Long-lived microbes in salt

Miss: a microbiologist friend of my mum calls some microbes sleeping beauties: do they really go to sleep for ages before being woken up by a kiss?

Credit Great Salt Lake Institute

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[1] Thanks to Dave R Clark and Maria Magliulo for valuable comments

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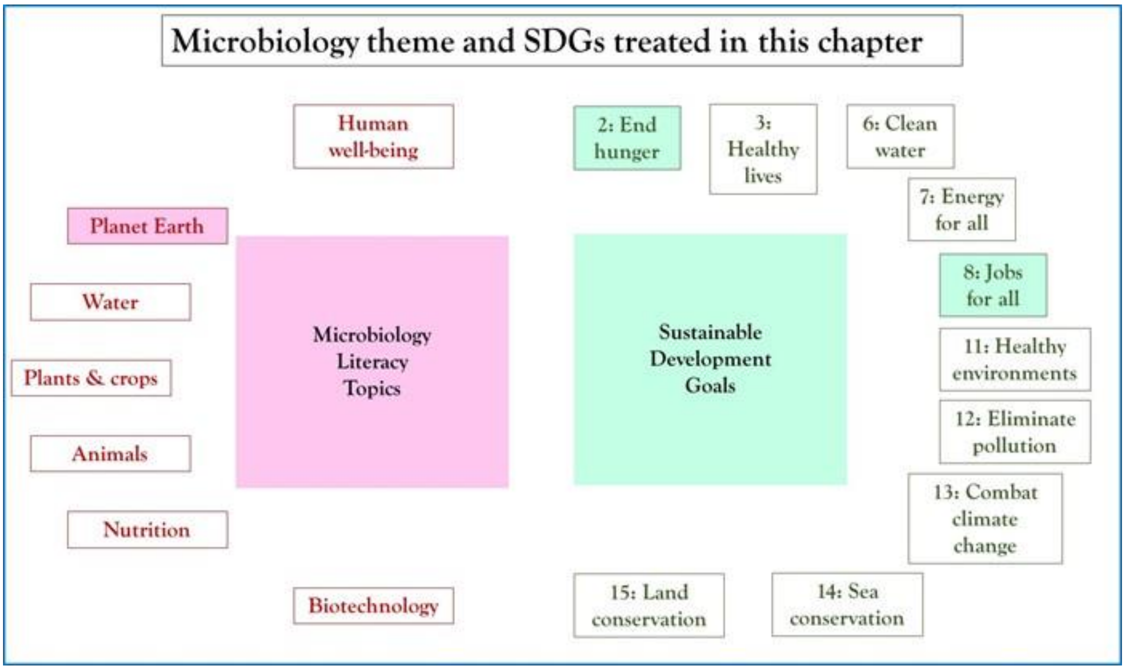
Storyline

Children are fascinated by microbes that flourish in extreme environments, such as the Great Salt Lake shown above. They are often even more excited to learn about the extreme longevity of some microbes, particularly those that may remain alive for millions of years in buried sediments. This idea is often met with some scepticism, leading to questions about how scientists can be certain of the antiquity of recovered microbes, which then provides an opportunity to discuss the scientific method. Children, therefore, get to weigh up diverse lines of evidence that come from different disciplines – from microbial physiology to geology – thereby seeing the interconnectedness of science. These topics can fuel a debate on, perhaps, the biggest scientific question of all – whether life exists, or has existed, beyond Earth.

The Microbiology- and Societal Context

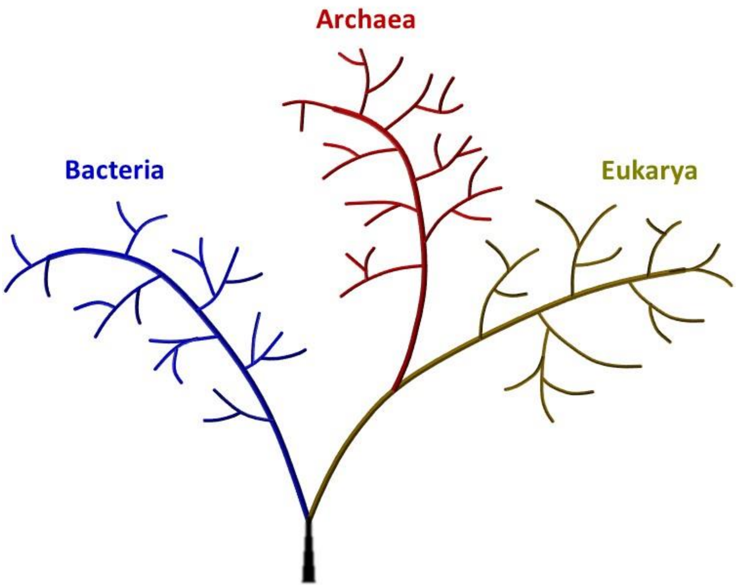
The microbiology: Mechanisms of adaptation to extremely saline environments and the environmental and physiological bases of longevity, linking to the field of astrobiology. *Sustainability issues:* The SDGs "end hunger" and "jobs for all" are alluded to, by considering issues such as fermented foods, biotechnological applications of halophilic (salt-loving) microbes, and the potential for very long-term data storage in the form of DNA cloned into halophilic microbes encased in salt.



Long-lived microbes in salt: the Microbiology

1. ***So Miss, do some microbes really go to sleep for ages before being woken up by a kiss?*** In a way this is true, but rather than use the word "sleep", a more accurate description is that they slow down their metabolism to extremely low levels, like hibernating bears or over-wintering roses, but I can explain that in more detail later. Also, it wouldn't normally be a kiss that revives them, but a change in their environment, such as rainfall in a very dry desert soil.

