UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

Colegio de Posgrados

Effect of *Saccharomyces boulardii* as complementary treatment of *Helicobacter pylori* infection on gut microbiome

Tesis en torno a una hipótesis o problema de investigación y su contrastación

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Trabajo de titulación de posgrado presentado como requisito
para la obtención del título de Magister en Microbiología

Quito, 25 de enero 2021

UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

COLEGIO DE POSGRADOS

HOJA DE APROBACIÓN DE TRABAJO DE TITULACIÓN

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DEDICATORIA

A mis padres por su apoyo, amor y cariño incondicional, a mi esposo Mauricio a mis hermanas María Fernanda y Diana por su respaldo, a mi familia, a mis amigas y a mis compañeros del Instituto de Microbiología de la Universidad San Francisco de Quito.

AGRADECIMIENTOS

A mis padres, mi esposo y mis hermanas.

A la Universidad San Francisco de Quito, al Instituto de Microbiología. A mi director de tesis Paúl Cárdenas, por su constante ayuda y paciencia. A María Belén Prado por su apoyo y enseñanzas y a Lucia Fiallos.

RESUMEN

En el tracto digestivo es donde se encuentra la mayor densidad de microorganismos (se estima que existen unas 1000 especies de bacterias) y juegan un papel importante en la salud de las personas ya que actúan como simbiontes y mutualistas con los humanos. La homeostasis intestinal puede sufrir alteraciones (disbiosis) por condiciones ambientales como el uso de medicamentos, alimentación, estilo de vida y otras más graves como el cáncer colorrectal y cáncer de estómago. *Helicobacter pylori* produce enfermedades asociadas al tracto digestivo como cáncer gástrico, inflamación del tejido linfoide asociado a la mucosa, úlceras gástricas y duodenales y además en los últimos años se ha encontrado que el tratamiento recomendado no es muy efectivo debido a la resistencia a antibióticos por lo que se ha utilizado *Saccharomyces boulardii* como terapia adyuvante ya que ayuda a la eliminación de *H. pylori*. En el presente estudio intentamos identificar los cambios en la microbiota bacteriana y en los efectos secundarios (dolor abdominal, diarrea) con el uso suplementario de *S. boulardii* en el tratamiento convencional de *H. pylori*, para esto 74 pacientes participaron en este estudio divididos de forma aleatoria en dos grupos: 32 recibieron el tratamiento convencional (40 mg de esomeprazol dos veces al día, 1 g de amoxicilina tres veces al día, 1 g de tinidazol una vez al día) y 33 participantes recibieron este tratamiento convencional complementado con probióticos (250 mg de *S. boulardii* tres veces al día) y 10 pacientes negativos para *H. pylori* que fueron considerados como controles. Se recolectaron muestras de heces de todos los participantes al inicio, durante y después del tratamiento (15 días después del final del tratamiento). En total, se recolectaron 3 muestras de heces por paciente. Se extrajo el ADN y se amplificó las regiones V3-V4 de la región de rRNA 16S y se secuenció con Illumina MiSeq, el análisis estadístico de los datos epidemiológicos recolectados a través de las encuestas realizadas a los participantes se realizó en el programa SPSS (2016.06)

y el procesamiento de los datos obtenidos de la secuenciación se realizó en el programa QIIME2 versión 2018.11. Para comparar los efectos secundarios de este estudio se utilizó la prueba paramétrica Chi cuadrado, y se pudo observar que el grupo de tratamiento convencional suplementado con *S. boulardii* (1.58 ± 0.5 p = 0.001) tuvo menos dolor abdominal en comparación con el grupo de tratamiento convencional (1.96 ± 0.2 p = 0.001) esto pudo deberse a que hubo una disminución de *Bacterioides*, *Parabacteroides* y la familia *Ruminococcaceae* en el grupo de tratamiento convencional. Por lo que el suplemento con *Saccharonyces boulardii* ayuda a disminuir los efectos secundarios de la terapia convencional con antibióticos.

Palabras clave: *Microbiota intestinal*, *Saccharomyces boulardii*, *Helicobacter pylori*, *beta-diversidad*

ABSTRACT

In the digestive tract is where the highest density of microorganisms is found (it is estimated that there are about 1000 species of bacteria) and they play an important role in people's health since they act as symbionts and mutualists with humans. Gut homeostasis can be altered (dysbiosis) by environmental conditions such as the use of medications, diet, lifestyle, and other more serious conditions such as colorectal cancer and stomach cancer. *Helicobacter pylori* produces diseases associated with the digestive tract such as gastric cancer, inflammation of the lymphoid tissue associated with the mucosa, gastric and duodenal ulcers, and in recent years it has also been found that the recommended treatment is not very effective due to resistance to antibiotics. *Saccharomyces boulardii* has been used as adjunctive therapy as it helps in the elimination of *H. pylori*. In the present study we tried to identify the changes in the bacterial microbiota and in the side effects (abdominal pain, diarrhea) with the supplementary use of *S. boulardii* in the conventional treatment of *H. pylori*, for this 74 patients participated in this study divided randomly in two groups: 32 received conventional treatment (40 mg of esomeprazole twice daily, 1 g of amoxicillin three times daily, 1 g of tinidazole once daily) and 33 participants received conventional treatment supplemented with probiotics (250 mg of *S. boulardii* three times a day) and 10 *H. pylori* negative patients who were considered as controls. Stool samples were collected from all participants at the beginning, during and after treatment (15 days after the end of treatment). In total, 3 stool samples were collected per patient. The DNA was extracted and the V3-V4 regions of the 16S rRNA region were amplified and sequenced with Illumina MiSeq, the statistical analysis of the epidemiological data collected through the surveys carried out with the participants was carried out in the SPSS (2016.06) and the processing of the data obtained from the sequencing was carried out in the QIIME2 version 2018.11 program. When comparing

the side effects of this study, Chi-square parametric test was used, and it can be seen that the conventional treatment group plus *S. boulardii* (1.58 ± 0.5 p = 0.001) had less abdominal pain compared to the conventional treatment group (1.96 ± 0.2 p = 0.001) this could be due to the fact that there was a decrease in *Baterioides*, *Parabacteroides* and the *Ruminococcaceae* family in the conventional treatment group. Supplement with *Saccharonyces boulardii* helpeds to reduce the side effects of conventional antibiotic therapy.

Key words: *gut microbiota, Saccharomyces boulardii, Helicobacter pylori,* beta-diversity.

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INTRODUCTION

The group of bacteria, viruses, eukaryotes, and archaea that are found in an established habitat is known as microbiota (Hooper, Midtvedt, Gordon, 2002; Cho, Martin, Blaser, 2012). The human microbiota is found in different areas of our body such as the respiratory, digestive and reproductive tracts, and skin (Qin, Li, Raes, Arumugam, Burgdorf, Manichanh, Nielsen, Pons, Levenez, Yamada, et al. 2010; Chen, Liu, Ling, Tong, and Xiang, 2012). The highest density of microorganisms in the digestive tract It is estimated that there are about 1000 species of bacteria) and play an important role in the health of people since they act as symbionts and mutualists (Del Campo-Moreno, Alarcón-Cavero, D'Auria, Delgado-Palacio, and Ferrer-Martínez, 2018). Gut microbiota helps in the protection against pathogens and to metabolize different compounds and to synthesize vitamin K and B (Koliarakis, Messaritakis, Nikolouzakis, Hamilos, Souglakos Tsiaoussis, 2019). Gut homeostasis can suffer alterations (dysbiosis) due to environmental conditions such as the use of medications, drug administration, food, lifestyle, which can cause diseases such as type I and II diabetes, obesity, kidney diseases, and more severe ones such as colorectal cancer and stomach cancer, (the third most common type of cancer worldwide) (Larsen, Vogensen, Van den Berg, et al. 2010; Arthur, Jobin, 2011; Ferlay, Soerjomataram, Dikshit, Eser, Mathers, Rebelo, Parkin, Forman, Bray, 2014).

Dysbiosis reported by *Helicobacter Pylori* treatments

Helicobacter pylori is a bacterium that is located in the epithelial lining of the stomach and is present in approximately 50% of the world's population (Liou, Fang, Chen, et al. 2016; Hooi, Lai, Ng, Suen, Underwood, Tanyingoh, Ng, et al, 2017), and the World Health Organization (WHO) has listed it as a high priority bacterium due to

antibiotic resistance. It produces diseases associated with the digestive tract such as gastric cancer, inflammation of the lymphoid tissue associated with the mucosa, gastric and duodenal ulcers (Doorakkers, Lagergren, Engstrand, Brusselaers, 2018; Suzuki, Esaki, Kusano, Ikehara, Gotoda, 2019).

Generally, people infected with *H. pylori* develop gastritis that occasionally has no clinical symptoms, however, it is considered a type I carcinogen by the International Agency for Research on Cancer (Zhang, Ning, Mayne, Moore, Li, Butcher, Deeke, Chen, Chiang, Wen, Mack, Stintzi, Figeys, 2016). The recommended treatment is 7 days course with clarithromycin (CAM), a proton pump inhibitor (PPI) as omeprazole and amoxicillin (AMPC) stops the growth of bacteria that can cause ulcers, however due to the high resistance of *H. pylori* against antibiotics the recommended first-line treatment is currently 15 days plus bismuth, the quadruple therapy (Malfertheiner, Megraud, O'Morain, Gisbert, Kuipers, Axon, Bazzoli, Gasbarrini, Atherton, Graham, Hunt. Moayyedi, Rokkas, Rugge, Selgrad, Suerbaum, Sugano, El-Omar, 2016; Choi, Kook, Kim, Cho, Lee, Kim, Nam, 2018). Being infected with *H. pylori* is considered high risk for developing gastric adenocarcinoma because it damages the gastric mucosa and the glandular tissue of the mucosa generating intestinal metaplasia, so the use of antibiotics to eradicate it is important since Doorakkers and collaborators showed that people who received antibiotic treatment decreased the chances of developing gastric adenocarcinoma (Cho, Choi, Kook, et al, 2013; Choi, Kook, Kim, Cho, Lee, Kim, Nam, 2018; Doorakkers, Lagergren, Engstrand, Brusselaers, 2018; Pichon, Burucoa, 2019).

Helicobacter pylori possess lectins that allow it to adhere to the mucous layer of the gastrointestinal tract that is formed of glycoproteins by causing this bacterium to bind to the antigens of the blood group of the mucous layer and altering the microbiota present (through the decrease of nutrients, changes in gastric pH and modifying the innate

immune response) which has been shown to help the formation of the mucous layer through the formation of mucin and also through the increase of goblet cells in mice (Sgouras, Trang, Yamaoka, 2015; Geerlings, Kostopoulos, de Vos, Belzer, 2018).

Epidemiology of *Helicobacter pylori* in Ecuador

The incidence of *Helicobacter pylori* varies significantly in each country. The World Health Organization (WHO) indicates that half of the population is infected with *H. pylori* (Yeh, Goldie, Kuntz, Ezzati, 2009). The infection prevalence in developed and industrialized countries is 80% and 40% respectively, (Malnick, Melzer, Attali, Duek, Yahav, 2014), this great difference is due to the fact that in developing countries there are inadequate hygienic practices, differences in socioeconomic quality of life, food health, absence of drinking safe water, in contrast, the transmission in developed countries is generally from person to person (Bellack, Koehoorn, Mac Nab, Morshed. 2017).

According to studies carried out by Hooi and collaborators in 2017 there is a prevalence of *Helicobacter pylori* in South America of 69.4%. In countries like Colombia and Brazil the prevalence is between 60 and 90% (Eshraghian, 2014), and in Ecuador is 72.2% this may because there are areas with lack of sanitation and urbanization, as well as lack of access to drinking safe water due to the low socioeconomic status of certain regions and mainly because of poor access to medications to treat this condition (Hooi, Lai, Ng, Suen, Underwood, Tanyingoh, Ng, et al, 2017).

Reports of the use of *Saccharomyces boulardii*

Saccharomyces boulardii has been widely used since the 1950s as a probiotic to treat infections that cause diarrhea in children and adults as it helps increase the production of cytokines and immunoglobulins (i.e immunoglobulin A against

Clostridioides difficile), influencing in the innate and adaptive immune response, (Villar-García, Hernández, Güerri-Fernández, González, Lerma, Guelar, Saenz, Sorlí, Montero, Horcajada, J. P., et al. 2015; Stier, Bischoff, 2016), it can also neutralize toxins produced by pathogenic microorganisms through their binding to these pathogens, and it is also known that this yeast at 37°C can increase the production of acetic acid helping in the inhibition of certain bacteria to treat gastrointestinal diseases such as ulcerative colitis, Crohn's disease, diarrhea due to antibiotics and infections due to *Clostridioides difficile* (Currò, Ianiro, Pecere, Bibbò, Cammarota, 2017; Offei, Vandecruys, De Graeve, Foulquié-Moreno, Thevelein, 2019). Although *Saccharomyces boulardii* has been widely used as a probiotic and in the treatment of diseases, there are no reports that it can cause serious complications in immunologically competent patients, however, according to Cohen et al., 19 cases of fungemia have been reported due to the consumption of *Saccharomyces boulardii* as a probiotic in immunocompromised patients (Cohen, Ranque, Raoult, 2013; Roy, Jessani, Rudramurthy, Gopalakrishnan, Dutta, Chakravarty, Jillwin, Chakrabarti, 2017).

In a meta-analysis study conducted by Zhou et al., a study was carried out to assess whether *S. boulardii* as an adjuvant therapy helps the elimination of *H. pylori*, in this study it was shown that there is an increase in the eradication rates of *H. pylori* and also *S.s boulardii* decreaseds side effects such as abdominal pain and diarrhea. *S. boulardii* has not only been used in humans but also in animals as a supplement for increasing calf weight and to help prevent diarrhea during the weaning process. Experiments have also been carried out to evaluate the protective effect of *S.s boulardii* on the intestinal mucosa and microbiota in mice that were induced colitis by sodium sulfate, resulting in a protective effect of the intestinal mucosa since it was observed that the microvilli were regenerated in the presence of *S. boulardii* and as for the microbiota it was observed that

there was a higher percentage of *Bacteroidetes* and a lower percentage of *Firmicutes* in relation to control mice, it also reduced levels of certain pro-inflammatory cytokines such as IL-6, IL-1β, and TNF-α (Dong, Jin-Pei1, Zheng, Yue, Wu, Ting, He, Qun, Teng, Gui-Gen, Wang, Hua-Hong. 2019; Villot, Ma, Renaud, Ghaffari, Gibson, Skidmore, Chevaux, Guan, Steele, 2019; Zhou, Chen, Li, Wan, Ai. 2019).

For that reason, the current study tries to identify the bacterial microbiota changes in the supplementary use of *S. boulardii* in patients that receive treatment for *H. pylori* infection.

METHODOLOGY

Participants

A total of 188 patients were evaluated for the study, of which 65 were considered for the trial (see exclusion criteria).

A 65 patients (18 to 55 years old) who presented symptoms of dyspepsia participated in the current study, the diagnosis was made through upper gastrointestinal-endoscopy and histopathology. *H. pylori* positive participants were selected for the clinical trial. In addition, *H. pylori* negative patients were used as control (10 controls were included). The collection of samples and the diagnosis was made at Axxis Hospitals Sangolquí (15 patients) and Ecuadorian Social Security Institute (IESS) of Sangolquí (50 patients) Ecuador.

The design of this trial was based as a simple-blind study. The participants were randomized into two groups: 32 received the conventional treatment (40 mg of esomeprazole twice a day, 1 g of amoxicillin three times a day, and 1 g of tinidazole once a day) for two weeks and 33 participants received the same conventional treatment supplemented with probiotics (250mg of *S. boulardii* three times a day) for two weeks.

The ethics committee of the Universidad de las Americas (CEBE-UDLA) reviewed and approved the protocol for this study.

Samples collection

Stool samples were collected from all participants at the beginning, during (for two weeks for all participants) and after treatment (15 days after the end of treatment). In total, 3 stool samples were collected per patient. In addition, a survey was conducted during the 15 days that the treatment with antibiotics lasted, to evaluate the improvement of the participants who belonged to the groups with conventional treatment group plus *S.*

boulardii and conventional treatment group. The stool samples were stored at -80 ° C for further analysis.

Inclusion criteria

As inclusion criteria, participants must have *H. pylori* gastritis symptoms and confirmation by upper gastrointestinal endoscopy and histopathology and must also have completed the assigned treatment. The age was 18 to 55 years old and do not have used antibiotics two weeks before the trial.

Exclusion criteria

Patients who do not comply with more than 70% of treatment, antibiotic o probiotics use in the last two weeks before, patients who present serious medical complications and participants who have taken antibiotics up to one month before entering the study were excluded.

