The contribution of transcriptomic and proteomic analysis in elucidating stress adaptation responses of Listeria monocytogenes

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The Contribution of Transcriptomic and Proteomic Analysis in Elucidating Stress Adaptation Responses of *Listeria monocytogenes*

Kamlesh A. Soni,1 Ramakrishna Nannapaneni,1 and Taurai Tasara2

Abstract

The foodborne transmission of *Listeria monocytogenes* requires physiological adaptation to various conditions, including the cold, osmotic, heat, acid, alkaline, and oxidative stresses, associated with food hygiene, processing, and preservation measures. We review the current knowledge on the molecular stress adaptation responses in *L. monocytogenes* cells as revealed through transcriptome, proteome, genetic, and physiological analysis. The adaptation of *L. monocytogenes* to stress exposure is achieved through global expression changes in a large number of cellular components. In addition, the cross-protection of *L. monocytogenes* exposed to different stress environments might be conferred through various cellular machineries that seem to be commonly activated by the different stresses. To assist in designing *L. monocytogenes* mitigation strategies for ready-to-eat food products, further experiments are warranted to specifically evaluate the effects of food composition, additives, preservatives, and processing technologies on the modulation of *L. monocytogenes* cellular components in response to specific stresses.

Introduction

*Listeria monocytogenes* is an important public health problem and food safety challenge. As a foodborne pathogen, this bacterium primarily targets immunosuppressed individuals, including the elderly, pregnant women, and newborns, leading to listeriosis, a disease that, although less frequent in occurrence (~2.4 cases/million), is associated with relatively high (averaging 20%–30%) mortality rates (Mead et al., 1999). *L. monocytogenes* is associated with significant food safety control problems due to its wide distribution in nature and its capacity to survive and grow on the food products despite frequent exposure to harsh environmental conditions associated with food processing and preservation measures.

Upon encountering different environmental stress challenges along the food supply chain, it is presumed that *L. monocytogenes* cells depend on various stress-sensing mechanisms that detect stress-associated molecular hardships. On sensing such impending molecular stress problems, appropriate signal transduction processes are activated, subsequently leading to the mobilization of necessary stress protection measures through modifications in gene expression and protein function activities. Insights into gene expression changes mobilized during stress adaptation responses have been recently gained through transcriptome and proteome stress analysis in this bacterium. This article provides a review of cold, heat, osmotic, acid, alkaline, and oxidative stress responses in this bacterium.

Cold Stress Adaptation Responses of *L. monocytogenes*

Numerous molecular hardships confront *L. monocytogenes* cells exposed to cold stress such as increased membrane rigidity, reduced protein and enzyme activity, slow transport and nutrient uptake processes, stalled gene expression processes, and protein damage and alteration. Altered expression of numerous gene transcripts and proteins was observed in *L. monocytogenes* cells adapted to cold exposure (Chan et al., 2007; Cacace et al., 2010). Functional classification of genes and proteins identified in these two studies revealed that cold stress adaptation genetic responses of this bacterium promote (1) specific and general stress protection; (2) membrane fluidity and function; (3) resumption of gene expression events; (4) protein folding and degradation; (5) assimilation of carbon sources and cold protective nutrients; (6) oxidative stress protection; (7) energy production, and (8) specific amino acid and lipid biosynthesis pathways. A number of cold stress adaptation mechanisms that have been experimentally validated in this bacterium are listed in Table 1 and Figure 1A.

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Table 1. An Overview of the *Listeria monocytogenes* Gene and Protein Systems Associated with Cold, Osmotic, Acid, Heat, Oxidative, and Alkaline Stress Adaptation Responses

