Survival of *Listeria monocytogenes* and *Salmonella* on surfaces found in the dry packinghouse environment and effectiveness of dry-cleaning processes on pathogen reduction

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Summary

In the packinghouse, commodities are subjected to a series of critical steps designed to reduce the microbial load and eliminate possible pathogens. Packing operations can involve wet or dry areas, and in some situations dry cleaning and sanitation. Validation of the dry sanitation is important since dry bacteria (present on surfaces as attached biofilm or free planktonic cells) have well-known cross resistance to other environmental stresses, including sanitation agents. For example, dry *Salmonella* cells have an increased heat tolerance compared with the hydrated cells. In this project we aim to determine the survival of dry surface biofilms of *L. monocytogenes* and *Salmonella* on surfaces and conditions typically found in a dry packing area, determine optimal dry-cleaning operations, and validate our findings in large-scale settings.

Objectives

1. Determine die-off curves of dry surface-formed biofilms and dried planktonic cells of *L. monocytogenes* and *Salmonella* (in single and mixed cultures with environmental isolates) on surfaces commonly found in the packinghouse.
2. Test the efficacy of commonly used dry cleaning and sanitation methods on dry surface biofilms and desiccated planktonic cells of *L. monocytogenes* and *Salmonella* on surfaces found in the packinghouse.
3. Validate, in pilot plant trials, the laboratory die-off rates of the surface-associated microflora isolated from the dry areas of the packinghouse, and the inactivation through dry cleaning/sanitation methodology.

Methods

To determine the die-off curves of planktonic cells on packinghouse surfaces, microorganisms were resuspended and droplet-inoculated in peach wash water (PW). The effect of fruit coating was tested in separate experiments by the addition of two commercial fruit coatings (A and B). To simulate the effect of organic matter on surfaces, cells were also resuspended and dried in Proteose Peptone 3. Dry surface biofilms were grown by inoculating 10 microliters of cultures on top of sterile polycarbonate filter membranes resting on the test surface coupons seeded with the appropriate agar containing chemically defined media. *Listeria* biofilms were grown for 48 hours and *Salmonella* for 24 hours. Inoculated coupons were stored at 20, 60 and 85% RH at 25°C and used for microbial enumeration.

Results to Date

For planktonic cell samples stored at 20% RH, the shortest survival was recorded for PW (less than 4 days for *Salmonella* and 8 days for *Listeria*). The presence of commercial fruit coatings increased survival for both pathogens stored at low relative humidity. The presence of organic matter had a similar effect (**Figure 1**). Recovery of planktonic cells inoculated on coupons and stored at 60 and 85% RH was dramatically decreased for both pathogens; bacteria survived for hours only.

In contrast, dried biofilms of *Listeria* survived the least time interval when stored at 20% RH. The presence of high relative humidity resulted in higher bacterial recovery from dried biofilms. Both pathogens survived for extended time when stored at 60 and 85% RH (**Figure 2, Figure 3**).

Benefits to the Industry

The die-off rates for *L. monocytogenes* and *Salmonella* were determined on inoculated coupons cut from surfaces commonly found in the packinghouse. The die-off curves of planktonic cells versus dried biofilms suggest that microorganisms have multiple cellular mechanisms for microbial adaptation in dry conditions. Our experiments included a range of environmental conditions that could be found in the dry area of the packinghouse. The long survival interval for dried *Listeria* and *Salmonella* biofilms emphasizes the importance of cleaning and sanitation of the dry area to prevent microbial attachment and subsequent biofilm formation.

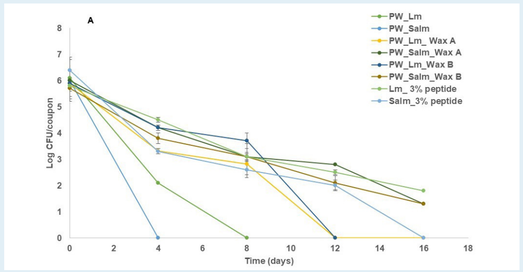

Figure 1.
Survival of *Salmonella* and *L. monocytogenes* planktonic cells (Log/CFU) dried at 20% RH. Cells were grown to stationary phase and then resuspended in fresh media (proteose peptone 3 [3% peptide]), peach extract (PW), and peach extract supplemented with fruit coating (wax) A or B.

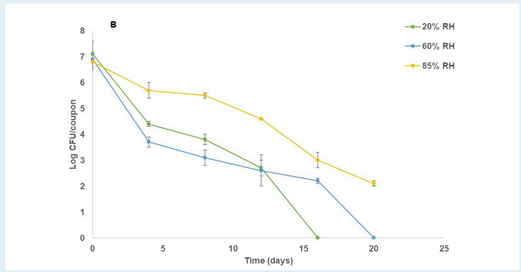

Figure 2.
Survival of *L. monocytogenes* dried biofilms (Log/CFU) stored in controlled relative humidity at 25°C. Cells were grown in chemically defined media to stationary phase and then grown as colony (dry biofilms) on PVC coupons.

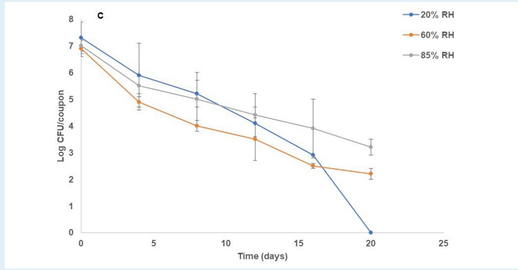

Figure 3.
Survival of *Salmonella* dried biofilms (Log/CFU) stored in controlled relative humidity at 25°C. Cells were grown in chemically defined media to stationary phase and then grown as colony (dry biofilms) on PVC coupons.