

**Patterns of horizontal gene transfer within microbial community succession in evaporative brine systems.**

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**Introduction:** As remnant ocean worlds (such as Mars) transition from wet to dry, their residual liquid waters eventually accumulate high concentrations of dissolved salts via evapoconcentration. Life in these evaporative brines must adapt to constantly changing and increasingly stressful environmental conditions; as the various salt species gradually become saturated, they precipitate one-by-one, drastically altering the geochemistry of the remaining brine [1]. We investigated how the microbial community coevolves with this geochemical succession in one terrestrial evaporative brine system: a solar salt harvesting facility in Chula Vista, California, where seawater is evaporated in a series of shallow ponds, forming a broad geochemical gradient from seawater to highly chaotropic, MgCl<sub>2</sub>-saturated brines.

**Microbial community succession:** Using environmental 16S amplicon sequencing, we discovered that the microbial community succession in these salterns was driven primarily by the water activity ( $a_w$ ) of the brines and took place in three distinct stages. The first stage ( $1 \geq a_w \geq 0.7$ ) was characterized by an explosion of abundance and diversity of closely related haloarchaea, which dominate hypersaline brines globally. The second stage ( $0.7 \geq a_w \geq 0.64$ ) was characterized by progressively more intense selection pressure and dwindling survival as water activity approached limit of life. At the very edge of the habitable window, the microbial community was dominated by a single taxon, *Haloquadratum walsbyi*. Finally, the conditions within the brines became too harsh for active microbial life. This third and final stage ( $a_w \leq 0.64$ ) was characterized by a ‘fossil’ microbial community: preserved cell remnants and exogenous genetic material [2].

**Evidence of horizontal gene transfer:** We used shotgun metagenomic sequencing to further investigate the surge of haloarchaeal microdiversity observed in stage 1, with particular interest given to the role of horizontal gene transfer (HGT). Haloarchaea—especially *Halorubrum* and *Haloarcula*—are known for frequent recombination and HGT, leading to high intragenus and intraspecific diversity [3, 4]. We therefore hypothesized that evidence of HGT found within the saltern metagenomes would correlate closely with haloarchaeal diversity. However, our preliminary results suggest instead that the surge in

microdiversity described in stage 1 actually lags the peak in HGT. The frequencies of genes annotated as either mobile genetic elements or HGT agents share a pronounced maximum c.  $a_w = 0.92$ , much earlier within the evaporitic geochemical succession than the peak in haloarchaeal diversity ( $a_w \approx 0.7$ ). This suggests that while HGT might initiate the surge of microdiversity, it is maintained and expanded by other ecological processes (e.g., niche partitioning or viral infection). Further investigation will focus on identifying specific genes that are frequent targets for HGT, and whether certain variants of those genes confer adaptive benefits to their hosts, allowing them to survive better in increasingly harsh, hypersaline brines.

**Broader significance:** Terrestrial evaporative brine systems are valuable analogues for Mars’s ancient receding oceans. On Mars, and other remnant ocean worlds, hypersaline brines could have been among the last refugia of life, making them promising targets in the search for extinct and extant extraterrestrial life. Understanding how life on Earth adapts to the constantly changing brine chemistries in terrestrial analogue environments is therefore essential to the search for life on Mars and elsewhere.

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