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Unravelling *Sideroxydans lithotrophicus* ES-1 Fe(II)-oxidizing pathway using transcriptomics and RT-qPCR

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Microaerophilic iron-oxidizing bacteria (FeOB) may catalyze most of the Fe(II) oxidation in suboxic circumneutral environments and connect iron and carbon cycling. Most known neutrophilic FeOB are obligate Fe(II)-oxidizers, and thus, the specificity of genes and proteins involved in the Fe(II) oxidation mechanism is not well-resolved. *Sideroxydans lithotrophicus* ES-1 contains genes to oxidize different substrates, including Fe(II) (*mtoA*, three *cyc2* gene copies) and sulfur (*sox*, *tsd*, *dsr*), making it an ideal model to study the genes that are specifically involved in Fe(II) oxidation. In our work, we performed transcriptome sequencing on ES-1 grown on either Fe(II) or thiosulfate at different growth phases to investigate the long-term response to substrates. Moreover, we also investigated ES-1's response to a short-term switch from thiosulfate oxidation to Fe(II) oxidation in a time-series within 90 min. Transcriptomic and RT-qPCR data showed all *cyc2* gene copies were expressed orders of magnitude higher than *mtoA*. The three *cyc2* genes were all Fe(II) responsive but differed in their responses in the long and short term experiments. Furthermore, Fe(II) oxidation by ES-1 corresponded to upregulation of reverse electron transport and carbon fixation-related genes, notably alternative complex III and RuBisCo genes. We also identified potential periplasmic electron carriers and a novel cytochrome-containing gene cluster that was Fe(II) responsive. These results demonstrate *cyc2* plays an important role in FeOB metabolism and can be used to indicate FeOB activity and the Fe(II) oxidation potential. However, to demonstrate Fe(II) oxidation activity may require examination of the full pathway which includes electron transport and CO_2 fixation. Our study updated the ES-1 Fe(II) oxidation pathway and provides perspectives on detecting environmental microbial Fe(II) oxidation activities in environments.