Systems Modelling of Lactose Intolerance

A Project Report

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THESIS CERTIFICATE

This is to certify that the thesis titled SYSTEMS LEVEL MODELING OF LACTOSE INTOLERANCE , submitted by Rajiv K (BE13B025), to the Indian Institute of Technology Madras, for the award of the degree of Dual Degree (B.Tech and M.Tech) in Biological Engineering, is a bonafide record of the research work done by him under my supervision. The contents of this thesis, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma.

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ABSTRACT

KEYWORDS: Genome Scale Models, Metabolic Modelling, Gut Microbiome, Flux Balance Analysis, Systems Biology, Lactose Intolerance

Lactose maldigestion and intolerance affect a large part of the world population. The underlying factors of lactose intolerance are not fully understood. But it is known that the microbe comminuty in the gut plays a major role in regulating the disease. To understand the metabolic interactions that drive the pathogenesis of Lactose intolerance (LI), a systems level analysis of the LI implicated gut microbes is essential.

A large number of microbes with different strain types occupy the human gut. They play a humungous role in the decomposition of indigestible dietary macro-nutrients. Changes in the composition of the gut microbiota can have effect in the human health. It has been shown that there is a complex interaction going on between microbe-microbe and microbe-host and microbe-diet, and unweaving this complex web still remains a herculean task. The emerging field of systems biology can help us better understand these interactions by making use of mathematical models along side high throughput data like metagenomics and transcriptomics.

This thesis attempts to study the metabolism underlying the pathogenesis of Lactose Intolerance through a computational study of the gut microbiome. Using Genome Scale Models (GEMs) of specific gut microbe species, we attemt to model the wokring of the gut microbiome and simulate the conditions pertaining to LI. Genome scale models of 8 microbes most assoiated with LI were combined with cell specific model of the small intestine. Using steady state metabolic modelling methods, i.e., COBRA (COnstraint

Based Reconstruction and Analysis), the integrated models of the gut microbiome and human small intestine epithilial cell were simulated under 2 dietary conditions after integrating 3 constraints specific to the disease. Metabolic models of known probiotics were added to the model to study the changes in fluxes of biomass. It was found that addition of probiotics reduces the excretion of short chain fatty acids and increases the flux of biomass.

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ABBREVIATIONS

LI - Lactose Intolerance

LP - Lactase Persistent

LNP - Latase Non-Persistent

GEM - GEnome scale Metabolic model

COBRA - COnstraint Based Reconstruction and Analysis

BL - Bifidobacterium Longum

BF - Bacteroides frigilis

CD - Clostridium Difficile

ER - Eubacterium Rectale

EC - Escherichia coli

LL - Lactococcus Lactis

LA - Lactobacillus Acidophilus

ST - Streptococcus Thermophylus

SIEC - Small Intestine Epithelial Cell

INTRODUCTION

Lactose Intolerance

Lactose is the main sugar in milk and therefore the main energy source for the newborn. Milk contains 4.8% lactose. Lactose is a disaccharide consisting of glucose and galactose (Zhong *et al.*, 2004).

