



SECOND GENOME
THE MICROBIOME COMPANY

Microbial Profiling Report Example

DIYgenomics

Project: MyGut, MyBiotic

Participant ID: P02

Date: MM/DD/20YY

Confidential

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Summary

Project: MyGut, MyBiotic

Participant ID: P02

Objective

The objective of this study is to evaluate the impact on the gut microbiome of an over the counter (OTC) probiotic and the potential efficacy in modulating the community of microbioata in the lower intestine as measured by analysis of stool samples.

Project Description

Using Second Genome's Microbiome Signature Discovery™ platform, microbial community analysis revealing the richness and diversity of the gut microbiome will be carried out assessing the shift in the gut microbiome in healthy individuals after 4 weeks of a daily dose of an OTC probiotic, e.g. Culterelle® (Lactobacillus GG).

Stool specimens were collected within 1 week (+ / - 3 days) prior to the initial dose of probiotic and during the last week of the intervention. These data were aggregated into a personalized analysis. A report was provided to each participant. The report describes the global shift in abundance identified in each individual and in each study group as well as a personalized profile including ratios pre and post intervention of Firmicutes:Bacteroidetes, Prevotella: Bacteroidetes, Helicobacter pylori, and the most abundant 10-15 bacterial taxa at the phylogenetic family level.

Main Findings

A change in the microbial composition of your gut, as represented by the stool samples you provided, was observed during the course of this study. The following genera displayed the greatest shift in our analysis: XXXXXX. At the phylum level, a statistically significant reduction in the ratio of (example Bacteroidetes to Firmicutes) was observed. While the majority of (example: Proteobacteria) belonging to the (Enterobacteriaceae) Family were unchanged, we did observe a decrease in the levels of (Eschericia coli) after probiotic treatment.

Demographic Metadata

Short Name	Study Participants	Timepoint	Age	Gender	BMI	Stool Form 1	Stool Form 2
P01_Pre	P01	Pre	28	Male	18	-	-
P01_Post	P01	Post	28	Male	18	-	-
P02_Pre	P02	Pre	35	Male	16	-	-
P02_Post	P02	Post	35	Male	16	-	-
P03_Pre	P03	Pre	48	Male	15	-	-
P03_Post	P03	Post	48	Male	15	-	-
P04_Pre	P04	Pre	35	Female	19	-	-
P04_Post	P04	Post	35	Female	19	-	-
P05_Pre	P05	Pre	28	Female	23	-	-
P05_Post	P05	Post	28	Female	24	-	-
P06_Pre	P06	Pre	58	Male	28	-	-
P06_Post	P06	Post	58	Male	28	-	-
P07_Pre	P07	Pre	30	Male	23	-	-
P07_Post	P07	Post	30	Male	21	-	-
P08_Pre	P08	Pre	22	Female	30	-	-
P08_Post	P08	Post	22	Female	30	-	-
P09_Pre	P09	Pre	54	Male	26	-	-
P09_Post	P09	Post	54	Male	26	-	-
P10_Pre	P10	Pre	43	Female	21	-	-
P10_Post	P10	Post	43	Female	20	-	-

Notes:

1. The short names are the participant id and timepoint concatenated. These short names will be used in the figures, tables, and supporting files.
2. Timepoint refers to pre and post probiotics.
3. Stool Form 1 and Stool Form 2 will be populated with data from the forms.

Chapter 1: Microbial Community Characterization for P02

General Observations

- The top 10 families for individual P02 will be shown in Chapter 1, as well as the number of different genera that were found in each of the two samples.

Supporting Figures	High-Resolution Image Location
<i>Figure 1-1</i>	<i>./P02/genus.richness.ps (+pdf)</i>
<i>Figure 1-2</i>	<i>./P02/family.barchart.ps (+pdf)</i>
	<i>Additional richness and diversity figures can be found for every taxonomic level (i.e. phylum, class, order, etc.) in the Overview folder (i.e. ./Overview/).</i>

Supporting Data Tables	Description
<i>./P02/genus.bt1.bacteria.richness.table.txt</i>	Taxon richness at the <i>genus</i> level, using bt1.table.txt
<i>./P02/genus.bt1.archaea.richness.table.txt</i>	Taxon richness at the <i>genus</i> level, using the bt1.table.txt
<i>./P02/family.barchart.table.txt</i>	Proportions of OTUs classified at the family rank for top 10 families



Figure 1-1

Archaea and bacteria taxon richness at the genus level, using the bt1 table.

