

Microbial Coenocline Associated with Geochemical Gradients at a Groundwater Discharge Zone

V.L. SHIROKOVA^{1*} AND F.G. FERRIS²

¹ University of Toronto, Toronto, Canada, vshi@geology.utoronto.ca

² University of Toronto, Toronto, Canada, grant.ferris@utoronto.ca

Abstract

A combined metagenomic, geochemical, and statistical investigation was used to characterize a groundwater spring with a dramatic electrochemical gradient. Eh, pH, precipitated iron, and sulfate concentration increased downstream of the source. The ferrihydrite saturation index, as well as ferrous iron, ferric iron, sulfide, ammonium, and nitrite concentration decreased downstream. A total of 672 clones were compiled into a cDNA library, with taxonomic identities across 9 phyla, including *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Chlorobi*, *Chloroflexi*, *Firmicutes*, *Nitrospirae*, *Planctomycetes*, and *Proteobacteria*. The variation in the relative abundance of the OTU phyla in response to geochemical parameters was modeled using coenoclines. *Alpha-proteobacteria* were the most sensitive to geochemical change, while *Acidobacteria* were least sensitive. Spearman's rank correlation coefficients were used to evaluate the relationship between geochemical variables and relative abundance of microbial OTU. Reduction-oxidation potential appears to be an overarching parameter in the stream, controlling the distribution of redox species and leading to strong segregation of microbial functional groups. Our study of Ogilvie Creek represents one of the first to combine geochemical, metagenomic, and statistical analysis in an effort to gain insight on the interrelationships between biogeochemical processes in natural systems.

Determining reactive thiol concentration in naturally occurring organic molecules

ELIZABETH M. SHOENFELT¹, CLARESTA M. JOE-WONG^{2*}, NYSSA M. CROMPTON³, EMILY A. JAYNE³, SATISH C. B. MYNENI³

¹ Department of Geosciences, Princeton University, Princeton, USA, eshoenfe@princeton.edu

² Departments of Geosciences & Chemistry, Princeton University, Princeton, USA, cmjoe@princeton.edu (*presenting author)

³ Departments of Geosciences & Chemistry, Princeton University, Princeton, USA

Thiols, components of soil and aquatic organic molecules, exhibit high affinity for soft Lewis acids such as Cd²⁺ and Hg²⁺ and play an important role in metal speciation in natural waters. However, thiols' low abundance, high reactivity, and structural similarity to other reduced-S ligands such as methionine make their detection difficult. We propose a novel method to measure thiol concentrations in natural systems using the water soluble, charged, thiol-sensitive fluorophore monobromo(trimethylammonio)bimane (qBBr), which fluoresces upon binding to a thiol. Once the sample's natural fluorescence is subtracted out, a solution's fluorescence intensity is proportional to the concentration of thiol-bound fluorophore. By measuring the fluorescence intensities of a series of solutions with fixed sample concentration and increasing fluorophore concentrations, the saturation point can be calculated, giving the sample's thiol concentration. This method accurately estimates thiol concentrations in pure thiol-containing solutions, dissolved organic matter (DOM), and microbial cell membranes. Moreover, the presence of other chemical species often found in natural systems does not influence this method's sensitivity.

When pure glutathione or cysteine solutions were examined, this method produced an estimated thiol concentration within 1.85% of the expected value at micromolar concentrations, and within 7.40% at nanomolar concentrations. Although species such as dissolved salts, carboxylic acids, and other organo-sulfide groups might affect the absolute fluorescence intensity of the thiol-bound fluorophore, we found that these species do not interfere with determining the thiol concentration. Testing glutathione solutions with dissolved magnesium chloride in 500-fold excess resulted in calculated saturation values accurate to 6.40%. Likewise, adding up to 15-fold excess malate, a surrogate for carboxylic acids common in DOM, does not affect accuracy. Similar tests show that qBBr does not react with other sulfur groups such as disulfides and thioesters.

Using the technique developed for model systems, thiol concentrations in the DOM pool and microbial biopolymers can be calculated. The cell membrane thiol concentrations of three model microorganisms common to natural systems—*Bacillus subtilis*, *Shewanella oneidensis*, and *Geobacter sulfurreducens*—were estimated. *G. sulfurreducens* exhibited the highest thiol concentration, and *B. subtilis* the lowest. The calculated thiol:dissolved organic carbon ratio in surface water DOM from the Pine Barrens in New Jersey is approximately 10⁻³-10⁻⁴. Estimation of thiol concentration is necessary to understand the role of thiols in contaminant and nutrient speciation and transformation, and this method offers a simple way to measure thiol concentrations in natural systems.