<table>
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<tbody>
<tr>
<td>LisRK</td>
<td>Two component system</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>Cotter <em>et al.</em> (1999); Kallipolitis and Ingmer (2001); Sleator and Hill (2005)</td>
</tr>
<tr>
<td>KpdED</td>
<td>Two component system</td>
<td></td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td>Kallipolitis and Ingmer (2001)</td>
</tr>
<tr>
<td>Lmo 1172</td>
<td>Putative two component system</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td>Chan <em>et al.</em> (2008)</td>
</tr>
<tr>
<td>Lmo 1060</td>
<td>Putative Two component system</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Chan <em>et al.</em> (2008)</td>
</tr>
<tr>
<td>RsbV</td>
<td>Anti-antisigma factor protein</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td>Chaturongakul and Boor (2004)</td>
</tr>
<tr>
<td>RsbT</td>
<td>Serine kinase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>Chaturongakul and Boor (2004)</td>
</tr>
<tr>
<td>SigB</td>
<td>Alternative sigma factor protein</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Chan <em>et al.</em> (2007); Giotis <em>et al.</em> (2008a); Raengpradub <em>et al.</em> (2008)</td>
</tr>
<tr>
<td>SigC</td>
<td>Alternative sigma factor protein</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
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<td></td>
<td>Chan <em>et al.</em> (2008); Zhang <em>et al.</em> (2005)</td>
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<tr>
<td>SigH</td>
<td>Alternative sigma factor protein</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>Chan <em>et al.</em> (2008); Rea <em>et al.</em> (2004)</td>
</tr>
<tr>
<td>SigL/RpoN</td>
<td>Alternative sigma factor protein</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>Chan <em>et al.</em> (2008); Okada <em>et al.</em> (2006); Raimann <em>et al.</em> (2009)</td>
</tr>
<tr>
<td>Fur</td>
<td>Transcription regulator</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td>Rea <em>et al.</em> (2004)</td>
</tr>
<tr>
<td>CtsR</td>
<td>Negative transcription regulator</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>Nair <em>et al.</em> (2000)</td>
</tr>
<tr>
<td>HrtC</td>
<td>Negative transcription regulator</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>Hu <em>et al.</em> (2007a)</td>
</tr>
<tr>
<td>MogR</td>
<td>Transcription repressor</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>van der Veen <em>et al.</em> (2009)</td>
</tr>
<tr>
<td>OppA</td>
<td>Oligopeptide permease</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>Borozee <em>et al.</em> (2000)</td>
</tr>
<tr>
<td>OpuC, Gbu</td>
<td>Osmolyte transporter</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Angelidis and Smith (2003a, 2003b)</td>
</tr>
<tr>
<td>ProBA</td>
<td>Proline synthesis enzyme system</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sleator <em>et al.</em> (2001)</td>
</tr>
<tr>
<td>GAD</td>
<td>Glutamate decarboxylase system</td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td>Cotter <em>et al.</em> (2001)</td>
</tr>
<tr>
<td>CspS</td>
<td>Cold shock domain proteins</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td>Loepe <em>et al.</em> (2010); Schmid <em>et al.</em> (2009)</td>
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<tr>
<td>Csps</td>
<td>RNA chaperone-</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>Christiansen <em>et al.</em> (2004)</td>
</tr>
<tr>
<td>Ctc</td>
<td>50S ribosomal protein L25</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td>Gardan <em>et al.</em> (2003b)</td>
</tr>
<tr>
<td>ClpB, ClpC, ClpE, ClpP</td>
<td>ATP dependent Clp chaperone</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td>Nair <em>et al.</em> (2000)</td>
</tr>
<tr>
<td>Fri</td>
<td>DNA binding protein of starved cells (Dps)</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Dussurget <em>et al.</em> (2005)</td>
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<tr>
<td>Catalase</td>
<td>Catalase</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Azizoglu and Kathariou (2010b)</td>
</tr>
<tr>
<td>SOD</td>
<td>Superoxide dismutase</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Archambaud <em>et al.</em> (2006)</td>
</tr>
<tr>
<td>ADI</td>
<td>Arginine deiminase system</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>Ryan <em>et al.</em> (2009)</td>
</tr>
<tr>
<td>Lmo0038</td>
<td>Putative argmatine deiminase</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>Chen <em>et al.</em> (2009)</td>
</tr>
<tr>
<td>Lmo1078</td>
<td>Putative UDP glucose synthetase</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>Chassaing and Auvray (2007)</td>
</tr>
<tr>
<td>Bdk system</td>
<td>Branched chain a-keto acid dehydrogenase enzyme complex</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Zhu <em>et al.</em> (2005)</td>
</tr>
<tr>
<td>F$<em>{0}$/F$</em>{1}$-ATPase</td>
<td>Proton efflux membrane ATPase</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>Cotter <em>et al.</em> (2000)</td>
</tr>
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</table>

+ = Gene confirmed to have significant functional contributions under these stress conditions.
Heat Stress Adaptation Responses of *L. monocytogenes*

