples, as seen in Figure 8. This suggests it can be used as a viable marker for health status, with any production indicating a healthy sample.

L-Tryptophan

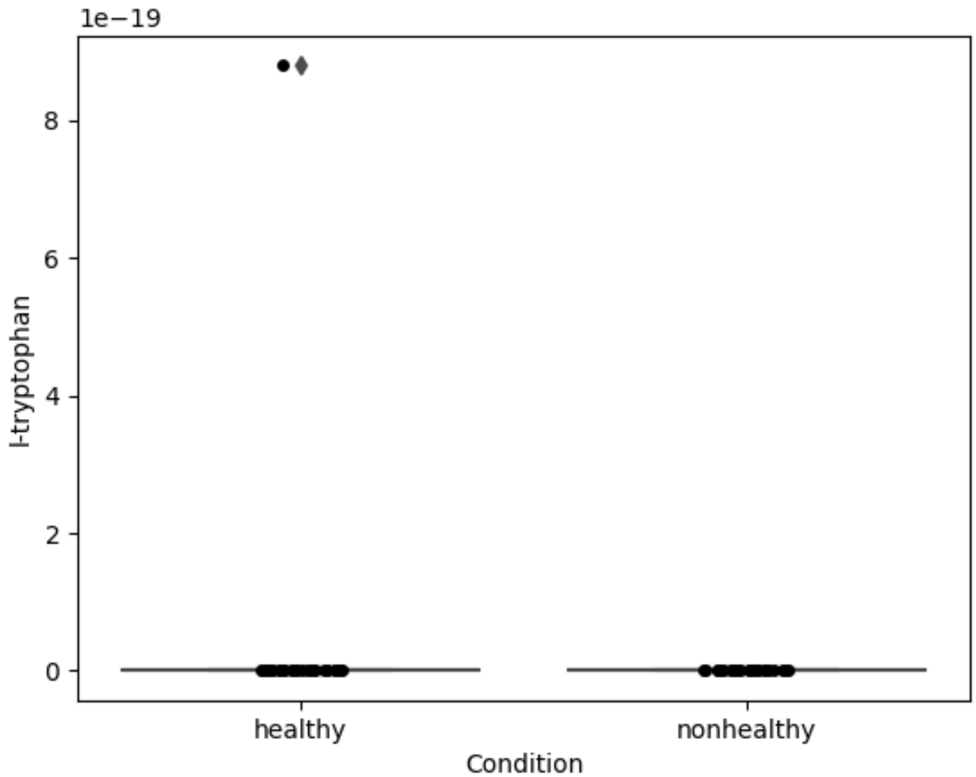

Figure 9: L-tryptophan production in healthy vs. nonhealthy samples

L-Tryptophan was found to be produced among some healthy samples, as seen in Figure 9. This suggests it can be used as a viable marker for health status, with any production indicating a healthy sample.

L-Phenylalanine.

