Experimental evolution of *Bacillus subtilis* 168 in the spaceflight environment

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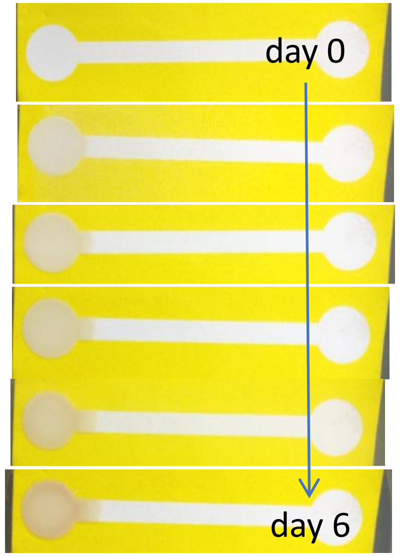
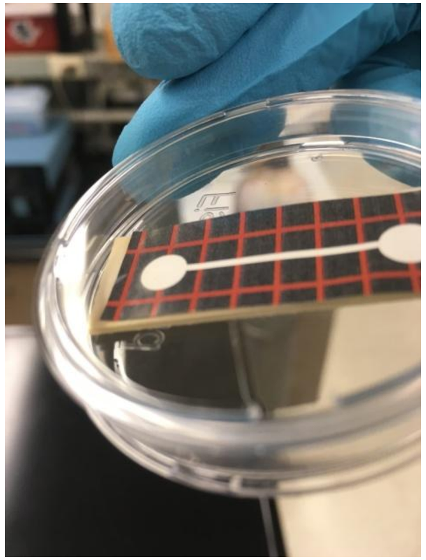


