

# Investigating the effect of capsaicin on Firmicute bacteria in the human gut

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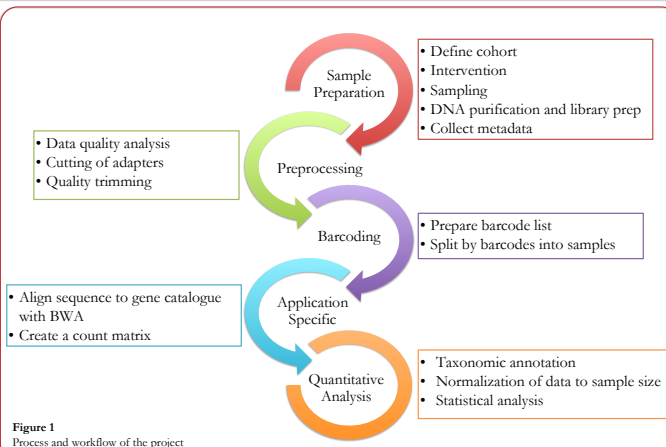
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## Introduction

Research on the human gut microbiota has become a major research area partly due to the association between the gut microbiota and diseases. Capsaicin is the active compound in chili peppers, and the focus on capsaicin has increased due to studies that suggest beneficial effects in controlling obesity and cardiovascular diseases (1,2). Only few studies of the effect of capsaicin on the microbiota have been published. One study with a 6-week controlled capsaicin feeding trial revealed an increased Firmicutes/Bacteroidetes ratio (4). This study analyzed fecal samples with bacterial 16S rRNA gene sequencing, which revealed the proportion of Firmicutes increased at different concentrations of capsaicin, whereas only a high concentration (10 mg/day) of capsaicin revealed a decrease of Bacteroidetes (4). These two phyla are considered to be the major phyla of a healthy microbiota, and studies have associated different distributions of these phyla with obesity and Crohn's disease (5).

Our aim is to investigate the effect of capsaicin on Firmicutes bacteria in the human gut based on the study by Kang, C. et al. We expect to see an increase of the Firmicutes in the gut after intake of chili.

## Process



**Figure 1**  
Process and workflow of the project

## Data and Methods

### Library Preparation

21 individuals, aged 20-35 participated in the study and were randomized in two groups; an intervention group and a control group. The intervention group had to intake a large amount of chili and collect a fecal sample prior to and after the intervention, whereas the control group had to maintain a normal diet. DNA were purified and isolated from the fecal samples, which were subsequently fragmented and quality analyzed. Next the library preparation was performed by ligation of adapters and addition of barcodes and primers to the fragments. The samples were sent for Illumina sequencing at BGI, China.

### Preprocessing

A quality analysis of the reads was performed using FastQC v0.11.5. The report lead to cutting of adapters and a minimum length of 30 bp.

### Barcoding

A barcode list was prepared, and the reads were splitted by barcodes into forward and reverse samples.

### Application Specific

The reads were mapped against a gene catalogue containing 3.9M genes using the Burrows-Wheeler alignment (BWA) v0.7.1. The mapped genes were combined in a count matrix.

### Quantitative Analysis

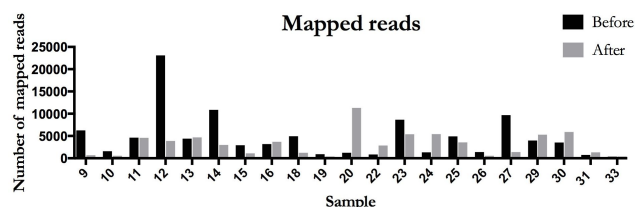
The genes in the count matrix were annotated to metagenomic species (MGS) and hereafter the phylum. The data was normalized by downsizing to sample size. t-test analysis was performed to identify significant changes in the levels of Firmicutes before and after the intervention with capsaicin.

### Reference List

- (1) Zhang, L.L. et al. *Activation of Transient Receptor Potential Vanilloid Type-1 Channel Prevents Adipogenesis and Obesity*. *Circulation Research* (2007): 100, 1063-1070.
- (2) Sharma, S.K., Vij, A.S., and Sharma, M. *Mechanisms and clinical uses of capsaicin*. *The European Journal of Pharmacology* (2013): 720, 55-62.
- (3) Baboota, R.K. et al. *Capsaicin-induced transcriptional changes in hypothalamus and alterations in gut microbial count in high fat diet fed mice*. *The Journal of Nutritional Biochemistry* (2014): 25, 893-902.
- (4) Kang, C. et al. *Healthy subjects differentially respond to dietary capsaicin correlating with the specific gut enterotypes*. *Journal of Clinical Endocrinology and Metabolism* (2016): epub ahead of print.
- (5) Qin, J. et al. *A Metagenome-Wide Association Study of Gut Microbiota in Type 2 Diabetes*. *Nature* (2012): 490, 55-60.

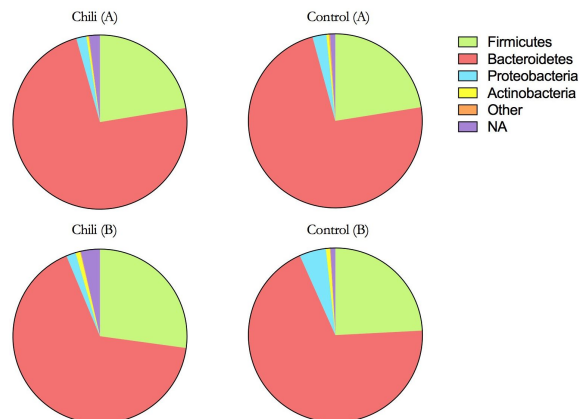
## Results and Discussion

The number of reads in the samples varied from 186 to 43,216 reads. The number of mapped reads were assessed and varied from 149 to 23,109 (figure 2).



**Figure 2**  
Sample size by number of mapped reads.

The number of Firmicutes and other major phylums were compared between samples before and after consumption of chili and compared with the control group. The statistical hypothesis t-test was used, and no significant changes (p-value below 0.05) in the distribution of phylums were observed (figure 3).



**Figure 3**  
Phylum distribution in the chili and control group.

### Study design

The intervention should have been conducted longer than 24 hours. Standardization and superior guidelines regarding exact amount of chili consumption by the intervention group would have been preferred.

### Library preparation

From the Bioanalyser, the quality of DNA in a number of samples was not good enough. The number of reads in the sample, and also mapped reads were very low for metagenomic data. From that it could be concluded that the depth and the coverage of the species in the sample were insufficient. This could be due to some space issues when processing the files, however it could also be that the 42 samples were prepared for sequencing by different people resulting in differences in the sample preparation method.

### Normalization

Rarefaction should only be used when low-abundant genes are not of interest and the sample sizes are equivalent. Downsizing to sample size allowed us to obtain the same sample size of each sample as all samples were normalised in percentage. This approach was chosen as the variance between the number of reads in the samples and mapped reads was high, and it could therefore not be justified to use other normalization methods.

### Distribution of Firmicutes

No significant changes were observed regarding the Firmicutes. This might be due to the short intervention time, sample preparation or to small chilli consumption. If there had been time, it would have been interesting to annotate the sequences to species instead of phylum. Annotating to species would have allowed us to investigate, if one specific specie increased or decreased due to elevated chili intake.

## Conclusion and Future work

No significant change in the distribution of Firmicutes was observed due to an elevated intake of chili within 24 hours. No changes were observed based on neither nationality, gender or age. The results are however not very reliable, since the intervention in general should have been conducted differently to exclude drawbacks. The intervention could profitably be conducted again with more standardisation.