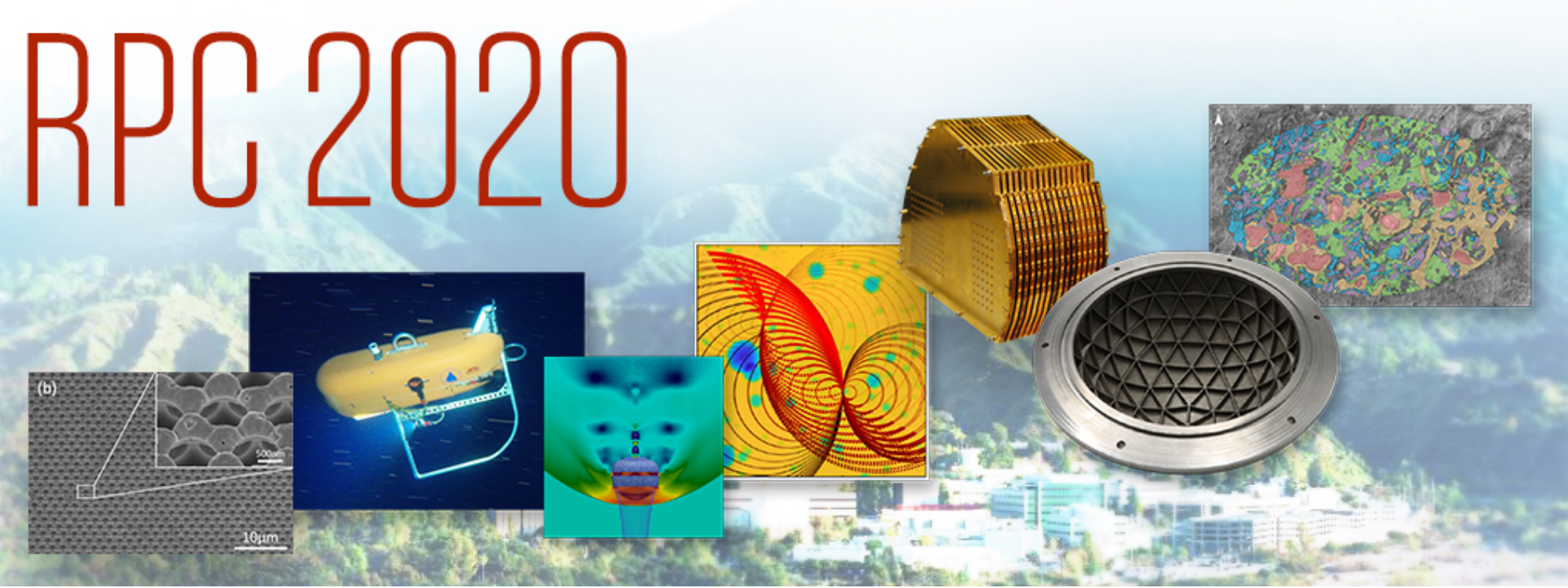


Virtual Research Presentation Conference

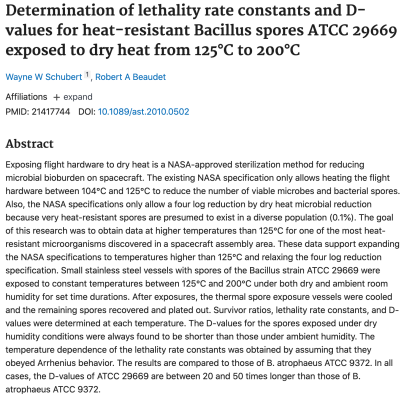
Testing the role of isotopic composition and heterogeneity in the heat tolerance of bacterial spores
Principal Investigator: Arman Seuylemezian (352N)
Co-Is: Matthew Koehler (Laboratory for Research in Complex Systems)
Program: Spontaneous Concept

Assigned Presentation #RPC-206

Jet Propulsion Laboratory
California Institute of Technology

Introduction

- Currently the gold standard microbial reduction modality that is implemented on spacecraft hardware is dry heat microbial reduction. This technique requires exposing a piece of hardware to a particular temperature in an enclosed environment for a set interval of time. The time and temperature requirements are determined based upon the desired level of reduction that would like to be achieved.
- Typically, a single model organism is chosen which is then subjected to extensive wet laboratory testing in order to establish the level of reduction that can be achieved at certain time and temperatures.



