Food Microbiology 58 (2016) 139–147

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Heat resistance of *Salmonella enterica* is increased by pre-adaptation to peanut oil or sub-lethal heat exposure

Karen Fong, Siyun Wang*

Food, Nutrition and Health, Faculty of Land and Food Systems, The University of British Columbia, 2205 East Mall, Vancouver, BC V6T 1Z4, Canada

ARTICLE INFO

Article history:
Received 20 November 2015
Received in revised form
10 April 2016
Accepted 15 April 2016
Available online 21 April 2016

Keywords:
Salmonella
Desiccation
Heat resistance
Gene expression
Cross-protection

ABSTRACT

Cross-protection represents a considerable challenge in the food industry where hurdled interventions are often employed to reduce *Salmonella* contamination. The heat resistance of *Salmonella* strains from five serotypes (i.e., Typhimurium, Enteritidis, Tennessee, Thompson and Hartford) at 70 °C was determined by measurement of viable cell populations before and after adaptation to two common stresses employed in low-water activity food processing, desiccation and sub-lethal heat treatment. Survival of *Salmonella* at 70 °C significantly increased ($p < 0.05$) following the six-day incubation in peanut oil (a_w 0.52 ± 0.00) and/or the exposure to a sub-lethal heat treatment at 45 °C for 3 min. Quantitative PCR revealed upregulation of two desiccation stress-related genes, *fadA* and *otsB*, following the peanut oil incubation, whereas heat treatment induced upregulation of a heat-resistance gene, *dnaK*. Invasion gene *invA* and alternative sigma factor *rpoE* were downregulated following either of the treatments. Interestingly, different *Salmonella* strains yielded different transcriptional profiles. The strain-specific resistance phenotypes and transcriptional profiles provided further insights into the mechanisms employed to tolerate desiccation and heat stresses in the food industry.

1. Introduction

Salmonella enterica is an enteric pathogen that causes foodborne salmonellosis in humans. Annually, it is estimated that 80.3 million cases of foodborne salmonellosis are reported worldwide, leading to 155,000 deaths (Majowicz et al., 2010). Although animal-derived food products (e.g., poultry and pork) are well-recognized sources of *Salmonella* (Andino and Hanning, 2014; Atterbury et al., 2007), outbreaks in a variety of low-water activity (a_w) food products have drawn cause for concern in recent years (CDC, 2007, 2009, 2013, 2014a, 2014b).

Survival of *Salmonella* in low-a_w environments requires substantial adaptations at the cellular level (Deng et al., 2012; Gruzdev et al., 2012; Li et al., 2012a). Upon the introduction of a environmental stressor, gene regulation plays a critical role in facilitating the survival under such stress (Gruzdev et al., 2012; Li et al., 2012a). These regulatory networks play interconnected roles in cellular stress response, which may lead to a phenomenon known as cross-protection (Wesche et al., 2009). Cross-protection occurs when an initial sub-lethal injury incurred as a result of encountering an environmental stress enhances the resistance to a stress incurred subsequently (Gruzdev et al., 2011). This anomaly is of particular concern in the low-a_w food industry, where food commodities commonly undergo a series of interventions in tandem to reduce the microbial load. In particular, drying (desiccation) treatments often take place prior to pasteurization (heat) (Finn et al., 2013; Woodroof, 1983). In the case of peanuts, mechanical drying normally takes place following harvest, which is accomplished with on-farm dryers that blow heated air (~35 °C) through the peanuts, drying them to moisture levels of approximately 7–10% to minimize aflatoxin contamination (Woodroof, 1983). Subsequently, sizing of in-shell or shelled nuts will take place according to sizing standards, followed by roasting steps (Kader and Thompson, 1992), which occur at temperatures between 70 and 75 °C (Shachar and Yaron, 2006). It is known that successive drying and roasting steps may facilitate the survival of bacterial pathogens during food processing, in the food product, and also upon consumption (Sirsat et al., 2011). Previous studies have suggested that cross-protection accounts for the lower infectious dose of *Salmonella* in low-a_w food commodities, as they may survive better in the presence of gastric acids and bile salts prior to colonization of the small intestine (Aviles et al., 2013b; Gruzdev et al., 2011; Stackhouse et al., 2012). In