2. ***Ah! I've heard about water bears that can survive being dried up – are they microbes?*** Water bears, also known as tardigrades, are amazing, but they are animals and not microbes. They are relatively large at ~0.5 mm and have at least 1000 cells that form differentiated body parts, like legs and a head. Microbes are found in all three domains of life. All Bacteria and Archaea are microbes, and there are a whole range of microbes that belong to the Eukarya (Eukaryotes). Remember that plants and animals (including sponges, tardigrades and humans) are a small terminal branch in the Eukaryote domain. The distinction between what is a microbe in the Eukaryote domain is a bit subjective, but key features include being single-celled or forming small clusters of cells with little cellular differentiation.

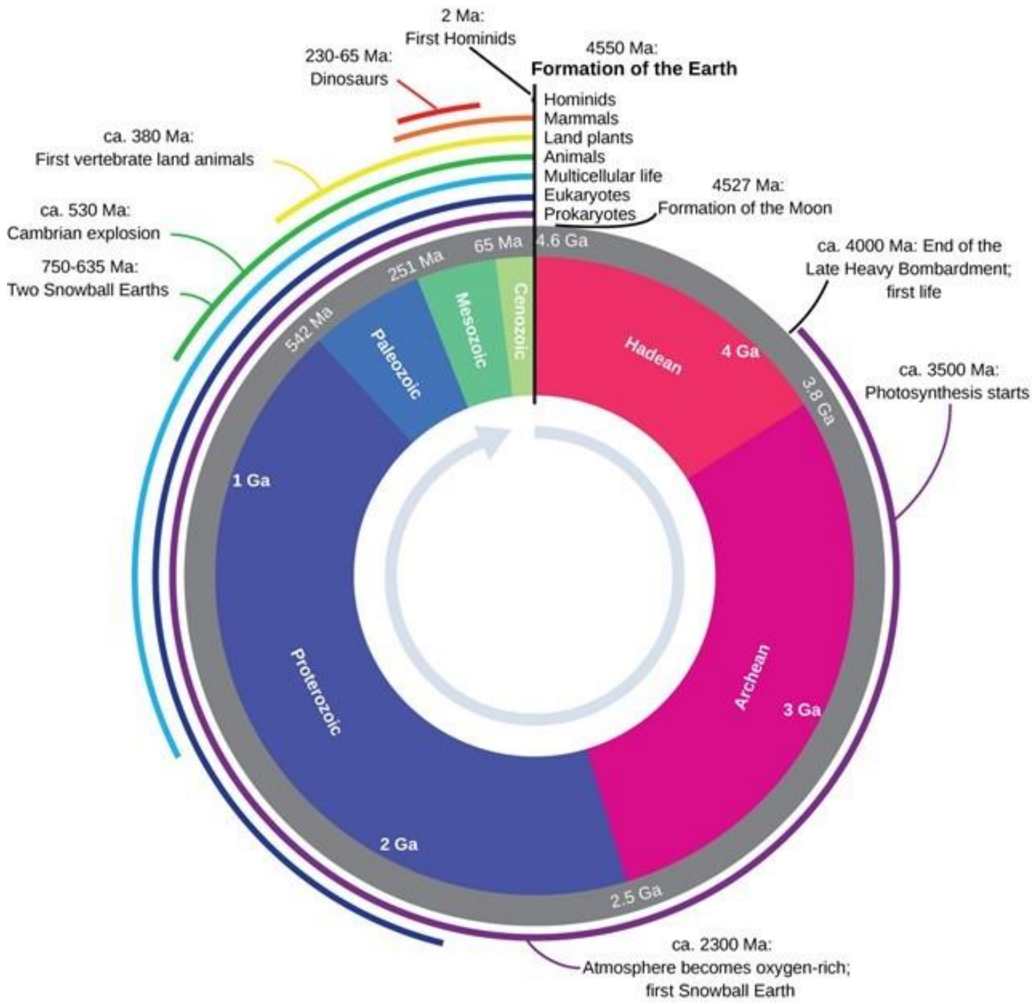


Schematic view of the tree of life, illustrating the Bacteria, Archaea and Eukarya as three distinct domains. Bacteria and Archaea, all of which are microbes, lack a membrane-bound nucleus which is a typical feature of Eukarya. However, Bacteria and Archaea also have distinguishing features, such as the structure of their membrane lipids. Eukarya include protists, which are a diverse group of microbes, as well as plants and animals. Such trees are built by comparing the nucleic acid sequences of genes that are common across all organisms, and there is still controversy about the exact placement of branches.

3. ***But can microbes live longer than tortoises?*** Many can – much longer. I believe there was a tortoise that lived for around 250 years, although no one is certain exactly when he was born. And some sponges, which are primitive animals, are believed to be 11,000 years old. But there are reports of Bacteria and Archaea that live for millions, possibly hundreds of millions of years.

4. ***Wow! That's a long time, but I can't even picture how long is that is?*** The next figure shows the timeline for the Earth and some of its main events. But let's try to relate some key events to the 24-hour clock, recognizing that there is uncertainty about the timings. If the Earth formed at 00:00 hours, then life originated at approximately 04:00 (3,800 million years). It then took until early afternoon (13:00, or 2,100,000 million years) before the first microbial Eukaryotes appeared, and we could see the first animals at 20:00 (760 million years; https://www.oum.ox.ac.uk/firstanimals/). The dinosaurs were around from 22:48 to 23:40 (230 to 65 million years). If we take a date of 4.4 million years for the arrival of the genus *Homo*, then our ancestors appeared just 1 minute and 18 seconds before midnight. Our own species, *Homo*

sapiens, which has been on the scene for a mere 1.9 seconds (0.1 million years), built the Egyptian pyramids less than one-tenth of a second before midnight (4,500 years ago).



