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ARTICLE

The Correlation between NaCl Adaptation and Heat Sensitivity of *Listeria monocytogenes*, a Foodborne Pathogen through Fresh and Processed Meat

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Abstract

This study examined the relationship between NaCl sensitivity and stress response of *Listeria monocytogenes*. Nine strains of *L. monocytogenes* (NCCP10805, NCCP10806, NCCP10807, NCCP10808, NCCP10809, NCCP10810, NCCP10811, NCCP10920 and NCCP 10943) were exposed to 0%, 1%, 2% and 4% NaCl, and then incubated at 60°C for 60 min to select strains that were heat-sensitized (HS) and non-sensitized (NS) by NaCl exposure. After heat challenge, *L. monocytogenes* strains were categorized as HS (NCCP 10805, NCCP10806, NCCP10807, NCCP10810, NCCP10811 and NCCP10920) or NS (NCCP10808, NCCP10809 and NCCP10943). Total mRNA was extracted from a HS strain (NCCP10811) and two NS strains (NCCP10808 and NCCP10809), and then cDNA was prepared to analyze the expression of genes (*inlA*, *inlB*, *opuC*, *betL*, *gbuB*, *osmC* and *ctc*) that may be altered in response to NaCl stress, by qRT-PCR. The expression levels of two invasion-related genes (*inlA* and *inlB*) and two stress response genes (*opuC* and *ctc*) were increased ($p<0.05$) in NS strains after NaCl exposure in an NaCl concentration-dependent manner. However, only *betL* expression was increased ($p<0.05$) in the HS strains. These results indicate that the effect of NaCl on heat sensitization of *L. monocytogenes* is strain dependent and that *opuC* and *ctc* may prevent NS *L. monocytogenes* strains from being heat sensitized by NaCl. Moreover, NaCl also increases the expression of invasion-related genes (*inlA* and *inlB*).

Keywords: *Listeria monocytogenes*, NaCl, heat sensitivity, transcriptome, invasion gene

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Introduction

Listeria monocytogenes is a gram-positive, facultative anaerobic bacterium that can proliferate at low temperatures (Walker *et al.*, 1990) and survive in diverse environments, including NaCl concentrations up to 10% (McClure *et al.*, 1989) and under acidic conditions (Cole *et al.*, 1990). *L. monocytogenes* is a invasive bacterium, which is able to invade the human epithelial cells (Galdiero *et al*., 1997). In addition, *L. monocytogenes* is a pathogen that causes listeriosis, which is associated with septicemia, stillbirth, abortion, etc. (Gillespie *et al.*, 2006). Listeriosis is usually linked to the consumption of raw milk, soft cheeses made from raw milk, smoked fish, and processed meat products (fermented sausages etc.), which are formulated with NaCl (Muhterem-Uyar *et al.*, 2015; Samelis and Metaxopoulos, 1999).

NaCl is used to improve the flavor of processed products and to preserve food products by damaging the contaminating bacterial cells (Breslin and Beauchamp, 1997; Sofos, 1984). However, the NaCl concentrations used in foods may not be sufficient to inactivate pathogenic bacteria, and thus contributes to increased pathogenicity and resistance of bacteria to various stresses such as salt, acid, and heat (Bae *et al.*, 2012; Garner *et al.*, 2006; Jo *et al.*, 2014). Phan-Thanh *et al.* (2000) found that *L. monocytogenes* adapted to an acidic environment (pH 5.2) for 2 h became resistant to heat and salt. In addition, NaCl-exposed *E. coli* O157:H7 NCCP11142 was heat resistant, and could survive at 50°C (Lee *et al.*, 2015). Also, Yoon *et al.* (2013) showed that heat resistance in *Salmonella*

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Typhimurium exposed to high NaCl concentration was increased.

Quantitative reverse trancription-PCR (qRT-PCR) was used to quantify the certain gene expression level (Livak and Schmittgen, 2001), and this method can be used to quantify gene expression levels by NaCl. For instance, *Staphylococcus aureus* upregulated the expression of genes related to biofilm formation when grown under high NaCl conditions (Rode *et al.*, 2007).

To identify the invasive capability of *L. monocytogenes*, invasion assay using various human epithelial cell lines was usually performed, and the invasion efficiency was influenced by several stresses (Garner *et al*., 2006; Lee *et al.*, 2013). Yoon *et al.* (2013) demonstrated that *S.* Typhimurium exposed to high NaCl concentration increased invasion efficiency into Caco-2 cells. In addition, Olesen *et al.* (2010) found that NaCl influences the invasiveness of *L. monocytogenes*.

