

Bacteriophage Lysis Of Physiologically Stressed Listeria Monocytogenes In A Simulated Seafood Processing Environment

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Abstract : In seafood processing plants, *Listeria monocytogenes* likely exists in a metabolically stressed state due to the nutrient-deficient environment, processing treatments such as heating, curing, drying, and freezing, and exposure to detergents and disinfectants. Stressed *L. monocytogenes* cells have been shown to be as pathogenic as unstressed cells. This study investigated lytic efficacy of (LiMN4L, LiMN4p, and LiMN17) which were previously characterized as virulent against physiologically stressed cells of three seafood borne *L. monocytogenes* strains (19CO9, 19DO3, and 19EO3). Physiologically compromised cells of *L. monocytogenes* strains were prepared by aging cultures in Trypticase Soy Broth at $15 \pm 1^\circ\text{C}$ for 72 h; heat injuring cultures at $54 \pm 1 - 55 \pm 1^\circ\text{C}$ for 40 - 60 min; salt-stressing cultures in Milli-Q water were incubated at $25 \pm 1^\circ\text{C}$ in darkness for three weeks; and incubating cultures in 9% (w/v) NaCl at $15 \pm 1^\circ\text{C}$ for 72 h. Low concentrations of physiologically compromised cells of three *L. monocytogenes* strains were challenged in vitro with high titre of three phages in separate experiments using Fish Broth medium (aqueous fish extract) at 15°C in order to mimic the environment of seafood processing plant. Each phage, when present at $\approx 9 \log_{10}$ PFU/ml, reduced late exponential phase cells of *L. monocytogenes* suspended in fish protein broth at $\approx 2-3 \log_{10}$ CFU/ml to a non-detectable level (< 10 CFU/ml). Each phage, when present at $\approx 8.5 \log_{10}$ PFU/ml, reduced both heat-injured cells present at $2.5-3.6 \log_{10}$ CFU/ml and starved cells that were showed coccoid shape, present at $\approx 2-3 \log_{10}$ CFU/ml to < 10 CFU/ml after 30 min. Phages also reduced salt-stressed cells present at $\approx 3 \log_{10}$ CFU/ml by $> 2 \log_{10}$. *L. monocytogenes* ($\approx 8 \log_{10}$ CFU/ml) were reduced to below the detection limit (1 CFU/ml) by the three successive phage infections over 16 h, indicating that emergence of spontaneous phage resistance was infrequent. The three virulent phages showed high decontamination potential for physiologically stressed *L. monocytogenes* strains from seafood processing environments.

Keywords : physiologically stressed *L. monocytogenes*, heat injured, seafood processing environment, virulent phage

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