

Study on Biocontrol of Phage-Resistant Bacterial Populations

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Thesis Summary

Bacteriophages (Phages) are viruses that are widely present in the environment. The use of phages could be promising for the control of food poisoning bacteria in improving food quality as well as in clinical therapy. However, faced with naturally existing phage predators, bacteria have evolved various mechanisms to resist phage infection and reproduction. Prolonged contact between phages and bacteria has been reported to lead to the emergence of bacterial population that are resistant to phage. Overcoming phage resistance remains a major challenge in phage therapy. This research explored a strategic method aimed at improving the effectiveness of bacteriophages in combating the foodborne pathogenic *Escherichia coli* O157:H7, with an emphasis on understanding the molecular biological basis of resistance to phages within *E. coli* populations.

Firstly, 3,909 single-gene deletion mutants of non-pathogenic *Escherichia coli* BW25113 from the Keio collection were screened to identify genes associated with phage resistance. The genes involved in resistance to phage were pinpointed by examining deletion mutants that increased susceptibility to the phage S127BCL3. Ten gene-deletion mutants (Δrpe , $\Delta yfcD$, $\Delta ybcH$, $\Delta yaiW$, Δgor , $\Delta ubiE$, $\Delta dnaK$, $\Delta tolR$, $\Delta priA$, and $\Delta tolA$) showed higher susceptibility to the phage than that of the parent strain BW25113. Notably, the $\Delta priA$ gene-deletion mutant exhibited significantly high susceptibility to phage S127BCL3. The gene *priA* encodes a primosomal replicative protein known as PriA, which is likely crucial for the mechanism of phage resistance. Furthermore, at low concentrations, PriA inhibitors exhibited combined effects with phages against *E. coli* O157:H7 and significantly reduced the rate of regrowth of phage-resistant cells. These results imply that the combined action of substances that inhibit PriA function and phages could serve as an effective biocontrol strategy.

Secondly, since phage resistance is governed by multiple genes through various mechanisms, the synergistic impacts of PriA inhibition and other resistance mechanisms targeting DnaK functions could lead to greater suppression of regrowth in phage-resistant populations. It has been reported that the chaperone protein DnaK involving in CRISPR Adaptation through the interaction with the Cas1–Cas2 complex and modulating its activity. Moreover, the $\Delta dnaK$ gene-deletion mutant also showed a phage susceptibility higher than that of the parent strain *E. coli* BW25113 in this study. A well-known plant flavonoid, myricetin, reported to inhibit the cellular function of DnaK was used in the study to examine the combined effects with PriA inhibition. The result showed that combined use of phages, DnaK inhibitor such as myricetin, and low doses of PriA inhibitor such as chloramphenicol could provide valuable insights for developing strategies effective to control phage resistance in pathogenic *E. coli* O157:H7.