CHARACTERIZATION OF OUTER SPACE RADIATION INDUCED CHANGES IN EXTREMOPHILES UTILIZING DEEP SPACE GATEWAY OPPORTUNITIES. K. Venkateswaran[1], C. Wang[2], D. Smith[3], C. Mason[4], K. Laundry[5], and P. Rettberg[6]. [1]Jet Propulsion Laboratory, California Institute of Technology, Pasadena, CA, USA (kjvenkat@jpl.nasa.gov); [2]Department of Pharmacology and Pharmaceutical Sciences, School of Pharmacy, University of Southern California, Los Angeles, CA, USA (clayw@usc.edu); [3]NASA-Ames, Space Biology Research Branch, Moffett Blvd, Mountain View, CA, USA (david.j.smith-3@nasa.gov); [4]Department of Physiology and Biophysics, Weill Cornell Medicine, New York, NY, USA (chm2042@med.cornell.edu), [5]Expeditionary and Special Programs Division, Liberty Biosecurity, Boston, MA, USA (klandry@libertybiosecurity.com); and [6]Deutsches Zentrum für Luft- und Raumfahrt e.V. (DLR), German Aerospace Center, Institute of Aerospace Medicine, Cologne, Germany (petra.rettberg@dlr.de).

Introduction: Early integration of science and exploration concerns into the design of the Deep Space Gateway (DSG) is essential to maximizing its science and exploration potential. The proposed concept, *characterization of outer space radiation induced changes in microbial extremophiles*, requires the DSG as infrastructure supplying power, communications, *etc.* to otherwise autonomous systems. Survival and proliferation of life beyond low earth orbit (LBLEO) can be accomplished by exposing extremophilic microorganisms in outer space radiation (OSR) conditions using DSG system. Extremophilic microbial survival, adaptation, biological functions, and molecular mechanisms associated with outer space radiation can be tested by exposing them onto DSG hardware (inside/outside) utilizing the traditional microbiology methods and state-of-the-art molecular biology techniques.

Exposure of Microbial Extremophiles Concept: The proposed OSR extremophiles concept is a flight experiment and hypothesis-driven research investigation, resulting from several "omics" approaches that would translate spaceflight derived data into new knowledge about microorganisms. With this approach NASA's Space Biology Program science element (microbiology) and its guiding questions can be addressed: (a) How the genetic, molecular, and biochemical processes of the OSR-tolerant extremophiles are influenced by the space environment and (b) What systems biology mechanisms and functional pathways are responsible for the enhanced virulence in spaceflight when compared to the ground controls. This concept directly responds to *understand the underlying mechanisms that control responses, adaptation and performance of microbes in space (e.g.: LBLEO) environments at the cellular, molecular and genomic level.* Microorganisms are known to drastically affect human health in a closed system therefore understanding their behavior in space environment (e.g.: biofilm formation, virulence) or reaction to sterilization technologies is critical to protecting crew. Generating empirical data set that can be used to set guidelines for assessing acute radiation risks is essential.

The data generated from DSG will be important for assessing both, the probability and mechanisms of survival, of microbial contaminants during future human exploration to the Mars and beyond. We can use the results to calculate the rates of inactivation of microbial species caused by the low pressure and high desiccation in simulation experiments as well as DSG conditions. Such empirical data sets will give broad insight on the ability of terrestrial microorganisms to survive in the DSG environment. The molecular analyses that can be employed in this project will detect "omics" changes in OSR extremophiles that may correlate with "omics" changes that occur in response to the need to adapt to the conditions in space. In addition, innovative capabilities of the analytical system in detecting subtle changes between microorganisms in different environments can be demonstrated. Such techniques can be applied to numerous future biologically oriented missions (e.g. life detection, instrument development, and astronaut health). At the end of the implementation of the concept, resulting data will enable to assess the probability and mechanisms of survival of microorganisms. These results can be used to calculate the rates of inactivation of microbial species caused by various aspects of space conditions. Overall the results will give further insight into the behavior of resistant microbes in space environments beyond LEO.

