

# Differential gene expression in a cross-feeding two-species model microbial community under simulated microgravity and deep-space radiation

Jessica L. Gonzalez<sup>1</sup> and Jessica A. Lee<sup>2</sup>

<sup>1</sup>Department of Biology, Florida International University

<sup>2</sup>Space Biosciences Research Branch, NASA Ames Research Center

Contact: jessica.a.lee@nasa.gov



## RESEARCH QUESTION

Microbes within multispecies communities produce and exchange metabolic products with each other. How do the microbial responses to spaceflight stressors change within metabolically interdependent microbes? The combined effect of microgravity and ionizing radiation on bacterial community response when species are interdependent through exchange of metabolites in a fluid medium is still underexplored. Our project investigates the combined effects of microgravity and ionizing radiation on gene expression of an actively metabolizing microbial community through simulation of galactic cosmic rays (GCRsim) and microgravity. Understanding how microbial responses change under spaceflight stressors such as microgravity and ionizing radiation could provide insight into the potential for cross-feeding microbial communities in bioregenerative life support applications in low-Earth orbit (LEO) missions and beyond.

## MODEL BACTERIAL COMMUNITY

The *E. coli*-*S. enterica* consortium is a bioengineered model microbial community for microbial mutualism

Our project utilizes a model microbial community in which microbial species (*Escherichia coli* and *Salmonella enterica*) are mutually codependent (Fig. 1). Each species relies on a metabolic product produced by the other.

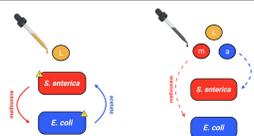


Figure 1. cross-feeding culture (left) vs non-cross-feeding culture (right). Created with BioRender.

Lactose minimal media (LMM) where lactose is the only carbon substrate → codependent microbes.

## MICROGRAVITY AND RADIATION ON MICROBES

### Radiation

Space radiation causes direct and indirect DNA damage

Ionizing radiation such as galactic cosmic rays (GCR) can cause direct DNA damage as highly charged subatomic particles interact with DNA, as well as indirect damage via generation of reactive oxygen species (ROS) through radiolysis in intracellular water (Fig. 2).

High-energy-charged particles lose energy in a small distance as it traverses through material. This causes greater deposits of radiation energy within a cell that yields more DNA damage.

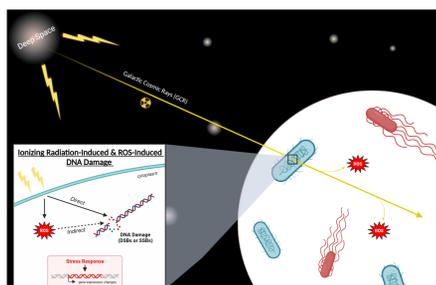


Figure 2. DNA Damage and gene expression responses to ionizing radiation and reactive oxygen species generation. Created with BioRender.

### Microgravity

Microgravity may slow interspecies mass-transfer and lead to accumulation of waste-products

In a fluid environment, microbes are subject to buoyancy-driven convective flow, creating a well-mixed environment. When microbes are well-mixed, they have access to metabolites nearby.

In the absence of gravity-dependent forces, extracellular mass transport depends on diffusion which is limited in low-shear, poorly mixed fluid environments. This results in substrate depletion or lack of access to substrate, as well as waste product accumulation, leading to a microbial stress response to starvation and acidic local environment.

### Combined effects of microgravity and radiation on gene expression

Microgravity may exacerbate community response to stress

The “weakest link” hypothesis poses that in a community of mutually interdependent organisms, direct damage to one organism indirectly impacts its dependent partner.

In a community that is exposed to GCR which damages organisms within the community, whether directly or indirectly, microgravity may exacerbate the community response to stress. Poor mixing inhibits the distribution of metabolites that would aid in damage mitigation of injured cells.

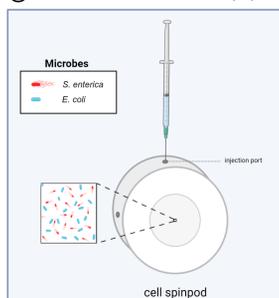
### How do radiation and microgravity affect metabolically interdependent microbial community gene expression?

