Poster

DETECTING THE UNDETECTABLE: A PMAXX™-QPCR APPROACH FOR VIABLE BUT NON-CULTURABLE CAMPYLOBACTER JEJUNI

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Aim. Campylobacter jejuni is one of the leading causes of foodborne bacterial gastroenteritis and is mainly associated with poultry products. Under environmental and food-processing stresses, this microorganism may enter a viable but non-culturable (VBNC) state, remaining metabolically active while escaping conventional culture-based detection. The aim of this study was to develop a PMAxx™-qPCR workflow for the selective detection and quantification of viable C. jejuni cells, including VBNC forms, and to establish the analytical basis for its subsequent validation in food matrices.

Methods. Eight scalar dilutions (10^8-10^1 UFC/mL) of C. jejuni ATCC 33291 were prepared in Bolton broth and enumerated by plating on modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA) after incubation at 41.5 ± 1°C for 48 h under microaerophilic conditions. TaqMan real-time PCR targeting the species-specific hipO gene was performed on DNA extracted by thermal lysis from the eight 10-fold serial dilutions and, in parallel, on eight 10-fold serial dilutions prepared from the DNA stock, corresponding to the DNA extracted from the initial bacterial suspension (10^8 UFC/mL). Primer/probe optimisation was performed by comparing two assay configurations: 900 nM forward primer/900 nM reverse primer/250 nM probe (900F/900R/250P) and 600 nM forward primer/600 nM reverse primer/250 nM probe (600F/600R/250P). The viability-PCR workflow included three aliquots: an untreated sample for total DNA, a PMAxx™-treated sample for apparently viable cells, and an internal sample processing control obtained after bacterial inactivation with hydrogen peroxide or heat, followed by PMAxx™ treatment. PMAxx™ concentrations of 25 and 50 µM, with enhancer and photoactivation, were selected for optimisation.

Results. The bacterial counts of the serially diluted cultures ranged from 3.3×10^8 to 13 CFU/mL. Both the primer/probe configurations yielded comparable mean Cq values and ΔCq across the serial dilutions, indicating similar performance. However, the 900F/900R/250P configuration provided the best overall analytical performance, including lower variability, improved fluorescence signal and positive amplification down to approximately 10^2 CFU/mL, with a mean Cq of 38.8. Standard curves were generated using serially diluted DNA stock and DNA extracted from the bacterial dilution series. Comparable Cq values were obtained at the corresponding dilution levels for both templates. The standard curve generated from the DNA stock showed a coefficient of determination (R^2) of 0.9987, a slope of −3.6, and an amplification efficiency of 87%, whereas the curve generated from the bacterial dilution series showed an R^2 of 0.9978, a slope of −3.7, and an amplification efficiency of 85.3%. Experimental evaluation of dead-cell signal suppression and VBNC detection is ongoing.

Conclusions. The optimised hipO-based qPCR showed suitable linearity, efficiency and sensitivity for the quantitative detection of C. jejuni. Its integration with PMAxx™ and an internal dead-cell processing control represents a promising strategy to improve the selective detection of viable cells that may be underestimated by culture-based methods. Further validation in artificially and naturally contaminated poultry matrices will determine its applicability for food-chain monitoring and microbiological risk assessment.