Proteins exposed to elevated heat stress are denatured, leading to aggregate formation and loss of function, including enzyme activity. Organisms adapting to heat stress must mobilize cellular mechanisms to restore membranes and nucleic acids functions, remove heat degraded proteins, and produce new proteins to restore metabolic functions lost through thermal stress destruction of proteins. Genomewide transcriptome analysis has revealed that heat stress responses in *L. monocytogenes* involve the recruitment of various genes with roles in heat shock and SOS responses, as well as cell division and cell wall synthesis (Hu et al., 2007a, 2007b; van der Veen et al., 2007). This heat shock response is associated with increased production of a specific set of proteins, the heat shock proteins (Hsps), which includes highly conserved chaperones and ATP-dependent proteases, involved in damaged protein refolding and degradation. *L. monocytogenes* contains two heat shock response protein groups: the class I and III proteins that are negatively controlled through HrcA and CsrR transcription repression mechanisms, and the σ^B^ controlled proteins, which include the class II stress response proteins, as well as class IV proteins with roles in general stress adaptation functions (Hu et al., 2007a, 2007b). The HrcA repressor regulon includes chaperonin proteins GroES, GroEL, and DnaK. The activation of these proteins at elevated temperature provides cellular protection by assisting in protein folding and assembling (Hu et al., 2007a). Further, the DnaK operon also includes the DnaJ and GrpE proteins, which are proposed to be important for stimulating the ATPase activity of DnaK (Liber et al., 1991). CsrR repressor-controlled ATP-dependent proteases are ClpP, ClpE, and ClpC, which degrade damaged or misfolded proteins, and ClpB, which reactivates the protein aggregates (Hu et al., 2007b). A ctsR-deleted mutant is more tolerant to heat stress consistent with CtsR in transcriptional repression of class III stress response gene expression (Hu et al., 2007a). Table 1 and Figure 1B include various protein systems so far shown to contribute to heat stress adaptation responses of *L. monocytogenes*. These include genes of two component signal transduction systems (hisR and kdpE) as well as various negative (hrcA, ctsR, and mogR) and positive (σ^B^ and σ^C^) transcriptional regulators (Kallipolitis and Ingmer, 2001; Zhang et al., 2005; Hu et al., 2007a, 2007b; van der Veen et al., 2009).

Proteome analysis of heat stress responses in *L. monocytogenes* cells revealed increased production of GroEL and DnaK, which is consistent with the role of these highly conserved Hsps, in removal of altered proteins in *L. monocytogenes* cells exposed to heat stress (Sokolovic et al., 1990; Morange et al., 1993). Another heat stress response proteome study identified the general stress response protein Ferritin (also referred as ferritin like proteins, Flp) to be the predominant heat stress-induced protein in this bacterium (Hebraud and Guzzo, 2000). The fri gene transcripts were found to be heat stress inducible, whereas fri-gene-deleted *L. monocytogenes* cells had increased heat stress sensitivity (Hebraud and Guzzo, 2000; Dussurget et al., 2005). Agoston et al. (2009) compared proteomes associated with mild and prolonged heat treatments on *L. monocytogenes* and discovered that a large number of metabolic proteins are suppressed by heat stress exposure. In addition, they also found that the Hsp DnaN, which is a beta subunit of DNA polymerase III, was highly induced in response to different heat shock treatments. Induction of DnaN as observed in this study thus suggests that *L. monocytogenes* also counteracts heat stress challenges by modulation of DNA replication processes apart from the elevated activity of protein folding and degradation mechanisms. Heat stress exposure also induces recruitment of SOS response and DNA repair genes, as well as PrfA-controlled virulence genes, while repressing the cell division and cell wall synthesis genes in line with suppressed cell division and cellular elongation observed under heat stress (van der Veen et al., 2007).

Osmotic Stress Responses of *L. monocytogenes*

The high level of sugars and sodium salts typically used as food preservers threaten bacterial cells with dehydration, which leads to increased intracellular solute concentrations and disruption of various biological functions. Proteome analysis has been used to study osmotolerance responses in *L. monocytogenes*. Eleven osmotic stress acclimation proteins in this bacterium were identified, which include the GbuA, AppA, Ctc, DnaK, HtrA, and OpuC proteins (Duche et al.,...
Specific osmotic stress adaptation protein systems currently known in this bacterium are listed in Table 1 and Figure 1C.

