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Phages for Food Safety: phage application against Yersinia (#663)

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Short Abstract

Introduction:
Yersiniosis is recognized globally as an important zoonotic disease. Consumption of raw or undercooked contaminated pork is the main source of this disease in humans. It can also be contracted through contact with someone who has handled a contaminated pork product or contact with infected animals or their feces, and, less commonly, by ingestion of contaminated milk or untreated water. *Yersinia enterocolitica*, the primary cause of yersiniosis, is one of the most important foodborne pathogens. Bacteriophages (phages) have been recognized as potential tools to control bacterial pathogens in food instead of antibiotics, the application of which in food is becoming increasingly restricted due to their limitations. In the current study, four virulent bacteriophages (phages) capable of infecting *Y. enterocolitica* were isolated and characterized. Our aim was to evaluate the effectiveness of *Yersinia* phages against *Y. enterocolitica* in pork products and on kitchen utensils.

Methods:
Phage isolation was performed using a standard enrichment method with two indicator bacteria, *Y. enterocolitica* O:3 strain 6471/76 and *Y. enterocolitica* O:9 strain Ruokola/71. For morphological analysis, phages (10^9 plaque-forming units [PFU]/ml of each phage) were negatively stained with 2% uranyl acetate, and electron micrographs were taken using JEM 1010 (JEOL, Tokyo, Japan) and JEM 1400 (JEOL) transmission electron microscopes at an accelerating voltage of 80 kV. To determine the burst sizes and latent periods of the phages, one-step growth curve analyses were performed. For phage genome characterization, sequencing was carried out using a MiSeq PE300 sequencer (Illumina) with a read length of 300 nucleotides. To evaluate the effectiveness of phage, we designed an experimental model of the food market environment: raw minced pork, ready-to-eat barbequed pork loin, and milk were purchased from grocery stores; cutting boards, knives, artificial hands were prepared.

Results:
Phage, fHe-Yen9-01 had the broadest host range (61.3% of strains, 65/106). It demonstrated a latent period of 35 min and a burst size of 33 plaque-forming units/cell, and was found to have a genome of 167,773 bp with 34.79% GC content. Phage treatment after bacterial inoculation of food samples, including raw pork (4 °C, 72 h), ready-to-eat pork (26 °C, 12 h), and milk (4 °C, 72 h), prevented bacterial growth throughout the experiments, with counts decreasing by 1-3 logs from the original levels of $2–4 \times 10^3$ CFU/g or ml. Similarly, when artificially contaminated kitchen utensils, such as wooden and plastic cutting boards and knives, and artificial hands, were treated with phages for 2 h, bacterial growth was effectively inhibited, with counts decreasing by 1-2 logs from the original levels of ca 10^4 CFU/ cm^2 or ml.

Conclusion:
To the best of our knowledge, this is the first report of the successful application of phages for the control of *Y. enterocolitica* growth in food and on kitchen utensils. This study emphasizes the potential benefits of applying phages to various objects, from food to kitchen utensils, for the control of foodborne infections.

Notes