Abstract: Gut microbiome associations with Inflammatory Bowel Disease (IBD) have been reported in multiple studies. However, the interaction between the active bacteria and the host gene expression has not yet been well understood. In this study, we analysed 185 biopsies of intestinal mucosa from subjects with 58 Crohn’s Disease (CD), 88 Ulcerative Colitis (UC) and 39 Controls. 16S rRNA gene amplicon sequencing from inflamed and Control biopsies was performed on extracted DNA and RNA, hereafter termed DNA-16S and RNA-16S, respectively. Host gene expression analysis was performed via RNAseq. DADA2 de-noided sequences were used for microbial differential abundance and activity analysis using metagenomeseq package in R statistical environment. DEseq2 package was used for the host RNAseq analysis. DNA-16S demonstrated one and four dynamically depleted Ribosomal Sequence Variants (RSVs) in CD and UC respectively, compared to the Controls. Whereas, five and six RSVs from RNA-16S were dynamically and differentially expressed in CD and UC respectively. Of those, Anaerostipes hadrus RSV was significantly depleted in UC in DNA-16S, in both CD and UC in RNA-16S. Following on these findings, we compared RNA-16S levels of A. hadrus to host RNAseq gene expressions. Termination of O-glycan biosynthesis pathway was significantly enriched, which could be an advantage of this organism to survive and prosper in this environment.

Methods

**Study Design:** Total of 185 biopsies of intestinal mucosa were collected (Table 1). Biopsy samples were obtained from inflamed tissues of adult IBD patients or healthy tissues of control subjects. Exclusion criteria was the use of antibiotics one month prior or during the GI investigation.

**Microbiota analysis:** DNA and RNA were extracted using AxyPrep DNA/RNA/Protein Mini kit (Iagien). Total RNA was reverse transcribed to cDNA. 16S rRNA V3-V4 hypervariable region was amplified using 341F and 805R primer set. Amplified DNA (DNA-16S) and cdNA (RNA-16S) were sequenced using Illumina MiSeq for 2x300 bp reads. DADA2 (Callahan et al., 2016) was used to denoise DNA-16S and RNA-16S sequences. Strain level taxonomy was assigned to Ribosomal Sequence Variants (RSVs) using StrainsSelect 2016. Differential test was conducted using R package, metagenomeseq.

**Host RNAseq analysis:** Aliquots of RNA samples were used for host transcriptome RNAseq using TruSeq Stranded mRNA Sample Prep Kit (Illumina) with Illumina HiSeq 4000 2x100bp reads. Quality filtered reads were aligned to the human genome (GRCh38) using HISat2 and a count table was generated using SUBREAD. Differential expression analysis on host RNAseq was performed using DEseq2 package (Love et al., 2014). Pathway enrichment analysis was conducted using R package, ReactomePA.

**Microbial composition of RNA-16S samples demonstrated a shift from DNA-16S samples**

![Figure 1](image1.png)

**Figure 1.** DNA-16S and RNA-16S data were compared using 102 samples which have both datasets. From the total of 486 RSVs, 262 were shared between two datasets, and 51 and 173 RSVs were unique to DNA-16S and RNA-16S, respectively. Principal Coordinate Analysis (PCoA) on Bray-Curtis dissimilarity of DNA-16S and RNA-16S data were compared using 102 samples which have both datasets.

**Microbial composition of RNA-16S samples demonstrated a shift from DNA-16S samples**

![Figure 2](image2.png)

**Figure 2.** Disease status (CD, UC or Control) was significantly associated with host gene expression profile (PERMANOVA p<0.001). Pathway enrichment analysis demonstrated significant change in IL-4/IL-15, Extracellular matrix organization, Collagen degradation, and IL-10 signaling pathways in both CD and UC patients compared to controls.

**Host RNAseq confirmed inflammation in CD and UC compared to Controls**

![Figure 3](image3.png)

**Figure 3.** Volcano plots depicting significance and effect size of each RSV. A. Abundance of A. hadrus RSV in DNA-16S and RNA-16S. This RSV was the only one dynamically depleted in both UC and CD compared to Controls in RNA-16S datasets. B. Volcano plots depicting significance and effect size of each RSV. A. hadrus metabolic activity was associated with depletion of O-glycan biosynthesis pathway.

![Figure 4](image4.png)

**Figure 4.** One to six RSVs were dynamically depleted or enriched in CD or UC (adjusted p-value <0.05, absolute log2 fold change >1 and non-zero sequence counts in at least 75% of the subjects in at least one group). A. Volcano plots depicting significance and effect size of each RSV. B. Abundance of A. hadrus RSV in DNA-16S and RNA-16S. This RSV was the only one dynamically depleted in both UC and CD compared to Controls in RNA-16S datasets.

**Anaerostipes hadrus metabolic activity was significantly depleted in both UC and CD**

![Figure 5](image5.png)

**Figure 5.** Termination of O-glycan biosynthesis pathway was significantly enriched by pathway enrichment analysis (adjusted p<0.002). Terminal modification of glycans is known to affect bacterial adhesion (Bais et al., 2012) and could serve as substrates for specific microbiota to provide nutritional advantage (Pacheco et al., 2012).

**Conclusions**

- Anaerostipes hadrus was identified in both UC and CD. The activity was associated with termination of O-glycan biosynthesis pathway, which could be an advantage of this organism to survive and prosper in this environment.

**References**


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