International Journal of Food Microbiology 219 (2016) 1–6

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

The isolation and identification of *Pantoea dispersa* strain JFS as a non-pathogenic surrogate for *Salmonella* Typhimurium phage type 42 in flour

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ARTICLE INFO

Article history:
Received 8 August 2015
Received in revised form 4 November 2015
Accepted 21 November 2015
Available online 22 November 2015

Keywords:
Salmonella Typhimurium
Flour
Pantoea dispersa
Surrogate
Heat treatment

ABSTRACT

Salmonella is a common pathogen which has been the cause of foodborne illness outbreaks implicating a variety of commodities, including low-moisture foods such as flour. *Salmonella* costs more than any other pathogen in the United States in terms of health care expenses and time of lost work. Heat treatment can be used to reduce *Salmonella* and other pathogens in flour to safe levels. However, in low-moisture foods, process times must be increased to achieve adequate lethality, possibly resulting in changes in the flour's functionality such as changes in the gluten quality, vitamin content, and the level of starch gelatinization. There is a need to determine the minimal heat treatment required to achieve desired lethality in flour and other low-moisture foods, with the goal of retaining the flour's functionality. Currently there is no published data about a nonpathogenic bacterial surrogate for *Salmonella* in flour. In this study, a surrogate, which closely matches the thermal death rate of *Salmonella* in flour, has been isolated. The surrogate was identified following an evaluation of thermal death curves of ten different strains of bacteria isolated from heat-treated flour and two nonpathogenic surrogates used in other commodities. Flour samples were inoculated with *Salmonella* or one of the twelve bacterial isolates, and then subjected to heat (70, 75, and 80 °C) for 12–60 min. The heat tolerance for each organism was determined by plating out at least four different time points for each temperature and comparing the death curve to those from *Salmonella*. The death curve from *Pantoea dispersa* was not statistically different ($p < 0.05$) than the death curve of *Salmonella*. This strain of *P. dispersa* (strain JFS) can be used as a conservative, slightly more heat resistant, surrogate for *Salmonella*. It can be used to verify the combination of heat and time necessary to kill *Salmonella* in flour using a commercial heat-treatment process.

1. Introduction

Eliminating or reducing pathogens to safe levels is one of the most important safety considerations when producing a new food product or process. However, a food manufacturer struggles with the difficult balance of applying adequate heat treatment to reduce the microbial load without resulting in excessive structural, functional, and sensory changes in the treated product. In low-moisture foods, heat transfer is slower and heat efficiency, with respect to lethality, is lower than in higher moisture products, resulting in a longer processing time and a higher chance of altering essential attributes of the product (Denyer et al., 2011). This change in heat transfer efficiency is due to the low amount of water in low-moisture products and the increased ratio of air to water in these products (Jay et al., 2005). This has made the development of a suitable thermal process especially difficult for low-moisture foods.

The ability to better test ingredients has brought to light the fact that pathogens are found in both high- and low-moisture ingredients. While high-moisture foods may contain more pathogens, low-moisture foods — defined by Jay et al. (2005) as foods having a water activity of <0.70, have recently been the source of several foodborne disease outbreaks around the world (Izurieta and Komitopoulou, 2012; McCallum et al., 2013; Neil et al., 2012; Zhang et al., 2007). For example, *Salmonella* Typhimurium phage type 42 (PT 42) was recently isolated from an outbreak in New Zealand. One of the factors that made this outbreak unique is that the suspected *Salmonella* strain was isolated from flour found in the consumers' homes (McCallum et al. 2013). Outbreaks such as this one have caused companies to start designing and using methods to lower the microbial load of specific pathogens in their low-moisture products.

One area of research that has received little attention is the development of heat treatment options for flour. Heat treatment is one way to ensure the safety of low-moisture foods. The implementation of the Food Safety and Modernization Act has mandated that food companies

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http://dx.doi.org/10.1016/j.ijfoodmicro.2015.11.012
0168-1605/

develop a plan to validate the efficacy of all food-processing methods in reducing pathogens to acceptable limits. Another problem that has prolonged the development of heat treatment processes for low-moisture foods is the notion that because bacteria cannot grow in low-moisture foods, they are not as much of a concern as in high-moisture foods. This misconception ignores the reality that low-moisture foods are often ingredients in high-moisture products, where conditions are suitable for growth. Complicating the process, microorganisms also exhibit a higher heat resistance in low-moisture foods. One of the original observations of the phenomenon was made by Goepfert et al. (1970) with *Salmonella*. This phenomenon has also been observed by several others (Fine and Gervais, 2005; Laroche et al., 2005; Villa-Rojas et al., 2013). This increase in heat resistance prohibits simply using the same surrogates for high-moisture and low-moisture products. These surrogates cannot be used without further testing to validate that they exhibit the same increase in heat tolerance as the pathogens of interest. The use of surrogates typically employed to validate high-moisture thermal processes in low-moisture thermal processes is not good practice and demonstrates the need for a non-pathogenic surrogate for use in low-moisture food product validation studies. The requirement to validate thermal processes and the fact that pathogens are present in low-moisture foods also reinforce the necessity that all surrogates should be validated in the product currently being tested. Surrogates have been used to verify the safety of food-processing systems for many years.

Salmonella is often a target pathogen of heat treatment processes. Of the fourteen most common pathogens, *Salmonella* has exhibited the greatest impact in terms of illness, loss of quality of life, number of hospitalizations, and number of deaths each year (Batz et al., 2012). One potential non-pathogenic surrogate organism for *Salmonella* in flour is *Enterococcus faecium*. *E. faecium* has been evaluated as a potential surrogate for *Salmonella* in both a high-moisture food (beef) and a low-moisture food (almonds) (Bianchini et al., 2012; Jeong et al., 2011; Ma et al., 2007). Another potential surrogate is *Escherichia coli* which has been used as a surrogate in high-moisture foods (Eblen et al. 2005).

