

## Microorganisms and the space sciences

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**ABSTRACT**—Microorganisms are relevant to NASA objectives in the areas of astrobiology, exobiology, planetary protection, and exploration research. The PI and co-I's have a strong record of collaboration and have been well funded by many agencies, including NASA. This earlier work has generated publications and preliminary results that could form the basis of multiple new efforts. The goal of this cluster was to bring together these individuals and focus the available preliminary data in an effort to obtain new external funding. As a result, six new proposals were initially submitted, with two funded. Follow-up efforts on the various proposals are ongoing.

### INTRODUCTION

The research cluster for this project comprises a team of individuals with overlapping interests, complementary areas of expertise, and strong experience in effective collaboration. Our joint interest in microorganisms and the space sciences began with efforts to understand the origins of life on Earth. To that end, astrobiologists are currently exploring various Earth-based ecosystems resembling those likely present on the early Earth (Research Area 1). However, understanding the origins of Earth life would be greatly advanced if comparisons could be drawn with life on another planet. And, of course, it is a critical goal of the space sciences to find evidence of such life, if it exists. Research Area 2, therefore, focuses on developing technology to search for extraterrestrial life, especially on Mars.

Identifying life on Mars—or any other planet—is complicated by issues of contamination, in that what is found might well have been transported from Earth. Research Area 3 addresses planetary protection concerns and focuses on identifying the types of organisms that might survive a journey to Mars and the radiation environment of that planet's surface. Microorganisms are an unavoidable, and in some cases necessary, part of any space voyage. Most are human-associated and may be pathogenic, while others may cause biofouling. Research Area 4 stems from the need to monitor the types and number of bacteria present aboard any spacecraft. By contrast, Research Area 5 focuses on monitoring self-contained systems for bacteria specifically used to treat human waste or facilitate plant growth.

### RESEARCH AREA 1: HORIZONTAL GENE TRANSFER IN MICROBIAL ECOSYSTEMS

The mineralized thermal springs at Cuatro Ciénegas (CC) near Coahuila, Mexico, are regarded by astrobiologists as a very good model for the types of ecosystems that existed on

the early Earth. Collaborator Janet Siefert, Ph.D., has been working at CC with astrobiologists from Arizona State University to test the role of phosphorus limitation on food webs dominated by microbial communities analogous to those that formed ancient stromatolite. The initial work suggests a key role for phosphorus in driving ecosystem evolution, especially with regard to horizontal gene transfer, because phosphorus limitation should affect the mode of transport for delivery of alien DNA. Marine-adapted members of the genus *Bacillus* have been found to play a key role in this environment. Separately, Fox's group has developed a novel *in vivo* system<sup>1</sup> for rapidly evaluating the ability of a cell to adapt laterally transferred material to some utilitarian purpose. In essence, this is an experimental system for studying horizontal gene transfer in the laboratory, and Fujita's group has been studying sporulation in *Bacillus*. A proposal was submitted to the National Science Foundation (NSF), the essence of which was to conduct simultaneous laboratory and real-world studies of lateral transfer in *Bacillus*. The effort would have had a large bioinformatics component as well with a major contribution from Yuriy Fofanov, Ph.D. This proposal was not funded, and a revised proposal with the narrower theme of characterizing the metagenome of *Bacillus* species at CC using extensive Solexa sequencing was recently submitted to the NSF Genes and Genomes program.

### RESEARCH AREA 2: SEARCHING FOR EXTANT LIFE ON MARS

While extinct life can be expected to leave diagenetic products that could be worthy targets for exploration, the tenacity characteristic of all life suggests that if it existed in the Martian past, there should be taxa today that are well-adapted—indeed, perfectly at home—in select Mars environments. The prospect of extant life forms on the Red Planet is further supported by the presence of Martian subsurface ice,<sup>2</sup> along with evidence from NASA's Mars Exploration Rovers (MER) that large bod-

ies of surface water may have existed in liquid form in Mars' recent geological past.<sup>3</sup>

