

Fate of *Escherichia coli* O157:H7 in Aquatic Environment and its Detection by GFP-Labeled Lysozyme Inactivated Bacteriophage

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Escherichia coli O157:H7 is a human pathogen causing severe illness including diarrhea and renal failure. Outbreaks of disease every year, urge us to clarify the ecology and fate of these pathogens in the environment. Some enteric pathogens have been shown to enter the viable but non-culturable (VBNC) state when exposed to potential environmental stress. The results from present study suggest that *E.coli* O157:H7 can also enter VBNC state when exposed to low temperature and sea water conditions. Under such conditions, they are no longer detectable by conventional bacteriological methods. The method capable of detecting *E. coli* O157:H7 in culturable as well as VBNC state in single assay still remains awaited. We describe herein the development and characterization of a novel assay that uses *E.coli* O157:H7 (*E.coli* O157:H7)-specific PP01 bacteriophage for the detection of *E.coli* O157:H7. The bacteriophage was labeled with green fluorescent protein (GFP) and was lysozyme-inactivated (PP01e⁻/GFP). When combined with nutrient uptake analysis, the PP01e⁻/GFP assay was capable of discriminatively detecting the culturable, viable but non-culturable (VBNC) and dead cells in the stress-induced aquatic environment. In contrast to conventional techniques (staining, direct viable count methods), which demonstrate the VBNC cells as the differential value of culturable cell count and total viable count, our assay enabled the direct detection and estimation of VBNC cells within 12 h. Since the proposed assay provides a direct and specific means of detection of *E. coli* O157:H7, it can be applied for tracking the fate of bacteria introduced in natural environments; for the detection of natural *E. coli* O157:H7 and finally for the identification of potential reservoirs of *E. coli* O157:H7 in the environment.