Results of the Micro-12 Flight Experiment: Effects of Microgravity on *Shewanella oneidensis* MR-1

Michael Dougherty (KBRWyle), Adam Deutschbauer (LBNL-EBI), Natalie Ball (KBRWyle), Fathi Karouia (KBRWyle), Morgan Price (LBNL-EBI), Jayashree Ray (LBNL-EBI), Adam Arkin (LBNL-EBI), John Hogan (NASA)

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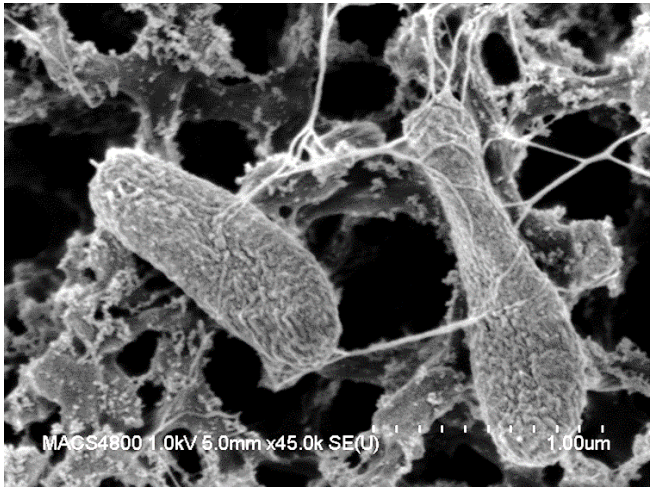
Experimental Organism: *Shewanella oneidensis* MR-1



- Facultative anaerobe, Gram -, ɣ-proteobacterium
- 20+ *Shewanella* strains sequenced
- Dissimilatory metal-reducing bacteria (DMRB)
- MR-1 = manganese reducing
- Discovered in Lake Oneida, NY in 1988 by Ken Nealson
- Cytochrome system well established; 39 c-type cytochromes, 32 previously unknown
- Multi-component, branching electron transport pathway
- High electron-acceptor versatility
- Can respire using metals such as iron, manganese, lead, uranium, mercury
- Can also respire nitrates, sulfates, chromates
- Abnormal protrusions linked to metal reduction – nanowires
- Easy to culture with genetic tools
- A model organism for extracellular electron transfer (EET) – both direct and indirect reduction of extracellular substrates

Rationale for Flight Experimentation

The effects of the space environment on the physiology of exoelectrogens remain unknown. Potential effects include:

Response to cellular stress – The space environment (e.g., increased mutation rates due to radiation exposure, microgravity, reduced mass-transfer) could result in increased cellular stress, which can compromise fitness and EET performance.

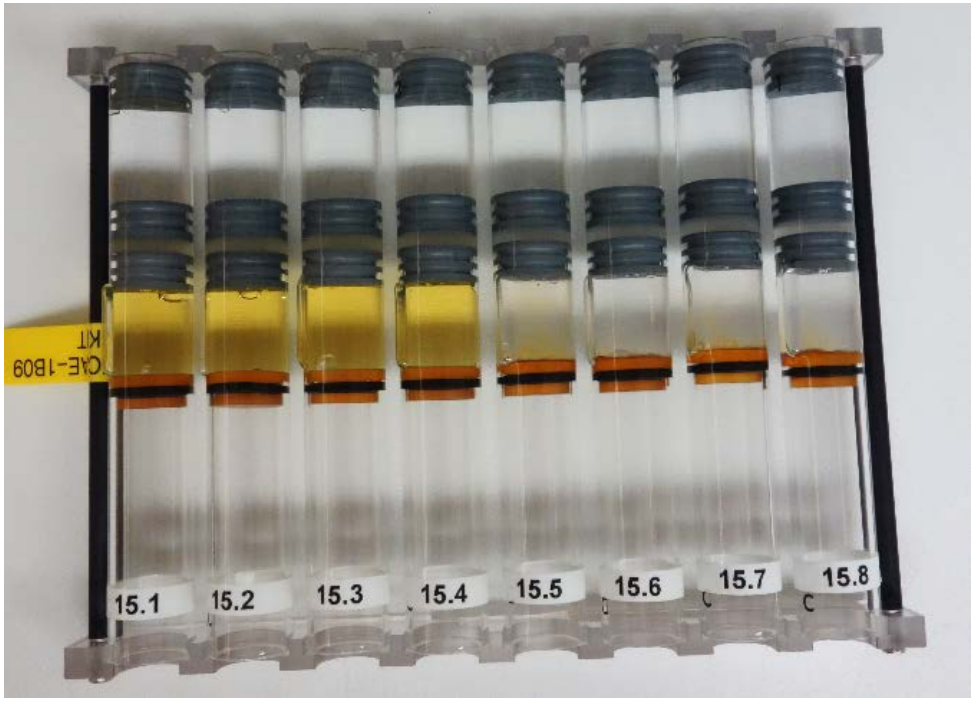
Changes in biofilm development – Biofilm formation on electrodes and mineral surfaces is often critical for efficient electron transport. If microbial association with these surfaces is compromised, EET will also be affected. Previous ISS experiments identified changes in *Pseudomonas* biofilm development including increased thickness and altered morphology.