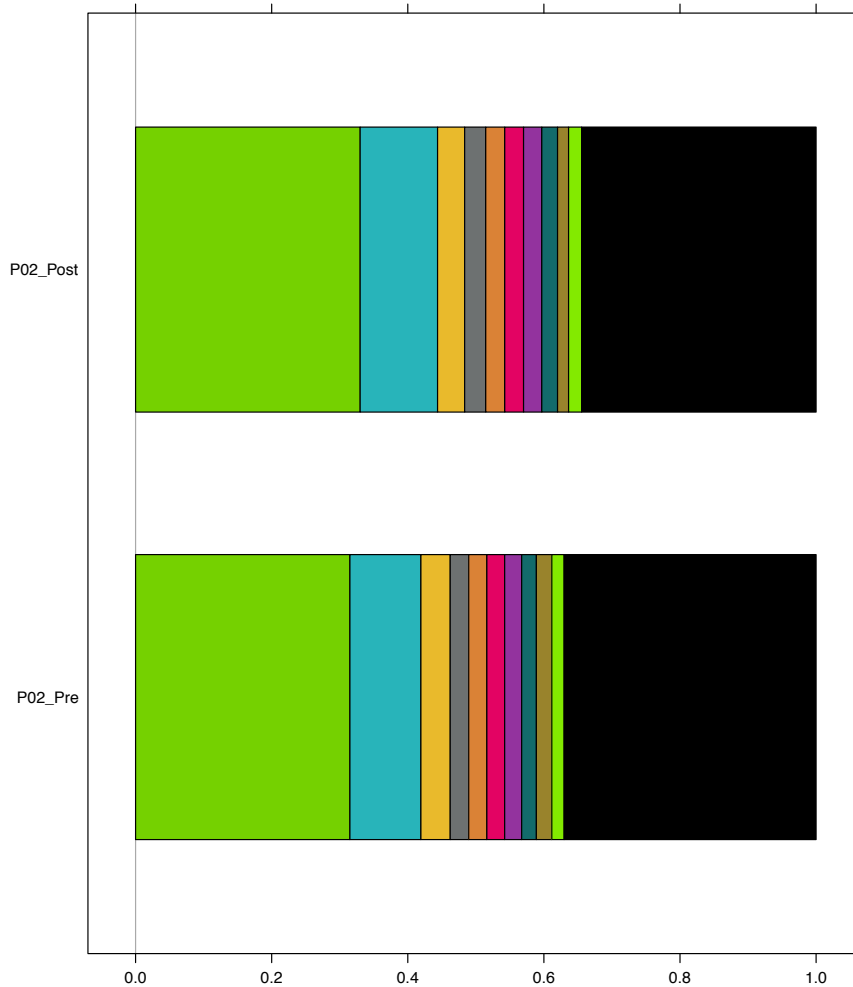


Table 1-1 Observations

1. This chart shows the number of different genera that were found in the same individual pre and post probiotics.
2. In this example, the P02_Pre sample has 457 different bacterial genera and 1 archaeal genera. Sample P02_Post has 423 different bacterial genera. The full list of these genera will be found in the supporting files.

Figure 1-2

Proportions of OTUs classified at the family rank. The bar chart considers the top 10 most rich families and an “Other” category which includes all other families (shown in black). The size of each color block in the family richness bar chart represents the number of detected OTUs in that family relative to the total number of OTUs detected in that sample. Note that the chart represents only the number of detected OTUs in a family, not the abundance of those OTUs. For example, in sample P02_Post, Lachnospiraceae OTUs accounted for 9.6% of the total sample’s OTUs.



Color	Domain	Phylum	Class	Order	Family
Green	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae
Cyan	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae
Orange	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae
Grey	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae
Pink	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae
Purple	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae
Teal	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Corynebacteriaceae
Brown	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae
Light Green	Bacteria	Firmicutes	Bacilli	Bacillales	Staphylococcaceae
Black	Bacteria	Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae
Black	Other				

Table 1-2 Observations

1. This chart shows the top 10 families in the individual’s pre and post sample. The top 10 families in this example represent approximately 63% of all of the samples’ OTUs.

