







Methodology

Effectiveness of phage phT4A incorporated in pullulan films against Escherichia coli

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Introduction

- Biopolymeric films of Despite the recent advances in food industries, microbial pullulan incorporating contamination remains a serious issue; phages obtained by casting method E. coli is one of the most important foodborne at 25°C pathogens;1 Sample: Phage Bacteria Bacteriophages have been recognized for their great Control: Collection of **Bacteria** Control: effectiveness in controlling bacterial pathogens in agro-Only Only aliquots over food industry;1 Phage phage Bacteria time and serial dilutions Their ubiquity, high specificity against a target host, selfreplication capacity, low inherent toxicity, easy and economical isolation and production make them Incubator at 25 °C during 24 h promising antibacterial candidates;2,3 Pour Plate Method - Bacteria quantification The incorporation of phages in food packaging can be an effective alternative to protect phages from environmental Double Layer Method

 Phage quantification challenges and improve their efficacy, allowing a slower and continuous release. After plates incubation **PURPOSE:** at 37 °C for 18-24 h Evaluate the effectiveness of phage phT4A **Results** incorporated in pullulan films against E. coli for future application in packaging materials In vitro - TSB In food - Milk 10 10 9 9 Log CFU/mL 8 8 Log CFU/mL E. coli Quantification 5 log 4 loa 7 7 6 6 **Bacteria Control** 5 5 Bacteria + Phage 4 4 3 3 0 6 12 18 24 0 6 18 12 24 Time (h) Time (h) 10 10 9 9 Log PFU/mL Log PFU/mL 8 **Phage Quantification** 8 7 7 Phage Control 6 6 5 - Bacteria + Phage 5 4 4 3 3 6 0 12 18 24 0 6 12 18 24 Time (h) Time (h) Conclusions
- Phage phT4A was 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 phage phT4A incorporated in pullulan films was demonstrated *in vitro* and artificially contaminated milk, with around 5 and 4 log CFU/mL of inactivation, respectively, 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.

References: 1. O'Sullivan, L. et al. Annual Review of Food Science and Technology 2019, 10:151–72; 2. Altamirano, F. L. G., & Barr, J. J. Clinical Microbiology Reviews 2019, 32 (2),1–25; 3. Principi, N. et al. Frontiers in Pharmacology 2019,10 (513),1–9.

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