

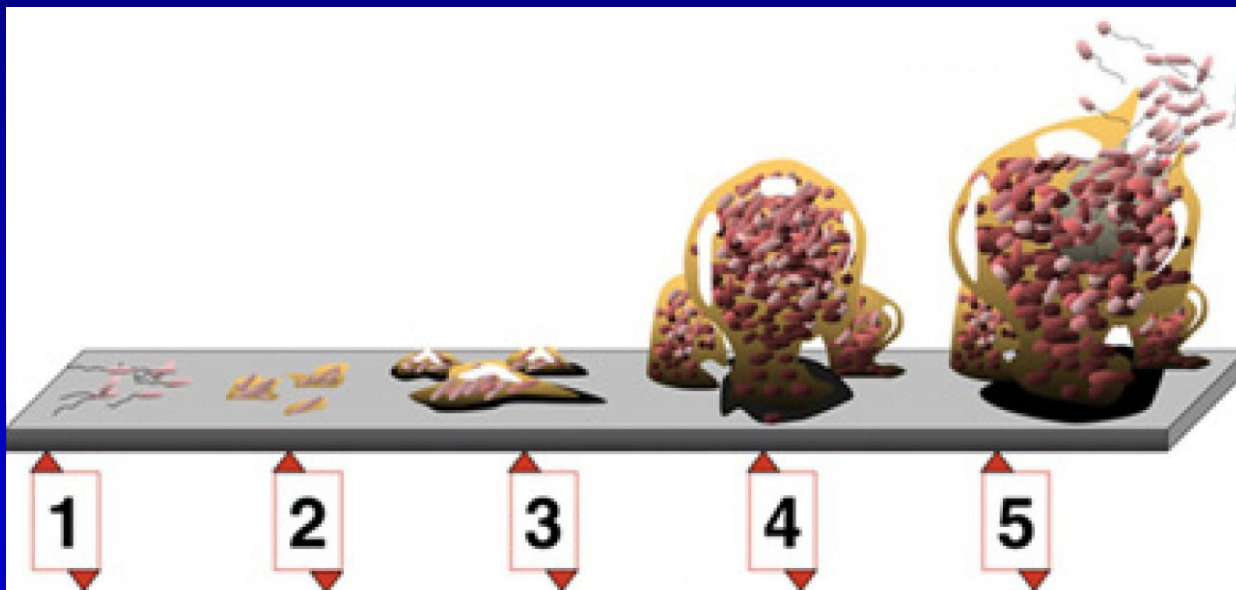
Efficacy of a novel bactericide for elimination of biofilm in food processing facilities

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Processing & technology**

Biofilm

Biofilms are single or multi layers of microorganisms embedded in their own extracellular polymeric substances (EPS) which associate with a solid surface



1. Initial attachment
2. Irreversible attachment
3. Microcolony
4. Maturation
5. Dispersion

Biofilm

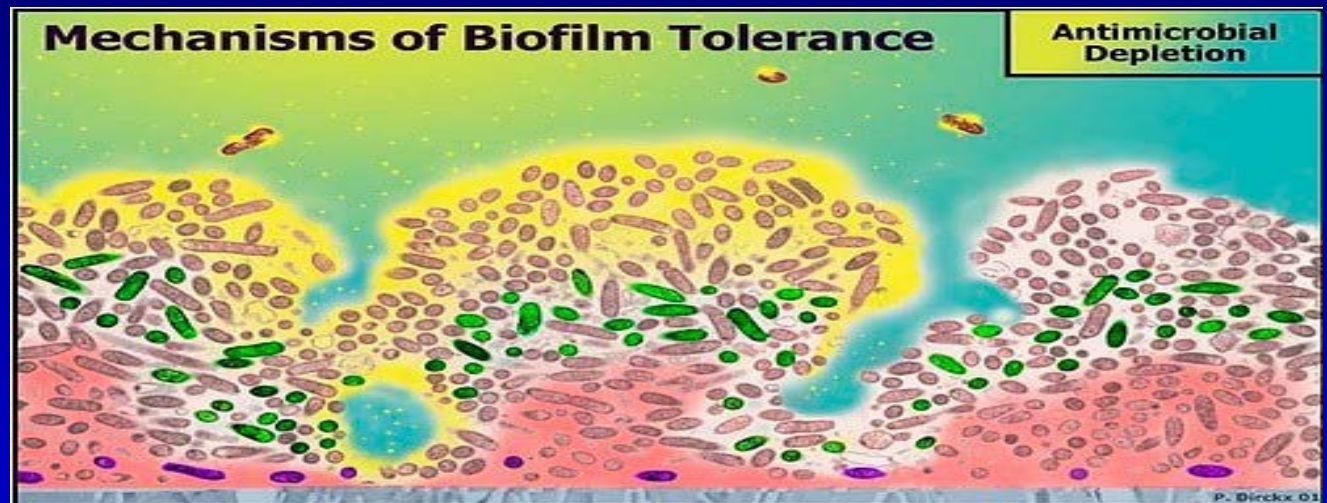
- Predominant matrix for bacterial growth
- ~ 80% of all bacterial infections are biofilm-associated



