Long-term multi-generational evolutionary studies of bacteria in the spaceflight environment (MVP-Cell-02)

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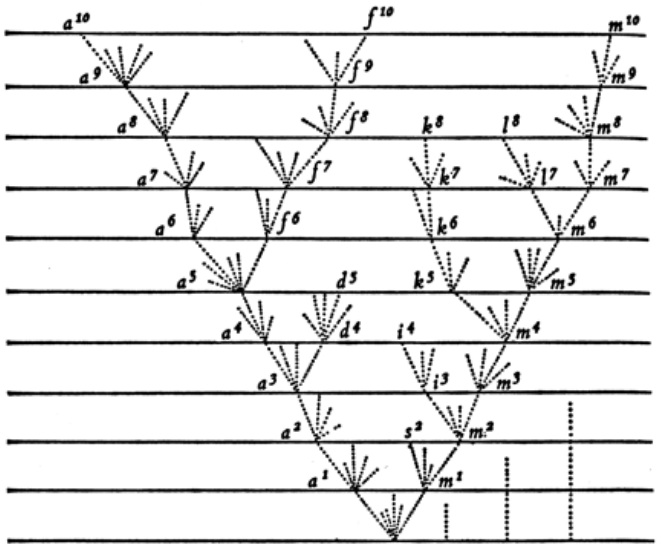
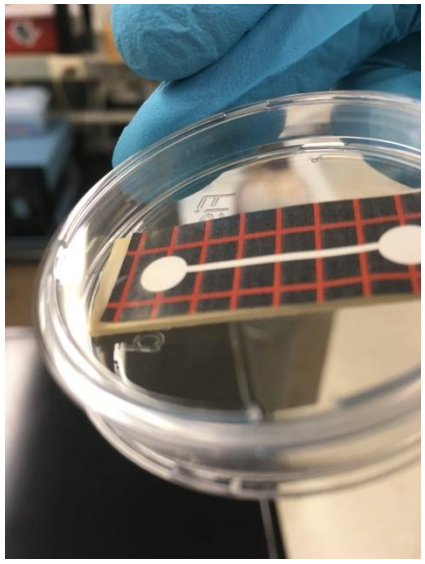
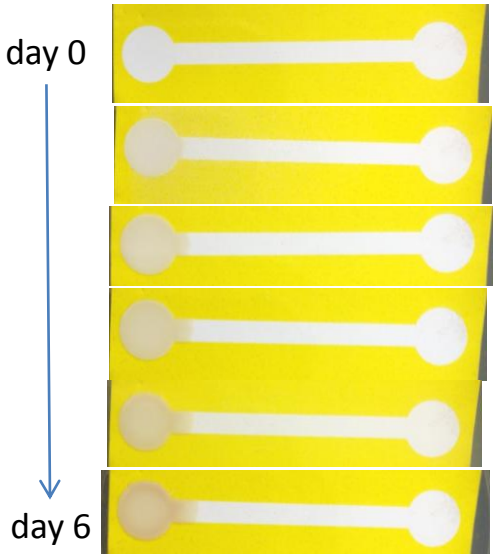






Evolution Matters

Spaceflight presents multiple selective pressures on, and growth opportunities for life, and microorganisms. Some examples include: **microgravity**, ionizing radiation, varying temperature / humidity / atmospheric gas composition, prolonged contact with novel materials, periodic disinfection, and prolonged contact with human hosts.

