

Influence of cold stress on the survival of *Listeria monocytogenes* Bug600 and ScottA in lethal alkali, acid and oxidative stress



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ABSTRACT

The objective of this study was to determine the influence of cold stress (4 °C) on the survival of *Listeria monocytogenes* Bug600 and ScottA in lethal alkali, acid and oxidative stress in broth and in distilled water. Four distinct patterns were observed: (1) Survival of cold stressed *L. monocytogenes* was increased by 1–4 logs in lethal alkali stress (pH 12.3 NaOH or KOH); (2) Survival of cold stressed *L. monocytogenes* was decreased by 1–4 logs in lethal acid stress (pH 1.3 HCl or H₃PO₄); (3) Survival of cold stressed *L. monocytogenes* was increased by 1–4 logs in lethal oxidative stress (1500 ppm H₂O₂); and (4) No difference in survival of cold stressed *L. monocytogenes* was observed in lethal oxidative stress by chlorine (1000 ppm NaOCl) compared to control. These patterns were also consistently observed for survival of *L. monocytogenes* cells in lethal alkali, acid or oxidative stress after adaptation of cold stress at 4 °C in low nutrient conditions (1/10 TSBYE). These findings demonstrate that cold stressed cells of *L. monocytogenes* Bug600 and ScottA have greater tolerance to lethal alkali and some oxidative stresses that are commonly employed by the food industries to kill this foodborne pathogen.

1. Introduction

Listeriosis, the foodborne illness caused by *Listeria monocytogenes*, has been linked to numerous outbreaks associated with a wide range of food products, such as raw fruits and vegetables, ready to eat salads, dairy products or processed meat products (Radoshevich, Lilliana, & Cossart, 2018). It primarily affects those with weakened immunity, such as pregnant women, old and young people, causing abortion and various clinically severe illnesses and high fatality rate of 25–30% (Buchanan, Gorris, Hayman, Jackson, & Whiting, 2017).

Controlling of *L. monocytogenes* in the food processing industries is considered challenging due its ubiquitous nature (Carpentier & Olivier, 2011). *L. monocytogenes* is able to withstand adverse environmental conditions including temperature from 0 °C to 45 °C, pH from 4.1 to 9.6 and salt concentrations up to 20% (Gandhi & Chikindas, 2007; Hamon, Bierne, & Cossart, 2006). Particularly, the ability to survive and grow at low temperatures makes *L. monocytogenes* a serious food safety and public health risk (Arguedas-Villa, Kovacevic, Allen, Stephan, & Tasara, 2014; Saldivar, Davis, Johnson, & Ricke, 2018). Stress adaptation in *L. monocytogenes* is one of a major phenomenon where sublethal stressed cells become resistant to lethal inactivation treatments (Bolton & Frank, 1999; Soni, Nannapaneni, & Tasara, 2011). Several studies indicated that the exposure of *L. monocytogenes* to sublethal acid, alkali and

oxidative stress are more tolerant to lethal levels of the same stress or different stresses (Hill, Cotter, Sleator, & Gahan, 2002; Lou & Yousef, 1997; Shen et al., 2016; Shen, Soni, & Nannapaneni, 2014).

Cold storage is frequently used in food industry to extend the shelf-life of food (Liu, Mou, & Su, 2016). Bacteria exhibit different physiological characteristics when exposed to cold stress for short or longer periods of time compared to non-cold stressed cells (Saldivar et al., 2018; Tasara & Stephan, 2006). For example, Vail, McMullen, and Jones (2012) have reported that *L. monocytogenes* cells increased their relative cell length when grown at 3 °C when compared to cells grown at 15 °C. In addition, *Streptococcus thermophilus* was more resistant to gastric juice or less resistance to disinfectants such as Quatricide and Clidox-S when exposed to 10 °C for 2 h (Fang, Lai, & Chou, 2013). *L. monocytogenes* and *Salmonella* Typhimurium cells were more resistant to chlorine dioxide and quaternary ammonium compounds when exposed to 15 °C for 3 h as compared to non-cold stressed cells (Lin, Chiang, Pan, & Chou, 2012).

Despite frequent use of cleaners and sanitizers, the elimination of *L. monocytogenes* from food processing and storage environments is challenging since it may withstand in lethal alkali, acid, oxidative and chilled stress conditions that cells may experience during cleaning and sanitation (Magalhães et al., 2016; Smith, 1996). Understanding the role of cold stress on the survival of *L. monocytogenes* cells when

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subsequently exposed to acid, alkali and oxidative stress is important with respect to eliminating this foodborne pathogen. Moreover, most of the previous studies compared the survival of cold stress adapted and non-adapted *L. monocytogenes* cells by performing the time-to-kill studies in nutrient rich broth models (i.e. BHI or TSBYE). However, cells of *L. monocytogenes* are exposed to lethal disinfectants in water during the cleaning and sanitation process in food processing plant. The cross-protection effect of cold stress adapted cells to lethal disinfectants in water has not been published. Therefore, the objective of this study was to investigate the influence of cold stress (4 °C for 5 min to 24 h) on the survival of *L. monocytogenes* Bug600 and ScottA cells in lethal alkali, acid and oxidative stress conditions in TSBYE and distilled water.

