Microbial community analysis to assess food safety of crops grown in the Veggie plant chambers on ISS

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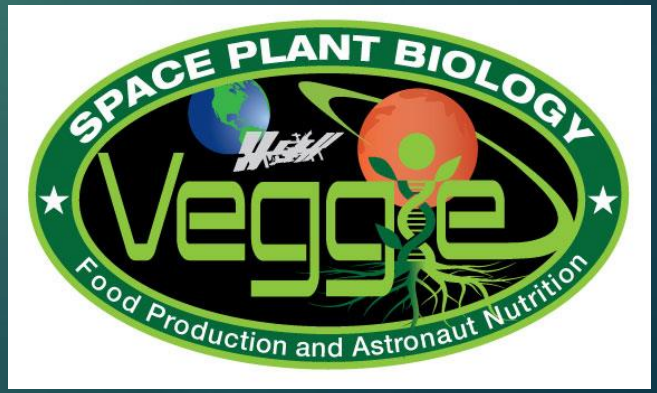


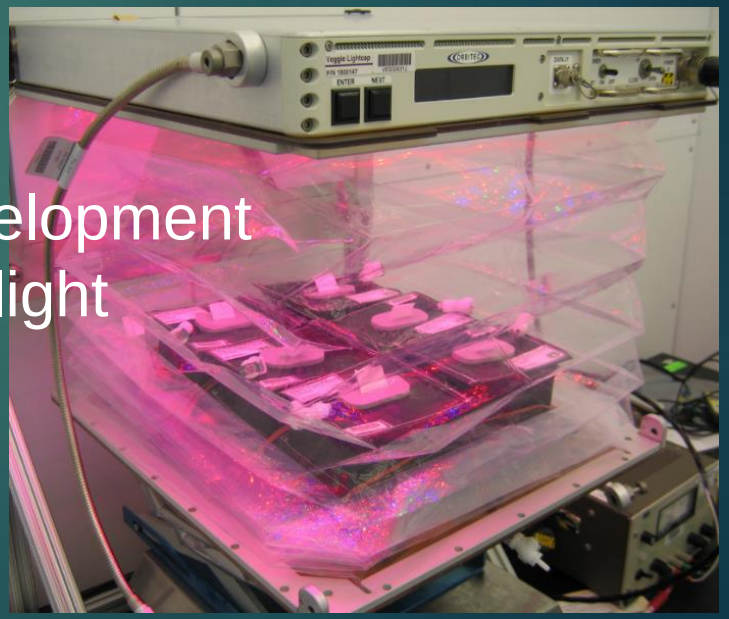
History

- Astronauts have extended stay in space
  - Requires adequate nutrition
  - Fresh, nutritional value items
- NASA has made efforts to grow and harvest healthy vegetables on ISS
  - Red lettuce “Outredgeous”
- Importance in methods used to grow fresh food
  - Safety emphasis

‘Outredgeous’
red romaine lettuce

History

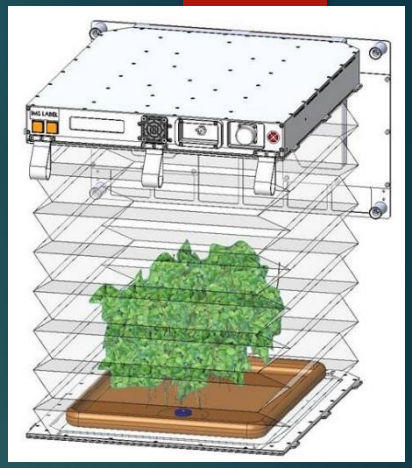
- Bacterial and fungal communities play important roles in plant health and growth systems
- Requirements & concerns being developed toward a healthy crop
  - Safety
  - Nutrition
- Provide insight into community development
  - Differences between ground & flight
  - Total microbial load
  - Microbial constituents



Goals

- To investigate and compare the microbial communities between flight and ground crops grown under similar conditions
- To compare two grow outs of flight crops
- To identify the presence of any plant or human associated microbes

Methods



- Red romaine lettuce (*Latuca sativa* cv Outredgeous) was grown & harvested on ISS in Veggie pillows under controlled conditions

- Parallel experiment was completed on ground under similar conditions
  - Temperature ~25 °C
  - Soils – arcillite w/ nutricote (7.5 g /L)
  - Water source