Impacts of microbes:

-Biologically-based support systems (*ISRU, closed-loop life support, food production)*

-Novel, self-contained ecosystems - the built environment

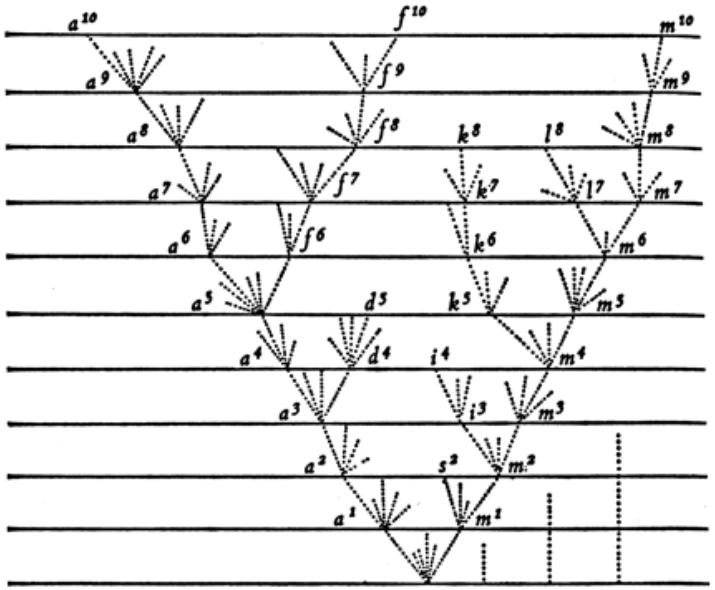
-Contamination, biofilms, virulence

-Stability and functionality of biological systems

Understanding microbial evolution is essential for long-term space travel:

-Rapidly evolve: Ubiquitous, large population sizes, short generation times

-They are coming whether we like it or not



Darwin, 1859

Evolution

1) Generation of new genetic information
2) Natural selection and neutral processes

Adaptation can be physiological and/or genetic – *Evolution* is the 'recorded change'

Classical Experimental Evolution (*e.g*. Richard Lenski):

-12 identical copies of *E. coli* started in 1988
-Transferred daily (~every 6.6 generations), in identical benign growth conditions (liquid culture)
-Now at 60,000 generations+, and still evolving

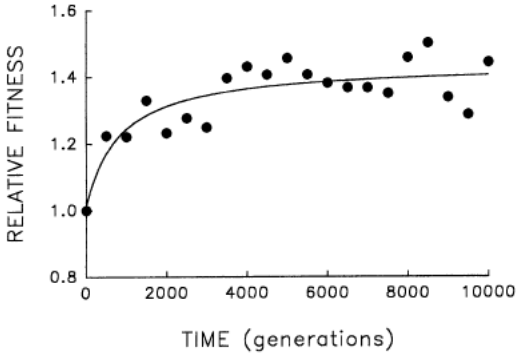


FIG. 4. Trajectory for mean fitness relative to the ancestor in one population of *E. coli* during 10,000 generations of experimental evolution. Each point is the mean of three assays. Curve is the best fit of a hyperbolic model.

Lenski and Travisano, PNAS 1994

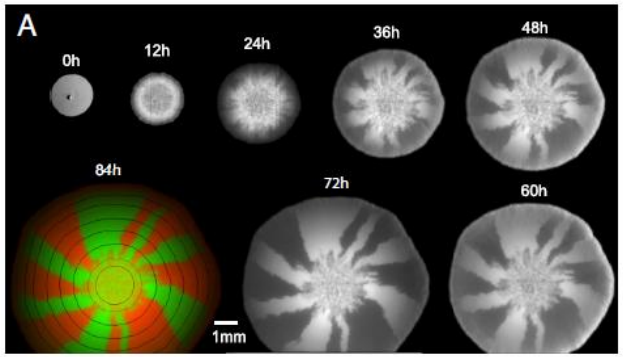
Not possible onboard ISS:
short experiment window, large mass/volume
astronaut time constraints

Evolutionary studies take time – how can we perform them in space?

