THESIS ABSTRACT

Comparative detection and enumeration strengths of Quantitative Real-Time PCR & FISH for Waterborne Bacterial Pathogens in Municipal Wastewater

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This study comparatively evaluates the detection and enumeration strengths of Real-Time PCR (RT PCR) and FISH, for selected bacterial pathogens in municipal wastewater. Both assays were performed using three primer and probe sets complementary to the same chromosomal virulence gene sequences. Primer & probe specificity was confirmed with DNA & fixed cells from each of the strains, while assay sensitivities were calculated using DNA & fixed cells from pure bacterial cultures as well as seeded wastewater samples. Detection limits calculated for the RT PCR assay were 25 to 303 *tir* gene copies for *Escherichia coli* O157:H7 and 3 x 10^4 to 293 x 10^7 *invA* gene copies for *Salmonella enterica*, using pure cultures and seeded wastewater samples, respectively. In spite of the confirmed specificity of the DNA hybridization probes with target nucleic acids, fluorescent signals from hybridized whole target cells were below the detection limit of the FISH assay, and consequently were not quantified. This research demonstrates both the utility of RT PCR in detecting bacterial pathogens and the need for further optimization with DNA-targeted FISH, using environmental samples.