Problem

Cells in biofilms are **more resistant** to cleaning and disinfection processes than their planktonic counterparts

- EPS
- Slow growth
- Gene expression



Development of a novel bactericide

- Effective, easy-to-use, cost-efficient, and environmentally friendly.
- Levulinic acid and sodium dodecyl sulfate (SDS) were tested in combination at different concentrations and temperatures (8 or 82°C) either as liquid or foam for their killing effects on human pathogens formed as biofilms.

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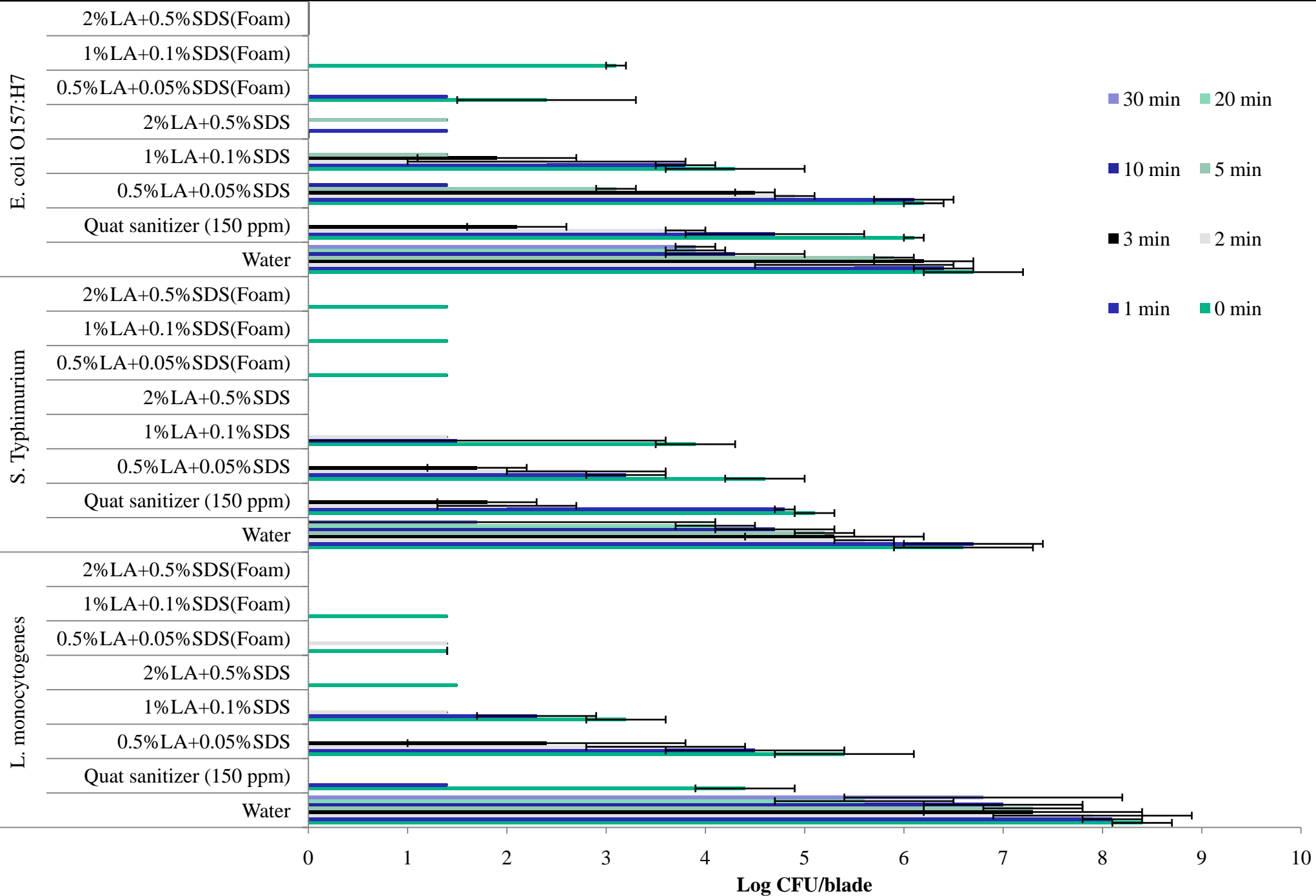
- U. S. Patent and Trademark Office. U.S. patent number 8,722,123. Issue date: May 13, 2014. Antimicrobial Composition and Use as Food Treatment.
- More than 10 papers regarding its application on dental, produce, poultry, and meat have been published.
- Marked by HealthPro Brands.

