DETECTION OF VIABLE ESCHERICHIA COLI IN ENVIRONMENTAL WATER

USING A COMBINED PROPIDIUM MONOAZIDE STAINING-REAL-TIME PCR

Yuan Yuan
Dr. Azlin Mustapha, Thesis Supervisor

ABSTRACT

The objective of this study was to test the *ycj*M gene of *E. coli* in a propidium monoazide (PMA)-qPCR assay, and to investigate its specificity and efficiency in detecting only viable *E. coli* in environmental water. *E. coli* were freshly grown and spiked into autoclaved tap water and other environmental water samples，followed by cell collection, PMA treatment, DNA isolation and qPCR detection. Results showed that *ycj*M primers could detect most of the *E. coli* strains but not *Shigella* strains. In peptone water, 5 µM PMA with a 10-min light exposure time, was efficient to inhibit the amplification of DNA from 10^5 CFU /mL dead *E. coli* cells, with a detection limit of 10^2 CFU/100 mL. While in tap and winter environmental waters, 10 µM PMA was required and as low as 10^3 CFU/100 mL viable cells could be detected, when 10^5 CFU/100 mL dead cells were present; in summer water samples, 10^2 CFU/10 mL viable cells could be detected after a 20 µM PMA treatment, in the presence of 10^4 CFU/ 10 mL dead cells. A significant and strong correlation was found between PMA-qPCR and the US Environmental Protection Agency (EPA) Standard Method 1603, without over or underestimation of PMA-qPCR compared with Method 1603. In conclusion, the PMA-qPCR could accurately and effectively differentiate viable *E. coli* from dead cells by suppressing the amplification of DNA from dead cells, although it required optimization steps to remove suspended solids in environmental water.