Processing of the samples

Extraction of DNA and Preparation of libraries and sequencing

DNA was extracted from the -80ºC stored stool samples with the MpBio kit (FastDNA SPIN Kit for Soil) according to the manufacturer's instructions. DNA concentration and purity were measured on a NanoVue Plus- Spectrophotometer with A260/280 1.8 and A260/230 2.0-2.2 purity values to assess carbohydrate contamination. Then a PCR was performed to amplify the V3-V4 regions 16S rRNA using Q5 master mix and barcode primers (Phil James, Elena Turek, 2015). Then an electrophoresis was performed for 30 minutes on 1.5% agarose gel, where the amplification was confirmed with the presence of a band of 200 kb. Multiplexing was performed with the Nextera kit and sequencing was performed at UNC at Chapel Hill using Illumina's MiSeq technology.

Statistical Analysis

A statistical analysis of the epidemiological data collected was carried out, in SPSS (2016.06). Comparisons were made between the conventional treatment group plus *S. boulardii* against conventional treatment group. Also, descriptive statistics such as frequency analysis, and hypothesis tests such as Chi square and ANOVA were performed.

Bioinformatic analysis

The data obtained from the sequencing was analyzed in Qiime2 version 2018.11 program (https://qiime2.org/) (Bunyavanich, et al., 2016; Caporaso, et al., 2010). The metadata in Keemei tool (Rideout et al., 2016). The metadata was generated by placing a code on each sample and compiling the patient information (age, sex, weight, body mass index) and the additional information that was collected, like economic situation, smoking. The metadata was exported in .tsv format.

The raw sequences that were sent by UNC were demultiplexed using Qiime2 and denoise was removed with (DADA2) (Callahan, B. et al., 2016). For demultiplexed, the command demux emp-single was used, since the data was processed as single end sequences and by means of the summarize command it was possible to obtain a summary of the characteristics of the sequences. To denoise using DADA2, the commands dada2 denoise-single were used and thus eliminate chimeric sequences that were produced in the Illumina sequencing, this could be visualized through the feature-table summarize and feature-table tabulate-seqs command and was obtain OTUs.

Alpha and Beta diversity analysis

The analyzed groups were based on the type of sample (conventional treatment group plus *S. boulardii* and conventional treatment group) for analysis of alpha (diversity within samples) and beta (diversity between samples) diversity. The first thing that was done to generate a phylogenetic diversity tree using q2-phylogeny and align-to-tree-

mafft-fasttree, and obtain a tree without a root using FastTree which allowed to eliminate variable sequences.

Then the *q2-diversity* plugin and the core-metrics-phylogenetic method were used to calculate the alpha diversity (Shannon's diversity index, Faith's Phylogenetic Diversity and Evenness) and beta diversity metrics (Bray-Curtis distance, unweighted UniFrac distance, weighted UniFrac distance), also principal coordinates analysis (PCoA) was done through Emperor and achieved using Feature-table.

Alpha rarefaction analysis

Alpha rarefaction analysis was realized to assess alpha diversity based on the depth of the sampling that was 400.

Taxonomics analysis

The Greengenes database (Almeida, Mitchell, Tarkowska and Fin, 2018) was used for taxonomic classification, for this We used Feature-table and res-seqs sequences (3000) and the command aligned-rep-seqs to align the tree and eliminate variable positions that cause noise.

Differential abundance analysis

For the differential abundance tests between groups, we used Analysis of composition of microbiomes (ANCOM) (Mandal, Van Treuren, White, Eggesbø, Knight & Peddada, 2015) then, the *q2-composition* the *add-pseudocount* plugin were used. so that the minimum frequency starts from 1 and not from 0. The q2-gneiss complement was used to find differences in the depth sequencing based on proportions, and get heatmaps. and to measure relative abundance. For this, taxa-bar-plots were used in .qzv format to be able to visualize the count of each taxonomic level that can reach the species level. The count for each OTU comes from the sum of each taxon.

RESULTS

DNA quality

The average concentration of the study DNA samples using MPbio was 174.18 ng/μL (range 25.5 - 799.5 ng / μL), the A260/280 ratio was 1.75 (range: 1.30-1.95) and A260/230 ratio was 0.25 (range: 0.019 - 0.82) indicating Good quality of the DNA extracted.

General patient data

Of the 131 patients who participated in the study, 74 were discarded because did not comply the inclusion criteria (see methodology for inclusion criteria). A total of 57 patients were includen in our study, of which 27 (47.37%) were men and 30 (52.63%) were women. In total, 108 samples were analyzed (collected before, during and after treatment) because not all participants gave 3 samples.

Additional information was collected that could influence the microbiota such as age, sex, body mass index, smoking habits, regular alcohol consumption, antibiotic treatments prior to the study.

The table 1, showed the analysis of epidemiological data, the mean age of the two study groups was 36 years and there were no significant differences, while in gastrointestinal problems there was a significant difference between the two groups obtained with descriptive statistics as frequency analysis (p=0.04). Neither group presented chronic diseases such as cancer.

Microbiome analysis

5198 (OTUS) and 2,953,824 Total frequency were obtained.

Alpha and Beta diversity

Alpha diversity

The alpha's diversity tests Faith's Phylogenetic Diversity and Evenness using non-parametric Kruskal-Wallis test had no significant differences for the conventional treatment group, conventional treatment group plus *S. boulardii* and without *H. pylori* (control) groups. The results are shown in Table 2.

Based on the Kruskal-Wallis non-parametric statistical value, there were no significant differences between the conventional treatment group and Conventional treatment group plus *S. boulardii* and without *H. pylori* groups (controls) for the two tests of alpha diversity analysis, Faith's Phylogenetic Diversity and Evenness, this was observed in Table 2 and 3.

Alpha diversity analysis with Faith's Phylogenetic Diversity and Evenness for the position sample parameter where M1 is the sample taken before treatment, M2 is the sample taken during treatment and M3 is the sample taken after treatment in the conventional treatment group and conventional treatment group plus *S. boulardii* did not show significant differences in any parameter ($p \geq 0.05$) except for M3 in evenness with a p-value of 0.041 that shows that there are significant differences between conventional treatment group and conventional treatment group plus *S. boulardii* within M3 (Table 4).

The box plot in Figure 1 and 2 does not show difference between the position sample, the (p value > 0.05) when performing the Kruskal-Wallis non-parametric test since the p values were M1 = 0.884, M2 = 0.624 and M3 = 0.294 (Figure 1) and M1 = 0.944, M2 = 0.562 and M3 = 0.071 (Figure 2), did not show significant differences.

There are no differences in alpha diversity between the position of the sample and the conventional treatment group and conventional treatment group plus *S. boulardii*.

The analyzes of Faith's Phylogenetic Diversity and Evenness for the conventional treatment group and conventional treatment group plus *S. boulardii* and without *H. pylori* (control) for the position of the sample M1, M2 and M3 did not have significant differences for any group (p value > 0.05).

Alpha rarefaction analysis

Alpha rarefaction is used to evaluate the sampling depth and by means of figures 3 and 4 of alpha diversity a sampling depth with respect to the Shannon index (Figure 3) of 50,000 was observed, even though the *Saccharomyces* (conventional treatment plus *S. boulardii*) curve increases by 200,000 but after it is leveled again, the same happened with the Faith's Phylogenetic Diversity index (Figure 4) the curve levels off at 50,000 but the without *H. pylori* curve increases to 340000 to then level off. The Shannon index calculated by Kruskal-Wallis statistics analysis resulted in a value of H = 1.05169 (P = 0.59105), the presence or absence of *S. boulardii* did not produce differences in Shannon index analysis.

Beta diversity analysis

The intestinal microbiota of patients who underwent conventional treatment group was compared with patients who underwent more probiotic conventional treatments group plus *S. boulardii* to analyze beta diversity based on the metrics of unweighted UniFrac distance and weighted UniFrac distance.

In Figure 5, weighted UniFrac PCoA (variance = 42.78) is observed for the conventional treatment group a slight grouping is observed while in unweighted UniFrac PCoA (variance = 25.49) no grouping is observed. The same is true for the conventional treatment group plus *S. boulardii* there is a slight clustering in weighted UniFrac PCoA but there is no clustering in unweighted UniFrac PCoA.

The weighted PCoA (left) of figure 6 shows a slight cluster formation of the *Saccharomyces* (conventional treatment group plus *S. boulardii*), without *H. pylori* and Without *Saccharomyces* groups (conventional treatment group), but there were also samples that were outside the cluster, in the figure on the right it can be seen that there was no cluster formation.

In figure 7 in the weighted PCoA a slight cluster formation can be observed between the three test groups, samples from the three groups outside the cluster can also be observed, while in the right PCoA graph of unweighted any cluster was formed. In the PCoA Figure 8 of unweighted (right) and weighted (left) distances, cluster formation was not observed for any of the sample groups, same as a Figure 9.

Figure 10 shows the PCoA graphs of unweighted (right) and weighted (left) of the conventional treatment group plus *S. boulardii* during all treatments (at the beginning of M1, during M2 and at the end of M3), it can be seen in the PCoA of unweighted (right) that no cluster was formed while in weighted PCoA (left) a slight cluster formation is observed where the three groups are grouped (M1, M2 and M3).

Unlike Figure 11, Figure 12 shows the PCoA graphs of distances of unweighted (right) and weighted (left) of the groups conventional treatment group at the beginning (M1), during (M2) and after (M3) of the treatment. that in the figure on the left there is a cluster formation where the three groups of samples are found at the beginning M1, during M2 and at the end M3 of the treatment and the same occurs in the graph on the right of the unweighted PCoA where the three groups are forming a cluster.

Relative abundance analysis

The taxonomic composition comparing the conventional treatment group plus *S. boulardii* and conventional treatment group at the phylum level in Figure 13 shows the most abundant phylum and in the two groups the phylum that is most predominant is that

of *Firmicutes*, followed by *Proteobacteria*, then *Bacteroidetes*, *Actinobacteria*, *Verrucomicrobia* and *Tenericutes*.

In figure 14 the taxa bar plots are shown and the conventional treatment group and conventional treatment group plus S. *boulardii* are compared at the beginning of the treatment (M1), the most predominant phylum can be observed, which are first *Firmicutes*, followed by *Proteobacteria*, after this *Bacteroidetes*, *Actinobacteria* and *Verrucomicrobia*.

In figure 15 the taxa bar plots are shown and the conventional treatment group and conventional treatment group plus *S. boulardii* are compared at the end of the treatment (M3), the most predominant phylum can be observed, which are first *Firmicutes*, followed by *Bacteroidetes*, then this *Actinobacteria*, *Proteobacteria*, *Tenericutes* and *Verrucomicrobia*.

Figure 16 shows the most predominant phyla of the conventional treatment group plus *S. boulardii*, are *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, *Tenericutes* and *Verrucomicrobia*, same as Figure 17.

Differential abundance analysis

A comparison of the microbiota of the conventional treatment group plus *S. boulardii* and conventional treatment group was carried out by means of the relative abundance using Gneiss. In Figure 18, the predominance of the *Bacteroides*, *Parabacteroides* and family *Ruminococcaceas* can be observed in the conventional treatment group, whereas in the conventional treatment group plus *S. boulardii* is dominated by the *Bacteroides*, *Parabacteroides* phylum and *Ruminococcaceas* family, but to a lesser extent.

In Figure 19 the conventional treatment group the most abundant taxa were from the genus *Blautia*, *Faecalibacterium*, *Bacteroides*, *Parabacteroides* and *Rumicoccus*

while in the conventional treatment group plus *S. boulardii* they were the same but to a lesser extent, this relative abundance is of the microbiota at the beginning of M1 treatment.

The differential abundance of the microbiota of the conventional treatment group plus *S. boulardii* and conventional treatment group during the M2 treatment, has the most abundant taxa in the the conventional treatment group plus *S. boulardii* to the genus *Bifidobacterium*, *Lactobacillus*, *Oscillospira*, *Bacteroide*s, these are in a very low proportion compared to the Without *Saccharomyces* group, this is seen in Figure 20.

At the end of the M3 treatment, the most abundant taxa in the conventional treatment group belong to the genera *Roseburia* (most abundant taxon), *Blautia*, *Faecalibacterium* and *Ruminococcus* (less abundant taxon), in the conventional treatment group plus *S. boulardii* there were the same taxa but in a smaller proportion, except for *Ruminococcus*, which is in a greater proportion in relation to the group conventional treatment group and also *Bifidobacterium*. This is shown in Figure 21.

Figure 22 shows the differential abundance between groups M1 and M3 within the conventional treatment group plus *S. boulardii*, the most abundant taxa in M1 are the family *Ruminococcaceae*, the genus *Blautia*, *Oscillospira* and of the order *Clostridiales* while in M3 the most abundant taxa are from the genus *Bifidobacterium*, *Acidaminococcus* and *Prevotella*, these were in a higher proportion than those found in M1.

Figure 23 shows the differential abundance between groups M1 and M3 within the conventional treatment group, the most abundant taxa in M1 is the genus *Escherichia*, *Bacteroides*, while in M3 the taxa Most abundant are of the genus *Escherichia* and *Coprococcus* but in a lower proportion in relation to M1.

DISCUSSION

In the current study we investigate the changes in the structure of the intestinal microbial community among *H. pylori* positive patients receiving conventional treatment with or without *S. boulardii.*

On alpha diversity, the Faith's test did not showed significant differences between the conventional treatment groups and the conventional treatment group plus *S. boulardii*, which indicates that the two groups have the same microbial diversity, and that *S. boulardii* did not increase or decrease intestinal diversity. Furthermore, the microbial diversity before, during and after the treatment did not have significant differences in the two groups, which coincides with the study carried out by Yap, T. and collaborators (Yap T. et al., 2016).

When performing the analysis of beta diversity, no significant differences were observed between the two groups (conventional treatment group and conventional treatment group plus *S. boulardii*) so that in the graphs obtained from the unweighted UniFrac distance and weighted UniFrac distance, cluster formation cannot be observed in certain graphs and it can also be observed that the samples are scattered and there is no cluster formation (Figure 4 and 6). This indicates that there are differences between the two study groups and the control group (without *H. pylori*) and before, during and after treatment (Figure 9). Our results are similar to the study by Yap. et al. (Yap T. et al., 2016) which demonstrated there are no differences in bacterial communities between groups.

The gastric microbiota in healthy patients at the phyla level is mainly composed of *Bacteroidetes*, *Actinobacteria, Proteobacteria* and *Firmicutes* and at the genus level *Fusobacterium, Streptococcus, Rothia, Prevotella* and *Veillonella* (Sheh A. et al., 2013; Nardone G. et al. , 2015), but in *H. pylori* positive patients a decrease in bacterial diversity

has been observed at the phylum level of *Proteobacteria, Firmicutes, Actinobacteria* and *Bacteroidetes* and at the genus *Streptococcus* (Bruno G. et al., 2018; Parsons B. et al., 2017). Furthermore, in the study carried out by Klymiuk et al., where groups of *H. pylori* positive and *H. pylori* negative samples were compared, it was found that the genera *Fusobacterium, Prevotella, Actinomyces*, *Veillonella*, *Neisseria, Granulicatella* and *Streptococcus* are significantly different between the two groups. In the present study with the relative abundance test, it was obtained at the phylum and genus level that the most common taxa present in the two study groups that are at the phylum level are *Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria, Verrucomicrobia* and *Tenericutes*. The phylum Firmicutes is known to aid in the production of short-chain fatty acids (SCFA) that provide energy to colon epithelia, (Polansky, O. et al., 2016) and has also been confirmed to modulate the immune response of T cells (Smith, P. et al., 2013). Within this phylum is the genus *Alistipes* that produces butyrate that is an SCFA and that helps reduce intestinal inflammation (Borton, M., 2017).

Three fecal microbiota enterotypes have been reported, *Bacteroides* (enterotype-1), *Prevotella* (enterotype-2) or *Ruminococcus* (enterotype-3), they were assigned in this way because in each enterotype there is a greater presence of these taxa (Arumugam, M. et al., 2011) however enterotype three was reconsidered and joined with enterotype-1. These two enterotypes were found in the relative abundance and differential abundance analyzes in this study. A low level of *Bacteroides* according to Zhou et al. is related to inflammatory bowel disease (IBD), this may be due to the fact that *Bacteroides* express polysaccharide A that helps the growth of regulatory T cells and also influences the expression of cytokines that protect against colitis (Round, J. et al., 2011) on the other hand, the enterotype-2 *Prevotella* is related to the decrease or degradation of mucin glycoproteins and is also known to be present in *H. pylori* positive patients

(Gorvitovskaia, A. et al., 2016). Therefore, in the conventional treatment groups at the end of treatment there is a decrease in the proportion of *Bacteroides* (Figure 22) compared to conventional treatment groups plus *S. boulardii*, so this may have influenced the reduction of secondary symptoms such as diarrhea and abdominal distention, and have maintained secondary symptoms in the conventional treatment groups.