Results

Inactivation of *S. Enteritidis* on biofilm by 3% levulinic acid plus 2% SDS as a foam at 21°C

Coupon material	Chemical solution	<i>Salmonella</i> Enteritidis counts (log CFU/cm ²) at minutes					
		0	1	2	5	10	20
Stainless steel	PBS, pH 7.2	7.3	7.7	8.0	7.2	8.0	7.3
	3% levulinic acid plus 2% SDS, pH 2.8	8.3	6.7	6.8	4.0	2.3	2.0
Polyvinyl chloride	PBS, pH 7.2	8.0	8.3	8.2	8.6	8.2	8.6
	3% levulinic acid plus 2% SDS, pH 2.8	5.8	4.9	3.1	3.0	3.2	1.0
Nitrile rubber	PBS, pH 7.2	7.4	7.6	7.6	7.5	7.4	7.2
	3% levulinic acid plus 2% SDS, pH 2.8	7.1	4.1	3.5	3.3	2.4	1.7
Glass	PBS, pH 7.2	8.0	8.5	7.7	7.9	7.8	7.9
	3% levulinic acid plus 2% SDS, pH 2.8	4.9	4.4	3.3	3.5	1.7	1.7
Ultra-high molecular weight polyethylene	PBS, pH 7.2	6.9	6.9	6.7	6.4	6.7	6.3
	3% levulinic acid plus 2% SDS, pH 2.8	5.4	4.6	2.9	2.3	1.7	1.7





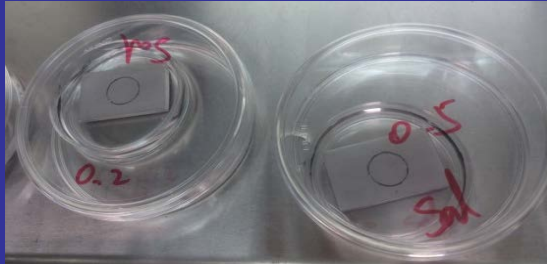


Before chemical treatment

1. The *Salmonella* isolation rate was 19% (19/100).
2. The fecal coliform population averaged 6.8 CFU/25 cm² log (ranged from 3 to 9.3 log CFU/25 cm²).
3. The total aerobic bacteria count averaged 7.9 log CFU/25 cm² (ranged from 5.7 to 9.9 log CFU/25 cm²).

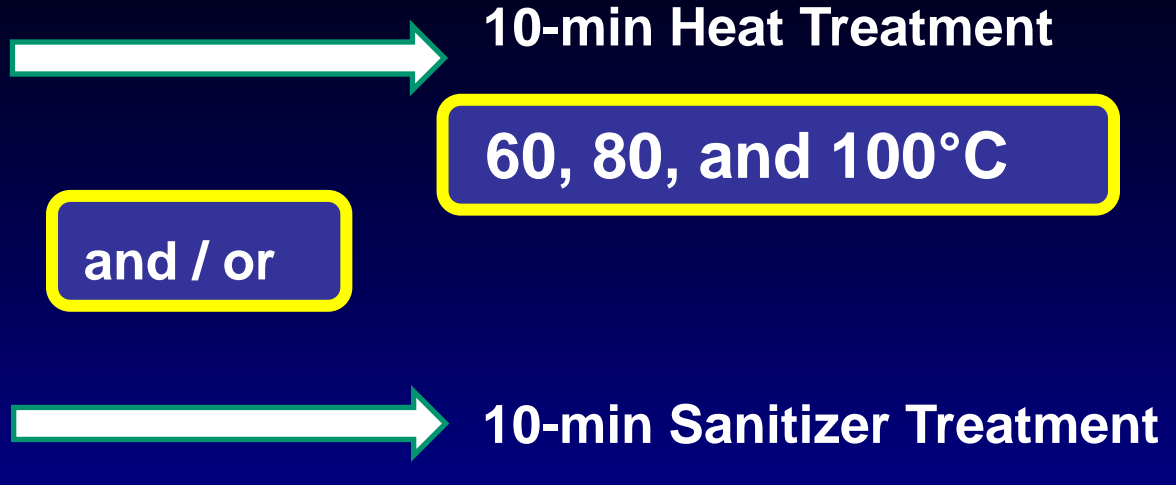
After treatment with 3% levulinic acid plus 2% SDS as a foam

