Salty Solutions: How Halophilic Archaea Can Help Spice Up Biotechnology

Philipp Kerpen*, Mahmoud Nasfat Azeez Rawajba**, Kirsti Rindal***

*Leipzig University, **Birzeit University, ***Universitetet i Bergen

SCAN ME

WHAT ARE HALOPHILIC ARCHAEA?

Halophilic archaea are a group of **extremophiles** that have adapted to **live in high-salt environments.** Some are able to **maintain intracellular osmotic balance** in highly saline conditions by producing or accumulating **compatible solutes**, which are small organic molecules that counteract the destabilizing effects of high salt concentrations on cellular macromolecules. Others follow a salt-in strategy. Additionally, have they developed some unique molecular mechanisms regarding their enzymes and membrane proteins (Li et al. 2021; DasSarma et al., 2015, Singh et al., 2017).

WHAT IS THEIR NATURAL HABITAT?

Halophilic archaea are found in different **highly saline environments**, including salt lakes, salt pans, and saline soils, with salt concentrations from just above sea level to saturation levels. The ideal growth conditions for different halophilic microorganisms are important to consider when studying their biotechnological applications (Li et al. 2021; DasSarma et al., 2015).

METHODS

Isolation and cultivation of halophilic microorganisms require specialized techniques due to the harsh growth conditions they require. Different methods have been used to isolate these organisms, including **enrichment culturing, dilution-to-extinction, and direct plating on solid media.**

One of the most common methods used for cultivating halophiles is the use of **high-salt agar media**, which typically contains between 15-30% (w/v) NaCl, depending on the target species. These media are supplemented with other nutrients such as peptone, yeast extract, and glucose to support microbial growth.

The use of **selective media**, which are tailored to favor the growth of specific halophilic microorganisms, can also be useful in the isolation and cultivation of these organisms. Selective media can be supplemented with antibiotics, dyes, or other substances that inhibit the growth of non-halophilic microorganisms.

Techniques such as **metagenomics**, which involves analyzing DNA extracted directly from environmental samples, can also be used to identify and isolate halophilic microorganisms. This approach has been particularly useful in uncovering novel species and enzymes with potential biotechnological applications.

Overall, the isolation and cultivation of halophilic microorganisms requires careful consideration of their unique growth requirements and specialized techniques. Advances in cultivation and genomic methods will continue to provide valuable insights into the diversity and potential of these organisms for various biotechnological applications (DasSarma et al., 2015; Aharon Oren, 2009).

Salt ponds with pink colored Haloarchaea on the edge of San Francisco Bay, near Fremont, California.

Salt deposits on the shores of the Dead Sea.

APPLICATIONS

ENZYMES

Halophilic archaea produce enzymes that **function optimally under high-salt conditions** and have **unique properties** that make them useful in various industrial applications, such as the production of detergents and bioremediation (DasSarma et al., 2015; Aharon Oren, 2009). They can produce proteases, amylases, lipases, esterases. Function in high-salt, high-temperature and in the presence of detergents. Stable enzymes. Unusual properties because of the environment in which they are produces. Practical in industrial applications (Aharon Oren, 2009).

SOLAR SALT

Halophilic archaea have been found to play an important role in the thousand years used procedures for production of solar salt, which is a type of salt produced by the evaporation of seawater under the sun.

It is found that the addition of halophilic archaea to salt ponds significantly increased the yield and quality of solar salt. Due to regulation of the pH, salinity, and mineral composition of the brine, which can affect the crystallization of halite, affecting salt quality. Furthermore, are the benthic microbial mats helpful as they seal off the bottom of evaporation ponds, preventing brine leakage (Aharon Oren, 2009).

FERMENTATION

Halophilic archaea are used to ferment high salt foods like fish sauce, pastes and pickles. This is a quick and cost-efficient process when these microorganisms are utilized (Aharon Oren, 2009).

DRUG DELIVERY SYSTEMS: GAS VESICLES

Gas vesicles are **gas-filled, protein-bound structures** found in some halophilic archaea, including *Haloferax mediterranei* and *Halobacterium salinarum.* These gas vesicles have attracted attention as potential drug delivery vehicles due to their **unique properties** such as biocompatibility, size tunability, and the ability to control their buoyancy. Gas vesicles can be purified from halophilic archaea and modified to express proteins or peptides on their surface for targeted delivery. For example, gas vesicles from H. mediterranei can be engineered to display a tumor-targeting peptide for cancer therapy (Pfeifer et al. (2021)). Additionally, these gas vesicles also have a potential use for imaging applications, such as magnetic resonance imaging (MRI) (Stokke et al. (2019)).

The use of gas vesicles in drug delivery systems is a promising area of research with potential for significant impact in the field of medicine.

