GENOMIC AND PHYSIOLOGICAL STUDIES ON EXTREMOPHILES: MODEL SYSTEMS FOR EXOBIOLOGY

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Abstract. The notion that extremophiles might provide model systems for exobiology and interplanetary transmission of life, has recently gained some acceptance. In this paper, we will examine the physiological properties, and the efficient DNA repair systems of thermophilic and desiccation resistant strains, and describe initial findings concerning the genomic sequence of a bacterium that can grow using carbon monoxide as its sole carbon and energy source.

NASA Goddard Space Flight Center (GSFC) has provided collaborative opportunities to measure the survival of two extremophiles exposed to space conditions and extreme UV (EUV) radiation. *Deinococcus radiodurans*, which is known to have the highest resistance of any cell to ionizing radiation, and the thermophilic bacterium PD3D (isolated by JDR and FTR from Yellowstone National Park) were selected because of their resistance to complete desiccation under high vacuum. For both microorganisms, desiccation in the high vacuum chamber at GSFC has very little effect on cell survival compared to the non-desiccated control.

Microbial cells were exposed to space vacuum and extreme UV separately and additively during a rocket flight on a Terrier Black Brant vehicle carrying a carrying the solar EUV spectrometer, SERTS. Following their exposure to space environment, we determined microbial survival using viable cell counts. Exposure to space vacuum alone (~ 10^{-6} Pa) decreased cell survival by 2 and 4 orders of magnitude for PS3D and *D. radiodurans* respectively. Exposure to EUV radiation

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additively decreased the survival of both organisms by an order of magnitude. This is the first measurement of the effect of EUV on cell survival. Our ongoing studies in the high vacuum chamber at the GSFC may reveal the molecular lesions inflicted by exposure of the cells to high vacuum, and the potential mechanisms involved in the repair of these lesions.

In order to explore the metabolism of a model thermophile that has minimal metabolic requirements, we have recently sequenced the compact genome of a sporulating bacterium, *Carboxydothermus hydrogenoformans*, which is able to propagate on a simple salts-only medium with the addition of a single gas, carbon monoxide, as its sole carbon and energy source. Preliminary analysis of the 2.1 megabase pair genome reveals that this extremely thermophilic, anaerobic microorganism has minimal sets of genes for complex cellular functions such as hydrogen production and sporulation.

We will determine whether these properties may provide these strains with margins of survival in space conditions, beyond the current limits for biological systems.

Introduction

More than a decade of research on the microbial flora of seemingly prohibitive, active geothermal areas such as undersea hydrothermal vents has revealed the existence of a wide variety of extraordinary microorganisms. It is now confirmed, for example, that thermophilic microbial growth can occur at temperatures up to 113 °C, and across a pH range from 0 to 13 at lower temperatures. The notion that these diverse microorganisms, termed extremophiles, might provide model systems for interplanetary transmission of life has recently gained some acceptance (Cady, 1998). The physiology and distribution of these organisms may have important implications for future spaceflight programs. They may be relics of historical transport processes that brought microbial life to Earth from neighboring planets (Cady et al., 2000). They also could cause contamination of other planets or satellites if they survive transit on manned or unmanned space vehicles.

We have carried out the isolation and characterization of novel radiation resistant microbial strains from hot springs in Yellowstone National Park containing elevated radon levels, and exposed to high fluxes of solar radiation. Exposure to hard vacuum and various types of radiation was used as a selective pressure to eliminate competing radiation sensitive microorganisms (DiRuggiero et al., submitted).

Thermophilic bacteria that are able to utilize CO under strictly anaerobic conditions, producing H_2 and CO_2 are of potential interest in astrobiology. Many volcanic exhalations contain high levels of CO (Giggenbach, 1980; Taran, 1988), and anaerobic, CO-utilizing, hydrogen-producing thermophiles can be isolated on the Kuril and Kermadec Islands, south of the Kamchatka Peninsula, in terrestrial hydrothermal springs (Bonch-Osmolovskaya et al., 1999). *C. hydrogenoformans,* the prototype for these strains, was characterized as a Gram positive thermophile with the potential for H_2 production (Svetlichny et al., 1991, 1994). Since then our colleagues have obtained isolates from deep sea hydrothermal vents (Sokolova et al., 2001) and hot springs in Yellowstone National Park, USA (Sokolova et al., in preparation).