* Corresponding author.
E-mail addresses: karen.fong@ubc.ca (K. Fong), siyun.wang@ubc.ca (S. Wang).

http://dx.doi.org/10.1016/j.fm.2016.04.004
0740-0020/

the present study, the heat resistance of *Salmonella* at 70 °C was evaluated following pre-adaptation to (i) desiccation in peanut oil ($a_w = 0.52 \pm 0.00$) for six days and/or (ii) a sub-lethal heat shock of 45 °C for 3 min in an effort to simulate the successive drying and roasting steps which *Salmonella* may undergo in low-a_w food processing. The temperature of 45 °C was chosen as the sub-lethal challenge temperature as it is the upper limit at which *Salmonella* can replicate (Montville and Matthews, 2005).

The expression of stress response and virulence genes after exposure to peanut oil ($a_w = 0.3$) demonstrated marked up or downregulation in an *S.* Enteritidis strain according to RNA-Seq data (Deng et al., 2012). However, gene expression patterns between different *Salmonella* strains may vary in response to the same desiccation or heat treatment, given the range of phenotypes previously observed in low-a_w foods (Fong and Wang, 2016). Further investigation on gene expression profiles of distinct *Salmonella* strains subjected to the same stressors encountered in low-a_w food processing may aid in development of tailored mitigation tools (Humphrey, 2004). To better understand the molecular mechanism underlying altered survival following pre-adaptation to desiccation in peanut oil or sub-lethal heat exposure, the expression of five genes (i.e., *fadA*, *otsB*, *rpoE*, *dnaK* and *invA*) was characterized in five strains of *Salmonella*. These genes are considered to be involved in *Salmonella* stress response and virulence (Gruzdev et al., 2012; Hsu-Ming et al., 2012; Li et al., 2012a; Sirsat et al., 2011), although their transcriptional profiles under desiccation and heat shock have not been elucidated in a variety of *Salmonella* serotypes yet.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Five strains of *Salmonella enterica* were used in this study: *S.* Enteritidis (FSL S5-523), *S.* Thompson (FSL S5-483) and *S.* Typhimurium (FSL S5-536) were human isolates, whereas *S.* Hartford (FSL R8-5223) and *S.* Tennessee (FSL R8-5221) were isolated from peanuts. *Escherichia coli* O157:H7 (EDL-933) was of human origin. Bacterial strains were maintained in Brain-Heart-Infusion (BHI) broth (BD/Difco, East Rutherford, N.J., USA) supplemented with 20% glycerol at −80 °C. Working stocks were maintained on Luria-Bertani (LB) agar (Amresco, Solon, Ohio, USA) at 4 °C for a maximum of four weeks. Prior to each experiment, single colonies were inoculated into 10 ml BHI broth in triplicates and the cultures were incubated at 37 °C at 170 rpm for 18 h to attain a final concentration of ~10^9 CFU/ml.

2.2. Heat challenge assays

To prepare for *Salmonella* cells pre-adapted to peanut oil (a_w 0.52 ± 0.00), 30 μl of overnight culture was transferred into three ml of fresh BHI broth (BD/Difco) in triplicates and incubated at 37 °C at 170 rpm until an optical density at 600 nm (OD_{600}) of 0.4 (mid-to late-exponential phase; ~10^8 CFU/ml) was attained. Subsequently, 100 μl of inoculum was transferred to a 30 ml disposable conical tube and air-dried for 0.5 h at 20 °C in a biological safety cabinet under constant airflow to enhance the stimulation of a stress response (i.e., as would normally occur in the natural environment prior to processing interventions). The non-evaporated, air-dried culture was then suspended in 9.90 ml of peanut oil to attain a final concentration of ~10^7 CFU/ml as determined by spreading onto the surface of Luria-Bertani (LB) agar (BD/Difco) in duplicates. Peanut oil suspensions were stored at 20 °C under closed conditions for six days to reach stationary phase (Fong and Wang, 2016).

