

Cytochrome c oxidase, NADH dehydrogenase, and manganese superoxide dismutase in photosynthetic outflow channels of continental hydrothermal springs

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Metals, such as copper (Cu) and manganese (Mn), are key reaction centers for many enzymes, and microbes must acquire and incorporate them from their environment. For hydrothermal systems, water-rock interactions dictate metal abundance. Thus, the presence of metal dependent enzymes and the microorganisms harboring them may be directly influenced by vent chemistry. We created shotgun metagenomes from four hydrothermal photosynthetic outflow channels (POCs) in Yellowstone National Park, “Happy Harfer” (HH), Boulder Spring (BS), and Rabbit Creek - green (RCG) and orange (RCO), that ranged in temperature from 47-68°C and pH from 6.5 - 9.3. We focused on three key aerobic-enzyme complexes, cytochrome c oxidase (COX) and NADH dehydrogenase, both Cu-binding enzymes, and manganese superoxide dismutase (MnSOD). COX and NADH dehydrogenase enzymes are essential in aerobic respiration as electron acceptors and donors [1, 2]. Using BLAST, we identified the COX sub unit-I and -II from *Synechococcus lividus* PCC 6715 (*S. lividus*) and *Chloroflexus aurantiacus* (*C. aurantiacus*) in HH and RCG at > 99.5% (*S. lividus*) and > 93% (*C. aurantiacus*) identity but absent in BS and RCO. NADH dehydrogenase from *S. lividus* in HH and RCG had an identity of 99.4% and < 73% in BS and RCO. Interestingly, the NADH dehydrogenase from *C. aurantiacus* was present in all locations except RCO. We detected the MnSOD enzyme, essential for mitigating oxidative stress [3], from *S. lividus* at 99.6% identity in all POCs except RCO. Notably, Cu and Mn concentrations remained below detection limits, 1.14×10^{-7} and 1.82×10^{-7} M respectively; except for HH, where the Mn concentration was $5.07 \pm 0.29 \times 10^{-7}$ M. Despite low metal concentrations, these enzymes using Cu and Mn are crucial for *S. lividus* and *C. aurantiacus* metabolism. Bioavailability is influenced by aqueous geochemical parameters such as pH which affects Cu and Mn speciation. This work suggests that microbial metabolism is strongly affected by the metallome and that trace metal chemistry is necessary to understand microbial abundance and distribution.

[1] Michel *et al.* (1998) *Annu. Rev. Biophys. Biomol. Struct.*, 27(1), 329-356. [2] Heikal *et al.* (2014) *Mol. Microbiol.*, 91(5), 950-964. [3] Sheng *et al.* (2014) *Chem. Rev.*, 114(7), 3854-3918.