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Oxygen-dependent effects of L-Cysteine and SigB on thermal tolerance of *Listeria monocytogenes* 10403S

Irem Soyler[1*], Mahide Muge Yilmaz Topcam[2,3], Kimon-Andreas Karatzas[4]

[1]University of Surrey, Department of Chemical and Process Engineering, Guildford, UK
[2]Akdeniz University, Department of Food Engineering, Antalya, Türkiye
[3]Royal Veterinary College, Department of Pathobiology and Population Sciences, London, UK
[4]University of Reading, Department of Food and Nutritional Sciences, Reading, UK

*Corresponding Author: Irem Soyler
E-mail: i.soyler@surrey.ac.uk
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Abstract: *Listeria monocytogenes* is a resilient foodborne pathogen capable of surviving diverse environmental stresses, including heat, oxidative, and osmotic conditions. The alternative sigma factor SigB plays a central role in mediating stress adaptation. However, its function under oxygen-limited conditions and in nutrient-rich environments remains insufficiently understood. L-cysteine, commonly present in food matrices, may influence bacterial stress tolerance by acting as both a metabolic signal and a precursor for antioxidant systems. This study investigated the effect of extracellular L-cysteine supplementation on the heat resistance of *L. monocytogenes* and evaluated the contribution of SigB under both aerobic and anaerobic conditions. Wild-type *L. monocytogenes* 10403S and an isogenic Δ*sigB* mutant were subjected to heat stress in defined medium supplemented with 1.57 mM L-cysteine. Bacterial survival was quantified and compared across strains and environmental conditions. L-cysteine supplementation significantly enhanced bacterial survival under anaerobic heat stress. Notably, the Δ*sigB* mutant exhibited greater resistance than the wild-type strain under these conditions. This observation suggests that L-cysteine-associated metabolic pathways may compensate, at least partially, for the absence of SigB-mediated stress regulation. The enhanced survival in the mutant strain points to alternative protective mechanisms, potentially linked to redox balance or sulphur metabolism. Overall, the findings demonstrate that L-cysteine availability, oxygen conditions, and SigB interact in a complex and context-dependent manner to influence heat stress survival in *L. monocytogenes*. These results highlight the importance of metabolic state in shaping bacterial stress responses and suggest that sulphur metabolism may serve as a key compensatory pathway under oxygen-limited conditions. A deeper understanding of these interactions could support the development of more effective strategies for controlling *L. monocytogenes* in food systems and processing environments.
Keywords: *Listeria monocytogenes*, heat stress resistance, SigB (σ^B), L-cysteine, anaerobic conditions.

L-Sistein ve SigB'nin *Listeria monocytogenes* 10403S'nin termal toleransı üzerindeki oksijene bağlı etkileri

Özet: *Listeria monocytogenes*, ısı, oksidatif ve ozmotik stresler dahil olmak üzere çok çeşitli çevresel koşullara dayanabilen önemli bir gıda kaynaklı patojendir. Alternatif sigma faktörü SigB, bakterinin stres adaptasyonunda merkezi bir rol oynamaktadır. Ancak oksijenin sınırlı olduğu koşullar ve besin açısından zengin ortamlardaki işlevi henüz tam olarak aydınlatılamamıştır. Et ve et ürünleri, süt ürünleri ve baklagillerinde yaygın olarak bulunan kükürt içeren bir amino asit olan L-sistein, hem metabolik bir sinyal molekülü hem de hücresel antioksidan sistemlerin öncüsü olarak stres toleransını etkileyebileceği önceki çalışmalarda gösterilmiştir. Bu çalışmada, hücre dışı L-sistein takviyesinin *L. monocytogenes*'in ısı direnci üzerindeki etkisi incelenmiş ve aerobik ile anaerobik büyüme koşulları altında SigB'nin rolü değerlendirilmiştir. Yabanıl tip *L. monocytogenes* 10403S suşu ile izojenik Δ*sigB* mutantı, 1,57 mM L-sistein ile takviye edilmiş tanımlı besiyerinde, kontrollü aerobik ve anaerobik koşullar altında 56°C'de ısı stresine maruz bırakılmıştır. Bakteriyel hayatta kalma oranı kantitatif olarak belirlenmiş ve elde edilen veriler farklı suşlar ve çevresel koşullar arasında karşılaştırmalı olarak analiz edilmiştir. Elde edilen bulgular, L-sistein takviyesinin özellikle anaerobik koşullarda uygulanan ısı stresi altında bakteriyel hayatta kalmayı anlamlı düzeyde artırdığını göstermiştir. Ayrıca, Δ*sigB* mutantının bu koşullarda yabanıl tip suşa kıyasla ısıl strese karşı daha yüksek direnç sergilediği belirlenmiştir. Bu sonuçlar, L-sistein metabolizması veya ilişkili biyokimyasal yolakların, SigB aracılı stres yanıtının yokluğunu kısmen telafi edebileceğini düşündürmektedir. Sonuç olarak, L-sistein mevcudiyeti, oksijen düzeyi ve SigB faktörü, *L. monocytogenes*'in ısı stresine karşı hayatta kalmasını bağlama bağlı olarak birlikte şekillendirmektedir. Bu etkileşimlerin daha iyi anlaşılması, gıda ürünleri ve işleme ortamlarında *L. monocytogenes*'in kontrolüne yönelik daha etkili ve hedefe yönelik stratejilerin geliştirilmesine katkı sağlayabilir.
Anahtar Kelimeler: *Listeria monocytogenes*, ısı stresi direnci, SigB (σ^B), L-sistein, anaerobik koşullar.

