NC STATE UNIVERSITY College of Agriculture and Life Sciences

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

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Solutions for TODAY
Planning for TOMORROW

May 6-9, 2019 | Rosemont, IL
Donald E. Stephens Convention Center

Outline

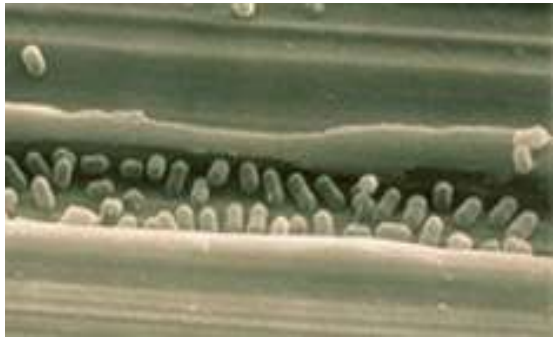
- **Biofilms and *Listeria* control**
  - Antimicrobial tolerance vs. resistance
  - Biofilm prevention and control strategies
- **What we know**
  - Not all biofilms are created equal
    - Impact of method characteristics (generation method, ecological diversity)
  - *L. monocytogenes* in isolation 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**

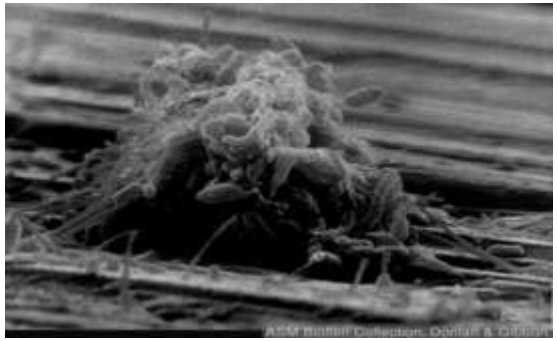
Biofilm definition

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?

BMC Genomics BioMed Central

Research article Open Access

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

Renato H Orsi[1], Mark L Borowsky[2,7], Peter Lauer[3], Sarah K Young[2], Chad Nusbaum[2], James E Galagan[2,4], Bruce W Birren[2], Reid A Ivy[1], Qi Sun[5], Lewis M Graves[6], Bala Swaminathan[6] and Martin Wiedmann*[1]

Address: [1]Department of Food Science, Cornell University, Ithaca, USA, [2]Genome Sequencing and Analysis Program, Broad Institute of MIT and Harvard, Cambridge, USA, [3]Anza Therapeutics, Concord, USA, [4]Department of Biomedical Engineering and Microbiology, Boston University, USA, [5]Computational Biology Services Unit, Center for Advanced Computing, Cornell University, Ithaca, USA, [6]Enteric Diseases Laboratory Branch, Division of Foodborne, Bacterial and Mycotic Diseases, Centers for Disease Control and Prevention, Atlanta, USA and [7]Department of Molecular Biology, Massachusetts General Hospital, Boston, USA

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".

Biofilm prevention and control resources

Critical Reviews in Food Science and Nutrition, 53:401–417 (2013)

ISSN: 1040-8398 / 1549-7852 online
DOI: 10.1080/10408398.2011.561378

Taylor & Francis

An Ecological Perspective of *Listeria monocytogenes* Biofilms in Food Processing Facilities

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doi:10.4315/0362-028X.JFP-16-311
Published 2019 by the International Association for Food Protection

Mini-Review

Biofilms and Meat Safety: A Mini-Review

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U.S. Department of Agriculture, Agricultural Research Service, U.S. Meat Animal Research Center, P.O. Box 166, State Spur 18D, Clay Center, Nebraska 68933, USA

MS 16-311: Received 3 July 2016/Accepted 20 September 2016/Published Online 2 January 2019

Food Control 44 (2014) 161–169
Contents lists available at ScienceDirect
Food Control
journal homepage: www.elsevier.com/locate/foodcont

Review

Persistence of foodborne pathogens and their control in primary and secondary food production chains

