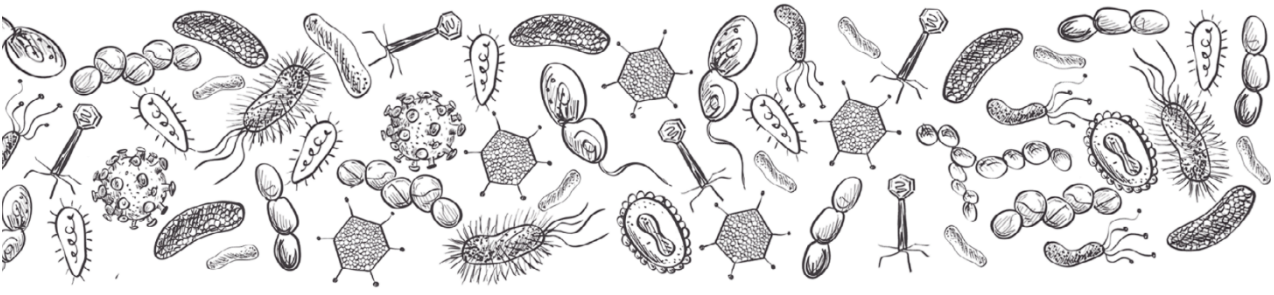
A clash of kings

Addendum

Summary



The human body harbors a large amount of microbial cells, called the microbiota. The majority of the microbiota is found in the human gastrointestinal tract, with the highest numbers in the large intestine. The gut microbiota plays an important role in host health, e.g. in absorption of nutrients and protection against pathogenic bacteria. The gut microbiota consists of microbes from different kingdoms: bacteria, viruses and fungi. Research has mainly focused on the bacterial part of the microbiota, thereby neglecting the viral and fungal component. Studies on the gut viruses and fungi show that they can be important in human health and therefore tools to research them are needed. *In vitro* gut fermentation models allow for the research on both microbial composition and functionality and could be helpful in the research on viruses and fungi in the human gut.

Bacteriophages make up the major part of the viruses found in the human gut. Bacteriophages are viruses that can kill bacteria and lytic bacteriophages can be used as therapy to combat bacterial infections. They are very specific, i.e. they have a small host-range, and this has as an advantage that the commensal bacterial community is not disrupted, as is the case with antibiotic therapy. With the rise of antimicrobial resistance, alternatives to antibiotic therapies should be explored. Bacteriophage therapy is a promising treatment strategy for bacterial infections, but more research is needed to design effective therapies. In **chapter 2** the importance of identification of bacteriophages before using them in therapy is described. This identification was done using Nanopore sequencing in combination with electron microscopy. It was also shown how bacteriophages can be isolated from waste water. After the identification, the survival of two bacteriophages (a phage against *E. coli* and phage J1 against *Lacticaseibacillus* [formerly *Lactobacillus*] *casei*) in the upper GI tract (stomach and small intestine) was investigated with the use of the *in vitro* model TIM-1. These experiments showed that bacteriophages are affected by passage through the upper GI tract, and also that the survival rate can differ between different phages. When phages are ingested in a fed-state, roughly 5-10% reaches the lower GI tract. The use of TIM-1 in the design of phage therapy could help with increasing the efficacy of the therapy by optimizing the dosage or exploring the use

of survival strategies, such as encapsulation, to enhance survival. For an effective bacteriophage therapy, after passage through the upper GI tract, bacteriophages need to survive and be effective in the complex microbial environment of the colon. In **chapter 3**, the survival and efficacy of bacteriophages in the colon and the effect on the bacterial community in the colon was studied with the use of the *in vitro* model TIM-2. An antibiotic-resistant *E. coli* was used in combination with a corresponding bacteriophage. The survival of the bacteriophage was tested in TIM-2 in different conditions: addition of only the phage, *E. coli* and the phage, and *E. coli* and multiple shots of the phage. The stability of the bacterial community was followed using 16S rRNA sequencing. It was shown that phage titers could be decreased by activity from the commensal microbiota. Levels of the phage host (here *E.coli*) were decreased in the interventions with the phage shot. Multiple shots did not seem to be more effective than a single shot. At the same time, the bacterial community was not disturbed and remained stable throughout the experiment, which is in stark contrast to treatment with antibiotics. This high specificity shows that phage therapy could be a promising alternative to antibiotic treatment in treating GI infections, because the microbial community is less affected.

In addition to viruses, also fungi and yeasts are present in the human gut. Comprehensive information on gut fungi is lacking, leading to missing insights on the mycobiome and its interaction with the gut bacterial community and the human host. In **chapter 4**, the use of TIM-2 as a tool for the study of the gut fungal community was investigated. In experiments with standard feeding (SIEM), bacteria and fungi were analyzed using 16S rRNA and ITS sequencing. The fungal community showed low diversity and a greater variability when compared to bacteria. Taxonomic classification showed that at the phylum-level *Ascomycota* and *Basidiomycota* dominated, while *Agaricus*, *Aspergillus, Candida, Penicillum, Malassezia, Saccharomyces, Aureobasidium, Mycosphaerella, Mucor* and *Clavispora* were the most abundant genera. In addition, dietary interventions (high carbohydrate, low carbohydrate and glucose as carbohydrate source) were carried out to see if this modulated the gut fungal community and it was shown that the

change of diet could influence the diversity. Overall, the experiments showed that the mycobiota could be modelled in TIM-2, however the low diversity and high variability make studying fungal, as compared to bacterial communities, much more challenging. Future research should focus on optimization of the stability of the fungal community to increase the strength of the results. The gut fungal community was further investigated in **chapter 5**, where the mycobiota of healthy individuals was analyzed. For this, on fecal samples of 163 individuals that were available from two separate studies, ITS2 and 16S rRNA sequencing was performed to analyze the fungal and bacterial microbiome, respectively, as well as their cross-kingdom interactions. The results showed a much lower fungal as compared to bacterial diversity. *Ascomycota* and *Basidiomycota* were dominant fungal phyla across all samples, but levels varied enormously between individuals. The ten most abundant fungal genera were *Saccharomyces, Candida, Dipodascus, Aureobasidium, Penicillium, Hanseniaspora, Agaricus, Debaryomyces, Aspergillus* and *Pichia,* and here also extensive inter-individual variation was observed. Correlations were made between bacteria and fungi, and only positive correlations were observed. To further investigate the importance of the observed correlations found, more research is needed to discriminate between gut colonizers and transient species. The interactions between the fungal and bacterial community in the gut were further analyzed in **chapter 6** with the use of TIM-2, where interventions with antibiotics and fungicide were carried out to investigate whether these two microbial communities were disrupted. The communities were analyzed with the use of next generation sequencing of the ITS2 region and the 16S rRNA gene. Also, production of SCFAs was followed during the interventions. Correlations between fungi and bacteria were calculated to investigate possible cross-kingdom interactions. It was shown that acute treatment with antibiotics or fungicides did not greatly alter the bacterial or fungal communities. SCFAs levels were lowered in samples treated with antifungals. Spearman correlations suggested that cross-kingdom interactions are present in the human gut, and that fungi and bacteria can influence each other. Further research is required to gain more insights in these interactions, and their molecular nature, and to determine the clinical relevance.