Schematic view of the age of the Earth and some of its major events. The Earth formed about 4.55 billion years ago (= 4,550 million years, 4,550,000,000 years = 4.55 × 10^9 years = 4.55 Giga-annons (Ga)). The symbol "Ma" stands for Mega-annons or million years. There are 1000 Ma in 1 Ga. The coloured lines on the outside indicate the start (and for dinosaurs the end) of the group's time on Earth. No one knows when the first cellular life formed. Here, an approximate start date of 4,000 million years is given. The first life forms were Prokaryotes, which literally means "before nucleus", and which is a collective name for the domains Bacteria and Archaea – the absence of a nucleus is not a very useful defining feature, and we now know that Bacteria and Archaea are distinct domains. The first appearance of single-celled Eukaryotes or Eukarya (literally "with nucleus") was about 2,100 million years. The timing of the appearance of hominids is contested.
Taken from: Fowler et al. (2013) https://openstax.org/books/concepts-biology/pages/b-geological-time

5. ***So where are these microbes sleeping?*** Microbes are abundant and almost everywhere on the planet's surface. One teaspoon of soil contains about a billion microbial cells and many thousands of species, some of which will be inactive and able to live for many years. For example, a microbiologist called Peter Sneath was able to grow bacteria from soil granules on the roots of plants housed in the Herbarium at Kew Gardens, England, that were dried in

1640. In fact, the majority of microbes in soil are dormant to some degree, where dormancy means a transient and reversible state of low metabolic activity.

But it's not just soils – every habitat will have sleeping microbes. Relatively recently, scientists have discovered microbial communities living in the deep subsurface (such as mud-rich sediments or basalt rocks down to several kilometres). Simple sources of energy, like sugars, that may be abundant on the surface, will have been used up in the deep subsurface, and so it is normal for microbes in such environments to grow very slowly, probably several thousand times slower than microbes growing in the laboratory. There's no light in these deep sediments to support photosynthesis, and microbes must subsist on buried organic matter, dead cells, or inorganic energy sources such as hydrogen.

6. ***Wow! So, thinking back to something I was taught in geography, are we talking here about the geosphere or the biosphere?*** What a great question! Humans need to define compartments to help their understanding, but the distinction between the "rock zone and "life zone" is blurry, and these deep sediments are both biosphere and geosphere combined.

7. ***And how can scientists be sure that microbes can live for millions of years?*** I sense some healthy scepticism here! Firstly, geologists can date sediments in different ways, such as measuring the decay of radioisotopes (forms of a chemical element in sediment minerals that change over time to a more stable form in a regular way), and combining this information with layering patterns (stratigraphy) of the rocks and their associated fossils.

Then, it is important to know about the geological history of the sediment under investigation, asking whether there has been contact with recent microbes. For example, movements in the Earth (from very slow movements to earthquakes) allow water and microbes from more recent environments to enter the seemingly ancient sediment. And it is vital, particularly when obtaining samples by drilling into sediments, to avoid contamination with microbes from the surface and from the fluid that is used to lubricate the drill. This is not trivial, and many checks must be in place, such as adding fluorescent bacteria-sized beads to the drilling fluid, and checking under the microscope that the control beads do not appear in sediment samples, or more recently by adding chemical tracers that must not be detected in drilled samples.

Then, it is important to show that the microbes are alive, for example by growing them in the laboratory. But most microbes are not adapted to rapid growth in media with lots of carbon and nutrients, which microbiologists usually use in their laboratories, so will not grow on agar plates. In this case, more subtle techniques are needed to measure activity, such as showing that microbes can take up nutrients, such as ammonia, amino acids and sugars, into their cells.

Finally, microbiologists identify the microbes found in ancient samples (e.g. by analysing the sequence of a highly conserved gene such as that coding for 16S rRNA, which is a kind of molecular identity card), and compare them to those found elsewhere in the world. If a particular type of microbe is repeatedly found in deep sediments, but rarely in other habitats, then it suggests (but does not prove) that it is particularly well adapted to that environment.

8. ***OK Miss, but where else do microbes live for a long time.*** Scientists have investigated all sorts of environments, such as ice cores and permanently frozen soils (permafrost), but my favourite is salt. If seawater or other sources of salty water are in a restricted area, like a lagoon or shallow basin (or, on a small scale, in a rock pool), then the rate of evaporation of water may be faster than the input of new water, leading to the seawater becoming even more salty. This

process is encouraged by hot, windy and dry conditions, and may continue until minerals start to precipitate from seawater.

First, you get calcium carbonate ($CaCO_3$; the main mineral that forms around taps and in kettles where you have hard water), then gypsum ($CaSO_4.2H_2O$; used in building, e.g. in plasterboard), followed by halite (NaCl; common salt or table salt), leaving behind a brine rich in potassium (K^+) and magnesium (Mg^{2+}) ions, which may also precipitate out with remaining chloride or sulfate ions. Seawater mainly consists of sodium (Na^+) and chloride (Cl^-) ions (see the next Table), so the main product is NaCl. We need lots of table salt for our food, so take advantage of this process to produce it by flowing seawater into shallow salt ponds or pans and allowing it to evaporate (illustrated in the next figures).