Bacterial Growth

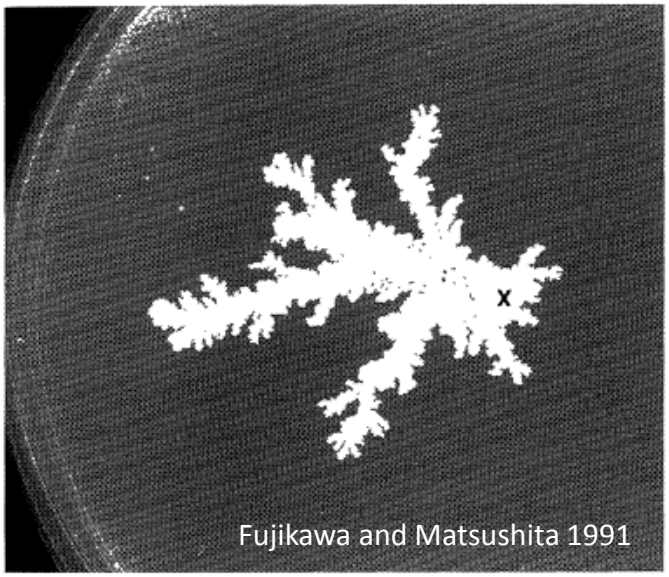
Evolution at the edge of a propagating colony: Population dynamics and drift of neutral alleles

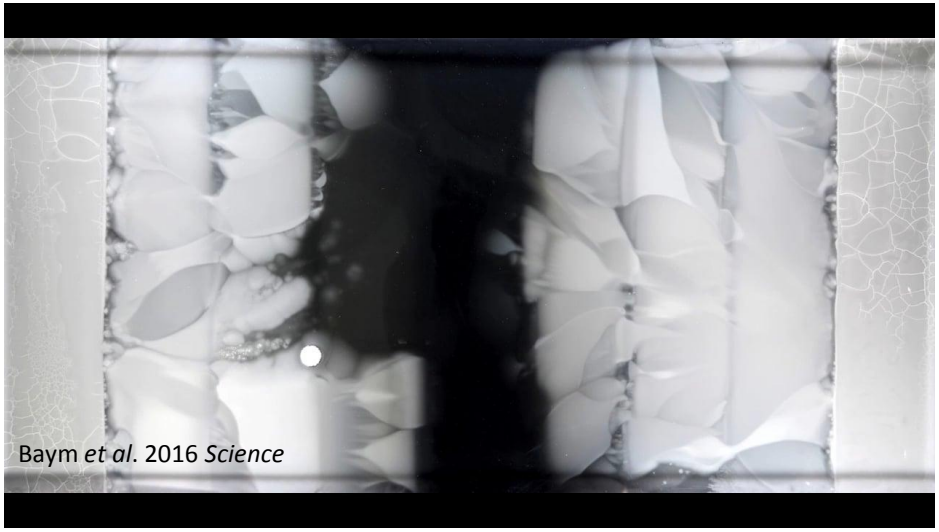
Hallatschek et al. 2007 PNAS



Directed colony growth against an environmental gradient

2 g/l [peptone] 0 g/l





Evolution of antibiotic resistance in space and time

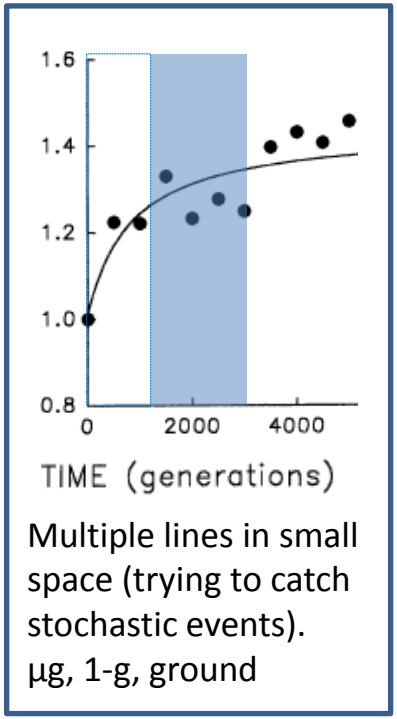
A propagating colony front is effectively in exponential or linear growth.
If constrained it can act as a continuous selective pressure.
higher growth rate = faster colony speed = higher fitness

Space Races – Experimental Evolution on ISS

PES membrane printed with hydrophobic wax and spotted with bacterial spores is overlain on capillary matting, to make growth tracks.