In this study, an analysis of the differential abundance of the conventional treatment groups plus *S. boulardii* and conventional treatment groups was also performed to determine which taxa are most abundant in each group at the phylum and gender level. In the conventional treatment groups plus *S. boulardii* the taxa that were found were *Bacteroides*, *Parabacteroides* and *Ruminococcaceas*, while in the conventional treatment groups the taxa were *Bacteroides*, *Parabacteroides* and the *Ruminococcaceae* family in greater proportion. This could be due to the fact that *S.s boulardii* helps to inhibit bacterial competitiveness producing a bacteriostatic effect (Song, H. et al., 2018).

To assess whether the differential abundance varies within the Saccharomyces group, a comparison was made at the beginning and at the end of the treatment and it was found that the taxa present at the beginning of the treatment are not the same as those found at the end of the treatment. At the beginning of the treatment, it was found that the most abundant taxa were from the *Ruminococcaceae* family, the genus *Blautia*, *Oscillospira* and the order *Clostridiales* all belonging to the phylum *Firmicutes*, while at the end of the treatment they were from the genus *Bifidobacterium* (phylum *Actinobacteria*) that has been established that can prevent antibiotic-associated diarrhea (Yap, T. et al., 2016)., Acidaminococcus (phylum Firmicutes) and Prevotella (phylum *Bacteroidetes*). The presence of *Bacteroidetes* and *Firmicutes* is associated with the regulation of lipids and bile acids, helping the host to maintain energy homeostasis (Turnbaugh, P. et al., 2006; Yap, T. et al., 2016). The *Clostridiales* order adapt to the

intestine using succinate generated by other bacteria to produce propionate that is an indicator of good intestinal health (Hosseini, E, et al., 2011).

The same previous procedure was performed with the conventional treatment groups and it was found that the most abundant taxa at the beginning of the treatment belong to the genus *Escherichia* (phylum Proteobacteria) and *Coprococcus* (phylum *Firmicutes*) and at the end of the treatment of the genus *Escherichia*, *Bacteroides*, (phylum *Bacteroidetes*) the taxa at the end of the treatment increased their proportion in relation to the start of the treatment. The genus *Escherichia* may have species that are pathogenic and can cause disease, but they generally help maintain a healthy digestive system because they aid in the production of vitamin K (Nardone G, et al., 2015).

When comparing the conventional treatment groups plus *S. boulardii* and conventional treatment groups plus *S. boulardii* at the start of treatment, it was found that the most abundant taxa are the genera *Blautia*, *Faecalibacterium*, *Bacteroides*, *Parabacteroides* and *Rumicoccus*, but in the conventional treatment groups plus *S. boulardii* these were in a lower proportion, the differential abundance of the samples taken during the treatment of the two groups it had the most abundant taxa of the *Bifidobacterium*, *Lactobacillus*, *Oscillospira*, *Bacteroides* genus, being more abundant in the conventional treatment groups plus *S. boulardii*, this may be an indication that the addition of the probiotic can change the composition of the microbiota. At the end of the treatment, the taxa that were found in a higher proportion belong to the group of conventional treatment groups are the taxa of the genus *Roseburia*, *Blautia*, *Faecalibacterium* and *Ruminococcus*, in the group of conventional treatment groups plus *S. boulardii* the same taxa were found to be in a much lower proportion, but the taxon Bifidobacterium was found. It can be seen that if there was a change in the intestinal microbiota at the end of the treatment in the two groups, the most evident difference is

the decrease in *Bacteroides* (genus *Ruminococcus*) and the appearance of the phylum *Firmicutes* (genus *Blautia*, *Faecalibacterium*, *Roseburia*). which is related to the increase in metabolic disorders since they are the producers of short chain fatty acids (SCFA) (Pourmasoumi, M. et al., 2019).

It must be taken into account that the presence of *H. pylori* in the intestine of a person is not indicative of disease, since virulence is related to the amount of *H. pylori* since peptic ulcers develop from a higher density at 10^5 CFU / g (Khulusi S. et al., 1995) therefore the administration of probiotics such as *Saccharomyces boulardii* will not completely eliminate *H. pylori*, but it does help to decrease it as prevents it from adhering and therefore decrease inflammation of the gastric mucosa (Song, H. et al., 2018). When comparing the side effects of this study, it can be seen that the conventional treatment groups plus *S. boulardii* (1.58 ± 0.5) had less abdominal pain compared to the conventional treatment groups (1.96 ± 0.2) with a *p*value = 0.001

In a study by Zhu, X. et al. valuating the efficacy of *Saccharomyces boulardii* combined with quadruple bismuth therapy as a treatment for *H. pylori* elimination, it was confirmed that side effects such as diarrhea and bloating were significantly less compared with the control group. These results are similar to those of this study where the side effects did have significant differences between the control and treatment groups with values of 2.0 ± 0.01 compared to 1.8 ± 0.4 respectively and with a *p* value = 0.001.

The eradication rate is very low in *H. pylori*, which is why many studies try to find new eradication strategies, one of these is the use of symbiotic microorganisms, one of these studies was carried out by Pourmasoumi, M., 2019 and found that the adverse effects with conventional treatments are reduced, but it cannot be effectively confirmed that these symbiotic microorganisms help to increase the eradication rate of *H pylori*, the same results were obtained with the study by Zhang et al. and the same results that were

obtained in this study (Zhang, M. et al. 2015). In addition, according to Shi X et al., The reduction of side effects occurs when probiotics are used before, during and after conventional treatment, which was the methodology followed for this study (Shi, X. et al. 2019).

It should be borne in mind that studies related to the microbiota and *H. pylori* and their treatment with antibiotics are usually inaccurate because the changes in the microbiome cannot be attributed to the absence of the pathogens because the antibiotics have a dramatic effect in the microbiota (Frost, F., Kacprowski, T., Rühlemann, M. et al. 2019).

CONCLUSIONS

• No significant differences in alpha diversity was observed between the conventional treatment groups and the conventional treatment group plus *S. boulardii*.

• The phyla and genera *Firmicutes, Proteobacteria*, *Bacteroidetes, Actinobacteria*, *Verrucomicrobia* and *Tenericutes* are the most common taxa in the two study groups.

• The decrease of the Bacteroides genus in the conventional treatment groups compared to the conventional treatment group plus *S. boulardii*, may have influenced the reduction of secondary symptoms such as diarrhea and abdominal distention.

• The addition of the probiotic *Saccharonyces boulardii* can change the composition of the gut microbiota.

• At the end of the treatment in the two groups there was a change in the intestinal microbiota, the most evident difference is the decrease of *Bacteroides* (genus *Ruminococcus*) and the appearance of the phylum *Firmicutes* (genus *Blautia*, *Faecalibacterium*, *Roseburia*) which is related to the increase of metabolic disorders due to the fact that they are producers of short chain fatty acids.

• The administration of probiotics such as *S. boulardii* will not completely eliminate *H. pylori* but it could reduce secondary symptoms since it prevents it from adhering and therefore reduces inflammation of the gastric mucosa.

REFERENCES

Arthur, J. C., Jobin, C. (2011). The struggle within: microbial influences on colorectal cancer. Inflammatory bowel diseases, 17(1), 396–409. doi:10.1002/ibd.21354

Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T., Mende, D., Fernandes, G., Tap, J., Bruls, T., Batto, J., Bertalan, M.., Borruel, N., Casellas, F., Fernandez, L., Gautier, L., Hansen, T., Hattori, M., Hayashi, T., Kleerebezem, M., Kurokawa, K., Leclerc, M., Levenez, F., Manichanh, C., Nielsen, H., Nielsen, T., Pons, N., Poulain, J., Qin, J., Sicheritz-Ponten, T., Tims, S., Torrents, D., Ugarte, E., Zoetendal, E., Wang, J., Guarner, F., Pedersen, O., De Vos, W., Brunak, S., Doré, J., Weissenbach, J., Ehrlich, S., Bork, P. (2011). Enterotypes of the human gut microbiome. Nature, 473(7346), 174–180. https://doi.org/10.1038/nature09944

Bellack NR, Koehoorn MW, Mac Nab YC, Morshed MG. (2017). A conceptual of water's role as a reservoir in Helicobacter pylori transmission: a review of the evidence. Epidemiol Infect 2006; 134:439-49

Brawner, K. M., Morrow, C. D., and Smith, P. D. (2014). Gastric microbiome and gastric cancer. Cancer journal (Sudbury, Mass.), 20(3), 211–216. doi:10.1097/PPO.000000000 0000043

Borton, M., Sabag-Daigle, A., Wu, J., Solden, L., O'Banion, B., Daly, R. A., Wolfe, R., Gonzalez, J., Wysocki, V., Ahmer, B., Wrighton, K. (2017). Chemical and pathogen-induced inflammation disrupt the murine intestinal microbiome. Microbiome, 5(1). https://doi.org/10.1186/s40168-017-0264-8

Bruno, G., Rocco, G., Zaccari, P., Porowska, B., Mascellino, M. T., & Severi, C. (2018). Helicobacter pylori infection and gastric dysbiosis: Can probiotics administration be useful to treat this condition? Canadian Journal of Infectious Diseases and Medical Microbiology, 2018. https://doi.org/10.1155/2018/6237239

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods, 13(7), 581–583. https://doi.org/10.1038/nmeth.3869

Cohen, L., Ranque, S., and Raoult, D. (2013). Saccharomyces cerevisiae boulardii transient fungemia after intravenous self-inoculation. Medical Mycology Case Reports, 2(1), 63–64. https://doi.org/10.1016/j.mmcr.2013.02.003

Currò, D., Ianiro, G., Pecere, S., Bibbò, S., Cammarota, G. (2017). Probiotics, fibre and herbal medicinal products for functional and inflammatory bowel disorders. Br J Pharmacol 174: 1426–1449. doi:10.1111/bph.13632

Chen, W., Liu, F., Ling, Z., Tong, X., & Xiang, C. (2012). Human intestinal lumen and mucosa-associated microbiota in patients with colorectal cancer. PloS one, 7(6), e39743. doi:10.1371/journal.pone.0039743

Choi, I. J., Kook, M. C., Kim, Y. I., Cho, S. J., Lee, J. Y., Kim, C. G., Nam, B. H. (2018). Helicobacter pylori therapy for the prevention of metachronous gastric cancer. New England Journal of Medicine, 378(12), 1085–1095. doi.org/10.1056/NEJMoa1708423

Cho, I., Martin, J., Blaser, MJ. (2012) The human microbiome: at the interface of health and disease. Nat Rev Genet. 2012; 13:260-70.

Cho, S. J., Choi, I. J., Kook, M. C., et al. (2013). Staging of intestinal- and diffuse-type gastric cancers with the OLGA and OLGIM staging systems. Aliment Pharmacol Ther 2013;38:1292-1302.

Del Campo-Moreno, R., Alarcón-Cavero, T., D'Auria, G., Delgado-Palacio, S., & Ferrer-Martínez, M. (2018, April 1). Microbiota and Human Health: characterization techniques and transference. Enfermedades Infecciosas y Microbiologia Clinica. Elsevier Doyma. doi.org/10.1016/j.eimc.2017.02.007

Dong, Jin-Pei1., Zheng., Yue., Wu., Ting., He., Qun., Teng., Gui-Gen., Wang., Hua-Hong. (2019, August). Protective effect of Saccharomyces boulardii on intestinal mucosal barrier of dextran sodium sulfate-induced colitis in mice. Chinese Medical Journal: August 20, 2019. Volume 132 - Issue 16 - p 1951–1958 doi: 10.1097/CM9.0000000000000364

Doorakkers, E., Lagergren, J., Engstrand, L., & Brusselaers, N. (2018, January 30). Helicobacter pylori eradication treatment and the risk of gastric adenocarcinoma in a Western population. Gut. BMJ Publishing Group. doi.org/10.1136/gutjnl-2017-315363

Eshraghian, A. (2014). Epidemiology of Helicobacter pylori infection among the healthy population in Iran and countries of the Eastern Mediterranean Region: A systematic review of prevalence and risk factors. World Journal of Gastroenterology: WJG, 20(46),17618–17625. http://doi.org/10.3748/wjg.v20.i46.17618

Ferlay, J., Soerjomataram, I., Dikshit, R., Eser, S., Mathers, C., Rebelo, M., Parkin, D.M., Forman, D., Bray, F. (2014, September 13). Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int. J. Cancer 2015, 136, E359–E386.

Frost, F., Kacprowski, T., Rühlemann, M. et al. Helicobacter pylori infection associates with fecal microbiota composition and diversity. Sci Rep 9, 20100 (2019). https://doi.org/10.1038/s41598-019-56631-4

Geerlings, S. Y., Kostopoulos, I., de Vos, W. M., & Belzer, C. (2018). Akkermansia muciniphila in the Human Gastrointestinal Tract: When, Where, and How? Microorganisms, 6(3), 75. doi:10.3390/microorganisms6030075

Gorvitovskaia, A., Holmes, S. P., & Huse, S. M. (2016). Interpreting prevotella and bacteroides as biomarkers of diet and lifestyle. Microbiome, 4. https://doi.org/10.1186/s40168-016-0160-7

Honda, K., Littman, DR. (2016, July 7). The microbiota in adaptive immune homeostasis and disease. Nature. 2016 Jul 7; 535(7610):75-84.

Hosseini, E., Grootaert, C., Verstraete, W., & Van de Wiele, T. (2011, May). Propionate as a health-promoting microbial metabolite in the human gut. Nutrition Reviews. https://doi.org/10.1111/j.1753-4887.2011.00388.x

Hooi, J. K. Y., Lai, W. Y., Ng, W. K., Suen, M. M. Y., Underwood, F. E., Tanyingoh, D., Ng, S. C. (2017). Global Prevalence of Helicobacter pylori Infection: Systematic Review and Meta-Analysis. Gastroenterology, 153(2), 420–429.doi.org/10.1053/j.gastro.2017.04 .022

Hooper, LV., Midtvedt, T., Gordon, JI. (2002) How host-microbial interactions shape the nutrient environment of the mammalian intestine. Annu Rev Nutr. 2002; 22:283–307.

Johansson, ME., Jakobsson, HE., Holmén-Larsson, J., Schütte, A., Ermund, A., Rodríguez-Piñeiro, AM., Arike, L., Wising, C., Svensson, F., Bäckhed, F., Hansson, GC. (2015, November 11). Normalization of Host Intestinal Mucus Layers Requires Long-Term Microbial Colonization. Cell Host Microbe. 2015 Nov 11; 18(5):582-92.

Juge, N. (2012, January). Microbial adhesins to gastrointestinal mucus. Trends in Microbiology. doi.org/10.1016/j.tim.2011.10.001

Klymiuk I., Bilgilier C., Stadlmann A., et al. The human gastric microbiome is predicated upon infection with Helicobacter pylori. Frontiers in Microbiology. 2017;8:p. 2508. doi: 10.3389/fmicb.2017.02508.

Khulusi S., Mendall M. A., Patel P., Levy J., Badve S., Northfield T. C. Helicobacter pylori infection density and gastric inflammation in duodenal ulcer and non-ulcer subjects. Gut. 1995;37(3):319–324. doi: 10.1136/gut.37.3.319.

Koliarakis, I., Messaritakis, I., Nikolouzakis, TK., Hamilos, G., Souglakos G., Tsiaoussis, J. (2019, August 19). Oral Bacteria and Intestinal Dysbiosis in Colorectal Cancer. International Journal of Molecular Sciences. doi.org/10.3390/ijms20174146

Larsen, N., Vogensen, FK., Van den Berg, FW., et al. (2010). Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. PLoS One. 2010; 5: e9085.

Liou, J.M., Fang, Y.J., Chen, C.C. et al. (2016). Concomitant, bismuth quadruple, and 14-day triple therapy in the first-line treatment of Helicobacter pylori: a multicentre, open-label, randomised trial. Lancet. 2016; 388: 2355–2365

Malfertheiner, P., Megraud, F., O'Morain, C. A., Gisbert, J. P., Kuipers, E. J., Axon, A.T., Bazzoli, F., Gasbarrini, A., Atherton, J., Graham, D. Y., Hunt. R., Moayyedi, P., Rokkas, T., Rugge, M., Selgrad, M., Suerbaum, S., Sugano, K., El-Omar, E. M. (2016, January). Management of Helicobacter pylori infection-the Maastricht V/Florence Consensus Report. Epub 2016, 66(1):6-30. doi:10.1136/gutjnl-2016-312288.

