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Survival of Salmonella Species on Stainless Steel Exposed to Dry Heat

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Abstract

Limited data is available regarding Salmonella survival on non-porous surfaces in dry conditions. In the dry goods industry Salmonella contamination is a serious concern since Salmonella is known to survive in several dry foods such as flour, nuts, and dried milk products. Common practice in many dry food manufacturing plants involves maintaining a dry environment for lengthy periods of several weeks followed by a wet cleaning and sanitation cycle. Salmonella were inoculated onto stainless steel carriers and exposed to several different dry and moist heat conditions. As the temperature increases, from 80℃ to 120℃, the number of Salmonella survivors decreases. The ability of Salmonella to survive in the presence of soil was also tested. Results of this study indicate dry heat can eliminate Salmonella from non-porous surfaces, such as stainless steel, but will require extended time periods and high temperatures.

Key words: Salmonella, dry heat, stainless steel

Introduction

Salmonella species are important food pathogens and are estimated to cause 19,586 hospitalizations and 378 deaths in the United States annually (Scallan et al. 2011). Although dried goods do not provide an optimal environment for pathogen growth and survival, Salmonella have been shown to survive in many types of dried goods such as flour, nuts, and dried milk products (McDonough and Hargrove 1968; VanCauwenberge et al. 1981; Archer et al. 1998; Doyle and Mazzotta 2000; Uesugi et al. 2006). There is also evidence that Salmonella can survive on non-porous hard surfaces such as stainless steel. S. enteritidis (pSB311) was found at 52℃ to have a D value of 10.80 min on glass and 9.79 min on stainless steel (Annear 1971; Dhir and Dodd 1995). Human isolates of S. enteritidis phage type 4 dried onto Formica squares with sterile horse blood had a D20℃ value of greater than 35 min and showed 45-53% survivors after 24 h (Humphrey et al. 1995).

In many dry food plants dry cleaning is conducted on a regular basis with a full wet clean conducted at longer intervals to minimize the addition of water to the environment (Umland et al. 2003). Dry cleaning consists of sweeping or vacuuming off the equipment without application of a sanitizing treatment. The lack of a sanitizing step allows bacterial contaminants that can survive dry conditions the opportunity to survive until the next production run and contaminate the product.

It is possible that dry heat could be used during dry cleaning to sanitize equipment without the introduction of moisture; however, there is limited data on the ability of Salmonella to survive dry conditions on non-porous hard surfaces. In order to further investigate the ability of dry heat to sanitize hard surfaces; this study examined the effect of different temperatures under dry heat conditions on a dried inoculum of Salmonella on stainless steel.

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Materials and Methods

Culture Preparation. Bacterial cultures of *Salmonella* Typhimurium ATCC 13311, *Salmonella* Choleraesuis ATCC 10708, *Salmonella* Enteritidis ATCC 13076, and *Salmonella* Enteritidis Phage Type 30 (PT30; ATCC BAA-1045), were incubated for at 35℃ for 24 h in brain heart infusion broth (BHIB; BD BBL, Sparks, MD, USA) prior to testing. A combined *Salmonella* inoculum was prepared by mixing equal parts of *S.* Typhimurium, *S.* Enteritidis and *S.* Choleraesuis. A 10% D-glucose (Calbiochem, EMD Biosciences, La Jolla, CA, USA) or a 10% lactose (BD BBL, Sparks, MD, USA) stock solution was prepared in phosphate buffered dilution water (Sigma-Aldrich, St. Louis MO, USA) and filter sterilized using a 0.22 µm membrane filter (Nalgene, Rochester,

NY, USA). Whole milk was purchased at a local store and sterilized by autoclaving at 121℃ for 12 min. The inoculum was prepared with 5% glucose, 5% lactose or 10% whole milk soil if indicated.

Stainless Steel Carrier Inoculation. Carriers were prepared by pipetting and spreading 20 µl of prepared inoculum onto one side of a 2.5 cm x 2.5 cm 304 grade brushed stainless steel carrier. Inoculated carriers were dried in a 35℃ incubator for 30 min. Time zero carriers were sampled immediately after being dried. Dried carriers were placed in a dry heat oven (VWR, Briston, CT) for temperatures at or above 60℃ or placed in an incubator set at 26 or 55℃. At various time intervals the carriers were removed and sampled.