The accumulation of osmolytes constitutes one of the main osmotic stress adaptation strategies in *L. monocytogenes*. The system, the Kdp-ATPase system, has been associated with osmotic stress protection roles in *L. monocytogenes* cells. The absence of the KdpE sensor and its regulator KdpD is asso-

**FIG. 1.** Graphical presentations depicting stress-affected cellular components and a selection of molecular stress adaptation responses documented in *Listeria monocytogenes* cells exposed to (A) cold stress, (B) heat stress, (C) osmotic stress, and (D) acid stress. Alterations are caused by stress exposure on cell envelope, nucleic acids (RNA and DNA), proteins, and metabolism. Such molecular alterations are presumably detected by different stress-sensing molecular systems involving cell envelope components, nucleic acids, and proteins leading to signal transduction events and subsequent activation of adaptive gene expression response regulators. Gene expression modulation leads to activation stress protection functions through increased protein synthesis and/or activity modulation.
associated with impaired \textit{L. monocytogenes} growth under NaCl osmotic stress (Kallipolitis and Ingmer, 2001). Proline is also another osmolyte shown to be exploited for osmoprotection in \textit{L. monocytogenes}. The disruption of the proBA locus encoding the ProA and ProB proteins was found to increase osmotic stress sensitivity of \textit{L. monocytogenes} cells, indicating that proline biosynthesis genes may also be crucial in osmotic stress adaptation of this bacterium (Sleator \textit{et al.}, 2001).

Single-gene deletions targeting \textit{clpC}, \textit{clpP}, and \textit{htrA} genes are all also associated with defective cellular growth under NaCl osmotic stress tolerance in this bacterium (Wonderling \textit{et al.}, 2004; van der Veen \textit{et al.}, 2007). These observations thus suggest that these protein chaperones are also vital in resolution of protein damages and restoration of cellular functions that get impaired by osmotic stress. An \textit{lmo1078}-gene-deleted \textit{L. monocytogenes} EGD-e strain was described, which is also defective in growth under NaCl osmotic stress (Chassaing and Auvray, 2007). \textit{Lmo1078} is a putative UDP-glucose phosphorylase. This protein has been proposed to facilitate cell wall and membrane lipid composition maintenance, and its functions seem important during osmotic and cold stress adaptation of \textit{L. monocytogenes} cells.

\textbf{FIG. 1. (Continued).}
Acid Stress Adaptation Responses of L. monocytogenes

The acid stress challenges faced by L. monocytogenes cells include organic acids used as food preservatives and contaminants as well as inorganic acids encountered within the gastrointestinal tracts of human hosts. The antibacterial mechanisms against organic acid stress are generally presumed to depend on their dissociation in the bacterial cell cytoplasm, leading to acidification as well as proton and anion influx. Proton influx inhibits the cellular ATP synthesis and catabolite transport capacities, whereas the acidification and ionic influx into the cytoplasm disrupts metabolic functions as well as induce damage to proteins, nucleic acids, and cell membranes. Table 1 and Figure 1D list various acid stress response protein systems confirmed in this bacterium.

Bowman et al. (2010) recently examined transcriptome responses to organic acid stress in L. monocytogenes. They found that organic acid salt stress exposure (21 mM sodium diacetate at pH 5.0) was associated with a broad range of gene expression changes, including an increased activation of σ8, PrfA, HrcA, and CtsR targeted regulons, as well as oxidative stress defenses, DNA repair, intermediary metabolism, cell-wall modification, and cofactor and fatty acid biosynthetic genes. A proteome analysis of L. monocytogenes ScottA cells exposed to food preservation organic acid salts also revealed an increased production of oxidoreductases and lipoproteins, and reduced expression of DNA-binding proteins, alpha amylase, and SecA (Mbandi et al., 2007). The repression of DNA-binding proteins (involved in transcriptional regulation), alpha amylase (involved in metabolizing complex carbon sources in readily available energy sources), and SecA (involved in translocation of proteins) may therefore be interpreted as downregulation of cell metabolic activity caused by organic acid stress exposure of L. monocytogenes cells.

