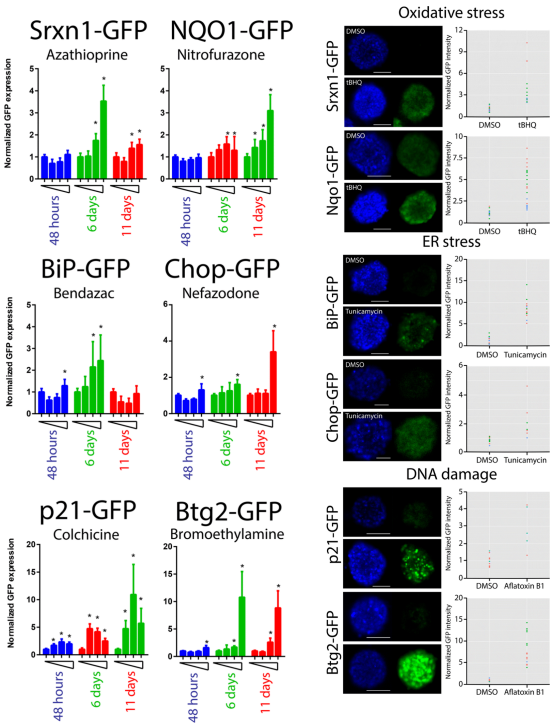
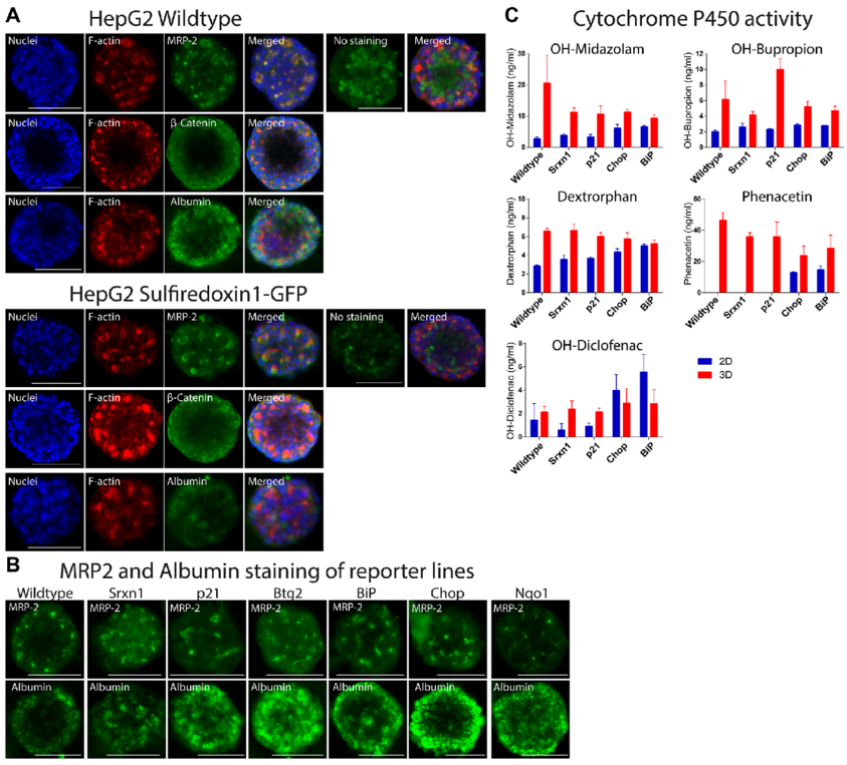
Possible Data Measurements

Stress - Stresslevels (Response Marker Detection)

The first type of biological data that can be collected involves stress response markers in the bacteria. (This measures how stressed the bacteria were during the flight. For this we use special bacteria that glow (with green or red light) when certain stress-related genes are turned on. After the flight, we check how much they glow or look at their gene activity to see how hard they had to work to survive.)