We hypothesize that the combined effects of microgravity and radiation on cross-feeding microbial community gene expression will differ than that of non-cross-feeding communities; cross-feeding communities will upregulate glucose catabolism genes in response to starvation stress and activate DNA damage repair genes, consistent with generalized starvation and acid stress response and DNA damage response pathways.

## METHODS

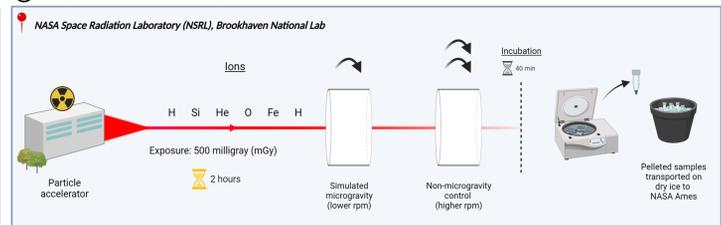
### Sample Preparation, Irradiation, and Extraction for RNA Sequencing

#### 1 Inoculation of Microbes into Cell Spinpods



- Crossfeeding community co-cultured in lactose minimal medium (LMM)
- Not crossfeeding community co-cultured in lactose minimal medium (LMM) + methionine + acetate

#### 2 Simplified Galactic Cosmic Ray Simulation (SimGCRsim)



#### 3 RNA Extraction, Library Preparation, and Sequencing

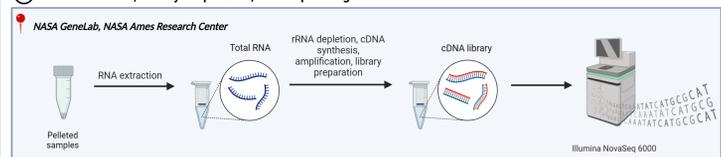


Figure 3. Sample preparation, irradiation, and extraction for RNA sequencing. Created with BioRender.

## RNA SEQUENCING PIPELINE CONSTRUCTION

Our goal for RNA sequencing is to understand how gene expression changes in actively metabolizing microbial communities when exposed to spaceflight stressors such as simulated microgravity and ionizing radiation as compares to their non-cross-feeding community control. However, RNA sequencing presents many challenges.

We constructed a workflow (Fig. 4) that would

- 1) adequately differentiate reads between the two species within our microbial coculture,
- 2) use tools that would be appropriate for prokaryotic organisms (e.g. aligners), and
- 3) provide us with sufficient resolution based on low-input samples.

