

**Pearl in the Mud: Genome Assembly
and Binning of a cold seep
Thiomargarita nelsonii cell and
Associated Epibionts from an
Environmental Metagenome**

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Sulfide-oxidizing *Thiomargarita*-like bacteria have been discovered in numerous locations on the ocean floor, including in oxygen minimum zone settings and at methane seeps. We used a genomic approach to investigate the physiology and ecology of *Thiomargarita nelsonii* that were growing on the shells of living gastropods from the Hydrate Ridge methane seep off of the coast of Oregon. The snail-attached cells appear to undergo an apparent dimorphism (elongate vs. budding) in their life cycle, elongating to almost a millimeter in length, and budding off spherical daughter cells. MDA amplification followed by Illumina sequencing of the daughter cells produced a metagenomic product that included both *T. nelsonii* and attached bacteria (epibionts). Metagenome reads were initially assembled and binned based on the tetranucleotide frequency of the resultant contigs. Then, the *Thiomargarita*-specific bins were further re-assembled using stricter assembly parameters and algorithms, and annotated via the Integrated Microbial Genomes pipeline.

The resulting draft genome shows evidence that *Thiomargarita nelsonii* has denitrification and ammonification pathways suspected to occur but not previously identified in genomes of large, vacuolated, sulfur-oxidizing bacteria. From the genome we were also able to construct a full sulfur oxidation pathway, and reveal enzymes present in the dataset thought to be responsible for the creation of the intracellular sulfur globules seen in *T. nelsonii*. The genes necessary for polyphosphate creation and hydrolysis were also observed. Polyphosphate metabolism is thought to play a role in the formation of phosphatic minerals that serve as important reservoirs in the marine phosphorous cycle. In addition to genes associated with carbon fixation, the *T. nelsonii* genome contains genes associated with an organoheterotrophic metabolism. As with genomes of the related sulfide-oxidizing *Beggiatoa*, the draft genome of *T. nelsonii* also contained substantial evidence for horizontal gene transfer with cyanobacteria.