

‘OMICS EXPLORATIONS OF DEEP HYDROCARBON HYDRATES. J. B. Glass¹, C. B. Kretz¹, J. Wu², P. Ranjan², D. Tsementzi³, K. Konstantinidis³, F. J. Stewart², B. L. Nunn⁴. ¹School of Earth and Atmospheric Sciences, ²School of Biology, ³College of Civil and Environmental Engineering, Georgia Institute of Technology, Atlanta, GA; ⁴Department of Genome Sciences, University of Washington, Seattle, WA. Correspondence: Jennifer.Glass@eas.gatech.edu

Introduction: The deep biosphere hosts what is arguably Earth’s largest reservoir of uncultivated microbes with potentially novel metabolisms. Methane serves as both a substrate for, and a product of, anaerobic microbial metabolisms in the anoxic subsurface. Continental margin sediments harbor thousands of gigatons of methane in frozen gas hydrates, possible analogs for hydrocarbon-based habitats on icy worlds.

Atribacteria. Microbial communities associated with methane hydrates are distinct from other deep sediment microbiomes, with particular enrichment in members of the JS-1 clade of the bacterial candidate phylum Atribacteria [1, 2]. Genomes of uncultivated terrestrial Atribacteria suggest that they are strict anaerobes that ferment sugars (OP-9 clade [3]) or organic acids (JS-1 clade [4]) to acetate and ethanol. Both OP-9 and JS-1 lineages have an outer membrane, in contrast to their closest phylogenetic relatives, the gram-positive Firmicutes. To date, only one partial genome (estimated 15% completeness) exists of a marine sediment Atribacteria bacterium [2]. Here, we report on the potential function of JS-1 bacteria that co-occur with methane hydrates.

Methods: We performed metagenomic and metaproteomic sequencing from 2–69 mbsf beneath Hydrate Ridge, offshore Oregon, at ODP Leg 204 Site 1244 (890 m water depth), where the gas hydrate stability zone extended from 45 to 124 mbsf. Geochemistry and 16S rRNA microbial community analysis were reported previously [5].

Results: The JS-1 genomic bin recovered from the deep subsurface core within the gas hydrate stability zone (E10-H5, 69 mbsf) had an estimated completeness of 69%, 2% contamination, and 35% GC content, similar to other partial JS-1 genomes (35-38% [2]). The partial genome contained one *rpoB* gene with 95% similarity to Atribacteria bacterium 34_128 from an oil reservoir [6]. It contained numerous tripartite ATP-independent periplasmic (TRAP) transporters, as well as amino acid transporters and peptidases. Limited sequences were recovered from metaproteomic sequencing almost all were transmembrane transport proteins. Expressed peptides includes a hit to an H⁺-translocating inorganic pyrophosphatase found on an operon likely involved in fermentation of aromatic amino acids to butyrate. These data suggest that deep subsurface Atribacteria associated with methane hydrates may perform metabolisms not yet found in the genomes of their near-surface and terrestrial relatives.

References: [1] Inagaki F. et al. (2006) *PNAS*, 103, 2815-2820. [2] Carr S.A. et al. (2015) *Front Microbiol*, 6, 872. [3] Dodsworth J.A. et al. (2013) *Nature Comm*, 4, 1854. [4] Nobu M.K. et al. (2015) *ISME J*, 10, 273-286. [5] Kretz C.B. et al (2015) *AbSciCon* 7208. [6] Hu P. et al (2016), *mBio*, 7, e01669-15.

Dedication: This study is dedicated to the memory of Katrina Edwards (1968-2014).

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