Compatible Solutes: Our and Their Protectants

Mummy: why do you smear cream on your face every day?

Photo by Karolina Grabowska from Pexels

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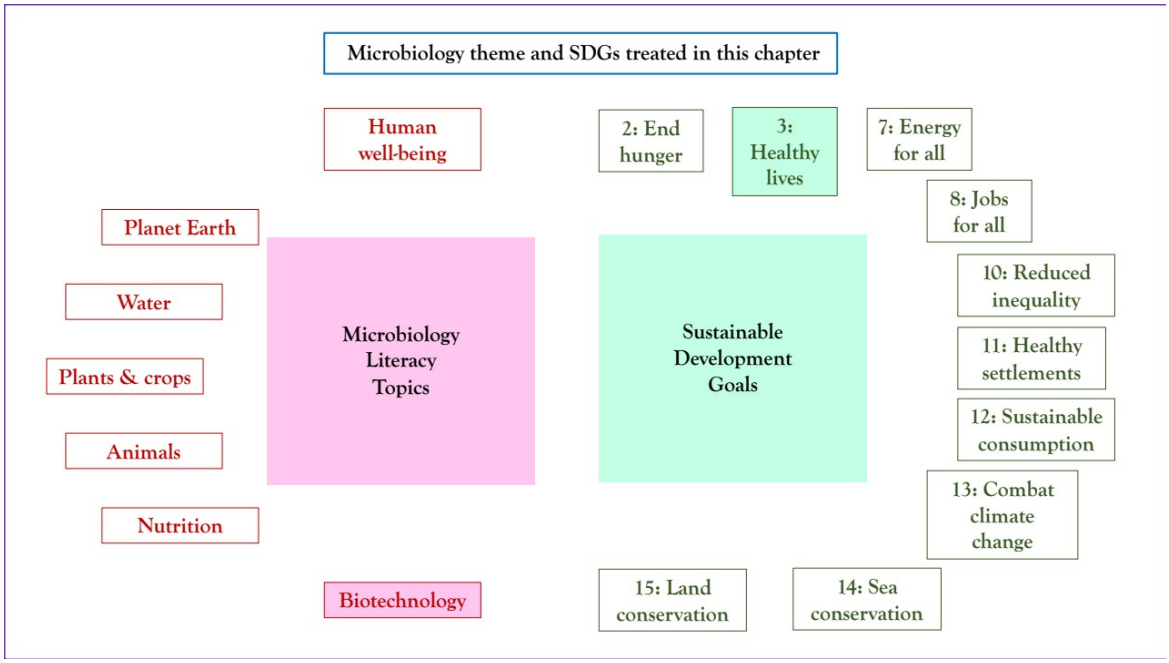
Compatible Solutes

Storyline

Microorganisms can thrive in hostile environments such as salt lakes and brines. Such saline waters are characterized by low water availability are therefore, like deserts, comparatively dry sites. To prevent water loss and avoid getting "pickled" by salt, microorganisms synthesize and amass compatible solutes inside the cell. Compatible solutes are water soluble, organic molecules that retain water inside the cell. Beside their function as moisturizers, compatible solutes are protectants against various stress factors. Compatible solutes protect proteins, membranes and entire cells against heat stress and damage caused by dryness, freezing, thawing and radiation. Compatible solutions, and ectoine in particular, have attracted the attention of the industry, which saw the potential for new health care products with improved protective properties. Today, customers will find numerous sunscreens and different cosmetics, including anti-aging products, based on the compatible solute ectoine. In addition, ectoine has become an active ingredient in health products with anti-inflammatory properties. Ectoine is particularly good at protecting skin tissue from inflammation and shows similar efficacy as antihistamines and corticosteroids. Compatible solutes may also prevent a certain type of protein misfolding called amyloids, which is associated with diseases such as Alzheimer's and prion-related diseases. Compatible solutes could help combat these diseases in the future. The ability of compatible solutes to stabilize proteins should also help vaccines be manufactured more effectively and stored and transported longer without refrigeration.

