Biofilm formation of wild-type and pressure-stressed *Listeria monocytogenes* at 7 and 25 °C and their sensitivity to quaternary ammonium compound

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USDA NIFA United States Department of Agriculture National Institute of Food and Agriculture

PBI Pressure BioSciences Inc.

ABSTRACT

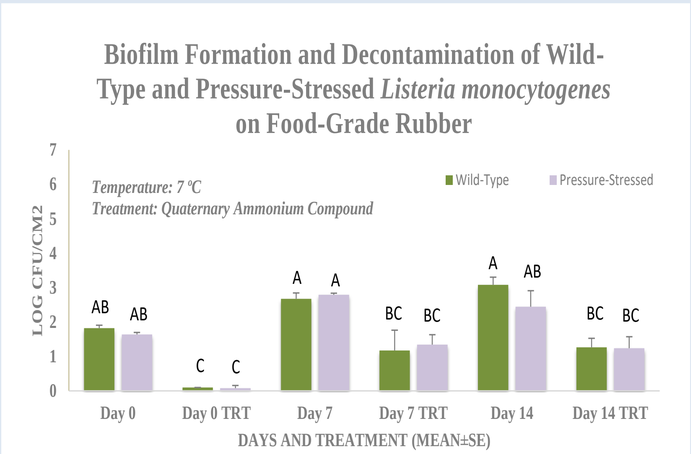
Introduction: Microbial biofilms are the main physiological mode of bacterial proliferation in food manufacturing and clinical settings and are estimated to be responsible for >80% of all bacterial infections. **Purpose:** Current study discusses the biofilm formation of *Listeria monocytogenes* on two abiotic surfaces and validates a decontamination intervention against wild-type and pressure-stressed phenotypes of the bacterium. **Methods:** Four strain mixture of *Listeria monocytogenes* were used for biofilm formation for up to 14 days. Biofilm formation/enumeration/decontamination was conducted on the surface of stainless steel (finish 2b) and rubber (Ethylene Propylene Diene Monomer) coupons at 7 and 25 ºC. After removal of loosely attached cells, samples were neutralized using D/E neutralizing broth and separated from coupons using sonication, prior to culture dependent analyses. Pressure-stressed *Listeria monocytogenes* were prepared by exposing the isolates to the sub-lethal elevated hydrostatic pressure of 15,000 PSI (approximately 100 MPa) for 15 minutes. The experiment was conducted in two biologically independent replications, as blocking factors of a randomized complete block design, with each block consist of three repetitions. The study was analyzed statistically by SAS, using a Tukey-adjusted mean separation. **Results:** In excess of 4.48 and 3.08 log CFU/cm^2 increase (P<0.05) in biofilms mass on stainless steel coupons were observed during 14 days for wild-type and pressure-stressed phenotypes of *Listeria monocytogenes*, respectively at 25 ºC. Treatment with a quaternary ammonium compound-based sanitizer on day 0 was responsible for 1.71 log CFU/cm^2 reduction (P<0.05) of the wild-type pathogen at 25ºC on stainless steel coupons while the same treatment was unable to reduce (P ≥0.05) the pathogen counts of one- and two-week mature biofilms. **Significance:** Wild-type and pressure-stressed phenotypes of *Listeria monocytogenes* exhibited similar biofilm formation capability and sensitivity to the sanitizer. A quaternary ammonium compound-based sanitizer, at the highest concentration recommended by the manufacturer, appears to be efficacious only against planktonic cells of the pathogen while exhibiting inability for the complete elimination of one- and two-week mature bacterial biofilms from rubber and stainless steel surfaces.