Chapter 2: Pre and Post Probiotic Comparison

General Observations

- This chapter will show differences between the pre and post samples in the same individual.

Supporting Figures	High-Resolution Image Location
Figure 2-1	./P02/Top200.differences.ps (+pdf)

Supporting Data Tables	Description
./P02/top100.circulartree.pdf	List of 100 families with the greatest abundance differences between pre-probiotic and post-probiotic.

Pre and Post Differences

Table 2-1. Pre and Post Differences			
	Pre-probiotic	Post-probiotic	Fold Change
OTU Count			
Prevotella / Bacteroidetes			
Bacteroidetes / Firmicutes			
Helicobacter Pylori ¹			
...			

Notes:

1. The *Helicobacter Pylori* counts refers to the sum of all strains detected within the *Helicobacter Pylori* species.

Table 2-1 Observations
<ol style="list-style-type: none"> 1. Here we will make observations about the difference between the pre and post samples from the sample individual. 2. Among other things, we will look at Bacteroidetes/Firmicutes ratio, Prevotella/ Bacteroidetes ratio, and <i>Helicobacter pylori</i> abundance in the baseline pre sample compared to the post.

Figure 2-1

Circular tree comparing pre-probiotic sample and post-probiotic samples (outer ring). From the over 30,000 taxa present in P02, 289 taxa (within 100 families) were selected as having the largest count difference between pre and post probiotic. 64 families showed an increase in count post-probiotic compared to pre-probiotic, while 36 showed a decrease in the post-probiotic sample. Dark blue indicates a 1000 count increase and dark red 1000 count decrease.



Figure 2-1 Observations

1. In Acidobacteria, Chloroflexi, and Planctomycetes, greater counts were observed in the post-probiotic sample.



Table 2-2. Annotations of Taxa with Most Increase in Post-probiotic					
Taxa ID	Phylum	Class	Order	Family	Post-probiotic count - pre-probiotic count
75097	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Chitinophagaceae	1000
57117	Planctomycetes	Planctomycea	Planctomycetales	Planctomycetaceae	9987
40426	Actinobacteria	Actinobacteria	Actinomycetales	Dermacoccaceae	9832
53658	Cyanobacteria	Nostocophycideae	Nostocales	Rivulariaceae	9821
1140	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Saccharospirillaceae	9784
66581	Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	9752
51673	Acidobacteria	Solibacteres	Solibacterales	Solibacteraceae	9732
...					

Table 2-3. Annotations of Taxa with Most Decrease in Post-probiotic					
Taxa ID	Phylum	Class	Order	Family	Post-probiotic count - pre-probiotic count
65953	Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	-1000
59075	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	-9887
45792	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	-9632
59313	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	-9621
...					

Chapter 3: Results Comparison of the Shift in the Gut Microbiome Pre and Post Probiotic

General Observations

- This chapter will show the whole microbiome analysis for all of the participants.

Supporting Figures	High-Resolution Image Location
Figure 3-1	<i>./Group/genus.richness.ps (+pdf)</i>
Figure 3-2	<i>./Group/family.barchart.ps (+pdf)</i>
	<i>Additional richness and diversity figures can be found for every taxonomic level (i.e. phylum, class, order, etc.) in the Overview folder (i.e. ./Group/).</i>
Figure 3-3	<i>./Group/at1.wunifrac.PCoA.ps (+pdf)</i>
Figure 3-4	<i>./Group/at1.wunifrac.HCAN.ps (+pdf)</i>
Figure 3-5	<i>./Group/bt1.unifrac.PCoA.ps (+pdf)</i>
Figure 3-6	<i>./Group/bt1.unifrac.HCAN.ps (+pdf)</i>

Supporting Data Tables	Description
<i>./Group/genus.bt1.bacteria.richness.table.txt</i>	Taxon richness at the <i>genus</i> level, using bt1.table.txt
<i>./Group/genus.bt1.archaea.richness.table.txt</i>	Taxon richness at the <i>genus</i> level, using the bt1.table.txt
<i>./Group/family.barchart.table.txt</i>	Proportions of OTUs classified at the family rank for top 9 families
<i>./Group/at1.table.txt</i>	Abundance metrics of taxa present in at least one of the samples, includes OTU taxonomic classification.
<i>./Group/at1.wunifrac.table.txt</i>	Weighted Unifrac distance matrix based on at1.table.txt
<i>./Group/bt1.table.txt</i>	Presence-Absence (binary metrics) of taxa present in at least one sample, includes OTU taxonomic classification
<i>./Group/bt1.unifrac.table.txt</i>	Unifrac distance matrix based on bt1.table.txt

Adonis Testing

Table 3-1. Adonis tests of significance (* indicates statistical significance reached)				
	Bins	Sample Counts	Abundance	P/A
Timepoint	Pre Post	10 10	0.002*	0.002*
Participant ID	P01-P10	2 per participant	0.566	0.364
Age	continuous	20	0.029*	0.021*
BMI	continuous	20	0.043*	0.126
...				

