

Unusual metabolic strategies identified in a hyperalkaliphilic microbial community associated with the serpentinization

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Serpentinization is a process whereby water reacts with ultramafic mantle rock (peridotite) to produce a new suite of minerals (including serpentine), ultrabasic fluid, molecular hydrogen and, under some conditions, reduced carbon in the form of methane. Serpentinizing systems are viewed as potential analogs for both early Earth and icy moons in the universe (e.g. Enceladus and Europa) where water was (is) present and highly reducing mineralogy was (is) likely widespread in an undifferentiated crust. Given that hydrogen is formed through the reactions, the systems have been implicated as locations that may have supported early microbial life on Earth and other stars.

A thermodynamic analysis based on the chemical disequilibrium has been applied to examine the habitability of serpentinization-associated settings, and the analysis has identified the potential microbial energy metabolisms that must allow microbial life to gain sufficient energy from the settings. However, since the Earth systems, including serpentinizing sites, are very complexed such types of analysis have the limitation to predict which habitable niches in the system are actually occupied by life. Namely, the varied geological structures and the heterogeneous distribution of mineral deposits create various chemical and physical gradients even in a given serpentinizing system (e.g. temperature, pH, redox potential, pressure, concentration of hydrogen, methane and other compounds including undetectable compounds at the surface of Earth), thus predicting microbial habitats only from geochemical, geological and mineralogical characteristics seen at the surface of the Earth is extremely difficult.

To identify the major microbial metabolic strategies that are actually functioning in the natural serpentinizing system, we have been focusing on analyzing the microbial communities that are associated with the site of active serpentinization in northern California, named The Cedars^{1,2}. The spring waters discharging from The Cedars serpentinization site feature extremely alkaline (pH = ~12), super reducing ($E_h = \sim -700$ mV) and have relatively high concentrations of Ca^{2+} , hydrogen and methane due to the active serpentinization reactions³. The spring waters are meteoric water in origin containing low concentrations of dissolved organic and inorganic carbon and undetectable levels of ammonium, phosphate and electron acceptors (dissolved oxygen, nitrate and sulfate). While plenty of hydrogen (energy source) has been continuously supplied to the system by the serpentinization reaction, combination of extremely high pH, lack of oxidants (electron acceptors), carbon and phosphorus sources creates a challenging environment for life to thrive, thus extremely low numbers of cells are present in the spring waters.

Here, I present the results of metagenomes, recovered draft genomes and metatranscriptomes from The Cedars serpentinization system⁴. The comprehensive omics analyses indicated that the serpentinizing system supported a community that was remarkable in its unprecedented metagenomic and genomic constitution and many of metabolic capabilities identified here were different from what we had predicated from the geochemical features of this system; The genomes of dominant organisms affiliated to the phylum OD1 were enigmatic, being very small, and lacking a number of genes needed for independent life, including ATP synthase, early-stage glycolysis genes, respiration-related genes, and key biosynthetic genes. The genome of the *Methanosarcinales* from this system also showed unusual features that have never been identified in the other methanogenic archaea. Many facts from the omics study

indicated that life strategies functioning in the serpentinizing system still remains elusive, thus this and perhaps other extreme settings for life may provide special opportunities for expanding the knowledge of the limitation and potential of the microbial habitability on Earth and other stars.

¹ Suzuki et al. (2013) PNAS 110 (38) 15336-15341.

² Suzuki et al. (2014) Nature Commun. 5, 3900

³ Morrill et al. (2013) GCA 109, 222-240

⁴ Suzuki et al. (2017) ISMEJ 11, 2584-2598

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