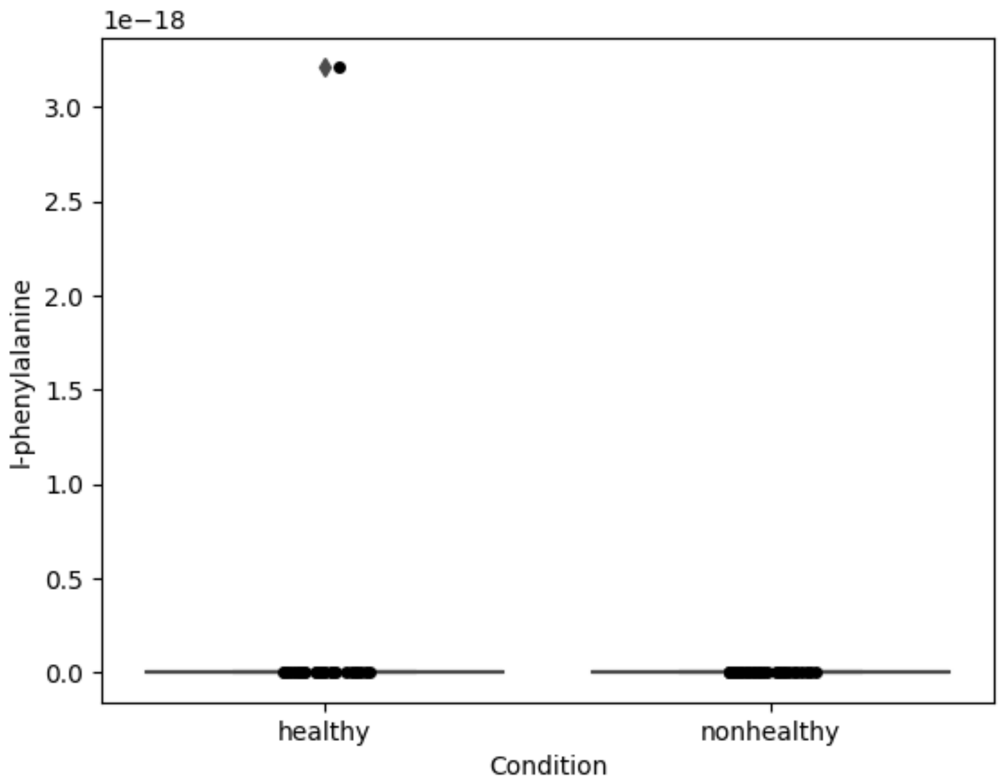

Figure 10: L-phenylalanine production in healthy vs. nonhealthy samples

L-Phenylalanine was found to be produced among some healthy samples, as seen in Figure 10. This suggests it can be used as a viable marker for health status, with any production indicating a healthy sample.

Other

Aside from the aforementioned categories, several other metabolites are correlated with health, including various amino acid derivatives. Some of these metabolites (2-arachidonoylglycerol, 1-oleoylglycerol, indole-3-propionate, and coprostanol) were not detected to be present in any of the 30 healthy or the 30 unhealthy samples analyzed. P-cresol, indole-3-acetate, and indole however were present in at least some of the considered samples and, in the case of p-cresol and indole showed a distinct pattern which can be associated with the health status of models.

2-Arachidonoylglycerol

2-Arachidonoyglycerol is known to be involved in stress regulation and the maintenance of the gut barrier integrity and permeability to nutrients (Srivastava et al., 2022). Low levels of this metabolite are associated with a nonhealthy state.

2-Arachidonoylglycerol was not present in any of the analyzed samples. This is possibly caused by the limitation of only adding species with an abundance above 0.001 to the community composition and thus having eliminated species which originally interacted with this metabolite.

1-Oleoylglycerol

High levels of 1-oleoylglycerol is known to have a detrimental effect on the host physiology. It is associated with an altered insulin response and as such, high levels are known to increase the risk of type 2 diabetes (Vangipurapu et al., 2020). As such, higher production of this metabolite is expected in nonhealthy samples when compared to healthy samples.

1-Oleoylglycerol was not present in any of the analyzed samples. This is possibly caused by the limitation of only adding species with an abundance above 0.001 to the community composition and thus having eliminated species which originally interacted with this metabolite.

Indole-3-Propionate

Indole-3-propionate is associated with beneficial effects for the human host. It is known to decrease inflammation and lipid peroxidation and is known to have a decreased level of production in association with several diseases, including colitis and diabetes (Konopelski & Mogilnicka, 2022). As such, healthy samples are expected to show higher production of this metabolite than nonhealthy samples.

Indole-3-propionate was not present in any of the analyzed samples. This is possibly caused by the limitation of only adding species with an abundance above 0.001

to the community composition and thus having eliminated species which originally interacted with this metabolite.

