# The developing infant gut microbiota: mathematical predictions of the effects of oligosaccharides

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# Summary

A complex community of microbes develops in the infant gut shortly after birth. We call this community the infant gut microbiota. The microbiota can influence the health of the infant, which makes the composition and function of the infant gut microbiota an important topic to study. It is not possible to directly study the development of the microbiota inside the infant, so we are limited to information from fecal samples and laboratory experiments. These can provide information on the composition of bacteria that leave the infant gut, and on the potential interactions between these bacteria, but we cannot assume that these observations are the same in the infant gut microbiota as it exists in the infant. Because it is so difficult to study, the processes and mechanisms that shape the microbiota also remain unclear. One technique that can provide insight into unseen processes such as these is mathematical modelling. Mathematical models can generate hypotheses and predictions about the inner workings of a system, such as the infant gut microbiota. In this thesis, we develop mathematical models to predict how the microbiota in the infant gut grows and interacts, and how it responds to oligosaccharides, a type of complex sugar.

The infant gut microbiota is composed of many species of microbes, including bacteria, archaea, and fungi. In this thesis we focus on the bacteria, which make up most of the microbiota. Previous studies have used the genomes of gut bacteria to predict what metabolic reactions they can do. These lists of metabolic reactions can be used to try to predict what metabolism a bacteria has under different circumstances, i.e. what metabolic reactions it uses. We have curated and refined these lists of reactions to better match the available data on how bacteria act in real life. The mathematical model we developed then predicts what metabolic reactions a bacterium uses in a particular environment with a technique called flux balance analysis. This lets the model make predictions both on how bacteria are influenced

by the environment and on how they influence the environment. In the model bacterial populations can influence their environment by taking up nutrients from it, by depositing other substances into it, and by growing. By applying this influence to the environment and repeating the technique, the model can make predictions for how the environment changes over time, and how the abundance and metabolism of bacteria changes over time. The model uses a grid to represent the gut environment, so that each bacterial population, nutrient, and metabolite has a particular location in the gut. This way it takes into account that different bacterial species may occur in different parts of the infant gut, and how the availability of nutrients may differ throughout the gut because of the influence of bacteria.

In this thesis, we use and further develop this model to make predictions on how various changes to the environment, such as the presence of oxygen, oligosaccharides, or antibiotic disturbances, influence the infant gut microbiota, their metabolism, and ultimately the infant.

In chapter 1 we first give an overview of the typical composition of the infant gut microbiota, and how this can change over time. We then describe our modelling approach in general, and flux balance analysis, the technique we use to model microbial metabolism, in particular. We explain how we use this technique to model not only a single bacterium but an entire microbiota. We also discuss how we model the potential variation over space and time in the infant gut microbiota.

In chapter 2 we introduce the mathematical model and its computational implementation, and use it to examine the effect of oxygen on the simulated infant gut bacteria. We show how the model can reproduce important parts of the metabolism of *Bifidobacterium* bacteria, and that it correctly predicts the major groups in the infant gut microbiota, but not the smaller groups. We also show that by introducing oxygen into the initial environment the model can reproduce the succession from *Escherichia coli* bacteria to *Bifidobacterium* bacteria that is observed in the real infant gut microbiota. The model can explain this through the different metabolisms of these species: while oxygen is abundant *E. coli* grows faster, but as oxygen depletes *Bifidobacterium* bacteria grow faster.

In chapter 3 we extend the model to also include different prebiotic oligosaccharides. Prebiotic oligosaccharides are complex sugars that cannot be digested by the infant, but can be digested by *Bifidobacterium* bacteria. These oligosaccharides are present in human milk, and also often added to infant formula. With the model we examine how oligosaccharides may affect not only the *Bifidobacterium* bacteria that

consume them, but also butyrate producing bacteria that consume the products of *Bifidobacterium* bacteria. These butyrate producing bacteria may play an important role in infant health. The model predicts that, in isolation, butyrate producing bacteria can co-exist with *Bifidobacterium* bacteria with or without oligosaccharides. However, the model predicts that in a complex ecosystem, like you might find in the infant gut, butyrate producing bacteria are outcompeted by other bacteria in most conditions. What bacterial species are effective competitors depends on whether oligosaccharides are present, and on what kind of oligosaccharide is present. The model predicts that the prebiotic milk oligosaccharide 2'-fucosyllactose can indirectly stimulate butyrate production within the infant gut microbiota, allowing them to outgrow their competitors. It can do so because *Bifidobacterium* produces propane-1,2-diol from 2'-fucosyllactose, which is consumed most efficiently by butyrate producers. These model predictions should be tested in laboratory experiments.

In chapter 4 we further extend the model with intestinal mucin. Mucin is produced by the gut wall, and consists largely of oligosaccharides that can be consumed by some bacteria. We also extend the model with extracellular digestion of oligosaccharides from both mucin and prebiotics. We aim to explain why the gut microbiota of breastfed infants consumes less mucin than the microbiota of non-breastfed infants, because the consumption of mucin may have negative health effects for the infant. The model predicts that the milk oligosaccharide 2'-fucosyllactose stimulates non-mucin consuming *Bifidobacterium* bacteria more than mucin-consuming *Bifidobacterium* bacteria. It further predicts that this happens because the mucin-consuming *Bifidobacterium* bacteria use extracellular digestion, and this causes the sugars they produce to be 'stolen' by other bacteria. This lets the non-mucin consuming *Bifidobacterium* bacteria outcompete the mucin consumers. We link these observations to the literature on 'public goods' that is available in the field of ecology.

In chapter 5 we examine how an antibiotic disturbance may affect the infant gut microbiota. We simulate an antibiotic disturbance in the model by increasing the probability for bacterial populations to die off on the simulated day 8 and 9 after birth, and then observe how the microbiota recovers. The model reproduces the reduced diversity of an antibiotic disturbance observed in the infant microbiota. The model further predicts that stronger disturbance of the microbiota by antibiotics that is observed in infants, compared to adults, may be partially explained by the antibiotics disturbing the natural succession that occurs in the early infant gut microbiota. Finally, the model predicts that prebiotic oligosaccharides in nutrition may lead to

a microbiota that returns to the original state more consistently after a disturbance, because the oligosaccharides consistently stimulate *Bifidobacterium* bacteria. However, the model does not reproduce the effect of a disturbance on the abundance of major bacterial groups correctly under most conditions. We give suggestions on how the model may be expanded to give a more complete view of the potential effects of antibiotic disturbances, and potentially function as a framework for future investigations.

Finally, in chapter 6 we place the model in a wider perspective. We first discuss limitations and future opportunities for the model, in particular how future versions may incorporate competition or cooperation between bacteria through antibiotics or acidification. We then compare our modelling approach with other approaches, and finally we discuss the direction of infant gut microbiota research in general. We predict that the microbiota of non-human mammals may provide further insights into the role of milk oligosaccharides in shaping the gut microbiota, and we also predict that a further standardisation of microbial modelling methods can lead to a better exchange of results between different studies.