1. The *Salmonella* isolation rate was 1% (1/100).
2. The fecal coliform population averaged 1.15 log CFU/25 cm² (ranged from 0.6 to 3.1 log CFU/25 cm²), a 5.6 log CFU/25 cm² reduction.
3. The aerobic bacteria count averaged 4.8 log CFU/cm² (ranged from 1.6 to 7.5 log CFU/25 cm²), a 3.2 log reduction.



- 72 h
- 100% RH
- 21°C

Biofilm Growth



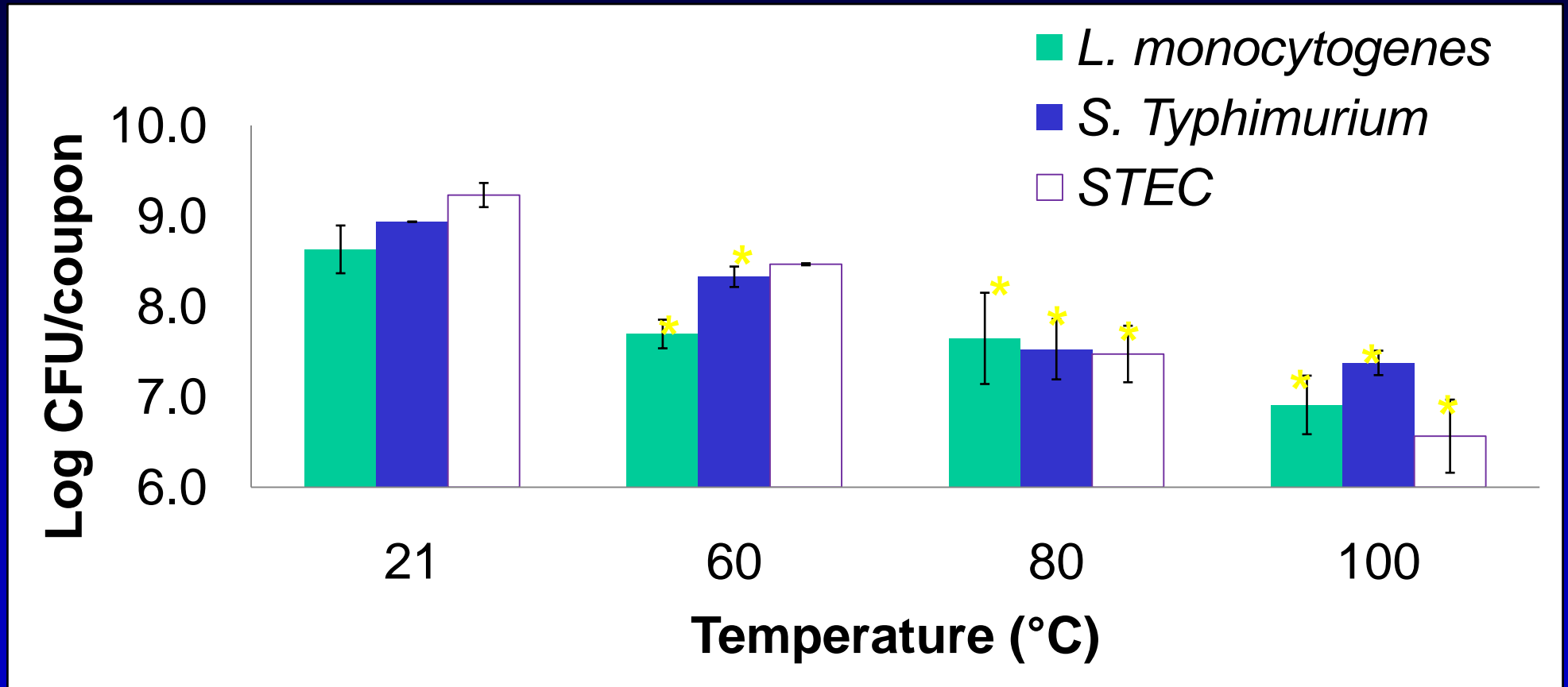
QAC, 150 ppm quaternary ammonium-based sanitizer;
LA, 3% lactic acid;
SHC, 100 ppm sodium hypochlorite;
HP, 2% hydrogen peroxide;
LVA, 3% levulinic acid;
SDS, 2% sodium dodecyl sulfate
0.5% LVA + 0.05% SDS
1% LVA + 0.1% SDS
3% LVA + 2% SDS

Acid Shock

Bacteria counts of selective agar plates and TSA of 72-h biofilms of *L. monocytogenes*, *S. Typhimurium*, and STEC formed on stainless steel after a 10-min treatment with 3% lactic acid (pH 2.2) or 3% levulinic acid (pH 2.7).