To prepare for *Salmonella* cells pre-adapted to a sub-lethal heat treatment, 50 μl volumes of overnight culture were added into sterile microcentrifuge tubes in triplicates and incubated at 45 °C for 3 min in a C1000 Touch™ thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA).

To prepare for *Salmonella* cells pre-adapted to both desiccation and sub-lethal heat treatment, 50 μl of the previously desiccation-treated *Salmonella* cells was subsequently subjected to the sub-lethal heat treatment at 45 °C for 3 min.

Briefly, 50 μl of the pre-adapted cell cultures were transferred to microcentrifuge tubes in triplicates and incubated at 70 °C for 1 min in a thermal cycler (Bio-Rad Laboratories). Subsequently, the cultures were diluted in phosphate-buffered saline (Amresco) and spread onto LB agar in duplicates. Colonies were counted after incubation at 37 °C for 24 ± 2 h.

2.3. RNA extraction and cDNA synthesis

RNA was extracted from cultures of the five *Salmonella* strains before and after the six-day incubation in peanut oil (a_w 0.52 ± 0.00) or 3 min sub-lethal heat exposure at 45 °C. To cease RNA synthesis and degradation, 500 μl of treated cultures was added to 500 μl of RNAprotect Bacteria Reagent (Qiagen, Valencia, CA, USA), followed by mixing for 10 s, incubation at room temperature for 10 min, and centrifugation at room temperature for 10 min at 5000 × *g*. The supernatant was subsequently decanted and cell pellets were stored at −80 °C. Total RNA isolation was performed using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA) and carried out according to the manufacturer's instructions. The genomic DNA was removed using the RNase-Free DNase Set (Qiagen, Valencia, CA, USA). RNA concentration and quality (A260/A280 and A260/A230, between 1.8 and 2.1) were measured using a Nanodrop spectrophotometer (Thermo Scientific, Waltham, MA, USA). The RNA samples were analyzed for integrity using the Bio-Analyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). Samples with an RNA integrity number (RIN) of ≥7 were used for subsequent analyses, with the exception of the samples positive for 23S ribosomal RNA (rRNA) intervening sequences. To detect for the presence of intervening sequences, *Salmonella* and *E. coli* O157:H7 cultures were prepared by inoculating a single colony into 10 ml BHI broth, followed by incubation at 37 °C at 170 rpm for 18 h. *E. coli* O157:H7 was used as a positive control as intervening sequences (IVSs) have not been previously reported in this organism (Pabbaraju et al., 2000). One μl aliquots from the overnight cultures were used as the template DNA in the PCR assay. Primers used for the detection of IVSs in the 23S ribosomal molecule at helices 25 and 45 are listed in Table 2. PCR was carried out for 35 cycles (Bio-Rad Laboratories) as described by Bhagwat et al. (2013): initial denaturation (1 min, 94 °C), three-step cycling: denaturation (1 min, 94 °C), annealing (1 min, 56 °C) and extension (1 min, 72 °C), followed by a final extension (3 min, 72 °C). The PCR amplicons were electrophoresed (Bio-Rad Laboratories) on 2% agarose (Amresco) and in 1× TAE buffer (Invitrogen, Waltham, Mass., USA). The visualization of the PCR products was carried out using the ChemiDoc MP System (Bio-Rad Laboratories).

Synthesis of complementary DNA (cDNA) from RNA was carried out using the Quantitect Reverse Transcription Kit (Qiagen) according to the manufacturer's instructions. "No-RT" controls, where nuclease-free water (Amresco) was used in place of the reverse transcriptase (RT), were also carried out to detect for the presence of genomic DNA.

2.4. Quantitative PCR

The relative expression of five genes was evaluated in response