Mining functional genes in the space environment and using synthetic biology approaches for effective metabolic engineering to manipulate electron flow and establishing the catalytic interface between the electrode and microbe will be critica for BES technology development.

Micro-12 Research Plan

- **Specific Aim 1 – measure EET**
  - Measure iron reduction rates for both soluble and insoluble iron substrates
  - Perform proteomic analysis to identify global expression changes under spaceflight conditions
- **Specific Aim 2 – biofilm development**
  - Characterize effects of microgravity on *Shewanella* biofilm development under EET growth conditions
- **Specific Aim 3 – fitness profiling**
  - Identify genes important for MR-1 physiology during spaceflight using
    - Mutant fitness profiling
    - Gene expression analysis via RNAseq

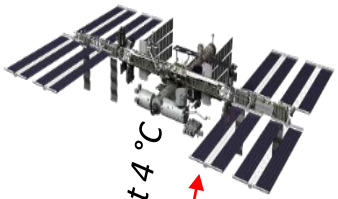
Flight Hardware – BioServe FPAs and GAPs



Fluid processing Apparatus (FPAs)

Group Activation Pack (GAP)

FLIGHT



Ambient and in cold bags at 4 °C

GAPs 1-4

- *4 time points*
- *4 replicates*
- *2 Media + HCl preservative*

GAPs 5-8

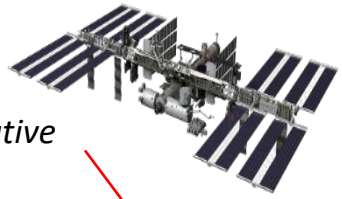
- *2 time points*
- *4 replicates*
- *2 Media + RNAprotect or B-PER*

GAPs 9-16

- *2 time points*
- *4 replicates*
- *4 Media/Substrate + PFA*

GAP 17-18

- *1 time point*
- *4 replicates*
- *4 Media + RNAprotect*



@ KSC or PI's Lab (3-5 days)

GROUND

Cells, media, and preservative inserted into FPAs

- Iron Reduction
- Omics
- Biofilm
- Fitness

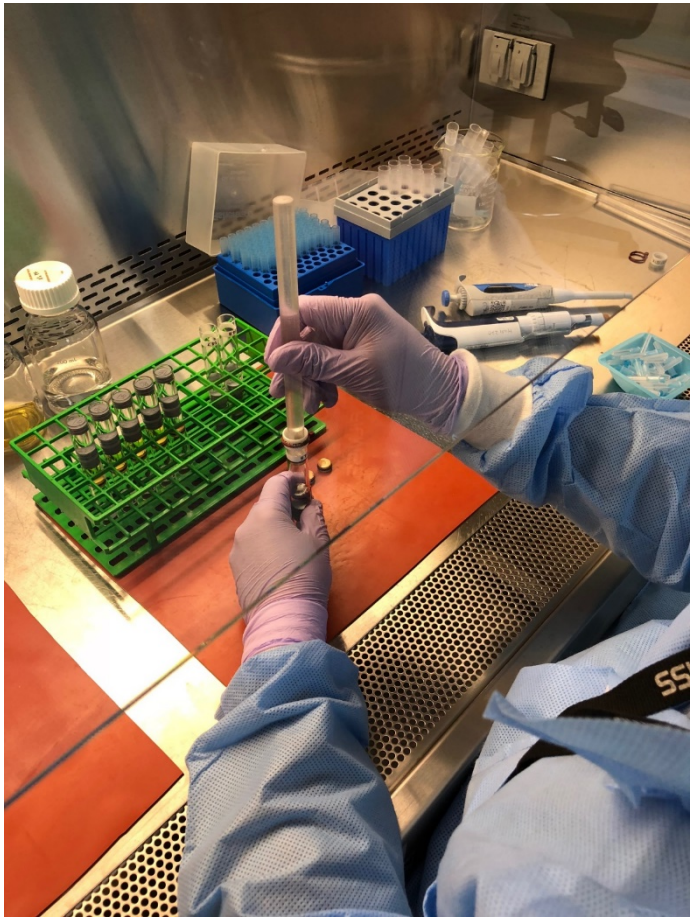
Same conditions as spaceflight
With 6 to 12 hrs delay