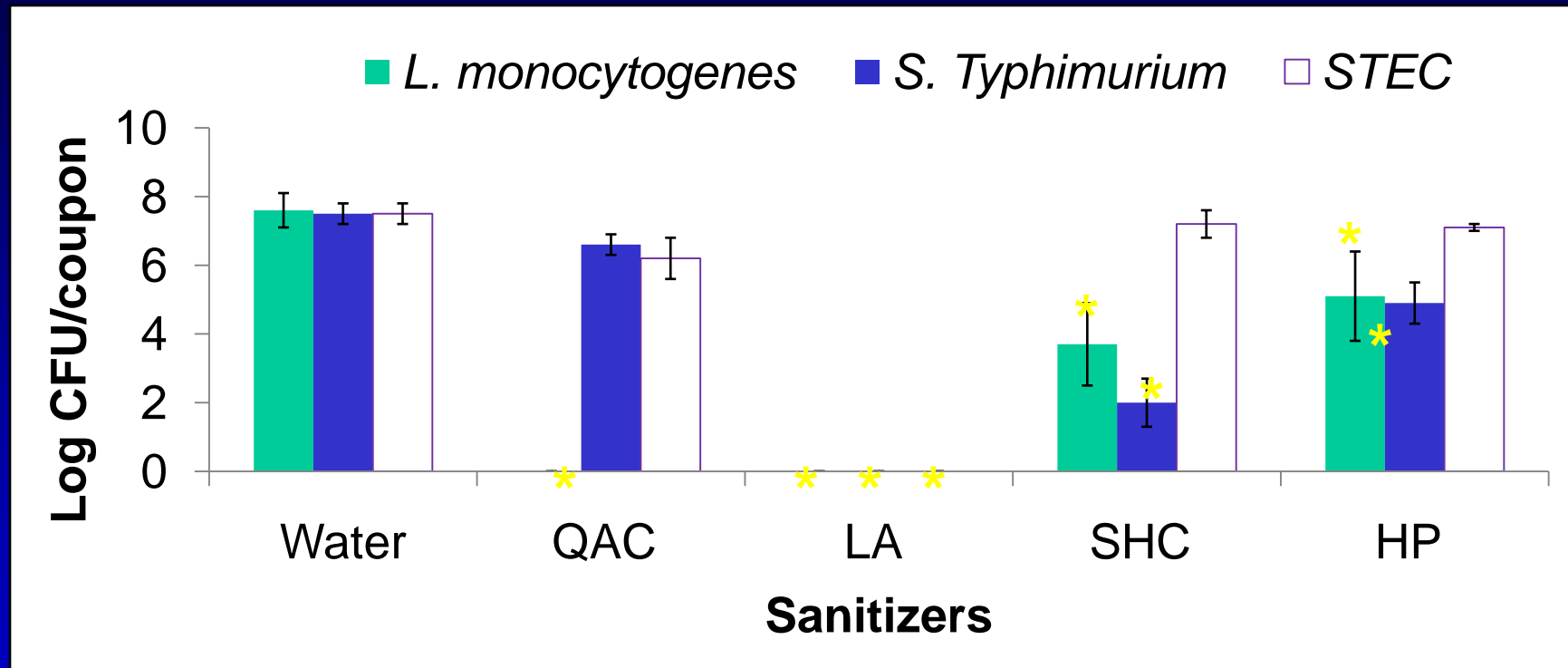
Pathogen	Acid (3%)	Count (log CFU/coupon)		% injured cells
		Selective agar plates	TSA	
<i>L. monocytogenes</i>	Water	8.6 ± 0.3	8.6 ± 0.2	0
	Lactic acid	2.1 ± 0.2	4.4 ± 0.2	52.3%
	Levulinic acid	8.3 ± 0.1	8.3 ± 0.2	0
<i>S. Typhimurium</i>	Water	8.9 ± 0.0	9.0 ± 0.0	1.1%
	Lactic acid	< 1.7	3.1 ± 0.0	> 45.2%
	Levulinic acid	4.4 ± 0.3	8.6 ± 0.2	48.8%
STEC	Water	9.2 ± 0.1	9.3 ± 0.1	1.1%
	Lactic acid	< 1.7	5.7 ± 0.1	> 70.2%
	Levulinic acid	6.4 ± 0.4	8.8 ± 0.1	27.3%

