A study of natural lytic *Listeria* phages with decontaminating properties for use in seafood processing plants

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Table of Contents

Abstract

Listeria monocytogenes is a major cause of illness, associated with seafood, therefore it is important to control this pathogen in seafood processing environments. Sporadic listeriosis outbreaks and seafood recalls indicate that current treatments to control this pathogen may be inadequate. The ability to adapt to harsh environmental conditions, develop resistance and form biofilms makes this environmental pathogen difficult to control using regular disinfectants. Bacteriophages (phages) could serve as effective alternative biocontrol agents. The main objective of this study was to isolate and characterize natural lytic *Listeria* specific phages and examine their effectiveness against *L. monocytogenes* under conditions mimicking those found in seafood processing environments.

Among a group of phages isolated from a seafood waste treatment unit, three phages (LiMN4L, LiMN4p and LiMN17) were selected based on plaque morphology and their source. The three phages were distinguished by morphology, efficiency of plating (EOP) in citrate agar and differences in EOP using different *L. monocytogenes* host strains. Three phages which were found as strictly virulent by whole genome sequence analysis, had broad host ranges at 15 °C and each phage also infected either *L. ivanovii* or *L. innocua*. These phages were unstable at 60 °C for 10 min suggesting psychrotrophic properties. The three phages showed low burst sizes indicating their potential suitability as passive biocontrol agents.

Low counts of *L. monocytogenes* strains (19O9, 19DO3 and 19EO3) in late exponential phase, metabolically injured/stressed by heat and salt, lysed by the three phages at 15 °C in 30 min. The results suggested that the three virulent phages may be good candidates as biocontrol agents against *L. monocytogenes* under conditions commonly found in seafood processing plants.

The phages LiMN4L, LiMN4p and LiMN17, used as single phage or a cocktail of three phages, lysed cells adhered to stainless steel conditioned with soluble fish protein and on clean stainless steel coupons (SSC). The phage cocktail also eradicated low cell counts of about 2 log CFU/cm^2 adhered to SSC surfaces in the presence of fish proteins at 15 °C in 15 min and no re-growth of cells was observed from phage infected surfaces. This study suggested that a biofilm matrix shielded the bacterial cells from phage infection as three consecutive repeat applications of phages did not efficiently lyse undisturbed biofilm cells. Biofilm cells, once removed from the surface, showed

similar to sensitivity to that of exponential phase planktonic cells. Therefore, disruption of the biofilm structure may be required for effective phage treatments.

Phages suspended in phosphate buffered saline survived refrigeration for at least twelve months and were stable for at least 6 h under likely application conditions such as ambient temperatures and under fluorescence lighting. The three phages, either individually or as a cocktail, showed a high lytic efficacy indicating their potential to serve as bio-decontaminating agents in seafood processing environments.