

Study on the isolation and characterization of *Clostridium perfringens* and its biocontrol by phages

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Title : Study on the isolation and characterization of *Clostridium perfringens* and its biocontrol by phages
(ウエルシュ菌の分離、同定とファージによるバイオコントロールに関する研究)

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Thesis Summary

Clostridium perfringens is one of the most important foodborne pathogens in developed countries. It causes severe food poisoning outbreaks worldwide, along with mortality and economic losses. Recently, bacteriophages (phages) have been investigated as an alternative tool to control pathogenic bacteria in the food industry. In this study, *C. perfringens* were isolated and characterized from chicken meat samples. Fifty-three chicken meat samples were collected from retail meat shops in Fukuoka City, Japan. Nineteen (35.85%) samples were contaminated with *C. perfringens*. All 19 isolates were positive for only alpha-toxin (*cpa*) gene, which verified them as *C. perfringens* type A. The *C. perfringens* enterotoxin (*cpe*) gene was not detected by PCR among the isolated *C. perfringens*. Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry analysis also demonstrated that all isolates are *C. perfringens*.

Secondly, six *C. perfringens* phages were successfully isolated from chicken meat samples. According to the host range and stability tests, phage CPQ1 showed high thermostability and the broadest host range. The electron micrograph image of this phage suggested that it belongs to the *Picovirinae* subfamily of the *Podoviridae* family. Nucleotide sequence analysis of the genomic DNA indicated the absence of any antibiotic resistance, toxin, or virulence genes. In broth, phage CPQ1 showed strong lytic activity with a low MOI of 1, decreasing the OD₆₀₀ of *C. perfringens* cell suspension from 0.2 to 0.02 at 37 °C in 2 h. In pasteurized milk and chicken meat, phage CPQ1 with an MOI of 10 also caused a significant decrease in viable counts of *C. perfringens* compared to the phageless control at both 24 °C and 37 °C. This is the first report on the application of phage to control *C. perfringens* in foods.

Thirdly, four more phages were isolated from chicken meat samples. Thereafter, these four phages and two phages previously isolated that display different host range patterns were morphologically and genetically characterized. Notably, the phages were stable at various pH values and in curry roux. Cocktails consisting of 6, 5, and 4 phages at the same concentration were examined to determine the most effective phage cocktail. The phage cocktail PC11, a 4-phage cocktail consisting of phages CPQ3, 7, 8, and 10, significantly decreased the viable count of *C. perfringens* to less than the lower detection limit up to 48 h at both 8 and 37 °C in broth and at 24 °C in curry roux. Overall, these studies suggest that all of *C. perfringens* isolates from chicken meat samples were positive for only alpha-toxin gene, and phage cocktail consisting of phages CPQ3, 7, 8, and 10 is a promising natural biocontrol agent against *C. perfringens* *in vitro* and in food.