Methods

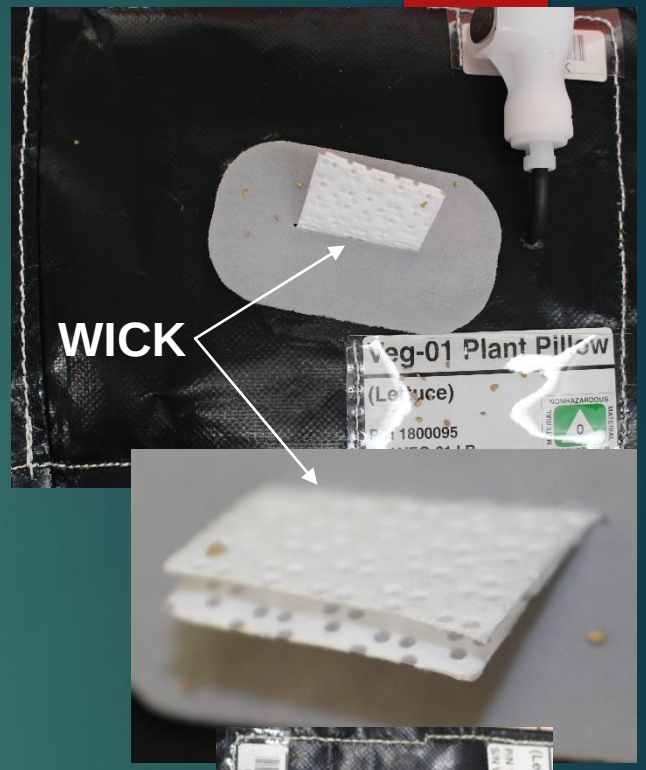
Post harvest:

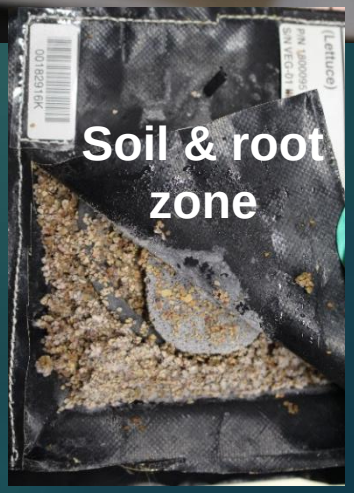
- Samples taken from both G & F
  - Plant surfaces, Root Zone
  - Wicks and Soil