Research Article

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1. Introduction

Listeria monocytogenes is a Gram-positive foodborne pathogen commonly found in natural and food-processing environments. It can survive and proliferate under diverse stressful conditions, including both aerobic and facultatively anaerobic settings (Bucur et al., 2018; O'Byrne & Karatzas, 2008). Its remarkable tolerance to a wide spectrum of stresses, spanning pH values from 5.5 to 9, temperatures from 0 to 45 °C, and osmotic stress up to 16% NaCl, renders it a persistent threat to food production (Clemente-Carazo et al., 2020; Bucur et al., 2018). This adaptability contributes to its ability to cause listeriosis, a severe illness with significant public health consequences. In England and Wales, listeriosis accounts for a median of 160 reported cases annually (2013–2022), while mortality rates remain high at 13.8% per 100,000 people in the European Union and 20–30% worldwide (UK HSA, 2025; Lee et al., 2019; Bucur et al., 2018; Xayarath et al., 2009). High-risk groups include pregnant women, where infections may cause fatal loss, and the elderly, in whom listeriosis can progress to sepsis and death (Wang et al., 2021). Beyond its health burden, listeriosis also imposes considerable economic costs through recalls and intensified safety interventions (Xayarath et al., 2009).

Heat treatment is one of the most widely used preservation methods in the food industry to eliminate pathogens and spoilage organisms (Bucur et al., 2018). Although proper thermal processing can inactivate *L. monocytogenes*, sublethal treatments may permit survival and subsequent regrowth, creating residual food safety risks (Gnanou Besse et al., 2000). The persistence of this pathogen in foods and processing environments, despite current decontamination measures, underscores the need for improved strategies (Bucur et al., 2018; Lin & Chou, 2004). A better understanding of the factors modulating thermal resistance is therefore critical. Current evidence suggests that three main variables influence heat tolerance in *L. monocytogenes*: nutrient composition, oxygen availability, and the activity of global stress regulators (Doyle et al., 2001).

The composition of the growth or heating medium plays a particularly important role in shaping stress responses. (Roberts et al., 2020). Cysteine-rich environments such as poultry, eggs, beef, and whole grains can affect bacterial behaviour by promoting redox homeostasis and increasing resistance to oxidative stress, both essential for the pathogen's survival under unfavourable food processing conditions (Turnbull & Surette, 2010; Górska-Warsewicz et al., 2018). Early studies demonstrated that L-cysteine supplementation facilitated the recovery of heat-injured cells (Knabel et al., 1990) and markedly increased the secretion of the virulence factor listeriolysin O (LLO) (Kouassi & Shelef, 1995). Similarly, Xayarath et al. (2009) and Yilmaz Topcam et al. (2025) suggested that L-cysteine protects against acid and oxidative stress. Given its abundance in food matrices prone to contamination, L-cysteine may represent an overlooked factor influencing bacterial survival during thermal processing. However, no recent studies have directly addressed the extracellular impact of L-cysteine on the heat resistance of *L. monocytogenes* 10403S.

As a facultative anaerobe, *L. monocytogenes* can grow under both oxygen-rich and oxygen-limited conditions (Couvert et al., 2019; Müller-Herbst et al., 2014). While oxygen availability does not significantly alter growth kinetics, it does modulate bacterial responses to environmental stresses (Couvert et al., 2019; Fu et al., 2015; Mols et al., 2009). Anaerobic conditions influence redox balance, energy metabolism, and stress signalling pathways (Roberts et al., 2020). Also, it was reported that oxygen limitation, in combination with nutrient availability, altered thermal resistance (Poimenidou et al., 2016; George & Peck, 1998). Yet, the interaction between oxygen deprivation and cysteine supplementation during heat stress remains largely unexplored.

Survival in hostile environments is also mediated by general stress response systems that regulate gene expression, protein repair, and antioxidant defences. A central regulator is the alternative sigma factor SigB, encoded by the *sigB* gene. SigB contributes to resistance against diverse stresses, including heat, acid, osmotic, and oxidative stress (NicAogáin & O'Byrne, 2016; Ferreira et al., 2001). Its role has been well documented in Gram-positive bacteria such as *Bacillus subtilis*, where Δ*sigB* displays markedly reduced heat resistance (Völker et al., 1999). In *L. monocytogenes*, deletion of *sigB* results in decreased survival under sublethal conditions and heightened sensitivity to acid and oxidative stress (Wemekamp-Kamphuis et al., 2004; Wiedmann et al., 1998). However, the contribution of *sigB* to heat resistance under anaerobic conditions and its potential interaction with cysteine availability remain poorly defined.

Based on this background, the present study aimed to: *(i)* investigate the effect of L-cysteine supplementation on the heat resistance of *L. monocytogenes*, *(ii)* determine the role of *sigB* in heat stress responses, and *(iii)* assess how aerobic versus anaerobic environments influence bacterial adaptation. Although heat treatment remains a cornerstone of food preservation, additional insights into how nutrients, oxygen status, and global stress regulators interact may inform more effective hurdle technologies. The findings of this study could therefore help improve the prediction and control of *L. monocytogenes* survival in food products and processing environments.