Other acid stress response proteomes induced mainly by exposure to hydrochloric acid have been determined in L. monocytogenes (Wemekamp-Kamphuis et al., 2004; Phan-Thanh and Jansch, 2006). The identified acid stress proteins indicate that proteins involved in respiration (enzyme dehydrogenases and reductases), osmolyte transport, protein folding and repair, general stress resistance, flagella synthesis, and metabolism are all recruited as part of acid stress responses in L. monocytogenes cells. Cellular proton permeability involves altering the composition of the membrane lipid bilayer. Along these lines, increased straight chain fatty acid concentrations and decreased levels of branched chain fatty acids were observed in cell membranes of L. monocytogenes cells exposed to acid stress (Giotis et al., 2007). Accelerated electron transfer through enhanced oxidation-reduction potential might be another mechanism employed by bacteria that are exposed to acid stress to dispel protons. The induction of dehydrogenases (GuaB, PduQ, and Lmo0560), reductases (YcgT), and respiratory enzymes associated with acid stress responses in L. monocytogenes cells may therefore also activate acid stress adaptive mechanisms, which involve active cellular proton efflux (Phan-Thanh and Jansch, 2006). Along the same lines, the F0F1 ATPase also plays an important role during L. monocytogenes acid stress adaptation, where it is thought to contribute to proton efflux events (Cotter et al., 2000). The glutamate decarboxylase (GAD) acid resistance system serves important acid stress adaptation roles in this bacterium (Cotter et al., 2001; Wemekamp-Kamphuis et al., 2004). The L. monocytogenes arginine deiminase (ADI) system includes enzymes encoded by arcA, arcB, and arcC, which are involved in conversion of arginine to ornithine, as well as the antiporter arcD, which transfers ornithine outside the cell in exchange for arginine (Ryan et al., 2009). An ArcR and ArcA deletion mutant showed impaired survival under mild (pH 3.5) and lethal (pH 4.8) acid stress conditions, respectively. The lmo0038, encoding a putative peptidylarginase deiminase family gene, has also been suggested to have acid stress adaptation functions in this bacterium (Chen et al., 2009). The TCS LisRK has been linked to acid stress response modulation probably through its role in sensing of acid stress-related environmental stimuli (Cotter et al., 1999; Sleator and Hill, 2005). In addition, L. monocytogenes cells lacking HtrA are acid stress sensitive consistent with the requirement of HtrA protease functions in removal of acid stress-damaged proteins (Wonderling et al., 2004).

L. monocytogenes Response to Alkaline Stress

Environmental L. monocytogenes strains inhabiting food-processing environments may frequently encounter sublethal alkaline stress associated with detergents and sanitizing agents applied as food hygiene measures. The adaptive strategies deployed in microorganisms during alkaline stress adaptation include metabolic changes to increase acid generation, as well as the induction of transporters and enzymes involved in proton retention, and cell surface modifications that promote cytoplasmic proton retention. Transcriptome analysis has revealed that as much as 390 gene transcripts in L. monocytogenes cells are differentially expressed in response to alkaline stress exposure. Genes mobilized for alkaline stress adaptation participate in general stress responses, solute transport, and various metabolic pathways (Giotis et al., 2010). In another study the proteome analysis of L. monocytogenes cells exposed to alkaline stress revealed the synthesis or over expression of a limited number of proteins as compared to the repressed expression of proportionally large number of proteins (Giotis et al., 2008b). Meanwhile, the stress protective chaperones DnaK and GroEL were also induced by alkaline stress exposure. By screening of a library of Tn917 lac insertional mutants in L. monocytogenes LO28, Gardan et al. (2003a) identified 12 mutant strains that were sensitive to alkaline stress, and these mutants were disrupted in genes encoding putative transporter proteins. At present there is, however, still less known about specific alkaline stress adaptation protein systems in this bacterium.

Oxidative Stress Adaptation Responses

L. monocytogenes cells must combat oxidative stress encountered in external environments and during host infection. In food environments, oxidative stress might develop due to atmospheric modification as well as by chemical reagents applied as detergents and disinfectants. Reactive oxygen species (ROS) are also produced as byproducts of metabolism and can accumulate due to respiratory chain impairment or metabolic alterations encountered in other stress situations (Bowman et al., 2010; Cacace et al., 2010). Oxidative stress is deleterious to various molecular processes and cellular components such as membranes, proteins, nucleic acids, and enzymes. To cope against oxidative stress, molecular
detoxification of ROS, as well as protein, membrane, and nucleic acid damage repair mechanisms must be activated.

