Fecal Microbiota Preservation: Examining the Effects on Microbial Composition 5-years Later

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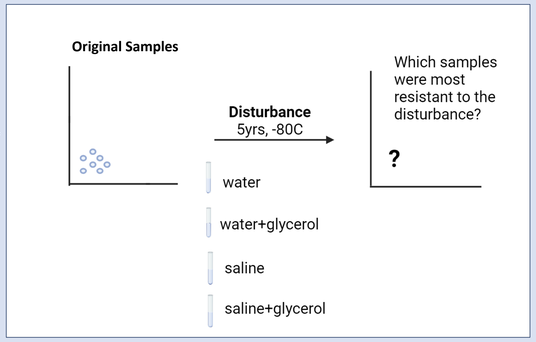
Objective

We examined fecal microbiota stored in 4 environments (water, water+glycerol, saline, saline+glycerol) at -80C for 5 years to determine which environment best supported the bacterial community's ability to resist change.

Introduction

Diversity and abundance of the microbial community in an individual's gut have a large impact on their health. When individuals go through intensive antibiotic treatments, antibiotics or the disease itself can significantly lower microbial diversity in the gastrointestinal system leading to complications and/or opportunistic infections such as C diff[1]. To restore the gut microbiome, sick patients can undergo a treatment called fecal microbiota transplant (FMT) [1]. This treatment involves donor fecal matter either from an individual or a stool bank. Stool bank storage of fecal matter does not ensure the fecal microbiota remains unchanged from the time of donation[2].

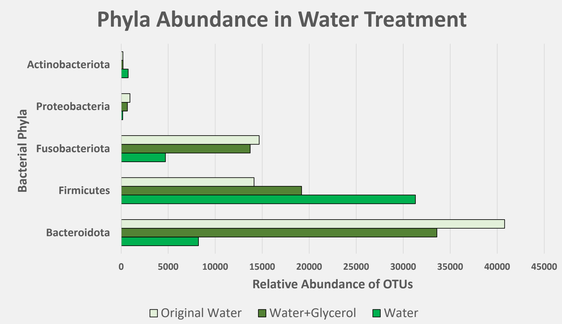
To ensure the microbial gut community stays abundant and diverse for FMTs, the handling time and preservation methods of the fecal matter used is important. The fecal microbiota must be able to resist changes to the microbial community during its storage to be of functional use to the recipient. It must maintain a similar microbial community as before storage.



Methods

- For **preservation methods**
  - Previous research group homogenized the fecal matter samples, from dogs, with sterile deionized water or 0.85% NaCl then partitioned. The fecal sample that was homogenized by deionized water, were split into preservation with or without 25% glycerol, same was done to the sample homogenized by 0.85% NaCl.
  - The two treatments (i.e., water only & saline only) were then used for immediate DNA extraction, then stored in -80C freezer
- To **extract DNA 5 years later**:
  - Used the QIAamp BiOstic Bacteremia DNA Kit (Qiagen) and followed the protocol to extract the DNA from the stored fecal matter samples.
- To **sequence DNA**:
  - Sequencing of the 16S rRNA v4-v5 region yielding paired-end reads was done by Wright Labs on a high throughput Illumina miSeq system.
- To **quality check and trim sequence data**:
  - Used the open access platform Nephele: Microbiome Analysis by NIH
  - Used DADA2 and QIIME2 paired-end FASTQ data and used taxa bar plots to analyze abundance of the different phyla present

Results



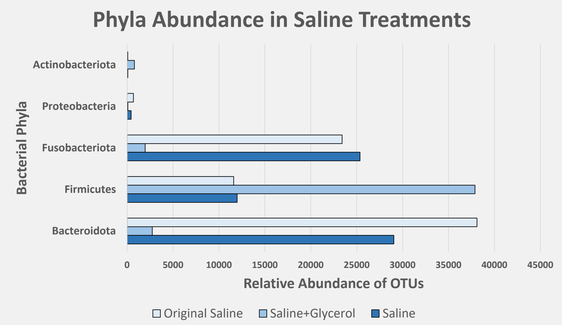


Figure 1. Analysis of the average relative abundance of bacterial phyla in the water treatments and the saline treatments (n=3). Original saline and original water represent samples pre-storage.