Data Analysis

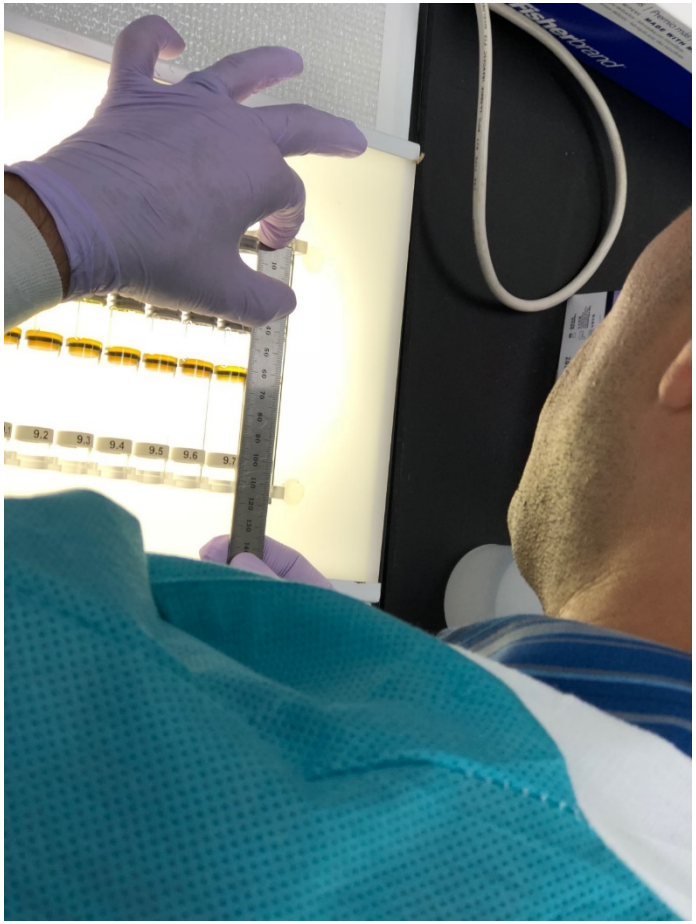
AIMS 1-3

Ground Control
(*GAPs* = 18)

