Assessment of Population Stability of *Salmonella enterica* in Matrices for use in Dry Inoculations

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Abstract

Background: Challenge tests associated with fresh produce generally utilize liquid bacterial inoculation methods. However, a recent salmonellosis outbreak in the U.S. was linked to peaches which were thought to be contaminated via fugitive dust from a nearby farm. This study examined the population stability of *Salmonella enterica* in different dry matrices to evaluate their use for dry transfer dust inoculations of produce.

Purpose: To examine the survival of *S. enterica* during storage of three different dry matrices for use in dry dust inoculations of produce.

Methods: A four-strain cocktail of *S. enterica* (10 mL) was inoculated into 50 g of silica (70-230 mesh, a_w=0.224), sand (50-70 mesh, a_w =0.228), or corn-cob small animal bedding (ground to 30 mesh, a_w =0.050). Matrices were mixed by hand and stored at 22°C/30%RH for 90 d. Every 7 d, the a_w of the matrices was measured and *S. enterica* was enumerated. Three independent trials were conducted with triplicate samples. Differences in a_w of the matrices or *S. enterica* population were statistically compared using Student's t-test; $p<0.05$ was considered significant.

Results: The population of *S. enterica* (10.88±0.54 log CFU/g) and a_w (0.740±0.150) of the three matrices were not significantly different post-inoculation. After 7 d storage, the a_w of the matrices equilibrated to their pre-inoculation values. After 28 d, the *S. enterica* populations in the silica and sand (9.44±0.38 and 9.70±0.01 log CFU/g, respectively) were significantly lower than in the bedding (10.74±0.07 log CFU/g). After 90 d, the population of the pathogen in bedding and sand (9.82±0.49 and 9.37±0.18 log CFU/g) were both significantly higher than in silica (8.59±0.46 log CFU/g). The *S. enterica* population was most stable in bedding during storage, only reducing by 1.06 log CFU/g after 90 d.

Conclusion: The results of this study suggest that silica, sand, and bedding may all be appropriate matrices for the dry inoculation of produce.

Introduction

- 2020: Salmonellosis outbreak linked to peaches in the U.S.
  - 101 cases from 17 states, 28 hospitalizations
- Outbreak strain (*S.* Enteritidis) not found on peaches, however *S.* Alachua was found on the peach tree leaves
- Outbreak strain was linked to a nearby poultry farming operation
  - "Fugitive dust" containing *S. enterica* from the poultry farm most likely contaminated the peach orchard

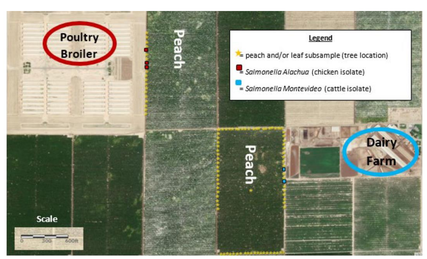

Figure 1: (left) Location of *Salmonella* spp. positive samples identified in the investigation which were adjacent to animal operations; (right) The implicated peaches in the outbreak.

Materials and Methods

- "Fugitive dust" matrices:
  - Silica gel (70-230 mesh)
  - Sand (50-70 mesh)
  - Corn-cob small animal bedding (30 mesh)
- Inoculation of matrices:
  - 4-strain *S. enterica* cocktail (Newport, Enteritidis, Agona, Alachua)
  - 10 mL of 11 log CFU/mL inoculated into 50 g matrix
  - Stirred by hand 5 min

- Storage:
  - 22°C, 30% relative humidity
  - 90 days
- a_w measurements:
  - 0, 7, 14, 21, 28, 60, and 90 days
  - 1 g sample, in triplicate, measured using an a_w meter

- Enumeration of *S. enterica*:
  - 0, 7, 14, 21, 28, 60, and 90 days
  - 1 g sample, in triplicate, homogenized with BPB 1:10 in a stomacher,
  - Homogenate plated onto BHIA
  - Data expressed as log CFU/g
- Statistical analysis:
  - Populations or a_w values (n=9) analyzed vis Student's t-test, α=0.05

