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Title of research project	Modelling the survival kinetics and probability of *Salmonella enterica* and enterohemorrhagic *Escherichia coli* under desiccation environment and on low water activity foods
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Name of principal research Investigator（PI）	Shigenobu Koseki

【Abstract】

We investigated the survival kinetics of *Salmonella enterica* and enterohemorrhagic *Escherichia coli* under various water activity (a_w) conditions to elucidate the net effect of a_w on pathogen survival kinetics and to pursue the development of a predictive model of pathogen survival as a function of a_w. Four serotypes of *S. enterica* (*S.* Stanley, *S.* Typhimurium, *S.* Chester, and *S.* Oranienburg) and three serotypes of enterohemorrhagic *E. coli* (*E. coli* O26, *E. coli* O111, and *E. coli* O157:H7) were examined. These bacterial strains were inoculated on a plastic plate surface at a constant relative humidity (22, 43, 58, 68, or 93% relative humidity (%RH), corresponding to the a_w) or on a surface of almond kernels (a_w 0.58), chocolate (a_w 0.43), radish sprout seeds (a_w 0.58), or cheddar cheese (a_w 0.93) at 5, 15, or 25°C for up to 11 months. Under most conditions, the survival kinetics were nonlinear with tailing regardless of the storage a_w, temperature, and bacterial strain. For all bacterial serotypes, there were no apparent differences in pathogen survival kinetics on the plastic surface at a given storage temperature between the tested relative humidity conditions, except for the 93%RH condition. Most bacterial serotypes were rapidly inactivated on cheddar cheese when stored at 5°C compared with their inactivation on chocolate, almonds and radish sprout seeds. Distinct trends in bacterial survival kinetics were also observed between almond kernels and radish sprout seeds, even though the a_w of these two foods was not significantly different. The survival kinetics of bacteria inoculated on the plastic plate surface showed little correspondence to those of bacteria inoculated on food matrices at an identical a_w. These results demonstrated that for low a_w foods and/or environments, a_w alone is insufficient to account for the survival kinetics of *S. enterica* and enterohemorrhagic *E. coli*.

Furthermore, we investigated a bacterial sample preparation procedure for single-cell studies. In the present study, we examined whether single bacterial cell obtained via 10-fold dilution followed a theoretical Poisson distribution. Four serotypes of *Salmonella enterica*, three serotypes of enterohaemorrhagic *Escherichia coli* and one serotype of *Listeria monocytogenes* were used as sample bacteria. An inoculum of each serotype was prepared via a 10-fold dilution series to obtain bacterial cell counts with mean values of one or two. To determine whether the experimentally obtained bacterial cell counts follow a theoretical Poisson distribution, a likelihood ratio test between the experimentally obtained cell counts and Poisson distribution which parameter estimated by maximum likelihood estimation (MLE) was conducted. The bacterial cell counts of each serotype sufficiently followed a Poisson distribution. Furthermore, to examine the validity of the parameters of Poisson distribution obtained from experimental bacterial cell counts, we compared these with the parameters of a

Poisson distribution that were estimated using random number generation via computer simulation. The Poisson distribution parameters experimentally obtained from bacterial cell counts were within the range of the parameters estimated using a computer simulation. These results demonstrate that the bacterial cell counts of each serotype obtained via 10-fold dilution followed a Poisson distribution. The fact that the frequency of bacterial cell counts follows a Poisson distribution at low number would be applied to some single-cell researches with a few bacterial cells. In particular, the procedure presented in this study enables us to develop an inactivation model at the single-cell level that can estimate the variability of number of bacterial survivors during the bacterial death process.

In addition, to describe the dispersion of the bacterial numbers as a stochastic variability, the Poisson distribution, as a representative probability distribution, was used to estimate variability of bacterial numbers during the inactivation process. We experimentally examined the bacterial death probability at the single-cell level under a desiccation environment, and further examined the changes in the probability distribution during the inactivation process using a computer simulation. Four serotypes of *Salmonella enterica* and three serotypes of enterohemorrhagic *Escherichia coli* were examined for survival evaluation. We prepared bacterial cells, whose numbers followed a Poisson distribution ($\lambda = 2$), and placed the bacterial cells into 96-well microplates. The microplates were stored under a desiccation environment at 10-20% relative humidity and at 5, 15, and 25°C. The survival or death of bacterial cells in a 96-well microplate was confirmed by adding tryptic soy broth as an enrichment culture after an arbitrary storage duration. The changes in the Poisson distribution parameter, which represents the variability of the bacterial survival number, was described by a gamma regression as a function of time. In addition, we simulated random changes in survival bacterial numbers by using a random number generator via computer simulation to determine whether the survival bacterial number followed the Poisson distribution during the bacterial death process. Over 83% of the simulated distributions followed a Poisson distribution. These results demonstrated that the variability of survival bacterial number could be described as a Poisson distribution by the developed model. Describing the survival bacterial number as a distribution provides a more realistic estimate compared with point estimates used in a deterministic approach. The probability models describing the variability of survival bacterial numbers as a probability distribution should prove useful to estimate the quantitative risk of bacterial survival during inactivation.