10-min Heat Treatment



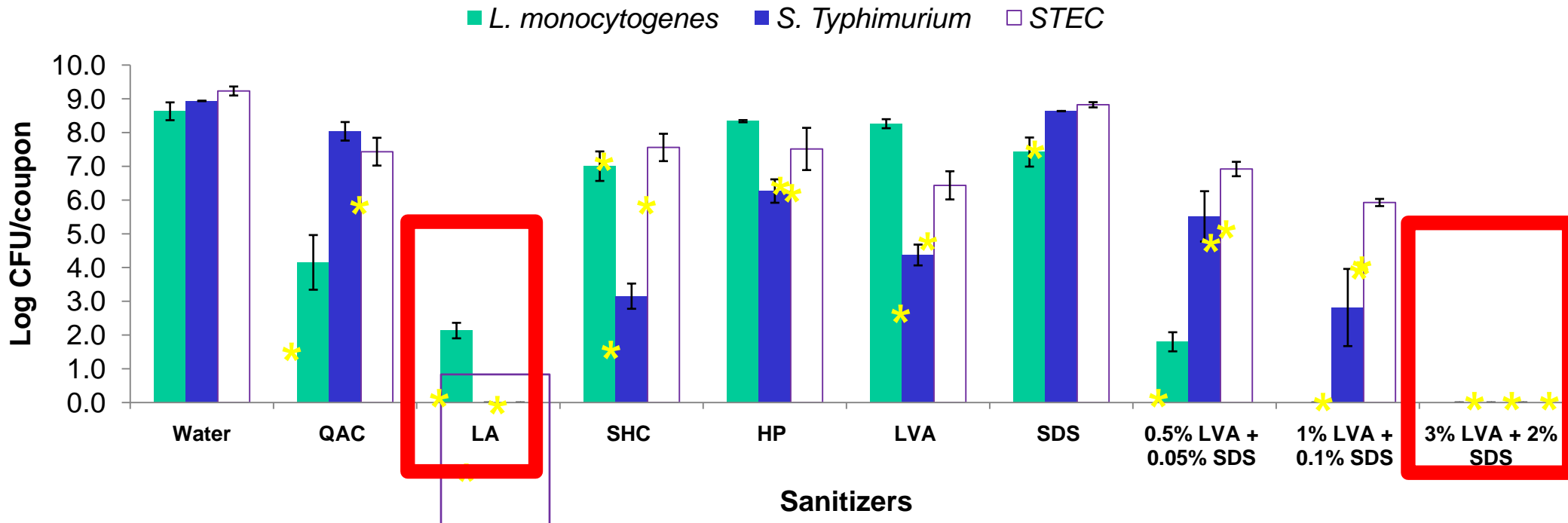
Inactivation of 72-h biofilms of *L. monocytogenes*, *S. Typhimurium*, and STEC formed on stainless steel after receiving 10-min heat treatment at 21, 60, 80 or 100°C.