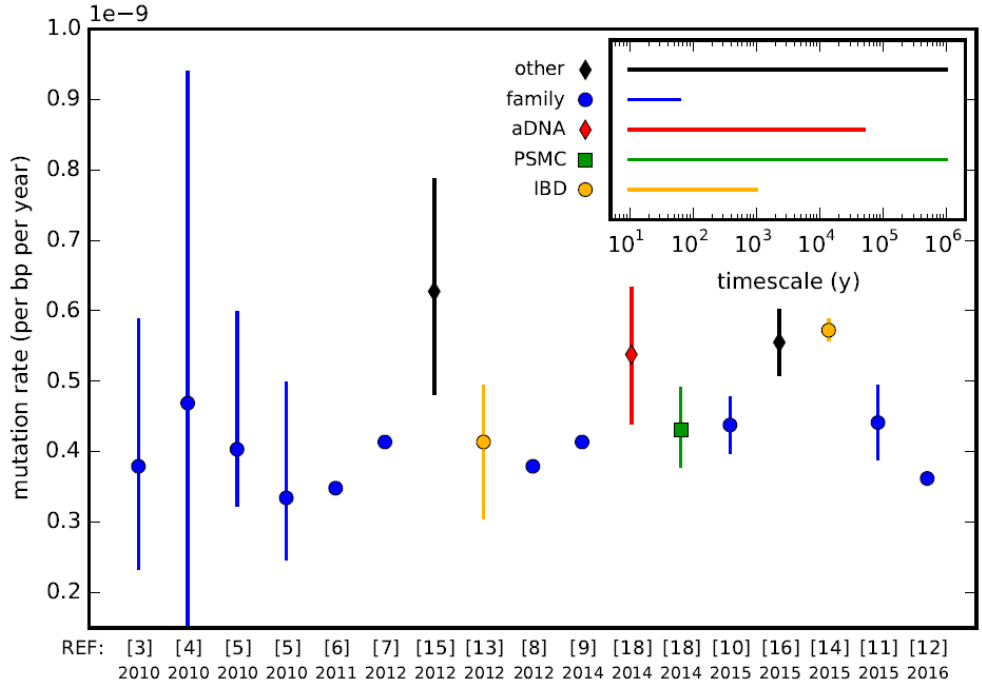
During exposure to extreme environmental conditions, bacteria such as Bacillus subtilis typically upregulate specific genes that are involved in dealing with cellular stress.

These genes include recA, which is important in DNA repair, groEL, which encodes a molecular chaperone that helps refold misfolded proteins, and sigB, which is a general stress response regulator. To detect these responses, we can use genetically engineered strains of B. subtilis that have fluorescent proteins such as GFP (green fluorescent protein) or RFP (red fluorescent protein) fused to the promoters of these stress response genes. After the flight, the Petri dishes or bacterial samples can be analyzed for fluorescence using either a plate reader (very unlikely to happen because it's too expensive) or a fluorescence microscope. The intensity of the fluorescence will correlate with the degree of gene expression, providing a quantitative measure of stress response.

An alternative (extreme) and more detailed approach involves extracting RNA from the bacterial cells after the flight and performing reverse transcription followed by quantitative PCR (RT-qPCR). This technique allows us to directly measure the mRNA levels of stress-related genes, offering insight into the transcriptional activity triggered by the stratospheric environment. This process requires access to a molecular biology laboratory after recovery of the payload.

Mutation Rate Assessment

The second type of data we can gather concerns mutation rates that may have occurred during the flight.



To assess this, we can use strains of B. subtilis that carry specific genes with known mutation profiles.

One approach is to use strains containing a mutated antibiotic resistance gene that can revert to a functional form through a single-point mutation.(easier way i think lol) After the flight, we can plate the bacteria on antibiotic-containing media and count the number of revertant colonies and then at the end we can compare our data with previous studied data! The frequency of these revertants will provide a relative measure of mutation rate.