Malnick, S. D. H., Melzer, E., Attali, M., Duek, G., & Yahav, J. (2014). Helicobacter pylori: Friend or foe? World Journal of Gastroenterology: WJG, 20(27), 8979–8985. doi: 10.3748/wjg.v20.i27.8979

Nardone G., Compare D. The human gastric microbiota: is it time to rethink the pathogenesis of stomach diseases? United European Gastroenterology Journal. 2015;3(3):255–260. doi: 10.1177/2050640614566846.

Offei, B., Vandecruys, P., De Graeve, S., Foulquié-Moreno, M. R., Thevelein, J. M. (2019, September). Unique genetic basis of the distinct antibiotic potency of high acetic acid production in the probiotic yeast Saccharomyces cerevisiae var. boulardii. Genome Res. 2019 Sep;29(9):1478-1494. doi: 10.1101/gr.243147.118.

Parsons B. N., Ijaz U. Z., D'Amore R., et al. Comparison of the human gastric microbiota in hypochlorhydric states arising as a result of Helicobacter pylori-induced

atrophic gastritis, autoimmune atrophic gastritis and proton pump inhibitor use. PLoS Pathogens. 2017;13(11) doi: 10.1371/journal.ppat.1006653.e1006653

Pichon, M., and Burucoa, C. (2019). Impact of the Gastro-Intestinal Bacterial Microbiome on Helicobacter-Associated Diseases. Healthcare (Basel, Switzerland), 7(1), 34. doi:10.3390/healthcare7010034

Polansky, O., Sekelova, Z., Faldynova, M., Sebkova, A., Sisak, F., & Rychlik, I. (2016). Important metabolic pathways and biological processes expressed by chicken cecal microbiota. Applied and Environmental Microbiology, 82(5), 1569–1576. https://doi.org/10.1128/AEM.03473-15

Pourmasoumi, M., Najafgholizadeh, A., Hadi, A., Mansour-Ghanaei, F., & Joukar, F. (2019, April 1). The effect of synbiotics in improving Helicobacter pylori eradication: A systematic review and meta-analysis. Complementary Therapies in Medicine. Churchill Livingstone. https://doi.org/10.1016/j.ctim.2019.01.005

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K.S., Manichanh, C., Nielsen, T., Pons, N., Levenez, F., Yamada, T., et al. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. Nature 2010, 464, 59–65.

Ray, K. (2011, November). Colorectal cancer: Fusobacterium nucleatum found in colon cancer tissue - Could an infection cause colorectal cancer? Nature Reviews Gastroenterology and Hepatology. doi.org/10.1038/nrgastro.2011.208

Roy, U., Jessani, L. G., Rudramurthy, S. M., Gopalakrishnan, R., Dutta, S., Chakravarty, C., Jillwin, J., Chakrabarti, A. (2017). Seven cases of Saccharomyces fungaemia related to use of probiotics. Mycoses, 60(6), 375–380. https://doi.org/10.1111/myc.12604

Round, J. L., Lee, S. M., Li, J., Tran, G., Jabri, B., Chatila, T. A., & Mazmanian, S. K. (2011). The toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. Science, 332(6032), 974–977. https://doi.org/10.1126/science .1206095

Sgouras, D. N., Trang, T. T., and Yamaoka, Y. (2015). Pathogenesis of Helicobacter pylori Infection. Helicobacter, 20 Suppl 1(0 1), 8–16. doi:10.1111/hel.12251

Sheh A., Fox J. G. The role of the gastrointestinal microbiome in Helicobacter pylori pathogenesis. Gut Microbes. 2013;4(6):505–531. doi: 10.4161/gmic.26205.

Shi, X., Zhang, J., Mo, L., Shi, J., Qin, M., & Huang, X. (2019). Efficacy and safety of probiotics in eradicating Helicobacter pylori: A network meta-analysis. Medicine, 98(15), e15180. https://doi.org/10.1097/MD.0000000000015180

Smith, P. M., Howitt, M. R., Panikov, N., Michaud, M., Gallini, C. A., Bohlooly-Y, M., … Garrett, W. S. (2013). The microbial metabolites, short-chain fatty acids, regulate colonic T reg cell homeostasis. Science, 341(6145), 569–573. https://doi.org/10.1126/science.1241165

Sommer, F., and Bäckhed, F. (2013, April). The gut microbiota-masters of host development and physiology. Nature Reviews Microbiology. doi.org/10.1038/nrmicro 2974

Song, H. Y., Zhou, L., Liu, D. Y., Yao, X. J., & Li, Y. (2018). What roles do probiotics play in the eradication of helicobacter pylori? current knowledge and ongoing research. Gastroenterology Research and Practice. Hindawi Limited. https://doi.org/10.1155/2018/9379480

Spiljar, M., Merkler, D., and Trajkovski, M. (2017). The Immune System Bridges the Gut Microbiota with Systemic Energy Homeostasis: Focus on TLRs, Mucosal Barrier, and SCFAs. Frontiers in immunology, 8, 1353. Doi:10.3389/fimmu.2017.01353

Stier, H., & Bischoff, S. C. (2016, September 13). Influence of Saccharomyces boulardii CNCM I-745 on the gut-associated immune system. Clinical and Experimental Gastroenterology. Dove Medical Press Ltd. Doi.org/10.2147/CEG.S111003

Suzuki, S., Esaki, M., Kusano, C., Ikehara, H., & Gotoda, T. (2019). Development of Helicobacter pylori treatment: How do we manage antimicrobial resistance?. World journal of gastroenterology, 25(16), 1907–1912. Doi:10.3748/wjg.v25.i16.1907

Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R., & Gordon, J. I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. Nature, 444(7122), 1027–1031. https://doi.org/10.1038/nature05414

Villar-García, J., Hernández, J. J., Güerri-Fernández, R., González, A., Lerma, E., Guelar, A., Saenz, D., Sorlí, L., Montero, M., Horcajada, J. P., et al. (2015). Effect of probiotics (Saccharomyces boulardii) on microbial translocation and inflammation in HIV-treated patients: a double-blind, randomized, placebo-controlled trial. J Acquir Immune Defic Syndr 68: 256–263. Doi:10.1097/QAI.0000000000000468

Villot, C., Ma, T., Renaud, D. L., Ghaffari, M. H., Gibson, D. J., Skidmore, A., Chevaux, E., Guan, L., Steele, M. A. (2019). Saccharomyces cerevisiae boulardii CNCM I-1079 affects health, growth, and fecal microbiota in milk-fed veal calves. Journal of Dairy Science, 102(8), 7011–7025. Doi.org/10.3168/jds.2018-16149

White, J. R., Winter, J. A., and Robinson, K. (2015). Differential inflammatory response to Helicobacter pylori infection: etiology and clinical outcomes. Journal of inflammation research, 8, 137–147. Doi:10.2147/JIR.S64888

Yap, T., Gan, H., Lee, Y., Leow, A., Azmi, A., Francois, F., Perez-Perez G., Loke M., Goh K., Vadivelu, J. (2016). Helicobacter pylori eradication causes perturbation of the human gut microbiome in young adults. PloS ONE, 11(3). https://doi.org/10.1371/journal.pone.0151893

Yeh, J. M., Goldie, S. J., Kuntz, K. M., & Ezzati, M. (2009). Effects of Helicobacter pylori infection and smoking on gastric cancer incidence in China: a population-level analysis of trends and projections. Cancer Causes & Control, 20(10), 2021–9. Doi:10.1007/s10552-009-9397-9

Zhang, M., Qian, W., Qin, Y., He, J., & Zhou, Y. (2015). Probiotics in Helicobacter pylori eradication therapy: A systematic review and meta-analysis. World Journal of Gastroenterology, 21(14), 4345–4357. https://doi.org/10.3748/wjg.v21.i14.4345

Zhang, X., Ning, Z., Mayne, J., Moore, J. I., Li, J., Butcher, J., Deeke, S. A., Chen, R., Chiang, C. K., Wen, M., Mack, D., Stintzi, A., Figeys, D. (2016). MetaPro-IQ: a universal metaproteomic approach to studying human and mouse gut microbiota. Microbiome, 4(1), 31. doi:10.1186/s40168-016-0176-z

Zhou, Y., & Zhi, F. (2016). Lower Level of Bacteroides in the Gut Microbiota Is Associated with Inflammatory Bowel Disease: A Meta-Analysis. BioMed Research International. Hindawi Limited. https://doi.org/10.1155/2016/5828959

Zhu, X., Du, J., Wu, J., Zhao, L., Meng, X., & Liu, G. (2017). Influence of *Saccharomyces boulardii* Sachets combined with bismuth quadruple therapy for initial *Helicobacter pylori* eradication. National Medical Journal of China, 97(30), 2353–2356. https://doi.org/10.3760/cma.j.issn.0376-2491.2017.30.008

Zhou, B. G., Chen, L. X., Li, B., Wan, L. Y., Ai, Y. W. (2019). Saccharomyces boulardii as an adjuvant therapy for Helicobacter pylori eradication: A systematic review and meta-analysis with trial sequential analysis. Helicobacter. 14.8. doi.org/10.1111/hel.12651

Table 1. Analysis of epidemiological data

	Conventional treatment group	**Conventional treatment group plus *S. boulardii***	***p*-value**
Age (years)	36 (22 - 54)	36 (22 - 54)	0,052
Female (%)	51	45,5	0,399
Male (%)	48,7	54,5	0,499
BMI (kg/m^2)	19,1	21,8	2,67
Smokinge (%)	8,1	10	0,289
Cancer (%)	0	0	0
Gastrointestinal problems (%)	70,2	51,4	0,04

Descriptive statistics as frequency analysis. Gastrointestinal problems are abdominal pain, diarrhea

Table 2. Results of Faith's Phylogenetic Diversity and Evenness analysis conventional treatment group and conventional treatment group plus *S. boulardii.*

	Faith's Phylogenetic Diversity	**Evenness**
H-value	1,864	0,944
***p*-value**	0,172	0,331

Kruskal-Wallis non-parametric statistical value

Table 3. Faith's Phylogenetic Diversity and Evenness analysis results for conventional treatment group and conventional treatment group plus *S. boulardii.*and without *H. pylori* (control) groups.

	Faith's Phylogenetic Diversity	**Evenness**
H-value	1,052	1,794
***p*-value**	0,591	0,407

Kruskal-Wallis non-parametric statistical value

Table 4. Results of Faith's Phylogenetic Diversity and Evenness analysis for the position of samples M1 (before treatment), M2 (during treatment) and M3 (after treatment) for the conventional treatment group and conventional treatment group plus *S. boulardii*

	Faith's Phylogenetic Diversity		**Evenness**	
Position Sample	**H-value**	***p*-value**	**H-value**	***p*-value**
M1	0,592	0,441	0,099	0,753
M2	0,000	0,976	0,188	0,664
M3	1,357	0,244	4,157	0,041*

Alpha diversity analysis with Faith's Phylogenetic Diversity and Evenness

Table 5. Results of Faith's Phylogenetic Diversity and Evenness analysis results for the position of samples M1 (before treatment), M2 (during treatment) and M3 (after treatment) for the conventional treatment group and conventional treatment group plus *S. boulardii* and without *H. pylori* (control)

	Faith's Phylogenetic Diversity		**Evenness**	
Position Sample	**H-value**	***p*-value**	**H-value**	***p*-value**
M1	0,246	0,884	0,114	0,944
M2	0,942	0,624	1,151	0,562
M3	1,099	0,294	3,261	0,071

Alpha diversity analysis with Faith's Phylogenetic Diversity and Evenness

Table 6. Results of Faith's Phylogenetic Diversity and Evenness analysis for the conventional treatment group and conventional treatment group plus *S. boulardii* and without *H. pylori* (control) groups for sample position M1, M2 and M3.

	Faith's Phylogenetic Diversity		**Evenness**	
Groups	**H-value**	***p*-value**	**H-value**	***p*-value**
Conventional treatment	3,292	0,348	0,238	0,971
Conventional treatment plus *S. boulardii*	0,422	0,809	4,595	0,100
Control without *H. pylori*	0,832	0,659	1,145	0,564

Alpha diversity analysis with Faith's Phylogenetic Diversity and Evenness

Figure 1. Box diagram of the Faith's Phylogenetic Diversity analysis for the position of samples M1 (before treatment), M2 (during treatment) and M3 (after treatment)

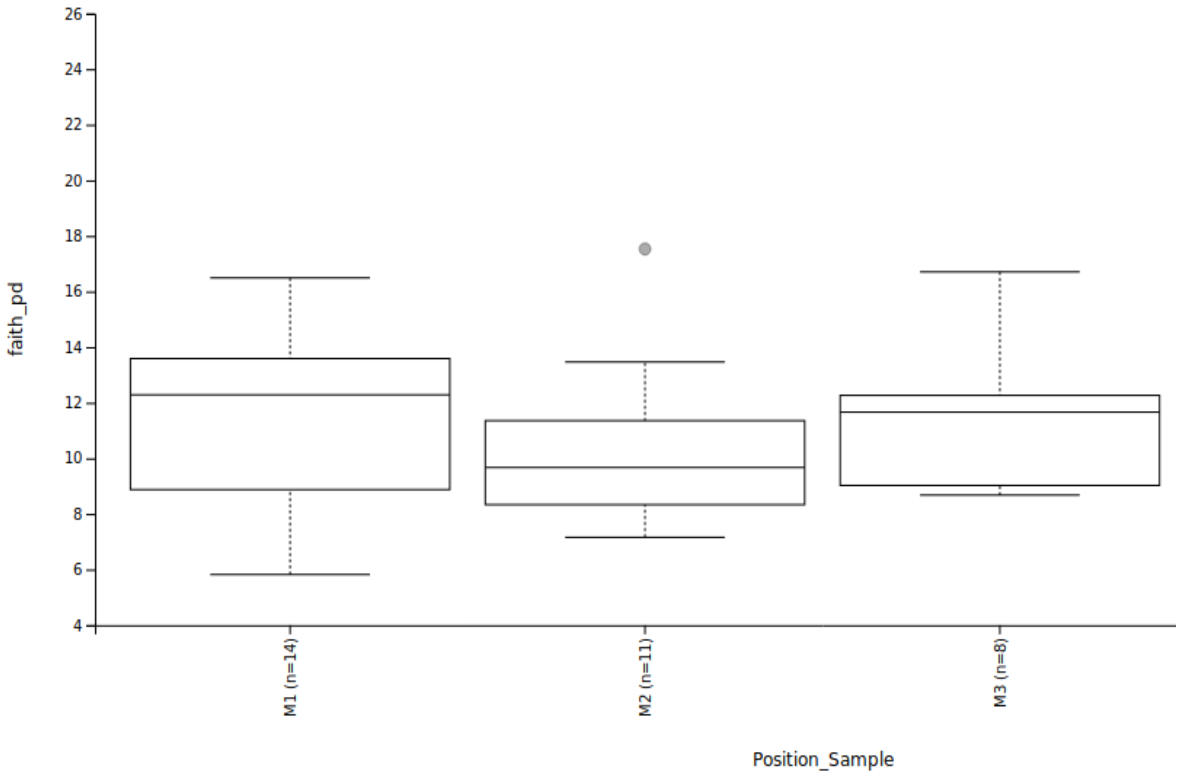


Figure 2. Box diagram of the Evenness analysis for the position of samples M1 (before treatment), M2 (during treatment) and M3 (after treatment)

