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GENOME SEQUENCES

Microbiota of the Hickey Run Tributary of the Anacostia River

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ABSTRACT Water from the Hickey Run Tributary of the Anacostia River is being collected quarterly (beginning August 2018) and analyzed to create high-resolution baseline taxonomic profiles of microbiota associated with this important aquatic ecosystem, which has a long history of exposure to residential and commercial effluents from Washington, DC. These United States National Arboretum Microbial Observatory data are available under NCBI BioProject number PRJNA498951.

When the *National Arboretum Act* became law in 1927, a national garden long envisioned by U.S. presidents was established as a research center to advance the scientific and economic prosperity of American agriculture (1). The mission of the United States National Arboretum (USNA) includes study and management of plants for long-term contributions to environmental sustainability and agricultural prosperity (1), a goal inextricably linked with water health. The 444 acres that comprise the USNA are situated between Mount Hamilton and the Anacostia River and are bisected by Hickey Run. Thus, study of microbiota from Hickey Run will provide valuable information about this aquatic ecosystem and contribute to our understanding of its intersection with the more than 16,000 cultivars, natural landscapes, and soils of the USNA. These data will also support a broader range of scientific inquiry, spanning objectives for sustainable stewardship of natural resources to public health surveillance of eukaryotic and bacterial pathogens.

Collection of 100 liters of water from each of 2 sites along Hickey Run (38.912341, −76.96536, and 38.9147, −76.966873) is being carried out quarterly using ultrafiltration according to previously described methods (2). To date, collections occurred on 28 August 2018, 26 November 2018, and 27 February 2019, with future collections planned for May and August of 2019. Metadata, including dates, times, location (latitude and longitude), temperature, additional site details, and photos, have been stored in the EpiCollect5 (3) project at the USNA Microbial Observatory, which will be made public upon completion of one full year of sample collection. Following ultrafiltration with Hemodialyzer Rexeed 25S filters (AsahiKasei, Chiyoda, Tokyo, Japan), using a Geopump peristaltic pump (Geotech, Denver, CO), filters are capped, bagged and stored at 4°C, until backflushing, centrifugation (using an Eppendorf 5810 instrument at 4,000 rpm for 10 min), and homogenization of filtrate. Aliquots of homogenate are then used for culture-independent DNA extraction (DNeasy Power soil kit; Qiagen, Germantown, MD) and enrichment of specific taxa, such as *Salmonella enterica* and *Escherichia coli*, according to methods described in the Bacteriological Analytical Manual (BAM) of the Food and Drug Administration (FDA) (4, 5) and for quasimetagenomic profiling (shotgun sequencing) of the 24-h point of enrichment (6–8). DNA was extracted using the DNeasy blood and tissue kit (Qiagen, Germantown, MD), and the library was prepared using the Nextera DNA Flex library prep kit (Illumina, San Diego, CA) according to the

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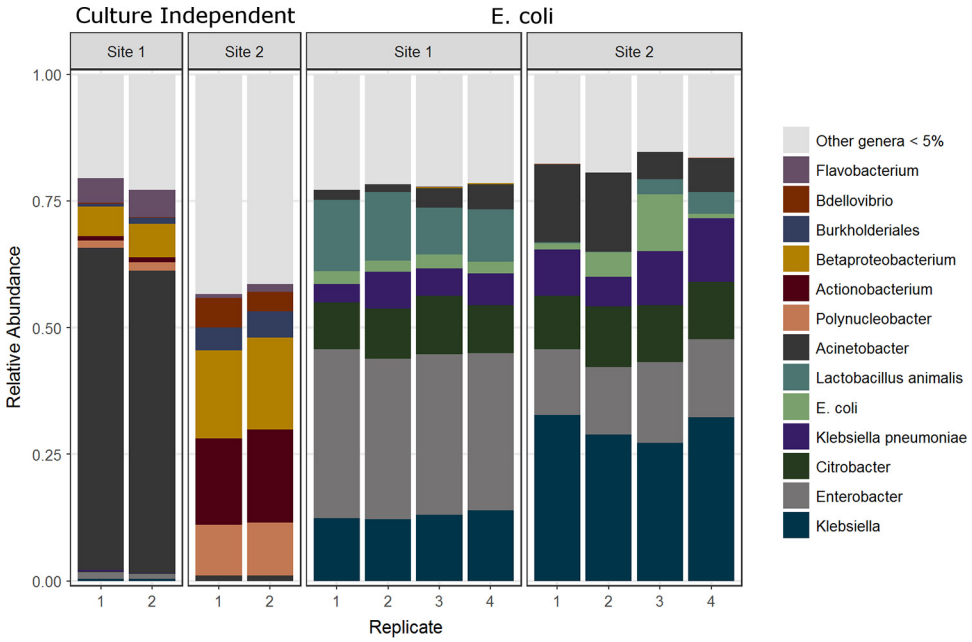

FIG 1 Microbial taxa from water collected at two sites along Hickey Run from DNA extractions of culture-independent and of 24-h *E. coli* enrichments.

manufacturers' specifications. Libraries were sequenced on an Illumina NextSeq 550 system using a NextSeq 500/550 high-output kit v2 (300 cycles, 2 × 150 bp).

Reads were trimmed with Trimmomatic (9), and preliminary taxonomic annotation was achieved using the CosmosID analytic pipeline (December 2018 database; Rockville, MD) (8, 10, 11). Phylotypes of *E. coli* were described using Center for Food Safety and Applied Nutrition (CFSAN) (FDA) in-house k-mer- and single-nucleotide polymorphism (SNP)-based pipelines (12). Additional boutique pipelines for description of protist species were used to annotate relevant eukaryotic species (13). Data from the 28 August 2018 sampling time point averaged 4.1 Gb (~27 million reads) per replicate.

The following bacterial taxa were observed (in order of relative abundance): *Acinetobacter* spp. (including *Acinetobacter junii*), *Bdellovibrio*, *Flavobacterium*, *Betaproteobacterium*, *Polynucleobacter*, *Burkholderidales*, *Enterobacter cloacae*, *Klebsiella* spp., *Lactobacillus animalis*, *Aeromonas caviae*, *Aeromonas hydrophilia*, and *Clostridium* spp (Fig. 1). As many as 34 official H and 112 official O antigens of *Escherichia coli* were observed, in addition to *Shigella sonnei wzx* and *wzy* genes. The following protists were described: *Dictyostelium citrinum*, *Thalassiosira*, *Paramecium biaurelia*, *Balantidium coli*, and *Balantidium hominis*, *Naegleria*, *Toxoplasma gondii*, *Saprolegnia parasitica*, *Chatonella*, and *Pseudo-nitzschia*.

Data availability. The USNA Microbial Observatory data from the 28 August 2018 collection time point are available under National Center for Biological Information (NCBI) BioProject number PRJNA498951 and SRA accession numbers SRR8126707 to SRR8126738. Data from subsequent time points will be added to the BioProject as completed.

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