Marianne Halberg Larsen [a], Marion Dalmasso [b], Hanne Ingmer [a], Solveig Langsrud [c], Mindaugas Malakauskas [d], Anneluise Mader [e], Trond Møretrø [c], Sonja Smole Možina [f], Kathrin Rychli [g], Martin Wagner [g], R. John Wallace [h], Jurgen Zentek [i], Kieran Jordan [b,*]
[a] Department of Veterinary Disease Biology, University of Copenhagen, Denmark
[b] Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland
[c] Nofima, The Norwegian Institute of Food, Fisheries and Aquaculture Research, Ås, Norway
[d] Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania
[e] Institute of Animal Nutrition, Freie Universität Berlin, Germany
[f] Department of Food Science and Technology, Biotechnical Faculty, University of Ljubljana, Slovenia
[g] Institute for Milk Hygiene, Milk Technology and Food Science, University of Veterinary Medicine Vienna, Austria
[h] Rowett Institute of Nutrition and Health, University of Aberdeen, Bucksburn, UK

Food Microbiology 45 (2015) 167–178
Contents lists available at ScienceDirect
Food Microbiology
journal homepage: www.elsevier.com/locate/fm

Biofilm-associated persistence of food-borne pathogens

A. Bridier [a], P. Sanchez-Vizuete [b,c], M. Guilbaud [b,c], J.-C. Piard [b,c], M. Naïtali [b,c], R. Briandet [b,c,*]
[a] Irstea, UR HBAN, Antony, France
[b] Inra, UMR 1319 Micalis, Jouy-en-Josas, France
[c] AgroParisTech, UMR Micalis, Massy, France

Antimicrobial tolerance vs. resistance

Scientific Status Summary

Resistance and Adaptation to Food Antimicrobials, Sanitizers, and Other Process Controls

This IFT Scientific Status Summary discusses the potential for microorganisms to become resistant to antimicrobials and sanitizers used in food processing.

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

Davidson PM, et al. 2002. Food Technology 56(11):69-78

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

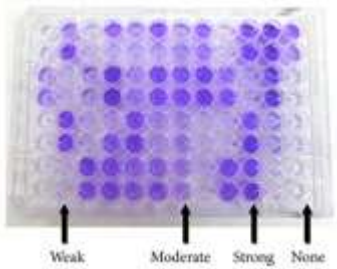
What we know

Experimental factors affecting biofilm development

1. Ecological diversity
2. Nutrients, time/temperature
3. Biofilm generation method
4. Surface characteristics (hydrophobicity, charge)

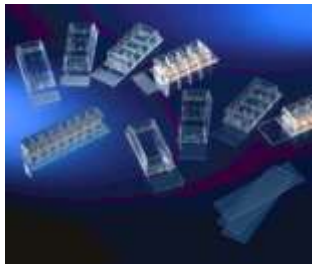
There are many ways to grow a biofilm

Static methods



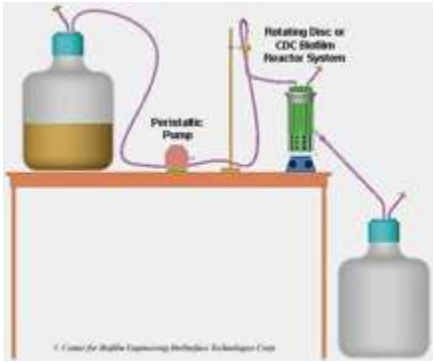
Microtiter plate assay

Static growth method



Chamber slide systems

Dynamic systems



Continuous flow stirred tank reactor

Take away: Method matters!

EPA/ASTM Approved Biofilm Methods

CBR 90 CDC Biofilm Reactor

EPA standards for biofilm claims

- EPA Microbiology Laboratory Branch (MLB) SOP MB-19, “Growing a Biofilm using the CDC Biofilm Reactor,” and
- EPA MLB SOP MB-20, “Single Tube Method for Determining the Efficacy of Disinfectants against Bacterial Biofilms.”