Gorski et al. (2008) found that best colonizing *L. monocytogenes* strains in oxidative food environments were those with an enhanced ability to tolerate oxidative stress exposure. Bacterial ROS detoxification systems include superoxide dismutase, catalase (Kat), and alkyl hydroperoxide (AhpCF) enzyme systems (Helmann et al., 2003). The Kat and superoxide dismutase have been shown to be important in oxidative stress protection of *L. monocytogenes* cells. The sod and kat null *L. monocytogenes* strains display poor aerobic growth phenotypes as well as increased oxidative stress sensitivity, poor macrophage survival, and virulence (Archambaud et al., 2006; Azzizoglu and Kathariou, 2010b). The Dps ferritin has also been shown to be important in oxidative stress protection of *L. monocytogenes*. The cells of this bacterium that are deleted in the ferritin protein encoding gene *fri* exhibit increased sensitivity to oxidative stress and poor macrophage infectivity (Dussurget et al., 2005). A ferric transcriptional regulator *per* deleted *L. monocytogenes* mutant was reported to exhibit increased sensitivity to hydrogen peroxide and poor aerobic growth (Rea et al., 2005). In addition, Per regulon determination in this study unveiled Per-dependent repression of numerous ROS detoxification genes, including the *kat*, *trxB*, *fur*, *fri*, and *hemA* genes. The increased peroxide sensitivity in *per* mutant cells was suggested to be a potential consequence of increased expression of some ROS defense genes that are otherwise normally controlled through Per repression.

### Role of σ Factors in *L. monocytogenes* Stress Adaptation

Proteins encoded and governed by global general stress response regulator σ factors play vital roles in protecting *L. monocytogenes* cells against various stress stimuli. σB is a positive regulator of several general and cold stress response genes, including *fri*, *opxA*, *opuCA*, and *lrrC* genes, with genetically confirmed cold stress adaptation roles in this bacterium (Chan et al., 2007). While recently defective cold growth phenotypes were also documented in *L. monocytogenes* mutant strains lacking genes for the σC, σA1, and σL (RpoN) alternative sigma factors, indicating that besides σB, these alternative sigma factors are also involved in regulation of cold adaptation processes in this bacterium (Chan et al., 2008; Raimann et al., 2009). The *L. monocytogenes* class II Hsps are positively controlled by σB, and increased heat stress sensitivity in a σB deletion mutant is consistent with σB-dependent transcriptional activation of the class II stress response genes in this bacterium (van der Veen et al., 2007). Apart from σB, the loss of σC function also increases *L. monocytogenes* sensitivity to a thermal treatment (Zhang et al., 2005).

Both σB and σC in *L. monocytogenes* are transcriptionally upregulated in response to elevated NaCl concentrations, whereas σA1- and σL-deleted mutants of this bacterium are NaCl salt-stress sensitive (Okada et al., 2008; Raimann et al., 2009). Based on transcriptomic profiling, σB-regulated genes in osmotic stress adaptation are associated with general stress responses, transcriptional regulation, cell transport, envelope modification, metabolism, protein synthesis and modification, as well as virulence-associated functions (Raengpradub et al., 2008). Genes positively controlled through σB also include osmolyte transporter genes (*opuCA* and *gbuA*) as well as *clpC* and *lfg* genes, which have all been genetically associated with osmotic stress adaptation (Christiansen et al., 2004; Abram et al., 2008; Raengpradub et al., 2008). The general stress response protein Ctc is vital in NaCl salt stress tolerance and the *ctc* gene in *L. monocytogenes* is preceded by a putative σB-dependent promoter (Gardan et al., 2003b).

Both σB and σC have also been functionally linked to regulation of the acid stress adaptation responses in this bacterium. *L. monocytogenes* cells deleted on σB and its regulators *rsBT* and *rsBV*, as well as σA1, have been found to display acid stress sensitive phenotypes (Chaturongakul and Boor, 2004; Wemekamp-Kamphuis et al., 2004; Raimann et al., 2009). σB is also a positive regulator of genes of the GAD and ADI acid stress response systems and its deletion diminishes expression of GAD and ADI genes (Cotter et al., 2001; Ryan et al., 2009). σB regulons in this bacterium have also been shown to include several oxidative stress response genes and σB-deleted mutant strains are significantly impaired in oxidative stress tolerance (Ferreira et al., 2001; Hain et al., 2008; Oliver et al., 2010). It has been recently described that the σB contribution to oxidative stress might also depend on strain genotype. Oliver et al. (2010) found that although σB loss induced oxidative stress sensitivity in strains of lineage I, II, and III B, this was not the case in a lineage IIIA strain. Such differences between the *L. monocytogenes* genomic lineages lead to a conclusion that such strain-specific differences might therefore also influence σB-dependent oxidative stress response gene regulation, in different genomic lineages of this bacterium.