Sample loading at KSC



Sample loading at KSC

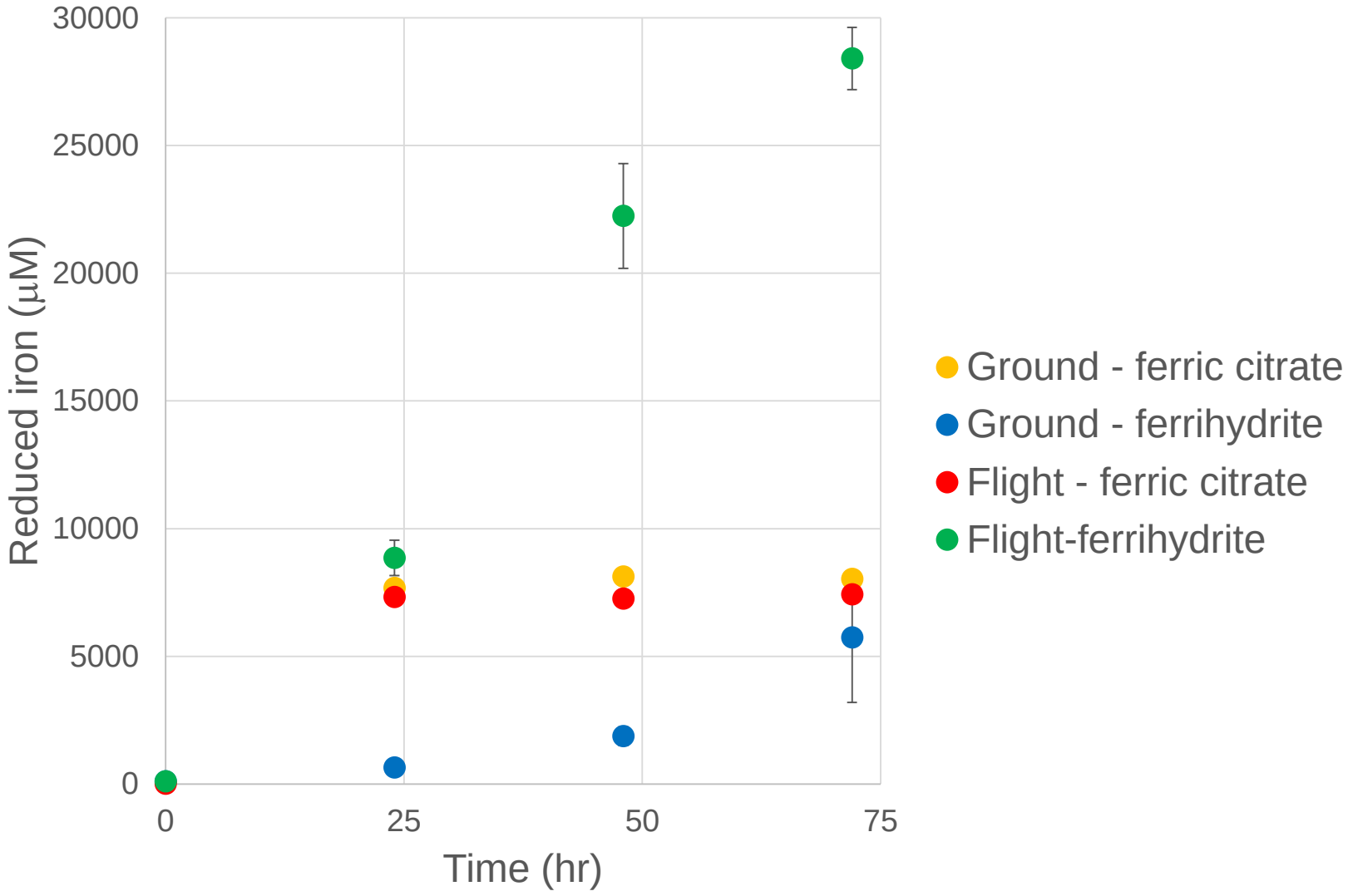


SpaceX-15 Launch

Flight experiment timeline

- Completed flight operations
  - Flight samples launched on SpaceX-15 on 6/29/18
  - The experiments were run on ISS from 7/29/18 – 8/1/18
  - Samples returned to Long Beach on 8/4/18 and to NASA Ames on 8/6/18
- Ground controls were repeated at NASA Ames between 9/5/18 and 10/17/18 due to a shipping issue with the original set of ground controls set up at KSC

