Biofilms and food safety: our current understanding and questions remaining to be answered

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Outline

• **Biofilms and *Listeria* control**
  – Antimicrobial tolerance vs. resistance
  – Biofilm prevention and control strategies

• **What we know**
  – Not all biofilms are created equal
    • Impact of method characteristics (generation method, ecological diversity)
  – *L. monocytogenes* in isolation is not the best biofilm model
  – Importance of cleaning prior to sanitizer application

• **Questions remaining to be answered**
  – Do persistent *L. monocytogenes* strains display increased survival in mixed-species biofilms?
  – Emerging biofilm control strategies

• **Conclusions**
Biofilm definition

**Biofilm**: surface adhered community of microbial cells **entrapped in an exopolymer matrix**. Act as reservoir for microorganisms thereby enhancing the likelihood of their survival in adverse environmental conditions.

**Components**
- Microbial cells
- Protein
- Carbohydrate
- Lipids
- Extracellular DNA

Dr. Amy Wong, UW Madison; ASM Biofilm Collection Donlan & Gibbon
How does *Listeria* persist in production environments?

**Conclusion**

“Our data support the hypothesis that the **2000 human listeriosis outbreak was caused by a *L. monocytogenes* strain that persisted in a food processing facility over 12 years** and show that genome sequencing is a valuable and feasible tool for retrospective epidemiolocal investigations”.

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**BMC Genomics**

*Short-term genome evolution of *Listeria monocytogenes* in a non-controlled environment*

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Biofilm prevention and control resources
Antimicrobial tolerance vs. resistance

“There is no evidence that proper use of sanitizers in food manufacturing will lead to development of resistant microorganisms”.

Implications of “sanitizer resistance”: an ongoing debate

In favor

• Tolerance of *Listeria monocytogenes* to Quaternary Ammonium Sanitizers Is Mediated by a Novel Efflux Pump Encoded by *emrE*

• Genetic characterization of plasmid-associated BAC resistance determinants in *L. monocytogenes*

• Tolerance to QAC disinfectants may enhance growth of *L. monocytogenes* in the food industry

Against

• Industrial disinfectants do not select for resistance in *L. monocytogenes* following long term exposure

• Failure of foodborne pathogens to develop resistance to sanitizers following repeated exposure to common sanitizers

• Resistance of Lm biofilms to sanitizing agents in a simulated food processing environment
What we know
Experimental factors affecting biofilm development

1. Ecological diversity
2. Nutrients, time/temperature
3. Biofilm generation method
4. Surface characteristics (hydrophobicity, charge)
There are many ways to grow a biofilm

Static methods
- Microtiter plate assay
- Static growth method
- Chamber slide systems

Dynamic systems
- Continuous flow stirred tank reactor

Take away: Method matters!
EPA/ASTM Approved Biofilm Methods

EPA standards for biofilm claims

- EPA Microbiology Laboratory Branch (MLB) SOP MB-19, “Growing a Biofilm using the CDC Biofilm Reactor,” and

ASTM Standards

- E2562-17: Standard Test Method for Quantification of Pseudomonas aeruginosa Biofilm Grown with High Shear and Continuous Flow using CDC Biofilm Reactor
Impact of method characteristics on chemical sanitizer efficacy

Method characteristics impact study outcomes
- Surface area to volume ratio
- Biofilm cell density
- Ecological diversity

Example:
- Two studies reporting peracetic acid dose (mg min l\(^{-1}\)) required for 3 \(\log_{10}\) reduction against \(P.\) aeruginosa
- 5,000 fold difference in dose
**L. monocytogenes** in isolation is not the best biofilm model

A. (Mosquera-Fernández, Rodríguez-López, Cabo, & Balsa-Canto, 2014)
B. (Olszewska, Zhao, & Doyle, 2016)
C. (Rieu et al., 2008)
Figure 1. *L. monocytogenes* does not form biofilms at 21°C on stainless steel using EPA/ASTM approved methods. **Confocal microscopy; Live/Dead stain.**
Working towards a more industrially relevant biofilm model

Dual-culture
- *Pseudomonas aeruginosa* (ATCC 15442)
- *Listeria monocytogenes* (persistent retail deli isolate)

Produced using CDC Biofilm Reactor at 21°C on stainless steel coupons

Moorman, Eric. Unpublished
Figure 2. Poor performance of chemical sanitizers against *L. monocytogenes* and *P. aeruginosa* dual-species biofilms at typical food contact surface concentrations for 1-minute per 40 CFR 180.940

EPA standard: 6 log$_{10}$ reduction
Figure 3. Efficacy of commercial quaternary ammonium compound sanitizer against dual species (*P. aeruginosa* and *L. monocytogenes*) biofilm.

EPA standard: 6 log\textsubscript{10} reduction
Figure 4. Inability of a commercial sanitizer (peracetic acid + hydrogen peroxide) to diffuse throughout entire biofilm illustrated by confocal laser scanning microscopy.
Questions remaining to be answered

• Are persistent strains of *L. monocytogenes* better suited to survive in mixed-species biofilms?
• Emerging strategies for biofilm prevention
  – Should we be rotating sanitizers?
## Sanitizer rotation

Table 1. USDA FSIS recommendation; one chemistry used on weekdays and a different chemistry over the weekend.

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Table 2. Alternating two product chemistries every other day.

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Sanitizer rotation on funding agencies radar

A good hygiene program may include changeouts of sanitization chemicals for areas (e.g., floor drains) that are susceptible to Lm contamination, but there is a lack of scientific publications to support or refute a rotating sanitization program. What is the effect of the sanitizing agent and its performance, and what other effects may mask what is really happening with the sanitizer? Are micro-environments and/or harborage sites being built up (e.g., due to hard-water scaling) that the sanitizer may even be contributing to or cannot overcome?
Questions remaining to be answered

• Are persistent strains of *L. monocytogenes* better suited to survive in mixed-species biofilms?
• Emerging strategies for biofilm prevention
  – Should we be rotating sanitizers?
  – Competitive exclusion
Questions remaining to be answered

- Are persistent strains of *L. monocytogenes* better suited to survive in mixed-species biofilms?
- Emerging strategies for biofilm prevention
  - Should we be rotating sanitizers?
  - Competitive exclusion
- Emerging strategies for biofilm control
  - Dual (or more) mode of action antimicrobials
  - Molecular methods for biofilm detection
Conclusions

- Biofilm method characteristics impact biofilm challenge studies.
- Biofilms don’t exist in pure culture. Too much of a reductionist approach. Work towards poly-microbial biofilms for applied research.
- Chemical sanitizers at food contact surface concentrations ineffective against spoilage and pathogenic bacteria in a biofilm state.
- Ineffectiveness of chemical sanitizers against biofilms highlights importance of effective cleaning prior to sanitizing.
- Experimental evidence needed to support or refute necessity of sanitizer rotation.
- **Importance of the basics.** *Listeria* control and biofilm control multifaceted issue that requires comprehensive food safety programs (GMPs, hygienic zoning, hygienic equipment design, seek and destroy environmental monitoring, etc.). 3-A sanitary standards next week.
References


Thank you!

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