ABSTRACT: Genomic and metagenomic techniques are being used to study temporal and spatial variability of bacterial and archaeal communities in the Hanford Site 300 Area in relation to hydrologic and geologic features. Although 16S rRNA analyses of groundwater microbial populations collected over a 9-month period from 300 Area wells has revealed dynamic microbial community membership, the environmental factors responsible for driving this behavior are less well understood. Shotgun metagenomic sequence has been collected from four wells extending across the breadth of the 300 Area Integrated Field Research Challenge area. Co-assembly of collected sequences yielded 11,119 contigs >5000bp in length. Contiguous assemblies were sorted into 105 putative taxonomic bins using emergent self-organizing maps of nucleotide composition data coupled with read abundance data. Taxonomic evaluation of the binned sequences is in good agreement with pyrotag analysis previously done on these samples. A metabolic reconstruction of the individual bins and the entire community is underway to elucidate microbial interactions in the community. Single cell genomic techniques were employed to identify metabolic roles for Pedobacter species that exhibited significant fluxes in relative abundance over this time period, ranging from 0-14% of the community. Cells were desorbed from Hanford formation sediments, isolated using Fluorescent Activated Cell Sorting (FACS), and then single-cell genomic DNA was amplified using Multiple Displacement Amplification (MDA). Screening of single cells via 16S rRNA analyses was used to identify four Pedobacter cells for further whole genome shotgun sequencing. Reconstructed genomes were annotated, and estimates of genome completeness were inferred from single-copy gene identifications; these values ranged from 20-75%. Further analyses of these microorganisms indicate that they are chemoheterotrophic, with the ability to degrade complex organic polymers such as xylan. In addition, they may tolerate more microaerophilic niches in the subsurface via the use of cytochrome cbb3-type terminal oxidases. These inferences suggest that possible fluctuations in organic carbon content in groundwater may play a role in driving abundance shifts in Pedobacter in the Hanford subsurface.

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