### Cross Protective Stress Responses

It has been shown through several studies that the exposure of *L. monocytogenes* to sublethal stress induces the development of stress-conditioned organisms, which are physiologically more tolerant to increased levels of the same or different stresses. For example, *L. monocytogenes* cells exposed to sublethal acid stress display increased resistance to higher acid stress levels as well as become more tolerant to heat and osmotic stress (Gahan et al., 1996). Acid- and cold-adapted cells of this bacterium are more protected from the effects of high hydrostatic pressure stress (Wemekamp-Kamphuis et al., 2002). *L. monocytogenes* cells preadapted by exposure to sublethal heat stress show enhanced osmotic and ethanol stress protection (Lou and Yousef, 1997). Taormina and Beuchat (2002) showed that alkaline stress-adapted *L. monocytogenes* cross-contaminating food products are more resistant to thermal food safety measures in comparison to those not previously exposed to sublethal alkaline stress conditions.

To date, investigators have identified various molecular mechanisms that appear to be crucial in mediation of cross protective stress responses in this bacterium. For instance, molecular adaptive challenge envisaged in cold environments, particularly freezing, include intracellular accumulation of ROS due to cold stress-induced metabolic alteration. A catalase-deficient *L. monocytogenes* F2365 mutant was recently described, which is impaired during cold growth (Azzizoglu and Kathariou, 2010b). In addition, mutants lacking some *csp* genes are also characterized by increased peroxide stress sensitivity and reduced host cell infectivity (Loepfe et al., 2010). Moreover, *L. monocytogenes* mechanisms to combat osmotic stress seem to also involve functions of the cold shock domain family proteins. A diminished osmotic stress...
tolerance phenotype is induced by cspD deletion in this bacterium (Schmidt et al., 2009). A csr null mutant was described vital to osmotic stress adaptation responses of L. monocytogenes cells, indicating that some CsrR-repressed heat stress proteins also contribute toward osmotic stress (Nair et al., 2000). Another heat stress repressor protein, HtrA, is also presumed to degrade misfolded or aggregated proteins that accumulate when L. monocytogenes cells are exposed to harsh environmental conditions such as high osmolality and low pH in addition to high temperatures (Wilson et al., 2006; van der Veen et al., 2007). Giotis et al. (2008b) reported induced expression DnaK and GroEL chaperones by alkaline stress and as such these proteins have very well-defined role in heat stress adaptation. Thus, based on above examples it is evident that a large number of proteins and regulatory mechanisms are involved in L. monocytogenes cross protective responses.

Conclusion and Future Perspectives

The development of more effective food preservation methods depend on an improved understanding of fundamental changes that are instituted at the gene expression level in cells of this bacterium when challenged with adverse environmental stress conditions (Tasara and Stephan, 2006). Although considerable progress has been made so far in understanding the L. monocytogenes stress response, one major drawback is that the majority of current information is based on broth models and not with actual food substrates. We therefore propose that more efforts should be invested in understanding the cellular stress response of L. monocytogenes at the molecular level in the presence of different food substrates and varying environmental conditions. It is hoped that with this further knowledge, the complexity and hierarchical nature of stress adaptation response mechanisms can be understood better, paving the way to the development of novel strategies that are more effective in combating the stress resistance properties of L. monocytogenes. Such future breakthroughs may include (1) novel ways to block the expression of transporter proteins that govern cold/osmotic stress adaptation to prevent the uptake of osmolyte molecules from food substrates to make L. monocytogenes cells susceptible to both cold and osmotic stress stimuli, and (2) identifying the antimicrobial compounds that suppress the induction of stress proteins of L. monocytogenes in food products.

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References


Azizoglu RO and Kathariou S. Inactivation of a cold-induced putative rna helicase gene of Listeria monocytogenes is accompanied by failure to grow at low temperatures but does not affect freeze-thaw tolerance. J Food Prot 2010a;73:1474–1479.


Chaturongakul S and Boor KJ. RsbT and RsbV contribute to sigmaB-dependent survival under environmental, energy, and intracellular stress conditions in Listeria monocytogenes. Appl Environ Microbiol 2004;70:5349–5356.


Cotter PD, Emerson N, Gahan CG, and Hill C. Identification and disruption of lisRK, a genetic locus encoding a two-component signal transduction system involved in stress tolerance and


Ferreira A, O’Byrne CP, and Boor KJ. Role of sigma(B) in heat, ethanol, acid, and oxidative stress resistance and during carbon starvation in Listeria monocytogenes. Appl Environ Microbiol 2001;67:4454–4457.


