2-phenethylamine (PEA) induces aggregation in *Acidiphilium* strains

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Iron-rich pelagic aggregates, also known as "iron snow", are hot spots for microbial interactions [1]. Fe(III)-reducers (Acidiphilium C61 and Acidocella C78) and Fe(II)-oxidizers (Acidithrix C25 and Ferrovum sp.) make up 49% of the total microbial community of iron snow collected from acidic coal mining lakes. These isolates can be used as model organisms to study microbial communication. 2-phenethylamine (PEA), a signalling molecule produced by Acidithrix C25, was previously shown to trigger aggregate formation in Acidiphilium C61 [2], however, its effect on other iron snow organisms, and the mechanisms of PEA-induced aggregation remain unclear. In this study, we tested the effects of increasing concentrations of PEA on different strains of Acidiphilium and on other members of the iron snow microbial community (Acidphilium C61, Acidocella C78, and Ferrovum sp.). Among all microorganisms tested, only Acidiphilium sp. formed aggregates. Based on literature studies, we hypothesized that PEA might be involved in the downregulation of the flagella biosynthesis. Interestingly, comparative RNAseq analysis of Acidiphilium C61 incubations with and without PEA showed the genes involved in flagella biosynthesis and activity were among the most upregulated differentially expressed genes (DEGs). Additionally, genes involved in energy production, respiration, and genetic processing were upregulated in the presence of PEA, thus implying that PEA potentially led to increased ATP formation, electron transfer, and ultimately enhanced growth of increases in cell growth of Acidiphilium C61 cells. Quantitative PCR of the bacterial 16S rRNA genes could show a slight increase in gene copies among Acidiphilium C61 cultures with increasing exogenous concentrations. Thus, our results show that the aggregation effect of PEA on the tested Acidiphilium C61 is not associated with flagellar motility but rather may be due to growth related factors.

[1] Lu *et al.* (2013). Appl Environ Microbiol, **79**, 4272–
4281. [2] Mori *et al.* (2017). ISME Journal, **11**, 1075–1086.