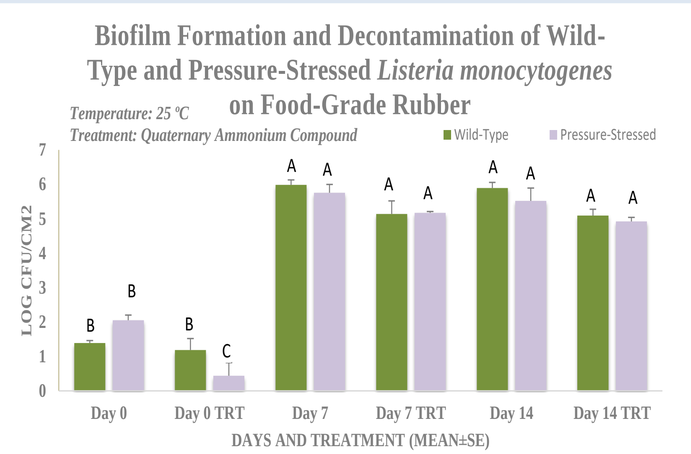
DESIGN, METHODS, & ANALYSES

- Two separate studies were conducted:
  - ✓ Four Strain mixture of Listeria monocytogenes (ATCC® numbers 51772, 51779, BAA-2657, 13932)
  - ✓ **Phenotypes**: Wild-type and Pressure-stressed
  - ✓ **Biofilm formation**: Days, 0, 1, 4, 7, 14
  - ✓ **Biofilm Treatments**: Days 0, 7, and 14
- Study was a **Randomized Complete Block Design** with:
  - ✓ Two biologically independent repetitions (*i.e.*, two **blocking factor**)
  - ✓ Each block, containing three **instrumental replications**
  - ✓ Each instrumental replication consisting of two **microbiological replications**

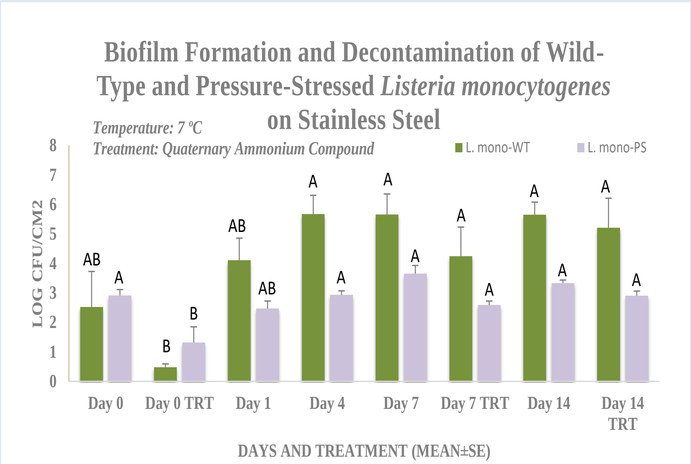
  (Thus each presented value is mean of at least 12 repetitions)

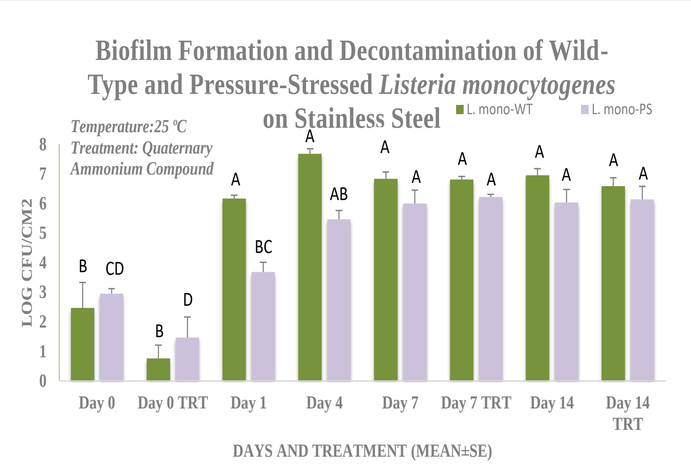
Decontamination of *Listeria monocytogenes* in planktonic and sessile stages on rubber coupons



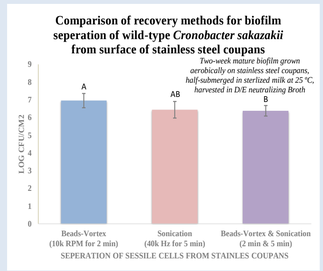


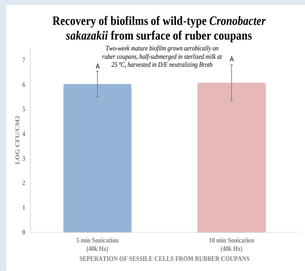
Decontamination of *Listeria monocytogenes* in planktonic and sessile stages on stainless steel coupons





Preliminary trial for biofilm removal





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