Therefore, the objective of this study was to evaluate the effect of NaCl on the heat sensitivity of *L. monocytogenes* and to identify the genes expressed relatively in heat-sensitized (HS) and non-sensitized (NS) strains to elucidate the correlation between NaCl and heat sensitivity in *L. monocytogenes*.

Materials and Methods

Preparation of inocula

Nine *L. monocytogenes* strains (NCCP10805, NCCP 10806, NCCP10807, NCCP10808, NCCP10809, NCCP 10810, NCCP10811, NCCP10920, and NCCP10943), listed in Table 1 were individually cultured in 10 mL of tryptic soy broth containing 0.6% yeast extract (TSBYE; Becton, Dickinson, and Company, USA) at 30°C for 24 h. Then, 0.1-mL aliquots of the cultures were transferred into 10 mL of fresh TSBYE and incubated at 30°C for 24 h. The cultures were centrifuged (1,912 *g*, 15 min, 4°C), and the cells were washed twice with phosphate-buffered saline (PBS, pH 7.4; 0.2 g of KH_2PO_4, 1.5 g of $Na_2HPO_4{\cdot}7H_2O$, 8.0 g of NaCl, and 0.2 g of KCl in 1 L of distilled water) and then diluted with PBS to obtain 4 Log CFU/mL.

Heat challenge

An aliquot (100 μL) of the inoculum was inoculated into 10 mL of TSBYE containing 0%, 1%, 2% and 4% NaCl, and incubated at 25°C for 24-48 h. The cells were then plated on tryptic soy agar plus 0.6% yeast extract (TSAYE; Becton, Dickinson, and Company, USA) supplemented with 0%, 1%, 2%, and 4% NaCl and incubated at 25°C for 48 h. After incubation, non-habituated *L. monocytogenes* (control) and NaCl-habituated *L. monocytogenes* cells (1-4%) growing on the plates were collected with a sterile bent glass rod, washed twice with PBS, and diluted with PBS to OD_{625}=0.1. Then,1 mL aliquots of the *L. monocytogenes* strains were inoculated into 9 mL of TSBYE preheated to 60°C in a water bath. To enumerate *L. monocytogenes* survival at 0, 20, 40 and 60 min, samples were removed at each time point, serially diluted with 0.1% buffered peptone water (BPW; Becton, Dickinson, and Company, USA), and plated on TSAYE. The plates were incubated at 30°C for 48 h. *L. monocytogenes* survival was expressed as $\mathrm{Log}(Y_t/Y_0)$, where Y_t is the cell count (Log CFU/mL) at time t and Y_0 is the initial cell count (Log CFU/mL). Based on the heat challenge results, the nine tested *L. monocytogenes* strains were categorized as heat-sensitive (HS) or non-sensitive (NS).

Table 1. General information of *Listeria monocytogenes* strains used in this study

Strain	Origin	Serotype
L. monocytogenes NCCP 10805	Poultry	1
L. monocytogenes NCCP 10806	Spinal fluid of man	2
L. monocytogenes NCCP 10807	Human	3a
L. monocytogenes NCCP 10808	Animal, Tissue (ruminant brain)	4a
L. monocytogenes NCCP 10809	Human	4b
L. monocytogenes NCCP 10810	Chicken	4c
L. monocytogenes NCCP 10811	Chicken	4e
L. monocytogenes NCCP 10920	Unknown	1/2a
L. monocytogenes NCCP 10943	Rabbit	1/2a

Transcriptional analysis and invasion assay

To determine the relative expression levels of genes that were related to virulence, and osmotic and general stresses (*inlA*, *inlB*, *opuC*, *betL*, *gbuB*, *osmC* and *ctc*; Table 2) after exposure to NaCl, 0.4 mL of HS and NS *L. monocytogenes* inocula were inoculated into 40 mL of TSBYE and incubated at 25°C to an OD_{625}=0.6. After incubation, 9-mL aliquots of the cultures were exposed to TSBYE plus 0%, 1%, 2% and 4% NaCl for 20 min. Then, 1.5-mL aliquots of the cultures were placed in microtubes and centrifuged at 5,000 *g* for 5 min. Then, 0.1 mL of lysozyme (10 mg/mL; Wako Pure Chemical Industries, Ltd., Japan) was added to the cell pellets and mixed vigorously. The mixture was incubated at 37°C for 15 min. After incubation, mRNA was extracted using the Qiagen RNeasy Mini Kit (Qiagen, Germany) and RNase-free DNase Set (Qia-