2. Materials and Methods

2.1 Bacterial strain and culture preparation

The bacterial strains used in this study were wild-type (WT) *Listeria monocytogenes* 10403S (serotype 1/2a) (Karatzas et al., 2010) and its isogenic Δ*sigB* null mutant (Wiedmann et al., 1998). Stock cultures were maintained at −80 °C in cryovials containing 70 µL of dimethyl sulfoxide (DMSO; Fisher Scientific, Loughborough, United Kingdom) per 930 µL of bacterial suspension. For experimental use, strains were revived by streaking onto Brain Heart Infusion (BHI) agar plates (Neogen, Heywood, United Kingdom) and incubated at 37 °C for 24 hours. Three well-isolated colonies from each plate were then transferred into 3 mL of sterile BHI broth and incubated at 37 °C with shaking at 120 rpm until reaching the stationary phase (~18 h). Stationary-phase cultures were used to inoculate 250 mL pre-sterilized Erlenmeyer flasks containing 20 mL of either defined medium (DM) or defined medium supplemented with 1.57 mM L-cysteine hydrochloride monohydrate (DMC), at a 1% (*v/v*) inoculum. Cultures were incubated aerobically at 37 °C with shaking (120 rpm) for 24 hours. For anaerobic preparation, stationary-phase starter cultures grown in BHI broth were transferred into universal tubes containing 20 mL of DM and DMC. These cultures were incubated for 24 hours at 37 °C under anaerobic conditions using a Whitley MG1000 anaerobic workstation (Don Whitley Scientific, United Kingdom).

2.2 Defined medium preparation

A defined broth medium (DM) was prepared in this study according to the method of Amezaga et al. (1995) to enable controlled manipulation of L-cysteine concentration. While the base DM contained 0.82 mM L-cysteine, a final concentration of 1.57 mM L-cysteine hydrochloride monohydrate (Sigma-Aldrich, Darmstadt, Germany) was achieved to create a cysteine-enriched variant (DMC). This concentration reflects the typical cysteine levels found in natural food sources such as beef, pork, and milk (~1 g/16 g nitrogen) (Pieniazek et al., 1975) and was confirmed to be non-inhibitory to *L. monocytogenes* growth (Yilmaz Topcam et al., 2025). To assess the effect of exogenous L-cysteine on heat resistance, overnight cultures were separately inoculated into DM and DMC to establish experimental cultures for subsequent assays.

2.3 Heat treatment assay

To assess thermal resistance, 1 mL aliquots of overnight bacterial cultures were transferred into pre-sterilized 1.5 mL Eppendorf tubes (Eppendorf, Sigma-Aldrich) and heated to 56 °C in a thermostatically controlled water bath. Samples were collected at 0 (pre-treatment), 10, 20, and 30 minutes. Following treatment, samples were serially diluted in 0.9% (w/v) Maximum Recovery Diluent (MRD; Oxoid, United Kingdom). Appropriate dilutions (approximately 10^{-6} CFU/mL) were spot-plated in 10 µL volumes onto BHI agar plates to determine viable cell counts. Plates were incubated at 37 °C for 24 hours, after which colony-forming units (CFU) were enumerated using a digital colony counter (Gallenkamp, United Kingdom). All experiments were conducted in biological triplicate using independently prepared cultures.

2.4 Statistical analysis

All experiments were conducted in three independent biological replicates, and each data point represents the mean of three technical replicates. Differences in the logarithmic values of viable cell counts were analysed between media types (DM vs. DMC), strains (WT vs. Δ*sigB*), and atmospheric conditions (aerobic vs. anaerobic) at each time point of heat treatment. Each time point was analysed independently using an unpaired Student's t-test, with p values < 0.05 considered statistically significant. To evaluate the effects of strain (WT vs. Δ*sigB*), medium type (DM and DMC), and their interaction on heat treatment resistance, a two-way ANOVA was performed. Tukey's multiple comparisons test was applied as a post hoc analysis. Data are presented as mean ± standard deviation (SD). All statistical analyses and figure generation were carried out using GraphPad Prism (version 10.1.0).

3. Results and discussion

3.1 L-cysteine impact on heat treatment resistance

The effect of heat treatment at 56 °C was evaluated in WT and Δ*sigB* strains of *L. monocytogenes* grown under aerobic and anaerobic conditions in two media, DM and DMC. Across all conditions, both strains showed a significant time-dependent reduction in viability ($p < 0.001$), confirming the robustness of heat as a lethal stress (Figure 1.).

Under aerobic conditions, WT survival patterns were similar in DM and DMC, with no significant differences at any time point. In Δ*sigB*, a transient increase in inactivation was noted in DMC at 20 minutes, suggesting a momentary sensitivity in the absence of SigB, but this effect did not persist during prolonged exposure.

In contrast, under anaerobic conditions, media-dependent differences became more apparent. For the WT strain, survival was consistently higher in DMC compared with DM after 10 minutes, with the strongest effect observed at later stages of heat treatment. This indicates that DMC conferred a measurable protective effect against heat stress in the absence of oxygen. The most likely explanation lies in the presence of L-cysteine, which plays a central role in thiol-based defences. Cysteine participates in thiolation and transsulfuration pathways that lead to the synthesis of homocysteine, methionine, and S-adenosylmethionine (Caballero Cerbon et al., 2024). These sulphur-containing molecules are essential for antioxidant defence, protein repair, and metabolic regulation (Smirnova et al., 2024). However, L-cysteine is prone to oxidation by molecular oxygen, a process accelerated in the presence of transition metals such as iron, reducing its antioxidant potential (Yang et al., 2010). Thus, under aerobic conditions its protective effect may be diminished, whereas in anaerobic environments, L-cysteine remains reduced and functions effectively as a redox buffer.

