Synergistic Actions of Bacteriophage and Bacterial Metabolite to Control Foodborne and Food Spoilage and Their Biofilm

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ABSTRACT:
1.Introduction: Foodborne disease and food spoilage are public health issues and food security. Significant disease outbreaks mostly linked to the presence of biofilm-forming pathogens. Recently, bacteriophagehave attracted attention as potential alternative agent to control pathogens related with food safety. In this study we combined bacteriophage and bacterial metabolites from *Phyllosphere* and *Actinomycetes* bacteria (*Pseudomonas fluorescens* JB3B and *Streptomyces thermocarboxydus* 18PM crude extracts) in treating pathogens and their biofilm was the aim of this present study. 2. Materials and Methods: Bacteriophage ETEC-phage-TG ($8.9 \pm 2.19 \times 10^8$ PFU/ml) and BC-VP ($1.28 \pm 0.29 \times 10^{11}$ PFU/ml) were isolated from artificial lake water from previous study. These two phages able to control *Bacillus cereus* (BC) and Enterotoxigenic *Escherichia coli* (ETEC). 3.Result: The combination of BC-VP with metabolite (*P. fluorescens* JB3B and *S. thermocarboxydus* 18PM) were able to inhibit (86.1%; 83.3%) and destruct (41%; 45.5%) biofilm formation of *B. cereus* respectively. We also assessed the synergy of bacteriophage ETEC-phage-TG and antibiofilm above showed promising activity against biofilm of ETEC with percentage of inhibition (81.9%; 76.4%) and percentage of destruction (54.1%; 44.4%). Application in various food, combination of BC-VP and bacterial metabolite extract (*P. fluorescens* JB3B; *S. thermocarboxydus* 18PM) were able to reduce *Bacillus cereus* in various foods such as mashed potato (99.6%; 99.4%) at cold temperature (4 °C) and (68.9%; 56.6%) at room temperature (28 °C), boiled pasta (99.5%; 99.4%) and (84.7%; 75.7%), also soymilk (96.9%; 96.7%) and (42.4%; 39.4%) respectively. While combination of ETEC-phage-TG and bacterial metabolite (*P. fluorescens* JB3B; *S. thermocarboxydus* 18PM) are potential in reducing ETEC at temperatures (4 °C and 28 °C) incubation in various foods such as bean sprouts (TFTC; TFTC) and (47.5%; 49.1%), chicken meat (TFTC; TFTC) and (58.1%; 54%), also minced beef (99.5%; 99.4%) and (41.1%; 28%). GC-MS analysis performed that oxalic acid, phenol, phenylethyl alcohol, N-hexadecanoic acid, and pyrolol[1,2-a]pyrazine-1,4-dione, hexadro-3-92-methylpropyl was the most active compound in *P. fluorescens* JB3B. 2,4-Di-tert-butylphenol, phenyl acetic acid, N-Hexadecanoic acid, pyrolol[1,2-a]pyrazine-1,4-dione, hexadro-3-92-methylpropyl, and Bis(2-ethylhexyl) phthalate was most active compound in the *S. thermocarboxydus* 18PM isolates. Molecular characterizations were performed by both phages were virulent bacteriophage. Also, no sign of antibiotic resistance gene and genes related to lytic cycle such as putative tail lysin and tail fiber, were annotated. 4. Conclusions: Therefore, we can conclude that the combination of bacteriophages and bacterial metabolite (antibiofilm) has potential to be used as biocontrol to control biofilm formed by foodborne and food spoilage bacteria.

KEYWORDS: Bacteriophage, Antibiofilm, Foodborne pathogen, Spoilage, Combination