

## The Effects of Sodium Chloride on the Physiological Characteristics of *Listeria monocytogenes*

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### Abstract

Sodium chloride is used to improve various properties of processed meat products, e.g., taste, preservation, water binding capacity, texture, meat batter viscosity, safety, and flavor; however, many studies have shown that sodium chloride increases the resistance of many foodborne pathogens to heat and acid. *Listeria monocytogenes* has been isolated from various ready-to-eat (RTE) meat and dairy products formulated with sodium chloride; therefore, the objective of this paper was to review the effects of sodium chloride on the physiological characteristics of *L. monocytogenes*. The exposure of *L. monocytogenes* to sodium chloride may increase biofilm formation on foods or food contact surfaces, virulence gene transcription, invasion of Caco-2 cells, and bacteriocin production, depending on *L. monocytogenes* strain and serotype as well as sodium chloride concentration. When *L. monocytogenes* cells were exposed to sodium chloride, their resistance to UV-C irradiation and freezing temperatures increased, but sodium chloride had no effect on their resistance to gamma irradiation. The morphological properties of *L. monocytogenes*, especially cell elongation and filament formation, also change in response to sodium chloride. These findings indicate that sodium chloride affects various physiological responses of *L. monocytogenes* and thus, the effect of sodium chloride on *L. monocytogenes* in RTE meat and dairy products needs to be considered with respect to food safety. Moreover, further studies of microbial risk assessment should be conducted to suggest an appropriate sodium chloride concentration in animal origin foods.

**Key words:** sodium chloride, *L. monocytogenes*, biofilm, stress response, resistance

### Introduction

*Listeria monocytogenes* is a gram-positive foodborne bacterium that is widely distributed in the environment, and it can grow even at refrigeration temperatures (Kathariou, 2002; Notermans *et al.*, 1998). *L. monocytogenes* is a major concern in the food industry because of the associated high mortality rate (20-30%) in susceptible populations; the virulence of the pathogen in human outbreaks is characterized by meningitis, miscarriage, gastroenteritis, and septicemia (Recourt *et al.*, 2000). The pathogen has been found in many habitats such as the human gastrointestinal tract, food, and abiotic surfaces of the food industry environment (Weis and Seeliger, 1975; Welshimer and Donker-Voet, 1971; Wong, 1998). Moreover, its presence in food processing environments can result in the post-processing contamination of ready-to-

eat (RTE) meat and poultry products, and contaminate at-risk products, e.g., soft cheeses and unpasteurized milk (McLauchlin *et al.*, 2004; Meng and Doyle, 1998; Sammarco *et al.*, 1997). Mead *et al.* (1999) estimated that *L. monocytogenes* caused 499 deaths every year in U.S. In Canada, there were multi-state *L. monocytogenes* outbreaks, which caused 20 deaths of 56 cases after RTE meat consumption in 2008 (PHAC, 2008). Fretz *et al.* (2010) reported that acid-cured cheeses caused 4 deaths of 14 outbreak cases due to *L. monocytogenes* contamination in Austria and Germany in 2009. Hence, various compounds (sodium chloride, sodium nitrite, sodium lactate, and sodium diacetate) are used to inhibit *L. monocytogenes* growth in RTE meat and dairy products (Glass and Doyle, 1989; Hornbaek *et al.*, 2006; Mbandi and Shelef, 2002).

*L. monocytogenes* possesses innate resistance to various preservative factors such as high salt concentration, low pH, and low storage temperature (Foegeding *et al.*, 1992; McClure *et al.*, 1989; Vasseur *et al.*, 1999); thus, *L. monocytogenes* can be isolated from fermented meat such as

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hard salami ( $a_w$ : 0.79-0.86, pH: 4.3-4.5) and high-salt dried ham, or cooked meats (Lado and Yousef, 2007; Wulff *et al.*, 2006). In addition, *L. monocytogenes* may be subjected to sublethal food processing interventions, resulting in an increased adaptive response (Hill *et al.*, 2002; Samelis *et al.*, 2003; Shadbolt *et al.*, 2001). Adaptive responses to hostile environmental conditions modify the physiological properties of foodborne pathogens such that they become more resistant to further stronger stresses (Abee and Wouters, 1999; Ryan *et al.*, 2008; Rychlik and Barrow, 2005). Shadbolt *et al.* (2001) showed that the exposure of *Escherichia coli* to acid stress (pH 3.5) followed by osmotic stress ( $a_w$  0.90) at 25°C was more lethal to *E. coli* than when exposure to the same stresses was reversed.

