

# Study on biocontrol and detection of *Campylobacter jejuni* using bacteriophage

ス ザー チー ルウィン

<https://hdl.handle.net/2324/7396297>

---

出版情報 : Kyushu University, 2025, 博士 (農学), 課程博士  
バージョン :  
権利関係 :



Name : ス ザー チー ルウィン (SU ZAR CHI LWIN)

Title : Study on biocontrol and detection of *Campylobacter jejuni* using bacteriophage  
(バクテリオファージを用いた *Campylobacter jejuni* のバイオコントロールと検出)

Category : Kou

### Thesis Summary

Campylobacteriosis, caused mainly by *Campylobacter jejuni* remains as one of the most common foodborne illnesses and presents significant public health concerns, particularly through its association with poultry products. With the growing threat of antibiotic resistance, innovative controls are needed for bacterial control and rapid detection. Bacteriophages (phages) offer as an effective alternative antibacterial in the recent years. Phage infection always begins with the attachment to the host bacterium with phage receptor binding proteins (RBPs) which can also serve as highly specific tools for detecting target bacteria. This study aimed to characterize *C. jejuni* phages for antimicrobial applications and to develop a rapid detection method suitable in food matrices.

Three *C. jejuni* phages, vB\_CjeM-PC10, vB\_CjeM-PC11 and vB\_CjeM-PC22 were primarily selected out of 26 phages based on their broad lytic host ranges. All three phages exhibited strong stability across the temperature range of 4 - 60°C and the pH range of 3 - 9. One-step growth analyses showed similar latent periods (30 min) for phages vB\_CjeM-PC10 and vB\_CjeM-PC11 with burst sizes of 7 and 12 PFU/cell, respectively. Phage vB\_CjeM-PC22 had a shorter latent period (20 min) and a larger burst size of 35 PFU/cell. Transmission electron microscopy showed all three phages are of the myovirus morphotype. Genomic sequencing showed that phage vB\_CjeM-PC10 possesses a linear dsDNA genome of 51,148 bp. Phages vB\_CjeM-PC11 and vB\_CjeM-PC22 contained the circular dsDNA genomes of 32,997 bp, and 32,543 bp, respectively, with 99% similarity between them, being distinct from phage vB\_CjeM-PC10. Based on the characteristics, phages vB\_CjeM-PC10 and vB\_CjeM-PC22 were selected for antibacterial activity evaluation. *C. jejuni* cells were infected with phages alone, cocktail, or in combination with 0.67 mmol/L EDTA and incubated for 48 h. At 42°C in broth, infection with phage cocktail at MOI 10, simultaneously lowered the viable cell counts by 1.6 log<sub>10</sub> after incubation for 48 h compared to the control, while co-treatment with EDTA enhanced the inhibition by 5.2 Log<sub>10</sub>. At 4°C, phage vB\_CjeM-PC10 alone reduced the viable counts by 3.1 log<sub>10</sub> after the incubation for 48 h in broth. On chicken skin, phage vB\_CjeM-PC10 reduced viable counts by 1.1 log<sub>10</sub> after 1 h of incubation and 2.3 log<sub>10</sub> after a 48-h incubation.

Genomic and proteomic analyses identified the tail protein (gp76) of phage vB\_CjeM-PC10 as a potential RBP (termed RBP<sub>PC10</sub>). A DNA fragment encoding RBP<sub>PC10</sub> was synthesized, fused with *mCherry* gene, cloned and expressed as a recombinant red-fluorescence RBP<sub>PC10</sub> protein (rRBP<sub>PC10</sub>). The rRBP<sub>PC10</sub> protein expression was confirmed by SDS-PAGE as a band of ~67 kDa. The functional analyses were carried out via fluorescence microscopy and spectrofluorometry. The optimal binding of the protein to *C. jejuni* cells was observed at 5 µmol/L and 15 min of incubation time. The protein was stable at pH ranging from 7 to 10 and the high and stable performance was observed at pH 8. The rRBP<sub>PC10</sub> exhibited binding to all the tested *C. jejuni* strains showed 36% higher binding range than host phage of the phage. Non-specific binding to other bacterial species was not observed. Fluorescence detection using the protein showed good linear correlation between fluorescence intensity and log cell concentration of *C. jejuni* ( $10^4$ - $10^8$  CFU/mL) with a lower detection limit of  $10^2$  CFU/mL within 30 min in broth and in chicken skin samples. Detection efficiency of rRBP<sub>PC10</sub> was analyzed in the bacterial mixture. The presence of non-target bacteria at concentrations  $10^7$  time higher than that of *C.jejuni* did not impair the detection performance of rRBP<sub>PC10</sub>.

This study successfully characterized *C. jejuni* phages with strong antibacterial activity and developed the RBP-based detection method for rapid and specific detection of *C. jejuni* in food-related environment.