Fecal Matter Transplants: Determining the Best Preservation Method for Fecal Matter

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Abstract

Fecal Matter Transplants, (FMT), are an effective, yet underutilized, treatment for *Clostridium difficile* infections. *C. diff* infections are characterized by a dysbiosis of colonic microbiota, usually caused by antibiotics, which allow the pathogen to proliferate and reach virulent levels. Despite having a 90% success rate, and patient reports of immediate improvement, FMTs are approved only as a last resort for C. diff infections; this is most likely due to strict restrictions put forth by the US Food and Drug Administration, which label FMTs as "experimental." This study aimed to create a comparison between microbial preservation methods to determine which method has the least detrimental effects on stool microbes to improve FMTs. To study the impact of preservation methods on the microbial components of fecal samples, stool samples were collected from dogs. Samples were homogenized with either sterile deionized water or 0.85% NaCl. The homogenized mixtures were then partitioned for immediate DNA extraction and sequencing or preservation with or without glycerol prior to -80C storage. After 60 days, DNA extraction and sequencing was again effectuated for comparison between initial samples, and preservation treatments. Preliminary results yielded 697,032 high quality sequences, averaging 252.4 bases in length. Sequences were truncated at 250 bases to maintain a 0.5% average expected error, which resulted in retainment of 82.08% of sequences. Data acquired will aid in determining the most efficient preservation method which in turn could be used to further improve FMTs and help fuel FMT related research in hopes of attenuating FDA restrictions.

Introduction

Clostridium difficile (C. diff) is an opportunistic pathogen known for being one of the main culprits of hospital acquired infections [1]. Infection usually occurs when a patient receives treatment by antibiotics which disrupt the normal symbiosis of the intestinal microbiota thus, allowing C. diff to grow unrestrained and become harmful [2]. Currently, the most common treatment used for CDIs is a distinct course of antibiotics specifically designed to disrupt the bacteria's growth; however, due to the overemployment of antibiotics, there is interest in an efficient alternative which does not require the use of antibiotics.

FMT's, are a simple procedure involving the introduction of healthy, donor stool into the colon of a patient suffering from CDI, usually via a colonoscopy [3]. The donor's blood serum and stool samples are thoroughly screened for the presence of potential infectious diseases, like Hepatitis in serum or parasites in stool, which could be transmitted through the transplant and further endanger the patient [4]. Donor stool should contain healthy levels of colon bacteria which, when introduced into the unhealthy patient, will help replenish normal intestinal bacteria thereby, regaining control over *C. diff*. Unfortunately, FMT's are employed only as a last resort treatment for patients with recurring CDIs. This could most likely be due to the current restrictions put forth by the US Food and Drug Administration (FDA) which labels FMT's as an "experimental treatment" and require special permissions to be acquired by physicians before conducting an FMT [5].

Currently, hospitals and stool banks do not test FMT stool samples beyond the aforementioned screening; samples which pass infectious screening are automatically assumed to be suitable [6]. Because there is no bacterial analysis or culturing involved, there is no way to know whether the flora initially present in the stool sample has remained unaffected during preservation. Current long-term preservation methods include the treatment of the stool sample with a liquid solution, which is then homogenized, filtered, and stored in sterile conditions at freezing temperatures [4]. When a stool sample is requested for use by a physician, the sample is thawed and immediately transplanted. Further research on the effectiveness of various preservation methods, when compared to the effects of each method to the flora present on the samples, would allow for the identification of the optimal preservation method; a method which allows for the least alterations to the flora in the sample.

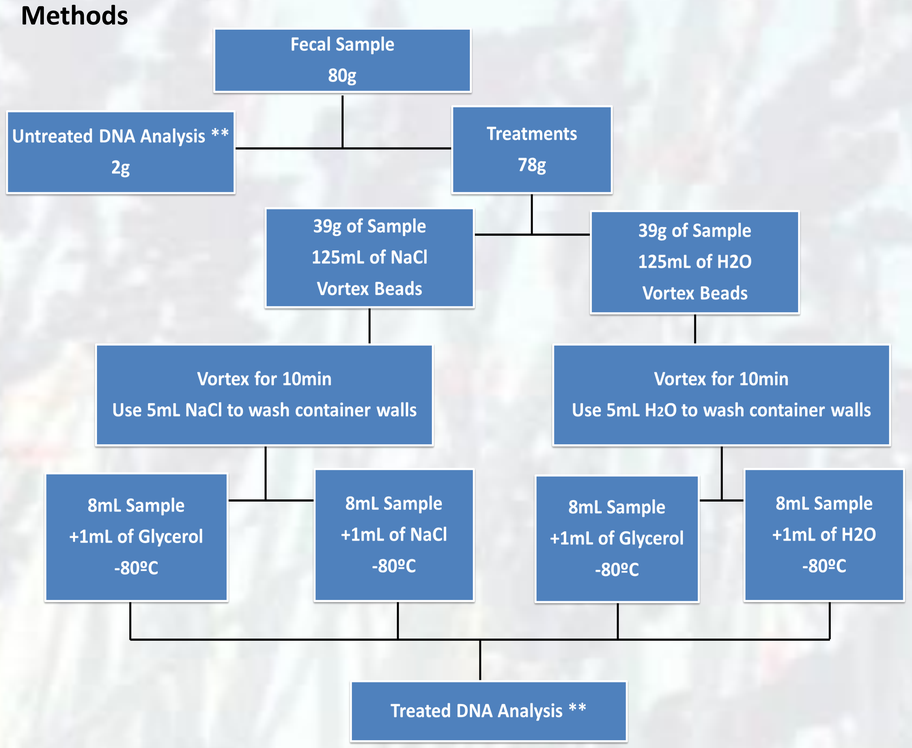
Study Aim

To determine the best method for fecal sample preservation which causes the least qualitative changes in microbiota

Methods Compared (-80C storage):

Purpose: To use the study results to provide stool banks, and health administrators with the most efficient preservation method to further refine FMTs in hopes of assuaging FDA restrictions, and increasing their use as an alternate treatment over antibiotic.

