Biofilm basics I

- Community of bacterial cells adherent to a surface or interface or each other, and encased in a self-produced extracellular matrix
- Extracellular matrix material could be:
  - Polysaccharides
  - Proteins
  - Extracellular DNA
- Environment, industrial equipment, medical devices, living organisms, food processing environments

Biofilm Basics II

- 99.9% of bacteria grow as biofilms
- Research over last 20 - 30 years (persistent infections)
- Numerous methods for growth, testing and measurement
- Factors to consider: media, temperature, inoculum density, agitation, flow rate, shear, substratum topography
Biofilm basics III

- Cells growing in biofilms are physiologically different from planktonic cells.
- These differences may account for their enhanced resistances to antibiotics, disinfectants, and host defenses.

Biofilm basics IV

Why biofilms

The lack of efficacy using aqueous sanitizers was attributed to bacterial attachment to inaccessible sites on the rind and/or biofilm formation (Anous et al., 2004, J. Food Prot, 67: 1876-1885)

Lack of efficacy using aqueous sanitizers

- $\text{H}_2\text{O}_2$ (1-5%), chlorine ($\geq 200$ ppm), SDS, sodium dioctyl succinate, sodium 2-ethylhexyl sulfate, trisodium phosphate, dodecyl-benzene sulfonylic acid (pH 2), phosphoric acid (pH 2), nisin-EDTA
- Log reductions: 0 - ~3 logs
- Decreased efficacy after 24 hours
Resistance to Antibacterial Agents

• A Consequence of Biofilm Formation?
• WHY and HOW: the great debate

Salmonella case study

<table>
<thead>
<tr>
<th>Reference</th>
<th>Organism</th>
<th>Antibiotic</th>
<th>MIC or MBC of planktonic phage (µg/ml)</th>
<th>Colon effective against biofilm phage (µg/ml)</th>
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<tr>
<td>215</td>
<td>S. aureus ATCC 1925-4</td>
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<td>P. pseudomallei</td>
<td>Oxolinic</td>
<td>8 (MBC)</td>
<td>80</td>
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<tr>
<td>114</td>
<td>Shigella sonnei 29644</td>
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6 Concentration required for 90% reduction.
6 Minimal biofilm eradication concentration.
6 Concentration required for 90% reduction.
6 Concentration required for >90% reduction.


Resistance to Irradiation

Salmonella biofilm formation: a recent observation

• Documented on various inert surfaces: glass, rubber, plastic, cement, stainless steel
• Two components of the extracellular matrix:
    • Surface fibers involved with adhesion
    • Expressed by Salmonella select other enterobacteriaceae
    • Often found in plant pathogens, newly discovered in Salmonella
    • Provides elasticity to cells packed together
State of foodborne pathogens on plant surfaces

- Not well characterized
- Aggregate formation?
- Native microflora present as biofilms. Human pathogens?

**Question 1**: Do *Salmonella* spp. exist as biofilms on the surfaces of cantaloupe melons?

Cantaloupe surfaces: a tangled net

- Epidermal cell surface ruptured by meshwork of raised tissue (netting)
- Netting consists of lenticels and phellum
- Guard cells non-functional
- Cuticular cracks-points of ingress for microorganisms

Methodology

- Two isolates utilized
- 10 µl spots deposited on marked areas or dip inoculation
- Spots contained $10^6$ or $10^3$ CFU
- Melons dried 2 h, then held at 22 or 10°C
- Samples collected
- Sections removed, fixed, dried, mounted, and examined using SEM

SEM (X2500) of Control (uninoculated) Cantaloupe stored at 10°C for (A) 48 or (B) 72 H, and 20°C for (C) 48 or (D) 72 H
SEM (2500X) of S. Poona Cells Inside the Netting

2 h drying at RT

72 h drying at RT

S. Poona following storage at 20°C for A) 2 h, B) 24 h, C) 48 h Note: extracellular matrix encapsulating cells, and D) 72 h
Residual populations of Salmonella Poona on artificially inoculated cantaloupes stored for 24 h at 4ºC or room temperature