In the meat industry, sodium chloride is used for its positive effects on water binding, texture, viscosity of meat batters, food safety, and flavor (Breslin and Beauchamp, 1997; Sofos, 1993). The exposure of *L. monocytogenes* to sodium chloride resulted in the pathogen becoming more resistant to subsequent stresses with the increased transcription levels of the genes involved in the uptake of glycine betaine/L-proline (Bae *et al.*, 2012; Skandamis *et al.*, 2008). The exposure of *L. monocytogenes* to high acidity and then osmotic stress increased its tolerance to acidity and osmolality through acid adaptation and osmo-adaptation, respectively (Faleiro *et al.*, 2003). Sodium chloride (5%) also increases biofilm formation by *L. monocytogenes* because of the production of extracellular polymeric substances rather than the accumulation of biofilm-forming cells (Pan *et al.*, 2010); however, the influence of sodium chloride on biofilm formation is concentration-dependent (Caly *et al.*, 2009).

*L. monocytogenes* enters, survives, and multiplies inside phagocytic and nonphagocytic cells (Vazquez-Boland *et al.*, 2001). The capability of *L. monocytogenes* to invade human epithelial cells was increased by sublethal sodium chloride (2.2%) (Conte *et al.*, 2000, 2002; Garner *et al.*, 2006).

Therefore, objective of this paper was to review the effect of sodium chloride on various physiological characteristics of *L. monocytogenes* that is found in RTE meat and dairy products.

### Biofilm formation

Biofilm is defined as a metabolically active matrix of bacterial cells and extracellular compounds (Kumar and Anand, 1998). Many types of bacteria are able to form

biofilms, which are found in a variety of food-related environments; however, the biofilms formed by foodborne pathogens represent a significant threat to food safety (Tarver, 2009). Thus, various methods to control or remove biofilms have been studied (Yoon and Sofos, 2008).

*L. monocytogenes* forms a biofilm on food contact surfaces such as stainless steel, plastic, and rubber in meat processing environments, and environmental factors may affect biofilm formation (Chavant *et al.*, 2002; Djordjevic *et al.*, 2002; Kim and Frank, 1995). The presence of various foods increases osmolality, which resulted in increased biofilm formation (Conlon *et al.*, 2002; Cramton *et al.*, 2001; Knobloch *et al.*, 2001). In addition, glucose synergistically increased the effect of sodium chloride on the formation of biofilm by *L. monocytogenes* (Pan *et al.*, 2010). Jensen *et al.* (2007) examined 18 strains of *L. monocytogenes* from different serotypes and origins to identify the relationship between sodium chloride in food and biofilm formation. The strains had similar growth patterns at 5 and 35°C, but the addition of sodium chloride caused the marked aggregation of 13 of these strains. This strain variation in biofilm formation was also suggested by Norwood and Gilmour (2001). Caly *et al.* (2009) also demonstrated strain variation for the adhesion of *L. monocytogenes* to polystyrene and stainless steel at 0% and 6% sodium chloride. Strain variation is also observed in *L. monocytogenes* to other stresses such as heat and acid (Lianou *et al.*, 2006). *L. monocytogenes* had lower bacterial adhesion at 11% sodium chloride compared to 0% and 6% sodium chloride because the expression of genes related to flagella formation was down-regulated in the presence of 11% sodium chloride. These results indicate that the effect of sodium chloride on biofilm formation also depends on its concentration (Caly *et al.*, 2009).

