Spring 2021

Effects on Bacterial Composition from Long-term Storage of Fecal Matter for Fecal Matter Transplant

Sabir Mali
Elizabethtown College, malis@etown.edu

Debra Wohl Ph.D.
Elizabethtown College, wohld@etown.edu

Recommended Citation

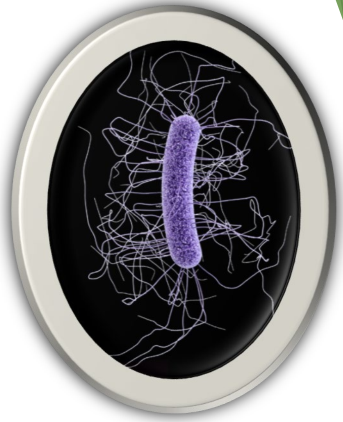
Mali, Sabir and Wohl, Debra Ph.D., "Effects on Bacterial Composition from Long-term Storage of Fecal Matter for Fecal Matter Transplant" (2021). *Biology: Student Scholarship & Creative Works*. 14.
https://jayscholar.etown.edu/biostu/14

Effects on Bacterial Composition from Long-term Storage of Fecal Matter for Fecal Matter Transplant

Sabir Mali, Debra Wohl Ph.D.

Elizabethtown College

Clostridium difficile (C. diff)



- Half a million infections in the United States each year, with an estimated 15,000 deaths.
- Symptoms include:
  - Severe diarrhea
  - Fever
  - Inflammation of the colon
- Risk factors:
  - According to CDC, 80 percent of the deaths associated with C. difficile occurred among Americans aged 65 years or older.
- In most cases *C. diff* infections occur when taking antibiotics for an extended period.
  - Lead to gastrointestinal dysbiosis, enabling opportunistic bacteria such as *Clostridium difficile* to cause infection.

What is Fecal Matter Transplant (FMT)?

- Transplanting stool from a healthy donor into the gastrointestinal tract of a patient.
  - To restore balance of bacteria in their gut.
- Also known as Bacteriotherapy.
- In 30 percent of treated individuals, the infection returns within a few days or weeks after finishing the antibiotic course.

Stool Banks

- For easy access to stool samples, many stool banks such OpenBiome have emerged to provide stool samples.
- Stool banks safely collect, store, and distribute stool product for treatment of *Clostridium difficile* infections.
- Stored donor stool samples are mixed with a glycerol buffer and stored at -80 °C, for a maximum of two years.
- There lack studies examining the bacterial composition, for such long-term storage, for fecal matter.

This Study

- This study aimed to compare the effects of freezing time on the composition of bacteria.
  - Find out if long-term freezing impacts bacterial composition.
  - Done using fecal matter obtained from dogs (*Canis lupus familiaris*).
- Null Hypothesis:
  - If all the samples were to be freezed at -80°C, whether for long-term or short-term, then the bacterial composition will not change, as all the samples will look similar to the original sample (t=0).

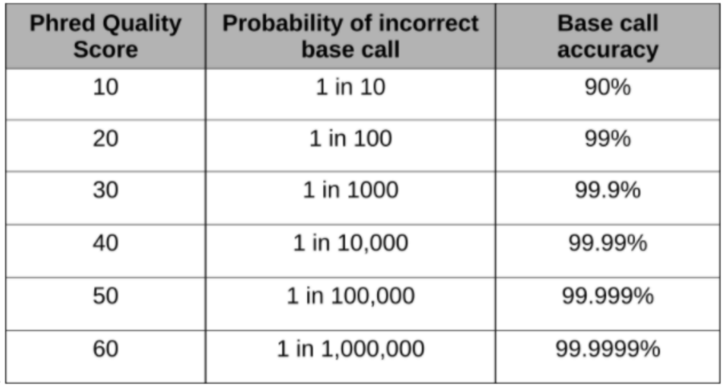
Alternative Hypothesis

1. If freezing or/and thawing impacts fecal matter composition, bacteria composition will appear similar for times 1 months, 1 year, and 2 years but will be different from the original composition (t=0).
2. If long-term storage in a freezer impacts bacterial composition then the diversity among the sample will vary, where the diversity in sample t=0 will be different from the other samples.