Table 2. Oligonucleotide primers used in the quantitative reverse transcription-PCR analysis

Gene	Primer	Sequence (5′ → 3′)	Reference
16s RNA	16s RNA-F	CTA CGC ATT TCA CCG CTA CA	This study
	16s RNA-R	GAG GGT CAT TGG AAA CTG GA	This study
inlA	*inlA*-F	GGT CTC ACA AAC AGA TCT AGA CCA AGT	Sue *et al.* (2004)
	inlA-R	TCA AGT ATT CCA CTC CAT CGA TAG ATT	Sue *et al.* (2004)
inlB	*inlB*-F	TGG GAG AGT AAC CCA ACC AC	This study
	inlB-R	CGT CCC TGC CTC TAC TTT TG	This study
opuC	*opuC*-F	CGG AAG ATC CCG TCA AAC TA	This study
	opuC-R	CGT CAT ATG TGG CAT CAA GC	This study
betL	*betL*-F	AAA CGA CAG GCG GAT CTT TA	This study
	betL-R	CTT GCT ATC CCT GCT TGG AG	This study
gbuB	*gbuB*-F	ATG ATG GCG GGT ATT AAC CA	This study
	gbuB-R	CAT TGC ACC GAT CAT TGA AG	This study
osmC	*osmC*-F	CTC CGT AAC CAG CAG CAA AT	This study
	osmC-R	TCT CTG CAC CAA CAG AGC TT	This study
ctc	*ctc*-F	CAG TTC GTG ACA ATG GTC GT	This study
	ctc-R	CCT TTA ACG GGT CCA CTT GA	This study

gen) according to the manufacturer's instruction. Total mRNA was quantified by using an Epoch™ Microplate Spectrophotometer (BioTek Instruments, Inc., USA). The relative expression levels of virulence-, osmotic stress-, and general stress-related genes were measured by qRT-PCR. cDNA was synthesized from the extracted mRNA by using the QuantiTect Reverse Transcription Kit (Qiagen) according to the manufacturer's instructions. The reaction mixture [24 μL; containing 12.5 μL of master mix, 6.5 μL of dH_2O, and 2.5 μL of forward and reverse primers (10 pmol/μL)] was prepared by using the Rotor-Gene SYBR Green PCR Kit (Qiagen) according to the manufacturer's protocol. Then, 1 μL of cDNA and 24 μL of the reaction mixture were added to a PCR strip. To determine the relative expression levels of the target genes, the data was analyzed using Rotor-Gene Q software (Qiagen). The mean threshold cycle (C_T) values were used for the transcriptional analysis, and 16s rRNA was used as the reference gene to determine relative gene expression levels.

A Caco-2 cell invasion assay was performed to compare the invasion efficiency of the HS and NS *L. monocytogenes* strains according to the method by Lee *et al.* (2012).

Statistical analysis

Each experiment was tried twice with two samples per trial (n=4). The data for heat challenge and gene expression level were analyzed by the mixed model procedure and the general linear model procedure of SAS® (version 9.3; SAS Institute Inc., USA), respectively. A pairwise *t*-test at α=0.05 was used for all mean comparisons.

Results and Discussion

After heat challenge of the nine *L. monocytogenes* strains that were exposed to various NaCl concentrations, the strains were categorized in Table 3 as HS (NCCP10805, NCCP10806, NCCP10807, NCCP10810, NCCP10811, and NCCP10920) or NS (NCCP10808, NCCP10809, and NCCP10943). This result indicates that the cross-protective effect of NaCl on *L. monocytogenes* against heat is strain dependent. Therefore, it was necessary to find out what caused this strain variation. Palumbo *et al.* (1995) also showed that the survivability of *L. monocytogenes* in liquid egg yolk increased when 10% and 20% salt were added, because the *D*-value was higher as the temperature of the liquid egg yolk increased. In addition, the *D*-value of *Salmonella* spp. grown in liquid egg yolk containing 10% salt was higher than that in plain egg yolk (Palumbo *et al.*, 1995). Lee *et al.* (2012) demonstrated that a mixture of 10 *L. monocytogenes* strains habituated by NaCl showed heat resistance, especially when they were exposed to sequentially higher NaCl concentrations (0%, 2%, 4%, and 6%). Kotrola and Conner (1997) showed that NaCl exposure increased the *D*-value of *E. coli* O157:H7 when grown at 52°C, 55°C, 57°C, and 60°C, indicating the increased survival of the bacterium. However, these studies did not identify the genes related to the cross-protection effect. Thus, we sought to analyze the gene expression levels of *L. monocytogenes* strains, exhibiting NaCl cross-protection to heat stress.

