Systems approach to study the biochemical factors driving the pathogenesis in Short Bowel Syndrome

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Dual Degree Project Report

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Abstract

Surgical removal of small intestine causes Short bowel syndrome or SBS. The procedure is done for various reasons, ranging from inflammations to tumours. It is characterized by bacterial overgrowth in the gut, leading to pathological consequences in small intestine and liver. In order to understand the underlaying biochemical mechanisms that drive the pathogenesis of SBS, a systems level analysis of the metabolism of the SBS-implicated gut microbes is essential. Additionally, interaction between these microbes among themselves, as well as, with the human host could aid in predicting optimal dietary interventions.

Genome scale metabolic models of the 9 SBS associated microbes were combined with cell specific metabolic model of the small intestine. Using steady-state metabolic modeling methods, i.e., COBRA (Constraint Based Reconstruction and Analysis) the integrated model of gut-microbes and small intestine was simulated under 11 dieatry constraints/conditions. The microbial growth rates were identified to be highly sensitive to the different diets. Integration of liver-specific metabolic model to the combined set-up will aid in better understating of SBS.

Short Bowel Syndrome

Short Bowel Syndrome (SBS) is a small intestinal disease arising from surgical removal of a section of the small intestine. This is done for a variety of reasons, ranging from inflammation to cancerous tumours. This causes malabsorption, and is charecterized by severe diarrhea. The patient is kept on a parenteral diet. SBS further leads to liver damage. [4]
It has been recently found that the altercations associated with SBS arise majorly because of changes in the gut microbiome composition associated with parenteral nutrition. Specifically, SBS causes an overgrowth of 2 bacterial phyla: Bacteroides, Firmicutes. Multi-variate analyses were performed on piglet model of SBS to find a panel of metabolites with a correlation analyses with the individual microbes could be performed. This way, a set of metabolites, measured non-invasively, were found which correlate with the bacterial composition in the gut. [2] Reconstruction details of the 9 bacterial models associated with SBS that we are using for our experiments are given in table 1.

Gut Microbiome

Co-evolution has forced mutiple species to forge a mutually benefical bond in various parts of the environment. This is seen virtually in every life form. Similiarly, mammals and the microbiota present in their guts have co-evolved and have become co-dependent on each other. The Gut microbiome is an essential part of the body; it is an 'acquired organ'. It allows and efficiates multiple essential processes in the human body. The stability of this relationship is dependent heavily on environmental and external factors.[5]
The gut microbiome is majorly composed of four phyla: Bacteroides, Firmicutes, Proteobacteria, and Actinobacteria. The first two are the most abundant of the gut microbiota. Though the exact composition of the gut microbiome varies from one person to another, even a delicate change in the composition of an individual gut composition will lead to disease and malfunction.[7]
The gut microbiome provides a variety of benefical functions. Some of them are: gleaning indigestible ingredients from food and synthesizing nutritional factors, such as vitamins; detoxifying the deleterious xenobiotics[8] and affecting the host metabotypes; development of a robust systemic and intestinal immune system; providing signals for epithelial renewal and maintaining gut integrity; and secretion of anti-microbial products[8], which negatively select against pathogenic bacteria through the develop- ment of colonization resistance.[5] When dysbiosis in the gut happens, it can cause and sustain disease states. It has been reported that when bacteroides composition in the gut increases, it promotes atherosclerosis through metabolism of dietary carnitine and phosphatidylcholine. This has been found to be positivly corelated with bacteroides, and associated with a western diet. [10] It has been clearly established that the gut microbiome is very crucial to the functioning of the human body itself. Thus, charecterizing the metabolic interactions between the microbiome and the gut is necessary to understand and predict the functional relationship between them. Various methods have been applied to charecterize the metabolic interactions.

Metabolic modelling of gut microbes

The gut microbiome has an estimated 10^{14} bacteria from thousands of phylotypes. Numerous model systems exist to study the links between gut microbiota and host metabolism, including in vitro cell culture models, in vitro gut models, ex vivo organ models, animal models, human in patient studies etc. To identify microbes responsible for fermentation of certain dietary carbon sources, C-13 isotopomer labeling has been recently combined with 16S rRNA based stable isotope probing [11]. A bottoms up manner for metabolic reconstructions has been well established and implemented. Pathways are assembled based on detailed information obtained from experimental studies and integrated into large-scale reconstruction.

