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Effects of Metallophores on Nitrogen Fixation by Methanogens using Mineral-associated Metals as cofactors

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Metallophores are small chemicals produced by microorganisms to acquire metals from proximal environments, either by directly complexing with dissolved metal or promoting solid metal-bearing mineral dissolution. In terms of nitrogen fixation, certain N_2-fixing bacteria, such as *Azotobacter vinelandii*, are known to produce metallophores to aid in the uptake of molybdenum (Mo) and iron (Fe) which are essential for nitrogen fixation. However, despite the presence of genes associated with potential metallophore production in several methanogenic species, the production of metallophores by methanogens, which are recognized as the earliest microbes capable of nitrogen fixation, has not been readily detected. Moreover, whether methanogens are able to use external metallophores for their nitrogen fixation remains uncertain, even though methanogens often coexist with other metallophore-producing microorganisms in natural environments.

Therefore, in this study, the model methanogen, *Methanoscrcina Acetovorans*, was cultured with two types of metallophores, catecholate protochelin and hydroxamate Desferrioxamine-B (DFOB), and molybdenite/basalt to investigate the effects of metallophore presence on growth and nitrogen fixation of methanogens with minerals as trace metal sources. Our preliminary results confirmed that *M. Aceticorans* can utilize Mo from molybdenite and trace metals from basalt, respectively, to benefit cell growth as well as nitrogen fixation. When molybdenite was provided as the sole source of Mo, the addition of either 100 μM protochelin or DFOB significantly delayed cell growth, whereas 10 μM protochelin or DFOB did not affect cell proliferation and nitrogen fixation. In contrast, at either concentration, protochelin or DFOB did not promote cell growth and nitrogen fixation when basalt was given as the only source of trace metals. This might be a combined result of metallophore-promoted mineral dissolution with subsequent metal release, possible metallophores toxicities, and metallophore complexation with dissolved metals (metal-metallophore complexes might not be directly usable by cells). This study provides insights into the metal acquisition from solids to fix nitrogen by methanogens collaborating with metallophore-producing microbes, and the evolution of nitrogen fixation from understanding the interaction between methanogens and metallophores.