Procedure

- DNA was extracted from fecal sample stored for 2 years.
- Extracted DNA samples from the following times were obtained.
  - t=0
  - 1 month
  - 1 year
- The V4 of 16S region of rRNA was amplified using PCR and sequenced, for all samples.
- Data were analyzed for changes in microbial composition across time using Qiime (Quantitative Insights Into Microbial Ecology).

Data: Phred Score



Phred Quality Score	Probability of incorrect base call	Base call accuracy
10	1 in 10	90%
20	1 in 100	99%
30	1 in 1000	99.9%
40	1 in 10,000	99.99%
50	1 in 100,000	99.999%
60	1 in 1,000,000	99.9999%

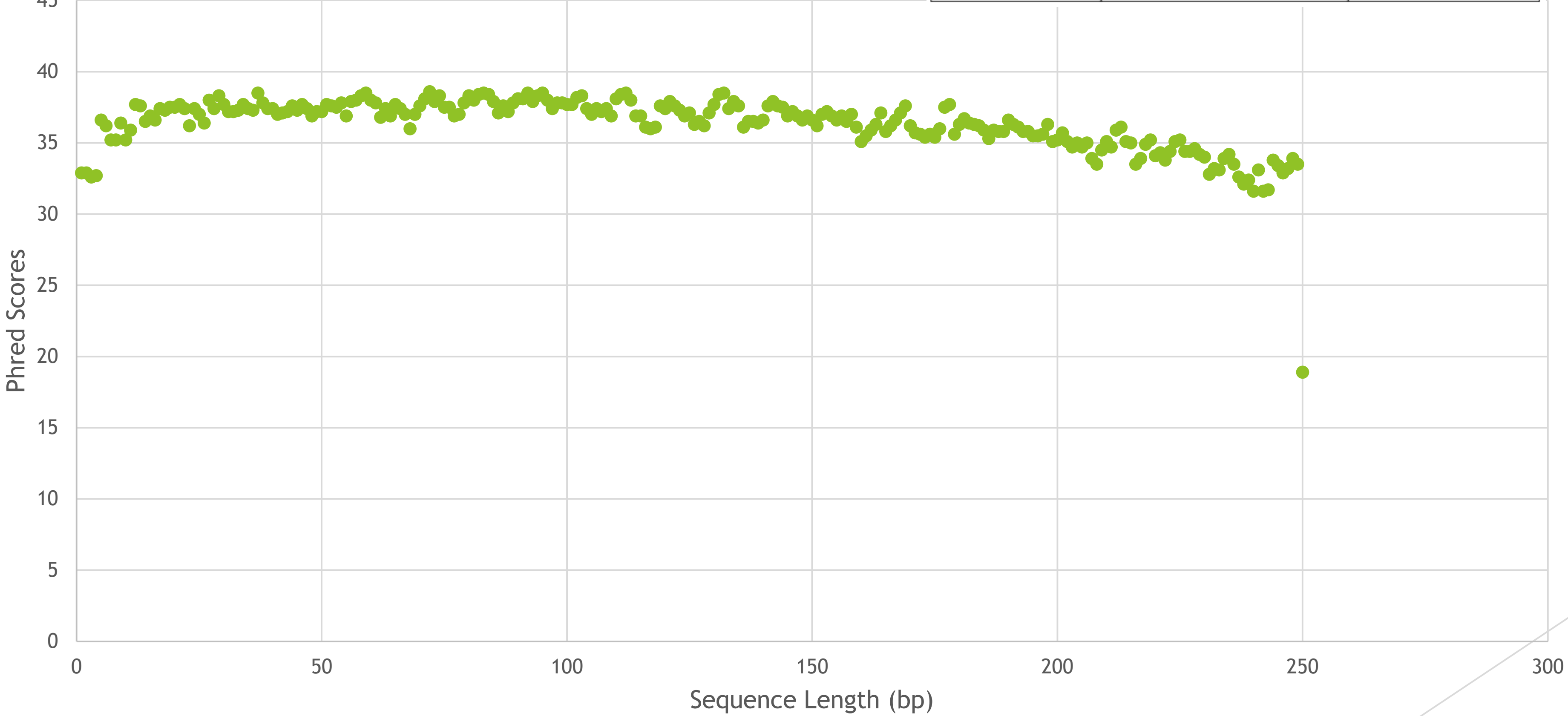


Figure 1. Phred Score for each base pair/nucleotide for 250 base pairs for all sequences.