Table 3-1 Observations
1. Here we will make observations concerning which factors were found to be significantly correlated with the microbiome and provide the p-values.

Figure 3-1

Archaea and bacteria taxon richness at the genus level, using the bt1 table.

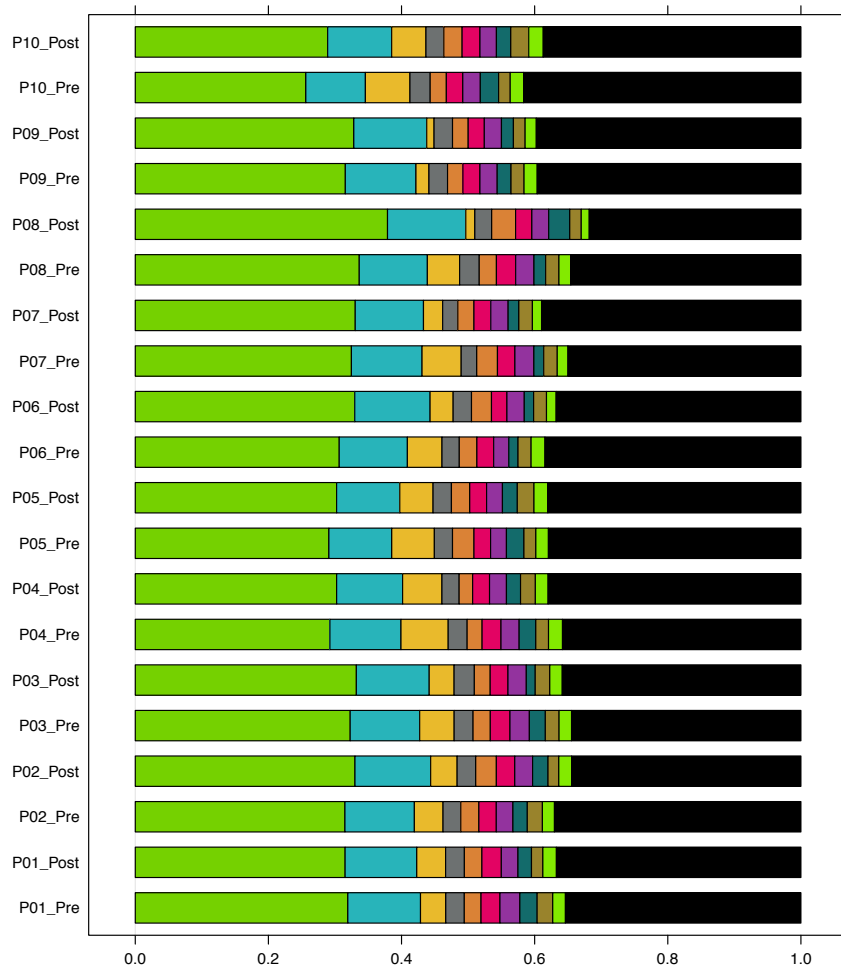


Figure 3-1 Observations

1. Here we will show the number of different genera in each of the samples.

Figure 3-2

Proportions of OTUs classified at the family rank. The bar chart considers the top 10 most rich families and an “Other” category which includes all other families (shown in black). The size of each color block in the family richness bar chart represents the number of detected OTUs in that family relative to the total number of OTUs detected in that sample. Note that the chart represents only the number of detected OTUs in a family, not the abundance of those OTUs. For example, in sample P05_post, Lachnospiraceae OTUs accounted for 9.6% of the total sample’s OTUs.