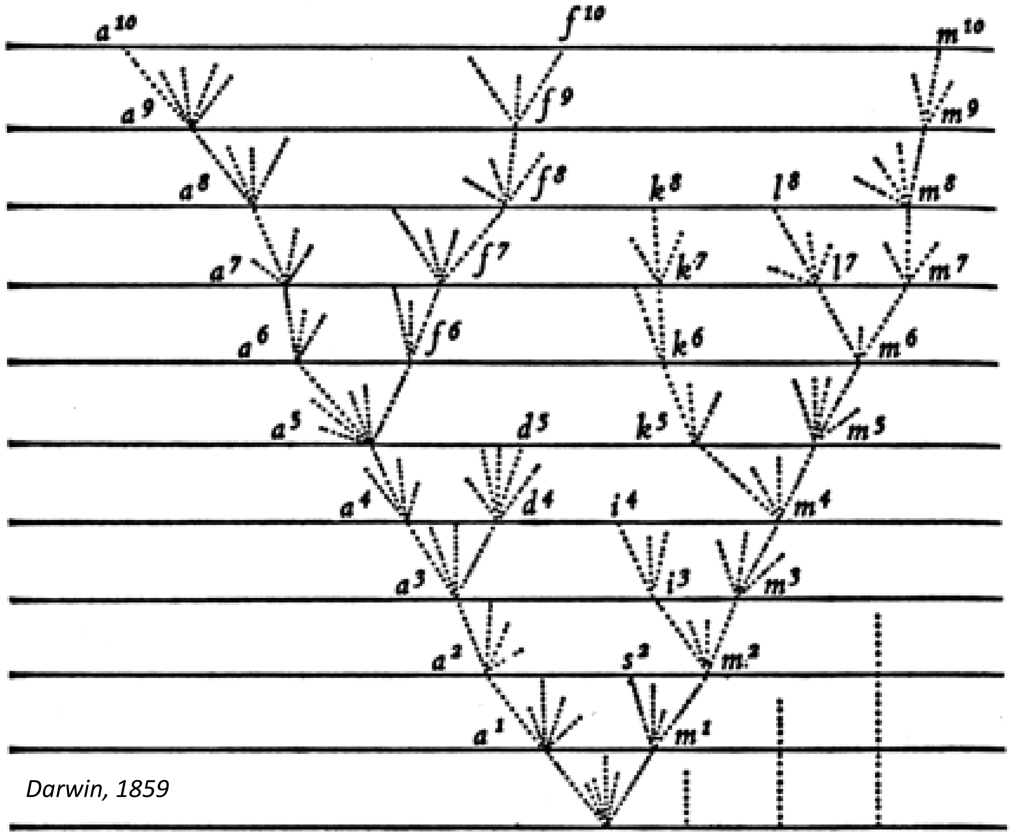


Evolution Matters

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

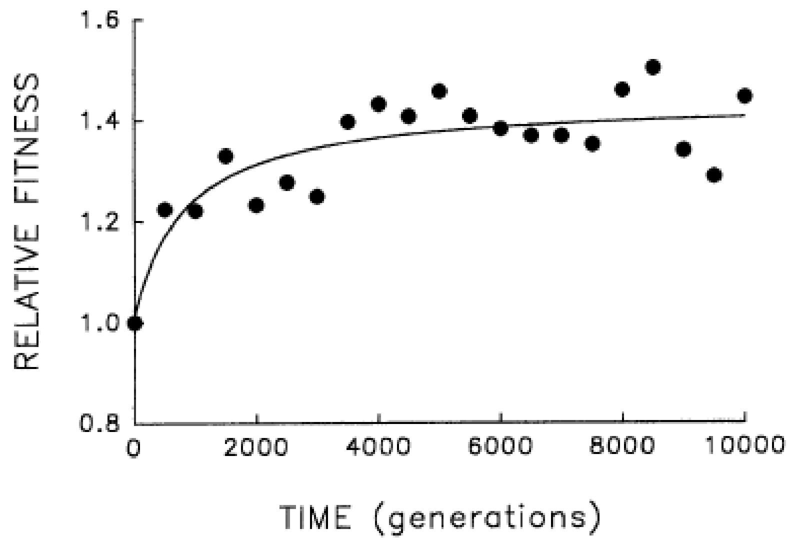


FIG. 4. Trajectory for mean fitness relative to the ancestor in one population of *E. coli* during 10,000 generations of experimental evolution. Each point is the mean of three assays. Curve is the best fit of a hyperbolic model.

Lenski and Travisano, PNAS 1994

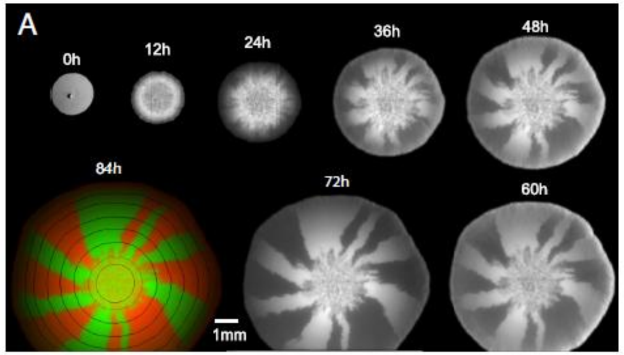
Not possible onboard ISS:
short experiment window, large mass/volume
astronaut time constraints

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

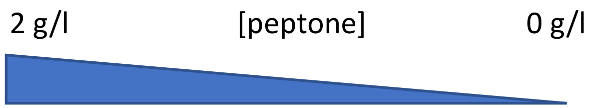
Bacterial Growth

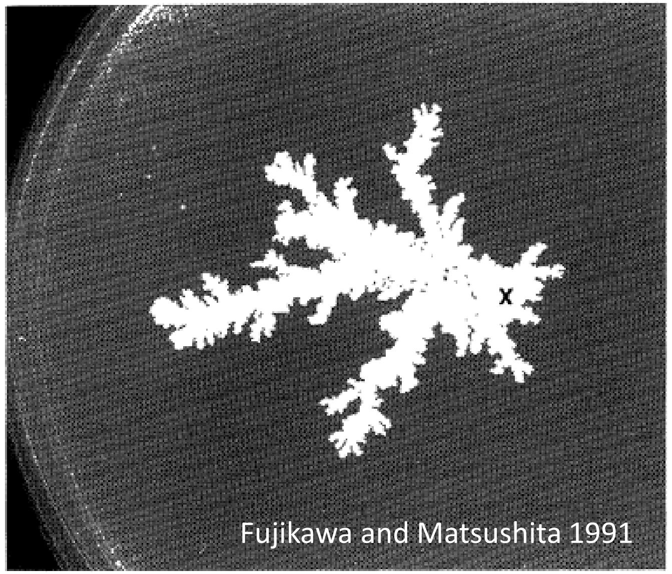
Evolution at the edge of a propagating colony: Population dynamics and drift of neutral alleles