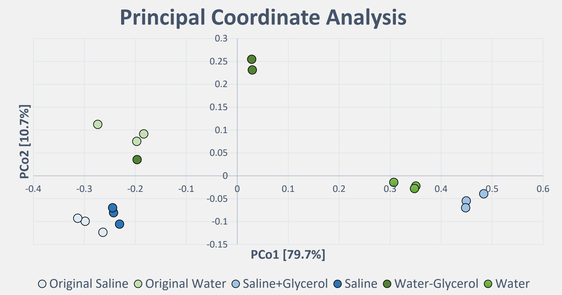


Figure 2. The PCoA Bray-Curtis analysis shows the dissimilarity between samples by proximity of one another. Samples that are closer together are more similar, while samples farther apart are more distinct.

Discussion

- Samples with most similar abundance and distribution of phyla (Fig. 1)
  - Original water and five-year water+glycerol treatments
  - Original saline and five-year saline treatments
- The phyla of bacteria most present across all samples were: Actinobacteriota, Proteobacteria, Fusobacteriota, Firmicutes, and Bacteroidota
- The original saline treatment and stored saline only treatment have high levels of Bacteroidota and Fusobacteriota, with lower levels of Firmicutes; the original water treatment and the water-glycerol treatment have relatively high Bacteroidota and Fusobacteriota levels
- PCoA analysis shows the saline only and original saline treatments have most similar composition of diversity and abundance (Fig. 2)
- The Firmicutes and Bacteroidetes are the most dominant phyla present in the human gut microbiota, while Proteobacteria and Actinobacteria are two other subdominant phyla with lower abundance; suggesting a healthy gut microbiota[3]
- The canine GI tract has similar phyla as the human gut microbiota, with the phylum Bacteroidota being the most abundant in canines[4]
- The phylum Bacteroidota had the highest abundance and is an important part of the microbiome in the fecal matter[3]
  - Bacteroidota is beneficial to the host by metabolizing polysaccharides and oligosaccharides, providing nutrition and vitamins to host and microbiota in the gut[5]

Conclusion

We can conclude fecal microbiota stored in the saline only treatment is most resistant to 5 years of storage at -80C indicated by having the most similar abundance and distribution of microbiota to the original sample

Future Studies

- Test cell viability of the fecal microbiota when stored with only saline compared to the fecal microbiota of original samples through analysis of cell function
- Analyze effect of treatments in preservation of human fecal matter samples

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References

1. Wilson, B. C., Vatanen, T., Cutfield, W. S., & O'Sullivan, J. M. (2019). The Super-Donor Phenomenon in Fecal Microbiota Transplantation. Frontiers in cellular and infection microbiology, 9, 2. https://doi.org/10.3389/fcimb.2019.0000

2. Wang, J. W., Kuo, C. H., Kuo, F. C., Wang, Y. K., Hsu, W. H., Yu, F. J., Hu, H. M., Hsu, P. I., Wang, J. Y., & Wu, D. C. (2019). Fecal microbiota transplantation: Review and update. *Journal of the Formosan Medical Association = Taiwan yi zhi*, *118 Suppl 1*, S23–S31. https://doi.org/10.1016/j.jfma.2018.08.011

3. Zafar, H. & Saier, M. H., Jr. (2021). Gut *Bacteroides* species in health and disease. *Gut microbes*, *13*(1), 1-20. https://doi.org/10.1080/19490976.2020.1848158

4. Pilla, R., & Suchodolski, J. S. (2020). The Role of the Canine Gut Microbiome and Metabolome in Health and Gastrointestinal Disease. *Frontiers in veterinary science*, *6*, 498. https://doi.org/10.3389/fvets.2019.00498

5. Magne, F., Gotteland, M., Gauthier, L., Zazueta, A., Pesoa, S., Navarrete, P., & Balamurugan, R. (2020). The Firmicutes/Bacteroidetes Ratio: A Relevant Marker of Gut Dysbiosis in Obese Patients? *Nutrients*, *12*(5), 1474. https://doi.org/10.3390/nu12051474