2. Materials and methods

2.1. Bacterial strains and growth conditions

The working stocks of *L. monocytogenes* EGD strain (Bug600) (serotype 1/2a, clinical, Institute Pasteur, France) and ScottA (serotype 4b, human clinical, FDA) were prepared in tryptic soy agar slants containing 0.6% yeast extract (TSAYE) and maintained at 4 °C. Overnight cultures were prepared by inoculating 10 ml of TSBYE (Bacto™) from the working stock prior to incubation at 37 °C for 18–20 h.

2.2. Preparation of lethal treatments

Due to the differences in the chemical nature of TSBYE and distilled water, different amounts of NaOH or HCl were required for achieving their correct lethal effects. Two lethal alkali treatments (pH 12.3) were prepared either by adding 350 µl of 4M NaOH (Fisher scientific) (5600 ppm) or 410 µl of 4M KOH (Fisher scientific) (9200 ppm) to 10 ml of TSBYE. To prepare lethal acid treatments (pH 1.3), either 451 µl of 9.1M HCl (Fisher scientific) (15000 ppm) or 712 µl of 4.3M H₃PO₄ (Fisher scientific) (30000 ppm) was added to 10 ml of TSBYE. Lethal oxidative stress by H₂O₂ was prepared by adding 294 µl of 1.5M H₂O₂ (Acros organics) to 10 ml of TSBYE (1500 ppm). Lethal oxidative stress by NaOCl was prepared by adding 122 µl of 1.1M NaOCl (Clorox) to 10 ml of TSBYE (1000 ppm). To prepare lethal treatments in distilled water, 180 µl of 4M NaOH (pH 12.3/5600 ppm), 175 µl of HCl (pH 1.3/5800 ppm) or 294 µl of H₂O₂ was added to 10 ml of distilled water.

2.3. Cold stress treatments

For the preparation of cold stressed cells, the overnight culture of *L. monocytogenes* that was grown at 37 °C was centrifuged for 5 min at 12,685 × g (Centrifuge- Eppendorf-Netheler-Hinz GmbH, Brinkmann Instruments Inc., Westbury, NY). After resuspending the cells in fresh TSBYE, 100 µl of the cell suspension was transferred to 9.9 ml of pre-chilled TSBYE to yield a cell concentration of 10⁷ CFU/ml. Cell suspensions were then incubated at 4 °C for 5 min, 1 h, 4 h (Short-term) or 24 h (Long-term) (Low temperature incubator-VWR, Scientific Products, Sheldon manufacturing Inc., Cornelius, OR). For the non-cold stressed control cells, 100 µl of the cell suspension was transferred to 9.9 ml of fresh TSBYE. These cells were immediately centrifuged at 12,685 × g for 5 min and resuspended in medium that contained lethal alkali, acid or oxidative stress challenges. For the preparation of cold stressed and non-cold stressed cells of *L. monocytogenes* at the low nutrient level, the 1/10 diluted TSBYE with sterile distilled water (1/10 TSBYE) was substituted for TSBYE.

2.4. Lethal stress treatments

For the lethal stress treatments in TSBYE, 1 ml aliquots of cold stressed and non-cold stressed *L. monocytogenes* cells were centrifuged at 12,685 × g for 5 min and the cell pellets were resuspended in 1 ml of TSBYE or distilled water that was adjusted to a pH of 12.3 with NaOH/

KOH (for lethal alkali stress) (Shen et al., 2016), a pH of 1.3 with HCl/H₃PO₄ (for lethal acid stress), 1500 ppm H₂O₂ (for lethal oxidative stress) or 1000 ppm NaOCl (for lethal oxidative stress) and incubated at 22 °C for 30 or 45 min.

2.5. Enumeration of viable *L. monocytogenes* cells after lethal stress

One ml aliquots of cold stressed and non-cold stressed cells were centrifuged to remove the lethal stress treatments and the cell pellets were resuspended in sterile physiological saline. After serial dilution in physiological saline, a 0.1 ml from each dilution was plated on TSAYE-EF (Tryptic soy agar containing yeast extract, esculin and ferric ammonium citrate) plates. To decrease the minimum detection limit to 10 CFU/ml, 1 ml of undiluted resuspended cell culture was spread plated on TSAYE-EF at each time point. Plates were then incubated at 37 °C for 48 h. *L. monocytogenes* CFU counts were transformed to log units using the equation: log₁₀ (x + 1) where x is CFU/ml. One was added to the CFU/ml values to allow the use of zero values in the analyses (Abeysundara, Nannapaneni, Soni, Sharma, & Mahmood, 2016; Salles et al., 2015).

2.6. Statistical analysis

Completely randomized designs with 3 replications and 5 treatment times were used to determine the effect of cold stress time on *L. monocytogenes* cell counts for each lethal stress condition at exposure time of 30 and 45 min. Tukey's honestly significant difference test ($P < 0.05$) was performed to separate means where significant differences occur at each lethal time interval (SPSS version 12.0, SPSS, Chicago, IL).