Hallatschek et al. 2007 PNAS

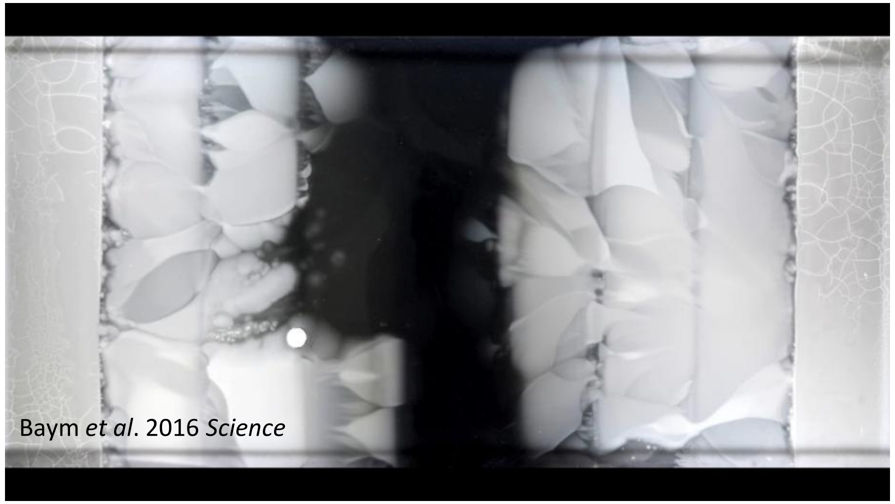


Directed colony growth against an environmental gradient





Fujikawa and Matsushita 1991


Baym *et al*. 2016 *Science*

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

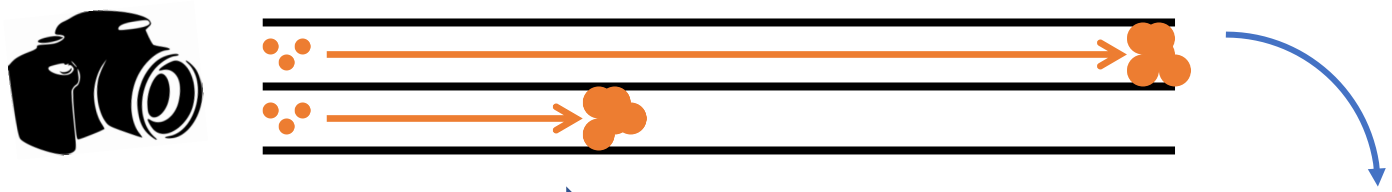
"Space Races" MVP-Cell-02: Experimental Evolution on ISS

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 for a "wild-type" or mutator strain germinate at one end, propagate along the PES path, biomass collected at the other end.

Small sample volume and mass allow high replication (n=7 per treatment)

Imaging monitors growth and 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 mutation rate (as detected in sequenced DNA samples) occur at different rates in space compared to Earth. Hypothesis 1b *(if 1-g replicate is available) Effects of microgravity on these processes are also different.*

Testable hypothesis 2

Rates of adaptive integration of exogenous DNA are different in space compared to Earth (compared to ground control).

