Luminescent Phage Based Most Probable Number Analysis of Environmental Samples

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Abstract

Bioluminescence is an incredibly powerful reporter system for evaluating the presence of microorganisms; its use allows for robust methods of cell quantification, metabolic activity, and detection. While the phenotype of bioluminescence is easily quantified, developing the means to introduce the necessary genes consistently to the desired organisms can be challenging, especially when applying this system to microbial detection strategies. Reliable detection of microbes using bioluminescence requires an efficient and specific delivery method for the DNA encoding bioluminescence genes to a target organism. Bacteriophage (phage) provide an evolutionarily viable route through which to target bacteria, as phage have evolved to specifically infect distinct species and cultivars of bacteria. Additionally, phage that have lysogenic capabilities apply a unique selective pressure to target bacteria, resulting in stable insertions of the phage genome over several bacterial generations. The presented research will summarize the coupling of lysogenic phage specific for pathogenic *Escherichia coli* O157:H7 and genes encoding bioluminescence systems to develop detection platforms. By recombining reporter genes into the phage genome, the resulting lysogenic infections produce target bacterial cells that display the bioluminescent phenotype. This process lends itself well to detection technologies in the food safety industry and environmental sampling methods as the infective events are restricted to only *E. coli* O157:H7, even in a complex microbial background. Detection is able to be made quantitative by applying a Most Probable Number (MPN) analysis to an enrichment of recombinant phage and target bacteria. MPN analysis results in a statistically significant estimation of the number of original cells in a sample based on binary growth data from series of dilutions. The introduction of commercially available MPN field tests has facilitated the development of quantitative, field-ready, phage-based detection technologies for pathogenic bacteria.

Keywords: bacteriophage, detection, MPN, environmental sampling

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