Heat stress is tightly linked with oxidative stress, as elevated temperatures disrupt cellular homeostasis and increase ROS generation (Y. Wang et al., 2021). In this context, L-cysteine may directly scavenge ROS or stabilize protein thiols, thereby preventing oxidative protein damage (Aryal et al., 2025). Protection may also be mediated through the CyuR regulatory pathway, which enhances bacterial heat resistance by modulating hydrogen sulphide (H_2S) production. H_2S functions both as a signalling molecule and as an antioxidant, contributing to protection against heat-induced oxidative stress (Rodionova et al., 2024). Similarly, the CymR regulon, which controls cysteine levels in the cell, detects oxidative stress through the thiolation of its cysteine residue in *Bacillus subtilis* (Even et al., 2006; Burguière et al., 2005), *Staphylococcus aureus* (Ji et al., 2012; Soutourina et al., 2009), and possibly *L. monocytogenes*.

More broadly, oxygen availability strongly influenced stress outcomes in our study. Aerobically grown cells showed higher growth rates and greater resistance to lethal heat stress, consistent with earlier reports linking aerobic metabolism to enhanced activation of antioxidant defences (Roberts et al., 2020; Bucur et al., 2018). Increased catalase activity in aerobically grown cells likely contributed to this protection, as catalase detoxifies hydrogen peroxide generated during heat stress (Yasser, 2022; Manso et al., 2020). In contrast, anaerobically grown cells exhibited weaker catalase induction and greater sensitivity, though in some cases anaerobic metabolism may also confer survival benefits (Roberts et al., 2020). The defined media minimized nutrient variability, enabling us to dissect how oxygen-dependent metabolic and enzymatic responses shape heat-stress tolerance (Müller-Herbst et al., 2014). These findings align with prior research showing that oxygen availability modulates protective mechanisms such as heat shock proteins and oxidative stress defences, which together determine *L. monocytogenes* survival under heat stress (Wiktorczyk-Kapischke et al., 2021; Shen et al., 2014).

3.2 Effect of *ΔsigB* deletion on L-cysteine–mediated heat stress response

At 30 minutes (Figure 2.), WT cells were significantly more inactivated than the Δ*sigB* in both DM and DMC under anaerobic conditions ($p < 0.01$). This indicates that deletion of *sigB* conferred partial resistance to heat stress, a result that was most pronounced when exogenous L-cysteine was present (DMC). Thus, the protective effect of DMC appeared to interact with *sigB* deletion, enhancing survival in anaerobic environments.

SigB is a global stress regulator that contributes to bacterial stress adaptation, including heat tolerance, but its role is highly context-dependent (Liu et al., 2019). Previous studies have shown variable effects of *sigB* deletion depending on temperature, oxygen availability, and strain background. For example, Ferreira et al. (2001) reported that *sigB* deletion had a negligible impact on survival at 50 °C under aerobic conditions. Moreover, under anaerobic conditions, strain-dependent outcomes were observed: in *L. monocytogenes* 10403S, *sigB* deletion had little effect on survival, whereas in EGD-e it caused increased sensitivity (Boura et al., 2016). They also demonstrated that in *L. monocytogenes* 10403S, SigB is essential for oxidative stress resistance under aerobic conditions, yet its deletion occasionally conferred hyper-resistance to hydrogen peroxide in stationary-phase cultures. These divergent findings suggest that compensatory pathways, or reduced oxidative burden in the absence of oxygen, can alter the regulatory importance of SigB.

Our findings expand this understanding by showing that in the presence of L-cysteine, Δ*sigB* not only tolerated heat stress but, in some cases, outperformed WT cells. This suggests that thiol-based metabolic buffering can substitute for SigB-dependent regulation (Crespo Tapia et al., 2020). L-cysteine, as a

precursor of glutathione, H_2S, and other thiol compounds, may generate alternative antioxidant defences that mitigate stress damage (Berude et al., 2024). These thiol-based systems overlap with SigB-dependent networks but may function independently when SigB is absent (Crespo Tapia et al., 2020). The protective effect of DMC is therefore best explained as the combined outcome of L-cysteine's redox-buffering capacity and its role as a metabolic signal. In anaerobic conditions, where oxidative degradation of cysteine is minimized, this effect is amplified. Thus, Δ*sigB* may exploit cysteine-derived pathways to compensate for its lack of SigB regulation, leading to paradoxical survival benefits.

Overall, these results highlight the complex interplay between oxygen, nutrient composition, and global stress regulators in shaping bacterial stress responses. While direct mechanistic links between SigB, cysteine metabolism, and heat resistance remain unresolved, our data indicate that cysteine-dependent buffering mechanisms can, under certain conditions, replace SigB-mediated protection. This observation underscores the need for targeted mechanistic studies to elucidate the contributions of metabolic and regulatory pathways to *L. monocytogenes* heat resistance.