Data: Number of Sequences

Number of Sequences Per Sample	
Sample Time	**Number of Sequences**
T=0	65,327
1 Month	85,757
1 Year	104,831
2 Year	86,701

Table 1. Number of sequences after 249 base pair trim, for each samples (t=0, 1 month, 1 year, and 2 year).

Data: Sequence versus Observed Species

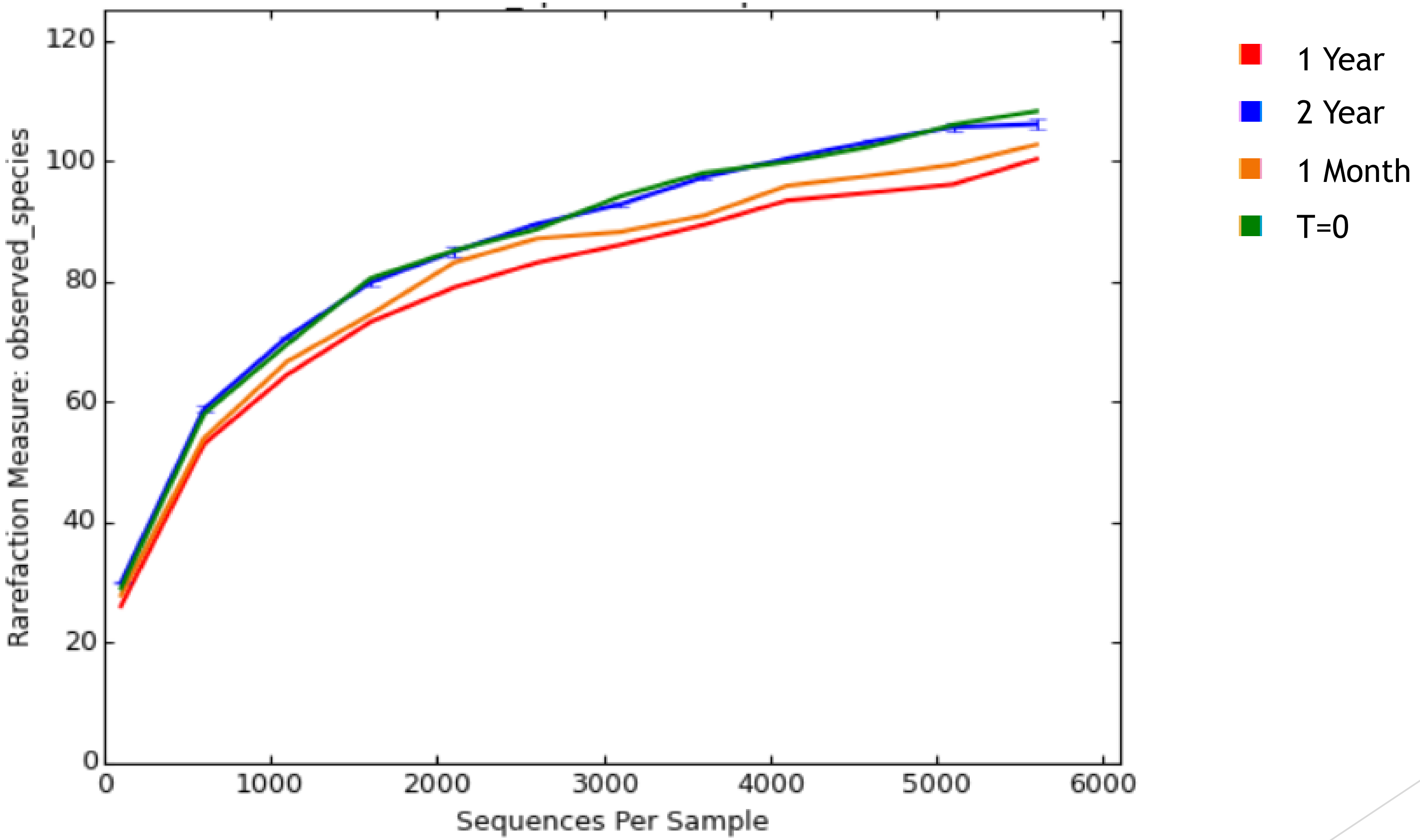


Figure 2. Observed species as sequences per sample increase for samples t=0, 1 month, 1 month, and 2 years.