Oliver HF, Orsi RH, Wiedmann M, and Boor KJ. Listeria monocytogenes [sigma]B has a small core regulon and a conserved
role in virulence but makes differential contributions to stress
tolerance across a diverse collection of strains. Appl Environ
Phan-Thanh L and Jansch L. Elucidation of mechanisms of acid
stress in Listeria monocytogenes by proteomic analysis. Methods
Raengpradub S, Wiedmann M, and Boor KJ. Comparative
analysis of the sigma B-dependent stress responses in Listeria
monocytogenes and Listeria innocua strains exposed to selected
Raimann E, Schmid B, Stephan R, and Tasara T. The alternative
sigma factor sigma(L) of L. monocytogenes promotes growth
under diverse environmental stresses. Foodborne Pathog Dis
Rea R, Hill C, and Gahan CG. Listeria monocytogenes PerR mu-
tants display a small-colony phenotype, increased sensitivity
to hydrogen peroxide, and significantly reduced murine vir-
Rea RB, Gahan CG, and Hill C. Disruption of putative regulatory
loci in Listeria monocytogenes demonstrates a significant role for
Ryan S, Begley M, Gahan CG, and Hill C. Molecular character-
ization of the arginine deiminase system in Listeria mono-
cytogenes: regulation and role in acid tolerance. Environ
Schmid B, Klumpp J, Raimann E, Loesner MJ, Stephan R, and
Tasara T. Role of cold shock proteins in growth of Listeria mono-
cytogenes under cold and osmotic stress conditions. Appl
Sleator RD, Gahan CG, and Hill C. Identification and disruption
of the proBA locus in Listeria monocytogenes: role of proline
biosynthesis in salt tolerance and murine infection. Appl Environ
Microbiol 2001;67:2571–2577.
Sleator RD and Hill C. A novel role for the LisRK two-compo-
nent regulatory system in Listerial osmotolerance. Clin Mi-
Sokolovic Z, Fuchs A, and Goebel W. Synthesis of species-spe-
cific stress proteins by virulent strains of Listeria mono-
Taormina PJ and Beuchat LR. Survival of Listeria monocytogenes
in commercial food-processing equipment cleaning solutions
and subsequent sensitivity to sanitizers and heat. J Appl Mi-
Tasara T and Stephan R. Cold stress tolerance of Listeria mono-
cytogenes: a review of molecular adaptive mechanisms and
vander Veen S, Abe T, de Vos WM, and Wells-Bennik MH.
Genome-wide screen for Listeria monocytogenes genes impor-
tant for growth at high temperatures. FEMS Microbiol Lett
vander Veen S, Hain T, Wouters JA, Hosain H, de Vos WM,
Abe T, Chakraborty T, and Wells-Bennik MH. The heat-
shock response of Listeria monocytogenes comprises genes in-
volved in heat shock, cell division, cell wall synthesis, and the
Wemekamp-Kamphuis HH, Karatzas AK, Wouters JA, and
Abe T. Enhanced levels of cold shock proteins in Listeria
monocytogenes LO28 upon exposure to low temperature and
high hydrostatic pressure. Appl Environ Microbiol 2002;68:
456–463.
Wemekamp-Kamphuis HH, Wouters JA, de Leeuw PP, Hain T,
Chakraborty T, and Abe T. Identification of sigma factor sigma B-controlled genes and their impact on acid stress,
high hydrostatic pressure, and freeze survival in Listeria
3466.
SA, King DS, Jones KF, and Hruby DE. Listeria monocytogenes
HtrA is necessary for resistance to cellular stress and
Wonderling LD, Wilkinson BJ, and Bayles DO. The htrA (degP)
gene of Listeria monocytogenes is essential for optimal
growth under stress conditions. Appl Environ Microbiol
Zhang C, Nietfeldt J, Zhang M, and Benson AK. Functional
consequences of genome evolution in Listeria monocytogenes:
the lmo0423 and lmo0422 genes encode sigmaC and LstR, a
7243–7253.
Zhu K, Bayles DO, Xiong A, Jayaswal RK, and Wilkinson BJ.
Precursor and temperature modulation of fatty acid composi-
tion and growth of Listeria monocytogenes cold-sensitive
mutants with transposon-interrupted branched-chain alpha-

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