To model a network of microbial models is much more complex, because the simplistic assumptions at the basis of FBA become challenging when aplied to model multi organism metabolic interactions.

Table 1: Bacteria associated with SBS. Shown are the model contents

Name	Reactions	Metabolites	Genes
Acidaminococcus fermentans	1090	903	646
Acidaminococcus intestini	994	827	599
Acidaminococcus sp. D21	994	827	599
Parabacteroides distasonis	1347	1044	796
Parabacteroides goldsteinii	1332	1050	944
Parabacteroides gordonii	1308	1029	831
Parabacteroides johnsonii	1282	980	803
Parabacteroides merdae	1303	990	774
Parabacteroides sp. D13	1212	984	877

Typically, the species are reconstructed separately and then joined through appropriate in silico methods[12]. A community objective function has to be defined to allow the optimization of multi species growth. In many cases this meant that the two species could consume simulated dietary inputs and exchange nutrients with each other through an in silico compartment simulating the intestinal lumen[13]. AGORA (Assembly of Gut Organims through Re- construction and Analysis) is a resource of genome scale reconstructions of 773 gut bacteria. Genome sale reconstruction is done with the help of experi- mental information. Since manual curation of indi- vual microbe is time consuming, they have deloped a method to propogate a change in a metabolic re- construction to other reconstructions too, thus ac- celerating the process[1].

Flux Balance Analysis

Flux balance analysis is a constraint based approach for analyzing the flow of metabolites through a metabolic network. FBA calculates the flow of metabolites through this metabolic network, thereby making it possible to predict the growth rate of an organism or the rate of production of a biotechnologically important metabolite. FBA is now a widely used approach for studying biochemical networks,[9] in particular, genome-scale metabolic network reconstructions which contain a structured information about all metabolites, reactions, genes, and the enzymes they encode. The constraints imposed are either in the form of metabolite input/outputs or as inequalities to define the boundaries of the system. For eg: in an anaerobic system, the flux of oxygen is constrained to be zero, while in an ideal aerobic system, the system is allowed to take in as much oxygen as it needs. Now, we have with us a set of linear equations and a set of constraints, giving us a constrained optimization problem. Since a typical cell will have hundreds of reactions and hundreds of metaolites, it is a herculian task to solve them by hand. These poblems are often solved with the help of computational tools. We use the COBRA toolbox in MATLAB to solve such problems.[9]

Metabolic reactions are represented as a stoichiometric matrix (S), Every row of this matrix represents one unique compound and every column represents one reaction. Each entry in the matrix denotes the stoichiometric coefficient of the metabolite participating in the corresponding reaction. It is positive when the metabolite is produced in the reaction, and negative when it is consumed. S is usually a sparse matrix, since each metabolite participates in a very limited set of reactions. In a typical system, the number variables under play will be more than the number equations available. Such a system is called an under-determined system. Such a system is solved by imposing additional constraints on the fluxes.

A vector of all the fluxes through a network is usually represented by the variable v. Then the system of mass balance equations at steady state is represented as $S.v = 0$.

FBA can thus be defined as the use of linear programming to solve the above equation given a set of upper and lower bounds on v and a linear combination of fluxes as an objective function.[9]

Small Intestine Model

A genome level reconstruction of a human small intestine has been done. Through extensive literature review, and manual curation of the model, the final human small intestine model was constructed with a total of 1282 reactions and 433 unique metabolites and 611 genes. [3]

In order to mimic SBS like constraints in the small intestine model, gene expression data capturing the SBS associated genes, i.e., differential gene expression was used.1346 upregulated and 678 downregulated genes have been established in SBS in zebra fish model. [4]
It has also been established that 71.4 percentage of human genes have atleast one zebrafish orthologue of th 26,206 protein coding genes. Similiarly, 69 percentage of zebrafish genes have atleast one human orthologue. Further, 47 percentage of the orthologous human genes share an one-one relationship with their zebrafish conterpart.[6] This data will be used to constrain the healthy gut microbe model for simulating SBS-like pathology.