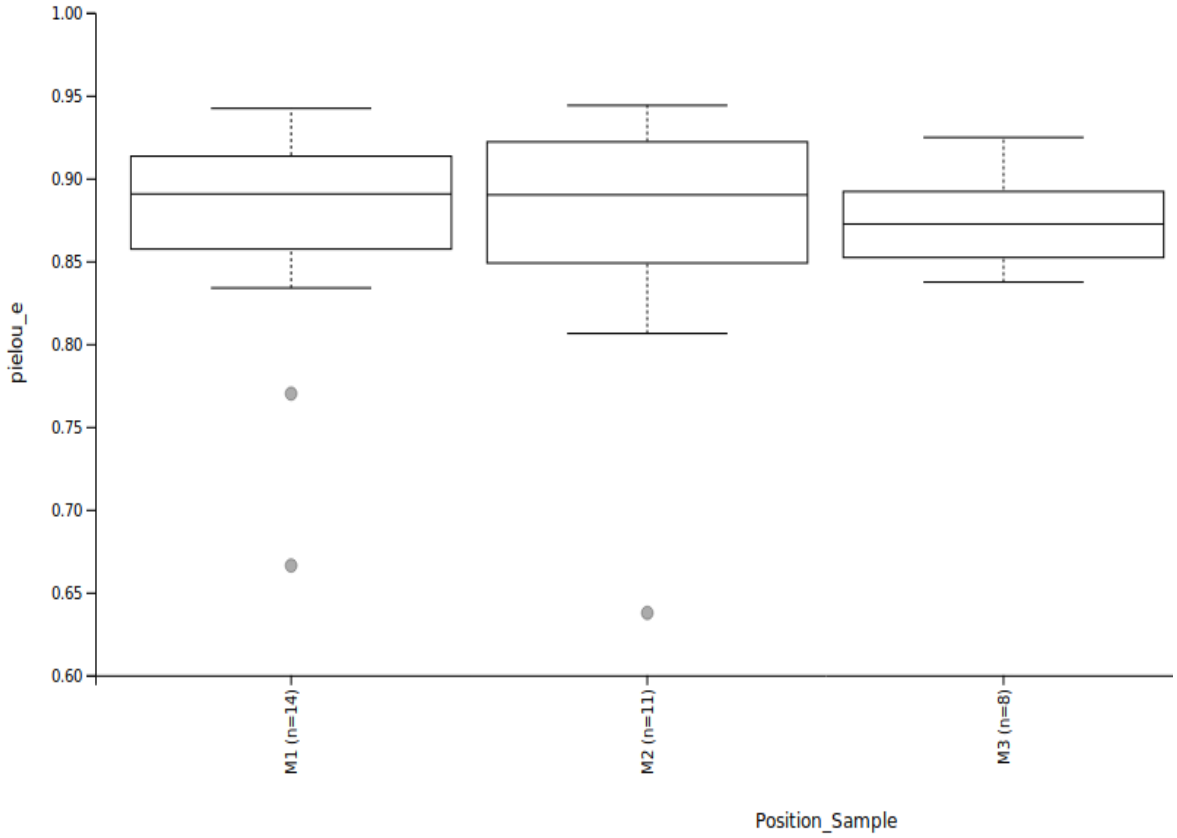
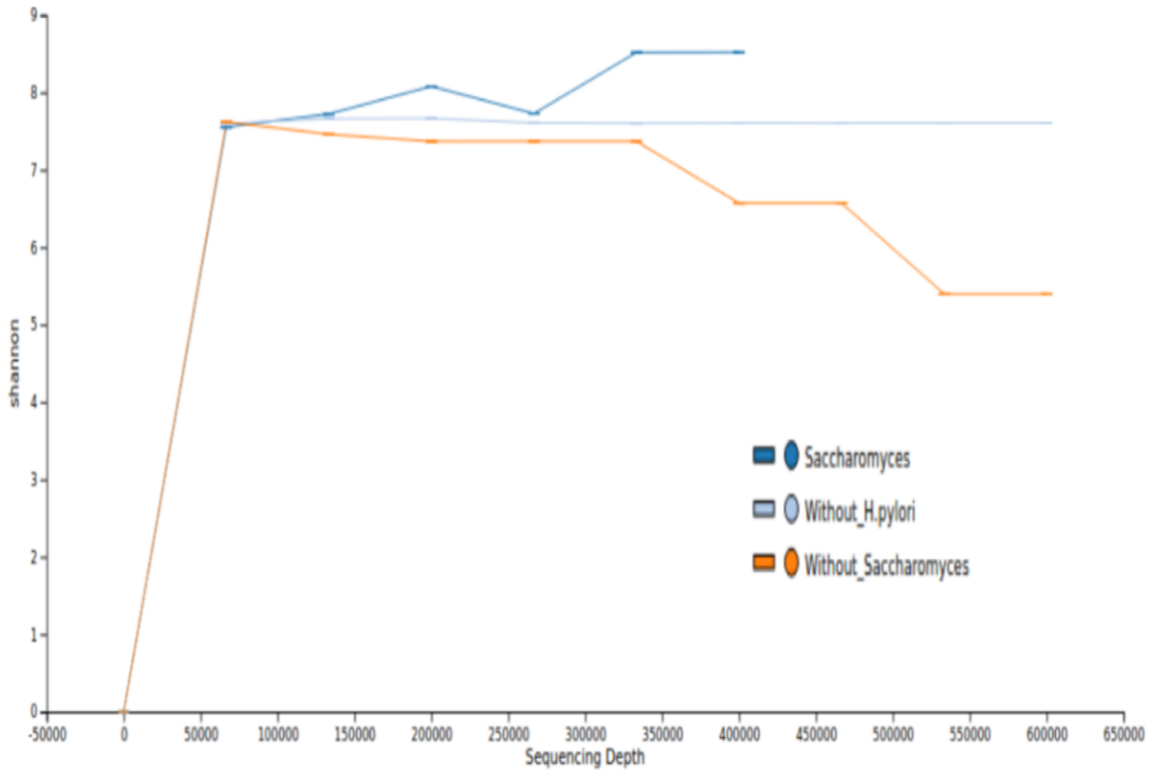
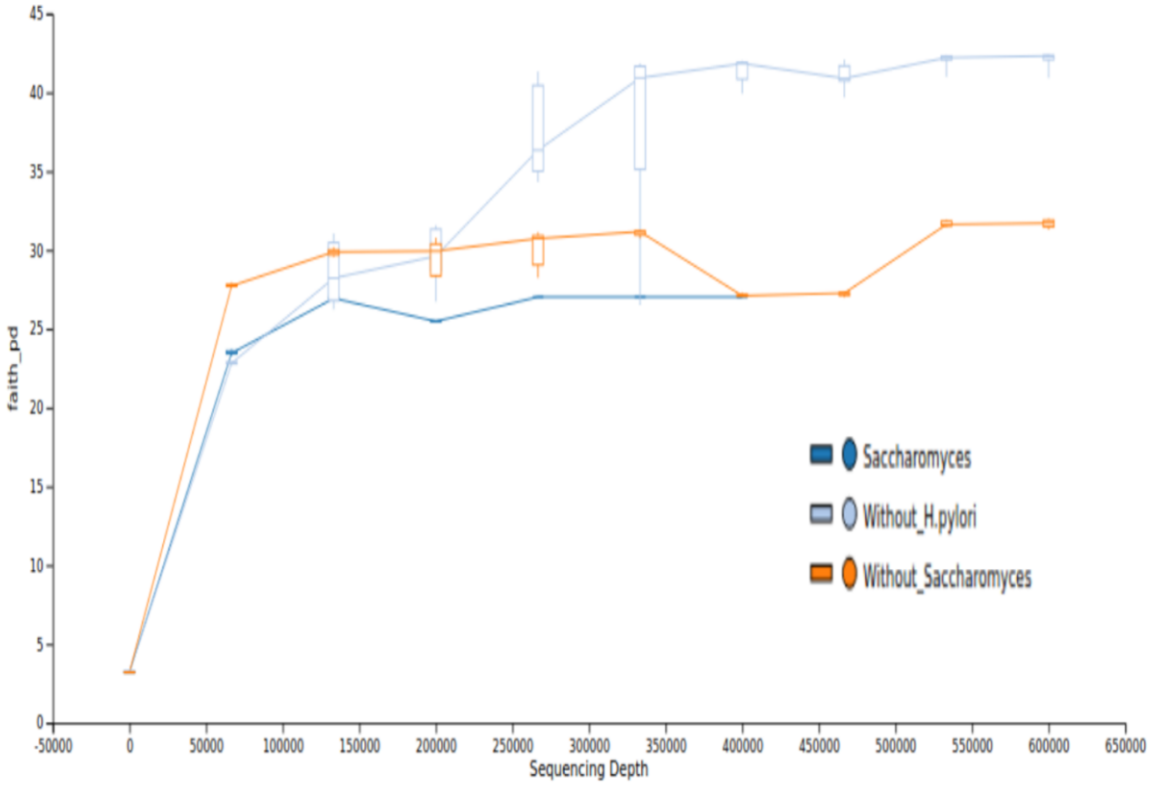


Figure 3. Alpha rarefaction curve using the Shannon index comparing the conventional treatment group and conventional treatment group plus *S. boulardii* and without *H. pylori* (control) groups.



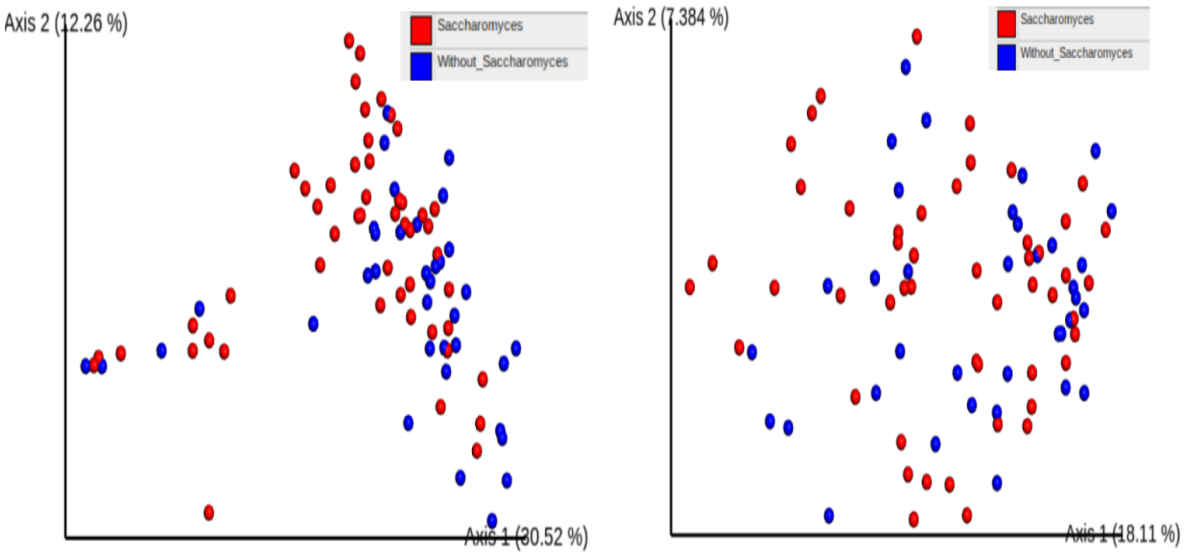
The blue points correspond to the conventional treatment group plus *S. boulardii*, the light blue points to the without *H. pylori* (control) group and the orange points to the the conventional treatment group

Figure 4. Alpha rarefaction curve using the de Faith's Phylogenetic Diversity index comparing the conventional treatment group and conventional treatment group plus *S. boulardii* and without *H. pylori* (control) groups.



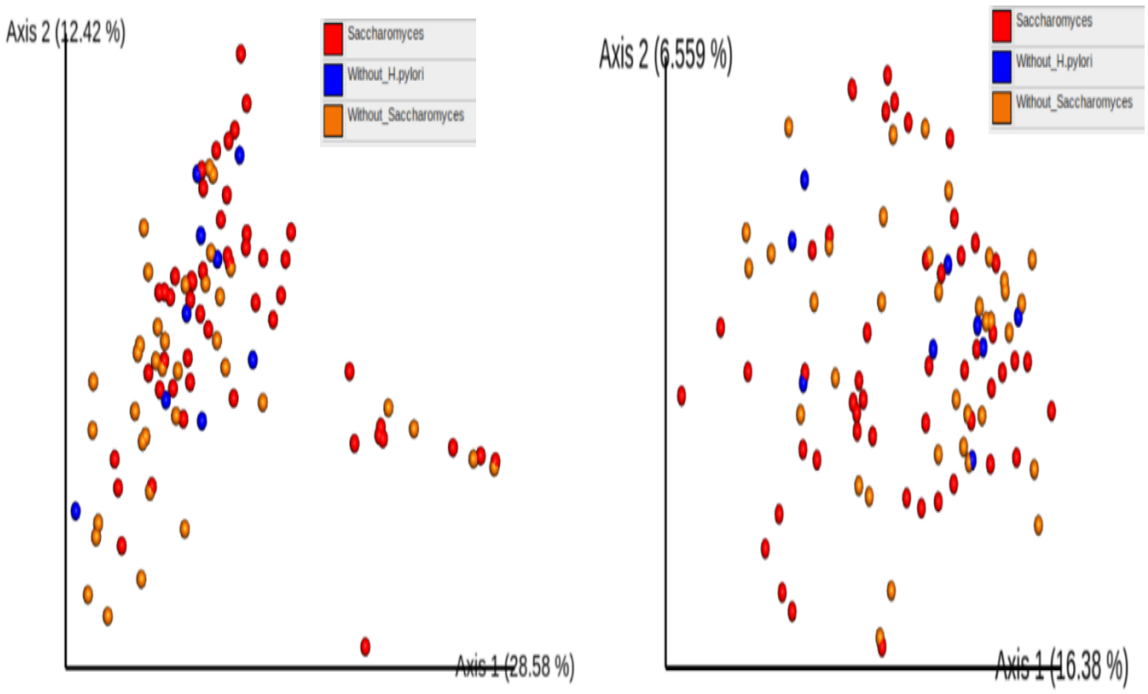
The blue points correspond to the conventional treatment group plus *S. boulardii*, the light blue points to the without *H. pylori* (control) group and the orange points to the the conventional treatment group

Figure 5. PCoA of unweighted (right) and weighted (left) distances of the 108 faecal samples.



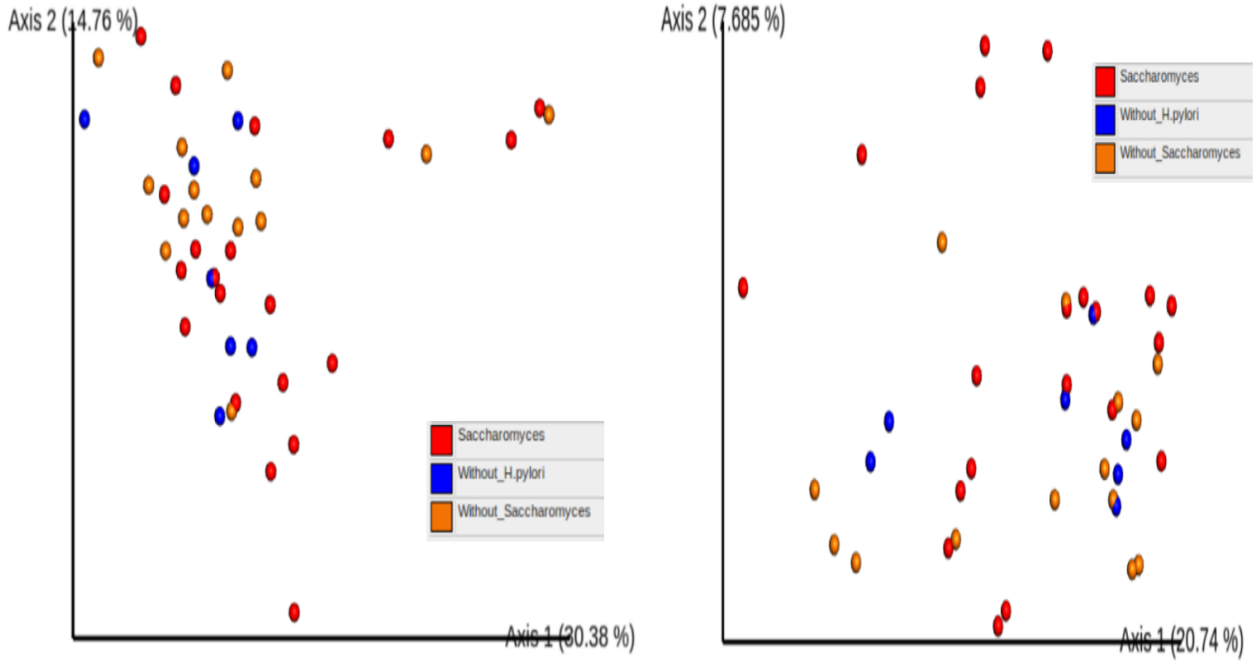
The red points correspond to the conventional treatment group plus *S. boulardii* and the blue points to the conventional treatment group.

Figure 6. PCoA of unweighted (right) and weighted (left) distances of the 108 faecal samples.