Carrier sampling. Immediately after exposure to the test condition carriers were placed in sterile 60 ml sample bottles (Nalgene, Rochester, NY, USA) containing PBDW and shaken on a rotary shaker (IKA, NC, USA) for 1 min at 250 rpm. Samples were serially diluted, plated on brain heart infusion agar (BHIA; BD BBL Becton Dickinson, Sparks, MD, USA) and incubated at 35℃ for 48 h. Each time point was tested in triplicate.

Data Analysis. The data was transformed from CFU/ml to CFU/carrier to account for the 10 or 25 ml of phosphate buffered dilution water used to recover the survivors. Log reductions were calculated by subtracting the log CFU/carrier for the time point from the log CFU/ carrier for the time zero time point. Statistical comparisons were completed using the ANOVA and Tukey's method within Minitab 16 (Minitab Inc., State College, PA, USA).

Results and Discussion

Survival of *Salmonella* cocktail at 26°C and 55°C. *Salmonella* populations showed a steady decline in survivors when dried on stainless steel carriers and stored at 26℃ (Fig.1). After 7 days at 26℃ the number of survivors dropped below the limit of detection (1.40 log CFU/carrier) and resulted in a 4.9 log reduction from initial levels. When carriers were held at 55℃ for 9 days a steady decline in survivors was noted until day 4; after day 4 surviving populations were maintained at approximately 3 log CFU/carrier. Carriers stored at 55℃ showed a 3.4 log reduction in *Salmonella* after 9 days. Heat shock proteins are known to facilitate survival at higher temperatures and this may have been the mechanism by which *Salmonella* was able to survive at a constant population after 4 days at 55℃ (Doyle and Mazzotta 2000; Foster 2007). As 26℃ is within the temperature growth range for *Salmonella* and therefore not likely to induce a stress response it is possible that no survival response was elicited and the organism was unable to survive desiccation. These results are in contrast to *Salmonella* survival in food which has been found to survive at room temperature in food such as almonds for 550 days or dried milk for 15 weeks (McDonough and Hargrove 1968; Uesugi et al. 2006). Food products have attributes found to offer heat resistance, such as lower moisture content, higher fat content, or lower pH level, and this may explain why *Salmonella* survival occurs within food but not on stainless steel (Moats et al. 1971; Doyle and Mazzotta 2000).

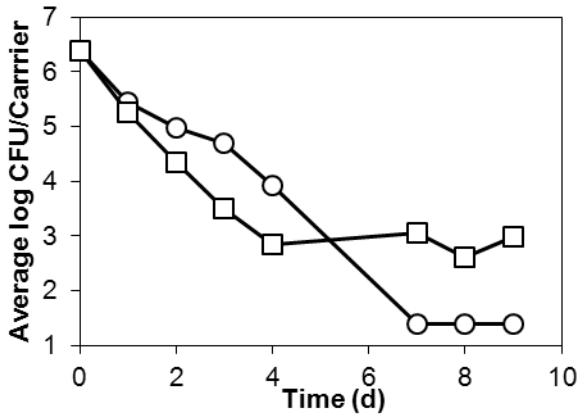

Figure. 1. Survival of *Salmonella* cocktail at 26°C (○) and 55°C (□).

Survival of *S.* Enteritidis PT30 and *Salmonella* cocktail at 80°C to 120°C. Following exposure to 80℃ there was a steady decline in the *Salmonella* cocktail population, survivors reaching a level of 4.2 log CFU/carrier after 4h (Fig. 2A). This corresponded to a 1.9 log reduction from the initial inoculum (Table 1).

Table 1. Comparison of log reductions between dry heat temperatures after 4 h*.

Temperature (℃)	*Salmonella* Cocktail	*S.* Enteritidis PT30
80	1.9 B	1.6 C
90	4.3 A	4.0 B
100	4.4 A	4.4 B
110†	-	6.2 A
120†	-	6.1 A

* Means within columns that do not share letters are significantly different (P<0.05).
† Temperatures only tested with *S.* Enteritidis PT30.

