Public Abstract
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Title:DETECTION OF VIABLE ESCHERICHIA COLI IN ENVIRONMENTAL WATER USING A COMBINED PROPIDIUM MONOAZIDE STAINING-REAL-TIME PCR

Escherichia coli is a common inhabitant of the intestinal tract of humans and warm-blooded animals. Thus, it is used as a principal fecal indicator bacterium, indicating sewage pollution and the possible existence of enteric pathogens in drinking water, recreational water and food. Recently, it was found that detecting traditional target genes of E. coli does not allow for differentiation of normal E. coli from “naturalized” E. coli, which exist in the external environment and not in feces of human or warm-blood animals. To solve this problem, the putative glucosyltransferase gene-ycjM gene can be used in Real-time polymerase chain reaction (PCR) to estimate the amount of normal E. coli. Real-time PCR is a technique that can amplify a single or a few copies of a piece of DNA across several orders of magnitude in several hours, thus achieving a rapid and sensitive detection of targets. Although PCR is able to provide a more accurate reflection of water quality and safety, it cannot differentiate dead cells from viable cells. However, by applying a chemical called propidium monoazide (PMA), DNA of dead cells will be bound and cannot be amplified in the subsequent PCR procedure. Thus, the objective of this study was to test a new self-designed primer set targeting the ycjM gene of E. coli in a PMA-qPCR assay, and to investigate its specificity and efficiency in detecting only viable E. coli in environmental water.
This research indicated that ,by testing nineteen different E. coli strains, including E. coli ATCC 25922, E. coli K12, E. coli O157 strains and strains from environmental sources, as well as Shigella.dysenteriae, S. flexneri and S. sonnei, ycjM primers were found to be more specific than primers based on other genes, . It showed that ycjM primers could detect most of the E. coli strains but did not amplify any DNA from Shigella strains.
To check the sensitivity of this PMA-PCR assay, four cultures of E. coli were respectively isolated from feces of cattle, deer, human and pig, freshly grown separately and then mixed together and serially diluted to log 2-6 CFU/mL. Dead cell suspensions were obtained by heating at 80 °C for 25 min. After being spiked with the cell suspension, tap water and other environmental water samples, including water from Lake of the Ozarks, Missouri River and Mississippi River were filtered or centrifuged for the cell collection. Samples were then treated with PMA, followed by DNA isolation and TaqMan® real-time PCR detection.
Results showed that in pure culture, 5 µM PMA with a 10-min light exposure was efficient to inhibit the amplification of DNA from log 5 CFU /mL dead E. coli cells, with a detection limit of log 2 CFU/100mL. In tap and winter environmental water, a higher PMA concentration of 10 µM was required and as low as log 3 CFU/100 mL viable cells could be detected, in the presence of log 5 CFU/100 mL dead cells; in water samples collected in summer, which is more turbid than that of winter, log 2 CFU/10 mL viable cells could be detected after a 20 µM PMA treatment, in the presence of log 4 CFU/10 mL dead cell by this optimized PMA-qPCR assay. Significant and strong correlations were found between the PMA-qPCR and Environment Protection Agency (EPA) Standard Method 1603, meaning no over or underestimation of PMA-qPCR compared with Method 1603.
In conclusion, PMA-qPCR targeting the ycjM gene, could indicate the amount of viable E. coli cells in environmental water, without showing false-positive results from dead cells, Shigella strains or E. coli that already exist in the environment. In addition, when comparing with the EPA standard culture-based method, no over or underestimation was shown by PMA-PCR, while it shortened the testing time from overnight to several hours. Thus, it provides a more accurate and effective way to estimate the microbiological quality of

water.