Evidence for ‘super’ survival over millions of years is growing, while evidence for survival over tens of thousands of years is almost unequivocal.

result in cell damage. Both desiccation and radiation can damage cells by the production of highly reactive forms of oxygen, and so microbes that cope with drying are generally also good at surviving high doses of radiation. H. salinarum has evolved mechanisms that make it one of the most radiation-resistant microbes known. Evidence is emerging that the high cellular concentrations of peptides and the minerals phosphate and manganese (and correspondingly low levels of iron), combine to protect cellular proteins. These proteins include enzymes that repair damaged nucleic acids, which, combined with other unusual haloarchael features, such as multiple copies of the chromosome and an efficient means of repairing and recombining DNA fragments, ensures genetic material stays intact. The carotenoids and high cellular concentrations of KCl also provide radiation protection.

Living in tiny brine inclusions in salt crystals
Although a crystal of common salt may look completely dry, up to 5% of its volume is liquid in the form of hundreds of brine inclusions, i.e. small reservoirs of salt-saturated brine surrounded by a solid matrix of NaCl. The pioneering work of Professor Bill Grant and others revealed that haloarchaea become trapped inside salt crystals, living in the brine inclusions. Hypersaline environments are dynamic systems that frequently dry up so this strategy employed by H. salinarum and friends enables them to survive within a small-scale aquatic environment until the rains come and dissolve the salt crystals, regenerating the brine lake.

Staying alive over geological time
But what happens to the haloarchaea if the rains don’t come and the salt starts to accumulate and ultimately gets buried? From laboratory experiments we know that haloarchaea, and H. salinarum in particular, can remain alive inside salt crystals for years. We cannot perform experiments for thousands of years, of course, so we sample directly from ancient brine salt deposits, taking great care to exclude external contaminants. Different research groups have independently and repeatedly isolated haloarchaea from ancient salt crystals, and evidence for ‘super’ survival over millions of years is growing, while evidence for survival over tens of thousands of years is almost unequivocal.

Over time, salt crystals may be buried, forming the salt deposits we mine today, and providing an environment conducive to long-term survival of entombed microbes by restricting the amount of radiation reaching the cells. In addition, the salt-saturated brine inclusions contain little oxygen, minimizing the creation of cell-damaging reactive oxygen compounds. Incidentally, H. salinarum can grow with or without oxygen.

What can H. salinarum feed on inside brine inclusions?
The repair of H. salinarum proteins and nucleic acids needs organic matter for energy. An obvious question is whether there is enough organic matter in the brine inclusions to keep H. salinarum alive for thousands of years. The brine inclusions are best considered relative to the size of the microbes that they are housing: a single cell of H. salinarum in a brine inclusion is equivalent to a water flea in a bucket of water. Also, there are often thousands of co-entombed microbial cells, including D. salina. In fact, remnants of this glycerol-packed green alga have been found in ancient brine inclusions by Tim Lowenstein’s group. There is a good supply of organic matter from D. salina and the dead cells of those haloarchaea that are less adept at surviving in brine inclusions, such as the square Haloquadratum walsbyi, to allow H. salinarum to stay alive.

Where next?
There are many open questions about the amount of energy needed, the nature of the environment and the cellular adaptations required to hold the Grim Reaper at bay for millions of years. It will be important to learn how different species of halophile interact, and how those interactions change over time in the closed system of a brine inclusion. Astrobiologists should be aware of H. salinarum’s long-term survival, as Mars once had an environment that was more conducive to life, including hypersaline brines that turned into salt deposits. Also, Jupiter’s moon Europa has a subterranean hypersaline seas. Therefore, if we are going to search for existing or former life on other planets, these salty environments should be prime targets.

Further reading

Left: Colonies of Halobacterium salinarum growing on salt-saturated agar plates. Matt N Ford
Right: Microscopic image from a natural hypersaline brine. Based on their morphology we can identify D. salina living alongside Haloquadratum walsbyi (flat square with gas vesicles). A rod-shaped microbe can also be seen, which may be Halobacterium salinarum. Mike Dyke-Smith

A brine inclusion containing D. salina and H. salinarum. Features of brine inclusions that enhance long-term survival
• Salt saturated
• Low oxygen concentration
• Water-saturated microbes as a source of carbon and energy

Features of D. salina and H. salinarum cells that enhance long-term survival
• High KCl concentration
• High manganese:iron ratio
• Manganese antioxidant complexes
• Multiple chromophores
• Carotenoids

Schematic illustration of where and how haloarchaea survive in salt crystals. From left to right: A laboratory-made crystal of NaCl encasing a haloarchaeal species. The orange colour is from haloarchaea. The redness of the halo (NaCl) crystal is due to the large number of brine inclusions shown in the second schematic. The third schematic illustrates a single, large brine inclusion, showing the scenario in which H. salinarum is entombed with the green alga D. salina (top left). The final picture shows a single cell of H. salinarum. These environmental or cellular features that enable the cells to survive over geological time are described in the boxes. Sizes for illustrative purposes only and pictures are not always drawn to scale. M. McGentry

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