When exposed to 90℃ and 100℃ there was a greater drop in survivors over 4h compared to 80℃. At 4h populations exposed to 90℃ declined to 2.4 log CFU/carrier which corresponded to a 4.3 log reduction. After 2h the samples at 100℃ had fallen below the limit of detection at 1.4 log CFU/carrier and achieved at least a 4.4 log reduction from the initial counts. Although the

inoculated carriers exposed to 90℃ and 100℃ showed similar results, only the carriers held at 100℃ resulted in population numbers falling below the limit of detection.

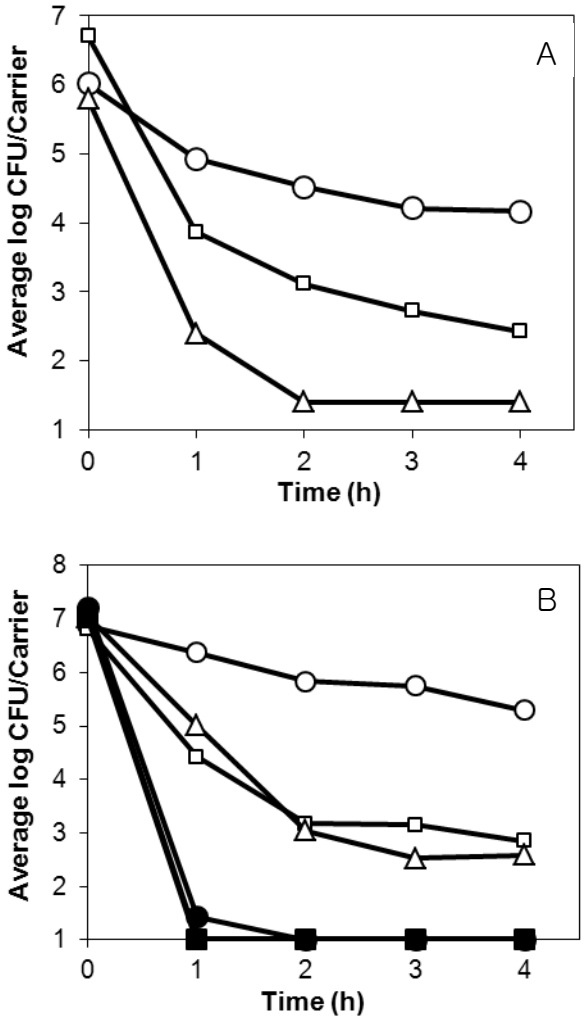

Figure 2. *Salmonella* cocktail (**A**), and *S.* Enteritidis PT30 (**B**), survival at 80°C (○), 90°C (□), 100°C (Δ), 110°C (●), and 120°C (■).

Similar observations were made with *S.* Enteritidis PT30 in that following 4h exposure to 80℃ a 1.6 log reduction was evident while at 90 and 100℃ a >3 log reduction was observed after 2h (Fig. 2B). At 110 and 120℃ populations of *S.* Enteritidis PT30 were reduced to levels at or below the limit of detection after 1h and exhibiting at least 6 log reduction. The data indicate that under the conditions tested *S.* Enteritidis PT30 does not differ from other *Salmonella* in its ability to survive extremes of dry heat. *Salmonella* Enteritidis PT30 was treated separately in this study as it has been shown to exhibit heat resistance properties during almond kernel processing (Abd et al. 2012; Harris et al. 2012).

Survival with glucose, lactose and whole milk soils. In a dry foods processing environment, equipment cleaning without the use of water may result in low level residual soil such as carbohydrate, protein and fat. Whole milk (10% in inoculum), 5% glucose solution and 5% lactose solution were used as carrier soils in this study. When glucose soil was used at either 100 or 120℃ dry heat, populations were reduced more rapidly than carriers without soil (Fig 3. A,B). For example, the *Salmonella* cocktail showed a >4 log reduction within 15 min at 100℃ in the presence of glucose, while the same reduction was achieved without soil after 45 min exposure. Other researchers have observed that when bacteria were immersed in various solutes that decrease water activity, for example a glucose-fructose mixture, there was an increase in heat resistance of bacteria with high temperature exposure (>70℃), but decrease in heat resistance at lower temperature (<65℃) (Mattick et al. 2001; Pflug 2001). Moreover, Moats et al. (1971) also found similar phenomenon of decreased heat resistance when *Salmonella* Anatum immersed in a 5% glucose solution was exposed to 55℃. The presence of a 5% glucose soil may have been sufficient to exert significant osmotic pressure on *Salmonella* causing dehydration and concomitant membrane structural alterations resulting in membrane shrinkage and contraction of the cytoplasm (Beney et al. 2004). It is possible that cell injury brought about by the osmotic stress of glucose soil rendered organisms highly susceptible to dry heat and brought about rapid and complete elimination.