Determination of lethality rate constants and D-values for heat-resistant Bacillus spores ATCC 29669 exposed to dry heat from 125°C to 200°C

Wayne W Schubert [1], Robert A Beaudet

Affiliations + expand

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Abstract

Exposing flight hardware to dry heat is a NASA-approved sterilization method for reducing microbial bioburden on spacecraft. The existing NASA specification only allows heating the flight hardware between 104°C and 125°C to reduce the number of viable microbes and bacterial spores. Also, the NASA specifications only allow a four log reduction by dry heat microbial reduction because very heat-resistant spores are presumed to exist in a diverse population (0.1%). The goal of this research was to obtain data at higher temperatures than 125°C for one of the most heat-resistant microorganisms discovered in a spacecraft assembly area. These data support expanding the NASA specifications to temperatures higher than 125°C and relaxing the four log reduction specification. Small stainless steel vessels with spores of the Bacillus strain ATCC 29669 were exposed to constant temperatures between 125°C and 200°C under both dry and ambient room humidity for set time durations. After exposures, the thermal spore exposure vessels were cooled and the remaining spores recovered and plated out. Survivor ratios, lethality rate constants, and D-values were determined at each temperature. The D-values for the spores exposed under dry humidity conditions were always found to be shorter than those under ambient humidity. The temperature dependence of the lethality rate constants was obtained by assuming that they obeyed Arrhenius behavior. The results are compared to those of B. atrophaeus ATCC 9372. In all cases, the D-values of ATCC 29669 are between 20 and 50 times longer than those of B. atrophaeus ATCC 9372.

- Limitations of this approach:
  - The model organism chosen may not be representative of the types of species present on the surface of interest.
  - This may lead to gross overestimations or underestimations of the true time and temperatures requirements needed to achieve the desired level of reduction thereby impacting flight project budget and timelines.

Dry Heat Microbial Reduction

With the advent of next generation sequencing technologies and our ever expanding understanding of the microbial diversity present in cleanroom environments, there is a need to ***identify*** and ***quantify*** the risk associated with the recovery of certain organisms and their likelihood of surviving interplanetary transfer.

Current State of Art: Our current understanding of dry heat resistance is limited by culture based techniques, as there are no methods for characterizing dry heat resistance using molecular information.

Relevance to NASA and JPL:

As planetary protection implementation shifts more towards molecular based assessments, our need to understand dry heat resistance using molecular datasets becomes increasingly critical. This study has established a framework based upon genomic data combined with culture based datasets to identify potential genes of interest that could further be used to establish a computational model to predict the dry heat resistance of unculturable microbes. In addition, we aimed to supplement this work with RNA-sequencing data which would validate the genes identified in this dataset and help strengthen the framework needed for model development.

Methodology

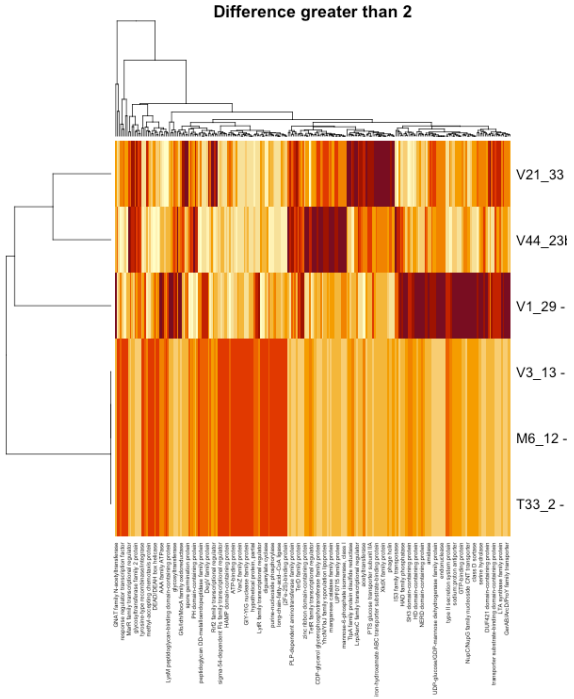
Experimental Description:

We combined existing genomic datasets of 7 different dry heat resistant bacterial species which were associated with the Viking mission alongside empirically collected culture based dry heat resistance data. Genomic annotations were subjected to statistical techniques in order to identify those genes exhibiting significantly different copy numbers across the various genomes. This was used as a proxy for the relative expression of these genes which will be further investigated using direct RNA sequencing. Statistical manipulations were carried out in R, along with already existing Python packages and annotation pipelines such as NCBI PGAP, and RAST.