"Wet and forget" Growth medium added, with or without exogenous DNA, spores germinate at one end, propagate along the PES path, biomass collected at the other end.

Small sample volume and mass allow high replication (mitigates reduced generations allowed in 20-60 day window).



Multiple lines in small space (trying to catch stochastic events).
µg, 1-g, ground

Imaging detects changes in growth rate over experimental timeframe

Post-experiment sample return → high-throughput sequencing of 'winners'

Genomics analysis identifies genetic changes in winner lines compared to ancestor

Research Questions

Testable hypothesis 1

Evolutionary processes, as measured by fitness (speed of growth in lanes, and changes in growth speed over the course of the experiment), and observed evolutionary changes (as detected in sequenced DNA samples) occur at different rates in microgravity compared to 1-g and Earth. Hypothesis 1b*: Effects of microgravity on these processes are also different and will be tested in flight with 1-g controls.*

Testable hypothesis 2

Rates of adaptive integration of exogenous DNA are different in microgravity compared to 1-g control and Earth).

Testable hypothesis 3

Targets of DNA-uptake under the treatment conditions will be non-random, with specific loci/pathways preferentially assimilated under space conditions.

Outcomes and Impact

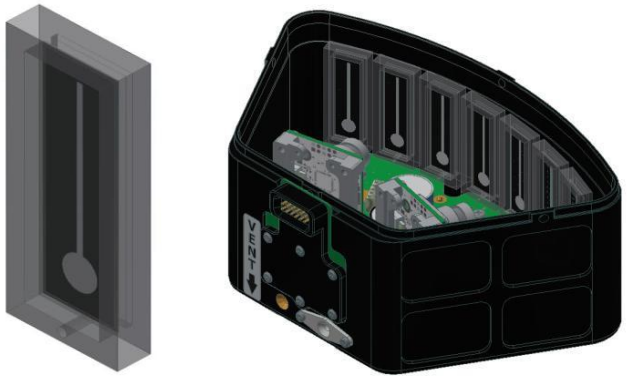
Advance understanding of the evolutionary processes and challenges facing biological systems in long-term space exploration and habitation.

-Changes in growth rate, mutation rate, targets of selection in space environment
-Specifically tease out gravitation effects on evolutionary process
-Naïve organisms evolutionary response to truly novel conditions (µg)

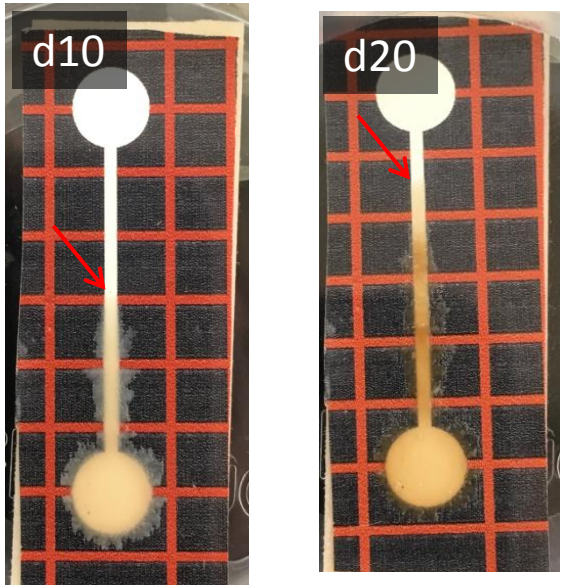
Space Races – Flight Experiment Summary

Specimen: *B. subtilis* sp. 168 wt (1A1) and *B. subtilis* sp. 168 mutator (1A24)
Experiment *g* level: 1 *g* and µ*g* on ISS; ground control
Experimental Treatments: 12 total treatment types. 2 strains in 1 *g* or µ*g*, same nutrient source, different DNA* availability: DNA free, low complexity DNA, high complexity DNA
Samples per insert/module/rotor: 1; with 7 inserts per module, 6 modules per rotor, for 42 samples per rotor (42 1-*g*, 42 u*g*)
Experimental Duration: TBD; minimum 20 days; prefer 60-90 days. At 20 days, estimated that 1,000 bacterial generations can occur with sufficient growth across surface.