WASTEWATER TREATMENT

Archaeal and bacterial halophiles can remove a wide range of pollutants, including heavy metals, organic compounds, and dyes, from hypersaline wastewater. These microorganisms can also degrade petroleum hydrocarbons, which are persistent and toxic pollutants commonly found in wastewater (Li et al. 2021).

During bioremediation, halophiles utilize various metabolic pathways to break down pollutants into less harmful or even beneficial substances. For instance, some halophiles use sulfate reduction pathways to convert sulfate ions to hydrogen sulfide, which can be easily removed from wastewater. Others use denitrification pathways to remove nitrogen compounds, such as nitrates and nitrites, from wastewater. In addition, halophiles can also produce extracellular enzymes that can degrade complex organic molecules into simpler molecules that can be easily removed from wastewater (Li et al. 2021).

In conclusion, halophilic microorganisms have shown great potential in the bioremediation of wastewater, and their ability to recycle and transform various elements and molecules makes them promising candidates for sustainable waste management (Li et al. 2021; Aharon Oren, 2009).

BIOFUELS

Biofuel production involves the conversion of organic matter into biofuels through anaerobic digestion, fermentation, and other metabolic pathways by the halophilic archaea. This process can produce hydrogen, methane, and ethanol from various sources such as glycerol, glucose, and lignocellulosic biomass (Kasirajan et al., 2021).

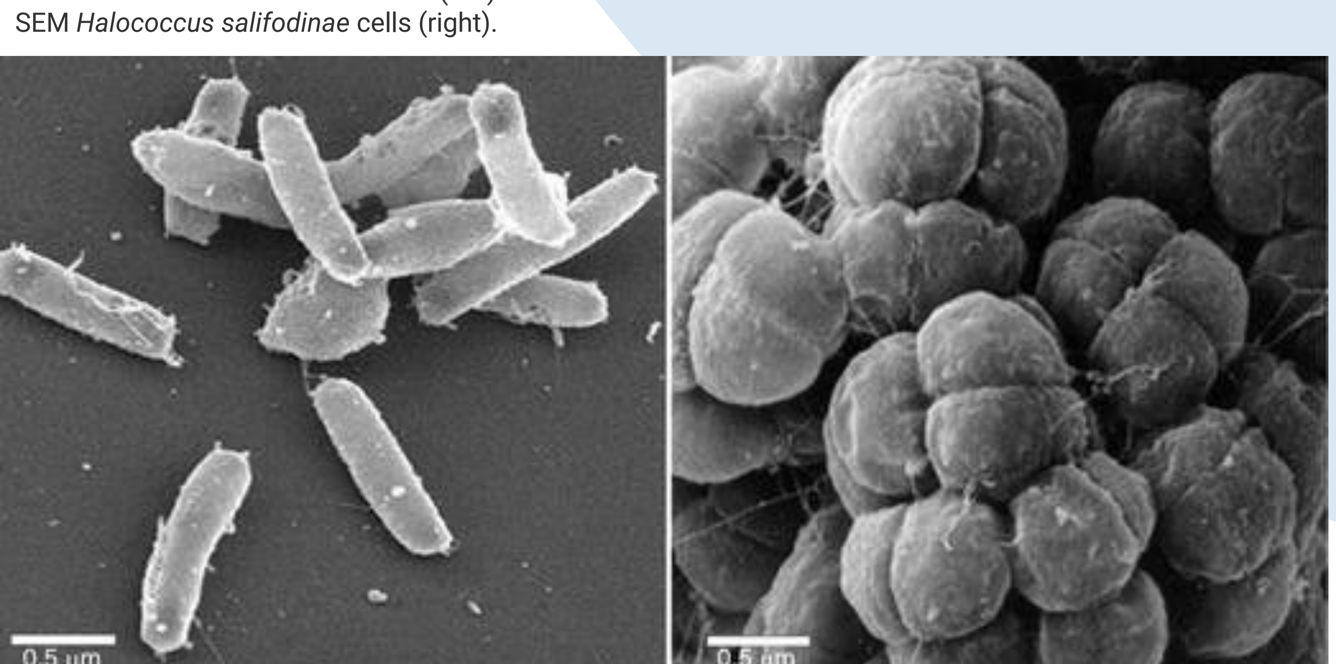
The production of hydrogen, for example, can be accomplished by Halobacterium salinarum through the use of light energy (DasSarma et al., 2015).

This organism, additionally, has been shown to produce large amounts of the energy-rich compound polyhydroxyalkanoate (PHA) that can be converted into biofuels such as biodiesel and biogas. In addition, some halophilic archaea, such as Haloferax mediterranei, have the ability to produce extracellular enzymes that can break down lignocellulosic biomass into simpler sugars that can be fermented into biofuels (Kasirajan et al., 2021).

