Exposing Microorganisms in the Stratosphere for Planetary Protection Project

Center Independent Research & Developments: KSC IRAD Program | Science Mission Directorate (SMD)

ABSTRACT

Earth's stratosphere is similar to the surface of Mars: rarified air which is dry, cold, and irradiated. *E-MIST* is a balloon payload that has 4 independently rotating skewers that hold known-quantities of *spore-forming bacteria* isolated from spacecraft assembly facilities at NASA. Knowing the survival profile of microbes in the stratosphere can uniquely contribute to *NASA Planetary Protection* for Mars.

Objectives

1. Collect *environmental data* in the stratosphere to understand factors impacting microbial survival.

2. Determine % of *surviving microbes* (compared to starting quantities).

3. Examine microbial **DNA mutations** induced by stratosphere exposure.

ANTICIPATED BENEFITS

To NASA funded missions:

NASA Astrobiology Roadmap: Broadening our knowledge both of the range of environments on Earth that are inhabitable by microbes and of their adaptation to these habitats will be critical for understanding how life might have established itself and survived in habitats beyond Earth.

•Astrobiology Roadmap <u>GOAL 5</u> – Understand the evolutionary mechanisms and environmental limits of life; *Objective 5.3* – Biochemical adaptations to extreme environments.

To NASA unfunded & planned missions:

E-MIST will carry spore-forming bacteria (extremophiles resistant to harsh conditions) that were previously isolated from



First E-MIST test flight in August 2014; next flight scheduled for September 2015

Table of Contents

Abstract 1
Anticipated Benefits1
Technology Maturity1
Realized Benefits2
Management Team 2
Technology Areas 2
Detailed Description3
U.S. Locations Working on this
Project
Details for Technology 1 6



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spacecraft assembly facilities at KSC. We know these microbes are traveling to Mars on NASA spacecraft assembled at KSC; our objective is to measure if they can survive once reaching the Red Planet.

Microbes must survive pressure ~1 to 10 mbar, temperatures from 0 to -100 °C, low water availability at < ~20% relative humidity, and high ionizing radiation levels). Knowing the survival profile of microbes in the stratosphere can uniquely contribute to *NASA Planetary Protection policies*. If a microbe can survive in the stratosphere, it can probably survive on the surface of Mars as well. Back here on Earth, the upper atmosphere *is a natural laboratory for mining genes that guard or restore radiation-damaged biomolecules*.

To other government agencies:

Space Technology Grand Challenges: Theme 1 – Economical Space Access (*Provide economical, reliable and safe access to space, opening the door for robust and frequent space research...*)

To the commercial space industry:

Next Generation Manufacturing Technologies (3D printed E-MIST hardware components)

To the nation:

An experiment intentionally exposing microorganisms to the Earth's stratosphere has not been flown before. Thus, our project is path-finding.

There are no existing data for the exposure of microbes to the stratosphere. *This study will produce foundational knowledge* for the limits of terrestrial life and the bacterial candidates most likely survive on Mars.



Management Team

Project Manager:

Nancy Zeitlin

Principal Investigator:

David Smith

Co-Investigators:

- Anthony Bharrat
- Nicole Dawkins
- Adam Dokos
- Leandro James
- Christina Khodadad
- Michael Lane
- Prital Thakrar

Technology Areas

Science Instruments, Observatories, and Sensor Systems (TA 8)

Continued on following page.

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DETAILED DESCRIPTION

Introduction: We designed, built and flew a self-contained payload, Exposing Microorganisms in the Stratosphere (E-MIST), on a large scientific balloon launched from New Mexico on 24 Aug 2014 [1]. The payload carried *Bacillus pumilus* SAFR-032, a highly-resilient spore-forming bacterial strain originally isolated from a NASA spacecraft assembly facility. Our balloon test flight evaluated microbiological procedures and overall performance of the novel payload. Measuring the endurance of spacecraft-associated microbes at extreme altitudes may help predict their response on the surface of Mars since the upper atmosphere also exerts a harsh combination of stresses on microbes (e.g., lower pressure, higher irradiation, desiccation and oxidation) [2].

Materials and Methods: Our payload (83.3 cm x 53.3 cm x 25.4 cm; mass 36 kg) mounted onto the exterior of a high altitude balloon gondola. Four independent "skewers" rotated 180° to expose samples to the stratosphere. During ascent or descent, the samples remained enclosed within dark cylinders at ~25 °C. Each skewer had a base plate holding ten separate aluminum coupons with *Bacillus pumilus* spores deposited on the surface. Before and after the flight, *B. pumilus* was sporulated, enumerated and harvested using previously described techniques [3–5].