*High-complexity environmental DNA = (DNA with high-informational content from native soil habitats of *B. subtilis* Low-complexity DNA = digested pUC plasmids or synthesized "nonsense" DNA

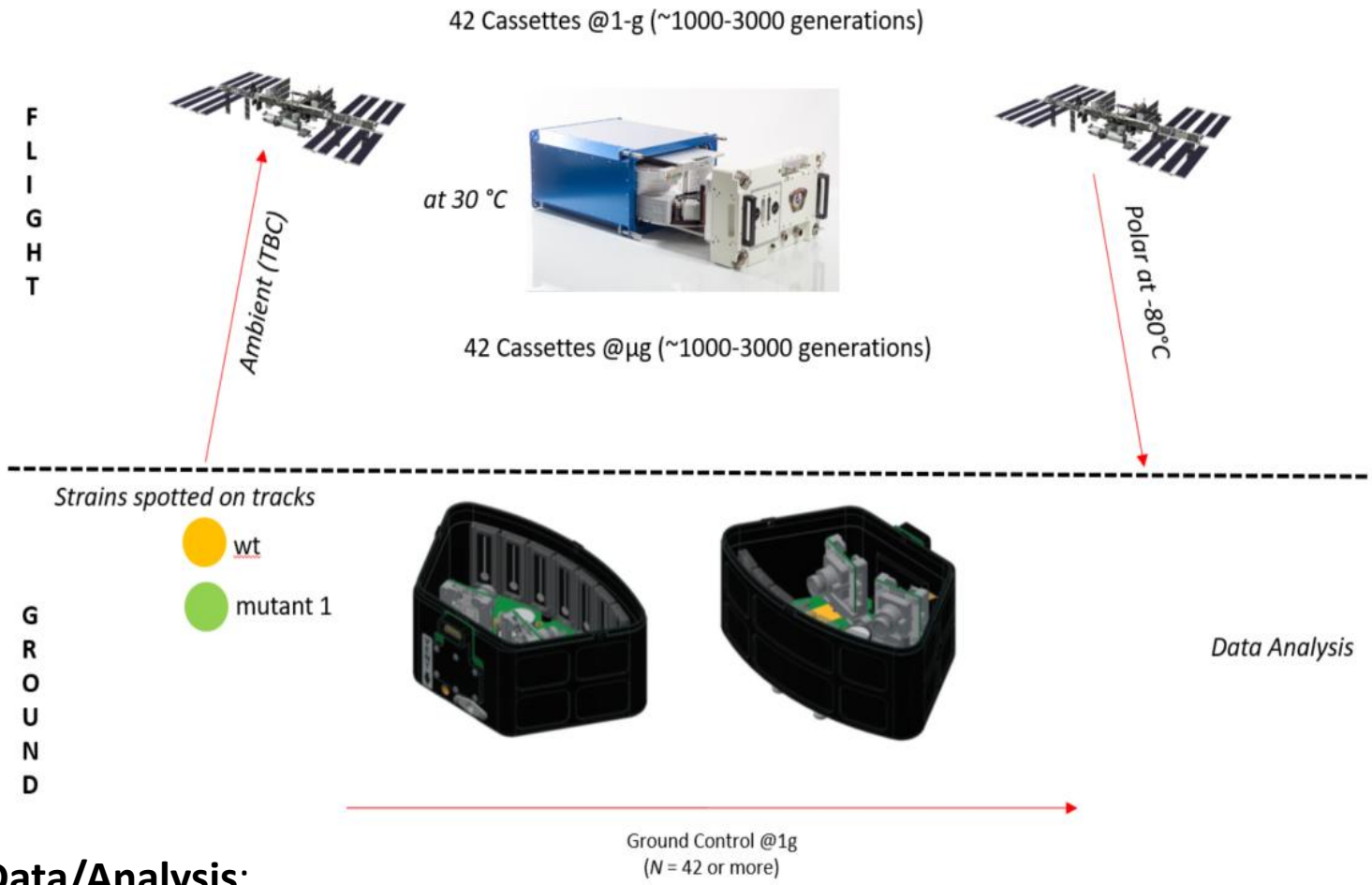


Single growth track (left), 7 per module (right), 6 modules per rotor, 2 rotors. Images courtesy Techshot



Propagation of growth from spores to d20

Space Races – Flight Experiment Summary



Data/Analysis:

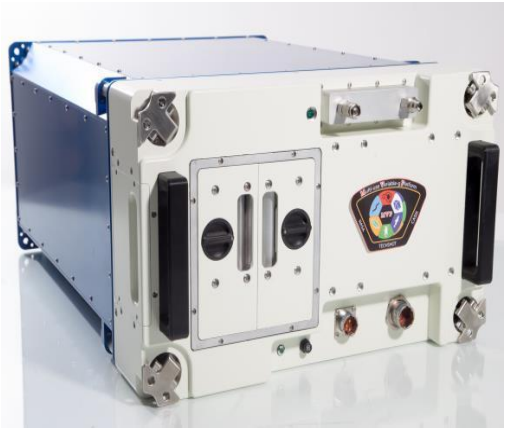
Imaging- **Daily images** to monitor speed and changes in propogation.

Sample return- For genomic sequencing of winners, and capture of evolved isolates. **Comparative genomics** with ancestral lines to identify targets and nature of any changes.

Hardware and Heritage

Flight Hardware:

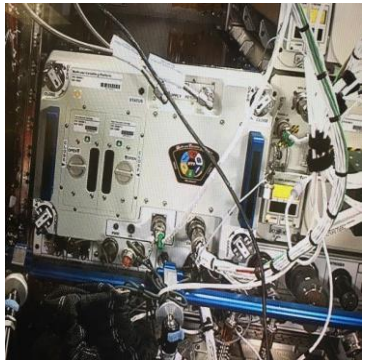
- MVP Facility (Techshot)
- 12 Cell Experiment Modules (CEM) and fluidics, customized for the Everroad Experiment (Techshot)