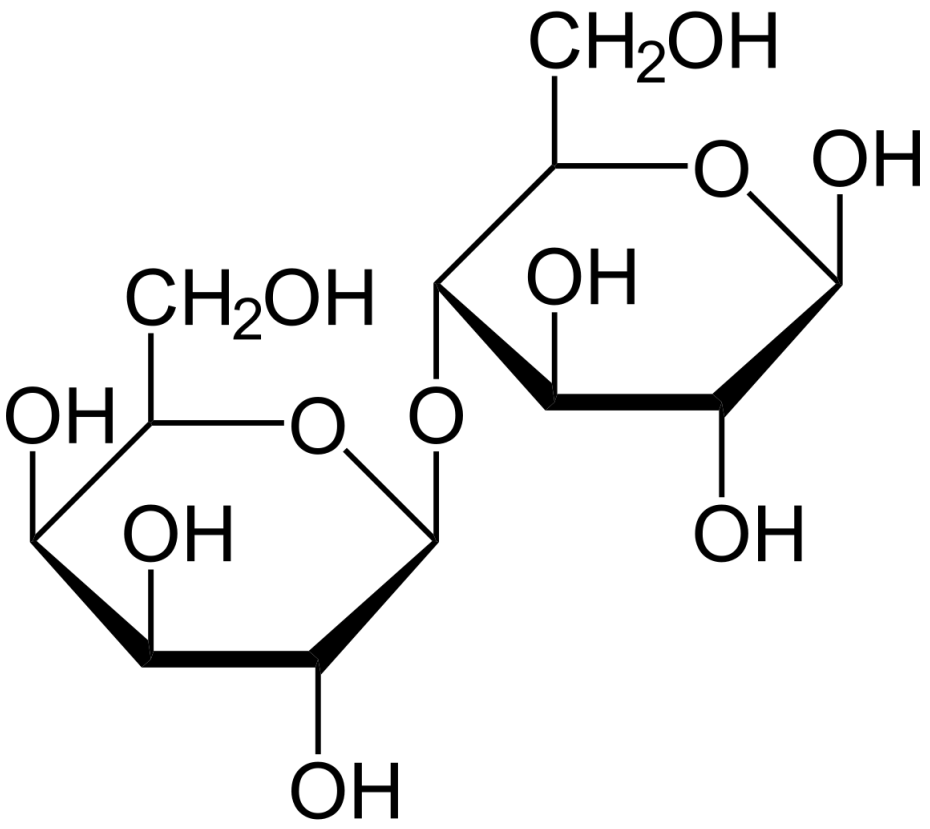

Figure 1.1: Structure of Lactose (Ref: wikipedia.org/lactose)

The inability to digest lactose in our small intestine results in a variety of symptoms including vomiting, diarreah, gloating etc. These symptoms are broadly called as lactose intolerance. Lactose Intolerance occurs because of a condition called lactase deficiency (Montalto *et al.*, 2006). In normal physiological conditions lactose is hydrolyzed by lactase also known as lactase-phlorizin hydrolase and under its systemic name lactose- galactosehydrolase (EC 3.2.1.108) (Shoaie, 2015), which is a brush-border membrane bound enzyme. Mutations in chromosome 2q21 resulted in sustenance in the production of lactase as human beings grew older. These mutations are beleived to have occured around 10000 years ago, when human beings settled down with dairy animals, and used milk as an alternative source of energy. Ever since, humans have been split into 2 groups: Lactase Persistent, those who can produce lactase, and Lactase Non-persistant, those who cannot produce lactase after a certain period of time (Szilagyi, 2015).

Gut Microbiome

The human microflora, known as "microbiota", includes bacteria, fungi, bacteriophages, and viruses and acts as an "organ" synergistically with the host, creating an ecosystem. It is able to colonize skin, the genitourinary system, the respiratory system, and, above all, the gut. Gut microbiota includes around a thousand different species and more than 15,000 different strains of bacteria, for a total weight of about 1 Kg (Shoaie, 2015)(Scaldaferri *et al.*, 2013). Stomach and small intestine are relatively poor of bacteria, whilst the colon hosts about $10^{12} - 10^{14}$ microorganisms (Shoaie, 2015). The bacterial taxonomix diversity varies with human gastro intestinal sites. The major phyla inhabiting the gut are bacteroides and firmicutes. Bacteroides phyla is gram negative and comprises of species like B. thetaiotamicron, Bacteroides spp (Scaldaferri *et al.*, 2013). They play a major role in polysaccharide breakdown. Firmicutes are gram positive and comprise of Clostridium, Eubacterium. Other less abundant phyla are Actinobacteria, Proteobacteria and Verrucomicobia (Scaldaferri *et al.*, 2013).

Composition of the gut microbiota varies across age, geogrpahy, diets, lifestyle,

diseases and antibiotic usage etc(Shoaie, 2015). The faecal samples are still the most common sources for gaining knowledge about the gut microbiota due to difficulties in obtaining samples from other parts of the intestinal tract.These colonised microbes in the human gut have dynamic and beneficial functions for the human body. Through their symbiotic relationships, the indigestible part of diet by human cells is assimilated (Sun and Chang, 2014).The gut microbiota affects the immune system by regulating immune homeostasis and autoimmunity and maintains the stability of the immune system by providing resistance against pathogens. Amino acids and xenobiotic metabolism, vitamin biosynthesis, microbial regulation of bile- acid metabolism and microbial metabolism of choline are some of the examples of microbial metabolic activities (Shoaie, 2015). Continuous exchange of bioactive molecules between the gut microflora and the host, and competition for resources have a huge influence on the health status of the host. For eg, Choline, which is taken in from the diet is absorbed by both the liver and gut microbes. This prevents the progression of multiple diseases like fatty liver disease and diabetes (Shoaie, 2015). Some metabolic functions, and possible disease associations of the major species of bacteria found in the gut are listed in Table 1.