	Seawater	Seawater at the onset of halite precipitation
Na^+	10.8	98.4
Mg^{2+}	1.3	14.5
Ca^{2+}	0.4	0.4
K^+	0.4	4.9
Cl^-	19.4	187.0
SO_4^{2-}	2.7	19.3
salinity	35	324.5

Table showing the concentration of the major cations (Na^+, Mg^{2+}, Ca^{2+}, K^+) and anions (Cl^-, SO_4^{2-}) in normal seawater and in seawater that has been concentrated so that halite (NaCl) is starting to precipitate (units are g/l). *Taken from Javor (1989).*

Satellite view of salt ponds in South San Francisco Bay, USA, 2002, showing vivid red, orange and green ponds in which seawater evaporates leaving behind salt (field of view is approximately 36 x 25 km). The colours are due to the pigments of the halophilic microbes in the brines of different salinity. The red/orange pigments are primarily carotenoids (a type of lipid) in haloarchaea and *Dunaliella salina* that inhabit the most hypersaline ponds. White ponds are where NaCl has fully come out of solution and exists as salt crystals. *Credit NASA* https://earthobservatory.nasa.gov/.

A crystalliser pond in a coastal sea-salt facility in Mallorca, Spain. The wind has blown crystals of halite (white, wavy bands marked with arrows) to the near corner, and the red colour of the brine is apparent.

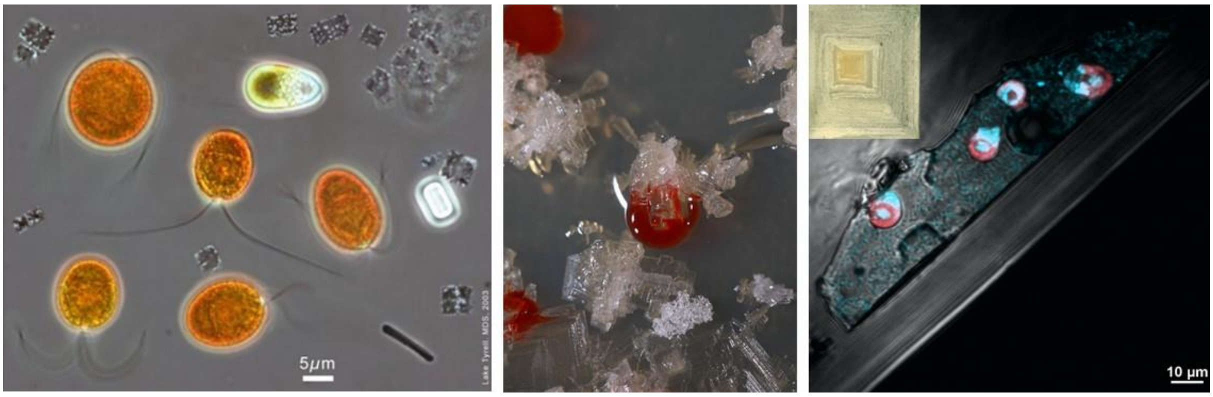
9**. *Is this the salt that I put on my fish and chips?*** That's right. In fact, salt is so much more than a condiment, it is used worldwide to preserve foods and improve their nutritional value by encouraging growth of beneficial microbes during fermentation.

Anyway, I digress. My main point is that as seawater becomes more concentrated the microbial community changes, resulting in the dominance of microbes that not only tolerate high concentrations of salt, but actually prefer living at extremely high salinity. And as salinity increases these salt-adapted cells can grow to such a high density that the brine becomes opaque and red, like a tomato soup (see the previous figures). Ultimately, as halite precipitates, forming cubic crystals, many of the microbes become trapped inside the halite, which is where the story about their longevity begins.

10**. *But isn't sea-salt a solid – I recall that in chemistry we were told that it was a cubic lattice of Na and Cl joined together, so how can microbes live in that?*** Ah, yes, I forgot to explain that little pockets of the liquid get trapped within the crystal lattice. These so-called *brine inclusions* are where the microbes live. It provides a safe haven for extremely halophilic microbes to escape from desiccation. Note that this trapped liquid is a highly saline brine, and it can make up to 5% of a halite crystal. In terms of relative size, a single microbial cell in a brine inclusion is equivalent to a water flea in a bucket of water, and there can be many hundreds of cells from different species trapped inside a single brine inclusion (see the next figure).

11. ***So, extremely salty water packed full of microbes dries up, but the salt isn't totally dry? Salt crystals have brine inclusions, and microbes live in these super-salty pools inside the crystals, right? But what types of microbes are these, because you taught us that salt was added to food to <u>stop</u> microbial growth – like that disgusting salted cod you gave us***? That salted cod was delicious! But that's beside the point. You are right that most microbes cannot grow at high concentrations of salt, especially most pathogens and food-spoilage agents. But there are microbes that can grow at very high salt concentrations. Although there are hundreds of species of Bacteria in seawater, there are few that grow at the salinity at which halite starts to precipitate out of solution.

By contrast, a group of Archaea, known formally as Halobacteria (though many prefer to call them by their more informative name of haloarchaea), dominate in salt-saturated brines. One species of Eukaryote, a single-celled algal species called *Dunaliella salina* (see next figure), thrives in hypersaline brines, but struggles to grow as halite starts to precipitate. So, different microbes have evolved to a preferred salinity for growth, just as they have optimal temperature and pH growth ranges, etc. Those microbes that grow optimally above seawater salinity are called halophiles. Most of the haloarchaea are extreme halophiles that cannot grow below a salinity of 100 g/l (about three times more saline than seawater), and they function perfectly in salt-saturated brines (about nine times more saline than seawater); that is when the halite is precipitating out.



