

Deciphering the global roles of cold shock proteins in *Listeria monocytogenes* nutrient metabolism, stress tolerance and virulence

Background:

L. monocytogenes has been identified as a significant food safety and public health concern. It has evolved an extensive array of mechanisms for coping with stress and adapting to changing environmental conditions, ensuring its virulence phenotype expression. Among these adaptation systems are cold shock proteins (Csps), low-affinity RNA or DNA-binding proteins, which facilitate rapid response to stress exposure. *L. monocytogenes* has three highly conserved *csp* genes, namely, *cspA*, *cspB*, and *cspD*.

WP1: *Listeria monocytogenes* cold shock proteins (CSP's): small proteins with a huge impact - a review

<https://www.mdpi.com/2076-2607/9/5/1061>

WP2: Roles of cold shock proteins in *Listeria monocytogenes* nutrient metabolism, stress tolerance and virulence

Role of cold shock proteins in growth of *Listeria monocytogenes* under cold and osmotic stress conditions

<https://pubmed.ncbi.nlm.nih.gov/19151183/>

Insight: CSP's are required for efficient cold and osmotic stress tolerance of *L. monocytogenes*. The hierarchies of their functional importance differ, depending on the environmental stress conditions: CspA>CspD>CspB in response to cold stress versus CspD>CspA/CspB in response to NaCl salt osmotic stress. The fact that Csps are promoting *L. monocytogenes* adaptation against both cold and NaCl stress has significant implications in view of practical food microbial control measures. The combined or sequential exposure of *L. monocytogenes* cells to these two stresses in food environments might inadvertently induce cross-protection responses.

Reduced host cell invasiveness and oxidative stress tolerance in double and triple *csp* gene family deletion mutants of *Listeria monocytogenes*

<https://pubmed.ncbi.nlm.nih.gov/20184451/>

Insight: The data of this study indicate that besides cold and osmotic stress adaptation roles, Csp family proteins also promote efficient host cell invasion and oxidative stress adaptation processes in *L. monocytogenes*.

Cold shock proteins contribute to the regulation of listeriolysin O production in *Listeria monocytogenes*

<https://pubmed.ncbi.nlm.nih.gov/23952475/>

Insight: Overall, these results indicate that Csps, in particular CspB, are important components of gene expression regulatory mechanisms that promote efficient pore-forming cytolysin listeriolysin (LLO) production and hence virulence responses of *L. monocytogenes*.

Cold-shock proteins affect desiccation tolerance, biofilm formation and motility in *Listeria monocytogenes*

<https://pubmed.ncbi.nlm.nih.gov/32505890/>

Insight: The role of Csps in desiccation tolerance and biofilm formation was investigated in *csp* mutants as well as twenty-one other wild-type (WT) strains. Mutants with a single ($\Delta cspA$) or multiple ($\Delta cspAB$, $\Delta cspAD$ and $\Delta cspABD$) deletions of *csp* genes, in a desiccation sensitive WT background (*L. monocytogenes* EGD-e) were immotile and exhibited an elevated desiccation tolerance compared to the parent strain.

Cold-Shock Domain Family Proteins (Csps) Are Involved in Regulation of Virulence, Cellular Aggregation, and Flagella-Based Motility in *Listeria monocytogenes*

<https://www.frontiersin.org/articles/10.3389/fcimb.2017.00453/full>

Insight: A *L. monocytogenes* mutant deleted in all three *csp* genes ($\Delta cspABD$) is attenuated with respect to human macrophage infection as well as virulence in a zebrafish infection model. Moreover, this mutant is incapable of aggregation and fails to express surface flagella or exhibit swarming motility. An evaluation of double *csp* gene deletion mutant ($\Delta cspBD$, $\Delta cspAD$ and $\Delta cspAB$) strains that produce single *csp* genes showed that there is redundancy as well as functional differences among the three *L. monocytogenes* Csps in their contributions to virulence, cellular aggregation, flagella production, and swarming motility.

Cold Shock Proteins Promote Nisin Tolerance in *Listeria monocytogenes* Through Modulation of Cell Envelope Modification Responses

<https://www.frontiersin.org/articles/10.3389/fmicb.2021.811939/full>

Insight: Without *csp* genes, a *L. monocytogenes* $\Delta cspABD$ mutant displayed severely compromised growth under nisin stress. Characterizing single ($\Delta cspA$, $\Delta cspB$, and $\Delta cspD$) and double ($\Delta cspBD$, $\Delta cspAD$, and $\Delta cspAB$) *csp* gene deletion mutants revealed a hierarchy ($cspD > cspB > cspA$) of importance in *csp* gene contributions toward the *L. monocytogenes* nisin tolerance phenotype. Individual eliminations of either *cspA* or *cspB* improved the nisin stress tolerance phenotype, suggesting that their expression has a curbing effect on the expression of nisin resistance functions through CspD. Gene expression analysis revealed that Csp deficiency altered the expression of DltA, MprF, and penicillin-binding protein-encoding genes. Furthermore, the $\Delta cspABD$ mutation induced an overall more electronegative cell surface, enhancing sensitivity to nisin and other cationic antimicrobials as well as the quaternary ammonium compound disinfectant benzalkonium chloride.

Deciphering the Global Roles of Cold Shock Proteins in *Listeria monocytogenes* Nutrient Metabolism and Stress Tolerance

<https://www.frontiersin.org/articles/10.3389/fmicb.2022.1057754/full>

Insight: Phenotype microarrays (PM) analysis showed that the absence of Csps in $\Delta cspABD$ reduces carbon (C-) source utilization capacity and increases *Lm* sensitivity to osmotic, pH, various chemical, and antimicrobial stress conditions. Single and double *csp* deletion mutants in different *Lm* genetic backgrounds were used to further dissect the roles of individual Csps in these phenotypes. Selected PM-based observations were further corroborated through targeted phenotypic assays, confirming that Csps are crucial in *Lm* for optimal utilization of various C-sources including rhamnose and glucose as well as tolerance against NaCl, β -phenylethylamine (PEA), and food relevant detergent stress conditions. Strain and genetic lineage background-based differences, divisions of labour, epistasis, and functional redundancies among the Csps were uncovered with respect to their roles in various processes including C-source utilization, cold, and PEA stress resistance. Finally, targeted transcriptome analysis was performed, revealing the activation of *csp* gene expression under defined stress conditions and the impact of Csps on expression regulation of selected rhamnose utilization genes. Overall, our study shows that Csps play important roles in nutrient utilization and stress responses in *Lm* strains, contributing to traits that are central to the public health and food safety impacts of this pathogen.