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Strain variability of *Salmonella enterica* and the central role of RpoS sigma factor in stress resistance and overall phenotype

This thesis is submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy

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Declaration

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

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Abstract

Salmonella is considered to be one of the most important causal agents of foodborne illness throughout the world. The ability of *Salmonella* to resist stress and survive under adverse conditions can vary between strains. This work examines the contribution of variable phenotypic properties to survival under mild and severe stress and how these are linked with genetic and functional variability of *rpoS* gene and RpoS status respectively in representative serovars of *Salmonella enterica* commonly associated with foodborne illness. The test strains were *Salmonella* serovars Anatum, Enteritidis (466), Enteritidis (496), Hadar, Heidelberg, Montevideo, Newport and Virchow and two Typhimurium strains previously characterised as either RpoS-positive or RpoS-negative.

Survival of *Salmonella* is affected by environmental conditions such as temperature, pH and water activity. Our main aim was with the use of ComBase database, to identify the major environmental factors affecting the growth of *Salmonella*. Through analysis of the data in the database we could conclude that inactivation of *Salmonella* in all systems was mainly dominated by temperature while pH and water activity (a_w) levels appeared to be less influential. Although predictions based on data from databases can be very useful, biological variability may affect the confidence limits of such predictions. Therefore, we proceeded with investigating the role of these parameters in survival of *Salmonella* taking also in account the variability between different strains.

Survival was compared in broth medium containing 9% NaCl, 20% NaCl, acetic acid pH 4.5 or lactic acid pH 3.5. Two phenotypically RpoS -negative strains (*S.* Heidelberg and *S.* Typhimurium 10) were consistently the most sensitive to all stresses but variability in survival was also seen among RpoS -positive strains. The most resistant strain depended on the type of stress with serovars Enteritidis (466) and Enteritidis (496) being the most resistant to acid and serovar Hadar the most resistant to salt. We also found a good correlation between acid

resistance and lysine decarboxylase activity which suggests that the lysine decarboxylase plays a significant role in acid resistance. The survival results suggest a considerable role of RpoS in stress resistance.

Strain variation was also observed in other phenotypes related to survival (motility, biofilm formation and sensitivity to bile salts). However, RpoS status did not seem to affect motility, while it was found to affect biofilm formation and resistance to bile salts. Interestingly enough *S.* Virchow, although it behaved overall as an RpoS positive serovar, it showed a significant sensitivity to bile salts.

Subsequently, we progressed with sequencing of the *rpoS* gene of all serovars and we confirmed that the two strains that were particularly sensitive to various stresses (*S.* Typhimurium and *S.* Heidelberg) harboured significant mutations in the *rpoS* gene. The sequencing of *rpoS* not only confirmed a link between RpoS and various phenotypic characteristics related to stress but also it also revealed a link with differences in the utilisation of carbon sources. We found that the RpoS-negative phenotype was linked with an increased growth under different carbon sources suggesting that a functional RpoS is a burden for growth which is in agreement with the Self Preservation and Nutritional Competence (SPANC) hypothesis previously shown in *E. coli.*

Strain differences established in broth systems can also apply to survival in food. Intra–species variability of *Salmonella* survival kinetics in *belacan,* a traditional Malaysian food suggested that factors additional to RpoS might play a role while one of these factors might be an unknown antimicrobial compound we found to exist in *belacan*.

In conclusion, this work has shown that RpoS is an important factor under adverse conditions but also under optimal conditions contributing greatly in strain variability that might not be taken in account when mathematical models are used.