Table 1.1: **Table 1:** Contribution of relevant gut microbiota to metabolism and diseases. (Ref: (Shoaie, 2015))

Species	**Phyla**	**Metabolic Role**	**Possible Disease Association**
Bacteroidetes spp.	Bacteroides	Polysaccharide breakdown	Obesity
B. thetaiotaomicron	Bacteroides	mucin degradation	Type 2 Diabetes
F. prausnitzii	Clostridium	Carbohydrate metabolism	Colorectal cancer
E. rectale	Clostridium	Acetate utilizer	Bloating, Diarrhoea

Involvement of Gut Bacteria in LI

From multiple experiments over the years we have confirmed that the gut microflora's composition has a huge effect on the causing and easing of symptoms associated with LI.

1. Patients who are LNP have been found to adapt to lactose over a period of time,

with an evident decrease in the intensity of symptoms with regular intake of lactose. This implies internal changes have taken place in the gut that allow the digestion of lactose (He *et al.*, 2007)(Sun and Chang, 2014).

2. Short Chain Fatty Acid (SCFA) concentrations were found to be different in healthy people and lactose intolerant people, suggesting key differences in digestive metabolism between the 2 groups (Francavilla *et al.*, 2012)(Montalto *et al.*, 2006).

3. Antibiotics that influence the composition of the gut microbiota have been found to have a beneficial effect in allevating the symptoms relating to LI (He *et al.*, 2007)(He *et al.*, 2008)(Bosi *et al.*, 2017)(Shoaie, 2015).

4. Even amongst people with LI, the intensity of symptoms are different, even with equal lactose ingestion and oro-cecal time (Montalto *et al.*, 2006)(Shoaie, 2015).

These evidences suggest that the gut microbiome plays a huge part in Lactose intolerance. Though there have been many clinical studies that aim to reduce the symptoms due to LI through probiotics, a computational study to understand the biochemical mechanism underlying LI have not been performed previously. In this study, we have developed a computational model of a human small intestine epithelial cell integrated with key microbes associated with LI to understand the disease.

Materials and Methods

Selection of relevant gut microbes to model LI

Flourescent In-situ Hybridization (FISH) method for analyzing relative composition of microbes in feacal samples (Zhong *et al.*, 2004) was performed to check the relative abundances of different organisms in patients with LI. From such studies 4 commensal (He *et al.*, 2007)(Pakdaman *et al.*, 2015)(Ritchie and Romanuk) and 4 pathogenic bacteria (Drouault, 2002)(Li *et al.*, 2012)(Scaldaferri *et al.*, 2013) were taken into consideration for building the integrated community model. The details of all the models are listed in table 2.

GEM models of Microbes and host

Assembly of Gut Organisms through Reconstruction and Analysis, or AGORA, is a resource of semi-automatically generated genome-scale metabolic reconstructions of

Table 2.1: **Table 2:** Details of gut bacteria and their Genome Scale Reconstructions from AGORA to model LI

Species	**Phyla**	**Genus**	**Genes**	**Reactions**	**Metabolites**
B. Fragilis	Bacteroidetes	Bacteroides	804	1242	1086
B. Longum	Actinobacteria	Bifidoacterium	548	1063	966
C. Difficile	Firmicutes	Clostreidiodes	889	1368	1158
E. coli	Proteobacteria	Escherichia	1243	1753	1315
E. rectale	Firmicutes	Eubacterium	700	1194	1050
L. acidophilus	Firmicutes	Lactobacillus	519	907	826
L. lactis	Firmicutes	Lactococcus	737	1230	1104
S. thermophylus	Firmicutes	Streptococcus	570	927	842

human gut bacteria (MagnÃžsdÃşttir *et al.*, 2017). By using draft Genome Scale Reconstructions from openly available sources, a metabolic network is assembled and the gaps are filled automatically, building a metabolic reconstruction which can carry flux through a defined biomass objective function. Then a semi-automatic quality check of the models are performed by reference to 236 publications and other published data. This was performed for 773 bacteria and is hosted for public access at vhm.uni.lu (Noronha *et al.*, 2018).

Genome Scale Reconstructions of the 8 microbes under consideration were retrieved from AGORA. In case of multiple models for a genus, the one with the maximum reactions was chosen. The details of all the 8 GEM models can be found in table 2.

A metabolic reconstruction of a human small intestinal epithilial cell has been reported (Sahoo and Thiele, 2013). This model was constructed using a bottom-up approach through extensive literature review and identification of previously unknown pathways in the global metabolic reconstruction.

Reconstruction of integrated host-microbes system

Constraint Based Reconstruction and Analysis is a method to simulate, analyse and predict a veriety of metabolic phenotypes from metabolic models (Thiele and Palsson, 2010).The conversion of a metabolic reconstruction into a condition-specific model includes the transformation of the biochemical reaction list into a mathematical matrix format, addition of serveral physio-chemical constraints and system boundaries (Gottstein *et al.*, 2016). The COBRA approach assumes steady state concentrations, that is, the change in metabolite concentrations over time is zero (Thiele and Palsson, 2010).