Testable hypothesis 3

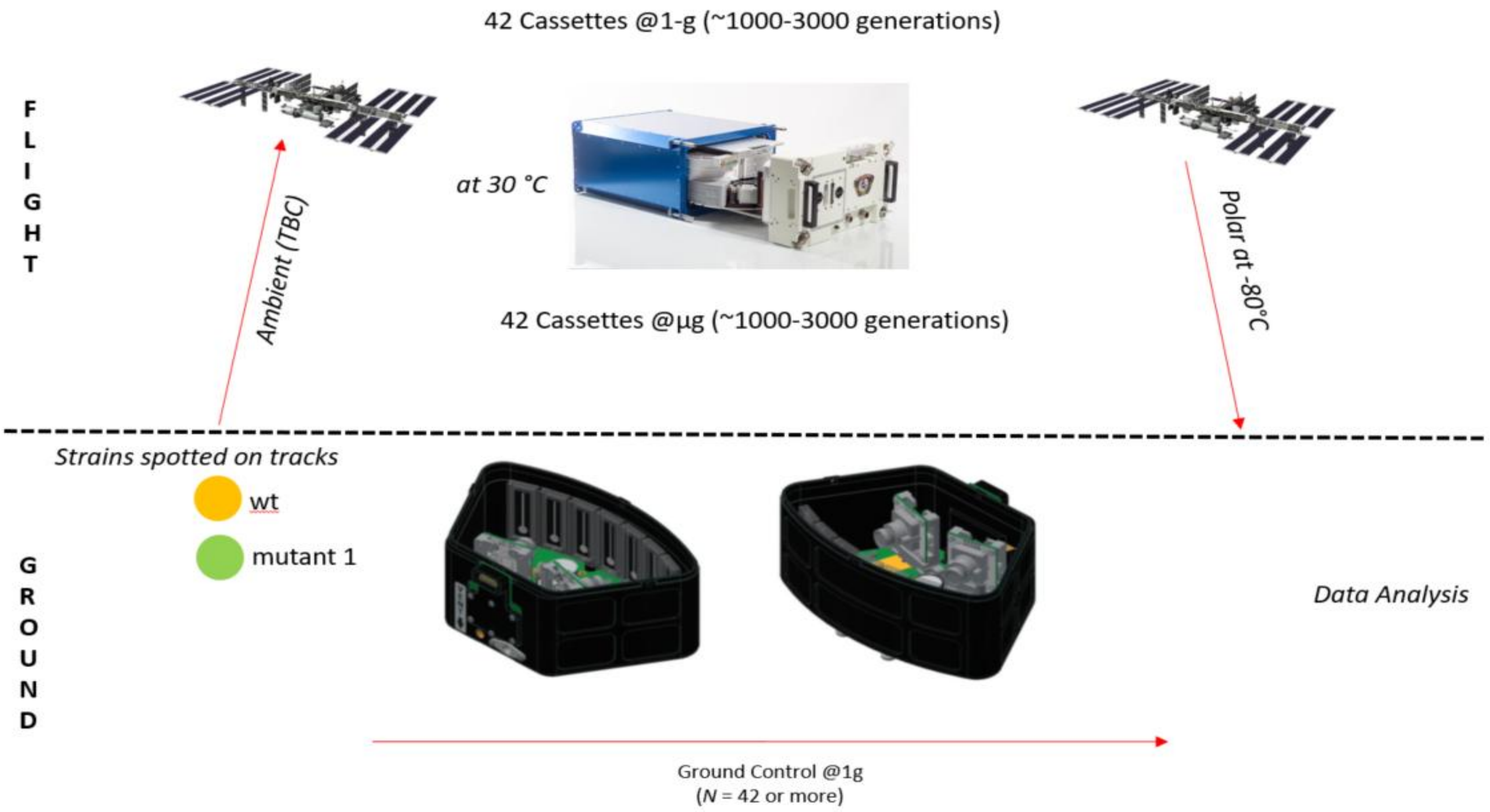
Targets of DNA-uptake under the treatment conditions will be non-random, with specific loci/pathways preferentially assimilated under space conditions.