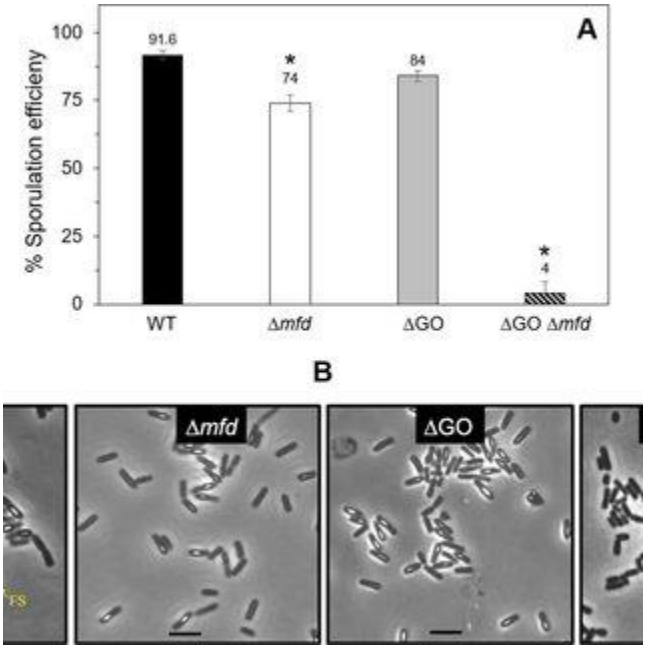
Another method involves sequencing selected genes before and after the flight to detect any point mutations or insertions/deletions.(would be the easiest way but 1.approach is more quality data i think) Genes such as rpoB, which encodes the beta subunit of RNA polymerase and is often used in mutation assays, are ideal candidates.

A third approach would be to perform a fluctuation test, similar to the classic Luria-Delbrück experiment, which allows us to determine mutation rates based on the statistical distribution of mutant colonies across multiple cultures.

Sporulation Efficiency Analysis

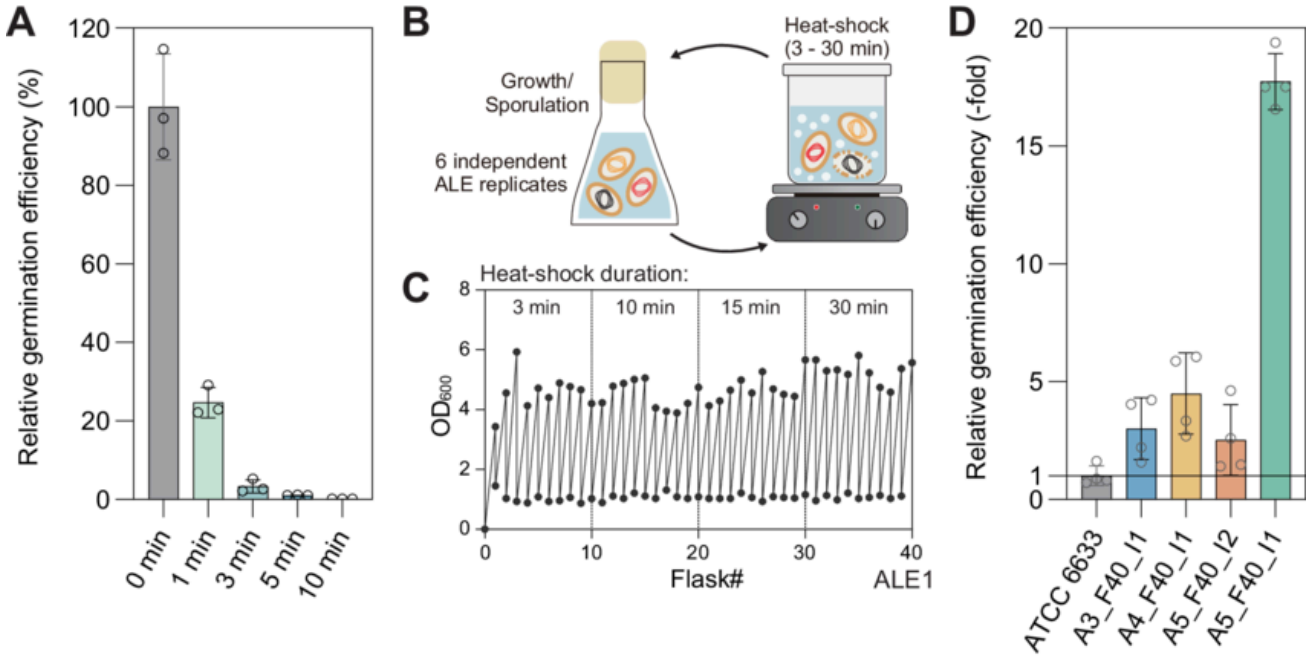
I put this here just to show it is possible but my teammate is working on this alr thats why i dont want to get specific on this

This checks how many bacteria turned into spores, which are their tough, survival forms. After the flight, we stain or heat them to count how many spores became. If lots of spores are found, it means the bacteria felt very stressed and tried to protect themselves.



