Veggie ISS Validation Test Results and Produce Consumption

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**VEGGIE Overview**

- Small Vegetable Production System – 0.15 m² growing area
- Flew to ISS on SpaceX-3 and was installed in Columbus module in May, 2014
- Initial experiments validated capabilities using ‘Outredgeous’ red romaine lettuce
- Samples returned Oct., 2014

VEGGIE was designed and built by Orbital Technologies Corporation (ORBITEC)
VEG-01 Analysis

- Fresh Mass
- Anthocyanin/Antioxidant/Phenolic Analysis
- Elemental analysis of plants and water
- Culturable microbial assessment:
  - Plants
  - Water
  - Pillow components
  - Identification of cultured microbes
- RNA sequencing/id of total microbial population
- Crew Questionnaire
- X ray tomography of pillows
Fresh Mass

<table>
<thead>
<tr>
<th></th>
<th>Flight</th>
<th>Ground</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Average FM</td>
<td>20.61 g</td>
<td>15.29 g</td>
</tr>
<tr>
<td>SD FM</td>
<td>11.66 g</td>
<td>9.60 g</td>
</tr>
<tr>
<td>Max</td>
<td>31.51 g</td>
<td>26.11 g</td>
</tr>
<tr>
<td>Min</td>
<td>8.31 g</td>
<td>2.81 g</td>
</tr>
</tbody>
</table>

Sample priority:
1. Microbial analysis
2. Anthocyanin/Antioxidant/Phenolic Analysis
3. Elemental analysis of plants

Only samples of >19 g could be used for all three.
Microbial Assessment of ISS-grown ‘Outredgeous’ Red Romaine Lettuce

• Specific pathogen screens: *E. coli*, *S. aureus*, *Salmonella* sp. not found on any plants.

• Aerobic plate counts less than limit for non-thermostabilized food on all flight plants and all but one ground plant (unexplained). (Note: there are no standards for space produce so these are the closest approximate standards)

• Total yeasts and molds all below limit except on one flight plant (plant C, the largest, slightly over).

• Bacterial and fungal species isolated appear to be typical station microbes. There were some differences in the community from the ground set. (details in backup slides)
NASA standards for non-thermostabilized food
Microbial Assessment continued…

• Measured microbial levels and composition do not indicate a threat to crew health
  • Previous ground testing showed naturally low microbial levels on lettuce

• Proposed that precautionary wiping of produce with a Pro-San® sanitizing wipe would further reduce microbial levels
Pro-San® Sanitizer for Vegetables

• Commercial citric acid-based produce sanitizer

• A Pro-San® wipe was developed for cleaning the Veggie hardware: already approved for spaceflight and used on ISS

• Studies were performed to determine the efficacy of using wipes containing Pro-San® to clean and disinfect.
  • Vegetables grown in vegetable production units like Veggie (these data are in backups).
  • Veggie hardware surfaces (these data are in backups).

• Wipes reduce levels by >4 orders of magnitude.
Produce Consumption Approval

- A splinter session was held at the HRP meeting to discuss Veggie results and a plan for consumption.
- Using on-board produce sanitizing wipes was tested.
- Presentations were made to the Flight Medicine Board and the Tri-board (Space Medicine, Biomedical Research & Environmental Sciences, and Human Systems Engineering & Development) at JSC and received approval.
- The Payload Safety Review panel approved.
- Crew was approved to consume produce with precautionary wiping.
Approved Safe-Handling Procedures

• Veggie seeds, media, wicks and pillows shall be clean and free of contaminants.
• Crew shall wear gloves when handling pillows and plants.
• Veggie hardware (bellows, etc.) shall be wiped with Pro-San wipes prior to installation of plant pillows and following growth.
• Tools shall be wiped before and after use.
• Produce shall be wiped at harvest according to produce-specific procedure.
Sanitizing Produce
Veg-01 Second Crop

- ½ the produce harvested for consumption, ½ for science
- Plants harvested Aug. 10, 2015 and eaten live on NASA TV
Thank you!

• Veggie and VEG-01 teams at KSC and ORBITEC

• Astronauts Steve Swanson, Rick Mastracchio, Scott Kelly, Kjell Lindgren

• Payload Operations and Integration Center

• Grace Douglas and Mark Ott

Veggie
Send more seeds!
We are hungry.

