

Comparative Genomic Analysis of Halophilic and Xerophilic Microbes to Elucidate Adaptions to Chaotropic and Low Water Activity Environments

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Abstract

Studies on the molecular mechanisms of microbial adaptation in chaotropic and low water activity (aw) environments are poorly understood. Chaotropic environments are characterized as salt rich, MgCl₂ and CaCl₂, which lowers the availability of water for biological processes. PATRIC, an integrated genomic browsing tool containing vast libraries of sequenced genomes, can help us identify unique genetic markers in chaophilic and xerophilic microbes. Halophilic microbes are characterized as obligate hypersaline with the ability to tolerate exposure to chaotropic agents. Microbes with the greatest tolerance in these extreme environments must have advanced adaptive methods. *Halobacterium salinarum* and *Haloquadratum walsbyi* are chaotolerant and well adapted to low water activity. *Haloquadratum walsbyi* is unique among the halophilics as having the highest tolerance for chaotropes and its square shape. Performing comparative genomics using fully sequenced halophilic archaea such as *Halobacterium salinarum* NRC-1, a model halophile, and *Haloquadratum walsbyi* C23, we were able to identify genes that confer adaptation to chaotropic and low aw environments, as well as individual adaptations that may be responsible for the varying levels of tolerance in chaotropic environments. Characterizing genes associated with chaotolerance and low aw adaptations can help elucidate the cellular functions that make these microbes unique. Chaotropic brines may be used as analogs to study the origin of life and the possibility of suitable environments hosting extremophilic microbes on other planets like the Martian brines and the icy moons of Europa; therefore, studying the microbiome of chaotropic environments are essential in the field of astrobiology.

COMPARATIVE GENOMIC ANALYSIS OF HALOPHILIC AND XEROPHILIC MICROBES TO ELUCIDATE ADAPTIONS TO CHAOTROPIC AND LOW WATER ACTIVITY ENVIRONMENTS



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Introduction

- Mechanisms of microbial adaptation in chaotropic and low water activity (a_w) environments are poorly understood.
- Chaotropes, like $MgCl_2$, disrupt hydrogen bonding in water molecules deteriorating hydrophobic properties of cellular membranes; causing cell death.
- Adaptations include salt-in and salt-out strategy. Salt-in strategy is the uptake of K^+ and Cl^- ions across the membrane and the salt-out method is the production of compatible solutes to become iso-osmotic with the cell's environment.⁶ must be able to retain water and conserve energy for biomolecule repair²
- Chaotropic sites with low a_w include the Dead Sea and $MgCl_2$ saturated deep-sea brine pools.³
- Water is essential for life functions and is expressed as a_w ². The a_w of pure water is one, sea water is 0.98, and chaotropic environments can be 0.4. Microbial life can be active at a a_w as low as 0.61 most can't survive below 0.9¹
- Haloquadratum walsbyi is unique due to its extremely high $MgCl_2$ tolerance and its square shape³
- We have identified several genes that may help elucidate the molecular mechanisms of adaptation in chaotropic and low a_w environments.
- Studying chaotropic environments is essential in the field of astrobiology.



Figure 1. Pink halophile bloom. Red carotenoids produce red pigment. Turns water pink

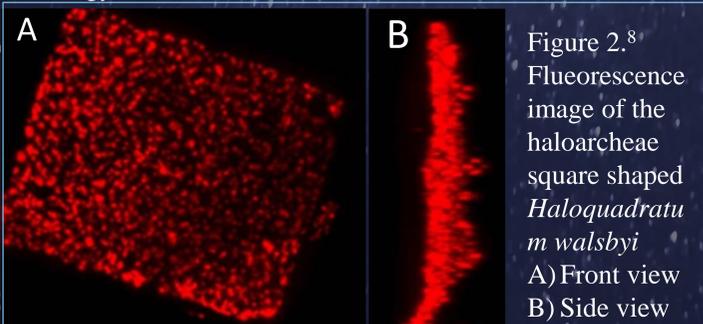


Figure 2.⁸ Fluorescence image of the haloarchae square shaped *Haloquadratum walsbyi*
A) Front view
B) Side view

Discussion

- An average nucleotide identity (ANI) reveals roughly a 66% similarity between the species.
- *Haloquadratum walsbyi* could be square in order to maximize surface area to absorb scarce nutrients like phosphate⁷
- The most unique feature in *Haloquadratum walsbyi* is the *hmu1* gene, a mammalian homologue. Like lungfish mucin found in the lungs; allows the fish to survive out of the water. It is characterized as a being negatively charged and serves as an aqueous shield that acts as a barrier to retain water⁷
- *H. walsbyi* contains the only genes specific to Mg^{2+} transport and eight times as many genes that code for Na^+/H^+ antiporters than *H. salinarum* suggesting much more salt pumping.