- Kits for preparing/freezing samples (Techshot)
- Cold Stowage for Orbit and Descent: MELFI, and POLAR. Temperature desired: -80C



Multi-use Variable-g Facility (MVP)

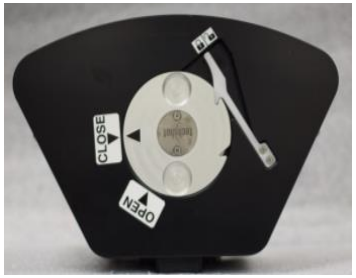
Flight Heritage:

- MVP Facility is currently installed on an EXPRESS rack of the ISS (US Lab, Destiny)
- MVP-FLY-01 (ARC) was successfully conducted in MVP during SpX-14 dock (April 2018). This experiment required video capture and constant downlink.
- Two MVP CEMs are part of a Techshot EVT after SpX-14 undock (May 2018)



MVP installation on ISS (SpX-14)

Cell Experiment Module (CEM) – Side view



Cell Experiment Module (CEM) – Top view

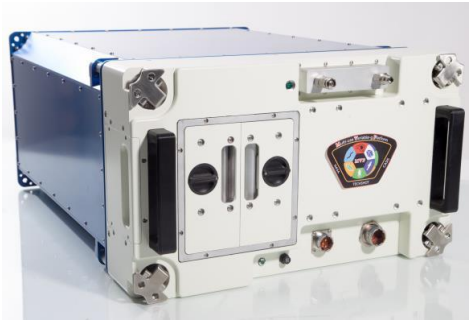
Images courtesy Techshot

Summary

Evolution matters – factors that constrain life and habitability in the universe, long-term human exploration, important life processes in space.