Among the *L. monocytogenes* serotypes, 1/2a, 1/2b and 4b strains are involved in the majority of human cases of listeriosis, with 4b strains accounting for the majority of human outbreaks (Kathariou, 2002). Moreover, serotype 1/2a is usually isolated from food-processing environments (Kathariou, 2002). When serotype 1/2a and 4b strains of *L. monocytogenes* grown at 0.5% and 7% sodium chloride were compared for biofilm formation, serotype 1/2a strains generally formed more biofilms than the 4b strains due to the production of extracellular polymeric substances instead of biofilm-forming cells (Pan *et al.*, 2010). However, most serotype 4b strains show a higher maximum growth rate than the 1/2a strains, indicating that growth rate is not directly related to biofilm

formation (Chae and Schraft, 2000; Djordjevic *et al.*, 2002; Pan *et al.*, 2010).

### Virulence

Olesen *et al.* (2010) compared the relative transcriptions of 2 virulence genes (*prfA* and *inlA*) and 2 virulence/stress response genes (*sigB* and *clpC*) in 3 *L. monocytogenes* strains (EGD-e: reference strain; O57: a more sodium chloride sensitive strain; 6869: a more sodium chloride tolerant strain) from liver pâtés with various sodium chloride concentrations. They found that a decrease in sodium chloride concentration (1.39-3.66%) of the liver pâtés increased the relative transcription levels of *prfA* in *L. monocytogenes* O57 and *sigB* in *L. monocytogenes* 6896, but reduced concentrations of sodium chloride did not change the relative transcription levels of *prfA*, *inlA*, *sigB*, or *clpC* in other strains. Kazmierczak *et al.* (2003) showed that the exposure of *L. monocytogenes* to 3% sodium chloride activated the alternative  $\sigma^B$  factor, which is encoded by *sigB*; the  $\sigma^B$  factor of *L. monocytogenes* is responsible for the stress response under acid and osmotic stresses, pathogenesis, and *prfA* transcription (Nadon *et al.*, 2002; Navarre and Schneewind, 1999). In addition, the activity of virulence factors such as catalase, superoxide dismutase, and listeriolysin O is generally influenced by sodium chloride (Myers *et al.*, 1993).

Sodium chloride is commonly added to RTE meat and poultry products, and it may influence the virulence of *L. monocytogenes* such as human epithelial cell invasion. When *L. monocytogenes* strains EGD-e and 4140 were exposed to 4.5% sodium chloride for 24 h, the genes related to cell invasion and intracellular life cycle were transcriptionally up-regulated (Olesen *et al.*, 2009). In addition, Garner *et al.* (2006) showed that sodium chloride shock and short-term adaptation (1 h) also induced the transcription of *L. monocytogenes* 10403S virulence gene and increased their invasion of Caco-2 cells. However, a study by Lee (2012) showed that 4% sodium chloride did not increase the invasion of Caco-2 cells by *L. monocytogenes* NCCP10943. This discrepancy with regard to the invasion of Caco-2 cells may be explained by strain variation as described by Lee (2012) and Olesen *et al.* (2009).

### Stress responses

Vogel *et al.* (2010) compared the survival of *L. monocytogenes* in physiological peptone saline (PPS) and in PPS supplemented with 5% sodium chloride. The addi-

tion of sodium chloride to PPS increased the resistance of *L. monocytogenes* to desiccation tolerance. This stress resistance may be caused by the high affinity of the glycine and betaine porters of *L. monocytogenes* because the porters allows *L. monocytogenes* to scavenge glycine and betaine in order to protect the pathogen from osmotic stress as well as cold stress (Mendum and Smith, 2002). Skandamis *et al.* (2009) showed that a 10-strain composite of *L. monocytogenes* had increased ( $p < 0.05$ ) resistance to lactic acid at pH after exposure to 3.5% sodium chloride. During cooking ground beef at 57.5°C and 62.5°C, *D*-values for *L. monocytogenes* increased as sodium chloride concentration increased up to 3% (Juneja *et al.*, 2013). The protective effect of sodium chloride on heat resistance can be explained by the ability of sodium chloride to decrease the water activity because dehydrated cytoplasm (plasmolysis) of the microbial cell leads to increased thermal stability of the intracellular enzymes and structural proteins (Corry, 1974; Leistner and Russell, 1991). Faleiro *et al.* (2003) exposed *L. monocytogenes* isolates from Portuguese cheese, meat and listeriosis to low pH and high sodium chloride concentration, and they found that the Portuguese cheese isolates were more resistant to the stresses than the other isolates because of their exposure to two sequential stresses, i.e., acidity during fermentation and osmotic stress during ripening. These results indicate that sodium chloride in RTE meat and dairy products may increase resistances of *L. monocytogenes* to various stresses.