When lactose soil was present for *S.* Enteritidis PT30, the log reductions were significantly ($P<0.05$) lower after 15 min than reductions observed with glucose soil. It was noted that glucose soiled carriers were subject to browning, most likely the result of Maillard reaction products produced through the reaction of glucose and residual amino acids from the BHIB. Glucose has a higher reactivity in a Maillard reaction than lactose, and products of Maillard reactions are known to be mutagenic and sometimes directly antimicrobial (Powrie 1986; Rufián - Hernares and Morales 2007; Mueller et al. 2011). Brands et al. (2000) found that *Salmonella* Typhimurium had a higher mutagenicity rate when treated with a solution of Maillard reaction products derived from a glucose solution rather than a lactose solution. More work will need to be done to investigate whether Maillard reaction products are impacting the survival of *Salmonella* on glucose treated carriers.

Population reductions of *Salmonella* cocktail in the presence of whole milk soil did not differ from the no-soil condition after 15 minutes. Lipids have been known to increase heat resistance in several bacterial species; this protective effect may explain why pathogens survived for slightly longer time periods in the presence of whole milk soil than other soils (Moats et al. 1971; Pflug 2001).

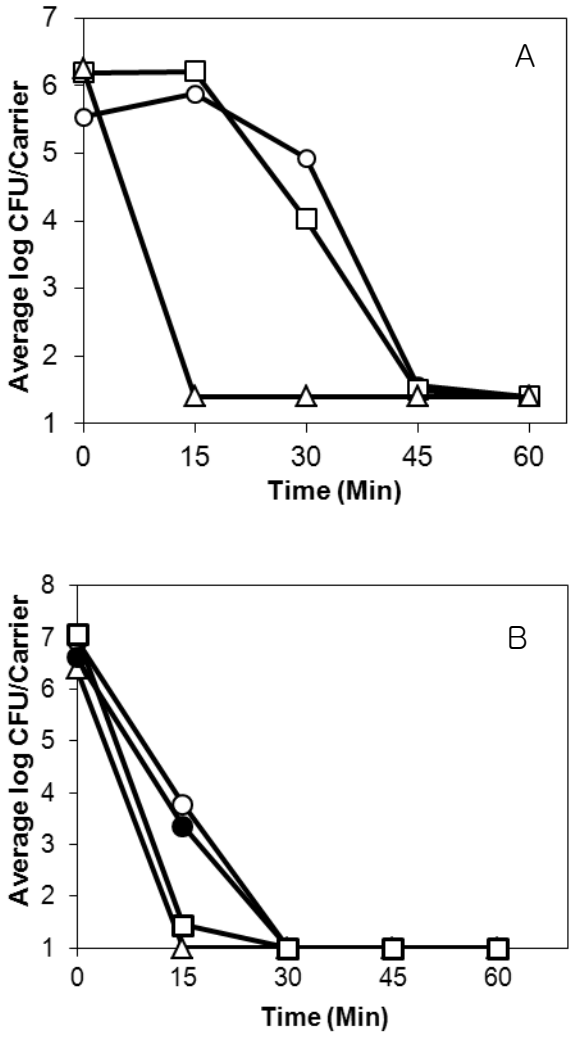

Figure 3. *Salmonella* cocktail at 100℃ (**A**), *S.* Enteritidis PT30 at 120℃ (**B**), survival in the absence (○) or presence of 10% whole milk (□), 5% lactose (●) or 5% glucose soil (Δ).

Results of this study indicate dry heat can eliminate *Salmonella* from non-porous surfaces, such as stainless steel, but will require extended time periods and high temperatures.

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