ASTM Standards

- E2562-17: Standard Test Method for Quantification of Pseudomonas aeruginosa Biofilm Grown with High Shear and Continuous Flow using CDC Biofilm Reactor
- E2871-13: Standard Test Method for Evaluating Disinfectant Efficacy Against Pseudomonas aeruginosa Biofilm Grown in CDC Biofilm Reactor Using Single Tube Method1

Impact of method characteristics on chemical sanitizer efficacy

AAC Accepted Manuscript Posted Online 25 February 2019
Antimicrob. Agents Chemother. doi:10.1128/AAC.00020-19

1 **Measuring Antimicrobial Efficacy against Biofilms: A Meta-Analysis**

2

3 Running Title: Measuring Antimicrobial Efficacy against Biofilms

4

5 Philip S. Stewart[1,2*] and Albert E. Parker[1,3]

6

7

8 Addresses

9 [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT

10 [2]Chemical and Biological Engineering, Montana State University, Bozeman, MT

11 [3]Mathematical Sciences, Montana State University, Bozeman, MT

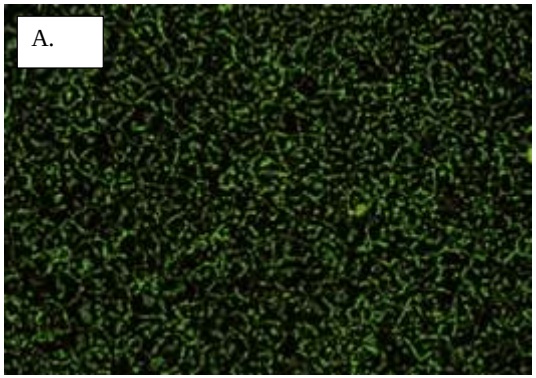
Method characteristics impact study outcomes

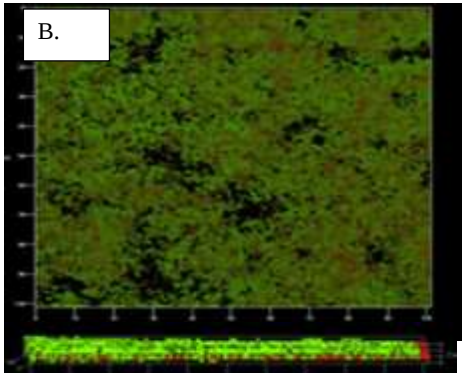
- Surface area to volume ratio
- Biofilm cell density
- Ecological diversity

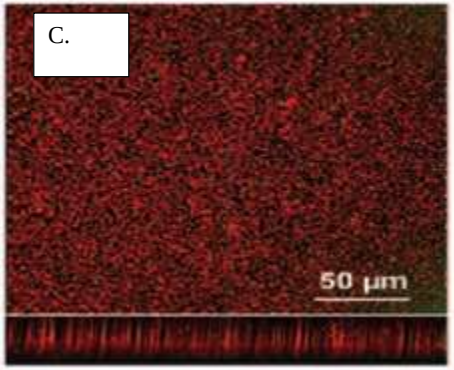
Example:

- 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

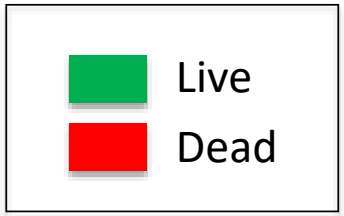
L. monocytogenes in isolation is not the best biofilm model