The underlying premise of our approach is that carbon-based life on any planet or moon will be able to select between stereoisomers or enantiomers of any given compound that has chiral centers. The approach builds upon the strategy used in the biology experiments of Project Viking during the 1970s—the exposure of environmental samples to nutrient compounds whose atoms can identify subsequent chemical changes resulting from metabolism—while circumventing limitations of those earlier efforts. In collaboration with Omicron, Inc., we have developed enantiomer compound pairs, including sugars, peptides, and nucleic acids of opposite chirality, and exposed these to common soil and JSC Mars 1 regolith stimulant. One of each compound pair is labeled with the stable carbon isotope, <sup>13</sup>C, and metabolic activity is detected by changes in the mass spectra of soil and regolith simulant extracts following incubation with the compounds. It has been shown that Earth-based life will consistently degrade one pair over the other.

The objective of the proposal was to collaborate with Biotex, Inc., and Stellar Exploration, Inc., to develop an instrument capable of detecting signs of extant Martian life using this chirality approach. The asymmetric elimination of one or more of the pairs would be an indication of biological activity. In the proposed project, we would move into the next stage, involving a breadboard instrument that can achieve the target technology readiness level (TRL-6). To this end, a collaboration was also arranged with OPTI-MS, Inc., to build an appropriate instrument around their NASA-funded miniaturized mass spectrometer. A proposal was submitted to the Mars Instrument Development Program but not funded. The reviews were generally positive, with the universal criticism that the instrument was not far enough along in its development. The current plan is to resubmit the proposal in December 2008 to the NASA's ASTID program which is more appropriate to the current TRL level.

### RESEARCH AREA 3: ORIGINS OF UNUSUAL SPORE RESISTANCE IN *BACILLUS PUMILUS*

The 1967 Outer Space Treaty obliges the United States to avoid harmful contamination of celestial bodies, including Mars, that might harbor life. Spacecraft that land on Mars but are not equipped with life-detection experiments must minimize the bioburden they bring to the planet. Thus, U.S. spacecraft are subjected to rigorous cleaning and must be assembled in a Class 100,000 clean room or better. If the mission involves life detection experiments, a sterilization process as good as or better than that applied to the landers used in the 1967 Viking missions must be applied. Among the organisms most likely to be resistant to sterilization are endospore-forming bacteria of the genera *Bacillus* and *Clostridium*. Hence, a spore assay was developed to evaluate the effectiveness of the sterilization process using *Bacillus subtilis* as the model organism.

While conducting a microbial census of NASA spacecraft assembly facilities, Kasthuri Venkateswaran, Ph.D., and colleagues of the Jet Propulsion Laboratory discovered that strict contamination-control measures did not provide an absolute

barrier to microbial contamination but instead established a series of selective “bottlenecks” sufficient to prevent the penetration or survival of all but the hardiest microbes into the interior assembly area.<sup>4</sup> The predominant isolates repeatedly found to have penetrated deepest within the cleanest parts of the spacecraft assembly facilities were spore-forming bacteria, especially strains of *Bacillus pumilus*, *B. nealsonii*, and *B. safiensis*,<sup>5</sup> whose spores are unusually resistant to UV light, gamma radiation, and other sterilants.

In 2006, an ISSO mini-grant allowed us to begin studying the origin of these elevated resistances, which included the sequencing of the genomes of two of these resistant strains.<sup>6</sup> As a result of these early efforts, Fox and Fofanov are key participants in a proposal recently submitted by JPL that, if funded, will create an astrobiology center to study microbial aspects of planetary protection. Our role in the proposed center will be to identify bacterial genes characteristic of *Bacillus* species isolated from clean rooms and able to survive key stresses, including UV and gamma ray radiation and hydrogen peroxide.

Three approaches will be utilized. First, Solexa sequencing technology will be used to obtain draft genome sequences of representative strains that have been isolated from clean rooms and shown to be unusually resistant to key stresses. Closely related non-resistant strains will also be sequenced. Bioinformatics tools will be used to identify genes that are associated with (not necessarily generative of) organisms exhibiting elevated resistances. Second, laboratory experiments will be undertaken to adapt a non-resistant *Bacillus* strain to high levels of UV resistance by exposing both vegetative cells and spores to elevated levels of UV over multiple generations. As adaptation occurs, we will re-sequence the genome to determine which genes or regulatory pathways are responsible for the elevated resistances. Finally, metagenomic studies on clean room populations will allow us to determine how prevalent resistance-associated genes are in the *Bacillus* populations and to more generally determine the relative abundance of functional genes of various types.

