Isolation, characterization and application of lytic bacteriophages against foodborne pathogens

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Title　：Isolation, characterization and application of lytic bacteriophages against foodborne pathogens
（食中毒細菌に対する溶菌ファージの分離、性質および利用に関する研究）

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Thesis Summary

Salmonella spp. has been recognized as an important foodborne pathogen worldwide. *S.* Enteritidis and *S.* Typhimurium are the two serovars most frequently associated with human infection. Salmonellosis is commonly linked with the consumption of contaminated foods, especially chicken products and egg. *Staphylococcus aureus* is also one of the major cause of food poisoning outbreaks since this pathogen can produce heat-stable enterotoxins. Contaminated milk and dairy products are known as important sources of staphylococcal food poisoning. Enterohaemorrhagic *Escherichia coli* O157:H7 has become one of the most important foodborne pathogen due to the severe illness caused by this pathogen such as haemolytic uraemic syndrome. Bacteriophages are bacterial viruses that only infect and kill specific bacterial strain or species. The application of bacteriophages is increasingly considered to be a novel strategy for biocontrol of foodborne pathogens. The susceptibility of bacterial strains to phages seems to differ from the strains in other countries. To isolate phages effective for biocontrol of domestic pathogens, bacterial strains isolated in Japan as hosts and domestic foods should be used.

This study focuses on the isolation and characterization of lytic bacteriophages for controlling *Salmonella, E. coli* O157:H7 and *S. aureus* in foods. Several phages specific to *Salmonella, E. coli* O157:H7 and *S. aureus* were successfully isolated from various food samples including chicken, pork, beef, and fish obtained in Fukuoka, Japan. The isolated phages were characterized by host range, electron microscopy, one-step growth, stability, and whole genome sequencing. Following the characterizations, potential phage candidates were selected and their effectiveness in reducing these pathogens were evaluated at different temperature conditions in foods. In case of *Salmonella*, the application of phage cocktail consisting of five different phages (STG2, STG5, STS9, SEG5, and SES8) yielded the significant reductions of viable counts of *S.* Enteritidis and *S.* Typhimurium on raw chicken breast. Treatment with single phage, PS5, also resulted in the significant reductions of *S.* Enteritidis and *S.* Typhimurium counts in various foods and temperatures tested. Interestingly, PS5 was characterized as a polyvalent phage that was able to significantly reduce viable counts of *E. coli* O157:H7 in foods as well. In case of *S. aureus,* a novel phage SA46-CTH2 showed the great efficacy on both planktonic and biofilm cells of *S. aureus*. In addition, the use of phage SA46-CTH2 in combination with nisin was found to be effective in minimizing the development of resistance to the phage in *S. aureus*.

The results in this study demonstrate that bacteriophages can be promising tools for biocontrol of foodborne pathogens. However, a variety of factors can affect the efficacy of phages, hence application protocol and storage conditions should be carefully designed according to the type of food.