Listeria develops reduced sanitizer sensitivity but not resistance at recommended sanitizer use levels

Contact

Martin Wiedmann
Cornell University
Department of Food Science
607-254-2838
mw16@cornell.edu

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Authors

Anna Sophia Harrand, Martin Wiedmann

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Summary

Listeria monocytogenes can persist in food processing facilities by hiding in difficult-to-clean places in which *Listeria* may only be exposed to dilute sanitizer concentrations. *Listeria* sanitizer tolerance might be due to mutations or resistance genes that can spread across populations, making *Listeria* control more challenging. To assess the occurrence of resistant *Listeria*, isolates collected from the pre- and post-harvest environments are being screened for sanitizer resistance. Further, the project will determine if repeated sanitizer exposure can lead to resistance to increasing sanitizer concentrations. WGS data and bioinformatic tools will be used to identify possible mutations or resistance genes. These data will provide tools for more rapid identification of resistant *Listeria* and will help in the design of improved sanitation strategies.

Benefits to the Industry

The data collected after completion of the project will include (i) frequencies of sanitizer tolerance to three sanitizers among *Listeria* isolates from produce packinghouses, fresh-cut produce facilities, retail, and the pre-harvest environment; (ii) maximum resistance to three sanitizers (BC, NaOCl and PAA) of wildtype strains as well as strains artificially exposed to these sanitizers; and (iii) genes, SNPs, and resistance mechanisms responsible for sanitizer tolerance. The information will allow for rapid detection of resistant *Listeria* isolates and will facilitate improved risk management practices to control *Listeria*. These data, as well as strains from this project will be available to the produce industry and will aid in developing improved sanitation strategies.

Objectives

1. Screen >500 *Listeria* spp. and *L. monocytogenes* isolates from packinghouses and fresh-cut operations for reduced sensitivity to key sanitizers, including quaternary ammonium compounds, sodium hypochlorite and peroxyacetic acid.
2. Perform whole genome sequencing (WGS) of *Listeria* spp. and *L. monocytogenes* strains identified as showing reduced sanitizer sensitivity to identify mutations and gene acquisitions responsible for reduced sanitizer sensitivity.
3. Expose selected strains with reduced sanitizer sensitivity to increasing sanitizer concentrations to determine the potential of these strains to become resistant to sanitizer levels close to the recommended use levels.

Methods

Sanitizer screening. Growth curves are generated for each *Listeria* isolate to identify the time point at which they reach early stationary phase to ensure isolates are in the same growth phase upon sanitizer exposure. Isolates are exposed to 300 ppm benzalkonium chloride (BC), 80 ppm peroxyacetic acid (PAA), or 500 ppm of sodium hypochlorite (NaOCl) for 30 sec, dilutions are spot plated on Brain-Heart Infusion agar and incubated for 24 h and 48 h. Isolates will be identified as tolerant and sensitive based on their log reductions.

Bioinformatics. Isolates will be screened for presence or absence of previously identified resistance genes (e.g., *bcr*ABC, *qacH*, SSI-2), and genomic comparisons will allow for identification of novel genes or single nucleotide polymorphisms (SNPs) associated with a resistant phenotype.

Results to Date

The assembled collection of produce-associated *Listeria* isolates is comprised of 588 isolates, including *L. monocytogenes* and other *Listeria* spp. from pre-harvest and post-harvest environments (**Figure 1**), representing at least 89 different *sigB* allelic types.

The mean log reductions from 24 h and 48 h for 329 isolates do not differ significantly when exposed to BC ($P = 0.07$), but are significantly different when exposed to PAA ($P < 0.005$) and NaOCl ($P < 0.005$) (**Figures 2–4**). The observed log reductions for most isolates (61–76%) fall within one standard deviation from the mean. However, subsets of isolates were identified as (i) "sensitive" (12–21%) with up to 3 log reductions above the mean, and (ii) "tolerant" (12–21%) with up to 3 log reductions below the mean.