### RESEARCH AREA 4: BACTERIAL IDENTIFICATION AND MONITORING

Microorganisms are inevitably associated with human space flight. The organisms most likely to be present are those that are routinely associated with humans. Additional organisms may enter the ecosystem from populations present in advanced life support (ALS) systems or on surfaces of specialized equipment. The most obvious problem posed by microorganisms is the risk of human disease. In addition, changes in bacterial populations may negatively impact an ALS system, and buildups of biofilms may damage or interfere with the performance of hardware. These risks are augmented by concerns that the low gravity/high background radiation environment may select for changes in the microorganisms' antibiotic sensitivity or pathogenicity over an extended mission. Experience to date suggests that if the structure of the microbial population remains largely unchanged and the populations remain at modest levels, the risk of problems is substantially mitigated.

Thus, the key countermeasure is to ensure that this remains the case. In order to accomplish this, it is essential to have appropriate technology to monitor the level and structure of microbial populations.

Prior to NASA's termination of numerous ongoing research projects relating to manned flight, Fox and Willson had been awarded funding to address these issues. In order to identify any organism, whether anticipated or not, and to monitor changes in population structure, a mass cataloging system was developed in collaboration with William Jackson, Ph.D. The approach is essentially as follows:

An RNA containing mass-labeled uracil is generated from an informative molecule, e.g., 16S rRNA, and specifically digested with a ribonuclease. Multiple oligoribonucleotides are released, some of which are unique to any particular organism. In lieu of sequence, the mass of each large oligoribonucleotide is determined and the list of masses generated is used to identify the organism by comparison to a database. Alternatively, in a mixed population it is likely that key signature masses can be used to monitor the relative numbers of various types of bacteria or to detect the presence of a specific organism of concern. When used with cloned 16S rRNAs, this method is extremely fast and effective.<sup>7</sup> It remains to test it with a spacecraft-compatible mass spectrometer, develop spacecraft compatible protocols, and to extend the approach to complex mixtures in which the goal will be to detect major population changes.

In the absence of any new NASA funding opportunities for work of this type, researchers responded to an Environmental Protection Agency request for methods of detecting specific cyanobacteria and cyanotoxins in clean water. Although the reviews were generally positive, funding was not obtained.

## RESEARCH AREA 5: MOLECULAR MARKERS TO MONITOR ADVANCED LIFE SUPPORT SYSTEMS

Advanced Life Support (ALS) systems that are being developed to regenerate resources needed to support the International Space Station and ultimately to sustain a lunar base or a voyage to Mars will likely utilize biological treatment systems. Likewise, hydroponic growth systems for plants will also contain microorganisms. If microorganisms escape these closed environments, they will add to the overall microbial burden in crew areas and likely increase the complexity of the microbial ecosystem. Thus, crew health may be put at risk. In order to determine whether escape is occurring, a monitoring system is required that distinguishes representative bacteria originating in these systems from potentially identical bacteria that may already have been present in the spacecraft environment. To address this type of problem, Fox and Willson have pioneered a novel method of marking bacterial strains.

In brief, it has been shown that unique inserts can be placed into a 5S rRNA gene carrying a substantial deletion. The resulting construct produces more than 10,000 copies of the modified 5S rRNA in each bacterial cell without being deleterious.<sup>8</sup> This technology is, however, of more general utility. Although the cutbacks in NASA funding have precluded a

proposal relating to ALS monitoring, we have instead sought funding for practical aspects of the technology. As a result, Fox, Willson, and Jackson submitted proposals through Biotex, Inc., to STTR programs at NSF and the National Institutes of Health (NIH). Phase 1 awards were obtained for both of these projects and larger Phase 2 requests will be submitted following completion of the Phase 1 efforts. One of these proposals seeks to take advantage of the high accumulation of modified RNA as a possible manufacturing system. The other seeks to explore the possibility of selecting useful RNAs from mixed populations of the insert carrying 5S rRNAs.

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