The Microbiology and Societal Context

The microbiology: Bacteria as source of active ingredients in health care products and cosmetics; more stable vaccines without cooling; compatible solutes from bacteria as starting point for the development of novel drugs against neurodegenerative diseases. Peripherally, for completeness of the storyline: osmosis-osmoregulation; life in extreme environments. Sustainability issue: health.



Compatible Solutes: the Microbiology

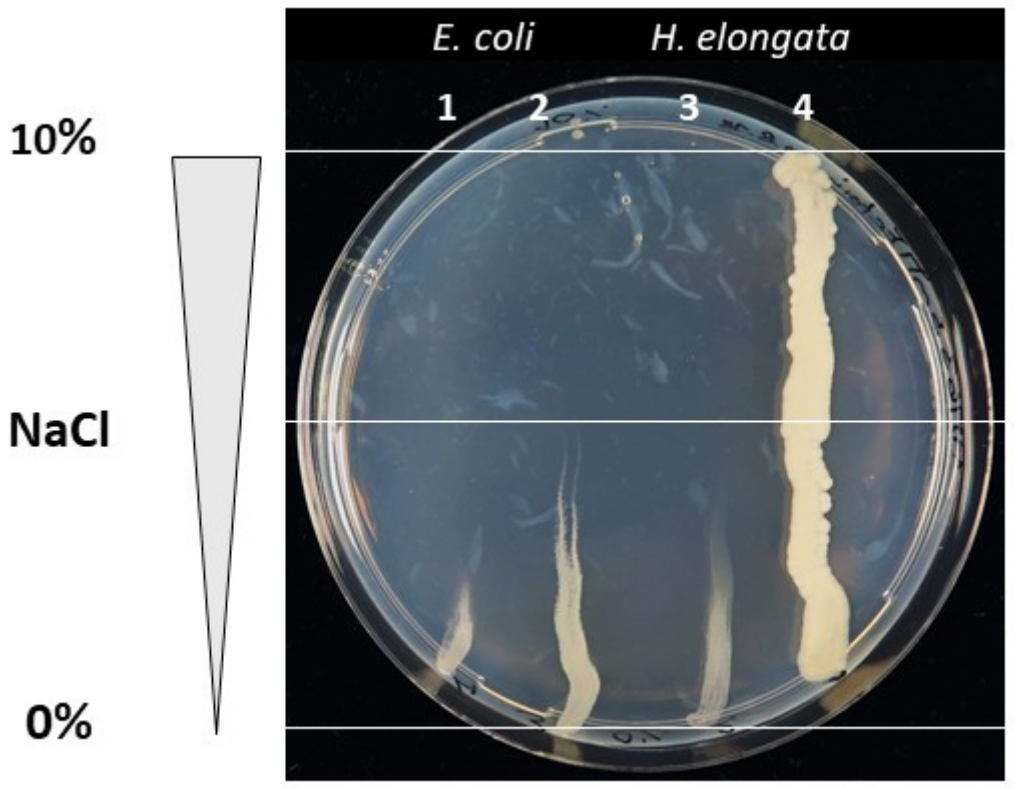
1. ***Salt lakes - A lot of water but dry like a cookie.*** People have known table salt (sodium chloride) for thousands of years as a vital ingredient in many of our foods. Since ancient times, table salt has also been used as a preservative to keep meat or fish from spoiling. Since those times, table salt has been produced by the evaporation of seawater (3 % (w/v) NaCl) in seawater salt works (salterns). Saltern workers noticed that as the salt content of the brine increased, the water turned reddish. Today we know that microorganisms cause the staining of the water.

Salt works on Île de Ré, France. Halophilic microorganisms thrive in salt-saturated (NaCl) water and cause the typical reddish discoloration. The reason for this is phenomenon the pigments in the membranes of the microorganisms.

These microorganisms became popular as research subjects with the beginning of space exploration and the search for life on other planets. The scientists wanted to find out what kind of life can exist in extremely salty conditions, such as those found in numerous places like the Great Salt Lake or the Dead Sea. It sounds surprising, but a salt lake, although it is made of water, is an extremely dry place and resembles more a desert. Indeed, a salt lake is dry like a cookie. Likewise, salted foods are dry as well and this tells us how NaCl acts as a preservative. NaCl lowers the content of freely available water, which is needed for all living organisms, and environments with a high salt content are like deserts hostile to life.

