

# Study on the Control of Foodborne Pathogens by Phages, Endolysin and Additives

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(ファージ、エンドリシンおよび食品添加物による食中毒細菌制御とその機構に関する研究)

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## Thesis Summary

Foodborne pathogens are a major cause of foodborne illness and pose a serious threat to food safety. A majority of pathogens are becoming more difficult to control due to their increasing antibiotic resistance. In addition, biofilm formation is another form of resistance mechanism that makes them tolerant to conventional chemical and physical treatments. Bacteriophages and phage-encoded endolysins are novel therapeutic agents that may provide a solution to the worldwide epidemic of antibiotic-resistant bacterial pathogens. The major purpose of this study is to determine the effects of phages and endolysins to control foodborne pathogens and their biofilms.

In the current study, phage FP43, isolated from bovine intestine, was shown to have wide host range against both non-pathogenic and pathogenic *E. coli*. It exhibited great efficacy in the inhibition and removal of a mixed biofilm of two enterohemorrhagic *E. coli* strains. Phage FP43 decreased the formation of biofilm, comprising *E. coli* O157:H7 and O91:H-, by 82.4% at 30 °C. Meanwhile, more than 60% of an established mixed biofilm was removed after a 6 h exposure to the phage, in which *E. coli* O157:H7 and total viable counts decreased by 2.07 and 1.93 log, respectively. Moreover, the combined treatment of phage FP43 and slightly acidic hypochlorous water (SAHW) showed effective on the removal of their biofilms on lettuce, which significantly decreased *E. coli* viable biofilm cells to under detection level.

Furthermore, the gene encoding LysSTG2, an endolysin from *Salmonella*-lytic bacteriophage STG2 belonging to the Peptidase\_M15 superfamily was cloned and a recombinant LysSTG2 was overexpressed in *E. coli*. The recombinant LysSTG2 showed strong lytic activity against *S. Typhimurium* cells. LysSTG2 (100 µg/mL) reduced the viability of intact *S. Typhimurium* planktonic cells by 1.2 log and that of the biofilm cells after 1-h treatment. Sequential treatment of SAHW with 40 mg/L available chlorine and LysSTG2 (100 µg/mL) killed more than 99.99% of *S. Typhimurium* biofilm cells. LysSTG2 was also used to control *Pseudomonas aeruginosa* and *P. putida*, which showed high susceptibility to LysSTG2. It significantly reduced the viable counts of both *P. aeruginosa* and *P. putida* in bottled water, chicken breast, salmon, and the biofilms formed on the surface of polystyrene resin and stainless steel in the presence of EDTA.

In conclusion, the results of this study showed that phages and phage-derived endolysins are very promising tools for controlling foodborne pathogens in foods and food contact surfaces. However, the efficacy of phage or endolysin treatment could be affected by many factors, such as target bacteria, food source structures and environment conditions. Therefore, the applications of phage and endolysin should be carefully designed for each treatment. Further studies will focus on the mechanism of endolysin to control bacteria and bacterial biofilms.