

Study on characterization and advanced utilization of Campylobacter lytic phages

黄, 弘鑫

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Name : HUANG HUNG-HSIN

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(*Campylobacter* 溶菌ファージの特性解明と高度利用に関する研究)

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Thesis Summary

Campylobacteriosis is notorious foodborne diseases worldwide, commonly caused by *Campylobacter coli* and *Campylobacter jejuni*. Although much efforts have been made to reduce the prevalence of *Campylobacter* contamination in chicken and raw milk, further researches are required to combat this pathogen. Hence, phage-based approaches are promising strategies to combat foodborne pathogens to date. This study aimed to isolate and characterize phages specific to *C. coli* from raw meat, and evaluate its bactericidal activity *in vitro* and in food.

Among the 16 *C. coli*-specific phages isolated from raw meat samples, phage CAM-P21 having the widest host range was used for further characterization such as morphology observation, one-step growth curve, pH stability, and genomic DNA sequencing. CAM-P21 had a symmetrical icosahedral head and a long, flexible, and non-contractile tail. It had a latent period of 60 min and a burst size of 20 PFU/cell. The phage was stable at temperatures ranging from 4 to 50 °C as well as at pH from 4 to 8. The genome of CAM-P21 was a linear dsDNA with 12,587 bp in length, and an average G+C content of 31.19%. It was predicted to encode for 18 open reading frames (ORFs) but no tRNA genes, among which 9 ORFs (50.0%) have putative functions and while the others were hypothetical proteins. Genes associated with antibiotic resistance, toxins, and virulence factors were not found in the genome of CAM-P21.

Lytic activity test in broth showed that phage CAM-P21 reduced the *C. coli* viable count by 2.1 and 2.7 and 1.7 log at 42, 37 and 8 °C, respectively. The phages with different host range were selected for the preparation of phage cocktail. The phage cocktail consisted of CAM-P16, CAM-P19, and CAM-P21 effectively reduced the viable counts and retarded the regrowth of *C. coli* in BHI broth. On raw meat at 8 °C, CAM-P21 reduced the viable count by 1 log after a 12-h incubation. The antibacterial effect of the phage cocktail was stronger than that of CAM-P21 alone, reducing the viable counts by 1.4 log on raw meat. Furthermore, in milk at 8 °C, CAM-P21 reduced the viable count of *C. coli* by 1.2 log after incubation for 24 h. Phage CAM-P21 was also effective against *C. coli* biofilm, reducing viable counts by 4.4 and 1.6 log at 42 and 8 °C, respectively. The phage cocktail was more effective on the reduction of the viable count than that of CAM-P21 alone at both temperatures. In addition, the combined effects of phage CAM-P21 and EDTA were investigated on the viability of *Campylobacter* in BHI broth at low temperatures followed by the optimum growth temperature. The re-growth of *C. coli* was significantly inhibited by the coexistence of 1.3 mM EDTA, and the viable counts of surviving *C. coli* after the incubation was about the same as the initial viable count.

In conclusion, this study demonstrated that a siphophage CAM-P21 and a phage cocktail containing CAM-P16, CAM-P19, and CAM-P21 could be a promising strategy to combat *C. coli* planktonic and biofilm cells in food products. The combined use of lytic phages and EDTA is also effective in inhibiting the re-growth

of phage-resistant bacterial *C. coli* cells. These results suggested that the phages are promising biocontrol agents against foodborne pathogen in food and food contact surfaces.