### Response to irradiation

The use of UV light irradiation has been recommended to eliminate foodborne pathogens on the surfaces of meat products (Wong *et al.*, 1998; Yousef and Marth, 1988). The bacterial responses to UV light exposure were influenced by the sublethal stresses, that bacteria previously experienced (Mendonca *et al.*, 2004). McKinney *et al.* (2009) exposed *L. monocytogenes* to distilled water, fresh brine (9% sodium chloride), spent brine (20.9% sodium chloride), 5% spent brine, 35% spent brine, and 55% spent brine, followed by UV light (253.7 nm) irradiation. They found the following order of reductions in *L. monocytogenes* cell counts: water > fresh brine > 5% spent brine > 35% spent brine > 55% spent brine > undiluted spent brine. Bernbom *et al.* (2011) also suggested that sodium chloride (5%) enhanced the resistance of *L. monocytogenes* on foods to UV-C irradiation (254 nm), because sodium chloride caused *L. monocytogenes* to adhere strongly to

surfaces, which may decrease the penetrating ability of UV-C irradiation (Bernbom *et al.*, 2011; Jensen *et al.*, 2007). However, *L. monocytogenes* strains did not exhibit resistance to low doses of gamma-irradiation (0-0.5 kGy) after exposure to sodium chloride (0-9%) (Yoon *et al.*, 2009). Similarly, Briggs and Yazdany (1970) also found that the presence of sodium chloride had no effect on the resistance of *Bacillus* spp. to gamma-irradiation.

### Resistance to bacteriocins

The use of bacteriocins has been suggested for the effective control of *L. monocytogenes* growth in RTE meat and dairy products, especially in combination with sodium chloride and low pH (Delves-Broughton, 1990; Muriana, 1996). *Lactococcus lactis* produces nisin, which is a highly active bacteriocin against gram-positive bacteria (Delves-Broughton, 1990).

In *Listeria* isolates from RTE meat and dairy products, the frequency ratio of nisin resistance was very high up to  $10^{-3}$ , depending on the isolates (Martinez *et al.*, 2005). *L. monocytogenes* developed resistance by producing exopolysaccharides and modifying the cell membrane, which prevents nisin pores from forming (Breuer and Radler, 1996; Breukink *et al.*, 1999; Crandall and Montville, 1998; Grade *et al.*, 2004; Mantovani and Russell, 2001). Hornbaek *et al.* (2006) observed that the antilisterial activity of leucocin 4010 and nisin decreased when 2.5% sodium chloride was added to the sausage meat. Boziaris *et al.* (2007) also showed that low to medium concentrations (1.75-4%) of sodium chloride decreased the antimicrobial activity of nisin (50 IU/mL) to *L. monocytogenes* Scott A. However, Ben Hammou *et al.* (2010) suggested that a combination of nisin and sodium chloride (0-12%) synergistically prevents the growth of *L. monocytogenes* in sheep natural casings. Besides, during the storage of raw buffalo meat mince for 16 d at 4°C, *L. monocytogenes* cell counts increased by up to 6.3 and 6.1 Log CFU/g for 400 IU nisin and 400 IU nisin + 2% sodium chloride treatments, respectively, indicating that the addition of sodium chloride did not affect the antilisterial activity of nisin (Pawar *et al.*, 2000). This discrepancy for the sodium chloride effect on the antilisterial effect of nisin may be explained by strain variation of *L. monocytogenes* (Buncic *et al.*, 2001). Harris *et al.* (1991) found that the addition of 2.5% and 3.5% sodium chloride increased antilisterial effect of nisin compared to 0.5% and 1.5% sodium chloride; however, there was no significant difference in its antilisterial effect between 0.5% and