80°C + Sanitizer Treatment

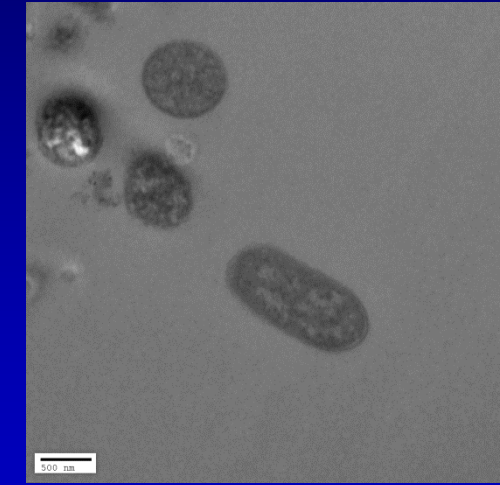
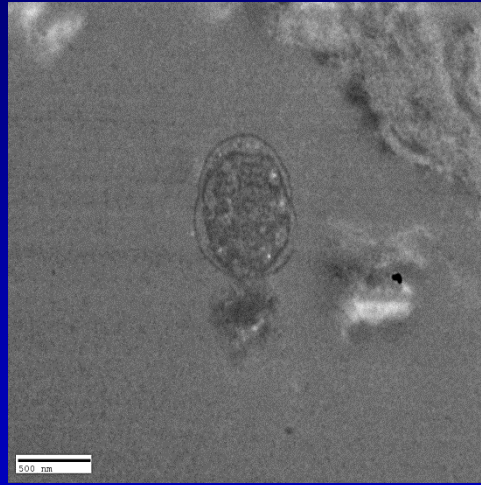
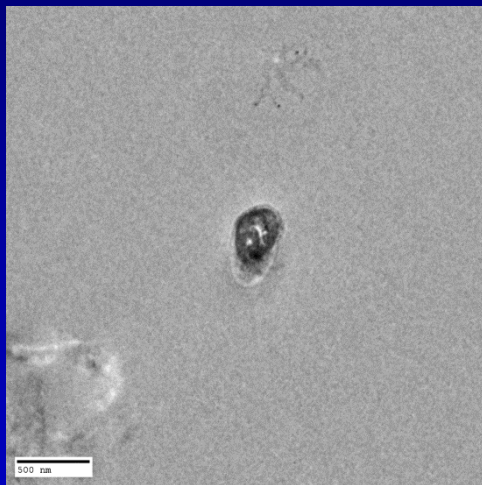
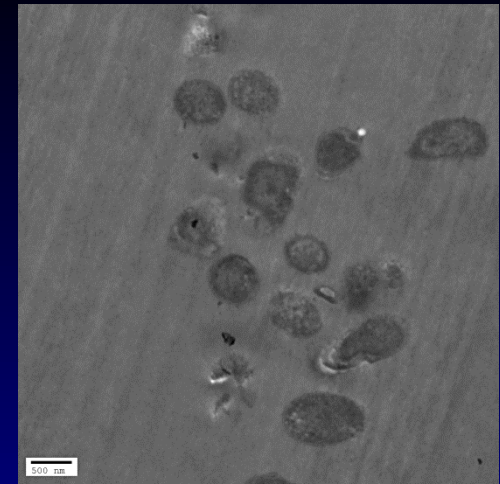
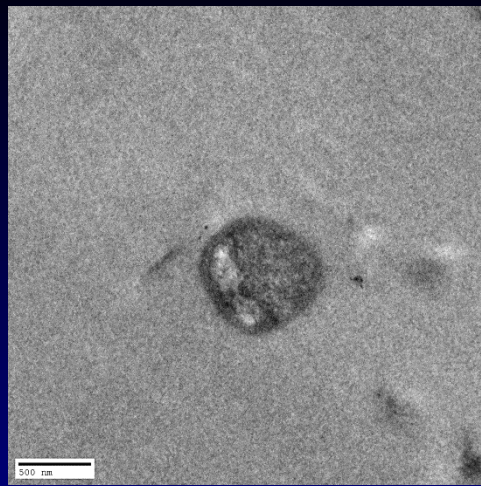
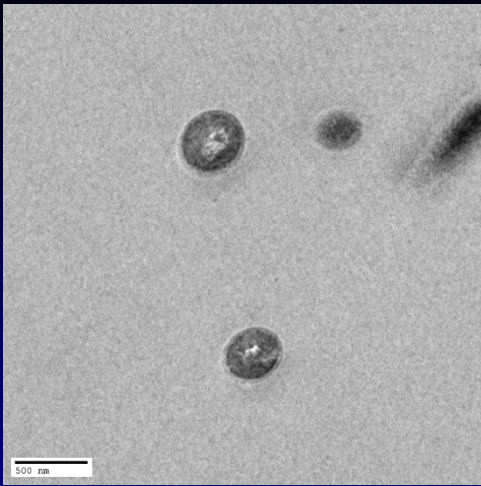


Inactivation of 72-h biofilms of *L. monocytogenes*, *S. Typhimurium*, and STEC formed on stainless steel after receiving a 10-min 80°C treatment and a subsequent 10-min sanitizer treatment.