Method

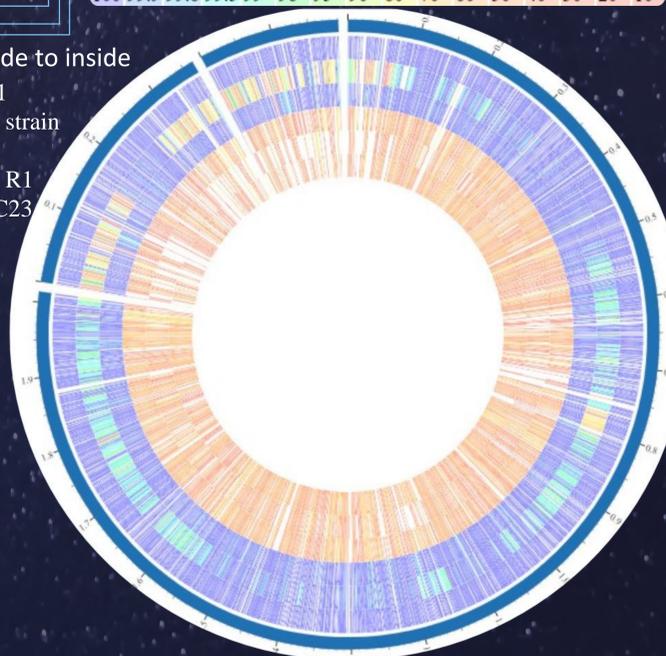
• Genomes of chao-tolerant microbes were analyzed using a comparative genomics approach to differentiate between essential and inconsequential genes to distinguish chaophilic and xerophilic (low a_w) adaptations.

Genomes of *Halobacterium salinarum* NRC-1 and *Haloquadratum walsbyi* C23, globally distributed halophiles have greatly different chaotolerances, and were compared using PATRIC⁹

List of tracks, from outside to inside

1. Halobacterium sp. NRC-1
2. Halobacterium salinarum strain 91-R6
3. Halobacterium salinarum R1
4. Haloquadratum walsbyi C23
5. Haloquadratum sp. J07HQX50
6. Haloquadratum walsbyi J07HQW1

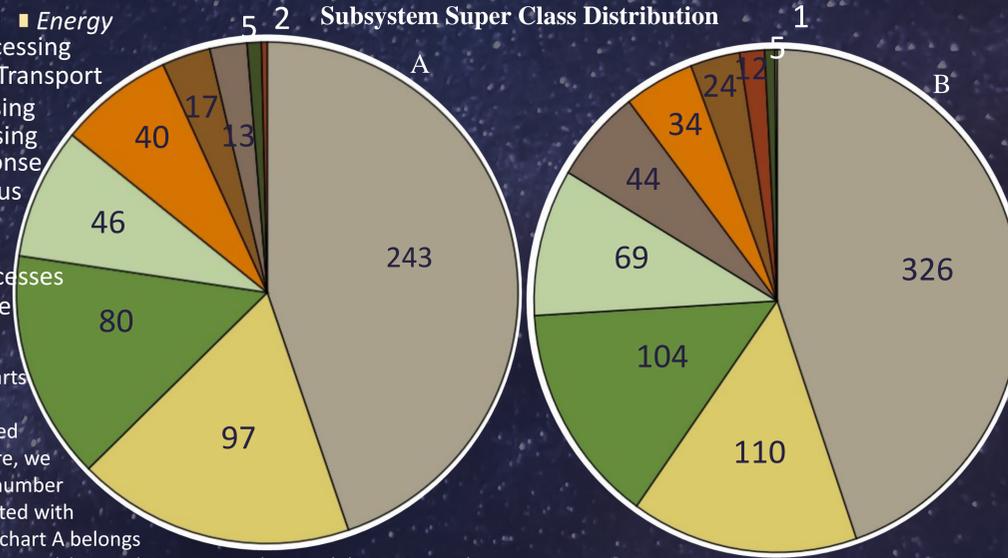
Figure 4. PATRIC tool used to visually compare protein sequence-based genomes. Reference genome is representative *H. salinarum* R1.