In the HS strain NCCP10811, the relative expression levels of osmotic stress- and general stress-related genes

Table 3. Reduction of cell counts [mean±SD; Log (Y_t/Y_0)] of nine *Listeria monocytogenes* strains, which were non-habituated (0% NaCl) or habituated to 1%, 2% and 4% NaCl during heat challenge at 60°C for 60 min

L. monocytogenes strains		Heating time (min)	NaCl concentration (%)			
			0	1	2	4
Heat-sensitized group (HS)	NCCP 10805	0	0.0±0.0^{A}	0.0±0.0^{A}	0.0±0.0^{A}	0.0±0.0^{A}
		20	-3.0±0.4^{A}	-3.2±0.1^{A}	-3.1±0.1^{A}	-3.2±0.4^{A}
		40	-3.6±0.2^{A}	-4.1±0.3^{B}	-3.5±0.3^{A}	-3.8±0.2^{AB}
		60	-4.1±0.3^{A}	-4.9±0.6^{B}	-4.1±0.7^{A}	-4.5±0.5^{AB}
	NCCP 10806	0	0.0±0.0^{A}	0.0±0.0^{A}	0.0±0.0^{A}	0.0±0.0^{A}
		20	-2.7±0.3^{A}	-3.0±0.1^{AB}	-3.1±0.3^{B}	-3.3±0.2^{AB}
		40	-3.3±0.3^{A}	-3.5±0.3^{A}	--3.5±0.3^{A}	-3.9±0.1^{B}
		60	-3.8±0.2^{A}	-4.5±0.3^{B}	-4.5±0.3^{C}	-4.3±0.3^{B}
	NCCP 10807	0	0.0±0.0^{A}	0.0±0.0^{A}	0.0±0.0^{A}	0.0±0.0^{A}
		20	-3.1±0.2^{A}	-3.4±0.2^{AB}	-3.6±0.2^{B}	-3.3±0.4^{AB}
		40	-3.8±0.4^{A}	-3.9±0.2^{A}	-3.9±0.2^{A}	-4.4±0.3^{B}
		60	-4.5±0.5^{A}	-4.9±0.4^{B}	-4.7±0.3^{AB}	-5.1±0.4^{B}
	NCCP 10810	0	0.0±0.0^{A}	0.0±0.0^{A}	0.0±0.0^{A}	0.0±0.0^{A}
		20	-2.9±0.2^{A}	-3.0±0.2^{A}	-3.2±0.1^{AB}	-3.4±0.3^{B}
		40	-3.5±0.2^{A}	-4.0±0.2^{B}	-3.6±0.4^{A}	-3.9±0.2^{B}
		60	-4.1±0.2^{A}	-4.3±0.2^{A}	-4.0±0.3^{A}	-4.8±0.4^{B}
	NCCP 10811	0	0.0±0.0^{A}	0.0±0.0^{A}	0.0±0.0^{A}	0.0±0.0^{A}
		20	-3.2±0.5^{AB}	-2.8±0.1^{A}	-3.5±0.7^{B}	-3.6±0.3^{B}
		40	-3.7±0.6^{AB}	-3.3±0.3^{A}	-3.9±0.7^{B}	-4.3±0.4^{B}
		60	-4.4±0.7^{A}	-4.0±0.4^{A}	-4.6±0.8^{A}	-5.2±0.3^{B}
	NCCP 10920	0	0.0±0.0^{A}	0.0±0.0^{A}	0.0±0.0^{A}	0.0±0.0^{A}
		20	-3.0±0.2^{A}	-3.1±0.2^{A}	-3.0±0.2^{A}	-3.3±0.1^{A}
		40	-3.5±0.3^{A}	-3.4±0.2^{A}	-3.9±0.5^{B}	-3.7±0.3^{AB}
		60	-3.7±0.2^{A}	-3.9±0.3^{A}	-4.5±0.6^{B}	-4.6±0.6^{B}
Non heat-sensitized group (NS)	NCCP 10808	0	0.0±0.0^{A}	0.0±0.0^{A}	0.0±0.0^{A}	0.0±0.0^{A}
		20	-3.1±0.2^{A}	-3.0±0.1^{A}	-3.2±0.4^{A}	-3.4±0.5^{A}
		40	-3.8±0.2^{AB}	-3.5±0.2^{A}	-4.2±0.1^{B}	-3.9±0.4^{AB}
		60	-4.0±0.2^{A}	-4.2±0.7^{A}	-4.3±0.1^{A}	-4.2±0.3^{A}
	NCCP 10809	0	0.0±0.0^{A}	0.0±0.0^{A}	0.0±0.0^{A}	0.0±0.0^{A}
		20	-4.1±0.2^{A}	-3.9±0.1^{A}	-3.6±0.7^{A}	-3.8±0.4^{A}
		40	-4.3±0.3^{A}	-4.4±0.3^{A}	-4.1±0.7^{A}	-4.1±0.2^{A}
		60	-5.1±0.3^{A}	-4.7±0.2^{A}	-5.1±0.8^{A}	-4.9±0.2^{A}
	NCCP 10943	0	0.0±0.0^{A}	0.0±0.0^{A}	0.0±0.0^{A}	0.0±0.0^{A}
		20	-3.0±0.3^{AB}	-3.0±0.1^{A}	-2.9±0.2^{A}	-3.2±0.1^{B}
		40	-3.5±0.0^{A}	-3.5±0.3^{A}	-3.5±0.2^{A}	-4.0±0.1^{B}
		60	-4.2±0.2^{A}	-4.4±0.3^{A}	-4.1±0.3^{A}	-4.3±0.3^{A}