2. ***How microorganisms stay wet in a desert.*** Still, life thrives in salt water, and in the early 20th century it was shown for the first time that the red coloration of salt water is caused by the blooming of microorganisms. Some of the salt loving (= halophilic) microorganisms are specialized for growth at high salinities from 15 % (w/v) NaCl to salt-saturated brines (> 35 % (w/v)), whereas others can adapt to fluctuating low and high salt concentrations. The different growth patterns are based on the two different ways microorganisms deal with salt in their environment. It is important to know that water can freely pass the membrane of any living cell, but the membrane keeps ions and larger molecules inside the cell (semi-permeable membrane). Increasing the salt concentration outside the cell leads to osmosis, which means cell water will flow to the outside until an equilibrium – the same concentration inside and outside the cell – is reached.

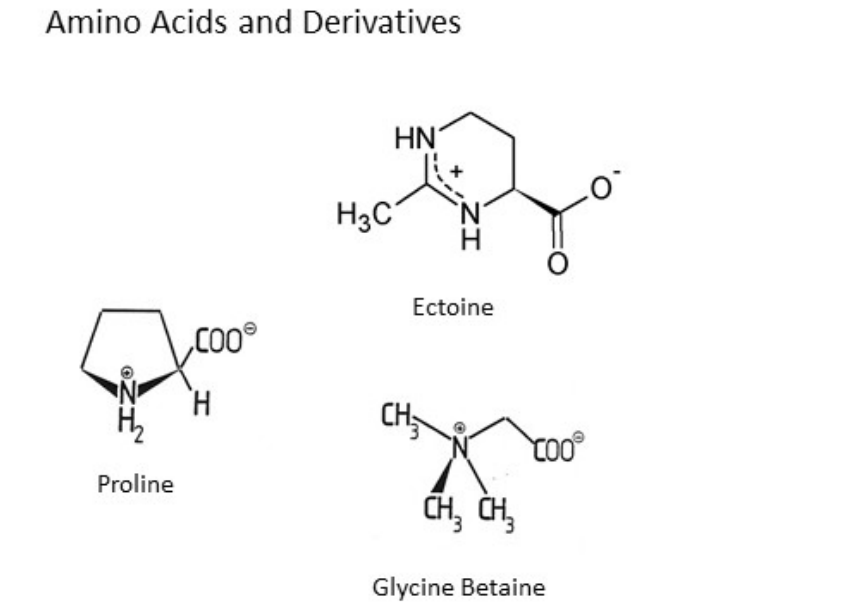
Extreme halophilic microorganisms retain water inside the cell (cytoplasm) by raising the salt (KCl) concentration inside the cell until the water loss has stopped. This adaptation mechanism is called "salt-in-cytoplasm" and cells employing this strategy have to adjust the interior protein chemistry of the cell to high salt concentration. As a result, "salt-in-cytoplasm" microorganisms are restricted to high saline environments. In contrast, the moderate halophilic microorganisms are more flexible and can adapt to fluctuating salinity levels. They synthesize and amass small water-soluble organic molecules inside their cell to cope with salt stress. Although these molecules are accumulated in high concentration in the cytoplasm, they do not interfere with the cell's metabolism and are therefore called *compatible solutes.*



Compatible solutes allow bacteria to grow even at high salt concentrations. The non-halophilic bacterium *Escherichia coli* and the salt-loving bacterium *Halomonas elongata* grow on a solid (agar) medium (the visible vertical streaks represent millions of bacteria that have grown and multiplied from just a few invisible bacteria streaked on the growth medium of the agar plate). The plate is a salt gradient plate: at the bottom, the medium contains 0 % salt (NaCl); towards the top, the salt concentration increases to 10 % (w/v) NaCl. The two *E. coli* strains (1, 2) and an *H. elongata* mutant (3) cannot accumulate compatible solutes and only grow-multiply at salt concentrations below 5 % (w/v) NaCl. The wild type of *H. elongata* (4) synthesizes the compatible solute ectoine and can even thrive at 10 % (w/v) NaCl and above.