Microbial Extremophiles: The spacecraft associated extremophiles have been reported to withstand several space related parameters including radiation recorded at high altitude (*1*) and outside International Space Station (ISS) conditions (*2-9*). It is hypothesized that spacecraft associated extremophiles would be the ideal candidates for surviving under DSG conditions since these extremophiles exhibited molecular tenacity and plasticity in surviving extreme space conditions for 18 months exposure time (*5, 10, 11*). Hence, exposing OSR extremophiles under LEO environment using DSG mission is important to understand their biological functions and characterizing likely survival mechanisms. Furthermore, virulence properties exhibited by fungal population need to be tested after exposing

them beyond LEO orbit (*12, 13*). Once these OSR extremophiles exhibit survival (*14, 15*), their proliferation inside the DSG spacecraft would enable developing biofilms and such phenomenon should be tested under beyond LEO environment. Subsequently, there is a need to develop countermeasures to eradicate or contain these OSR extremophiles without human intervention for long duration missions. An understanding of the mechanisms of resistance in OSR extremophiles will help design Life Support and Habitation (LSH) mission systems that provide a harsher environment to microbes protecting astronaut health. Since the genomes, transcriptomes, and base modification systems (epigenomes) of all the OSR extremophiles included are already available (*16, 17*) or will be in early 2018, their comparison with matched, DSG-exposed species will facilitate recognition on a molecular level of the resistance mechanisms in microbes.

Microbiome of Closed Systems: As recommended by the National Research Council Decadal Survey, generating microbial census of surfaces of the closed system is needed using traditional culture-based methods, molecular microbial community analysis techniques, and bioinformatic computational modeling. The proposed DSG-microbiome analyses will provide significant insight into spaceflight-induced changes in the populations of beneficial and potentially harmful microbes. This approach would also provide both mechanistic understanding of these changes, for example cataloging population changes and mapping/linking these to environmental niche and genomic changes, as well as insight into practical countermeasures for mitigating risks to humans and environmental systems. Leveraging results of the NASA-funded Microbial Tracking experiments and also accounting expertise gained from the Mars Program funded projects would allow to analyze samples collected from DSG modules. The DSG microbiome database will augment NASA GeneLab program with which NASA will acquire ability to accurately and confidently assess the status of microbes associated with closed habitation and crew health maintenance. In addition to overall microbial profiles, this approach will determine which microbial taxa pose particular threats to crew health. Furthermore, the DSG-microbiome concept will enable NASA to resolve applicable NASA-Human Research Program integrated research plan risks.

Significance: The aims of the OSR concept are to perform biological research intended at preparing for future human exploration missions. As stated in the NASA Space Biology objectives, the OSR concept is related to fundamental research–gaining knowledge of spaceflight alterations in the microorganisms isolated from ISS to improve life on Earth. Understanding the molecular mechanisms in the spaceflight microorganisms might reveal the presence of potential stress-induced biomolecules and adaptations that are essential to adapt to beyond LEO conditions. Such stress-induced biological system modifications could be identified and applied (early diagnostics and superior countermeasure development) to improve crew health as well as recognize secondary metabolites that are useful compounds for the biotech industry (antibacterial, novel pharmaceuticals, biosynthetic gene clusters, etc.). Similarly, identification of stress-induced biomolecules that are antimicrobials will facilitate maintaining crew health and their closed habitat system for the future human exploration.

The OSR concept will serve several purposes by investigating the common underlying molecular networks, pathways, and mechanisms of life in space-exposed microorganisms (and compare with Earth counterparts) which are important to understanding human health and environment in space, and will translate space-derived knowledge to address specific human health conditions and environments here on Earth. Therefore, "omics" data of the targeted spaceflight microorganisms and ground-based investigations with direct translational research connections proposed in the OSR concept will directly address the important key priorities for the DSG and for the NASA Space Biology mission(s).

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