$^{A-C}$Different letters in a same row mean significantly different at $p<0.05$.

(*inlA*, *inlB*, *opuC* and *ctc*) were not significantly increased by increasing NaCl concentrations ($p>0.05$) (Table 4; Fig. 1). Conversely, the relative expression levels of *betL*, *gbuB* and *osmC*, osmotic stress-related gene, increased as NaCl concentration increased ($p<0.05$) (Table 4; Fig. 1). However, in two of the NS strains (NCCP10808 and NCCP 10809), the relative expression levels of analyzed genes (*inlA*, *inlB*, *opuC* and *ctc*) were increased as the concentration of NaCl increased ($p<0.05$) (Table 4; Fig. 2). Osmotic-stress related genes are expressed as a response to osmotic stress conditions. In particular, *inlA* and *inlB* expression levels were much higher in the NS strains ($p<0.05$) than in the HS strain as the NaCl concentration increased. Therefore, the invasiveness of *L. monocytogenes* exposed to a high concentration of NaCl would likely increase. However, the invasion efficiency of the NS and HS *L. monocytogenes* strains in Caco-2 cells was not different (data not shown). Lee *et al.* (2012) also showed that exposure to NaCl did not affect human epithelial cell invasion of *L. monocytogenes*. These results indicate that there may be a threshold for *inlA* and *inlB* gene expression required for efficient *L. monocytogenes* invasion, and

Table 4. The relative gene expression levels (mean±SD) of *Listeria monocytogenes* adapted NaCl 1%, 2% and 4%