Concept Summary

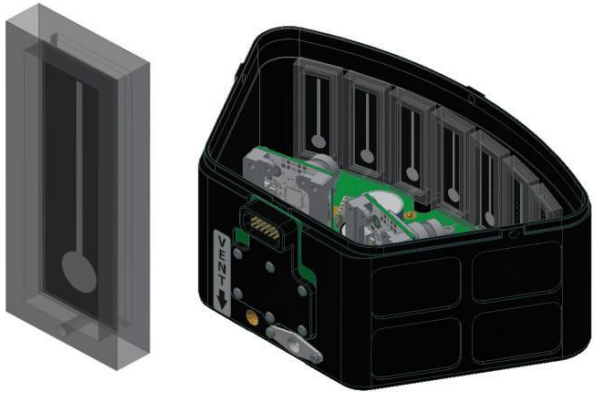
- Grow bacteria along a solid surface in µ*g* onboard ISS for 1000+ generations. Monitor growth speed and changes by imaging.
- Sequence evolved strains from µ*g*, 1-*g* control, and compare to the starting sequence. Identify fitness and genetic changes in µ*g*.



Multi-use Variable-g Facility (MVP)

Alignment to NASA goals

- Prioritized Research Areas P1 and P2 of the Decadal Survey
- Guiding questions MB-1, MB-6 and DEV-9 of the Microbiology, and the Developmental, Reproductive and Evolutionary Biology elements of the Space Biology Science Plan.



Schematic of a growth insert (left) and experimental module in the MVP with an array of inserts and two cameras (right).

Impact and importance of ISS

- ISS ideal for long-term study, with 1-*g* control, sample return
- Evolution in response to truly unique environment, how evolution works, targets, and processes.
- Understanding of how life adapts to the spaceflight environment.

Thank You!

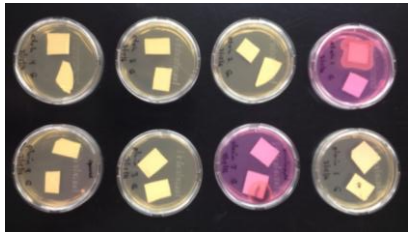
Science Team (ARC):

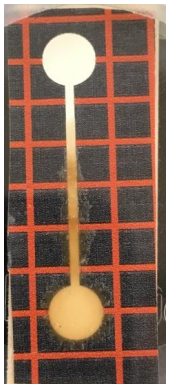
Brad Bebout
Angela Detweiler
Jessica Koehne
Tony Ricco

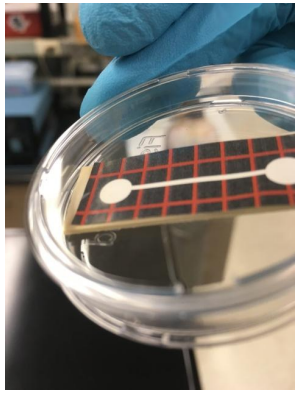
Implementation team (ARC):

Fathi Karouia
Kevin Martin
David Smith

ARC Space Biology Team







techshot

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Alignment with NRC Recommendations