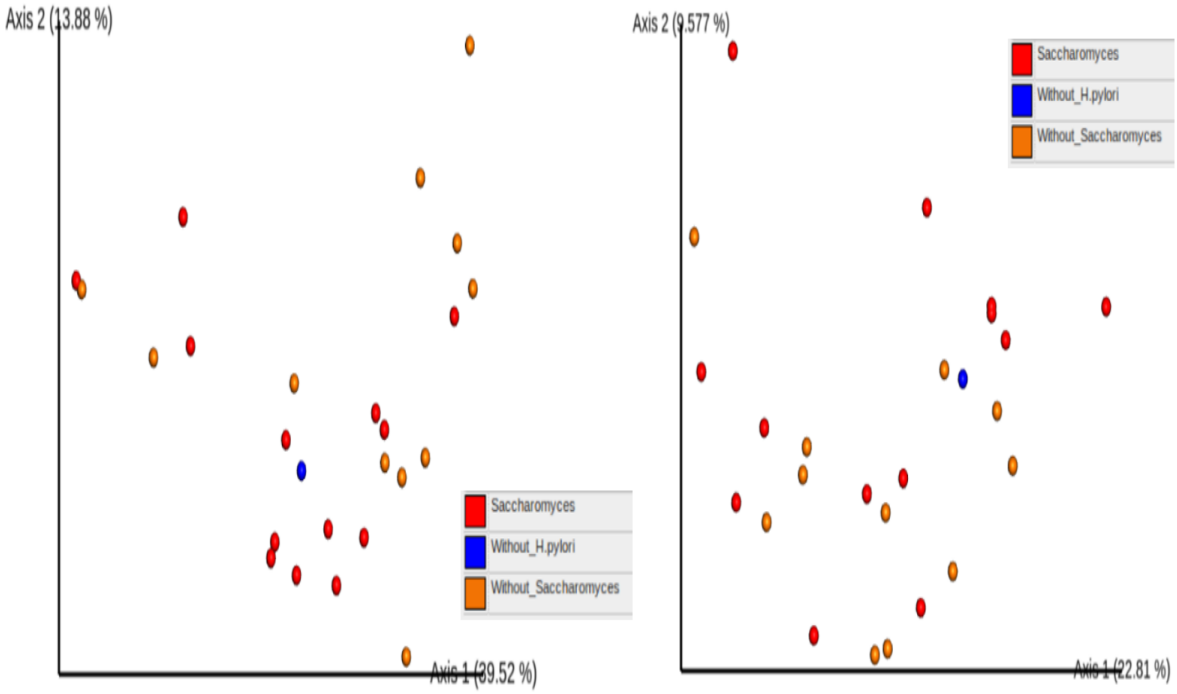
The red points correspond to the conventional treatment group plus *S. boulardii*, the blue points to the without *H. pylori* (control) group and the orange points to the the conventional treatment group.

Figure 7. PCoA of unweighted (right) and weighted (left) distances of the fecal samples taken at the start of treatment (M1).



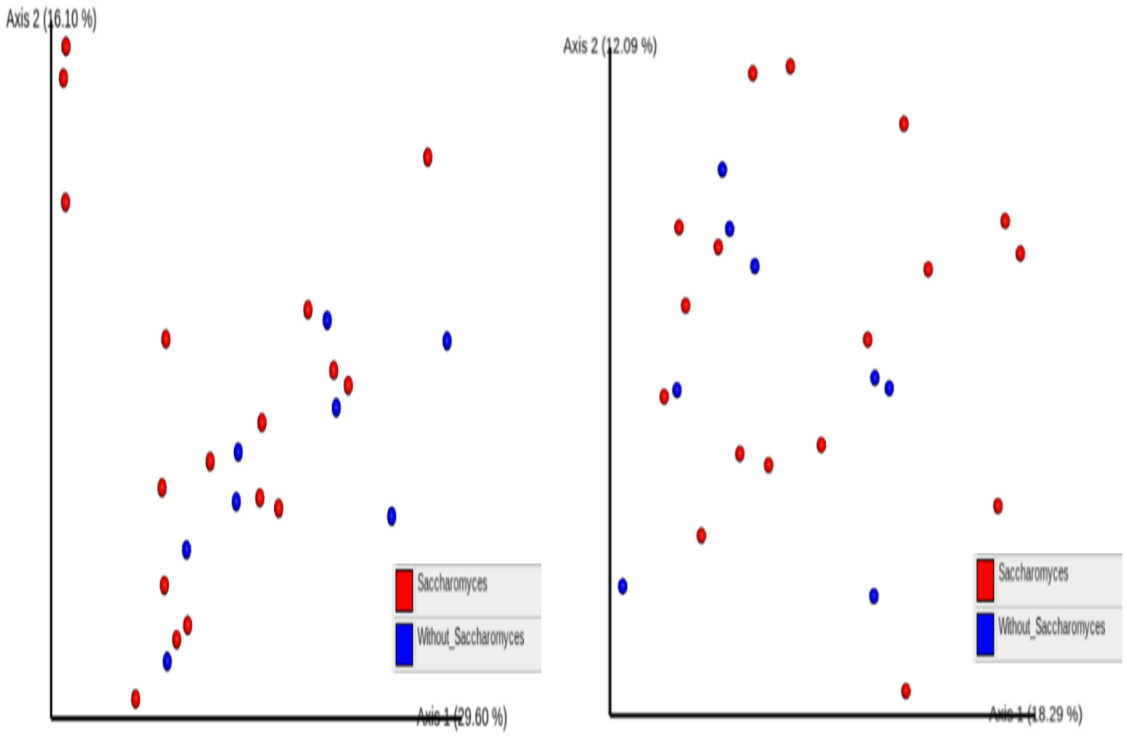
The red points correspond to the conventional treatment group plus *S. boulardii*, the blue points to the without *H. pylori* (control) group and the orange points to the conventional treatment group.

Figure 8. PCoA of unweighted (right) and weighted (left) distances from faecal samples taken during treatment (M2).



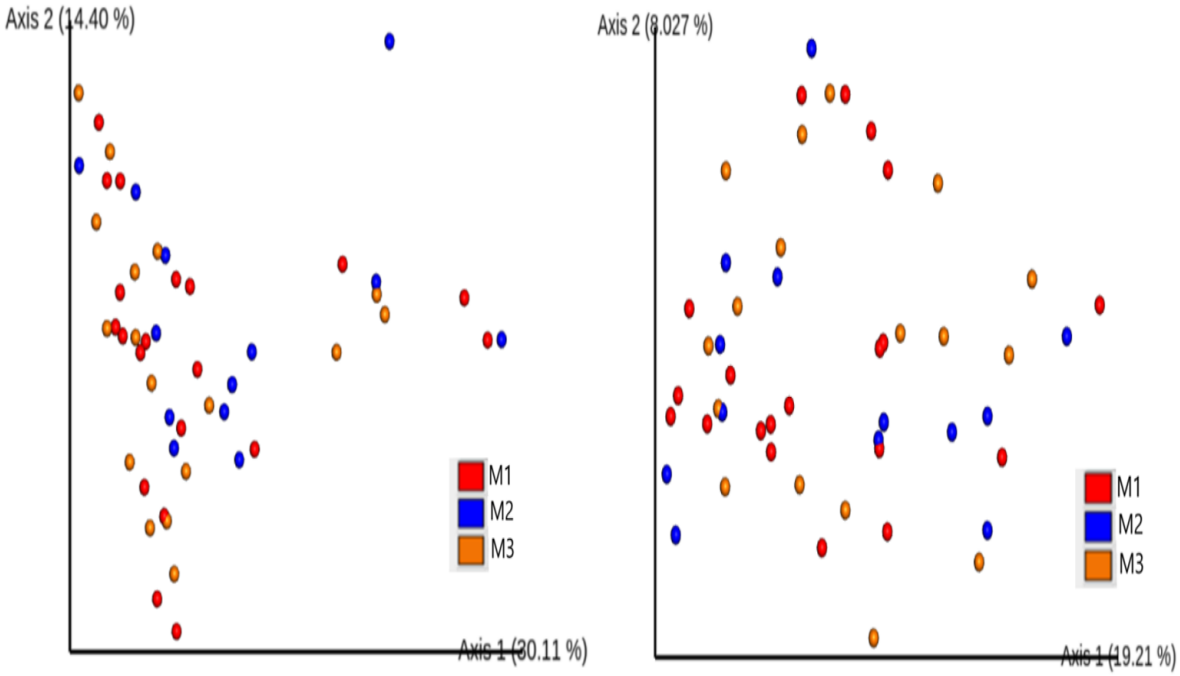
The red points correspond to the conventional treatment group plus *S. boulardii*, the blue points to the without *H. pylori* group and the orange points to the conventional treatment group.

Figure 9. PCoA of unweighted (right) and weighted (left) distances from faecal samples taken after treatment (M3).



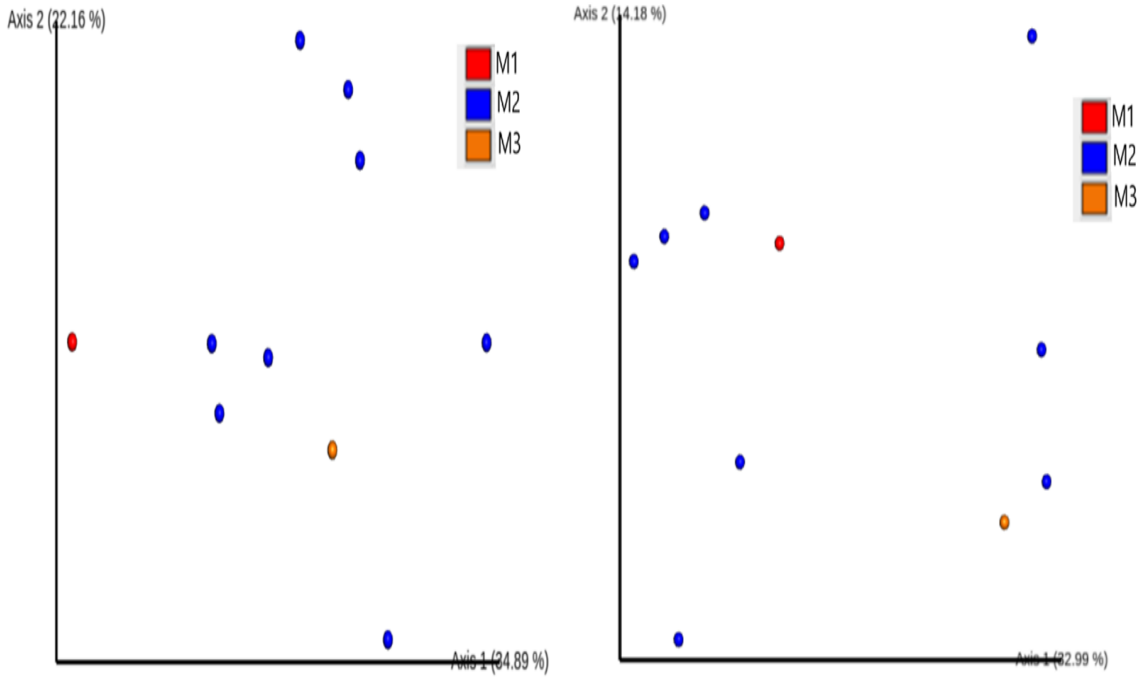
The red points correspond to the correspond to the conventional treatment group plus *S. boulardii*, the blue points to the correspond to the conventional treatment group.

Figure 10. PCoA of unweighted (right) and weighted (left) distances of the stool samples from the conventional treatment group plus *S. boulardii* at the beginning (M1), during (M2) and after (M3) of the treatment.



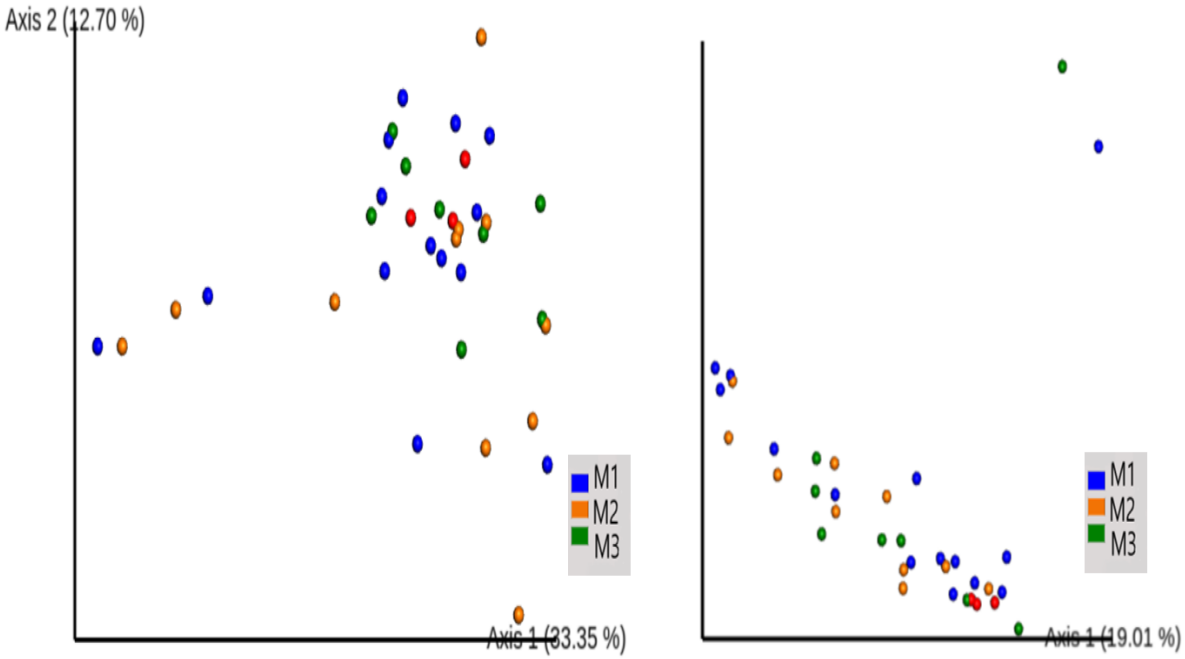
Red points correspond to the start of treatment (M1), blue points correspond to samples during treatment (M2) and orange points correspond to the end of treatment (M3)

Figure 11. PCoA of unweighted (right) and weighted (left) distances of stool samples from the without *H. pylori* (control)group at the beginning (M1), during (M2) and after (M3) of the treatment.



Red points correspond to the start of treatment (M1), blue points correspond to samples during treatment (M2) and orange points correspond to the end of treatment (M3)

Figure 12. PCoA of unweighted (right) and weighted (left) distances of the stool samples from the conventional treatment group at the beginning (M1), during (M2) and after (M3) of the treatment.



The blue points correspond to the beginning of the treatment (M1), the orange points correspond to samples during the treatment (M2) and green points correspond to the end of the treatment (M3)

Figure 13. Taxa bar plots comparing the conventional treatment group and conventional treatment group plus *S. boulardii* at the taxonomic level of 2.

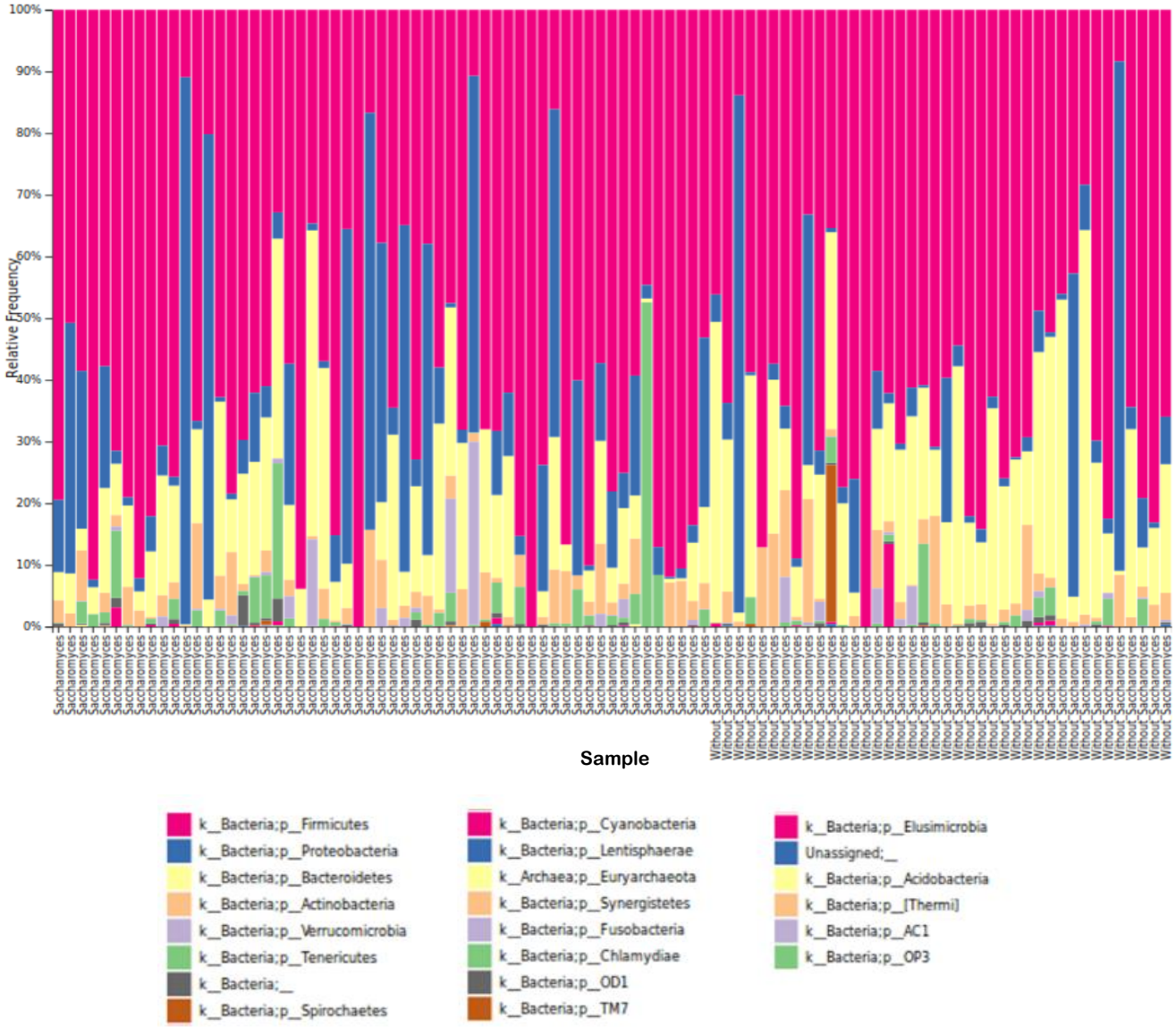


Figure 14. Taxa bar plots starting treatment (M1) comparing conventional treatment group and conventional treatment group plus *S. boulardii* at the taxonomic level of 2.

