B-SURE: Microgravity–induced changes in gene expression of *Vibrio natriegens* and *Pseudomonas putida* engineered for space bioproduction

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Background

Unique challenges to human health in space:

- Microgravity, radiation, limited access to Earth, and a confined environment have unprecedented effects on human health

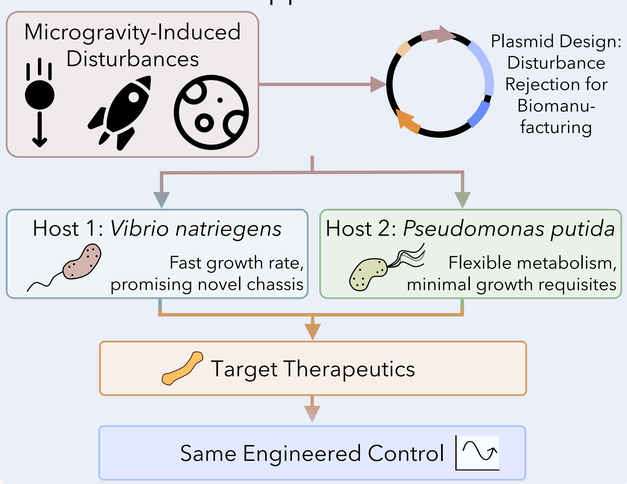
Current medication options are unrealistic for long term space exploration:

- Medicine stocks are reliant on resupply from Earth
- Pharmaceuticals degrade, and chemical production of medication is costly and inflexible

Biomanufacturing therapeutics using synthetic biology as a novel approach to providing healthcare in space:

- A synthetic biology approach is adaptable and requires low input
- Microgravity can alter bacterial gene expression, therefore tight regulation is crucial for bioproduction
- Differential gene expression is not well understood for different biomanufacturing hosts in spaceflight

Approach



Methods

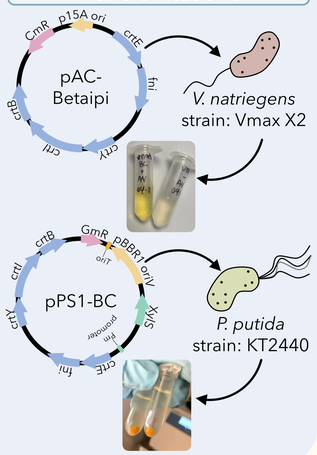
Product Selection

β-carotene

- Vitamin A precursor
- Antioxidant
- Supports eye health
- Colorimetric

Spaceflight

Transformations



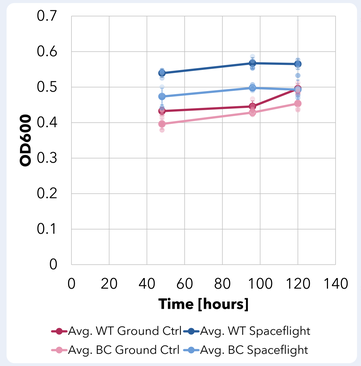
RNA Analysis

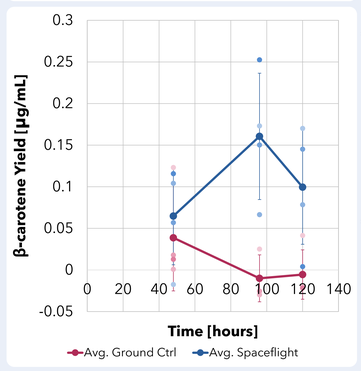
RNA Extractions → QC & Quantify → To Interdisciplinary Center for Biotechnology Research (ICBR)