- Heterotrophic bacterial & fungal plate counts

- Media specific screening
  - *E. coli*
  - *Salmonella*
  - *Staphylococcus*

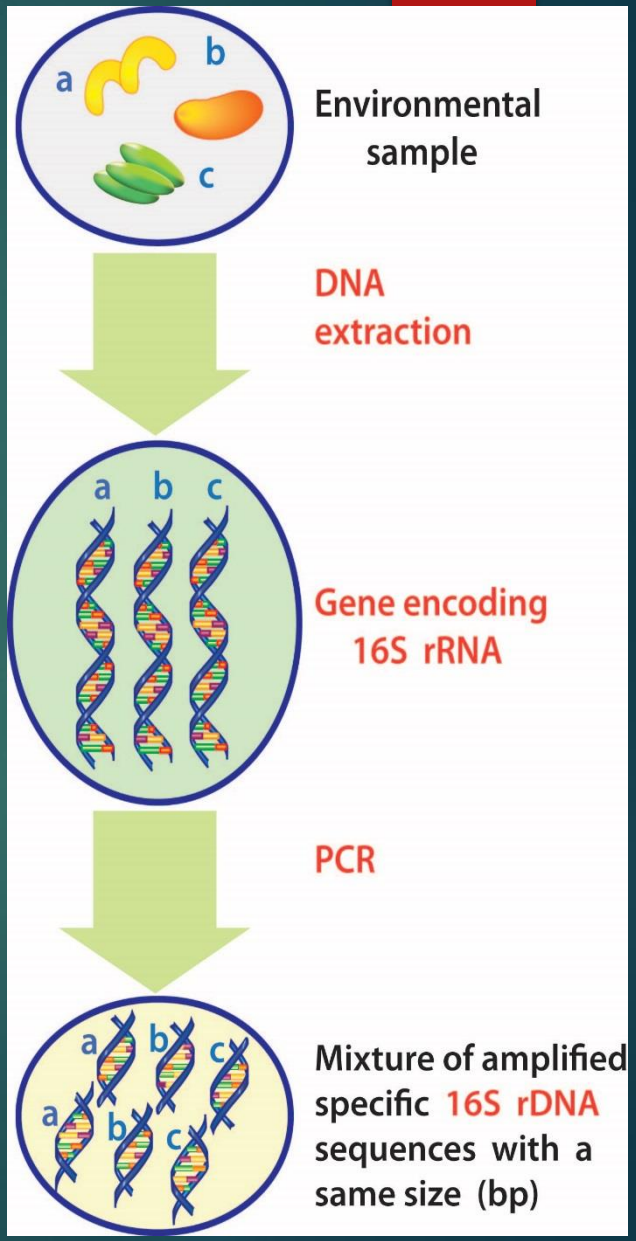
- Identification



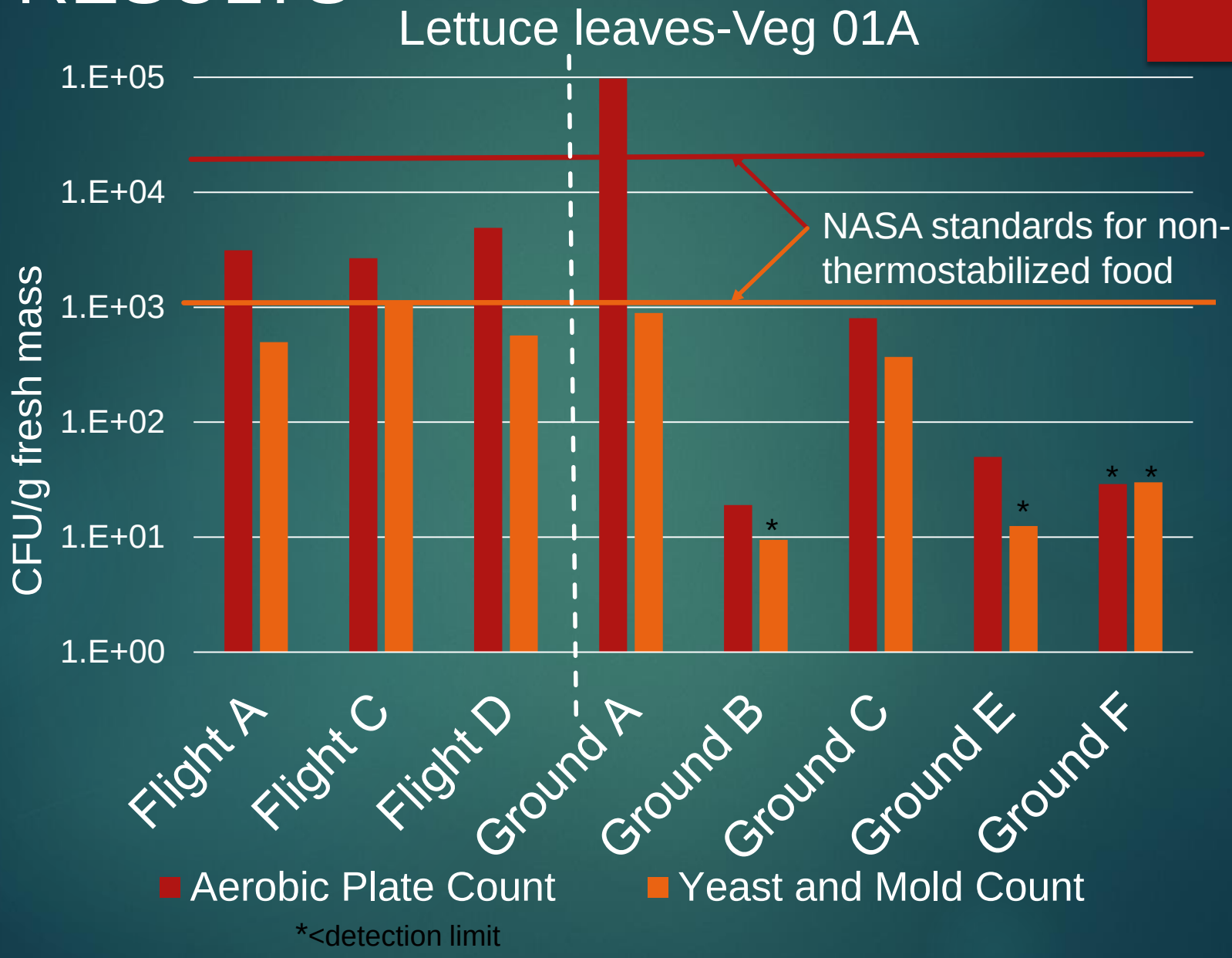


