

Virtual Research Presentation Conference

Constraining Extreme Environment Adaptation Pathways: Tracing Genetic Divergence within Halophilic Microorganisms

Principal Investigator: Dr. Scott M. Perl, Geobiology and Astrobiology (397D) Co-I: Dr. Chhandak Basu, Dept. of Biology, CSUN Assigned Presentation # 094



Jet Propulsion Laboratory California Institute of Technology

Introduction

Abstract

The adaptations of life to survive in extreme saline and hypersaline ecologies yield survival pathways that in its beginning phases completely eradicate any evidence of the majority of microbial species and lower the diversity of biological communities significantly compared to typical marine environments.

- How can the utilized adaptations of halophilic microorganisms ("halophiles") to their extreme environments be used to understand their evolution over geologic time?
- How can halophilic microbial communities residing in hypersaline environments on Earth tell us about biosignature detection for extant life in Martian and Europa in their respective analogue geochemical environments?



Fig 1. (Adopted from Fig. 1c from Perl & Baxter, 2020). Arial view of the salinity gradients of the Great Salt Lake, Utah, United States.

Problem Description How can we understand the adaptations of life "as we don't know it" without a evolutionary baseline? What adaptations are needed for extreme life on Earth that we can build a baseline for? What gene expressions are needed for biology outside of Earth?





- New interest in extant life on Mars as well as continued searches for life on the ocean worlds need a context for how adaptative that biology would need to be in order for it to be detected. Discovering a second sign of biology is equally important to understanding it's point in evolution.
- SOA: This is a novel methodology that has never been used in a planetary context. We are applying what we consider "early" evolutionary adaptation pathways for microbial survival to how biology could indeed survive during the loss of surface water on Mars and in the ocean-ice interface on Europa.
- This is relevant to NASA and JPL due to the impacts for life detection and biological validation work
- · Results will enable recommendations for in-situ sample analyses, "taxis-driven" experiments (in-situ and laboratory), and validation experiments.
- ٠ Strengthens JPL's broad expertise in astrobiology, life detection, state-of-the-art laboratory SOPs, and new tools for genomics and microbiology investigations.
- Provides NASA with a biology-driven approach to future payload designs and mission formulation

We will investigate the active and non-active gene expressions of halophilic bacteria from the genomic metadata from previous field investigations to understand these adaptations for planetary insight.

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Methodology

- We extracted the specific genes responsible for an array of adaptation processes all are related to geochemical and saline environments that occurred during the transition from ancient to modern Mars and that are likely needed for modern Europa.
- Some notable adaptation processes we found and grouped together for halophilic life were:
 - Na-buffering (for life in high salt)
 - UV-C resistance (for photobiology)
 - Arsenic resistance (for "hostile" chemistries)
 - (and dozens more that are related/complementary)



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Results 1/3

- While the original samples were collected and DNA extracted some time ago (Perl & Baxter, 2020; Perl et al. 2020), the metadata can be enhanced and compared to several BLAST and metagenomic databases for which genes need to be expressed (activated) in order for metabolism to be maintained.
- Future work will do this same process in the lab with extracted RNA and under planetary conditions (T, salinity, and UV-C).

Fig 1. Domain-level analysis of 16S rRNA gene sequences which provided us with the metadata for gene expression



Figs 2,3. Specieslevel OTUs from Fig. 1 which allowed us to build our database based on species-driven adaptations, how they are preserved in the evaporite record, and their activated genes used for survival in hypersaline settings



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Results 2/3

- From the species-level data, the taxonomic units are compared against our databases for filtering of only genes that correspond to specific adaptations (Nabuffering for halophiles are shown here).
- These genes then are matched to other 16S rRNA sequences for metabolism overlaps, then filtered again for what is not present in our sites.
- Expressed genes for Nabuffering also include arsenic resistance expressions even though the original in-situ site does not contain these chemistries



Fig 4. EC distributions for chosen OTU sequences



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Fig 5. Activated genes in halophiles for photoprotection (UV) ability

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Results 3/3

- Our final heat map from our insitu samples show which halophilic microorganism genes are activated (expressed) in the matrix compared to BLAST and KEGG databases. This gives us an idea about how commonplace some genomic expressions are.
- Our future proposals will expand on this database from new in-situ samples and allow us to create a library of extreme environment microorganisms, and their genes that would need to be activated in order to survive on ancient or modern Mars as well as Europa's frozen climate.

Fig 7. Total heat map of the top halophilic species with respect to our samples. Upregulated genes (red bars indicative of high relative expression) show which species contain this active gene. Downregulated genes (green bars = low expression) are present and likely used for other processes but not halophilic-driven ones.