Results

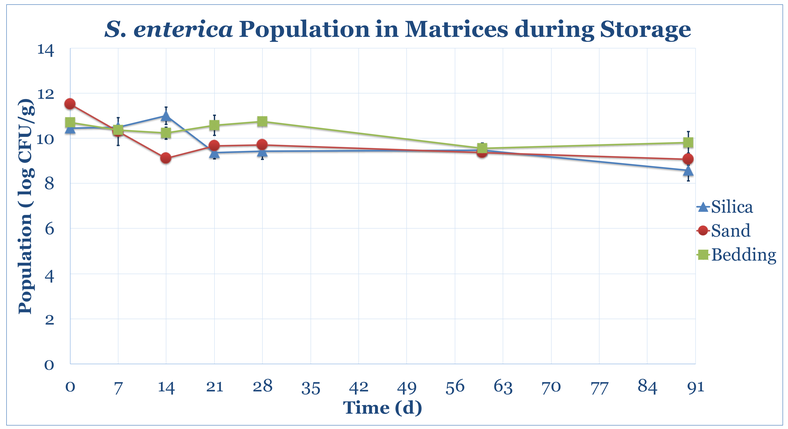

Figure 2. The population (log CFU/g) of *S. enterica* in the three "fugitive dust" matrices during storage at 22°C and 33% relative humidity for 90 days. Data are mean values ± standard deviation (n=9).

← Summary
- After 21 and 28 days, the population of *S. enterica* in the bedding was significantly higher than the other two matrices.
- After 90 days, the populations in the bedding and sand matrices were significantly higher than in the silica matrix.
- *S.* enterica reduced in population by only 1.06 log CFU/g in the bedding.

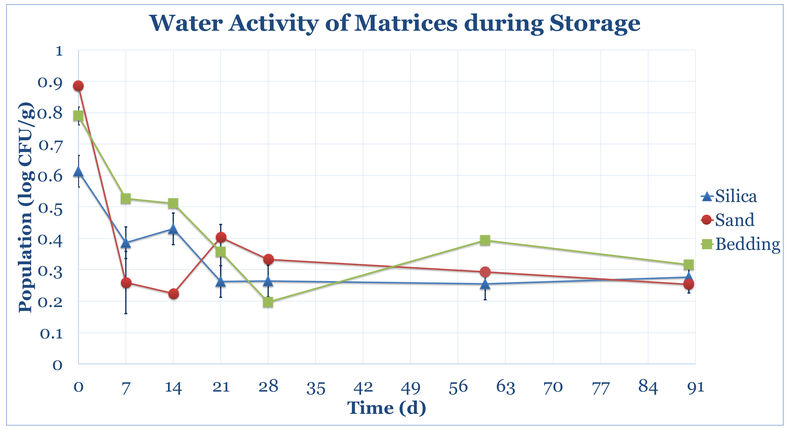

Figure 3. The water activity (a_w) of the three "fugitive dust" matrices during storage at 22°C and 33% relative humidity for 90 days. Data are mean values ± standard deviation (n=9).

← Summary
- Prior to inoculation, the a_w values of the silica, sand, and bedding matrices were 0.224, 0.228, and 0.509, respectively.
- a_w values decreased during the first 14 days of storage.
- The a_w values of the sand and silica did not significantly change from 28 to 90 days; the a_w of the bedding fluctuated during the same timeframe.

Conclusion

- *S. enterica* survived in three different low a_w matrices for 90 days with minimal decrease in population.
- This study identified possible matrices that can be used to mimic dry dust inoculation of produce, including peaches.
- Future steps: assessment of *S. enterica* survival in larger batches of inoculated matrix during storage.

Acknowledgements

This work was supported in part by grant U19FD005322 from the U.S. Food and Drug Administration to the Illinois Institute of Technology. B. Khouja and M. Fay were supported by the Oak Ridge Institute for Science and Education Research Participation Program to the U.S. Food and Drug Administration.