Genes	NaCl (%)	NCCP 10808	NCCP 10809	NCCP 10811
inlA	0	1.00±0.00^{B}	1.00±0.00^{B}	1.00±0.00^{A}
	1	1.14±0.42^{B}	1.45±0.40^{B}	1.49±0.57^{A}
	2	3.28±0.64^{B}	4.12±1.24^{A}	1.40±0.03^{A}
	4	6.54±2.87^{A}	4.29±1.36^{A}	1.85±0.64^{A}
inlB	0	1.00±0.00^{C}	1.00±0.00^{C}	1.00±0.00^{A}
	1	4.05±3.36^{BC}	1.52±0.60^{BC}	1.09±0.03^{A}
	2	7.21±3.31^{B}	5.06±1.12^{A}	1.50±0.10^{A}
	4	14.08±1.85^{A}	4.70±1.11^{AB}	1.50±0.52^{A}
opuC	0	1.00±0.00^{C}	1.00±0.00^{B}	1.00±0.00^{A}
	1	2.55±1.08^{B}	1.51±0.17^{B}	1.56±0.28^{A}
	2	3.89±0.61^{B}	4.02±0.08^{A}	2.08±0.23^{A}
	4	7.37±0.92^{A}	3.67±1.46^{A}	2.27±0.08^{A}
betL	0	1.00±0.00^{B}	1.00±0.00^{B}	1.00±0.00^{B}
	1	1.56±0.08^{B}	1.60±0.08^{B}	1.52±0.06^{B}
	2	1.83±0.66^{AB}	4.01±0.73^{A}	3.46±0.33^{A}
	4	3.45±2.20^{A}	3.77±0.30^{A}	4.09±1.14^{A}
gbuB	0	1.00±0.00^{A}	1.00±0.00^{C}	1.00±0.00^{B}
	1	1.66±0.49^{A}	2.25±0.43^{AB}	1.78±0.01^{B}
	2	1.72±0.36^{A}	2.91±0.83^{A}	2.71±0.43^{A}
	4	1.51±0.13^{A}	1.64±0.25^{BC}	1.74±0.19^{B}
osmC	0	1.00±0.00^{A}	1.00±0.00^{C}	1.00±0.00^{B}
	1	1.13±0.04^{A}	1.27±0.19^{BC}	1.39±0.09^{B}
	2	1.37±0.93^{A}	1.81±0.00^{AB}	2.15±0.07^{A}
	4	1.46±0.62^{A}	2.14±0.11^{A}	2.46±0.10^{A}
ctc	0	1.00±0.00^{B}	1.00±0.00^{C}	1.00±0.00^{A}
	1	1.73±0.48^{B}	1.72±0.53^{C}	0.99±0.23^{A}
	2	2.47±0.24^{B}	4.97±2.16^{B}	1.72±0.24^{A}
	4	6.74±3.13^{A}	8.85±2.69^{A}	2.08±0.28^{A}

$^{A-C}$Different letters in a same column mean significantly different at $p<0.05$.

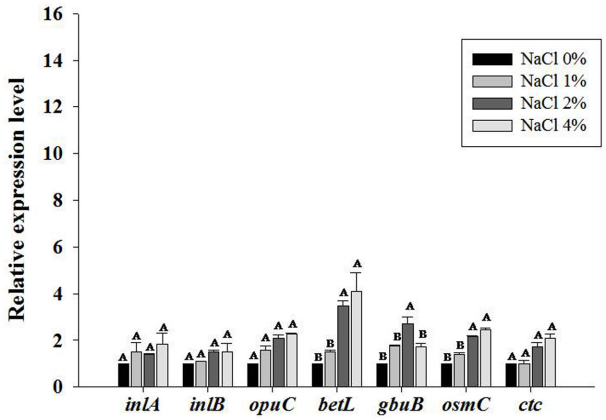

Fig. 1. Relative expression levels of stress response genes and pathogenicity related genes of HS strain of *Listeria monocytogenes* (*L. monocytogenes* NCCP10811) which were exposed to NaCl (tryptic soy broth with 0.6% yeast extract plus 0%, 1%, 2% and 4% NaCl). ^{A,B}Different letters indicate significantly different at $p<0.05$.

invasion efficiency may not be affected by *inlA* and *inlB* expression above the threshold. In other studies, the expression levels of *betL*, *gbu* and the *opuC* operon were increased as an adaptation to osmotic stress (Angelidis and Smith, 2003; Ko and Smith, 1999). Bae *et al.* (2012) showed that several transporters associated with the uptake of glycine and betaine were upregulated at 1.2% NaCl, which is a salt concentration commonly used in many RTE foods. In addition, the accumulation of *inlA*, *opuC* and *opuA* increased within 5 min when *L. monocytogenes* was exposed to osmotic stress when compared to the levels in the control, which was not exposed to osmotic stress (Sue *et al.*, 2004). A study by Gardan *et al.* (2003) showed that *ctc*, a *L. monocytogenes* gene related to general stress, was expressed at higher levels under high osmolarity conditions when there were no osmoprotectants, including glycine and betaine. Duche *et al.* (2002) showed that salt shock proteins (Ssp) in *L. monocytogenes* rapidly increased after exposure to salt stress, and Ssp overexpression was retained several hours after shifting back to normal conditions.