Left: Microscopic image from the hypersaline Lake Tyrell, Australia (salinity >20% w/v), in which we can identify two microbes based on their morphology. The large, orange microbes with two flagella belong to the Eukaryotic photosynthetic algal species, *Dunaliella salina.* This species is grown commercially for the carotenoid, β-carotene (the source of its pigmentation), which is a natural food colorant and pre-cursor to vitamin A. The flat, square cells are the haloarchaeon, *Haloquadratum walsbyi,* which has inflatable gas pockets (seen as white patches) that allow flotation to the surface, probably to enable it to acquire oxygen (scale bar is 5 μm). There is a rod-shaped cell (bottom right), but it cannot be identified based on morphology alone. *Credit Mike Dyall-Smith*

Middle: Colonies of the haloarchaeon, *Halobacterium salinarum*, growing on an agar plate with added organic compounds and high concentrations of salt. The red colonies remain luxuriant despite the precipitation of halite, indicating this species' extreme adaptation to high salinity. *Halobacterium salinarum* and *Haloquadratum walsbyi* are both extreme obligate halophiles. *Credit Matt W Ford, www.pandasthumb.org*

Right: The inset shows a laboratory-made halite crystal (about 1 cm across) in which haloarchaea were entombed. The crystal is cloudy due to hundreds of brine inclusions. The main image shows a single brine inclusion from another laboratory-made halite crystal in which both *Dunaliella salina* and *Halobacterium salinarum* were entombed. Four cells of *Dunaliella salina* can be seen, with their chloroplasts staining red and DNA staining blue. Hundreds of *Halobacterium salinarum* cells (tiny blue rods, approximately 2 μm long) are just about visible. *Credit Nora Georgiev, Maria Magliulo and Philippe Laissue*

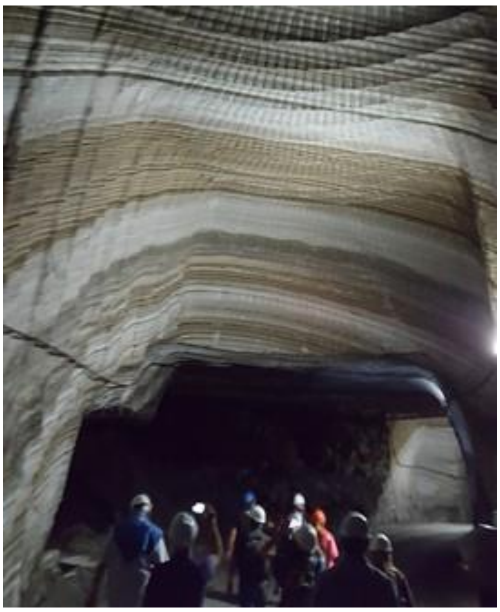
12. ***But you taught us about osmosis. Why doesn't all the water move from the inside of the cell to the outside?*** This is exactly what would happen to an organism that was not adapted to growing at high salinity. Haloarchaea balance the salinity either side of their semi-permeable cell membrane by bringing salt into the cell to the same concentration as it is outside, thereby preventing loss of water from the cell by osmosis.

They are quite unusual in this respect, and it means that their macromolecules, such as proteins, have evolved to function in this extremely saline cytoplasm, and generally cannot function at low salinity, which is why they are called obligate extreme halophiles. It is these haloarchaea that have been found alive in ancient halite.

13. ***What do you mean by ancient halite?*** Sometimes salt can form on a large scale. For example, between 5.96 and 5.55 million years ago the Mediterranean Sea was partially disconnected from the Atlantic Ocean. Since evaporation of seawater then exceeded inflow from the Atlantic and rivers, this resulted in the formation of a large-scale hypersaline sea and massive formation of salt deposits throughout the Mediterranean, which are now overlain by other, muddy sediments.

In places, like Sicily, there are deposits where NaCl is mined (see next figure), but most of the salt lies under the sea floor. This is just one example, but there are ancient salt deposits of different ages across the world.

Haloarchaea have been grown from many different salt deposits from thousands to hundreds of millions of years in age. Not all studies claim that the haloarchaea were as old as the salt, perhaps because the authors cannot be certain that the salt hasn't received some input of water since it was buried, but some studies do surmise that long-term survival is the most likely scenario. Improvements in the procedure for disinfecting the surface of halite crystals has given more confidence to these findings, as well as repeated isolation, especially of haloarchaea belonging to the genus *Halobacterium*.



A cavern in the Realmonte salt mine in Sicily, which was deposited during the Messinian Salinty Crisis 5.96 to 5.55 million years ago. The bedding pattern with layers of salt (white) and clay / clay-rich salt (brown) is clearly visible.

14*. **How do, or should I say "might", haloarchaea survive for millions of years**?* This is the big question. In addition to ensuring that halite has not been altered since burial, that negative controls have been included, and that the crystals' disinfection regime has been rigorously tested, it is important to consider what usually kills microbes, and whether/how *Halobacterium* species that have been repeatedly grown from ancient salt can escape death.

15. I've never even thought about what kills microbes other than antibiotics. How do they die? This is not as easy to answer as it might appear. Extreme temperatures can denature proteins and disrupt lipid membranes, but when looking for signs of ancient life, very deeply buried, and thus heated, salt is avoided. There are no known protists that can survive and eat the haloarchaea inside halite, so they can most likely be ruled out. Just like humans, haloarchaea have their own viruses that can lead to cell lysis and death.