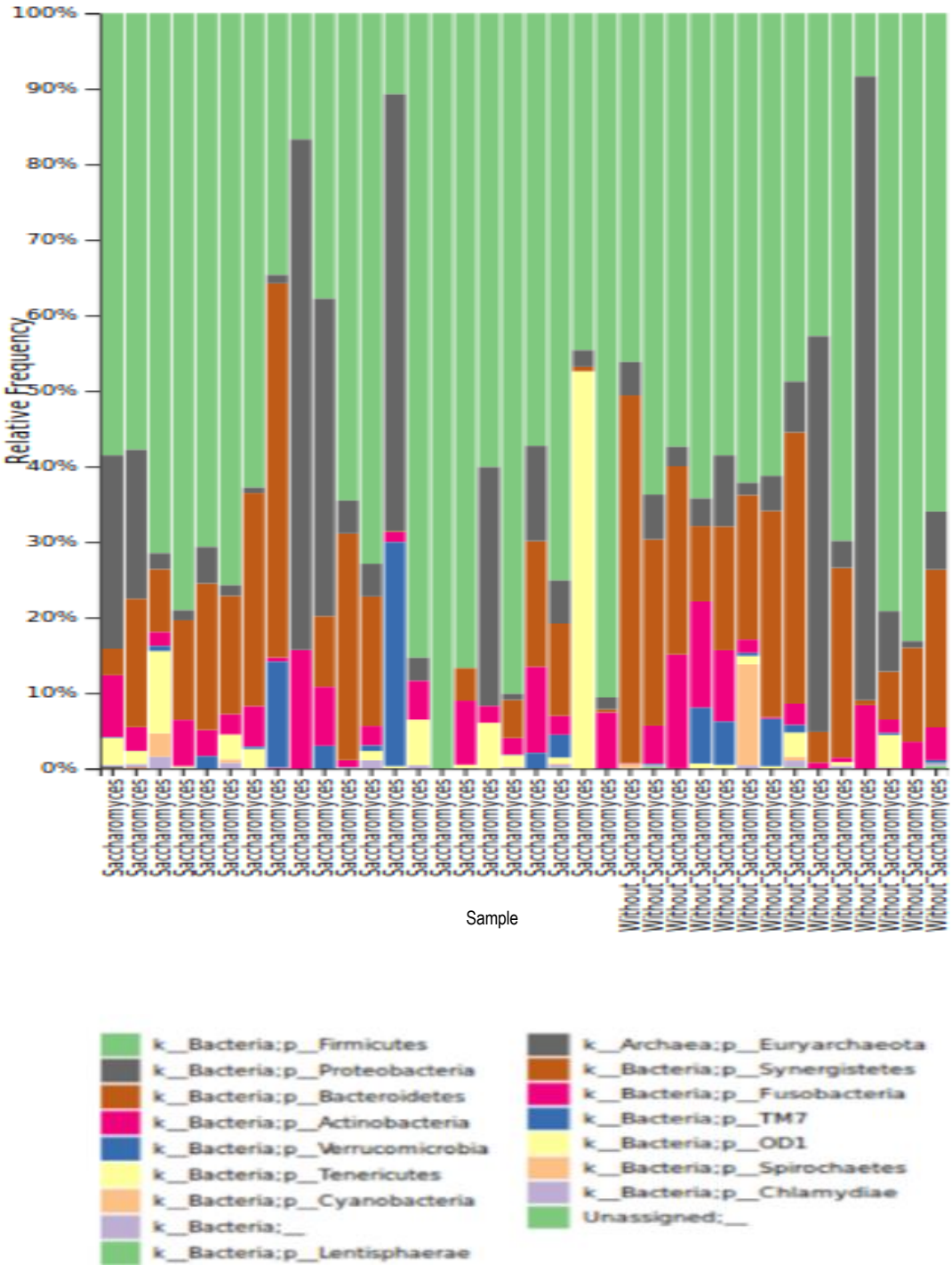
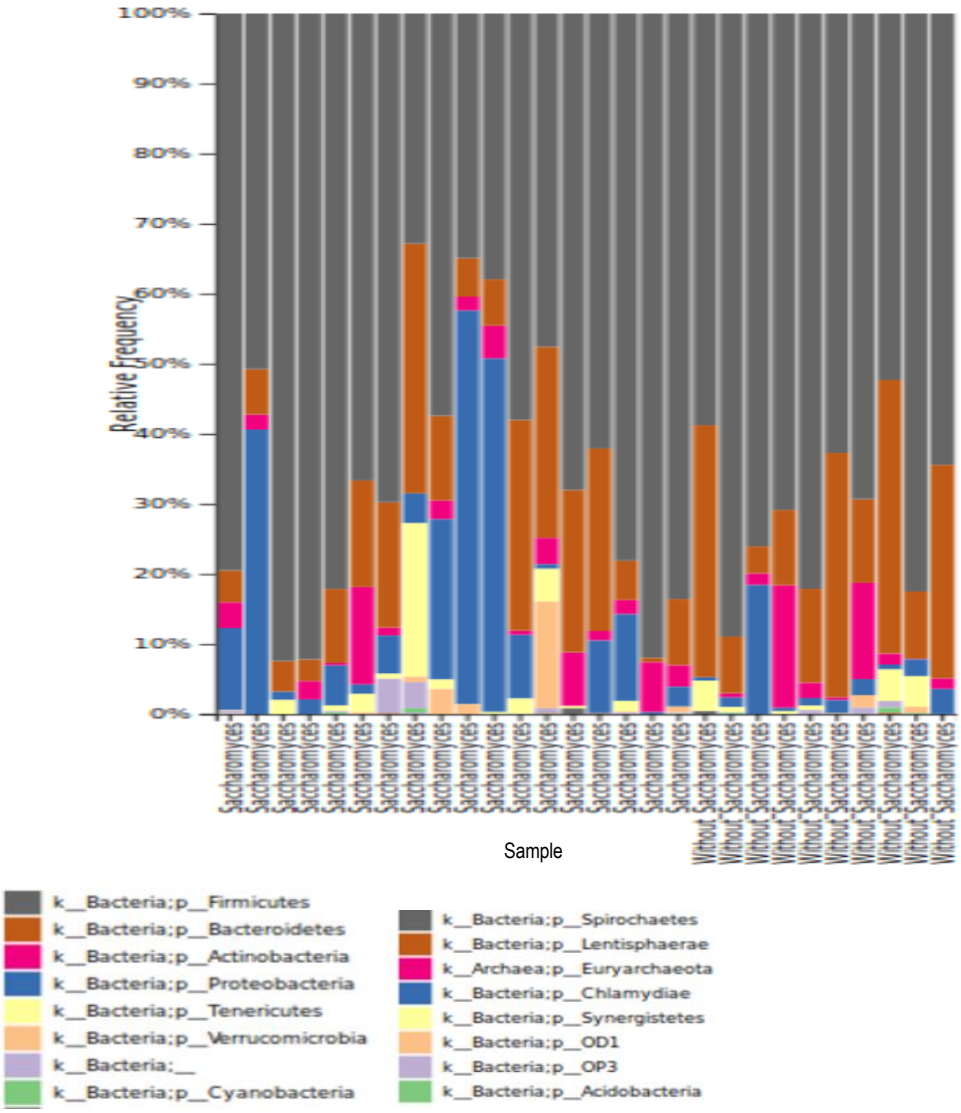


Figure 15. Taxa bar plots at the end of treatment (M3) comparing the conventional treatment group and conventional treatment group plus *S. boulardii* at the taxonomic level of 2.



igure 16. Taxa bar plots of taxa of the conventional treatment group plus *S. boulardii* at taxonomic level 2

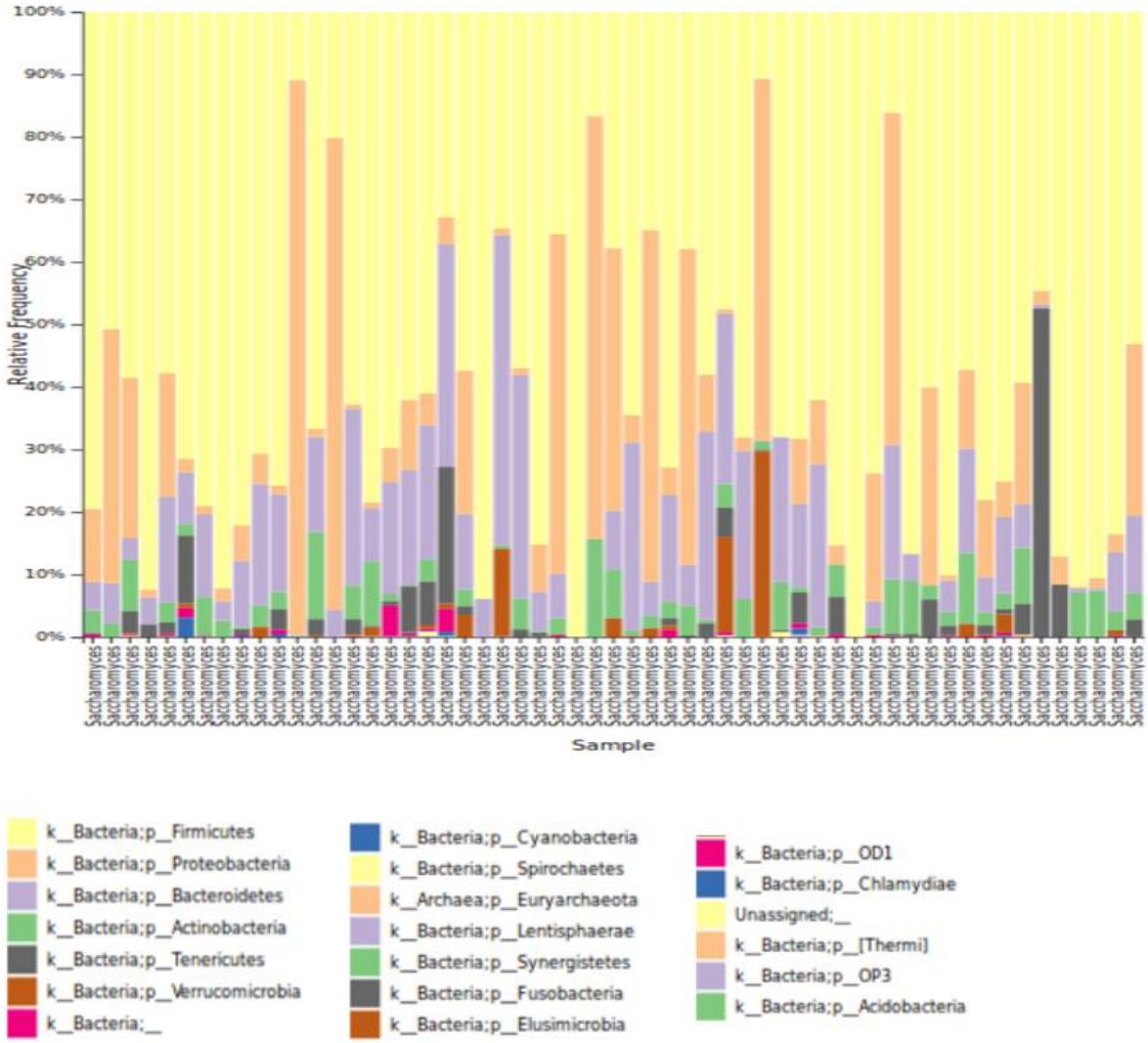


Figure 17. Taxa bar plots of taxa from the conventional treatment group at taxonomic level 2.

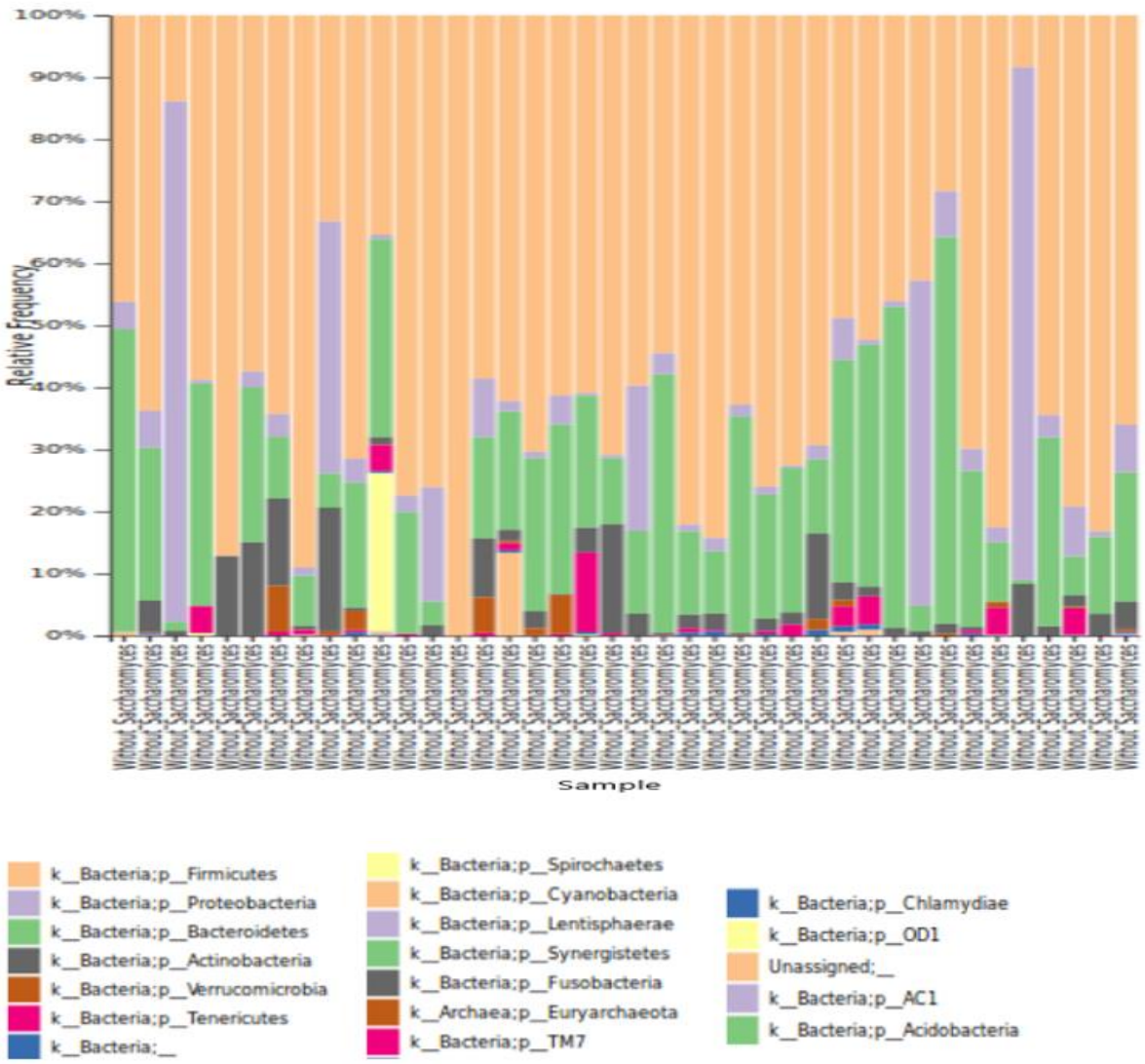
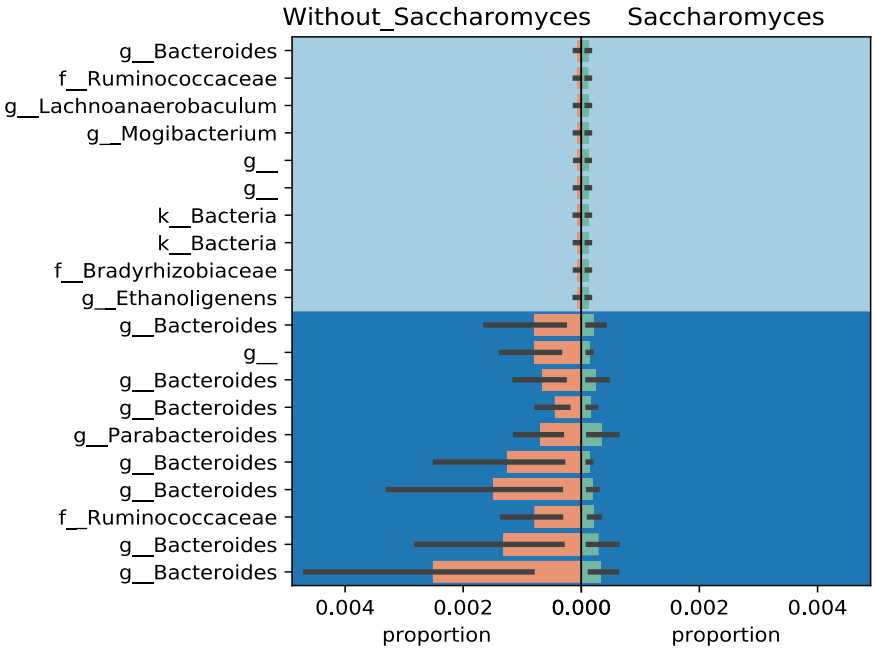
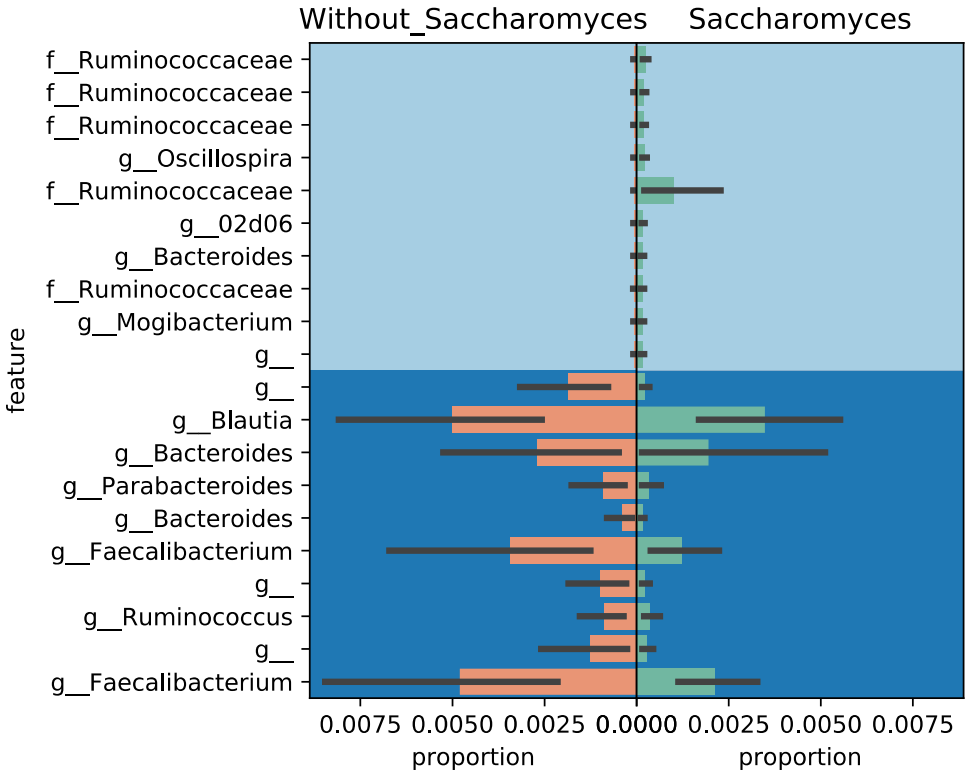