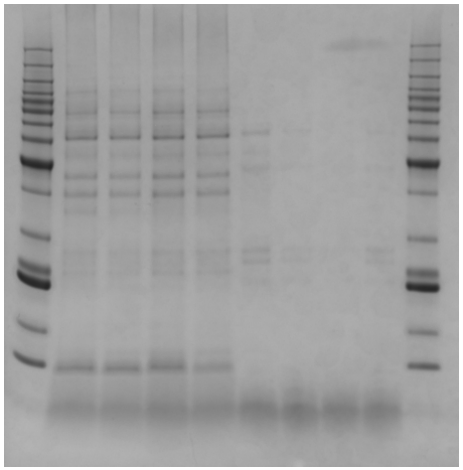
Ferrozine Assay – Iron reduction rates



Protein Extraction Data

Representative SDS-PAGE gels

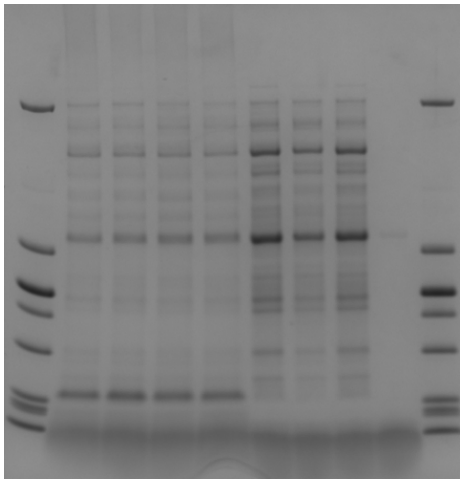
Flight



ferric citrate | ferrihydrite

Time point (hr)	Media	Protein extracted/FPA (μg)
48	ferric citrate	62±3
72	ferric citrate	61±3
48	ferrihydrite	10±2
72	ferrihydrite	4.9±0.2