1.5% of sodium chloride. This finding suggests that the effect of sodium chloride on the antilisterial activity of nisin may depend on the concentrations of sodium chloride and nisin. Bouttefroy *et al.* (2000) also suggested that the antilisterial effect of nisin depended on sodium chloride concentration. Accordingly, it could be hypothesized that the resistance of *L. monocytogenes* to bacteriocins could be influenced by sodium chloride in RTE meat and dairy products.

### Cell morphology

Although many studies have been conducted to elucidate the effects of sodium chloride on the physiological characteristics of *L. monocytogenes* (Kuzhiyil *et al.*, 2012; McKinney *et al.*, 2009; Olesen *et al.*, 2009), little attention has been paid to the effect of sodium chloride on cellular morphology, which may influence the physiological properties of *L. monocytogenes* (Zaika and Fanelli, 2003).

When *L. monocytogenes* cells were exposed to 5-10% sodium chloride at 25°C for 6 d, the cells elongated (Hazeleger *et al.*, 2006). The cell surface of *L. monocytogenes* strains Scott A and LO28 becomes strongly hydrophilic following the addition of sodium chloride to acidic condition, which may alter the adhesion properties of the pathogen (Bereksi *et al.*, 2002). Zaika and Fanelli (2003) showed that *L. monocytogenes* cells became longer and thicker at high levels (6% and 7.5%) of sodium chloride. When *L. monocytogenes* was challenged with 1.46% to 8.77% sodium chloride, the filament was formed with increasing length as the sodium chloride concentration increased (Isom *et al.*, 1995). Similarly, Zarei *et al.* (2012) evaluated the effects of various concentrations of sodium chloride (1-9%) and potassium chloride (1-11%) on the morphology of *L. monocytogenes*. They found that filament formation with increased filament length became apparent in 5% sodium chloride and 7% potassium chloride (Zarei *et al.*, 2012). These findings were also observed in *Salmonella* at low water activity and low temperature (Mattick *et al.*, 2000, 2003).

*L. monocytogenes* forms a bacterial filament when it cannot complete cell division (Hazeleger *et al.*, 2006). The presence of an extensive filament increases optical absorbance without a corresponding increase in the number of viable cell counts because the filament appears as a single colony on an agar plate (Gill *et al.*, 2007). However, the filament can divide quickly to increase the number of cells substantially under optimum growth condition (Jones *et al.*, 2004; Mattick *et al.*, 2003). Hence, the risk

of *L. monocytogenes* in RTE meat products containing high sodium chloride concentrations may be under-estimated under high sodium chloride concentrations because of the presence of numerous filamentous structures (Bereksi *et al.*, 2002).

### Conclusion

In processed meat products, sodium chloride is used widely to improve taste, preservation, water binding, texture, viscosity of meat batters, food safety, and flavor. However, many studies have shown that the presence of sodium chloride in RTE meat and dairy products may increase the resistance of *L. monocytogenes* to diverse forms of stress. *L. monocytogenes* is the high risk food-borne pathogen isolated from RTE meat and dairy products. In this review, the exposure of *L. monocytogenes* to sodium chloride may influence biofilm formation on food surfaces and its virulence, especially for human epithelial cell invasion. The exposure of the pathogen to sodium chloride caused morphological changes to *L. monocytogenes* cells. Moreover, sodium chloride may protect *L. monocytogenes* cells in RTE meat and dairy products from UV-C irradiation, osmotic stress, freezing temperatures, and bacteriocins. However, the adaptive responses of *L. monocytogenes* to sodium chloride depend on sodium chloride concentration and strain. Therefore, the various responses of *L. monocytogenes* to sodium chloride need to be considered to improve the food safety of RTE meat and dairy products. Moreover, the microbial risk assessment for animal origin food with different sodium chloride concentrations is necessary to suggest an appropriate sodium concentration in the food.

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