

## **Meta'omics reveal insights into microbial Fe(II) oxidation coupled to nitrate reduction in freshwater enrichment cultures**

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Nitrate-dependent iron(II) oxidation (NRFeOx) has been recognized as an environmentally important process in many freshwater ecosystems. However, examples of autotrophic NRFeOx bacteria are very rare and their physiology and metabolic mechanisms underlying the process are still largely unknown. For two autotrophic enrichment cultures, culture KS and the novel culture BP, both originating from a freshwater pond in Bremen, Northern Germany, meta'omics (metagenomics, metatranscriptomics and metaproteomics) was applied to study metabolic pathways involved in NRFeOx under both heterotrophic and autotrophic conditions. Metagenomics was used to assemble draft genomes, including a genome of the potential key Fe(II) oxidizer in culture KS, affiliating with *Gallionellaceae*. Under autotrophic conditions, metatranscriptomics and metaproteomics revealed 9084/1801 significantly up-/down-regulated transcripts and 74/618 significantly up-/down-regulated proteins of culture KS. Under autotrophic conditions, upregulated transcripts of *Gallionellaceae* were involved in metal oxidation (e.g. *mtaA*, *mtbB*), denitrification (e.g. *napGHI*, *nirK*), carbon fixation (e.g. *rbcL*) and oxidative phosphorylation (e.g. respiratory chain complexes I, II, III, IV, V). These results suggest that *Gallionellaceae* in culture KS couple nitrate reduction to Fe(II) oxidation while fixing CO<sub>2</sub>. In addition, the findings point towards a microaerophilic lifestyle, yet *Gallionellaceae* is surviving under anoxic conditions. Current metagenomic analysis of culture BP help to reveal metabolic mechanisms of another *Gallionellaceae* organism which possesses different denitrification genes (e.g. *nirS*, *norBC*). While genes homologues of *mtaA* and *mtbB* were so far not detected in culture BP, metatranscriptomics and metaproteomics might reveal metal oxidation mechanisms. This study uncovers survival strategies of NRFeOx in freshwater ecosystem.