A customizable stool ex vivo assay for detecting microbiome and metabolic changes in the gut

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Introduction

Significance:
There are limited methods that allow for the investigation of the individual gut microbiome and its functionality in a clinical setting.

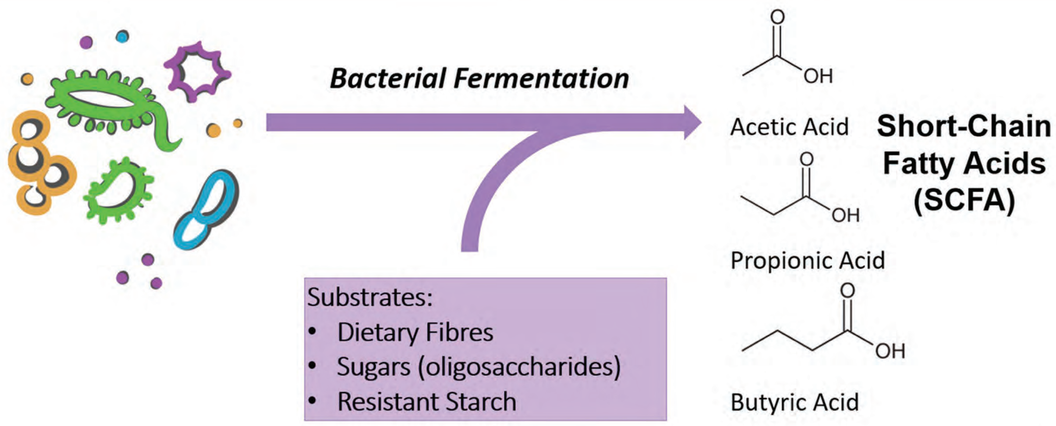

Figure 1. Bacterial fermentation in the gut produces short-chain fatty acids (SCFAs). Indigestible carbohydrates and oligosaccharides can be used for SCFA production by bacteria found in the gut. The most abundant SCFAs produced are acetic, butyric, and propionic acid. (PMID: 33764858, 32082260, 33723382)

Methods

- Human or C57BL/6Cr mice stool were inoculated at 0.5% w/v in 7.5% w/v brain heart infusion broth with the addition of 0.5% w/v substrates. Negative controls contained phosphate-buffered saline (PBS) alone.
- Baseline was collected while remainder of samples were incubated for 24 hours anaerobically, followed by sample collection.
- Microbiome composition and SCFA were determined by 16S rRNA sequencing and gas chromatography-tandem mass spectrometry (GC-MS/MS), respectively.

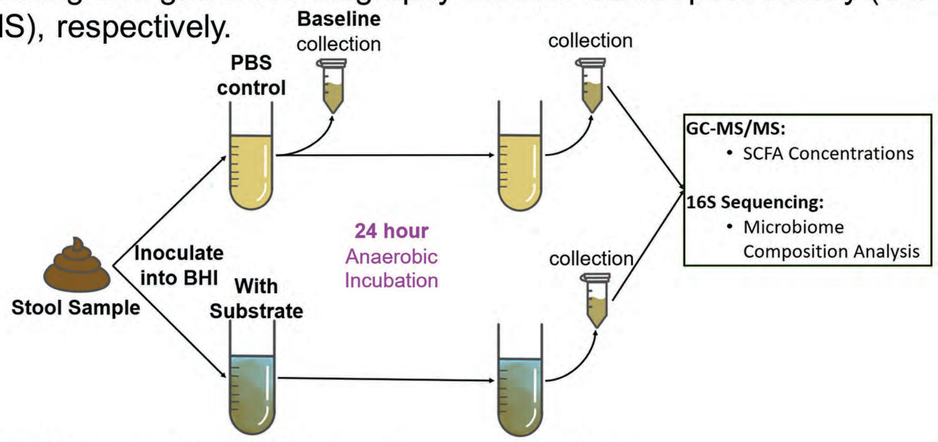

Figure 2. An overview of the *ex vivo* assay setup.

Conclusion

- This *ex vivo* assay is:
  - Able to differentiate between individual gut microbiome signatures
  - Reproducible
  - Sensitive to microbiome compositional changes
- This *ex vivo* assay has the potential of being utilized as a drug testing platform and method in determining influence of differing diets on individual gut microbiome and SCFA concentration for clinical use.

Acknowledgements

- GC-MS/MS for SCFA analyses were performed by Analytical Core for Metabolomics and Nutrition
- Sequencing of 16S libraries were done by UBC Bioinformatics and Sequencing Consortium
- We thank Christine Yanta and Dr. Alana Schick for performing the bioinformatics analyses

Results

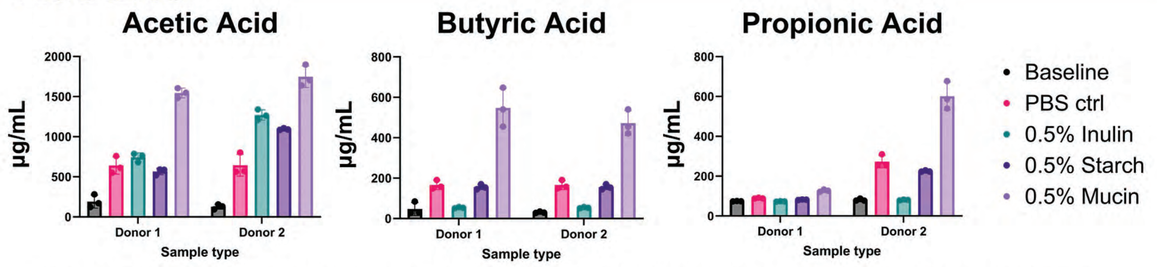

Figure 3. Assay identifies different SCFA signatures from various human donors. Following the general *ex vivo* assay set up (Fig. 2), two different human stool donors were inoculated and spiked with PBS as a control or with inulin, starch, and mucin. Three technical replicates of each condition were performed.