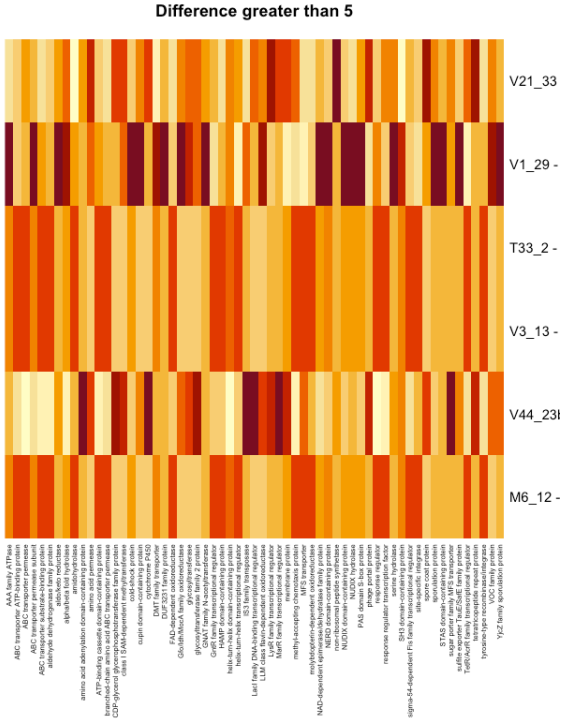
Strain Name	D-value at 125C (minutes)
M6-12	59.04
V44-23'b	74.28
V3-13	115.13
T33-2	255.84
V1-29	328.94
V21-33	460.52
ATCC 27380	8340

Innovation:

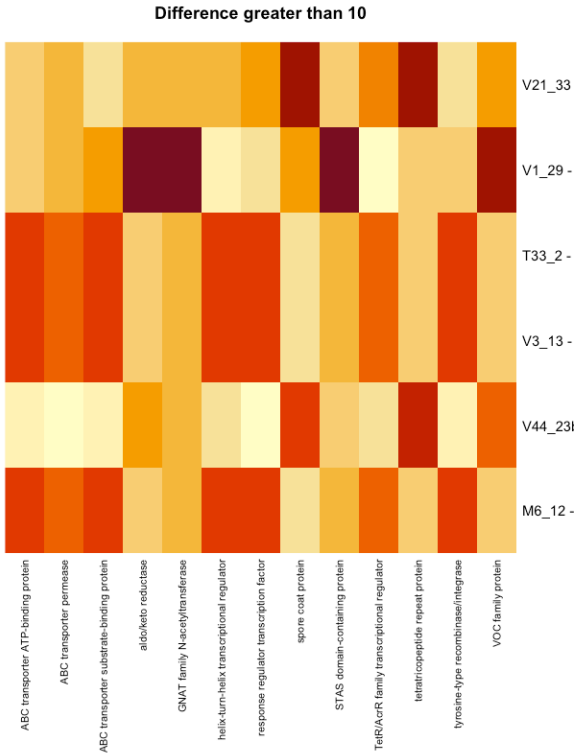
We aim to develop a framework by which a computational model could be built to predict the dry heat resistance capabilities of bacterial organisms based purely on genomic characteristics such as the presence/absence of certain genes or more subtle identifiers such a SNP (single nucleotide polymorphisms). This approach will be the first attempt to understand a complex multi-layered process such as dry heat resistance strictly from a molecular perspective.



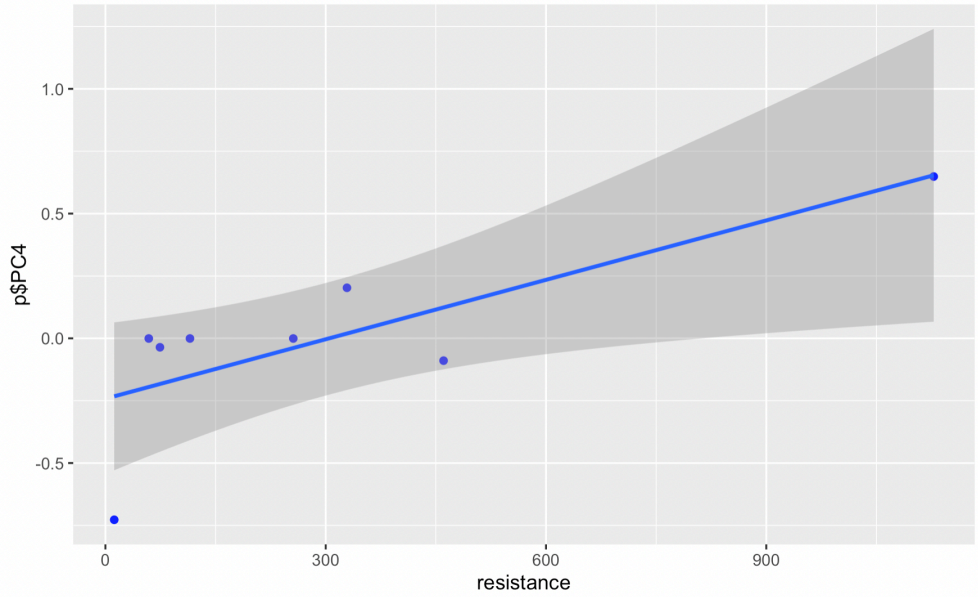
- Genes containing a minimum difference of 2 copy numbers across 6 different dry heat resistant bacterial strains isolated from spacecraft assembly environments.