Methods

Assembly of Gut Microbes using Reconstruction and analysis (AGORA) has a resource of 773 gut microbes, reconstructed and tested.[1] From them, our bacteroides were taken and converted into mat format for use in COBRA toolbox. They were grown in different diets to find where they grow the most. These microbes are then integrated with the SIEC model, and the corresponding diet is fed, and the growth rates are calculated. The fluxes of various metabolites associated with SBS and the metabolites which are malabsorbed by the small intestine are studied keenly to find out if there are any metabolic factors driving SBS pathogenesis. A workflow of the procedure is given in figure 1.

Results

The 9 microbe models associated with SBS were grown on 9 different diets. The maximum growth rates were found in western and high fiber diets. The growth rates of the 9 organisms in a high fiber diet and in a western diet are plotted in figure 2 and 3. The same growth rates have been reported earlier[1].

Discussion and future work

Individual charecterization of microbe-metabolite interactions in the gut are sverely limited, because of co-evolution and co-dependence. A systems level charecterization of the interactions provides us a clearer picture. The next step of the project would be to integrate these 9 organisms with the small intestine model, and grow them in the high fiber diet. Further, mapping zebrafish genes with the latest RECON will help us in computationally analyzing the genetic factors involved.

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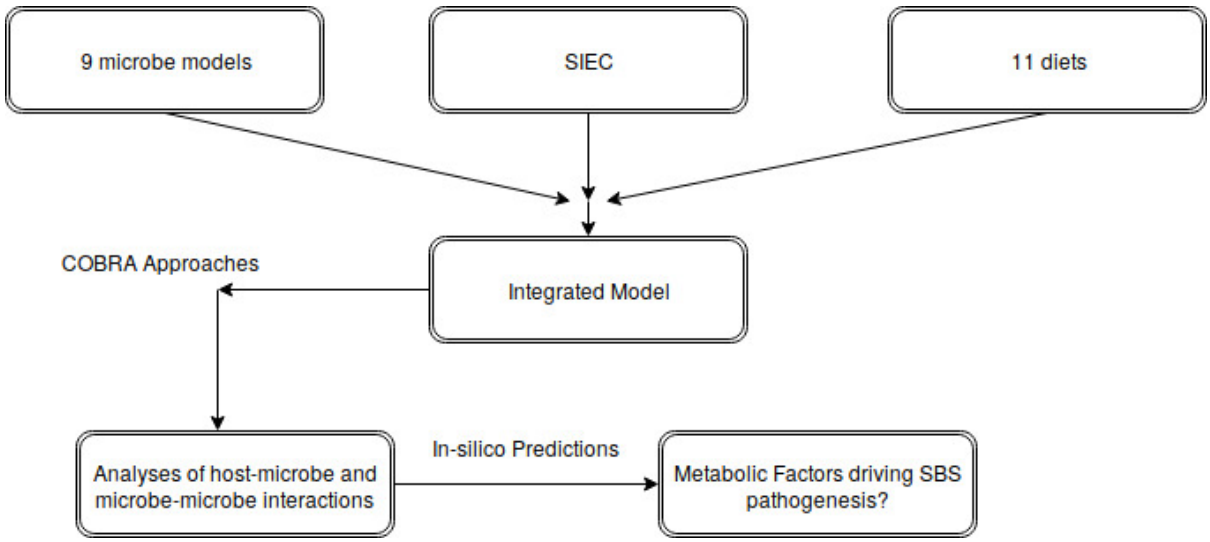

