

Metabolomic profiling of *Shewanella oneidensis* MR-1 and *Sideroxydans* CL-21 grown in co-culture

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Microbial-mediator interactions are thought to greatly influence a variety of redox active metabolic processes between iron-oxidizing bacteria (FeOB) and iron-reducing bacteria (FeRB) in natural environments, however, the exact mechanisms through which these interactions occur are not fully understood. To gain insight into the complex nature of microbial co-existence, we seek to identify chemical mediators that may elicit an advantage when mixed populations of FeOB and FeRB that rely on each other's product formation co-occur in natural environments with fluctuating redox conditions. Supernatant exchange experiments were used to elucidate the effects of chemical mediators produced by either the autotrophic, microaerophilic, FeOB *Sideroxydans* CL-21 or the heterotrophic, FeRB *Shewanella oneidensis* MR-1 on each other. Addition of *S. oneidensis* cell-free supernatant to *Sideroxydans* CL-21 batch cultures increased Fe(II) oxidation rates (6.31 $\mu\text{M hr}^{-1}$) in comparison to control incubations (2.83 $\mu\text{M hr}^{-1}$). Interestingly, *S. oneidensis* MR-1 Fe(III) reduction rates in incubations supplemented with *Sideroxydans* CL-21 cell-free supernatant (9.42 $\mu\text{M hr}^{-1}$) were only slightly enhanced compared to control incubations (7.88 $\mu\text{M hr}^{-1}$). Comparative metabolomic profiling led to the identification of four potential chemical mediators. In an effort to understand the impact of exposure to the partner's metabolome, current work involves pinpointing which of our candidate compounds functions as the chemical mediator and subsequent bioassays to test how these microbes can take advantage of their partner's metabolome. To further understand advantages of concomitant growth, we established working co-culture systems with the *Sideroxydans* CL-21 and *S. oneidensis* MR-1 growing under an atmosphere of 78%N₂:20%CO₂:2%O₂ with constant flushing. 16S rRNA gene copy numbers of *S. oneidensis* MR-1 were enhanced 2.5-fold when grown in co-culture with *Sideroxydans* CL-21 during the first 6 days of incubation, thus implying physical contact between the FeOB and FeRB positively influences the growth. While we can infer that *S. oneidensis* and *Sideroxydans* CL-21 depend on each other in co-culture conditions because they need each other's Fe products, heat maps of comparative metabolomes show the occurrence of specific compounds unique to co-culture conditions. Transcriptome analyses of our co-culture system are currently underway to determine how mediators alter the redox active metabolic process via activation or deactivation of specific gene clusters as well as mediator production and uptake.