We use COBRA Toolbox, a MATLAB extension to perform COBRA operations (MATLAB, 2017). The GEM models of the 8 organisms were downloaded from VMH and were merged with the GEM model of the human small intestine epithelial cell. In this method of integration, we constructed a common compartment called the lumen

(*[u]*), through which the microbes and host exchange metabolites (Heinken and Thiele, 2015). Also, each microbe and the host have their own extracellular compartment (*e*) through which they exchange metabolites with the environment (Aw and Fukuda, 2015)(Heinken and Thiele, 2015)(Roell *et al.*, 2019). Dietary metabolites are added to the *[u]* compartment, from which every other model including the host, take their share. The secretion products and end products from the bacterial models are again transported to the lumen compartment. The secretion products and end products from the host sIEC model are transported to a separate compartment called body fluids *[b]*. This transport is unidirectional. Fig 2 is a representative image outlying the integrated model.

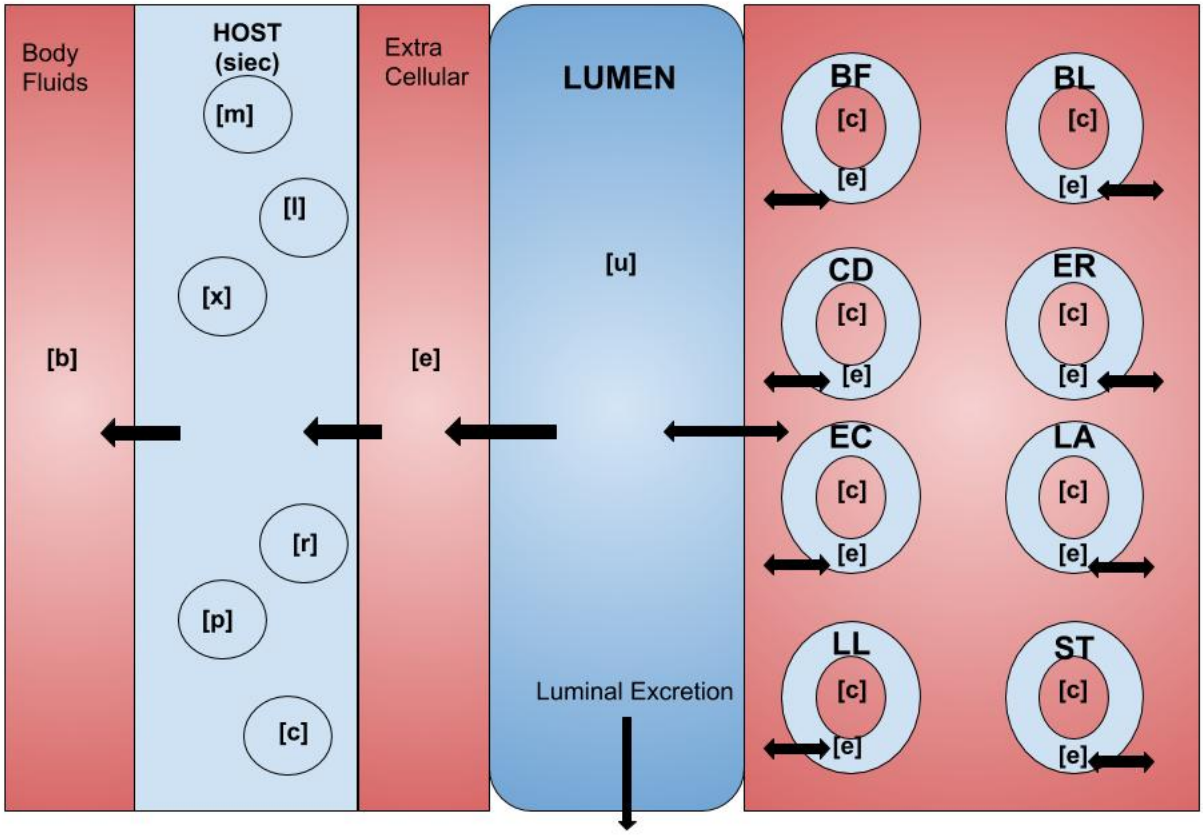

Figure 2.1: Representative model of the integrated Host-Microbe system

Flux Balance Analysis

Flux Balance Analysis (FBA) is a method used to study the behaviour of phenotypes of a GEM in a COBRA model. These networks or reconstructions consist of all the known metabolic reactions in an organism and the genes that encode each enzyme. FBA uses linear optimization algorithms to find the fluxes of all the reactions present in any reconstruction. It involves carrying out a steady state analysis, using the stoichiometric matrix for the system in question (Orth *et al.*, 2010). The biological cell network is represented by a stoichiometric matrix **S** of dimension $m \times n$. Each row of this matrix corresponds to a unique metabolite. There are m such metabolites in the system. Every column in that matrix represents a new reaction. The values of this matrix are filled based on the stoichiometric coefficients in their corresponding reactions. The flux through all the reactions in the network is represented by the vector v of length n (n number of reactions). If c is a vector representing the objective function composition in terms of fluxes, and LB and UB are the lower bound and upper bound constraints placed on all the reactions, then FBA can be rewritten as:

$$max_v c^T \cdot v,$$

such that

$$S \cdot v = 0$$

and

$$LB \leq v \geq UB$$

Figure 3 shows the conceptual basis of FBA with constraints. The addition of constraints restricts the possible solution set in the *n*-dimensional space, where *n* is the number of reactions, whereas it previously is unrestricted (Thiele and Palsson, 2010). These constraints form the basis of constraint based analysis and some examples of these constraints are steady state constraints, maximal flux constraints, dietary input constraints. Although constraints define a range of solutions, it is still possible to identify and analyze single points within the solution space. For example, we may be interested in