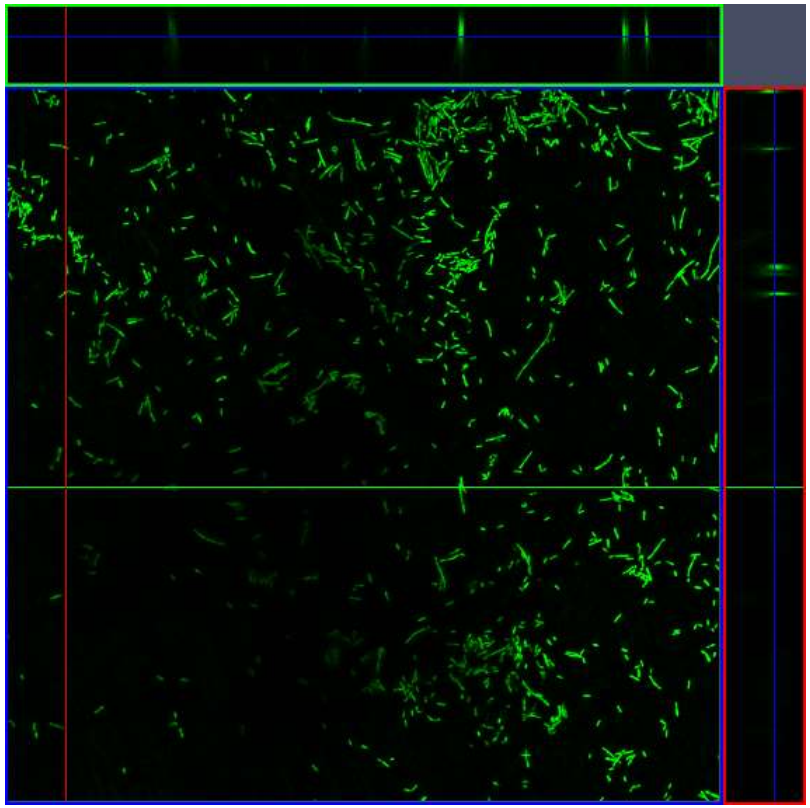


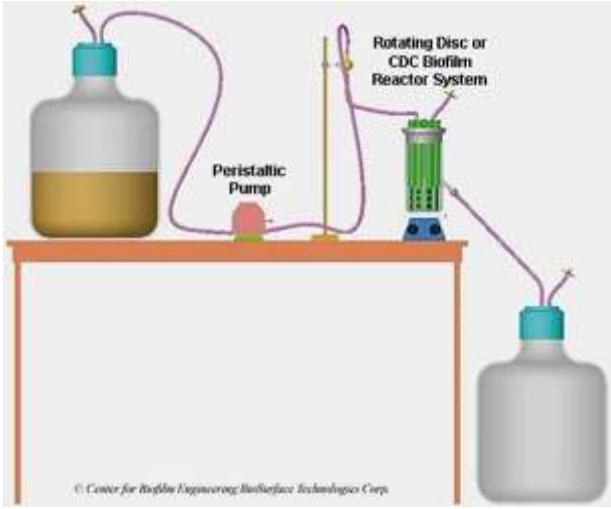


Static



A. (Mosquera-Fernández, Rodríguez-López, Cabo, & Balsa-Canto, 2014)
B. (Olszewska, Zhao, & Doyle, 2016)
C. (Rieu et al., 2008)





CDC Biofilm Reactor

Figure 1. *L. monocytogenes* does not form biofilms at 21°C on stainless steel using EPA/ASTM approved methods.
**Confocal microscopy; Live/Dead stain.