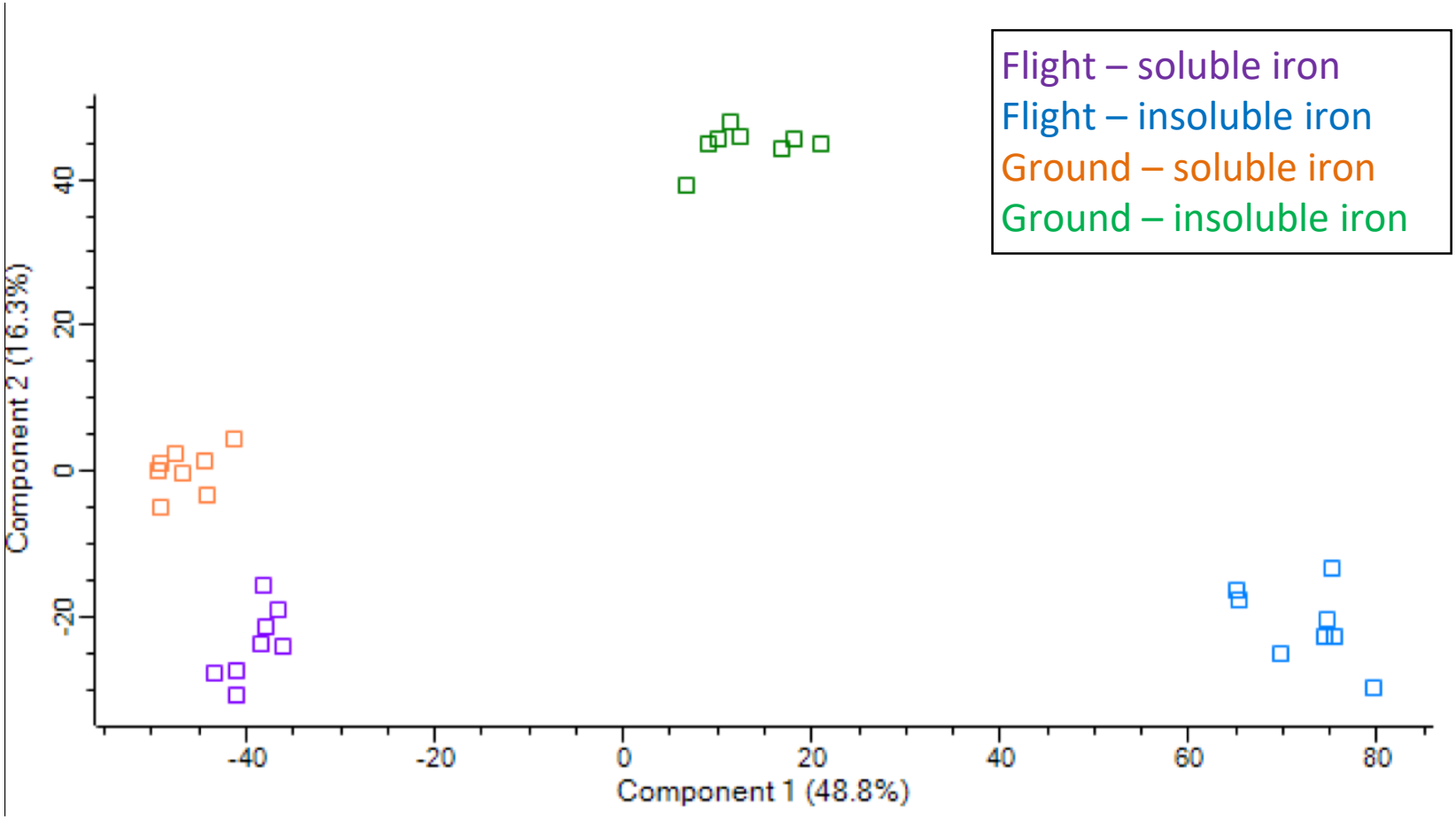
Ground



Time point (hr)	Media	Protein extracted/FPA (μg)
48	ferric citrate	51±5
72	ferric citrate	51±6
48	ferrihydrite	41±24
72	ferrihydrite	54±4

- Proteomics analysis
  - SWATH-MS was performed at UCSF Sandler-Moore Mass Spectrometry Facility
  - Samples (10 μg) were prepared via SDS-PAGE and in-gel trypsin digestion, then split:
    - Data Dependent Analysis MS acquisition (1 μg)
    - Data Independent Acquisition library pool (2 μg)
    - Data Independent Acquisition to generate SWATH-MS data (5 μg)
  - MS data was acquired on a 5600 TripleTOF Mass Spec operating in DIA mode

Principal Component Analysis



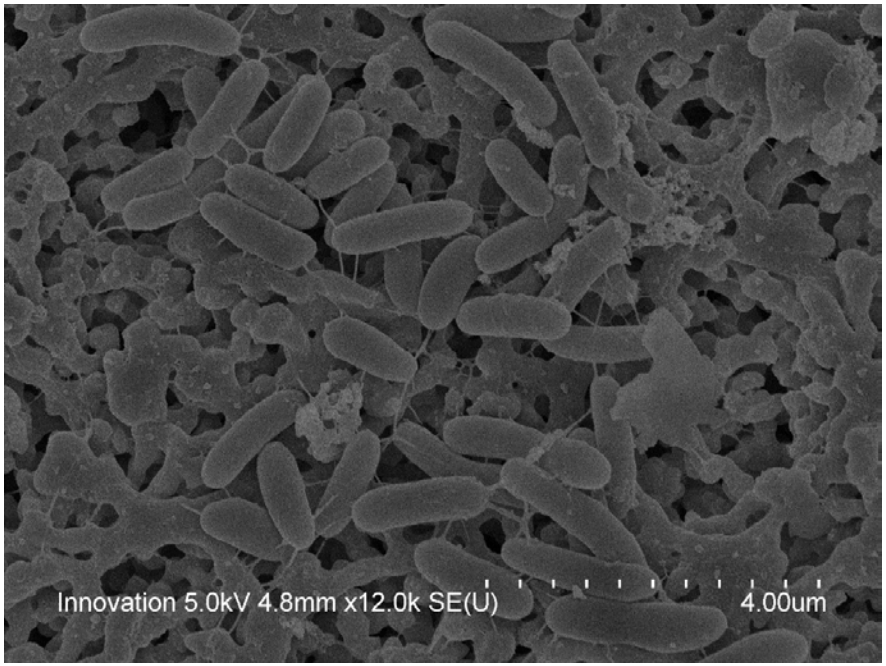
Top 20 differentially expressed protein – flight vs. ground

Student's T-test Difference	Protein Gene	Description
-8.325030979	DI594_12935	
8.303729056	modA	ABC-type molybdate uptake system
-7.486366503	pstS	ABC-type phosphate uptake system
-7.308873683	pstS	ABC-type phosphate uptake system
7.161167278	adhB	alcohol dehydrogenase II
-6.904020761	SO_1557	outer membrane phosphoporin
-6.820982377	DI594_12950	
-6.287595604	glgC	glucose-1-phosphate adenylyltransferase
6.081191622	DI594_05630	
-5.8603965	DI594_16500	
5.753818957	SO_3967	orphan ABC-type transport system
-5.618806421	sucB	2-oxoglutarate dehydrogenase complex
-5.523222908	DI594_21070	
-5.464073885	DI594_13815	
-5.319727331	proQ	activator protein
-5.221296861	rplQ	50S ribosomal protein L17
-5.027110845	DI594_10865	
-4.779675667	DI594_18230	
-4.737512954	SO_4129	putative negative regulator of univalent cation permeability
-4.724377465	DI594_13990	