One way to measure sporulation efficiency in *Bacillus subtilis* is by directly observing the bacteria under a microscope after staining. This method allows us to visually distinguish spores from regular cells. First, the bacterial sample is stained using a special dye called malachite green. This dye only stains the spores because their tough outer shell allows them to retain the dye when heated. A second dye, like safranin, is added to stain the remaining vegetative (non-spore) cells pink or red. After staining, we place the sample on a glass slide and examine it under a compound light microscope. Spores appear green, while the rest of the cells appear pink. By counting how many green spores we see compared to pink cells, we can calculate what percentage of the population turned into spores. This method gives us a clear visual confirmation of sporulation and helps us directly compare the effect of different conditions on spore formation.

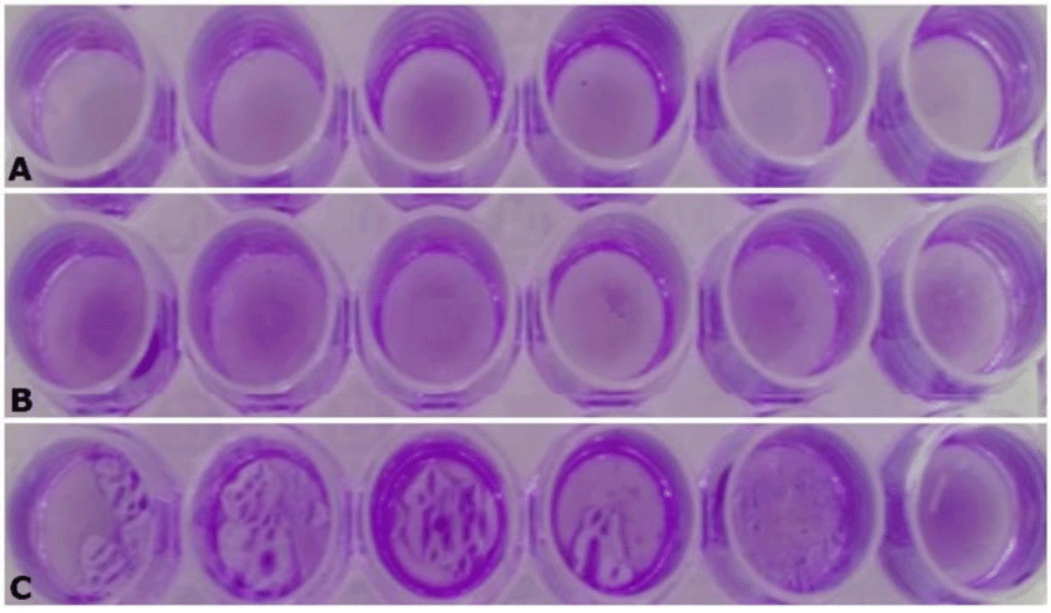
If a microscope is not available, we can still measure sporulation efficiency using the heat-shock method.



In this technique, we rely on the fact that spores are resistant to heat, while vegetative cells are not. First, we take a sample of the bacterial culture and expose it to high heat, typically around 80 degrees Celsius for about 10 minutes. This kills all the regular cells but leaves the spores alive. After the heat treatment, we spread the sample on an agar plate and incubated it. Only the spores will survive the heat and grow into colonies. After a day or two, we count how many colonies appear. This number tells us how many spores were present in our original sample. By comparing this to the total number of bacteria before the heat treatment, we can estimate the efficiency of sporulation. This method does not require any special equipment and still gives reliable results.

Biofilm Formation Evaluation

The fourth category of biological data we may want to explore is the ability of the bacteria to form biofilms following the stratospheric flight.



Biofilm formation is a common response to stress and can be an indicator of changes in bacterial physiology.

After the flight, bacterial samples can be grown in multi-well plates or on small inert substrates such as glass or plastic slides placed within the Petri dish before launch. To assess the amount of biofilm produced, we can perform a crystal violet assay.

In this method, the bacteria are allowed to grow and be to the surface, after which the non-adherent cells are washed away and the remaining biofilm is stained with crystal violet dye. The bound dye is then solubilized using ethanol (dont drink btw not healthy just saying) or acetic acid, and the absorbance is measured using a spectrophotometer. A higher absorbance indicates more extensive biofilm formation. (simple method, cheap and pro data) Using light microscopy may reveal structural differences in the biofilm between flight-exposed and control samples which is more DATA!!!.