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		-0.4857	-0.4857	-0.4857	-0.4857	-0.0702	2 0129	Spirochaeta thermophila
	- H	-0.5223	-0.5223	-0.5223	-0.5223	0.1153	1.9737	Pediococcus pentosaceus
	1*	-0.5395	-0.5395	-0.5395	-0.5395	0.2111	1.947	Methanosaeta harundinacea
	1 1	-0.4832	-0.4832	-0.0819	-0.4832	-0.4832	2.0148	Laceyella sacchari
	1 4,	-0.4082	-0.4082	-0.4082	-0.4082	-0.4082	2.0412	Elusimicrobium minutum
	1 11	-0.4082	-0.4082	-0.4082	-0.4082	-0.4082	2.0412	Flexistipes sinusarabici
	1 4	-0.4082	-0.4082	-0.4082	-0.4082	-0.4082	2.0412	Bacteriovorax stolpii
	1 1	-0.4082	-0.4082	-0.4082	-0.4082	-0.4082	2.0412	Fervidobacterium nodosum
-	1 1	-0.4082	-0.4082	-0.4082	-0.4082	-0.4082	2.0412	Candidatus Mancarchaeum acidiphilum
e	1 1	-0.4082	-0.4082	-0.4082	-0.4082	-0.4082	2.0412	Devosia ginsengisoli
•	п І	-0.4082	-0.4082	-0.4082	-0.4082	-0.4082	2.0412	Acinetobacter schindleri
h		-0.4082	-0.4082	-0.4082	-0.4082	-0.4082	2.0412	Paenibacilius brasilensis Methonothormus femidus
		-0.4082	-0.4082	-0.4082	-0.4082	-0.4082	2.0412	Chandramerinus rervidus
		-0.4062	-0.4062	-0.4062	-0.4062	-0.4062	2.0412	Sediminienirocheeta emeradinee
		-0.4082	-0.4082	-0.4082	-0.4082	-0.4082	2.0412	Sphaerotilue natane
		-0.4082	-0.4082	-0.4082	-0.4082	-0.4082	2.0412	Oceanithermus profundus
~		-0.58	-0.58	-0.58	-0.58	1.854	0.4659	Azotobacter vinelandii
S		-0.4082	-0.4082	-0.4082	-0.4082	2 0412	-0.4082	Luteimonas sp. JM171
-		-0.4082	-0.4082	-0.4082	-0.4082	2.0412	-0.4082	Bacteroides uniformis
Δ		-0.4082	-0.4082	-0.4082	-0.4082	2.0412	-0.4082	Planctomycetes bacterium FF011L
C	4	-0.4082	-0.4082	-0.4082	-0.4082	2.0412	-0.4082	Neisseria mucosa
1		-0.4082	-0.4082	-0.4082	-0.4082	2.0412	-0.4082	Acidovorax avenae
n		-0.4082	-0.4082	-0.4082	-0.4082	2.0412	-0.4082	Capnocytophaga ochracea
••		-0.4082	-0.4082	-0.4082	-0.4082	2.0412	-0.4082	Yoonia vestfoldensis
0		-0.4082	-0.4082	-0.4082	-0.4082	2.0412	-0.4082	Candidatus Desulfovibrio trichonymphae
e		-0.4082	-0.4082	-0.4082	-0.4082	2.0412	-0.4082	Actinotignum schaalii
		-0.4082	-0.4082	-0.4082	-0.4082	2.0412	-0.4082	Glaciecola nitratireducens
c		-0.4082	-0.4082	-0.4082	-0.4082	2.0412	-0.4082	Clostridium sp. JN500901
0	I '	-0.4082	-0.4082	-0.4082	-0.4082	2.0412	-0.4082	verrucomicrobium spinosum
- 1		-0.8827	0.7382	0.5/21	1.338	-0.8827	-0.8827	Roseburia intestinalis
11		-0.6433	1.15/4	-0.6433	1.4159	-0.6433	-0.6433	Fusobacterium nucleatum
.,	I — _ `	-0.6346	0.9802	-0.6346	1.0083	-0.5345	-0.5345	Allsupes finegoldii Moravella celoensia
nr 🛛		0.0373	0.0997	-0.1632	2.002	-1.0041	0.2272	l actobacillus fermentum
л	11 4 7	-0.507	0.0307	-0.67	1 9907	-0.55	-0.5375	Drevotella orie
	11 [16]	-0.0007	-0.1582	-0.000	2.0256	-0.000	-0.0007	Lactobacillue rhampoeue
Dt –		-0.0259	-0.4948	-0.4948	2 005	-0.4948	-0.4948	Haemonhilus narahaemolyticus
~	Irl U.	-0.4082	-0.4082	-0.4082	2 0412	-0.4082	-0.4082	Campylobacter showae
	אר ווו	-0.4082	-0.4082	-0.4082	2.0412	-0.4082	-0.4082	Inhella inkvongensis
	111 1.	-0.2205	-0.453	-0.453	2.0324	-0.453	-0.453	Clostridium pasteurianum
	11 1	-0.2289	-0.4511	-0.4511	2.0332	-0.4511	-0.4511	Listeria ivanovii
		1.3497	1.165	-0.7702	-0.204	-0.7702	-0.7702	Bacillus krulwichiae
		-0.4082	2.0412	-0.4082	-0.4082	-0.4082	-0.4082	Belliella baltica
	ч і	-0.4082	-0.4082	2.0412	-0.4082	-0.4082	-0.4082	Vibrio alginolyticus
		-0.4082	-0.4082	2.0412	-0.4082	-0.4082	-0.4082	Vibrio tubiashii
	۰ Y	-0.4082	-0.4082	2.0412	-0.4082	-0.4082	-0.4082	Vibrio diabolicus
		1.9987	-0.5013	-0.5013	0.0066	-0.5013	-0.5013	Desultovibrio piger
		1.9563	-0.534	0.1796	-0.534	-0.534	-0.534	Paratiavitalea soli
	_	1.8641	-0.6991	0.2426	-0.0093	-0.6991	-0.6991	virgibacilius necropolis
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Publications

- Perl, S.M. & Basu, C. (in-prep) Using Genomic Expression a Biomarker for Adaptation and Survivability. *To be submitted to Astrobiology*
- Perl, S.M., Basu, C., Baxter, B.K., Celestian, A.J. (in-prep for AbSciCon) Constraining Halophilic Gene Expression for Ancient Mars Surviveability *To be submitted to the Mars Habitability session for the 2021 Astrobiology Science Conference*

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