Live
Dead

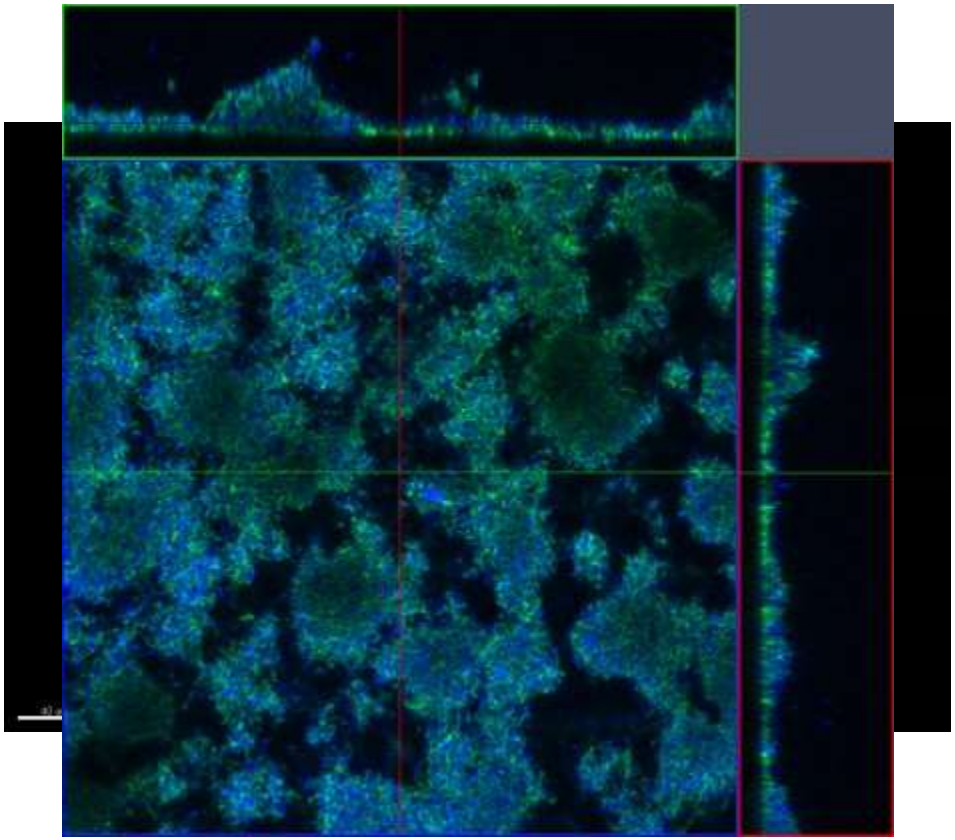
Moorman, Eric. Unpublished

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

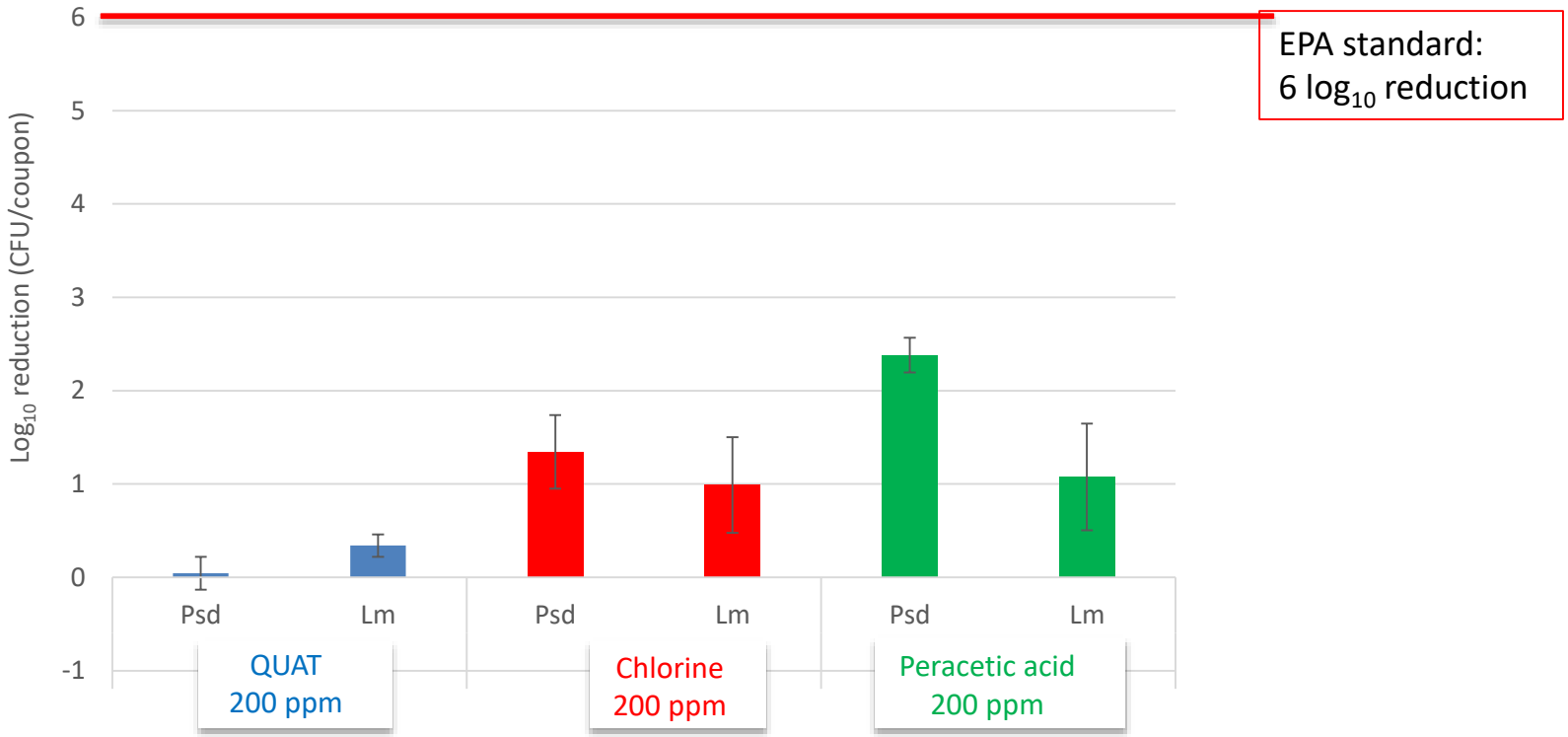


Figure 2. Poor performance of chemical sanitizers against *L. monocytogenes* and *P. aeruginosa* dual-species biofilms at typical food contact surface concentrations for 1-minute per **40 CFR 180.940**

