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UNIVERSITI MALAYSIA SABAH Transforming Ideas into Reality

Halophiles

IB6063 Molecular Microbiology
Dorathy anak Jampi
MZ1612006T

Introduction

Halophiles
- extremophilic microorganisms that grow optimally at high salt concentrations (Siglioccolo, Paiardini, Piscitelli, & Pascarella, 2011).

Mainly found in marine salterns and hypersaline lakes, such as the Great Salt Lake and the Dead Sea (Oren, 2008)

The Red Sea was named after halobacterium that turns the water red during massive blooms (Sharma, n.d.)

"Red herring" is the foul smell of salted meats spoiled by halobacterium


Dead Sea


Red Sea


Great Salt Lake

Types of halophiles


Halobacteriaceae

Extreme halophiles
- grow optimally in media contained 1.5-4.0 M salt
- *Halobacteriaceae* and *Salinibacter ruber*

Moderate halophiles
- grow optimally in media contained 0.5-2.5 M salt
- In genera *Desulfovibrio, Desulfocella, Desulfohalobium Desulfotomaculum*

Halotolerant
- not exhibit absolute requirement for salt for growth but grow well at high salt concentrations (above 2.5M salt = extreme halotolerant)
- Bacteria genera *Alteromonas, Lactobacillus, Bacillus, Myxococcus, Pediococcus*
- Fungi such as *Debaromyces, Hansenula*

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Effects of high salinity to non-halophiles

Plasmolysis

When an organism is placed in a high salinity solution, water inside the organism will diffuse to the water outside the organism to restore the balance due to osmotic stress.

Water move from high concentration region to low concentration region

Hyperosmotic stress will cause cell to dehydrate and shrink (plasmolysis) (Wharton, 2007).

Conformational stability of proteins

Destabilizing the folded form and nonfolded form of proteins (Ebel, Madern, & Zaccai, 2009).

Applications

- Red color from carotenoid compounds of bacteriorhodopsin can be used for **food coloring**
- halophilic organisms can be used on soy sauce and Thai fish sauce **fermentation**
- in organic-osmolyte strategy, the compatible solutes can be used in protein and cell **protectants** in variety of industrial uses.
- the bacteriorhodopsin can be used in **bioelectronics** as optical switches and photocurrent generators
- incorporation of genetically engineering halophilic enzyme encoding DNA into crops to allow for **salt tolerance**
- **waste water treatment** such as hypersaline waste brines contaminated with various organics

Adaptation strategies

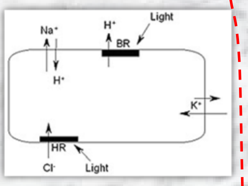
The "salt-in" strategy

the accumulation of high concentrations of inorganic ions in the cytoplasm (Oren, 2006).

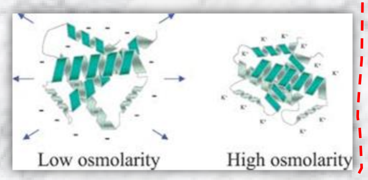
the accumulation of potassium and/or sodium in the cytoplasm causes cytoplasm to be exposed to an increased ionic strength

the interior protein chemistry of the cell will adapt to the high salt concentration

achieved thermodynamic adjustment



Low pI value of halophilic proteins



In order to adapt to the ionic cytoplasm, enzymatic machinery proteins of halophiles contain more acidic amino acids than basic residues (Kunte, 2009)

Increase in acidic residues (glutamate, aspartate) and decrease in lysines (Kiraga et al., 2007; Siddiqui & Thomas, 2008)

Predominance of charged amino acids on enzymes and ribosomes surface stabilize the hydration shell

Thus, halophile that employed salt-in cytoplasm strategy exhibit restricted growth in highly saline environments (Kunte, 2009)

However, in low salinity environment, the excess negative charged ions will destabilize the structure of molecule due to repulsion when the shielding cations are removed

Twin-arginine translocation (Tat) pathway

Sec pathway: proteins that can fold after translocation
Tat pathway: proteins that fold before translocation

Tat pathway is energetically expensive that most prokaryotes more than 90% of proteins are Sec dependent (Siddiqui & Thomas, 2008)

hydrophobic interactions are stronger at high cytoplasmic salt concentration with high ionic strength

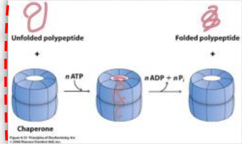
Produced folded proteins are incompatible with Sec pathway

Proteins fold very rapidly in the cytoplasm using salt-in strategy

Chaperonin GroEL homologue (hpGroEL)

Heat shock proteins (Hsp) called chaperonin and GroEL involved in folding, assembly and transport newly synthesized proteins to be exported across the cytoplasmic membrane

Under stress, halophile induce sets of Hsps that prevent aggregation of denatured cellular proteins and promote the refolding of partially damaged cellular proteins (Tokunaga, Miyawaki, Shiraishi, & Tokunaga, 1997).



Organic-osmolyte strategy

As the non-ionic, water-soluble compounds do not interrupt the metabolism even at high cytoplasmic concentration, thus named compatible solutes

Halophile that employed compatible solutes strategy can growth in low salinity environments (Kunte, 2009)

The halophile preserve the same enzymatic machinery as non-halophile with minor adjustments of interior proteins that slightly more acidic that in *Escherichia coli*

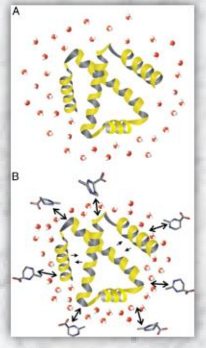
also known as compatible solutes

organisms keep the cytoplasm free of NaCl so that the cell interior remains basically unchanged (Kunte, 2009)

reduced of chemical potential of the cell water by accumulation of uncharged (zwitterionic), highly water-soluble, organic solutes

halophiles accumulate organic compounds by *de novo* synthesis or by uptake from surrounding environment
- *de novo* synthesized of solutes is energetically expensive
- synthesis of one compatible solute (ectoine or glycine–betaine) requires about 40 ATP-equivalents
- compared to salt-in strategy that requires 1 ATP to transport two K^+ and two Cl^- from the environment

organic compounds: sugars, polyols, amino acids, and/or amino acid derivatives



Comparative analysis of proteomes of halophiles and non-halophiles

- low hydrophobicity
- over-representation of acidic residues, especially Asp
- under-representation of Cys
- lower propensities for helix formation
- higher propensities for coil structure (Paul, Bag, Das, Harvill, & Dutta, 2008)

Conclusion

Adaptation strategy
- Less energy
- Expensive energy
- Extreme environment
- Benefit human
- Further study
- Applications

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