Rapid detection of Shiga toxin producing *Eschericia coli* using Vero cell-based assay

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Abstract

In this study, we investigated if CARD (Cell-based assay for rapid high-throughput detection of Shiga-toxin producing Escherichia coli) could be used for rapid screening and detection of Shiga-toxin producing Escherichia coli (STEC) by measuring release of lactate dehydrogenase from Vero epithelial kidney cells as a biomarker for cytotoxicity to differentiate these from non-STEC. After evaluating two phage induction treatments, four growth media, and two cell culture platforms, uninduced bacterial cells resuspended in Luria-Bertani broth (LB) were found to be suitable for building 3D CARD in generating high percent cytotoxicity similar to induced cells by Mitomycin C (2 ug/ml) and ciprofloxacin (100 ng/ml) treatment. CARD can detect STEC after 6 h post infection with percent cytotoxicity ranging from 33%-79%, which is 10 h faster than the traditional 2D platform we modified previously, when tested against known laboratory strains of Shiga-toxin producing Escherichia coli. The percent cytotoxicity against non-STEC, Salmonella, and Listeria spp. is below the established cytotoxicity cutoff value of 15%, showing high specificity against STEC. Ground beef samples (n=27) were tested for application potential, and CARD detected STEC with 38-46 percent cytotoxicity in artificially contaminated samples. Isolates (n=42) were further confirmed by stx1 and stx2 gene-specific multiplex PCR and traditional plating method on SMAC. CARD-based identification of samples resulted low false-positive results. This is the first screening technology against viable STEC relying on host pathogen interaction that can compliment other genomic and proteomic detection in isolating and detecting STEC from food samples as a preventative control for foodborne outbreaks.

Keywords— *Cytotoxicity; Food sample; multiplex-PCR; Pathogen detection*

Professional Biography

Celina To received her B.S. in Food Science at California Polytechnic State University. She is currently pursuing her M.S. at Purdue University with research focus in pathogen and biotoxin detection in the Molecular Food Microbiology Laboratory under the supervision of Dr. Arun Bhunia. With a passion for food safety and quality assurance, she has competed in the Institute of Food Technologists (IFT) team product development competitions and received additional training from previous internships at Hilmar Cheese Company and NASA Johnson Space Center.



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