

Global profiling by UPLC-Orbitrap Fusion-MS

Cecal contents (~ 50 mg) from conventional and germ-free (GF) mice or freeze dried human stool (~ 30 mg) were mixed with 1 mL of ice cold 80% methanol (v/v) containing 0.1% formic acid (v/v). Each mixture was homogenized with 1 mm zirconium beads using a BeadBlaster™ 24 (Benchmark Scientific, Edison, NJ, USA) homogenizer. All samples were homogenized according to the program parameters: 6500 - 1×30 - 005 (×3). After vortexing, samples were sonicated for 20 min in an ice water bath, prior to centrifugation at 20,000 × g for 20 min at 4 °C. The supernatants were collected, dried in a Savant SpeedVac (Thermo Scientific, Waltham, MA, USA), and reconstituted in 100 µL of 3% methanol (v/v) containing 1 µM chlorpropamide (internal standard). Samples were then analyzed using a Vanquish UHPLC system connected to an Orbitrap Fusion Tribrid MS (Thermo Fisher Scientific, Waltham, MA, USA) with a BEH C18 column (2.1 × 100 mm, 1.7 µm particle size; Waters). Solvent A was HPLC-grade water with 0.1% formic acid, and solvent B was HPLC-grade acetonitrile with 0.1% formic acid. The initial condition was 97% A and 3% B, increasing to 45% B at 10 min and 75% B at 12 min, where it was held at 75% B until 17.5 min before returning to the initial conditions. Differential analyses were performed by the use of Compound Discoverer (Thermo Fisher Scientific, Waltham, MA, USA) or in the case of human fecal analysis, MS-DIAL and its database was used in the data analyses.^{1,2}

References

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- (2) Tsugawa, H.; Nakabayashi, R.; Mori, T.; Yamada, Y.; Takahashi, M.; Rai, A.; Sugiyama, R.; Yamamoto, H.; Nakaya, T.; Yamazaki, M.; Kooke, R.; Bac-Molenaar, J. A.; Oztolan-Erol, N.; Keurentjes, J. J. B.; Arita, M.; Saito, K., A cheminformatics approach to characterize metabolomes in stable-isotope-labeled organisms (vol 16, pg 295, 2019). *Nat Methods* **2019**, 16, (5), 446-446.