Outcomes and Impact

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

-Changes in mutation rate, targets of selection in space environment
-Specifically tease out gravitation effects on evolutionary process

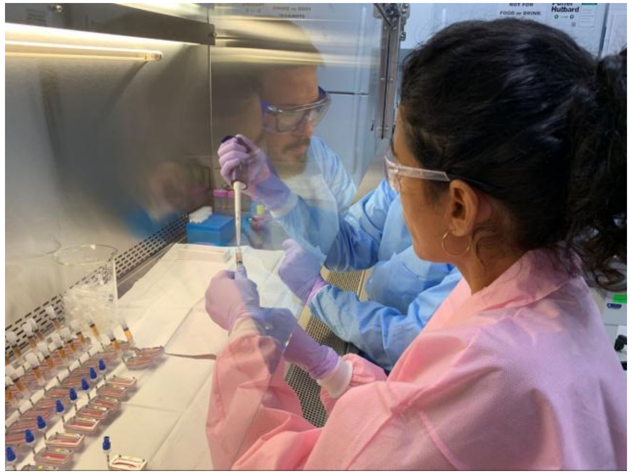
Flight Experiment Concept



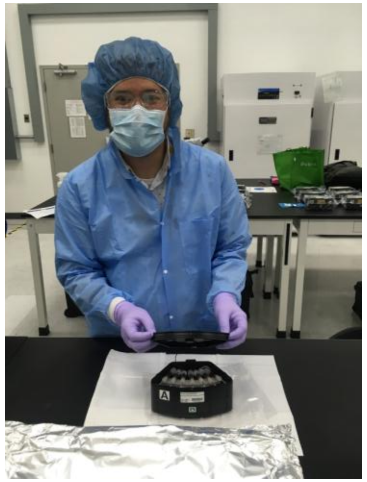
Flight Experiment Summary

- Launched aboard SpX-18 on July 25, 2019.
- Science operations in MVP began on August 27, 2019, ended September 15, 2019.
- Ground Control began at Techshot August 30, 2019, ended September 18, 2019.
- On station anomalies limited science return (several replicates and all imaging lost); ground control proceeded with minimal issues.
- Low-res video captures indicate biology still met success criteria for germination and contamination on ISS. **0% contamination!**
- **Experimental matrix with n=3 for all treatments was maintained.**
- Growth rate analysis not possible for flight, but sample return is hopeful in the coming months to allow science return for genomics.