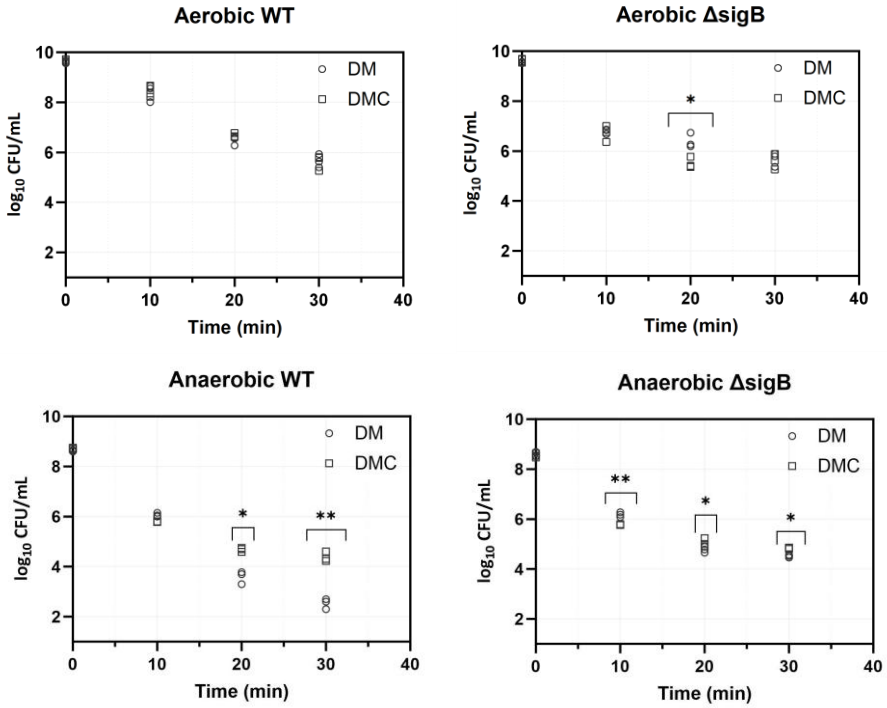

Figure 1. Survival kinetics of WT and ΔsigB strains under heat treatment at 56 °C in DM and DMC. Viable cell counts were determined at 0, 10, 20, and 30 minutes and expressed as $\log_{10}$ CFU/mL. Data are presented as mean ± SD from three independent biological replicates, with each replicate displayed individually in the figure. (*p < 0.05, **p < 0.01).

*Şekil 1. DM ve DMC ortamlarında 56°C'de ısıtma işlemine tabi tutulan WT ve ΔsigB suşlarının hayatta kalma kinetiği. Canlı hücre sayıları 0, 10, 20 ve 30. dakikalarda belirlenmiş ve $\log_{10}$ CFU/mL olarak ifade edilmiştir. Veriler, üç bağımsız biyolojik tekrardan elde edilen ortalama ± standart sapma olarak sunulmuştur ve her tekrar ayrı ayrı şekilde gösterilmiştir. (*p < 0,05, **p < 0,01).*

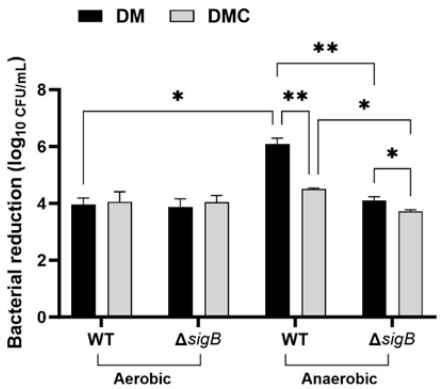

Figure 2. Log reduction of WT and ΔsigB strains after 30 minutes of heat treatment at 56 °C under aerobic and anaerobic conditions. Viable cell counts were determined and expressed as $\log_{10}$ CFU/mL. Data represent the mean ± SD of three biological replicates. Statistical significance was assessed by two-way ANOVA followed by Tukey's multiple comparisons test (*p < 0.05, **p < 0.01).

*Şekil 2. WT ve ΔsigB suşlarının 56°C'de 30 dakika süreyle aerobik ve anaerobik koşullar altında ısıtılmasından sonraki logaritmik azalma değerleri. Canlı hücre sayıları belirlenmiş ve $\log_{10}$ CFU/mL olarak ifade edilmiştir. Veriler, üç biyolojik tekrarın ortalama ± standart sapmasını temsil etmektedir. İstatistiksel anlamlılık, iki yönlü ANOVA ve ardından Tukey çoklu karşılaştırma testi ile değerlendirilmiştir (*p < 0,05, **p < 0,01).*

4. Conclusion

This study demonstrates that oxygen availability, medium composition, and global stress regulation collectively shape the heat resistance of *L. monocytogenes*. Supplementation with L-cysteine in defined medium conferred a measurable protective effect under anaerobic conditions, likely due to its role as a thiol-based antioxidant and metabolic signal. Interestingly, deletion of *sigB* unexpectedly enhanced survival in the presence of L-cysteine, suggesting that cysteine-dependent pathways can partially compensate for the absence of SigB-mediated regulation. These findings reinforce the notion that the role of SigB in stress resistance is highly context-dependent, influenced by oxygen status and nutrient environment.

From a food safety perspective, the results highlight the complexity of bacterial adaptation under processing-relevant stresses. In oxygen-limited environments such as vacuum-packaged or modified-atmosphere foods, sulphur-containing compounds could alter *L. monocytogenes* heat resistance in ways that are not predicted by standard aerobic models. Given that many high-risk foods inherently contain cysteine or cysteine-rich components such as dairy products, meat matrices and protein-enriched ready-to-eat items, our findings indicate that cysteine availability may inadvertently enhance thermal tolerance and should therefore be incorporated into assessments of *Listeria* survival within real food systems. The interplay between metabolic buffering and global stress regulation should therefore be considered when designing control strategies, particularly for thermal treatments. Future research should further dissect the mechanistic links between cysteine metabolism, redox buffering, and regulatory pathways, to better predict bacterial persistence under combined food processing stresses.