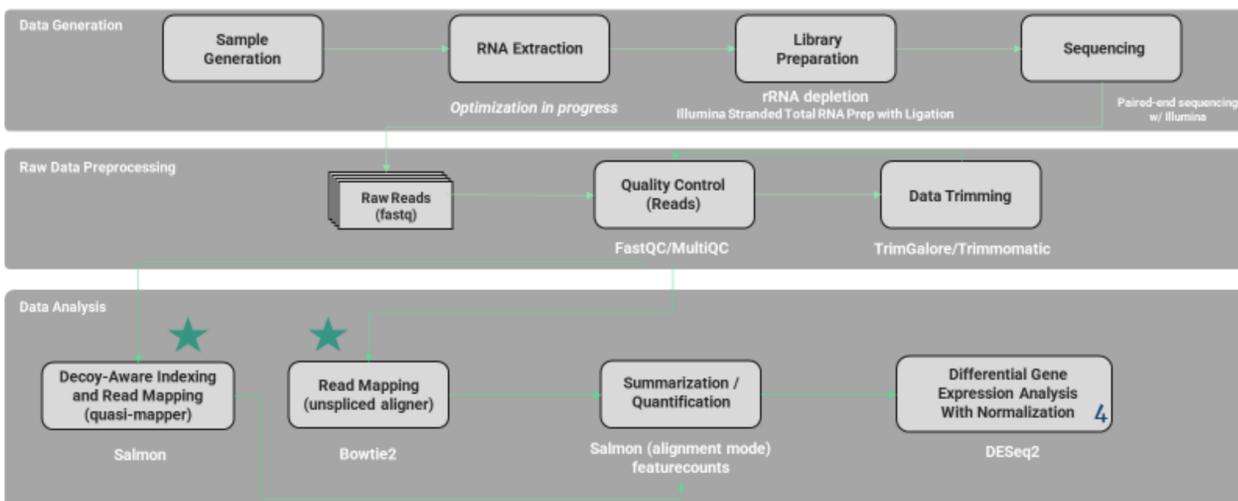


Figure 4. RNA Sequencing Pipeline from data generation to data analysis.

## PREDICTED RESULTS BASED ON LITERATURE REVIEW

Literature review shows microbial gene expression changes upon microgravity and radiation exposure

Gene expression is regulated by micro environmental factors (internal to the cell) and macro environmental factors (external stimuli and stressors). Microgravity and ionizing radiation have been shown to effect gene expression in microbes and other organisms (Table 1).

Changes in gene expression in cross-feeding microbial community exposed to both microgravity and radiation as compared to non-cross-feeding and non-microgravity controls are expected. Generalized acid/starvation stress responses have been seen in cocultures of *S. enterica* and *E. coli*.

Microbes in monoculture when exposed to space conditions or microgravity have exhibited upregulation of several stress response genes including those related to starvation.

Bacteria irradiated with single ions (such as Fe or H) in a low-dose for one day exhibited downregulation of oxidative stress genes.

Table 1. Differential gene expression results from prior studies involving metabolically interdependent microbes and/or microbes exposed to spaceflight stressors

Organism	Spaceflight Stressors	Experimental Conditions		DGE Results					
		Radiation	Microgravity	Ground	Simulated (LSMAG)	Space	Upregulation	Downregulation	Compared against?
<i>S. enterica</i>	Monoculture, Coculture, or Other						plasmid activity, galactose use, leucine biosynthesis, quorum sensing	acetate utilization, alanine metabolism	Monoculture
<i>E. coli</i>	Coculture						transport and catabolism functions, genes associated w/ cryptic prophages	motility, chemotaxis	
<i>E. coli</i>	Monoculture						thiGHS genes, dps, crp, glgG and nac, poxB, glucose catabolism activation		Ground
<i>E. coli</i>	Monoculture						(50) stress-response genes		Ground
<i>S. enterica</i> serovar Typhimurium	Monoculture							hfq	Ground
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<i>E. coli</i> DH10B	Monoculture						gluconeogenesis enzymes - 15 days Fe or H (Table 1); oxidative stress genes (sodC and katE) - 15 days Fe or H; markers of oxidative stress ex: iron-sulfur cluster assembly proteins, DNA-binding transcriptional dual regulator, and an iron-binding and storage protein - 15 days H	oxidative stress genes (sodC and katE) - 1 day Fe; markers of oxidative stress ex: iron-sulfur cluster assembly proteins, DNA-binding transcriptional dual regulator, and an iron-binding and storage protein - 1 day Fe	
<i>P. aeruginosa</i>	Monoculture						AigU		Random gravity (RG) and normal gravity (NG) control

The combination of short-term ionizing radiation exposure and microgravity in metabolically interdependent microbial communities may yield a significant effect on gene expression. We expect to see an increased upregulation of starvation stress response genes in cross-feeding communities in microgravity caused by the decreased mass transport of substrate vital to survival. Radiation exposure may lead to the activation of genes related to DNA damage repair and oxidative stress pathways.

## FUTURE DIRECTIONS

We plan to have RNA sequencing data for differential gene expression analysis in hand by early 2024. Our gene expression analysis would serve to inform new hypotheses in future space biology experiments related to metabolically-interdependent microbial communities under simulated or actual spaceflight conditions. In the future, we hope to expand upon our experiment and propose a project to observe and analyze gene expression changes in metabolically active microbial communities under spaceflight stressors over long evolutionary time scales.