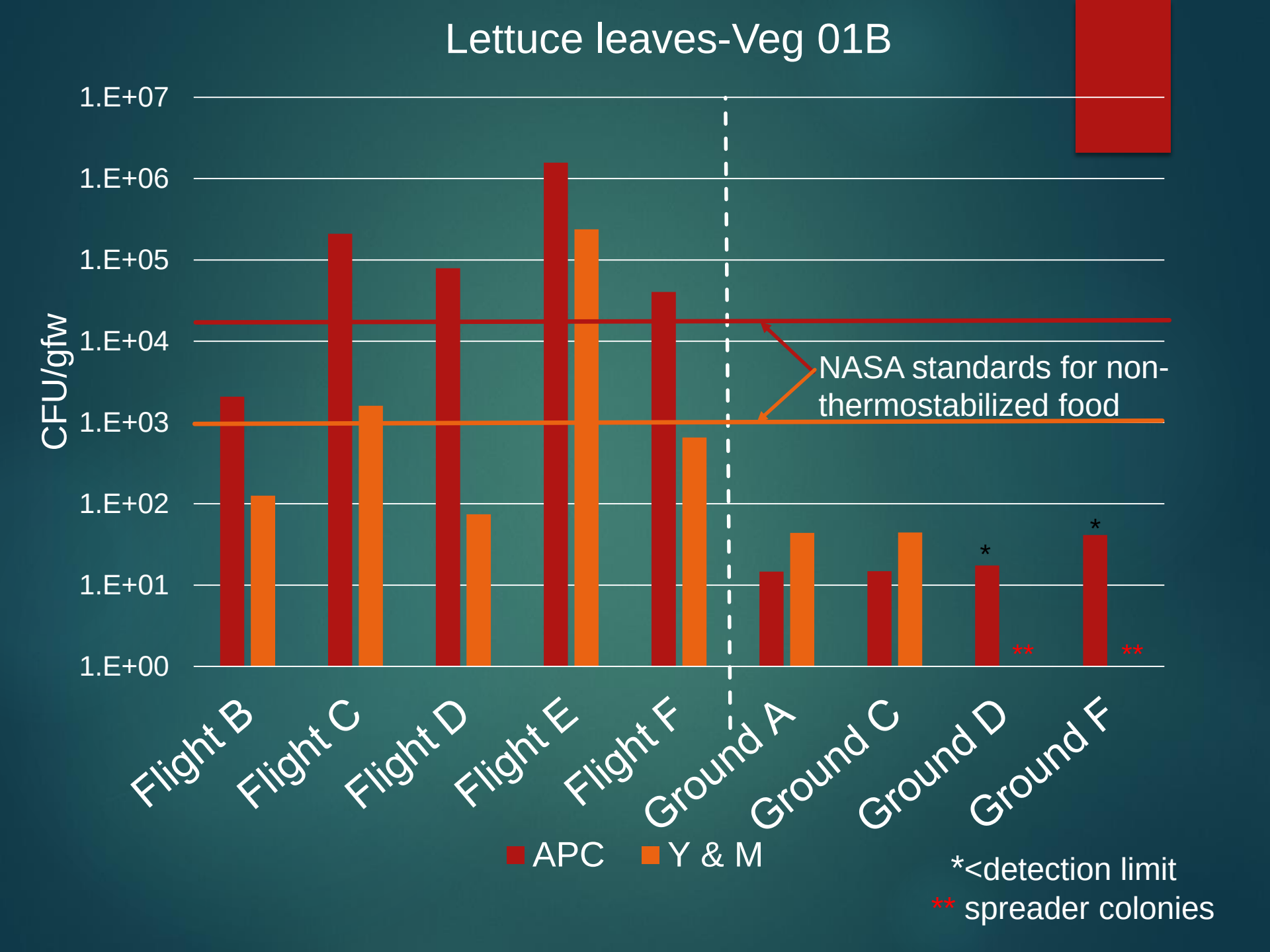
Methods

- PCR completed using barcoded primers
  - Bacterial 16S rRNA gene
  - Fungal ITS region
- Sequenced with MiSeq NGS
- Preliminary data analysis
  - GreenGenes bacterial database
  - UNITE fungal database

