

Summary Reports on Previous 2003 and 2004 Projects

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Effects of Simulated Microgravity on Microbial Gene Expression

STUDIES OF THE EFFECTS OF SIMULATED MICROGRAVITY ON MICROBIAL gene expression are ongoing. Initial ISSO funding for the project was used to support postdoctoral fellow Dr. Gary Schultz. After the first year, he was replaced by Dr. Don Tucker. Dr. Tucker is currently a USRA Fellow working with Dr. Duane Pierson at NASA-JSC. Although the ongoing current research is a continuation of these earlier projects, it should be emphasized that the initial results obtained contributed to the successful effort by Dr. George E. Fox and Dr. Richard Willson to obtain major funding from the National Space Biomedical Research Institute (NSBRI). That funding was responsible for multiple publications. Ongoing work on the current PDAF provided significant additional preliminary results sufficient to obtain a current award from the Office of Biological and Physical Research to Drs. Fox and Willson.

Characterization of Evolving Bacterial Populations

ONE OF THE MAJOR GOALS ON NASA'S ASTROBIOLOGY ROADMAP is to understand how life evolves on the molecular, organismal, and ecosystem levels. Such knowledge, we believe, will provide a better understanding of how life might have evolved on planets other than Earth and how Earth life might respond to environmental changes in the future. The capabilities of laboratories under the supervision of Dr. Michael Travisano and Dr. George E. Fox were combined to study morphological diversification in the bacterium *Escherichia coli*. The proposed plan demonstrates the feasibility of their approach, enabling submission of several grant proposals.

Project Premises

When propagated in a simple heterogeneous environment, *E. coli* populations founded from a single ancestral genotype undergo explosive morphological diversification. A striking feature of the evolved morphs is their niche specificity; the ancestral SM morphs (smooth morphology on agar plates) colonize the broth phase. The evolved WS morphs (wrinkled colony morphology on agar plates) form a biofilm at the air-broth interface, and the evolved FS morphs (fuzzy colony morphology on agar plates) colonize the vial bottom. The serendipitous corre-

spondence between colony morphology and niche preference permits real-time ecological and evolutionary dynamics to be determined by scoring changes in the frequency of colony phenotype.

Project Goals

The proposed study was to have been divided into three distinct areas: long-term selection, phenotypic characterization, and genetic characterization of the selected cultures. The long-term selection would have involved daily propagation of replicate cultures into one of two environments, with different treatments varying in spatial heterogeneity. During and subsequent to the selection, the cultures would have been assayed for phenotypic and genotypic changes. At interesting points in the selection, cultures would have been subjected to gene expression profiling and, ultimately, DNA sequencing of relevant regions of the genome.

Results

Experimental systems were conceptualized and the assays established. Preliminary results of the project were included as part of a proposal to the NASA Astrobiology Center submitted in early 2003 but were not funded. Subsequent discussions of the project with Dr. Kasthuri Venkateswaran of the Jet Propulsion Laboratory led to two proposals in which we would have used the project's underlying evolutionary approach to understand the source of the unusual resistance properties associated with certain spores isolated from NASA's spacecraft assembly facilities. These proposals were not funded, but a new effort is in the planning stages.

Funding and Proposals

Venkateswaran, K. and G. E. Fox. "Microbial Ecological Perspectives of Space-Exposed Microbes: A Genetic Approach," NASA-Human Support Technology, April 1, 2005–March 31, 2008, Total UH Costs: \$120,000. (*Not funded.*)

Rapid Identification of Unexpected Bacterial Pathogens in Space Environments

CREW HEALTH IS A DOMINANT ISSUE IN MANNED SPACE FLIGHT. Microbiological concerns have, in the past, emerged as determinants of flight readiness on at least two occasions, and biofilm problems have occurred on several long-duration missions. As mission duration and re-supply intervals increase further, it will be necessary to rely on advanced life support systems which

incorporate both biological and physical-chemical recycling methods for air and water that also provide food for the crew. It would be especially desirable if a microbial monitoring system were in place that could readily identify the problem with organisms if an in-flight incident were to occur. Such a monitoring technology must detect many organisms of known concern as well as unanticipated problem organisms. The technology must also be subject to miniaturization, and it need be highly automated. It is widely believed that a microbial detection system cannot be designed without prior knowledge of what is to be detected. The proposed work would implement a novel approach to array design that can overcome this problem.

Project Premises

The project had two underlying premises. First, it was argued that 16S ribosomal RNA carries within its sequence sufficient information to determine the genetic affinity of any bacterium, whether novel or not, relative to known species and genera. Second, researchers hypothesized that this information would, in fact, be represented as a “signature” in collections of small sequence segments contained in the 16S *r*RNA.

Project Goals

Two alternative approaches were suggested for the use of signature information to rapidly characterize the phylogenetic position (i.e., the identity) of any unknown bacterium. The first of these would utilize a hybridization array approach. The signature implications of a large number of oligonucleotides, e.g., all 10-mers, would be needed. Thus, it was necessary to develop software that would allow us to determine for each 10-mer which phylogenetic grouping (e.g., species, genera, etc.) obtains the most meaningful signal. A set of the most discriminating sequences, which encompasses the entire phylogenetic scheme, would form the basis for array design.

Results

This project and the ISSO post-doctoral award contributed key preliminary results that together led to the successful National Space Biomedical Research Institute proposal ultimately renewed twice. It now remains funded as part of NASA’s Exploration Research Program. In addition, this work preceded several grant applications in the area of biodefense that Dr. Fox, Dr. Willson and Dr. Yuriy Folfanov have submitted. This effort includes a currently funded award from the Department of Homeland Security. The key research finding of the project, the existence of large numbers of 16S *r*RNA signature oligonucleotides, resulted in a 2002 publication in *Bioinformatics*:

Zhang, Z., R. C. Willson, and G. E. Fox. “Identification of Characteristic Oligonucleotides in the 16S Ribosomal RNA Sequence Dataset,” *Bioinformatics* 18 (2002): 244-50.

Publications

Warmflash D., M. Larios-Sanz, J. Jones, G. E. Fox, and D. S. McKay. “Assessing the Biohazard Potential of Putative Martian Organisms for Exploration Class Human Space Missions,” *Aviation. Sci. Environ. Med.* (Accepted pending revision.)



PROTECTION—Dr. David Warmflash, Post-Doctoral Aerospace Fellow, is associated both with TCSUH and the Department of Biology and Biochemistry. He is investigating potential biohazards one supposes may be found on Mars in anticipation of dangers or foreign substances that may affect spacecraft landing on Mars or flying in the martian atmosphere. NASA invests considerable research to protect instruments and human life from space biohazards.