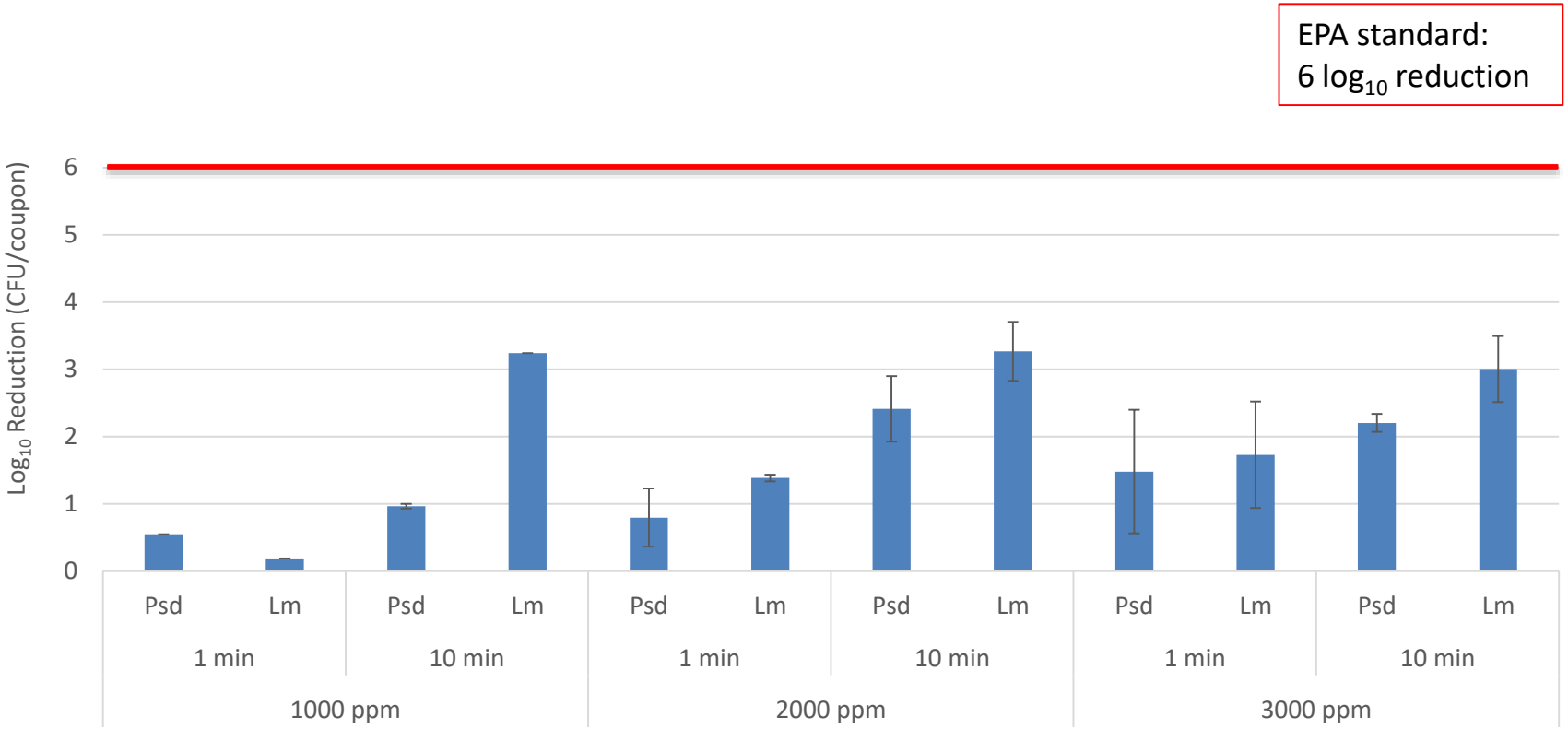
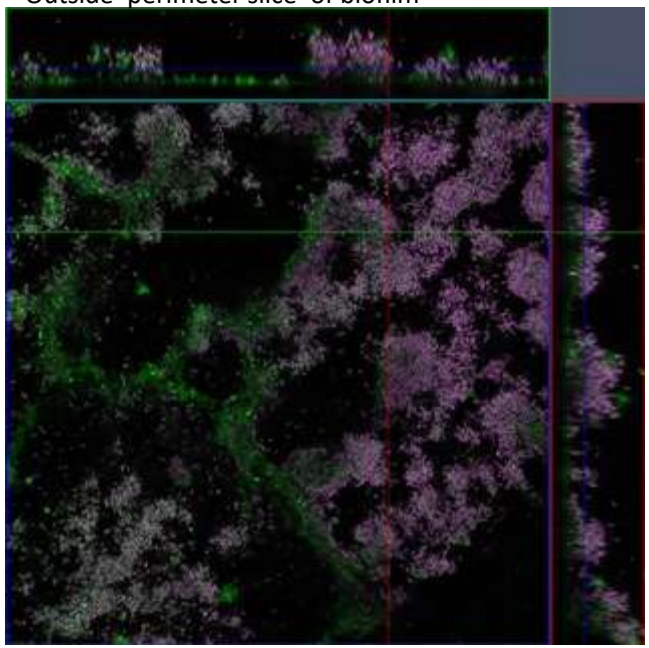