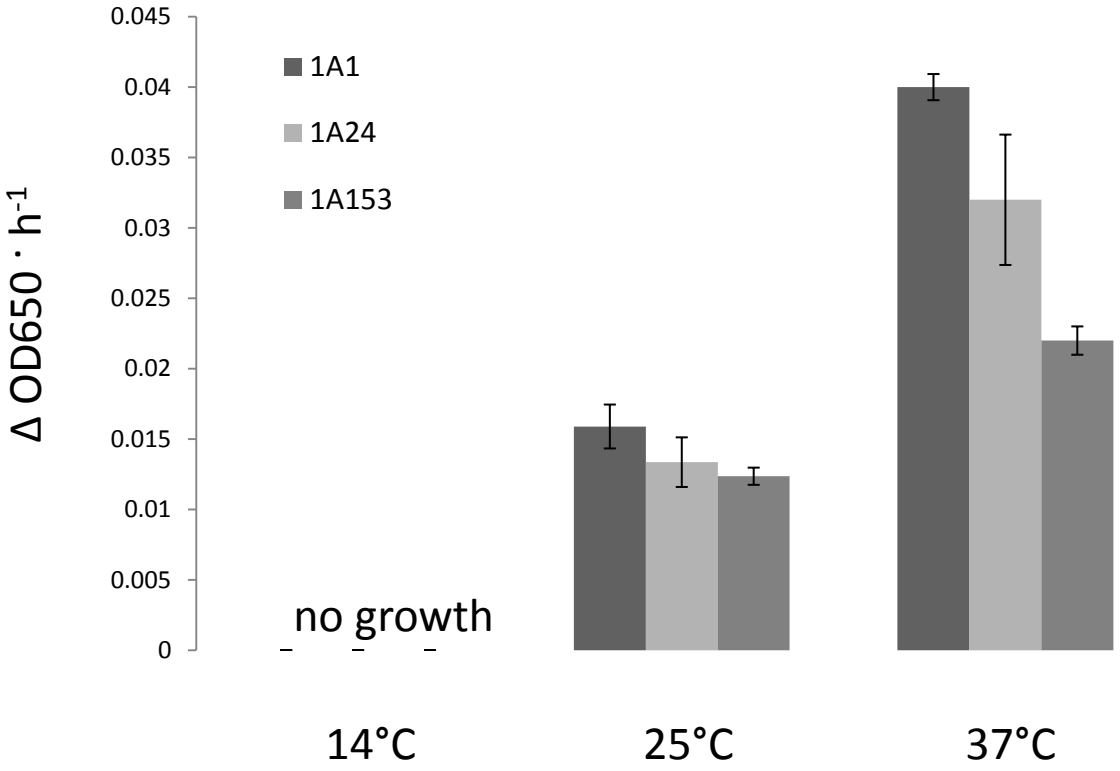
The experimental evolution of microbes in space over multiple generations directly aligns with Prioritized Research Area P1 in the 2011 Decadal Survey on Biological and Physical Sciences (Recapturing a Future for Space Exploration: Life and Physical Sciences Research for a New Era), specifically to:

"Capitalize on the technological maturity, low cost, and speed of **genomic analyses** and the **rapid generation time of microbes to monitor the evolution of microbial genomic changes in response to the selective pressures present in the spaceflight environment**", and in "**understanding the influence of the spaceflight environment on defined microbial populations**."

Additionally, this work more generally aligns with Prioritized Research Area P2, by exploring the "responses to individual components of spaceflight environments, such as **altered gravity**…", and by "…analyzing plant and microbial growth and physiological responses to the multiple stimuli encountered in spaceflight environment."

Preliminary Testing – Flight Definition

Strain selection, media, growth



1A1 – *B. subtilis* 168 ancestor, 'wt', *trpC2*

1A24 – *dnaN34 iluA1 metB5* – mutator strain (beta-clamp DNA pol III)

1A153 – *sigD2 lys trpC2* – non-motile (no flagella)

24-well plate assay, n=3

swimming vs. swarming motility vs. non-motile mutant
temperature effects on mutator

Preliminary Testing – Flight Definition

20 days growth in flight-like conditions on <1 mL dH2O rehydration

Buffered SMB medium with catabolic repressors.

Dried spores applied to dry membranes. Stasis protocols confirmed.

Rehydration of dried media and germination