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Metagenomes and metagenome-assembled genomes from *ex vivo* fecal incubations of six unique donors

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ABSTRACT We present a donor-specific collection of 78 metagenomes (13/donor) and 143 metagenome-assembled genomes (MAGs), representing the gut microbiomes of six healthy adult human donors. In addition to adding to the catalog of publicly available human gut MAGs, this resource permits a genome-resolved look into microbial co-occurrence across six individuals.

KEYWORDS metagenomics, gut microbiome, MAG, *ex vivo*

The gut microbiome is critical to human health and can both transform and be transformed by food components and bioactive products (1–3). Yet, inter-individual differences in gut microbiome composition can affect the metabolism of these compounds (4–7). We used incubations of fecal samples from six adult donors to assess the effects of selected plant extracts on the human gut microbiome.

Fecal samples were collected with prior consent from six healthy adult donors aged 29–40 (three male and three female) meeting the following criteria: BMI < 30, not pregnant or lactating, no cancer or GI disorders, non-smokers, alcohol use < 3 servings/day, no medications for allergies or psychological conditions, and no antibiotic, prebiotic, or probiotic use within 3 months preceding donation following IRB approval by the Ethics Committee of the University Hospital Ghent (BC-09977). Anaerobic incubations of fecal sample slurries were conducted by Cryptobiotix SA (Ghent, Belgium) using the SIFR *ex vivo* colonic simulation technology [5 mL sample volume incubated under continuous agitation (140 rpm) at 37°C in individual, sealed, small volume anaerobic bioreactors] (8) for 48 hours. Samples (spun-down pellets, n = 13 per/donor) were anaerobically collected representing the inoculum, media-only incubation, and incubations containing 3 g/L of 11 different plant extracts used as spices or traditional medicines. Analysis of the specific effects of the extracts on the gut microbiomes will be presented elsewhere (9).

DNA extraction, library preparation, and shotgun metagenomic sequencing were conducted by CosmosID (Germantown, MD, USA). DNA was isolated using the Qiagen DNeasy PowerSoil Pro Kit (Qiagen, Germantown, MD, USA). DNA sequencing libraries (1 ng input) were prepared using the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA, USA). IDT Unique Dual Indexes (IDT, Coralville, IA, USA) were added to each sample followed by 12 cycles of PCR. Libraries were purified using AMpure Magnetic Beads (Beckman Coulter), eluted in Qiagen EB buffer, and quantified using Qubit dsDNA HS Assay Kit (Thermofisher Scientific) Libraries were sequenced on an Illumina HiSeq X (2 × 150 bp) to a target depth of 3 million reads per sample. Links to raw reads and metadata are given in Table 1.

Unless specified, all software used default parameters. Adapter removal and quality trimming were done using BBDuk v.38.79 (10) (k = 31, hdist = 1, ftm = 5; qtrim = r, and trimq = 10). Trimmed read sets (n = 13/donor) were used as input for donor-specific

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TABLE 1 Links to sequence data and associated metadata files

Data description	Data type	Repository link	File names
		Raw sequencing data	
Raw shotgun metagenomic sequencing reads (150 bp PE, Illumina), *n* = 78	Fastq files (.fastq.gz)	NCBI BioProject PRJNA961974	Individual file links in metadata table below
Metagenome sample and sequencing metadata and statistics including links to individual SRA objects	Excel spreadsheet (.xlsx)	Ag Data Commons https://doi.org/10.15482/USDA.ADC/1529438	Metagenome metadata table
		Metagenome assembled genomes (MAGs)	
MAG fasta files (*n* = 143)	Fasta files (.fa)	NCBI BioProject PRJNA961974	Individual file links in metadata table below
MAG binning, quality, and taxonomy metadata including links to individual NCBI BioSample objects	Excel spreadsheet (.xlsx)	Ag Data Commons https://doi.org/10.15482/USDA.ADC/1529438	MAG metadata table

coassemblies (*n* = 6) using MEGAHIT v1.2.9 (11). For each assembly, contigs were binned using Metabat2 v.2.12.1 (12) and SemiBin2 v.1.5.1 (13, 14), using coverage profiles generated with BBMap v.38.79 (10) and Bowtie2 v.2.5.1 (15), respectively. DASTool v.1.1.6 (16) was used to select the best bin set for each donor's coassembly. Bin quality and completeness were estimated using CheckM v.1.2.2 (17), and taxonomy was assigned using GTDB-Tk v2.2.4 (18) with the GTDB r207v2 database (19). Following MIMAG standards (20), two MAGs are high-quality draft (completion > 90%; contamination < 5%; all rRNA genes, 18+ tRNA), with all others medium quality (completion ≥ 50%; contamination < 10%). Of these, 79 are medium quality due only to missing or incomplete rRNA genes. Bins were annotated with the NCBI Prokaryotic Genome Annotation Pipeline. Table 1 contains links to MAGs and metadata.

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DATA AVAILABILITY

MAG sequence data and raw metagenomic sequencing reads are available in the NCBI BioProject/SRA databases under accession number PRJNA961974. Tables with individual accession numbers and links for metagenomes and MAGs and extended metadata including sequencing and assembly metrics and taxonomic information are available in the USDA Ag Data Commons: https://doi.org/10.15482/USDA.ADC/1529438. Links and descriptions can be found in Table 1.

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