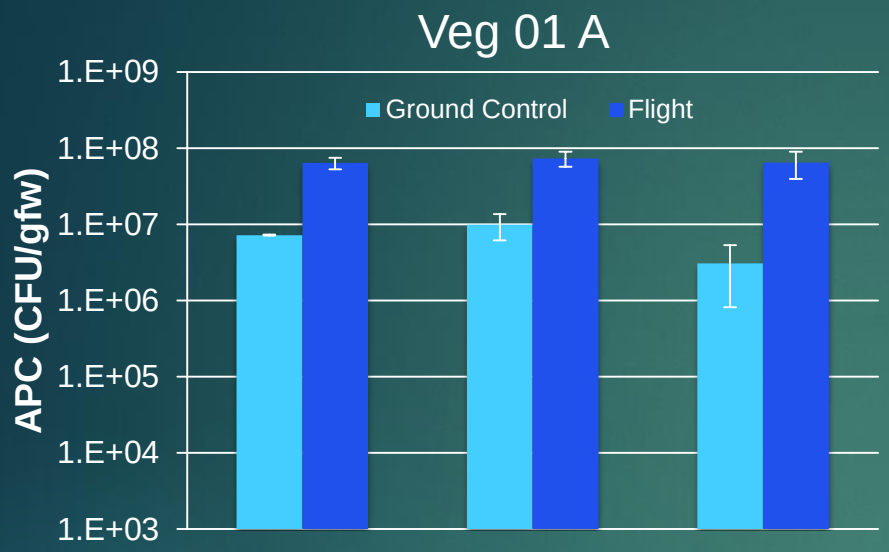


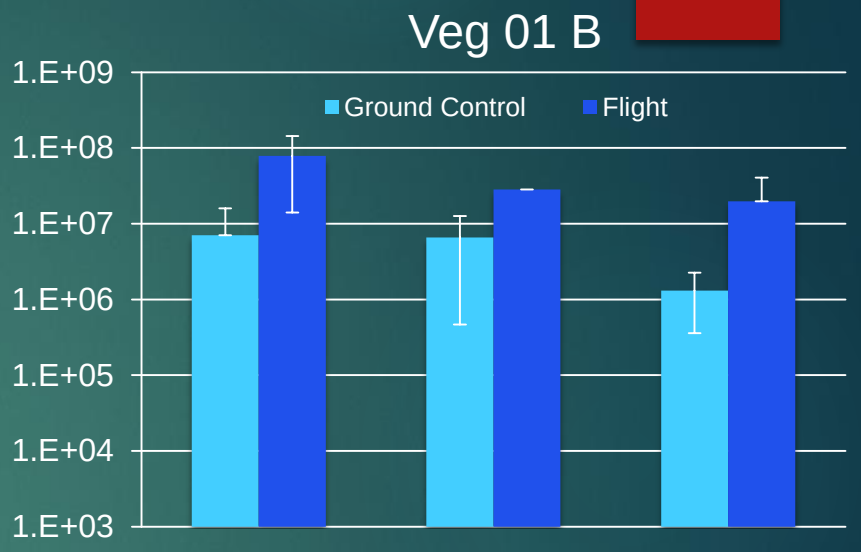
RESULTS

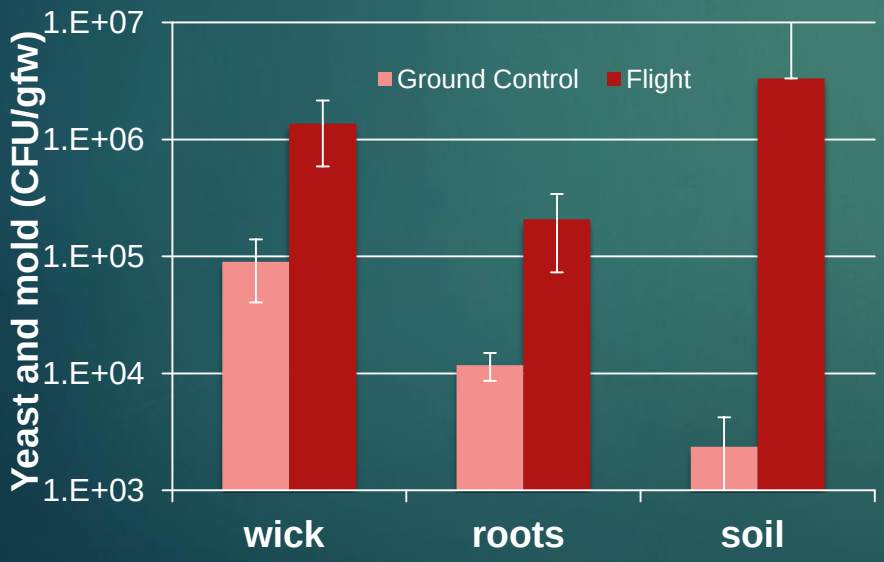

Lettuce leaves-Veg 01A
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
CFU/g fresh mass
NASA standards for non-thermostabilized food
*
*
* *
Flight A
Flight C
Flight D
Ground A
Ground B
Ground C
Ground E
Ground F
Aerobic Plate Count
Yeast and Mold Count
*<detection limit

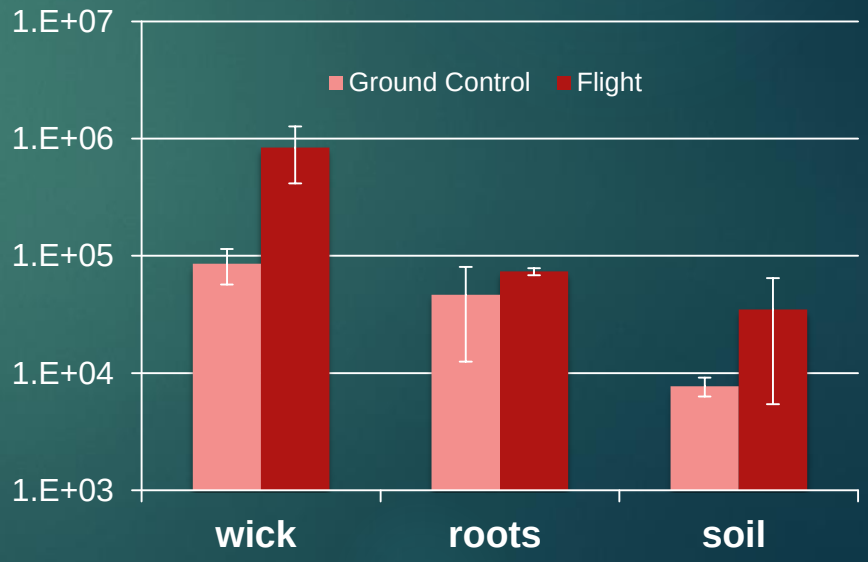

Lettuce leaves-Veg 01B
1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
CFU/gfw
NASA standards for non-thermostabilized food
Flight B
Flight C
Flight D
Flight E
Flight F
Ground A
Ground C
Ground D
Ground F
APC
Y & M
*<detection limit
** spreader colonies