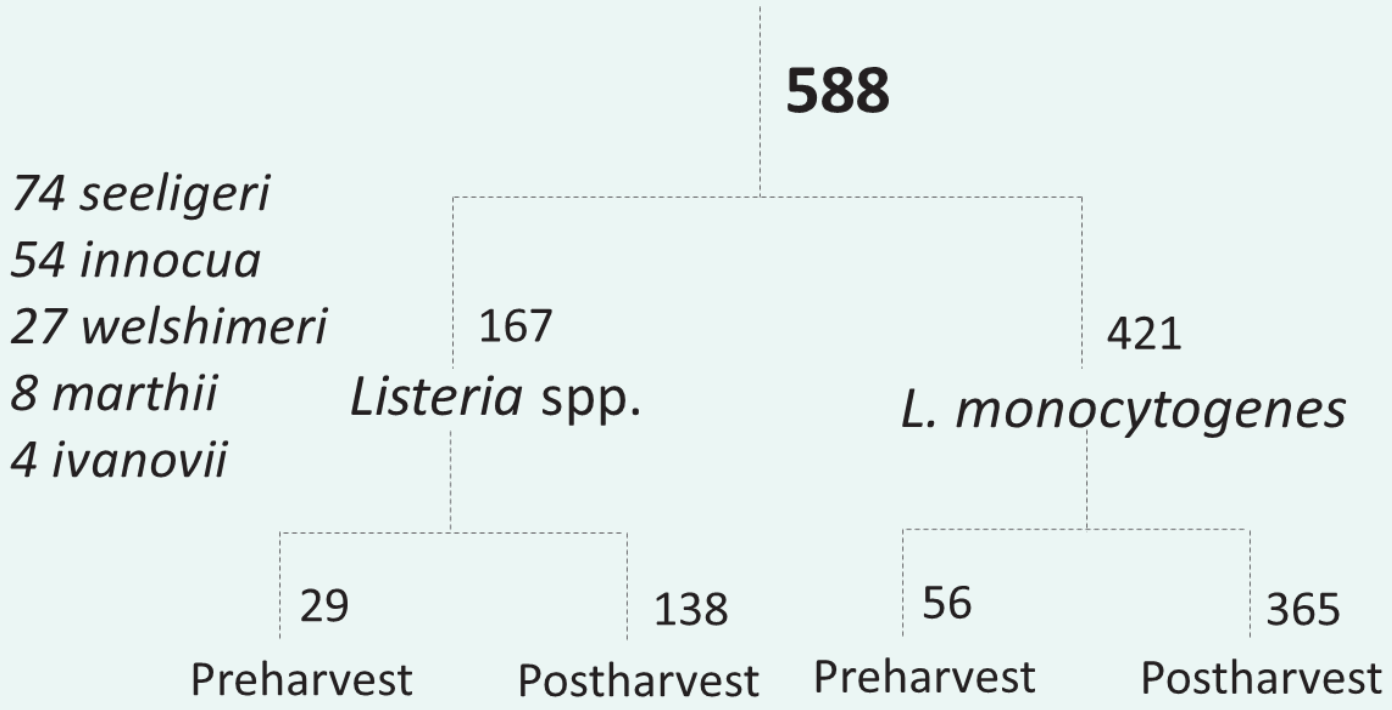
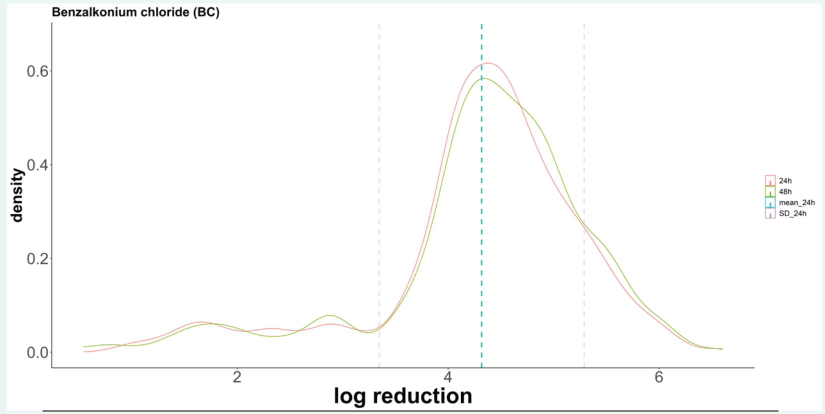
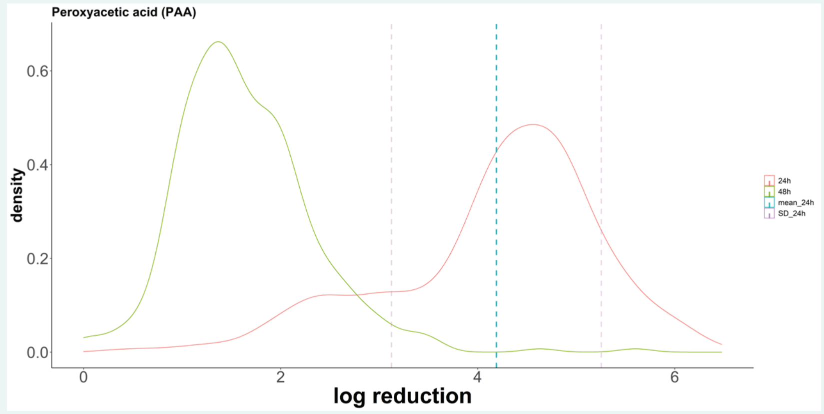