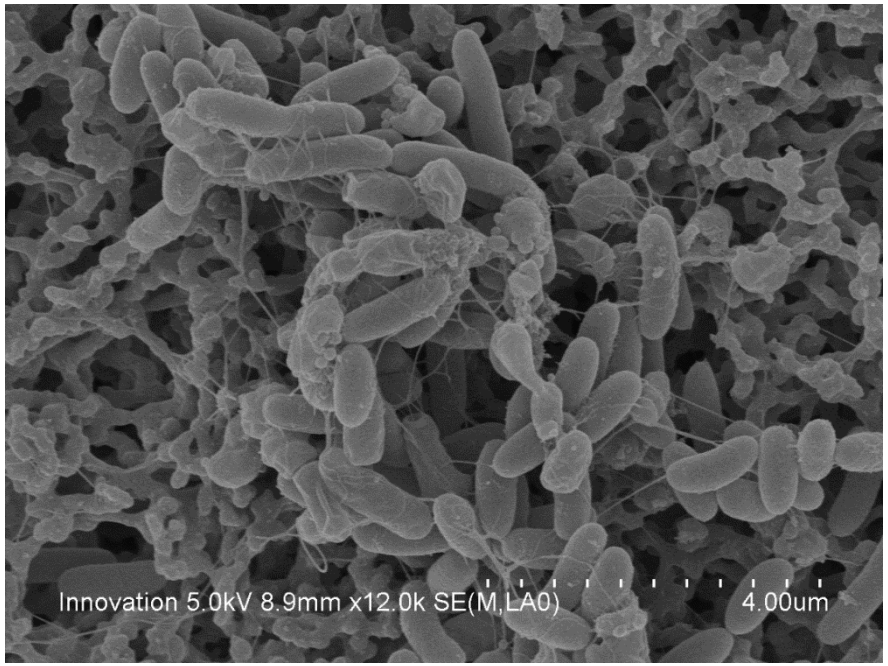
Top 20 differentially expressed protein – soluble iron vs. insoluble iron

Student's T-test Difference	Protein Gene	Description
10.06576131	atpF	ATP synthase
9.799007552	DI594_16565	
9.484404124	DI594_17930	
9.275180392	ttpC	TonB2 energy transduction system
8.978224169	tatA	twin arginine protein translocation system
8.284830968	DI594_09670	
8.250644198	SO_1824	TonB2 energy transduction system
7.902544744	SO_0548	histone-like DNA-binding protein
7.743248981	SO_4365	
7.729232309	DI594_13205	
7.703953533	rplV	50S ribosomal protein L22
7.676423252	DI594_18465	
7.670108989	DI594_07725	
7.532115861	rplJ	50S ribosomal protein L10
7.179908784	yceI	base-induced periplasmic protein
7.174606971	nusG	transcription termination factor
7.170750103	rpsT	30S ribosomal protein S20
7.121521336	DI594_07710	
6.93020412	DI594_13710	
6.791899106	fdhA	formate dehydrogenase