RESULTS


Veg 01 A
APC (CFU/gfw)
1.E+09
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
Ground Control
Flight


Veg 01 B
1.E+09
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
Ground Control
Flight

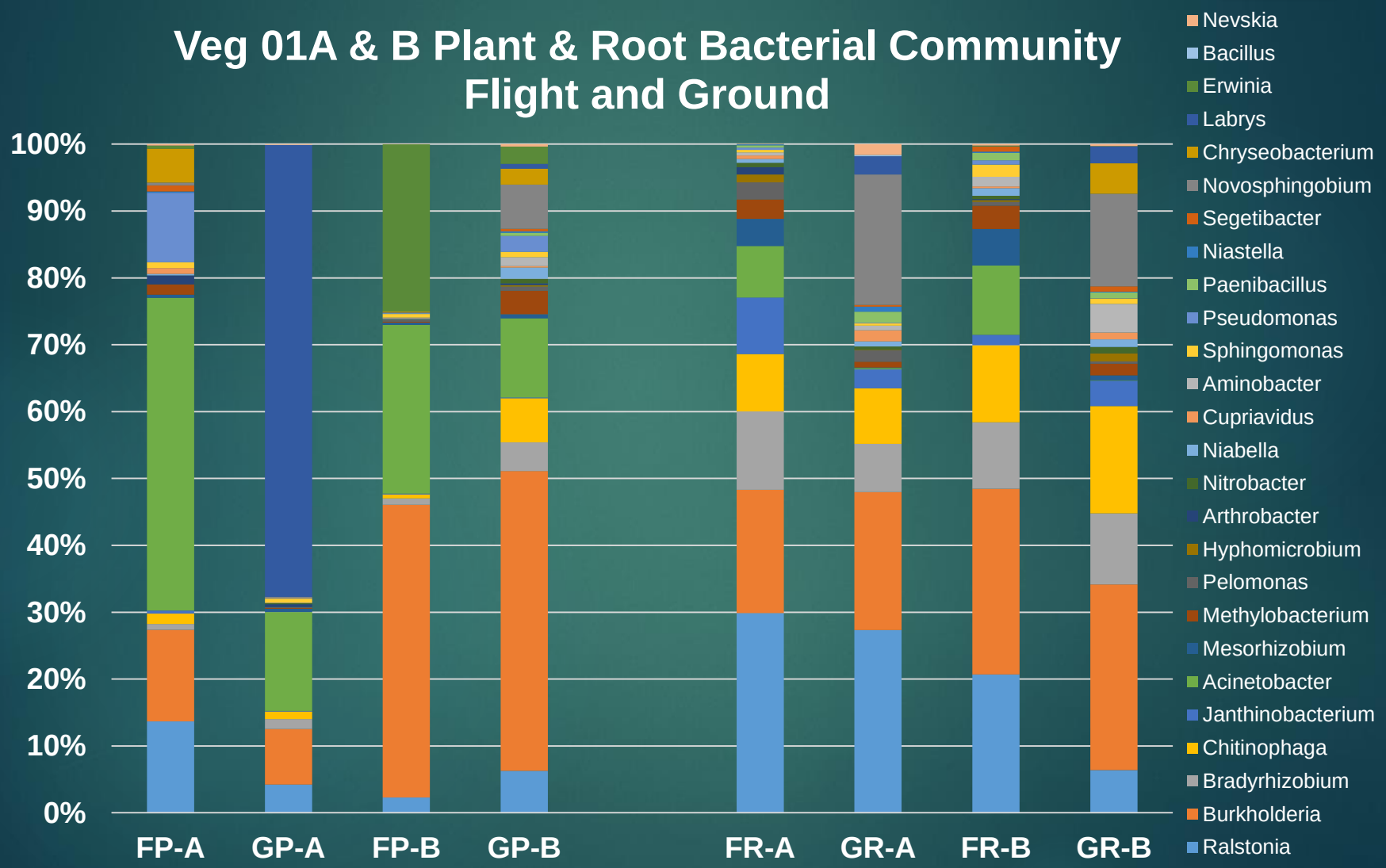

Yeast and mold (CFU/gfw)
1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
Ground Control
Flight
wick
roots
soil


1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
Ground Control
Flight
wick
roots
soil

