EFFECT OF TEMPERATURE AND WATER ACTIVITY ON THE RECOVERY OF *S.TYPHIMURIUM* DT104 IN A BROTH SYSTEM

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S.typhimurium DT104, relative humidity, chilling

Background

Chilling is recommended as a CCP in a number of generic HACCP plans for beef (Anon, 1999) and is presently included in the working HACCP plans of a number of beef plants in the USA (Bacon *et al.* 1999). However, reductions in bacterial counts are not consistently achieved under commercial chilling conditions. Data shows that bacterial numbers can increase, decrease or remain unchanged (Nutsch, *et al.* 1997). The factors responsible extrinsically are temperature, air speed and relative humidity with the main intrinsic factor being water activity (a_w). *S.Typhimurium* DT104 is an emerging pathogen primarily associated with cattle but has spread to a range of food animals, especially pigs and chickens. As a result of its importance to the food industry it will be used in this study.

Objective

The objective of this study was to evaluate the effect of temperature and water activity on the growth and survival of *S.typhimurium* DT104 stationary and exponential phase cells in a model broth system

Methods

S.typhimurium DT104 was used as the test organism. *Salmonella* strains were recovered from storage at -20°C on Protect beads (Mast Diagnostics) and incubated in BHI (Oxoid) for appropriate times and temperatures to yield cells in the stationary and exponential phase of growth. A model broth system was devised in which glycerol was used as a humectant to produce BHI broths over a range of a_w values. These broths were adjusted with glycerol (16, 32, 64 and 96% [vol/vol]) corresponding to a_w values of 0.79, 0.86, 0.92 and 0.96 with BHI alone serving as a control (Mattick *et al.* 2000). The a_w of the broths was measured using an Aqualab CX-3T (Labcell, Basingstoke, Hampshire, United Kingdom) water activity meter at 4°C, 10°C and 25°C.
One ml of a 6 and 8 $\log_{10}$ cfu/ml stationary and exponential phase cultures, respectively, were inoculated into broths at different a_w values. Uninoculated BHI served as a control. The broths were incubated for a 72 hour period at temperatures of 4°C, 10°C and 25°C. At 24 hour intervals a 1ml aliquot was removed from each sample and serially diluted in 9ml volumes of maximum recovery diluent (Oxoid). Aliquots (0.1ml) of appropriate dilutions were inoculated (using the pour plate technique) onto XLD (Oxoid) *Salmonella* selective agar and incubated for 24 hours at 37°C. This procedure was repeated on TSA agar for the enumeration of stressed *Salmonella* cells. A recovery technique was used in which cells were inoculated onto the surface of TSA which were incubated at 30°C for 2 hours, after which they were overpoured with XLD and then incubated at 37°C for 24 hours. All samples were plated and the experiments were replicated three times.

Results

The data in Table 1 show that both temperature and a_w had a major effect on the survival of the pathogen after incubation for 72 h. In general, reductions in counts were highest when a_w values were lowest. Temperature also had an effect and with an increase from 4 to 25°C the influence of water activity was reduced. The effect of temperature was also reflected in the controls in BHI. Differences between stationary and exponential cells were variable. At 4°C a difference in cell types was only evident when the a_w was 0.79, where losses in viability were greatest in the exponential cells. When the temperature was at 10°C the greatest reductions were in stationary phase cells at a_ws of 0.79, 0.86 and 0.92. Finally, at 25°C and an a_w of 0.79 exponential cells were most affected but this was reversed at values of 0.86 and 0.92 where stationary phase cells showed the largest reductions. It was also noted that the increases that occurred at an a_w of 0.96 and in the control were higher in stationary phase cells
In order to determine the difference between cell injury and cell death the recovery of *S. typhimurium* DT104 after exposure to different a_w and temperatures was observed and the results are shown in Table 2. The data show that high levels of cell recovery were possible for all treatments. This indicated that much of the reductions, shown in Table 1 were not related to cell death. The level of cell injury in the controls was of particular interest especially at 25°C which in Table 1 showed a net increase in growth. This was taken as an indication of the inhibitory nature of the XLD medium for both stationary and exponential phase cells.

Table 1: Mean $\log_{10}$ cfu/ml reduction or increase in counts of stationary and exponential cells of *Salmonella typhimurium* DT104 after 72 h incubation in 4, 10 and 25°C in broths at different a_w values

Temp. (°C)	4		10		25	
Cell types / a_w	Stat.	Exp.	Stat.	Exp.	Stat.	Exp.
0.79	-1.59	-2.46	-2.0	-0.58	-0.84	-1.91
0.86	-2.19	-2.31	-1.64	-0.69	-1.44	-0.49
0.92	-1.11	-1.27	-1.17	-0.47	-0.39	+0.90
0.96	-0.56	-1.04	+0.30	+0.14	+0.65	+0.08
Control (BHI)	-0.24	+0.11	+1.77	+1.87	+3.38	+2.21

Table 2: Mean recovery (log_{10} cfu/ml) of stressed stationary and exponential cells of *S. typhimurium* DT104 after incubation for 72 h in broths at different a_w values at 4, 10 and 25°C