3. Results

3.1. Influence of cold stress in TSBYE on survival of *L. monocytogenes* Bug600 in lethal alkali stress by NaOH, acid stress by HCl and oxidative stress by H₂O₂

The initial cell counts of 7 log CFU/ml of *L. monocytogenes* Bug600 did not change significantly over storage time from 5 min to 24 h in TSBYE ($P > 0.05$) at 4 °C (data not shown). *L. monocytogenes* Bug600 counts were 1–2 logs greater ($P < 0.05$) in 4 h and 24 h cold stressed cells at 30 min of lethal alkali stress in TSBYE when compared to cells that had been cold stressed for 5 min or 1 h and non-cold stressed control cells (Fig. 1A). The 4 h and 24 h cold stressed *L. monocytogenes* Bug600 cells had 2.5–3.5 log counts after 45 min of lethal alkali stress in TSBYE while the other cold stressed and control cells were not detectable under those conditions. Similar pattern was observed with respect to the survival of *L. monocytogenes* Bug600 cells when exposed to lethal alkali stress in distilled water (Fig. 1B). The cold stressed cells at 4 °C for 4 h or 24 h survived 2–3 logs greater ($P < 0.05$) at 30 min of lethal alkali stress in distilled water than the other cold stressed and non-cold stressed control cells. None of the cold stressed and control cells of *L. monocytogenes* Bug600 were detectable at 45 min of lethal alkali stress in distilled water.

Twenty-four hour cold stressed *L. monocytogenes* Bug600 cells that were exposed 30 min of lethal acid in TSBYE were not detectable ($P < 0.05$) while cells that were cold stressed for 1 and 4 h and control cells had 3.5 log counts under those conditions (Fig. 1C). The cold stressed and control of *L. monocytogenes* Bug600 cells did not survive after 45 min of exposure to lethal acid stress in TSBYE. Similarly, the survival of cold stressed *L. monocytogenes* Bug600 cells at 4 °C for 24 h was decreased by 3–4 logs when exposed to 30 min of lethal acid stress in distilled water as compared to other cold stress treatments and control cells (one-way ANOVA, $P = 0.000$) (Fig. 1D). None of the cold stressed and control *L. monocytogenes* Bug600 cells were detectable at 45 min of lethal acid stress in water.

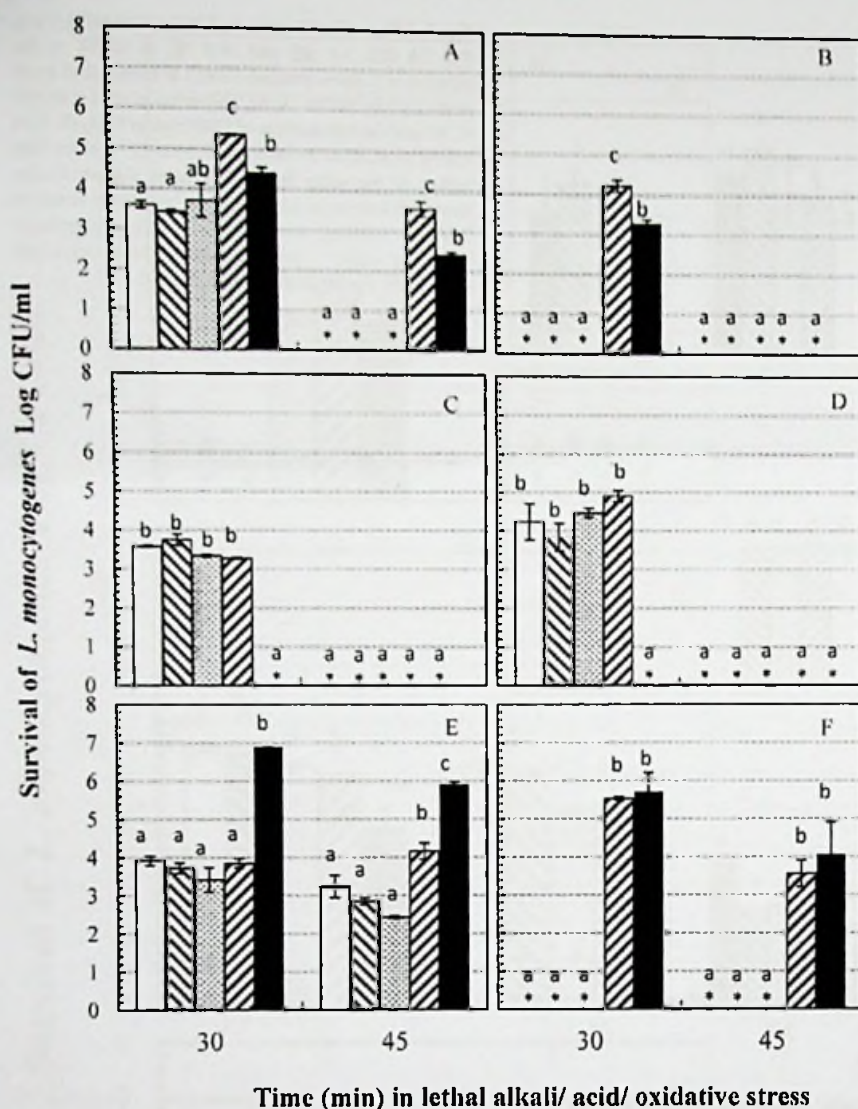


Fig. 1. Effect of cold stress at 4 °C for 0 min (□), 5 min (▤), 1 h (▥), 4 h (▧) and 24 h (▨) in TSBYE on the survival of *L. monocytogenes* Bug600 in lethal alkali stress of pH 12.3 by NaOH (A, B), acid stress of pH 1.3 by HCl (C, D) and oxidative stress of 1500 ppm of H₂O₂ (E, F) in TSBYE (A, C, E) or in water (B, D, F) at 22 °C. Note: Bars belong to the same lethal time point sharing similar lowercase letters are not significantly different based on One-way ANOVA and Tukey's Honestly Significant Difference tests. * indicate survival below detection limit. Error bars indicate standard error.

