Evaluation of Low-Pressure Cold Plasma for Disinfection of ISS Grown Produce and Metallic Instrumentation

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Microbes on ISS

NASA astronauts Scott Kelly and Kjell Lindgren take a bite of red romaine lettuce grown in Veggie. Photo credit NASA

- Food
- Instruments
- Potable water system
- Fluid delivery systems
- Surfaces
Disinfection using plasma

- Shown to be effective at precision cleaning aerospace hardware at Kennedy Space Center.
- Relatively new technology being investigated for disinfecting agricultural commodities and medical instruments.
- Plasma cleaning is a dry, non-thermal process, which can provide broad-spectrum antimicrobial activity.
- Microgravity compatible since cold plasma uses no liquids and is able to penetrate even the smallest cracks and crevices.
Objectives

• Determine plasma conditions, i.e. vacuum pressure and duration of plasma treatment and any effect on plant tissues.

• Evaluate the efficacy of plasma treatment for produce disinfection.

• Evaluate the efficacy of plasma treatment for disinfection/sterilization of solid items such as utensils and medical supplies.
Background

Gas is excited by energy supplied in a vacuum. Reactive species and UV are generated dependent on source gas.

- Oxidation of biomolecules
- Damage to DNA
- "Sandblasting" effect
Optimization

- Conditions for metallic coupons were based on previous precision cleaning techniques.
- Moisture present in the produce required adjustments to the low pressure settings to be able to maintain plasma and the integrity of the item for the duration of testing.

<table>
<thead>
<tr>
<th>Item</th>
<th>Pressure (mbar)</th>
<th>Quantity/Run</th>
<th>Exposure Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metallic Coupons</td>
<td>0.10</td>
<td>Up to 10</td>
<td>5, 10, 15, 30, 60</td>
</tr>
<tr>
<td>Cherry Tomatoes</td>
<td>0.60 and 0.80</td>
<td>5</td>
<td>5, 10, 15</td>
</tr>
<tr>
<td>Radishes</td>
<td>0.80</td>
<td>3</td>
<td>5, 10, 15</td>
</tr>
<tr>
<td>Peppers</td>
<td>0.80</td>
<td>2</td>
<td>5, 10, 15</td>
</tr>
<tr>
<td>Cabbage</td>
<td>Could not be determined</td>
<td>2</td>
<td>None</td>
</tr>
</tbody>
</table>
Metal Surfaces

- Coupons were inoculated with ~$10^7$ *Bacillus pumilus* spores or *E. coli* cells per coupon.

- Coupons exposed to plasma at different exposure times, up to 60 min. Controls were subjected to low pressure only.

- Analysis done by:
  - Scanning electron microscopy (SEM) imaging (*B. pumilus*)
  - Calculation of log reduction using Most Probable Number technique (*B. pumilus*)
  - Plate counts (*E. coli*)
Results (SEM) Plasma treatments

• Smaller in size
• Surfaces are smooth and pitted. Spore coat proteins affected?
• Extracellular material evident after 10 and 15 min treatment.
30 and 60 minute treatment.
Results. Viability

- Maximum log reduction is achieved with 10 minute treatment for *E. coli* and *B. pumilus*
  - Decimal reduction value=1.9 minutes for *B. pumilus*. Treatment time for a single Log\(_{10}\) reduction calculated.
- Theoretically 15 min should reduce~ 7.5 Log\(_{10}\) Bacillus spores
Produce

- Produce grown in controlled environment chambers at KSC.
- Selected candidate crops for Veggie VPU.
- Inoculated with ~ $10^7$ *E. coli* cells/piece.
- Exposure times tested were 0 (low pressure for 15 min), 5, 10 and 15 minutes.
- Analysis done by:
  - calculation of bacteria log reduction using plate counts
- No changes in temperature were observed when moisture was not present.
- Freezing or tissue damage was detected when water was present.
RADISHES

✓ Did not present any damage that could lead to test failing

![Graph showing E. coli reduction on radish](image)

- Reduction (Log_{10} CFU)
- Exposure time (minutes)

<table>
<thead>
<tr>
<th>Exposure Time (Minutes)</th>
<th>Reduction (Log_{10} CFU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.48</td>
</tr>
<tr>
<td>5</td>
<td>1.48</td>
</tr>
<tr>
<td>10</td>
<td>1.73</td>
</tr>
<tr>
<td>15</td>
<td>1.76</td>
</tr>
</tbody>
</table>
PEPPERS

Open peppers could lead to test failure and skin damage.

![Image of peppers]

**Graph: E. coli reduction on Pepper**

- **Reduction (Log 10 CFU)**
- **Exposure time (minutes)**

- 0 minutes: 0.25
- 5 minutes: 0.71
- 10 minutes: 1.26
- 15 minutes: 1.02
TOMATOES

✓ If tomato was very ripe and water detected through removed stalk considerable damage was observed.
CHINESE CABBAGE

- Failed due to water content
- Significant tissue damage
Potable Water Dispenser (PWD) Needle

• Successfully disinfected a piece of spaceflight hardware, the Potable Water Dispenser (PWD) needle that is used on the International Space Station (ISS). The needle is used by astronauts to rehydrate food packaging.

• The PWD needle was inoculated with a challenge organism inside the body of the needle and disinfected with cold plasma.
Conclusions

- Plasma is effective in killing spores of *B. pumilus and E. coli cells* on solid surfaces (>5.5 log reduction) within 10 minutes of exposure time.
- Treatment was less effective on reducing *E. coli* on produce.
- Low pressure cold plasma can effect plant tissues i.e. quality.
Future work

• Test efficacy of process on solid items inoculated with the fungus *Aspergillus niger*.

• Test sterilization of a variety of solid items inoculated with both test organisms.
  • ISS Potable water dispenser needle
  • Medical tools (ex. Hemostat, scalpel)
  • Plastic 3-D printed items
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