Ectoine

A survival tool for microorganisms living in the extreme

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Extreme microorganisms? What does this mean?

Yes exactly, but they are called extremophiles literally meaning ‘extreme-loving’. These organisms thrive in what we would call extreme environments. To define what extreme is we need to consider what our ideal environment is? 22 °C, sunshine and a cool breeze perhaps? Now imagine living in boiling acid or in a pitch black oxygenless swamp. Not quite pleasant for us but for extremophiles ideal. Extreme environments include high or low temperatures, extreme pressure or drastically salty conditions.¹

What does ectoine have to do with this?

In one sentence: Ectoine is a compatible solute in halophilic organisms. Now let’s take this fancy sentence apart. Halophilic organisms (halophiles) live in environments with high salinity levels. It was believed to be impossible to live in such hypersaline environments, but the discovery of microorganisms inhabiting these environments in the beginning of the twentieth century suggested otherwise.¹ Ectoine was first discovered in the extreme microorganism *Halorhodospira halochloris*, which resides in the salt lakes of Wadi Natrun (Egypt). The air humidity is so dry and salt concentration so high that plants and animals avoid this place and yet halophiles are able to survive these conditions.² What is their secret? (Hinting at ectoine here)

The biggest challenge for halophilic organisms is to balance the osmotic stress exerted by the environment. Most halophiles don’t possess systems for an active transport of water so they have to find ways to exclude salt from their cytoplasm to avoid being ‘salted out’. They have come up with an ingenious strategy in which they adapt themselves to the saline environment by accumulating high solute concentrations in their cytoplasm, preventing water in the cell from leaving down the concentration gradient.² There are basically two mechanisms, namely the ‘salt-in-cytoplasm’ mechanism and the ‘organic osmolyte’ mechanism.³

Organisms following the ‘salt-in-cytoplasm’ mechanism, mainly *Halobacteriaceae* (haloarchea), accumulate potassium chloride in their cytoplasm to achieve osmotic equilibrium with the environment. In other words, the potassium chloride concentration in the cytoplasm of the microorganism is equal to the external concentration of sodium chloride. This mechanism however, requires a number of physiological changes to proteins in the cell to adapt to the high internal potassium chloride concentration.³
The organic osmolyte mechanism is suitable for a wider range of saline concentrations and does not require physiological changes to the cell's interior. Much more practical than the ‘salt-in-cytoplasm’ mechanism! Organisms following this mechanism accumulate water soluble organic compounds which do not interfere the cell’s metabolic processes.¹ These so called compatible solutes include amino acids, sugars and polyols.²

Now we have come to the point where we can discuss ectoine. Going back to our fancy sentence one would deduce that ectoine must either be an amino acid, sugar or polyol. Take a look at the structure of ectoine below (Figure 1). Which of the three is it?

![Structure of ectoine](image)

Figure 1: Structure of ectoine 1, 4, 5, 6-tetrahydro-2-methyl-4-pyridine carboxylic acid.

If you placed your bets on amino acid, you are completely correct! Ectoine is a cyclic amino acid.²

**Okay. How does the structure of ectoine relate to its function?**

First off, ectoine is a zwitterionic species and contains three polar groups, indicated by the red boxes in Figure 1. The polar groups and the positive and negative charges in the structure induce a strong hygroscopic effect which in turn enhances and stabilizes the tetrahedral water structure. In essence the water is bound through hydrogen bonding to the ectoine molecule. This ectoine water complex (Figure 2) keeps the cell from drying out.⁴

![Ectoine water complex](image)

Figure 2: Ectoine water complex⁵
These ectoine water complexes increase the number of water molecules around a protein and thereby form a spherical protective shield which stabilizes the whole protein structure (Figure 3).  

Even a cell membrane (lipid bilayer) is stabilized in the presence of ectoine. An ectoine solution increases the number of hydrophilic interactions of water with the hydrophilic head groups which stabilizes the hydrophobic interactions of the non-polar tail (Figure 4). As a result the cell membrane fluidity is increased. These water binding properties of ectoine are the reason why extremophiles can survive in dry and saline environments.
How is ectoine synthesized by the cell?

The biosynthesis of ectoine was determined with the help of $^{13}$C-labeling techniques and proceeds via the pathway in Figure 5. The pathway starts with the phosphorylation of the amino acid L-aspartate in the presence of the enzyme aspartokinase and ends with the conversion of diaminobutanoate to ectoine via ectoine synthase.  

![Pathway for the biosynthesis of ectoine](image)

Figure 5: Pathway for the biosynthesis of ectoine
We said before that the ‘organic osmolyte’ mechanism is suitable for a wide range of salinities. This suggests the halophile is able to adapt to different salinity levels. How is this done?

As the salinity increases for example evaporation, the molecule just produces more ectoine. In the case of heavy rainfall or flooding it leads to a rapid decrease in salinity. This causes the halophile to release ectoine. It is not exactly known how the production and release of ectoine is regulated, but evidence suggests that a transcription regulator is in play.\(^7\)

**There must be a reason for all this research done on ectoine. What can we gain from this?**

Ectoine wasn’t always as exciting as it is today. Initially it was difficult to carry out research on ectoine as it was always found in small quantities. An industrial production of ectoine in larger quantities was possible, albeit expensive, and the uncertainty of finding commercial use for ectoine was just too great. But once ectoine was found useful in cosmetics, the search for a suitable microorganism which could synthesize ectoine at large scales began. Ideally the microorganism should respond well to large changes in salinity and be able to build up and release ectoine quickly.\(^1\)

Today, the bacterium *Halomonas elongata*, is used by bitop AG to produce ectoine by a process of ‘bacterial milking’ (Figure 6). Indeed it is in principle quite similar to milking a cow! First the strain has to be grown. This is done by a fermentation process in a vessel containing 15-20 % sodium chloride at temperatures between 25 °C and 40 °C. A cross flow filtration then reduces the volume of the culture. Part of the culture is returned to the fermentation vessel and the rest is transferred to the next step. The bacteria in this culture experience an osmotic down shock which releases ectoine. The ectoine is then separated from the bacteria. The bacteria return to the fermentation vessel while the ectoine is further purified through electro-dialysis, chromatography, freeze-drying, and crystallisation. Through this process ectoine with a purity of more than 99 % is yielded.\(^1\)

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\(^7\) Evidence suggests that a transcription regulator is in play.

\(^1\) Figure 6: Industrial production of ectoine; ‘bacterial milking’
Now that ectoine can be produced in large quantities all that is left is to find use for it! Not just in cosmetic products which stimulated the production of ectoine in large scales, but ectoine has also found to be advantageous in therapeutic and medicinal products.

- Eye drops containing ectoine (Triofan®) are effective in preventing the eyes from drying out and helps fluidize the tear fluid lipid layer.¹⁰
- Ectoine nasal sprays (Triofan® naturel and Triofan® Heutschnupfen) are used in the treatment of allergic rhinitis and rhinoconjunctivitis symptoms.⁹
- Mouth and throat sprays with ectoine are used to treat pharyngitis/laryngitis.¹⁰
- Ectoine containing creams are used in the treatment of atopic dermatitis.¹¹
- Ectoine is even found in ‘anti-aging’ creams as it hydrates the skin and improves its elasticity and surface structure.¹²

And this is just the beginning!

Bibliography


[10] Müll et al., 2016. "Efficacy and tolerability of an ectoine mouth and throat spray compared with those of saline lozenges in the treatment of acute pharyngitis and/or laryngitis: a prospective, controlled, observational clinical trial"