Figure 1: Workflow of the approach

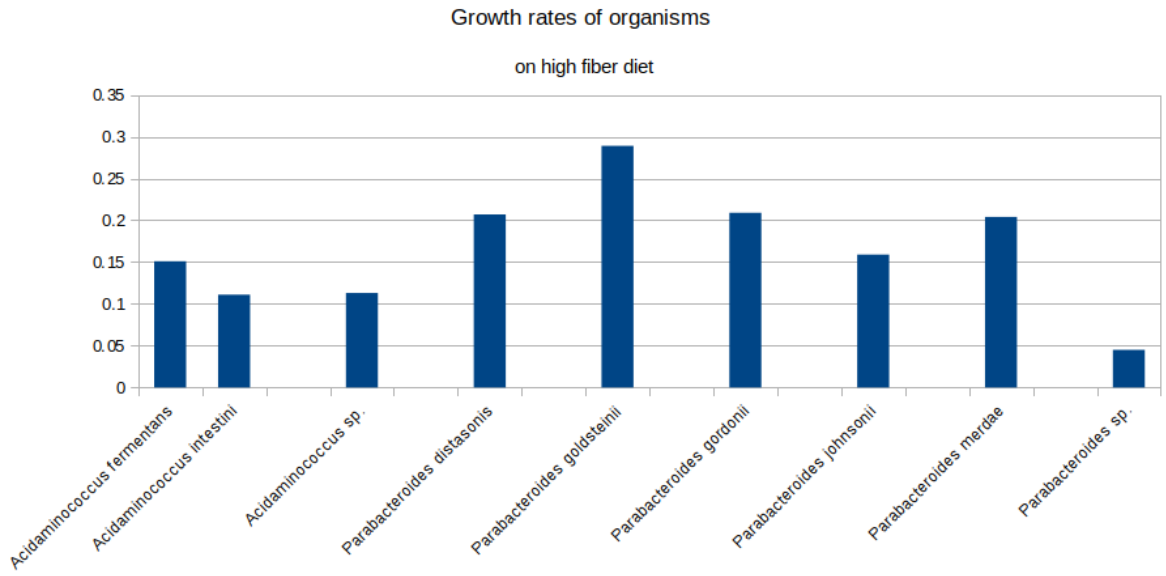

Figure 2: Growth rates of the metabolic models in high fiber diet (h^{-1})

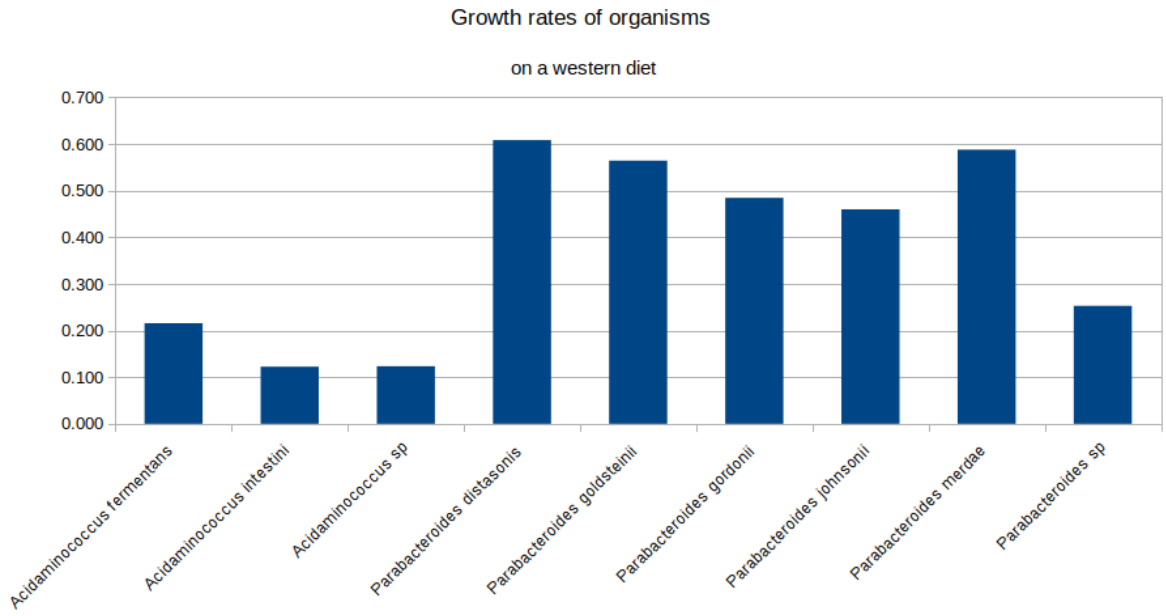

Figure 3: Growth rates of the metabolic models in western diet (h^{-1})