Study on the heat and acid tolerance and underlying mechanisms in Salmonella in the presence of sucrose

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Title　：Study on the heat and acid tolerance and underlying mechanisms in *Salmonella* in the presence of sucrose
（ショ糖存在下におけるサルモネラの熱および酸耐性とその機構に関する研究）

Category：Kou

Thesis Summary

Salmonella spp. is one of the major causes of foodborne diseases of a worldwide concern. Recently, a serious attention has been paid on salmonellosis caused by low moisture foods (LMFs). *Salmonella* in these LMFs prolonged survival, enhanced heat and acid tolerance. Sucrose has been implicated with many LMFs like chocolate and peanut butter and was reported to induce high heat tolerance in *Salmonella*. Therefore, the present study was focused on the heat and acid tolerance and underlying mechanisms in *S.* Typhimurium in the presence of sucrose.

Following heat treatment at 60 °C for 5 min, viable cell counts on Trypticase Soy Agar (TSA) of the cells grown in Trypticase Soy Broth (TSB) supplemented with 35 % (w/v) sucrose (35TSBS^{+}) for 24 h and resuspended in the same medium were 3-Log higher than those grown and resuspended in TSB without sucrose, and 1-Log higher than the cells grown in TSB and resuspended in TSB with 35 % sucrose. Viability of the cells directly transferred from TSB to preheated TSB with sucrose was positively correlated with sucrose concentration. These results suggest that under sucrose-induced low water activity (a_w), *Salmonella* increased heat tolerance in a multiple-stage manner. DNA microarray analysis identified sixteen up-regulated genes involved in cobalamin biosynthesis in the cells grown in the presence of 35 % sucrose. Deletion of the *pocR* gene, which positively regulates cobalamin biosynthesis, resulted in suppression of the improvement in heat tolerance of *S.* Typhimurium under sucrose-induced low a_w, suggesting potential contribution of this gene in increasing heat tolerance of *S.* Typhimurium.

The acid tolerance as well as global gene transcriptions in heat-injured *S.* Typhimurium in the presence of sucrose, were investigated as the first step of its pathogenicity evaluation. Growth broth with an initial pH of 6.0 and heat treatment at 60 °C for 5 min were identified as the conditions best suited for

generating injured cells. While the cells grown in TSB showed a logarithmic decrease on cell viability (~0.2 Log CFU/min) within simulated gastric fluid (SGF), the cells grown in 25TSBS$^+$ or 35TSBS$^+$ drastically lost viability in SGF within 1 min. These acid-sensitive cells grown in 25TSBS$^+$ or 35TSBS$^+$ strongly induced acid tolerance immediately after heat treatment. The acquired acid tolerance further increased during incubation after heat treatment, even in the presence of sucrose. However, the acid tolerance induced by heat treatment or subsequent incubation seems to be distinct from major existing mechanisms. The acid sensitivity of non-injured cells grown in 25TSBS$^+$ or 35TSBS$^+$ may largely due to the synergistic effect of acid and simultaneous osmotic shock as suggested by the DNA microarray analyses. The cause of elevated acid tolerance immediately after heat treatment is unclear but probably implicated with some heat-induced damage on membrane proton pump. The increase in acid tolerance during incubation after heat treatment seems to be a genetically controlled process, including the expression of *pspABCDEFG* that help cells maintain proton motive force, the expression of *dnaK*, *yccV*, *ibpA*, *grpE*, *ibpB* and *groEL* encoding heat shock proteins like chaperonins that capable of repairing acid-denatured proteins, and the repressed expression of *citDEFGX* that contribute to intracellular accumulation of citrate which can buffer the intracellular pH toward 6.4 and thus resist acid shock. Aside from the elevated acid tolerance, an almost overall expression of virulence factors at transcriptional level was increased in heat-injured cells during recovery in 35TSBS$^+$. These results develop the understanding of the pathogenicity in *Salmonella* in sucrose-containing LMFs.

To conclude, these knowledges would help understand the heat and acid tolerance of *Salmonella* in the presence of sucrose. The results of transcriptomic analysis serve as a basis for future study that eventually contributing to a better microbial control in the food industry.