But otherwise, a main contributor to cell death are reactive oxygen species, which are oxygen (O_2) intermediates, most of which are free radicals. This is the mechanism by which bleach kills microbes, but rather than one big dose, like when you put bleach down the sink, the microbes will get lots of small doses.

16. Are free radicals what can cause cancer? Exactly! Technically, free radicals are atoms without a full complement of paired electrons in their outer shell. A range of reactive oxygen species form during metabolism, such as when an oxygen molecule is reduced to superoxide. The main point for this story is that resultant reactive oxygen species are very unstable and damage biological macromolecules such as DNA, proteins and lipids. The main source of reactive oxygen species is radiation, which can also damage macromolecules directly. The amount of radiation received by an organism in a particular environment is a function of time.

So, to be able to survive for thousands or millions of years, a microbial species must fulfil several criteria: a) it should be in an environment where reactive oxygen species are less likely to form; b) it should have evolved systems to protect its macromolecules against radiation and reactive oxygen species; c) if cellular macromolecules are damaged, then it needs to be able to repair them; d) it needs enough energy to stay alive, and must grow as efficiently as possible on the available carbon and energy sources.

17. And do Halobacterium species manage to do all these things? Let's consider each of the criteria in turn.

a) "an environment where reactive oxygen species are less likely to form" There is radiation all around us, which is why, when physicists carry out studies into low-background radiation, including the search for Dark Matter, they set up labs underground, including in salt mines, because salt deposits block the background radiation (https://www.boulby.stfc.ac.uk/Pages/home.aspx). Indeed, it has been shown that haloarchaea inside halite crystals survive radiation better than those exposed directly. Also, when haloarchaea use compounds, like sugars or amino acids, as an energy source, they simultaneously consume oxygen (just as humans carry out aerobic respiration). Therefore, the oxygen concentration inside brine inclusions will decrease, and so reduce the likelihood of reactive oxygen species forming (see next figure).

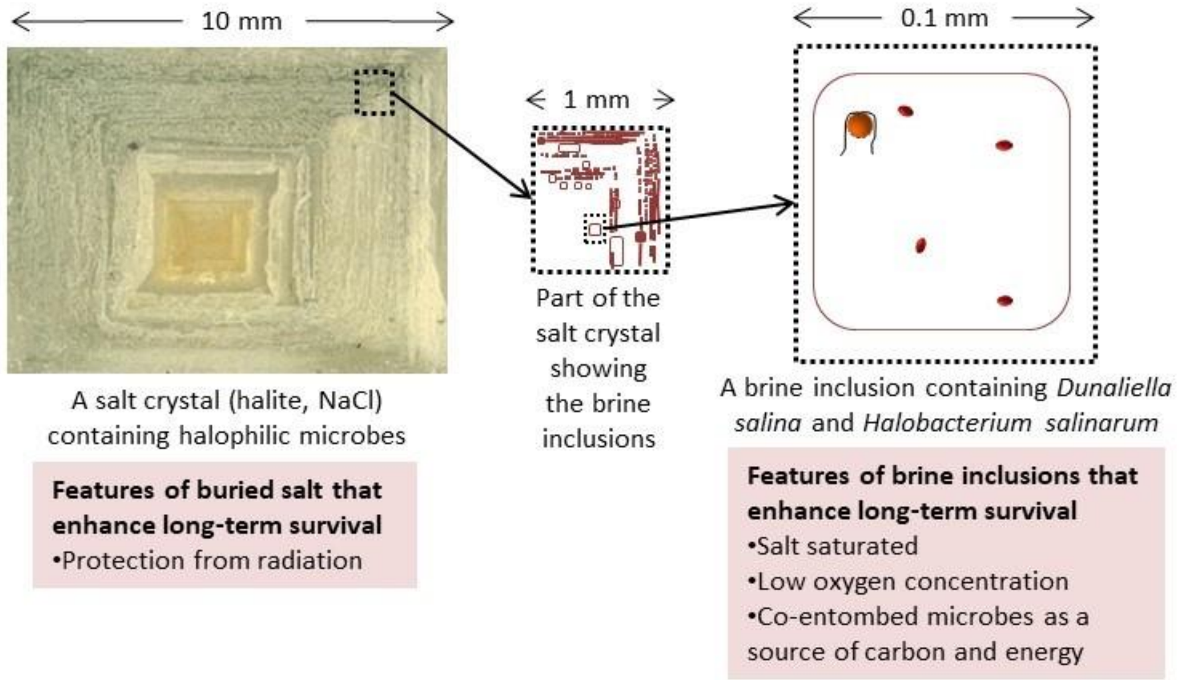
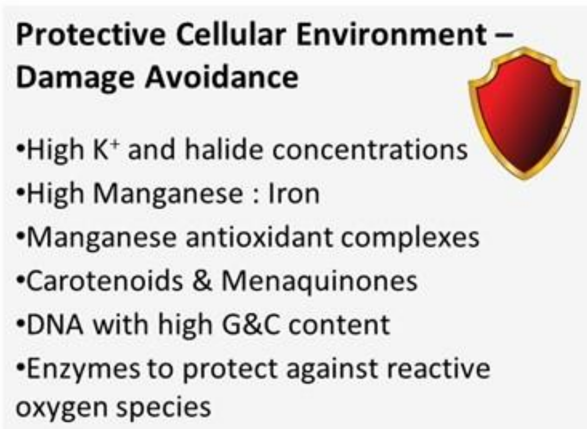


Illustration of how living in salt protects against reactive oxygen species, and can encourage long-term survival

b) "evolved systems to protect its macromolecules against radiation and reactive oxygen species". It is said that prevention is better than cure, and this is certainly the evolutionary strategy adopted by *Halobacterium salinarum*, the most studied species of *Halobacterium*. A cocktail of radiation damage-avoidance strategies are illustrated in the next figure, but two are probably most important. First is the formation of manganese complexes, mainly of peptides (several amino acids joined together) bound to manganese and orthophosphate, which catalytically remove reactive oxygen species, thereby protecting the cellular proteins. Second is the abundance of carotenoids and other mostly water-repellent molecules that sit within the lipid bilayer of the cell membrane. These molecules have an abundance of carbon-carbon double bonds that serve to scavenge and deactivate reactive oxygen species.



