

## Mutualism in Fe-cycling co-cultures is more driven by interspecies signaling than Fe processing

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The coexistence of microaerophilic Fe-oxidizing and anaerobic Fe-reducing microbes in habitats with fluctuating redox conditions is a prime example of mutualism in nature, where both organisms profit from the Fe produced by the partner. However, co-cultivation of these microbes is very challenging due to their contrasting growth requirements. To elucidate the interaction mechanisms of two model Fe-cycling microbes, *Sideroxydans* CL21 and *Shewanella oneidensis*, we designed a series of cell-free supernatant exchange and liquid co-culture batch experiments. We found that activity and growth of both species were enhanced when exposed to the exometabolome of the partner, such that rates of *Sid.* CL21 Fe oxidation increased 28%, rates of *S. oneidensis* Fe reduction increased 50%, and 16S rRNA gene copies increased 1-2 orders of magnitude, respectively. Liquid co-culture batch incubations showed growth of both organisms was stimulated when grown in contact with its partner, with 16S rRNA gene copies increasing 1-2 orders of magnitude over 6 days in single species and co-culture incubations. We also observed specific changes in the transcription expression patterns of co-cultures compared to single species incubations. RNAseq analyses of these incubations revealed 14-20% of total genes were differentially expressed (DEGs) in *Sid.* CL21 and *S. oneidensis*, respectively. The overall transcriptome profiles indicated that only a few genes involved in Fe-cycling were upregulated in both species when grown in co-culture. Instead, the most upregulated DEGs in *Sid.* CL21 were involved in biopolymer and lipoprotein transport, while genes involved in biosynthesis of flagella, hydrogenases, and dehydrogenases were downregulated. The most upregulated DEGs in *S. oneidensis* were involved in tungstate and zinc transport and in the synthesis and degradation of putrescine, a signalling molecule, that was also identified using targeted metabolomics in supernatant exchange cultures. Furthermore, untargeted metabolomic profiling revealed that *S. oneidensis* shapes the metabolome of the co-culture. Taken together, our results show that interactions of different functional groups that depend on each other's end product is orchestrated by signalling molecules stimulating inter-species biofilm formation during Fe-cycling in nature.