

Elusive Freshwater Chemolithotrophs: Metagenome Assembled Genomes (MAGs) provide insight into Archaeal and Bacterial Nitrifiers from the Laurentian Great Lakes.

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Introduction: While historically understudied, freshwater lakes are important to global biogeochemical cycling. Lakes output roughly 6-16% of naturally occurring methane globally [1] more than all oceanic emissions, and they bury twice the organic carbon of the oceans [2]. Understanding the ecology and physiology of freshwater microorganisms is therefore paramount in understanding global carbon cycling.

The Laurentian Great Lakes provide a compelling system for investigation of freshwater environments and their microbiology. Their size allows ocean-like dynamics with two hundred year long residence time for Lake Superior [3] comparable to the Atlantic Ocean [4]. In comparison to many smaller inland lakes, the Great Lakes are oligotrophic, and tend to be less productive. Large lakes around the world, including the Great Lakes, have experienced changing nutrient stoichiometry over recent decades, leading to increased nitrogen availability and exacerbating phosphorus limitation [5]. Understanding how Great Lakes microbial ecology differs from smaller freshwater systems and from marine systems could provide insight into the rules that structure aquatic microbial communities.

Chemolithotrophs that utilize the conversion of ammonia to nitrite as a source of energy are major contributors to the nitrogen and carbon cycles. As ‘gatekeepers’ in the nitrogen cycle, ammonia oxidizing (AO) microbes catalyze the rate limiting step in the conversion from ammonia to nitrate. AO microorganisms, particularly AO archaea are also contributors to the carbon cycle, as their abundance within freshwater and marine systems [6] implicates them as major contributors to carbon fixation. Bacteria were long thought to be the dominant AO microbes, since discovered by Winogradsky in the late 19th century. But recent assessment of abundance of AO archaea and the pervasiveness of the archaeal monooxygenase gene in aquatic and soil systems has reversed this belief. Marine and soil AO archaea have been isolated in culture, supporting the creation of the Thaumarcheota phylum. Freshwater AO archaea have so far evaded pure culture, and so are less well understood.

Methods: Metagenome assembled genomes (MAGs) allow insight into gene content, metabolic potential and phylogeny of microorganisms that are not currently in culture. Community genome DNA was shotgun sequenced from surface samples collected from each of the five Laurentian Great Lakes on the

R/V Lake Guardian during spring 2012. MAGs were reconstructed from co-assemblies of the resulting metagenomes using the CONCOCT [7] binning algorithm and manual curation using Anvi’o visualization platform [8]. MAGs were annotated and genes were identified and sorted into pathways via Interproscan [9] and phyloresponder [10] was used to place MAGs on phylogenetic trees based on 15 ribosomal proteins.

Results: Here we examine MAGs of ammonia and nitrite oxidizing microorganisms recovered from Laurentian Great Lakes spring surface samples. MAGs assigned to bacterial Nitrospirae and Nitrosomonadaceae, as well as archaeal Thaumarcheota were investigated. The MAGs were found to be 91%, 98% and 64% complete with GC content of 40.49%, 57.81% and 31.81% respectively. Most interesting results came from the Thaumarcheota MAG. The presence of cobalamin biosynthesis and complete urea utilization pathways was intriguing, as both features are not conserved across Thaumarcheota. A nitrous oxide reductase was also found, suggesting nitrifier denitrification. Our Thaumarcheota MAG was found to be most closely related to *Candidatus Nitrosoarchaeum limnia*, a low salinity AO archaea isolated from San Francisco Bay, also the most interesting of the placements as the phylogenetic placement of freshwater AO archaea has not yet been established.

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