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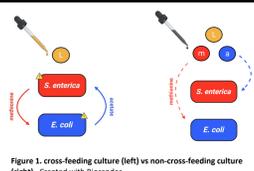


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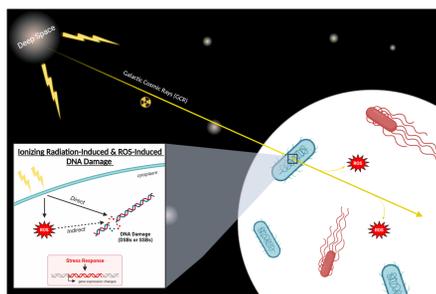


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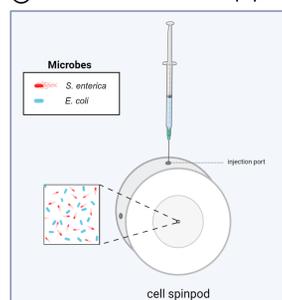
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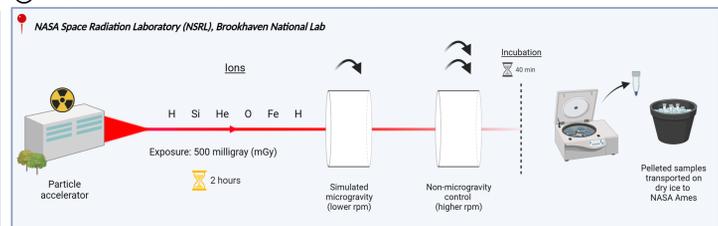
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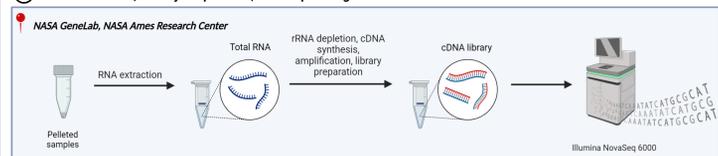


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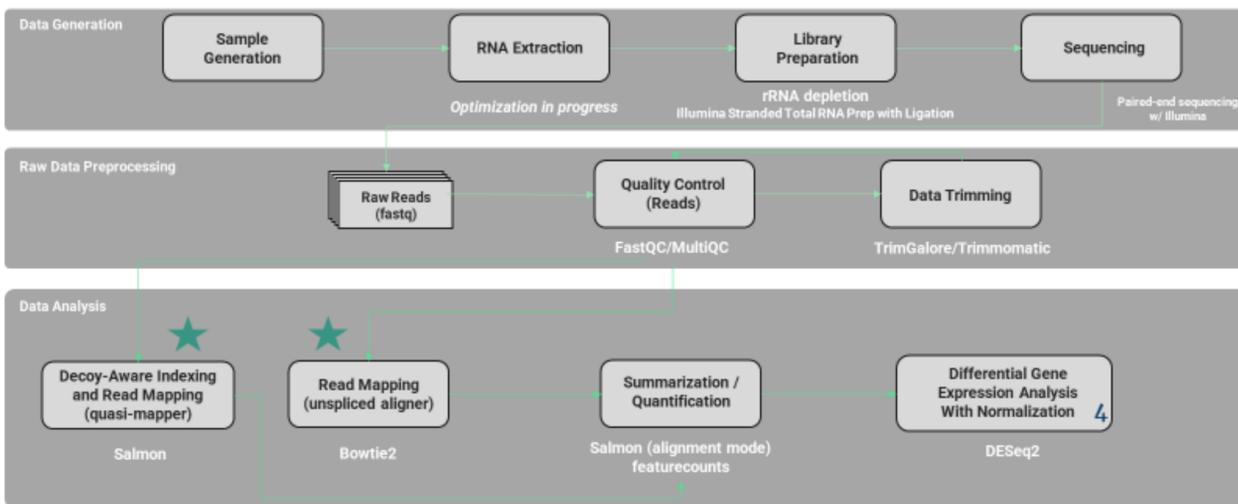


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