medical sciences forum

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Abstract

Effectiveness of Phage phT4A Incorporated in Pullulan Films against *Escherichia coli* †

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† Presented at the 2nd International Electronic Conference on Antibiotics—Drugs for Superbugs: Antibiotic Discovery, Modes of Action And Mechanisms of Resistance, 15–30 June 2022; Available online: https://eca2022.sciforum.net/.

Keywords: Bacteriophages; Foodborne bacteria; films; food safety

Antimicrobial Discovery, Development & Optimization. Despite the recent advances achieved in food industries to fulfil the growing consumer demand for high quality and safe food, microbial contamination remains a serious issue. Bacteriophages (phages, viruses that only infect bacteria) have been recognized for their great effectiveness in controlling bacterial pathogens in agro-food industry. Phages exhibit important features that make them promising antibacterial candidates, such as their ubiquity, high specificity against a target host, self-replication capacity while their hosts are present, low inherent toxicity, easy and economical isolation and production, and a long shelf life. The incorporation of phages in food packaging can be an effective alternative to protect phages from environmental challenges and improve its efficacy, allowing slower and continuous release. *Escherichia coli* is one of the most important foodborne pathogens. The purpose of the study was to evaluate the efficiency of biopolymeric films incorporating phage phT4A for future application in packaging materials. The inactivation of *E. coli* was first assessed in vitro (liquid culture medium) and ex vivo (milk) with free phage phT4A. The in vitro results showed that phage phT4A was effective to inactivate *E. coli* with a reduction of about 6 log colony-forming unit per millilitre (CFU/mL) for all tested values of multiplicity of infection (MOI). In milk, a more complex matrix, a maximum inactivation of about 4 log CFU/mL was obtained after 8 h of incubation (MOI of 10 and 100). Phages were successfully incorporated in the pullulan films increasing its concentration in solution until it remains stable until the end of the assays. The antibacterial efficacy of pullulan films was demonstrated in vitro and artificially contaminated milk, with 5-3 log CFU/mL of inactivation during first 12 h of incubation. Overall, pullulan-based films incorporating phages constitute a simple approach to preserve the activity of phages in order to improve food safety.

Author Contributions:

Funding:

Institutional Review Board Statement:

Informed Consent Statement:

Data Availability Statement:

Conflicts of Interest:

Citation: Braz, M.; Pereira, C.; Almeida, A.; Freire, C.S.R. Effectiveness of Phage phT4A Incorporated in Pullulan Films against *Escherichia coli*. *Med. Sci. Forum* **2022**, *2*, x. https://doi.org/10.3390/xxxxx

Academic Editor(s):

Published: date

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