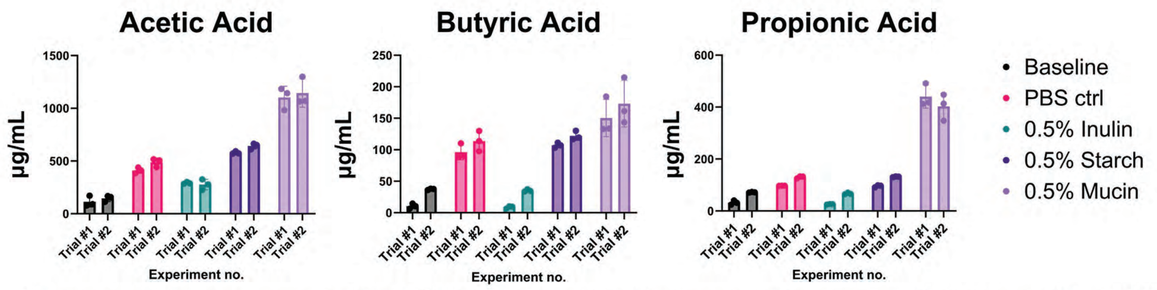

Figure 4. Assay reproducibility. Following the general setup of the assay (Fig. 2), C57BL/6Cr mice stool samples were analyzed in two independent experiments where samples from the same donor spiked with PBS as a control or with inulin, starch, or mucin. Three technical replicates of each condition were performed and all samples were analyzed with GC-MS/MS for SCFA concentrations. Two separate trials of the same stool sample were performed.

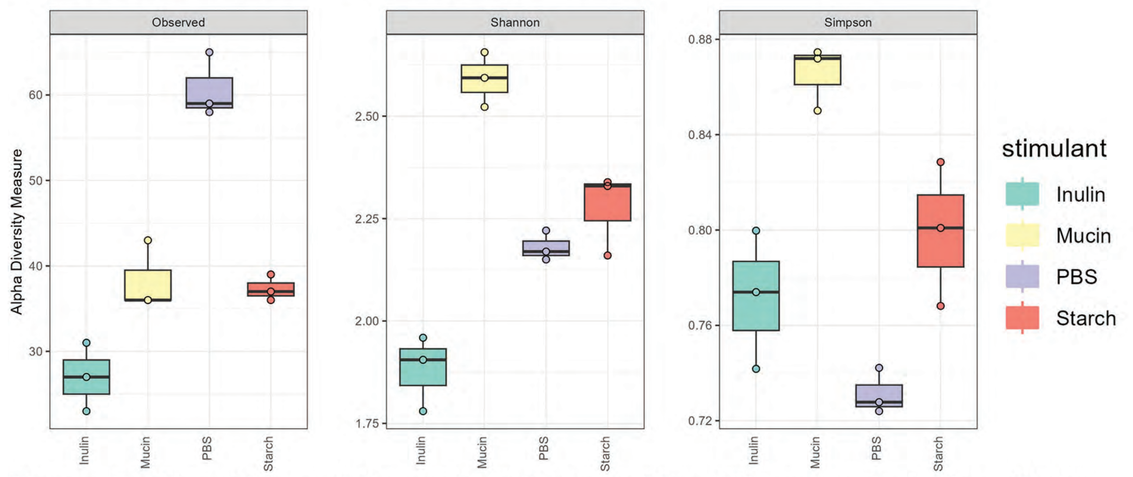

Figure 5. Microbiome richness is influenced by different substrates. Alpha diversity of inoculated C57BL/6Cr mice stool microbiome processed in the assay in Fig. 4 are shown. Relative to the PBS control, stimulation with inulin shows the greatest effect whereas mucin and starch show minimal impact.

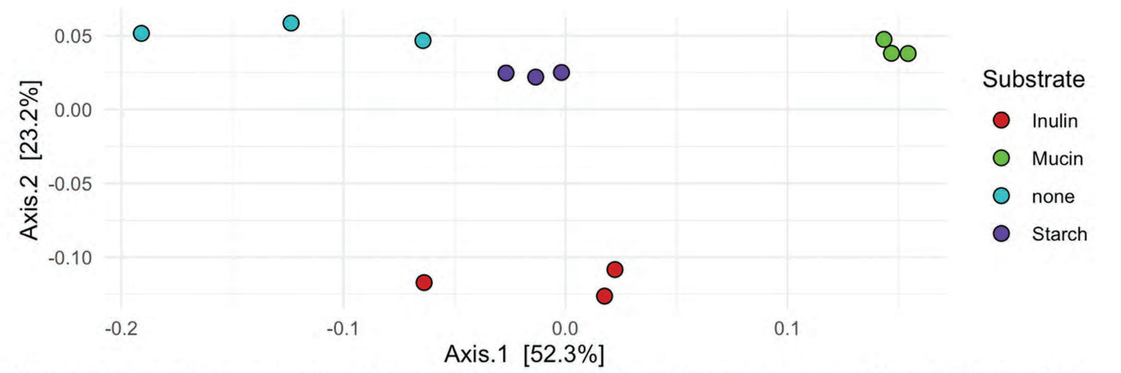

Figure 6. Variations are observed in the microbiome upon stimulation with different substrates. Analysis of the C57BL/6Cr mice stool inoculated in the assay in Fig. 4 are shown. Principle Coordinates Analysis plot (Weighted Unifrac) shows there is clustering of samples treated with the same substrate. Inulin shows greatest impact on beta diversity.