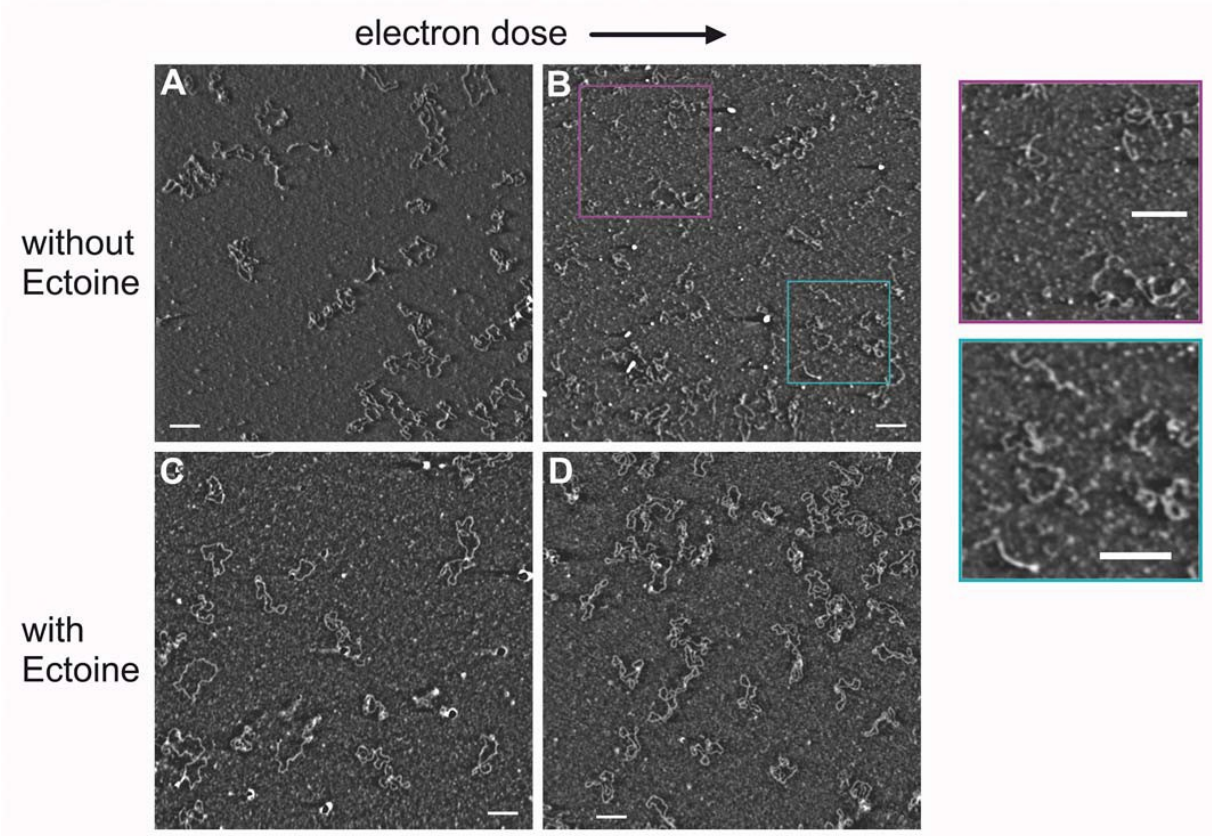
3. ***Compatible Solutes – A shield against hostile stress.*** What is a compatible solute? Chemically, compatible solutes are quite diverse and belong to different substance classes. Compatible solutes are often from the classes of amino acids and derivatives, sugars, polyols, and sugar-polyols. Compatible solutes that are widely distributed in nature and commonly found in bacteria include the amino acid proline, the amino acid derivatives ectoine and glycine-betaine, the sugar trehalose, and the sugar-polyol glucosylglycerol. The polyol glycerol is a well-known compatible solute in fungi and algae. The amine oxide trimethylamine *N* oxide (TMAO) can be found in mollusks (squids, mussels), crustaceans (crayfish), and fish. Despite

their differences in structure and composition, compatible solutes can be characterized as uncharged (nonionic, zwitterionic), but polar and highly water-soluble molecules.