Temp. (°C)	4		10		25	
Cell types / a_w	Stat.	Exp.	Stat.	Exp.	Stat.	Exp.
0.79	0.71	0.84	0.85	0.44	0.64	0.82
0.86	1.10	0.82	0.73	0.88	0.84	0.99
0.92	0.99	1.71	0.82	1.81	1.44	0.91
0.96	0.80	1.52	0.87	1.11	1.29	0.21
Control (BHI)	0.10	0.21	0.14	0.08	0.86	0.45

Discussion

In the present study *S. typhimurium* DT104 survived at low a_w values combined with low temperature, an observation also made by Mattick *et al.* (2000). Reductions in counts occurred under these conditions. The data in Table 2 however, indicated that even at the extremes of aw (0.79) and temperature (4°C) recovery was substantial but that the synergy between the two stresses had been bactericidal. In the a_w range from 0.92 to 0.96 at 4°C reductions were less, but of greatest importance was the almost complete recovery of the cells from injury (Table 2). The a_w of the surface of fresh meat is controlled by the chilling conditions (Rosset, 1982). In the initial stages of chilling the lower the temperature and the higher the air velocity, the higher the evaporative water losses from the meat surface. This loss of surface water leads to a decrease in the a_w of the meat. When the meat surface water content falls below 85%, the a_w is about 0.95, at which level bacterial growth ceases because cells on the meat surface are deprived of sufficient moisture for growth.

Under conditions of low temperature and a_w bacterial cells are still viable but stressed. The a_w subjects the cells to an osmotic stress, while the low temperatures result in cells that are cold shocked. Experiments with *E. coli*, *Salmonella* and other gram negative organisms have shown that there is a synergistic effect between a_w, low temperature and pH (Presser *et al.* 1998). At the pH of fresh meat entering the chill, (6.0+) until the ultimate pH is reached (5.4-5.5) *E. coli* at an a_w of 0.95 and a temperature of 5°C will survive for at least 48 h without a significant decrease in viability (Clavero and Beuchat, 1996). A similar relationship between a_w and low temperature has also been observed in the survival of *Salmonella typhimurium* (Li and Torres, 1993). The cells, while viable, are injured but are capable of recovery when incubated in suitable media. It should also be noted that in nature cells on the hide of animals, and therefore on beef carcasses, are in stationary phase (Sheridan and McDowell, 1998). While these cells are more resistant to adverse conditions than exponential cells, they respond to cold or osmotic stimuli in the same way and assist cells such as *E. coli* to survive in non-growth but stressful conditions (Palumbo *et al.* 1997; Clavero and Beuchat, 1996).

Conclusion

Although it may not be possible to directly extrapolate the present data to predict the survival of *S. typhimurium* DT104 on beef carcass surfaces, it strongly suggests that if this pathogen were present on the surface it could recover from the stresses imposed during carcass refrigeration. The importance of these results may be related to bacterial contamination on fresh meat surfaces during chilling.

References

Anon, (1999). Generic HACCP model for beef slaughter. US Department of Agriculture, Food Safety and Inspection Service, 1-40.

Bacon, R.T., Sofos, J.N., Belk, K.E., Reagan, J.O. and Smith, G.C. (1999). Commercial evaluation of multiple-sequential interventions for decontamination of beef carcasses. Colorado State University Beef Programme Report.

Clavero, M.R. and Beuchat, L.R. (1996). Survival of *Escherichia coli* O157:H7 in broth and processed salami as influenced by pH water activity and temperature and suitability of media for its recovery. Applied and Environmental Microbiology, 62, 2735-2740.

Li, K.Y. and Torres, J.A. (1993). Water activity relationships for selected mesophiles and psychrotrophs at refrigeration temperature. Journal of Food Protection, 56, 612-615.

Mattick, K.L., Jorgensen, F.,Legan, J.D., Cole, M.B., Porter, J., Lappin-Scott, H.M. and Humphrey, T.J. Survival and filamentation of *Salmonella enterica* Serovar Enteritidis PT4 and *Salmonella enterica* Serovar Typhimurium DT104 at low water activity. Applied and Environmental Microbiolgy, 66, 1274-1279.

Nutsch, A.L., Randall, K., Phebus, R.K., Riemann, M.J., Schafer, D.E., Boyer, J.E., Craig Wilson, R., Leising, J.D. and Kastner, C.L. (1997). Evaluation of a steam pasteurization process in a commercial beef processing facility. Journal of Food Protection, 485-492.

Palumbo, S.A., Pickard, A. and Call, J.E. (1997). Population changes and verotoxin production of enterohemorrhagic *Escherichia coli* strains inoculated in milk and ground beef held at low temperatures. Journal of Food Protection, 60, 746-750.

Presser, K.A., Ross, T. and Ratkowsky, D.A. (1998). Modelling the growth limits (growth/no growth interface) of *Escherichia coli* as a function of temperature, pH, lactic acid concentration and water activity. Applied and Environmental Microbiology, 64, 1770-1773.

Rosset, R., (1982). Chilling, freezing and thawing. In: Meat Microbiology, Ed. M.H.Brown, 265-318, Applied Science publishers, London.

Sheridan, J.J. and McDowell, D.A. (1998). Factors affecting the emergence of pathogens on foods. Meat Science, 49, S151-S167.