Color	Domain	Phylum	Class	Order	Family
Green	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae
Cyan	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae
Orange	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae
Grey	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae
Red	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae
Purple	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae
Teal	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Corynebacteriaceae
Brown	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae
Yellow-Green	Bacteria	Firmicutes	Bacilli	Bacillales	Staphylococcaceae
Black	Bacteria	Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae
Black	Other				

Figure 3-2 Observations

1. Here we will show the top 10 families in each of the samples.

Figure 3-3

PCoA based on Weighted Unifrac distance between samples given abundance of 37328 taxa present in at least one sample. Axis 1: 62% of variation explained. Axis 2: 11% of variation explained.

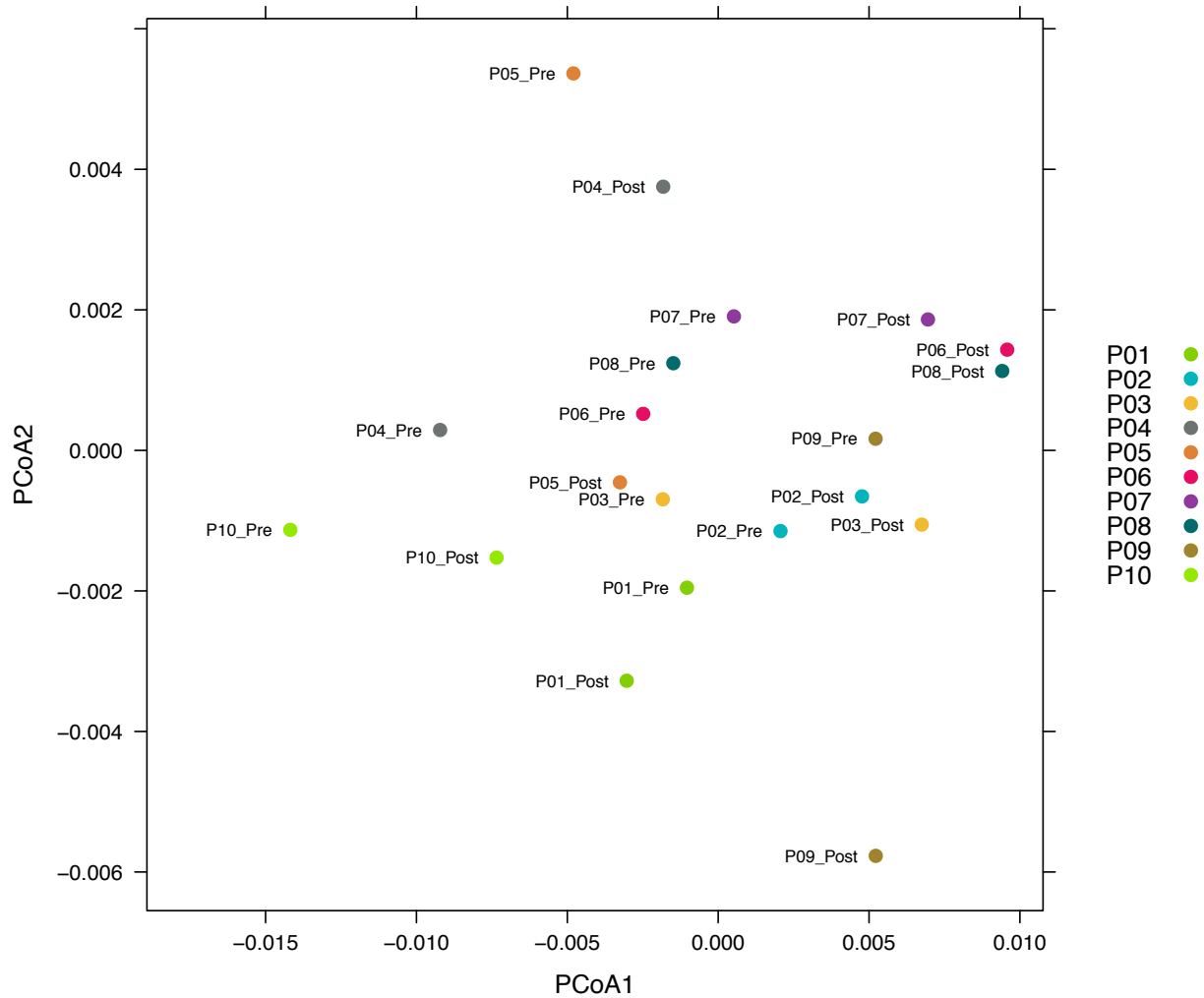


Figure 3-3 Observations

1. This ordination is based on the abundance levels for all of the OTUs found in each participant.
2. This figure is a 2D representation of the microbial community dissimilarity between samples. The distances between samples indicates the amount of similarity or difference of their microbial community composition..

