RESISTANCE OF SALMONELLAE TO HEAT, HIGH-PRESSURE AND IRRADIATION STRESS

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Background

Salmonella is a member of the family Enterobacteriacae. These pathogenic bacteria constitute the second largest cause of bacterial gastroenteritis in the developed world and are mainly associated with flesh foods. Owing to the widespread occurrence of Salmonellosis considerable attention has been given in recent years to the destruction or control of the responsible microorganism. Three such treatments under consideration in this investigation include heat, high-pressure and irradiation. Heat treatment is the conventional method used throughout industry to eradicate Salmonellae from food. High-hydrostatic pressure is a promising alternative to heat pasteurisation for preservation of minimally processed foods. Food irradiation is a processing technology that has been shown to be a wholesome process and an alternative to heat treatment.

Objective

It is well established that a species of food borne pathogen contains strains that can vary in their tolerance to an applied stress, such as heat, irradiation or high-pressure. Salmonellae are certainly sensitive to these stresses but that sensitivity can vary greatly. Different serovars of Salmonellae have been found to vary in their heat tolerance e.g. *Salmonella* Senftenberg 775W is considered the serovar with the greatest resistance to heat, up to 30 times higher then *S.* Typhimurium (Ng. *et al.* 1969). Variations between serovars to irradiation (Monk *et al.* 1995) and high-pressure treatment (Patterson *et al.* 1995) have also been noted. Some of the factors that influence microbial sensitivity are inherent resistance of the serovar, stage of growth and microbial load. This investigation will examine the resistance of a catalogue of forty isolates to injury caused by heat, high-pressure stress and irradiation stress with the aim of categorising the organisms on their ability to withstand the processing stress.

Methods

Forty isolates of *Salmonella*, representing 33 different serovars, were obtained from local sources. Prior calculation of D_{10} values for each stress was used to define the treatment that would produce a 3-log reduction in microbial load. The heating technique employed was an adaptation of Gaze *et al.* (1989), whereby glass universal bottles (25ml volume) containing 9.9ml of filter sterilised 24hr spent tryptone soy broth plus 0.6% yeast extract (TSBYE) were immersed in a stirred water bath and allowed to reach the test temperature 57°C. Each bottle was inoculated with 100µl of the 24hr stationary phase culture. After 14min heating time a 1ml aliquot was removed and dispensed into 9ml of ice-cold maximum recovery diluent (MRD). This had the immediate effect of stopping any heating injuries. Each isolate was heat stressed in triplicate.

For high-pressure treatment an adaptation of the packaging method used by Linton *et al.* (2001) was used. Five ml of a 24hr stationary phase culture was packaged in sterile polyethylene/polyamid pouches and subjected to 350 MPa for 10mins at 20°C. Each serovars was treated in triplicate.

When irradiating serovars 5ml of a 24hr stationary phase culture was packaged in sterile polyethylene/polyamid pouches and irradiated with 1.5kGy gamma rays from a cobalt 60 source. Each serovars was treated in triplicate.

After all treatments serovars were plated onto a recovery agar tryptone soy agar plus 0.6% yeast extract (TSAYE) and a selective agar brilliant green agar (BGA) using a spiral plater (WASP, Don Whitley Scientific). Incubation was at 37°C for 48 hours prior to counting using the Protocol plate counter.

Results and discussion

Responses of the forty serovars to the three stresses applied varied considerably. Most serovars showed similar counts on selective (BGA) and non-selective (TSAYE) media after exposure to irradiation implying injuries were either minor or lethal with few injured cells unable to recover on BGA. However this stress also gave the highest level of standard deviation (sd) from the mean (± 0.76 $\log^{10}$ cfu) reduction in total counts post stress. Heat stress resulted in the greatest number of injured cells, with the difference in recoveries between the two medias showing the largest difference. High-pressure treatment gave the most consistent results indicating a relatively uniform resistance to this stress in the population studied. The sd for heat and high-pressure was very similar being 0.51 and 0.48 $\log^{10}$ cfu respectively.

