The abundance and taxonomy of microbes in our meals for three diet types

Qianwen Luo

Abstract

This tutorial was based off of the paper The microbes we eat: abundance and taxonomy of microbes consumed in a day's worth of meals for three diet types. The article provides those interested in bioinformatics, food science, or microbiology information about how to test the abundance and taxonomy of microbes in our daily meals. The step by step procedures covers how the author run this experiment.

Source:
Lang JM, Eisen JA, Zivkovic AM. (2014) The microbes we eat: abundance and taxonomy of microbes consumed in a day's worth of meals for three diet types. PeerJ 2:e659. https://peerj.com/articles/659/

Citation: Qianwen Luo The abundance and taxonomy of microbes in our meals for three diet types. **protocols.io** dx.doi.org/10.17504/protocols.io.etpbemn
Published: 04 Apr 2016

Before start

Make sure you have QIIME installed.

Protocol

Step 1.

Meal (three diet types) preparation
All food was purchased and preparaed in standard American home kitchen and wash the dishes and cooking instruments with non-antibacterial dish washing detergent. Washed hands with non-antibacterial hand soap during meal preparation. All meals were prepared based on specfic recipes.

Three diet types of meal:
1: the Average American (AMERICAN): focused on convenience foods;
2: USDA recommended (USDA): enphasizing fruits and vegetables, lean meat, dairy, and whole grains
3: Vegan (VEGAN): excluding all animal products.

Step 2.

Blended food
After food preparation, weighted the food on a digital scale and transferred to a blender, and needed around 13 min to blended. Collected 4 ml aliquots of the blended meal composite and label it as AMERICAN, USDA, and VEGAN. Stored the samples in -80 °C until analysis.

AMOUNT
4 ml Additional info:

Step 3.

Diet Design

The target calories were 2,200 which was according to the average number of calories consumed by an average American per day. The AMERICAN meal plan had 2,268 calories, USDA meal plan had 2,260 calories, and VEGAN meal plan had 2,264 calories.

LINK:
http://www.cdc.gov/nchs/data/nhanes/databriefs/adultweight.pdf

NOTES
Stanton Burnton 04 Apr 2016
The information of average American is from National Health and Nutrition Examination Survey
The daily calorie intake per day respect to the weight was determined by USDA My Plate SuperTracker tool

Step 4.

Microbial community analysis
Counts the aerobic, anaerobia, yeast and mold plate for the three types of meal and table the results. For the "Meal" column, has three choices: breakfast, lunch, and dinner. The counting unit of plate is colony forming units (CFU) per gram.

Dietary pattern	Meal	Aerobic plate count	Anaerobic plate count	Yeast count	Mold count	Total count
AMERICAN						
USDA						
VEGAN						

NOTES
Stanton Burnton 04 Apr 2016
Microbial plate counts were performed by Covance Laboratories(Convance INC., Madison, WI)
Aerobic plate counts were performed by SPCM:7.
Anaerobic plate counts were performed by APCM:5.
The Yeast and mold counts were performed by Chapter 23 of the FDA's Bacteriological Analytical Manual.

Step 5.

Found the taxonomic composition of the microbes in three types of meals.

The Power Food Microbial DNA Isolation Kit was used to found taxonomic composition by amplification and sequencing of 16S rDNA (checked the script link and installed QIIME). When using the QIIME pipeline, make sure the format for the analyzed sequences are in fasta format.

The primer sequences and detailed PCR protocols are found in:
https://github.com/hollybik/protocols/blob/master/16S_rRNA_twostep_PCR.tex

The script:
https://github.com/gjospin/scripts/blob/master/Demul_trim_prep.pl

Step 6.

Statistical analyses

Using python scripts implemented in QIIME, script can be found in
http://nbviewer.ipython.org/gist/jennomics/c6fe5e113525c6aa8add

Analyze the difference in overall microbial community composition in different type of meals.
(beta diversity through plots.py script)

Using ANOVA as implemented to test the significance of overall microbial composition of three types of meal.
(compare_categories.py.script)

Using non-parameteric Kruskal-Wallis to test the significant differences in taxonomic composition.
(script in compare_alpha_diversity.py)

The group_significance.py.script used to test the variation in frequency of individual OTUs.

Step 7.

Visible the results
Using biplot function to plot the family-level OTUs in PCoA space alongside each meal.
(script: make_emperor.py)

LINK:
http://nbviewer.ipython.org/gist/jennomics/c6fe5e113525c6aa8add

Step 8.

Step 8: Make metagenome prediction with PICRUSt
1.Clustered the 16S rDNA sequences into a collection of OTUs
(pick_closed_reference_otus.py script)

2.Normalized the 16S rRNA gene copy numbers
(normalize_by_copy_number.py script)

3.Normalized the OUT table
(predict_metagenomes.py script)

4.Used STAMP to test the significant functional differences in three types of meal, the output is .biom table.