identifying which point corresponds to the maximum growth rate or to maximum ATP production of an organism, given its particular set of constraints. FBA is one method for identifying such optimal points within a constrained space (Orth *et al.*, 2010). This optimization can be carried out with the help of solvers available for COBRA toolbox. We used Gurobi toolbox Gurobi Optimization (2018) available for MATLAB to perform these optimizations.

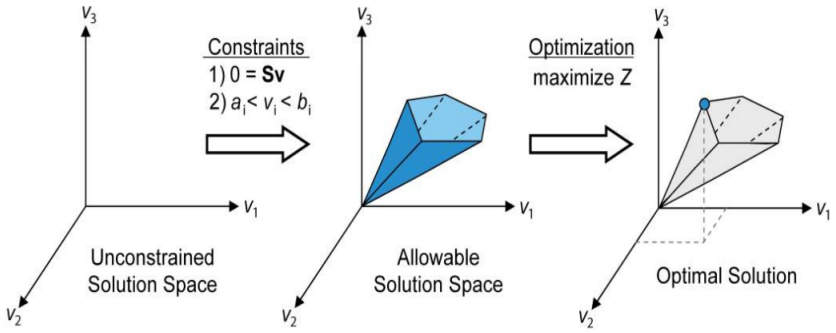

Figure 2.2: Representation of Flux Balance Analysis in a 3-D space

Addition of constraints

To simulate conditions similiar to that of a mamallian gut with LI, 4 layers of constraints were imposed.

Microbial Growth Rates

(Zhong *et al.*, 2004) and (Francavilla *et al.*, 2012) have investigated the gut metabolome of subjects with LI. Allergic patients have been found to have unusual gut microbiome with low numbers of lactobacilli/bifidobacterium, and high levels of clostridium and E.coli. The cells present in the feces were enumerated using Fluorescent Insitu Hybridization (FISH) method, using healthy, age-gender matched patients as control. After data analysis they tabulated the mean increase/decrease in the microbial populations in allergic infants. Using these data, the biomass fluxes of the organisms under our study were constrained. The ratios and ranges by which each organism's biomass fluxes were constrained to are available in Supplementary information.

Enterocyte Metabolite Fluxes

(Francavilla *et al.*, 2012) have calculated the concentration of several metabolites in the feces of patients with LI. Water soluble fraction of the patient's feces was analyzed through 1H NMR spectroscopy, and multiple volatile organic compounds were profiled through gas chromatography mass spectrometry. Through this, the concentration of several volatile compounds in the feces of patients with and without LI have been tabulated. They have also measured the median concentrations for all these compounds, along with the standard error. Using these as ranges, the upper and lower bound fluxes of the excretion reactions of the corresponding metabolites have been fixed in our model. The constraints placed on these fluxes are available in Supplementary Information S2.

Enterocyte Lactose Breakdown Pathway

The most important charecteristic of a patient with Lactose Intolerance is the inability of the gut to breakdown lactose. This reaction has been modelled in the gut and has been has been named as '*LACZ*' in the latest RECON 3D model of the human metabolism (E *et al.*, 2018). To simulate conditions similar to LI, this reaction was blocked, that is, the flux passing through this reaction is constrained to zero.

