Determination of viable-but-non-culturable *Campylobacter jejuni* in chicken using quantitative PCR combined with propidium monoazide pretreatment

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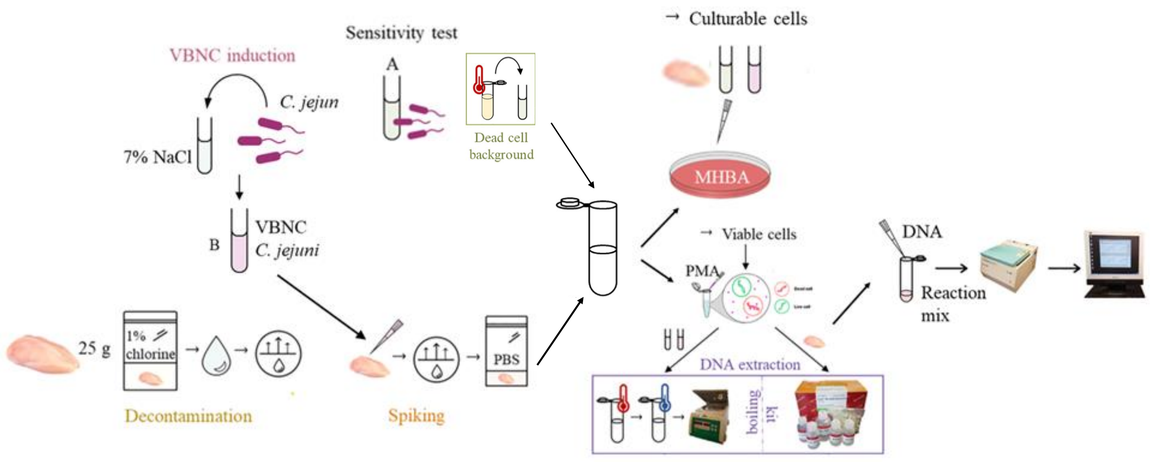
INTRODUCTION

- *Campylobacter* is responsible for the most frequently reported foodborne gastrointestinal infection in the world.
- *C. jejuni* can enter a viable but non-culturable (VBNC) state upon exposure to various stress conditions, such as low temperature, oxygen, acid treatment, and high osmotic pressure.
- VBNC *C. jejuni* can pose risks to food safety and public health because they cannot be detected using the routine microbiological plating assay but resuscitate under favorable conditions to develop virulence.
- DNA-intercalating dye, such as propidium monoazide (PMA), can be used to inhibit the amplification of DNA present in the dead cells.
- The combination of PMA and quantitative PCR can be used to quantify viable cells in bacterial population.

OBJECTIVE

- To quantify viable cells in pure cultures of *C. jejuni* in the background of dead cells.
- To induce VBNC *C. jejuni* under osmotic pressure and monitor the induction process
- To quantify the number of VBNC cells in artificially contaminated chicken meat.

EXPERIMENTAL DESIGN



RESULTS

1 Optimization of PMA concentration

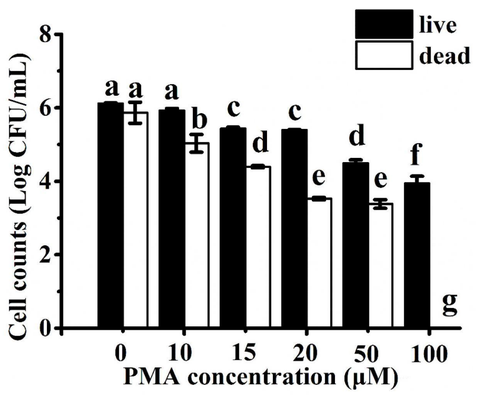


Fig. 1. *Campylobacter jejuni* cell counts estimated using Ct values with qPCR after treatment with different concentrations of PMA. Live and heat-inactivated cells at 6 log CFU/mL were treated and tested separately.

2 Specificity and sensitivity of PMA-qPCR assay for *C. jejuni* pure culture

Table 1. Bacterial strains used for the specificity test of qPCR.

Bacterial species	Strain	Source	PMA-qPCR result
Campylobacter jejuni	ACTC 33560	Bovine feces	+
	F38011	Human clinical isolate	+
	1658	Human clinical isolate	+
	NCTC 11168	Human clinical isolate	+
	81-116	Human clinical isolate	+
Campylobacter coli	RM 1875	Human clinical isolate	
	RM 2228	Human clinical isolate	-
	RM 5611	Human clinical isolate	-
Escherichia coli	O103:H2	Bovine feces	-
	O118:H16	Bovine feces	-
Salmonella enteritis	OEA2699	Human clinical isolate	-
	3512H	Human clinical isolate	-
Listeria monocytogenes	SEA 15B88	Human clinical isolate	-
	15B98	Human clinical isolate	-
Pseudomonas aeruginosa	H288	Human clinical isolate	-

