The Inner-Colonic Bacterial Communities of the Human Gut are Unique and Profoundly Different than those of Stool Samples

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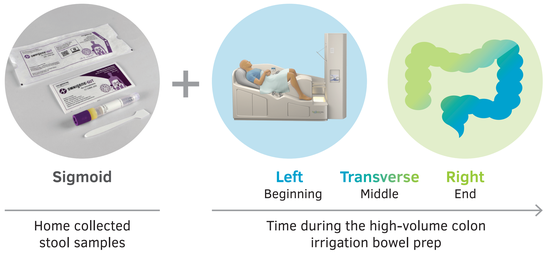
Introduction:

- The gut microbiota is a major factor in managing health and disease.
- There are numerous studies discussing the gut microbiome, however, they are mainly based on stool samples.
- Here we compare the microbial diversity of stool samples versus inner-colonic effluent samples collected prospectively during high-volume colon irrigation.

Methods:

- Samples were collected from 11 participants (mean age: 66, min–41, max-78) during an IRB-approved clinical trial. All patients underwent high-volume colon irrigation before screening colonoscopy.
- The inner-colonic samples (n=35) were collected three times during the prep as changes were noted in effluent appearance, to represent the left, transverse, and right colon. Stool samples were collected by patients in their home (n=11) using standad collection kit.
- Samples were immediately stabilized and stored following standard procedures and analyzed by PCR amplicon for bacterial rRNA, 16SV4.

Figure 1 - Study design for comparing microbial communities between home collected stool samples with inner-colonic microbiome communities collected during a high-volume colon irrigation bowel prep.



Results:

- Bacterial communities detected in inner-colonic samples were significantly different that those of stool samples.
- Of all bacterial strains detected, a third of the strains (n=344) were unique to the inner-colonic samples, with a unique contribution from the different colon biogeographic locations.
- We detected a decrease in the species abundance of *firmicutes* and increase in *proteobacteria* when comparing stool to inner-colonic samples, without a significant change in number of *bacteriodetes* species. Species of other phyla were more abundant in the inner-colonic samples.
- There was a clear biogeography gradient of bacteria along the colon, where the overall microbial alpha diversity in each sample decreases towards the proximal colon.
- The phylogeny of the microbiota community in all stool samples was relatively similar regardless of the patient.

Discussion

- There are distinctive and large differences between stool and inner-colonic samples.
- For most patients there is a detectable phylogenetic progression with a predictable, yet personal, trajectory.
- The phylogenetic spread in the multivariance space of the weighted analysis explains approximately 50% of the population diversity of the inner-colonic samples diversity (n=35) and is much wider than the phylogenetic difference of the stool samples.
- The patented process of collection of microbial samples using a high-volume colon irrigation is currently the only way of obtaining microbiome information from within the colon, non-invasively, and without the effects of oral prep purgatives.
- Obtaining information from inner-colonic microbioe communities can be imperative for developing personalized medicine.

Figure 2 - Inner sample diversity of the microbiome communities of the different samples (alpha diversity by Shannon index). Sample types: stool (n=11), three inner-colonic samples representing the left colon ("Effluent-1", n=13), transverse colon ("Effluent-2", n=11) and right colon ("Effluent-3", n=11).

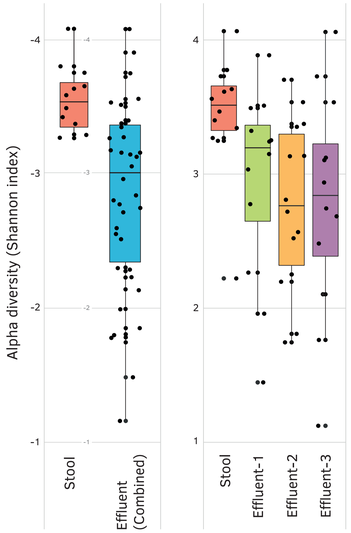


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Figure 3 - Phylogenetic species diversity between samples (beta diversity) (n=46), represented by PCoA weighted and unweighted Unifrac analysis.Sample types: stool (n=11), three inner-colonic samples representing the left colon ("Effluent-1", n=13), transverse colon ("Effluent-2", n=11) and right colon ("Effluent-3", n=11).

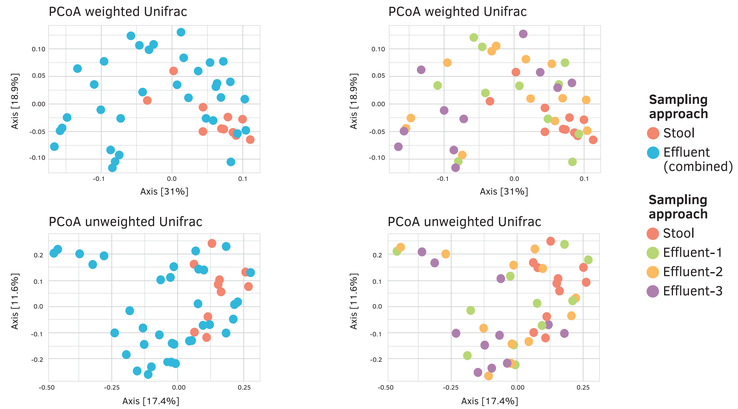
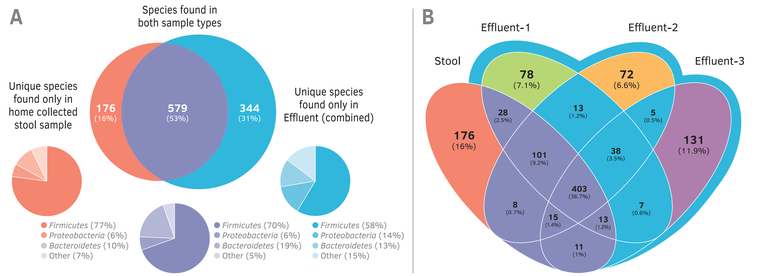
Figure 4 - A. A Venn diagram of the unique and shared species between stool and inner-colonic samples (by species presence and absence) and the species distribution of each group of species in the phyla taxonomic rank (n=11 for stool samples, n=35 for inner-colonic samples). B. A Venn diagram of the presence of species in stool and three different colonic segments. Sample types: Stool (n=11), left colon ("Effluent-1", n=13), transverse colon ("Effluent-2", n=11) and right colon ("Effluent-3", n=11).