Study on the mechanism for heat resistance and recovery from thermal damage in Salmonella

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Title　：Study on the mechanism for heat resistance and recovery from thermal damage in *Salmonella*

（サルモネラの耐熱性および熱損傷回復機構に関する研究）

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Thesis Summary

Heat treatment is the core technology of decontamination, food preservation and production for many years. Temperature is well known to be one of the important factors that can be applied by the food processor to render food products commercially sterile, which is the state of food free from the growth of pathogenic and spoilage microorganisms during the distribution and shelf-life of the product. After microorganisms are subjected to harsh conditions, including high temperature, some of the cells enter the state of sublethal injury. The existence of injured microorganisms in food and their recovery at the distribution stage are major problems in food hygiene. *Salmonella* spp. are major foodborne pathogens that cause thousands of incidents of foodborne diseases through their consumption of many kinds of food, including eggs, milk, meat, vegetables, fruits, and their processed food products. To clarify the mechanism of heat resistance and recovery from heat injury in *Salmonella* Typhimurium, the role of potential genes during the recovery from heat injury in TSB or TSB in the presence of 4 and 8% NaCl, were investigated in this thesis.

During recovery from heat injury in a rich medium, increase in the transcription levels of phage shock protein (*psp*) genes has been shown by the previous DNA microarray analysis. The increase in transcription levels of *pspABCDEFG* during the recovery process was confirmed by qRT-PCR. The *pspA* deletion mutant (*ΔpspA*) showed slightly lower viable counts and membrane potential than those of the wild-type strain during recovery. On the other hand, there was no significant difference in the viable counts between *pspA*-overexpressing strain (*S.* Typhimurium pBAD30/pspA(+)) and the control strains of *S.* Typhimurium pBAD30/pspA(-) and *S.* Typhimurium pBAD30(+) during recovery. It seems that a lack of PspA protein alone somewhat affects the recovery of

S. Typhimurium from heat injury, but overexpression of PspA alone is not sufficient to promote recovery.

After cultivation in TSB containing 4% and 8% NaCl, *S*. Typhimurium cells were heated at 60°C for 20 min. After the heat treatment, total viable counts including intact cells and injured but recoverable cells determined by the plating method using TSA were significantly higher than those of the cells cultured in TSB including 0.5% NaCl. After cultivation in TSB with 4% and 8% NaCl, 193 and 252 genes were up-regulated, and 173 and 211 genes were down-regulated, respectively, compared with those in TSB. During recovery culture, transcription was up-regulated in 716 and 509 genes, and down-regulated in 650 and 418 genes in the cells in 4% and 8% NaCl, respectively. Transcription of many genes involved in the colanic acid synthesis largely increased after cultivation in the presence of NaCl. The amount of colanic acid significantly increased when the cells were cultivated in a medium with 4% NaCl, compared to that without NaCl. After recovery culture for 3 h, transcription of the genes involved in the phage shock response strongly up-regulated, suggesting the contribution of these genes in recovery process of heat injured cells in the presence of 4 and 8% NaCl.

The results obtained in this study provide valuable information to elucidate the mechanism of increase in heat resistance and recovery from the thermal injury in the presence of NaCl. These findings help to develop approaches to kill pathogens and decrease the microbial food safety risks in foods including NaCl.