Analysis of variance was applied and grouped the forty isolates into five main clusters, with one serovars outlying the groups based on a very high sensitivity to high-pressure. *S.* Ruiru gave almost 100-fold fewer survivors following the application of 350 MPa for 10mins at 20°C. The reason for this sensitivity will now be investigated since increasing the sensitivity of all serovars to this level would greatly increase the efficacy of the method in producing safe products. A summary of the cluster analysis is illustrated in Table 1.

It has been suggested that the action of pressure inactivation is similar to thermal inactivation in that more than a single factor is responsible for the death of bacterial cells (Metrick *et al.* 1989). Two internationally important human pathogens have been identified as having higher resistance to both of these applied stresses *S.* Typhimurium DT104 and *S.* Enteritidis PT4. However, isolate E210 *S.* Typhimurium was the most sensitive of all 40 isolates studied with regard to the heating process, but showed no unusual sensitivity to high-pressure treatment. Thus it would seem that resistance to the applied stresses is inherent within each serovars. Further investigation will aim to identify which features could possibly provide this increased protection

A group of 6 S. Typhimurium isolates further illustrates the basis of this study (Table 2). The results showed that this serovars is sensitive to irradiation but heat resistance is more variable within the group, allowing resistance/repair mechanisms to be subsequently repaired.

Having grouped the isolates based on their resistance to the stresses, specific representatives of the 40 isolates will now be selected for further study in order to understand the basis of their ability to resist, or rapidly recover from, the processing stresses applied. A local isolate of *S.* Alachua (E88) will be of interest as it proved to be the isolate most sensitive to all three of the stresses applied.

Conclusions

Each *Salmonella* isolate is unique in its inherent genetic ability to withstand the heat, high-pressure and irradiation stresses applied. The variability in resistance detailed in this study is in agreement with previous investigations. The cluster analysis identified different groupings of isolates in terms of resistance to the applied stress. Results imply that each of the three stress have a different mode of action hence will have correspondingly different mechanisms of resistance. Further investigations aim to understand the basis of their ability to resist, or rapidly recover from, the processing stress applied. Thus the most rapid methodology for recovering Salmonellae subsequent to three specific stresses will be designed and tested. This will allow the agri-food industry to ensure high standards of hygienic quality whilst minimising product-holding times.

Pertinent literature

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Table 1. Summary of cluster analysis of *Salmonella* isolates subjected to three stresses heat, high-pressure and irradiation.

Resistance	3 Stresses	Heat & Pressure	Heat & Irradiation	Pressure & Irradiation
Least resistant	E88 S. Alachua	E33 *S.* Taksony	E170 *S.* Havana	E202 *S.* Ruiru
		E68 *S.* Schwarzengrund		E86 *S.* Corvallis
		E179 *S.* Chandans		E76 *S.* Meleagridis
	E102 S. Mbandaka	PP00/12 *S.* Typhimurium		E1 *S.* Typhimurium
		E83 *S.* Berta		
		E180 *S.* Liverpool	E164 *S.* Give	E92 *S.* Roterberg
		E66 *S.* Anatum	E52 *S.* Muenster	E19 *S.* Enteritidis
				E191 *S.* Cubana
Most resistant		SE00/93 *S.* Typhimurium DT104	No. 242 *S.* Dublin	E182 *S.* Java
		No. 220 *S.* Enteritidis PT4	No. 244 *S.* Dublin	E165 *S.* Agona

Table 2. Loss of viability in resting cells of six isolates of *S.* Typhimurium exposed to gamma irradiation or heat.

Isolate	Log_{10} change Irradiation	Log_{10} change Heat
E1	-2.55	-4.22
E210	-2.72	-5.46
E201	-2.95	-3.65
SE00/93	-3.14	-3.40
PP00/12	-3.57	-4.05
SE00/79	-3.58	-3.98