-Rick Mastracchio

• NASA Space Life and Physical Sciences, Human Research Program, and ISS Program
Backup Information
## Microbial counts on Veggie lettuce samples

<table>
<thead>
<tr>
<th>Plant Pillow</th>
<th>Flight Plants</th>
<th>Ground Control Plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant weight (g)</td>
<td>A</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>8.6</td>
<td>7.6</td>
</tr>
<tr>
<td>Aerobic Plate Count (cfu gfw⁻¹)</td>
<td>3120</td>
<td>2670</td>
</tr>
<tr>
<td>Yeast and Mold Count (cfu gfw⁻¹)</td>
<td>497</td>
<td>1120</td>
</tr>
<tr>
<td>E. coli, S. aureus, Salmonella sp.</td>
<td>&lt; DL**</td>
<td>&lt; DL</td>
</tr>
<tr>
<td>Microscopic Cell Count gfw⁻¹</td>
<td>8.45 x 10⁷</td>
<td>8.79 x 10⁷</td>
</tr>
</tbody>
</table>

**Below detection limit.
## Identification of isolates on Veggie lettuce

<table>
<thead>
<tr>
<th>Flight Plants</th>
<th>Ground Control Plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pillow</td>
<td>A</td>
</tr>
<tr>
<td>Bacteria</td>
<td>C</td>
</tr>
<tr>
<td>Bacteria</td>
<td>D</td>
</tr>
<tr>
<td>Bacteria</td>
<td>A</td>
</tr>
<tr>
<td>Bacteria</td>
<td>B</td>
</tr>
<tr>
<td>Bacteria</td>
<td>C</td>
</tr>
<tr>
<td>Bacteria</td>
<td>E</td>
</tr>
<tr>
<td>Bacteria</td>
<td>F</td>
</tr>
<tr>
<td>Fungi</td>
<td>1, 3, 4</td>
</tr>
<tr>
<td>Fungi</td>
<td>4</td>
</tr>
<tr>
<td>Fungi</td>
<td>5</td>
</tr>
<tr>
<td>Fungi</td>
<td>4</td>
</tr>
<tr>
<td>Fungi</td>
<td>none</td>
</tr>
<tr>
<td>Fungi</td>
<td>2</td>
</tr>
<tr>
<td>Fungi</td>
<td>none</td>
</tr>
<tr>
<td>Fungi</td>
<td>none</td>
</tr>
</tbody>
</table>

*Numbers indicate isolate identification from list below

### Bacteria
1. *Burkholderia cepacia*
2. *Burkholderia fungorum*
3. *Ralstonia insidiosa*
4. *Curtobacterium flaccumfaciens*
5. *Acinetobacter genospecies 3*
6. *Ralstonia picketti*
7. *Arthrobacter ilicis*
8. *Sphingomonas parapaucimobilis.*
9. *Flavobacterium columnare (water)*

### Yeast and filamentous fungi.
1. *Spordiobolus pararoseus*
2. *Cryptococcus albidus var diffluent*
3. *Rhodotorula aurantiaca B*
4. *Rhodotorula glutinis*
5. *Rhodotorula achemiour*
6. *Penicillium sp (presumptively identified by microscopy)*
7. *Aspergillus sp. (presumptively identified by microscopy)*
Produce Sanitation Validation: Ground

Vegetables:
• Two types of produce with very different surface topographies were tested: ‘Outrerdgeous’ red romaine lettuce and radish.

Challenge inoculation:
• Produce was inoculated with known levels of *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 4495562), and *Pseudomonas aeruginosa* (ATCC 3513563).

Sanitizing wipes:
• Polypropylene crew wipes (Kimberly Clark) were used to coincide with materials currently on ISS. Wipes were saturated with a 1% solution of Microcide® Pro-San sanitizer. Controls included water saturated wipes and no cleansing treatment.
The numbers above each bar on the graphs indicate the \( \log_{10} \) reduction of total aerobic bacteria (APC) and \( E. coli \) (EC) compared to controls. (e.g. 1 \( \log_{10} \)=90% reduction in counts)

- The Pro-San sanitization procedure reduced the average APC by 1.18 log per gram on the radish and EC was reduced by 2.44 log.

- Pro-San sanitization achieved a 5.25 log reduction in APC and a 4.37 log reduction in EC on lettuce.