Some strategies seen in *Halobacterium* species to help protect damage to the cell by radiation, desiccation and reactive oxygen species

c) “if cellular macromolecules are damaged, then it needs to be able to repair them”. *Halobacterium salinarum* has a suite of repair proteins, but it is also highly polyploid, often with more than 20 copies of its genome. Until relatively recently, it was not known that microbes could be so extremely polyploid. It has been suggested that by having multiple copies of the genome, if there is damage to part of that genome, then there will always be a template of the original to allow faithful repair of the DNA.

d) “it needs enough energy to stay alive and must grow as efficiently as possible on the available carbon and energy sources”. The first important point is that brine inclusions, which already have a low oxygen concentration due to the poor solubility of oxygen in salt-saturated brine, will become more depleted in oxygen as it is used up by aerobic respiration. *Halobacterium salinarum* and relatives can switch metabolism from aerobic respiration to either fermentation or anaerobic respiration, and so continue to function in the brine inclusions. They also require organic matter for energy, in order to maintain an energized cellular membrane and allow repair of any damaged macromolecules, among other functions that just keep the cell alive without it growing.

But a big question is whether there is enough organic matter in the brine inclusions to keep *Halobacterium* species alive for thousands of years? Remember that I told you that there may be hundreds of cells in a brine inclusion? It is likely *Halobacterium salinarum* feeds on the dead cells of its co-entombed “friends”, including members of the same species, that did not survive so well in halite. There are often thousands of co-entombed microbial cells in halite, including *Dunaliella salina,* which does not survive but provides a source of energy, carbon and other nutrients. It has been calculated by Aharon Oren that one cell of *Dunaliella salina* would provide enough carbon and energy for a single miniaturized haloarchaeal cell to survive for 12 million years. That’s another feature of *Halobacterium* species – they can form energy-saving miniature cells.

So, as you can see, there are many different lines of evidence to support the theory of haloarchaeal survival over millions of years, and it has led some to propose that similarly adapted microbes would be good candidates for finding life elsewhere in the universe.

18. Really – where would they live? No one knows whether life ever existed, or exists now, beyond Earth, but astrobiologists, chemists and physicists are looking for signs of life throughout our solar system. Mars is the planet that attracts most interest, because it once had a climate that was much more conducive to the formation and evolution of life as we know it. Any remnants of life are likely to be underground where there is no exposure to the intense radiation and oxidizing minerals found on the surface. Halite has been found in meteorites, indicative of its presence on Mars. Along with other buried salt minerals with fluid inclusions, halite is of particular interest given what we have learned about its capacity to preserve haloarchaea and their bio-signatures (e.g. DNA and lipids).

19. All this stuff about long-term survival is fascinating, but I’ve just realized that we must be eating haloarchaea when we eat sea salt – is that right? Yes, that’s absolutely right. Remember you’re imbibing microbes all the time anyway, mostly on uncooked food, like salads and fresh fruit.

Some haloarchaea play a major role in breaking down biopolymers, especially proteins, in salty environments, such as those used in the production of fish sauces. When we consume these sauces, many other salty fermented foods, or sea salt, we consume haloarchaea! We’ve been doing this for millennia and there is no evidence that haloarchaea do any harm, and who knows they may be beneficial in some way. In any case, they are found among our gut microbes. Whether or not they are normal inhabitants of our gut, and are really part of us, or just passing through, remains to be seen.

Relevance for Sustainable Development Goals and Grand Challenges
(https://sdgs.un.org/2030agenda)

Microbial survival inside halite and the activities of the main microbes found in hypersaline environments relate to the SDGs:

- **Goal 2. End hunger, achieve food security and improved nutrition and promote sustainable agriculture** Fermented foods were probably a key factor in human development and expansion, because of the improved preservation and nutritional value. Halophiles are very important in diverse fermented foods ranging from soy sauce to cheese, and haloarchaea specifically play a role in hydrolyzing proteins to allow downstream fermentation. Occasionally, this can be problematic, leading to the spoilage of salted fish, with the formation of smelly red patches. Entombed haloarchaea may also change the profile of volatile organic compounds in sea salt, which in turn may affect its flavour.
- **Goal 8. Promote sustained, inclusive and sustainable economic growth, full and productive employment and decent work for all** (*promote economic growth, productivity and innovation, enterprise and employment creation)*. There is a lot of interest in the potential for biotechnological products from halophiles. The best realized example is the osmolyte, ectoine, a small metabolite synthesized by many halophilic Bacteria that is compatible with cellular metabolism largely due its capacity to keep proteins hydrated. It is this property that has led to ectoine being incorporated into many skin-care products. The Eukaryotic microalga, *Dunaliella salina,* is cultivated in outdoor, hypersaline ponds to extract its commercially valuable β-carotene. There is interest in using haloarchaea as a source of polydroxyalkanoates, which can be used as biodegradable plastics. Also, bacteriorhodopsin, the transmembrane protein that is an outwardly directed proton pump driven by light, has been exploited in diverse ways, notably by its incorporation into identity cards, to restrict the possibility of forgery.