Methods



**** Methods for DNA Analysis:**

-- Extraction: UltraClean Microbial DNA Isolation Kit (MO Bio Laboratories, Inc.)

-- PCR Amplification: PTC-200 Peltier Thermal Cycler (Bio-Rad Laboratories, Inc., Hercules, CA)

--Sequencing: Illumina sequencing by Wright Labs, Inc.

--Analysis: Qualitative Insights into Microbial Ecology open-source bioinformatics platform (QIIME)

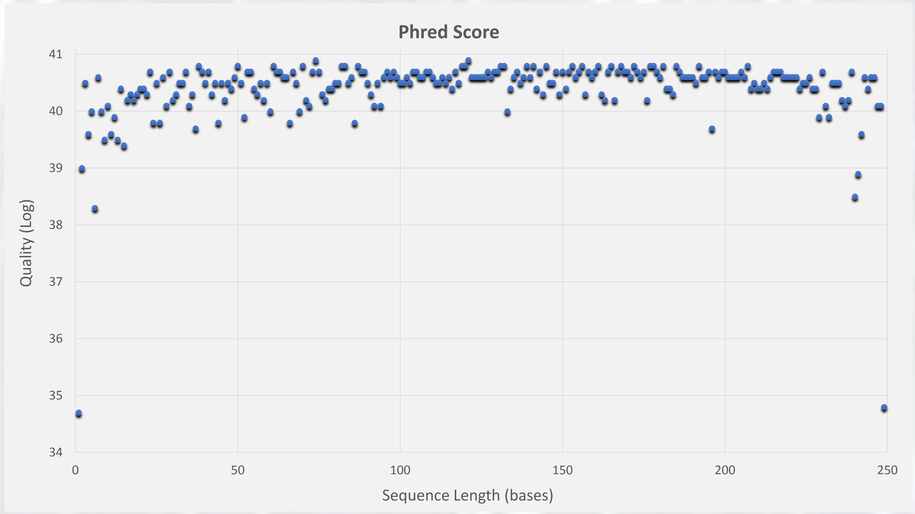
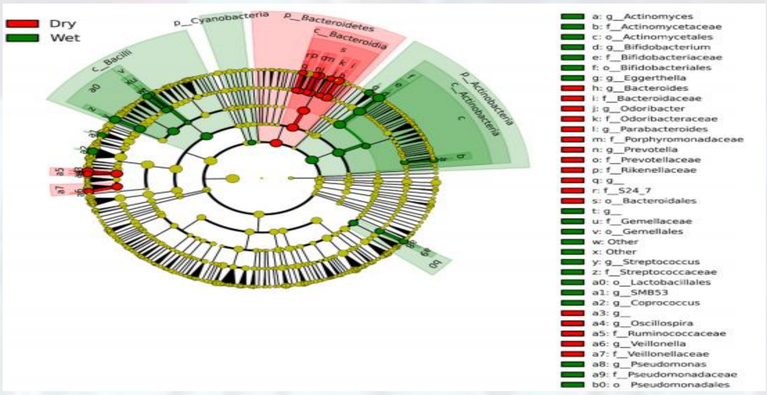


Figure 1. Quality assessment of sequenced data. A Phred score of 30 indicates that the probability of a correct base call is 99.9%.



Supplemental Figure 1. Cladogram representing taxa enriched under various Wet vs Dry Lyophilizing treatments by Lewis et al. [7]

Results and Discussion

Preliminary results from this study yielded a 697,032 high quality sequences totaling 175.9 million bases. Sequences were truncated at 250 bases in order to maintain a 0.5% error; this resulted in retainment of 82.08% of the data acquired. A 0.5% error allowed for high quality data by lowering the probability of an incorrect base in a sequence to one base in every 200 bases. This also allowed for Phred scores between 34.7 and 42; a Phred score of 30 indicates the probability of a correct base call is 99.9% (**Fig. 1**).

Further analysis of sequence data will compare original untreated samples to samples stored for 3 weeks at -80C diluted with water or saline and either with or without the cryoprotectant glycerol. Additionally, like Supplemental Figure 1, we will compare taxonomic diversity to determine sample relatedness, and to determine whether or not these particular preservation methods enrich for particular species.

Greater understanding of an optimal microflora for normal colonic functioning is the focus of many phylogenetic studies. Identifying optimal species or community composition could allow for more personalized stool screening with the goal of improving FMTs through appropriate preservation method. For example, Lewis et al. found employing wet lyophilization enriched Actinobacteria, while dry lyophilization favors Bacteroidetes [7]. We hope data acquired will aid in determining the most efficient preservation method which in turn could be used to further improve FMTs and help fuel FMT related research in hopes of attenuating FDA restrictions.

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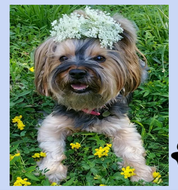
Bart (L) and Ernestine (R)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