Diets

The integrated model was fed on 2 different diets (Thiele *et al.*, 2013), an European diet and a high fiber diet. The 2 different diets have varying amounts of carbohydrates, proteins, lipids, and alcohol. These diets were taken from the Virtual Metabolic Human webisite (Noronha *et al.*, 2018). These diets were fed into the lumen compartment of the model [u]. The details of the diets and the matlab scripts for their addition can be found in Supplementary Information.

Addition of probiotics

Probiotics are live bacteria or yeast that complement the existing gut microflora by (1) secretion of anti-microbial substances, (2) competitive adherence to the mucosa and epithelium, (3) modulation of intestinal permeability, and (4) strengthening of the immune system (Ritchie and Romanuk). For LI in particular, there has been extensive study of the use of probiotics to ease the symptoms associated with LI. In addition to the removal of lactose in the small intestine, the effect of probiotics in patients with LI can also be explained at the level of colonic fermentation. They remove SCFA by using them as energy sources in an anaerobic respiration and converting them into bacterial mass. For the purpose of this study we used 2 well established probiotics (Ritchie and Romanuk) Lactobacillus casei (firmicutes) and Bifidobacterium breve (actinobacteria) to study the effect on the enterocyte biomass flux and the rate of excretion of SCFA into the blood from the system.

Results

Bacterial Growth Rates

After integrating the 8 bacterial models with the Small Intestine Epithelial Cell model, We added two diet constraints and optimized for the biomass flux of the small intestine. The biomass fluxes of all the organisms are shown below.

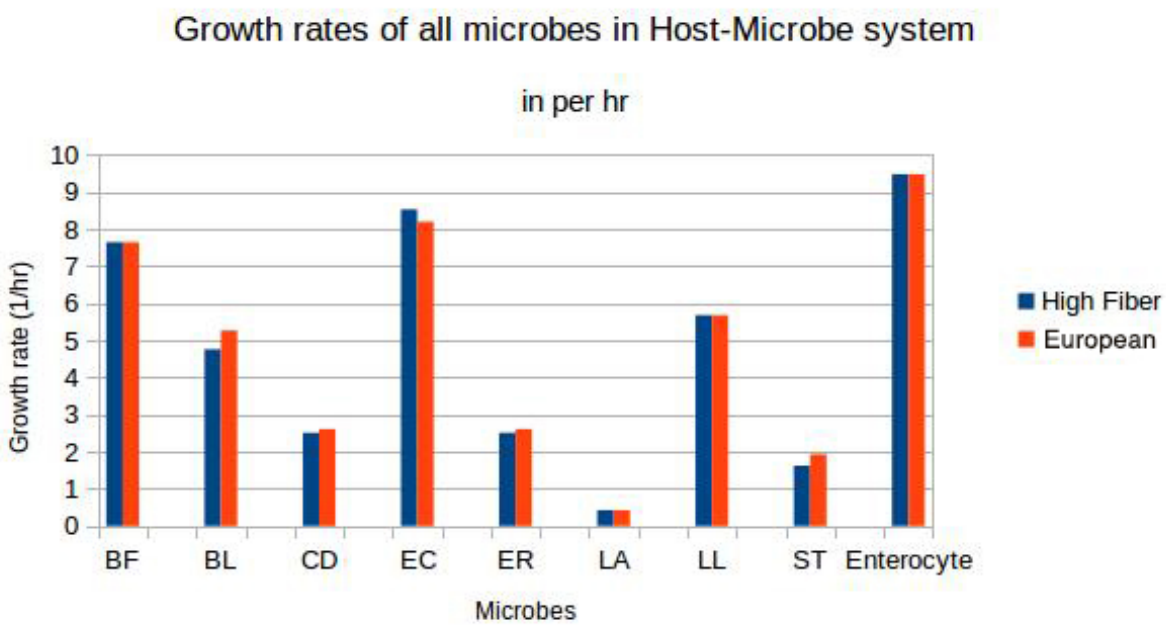

Figure 3.1: Flux through biomass of all EM models in 2 diet conditions

After adding the constraints needed to simulate conditions similiar to the gut of a patient with LI, we again optimized the model to maximize flux through biomass of small intestine. The biomass fluxes of all organisms are shown below.