Examples of compatible solutes in bacteria. Shown are the sugar and sugar derivative trehalose and glucosylglycerol and the amino acid proline. The amino acid derivatives glycine betaine and ectoine are widespread in halophilic bacteria.

4. ***Compatible solutes are beneficial for bacterial cells not only as osmoregulatory molecules to retain water in the cell, but also as protectants of proteins, membranes, nucleic and entire cells against various types of stress.*** Compatible solutes can protect proteins and cells against the damage caused by freezing and thawing, drying, and high temperatures. Some compatible solutes, such as ectoine, have additional protective properties compared to other compatible solutes, which are beneficial not only to bacteria, but also to animal and human cells. Ectoine stabilizes whole human cells against stresses such as ultraviolet (UV) radiation, inflammation caused by nanoparticles or cytotoxins. Recent research revealed that ectoine is also a protective agent for the nucleic acid DNA against ionizing radiation.



Compatible Solute ectoine protects DNA from ionizing radiation. DNA from bacteria (plasmids = small, circular DNA) were irradiated with electrons and examined with a microscope (atomic force microscopy) after irradiation. At low doses of radiation, DNA remains intact (A, C). At high radiation, DNA without ectoine is damaged (B, short linear DNA fragments). In contrast, DNA in ectoine solution is protected from radiation and remains in its native, circular shape (D).

5. ***Compatible solutes for purchase - From daily life products to healthcare.*** The beneficial properties of compatible solutes, and of ectoine in particular, attracted industry, which saw the potential to launch novel and improved health products with compatible solutes as active ingredients. The first commercial use of ectoine was as an ingredient in skin care products and cosmetics and today customers will find numerous sunscreens and anti-aging products from various brands in which ectoine is widely used.

Over the years, ectoine has become an active ingredient in health products with anti-inflammatory properties and ectoine is especially effective in protecting different types of skin tissue and skin cells (epithelia). For the nasal and eye epithelia in allergic eye-inflammation (conjunctivitis), nasal sprays and eye drops with ectoine effectively relieved the characteristic symptoms. Importantly, ectoine showed an efficacy similar to those of antihistamines and corticosteroids, but without the typical side effects.

Many ectoine-based health products for the treatment of allergies and skin inflammation like eczema (atopic dermatitis) and rhinosinusitis have been developed and successfully tested in clinical trials, and are available on the market today.

Ectoine-based medical products. Ectoine-based healthcare products on the market include allergy nasal sprays and allergy eye drops, eye drops and nasal sprays for the treatment of dry eyes and dry nose, products for the treatment of coughs and rhinitis, and dermatological creams for the treatment of atopic dermatitis and other inflammatory skin conditions

As mentioned, compatible solutes protect proteins from misfolding caused by heat or freezing. It was shown that compatible solutes can also prevent a certain type of protein misfolding that produces so-called amyloids. The formation of amyloids is associated with neurodegenerative diseases such as Alzheimer's disease. Compatible solutes may serve as a promising starting point for the development of novel compounds that prevent amyloid formation and may be used in the future to treat neurodegenerative diseases such as Alzheimer's, Parkinson's, or Huntington's as well as prion-related diseases.

6. ***Compatible solutes and vaccine protection.*** The ability of compatible solutes to stabilize proteins should also help vaccines be manufactured more effectively and stored and transported longer without refrigeration. Solutes are already advantageous at the beginning of the vaccine production. Vaccines are often produced using recombinant DNA technology, which requires expression of the protein antigen or an antibody in a suitable host organism. Cells that are used on a regular basis as hosts are bacteria such as *Escherichia coli* or eukaryotes such as yeast. These hosts have the advantage that they grow fast and allow production on a large scale. However, synthesizing a protein in foreign host can lead to its misfolding, and its recognition and subsequent degradation by specific enzymes (proteases) of the host cell. To reduce misfolding, compatible solutes are added to the cells during vaccine synthesis. Compatible solutes induce proper folding of the growing protein and thereby prevent degradation of the newly synthesized vaccine.