L. monocytogenes Bug600 cells that were cold stressed at 4 °C for 4 h or 24 h had 1-3 log greater counts after 30 and 45 min exposure to lethal oxidative stress in TSBYE than other cold stress and control cells ($P < 0.05$) (Fig. 1E). Similarly, cold stressed *L. monocytogenes* Bug600 cells at 4 °C for 4 h and 24 h survived by at least 4.5 logs greater in 30 min or 2.5 logs greater in 45 min of lethal oxidative stress in distilled water as compared to other cold stressed and control cells which were non-detectable (Fig. 1F).

3.2. Influence of cold stress in TSBYE on survival of *L. monocytogenes* ScottA in lethal alkali stress by NaOH, acid stress by HCl and oxidative stress by H₂O₂

The initial cell counts of 7 log CFU/ml of *L. monocytogenes* ScottA did not change significantly after exposure to cold stress at 4 °C in TSBYE for times ranging from 5 min to 24 h ($P > 0.05$) (data not shown).

L. monocytogenes ScottA cell counts after 4 and 24 h cold stress was 1 log greater ($P < 0.05$) after 30 min exposure to lethal alkali stress in TSBYE than *L. monocytogenes* that were cold stressed for 5 min or 1 h and control cells (Fig. 2A). *L. monocytogenes* ScottA cells that were cold stressed for 4 and 24 h had 4 log counts after 45 min exposure to lethal alkali stress in TSBYE while other cold stressed and control cells were not detectable. Similarly, *L. monocytogenes* Scott A cold stressed cells for

4 h and 24 h survived 1-1.5 logs greater after 30 min exposure to lethal alkali stress in distilled water as compared to other cold stressed and control cells (Fig. 2B). The 4 h and 24 h cold stressed *L. monocytogenes* ScottA cells survived 2.5-3.5 logs greater after 45 min exposure to lethal alkali stress in distilled water while other cold stressed and control cells were not detectable.

L. monocytogenes ScottA cells that were cold stressed at 4 °C for 4 h decreased by 1.5 logs and 24 h cold stressed cells were decreased by at least 3.5 logs after exposure to lethal acid stress in TSBYE for 30 min when compared to 5 min and 1 h cold stressed or control cells ($P < 0.05$) (Fig. 2C). None of the cold stressed and control cells were detectable at 45 min of lethal acid stress in TSBYE. The survival of *L. monocytogenes* ScottA cold stressed cells at 4 °C for 24 h were decreased by 2.5 logs after 30 min of exposure to lethal acid stress in distilled water while the survival of other cold stressed and control cells were not detectable (Fig. 2D). The survival of all the cold stressed and control *L. monocytogenes* ScottA cells were not detectable in 45 min of lethal acid stress in distilled water.

L. monocytogenes ScottA cells that were cold stressed at 4 °C for 24 h had 2-2.5 logs greater counts ($P < 0.05$) after exposure to 30 min of lethal oxidative stress in TSBYE than cells that were cold stressed for 5 min, 1 h and 4 h or control cells (Fig. 2E). The counts in *L. monocytogenes* ScottA cells that were cold stressed for 4 h and 24 h were at