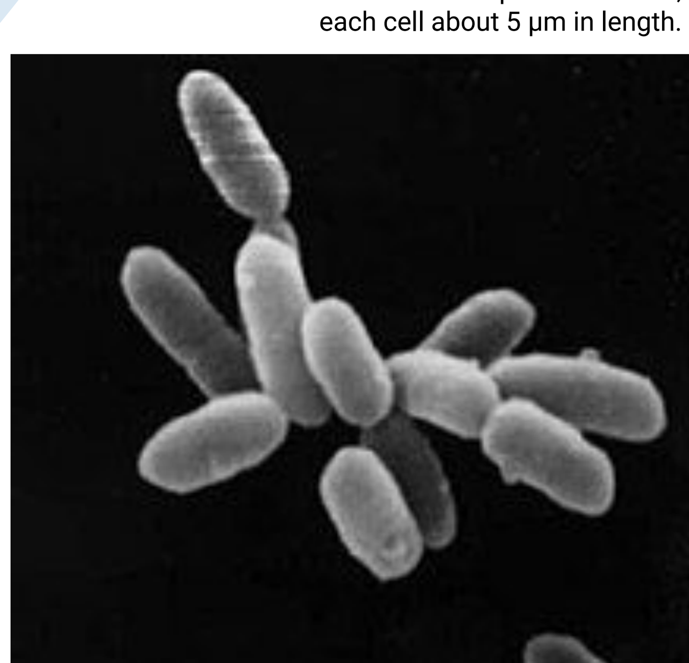
BIODEGRADABLE POLYMERS AND EXOPOLYSACCHARIDES

Polyhydroxyalkanoates and exopolysaccharides produced by halophilic archaea are biodegradable and have the potential to replace commercial non-degradable plastics and polymers. Many different species can produce these polymers (Singh et al, 2017).

SEM *Halobacterium salinarum* cells (left).
SEM *Halococcus salifodinae* cells (right).



Halobacterium sp. strain NRC-1, each cell about 5 µm in length.



REFERENCES

Daoud, L., & Ali, M. B. (2020). Halophilic microorganisms: interesting group of extremophiles with important applications in biotechnology and environment. In *Physiological and biotechnological aspects of extremophiles* (pp. 51-64). Academic Press.
Kumar, V., & Tiwari, S. K. (2019). Halocin diversity among Halophilic Archaea and their applications. *Microbial Diversity in Ecosystem Sustainability and Biotechnological Applications: Volume 1. Microbial Diversity in Normal & Extreme Environments*, 497-532.
Li, J., Gao, Y., Dong, H., & Sheng, G. P. (2022). Haloarchaea, excellent candidates for removing pollutants from hypersaline wastewater. *Trends in Biotechnology*, *40*(2), 226-239.
Obruča, S., Dvořák, P., Sedláček, P., Koller, M., Sedlář, K., Pernicová, I., & Šafránek, D. (2022). Polyhydroxyalkanoates synthesis by halophiles and thermophiles: towards sustainable production of microbial bioplastics. *Biotechnology Advances*, 107906.
Corral, P., Amoozegar, M. A., & Ventosa, A. (2019). Halophiles and their biomolecules: recent advances and future applications in biomedicine. *Marine drugs*, *18*(1), 33.
Anshuman, K. P. (2023). Halophiles and its biofilm for hyper saline waste water treatment. *Current Research in Life Sciences*, 6.
Amoozegar, M. A., Safarpour, A., Noghabi, K. A., Bakhtiary, T., & Ventosa, A. (2019). Halophiles and their vast potential in biofuel production. *Frontiers in microbiology*, *10*, 1895.
Lakshmi Kasirajan, Julie Maupin-Furlow (2021): Halophilic archaea and their potential to generate renewable fuels and chemicals. In: *Biotechnological Bioengineering* (118(3)), S. 1066–1090.
Oren, Aharon (2010): Industrial and environmental applications of halophilic microorganisms. In: *Environmental technology* 31 (8-9), S. 825–834.
Pfeifer, Kevin; Ergal, İpek; Koller, Martin; Basen, Mirko; Schuster, Bernhard; Rittmann, Simon K-M R. (2021): Archaea Biotechnology. In: *Biotechnology advances* 47, S. 107668.
Shiladitya DasSarma; Priya DasSarma (2015): Halophiles and their enzymes: Negativity put to good use. In: *Current Opinion Microbiology* (25), S. 120–126.
Singh, Aparna; Singh, Anil K. (2017): Haloarchaea: worth exploring for their biotechnological potential. In: Biotechnology letters 39 (12), S. 1793–1800