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Food Microbiology 2018: Induction of a viable but non-culturable state in Salmonella Typhimurium is correlated with free radicals generated by Thermosensation.- Hongmei Liao- Jiangnan University

Hongmei Liao*

School of Food Science and Technology, Collaborative Innovation Centre of Food Safety and Quality Control in Jiangsu Province, Jiangnan University, China

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***Corresponding author:** Hongmei Liao

hmeiliao@jiangnan.edu.cn

School of Food Science and Technology, Collaborative Innovation Centre of Food Safety and Quality Control in Jiangsu Province, Jiangnan University

Tel: +03366720143

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Objective

The objective of this work was to analyze the effects of Thermosensation (TS) on the induction of a viable but non-culturable (VBNC) state in Salmonella Typhimurium and to examine incubation factors affecting subsequent resuscitation of cells. A TS treatment of 380 W at 53°C for 30 min induced the VBNC state in S. Typhimurium cells in beef peptone yeast (BPY) broth, apple/carrot juice, physiological saline, and phosphate buffer solution. Addition of Tween 20 hindered resuscitation compared to the use of BPY medium alone. The optimal growth temperature (i.e. 37°C) was the most suitable temperature to resuscitate cells from the VBNC state. The VBNC incidence index decreased with the addition of sodium pyruvate during TS treatment, as it accelerated resuscitation. Hence, the kinds and amounts of free radicals generated during TS treatment should be analyzed in the future.

Introduction

Foodborne pathogens have a notable effect on human health and can cause enormous financial losses. In 2015, 26 European countries reported a total of 4362 foodborne outbreaks, which caused 45 874 cases of foodborne illnesses, 3892 hospitalizations, and 17 deaths (EFSA and ECDPC 2016). Most outbreaks were caused by bacterial agents (33.7% of all outbreaks), in particular Salmonella (21.8% of all outbreaks). Among Salmonella serovars, S. enteritidis and S. In Canada it was estimated that non-typhoidal Salmonella accounted for ~41% of 4 million cases of foodborne illnesses every year, and the annual cost of foodborne illnesses is enormous.non-conventional sterilization technologies, such as ultra-high hydrostatic pressure, pulsed electric field, and thermosensation (TS), have gained great popularity due to their potential to produce products with higher quality and a fresh-like taste, and the need for less energy to operate.

The sterilizing mechanism of ultrasound is based on chemicals (including the effects of free radicals) and mechanical effects. Ultrasound enhanced the sensitivity of microorganisms to heat, pressure, and low pH due to the induction of acoustic cavitation and other changes in cell membranes.

Salmonella Typhimurium, as one of the most important foodborne pathogens worldwide, can enter the VBNC state on exposure to starvation (Caro et al.1999) and low-temperature conditions (Oliver, Dagher and Linden 2005). For example, the VBNC state of S. Typhimurium was observed when cells were incubated at low (5°C) temperature and exposed to a mixture of chlorine and chloramines (Oliver, Dagher and Linden 2005). To the best of our knowledge, there is no report on the induction of the VBNC state in S.

Materials and Methods

Bacterial strain and growth conditions

Salmonella Typhimurium (CMCC 50115) was obtained from China Microbiological Culture Collection Center (CMCC, Beijing, China). Bacteria were obtained from BPY broth and were diluted with sterilized BPY broth to obtain a working solution with a final concentration of 107 CFU ml-1. The working solution was stored in a refrigerator at 4°C prior to TS treatments. It was cultured in beef peptone yeast (BPY) broth (containing peptone, 10 g l-1; beef extract, 5 g l-1; yeast powder, 5 g l-1; glucose, 5 g l-1; NaCl, 5 g l-1; pH 7.0) at 37°C.

The ultrasound probe was submerged 20 mm below the surface of the sample, and the distance between the tip of the probe and the bottom of the vessel was 25 mm. In order to keep the temperature, stable during ultrasound processing, each sample unit (60 ml) was contained in a double-walled cylindrical vessel in a surrounding water bath. Once the set temperature was reached, the ultrasound treatment began.

The double-walled cylindrical vessel was soaked in 75% alcohol for at least 15 min, washed three times with sterile water, and then washed with untreated S. Typhimurium BPY broth suspension. At the end of the treatment, samples were collected immediately for RNA/DNA extraction.

Determination of Viable and VBNC Cells by RT-qPCR

The numbers of viable, total, and VBNC S. Typhimurium were quantified by reverse transcription real-time quantitative polymerase chain reaction/real-time quantitative polymerase chain reaction (RT-qPCR/qPCR), according to the method of Jiang et al. (2013) with some modifications. RNA samples underwent reverse transcription using the PrimeScript RT reagent Kit with gDNA Eraser (Takara, Kusatsu, Japan) immediately after RNA extraction. DNA and cDNA samples were stored at -20°C prior to qPCR/RT-qPCR analysis.

Samples were serially diluted in sterile physiological saline and plate counts were determined on BPY medium after incubation at 37°C for 24 h. Standard curves were analyzed. The VBNC cell number of S. Typhimurium was calculated using the following formula:

VBNC cell number viable cell number-culturable cell number

VBNC incidence indexes of S.Typhimurium were calculated with the following formula:

VBNC incidence index=VBNC cell number/viable cell number×103

Results

A large fraction of S. Typhimurium cells at stationary phase emitted clear and strong green fluorescence, the observation indicating the entrance of SYTO 9 but the exclusion of PI in stationary cells, further indicating cell membrane integrity. The results showed a fraction of S. Typhimurium cells emitted red fluorescence resulting from damage of membrane while a few cells emitted green fluorescence demonstrating remaining viability. Since culturable cells could not be detected by the plate counting method (not shown in Results) in this situation, cells in a VBNC state existed and were revealed by this qualitative detection method.

The RNA quality consisted of two aspects: its integrity and purity, two bands of 23S and 16S RNA were visible in the agarose gel electrophoresis profile, indicating RNA integrity. Total RNA extracts of S. Typhimurium were confirmed with the quality of OD260/280 and OD260/230 ratios of more than 1.8 (data not shown). In addition, the number of untreated viable cells of CK samples detected by RT-qPCR was consistent with the number of culturable cells measured by the plate counting method.

The numbers of culturable cells were under the detection limit after 25 and 30 min of treatment, and there were 2.26 and 2.84 log CFU ml-1 VBNC cells detected by RT-qPCR, respectively. To ensure that all viable cells were in the VBNC state, a TS treatment of 380 W at 53°C for 30 min was used to induce the VBNC state in S. Typhimurium for the following experiments.

Note: This work is partly presented at 3rd International Conference on Food Microbiology & Nutrition November 29-30, 2018 Dublin, Ireland.