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Survival Strategies Used by *Salmonella* to Persist in Dry and Low-Moisture Processing Environments

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Introduction

Salmonella contamination in dry and low-moisture food processing environments is a food safety concern recognized by the FDA, with the organism being responsible for an estimated 94% of recalls of low water activity foods in the United States (Santillana Farakos and Frank 2014). *Salmonella* makes its way into dry and low-moisture processing environments in ways that are similar to what is seen in higher moisture processing environments, including via raw materials (e.g., packaging materials, ingredients), air (e.g., air handling systems, receiving areas), water (e.g., sanitation), equipment or tools, employees or visitors, and pests (Beuchat *et al.* 2011), as shown in Figure 1.

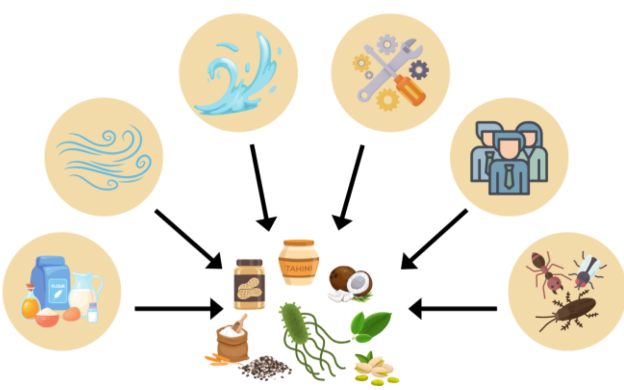

Figure 1. Routes of contamination of low water activity products by *Salmonella*.

For a microorganism to grow in a food processing environment, there must be enough available moisture for the organism to use. In a dry or low-moisture processing environment, there are typically not enough consistent sources of moisture for microorganisms to grow; however, *Salmonella* has shown the ability to adapt to this low-moisture stress and survive for long periods of time, sometimes gaining the ability to withstand other stressors as well (termed "cross-tolerance") (Finn *et al.* 2013).

Adaptive Behaviors Used by *Salmonella*

Salmonella has been shown to use several behaviors to adapt to stressors and survive in dry or low-moisture environments, including accumulation of osmoprotectants, up-regulation of outer membrane porins, alteration of gene expression, rRNA degradation, entering a viable but not culturable state, filamentation, and biofilm formation (Finn et al. 2013).

Osmoprotectants

When *Salmonella* is exposed to a low water activity environment, the organism needs to balance the osmolarity of its internal cell composition with that of the external environment (Malakar *et al.* 2022). In a dry or low-moisture environment, the solute concentration on the outside of the bacterial cell is higher than inside (termed "hypertonic"), often resulting in extreme water loss and shriveling of the organism (Figure 2).

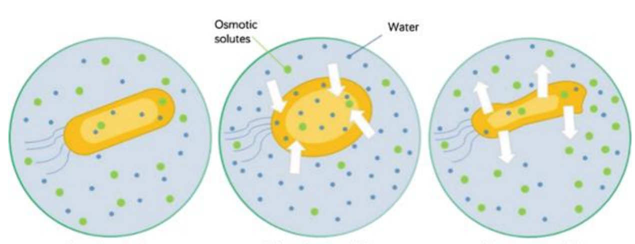

Figure 2. Types of osmotic balance conditions (Malakar *et al.* 2022).

One of the ways *Salmonella* prevents this water loss is by using osmoprotectants (Finn *et al*. 2013, Li *et al.* 2012). Osmoprotectants are small, neutral molecules that are non-toxic at high concentrations. *Salmonella* can increase the internal amount of osmoprotectants by either synthesizing them internally or by transporting them from the environment into the cell. *Salmonella* can do this in as little as six minutes after the introduction of the stressor (Balaji *et al.* 2005).

Outer Membrane Porins (OMPs)

OMPs are proteins that extend across the outer membrane of the bacterial cell that help promote the passive diffusion of osmoprotectants into the cell (Figure 3).

