Cell Concentration Dependency of Survival on Drying in *Salmonella* Species

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Abstract

Salmonella enterica is well-known for its ability to survive drying and persist in low-moisture environments. Growth as sessile cells, quorum sensing, and biofilm formation have been examined for possible links, however, the actual mechanisms involved remain elusive.

The purpose of this study was to determine the effect of initial cell concentration or soluble quorum sensing compounds on the desiccation survival of *Salmonella* when grown as sessile cells.

Salmonella was grown on TSBYE with agar and harvested using BPW. Cell suspensions (11.08±0.10 log CFU/mL) were divided into portions and treated as follows: one portion centrifuged and filtered to remove cells to create conditioned BPW (C-BPW) and a second portion heat-treated to kill cells (K-BPW). C-BPW, K-BPW, or control BPW were used to serially dilute the original harvested *Salmonella*. Cells (0.1 mL) were applied to membrane filters at concentrations of approximately 10 (undiluted), 8, 6 or 4 log CFU, dried (ambient) for 1 d, then stored at 25°C/33% RH. Cells were recovered from filters using BPW and cultivated on TSAYE. Data were compared via ANOVA ($p \leq 0.05$).

After 1 and 7 d storage, populations of undiluted *Salmonella* did not significantly change with an overall decrease of 0.25-0.50 log. When the initial *Salmonella* population was decreased, survival decreased compared to undiluted when cells were diluted in BPW or C-BPW but not when diluted with K-BPW. At the lowest dilution (4 log CFU), losses at 1 d were 1.97±0.09 for C-BPW, 1.30±0.50 for BPW, but only 0.33±0.06 log CFU for K-BPW. After 7 d, losses increased for cells diluted in C-BPW and BPW (>4 log CFU), whereas those diluted in K-BPW lost only 0.40±0.01 log CFU.

Survival of *Salmonella* during drying appears cell concentration dependent and may be aided by specific non-soluble components associated with harvested cells.

Introduction

Although *Salmonella* will not grow in low water activity foods, their desiccation resistance and persistence in low-moisture environments creates challenges to food safety and sanitation. Indeed, our previous research indicates that once dried, *Salmonella* displays destruction kinetics under what would typically be considered stressful conditions that differ little from more optimal conditions. For example, once dried, survival at low pH is similar to survival at neutral pH. Similarly, when applied as a dry inoculum to spices containing high levels of antimicrobial compounds resulted in survival not dissimilar to survival without the presence of such compounds. Consequently, events prior to and during drying clearly play an important role in subsequent survival once *Salmonella* is dried.

Currently, literature indicates *Salmonella* will survive drying at a higher level when grown as sessile vs planktonic cells. This was also demonstrated with spices by Hildebrandt et al, who also noted that survival on drying was improved at higher initial cell concentrations. Survival at higher cell concentrations may point to the presence of some quorum sensing compounds produced during sessile cell growth. The biological mechanism for greater survival as sessile cells has not been elucidated, however, a perusal of the literature indicates biofilm formation with the presence of surface polysaccharides, or the presence of quorum sensing compounds may play a role in this survival. The presence of novel catalase produced when *Salmonella* is grown as sessile cells has also been explored. Our current research focuses on the survival of *Salmonella* during desiccation when grown as sessile cells, in an effort to elucidate the mechanisms by which survival is improved.

Materials and Methods

Determination of cell concentration and/or presence of quorum sensing compound effect

1) Inoculate Tryptic Soy Broth (TSB; individually) with isolated colonies of *Salmonella* Agona and Typhimurium and grow to stationary phase.

2) Transfer (0.1 mL) to Tryptic Soy Agar with yeast extract (TSAYE) and spread for lawn grown (37oC, 24 h).

3) Harvest cells with Buffered Peptone Water (BPW) and divide into two portions.
A) Centrifuge and filter sterilize to create "conditioned media" (C-BPW)
B) Remaining live cells (11 log CFU/mL): dilute 9, 7, and 5 log CFU/mL with BPW (control)
- **BPW (control)**
- **C-BPW (conditioned media)**

4) Place 0.1 mL of original culture plus dilutions on 0.2 µM cellulose filters (final concentrations: 10, 8, 6, and 4 log CFU per filter).

5) Determine initial populations and dry 24 h at ambient temperature in biological cabinet; determine 24 h populations.

6) Store remaining filters at 25oC, 33% RH, and enumerate after 7, 14, and 28 d.

Determination of effect of killed cells on survival of *Salmonella*

1) Inoculate TSB, individually with isolated colonies of *Salmonella* Tennessee, Agona, Oranienburg, Anatum, Reading, and Typhimurium and grow to stationary phase.

2) Transfer (0.1 mL) to TSAYE and spread for lawn grown (37oC, 24 h).