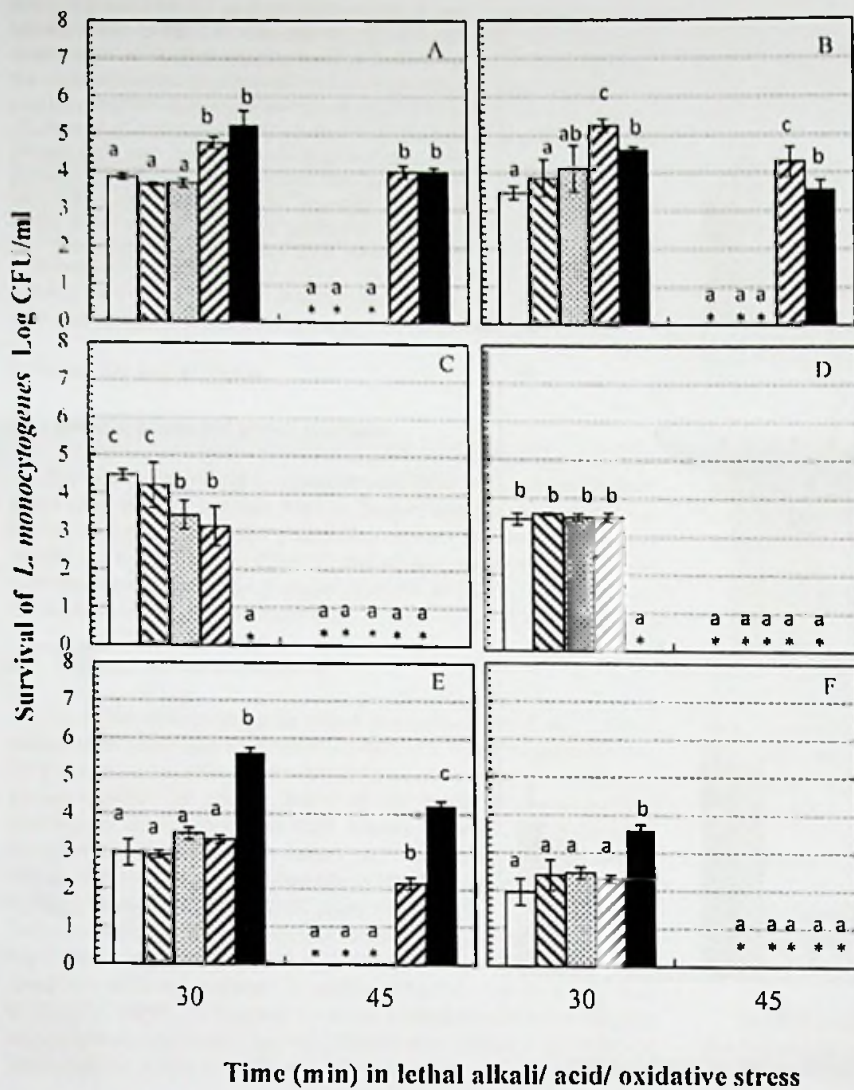


Fig. 2. Effect of cold stress at 4 °C for 0 min (□), 5 min (▨), 1 h (▩), 4 h (▧) and 24 h (■) in TSBYE on the survival of *L. monocytogenes* ScottA in lethal alkali stress of pH 12.3 by NaOH (A, B), acid stress of pH 1.3 by HCl (C, D) and oxidative stress of 1500 ppm of H₂O₂ (E, F) in TSBYE (A, C, E) or in water (B, D, F) at 22 °C. Note: Bars belong to the same lethal time point sharing similar lowercase letters are not significantly different based on One-way ANOVA and Tukey's Honestly Significant Difference tests. * indicate survival below detection limit. Error bars indicate standard error.

least 1–3 logs greater after 45 min exposure to lethal oxidative stress in TSBYE than the other cold stressed and control cells which were not detectable. Similarly, the cold stressed *L. monocytogenes* ScottA cells at 4 °C for 24 h survived 1–1.5 logs greater ($P < 0.05$) after 30 min exposure to lethal oxidative stress in distilled water as compared to other cold stressed and control cells (Fig. 2F). Survival in all the cold stressed and control cells of *L. monocytogenes* ScottA were not detectable in 45 min of lethal oxidative stress in distilled water.

In summary, survival of *L. monocytogenes* ScottA cold stressed cells at 4 °C for 4 h or 24 h in TSBYE was significantly greater in lethal alkali stress by NaOH or lethal oxidative stress by H₂O₂, but was significantly decreased in lethal acid stress by HCl as compared to other cold stressed and control cells.

3.3. Influence of cold stress in TSBYE on survival of *L. monocytogenes* Bug600 in lethal alkali stress by KOH (pH 12.3), lethal acid stress by H₃PO₄ (pH 1.3) and lethal oxidative stress by NaOCl (1500 ppm)

L. monocytogenes Bug600 cells that were cold stressed at 4 °C for 1 h, 4 h or 24 h had at least 2–3 logs greater counts ($P < 0.05$) after 30 min lethal alkali stress in TSBYE when compared to the cells that were cold stressed for 5 min and the control non-cold stressed cells which were not detectable (Fig. 3A). All the cold stressed and control cells were not

detectable after 45 min of lethal alkali stress. *L. monocytogenes* Bug600 cells that were cold stressed at 4 °C for 24 h had 1–1.5 logs lower counts ($P < 0.05$) after exposure to 30 min of lethal acid stress in TSBYE than 5 min, 1 h and 4 h cold stressed and control cells (Fig. 3B). *L. monocytogenes* Bug600 cells that were cold stressed at 4 °C for 24 h had at least 2.5–3 logs lower counts after 45 min exposure to lethal acid stress in TSBYE when compared to cold stressed cells for 5 min, 1 h and 4 h or control cell which were not detectable under the same conditions. No differences in cell counts existed ($P > 0.05$) between cells that were cold stressed at 4 °C for 5 min, 1 h, 4 h or 24 h and non-cold stressed control cells after exposure to lethal oxidative stress in TSBYE with 3 log and 2 log counts for all treatments after 30 min and 45 min exposure to lethal oxidative stress in TSBYE (Fig. 3C).

In summary, survival of *L. monocytogenes* Bug600 cold stressed cells for 4 °C for 1 h, 4 h or 24 h in TSBYE was significantly higher in lethal alkali stress by KOH, but was significantly decreased in lethal acid stress by H₃PO₄ in TSBYE as compared to other cold stressed and control cells. However, there were no differences in survival among the cold stressed and non-cold stressed *L. monocytogenes* Bug600 cells in lethal oxidative stress by chlorine in TSBYE.