(Starting \( E.coli \) inoculum on radish= \(~6 \times 10^7\), lettuce=\(~4 \times 10^7\))
Materials and Methods-Surface Sanitization

Surfaces:
- Two different Veggie surface materials were tested; Teflon Coated Kevlar (TCK) which is used on Veggie pillows and the clear bellows material.

Challenge inoculation:
- Plastics were inoculated with *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 4495562), and *Pseudomonas aeruginosa* (ATCC 3513563).

Sanitizing wipes:
- Two different wipe materials were tested for plastic surface sanitization. Initially a product called Mighty Wipes™ was used. These are low lint, clean room wipes.
- Subsequently, polypropylene crew wipes (Kimberly Clark) were used to coincide with materials currently approved for use on ISS. Wipes were saturated with a 1% solution of Microcide™ Pro-San sanitizer. Controls included water saturated wipes and no cleansing treatment.
### Surface Sanitizing

Log reduction (from untreated surfaces) in microbial counts (CFU) after 30 sec. wipe (Mighty Wipe™) with sterile water or 1% sanitizer. (Two surfaces were tested based on materials used for the Veggie hardware.)

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Teflon Coated Kevlar</th>
<th>Clear Plastic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water</td>
<td>Sanitizer</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>1.219</td>
<td>5.079</td>
</tr>
<tr>
<td>S. aureus</td>
<td>2.592</td>
<td>6.767</td>
</tr>
<tr>
<td>E. coli</td>
<td>3.169</td>
<td>6.614</td>
</tr>
</tbody>
</table>

Log reduction (from untreated surfaces) in microbial counts (CFU) after 30 sec. wipe with sterile water or 1% Pro-San® saturated polypropylene wipes.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Teflon Coated Kevlar</th>
<th>Clear Plastic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water</td>
<td>Sanitizer</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>0</td>
<td>4.182</td>
</tr>
<tr>
<td>S. aureus</td>
<td>3.725</td>
<td>3.957</td>
</tr>
<tr>
<td>E. coli</td>
<td>No growth detected on any treatments*</td>
<td></td>
</tr>
</tbody>
</table>

*In this test *E. coli* apparently did not survive the desiccation on the plastic surface.
Surface Sanitizing Summary

• The sanitizer was effective in reducing the number of challenge bacteria by 4 to >6 orders of magnitude on the Teflon Coated Kevlar (TCK) and clear plastic when assayed using surface swabbing.

• Dry wipe material type had an effect on the reduction of bacteria from both surfaces.

• Cleaning with water saturated wipes reduced the numbers of bacteria on surfaces however, microorganisms could survive on the wipes after 48 hr of storage while sanitizer soaked wipes exhibited a biocidal effect with no to few bacteria surviving after use and storage (data not shown).
Produce Preparation for ISS

This procedure is intended to provide the basic rules & standards applicable to the handling and preparation of fresh food for stowage – Space Food Systems

• Product shall be clean and free from foreign matter, approved for food use and have typical odor, color and flavor.

• Product shall be in excellent condition at time of use and from the freshest lot available.

• Clean produce thoroughly in flowing potable water and drain.

• Dip products into 200ppm chlorine solution for a minimum of 30 seconds.

• Let products air dry on a clean dry surface.

• Bag and pack for flight.
Anthocyanins / Antioxidants / Phenolics

- Anthocyanins were the same between ground and flight.
- Antioxidants and Phenolics were slightly higher in flight plants.

**Graphs:**
- **Antioxidant Capacity (ORAC):**
  - Ground vs. Flight Plants
  - Units: µmol TE/g
  - Ground: 750 ± 25
  - Flight: 1050 ± 15

- **Total Phenolic Content:**
  - Ground vs. Flight Plants
  - Units: mg/g DW
  - Ground: 20 ± 1
  - Flight: 35 ± 3
Elemental Analysis

- Iron levels were identical and Calcium, Molybdenum and Phosphorus were similar between flight and ground.
- Boron, Copper, Magnesium, Manganese, Sodium and Sulfur were slightly higher in flight plants.
- Potassium was slightly higher in ground plants.
- Nickel and Zinc were significantly higher in flight plants.
- Flight water had low levels of Sodium, Potassium, Chlorine, Sulfate and Nitrate at the start; ground water had Fluorine, Chlorine, Sulfate and Nitrate.