BACTERIAL SURVIVAL AND GROWTH IN DENSE BRINES, DELIQUESCENT LIQUIDS, AND CRYSTAL FLUID INCLUSIONS. M. A. Schneegurt[1], H. Z. Zbeeb[1], R. M. Cesur, MD Joad[1], H. H. Zayed[1], I. M. Ansari, A. Mahdi[1], T. M. Luhring[1], F. Chen[2], and B. C. Clark[3], [1]Department of Biological Sciences, Wichita State University, 1845 Fairmount Street, Wichita, Kansas 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.

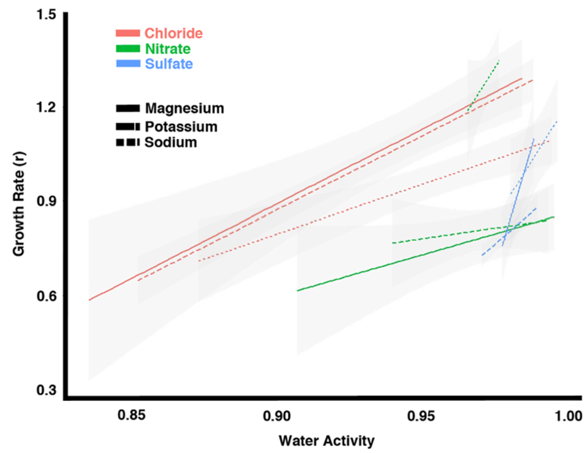
Life in brine: Liquid water on cold arid worlds needs to be salty, relying on high solute concentrations to depress the freezing point of water. Life requires liquid water, so growth tolerance to dense brines broadens the range of potentially habitable regions and periods [1]. Sulfate and chloride salts are important constituents of Mars regolith, with Ca, Fe, Mg, and Na counterions [2], while brine pockets and oceans on icy worlds may have similar salts. Significant concentrations of (per)chlorate salts are present on Mars with eutectic temperatures as low as –69 °C for $Mg(ClO_4)_2$. Icy worlds may maintain liquid water with high concentrations of ammonia.

Microbial tolerances to NaCl and carbonates have been studied far more than other salts. No single quality of these salts or ions appears to determine which will inhibit microbial growth, although it is clear that water activity (a_w) and ionic strength are important. Here we have measured growth in a series of salts, testing an iterative matrix of ions with different physical qualities.

When brines dissipate on arid worlds, the evaporites formed can entrap microbial cells within fluid inclusions and between crystals. Microbes tolerant to saturated brines may survive for extended periods in these liquid refugia. As more humid conditions occur, hygroscopic evaporites can deliquesce, creating liquid brines. This process may occur on Mars, particularly for (per)chlorate salts.

Bacterial isolates from analogue sites: Our bacteria were isolated from natural environments rich in NaCl (Great Salt Plains, OK) or $MgSO_4$ (Hot Lake, WA) [3]. The collections include *Bacillus*, *Halomonas,* *Marinococcus,* *Nesterenkonia*, *Planococcus*, and *Virgibacillus* isolates that grow at ≥10% NaCl (1.7 M), ≥50% (~2.0 M) $MgSO_4$, and >20% (~2.0 M) Na chlorate [3]. Salt Plains (SP) medium was supplemented with various salts at increasing concentrations to saturation.

Tolerance to an iterative matrix of ion pairs: Over 4000 time-series were fit to logistic growth curves of 18 salinotolerant bacteria to estimate the intrinsic rate of population increase (r) and maximum culture density (K) in the presence of an iterative matrix of all combinations (N = 1051) of 3 cations (Mg, K, Na) and 3 anions (Cl, NO_3, SO_4) and their physical qualities. Lower a_w led to lower growth rates (P < 2.2e-16) across all salts. Further, r was affected by salt-specific responses from ion pairing (anion × cation interaction, P < 2.2e-16). Responses appear to cluster by anion (P < 9.1e-15). No 3-way interactions were significant with a_w.

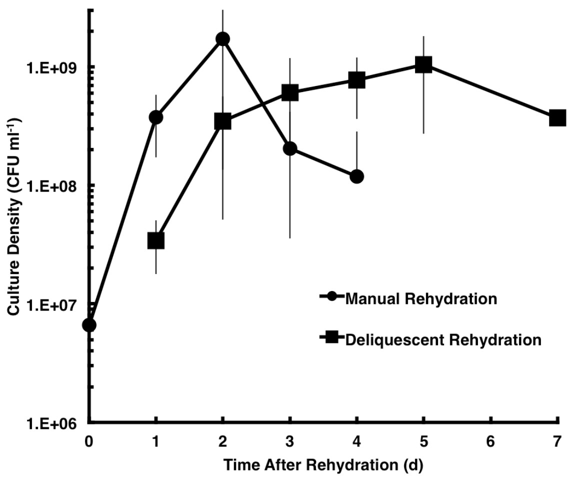


Effect of water activity on the growth rates of 18 salinotolerant bacteria over a range of permissible concentrations for salts (to saturation) in an iterative matrix of ion pairs

Degree of saturation and ionic strength similarly gave significant correlations between r and ion-pair interactions (P = 0.007). Models showed 2-way interactions of ionic strength with anions (P = 0.0008) and cations with anions (P <0.0001), but not for ionic strength with cations (P = 0.15). Degree of saturation had a more complex 3-way interaction with anions and cations (P <0.003), indicating that the effects of degree of saturation on r were salt-specific. Ions and salts affect bacterial growth differentially across gradients of solution qualities with no single physical quality adequately accounting for the observed solute-specific effects on bacterial growth.