Figure 3-4

Hierarchical Clustering (average linkage) based on Weighted Unifrac distance between samples given abundance of 37328 taxa present in at least one sample.

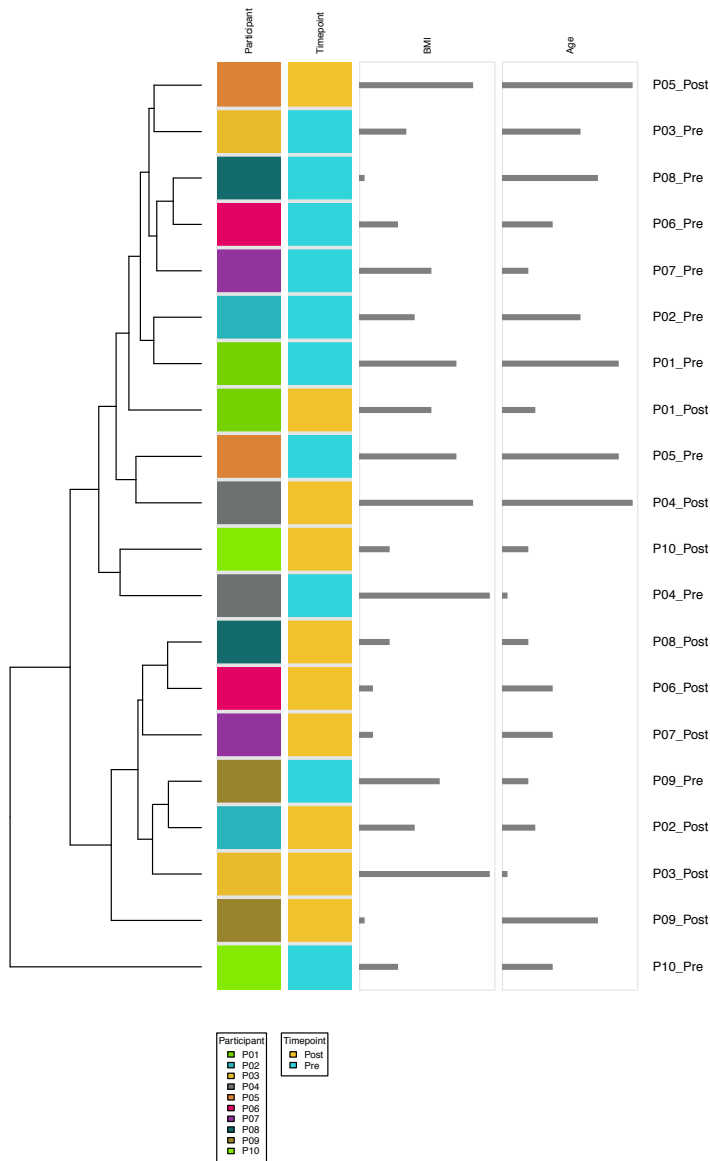


Figure 3-4 Observations

1. This figure shows the same information as in Figure 3-3, but in a dendrogram form. BMI and age are also displayed on the right.
2. We will make observations such as: all high BMI participants clustered together.

Figure 3-5

PCoA based on Unweighted Unifrac distance between samples given presence/absence of 37328 taxa present in at least one sample. Axis 1: 56% of variation explained. Axis 2: 21% of variation explained.