Results: Rank (Phylum)

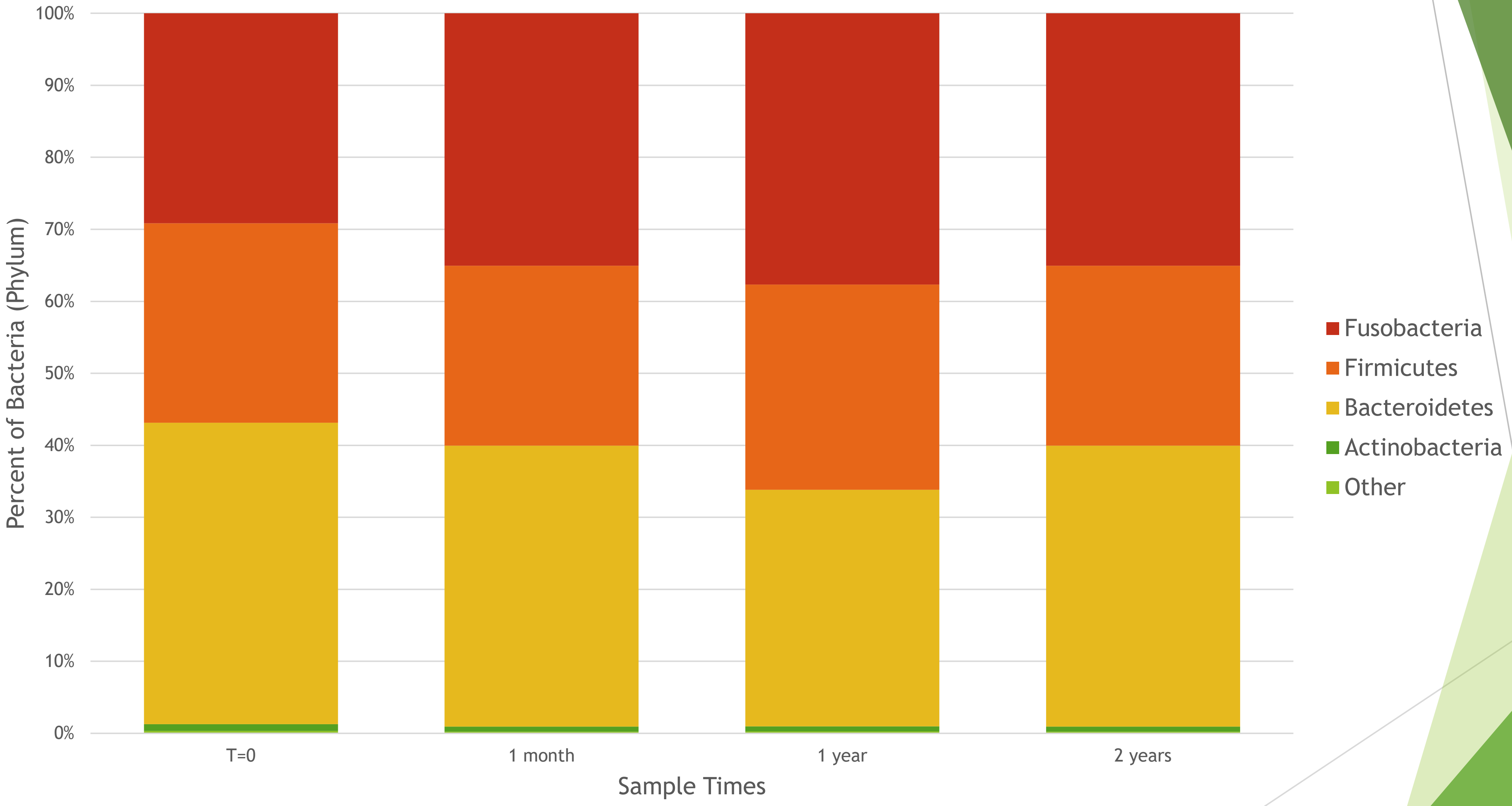
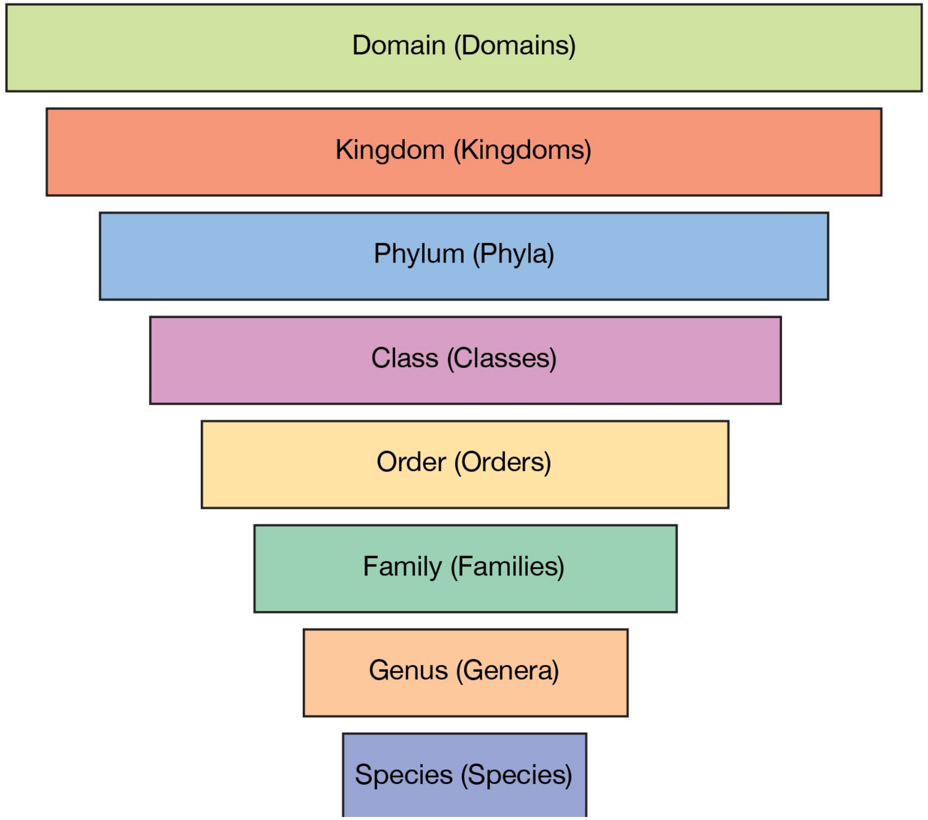


