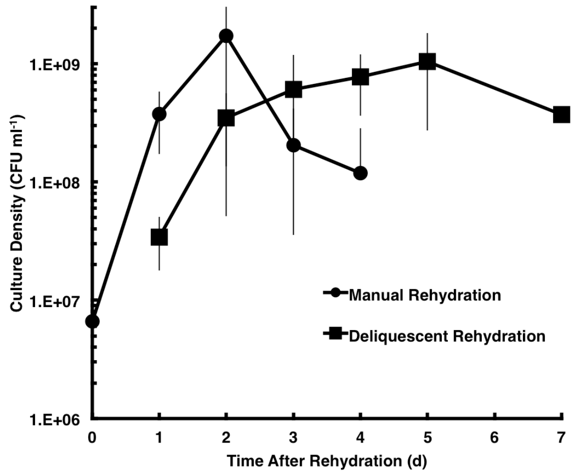
BACTERIAL SURVIVAL AND GROWTH IN FLUID INCLUSIONS AND DELIQUESCENT BRINES OF SALT EVAPORITES RELEVANT TO COLD ARID WORLDS. R. M. Cesur[1], I. M. Ansari[1], C. R. Stewart[1], M. A. Schneegurt[1], F. Chen[2], and B. C. Clark[3], [1]Department of Biological Sciences, Wichita State University, 1845 Fairmount St., Wichita, KS 67260, USA, mark.schneegurt@wichita.edu, [2]Jet Propulsion Laboratory, 4800 Oak Grove Dr., Pasadena, CA 91109, USA, [3]Space Science Institute, 4765 Walnut St., Boulder, CO 80301, USA

Salt evaporites as refugia: As a world becomes drier due to climate change, evaporite minerals will form from surface waters. Microbes that become entrapped in evaporites may survive in fluid inclusions within salt crystals. These dense brines may be the last remaining aqueous environments. Native microbes on aridifying worlds may become better adapted to life within salt crystals. When more humid conditions prevail, hygroscopic salts may deliquesce, forming dense brines by absorbing moisture from the atmosphere. The liquid water required by life may be formed at times on Mars through the deliquescence of salts near its hyperarid surface [1]. The deliquescent salts on Mars include chlorides, (per)chlorates, and sulfates of Ca, Fe, Mg, and Na [2]. Current surface conditions may only support bulk deliquescence of perchlorate salts, but these brines have thus far been incompatible with life at eutectic concentrations.

We have previously reported strong bacterial growth in saturated $MgSO_4$ (~67% w/v, as heptahydrate) at 25 °C [3]. Copious growth, albeit slow, was observed at the eutectics for $MgSO_4$ (17 wt% at –4 °C) and $KClO_3$ (3 wt% at –3 °C). On cold worlds, freezing-point depression by high solute concentrations expands the range of habitability in time and space.

Deliquescent growth: Here we have investigated the survival and growth, at 25 °C under deliquescing conditions, of salinotolerant bacteria (*Bacillus, Halomonas*, *Marinococcus*, and *Nesterenkonia*) from epsomic Hot Lake, WA, and the haline Great Salt Plains, OK [4]. Aliquots (50 μl) of bacterial cultures in SP growth medium supplemented with 2 M (~50% w/v) $MgSO_4$ were desiccated under vacuum in small cups. The culture evaporites were then incubated in sealed containers with a pool of 2 M $MgSO_4$ brine at the bottom, such that the water activity of the brine controlled the humidity of the headspace. This wetted the culture evaporites through deliquescence. Culture density was measured by serial dilution and plating on SP medium supplemented with 10% NaCl. Bacteria survive desiccation and begin to grow robustly once rehydrated by manual addition of water or by humidity alone, as shown here for *Halomonas* sp. str. HL12. Note that samples were not taken until deliquescence had fully rehydrated the evaporites and that cultures passed through supersaturated phases. Cells readily survived several cycles of drying and deliquescent rewetting, growing during wet phases of the process.