- Library Prep
- Illumina Sequencing
- Mapping & QC
- Preliminary Analysis

Results: *Vibrio natriegens*

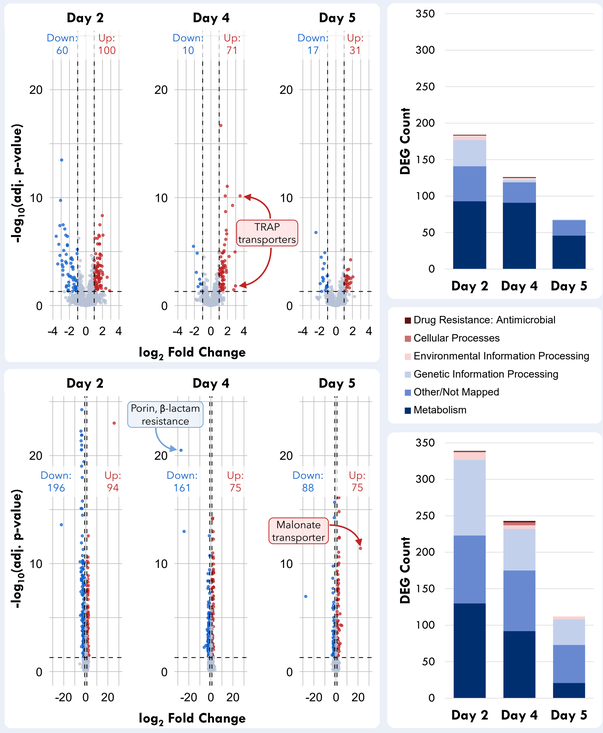
Spaceflight Characterizations





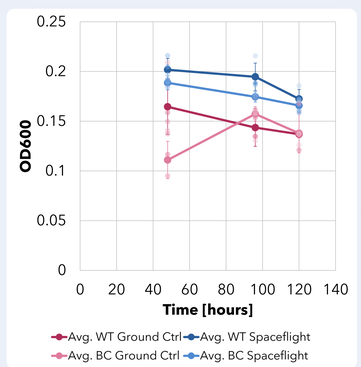
- In spaceflight, both WT and BC strains grew significantly more.
- β-carotene production significantly increases in true microgravity.
- The number of differentially expressed genes (DEGs) decreased over time.
- Highly differentially expressed genes include nitrogen fixation protein, starvation-inducible DNA-binding protein, and permease.
- Highly significant ($p<1x10^{-5}$) include flagellin, maltoporin, and copper chaperone protein.

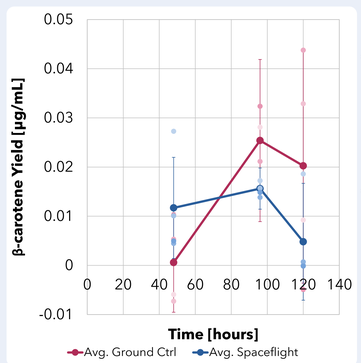
Differential Gene Expression



Results: *Pseudomonas putida*

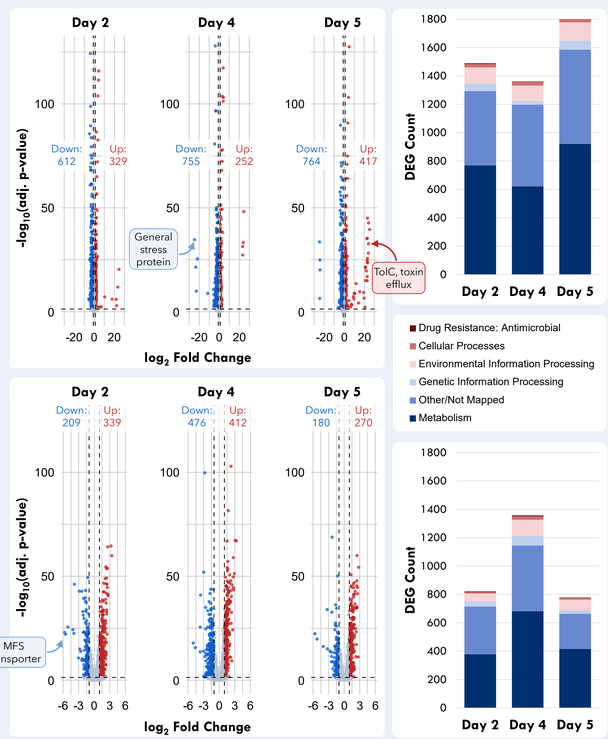
Spaceflight Characterizations





- In spaceflight, both WT and BC strains grew significantly more.
- β-carotene production is highly variable in both spaceflight and ground controls.
- β-carotene yield decreased after time, likely due to limited resources.
- There is no clear pattern to number of DEGs over time.
- Highly differentially expressed genes include nitrite reductase and siderophores.
- Highly significant ($p<1x10^{-5}$) include permease and dehydrogenases.

Differential Gene Expression



Discussion and Future Work

V. natriegens

- Optical density is higher than other species in microgravity and does not decrease.
- β-carotene production is higher in spaceflight cultures compared to ground controls.
- Up- and down-regulated genes included mostly metabolic genes, including carbohydrate metabolism and microbial metabolism in diverse environments.

P. putida

- *P. putida* shows an increase in growth between spaceflight samples and ground controls.
- β-carotene production is low in spaceflight samples with a slightly higher yield in ground controls.
- Up- and down-regulated genes included mostly metabolic genes, including amino acid and carbohydrate metabolism, xenobiotics metabolism, and microbial metabolism in diverse environments.

Future work

- V. natriegens was re-engineered for improved β-carotene production and sent to the International Space Station. Samples will be analyzed once they are returned to Earth.
- Comparisons in DEGs between wild type and engineered organisms will be examined.
- Gene pathways that are under-annotated in KEGG will be examined and added to correct exiting gene pathway categories.

References

1. Du, B. *et al.* Evaluation of physical and chemical changes in pharmaceuticals flown on space missions. *AAPS J.* **13**, 299–308 (2011).
2. Kast, J., Yu, Y., Seubert, C. N., Wotring, V. E. & Derendorf, H. Drugs in space: Pharmacokinetics and pharmacodynamics in astronauts. *Eur. J. Pharm. Sci.* **109S**, S2–S8 (2017).
3. Zea, L. *et al.* Phenotypic Changes Exhibited by E. coli Cultured in Space. *Front. Microbiol.* **8**, 1598 (2017).
4. 69. Cunningham, F. X. & Gantt, E. A study in scarlet: enzymes of ketocarotenoid biosynthesis in the flowers of Adonis aestivalis. *Plant J.* **41**, 478–492 (2005).
5. Sánchez-Pascuala, A., Fernández-Cabezón, L., de Lorenzo, V. & Nikel, P. I. Functional implementation of a linear glycolysis for sugar catabolism in Pseudomonas putida. *Metab. Eng.* **54**, 200–211 (2019).

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