In conclusion, the effect of NaCl on heat-sensitization of *L. monocytogenes* is strain-dependent, and *opuC* and *ctc*

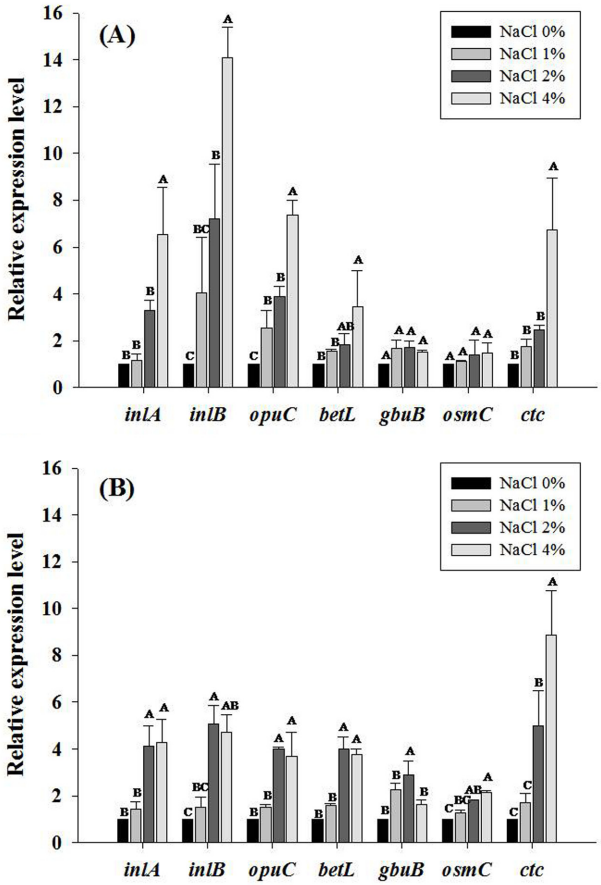

Fig. 2. Relative expression levels of stress response genes and pathogenicity related genes of NS strains of *Listeria monocytogenes* (A: *L. monocytogenes* NCCP10808; B: *L. monocytogenes* NCCP10809) which were exposed to NaCl (tryptic soy broth with 0.6% yeast extract plus 0%, 1%, 2% and 4% NaCl). $^{A-C}$Different letters indicate significantly different at $p<0.05$.

may play a role in preventing heat-sensitization by NaCl in NS *L. monocytogenes* strains. In addition, NaCl exposure also increased the expression of invasion-related genes (*inlA* and *inlB*) in NS *L. monocytogenes*.

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References

1. Angelidis, A. S. and Smith, G. M. (2003) Three transporters mediate uptake of glycine betaine and carnitine by *Listeria monocytogenes* in response to hyperosmotic stress. *Appl. Environ. Microb.* **69**, 1013-1022.
2. Bae, D., Liu, C., Zhang, T., Jones, M., Peterson, S. N., and Wang, C. (2012) Global gene expression of *Listeria monocytogenes* to salt stress. *J. Food Prot.* **75**, 906-912.
3. Breslin, P. A. S. and Beauchamp, G. K. (1997) Salt enhances flavour by suppressing bitterness. *Nature* **387**, 563.
4. Cole, M. B., Jones, M. V., and Holyoak, C. (1990) The effect of pH, salt concentration and temperature on the survival and growth of *Listeria monocytogenes*. *J. Appl. Microbiol.* **69**, 63-72.
5. Duche, O., Tremoulet, F., Glaser, P., and Labadie, J. (2002) Salt stress proteins induced in *Listeria monocytogenes*. *Appl. Environ. Microb.* **68**, 1491-1498.
6. Galdiero, E., D'Isanto, M., and Aliberti, F. (1997) Effect of saline concentration, pH and growth temperature on the invasive capacity of *Listeria monocytogenes*. *Res. Mcrobiol.* **148**, 305-313.
7. Gardan, R., Duche, O., Leroy-Setrin, S., and Labadie, J. (2003) Role of *ctc* from *Listeria monocytogenes* in osmotolerance. *Appl. Environ. Microb.* **69**, 154-161.
8. Garner, M. R., James, K. E., Callahan, M. C., Wiedmann, M., and Boor, K. J. (2006) Exposure to salt and organic acids increases the ability of *Listeria monocytogenes* to invade Caco-2 cells but decreases its ability to survive gastric stress. *Appl. Environ. Microb.* **72**, 5384-5395.
9. Gillespie, I. A., McLauchlin, J., Grant, K. A., Little, C. L., Mithani, V., Penman, C., Lane, C., and Regan, M. (2006) Changing pattern of human listeriosis, England and Wales, 2001-2004. *Emerg. Infect. Dis.* **12**, 1361-1366.
10. Jo, H., Park, B., Oh, M., Gwak, E., Lee, H., Lee, S., and Yoon, Y. (2014) Probabilistic models to predict the growth initiation time for *Pseudomonas* spp. in processed meats formulated with NaCl and $NaNO_2$. *Korean J. Food Sci. An.* **34**, 736-741.
11. Ko, R. and Smith, L. T. (1999) Identification of an ATP-driven, osmoregulated glycine betaine transport system in *Listeria monocytogenes*. *Appl. Environ. Microb.* **65**, 4040-4048.
12. Kotrola, J. S. and Conner, D. E. (1997) Heat inactivation of *Escherichia coli* O157:H7 in turkey meat as affected by sodium chloride, sodium lactate, polyphosphate, and fat content. *J. Food Prot.* **60**, 898-902.
13. Lee, H., Lee, S., Kim, S., Ha, J., Lee, J., Choi, K.-H., and Yoon, Y. (2015) NaCl influences thermal resistance and cell morphology of *Escherichia coli* strains. *J. Food Safety* **36**, 62-68.
14. Lee, J., Yoon, H., Lee, S., Lee, H., and Yoon, Y. (2013) Effect of fat contents on thermal resistance, antibiotic sensitivity, and Caco-2 cell invasion of *Listeria monocytogenes*. *Korean J. Food Sci. An.* **33**, 481-486.
15. Lee, J., Yoon, H., Lee, S., and Yoon, Y. (2012) Effect of NaCl on thermal resistance, antibiotic resistance, and human epithelial cell invasion of *Listeria monocytogenes*. *Korean J. Food Sci. An.* **32**, 545-552.
16. Livak, K. J. and Schmittgen, T. D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the method. *Methods* **25**, 402-408.
17. McClure, P. J., Roberts, T. A., and Otto Oguru, P. (1989) Comparison of the effects of sodium chloride, pH and tempera-