Figure 1. The isolate collection comprises 588 *Listeria* isolates, including *L. monocytogenes* and *L.* spp. isolates from pre-harvest and post-harvest environments. Numbers indicate number of isolates.



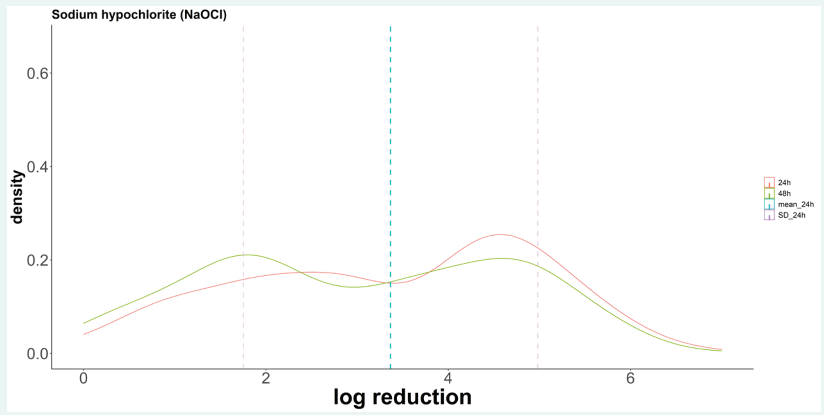
	Mean	Standard deviation	Range for "sensitive" isolates (percent of isolates)	Range for "tolerant" isolates (percent of isolates)
24 h	4.32 log	±0.97 log	5.29 to 6.60 log (12%)	1.00 to 3.35 log (12%)
48 h	4.35 log	±0.99 log	5.35 to 6.52 log (12%)	0.54 to 3.36 log (12%)

Figure 2. Density plot is representing the distribution of 329 *Listeria* isolates (y-axis) and the log reductions (x-axis) after exposure to 300 ppm benzalkonium chloride. Log reductions were determined after 24 h (red line) and 48 h (green line) of incubation. Blue line indicates the population mean of 24 h, and purple lines indicate the standard deviations of 24 h.



	Mean	Standard deviation	Range for "sensitive" isolates (percent of isolates)	Range for "tolerant" isolates (percent of isolates)
24 h	4.19 log	±1.07 log	5.25 to 6.48 log (13%)	0.40 to 3.12 log (17%)
48 h	1.64 log	±0.69 log	5.60 to 2.33 log (12%)	0.00 to 0.95 log (11%)

Figure 3. Density plot is representing the distribution of 329 *Listeria* isolates (y-axis) and the log reductions (x-axis) after exposure to 80 ppm peroxyacetic acid for 30 sec. Log reductions were determined after 24 h (red line) and 48 h (green line) of incubation. Blue line indicates the population mean of 24 h and purple lines the standard deviations of 24 h.



	Mean	Standard deviation	Range for "sensitive" isolates (percent of isolates)	Range for "tolerant" isolates (percent of isolates)
24 h	3.37 log	±1.62 log	4.98 to 6.78 log (21%)	0.00 to 1.75 log (18%)
48 h	3.04 log	±1.67 log	4.71 to 7.00 log (18%)	0.00 to 1.37 log (21%)

Figure 4. Density plot is representing the distribution of 329 *Listeria* isolates (y-axis) and the log reductions (x-axis) after exposure to 500 ppm sodium hypochlorite for 30 sec. Log reductions were determined after 24 h (red line) and 48 h (green line) of incubation. Blue line indicates the population mean of 24 h and purple lines the standard deviations of 24 h.