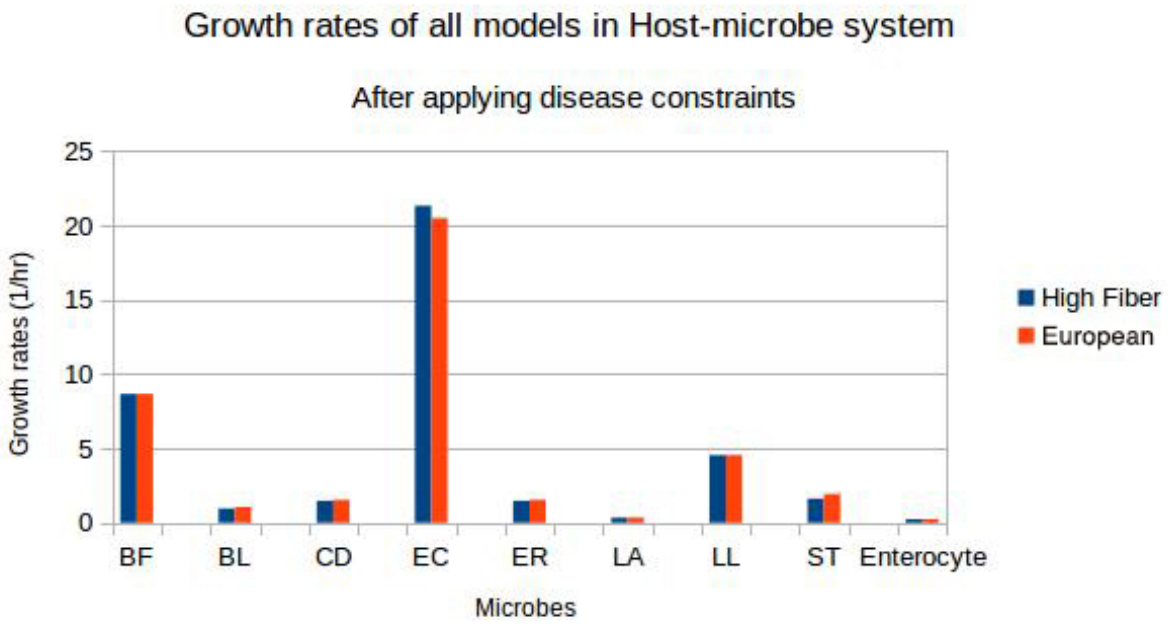

Figure 3.2: Flux through biomass of all GEM models in 2 diet conditions with LI constraints

The flux through biomass reaction falls close to zero in these conditions, which suggests that the applied conditions are not suitable for the growth of epithelial cells.

Effect of Probiotics

Significant improvement in the flux through biomass was observed after integrating genome scale models of Bifidobaterium breve and lactobacillus casei to the existing model in a high fiber diet condition. The flux through biomass increased by **23%** after addition of the known probiotic models. Fig 3.2 shows the difference in the biomass flux of SIEC after adding probiotics.

Further, we analysed the flux of SCFA being excreted from the system into the blood. We can see that the rate of excretion of SCFA decreases, thereby explaining the reduction in symptoms arising due to LI. Fig 3.3 shows the reduction in SCFA excretion.