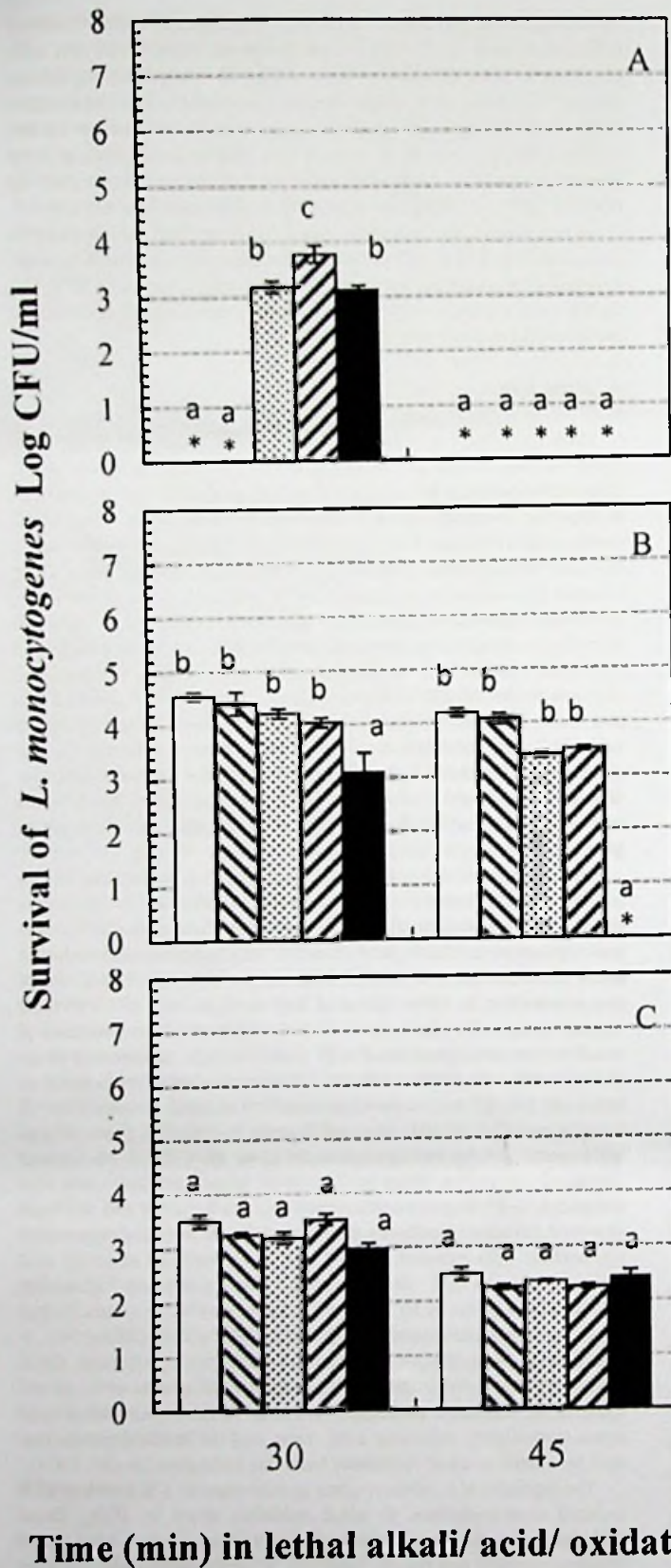


Fig. 3. Effect of cold stress at 4 °C 0 min (□), 5 min (▨), 1 h (▩), 4 h (▧) and 24 h (■) in TSBYE on the survival of *L. monocytogenes* Bug600 in lethal stresses alkali stress of pH 12.3 by KOH in TSBYE (A), acid stress of pH 1.3 by H₃PO₄ in TSBYE (B) and oxidative stress of 1000 ppm of NaOCl in TSBYE (C) at 22 °C. Note: Bars belong to the same lethal time point sharing similar lowercase letters are not significantly different based on One-way ANOVA and Tukey's Honestly Significant Difference tests. * indicate survival below detection limit. Error bars indicate standard error.