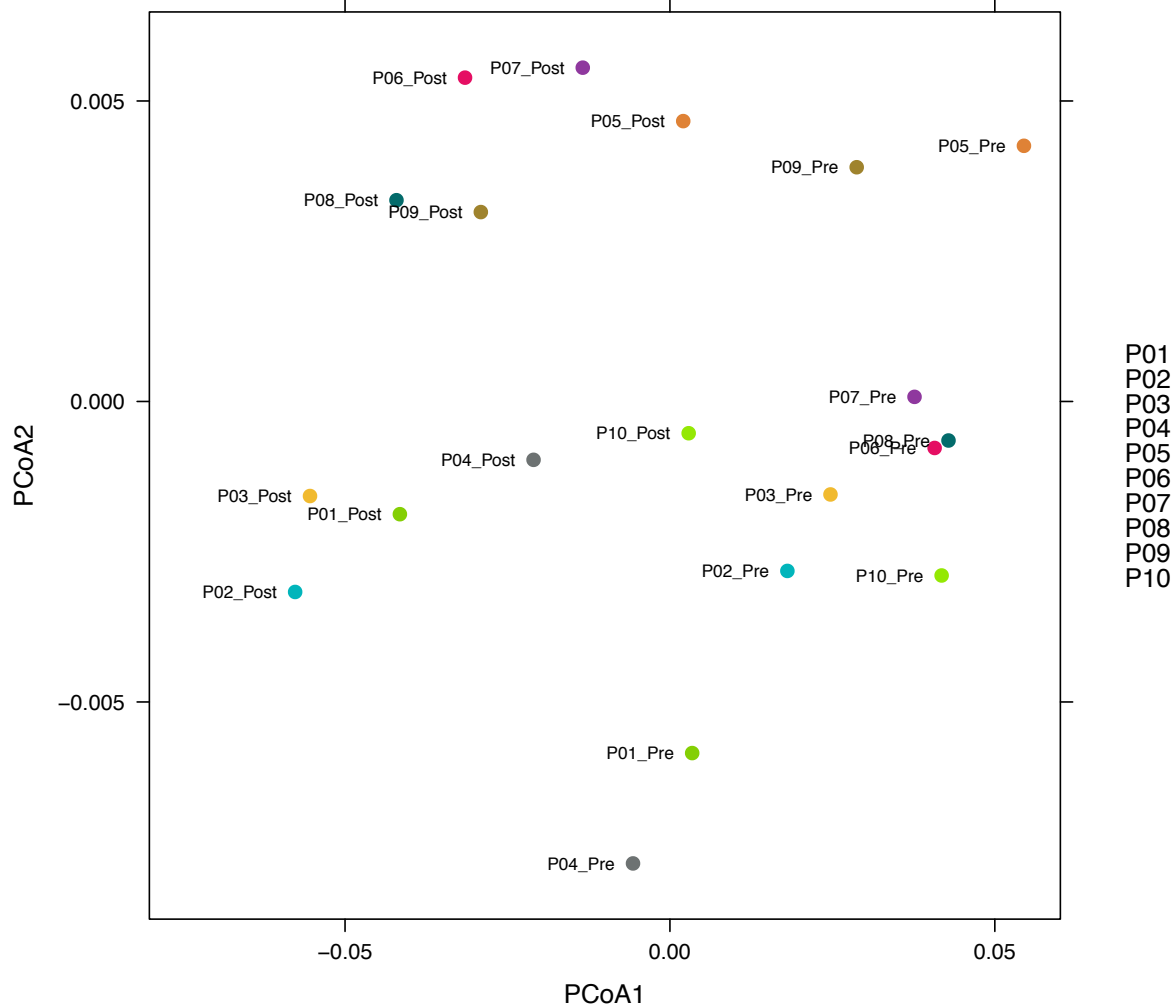


Figure 3-5 Observations

1. This ordination is based on the binary (presence or absence) data for the OTUs found in all samples.
2. Each sample is colored according to participant id number. Each point is labeled with a short name corresponding to participant number and the timepoint (pre or post probiotics).

Figure 3-6

Hierarchical Clustering (average linkage) based on Unweighted Unifrac distance between samples given presence/absence of 37328 taxa present in at least one sample.

