Sticking together: Inter-species aggregation of bacteria isolated from iron snow is controlled by chemical signaling

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Small groups of iron oxidizing bacteria (FeOB) and iron reducing bacteria (FeRB) dominate pelagic iron-rich aggregates ('iron snow') formed at the redoxcline in an acidic lignite mine lake. It was suggested that FeRB profit both from the carbon fixed by the FeOB and from the presence of the newly formed Fe(III)-minerals in the iron snow. Since the iron snow sinks rapidly through the water clumn, the FeRB have limited time to find their carbon and energy source. Thus, we tried to elucidate the potential chemical interaction between FeOB and FeRB within iron snow. We isolated Acidithrix, the dominant FeOB, and Acidiphilium, the dominant heterotrophic FeRB, from iron snow aggregates and investigated interspecies chemical cross talk through cell-free supernatant exchange experiments and metabolomics profiling to elucidate potential allelochemicals mediating their interaction. Supernatant exchange experiments revealed supplementation with *Acidiphilium* cell-free supernatants resulted in faster rates of Fe(II) cunsumption (from 3.39 mM day⁻¹ to 5.72 mM day⁻¹) as well as precipitation of brownish, insoluble Fe(III)-minerals (schwertmannite) in Acidithrix incubations. In addition, macroscopic cell aggregates of Acidiphilium were observed after 5 days following supplementation with Acidithrix cell-free supernatant. GC/MS analysis of bacterial extracellular products and metabolomics profiling suggested that an arylamine compound produced by Acidithrix triggers aggregation of Acidiphilium cells. Acidiphilium cultures supplemented with the arylamine triggered faster growth and cell aggregation, suggesting the arylamine produced by Acidithrix functions as the allelochemical which signals *Acidiphilium* to associate with iron snow. These results indicate interspecies chemical interactions between key organisms in pelagic iron snow aggregates can help the organisms to colonize, shape and transform the distinct spatial network they inhabit within the redoxcline.