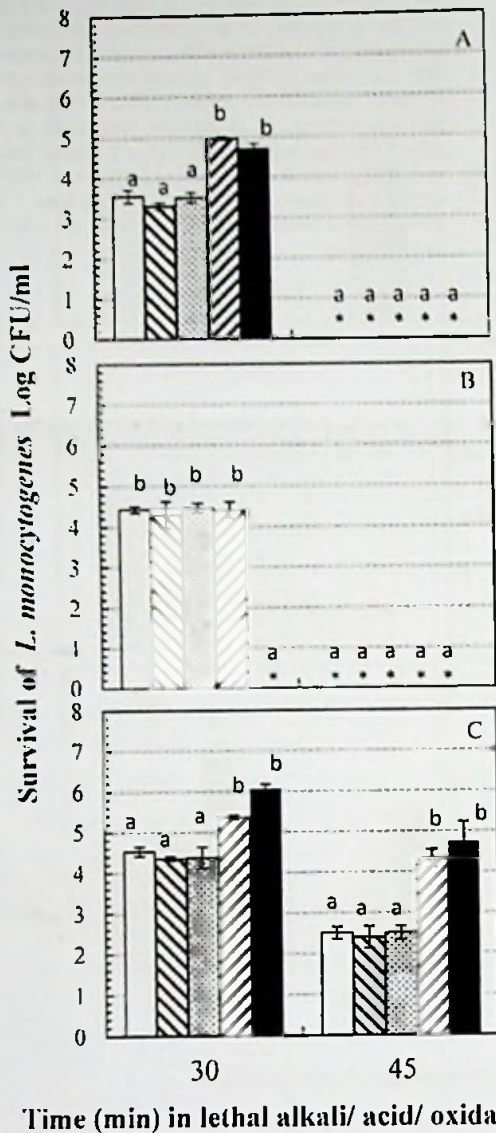


Fig. 4. Effect of cold stress at 4 °C for 0 min (□), 5 min (▨), 1 h (▧), 4 h (▩) and 24 h (■) in 1/10 TSBYE on the survival of *L. monocytogenes* Bug600 in lethal alkali stress of pH 12.3 by NaOH in TSBYE (A), in lethal acid stress of pH 1.3 by HCl in TSBYE (B), and in lethal oxidative stress of 1500 ppm of H₂O₂ in TSBYE (C) at 22 °C. Note: Bars belong to the same lethal time point sharing similar lowercase letters are not significantly different based on One-way ANOVA and Tukey's Honestly Significant Difference tests. * indicate survival below detection limit. Error bars indicate standard error.

3.4. Influence of cold stress in 1/10 TSBYE on survival of *L. monocytogenes* Bug600 in lethal alkali stress by NaOH (pH 12.3), lethal acid stress by HCl (pH 1.3) and lethal oxidative stress by H₂O₂ (1500 ppm)

L. monocytogenes Bug600 cells prepared in 1/10 TSBYE at 4 °C for 4 h or 24 h had 1–1.5 log greater counts ($P < 0.05$) after 30 min exposure to lethal alkali stress in TSBYE as compared to other cold stressed and non-cold stressed control cells (Fig. 4A). Survival in all the cold stressed and non-cold stressed control cells were not detectable after 45 min exposure to lethal alkali stress in TSBYE. *L. monocytogenes* Bug600 cold stressed cells prepared in 1/10 TSBYE at 4 °C for 24 h had at least 3.5 logs lesser counts ($P < 0.05$) after exposure to 30 min of lethal acid stress in TSBYE than the cells that were cold stressed for 5 min, 1 h and 4 h and control cells which were not detectable ($P < 0.05$) (Fig. 4B).

All cold stressed and control cells were not detectable after 45 min of lethal acid stress in TSBYE. *L. monocytogenes* Bug600 cells that were prepared in 1/10 TSBYE at 4 °C for 4 h or 24 h had 1–1.5 log greater counts ($P < 0.05$) after 30 min exposure to lethal oxidative stress using H₂O₂ in TSBYE than cells that were cold stressed for 5 min or 1 h and control cells (Fig. 4C). Both 4 h and 24 h cold stressed cells had 2 log greater counts ($P < 0.05$) after exposure to 45 min oxidative stress by H₂O₂ in TSBYE as compared other cold stressed and control cells.

In summary, *L. monocytogenes* Bug600 cold stressed cells prepared in 1/10 TSBYE at 4 °C for 4 h or 24 h showed significantly higher survival in lethal alkali stress by NaOH or in lethal oxidative stress by H₂O₂, but significantly decreased survival in lethal acid stress by HCl as compared to other cold stressed and control cells.

4. Discussion

Cold stress for 1 h, 4 h or 24 h increased the survival of *L. monocytogenes* Bug600 and ScottA in lethal alkali stress by NaOH or KOH. This indicates that cold stress may induce cross-resistance to lethal alkali stress in *L. monocytogenes*. Such induction of alkali stress adaptation during a short exposure to cold stress may prevent the cold-stressed cells of *L. monocytogenes* Bug600 and ScottA from being killed by alkali cleaners and sanitizers containing NaOH and KOH. Cross-protection to lethal alkali stress upon exposure to cold stress may be due to induction of common genes and proteins that are essential to regulate both cold and alkali stresses in *L. monocytogenes* (Beales, 2004). For example, previous studies by Liu, Graham, Begelow, Morse, & Wilkinson (2002) and Giotis et al. (2010) reported that *cat* and *sod* genes are expressed under both cold and alkali stress conditions in *L. monocytogenes*. Further confirmation is needed by comprehensive genomic and proteomic studies will help elucidate the cross-protection mechanism that governs both cold stress and alkali stress in *L. monocytogenes* and the group of genes that are regulated under these conditions.