Figure 18. Gneiss proportion plot of differential abundance between conventional treatment group plus *S. boulardii* and conventional treatment group.



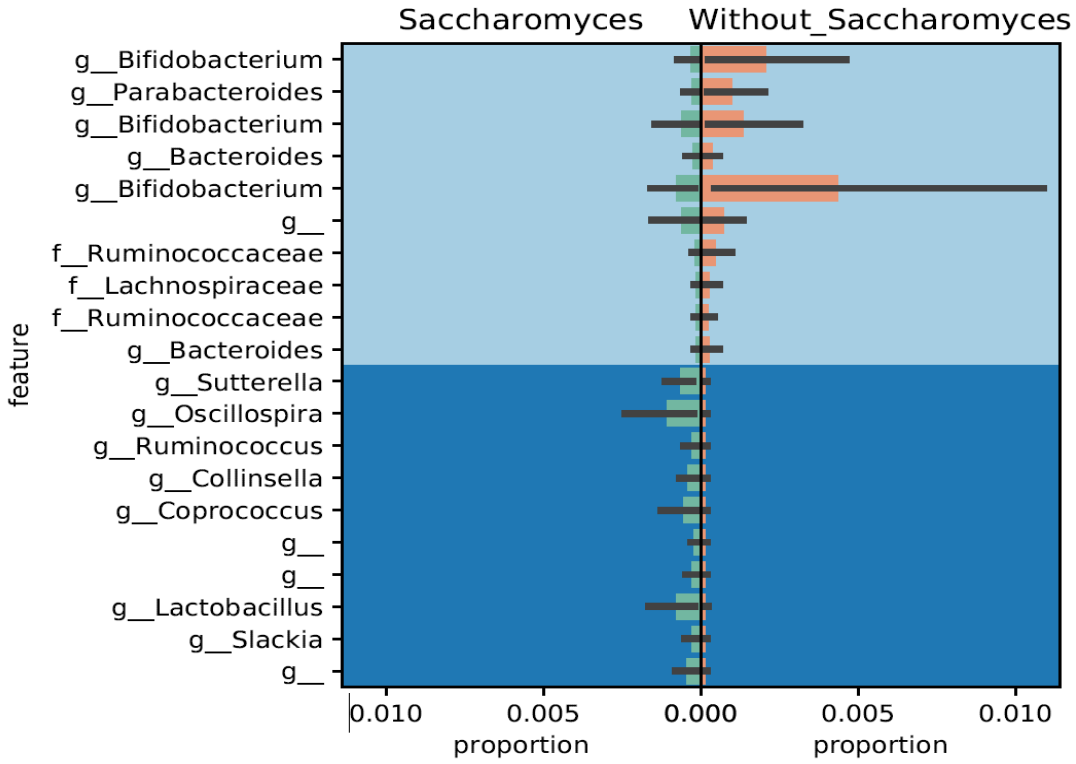
The orange bar represents the increase in relative abundance of taxa from the conventional treatment group, the green bar represents the increase in relative abundance of taxa from the conventional treatment group plus *S. boulardii*, the black line represents the margin of error.

Figure 19. Gneiss proportion plot of differential abundance between conventional treatment group plus *S. boulardii* and conventional treatment group at the beginning of the treatment (M1).



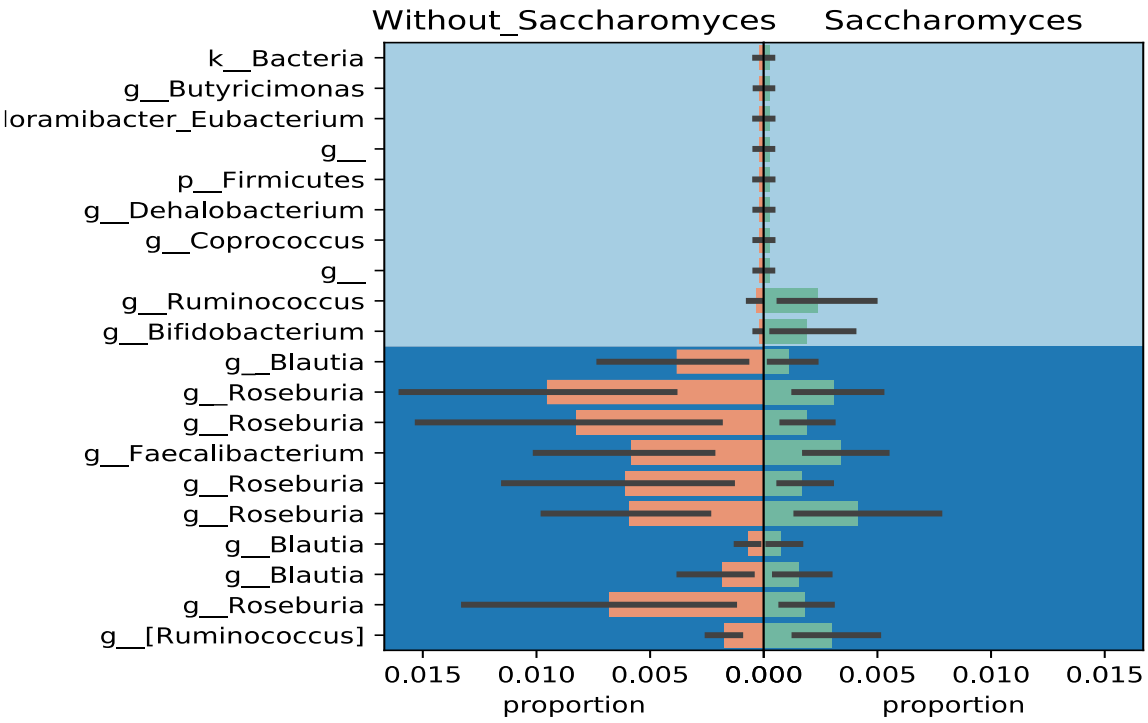
The orange bar represents the increase in relative abundance of taxa from the conventional treatment group, the green bar represents the increase in relative abundance of taxa from the conventional treatment group plus *S. boulardii*, the black line represents the margin of error.

Figure 20. Gneiss proportion plot of differential abundance between conventional treatment group plus *S. boulardii* and conventional treatment group during of the treatment (M2).



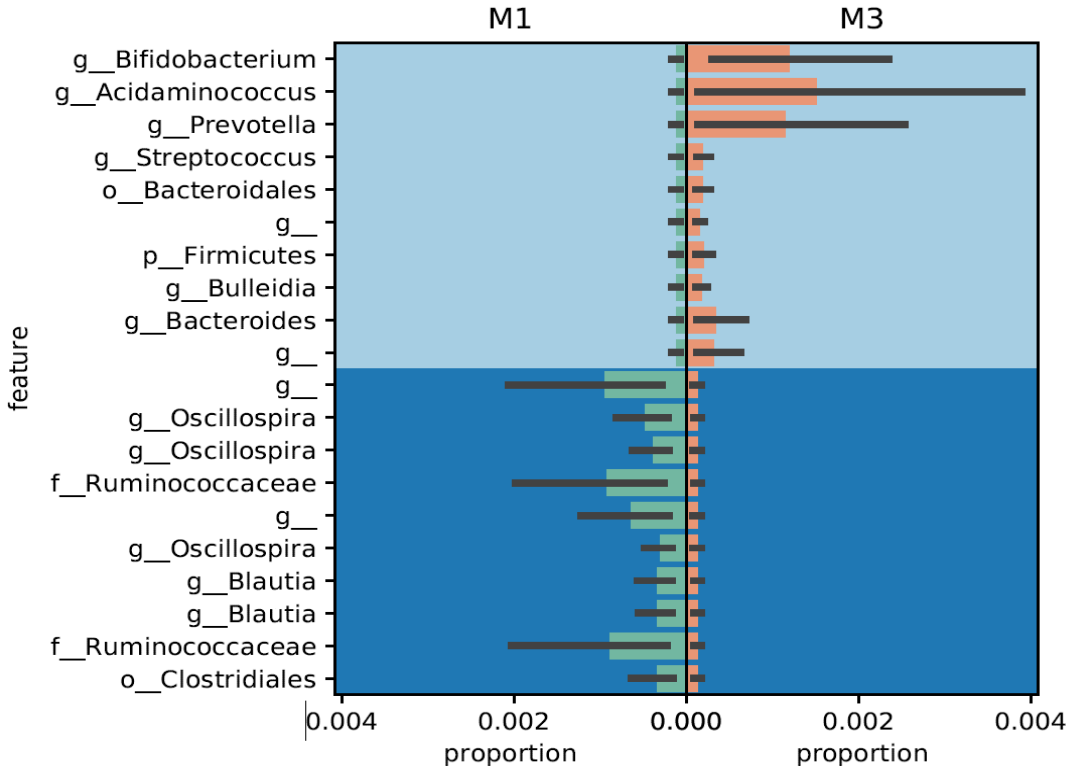
The orange bar represents the increase in relative abundance of taxa from the conventional treatment group, the green bar represents the increase in relative abundance of taxa from the conventional treatment group plus *S. boulardii*, the black line represents the margin of error.

Figure 21. Gneiss proportion plot of differential abundance between conventional treatment group plus *S. boulardii* and conventional treatment group at the end of treatment (M3).



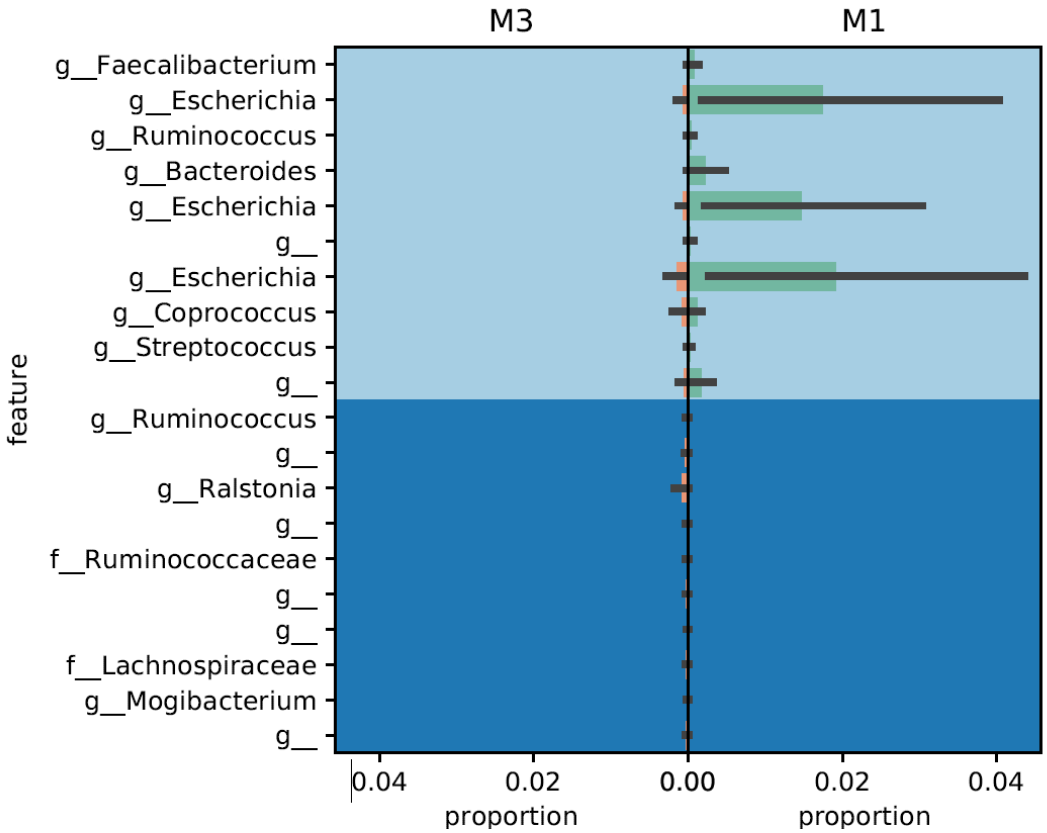
The orange bar represents the increase in relative abundance of taxa from the conventional treatment group, the green bar represents the increase in relative abundance of taxa from the conventional treatment group plus *S. boulardii*, the black line represents the margin of error.

Figure 22. Gneiss proportion plot of differential abundance between M1 (begin of treatment) and M3 (end of treatment) of conventional treatment group plus *S. boulardii*.



The orange bar represents the increase in the relative abundance of the taxa in group M1 (beginning of the treatment), the green bar represents the increase in the relative abundance of the taxa in the group M3 (final treatment samples), the black line represents the margin of error.

Figure 23. Gneiss proportion plot of differential abundance between M1 (begin of treatment) and M3 (end of treatment) of conventional treatment group.



The orange bar represents the increase in the relative abundance of the taxa in group M1 (beginning of the treatment), the green bar represents the increase in the relative abundance of the taxa in the group M3 (final treatment samples), the black line represents the margin of error.