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**HMMF Metabolomics**

Summary of General Methods

Updated: August 2023

**Metabolomics Overview**

The fecal metabolome was analyzed across four mass spectrometry platforms to capture quantitative and qualitative levels of gut-derived metabolites with varying physiochemical properties such as hydrophobicity, size, and charge. The DFI Host-Microbe Metabolomics Facility (DFI-HMMF) routinely studies fecal material with the proposed methods and analysis pipelines. In brief, metabolites are extracted with organic solvent, dried down and resuspended for direct analyses or derivatization. Previously, all compounds have been validated by the DFI-HMMF through retention time and fragmentation comparison to standards and available databases. Compounds were chosen based on known host-microbe mechanisms or the compound level in fecal material was shown to significantly vary across patient populations indicating a potential role in health. Gas chromatography-mass spectrometry (GC-MS) was used to detect compounds following derivatization with pentafluorobenzyl bromide (PFBBr)1 and trimethylsilyl-methoxamine (TMS-MOX)2,3 in two separate reactions. SCFAs (acetate, butyrate, propionate), lactate, and succinate will be quantitatively analyzed following PFB derivatization and detection by negative collision induced-gas chromatography-mass spectrometry ((-)CI-GC-MS, Agilent 8890). Additional PFB-derivatized compounds within the SCFA, branched chain fatty acid, amino acid, aromatic, hydroxylated fatty acid, organic acid, and indole compound subclasses will be studied by normalized peak area. Positive ion electron impact-GC-MS ((+)EI-GC-MS, Agilent 7890B) will be used to detect 169 molecules in the organic acid, carbohydrate, TCA intermediate, sterol, amino acid, indole and fatty acid subclasses following TMS-MOX derivatization. With the use of negative mode liquid chromatography-electrospray ionization-quadrupole time-of-flight-MS ((-)LC-ESI-QTOF-MS, Agilent 6546), bile acids from the primary, secondary and glyco/tauro-conjugated subclasses will be analyzed from fecal material.4 In addition to retention time validation, the standard intact and fragment masses are routinely detected with differences < 5 ppm compared to calculated values. Positive mode LC-triple quadrupole-MS ((+)LC-ESI-QQQ-MS, Agilent 6547) will be used to analyze indole and tryptophan catabolites. Tryptophan is an essential amino acid that is ingested in the host diet. Conversion by microbes and the host result in biologically active compounds such as serotonin, kynurenine, and melatonin. These metabolic products impact the local gut environment as well as systemically with many capable of crossing the blood-brain-barrier.

References

1. Haak, B. W., Littmann, E. R., Chaubard, J.-L., Pickard, A. J., Fontana, E., Adhi, F., Gyaltshen, Y., Ling, L., Morjaria, S. M., Peled, J. U., van den Brink, M. R., Geyer, A. I., Cross, J. R., Pamer, E. G., & Taur, Y. (2018). Impact of gut colonization with butyrate-producing microbiota on respiratory viral infection following allo-HCT. *Blood*, *131*(26), 2978–2986. https://doi.org/10.1182/blood-2018-01-828996
2. Shan, J., Peng, L., Qian, W., Xie, T., Kang, A., Gao, B., & Di, L. (2018). Integrated Serum and Fecal Metabolomics Study of Collagen-Induced Arthritis Rats and the Therapeutic Effects of the Zushima Tablet. *Frontiers in Pharmacology*, *9*, 891. https://doi.org/10.3389/fphar.2018.00891
3. Fiehn, O. (2016). Metabolomics by Gas Chromatography-Mass Spectrometry: Combined Targeted and Untargeted Profiling. *Current Protocols in Molecular Biology, 114*, 30.4.1–30.4.32. https://doi.org/10.1002/0471142727.mb3004s114
4. Gómez, C., Stücheli, S., Kratschmar, D. V., Bouitbir, J., & Odermatt, A. (2020). Development and Validation of a Highly Sensitive LC-MS/MS Method for the Analysis of Bile Acids in Serum, Plasma, and Liver Tissue Samples. *Metabolites*, *10*(7). https://doi.org/10.3390/metabo10070282