# LAGSAL. Effect of sublethal thermal injury on the survival and re-growth of *Salmonella* Enteritidis.

Marina Muñoz Cuevas

*Salmonella enterica* serovar Enteritidis is the cause of a worldwide increase in human salmonellosis. During the last three decades *Salmonella* Enteritidis was involved in a number of food poisoning outbreaks mainly associated with the consumption of meats and egg products. In recent years there has been an increase in consumer demand for fresh, minimally processed foods, but foodborne disease linked to ready-to-eat food has also increased. The Commission Regulation (EC) No. 2073/2005 and No. 1441/2007 on microbiological criteria for foodstuffs has established a series of food safety rules for ready to eat products. An increased interest in “hurdle technology” has been observed because of the increased market demand for minimally processed foods. Hurdle technology employs the combinations of various antibacterial treatments to limit the growth of spoilage bacteria, improving the microbial safety and maintaining the sensory and nutritional quality of food. Among these hurdles, mild heat treatment, low temperature, water activity, acidity, etc have been used for centuries. However, these treatments could leave cells damaged, but not inactivated, so they may be able to grow in favourable environmental conditions. Factors such as temperature of incubation and culture medium influence the capacity of cells to repair heat damage. The objective of this study was to determine the effect of adding NaCl during the sub-lethal heat treatment of Salmonella on adaptation to the subsequent growth environment. We used automated optical density (OD) measurements to quantify the variability of the cells response to stress. The results were validated with plate count measurements and micro-array data which showed significant changes at the molecular level.

Stationary phase cultures of *Salmonella enterica* subs. enterica serovar Enteritidis phage type 4 were used in the experiments. Tubes containing 10 ml of tryptone soya broth plus 0.3% yeast extract (TSYB) with NaCl concentrations of 0 or 8% (w/w) were used as heating media. The heat treatments were carried out in a water bath at 60°C for 30s. Tubes were submerged in the water bath to preheat and then injected with 100 µl cell suspension directly into the liquid using a precision syringe fitted with a long sterile needle. After the heating time, the tubes were removed from the bath and cooled rapidly in ice water. Appropriate dilutions of heated samples were inoculated into five tubes of each recovery medium. Recovery media consisted of TSYB containing NaCl concentrations from 0 to 9% (w/w). Tubes were incubated at 30°C. OD, plate count and micro-arrays measurements were some of the techniques used in this study.

## Probability of growth decreases with added salt in the recovery medium.

Most probable number was used to estimate the fraction of cells that are able to grow in TSYB with added NaCl, concentrations of which range from 0 to 9%. The probability of growth was close to 1 for less than 8% NaCl, but decreased for concentrations higher than 8%. We chose 8% NaCl for our study, which is a sufficiently high concentration to see the effect of osmotic stress, without decreasing the probability of growth in the recovery medium to a level where we could not acquire sufficient information on the kinetics of the survivors.

**Effect of the pre-inoculation osmotic stress on recovery.**

Detection times were plotted against the logarithm of the inoculum values. Datasets were generated for a range of the inoculum levels and an F-test showed that the data could be merged. F-test showed no significant difference between the slopes of the curves, but the intercept is significantly different (at 0.05 level) if the cells were heated without salt in the heating medium. The doubling time in 8% NaCl in the recovery medium was 12 hours whether heated in media with or without 8% NaCl. Without NaCl in the heating medium, the cells needed significantly longer time (ca 50 - 60 hrs) to reach the detection level in the Bioscreen wells.

The observation above was validated with viable count experiments from two different inoculum levels. From the lower inoculum, cells heated without NaCl in the heating medium needed up to two more days to reach exponential phase in the recovery medium containing 8% added NaCl, than when the heating medium contained the same NaCl concentration. From the higher inoculum it was 1.5 days.

**Gene Expression in *Salmonella* Enteritidis following exposure to heat treatment in salted medium**

The aim of this study was to explore differential gene expression in S. Enteritidis following exposure to heat treatment in salted or unsalted media using microarrays.

No RNA could be recovered in the experiments where the heat treated cells were recovered in TSYB with 8% NaCl. The following analysis is a comparison of the effect of NaCl in the heating medium for cells recovered in TSYB. Out of 3029 probes considered in the ANOVA, 640 genes were found to be significantly differentially regulated when NaCl was added to the heated menstrum. They included genes known to be linked to osmotic stress such as those corresponding to the production of the osmoprotectant trehalose, ostA and otsB, the porin regulator ompR, the global regulator H-NS and the hypothetical periplasmic protein YceI.

Our study in LAGSAL highlights the ambiguity of the concept of “quantification of injury”. Namely, combined treatment may cause more structural changes in the cell than one treatment alone, however if the subsequent growth environment is not optimal but contains elements of the inactivating environment, the cells could be more suitable to adapt and re-grow there.

Using OD data for survival studies has both its advantages and disadvantages. The advantage of this method as opposed to the viable count curves was that we could gain information on the variability of the bacterial responses due to the history effect, which is apparent in the initial decline period. The disadvantage is that the history effect has high uncertainty and it is practically impossible to separate two sources of the detection time variability: the history effect and the inevitable error in the dilution of the inoculum.