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Investigating iron oxidation marker genes in isolates and microcosms

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Iron oxidizing microorganisms play important roles in environmental biogeochemical cycling and industrial processes. To understand and harness these roles, we need to know the genes/pathways involved. Iron oxidizers use extracellular electron transfer (EET) pathways to oxidize iron at the cell surface; EET allows for oxidation of solid substrates, including iron minerals like magnetite and non-ferrous substrates like biochar. We investigated iron oxidation/EET pathways via a combination of isolate and environmental studies. We showed that *Sideroxydans lithotrophicus* ES-1 can grow on dissolved iron, ferrous minerals (smectite and magnetite), thiosulfate or biochar as electron donors [1-3]. Gene and protein expression data support the involvement of *cyc2*/Cyc2 in oxidation of dissolved iron while *mtoAB*/MtoAB is expressed for oxidation of solid substrates (Fe minerals and biochar), suggesting that multiheme cytochromes are important for oxidizing solid substrates. Another isolate, *Leptothrix cholodnii* SP-6 does not encode *cyc2* and *mto* genes, giving the opportunity to study novel iron oxidation pathways. Proteomic analyses suggest involvement of a large porin-cytochrome complex (PCC3), a set of periplasmic cytochromes (Irc), and possibly multicopper oxidases (MCOs) previously only implicated in manganese oxidation. We aim to apply our knowledge of iron oxidation genes to detecting iron oxidation in the environment; to test this, we performed transcriptomics of iron microbial mat microcosms, which include environmental representatives from *Sideroxydans* and *Leptothrix*, and other less-characterized iron mat inhabitants. The microcosms showed strong upregulation of iron oxidation genes, including *cyc2* and *mtoA,* after addition of Fe(II), giving further evidence that these genes are involved in environmental iron oxidation. We also aim to apply our understanding of iron oxidation to broader applications. The products of iron oxidation are iron oxyhydroxide minerals with affinity for other metals and elements, including rare earth elements (REEs). Understanding the full spectrum of iron oxidation mechanisms gives us options for using these enzymes in REE recovery and as structural support within engineered living materials.

[1] Keffer, Zhou, Rushworth, Yu & Chan (2025), *Appl. Environ. Microbiol.* 91, e01865-24.

[2] Zhou, Kupper, Catalano, Thompson & Chan (2022), *Environ. Sci. Technol.* 56, 17443–17453.

[3] Zhou, Keffer, Polson & Chan (2022), *Appl. Environ. Microbiol.* 88, e01595-21.