It has also been shown that the shelf life and stability of the vaccine increases if compatible solutes were present in the host cell during synthesis, which leads to another problem: the loss of vaccine efficacy during transport and storage. Often antibodies and

protein-antigens are unstable at room temperature and are therefore transported or stored at low temperatures. Unfortunately, some vaccines lose their efficacy when frozen or frozen and thawed. Compatible solutes such as trehalose, glycerol or ectoines are known to stabilize the natural function of proteins for months at temperatures of -20 °C or below. Compatible solutes can also help eliminate the need to freeze vaccines and protect them in the absence of a cold chain. Many compatible solutes protect labile proteins from heat- or warmth-induced damage, and glycerol, for example, has been shown to stabilize anthrax vaccine against inactivation at room temperature. To store vaccines at room temperature, to protect them from high temperatures during shipment, and to make shipping and distribution easier, attempts are made to stabilize vaccines by drying them into powder form. This is done by a method called freeze-drying. Although freeze-drying is considered gentle on proteins or whole cells, antigens or antibodies can lose their efficacy during this process. Again, compatible solutes can help stabilizing vaccines, and trehalose, for example, has been shown to effectively protect influenza vaccines during the drying process.

Relevance for Sustainable Development Goals and Grand Challenges

- **Goal 3. Ensure healthy lives and promote well-being for all at all ages.** Because compatible solutes are able to protect and hence stabilize pharmaceutical products from various stresses during production, formulation, transportation and on the shelf, they can reduce medicament and hence health costs, and enable medicaments to be more widely available, particularly in warm climates needing cold chains. In addition, they seem to have pharmacological activities in their own right, so may themselves become significant medicaments in future.

The Evidence Base, Further Reading and Teaching Aids

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Glossary

Antibody: is a protein of the immune system that recognize foreign components such as bacteria or viruses and help destroy them.
Antigen: a molecule on the outside of bacteria or viruses to which components of the immune system, such as antibodies, can bind.
Brine: is water with a high salt concentration, usually well above that of seawater, up to salt-saturated water.
Cytoplasm: all components of the cell that are enclosed by the cytoplasmic membrane, with the exception of the nucleus.
Cytoplasmic membrane: is a thin lipid layer of the cell that separates the cytoplasm from the environment.
Ionizing radiation: is able to remove electrons from atoms or molecules. This creates positively charged ions and breaks chemical bonds, causing damage to DNA and proteins.
Metabolism: comprises all biochemical reactions in a cell.

Microorganism: is a microscopic (cannot be seen by the naked eye) small organisms that exists as a single-cell or a colony of cells. All Archaea and Bacteria and many single-celled Eukaryotes are microorganisms.
Osmoregulation: describes the regulation of water and salt concentration in an organism.
Protein: is a polymeric molecule consisting of one or more polypeptides. Peptides are made of amino acids.
Salinity: describes the amount of salt dissolved in water. Salinity is often measured in grams of salt per volume of water, with the volume expressed in L (liter) or 100 mL (0.1 L). Grams per 100 mL is equal to % and the percent sign is then labeled w/v, which stands for weight (g) per volume (100 mL). Seawater contains about 3 g of salt per 100 mL and the notation in percent would be 3% (w/v).
Sodium chloride: is an ionic compound commonly known as salt. Its chemical formula is NaCl, consisting of sodium (Na^+) and chloride (Cl^-) ions in a ratio of 1:1. Sodium chloride is mainly responsible for the salinity of sea water and it is used as table salt.
Zwitterion: In chemistry, a zwitterion, also called an inner salt, is a molecule that contains an equal number of positively- and negatively-charged functional groups. With amino acids, for example, in solution a chemical equilibrium will be established between the "parent" molecule and the zwitterion.