Figure 3. Efficacy of commercial quaternary ammonium compound sanitizer against dual species (*P. aeruginosa* and *L. monocytogenes*) biofilm.

Visual evidence of why cleaning is important

A.
Outside 'perimeter slice' of biofilm



B.
Middle slice of biofilm

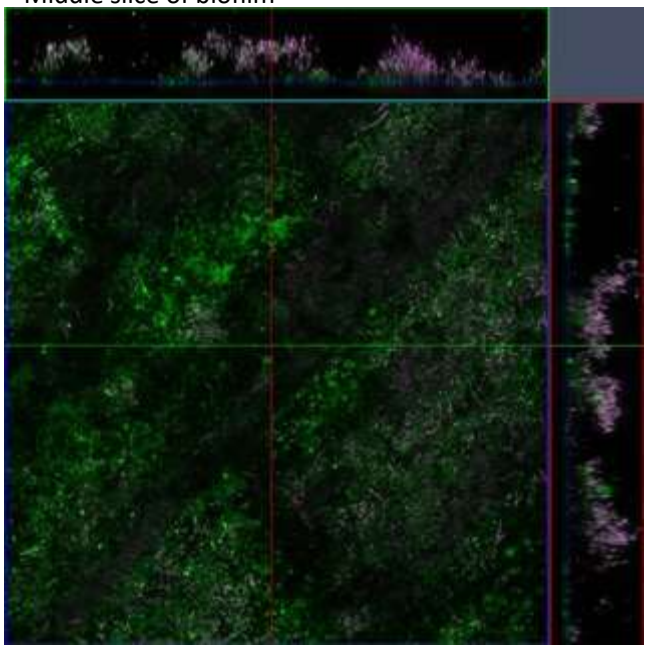
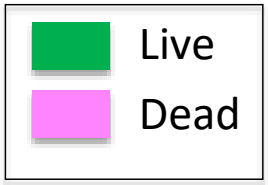


Figure 4. Inability of a commercial sanitizer (peracetic acid + hydrogen peroxide) to diffuse throughout entire biofilm illustrated by confocal laser scanning microscopy.



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.

	Day						
	1	2	3	4	5	6	7
Product A	A	A	A	A	A	A	A
Product B	B	B	B	B	B	B	B
Sanitizer Rotation	A	A	A	A	A	B	B

Table 2. Alternating two product chemistries every other day.

	Day						
	1	2	3	4	5	6	7
Product A	A	A	A	A	A	A	A
Product B	B	B	B	B	B	B	B
Sanitizer Rotation	A	B	A	B	A	B	A

Sanitizer rotation on funding agencies radar

cps CENTER for PRODUCE SAFETY | 2019 Request for Proposals

GRANTS PROGRAM: CENTER FOR PRODUCE SAFETY

2019 RFP Research Priorities Summary – September 11, 2018

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

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Thank you!

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