  The red pigmentation of many extreme halophiles warms the hypersaline brine in salt pans by absorbing solar radiation, leading to more rapid salt production.

  A fascinating application of *Halobacterium salinarum* and relatives is very long-term digital data storage. DNA has great potential as a very high-density storage medium. A pre-print, which has not yet been peer-reviewed, describes the incorporation of DNA that codes for 3D images into *Halobacterium salinarum,* followed by entombment of the archaeon in halite. The authors argue that the extreme longevity of haloarchaea provides a means of preservation that surpasses the currently most long-lived storage medium, i.e. paper!

Potential Implications for Decisions

This Topic Framework does not obviously impinge on decision-making processes. However, issues from the above section on the wider global context merit consideration, not least the value that we put on conservation of hypersaline habitats. The wider benefits of such environments and the threats they face are discussed in detail by Paul and Mormile (2017).

Pupil Participation

1. It would be valuable to hold a discussion on the perception of some people that it is dangerous to revive microbes from ancient environments. Pupils could discuss when this might be the case, why it isn't the case for the vast majority of microbes, the laboratory procedures that ensure safety and the degree to which so-called "ancient microbes" continuously re-seed the surface environments (e.g. via tectonic activity, uplift, dissolution of salt, mining, drilling). The discussion could be extended to issues of contaminating other planets with microbes, the possibility of panspermia, or even the strategies for attempting to recover extraterrestrial microbes.
2. Pupils could think about where saline environments (other than seawater) are found on Earth (e.g. salt springs, hypersaline lakes like the Dead Sea, and land affected by salt mining), and where the salt came from. This can lead to a discussion of weathering of rocks and the release of minerals by flowing water, and how surface water bodies may be connected with buried salt deposits (as well as the microbes they house).
3. Addressing the question "why are very similar haloarchaea found in coastal salt pans across the globe?" can stretch older pupils to think about how microbes move from place to place. In this case, seawater is a barrier for many haloarchaea as it is not sufficiently salty, so perhaps they are transported inside salt crystals by birds or wind.
4. Pupils could draw out the solar system, or the moons around Saturn and Jupiter, and indicate/discuss where they would look for signs of life based on what they know about the limits of life on Earth and the conditions on/in the planets/moons. The discussion could consider salinity, salt types, temperature, radiation exposure, and how these may change with depth in the planet or moon.
5. Older pupils could research different biotechnological applications of halophiles as a group, presenting their results in different formats. The roles of group members may include exploring: the market or potential market for the halophile-derived product or process, methods to improve the biotechnological process, the taxonomy/ecology of the microbes involved, and physiological/biochemical basis of the biotechnological application in the microbes.

The Evidence Base, Further Reading and Teaching Aids

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Glossary

16S rRNA. Refers to 16S ribosomal ribonucleic acid, where the “16S” refers to the size of the molecule, with “S” representing the sedimentation coefficient. It is a vital component of the ribosome, and specifically the smaller subunit, playing a role in protein synthesis (translation of messenger RNA into polypeptides). Its abundance in the cell and its high degree of sequence conservation made it the ideal target for examining the evolution of species. Nowadays, the *gene(s)* encoding 16S rRNA are usually sequenced following amplification by the polymerase chain reaction.

Aerobic respiration. The formation of energy as ATP (adenosine triphosphate) via the electron transport chain, using molecular oxygen (O_2) as a terminal electron acceptor. The electron transport chain results in a proton gradient, which drives ATP synthesis, across the cell membrane of Bacteria and Archaea, and the mitochondrial membrane of Eukarya.

Anaerobic respiration. The formation of energy as ATP (adenosine triphosphate) via the electron transport chain, using molecules other than as a terminal electron acceptor. In haloarchaea these molecules include nitrate, fumarate and dimethylsulfoxide.

Fermentation. In the biochemical sense, the formation of energy as ATP (adenosine triphosphate), usually under anaerobic conditions, using a chemical substrate that is both oxidized and reduced by enzymatic activity. The substrate that is fermented in *Halobacterium salinarum* is the amino acid, arginine.

Genome. All of the DNA found in an organism. In Bacteria and Archaea there is generally a large chromosome (usually circular), and other extra-chromosomal elements such as plasmids. In some haloarchaea it is difficult to distinguish between a large plasmid and an additional small chromosome.

Media (singular "medium"). Refers to growth media, which are solutions containing a mix of nutrients that will enable the growth of microbes, sometimes solidified with the polysaccharide agar to allow growth of colonies on plates.

Microbial community. Mixture of two or more (often many more) species of microbes.

Organic matter. Carbon-based material, such as sugars, fats, proteins and nucleic acids.

Panspermia. The theory that life on Earth originated from elsewhere in the universe.

Polyploid. Possessing multiple copies of the genome.

Protein. Large biomolecules consisting of one or more long chains of amino acids. There are often thousands of different types of protein in a microbial cells. Most are enzymes, catalyzing reactions that break down or synthesis molecules within the cell. Some have structural or regulating roles, while others transport molecules into and out of the cell.

Protists. A diverse group of Eukaryotic microorganisms that are not Fungi.

Reduced (opposite of oxidized). A molecule that has gained electrons is *reduced*.

Sediment. Solid material that is moved and deposited in a new location; or in the case of salts, salt minerals that form by evaporation of saline water. Sediments can consist of rocks, minerals, organic matter, and microbes.

Superoxide. The superoxide anion with the formula O_2^-.