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

Authors : Geevika J. Ganegama Arachchi, Steve H. Flint, Lynn McIntyre, Cristina D. Cruz, Beatrice M. Dias-Wanigasekera, Craig Billington, J. Andrew Hudson, Anthony N. Mutukumira

Abstract: In seafood processing plants, Listeriamonocytogenes(L. 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. monocytogenesstrains (19CO9, 19DO3, and 19EO3). Physiologically compromised cells of L. monocytogenesstrains were prepared by aging cultures in Trypticase Soy Broth at 15±1°C for 72 h; heat injuringcultures at 54±1 - 55±1°C for 40 - 60 min; salt-stressing cultures in Milli-Q water were incubated at 25±1°C in darkness for three weeks; and incubating cultures in 9% (w/v) NaCl at 15±1°C for 72 h. Low concentrations of physiologically compromised cells of three L. monocytogenesstrainswere challenged in vitrowith 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 ≈9 log10 PFU/ml, reduced late exponential phase cells of L. monocytogenes suspended in fish protein broth at ≈2-3 log10 CFU/ml to a non-detectable level (< 10 CFU/ml). Each phage, when present at ≈8.5 log10 PFU/ml, reduced both heat-injured cells present at 2.5-3.6 log10 CFU/ml and starved cells that were showed coccoid shape, present at ≈2-3 log10 CFU/ml to < 10 CFU/ml after 30 min. Phages also reduced salt-stressed cellspresent at ≈3 log10 CFU/ml by > 2 log10. L. monocytogenes (≈8 log10 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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