Exposure to 4 °C for 24 h made *L. monocytogenes* Bug600 and ScottA less resistance to lethal acid stress by HCl or H₃PO₄. Ivy, Weidmann & Boor (2012) reported that *L. monocytogenes* that was grown at 7 °C was less resistant to artificial gastric fluid or acidified brain heart infusion broth when compared to control cells that were grown at 37 °C. There was a reduction in transcription of key regulons such as σ^B , σ^L in *L. monocytogenes* cells when grown at 7 °C which could have resulted in sensitivity to acid stress. Similar to these findings, the survival of *Escherichia coli* cold stressed cells at 4 °C was reduced when exposed to lethal pH 2.0 HCl as compared to control (Leenanon & Drake, 2001). Lin, Yu, and Chou (2004) reported that the exponential phase cells of *Vibrio parahaemolyticus* exposed to 20 °C or 15 °C for 2–4 h showed decreased survival in lethal acid stress by lactic acid and acetic acid compared to 37 °C grown cells. However, this behavior has not been observed in other foodborne pathogens. For example, *Streptococcus thermophilus* cells exposed to 10 °C for 2–4 h survived in lethal acid stress than non-cold stressed cells. Also, *Salmonella* Enteritidis (ATCC13076) grown at 10 °C did not differ in survival in gastric fluid at pH 2.0 compared to that of cells grown at 37 °C (Fang, Chiang, Hsu, & Chou, 2012; Shah, Desai, Chen, Stevens, & Weimer, 2013; Yang, Khoo, Zheng, Chung, & Yuk, 2014). The induction of greater or lesser resistance in foodborne pathogenic bacteria to subsequent lethal acid stress immediately following cold stress may be strain dependent or may be absent in some foodborne bacterial pathogens (Beales, 2004).

The exposure of *L. monocytogenes* to cold stress at 4 °C for 4 h or 24 h induced cross-protection to lethal oxidative stress by H₂O₂. These findings are consistent with those of Liu, Graham, Bigelow, Morse, and Wilkinson (2002) and Lœpfe, Raimann, & Tasara (2010) who reported *L. monocytogenes* is likely be exposed to mild oxidative stress grown at 4 °C. Pre-exposure to mild oxidative stress increased the survival of *L. monocytogenes* in lethal oxidative stress conditions (Abeysundara et al., 2016; Bansal, Nannapaneni, Sharma, & Kiess, 2018; Lou & Yousef, 1997). Liu et al. (2002) reported a greater level of *flp* transcription in *L.*

monocytogenes at 10 °C than at 37 °C. In *L. monocytogenes*, Flp (Ferritin-like proteins) protect cells against oxidative stress and general stress protein Ctc is induced in cold and oxidative stresses (Gardan, Duché, Leroy-Sétrin & Labadie, 2003 and Schuid et al., 2009). *L. monocytogenes* also produced a greater concentration of antioxidant enzymes such as catalase and superoxide dismutase at 4 °C when compared to 37 °C (Cacace et al., 2010). These results suggest that high antioxidant enzyme production in cold stressed *L. monocytogenes* may contribute to their cross-resistance to lethal concentrations of H₂O₂. In contrast to these findings, *Vibrio parahaemolyticus* was susceptible to 1000 ppm of H₂O₂ when pre-exposed to 20 °C or 15 °C for 2 h or 4 h compared to cells that were grown at 37 °C (Lim et al., 2004).

Even though cold stress at 4 °C induced cross-protection against oxidative stress caused by H₂O₂ in *L. monocytogenes*, no such cross-protection was observed against oxidative stress by NaOCl. NaOCl is commonly used as a disinfectant in food processing plants in which OCl⁻ ion is the main active component that is responsible for inducing oxidative stress against microorganisms (Fukuzaki, 2006). This differs from H₂O₂ which induces oxidative stress through peroxide free radicals. Therefore, the differences in mechanism between NaOCl and H₂O₂ may cause the induction of cross-protection against H₂O₂ but not against NaOCl in *L. monocytogenes* following exposure to cold stress for 4 h–24 h.

L. monocytogenes may be exposed to different nutrient levels depending on the food processing environment in which these cells persist. Therefore, we determined the effects of cold stress at 4 °C from 5 min to 24 h in low nutrient conditions (1/10 TSBYE by diluted growth media) on the survival of *L. monocytogenes* Bug600 in lethal alkali, acid and oxidative stress conditions. Such cold stressed cells also consistently showed increased survival in lethal NaOH and H₂O₂ stress conditions but decreased survival in lethal HCl stress condition compared to non-cold stressed control cells. These findings suggest that even in low nutrient conditions, *L. monocytogenes* may induce cross-protection against certain lethal stresses upon exposure to cold stress.

In conclusion, a shorter exposure to cold stress at 4 °C in TSBYE or 1/10 TSBYE led to increased survival of *L. monocytogenes* Bug600 and ScottA in lethal alkali (by NaOH, KOH) and oxidative (by H₂O₂) stress but decreased survival in lethal acid (by HCl and H₃PO₄) stress when compared to control non-cold stressed cells. *L. monocytogenes* Bug600 cells that were cold stress did not induce such cross-protection against oxidative stress by NaOCl. These survival patterns were found in lethal disinfectants in prepared in either TSBYE or distilled water. Therefore, these findings indicate acid based cleaners and sanitizers may be more effective at controlling cold stressed *L. monocytogenes* Bug600 and ScottA cells when compared to alkali or H₂O₂ based cleaners and sanitizers. Future work will be conducted to determine the effects of long term exposure to cold-stress on the survival of *L. monocytogenes* in lethal alkali, acid and oxidative stress conditions.

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