In this paper, we describe the design and construction of a device that allows for exposure of microorganisms to space vacuum and extreme ultraviolet, and an initial sounding rocket flight to test the device and to determine the vacuum and radiation resistance of an isolate from Yellowstone National Park. The initial results of genomic investigation of an extreme thermophile *Carboxydothermus hydrogenoformans* .that is able to grow with carbon monoxide as sole energy and carbon source are described.

Materials and methods

The cells were exposed to space vacuum and extreme UV separately and additively during a rocket flight on a Terrier Black Brant vehicle carrying a SERTS telescope. Two 0.22 μm pore size circular polycarbonate filters carrying 10^8 cells in a monolayer were placed on each cell holder, one of the filters being kept in the dark at all time. The cell holders were mounted onto cylinders in which the incident light from the sun was passed through an thin aluminum window and concentrated using a focusing mirror with multilayer coatings to enhance its EUV reflectance. The cell holders were mounted onto cylinders in which the incident light from the sun was filtered through an 0.2 uM thick aluminum window and concentrated using a grazing incidence focusing mirror treated with multiple coatings to increase its EUV reflectance. The temperature was monitored in real time. The rocket reached an altitude of 304 km in 283.5 s, and the shutter was open for 441 s. The calculated dose at 30.4 nm was $6x10^{12}$ photons/cm^2. The combination of filter transmittance, mirror reflectance and solar spectrum assured that only EUV radiation at wavelengths shorter than 34 nm reached the top layer of test cells during the rocket flight. The second sample in each holder was subject to the same vacuum regime as the first, but was shielded from any EUV exposure. The rocket payload reached an altitude of 304 km in 283.5 s, and remained oriented toward the sun for a total of 395 s. Taking into account the measured solar EUV irradiance for the flight date, properties of the optical components, and varying atmospheric extinction rates during the flight trajectory, the total dose that was experienced by

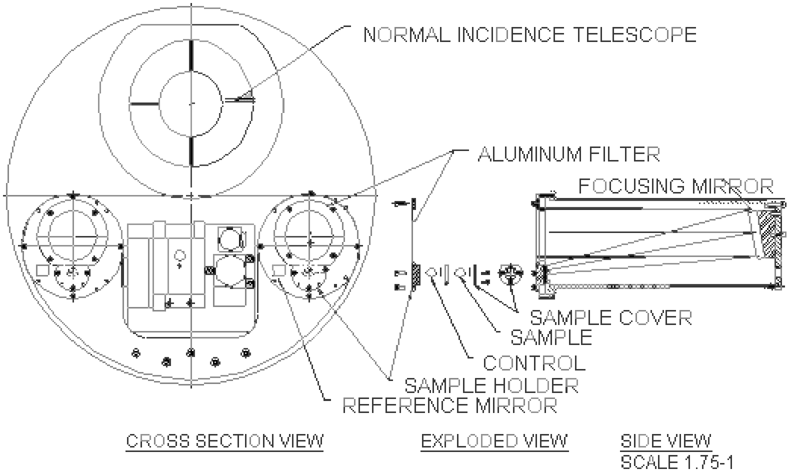

Fig.1 Layout of cell irradiation devices flown on the SERTS sounding rocket. The Cross-Section View (left) depicts the front face of the rocket payload; the large telescope on top fed the solar EUV spectrometer that was the principal experiment package carried, the lower box contained pointing-control optics and an EUV irradiance monitor, while the two devices on either side carried the biological samples. The Side View (right) shows the incoming solar radiation passing through an Aluminum filter onto a multilayer-coated concave mirror which then concentrates the light onto the top layer of cells. The Exploded View (center) indicates how a control sample that experienced the same vacuum and temperature regime without EUV irradiation was mounted just behind the exposed cells. Reference mirrors allowed optical alignment so that full sample illumination occurred whenever the rocket payload was properly pointed at the sun.

the exposed cell samples was $6x10^{12}$ photon/cm^2, which corresponds to an energy dose of 0.4 J/m^2. Within two hours of launch, the payload was recovered.The cell holders were removed immediately, and placed into sterilized bags for shipment to the University of Maryland where testing of viability was carried out on arrival.

Results and Discussion

For a microbial cell to survive lengthy exposure in space, it requires the ability to repair extensive genetic and biochemical lesions caused by complete desiccation under vacuum, temperature extremes, and irradiation across a wide range of wavelengths. Despite the isolation of some 24 genera of extreme thermophiles, we know very little about the strategies of any thermophiles for maintaining the integrity of its genetic material during exposure to hard vacuum and full spectrum solar radiation. In previous experiments, we have observed that quiescent, extremely thermophilic microorganisms can survive long periods of storage and desiccation and are able to survive high doses of ionizing radiation in laboratory conditions (DiRuggiero et al., 1996, submitted).