Tolerance to ammonia: Icy ocean worlds at the low cryogenic temperatures (–100 °C) may rely on eutectic ammonia-water mixtures (34 wt% NH_4^+) to remain liquid, since all brines freeze below –80 °C. Substantial tolerance to $(NH_4)_2SO_4$ at ~53% w/v (11 wt% NH_4^+) was observed in soils and for salinotolerant microbial isolates.

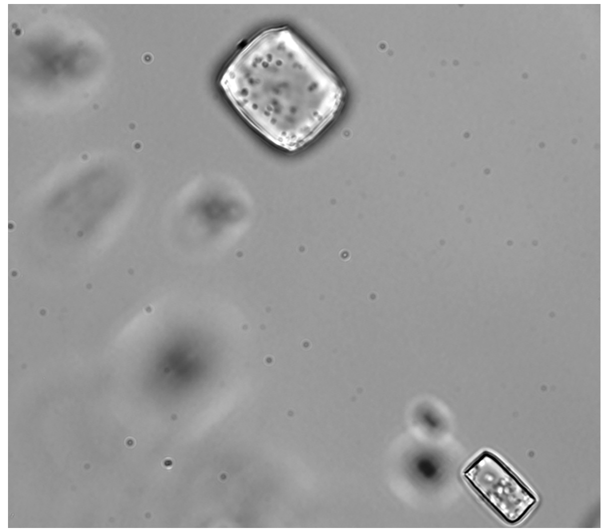
Growth in Deliquescent Liquids: Salinotolerant bacteria grown in brines of $MgSO_4$, NaCl, and $NaClO_3$ were vacuum-desiccated in small cups, which were then incubated in sealed containers with a pool of matching brine at the bottom. The evaporites rehydrated by humidity, deliquesced to liquid and microbial growth was monitored. Bacteria survived desiccation and renewed robust growth once rehydrated by manual addition of water or by humidity alone, as shown here for *Halomonas* in 2 M $MgSO_4$. Cells readily survived several cycles of drying and deliquescent rewetting, exhibiting growth during wet phases of the cycles. Similar results were obtained using SP medium supplemented with 10% NaCl or 20% $NaClO_3$. Note that the eutectic conditions are 17 wt% at –4 °C, 27 wt% at –21 °C, and 39 wt% at –23 °C for $MgSO_4$, NaCl, and $NaClO_3$, respectively.



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

Entrapment in salt crystals: Primary crystals (~1-mm^3) of NaCl were formed by air-drying from *Halomonas* cultures grown in 10% NaCl that was diluted 10-fold with a saturated NaCl solution. Cells entrapped in fluid inclusions were readily observed microscopically. Viable cells were culturable from individual crystals that were surface-sterilized. These crystals deliquesce and create brines suitable for microbial proliferation. Similar results were obtained for *Marinococcus* and with $MgSO_4$ evaporites.

Last refugia for life: The four most plausible wet environments to harbor extant life on Mars are caves, evaporites, ices, and the subsurface [4]; 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 [5]. Since evaporite minerals can be hygroscopic, should conditions become more humid, 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.



Micrograph of Halomonas *cells within fluid inclusions of a NaCl crystal*

Acknowledgements: We appreciate the technical assistance of Amer Al Soudi, Meris Carte, J. Alden Consolver, Casper Fredsgaard, Robert Goldstein, William Hendry, Shannon Howell, Tyler Nolan, Alexander Ratcliffe, Nayan Shrestha, Andrew Swindle, and Ali Taleb.

Funding: Supported by Wichita State University and awards from NASA, Research Opportunities in Space and Earth Science (ROSES), Planetary Protection Research (09-PPR09-0004 and 14-PPR14-2-0002) and Kansas INBRE NIGMS NIH (P20 GM103418).

References: [1] Rummel JD *et al.* (2014) *Astrobiology* 14:887; [2] Clark BC *et al.* (2005) *Earth Planet Sci Lett* 240:73, Clark BC & Kounaves SP (2016) *Int J Astrobiol* 15:311; Thomas NH *et al.* (2019) *Geophys Res Lett* 46:10754; [3] Caton TM *et al.* (2004) *Microb Ecol* 48:449, Crisler JD *et al.* (2012) *Astrobiology* 12:98, Kilmer BR *et al.* (2014) *Int J Astrobiol* 13:69, Al Soudi AF *et al.* (2017) *Int J Astrobiol* 16:229, Wilks JM *et al.* (2019) *Int J Astrobiol* 18:502; [4] Carrier BL *et al.* (2020) *Astrobiology* 20:785; [5] Rothschild LJ *et al.* (1994) *J Phycol* 30:431; Vreeland RH *et al.* (1998) *Extremophiles* 2:321