P-Cresol

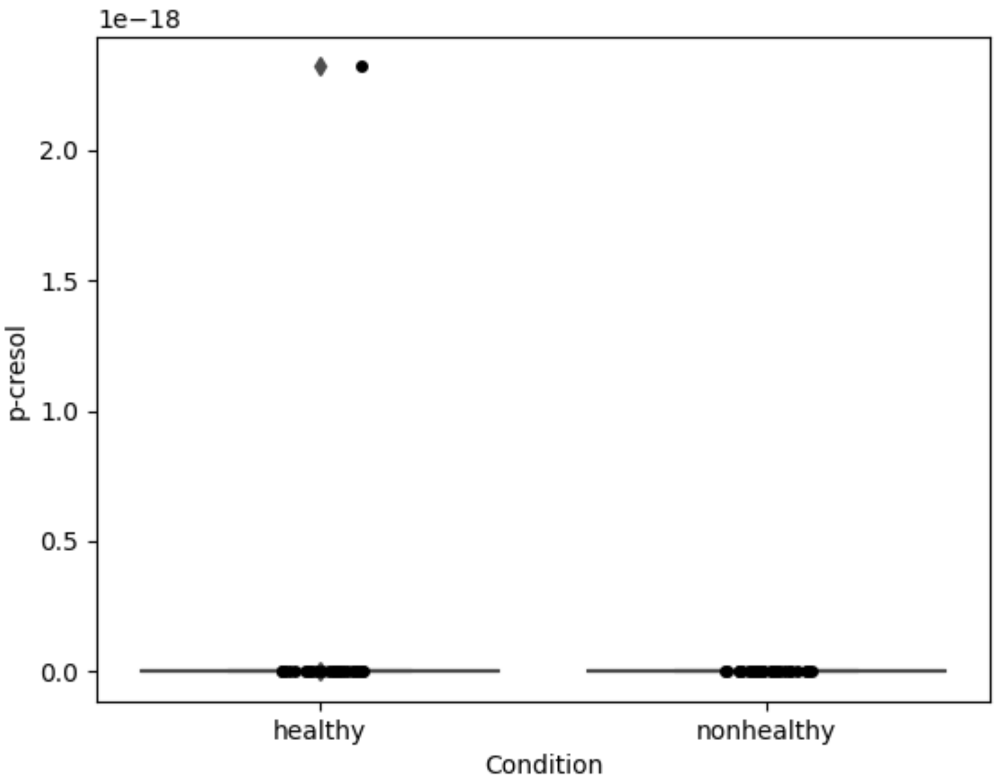

Figure 11: P-cresol production in healthy vs. nonhealthy samples

P-Cresol is associated with a number of diseases, including colorectal cancer, due to its place in the pathway towards a number of genotoxins (Al Hinai et al., 2019; Candeliere et al., 2022). As these detrimental effects are heavily associated with the buildup of this metabolite, healthy samples are expected to have lower a production of p-cresol than nonhealthy samples.

P-Cresol was detected in all 60 samples. However, two samples in the healthy group carried a positive flux, as seen in Figure 11. This suggests it can be used as a viable marker for health status, with any production indicating a healthy sample.

Indole-3-Acetate

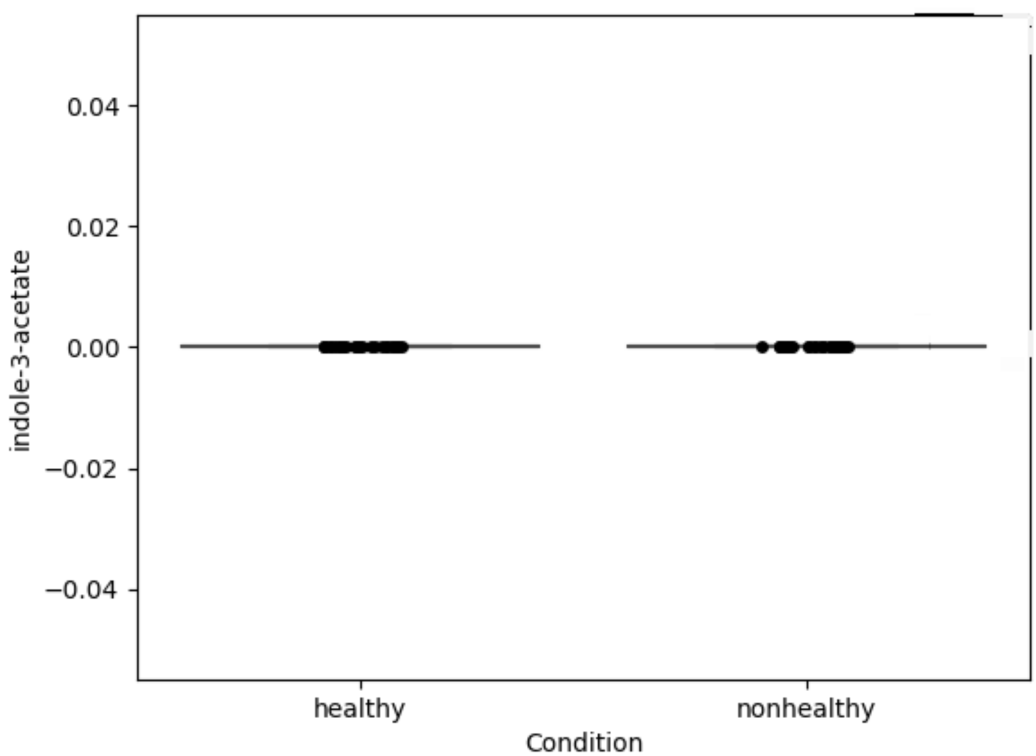

Figure 12: Indole-3-acetate production in healthy vs. nonhealthy samples

Indole-3-acetate is known to have anti-inflammatory and anti-oxidative effects and is overall seen as beneficial when present in moderation (Ji et al., 2020). As such, it is expected to have higher production in healthy samples than in nonhealthy samples.