Figure 3. Percent of Bacteria in the Phylum bacteria Taxonomy for each samples.

Bacterial Taxonomy



Domain (Domains)

Kingdom (Kingdoms)

Phylum (Phyla)

Class (Classes)

Order (Orders)

Family (Families)

Genus (Genera)

Species (Species)

Figure 4. Chart showcasing different taxonomic ranks for classifying an organism; in this case bacteria.

Rank (Family)

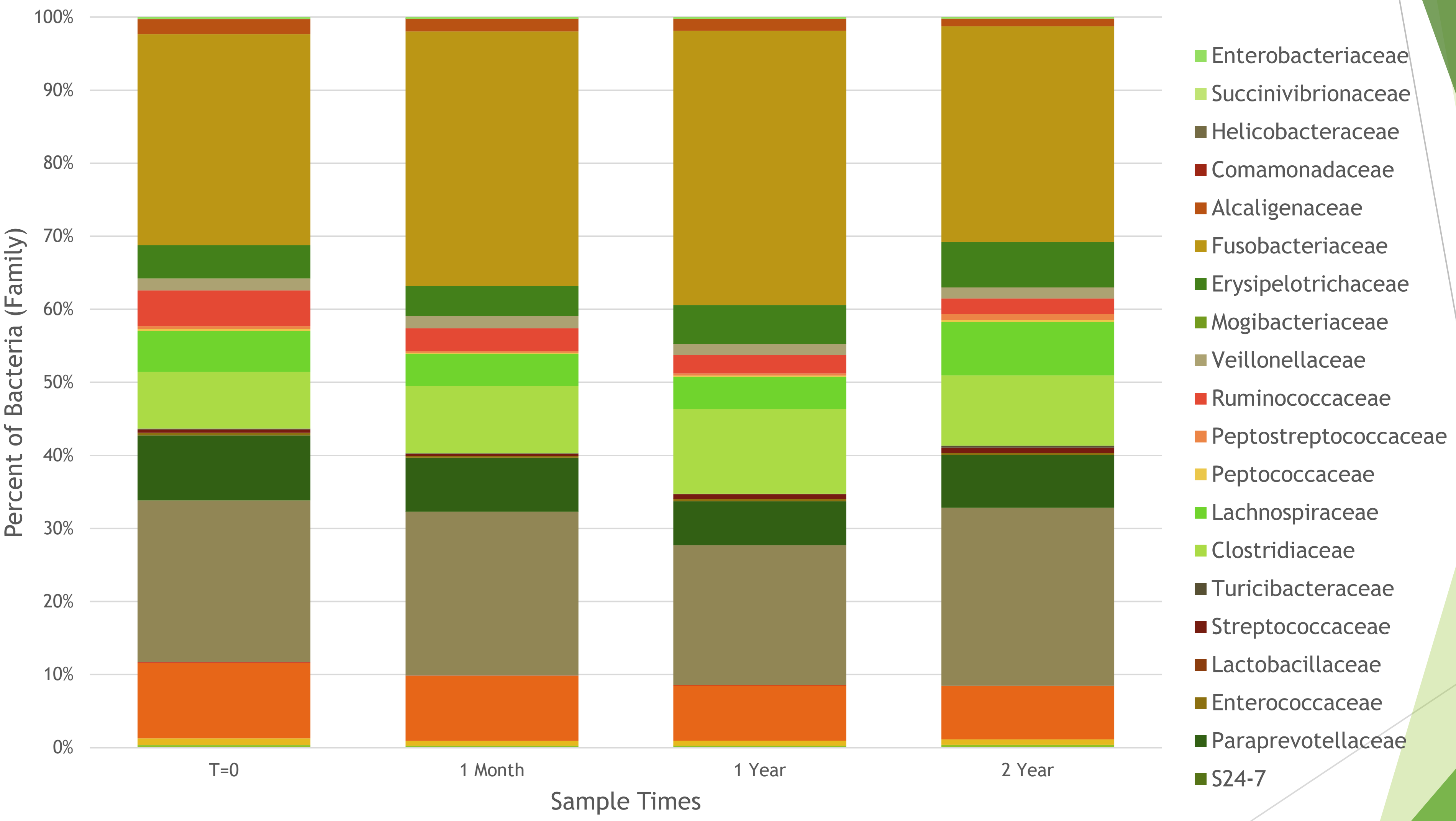


Figure 5. Percent of bacteria in the family taxonomy for each samples.

Discussion

- The Phred score for the number of nucleotides had a score of 35 and above.
  - This suggests the identification of the nucleobases generated by automated DNA sequencing was correct 99.99% of the time.
- For the number of sequencing per sample, times 1 year had a relatively high number of sequences, where t=0 was low.
  - The extracted sample size varied due to different people extracting the DNA with different amounts fecal matter samples.
- From the data, there were no significant changes to the bacterial composition after the following period of storage.

Discussion

- Three primary bacteria present in the Phylum level were the *Bacteroidetes*, *Fusobacteria*, and *Firmicutes*.
- According to Abdallah et al., these bacteria are found in abundant amounts in gut.
  - *Bacteroidetes* help digest carbohydrates via a series of metabolic pathways. Also, possess genes that encode enzyme to break down starch.
  - *Firmicutes* are well known to produce butyrate which is a fatty acid that supports both immune regulation as well as proper functioning of our intestinal lining.