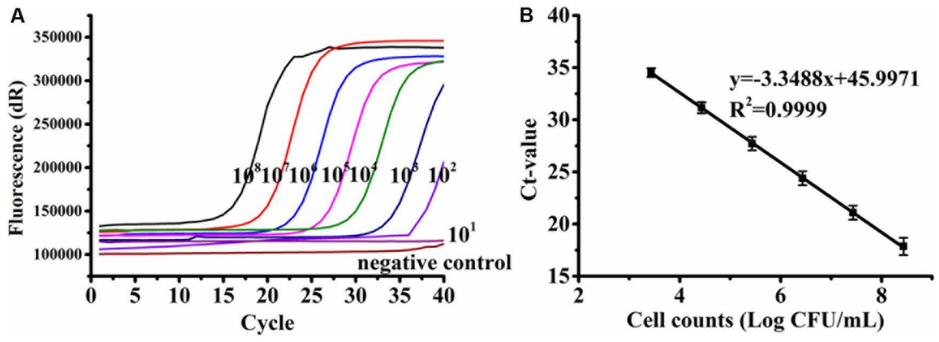


Fig. 2. Representative amplification curves (A) and standard curve (B) generated from 10-fold serial dilutions of viable *C. jejuni* F38011 cells ranging from 2.43 to 8.43 log CFU/mL in the background of 6 log CFU/mLof dead cells.

3 Induction of VBNC *C. jejuni* by osmotic stress

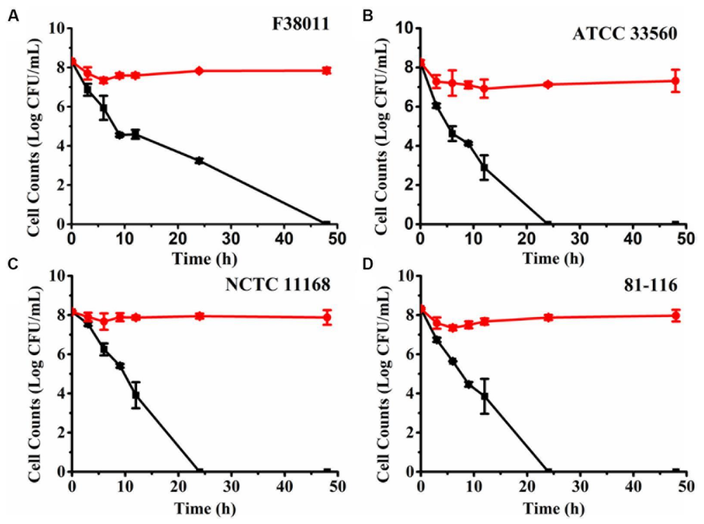


Fig. 3. Induction of viable but non-culturable (VBNC) *C. jejuni* F38011 (A), ATCC 33560 (B), NCTC 11168 (C) and 81-116 (D) under osmotic pressure in 7% (w/v) NaCl solution. Red circles (●) represent the viable cell counts quantified using PMA with real-time polymerase chain reaction (qPCR), while black squares (■) represent culturable cell counts determined using the plating assay. The difference between viable cells and culturable cells was considered as VBNC bacterial cells.

4 Quantification of VBNC *C. jejuni* in poultry products using PMA-qPCR

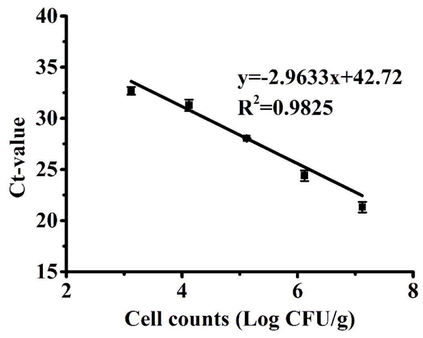


Fig. 4. Standard curves produced from 10-fold serial dilutions of the VBNC cocktail of *C. jejuni* cells ranging from 3.12 to 7.12 log CFU/g recovered from chicken samples.

CONCLUSIONS

- The optimized concentration of PMA (20 mM) could significantly inhibit the amplification of dead cells by qPCR with no significant interference on the amplification of viable cell DNA.
- PMA-qPCR was highly specific to *C. jejuni* with a limit of detection (LOD) of 2.43 log CFU/mL in pure bacterial culture.
- A standard curve for *C. jejuni* cell concentrations was established with the correlation coefficient of 0.9999 at the linear range of 3.43 to 8.43 log CFU/mL.
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- Induction of *C. jejuni* into the VBNC state by osmotic stress (i.e., 7% NaCl) was rapid (<48 h) and effective (>10% population).
- The LOD of PMA-qPCR for VBNC *C. jejuni* exogenously applied to chicken breasts was 3.12 log CFU/g.
- This technique can give insight into the prevalence of VBNC *Campylobacter* in the environment and agri-food production system.

REFERENCE

Lv et al. "Detection and quantification of viable but non-culturable *Campylobacter jejuni*." *Front. Microbiol.* (2020) 10: 2920.

Petersen et al. "Rapid determination of viable but non-culturable *Campylobacter jejuni* in food products by loop-mediated isothermal amplification coupling propidium monoazide treatment.“ *Int. J. Food Microbiol.* (2021) 351: 109263.

ACKOWNAGEMENT

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