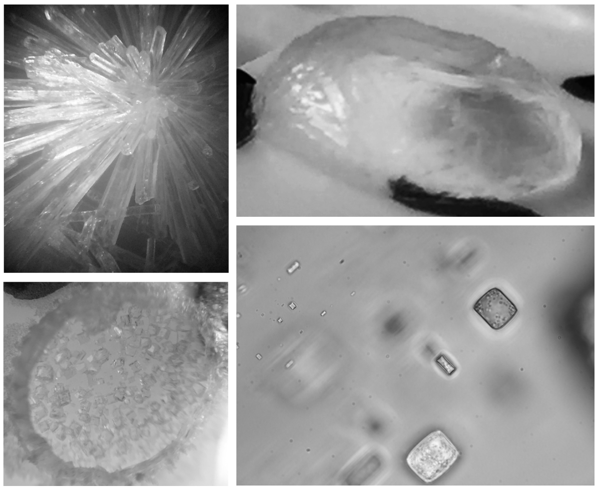


Growth of Halomonas *sp. str. HL12 after manual and deliquescent rehydration of desiccated evaporites of cultures grown in the presence of 2 M* $MgSO_4$

Similar experiments were performed using SP medium supplemented with 10% NaCl or 20% $NaClO_3$. Drying and deliquescence followed patterns resembling those of $MgSO_4$; $NaClO_3$ was the most hygroscopic, rehydrating even below 0 °C. Robust growth of *Halomonas* sp. str. HL12 was observed once dried evaporites had been rehydrated by the humidity within the sealed containers. Note that the eutectic conditions are 27 wt% at –21 °C and 39 wt% at –23 °C for NaCl and $NaClO_3$, respectively.

Living in salt crystals: Bacteria are known to remain viable inside of brine inclusions within individual salt crystals for years [5]. Previous reports claim that microbes have been cultivated from rock salt after millions of years. The stable brine inclusions that entomb cells will not dehydrate, thereby maintaining potentially habitable environments on cold arid worlds. The limited studies of microbes entrapped within laboratory-grown crystals thus far, have not determined the level of metabolic activity or physiological processes occurring in entrapped cells.

Here we have examined salt crystals grown in the laboratory by desiccating hypersaline bacterial cultures. Discrete 1-mm^3 primary crystals of NaCl were formed from dense brine (a culture grown in 10% NaCl that was diluted 10-fold with a saturated NaCl solution). Cells entrapped in fluid inclusions were readily observed microscopically. Individual crystals were surface-sterilized and dissolved in 10% NaCl solution to release cells for serial dilution and plating on SP medium supplemented with 10% NaCl. Viable cells of *Halomonas* and *Marinococcus* were recoverable. These crystals deliquesce and create brines suitable for microbial proliferation.



Images of salt evaporites. a, monoclinic epsomite crystals; b, epsomite hemispheroid; c, NaCl crystals; d, Halomonas *cells in fluid inclusions of NaCl crystals*

Similar experiments were performed using $MgSO_4$ and $NaClO_3$. Typically $MgSO_4$ cultures dried at 25 °C, did not form the slender monoclinic crystals produced when a warm supersaturated $MgSO_4$ solution is held at 4 °C. Instead, brines of $MgSO_4$ formed hemispheroids, retaining an interior salt slurry for weeks under standard conditions; these domes could be further dried in a desiccator (as for the deliquescence studies above). Hemispheroids also were observed when $NaClO_3$ brines were dried. Monoclinic epsomite crystals formed fluid inclusions that contained entrapped cells, which were observed microscopically.

Last refugia for life: The four most plausible wet environments to harbor extant life on Mars are caves, evaporites, ices, and the subsurface [6]; each of these involve salt brines, precipitates, efflorescences, and/or evaporite minerals. As climate change causes worlds to aridify, successful microbes might adapt to the scarcity of water and its increasing salinity. The last habitable water may be within evaporite minerals, eventually leaving only the steadfast brines within salt crystal inclusions. Since evaporite minerals can be hygroscopic, should conditions become more humid, hygroscopic evaporite minerals may deliquesce to brines, the first habitable wet environments. Further, entrapped microbes are protected during dispersion in the wind and crystals may deposit in locations humid enough for deliquescent brine to form.

Relevance to missions: The near-surface environment of Mars may be humid enough at certain times, to form deliquescent brines from the hygroscopic salts in the regolith. Microbes that survive drying and then grow in deliquescent brines are more likely to pose a forward planetary protection risk to Mars, should they contaminate spacecraft. While we specifically have investigated microbial tolerance to the harsh chemical conditions of cold arid worlds, to be successful, microorganisms would need to tolerate a range of physical and chemical challenges.

The evolutionary advantages of salinity tolerance and entrapment in crystals on cold arid worlds, suggest that evaporite minerals make prime targets for missions to detect extant life on Mars [6]. Furthermore, surface deposits on solar system bodies such as Ceres may be evaporite minerals from briny seas buried by ices. Salt crystals may persist in sublimation lag from arid polar caps or shadowed craters, lyophilized brine spray from icy worlds, or the exposed remnants of cryovolcanic events. When suspended aloft in the atmosphere, salt crystals may retain habitable water under dry or harsh conditions on cloudy worlds. On diverse worlds, life may seek the protection and steadfast habitable water of salt crystals.

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