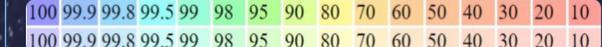
Results

- Metabolism
- Energy
- Protein processing
- Membrane Transport
- RNA Processing
- DNA Processing
- Stress Response
- Miscellaneous
- Cellular Processes
- Cell Envelope

Figure 3. Pie Charts of subsystem superclass's linked to genomes. Here, we can look at the number of genes associated with subsystems. Pie chart A belongs to *Haloquadratum walsbyi*, and B corresponds to *Halobacterium salinarum*



Proteome Comparison



Gene Tables

Gene	Product	Purpose	Genes
<i>znTa, yvgX, cpx</i>	Cu transporter	Osmotic stress	3
<i>caa, cat3</i>	Cation transport	Osmotic stress	5
<i>cbiN, cbiO2...</i>	Co transporter	Osmotic stress	4
<i>che1, cheD...</i>	Chemotaxis receptor	Motility	5
<i>ndhG2, ndhG3</i>	Na^+/H^+ antiporter	Osmotic stress	2
<i>kdpC,B,A</i>	K^+ uptake	Osmotic stress	10
<i>Bop</i>	Bacteriorhodopsin	Pigments/ ion pump	1
<i>hop</i>	Halorodopsin	Pigments/ ion pump	1
<i>dinF, dip1,2, ppe, mutS3,2,1</i>	DNA cleavage, mismatch repair, etc	General DNA repair	13
<i>gspE3...</i>	Flagella proteins	Flagella synthesis	9
<i>pstB1,2, pstA1,2...</i>	Phosphate utilization	Phosphate uptake/metblsm	33

Summary

Unique adaptation in *H. walsbyi* is the ability to produce an aqueous shield, halomucin, and Mg^+ pumps. *H. walsbyi* also has eight times as many ion antiporters. *H. walsbyi* has anion transporters, while *H. salinarum* has cation transporters. Unique to *H. salinarum* is motility and twice as many K^+ uptake pumps and bacteriorhodopsin.

Haloquadratum walsbyi

Gene	Product	Purpose	#
<i>copA, cadA</i>	Cu transporter	Osmotic stress	2
<i>mgtE1, mgtE2</i>	Mg/Co/Ni transporter	Osmotic stress	2
<i>arsA1, arsA2...</i>	Anion transporter	Osmotic stress	3
<i>Csp1, csp2...</i>	Cold shock protein	Stress response	3
<i>grpE, hipX...</i>	Heat shock protein	Stress response	3
<i>trkA6, trkH...</i>	K^+ uptake	Osmotic stress	5
<i>phaC, phaD3...</i>	Na^+/H^+ antiporter	Osmotic stress	16
<i>amb</i>	Ammonia transporter	Osmotic stress	1
<i>bop1</i>	Bacteriorhodopsin	Pigment/ion pump	1
<i>mutL1,2, ...</i>	repair proteins	General DNA repair	10
<i>ptsl...</i>	Phosphate utilization	Phosphate uptake/metabolism	24
<i>hmu</i>	Halomucin	Water retention	1

Table 1. Gene tables of genes unique to each organism are displayed with the number of genes associated with a subsystem

Future Directions

- Adaptive laboratory evolution to identify genes associated with magnesium tolerance
- Genome surveys of chaotropic environments to characterize other chaophilic and xerophilic microbes; likewise, further study of pathways that allow for chao/xerotolerance
- characterize the role of the *hmu* gene in a_w or chaotropic adaptation
- Chaotropic brines can serve as analogs to extraterrestrial locations, such as Martian brines and the subsurface oceans of several moons in our solar system⁴. Further Study may elucidate the possibility of life in extraterrestrial brines found on Mars and Europa thereby extending the limit of life on Earth and the and the universe.

- References
- ¹Lever, Mark A. (2016). *Frontiers In Microbiology*. 6:1505
 - ²W.D. Grant (2004). *The Royal Society*. 359: 1249-1267.
 - ³Boetius and Joye (2009). *Science*. 324: 1523-1525.
 - ⁴Antunes, et al. (2011). *Astrobiology: Current, Evolving and Emerging Perspectives*
 - ⁵Oren, Aharon. (1998). *Instruments, Methods, and Missions for Astrobiology*
 - ⁶Merlino, et al. (2018). *FEMS Microbiology Ecology*. 94:7
 - ⁷Bollhuis, et al. (2006). *GMC Genomics*. 7: 169
 - ⁸Zenke, et al. (2015). *Frontiers In Microbiology*. 6:244.
 - ⁹Brettin, et al. (2015). *Sci Rep*. 5:8365