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Authors Contributions

I.S.: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. M.M.Y.T.: Methodology, Writing – review & editing, Supervision. K.A.K.: Writing – review & editing, Supervision, Resources, Project administration, Formal analysis, Conceptualization.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Conflicts of Interest

The authors declare no conflict of interest.

Ethical Statement

This study did not involve human participants or animal experiments.

References

Amezaga, M.-R., Davidson, I., Mclaggan, D., Verheul, A., Abee2, T., & Booth', I. R. (1995). The role of peptide metabolism in the growth of *Listeria monocytogenes* ATCC 23074 at high osmolarity. *Microbiology, 141*(1), 41-49. https://doi.org/10.1099/00221287-141-1-41.

Aryal, B., Kwakye, J., Ariyo, O. W., Ghareeb, A. F. A., Milfort, M. C., Fuller, A. L., Khatiwada, S., Rekaya, R., & Aggrey, S. E. (2025). Major Oxidative and Antioxidant Mechanisms During Heat Stress-Induced Oxidative Stress in Chickens. *Antioxidants*, *14*(4), 471. https://doi.org/10.3390/antiox14040471

Berude, J. C., Kennouche, P., Reniere, M. L., & Portnoy, D. A. (2024). *Listeria monocytogenes* utilizes glutathione and limited inorganic sulfur compounds as sources of essential cysteine. *Infection and Immunity*, *92*(3), 1-18. https://doi.org/10.1128/iai.00422-23

Boura, M., Keating, C., Royet, K., Paudyal, R., O'Donoghue, B., O'Byrne, C. P., & Karatzas, K. A. G. (2016). Loss of sigb in *listeria monocytogenes* strains egde and 10403s confers hyperresistance to hydrogen peroxide in stationary phase under aerobic conditions. *Applied and Environmental Microbiology*, *82*(15), 4584–4591. https://doi.org/10.1128/AEM.00709-16

Bucur, F. I., Grigore-Gurgu, L., Crauwels, P., Riedel, C. U., & Nicolau, A. I. (2018). Resistance of *Listeria monocytogenes* to Stress Conditions Encountered in Food and food processing environments. *Frontiers in Microbiology, 9,* 2700. https://doi.org/10.3389/fmicb.2018.02700

Burguière, P., Fert, J., Guillouard, I., Auger, S., Danchin, A., & Martin-Verstraete, I. (2005). Regulation of the *Bacillus subtilis ytmI* operon, involved in sulfur metabolism. *Journal of Bacteriology*, *187*(17), 6019–6030. https://doi.org/10.1128/JB.187.17.6019-6030.2005

Caballero Cerbon, D. A., Gebhard, L., Dokuyucu, R., Ertl, T., Härtl, S., Mazhar, A., & Weuster-Botz, D. (2024). Challenges and Advances in the Bioproduction of L-Cysteine. *Molecules, 29*(2), 486. https://doi.org/10.3390/molecules29020486

Clemente-Carazo, M., Cebrian, G., Garre, A., & Palop, A. (2020). Variability in the heat resistance of *Listeria monocytogenes* under dynamic conditions can be more relevant than that evidenced by isothermal treatments. *Food Research International*, *137*, 109538. https://doi.org/10.1016/j.foodres.2020.109538

Couvert, O., Divanac'h, M. L., Lochardet, A., Thuault, D., & Huchet, V. (2019). Modelling the effect of oxygen concentration on bacterial growth rates. *Food Microbiology*, *77*, 21–25. https://doi.org/10.1016/j.fm.2018.08.005

Crespo Tapia, N., Dorey, A. L., Gahan, C. G. M., den Besten, H. M. W., O'Byrne, C. P., & Abee, T. (2020). Different carbon sources result in differential activation of sigma B and stress resistance in *Listeria monocytogenes*. *International Journal of Food Microbiology*, *320*. https://doi.org/10.1016/j.ijfoodmicro.2019.108504

Doyle, M. E., Mazzotta, A. S., Wang, T., Wiseman, D. W., and, §, & Scott, V. N. (2001). Heat Resistance of *Listeria monocytogenes*. *Journal of Food Protection, 64*(3), 410-429. https://doi.org/10.4315/0362-028x-64.3.410.

Even, S., Burguière, P., Auger, S., Soutourina, O., Danchin, A., & Martin-Verstraete, I. (2006). Global control of cysteine metabolism by CymR in *Bacillus subtilis*. *Journal of Bacteriology*, *188*(6), 2184–2197. https://doi.org/10.1128/JB.188.6.2184-2197.2006

Ferreira, A., O'Byrne, C. P., & Boor, K. J. (2001). Role of σB in Heat, Ethanol, Acid, and Oxidative Stress Resistance and during Carbon Starvation in *Listeria monocytogenes*. *Applied and Environmental Microbiology*, *67*(10), 4454–4457. https://doi.org/10.1128/AEM.67.10.4454-4457.2001

Fu, H., Yuan, J., & Gao, H. (2015). Microbial oxidative stress response: Novel insights from environmental facultative anaerobic bacteria. *Archives of Biochemistry and Biophysics, 584,* 28–35. https://doi.org/10.1016/j.abb.2015.08.012

George, S. M., & Peck, M. W. (1998). Redox potential affects the measured heat resistance of *Escherichia coli* O157:H7 independently of oxygen concentration. *Letters in Applied Microbiology, 27*(6), 313-317. https://doi.org/10.1046/j.1472-765x.1998.00466.x.

