Extensive genomic sampling of subsurface microbes provides a new view of the phylogeny and metabolic potential of the bacterial Candidate Phyla OD1 and OP11

Christopher Brown (ctb@berkeley.edu) - UC Berkeley, Jillian F. Banfield (PI).

Our understanding of subsurface microbial communities is limited due to the predominance of members of candidate phyla, which lack cultivated representatives. In particular, members of the bacterial Candidate Phyla OD1 and OP11 are frequently identified in the subsurface. However, the extent of their phylogenetic diversity is largely unknown, and their metabolism inferred only from relatively sparse genomic sampling.

Groundwater microbial communities recovered on 0.2 and 0.1 µm filters from a well at the Rifle Integrated Field Research Challenge site were analyzed prior to and during an acetate amendment. Community genomic DNA was extracted from the filters, sequenced, and assembled. 16S rRNA gene sequences were recovered from assembled data and validated using read mapping-based curation methods. Insertions within 16S rRNA genes were discovered from reference DNA sequence and RNA structural alignments. All 16S rRNA genes were aligned and used to infer a phylogenetic tree. Metagenome scaffolds were binned into genomes based on taxonomic assignment, coverage, nucleotide composition, and time series abundance patterns, allowing for a robust metabolic analysis of uncultivated microbial community members.

Phylogenetic analysis of 1,513 assembled 16S rRNA genes identified ~350 sequence types from members of the OD1, and ~250 sequence types from OP11. On average, these sequences share only 87-88% similarity with reference sequences. Within the complete dataset, 37 bacterial sequences contain large insertions (~500 nucleotides), the majority belonging to the OD1 or OP11 radiations. Most large insertions encode an endonuclease and are predicted to contain either a group I or group II catalytic intron, suggesting that they are self-splicing. Complete genomes for four members of these phyla, along with numerous (250+) genome bins reveal an absence of respiratory pathways, CRISPR/Cas systems, polar flagella, and lipid A synthesis.

Recovery of 16S rRNA genes from groundwater-associated bacteria, and removal of insertion sequences, greatly improves resolution of the OD1 and OP11 phyla, enabling classification at deeper taxonomic levels. The current study greatly expands the phylogenetic distribution of large bacterial 16S rRNA insertion sequences (previously only observed in the Thiotrichaceae). Complete genomes and extensive genome binning confirm the small size of OD1 and OP11 genomes (<1 MB) and their fermentation-based metabolism, whereas enrichment in filtrates suggests small cell size. Future work will overlay the metabolism of additional phyla represented in this complex environment and, combined with time-series analysis, will improve our understanding of the biogeochemical roles of uncultivated subsurface microorganisms.