- Genes containing a minimum difference of 5 copy numbers across 6 different dry heat resistant bacterial strains isolated from spacecraft assembly environments.



- Genes containing a minimum difference of 10 copy numbers across 6 different dry heat resistant bacterial strains isolated from spacecraft assembly environments.



- Linear regression analysis of principle component 4 (containing several different genes) demonstrating a positive correlation with increasing levels of dry heat resistance amongst 7 different bacterial strains isolated from spacecraft assembly environments

Next Steps

- Perform differential transcriptome analysis of 7 different dry heat resistant bacterial strains at 5 different time points using RNA-sequencing to characterize expression differences throughout the sporulation and germination processes.
- Publish the review paper in Applied and Environmental Microbiology discussing the current state of the state in our understanding of dry heat resistance in an effort to guide the scientific community towards developing computational tools to assess the degree of dry heat resistance exhibited by spore forming microorganisms.
- Analyze and publish the results of RNA sequencing experiment in collaboration with Liberty Biosecurity.

Regulon	Description	Number of genes[b]	Differentially expressed[c]		
			%	#up	#down
sigB	General stress response	151	30	14	31
sigD	Regulation of flagella, motility, chemotaxis, and autolysis	24	33	0	27
sigE	Sporulation (early mother cell-specific)	176	9	8	7
sigF	Sporulation (early forespore-specific)	63	11	2	5
sigG	Sporulation (late forespore-specific)	108	10	3	8
sigH	Transcription of early stationary phase genes (sporulation, competence)	37	43	1	14
sigI	Control of a class of heat shock genes	6	50	0	3
sigK	Sporulation (late mother cell-specific)	103	7	2	4
sigL	Utilization of arginine, acetoin, and fructose; required for cold adaptation	23	52	0	12
sigM	ECF-type sigma factor responsible for intrinsic resistance against beta-lactam antibiotics	69	35	4	13
sigO-rsoA	Two-subunit sigma factor	5	20	1	0
sigV	ECF-type sigma factor; response to lysozyme	4	0	0	0
sigW	ECF-type sigma factor; activated by alkaline shock, polymyxin B, vancomycin, cephalosporin C, D-cycloserine, and triton X-100	65	54	28	4
sigX	ECF-type sigma factor; cell surface properties	29	45	1	9
sigY	ECF-type sigma factor; maintenance of the SPβ prophage	7	0	0	0
ylaC	ECF-type sigma factor; response to oxidative stress	4	0	0	0
xpf	PBSX phage RNA polymerase sigma factor	10	50	0	5

[a] *Classification and description according to SubtiWiki and (Souza et al., 2014).*
[b] *Number of genes within the regulon.*
[c] *The numbers of up- (#up) and downregulated (#down) genes only include genes that have the same expression direction (up/downregulated) at all three time points. The percentage of differentially expressed genes includes all genes.*

Publications in works

- The genomic underpinnings of dry heat resistance in bacteria: Towards computational approaches to understanding dry heat resistance. Izabella Zamora, Peter Setlow, Kasthuri Venkateswaran, Chris Rodriguez, Helena Yee-Wah Chan, Ahmed Mohamed, Matthew Koehler, Rüdiger Pukall, Wayne Nicholson, Arman Seuylemezian. Applied and Environmental Microbiology. (In preparation)
- Transcriptomic profiles of 9 highly dry heat resistance bacterial endospores previously isolated from the Viking mission. Izabella Zamora, Timothy Avery, Daniel Vera, Kyle Landry, Arman Seuylemezian. Frontiers in Microbiology. (In preparation)