Major payload components were a lithium-ion battery, an ultraviolet (UV) radiometer (400 to 230 nm), humidity and temperatures sensors, and a flight computer. During the test flight, samples remained in a sealed position until the payload reached the lower stratosphere (~ 20 km above sea level). Next, the flight computer rotated the skewers into the outside air. After a short rotation demonstration (2 seconds), all skewers reverted to the closed position for the remainder of the flight. The payload

Technology Areas (cont.)

- Robotics and Autonomous Systems (TA 4)
- Remote Sensing Instruments and Sensors (TA 8.1)

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continued floating at an altitude of 37.6 km for 4 hours before beginning a 23 minute descent on parachute.

Results and Discussion: Our first test flight examined unknowns associated with sample transportation, gondola installation, balloon ascent/descent, and time lingering in the New Mexico desert awaiting payload launch and recovery. We created a batch of experimental control coupons (each containing approximately 1 x 10⁶ spores) used throughout the investigation for ground and flight test purposes. Several treatment categories were evaluated: Lab Ground Coupons (kept in the KSC laboratory); Transported Ground Coupons (traveled to New Mexico and back but not installed in payload); and Flight Coupons (flown). A subset of coupons from each treatment category were processed, resulting in statistically equivalent viability (Kruskal–Wallis rank-sum test at a 95% confidence level). Taken together, nearly identical viability from all coupons indicate that balloon flight operations and payload procedures did not influence spore survival. A negative control (blank, sterile coupon) was also flown to verify payload seals prevented outside contamination.

A species-specific inactivation model that predicts the persistence of microbes on the surface of Mars is one of many possible outcomes from balloon experiments in the stratosphere. The simplicity of the payload design lends itself to customization. Future investigators can easily reconfigure the sample base plate to accommodate other categories of microorganisms or molecules relevant to the Planetary Protection community. If future flights exposed microbes for hours, we would expect to see a rapid inactivation. Smith et al. [6] simulated stratospheric conditions and measured a 99.9% loss of viable *Bacillus subtilis* spores after only 6 hours of direct UV irradiation. Earth's stratosphere is extremely dry, cold, irradiated, and hypobaric, and it may be useful for microorganisms isolated from NASA spacecraft assembly facilities to be evaluated in this accessible and robust Mars analog environment.

A second, science test flight launching from Ft. Sumner, NM, is scheduled for September 2015.

References: [1] D. J. Smith et al. (2014) *Gravitational and Space Research, 2,* 70–80. [2] D. J. Smith (2013) *Astrobiology, 13,* 981–990. [3] P. A. Vaishampayan et al. (2012) *Astrobiology, 12,* 487–497. [4] R. L. Mancinelli and M. Klovstad (2000) *Planetary and Space Science, 48,* 1093–1097. [5] R. Moeller et al. (2012) *Astrobiology, 12,* 457–468. [6] D. J. Smith et al. (2011) *Aerobiologia, 27,* 319–332.

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U.S. LOCATIONS WORKING ON THIS PROJECT



• Ames Research Center

Other Organizations Performing Work:

• Engineering Services Contract

Contributing Partners:

California Institute of Technology / NASA Jet Propulsion Laboratory

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Page 5

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 - (http://www.aerospaceamerica.org/Documents/Aerospace%20America%20PDFs%202015//
- astrobiology news story
 - (http://astrobiology.com/2014/09/nasas-exposing-microorganisms-in-the-stratosphereexperiment-soars.html)

Publications

- GSR publication
 - (http://gravitationalandspacebiology.org/index.php/journal/article/viewFile/661/693)

DETAILS FOR TECHNOLOGY 1

Technology Title

Strat-X

Technology Description

This technology is categorized as a hardware system for unmanned flight

Experiments in space can be expensive and infrequent, but Earth's upper atmosphere is accessible via large scientific balloons, and can be used to address many of the same fundamental questions. Scientific balloons are made of a thin polyethylene film inflated with helium, and can carry atmospheric sampling instruments on a gondola suspended underneath the balloon that eventually is returned to the surface on a parachute. For stratospheric flights between 30 and 40 km above sea level, balloons typically reach the float altitude 23 hours after launch, and travel in the direction of the prevailing winds.

Autonomous technologies enabling stratospheric exposure experiments are lacking. As a result, a standalone enclosure system was developed that opens and closes at stratospheric altitudes,

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Page 6

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Capabilities Provided

Strat-X has a flexible design with removable panels for hardware integration, a control board for autonomous operations, and four doors for timed exposures aloft. The housing of the entire Strat-X system is composed of an 80/20 (aluminum alloy) frame and white powder-coated aluminum panels. The four independent skewers can rotate 180° to expose samples to the outside environment, or enclose samples and maintain ambient conditions inside Strat-X. Each cylindrical skewer is housed in a frame laced with Shuttle Nomex Felt Reusable Surface Insulation (FRSI) to shield the samples from light.

exterior of balloon gondolas. Strat-X has removable panels for hardware integration, and four

independently rotating "skewers" that hold experimental samples.

Performance Metrics



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Page 7

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