

Mineral-Microbe interaction –Identification of extracellular electron transfer mechanisms by using MetaOmics approach

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Microbial reduction of solid minerals such as iron-oxides and manganese-oxides is known to occur as a result of microbial extracellular electron transfer (EET). These electron transfer activities coupled with the oxidation of organic carbon or hydrogen are considered to play important roles within complex microbial communities in soil, sediment, and the deep subsurface environments. The surface redox potentials of solid-phase oxide minerals are known to be highly variable because of variations in the crystal structure, pH, salinity, and levels of hydration, posing major challenges for EET-capable microbes to adapt in the habitats. In fact, the *in situ* redox potentials of solid-state electron acceptors have been regarded as significant geochemical variables linked to the regulation of functional genes in the EET-capable microbes. However, mechanisms that the microbes use to perform respiration of insoluble metal-oxide minerals showing different surface redox potentials are complex and are not yet well understood.

Here, we used bioelectrochemical systems to systematically evaluate the effects of surface redox potentials (e.g. +100 mV, -50 mV, -200 mV vs SHE) on EET-active microbial community development and the overall electron transfer rates. The results clearly indicate that faster biocatalytic rates were observed under electropositive electrode surface potentials than the electronegative potentials. Temporal 16S rRNA-based microbial community analyses showed that *Geobacter* phylotypes were highly diverse and apparently dependent on the surface potentials. The well-known dissimilatory metal-reducing microbes affiliated with *Geobacter metallireducens* clade were associated with lower surface potentials and less current generation, whereas *Geobacter* subsurface clades were associated with higher surface potentials and greater current generation. These results suggest that surface potentials provide a strong selective pressure, at the species and strain level, for solid mineral respirators throughout the EET-active community development.

Next, we show metabolic and transcriptional responses of the EET-active microbial communities established on the three different poised electrodes to changes in the surface redox potentials. Combination of genome-centric stimulus-induced metatranscriptomics and metabolic pathway investigation revealed that nine *Geobacter* / *Pelobacter* microbes performed EET activity differently according to their preferable surface potentials and substrates. While the *Geobacter* / *Pelobacter* microbes coded numerous numbers of multi-heme c-type cytochromes and conductive e-pili, wide variations in gene expression were seen in response to altering surrounding substrates and surface potentials, accelerating EET via poised electrode or limiting EET via an open circuit system. These flexible responses suggest that a wide variety of EET-active microbes utilizing diverse EET mechanisms may work together to provide such EET-active communities with an impressive ability to handle major changes in surface potential and carbon source availability.

Conclusively, these results presented here offer a new perspective on the complexity of the EET-active members in the microbial communities that were adapted to the different surface redox conditions, and some initial insights into how they may regulate community function and provide adaptability to the different minerals on the earth. Overall, these results have opened a door toward unraveling the diverse microbial life within the various geological settings especially in the mineral-rich subsurface environments.

Keywords: Metatranscriptomics, Electromicrobiology, Redox potential