Sequencing Human Intestinal Tract bacteria before and after gastric bypass surgery

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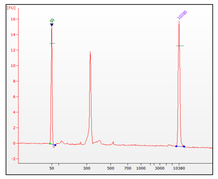
Background:

Roux-En-Y Gastric Bypass (RYGB) surgery has become a commonly used procedure to help morbidly obese patients lose weight. The RYGB procedure results in the creation of a small stomach, called the "Roux limb", which is then attached to the distal end of the small intestine. This causes the patient to feel full more quickly, preventing the patient from consuming a high number of calories. The procedure results in extreme weight loss within weeks. Of particular interest is the disappearance of Type 2 Diabetes Mellitus (T2DM) within days of the procedure. Little is known about how the procedure works biochemically to alleviate the markers of obesity in the body and how the procedure alleviates T2DM so quickly. Recent evidence has shown that the composition of the human intestinal microbial communities may contribute significantly to the acquisition and the severity of disease, particularly obesity and T2DM. The composition of the intestinal microbiologic flora has been shown to be different in obese and normal weight patients. The goal of this study was to further explore how the microbial community progresses in patients before and after they undergo the RYGB procedure.

Methods:

Eleven adult subjects with morbid obesity (body mass index ≥ 40 kg/m^2) and T2DM underwent RYGB surgery. Stool samples were collected 2 weeks prior to surgery (Day -14) and multiple time points after the surgery (Day +30, +90, and +180). Genomic DNA was isolated using a modified QIAamp DNA Stool Mini Kit procedure which included mechanical lysing. The V5 and V6 regions of the 16S gene were amplified in a PCR reaction using custom primers (see Figure 1) which contained barcode sequences and the adaptors necessary for sequencing. The PCR reactions were cleaned using an AMPure XP bead cleanup. Samples were pooled together in equimolar ratios at 26 pM. Samples were enriched on the IonTorrent OneTouch using an Ion OneTouch 200 Template Kit v2 DL. Samples were sequenced on the IonTorrent Personal Genome Machine (PGM) using an Ion PGM 300 Sequencing Kit on an Ion 314 Chip.



Bioanalyzer electropherogram of purified DNA amplicon at correct size of ~340 bp.

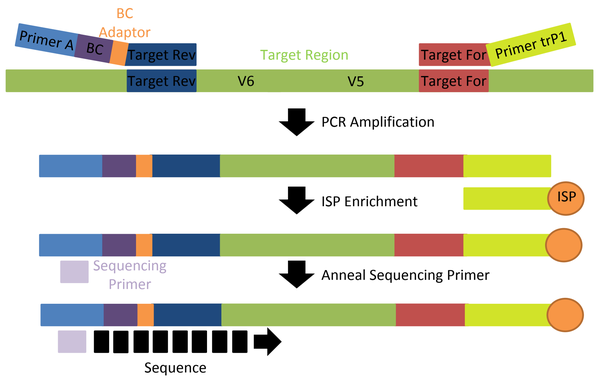


Figure 1:

Figure 1 is a pictorial representation of the PCR amplification and Ion Sphere Particle (ISP) Enrichment procedure to make DNA libraries for sequencing. Custom primers (Target Rev and Target For) targeted the V6 and V5 hypervariable regions of the bacterial 16S gene. These primers had additional primers (Primer A and Primer trP1) and barcodes (BC, BC Adaptor) attached which are necessary for sequencing.

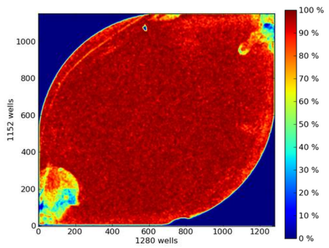


Figure 2:

Heatmap representing the percent loading of ISP's across an Ion 314 chip with an average loading density of ~93%. ISP's are loaded on to a chip and then centrifuged to secure ISP's into individual wells (see Figure 3). Standard loading density >60%.

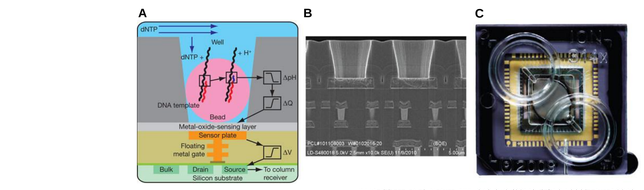


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Figure 3:

A. Schematic of sequencing steps. dNTP's are flowed across the chip sequentially. When incorporated into the growing template strand on an ISP, a H+ ion is released. The chip detects a drop in pH, and converts this into an electrical signal, which the sequencer then detects. **B.** Electron micrograph of individual wells on an Ion chip with underlying electronic sensors. **C.** Image of a standard Ion 314 chip.