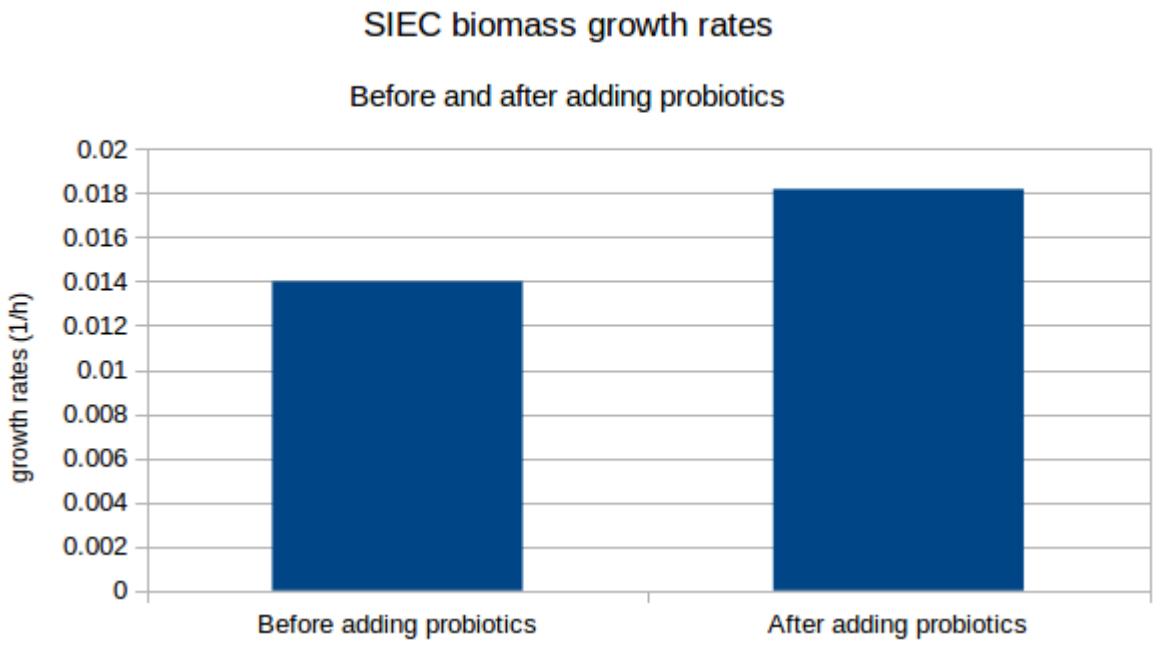

Figure 3.3: Flux through SIEC biomass flux before and after adding probiotics

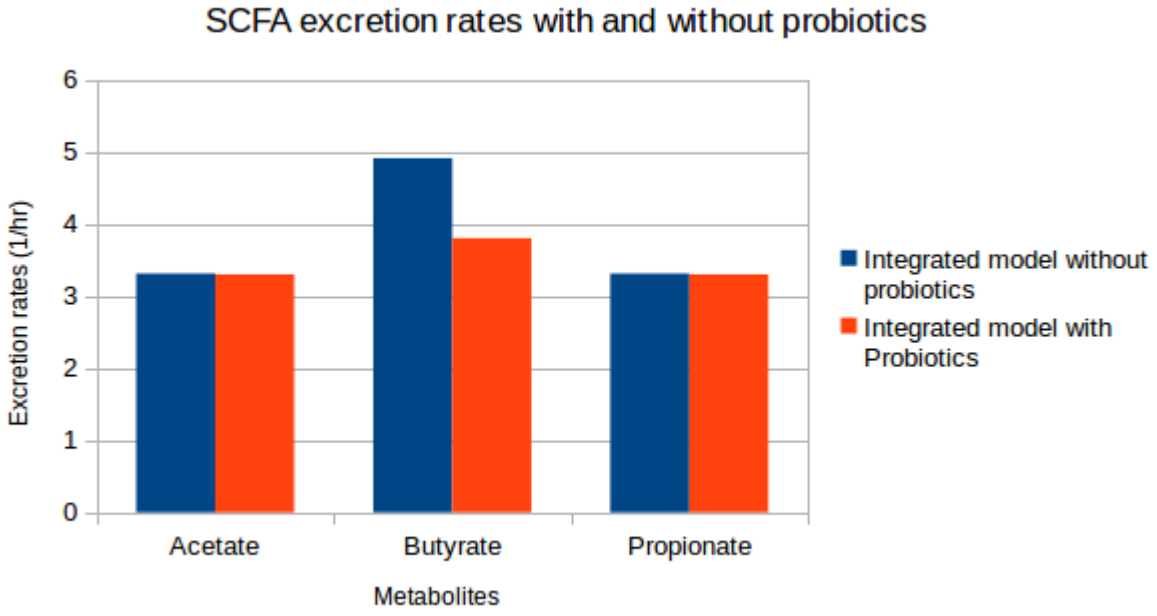

Figure 3.4: Flux through SCFA excretion to body fluids in high fiber diet conditions with LI constraints

Discussion

In this study we have developed an integrated model to simulate the conditions similar to that of a human gut with lactose intolerance. To achieve this, we integrated GEM models of 8 organisms most associated with LI, either through its commensality or its pathogenicity to the human small intestine, with a GEM model of the human small intestine epithilial cell. The constraints applied to simulate the conditions are based on a study which focuses on patients living in a small geographical area. The human gut microbiota's composition varies from place to place, depending on the food habits, climatic conditions etc.

Also we were able to determine the commensality/pathogenecity of all organisms used in the study based on their effect on the flux through biomass reaction in the human epithilial cell model. Calculating the fluxes for every pair-wise model of a bacteria and the epithilial cell model reveals that pathogenic bacteria lower the growth rate of the epithelial cell model to a much larger extent.

Supplementary Information

All codes, models and data mentioned above are available at https://github.com/RajivHeisenberg/Syster
Modelling-Lactose-Intolerance.

Table S1: Differences in excretion fluxes of metabolites between healthy and diseased patients.

Table S2: Differences in bacterial concentrations between healthy and diseased patients.

High Fiber Diet: Matlab script to add High fiber diet specified by VMH to the model.

European Diet: Matlab script to add a western diet specified by VMH to the model.

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