3) Harvest cells with BPW and combine equal volumes for cocktail; divide into two portions.
A) Killed cells: heat treatment, determine cell death (K-BPW)
B) Remaining live cells (log 11 CFU/mL): dilute 9, 7, and 5 log CFU/mL with BPW (control)
- **BPW (control)**
- **BPW + BSA (with Bovine Serum Albumin, BSA; 90 mg/mL)**
- **K-BPW (Killed cells in BPW)**

4) Follow steps 4-6 above.

Results and Discussion

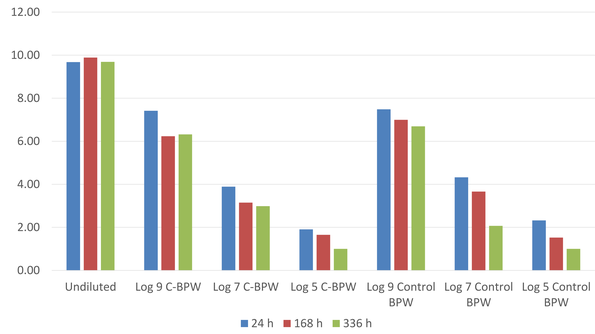

Figure 1. Effect of conditioned BPW on recovery of *Salmonella* Agona when desiccated.

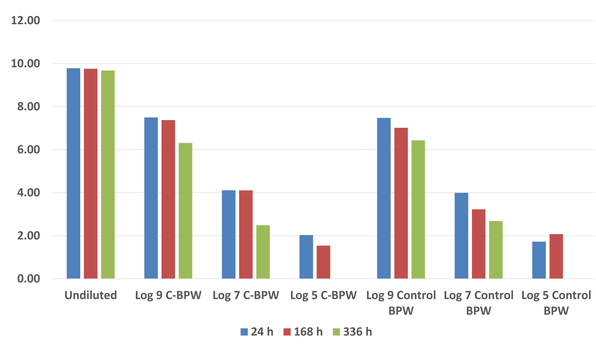

Figure 2. Effect of conditioned BPW on recovery of *Salmonella* Typhimurium when desiccated.

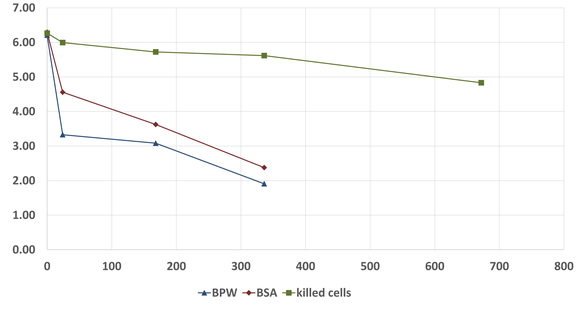

Figure 3. Survival of *Salmonella* after desiccation when diluted.

As shown in Figures 1 and 2, there was no effect of conditioned media (C-BPW) on the recovery of *Salmonella* after 24 h drying or on further storage under dry conditions. For both C-BPW and BPW, recovery decreased with decreasing initial cell concentration. This observation can also be made from Table 1, where recovery and loss over time are significantly higher at the lowest initial cell concentration. The loss of undiluted cells on drying was only 0.23 log, whereas the loss for cultures diluted to log 4 was over 2 logs when diluted in BPW or in BPW with BSA. However, when cells were diluted with killed cells, recovery of live *Salmonella* approximated that of undiluted cells. This effect is shown most clearly in Figure 3, where *Salmonella* diluted with killed cells displays much higher survival and recovery than cells diluted with either BPW alone or BPW + BSA.

Table 1. Initial loss of *Salmonella* on drying and rate of decline on storage.

Condition	Initial concentration (log CFU/filter)	24 h loss (log CFU/filter)	Rate of decline (log CFU/filter per d)
Undiluted	10	-0.23	-0.0013
BPW	8	-0.23	-0.0039
BPW + BSA	8	-0.41	-0.0030
K-BPW	8	-0.26	-0.0023
BPW	6	-2.88	-0.0046
BPW + BSA	6	-1.74	-0.0070
K-BPW	6	-0.27	-0.0018
BPW	4	-2.43	-0.0052
BPW + BSA	4	-2.05	-0.0048
K-BPW	4	-0.86	-0.0012

Conclusion

Survival of *Salmonella* during desiccation and storage as dried cells appears to be related to the initial cell concentration. This does not appear related to any soluble component, as use of a "conditioned" recovery media did not improve recovery. However, the addition of killed cells dramatically improved recovery resulting in loss of cells that was no different from undiluted cells. Consequently, survival during drying appears cell concentration dependent and may be aided by specific non-soluble components associated with harvested cells.