Indole-3-acetate produced no flux across any of the samples analyzed, as seen in Figure 12, however, it was detected in all 60 samples. This lack of production may stem from the exclusion of species responsible for interactions with indole-3-acetate from the model due to low relative abundances.

Indole

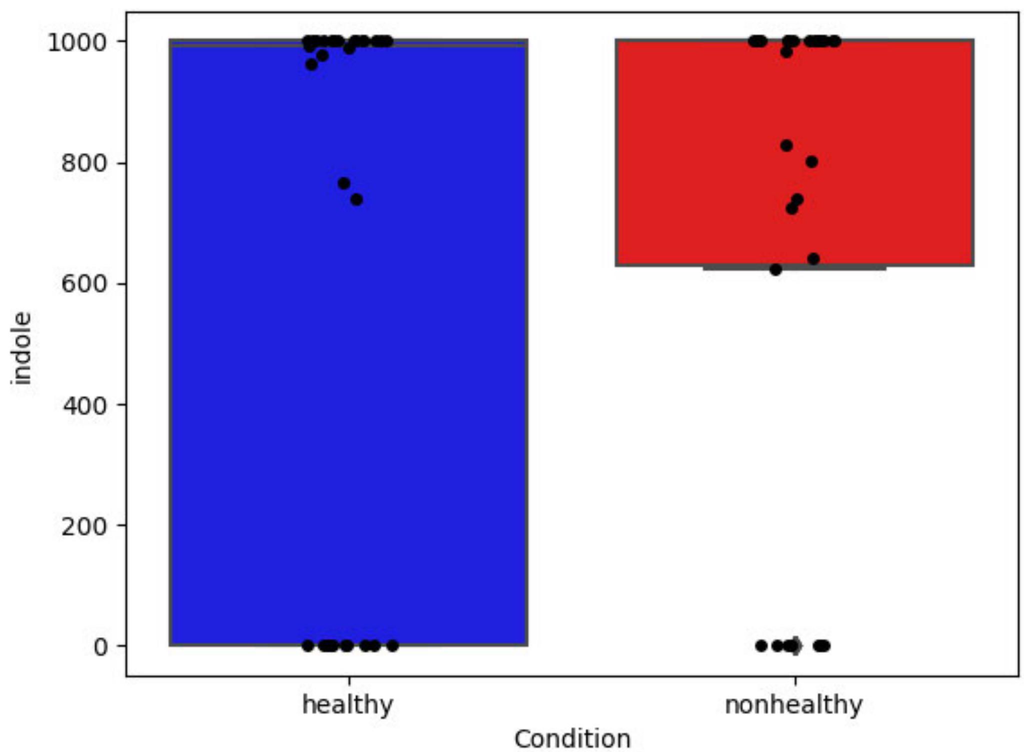

Figure 13: Indole production in healthy vs. nonhealthy samples

Indole is associated with decreased levels of inflammation and has positive effects on liver and GI homeostasis (Konopelski & Mogilnicka, 2022). In high levels, however, indole can have a toxic effect on the host (Al Hinai et al., 2019; Candeliere et al., 2022).

As such, production is expected to remain in a clear range within healthy samples and be higher in nonhealthy samples.

Indole was found over a distinct range, seen in Figure 13. While still unreliable as results beyond a pattern, the fecal exchange reaction of indole was, on average, produced with a higher average flux in nonhealthy samples than in healthy samples, with a much wider distribution among healthy samples overall. While this cannot be used as a standalone marker, it may be used as support to indicate a specific health status of a given sample.

Coprostanol

Coprostanol is a common product of cholesterol metabolism, known to be carried out by the gut microbiome (Juste & Gérard, 2021). It is associated with lower overall cholesterol levels and is thus expected to show higher production in healthy samples than in nonhealthy samples.

Coprostanol was not detected in any of the models. This is possibly caused by the limitation of only adding species with an abundance above 0.001 to the community composition and thus having eliminated species which originally interacted with this metabolite.

DISCUSSION AND CONCLUSIONS

In this study, we aimed to computationally investigate the capacity of gut microbiomes to produce a number of key metabolites implicated in healthy and disease. WE did this by using species-resolved computational GEMs of metabolism. These models allow us to computationally infer whether a microbiome sample is able to produce these key metabolites and to what extent. Our analyses identified a number of differences in the production level of these metabolites between healthy and non-healthy subjects.