SpX-18 launch. Photo: Kevin Martin



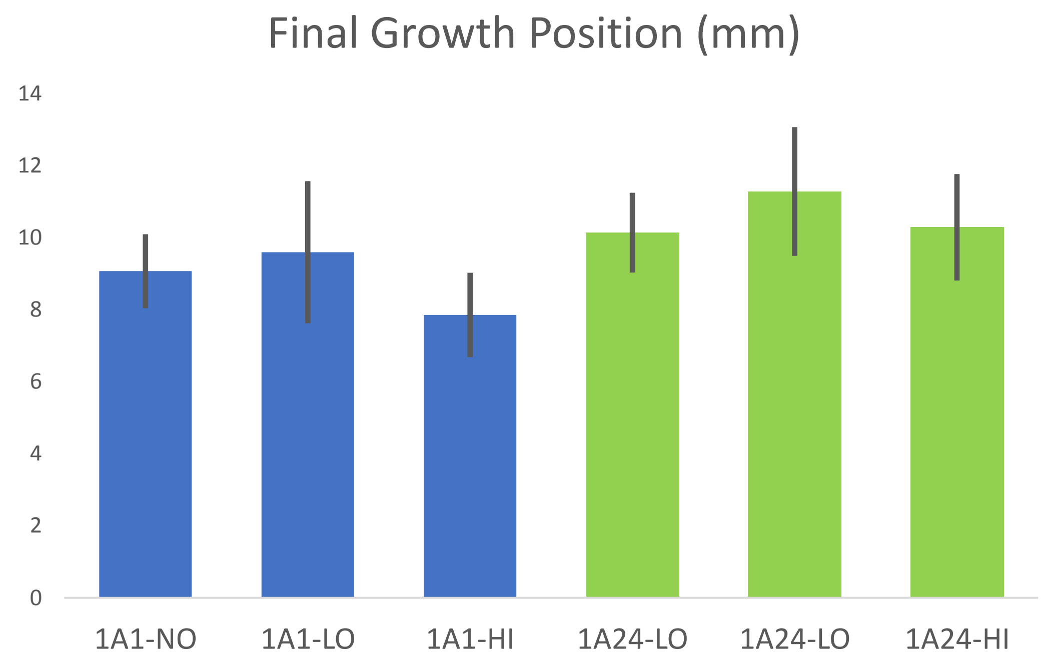
Flight build

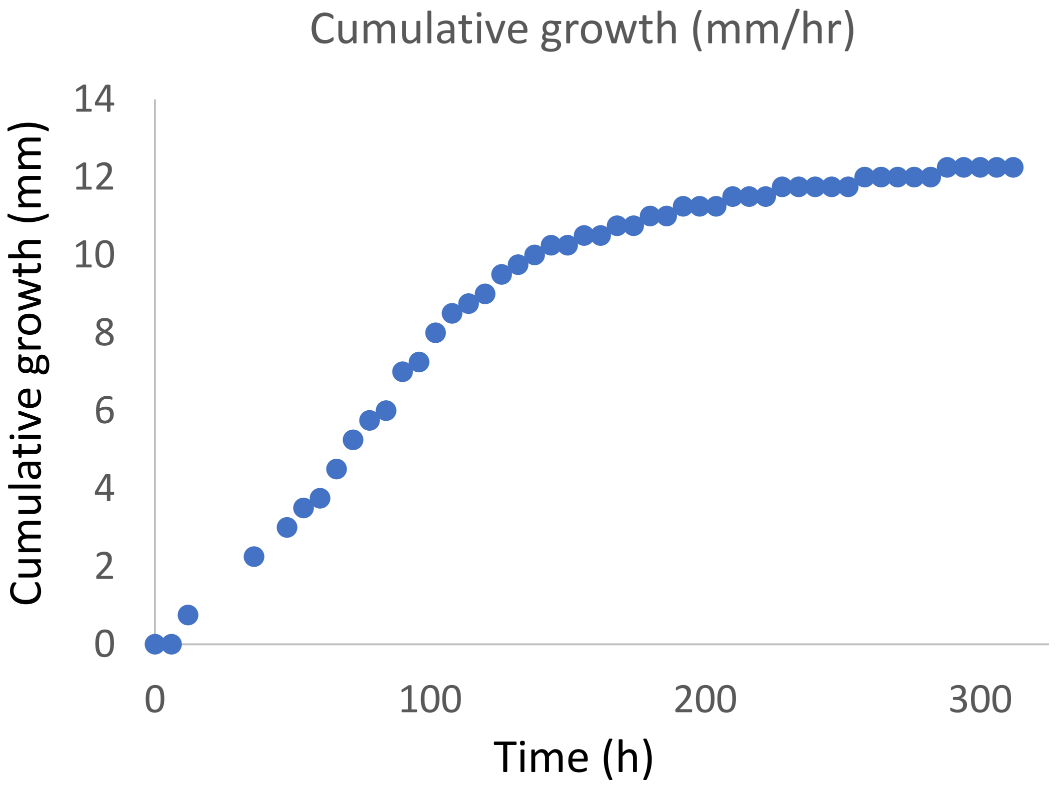


Techshot Multi-use Variable-g Facility (MVP)

Flight Experiment Summary

- Evolution science return on flight samples requires… flight samples. n=3 preserved.
- Ground control almost flawless, have good matrix and imaging/growth rate data (figures here).
- Indications of by strain and by DNA effects.





Total growth distance (mm) of the 6 treatments over 18 days for the ground control based on imaging (above). By strain differences seen, with some suggestion of a DNA effect.

Cumulative growth of cassette 1A24-LO-GC-4 in mm (left) at 6-hour and 0.25 mm resolution over 300+ hours showing the linear growth as predicted. Likely space-constrained early, nutrient-constrained late.

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*.

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.

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.

‘Space Races’ MVP-Cell-02

- **Highly controlled and replicated experimental design for experimental evolution in space.**
- **Flight proven biology platform for ‘gravity as a gradient’ research program. Adaptable to other organisms (e.g. algae, yeast, other ISS-relevant microbes).**
- **Pending genomics results will provide invaluable science return despite imaging loss.**

Thank You!

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