Gnanou Besse, N., Dubois Brissonnet, F., Lafarge, V., & Leclerc, V. (2000). Effect of various environmental parameters on the recovery of sublethally salt-damaged and acid-damaged *Listeria monocytogenes*. *Journal of Applied Microbiology*, *89*(6), 944–950. https://doi.org/10.1046/j.1365-2672.2000.01195.x

Górska-Warsewicz, H., Laskowski, W., Kulykovets, O., Kudlińska-Chylak, A., Czeczotko, M., & Rejman, K. (2018). Food products as sources of protein and amino acids—The case of Poland. *Nutrients, 10*(12), 1977. https://doi.org/10.3390/nu10121977

Ji, Q., Zhang, L., Sun, F., Deng, X., Liang, H., Bae, T., & He, C. (2012). *Staphylococcus aureus* CymR is a new thiol-based oxidation-sensing regulator of stress resistance and oxidative response. *Journal of Biological Chemistry*, *287*(25), 21102–21109. https://doi.org/10.1074/jbc.M112.359737

Karatzas, K. A. G., Brennan, O., Heavin, S., Morrissey, J., & O'Byrne, C. P. (2010). Intracellular accumulation of high levels of γ-aminobutyrate by *Listeria monocytogenes* 10403S in response to low pH: Uncoupling of γ-aminobutyrate synthesis from efflux in a chemically defined medium. *Applied and Environmental Microbiology*, *76*(11), 3529–3537. https://doi.org/10.1128/AEM.03063-09

Knabel, S. J., Walker, H. W., Hartman, P. A., & Mendonca, A. F. (1990). Effects of Growth Temperature and Strictly Anaerobic Recovery on the Survival of *Listeria monocytogenes* during Pasteurization. *Applied and Environmental Microbiology*, *56*(2), 370-376. https://doi.org/10.1128/aem.56.2.370-376.1990.

Kouassi, Y., & Shelef, L. A. (1995). Listeriolysin 0 secretion by *Listeria monocytogenes* in the presence of cysteine and sorbate. *Letters in Applied Microbiology, 20*(5), 295-299. https://doi.org/10.1111/j.1472-765x.1995.tb00449.x.

Lee, B. H., Cole, S., Badel-Berchoux, S., Guillier, L., Felix, B., Krezdorn, N., & Piveteau, P. (2019). Biofilm formation of *Listeria monocytogenes* strains under food processing environments and pan-genome-wide association study. *Frontiers in Microbiology*, *10*, 2698. https://doi.org/10.3389/fmicb.2019.02698

Lin, Y. D., & Chou, C. C. (2004). Effect of heat shock on thermal tolerance and susceptibility of *Listeria monocytogenes* to other environmental stresses. *Food Microbiology*, *21*(5), 605-610. https://doi.org/10.1016/j.fm.2003.10.007.

Liu, Y., Orsi, R. H., Gaballa, A., Wiedmann, M., Boor, K. J., & Guariglia-Oropeza, V. (2019). Systematic review of the *Listeria monocytogenes* σ regulon supports a role in stress response, virulence and metabolism. *Future Microbiology*, *14*(9), 801–828. https://doi.org/10.2217/fmb-2019-0072

Manso, B., Melero, B., Stessl, B., Jaime, I., Wagner, M., Rovira, J., & Rodríguez-Lázaro, D. (2020). The response to oxidative stress in *Listeria monocytogenes* is temperature dependent. *Microorganisms*, *8*(4), 521. https://doi.org/10.3390/microorganisms8040521.

Mols, M., Pier, I., Zwietering, M. H., & Abee, T. (2009). The impact of oxygen availability on stress survival and radical formation of *Bacillus cereus*. *International Journal of Food Microbiology*, *135*(3), 303–311. https://doi.org/10.1016/j.ijfoodmicro.2009.09.002

Müller-Herbst, S., Wüstner, S., Mühlig, A., Eder, D., Fuchs, T. M., Held, C., Ehrenreich, A., & Scherer, S. (2014). Identification of genes essential for anaerobic growth of *Listeria monocytogenes*. *Microbiology*, *160*(4), 752–765. https://doi.org/10.1099/mic.0.075242-0

NicAogáin, K., & O'Byrne, C. P. (2016). The role of stress and stress adaptations in determining the fate of the bacterial pathogen *Listeria monocytogenes* in the food chain. *Frontiers in Microbiology*, *7*, 1865. https://doi.org/10.3389/fmicb.2016.01865

O'Byrne, C. P., & Karatzas, K. A. G. (2008). The Role of Sigma B (σB) in the Stress Adaptations of *Listeria monocytogenes*: Overlaps Between Stress Adaptation and Virulence. *Advances in Applied Microbiology, 65,* 115-140. https://doi.org/10.1016/S0065-2164(08)00605-9

Pieniazek Danuta, Grabarek Z., & Rakowska Maria. (1975). Quantitative Determination of the Content of Available Methionine and Cysteine in Food Proteins. *Nutrition & Metabolism, 18*, 16–22. https://doi.org/10.1159/000175569. PMID: 1172609.

Poimenidou, S. V., Chatzithoma, D. N., Nychas, G. J., & Skandamis, P. N. (2016). Adaptive response of *Listeria monocytogenes* to heat, salinity and low pH, after habituation on cherry tomatoes and lettuce leaves. *PLoS One*, *11*(10), e0165746. https://doi.org/10.1371/journal.pone.0165746.