ture on the growth of *Listeria monocytogenes* on gradient plates and in liquid medium. *Lett. Appl. Microbiol.* **9**, 95-99.
18. Muhterem-Uyar, M., Dalmasso, M., Bolocan, A. S., Hernandez, M., Kapetanakou, A. E., Kuchta, T., Manios, S. G., Melero, B., Minarovièová, J., Nicolau, A. I., Rovira, J., Skandamis, P. N., Jordan, K., Rodríguez-Lázaro, D., Stessl, B., and Wagner, M. (2015) Environmental sampling for *Listeria monocytogenes* control in food processing facilities reveals three contamination scenarios. *Food Control* **51**, 94-107.
19. Olesen, I., Thorsen, L., and Jespersen, L. (2010) Relative transcription of *Listeria monocytogenes* virulence genes in liver pâtés with varying NaCl content. *Int. J. Food Microbiol.* **141**, S60-68.
20. Palumbo, M. S., Beers, S. M., Bhaduri, S., and Palumbo, S. A. (1995) Thermal resistance of *Salmoncella* spp. and *Listeria monocytogenes* in liquid egg yolk and egg yolk products. *J. Food Prot.* **58**, 960-966.
21. Phan-Thanh, L., Mahouin, F., and Alige, S. (2000) Acid responses of *Listeria monocytogenes*. *Int. J. Food Microbiol.* **55**, 121-126.
22. Rode, T. M., Langsrud, S., Holck, A., and Møretrø, T. (2007) Different patterns of biofilm formation in *Staphylococcus aureus* under food-related stress conditions. *Int. J. Food Microbiol.* **116**, 372-383.
23. Samelis, J. and Metaxopoulos, J. (1999) Incidence and principal sources of *Listeria* spp. and *Listeria monocytogenes* contamination in processed meats and a meat processing plant. *Food Microbiol.* **16**, 465-477.
24. Sofos, J. N. (1984) Antimicrobial effects of sodium and other ions in foods: A review. *J. Food Safety* **6**, 45-78.
25. Sue, D., Fink, D., Wiedmann, M., and Boor, K. J. (2004) σ^B-dependent gene induction and expression in *Listeria monocytogenes* during osmotic and acid stress conditions simulating the intestinal environment. *Microbiol.* **150**, 3843-3855.
26. Walker, S. J., Archer, P., and Banks, J. G. (1990) Growth of *Listeria monocytogenes* at refrigeration temperatures. *J. Appl. Bacteriol.* **68**, 157-162.
27. Yoon, H., Park, B.-Y., Oh, M.-H., Choi, K.-H., and Yoon, Y. (2013) Effect of NaCl on heat resistance, antibiotic susceptibility, and Caco-2 cell invasion of *Salmonella*. *BioMed Res. Int.* Article ID 274096.