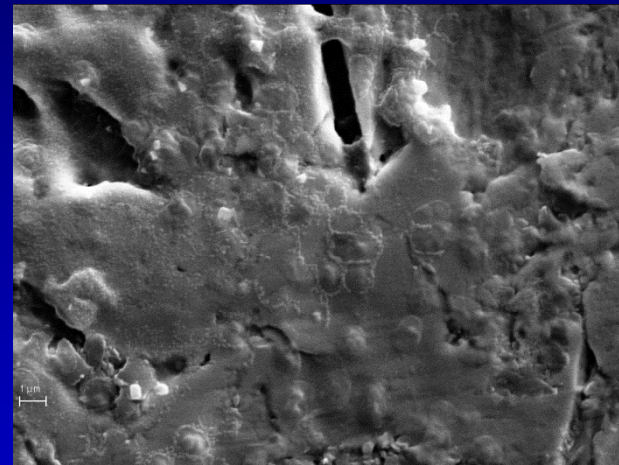
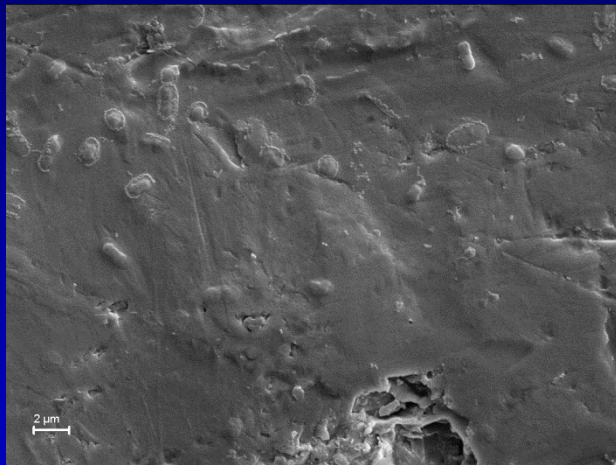
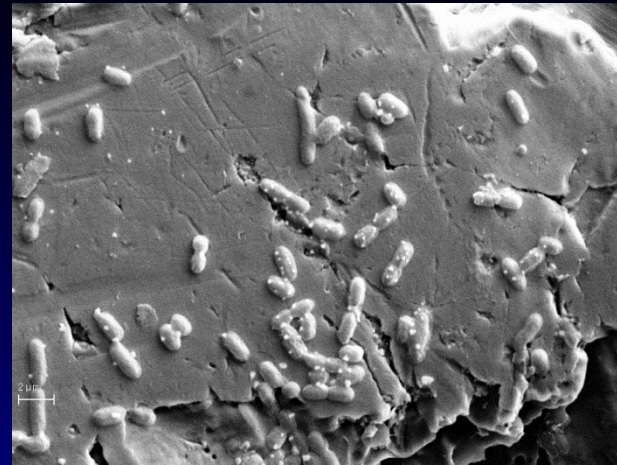
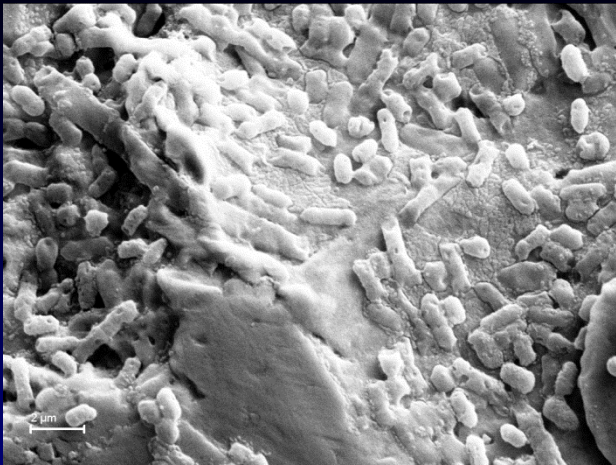
10-min Sanitizer Treatment



Inactivation of 72-h biofilms of *L. monocytogenes*, *S. Typhimurium*, and STEC formed on stainless steel after receiving a 10-min sanitizer treatment.



Representative images made by TEM of biofilms formed by *Listeria monocytogenes* (A, B), *Salmonella* (C, D), and STEC (E, F) after a 10-min treatment with water (control, A, C, E), and 0.5% levulinic acid + 0.05% SDS (B, D, F).



Representative images made by SEM of biofilms formed by *Salmonella* after a 10-min treatment with water (control, A), 0.5% levulinic acid + 0.05% SDS (B), 1% levulinic acid + 0.5% SDS (C), and 3% levulinic acid + 2% SDS (D).

Conclusions

- All foodborne pathogens as tested, including *E. coli* O157:H7, *Salmonella*, *L. monocytogenes* have the potential to form the biofilms on the surface of materials that commonly used in the processing facilities.
- Levulinic acid-based sanitizer either in liquid or in foam has strong ability to remove or eliminate the biofilms.

Acknowledgements

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