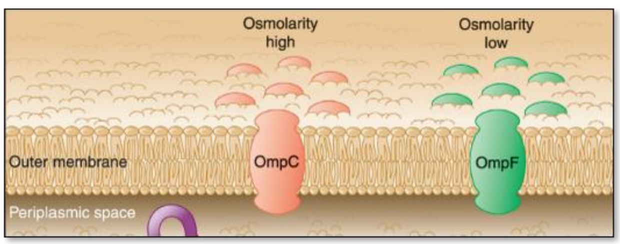

Figure 3. The outer membrane porins OmpC and OmpF (Kenney 2006).

Under dry or low-moisture stress, *Salmonella* expresses two OMPs to bring osmoprotectants into the cell within twelve minutes of the stressor being introduced (Balaji *et al.* 2005, Feng *et al.* 2003, Wang *et al.* 2012).

Alternative Sigma Factors

Salmonella possesses a variety of genes that, when expressed, will result in the production of molecules (e.g., proteins) that provide advantages to the cell under certain environmental conditions or stressors. The expression of these stress response genes is dependent on the specific environment the cell is in and is regulated by alternative sigma factors. When *Salmonella* is stressed in a low-moisture environment, alternative sigma factors will up-regulate the expression of genes that allow *Salmonella* to survive (McMeechan *et al.* 2007).

Ribosomal RNA (rRNA) Degradation

rRNA is essential to genetic expression, and therefore the function, of a living organism (Basturea *et al.* 2012, Deng *et al.* 2012). Under stress, *Salmonella* has been shown to increase rRNA degradation, which is the removal of rRNA components that are not immediately functionally required under an unfavorable environment. The cell may then enter a partially dormant state, wherein it is still metabolically active but has been shown to express about 5% of its total genes to modulate its environment to prolong survival in dry or low-moisture environments (Lewis 2007, Deng *et al.* 2012).

Viable But Not Culturable (VBNC) State

Under certain stress conditions, *Salmonella* has been shown to induce a “viable but not culturable” state (Oliver 2010, Gruzdev *et al.* 2012, Jayeola *et al.* 2022). Organisms in a VBNC state are metabolically inactive while in an unfavorable environment. Though inactive, the organism can preserve viability and regrow when resuscitated (Gupte *et al.* 2003), such as when reintroduced to a high-moisture environment.

Filamentation

Filamentous *Salmonella* cells are produced in response to environmental stress, preventing normal cellular division (Pratt *et al.* 2012). Formation of these filamentous cells presents a challenge in the processing environment as it adds to *Salmonella*’s biomass without increasing the detectable number of individual cells, which means that cell counts may underestimate how many cells are present. When *Salmonella’*s environment becomes favorable, these filamentous cells can rapidly divide to become viable cells that can potentially cause human illness (Mattick *et al.* 2000).

Biofilm Formation

Under certain conditions, *Salmonella* may produce biofilms to protect against excessive water loss under stress (White *et al.* 2006, Stocki *et al.* 2007). The adherence ability of *Salmonella* biofilms may be reduced depending on the surface (Giaouris and Nychas 2006); however, biofilm production has been shown to offer cross-protection against other environmental stressors (e.g. pH and heat).

Application to Food Processing Environments

Consideration of how a low water activity state was achieved (e.g., through thermal or non-thermal drying methods) may help predict trends in *Salmonella* behaviors observed in processing environments, such as increased resistance to heat or sanitizers and proficiency of biofilm formation. Incorporation of this information into hazard analyses and control plans may allow for the optimization of production practices aimed at mitigating the organism's survival.

Additional research is necessary to better understand ways *Salmonella* adapts to these environments for the purpose of developing effective mitigation strategies through a combination of bench-style, applied, and intervention-specific experiments. Research using methods to simulate food industry environments is needed to provide a better understanding of how specific *Salmonella* serovars behave in commodity-specific production settings (e.g., *Salmonella enterica* serotype Typhimurium in dried coconut, *Salmonella enterica* serotype Senftenberg in peanut butter). Food businesses should also work to collaborate with university and Cooperative Extension services to use experiential data collected at industry scale (such as from environmental monitoring programs or monitoring data) to predict and adapt in real-time to the changing landscape of *Salmonella* survival in dry and low-moisture environments.

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