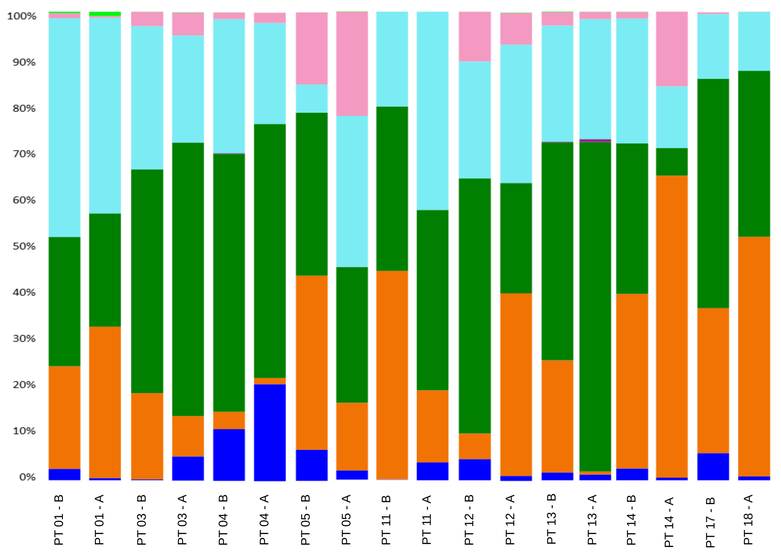

Figure 4:

Figure 4 shows the phyla level breakdown of the total gut flora in each patient 14 days before (**B**) the RYGB procedure and 30 days after (**A**) the RYGB procedure. The graph was generated using the Quantitative Insights Into Microbial Ecology (QIIME) software package. The majority of the changes observed were in the two prominent phyla groups of the intestinal tract, Bacteroidetes and Firmicutes.

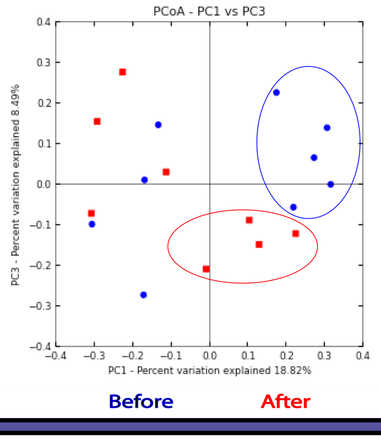

Figure 5:

2D Principal Coordinate Analysis (PCoA) plot generated to visualize sample clusters using the QIIME software. Clustering was observed in samples taken at the same time point.

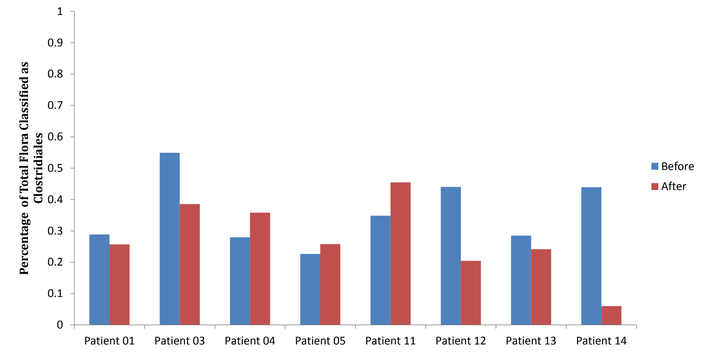

Figure 6:

Figure 6 expands on the observed difference in Firmucutes from Figure 4. The changes observed in the phyla Firmucutes before and after RYGB is largely associated with the changes in the class Clostridales (Figure 6). In all but three patients, the percentage of total flora classified as Clostridiales decreased after surgery.

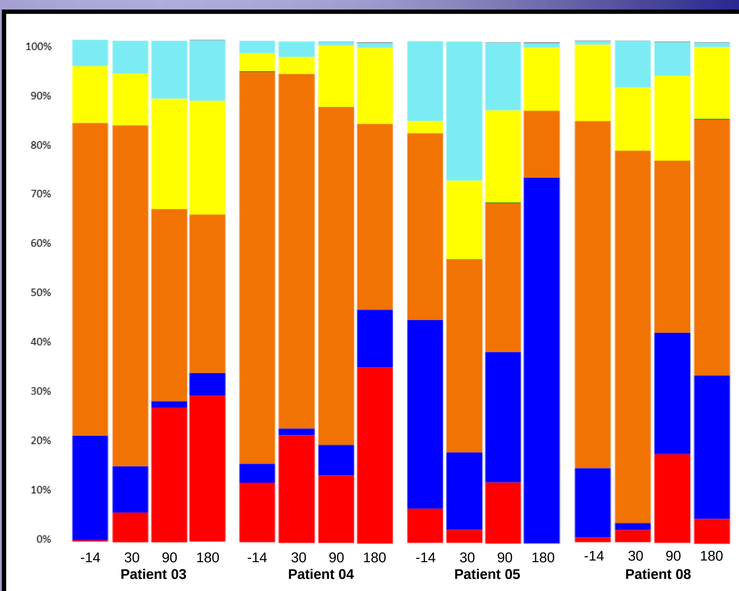

Figure 7:

Figure 7 shows the gut flora in four patients at four time points: 14 days pre-surgery (-14), and 30 days, 90 days, and 180 days post-surgery (30, 90, 180).

Conclusion:

While we detected changes in the phyla Bacteroidetes and Firmicutes after the RYGB surgery, the differences were not consistent across all patients. One theory that could lead to these inconsistencies in gut flora changes across the patients is that there may be several other factors involved such as patient's diet or medication history. Several studies suggest these factors may contribute significantly to the makeup of the gut flora. We plan on sequencing the remaining samples collected 90 and 180 days post-surgery to observe if the flora continues to change. Future studies may also include more biographic information such as diet, medication, or geography information.

References:

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