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<th>Room temperature (log CFU/cm²)</th>
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<tr>
<td></td>
<td>XLT-4</td>
<td>TSA with XLT-4 overlay</td>
</tr>
<tr>
<td>2 h Control</td>
<td>4.81 ± 0.43 A</td>
<td>5.40 ± 0.42 A</td>
</tr>
<tr>
<td>24 h Control</td>
<td>4.18 ± 0.56 AC</td>
<td>4.95 ± 0.33 AC</td>
</tr>
<tr>
<td>24 h 200 ppm chlorine (RT for 20 min)</td>
<td>3.36 ± 0.15 C</td>
<td>3.79 ± 0.03 C</td>
</tr>
<tr>
<td>24 h Acidic electrolyzed water (RT for 20 min)</td>
<td>3.20 ± 0.22 C</td>
<td>3.85 ± 0.10 C</td>
</tr>
<tr>
<td>24 h Basic electrolyzed water (RT for 20 min)</td>
<td>3.57 ± 0.60 C</td>
<td>4.25 ± 0.50 AC</td>
</tr>
<tr>
<td>24 h tap water (76ºC for 3 min)</td>
<td>0.04 ± 0.02² E</td>
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</tr>
<tr>
<td>24 h tap water (RT for 20 min)</td>
<td>3.22 ± 0.37 C</td>
<td>4.59 ± 0.38 AC</td>
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Residual populations of Salmonella Poona on artificially inoculated cantaloupes stored for 48 h at 4ºC or room temperature

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<tr>
<td>48 h Control</td>
<td>4.05 ± 0.70 A</td>
<td>4.56 ± 0.23 A</td>
</tr>
<tr>
<td>48 h 200 ppm Chlorine (RT for 20 min)</td>
<td>2.81 ± 0.39 C</td>
<td>3.70 ± 0.33 AC</td>
</tr>
<tr>
<td>48 h Acidic electrolyzed water (RT for 20 min)</td>
<td>3.67 ± 0.49 AC</td>
<td>4.39 ± 0.46 AC</td>
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<td>48 h Basic electrolyzed water (RT for 20 min)</td>
<td>3.52 ± 0.46 AC</td>
<td>4.17 ± 0.54 AC</td>
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<td>3.36 ± 0.11 AC</td>
<td>4.40 ± 0.19 AC</td>
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**Observations**

- Minimal biofilm detected on control melons
- All isolates produce biofilm following introduction
- Fibrillar material visible after 2 h
- Biofilm formation occurs rapidly, polymer visible after 24 h, regardless of temperature
- Sheet material likely provides protective cover
- Sanitizers unlikely to inactivate established cells
- Hot water resulted in total inactivation (not limited by biofilm)

**CONCLUSIONS**

- Biofilm formation and attachment of cells to inaccessible sites within the netting are likely responsible for the consistent findings among different laboratories that aqueous sanitizers are not effective at removing (or inactivating) bacteria on the surfaces of cantaloupes.
- Future research must address the fact that pathogens adhering to produce exist within biofilms that protect the cells from sanitizing solutions.
- Sanitizing treatments that are able to penetrate biofilms including surface treatments such as thermal pasteurization (Annous et al. 2004) or vapor-phase treatments such as chlorine dioxide gas may be potentially useful in food applications.

**Biofilm formation by Other Foodborne Pathogens**
SEM of *Salmonella* on mung bean seed

SEM of treated mung bean seed showing deformed cells of *Salmonella* within biofilms following hot water

SEM of *Salmonella* inoculated sprouts showing attachment and biofilm formation within the crevices on the root (A) and the stem (B) surfaces of mung bean sprout.

SEM of gaseous chlorine dioxide treated sprouts showing deformed cells of *Salmonella* spp. within biofilms on sprout surface.
**E. coli O157:H7 on lettuce**

![Image of E. coli O157:H7 on lettuce]

**Tomato: A) Stem scar,  B) Flower end**

![Image of tomato stem and flower end]

**E. Coli O157:H7 in Apple Calyx**

![Image of E. coli O157:H7 in Apple Calyx]

**E. Coli O157:H7 in Apple Stem**

![Image of E. coli O157:H7 in Apple Stem]
E. coli O157:H7 on Apple Surface

Biofilm formation by Shigella boydii on parsley leaf

E. coli O157:H7 on SS

Salmonella Poona on SS
Thank You