

GENOMIC ANALYSIS OF A HYPERALKALIPHILIC BACTERIUM IN AN ANTHROPOGENIC SERPENTINIZING SPRING ANALOGUE. J. I. Ohlsson¹, E. D. Becraft², S. M. Barmann³ and W. D. Swingle⁴, ¹Northern Illinois University, DeKalb, IL, USA, ²Bigelow Laboratory of Ocean Sciences, Boothbay, MN, USA; *Corresponding author: wswingle@niu.edu First author: johlsson@niu.edu

Introduction: In the pursuit of detecting life and habitats where life may form, it is necessary to know the physical limits of living organisms [1]. Microbes are known to grow at a variety of extremes on Earth, including freezing and boiling temperatures, high and low pressures, extreme salinity and highly corrosive pH. Acidophiles are well documented, and haloalkaliphiles – growing at alkaline pH by exploiting cations from available salts – have been studied to a lesser degree [2,3]. In recent years, bacterial species have also been characterized from hyperalkaline nonsaline sites, primarily serpentinizing springs [4,5,6]. These nonhalophilic alkaliphiles have to contend with pH above 10 without the readily available salt ions that haloalkaliphiles utilize. Unfortunately, current literature does not describe a physiological mechanism for extreme alkaline tolerance in freshwater systems.

This study focuses on a hyperalkaliphilic bacterium found in Indian Creek, Chicago, Illinois, USA [7] – a canal running through a steel mill slag landfill, which shows a surface water pH ≥ 13 near the main slag pile. The elevated pH and ubiquitous CaCO₃ precipitate at this anthropogenic pollution site mimics the conditions of serpentinizing springs, though the alkalinity is produced from weathering of the slag rather than groundwater interaction with olivine minerals. The species was first detected in ribosomal 16S RNA gene sequences obtained from sediment samples and classified as belonging to the Betaproteobacterial genus *Hydrogenophaga*. Recently several related species have been identified in natural serpentinizing spring systems [4,5,6].

Results: The Indian Creek *Hydrogenophaga* genome was reconstructed from metagenomic shotgun sequence using machine learning-based binning in WEKA [8] and Anvi'o [9], followed by *de novo* genome assembly in SPAdes [10]. This yielded a candidate genome of 255 contigs totaling 2.4 Mbp, which matches the chromosome size of related sequenced species, and contains 100% of a set of 139 conserved single-copy genes, signifying a complete core bacterial genome.

Bioinformatic analysis of the candidate genome via automatic annotation using RAST [11] and KAAS [12] reveals an apparent lack of the Na⁺/H⁺ antiporter proteins responsible for intracellular pH regulation in haloalkaliphiles [2], and other proteins implicated in

bacterial alkaline resistance are being investigated. Comparison with haloalkaliphilic genomes suggests that nonsaline alkaliphiles have novel methods of alkaline resistance.

Outlook: Characterization of this novel steel slag *Hydrogenophaga* will yield insight into the metabolic capacity of a major constituent of the most alkaline microbial habitat recorded to date. It highlights the need for a more detailed understanding of bacterial adaptation to hyperalkaline conditions. Describing its non-Na⁺-based alkaline resistance will have implications both for the study of microbes in high-pH systems on Earth and for the detection of extraterrestrial environments suitable to the evolution of bacteria-like life.

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