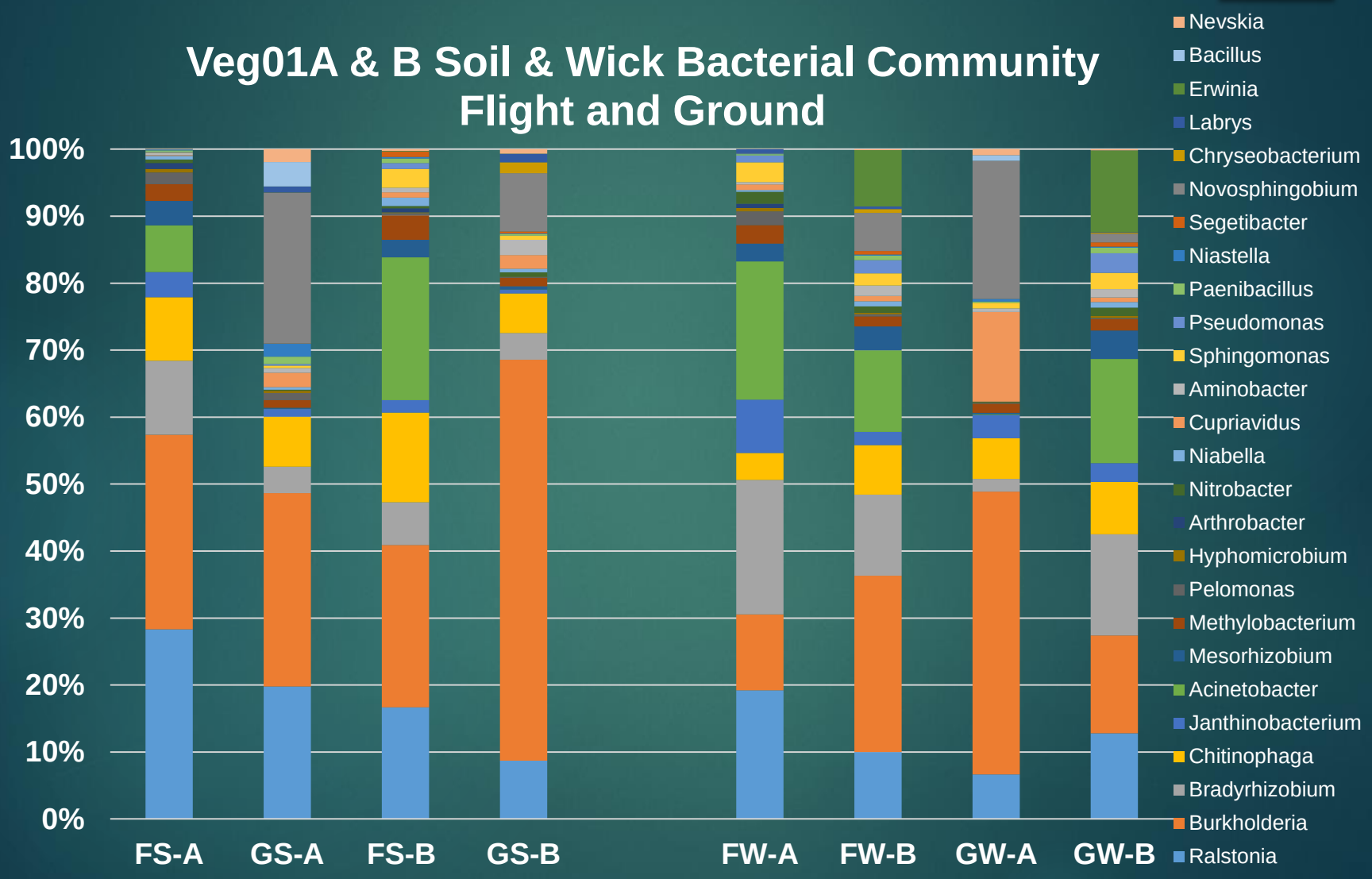
Results-Bacterial Community ID

Sample ID	Average Number of Species Identified		Shannon Species Diversity	
	Veg01A	Veg01B	Veg01A	Veg01B
Flight - Root	334±9.9	298±50.9	2.46±0.002	2.66±0.3
Flight - Soil	333±1.4	280±32.5	2.41±0.08	2.76±0.03
Flight - Wick	351.5±20.5	284	2.48±0.06	2.77
Flight - Plant	82.5±40.3	123.2±44.7	0.517±0.02	0.8±0.2
Ground - Root	68	329.0±49.5	2.291	2.16±0.17
Ground - Soil	390±22.6	410	2.207±0.08	2.27
Ground - Wick	304±53.7	408	1.587±0.23	2.77
Ground - Plant	61.4±16.6	72.3±18.5	0.462±0.02	0.5±0.0