Roberts, B. N., Chakravarty, D., Gardner, J. C., Ricke, S. C., & Donaldson, J. R. (2020). *Listeria monocytogenes* response to anaerobic environments. *Pathogens*, *9*(3). https://doi.org/10.3390/pathogens9030210

Rodionova, I. A., Lim, H. G., Gao, Y., Rodionov, D. A., Hutchison, Y., Szubin, R., & Palsson, B. O. (2024). CyuR is a dual regulator for L-cysteine dependent antimicrobial resistance in *Escherichia coli*. *Communications Biology*, *7*(1), 1160. https://doi.org/10.1038/s42003-024-06831-0

Shen, Q., Jangam, P. M., Soni, K. A., Nannapaneni, R., Schilling, W., & Silva, J. L. (2014). Low, medium, and high heat tolerant strains of *Listeria monocytogenes* and increased heat stress resistance after exposure to sublethal heat. *Journal of Food Protection*, *77*(8), 1298–1307. https://doi.org/10.4315/0362-028X.JFP-13-423

Smirnova, G., Tyulenev, A., Sutormina, L., Kalashnikova, T., Muzyka, N., Ushakov, V., & Oktyabrsky, O. (2024). Regulation of cysteine homeostasis and its effect on *Escherichia coli* sensitivity to ciprofloxacin in LB medium. *International Journal of Molecular Sciences*, *25*(8), 4424. https://doi.org/10.3390/ijms25084424.

Soutourina, O., Poupel, O., Coppée, J. Y., Danchin, A., Msadek, T., & Martin-Verstraete, I. (2009). CymR, the master regulator of cysteine metabolism in *Staphylococcus aureus*, controls host sulphur source utilization and plays a role in biofilm formation. *Molecular Microbiology*, *73*(2), 194–211. https://doi.org/10.1111/j.1365-2958.2009.06760.x

Turnbull, A. L., & Surette, M. G. (2010). Cysteine biosynthesis, oxidative stress and antibiotic resistance in *Salmonella typhimurium*. *Research in Microbiology*, *161*(8), 643–650. https://doi.org/10.1016/j.resmic.2010.06.004

UK Health Security Agency. (2025, May 8). *Listeriosis in England and Wales: Summary for 2022* (Surveillance report). UK Health Security Agency. https://www.gov.uk/government/publications/listeria-monocytogenes-surveillance-reports/listeriosis-in-england-and-wales-summary-for-2022

Wang, Y., Wang, H. L., Xing, G. D., Qian, Y., Zhong, J. F., & Chen, K. L. (2021). S-allyl cysteine ameliorates heat stress-induced oxidative stress by activating Nrf2/HO-1 signaling pathway in BMECs. *Toxicology and Applied Pharmacology*, *416.* https://doi.org/10.1016/j.taap.2021.115469

Wang, Z., Tao, X., Liu, S., Zhao, Y., & Yang, X. (2021). An update review on *Listeria* infection in pregnancy. *Infection and drug resistance*, 1967-1978. https://doi.org/10.2147/IDR.S313675.

Wemekamp-Kamphuis, H. H., Wouters, J. A., De Leeuw, P. P. L. A., Hain, T., Chakraborty, T., & Abee, T. (2004). Identification of sigma factor σB-controlled genes and their impact on acid stress, high hydrostatic pressure, and freeze survival in *Listeria monocytogenes* EGD-e. *Applied and Environmental Microbiology*, *70*(6), 3457–3466. https://doi.org/10.1128/AEM.70.6.3457-3466.2004

Wiedmann, M., Arvik, T. J., Hurley, R. J., & Boor, K. J. (1998). General Stress Transcription Factor B and Its Role in Acid Tolerance and Virulence of *Listeria monocytogenes*. *Journal of Bacteriology, 180*(14), 3650-3656. https://doi.org/10.1128/JB.180.14.3650-3656.1998.

Wiktorczyk-Kapischke, N., Skowron, K., Grudlewska-Buda, K., Wałecka-Zacharska, E., Korkus, J., & Gospodarek-Komkowska, E. (2021). Adaptive Response of *Listeria monocytogenes* to the Stress Factors in the Food Processing Environment. *Frontiers in Microbiology, 12, 710085*. https://doi.org/10.3389/fmicb.2021.710085

Xayarath, B., Marquis, H., Port, G. C., & Freitag, N. E. (2009). *Listeria monocytogenes* CtaP is a multifunctional cysteine transport-associated protein required for bacterial pathogenesis. *Molecular Microbiology*, *74*(4), 956–973. https://doi.org/10.1111/j.1365-2958.2009.06910.x

Yang, J., Duan, X., Landry, A. P., & Ding, H. (2010). Oxygen is required for the l-cysteine-mediated decomposition of protein-bound dinitrosyl-iron complexes. *Free Radical Biology and Medicine*, *49*(2), 268–274. https://doi.org/10.1016/j.freeradbiomed.2010.04.012

Yilmaz Topcam, M. M., Balagiannis, D. P., & Karatzas, K. A. G. (2025). Impact of c-di-AMP Accumulation, L-cysteine, and Oxygen on Catalase Activity and Oxidative Stress Resistance of *Listeria monocytogenes* 10403S. *Microorganisms*, *13*(6). https://doi.org/10.3390/microorganisms13061400