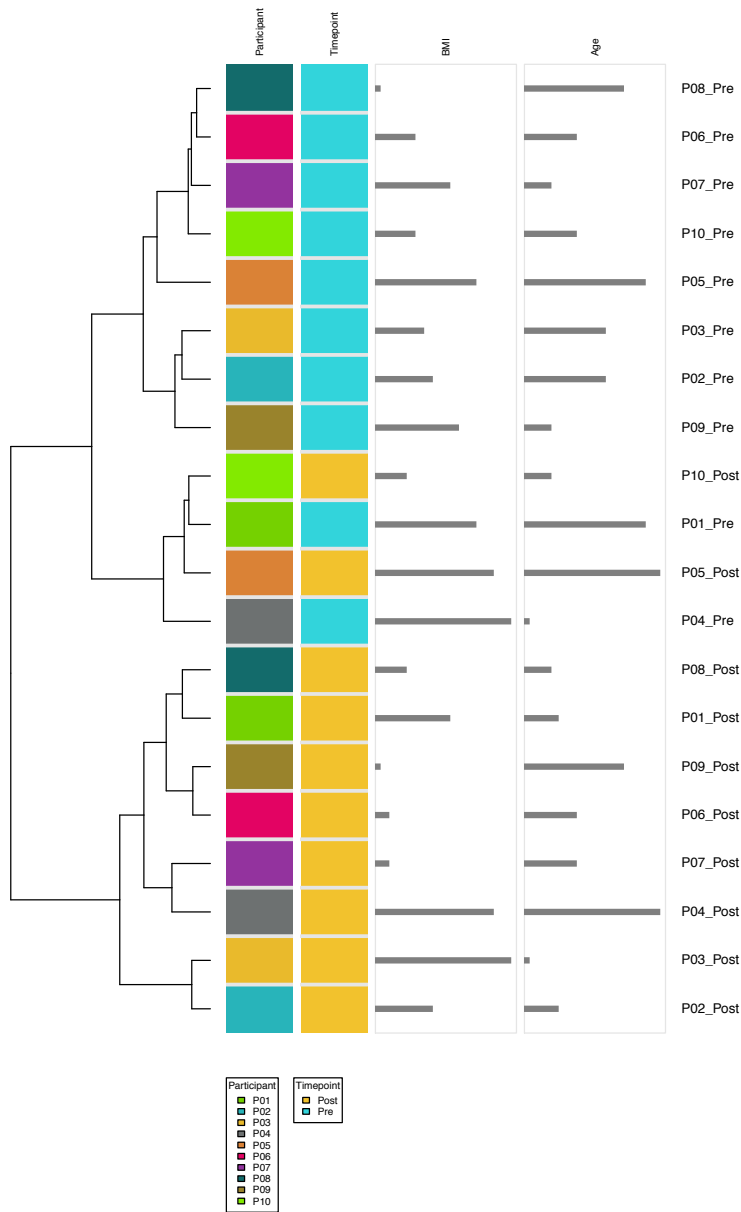


Figure 3-6 Observations

1. This figure shows the same information as in Figure 3-5, but in a dendrogram form.

Appendix:

Materials and Methods

Pre Analytic: Sample collection, shipping, storage, DNA isolation

Analytic: sample preparation, assay, quality control

Post Analytic: Bioinformatics

Appendix: References

Predictive Analysis for Microarrays (PAM)

Diagnosis of multiple cancer types by shrunken centroids of gene expression. Tibshirani, R., et al., PNAS 2002 99: 6567-6572.

Unifrac and Weighted Unifrac

UniFrac - An Online Tool for Comparing Microbial Community Diversity in a Phylogenetic Context. Lozupone, et al., BMC Bioinformatics 2006, 7: 371.

Prevotella Bacteroidetes Ratio

An obesity-associated gut microbiome with increased capacity for energy harvest. Turnbaugh, P., et al., Nature 444, 1027-1031 (21 December 2006).

Bacteroidetes Firmicutes Ratio

Microbial ecology: human gut microbes associated with obesity. Ley R.E., Turnbaugh P., Klein S., Gordon J.I., Nature 2006, 444:1022-1023.

The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age. Mariat, D., et al., BMC Microbiology 2009, 9:123.

Helicobacter Pylori

Helicobacter pylori persistence: biology and disease. Blaser M.J. and Atherton J.C., J Clin Invest 2004 113: 321-333.

Who are we? Indigenous microbes and the ecology of human diseases. Blaser, M. J., EMBO Reports 2006, 7 (10): 956-60.

Clostridium Difficile

Changes in predominant bacterial populations in human faeces with age and with Clostridium difficile infection. Hopkins M.J. and Macfarlane G.T., J Med Microbiol 2002, 51:448-454.

Human Microbial Ecology

Microbial ecology of the gastrointestinal tract. Savage D.C., Annu Rev Microbiol 1977, 31:107-133.

Molecular ecological analysis of the gastrointestinal microbiota: a review. Zoetendal E.G., et al., J Nutr 2004, 134:465-472.

Diversity of the human intestinal microbial flora. Eckburg P.B., et al., Science 2005, 308:1635-1638.