  - *Fusobacteria* activate host inflammatory responses designed to protect against pathogens.

Discussion

- At the family rank, *Fusobacteriaceae*, *Prevotellaceae*, and *Clostridiales* were found to be the most abundant.
  - *Fusobacteriaceae* produce organic acids to which are important for metabolism.
  - *Prevotellaceae* play an important role in human microbiota as they help breakdown protein and carbohydrate from food.
  - *Clostridiales* biosynthesis and release of serotonin.
- The observed species over sequences per sample graph showed a positive relationship.
  - The 2 year sample versus the t=0 sample were almost identical.

Conclusion

- From the results, we failed to reject the null hypothesis.
  - There was little change from freezing to the bacteria composition, relative percent of the bacteria, and the diversity from freezing, among all samples.
- Therefore, the data from the study showed promising results for fecal matter transplant, if one wishes to use stool bank donor samples stored for up to 2 years.

Future Studies

- Performing a similar study but using human fecal matter should yield more accurate representation of the human flora.

- A study looking at the maximum period of bacterial storage until the bacteria composition is affected.

Acknowledgments

- Dr. Bridge's dogs: Bart and Ernestine
- Earlier research students who did some of this work – Yanellis Bonano, Cecelia Martin
- Elizabethtown College & Department of Biological & Environmental Sciences for funding

Thank you

Questions?