

Project ID: DFI – HMMF – 708

Submitter, Submitter PI: Jeffrey Dewey, Dion Antonopoulos

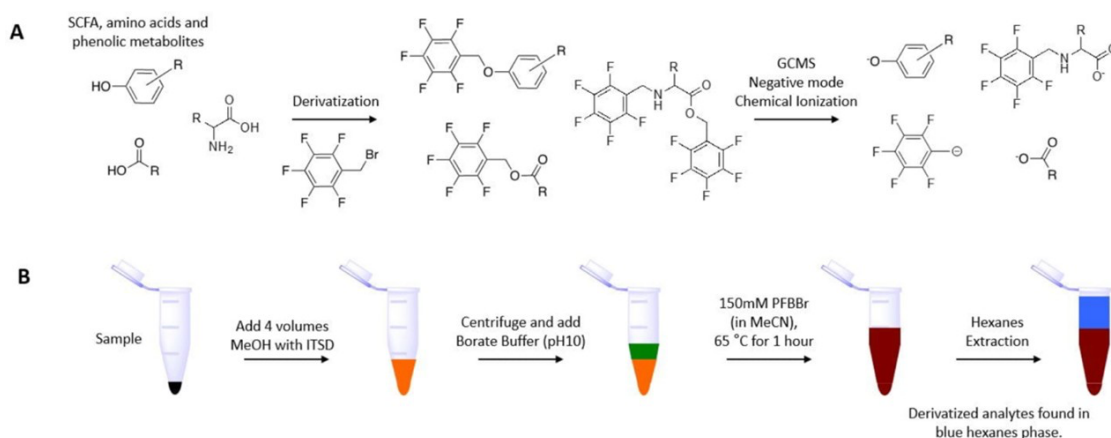
Panel(s) Requested: PFBBR(SCFA)

Project Completed: March 31, 2023

Experiment Details

PFBBR Panel

a. Chemistry and sample processing overview



Standard PFBBR Panel Sample Derivatization and Metabolite Extraction. A.) Carboxylic acids, primary amines, phenolic hydroxyls and other nucleophilic chemical functionalities are derivatized with pentafluorobenzyl-bromide (PFBBR). The compounds are subjected to gas chromatography mass spectrometry (GCMS) with chemical ionization (CI) and negative mode detection. B.) Sample preparation workflow for metabolite extraction, derivatization and liquid-liquid extraction prior to analyses.

b. Instrumentation used for analyses

- i. Agilent 8890/5977B and 7890B/5977B GCMS with chemical ionization (CI)

c. Files reported

- i. Quantitative files: Quantitative values are calculated after raw peak normalization with paired internal standards (Ex. Raw peak area for butyrate/raw peak area of internal standard d₇-butyrate). The 10-point line of the metabolite specific calibration curve is then used to calculate the endogenous concentration for each compound. Values are reported as **mM** concentration.
 1. Excel file of quantitative values
 2. Bar plot (mM)
- ii. Qualitative files: Qualitative values are calculated from the raw peak area of the endogenous compound normalized to the median raw peak area of two internal standards.
 1. Excel file of qualitative values
 2. Heatmap (coloration is the log₂fold change of the median-normalized peak area for each compound. Grey indicates samples with raw peak areas below standard detection level. The last page of the document includes compounds that were not detected.

Contact

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HMMF Website

HMMF <https://voices.uchicago.edu/dfihmmf/>

HMMF Quality Control

Quality control samples:

- Control biological samples: These samples are used to evaluate all HMMF processes. Specifically, metabolite extraction efficiency and instrument performance. The samples are extracted with solvent containing known internal standard (IS) concentrations. These samples are processed and analyzed alongside project runs. Recovery, retention time and %CV are calculated for ISs.
- Method blanks (Labeled “MB” in datasets): These samples do not contain any metabolites and are included in datasets to indicate instrument noise for the m/z at the retention time extracted.
- PooledQC: A small aliquot of submitter samples are combined to create a PooledQC sample that is run to determine if the matrix submitted impacts processes.