Biofilm Data – MCE membrane



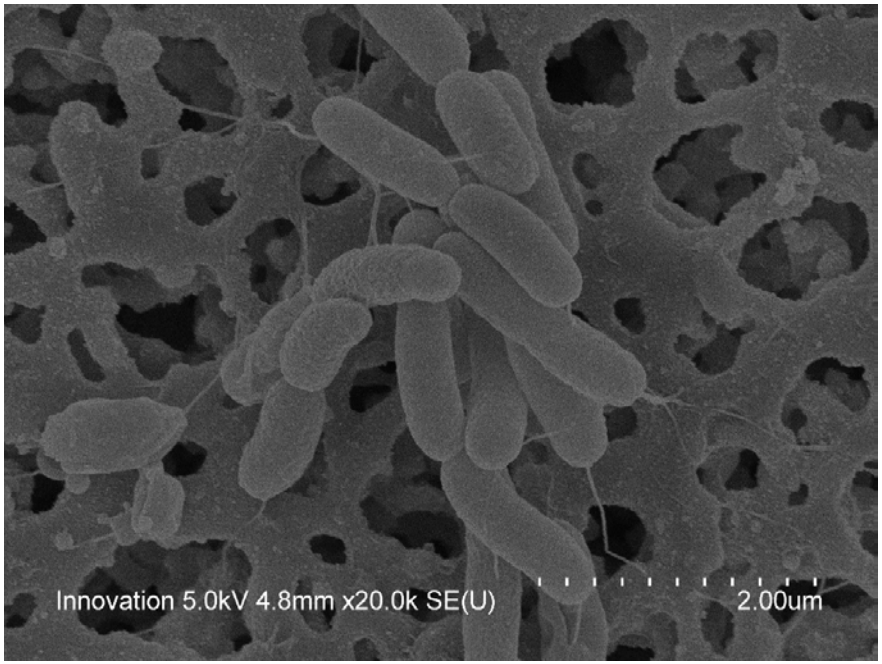
Ground control



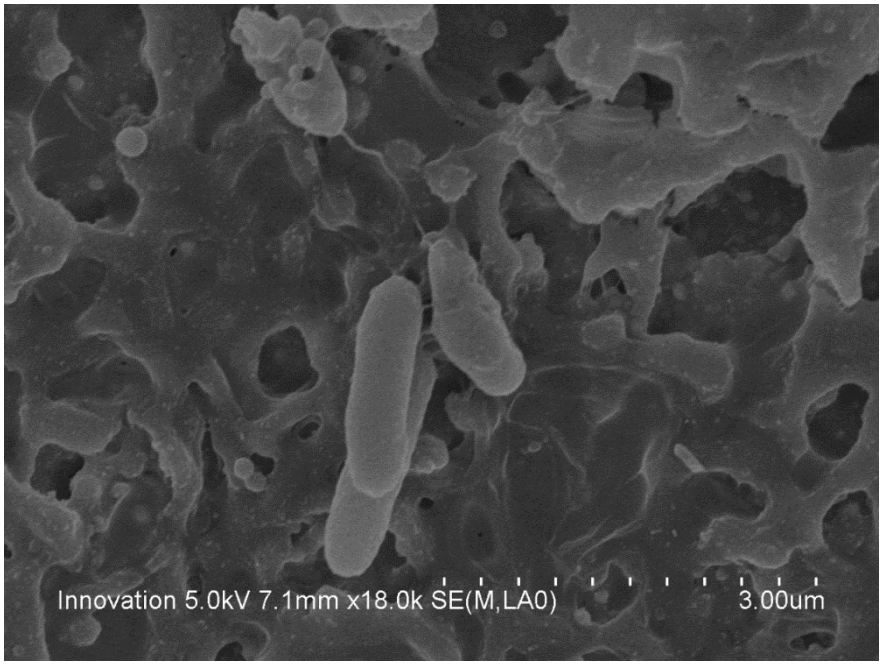
Flight sample

72 hours – lactate + ferric citrate

Biofilm Data – Fe-impregnated-MCE membrane



Ground control



Flight sample

72 hours – lactate + ferrihydrite

Mutant fitness analysis – genes identified with effects on fitness during spaceflight

Gene	Description	Fitness Cons.
SO0700	hypothetical protein	1.4
SO0902	NADH:ubiquinone oxidoreductase, Na translocating	-1.2, -1.5
SO0903	NADH:ubiquinone oxidoreductase, Na translocating	-1.4, -1.6
SO0905	NADH:ubiquinone oxidoreductase, Na translocating	-1.7, -1.6
SO0906	NADH:ubiquinone oxidoreductase, Na translocating	variable
SO4520	oxygen-indpendent coproporphyrinogen III oxidase, putative	-1.4, -1.3
SO4719	conserved hypothetical protein	-1.3

- Conclusions
  - A significant increase in the rate of reduction of insoluble ferrihydrite was noted in the flight samples
  - No difference was seen with soluble ferric citrate reduction rates between ground controls and flight samples
  - Many differentially expressed proteins were identified via proteomic analysis – the biological significance of these is unclear
  - No significant difference in biofilm formation has been found in comparing flight samples to ground controls
  - Mutant fitness analysis identified a handful of deletions with small effects on fitness in microgravity

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