We were able to identify some patterns that were indeed consistent with previous reports. For example, acetate, butyrate, and propionate were seen to have higher production among healthy samples than among nonhealthy samples; taurodeoxycholic acid was seen to be used within healthy samples and produced in nonhealthy samples, reacting a clear distinction between the two; indole had a lower average production among healthy samples than among nonhealthy samples.

We additionally, had some metabolites which were present in one group of models analyzed – healthy or nonhealthy – but not the other implying that one of the groups cannot produce that metabolite under any conditions. These include l-glutamate, l-tryptophan, and l-phenylalanine which were all only found to be produced among healthy samples.

Other metabolites were seen to be present in more samples within one group than the other, making it likely that their production indicates a specific health status for the analyzed model when taken in consideration with other factors. These include allolithocholic acid and allodeoxycholic acid which were detected to exist and be produced in a larger number of healthy samples than in nonhealthy samples, and deoxycholic acid which was present in all models but produced in a larger number of healthy samples when compared to nonhealthy samples.

It is important to acknowledge some limitations of our studies. We did not account for all GEMs of all species and strains found to be present in a sample according to its taxonomic profiling results, because no GEM existed in the AGORA database for some taxa in the samples. This means that while our models are close approximations, they are not complete and miss the metabolic functionalities for species with no GEMs. Furthermore, only GEMs for taxa with an abundance greater than 0.001 were included in the model, leading to exclusions in the interactions between these less abundant taxa within the community, further limiting how closely our models imitate the sample microbiome.

While our models were able to capture general qualitative patterns associated with many of the considered metabolites, we did not evaluate production levels quantitatively. This will be necessary for further investigation of the topics proposed herein. Further, our models capture metabolic interactions between members of the microbial community but lack the means to account for interactions via other means, such as toxins, quorum sensing, and physical interactions.

Lastly, with thirty healthy and thirty nonhealthy samples obtained from the same general geographic location, our sample size and scope is limited and results cannot necessarily be generalized to a universal model. Therefore, this study should rather only be considered as a proof-of-concept. The methods used in our analysis could nonetheless be utilized to tease out the contribution of individual species present in a microbiome sample to the overall metabolic capabilities of that sample under a given dietary regimen.

Overall, our studies provide a road map for computationally investigating the metabolic capabilities of the gut microbiota in the production of metabolites that are implicated in health and disease. We envision that this study could be used a basis for a more rigorous investigation of a healthy human gut microbiota .

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FUTURE DIRECTION

Using the patterns reaffirmed in this work, steps can be taken towards a quantitative definition of thresholds for marker metabolites. These quantitative limits can then be applied to discover essentials sets of taxa required to have and maintain a healthy level of metabolite production. Further, overlaps within minimal sets of species able to maintain these production levels can be used to identify redundancies in place within the microbiome in the form of taxa able to perform similar functions to maintain healthy metabolic processing.

Due to the inaccuracies present within the analyzed models, these results are merely indicative of possible patterns present in healthy and nonhealthy samples. By normalizing the abundance values of species within each sample such that their sum is equal to one, this problem can be corrected and a much more accurate view of the flux distributions within each set can be obtained. This would allow the setting of discrete threshold values for the fluxes of individual metabolites, permitting their use as parameters in the extraction of a minimal set of active species which would allow for an optimal utilization of resources such that the sample microbiome can stay within these limits.

By taking into account alternate solutions to the system, providing several sets of possible optimal species distributions for each sample, a more generalizable conclusion could be reached by assessing any commonalities between possibilities within the same sample as well as between samples of a given cohort.

Commonalities in these discovered sets of species would suggest a basic, generalizable composition of the microbiome necessary to maintain a healthy status as well as alternative compositions which could compensate for the loss or suppression of part of the required species. Further analysis could lead to the extraction of specific core functions which must be preserved, allowing the development of compensatory microbiome compositions which could stave off the effects of any disruptions to this environment.

Future planned work includes the correction of GEMs such that they are able to use a standard diet for a given region corresponding to the origin of the sample being analyzed while continuing to maintain a biomass flux between 0.4 and 1.0. With this correction, as well as corrections to the pipeline generating community models such that metabolites are capable of correct optimization and do not erroneously return the maximal or minimal permitted fluxes for their given fecal exchange reaction, more accurate parameters can be set from which a minimal set of essential species can be generated.

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