Quality control ions:

- All submitted samples are extracted with solvent that includes deuterated or heavy atom standards with known concentrations to evaluate metabolite extraction efficiency and instrument performance.
- Quality control ions are tracked in all control samples and the submitted samples.

Methods

Metabolite Extraction from Fecal/Cecal Material

Extraction solvent (80% methanol spiked with internal standards and stored at -80 °C) was added to pre-weighed fecal/cecal samples at a ratio of 100 mg of material/mL of extraction solvent in beadruptor tubes (Fisherbrand; 15-340-154). Samples were homogenized at 4 °C on a Bead Mill 24 Homogenizer (Fisher; 15-340-163), set at 1.6 m/s with 6 thirty-second cycles, 5 seconds off per cycle. Samples were then centrifuged at -10 °C, 20,000 x g for 15 min and the supernatant was used for subsequent metabolomic analysis.

Metabolite Extraction from Plasma/Serum/Culture Supernatant

Samples were incubated at -80 °C for at least one hour, or up to overnight. Extraction solvent (4 volumes of 100% methanol spiked with internal standards and stored at -80 °C) was added to the liquid sample (1 volume) in a microcentrifuge tube. Tubes were then centrifuged at -10 °C, 20,000 x g for 15 min and supernatant was used for subsequent metabolomic analysis.

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Metabolite Analysis using GC-nCI-MS and PFBBBr Derivatization

Short chain fatty acids were derivatized as described by Haak *et al.* with the following modifications.¹ The metabolite extract (100 μ L) was added to 100 μ L of 100 mM borate buffer (pH 10) (Thermo Fisher, 28341), 400 μ L of 100 mM pentafluorobenzyl bromide (Millipore Sigma; 90257) in Acetonitrile (Fisher; A955-4), and 400 μ L of *n*-hexane (Acros Organics; 160780010) in a capped mass spec autosampler vial (Microliter; 09-1200). Samples were heated in a thermomixer C (Eppendorf) to 65 °C for 1 hour while shaking at 1300 rpm. After cooling to RT, samples were centrifuged at 4 °C, 2000 x *g* for 5 min, allowing phase separation. The hexanes phase (100 μ L) (top layer) was transferred to an autosampler vial containing a glass insert and the vial was sealed. Another 100 μ L of the hexanes phase was diluted with 900 μ L of *n*-hexane in an autosampler vial. Concentrated and dilute samples were analyzed using a GC-MS (Agilent 7890A GC system, Agilent 5975C MS detector) operating in negative chemical ionization mode, using a HP-5MSUI column (30 m x 0.25 mm, 0.25 μ m; Agilent Technologies 19091S-433UI), methane as the reagent gas (99.999% pure) and 1 μ L split injection (1:10 split ratio). Oven ramp parameters: 1 min hold at 60 °C, 25 °C per min up to 300 °C with a 2.5 min hold at 300 °C. Inlet temperature was 280 °C and transfer line was 310 °C. A 10-point calibration curve was prepared with acetate (100 mM), propionate (25 mM), butyrate (12.5 mM), and succinate (50 mM), with 9 subsequent 2x serial dilutions. Data analysis was performed using MassHunter Quantitative Analysis software (version B.10, Agilent Technologies) and confirmed by comparison to authentic standards. Normalized peak areas were calculated by dividing raw peak areas of targeted analytes by averaged raw peak areas of internal standards.

1. Haak BW, Littmann ER, Chaubard JL, Pickard AJ, Fontana E, Adhi F, Gyaltsen Y, Ling L, Morjaria SM, Peled JU, van den Brink MR, Geyer AI, Cross JR, Pamer EG, Taur Y. Impact of gut colonization with butyrate-producing microbiota on respiratory viral infection following allo-HCT. *Blood*. 2018 Jun 28; 131(26):2978-2986. doi: 10.1182/blood-2018-01-828996. Epub 2018 Apr 19. PMID: 29674425; PMCID: PMC602463