Results


Veg 01A & B Plant & Root Bacterial Community
Flight and Ground
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
FP-A
GP-A
FP-B
GP-B
FR-A
GR-A
FR-B
GR-B
Nevskia
Bacillus
Erwinia
Labrys
Chryseobacterium
Novosphingobium
Segetibacter
Niastella
Paenibacillus
Pseudomonas
Sphingomonas
Aminobacter
Cupriavidus
Niabella
Nitrobacter
Arthrobacter
Hyphomicrobium
Pelomonas
Methylobacterium
Mesorhizobium
Acinetobacter
Janthinobacterium
Chitinophaga
Bradyrhizobium
Burkholderia
Ralstonia

Results


Veg01A & B Soil & Wick Bacterial Community
Flight and Ground
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
FS-A
GS-A
FS-B
GS-B
FW-A
FW-B
GW-A
GW-B
Nevskia
Bacillus
Erwinia
Labrys
Chryseobacterium
Novosphingobium
Segetibacter
Niastella
Paenibacillus
Pseudomonas
Sphingomonas
Aminobacter
Cupriavidus
Niabella
Nitrobacter
Arthrobacter
Hyphomicrobium
Pelomonas
Methylobacterium
Mesorhizobium
Acinetobacter
Janthinobacterium
Chitinophaga
Bradyrhizobium
Burkholderia
Ralstonia

Fungal Species Detected
Plant & Root

	Veg 01A	Veg 01B
Flight Plant	*Spordiobolus pararoseus* *Rhodotorula aurantiaca* *Rhodotorula glutinis* *Rhodosporidium fluviale* *Penicillium sp*	*Rhodotorula mucilaginosa* *Rhodosporidium sp* *Cryptococcus albidus var. diffluens* *Sporidiobolus ruineniae* *Fusarium oxysporum* *Aspergillus niger*
Ground Plant	*Rhodotorula glutinis* *Rhodosporidium fluviale* *Cryptococcus albidus var. diffluent* *Aspergillus sp.*	*Rhodotorula mucilaginosa* *Penicillium rubrum* *Aspergillus niger*
Flight Root	*Exophiala exophilae* *Rhodosporidium fluviale* *Rhodotorula glutinis* *Filobasidium unigluttulatum* *Rhodotorula mucilaginosa* *Rhodosporidium dibovatum*	*Fusarium oxysporum* *Aspergillus phoenicis* *Penicillium chrysogenum* *Alternaria tenuissima*
Ground Root	*Rhodotorula mucilaginosa*	*Aspergillus awamori* *Exophiala jeanselmei* *Exophiala oligosperma* *Penicillium oxalicum* *Penicillium sp.* *Penicillium rubrum* *Fusarium oxysporum* *Penicillium chrysogenum*

Fungal Species Detected
Soil Media & Wick

	Veg 01A	Veg 01B
Flight Soil Media	*Exophiala exophilae* *Rhodosporidium fluviale* *Rhodotorula glutinis* *Filobasidium unigluttulatum* *Rhodotorula mucilaginosa* *Rhodosporidium dibovatum* *Exophiala jeanselmei*	*Penicillium chrysogenum* *Fusarium oxysporum* *Aspergillus phoenicis* *Byssochlamys spectabilis* *Trichoderma moravicum* *Tomentella sp* *Verticillium leptobactrum*
Ground Soil Media	*Rhodosporidium fluviale* *Rhodotorula mucilaginosa*	*Aspergillus awamori* *Aspergillus sydowii* *Exophiala jeanselmei* *Penicillium chrysogenum* *Penicillium sp.* *Penicillium rubrum* *Purpureocillium lilacinum* *Fusarium oxysporum* *Penicillium oxalicum* *Exophiala oligosperma*
Flight Wick	*Exophiala exophilae* *Rhodosporidium fluviale* *Rhodotorula glutinis* *Rhodotorula mucilaginosa* *Rhodosporidium dibovatum*	*Penicillium chrysogenum* *Fusarium oxysporum* *Aspergillus phoenicis*
Ground Wick	*Rhodotorula mucilaginosa* *Rhodosporidium dibovatum*	*Penicillium chrysogenum* *Aspergillus awamori* *Exophiala jeanselmei* *Alternaria tenuissima* *Penicillium oxalicum*

Conclusions

- Specific pathogen screens: *E. coli, S. aureus, Salmonella* sp. were not detected on any plants.
- Aerobic plate counts were less than the limit for non-thermostabilized food on all flight plants and all but one ground plant.
- Total yeasts and molds all below limit except on one flight plant (plant C, the largest, slightly over).
- Fab five species – commonly found and previously identified on the ISS
- Many species also previously identified in ISS potable water source
- Many species normally associated with soils and plants
- Identified species are opportunistic species and not found to be harmful to healthy individuals

What's Next?

- Continue to look at the communities to characterize each community type.
- Evaluate additional plants to be grown aboard ISS for characterization, food safety, and nutritional value
- Investigate the functional structure of microbial communities in plants.

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INTERNS

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QUESTIONS