While there have been numerous studies conducted to find surrogates for *Salmonella* in high-moisture foods (such as chicken and beef), there have been relatively few studies conducted in the area of low-moisture foods — and none regarding wheat flour. This study will fill the current void of research that exists in the area of heat-related lethality of *Salmonella* in flour. The purpose of this study was to test nonpathogenic organisms as potential surrogates for *Salmonella* Typhimurium PT 42 in wheat flour.

2. Materials and methods

2.1. Bacterial strains

Salmonella Typhimurium PT 42 was received from The Institute of Environmental Science and Research (Porirua, New Zealand), an isolate from a New Zealand outbreak of salmonellosis in flour in 2008 (Izurieta and Komitopoulou, 2012). Once received, the *Salmonella* stock cultures were maintained at −40 °C. Cultures used during the experiment were maintained on Columbia agar plates (Hardy Diagnostics, CA; USA). *E. coli* 3A-I and *E. faecium* 2B-I were used from stock cultures kept at −40 °C (Brigham Young University Food Microbiology Culture Collection; Utah, USA). The flour isolates were collected from flour that was subjected to 7 min of heat treatment at 75 °C. Once subjected to heat-treatment, the samples were plated on Columbia agar and incubated at 35 °C for 48 h. After 48 h, the colonies were grouped by colony morphology and gram stain. Once grouped, the cultures were maintained on Columbia plates for the duration of the experiment. Isolated colonies were grown overnight in Columbia broth at 37 °C and then transferred to Columbia broth with 10% glycerol as stock cultures and frozen at −40 °C.

2.2. Preparation of cultures

Twelve different strains of bacteria, comprising *E. coli*, *E. faecium*, and ten different isolates from flour (identified as A–J) were evaluated as potential surrogates for *Salmonella*. Cultures were streaked onto Columbia agar and incubated 24 h at 37 °C to obtain a uniform lawn containing the maximum amount of stationary phase colonies (Martinez et al., 2003; Smith and Marks, 2015). The bacterial lawn was then harvested by scraping the lawn plate. The cells were then suspended in 3 mL of warmed Maximum Recovery Diluent (MRD, 1% peptone 8% NaCl per liter) (Komitopoulou and Penaloza, 2009), and vortexed for 10 s to homogenize the inoculum.

2.3. Procedure for inoculating flour

There was no detectable population of *Salmonella* in flour samples used for the test by plating the samples on Xylose Lysine Deoxycholate Agar (XLD, Becton Dickinson, MD; USA) with a limit of detection of 10^{-1} CFU/g. Samples were plated at the 10^{-1} dilution. The dilution was made with MRD and the plates were incubated for 48 h at 35 °C. No noticeable growth was observed on the plates after incubation.

A modified method from Bookwalter et al. (1980) was used to inoculate the flour. An enriched pastry flour (9% protein) used in the study was obtained from General Mills (Cameo Brand, General Mills, MN; USA). The flour was irradiated at a level of 10 kGy. Irradiated flour (120 g) was mixed for 30 s on the lowest setting of a kitchen stand mixer in a cooled autoclaved bowl. Inoculum (3 mL), standardized to 10^9 CFU/mL, was pipetted into the flour and allowed to mix on the lowest setting for 5 min. Inoculated flour was then placed in sterile quart size ceramic grinding mill jars (US Stoneware, OH; USA) with 950 g of 6.35 mm ceramic beads in each jar; the jars were then rolled for 30 min on a jar mill (US Stoneware, OH; USA) at 60 rpm. After mixing, the contents of the jars were screened through a sterile nr 4 sieve to remove mixing beads from the flour. The flour was packaged into sterile sample bags and stored at 4 °C for one week to allow for stabilization of the bacteria (Beuchat and Scouten, 2002; Jeong et al., 2012). Homogeneity of inoculum in flour samples was confirmed by plating of multiple samples from throughout the inoculated flour mixture.

Water activity of flour samples was determined using the chilled mirror technique of an Aqualab Series 3TE (Decagon Devices, WA; USA).

2.4. Heat treatment

A modified method from Izurieta and Komitopoulou (2012) was used for heat treating the samples. Samples of 5 g inoculated flour were weighed into individual sterile 2 mL screw top glass vials containing a septum in the cap then placed in the center of an agitation water bath. Type-T thermocouples monitored the temperature of the flour during treatment. Thermocouples were placed in the geometric center of the flour. An inoculated flour sample representing each strain of bacteria was tested at 75 °C for 0–40 min in ten minute increments. The temperature come-up time in the vials ranged from 7 to 10 min. The D-values of the potential surrogates were compared to the D-values obtained for *S.* Typhimurium PT 42. After comparison of the D-values at 75 °C, flour isolates A and F were selected and tested against *S.* Typhimurium PT 42 at 70° and 80 °C for 0–60 min depending on the temperature. Three replicates of the inoculum were performed at each temperature. The timing of the treatments began when the slowest heating vial reached target temperature (Izurieta and Komitopoulou, 2012). Once heat treatment was finished, the vials were removed from the water bath and placed on ice until completely cool.

2.5. Comparison of Salmonella and potential surrogate survival

Once cooled, the flour was unloaded from vials and placed into sterile 18 × 150 mm test tubes. MRD was used to make serial dilutions,