Two polycarbonate filters carrying 10^8 cells in a monolayer were placed on each cell holder. One filter in each pair was kept in the dark at all times as an unirradiated control, Following their exposure to the space environment, we determined microbial survival using viable cell counts. Exposure to space vacuum alone (~10^{-6} Pa) decreased cell survival by approximately 2 and 4 orders of magnitude for PS3D and *D. radiodurans* respectively.

We have recently isolated several thermophilic, iron reducing bacteria from both terrestrial and marine geothermal environments that have similar growth physiology on CO, indicating that they may have common features of CO-utilization, whilst using different electron acceptors.

Carboxydothermus hydrogenoformans was the first strictly carboxydotrophic strain to be described, with an optimal growth temperature of 72 °C (Svetlichny et al., 1991). Later, another species, *Carboxydothermus restrictus* was isolated from hydrothermal outlets on Raoul Island on the Kermadec Archipelago (Svetlichny et al., 1994). These strains grow optimally at 75 °C, using CO as an energy and carbon source, with H_2 and CO_2 as the only end detectable products. Chemolithotrophic growth by means of CO oxidation is coupled to hydrogen and carbon dioxide formation according to the following equation:

$$CO + H_2O \longrightarrow CO_2 + H_2 \quad \Delta G = -20\ kJ/mole$$

The sequence of *Carboxydothermus hydrogenoformans* confirmed our earlier observation that the strain was capable of spore formation, for it has a set of genes encoding for each of the major stages of endospore formation. In addition, since we had described two cooS genes encoding the catalytic subunits of carbon monoxide dehydrogenase (CODH), detected by random sequencing (Gonzalez and Robb, 2001), it was surprising to discover three additional cooS genes, indicating that the genome has the coding potential for a total of five CODHs. The genome also has a relatively high proportion (ca 30%) of open reading frames that have primary similarity to genes in methanogenic Archaea. This provides support for our hypothesis that states that the carboxydotrophic bacteria are frequently closely associated with methanogenic Archaea, allowing the exchange of metabolites, such as CO, but also leading to rather frequent lateral gene transfer between these examples of different Domains of Life.

Conclusions

NASA Goddard Space Flight Center (GSFC) has provided collaborative opportunities to measure the survival of two extremophiles exposed to space conditions and extreme UV (EUV) radiation. *Deinococcus radiodurans*, which is known to have the highest resistance of any cell to ionizing radiation, and the thermophilic bacterium PD3D (isolated by JDR and FTR from Yellowstone National Park) were selected because of their resistance to complete desiccation under high vacuum. For both microorganisms, desiccation in the high vacuum chamber at GSFC has very little effect on cell survival compared to the non-desiccated control. The most interesting result from this experiment is that exposure to EUV radiation additively decreased the survival of both organisms by an order of magnitude. This is the first measurement of the effect of EUV on cell survival. Our ongoing studies in the high vacuum chamber at the GSFC may reveal the molecular lesions inflicted by exposure of the cells to high vacuum, and the potential mechanisms involved in the repair of these lesions. Similar experiments are in progress with a synchrotron to assess the molecular damage sustained during exposure to monochromatic EUV.

The phenomenon of living cells propagating with a single gas, CO, as sole carbon and energy source has been subject to deeper analysis at the genomic level. The multiple genes encoding CODHs in a single bacterium may allow this strain to survive across a wide range of CO concentrations, and to contribute H2 to a number of anaerobic microorganisms whose primary energy conservation reaction is either sulfur or sulfate reduction (Hagen et al., 2000).

The contributions of extremophiles to biotechnology are tangible. Already, extremophile cells or their enzymes are widely used in industrial processes such as paper pulp bleaching, baking, biodegradation of toxic compounds, and clinical applications such as cancer detection, drug screening, forensic detection and blood chemistry screening. The most widely known application of thermostable enzymes in biotechnology is the use of thermostable DNA polymerases in the polymerase chain reaction (PCR), a universally applied technology that earned its inventor, Kary Mullis, the Nobel Prize. The first, and most valuable of several enzymes used in this reaction, Taq polymerase, with a market value of “hundreds of millions of dollars” was obtained from the extreme thermophile, *Thermus aquaticus*, isolated from Octopus Spring in Yellowstone National Park.

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