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Viable metabolisms in a simulated martian chemical environment

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Introduction: Liquid water may have been present on the surface of Mars during the Noachian. However, on modern day Mars water could only persist as brines in the near subsurface (Martín-Torres et al. 2015, Chevrier and Rivera-Valentin 2012, McEwen 2011, Ojha et al. 2015). The local lithologies would exert a strong effect on the chemistries of these brines, which would in turn influence the redox-oxidation reactions that could drive microbial metabolism (Schwenzer et al. 2016). Multiple metabolisms may be theoretically viable within subsurface martian brines (Nixon et al. 2013). In order to establish which of these are capable of supporting persistent growth, and any biosignatures that would subsequently be formed, an enrichment series was performed in a simulated martian chemical environment. The simulated martian environment was established using a newly developed geological simulant based on the chemistry of Rocknest (Ramkissoon et al. 2017) (wt % - Na₂O 3.36, MgO 6.12, Al₂O₃ 10.08, SiO₂ 44.17, P₂O₅ 0.56, SO₃ 3.72, Cl 0.1, K₂O 2.38, CaO 7.74, TiO₂ 0.73, Cr₂O₃ 0.17, MnO 0.24, FeO 18.81, Fe₂O₃ 1.76) and the associated brine, derived from the simulant using thermochemical modelling (Ramkissoon et al. 2018, Gellert et al. 2013, Gellert et al. 2006, Bridges and Warren 2006). These enrichments were performed using sediment collected from Pyefleet mudflats in the Colne estuary (Essex, UK), shown to contain a microbial community with a diverse metabolic potential (McKew et al. 2013).

Methodology: Enrichments were established by adding 5 g of sediment to 40 ml of the modelled brine containing 5g of the simulant material and 11 µM ammonium acetate (final concentration), a Mars relevant carbon source (Boston et al. 1992). The headspace was 1 bar of H₂/CO₂ (80:20). The enrichment was incubated for twenty days, after which 1 % was transferred into 10 ml of the modelled brines containing 10 g of simulant material. This provided a 1:1 ratio, akin to the water to rock ratio in rock pores, which will be used in future continuous culture experiments. The enrichment series was subcultured seven times to dilute the nutrients supplied by the estuary sediment, in order to select for a community capable of growth in the defined chemical environment. The chemical composition of the brines throughout the enrichment series was assessed using ICP-MS and ICP-OES in order to identify the shifts in chemistry with regards to the estuarine-derived nutrients. Changes in the composition of the enriched community were assessed by amplicon sequencing 16S rRNA genes amplified from DNA extracted from each stage of the enrichment. The metabolic profile of the enriched community was characterised by screening the DNA for functional genes relating to methanogenesis (*mcrA*), autotrophy (*cbbL*), denitrification (*nosZ*, *nod*, *narG*) and sulfate reduction (*dsrB*).

Results: The simulant and the brine derived from Rocknest were able to support microbial growth, which indicates that proposed aqueous environments in the martian subsurface could possess a chemistry capable of supporting specific metabolisms. The diversity of the enriched microbial community decreased over the course of the enrichment, with the defined chemical environment selecting for specific organisms and metabolisms. Specifically, sulfate-reducing bacteria and methanogens were shown to increase in abundance. This was reflected in the functional gene and 16S rRNA gene profile. Isolation work demonstrated that members of the microbial community were incapable of utilizing the supplied acetate or carbon dioxide for growth. This therefore suggests that they acquired alternate carbon sources, for example, from exuded carbon compounds produced by other microbes or through necrophagy.

Implications: The results produced from this enrichment series show that sulfate reduction and methanogenesis are viable metabolisms in our simulated martian chemical environment. This work also shows the value of considering community dynamics, i.e., syntrophy and inter-species interactions, as opposed to studying individual organisms in isolation when assessing viability and any associated impacts on the chemical environment, including the formation of biosignatures.

Future work: The next steps will be assessing differences in growth dynamics and community composition in chemical environments defined by simulants specified to exhibit the chemistries of other distinct martian regoliths. This will be investigated using a continuous culture approach to more accurately simulate lacustrine environments, allowing us to identify geochemical changes that occur as a result of microbial life, which could then potentially be used as biosignatures.

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