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Research article

Integrative analysis of transcriptomic and immunoproteomic data reveals stress response mechanisms in *Listeria monocytogenes*

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ABSTRACT

Listeria monocytogenes is a significant concern in the food industry due to its association with outbreaks of listeriosis, particularly affecting vulnerable populations. High-throughput technologies such as RNA sequencing (RNA-seq) and proteomics offer valuable insights into the molecular responses of *L. monocytogenes* to stress environments. In this study, a combined transcriptomic and immunoproteomic approach was applied to explore the stress response mechanisms of the *L. monocytogenes* strain ST7, which was responsible for an outbreak in central Italy. The bacterium was exposed to both optimal conditions and a stress environment representative of pork product matrices (pH 5.5; 7 % NaCl) and thermal abuse prior to consumption (12 °C).Transcriptomic analysis revealed variations in gene expression related to pathogenesis, stress responses, and virulence factors under different environmental conditions. Transcriptomic analysis of *Listeria* involves studying the complete set of RNA transcripts produced by the bacterium under various conditions or during different stages of its lifecycle. It can provide insights into its pathogenicity and virulence mechanisms. Immunoproteomic analysis identified proteins involved in stress response pathways, including oxidoreductases and DNA repair enzymes, uniquely expressed under stress conditions. Furthermore, the study highlighted proteins linked to antibiotic resistance and cell wall biosynthesis. By delineating specific proteins crucial in the stress response pathways, these findings not only deepen our comprehension of *L. monocytogenes* biology but also pave the way for designing more targeted mitigation strategies to safeguard food safety and public health effectively.

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1. Introduction

Listeria monocytogenes is a pathogenic bacterium, that presents a significant hazard to the food industry, due to its association with listeriosis, a potentially fatal disease, that primarily affects vulnerable populations including pregnant women, new-borns, the elderly and immunocompromised individuals [1]. Preventing food contamination is critical to controlling listeriosis, given the microorganism's ubiquity and ability to persist in diverse environments, including soil and water. Contaminated ready-to-eat (RTE) foods, such as meat products and cheeses, are common sources of *L. monocytogenes* outbreaks [2]. What makes L. *monocytogenes* particularly concerning is its ability to withstand a range of environmental stresses, including low temperatures, acidic conditions, and high osmolarity, which are commonly encountered in food processing and storage environments. *L. monocytogenes,* being a psychrotolerant bacterium, can survive refrigeration temperatures by reducing metabolic rates and changing its membrane composition, increasing the concentration of unsaturated fatty acids. Moreover it increases the encoding of cold shock proteins (Csps), and the uptake of cryoprotective compounds [3].

Furthermore, *L. monocytogenes* survives acidic environments, especially mild acid stress (pH 5.0), utilizing mechanisms such as the glutamic acid decarboxylase (GAD) system and the arginine and agmatine deiminases (ADI and AgDI) to maintain the bactierial cell homeostasis [4,5].

L. monocytogenes accumulates osmolytes, facilitated by the upregulation of the *opuCABCD* operon systems, which is involved in carnitine transport [6].

Inadequate cleaning and sanitation practices in food processing facilities, coupled with ineffective preventive strategies, contribute to the pathogen's survival and persistence in these environments [7].

High-throughput technologies as RNA sequencing (RNA-seq) and proteomics have revolutionized the field of molecular biology by providing comprehensive insights into gene expression and protein profiles.

The transcriptome includes the complete set of transcripts present in a microbial cell, along with their abundance, as a function ofspecific physiological condition or growth phase. Moreover, transcriptome analysis is useful for understanding how genes are correlated and activated in response to external factors.

RNA-seq is a powerful tool for transcriptomic analysis. It enables the quantification of transcripts expression levels and the identification of differentially expressed genes in a biological system.

By using this technology, it is possible to classify different species of transcripts, such as mRNAs, small RNA, coding and non-coding RNAs. Additionally, it allows for the identification of new transcripts, post translational modifications (PTMs) and genes transcriptional structure, such as splicing patterns, genes start sites and 5′ and 3' ends [8].

However, RNA-seq also has drawbacks. One of the main challenges is the accuracy of quantification and detection of low abundant transcripts: in fact their expression level might fall below the RNA-seq detection threshold. Another challenge arises with non-coding variants: RNA-seq may not fully capture how these variants affect splicing and gene expression when involved in specific subtle regulatory complex. Some RNA transcripts have a short half-life, making them difficult to detect in RNA-seq studies due to their rapid degradation in the sample. Additionally, RNA-seq presents statistical challenges because the data are discrete and over dispersed, necessitating the creation of specialized analysis methods [9].

This technique was employed to investigate how *L. monocytogenes* survives in the gastrointestinal (GI) tract adapting itself to anaerobic, acidic, and bile conditions. Results revealed variations in transcripts related to pathogenesis, cell wall proteins, DNA repair, transcription factors, and stress responses. Anaerobic and acidic conditions altered transcript levels for virulence factors and sensory kinases [10].

Transcriptomic analysis was performed to study the transcriptional response of *L. monocytogenes* strains 6179 (ST121) and R479a (ST8) to oxidative and acid stresses by analysing their chromosomal and plasmid transcriptomes, particularly focusing on the *σB* regulon [11].

Transcriptomic analysis of *L. monocytogenes* has provided valuable insights into the bacterium's response to various stress conditions, including bile, anaerobiosis, and pulsed electric field (PEF) treatment. A study analysing the response of *L. monocytogenes* to bile under aerobic and anaerobic conditions identified variations in transcript levels for genes responsible for pathogenesis, cell wall associated proteins, DNA repair, transcription factors, and stress responses [10]. Another study investigated the response of *L. monocytogenes* to PEF treatment and identified differentially expressed genes involved in stress response, energy metabolism, and cell wall biosynthesis [11].

Furthermore, temporal transcriptomic analysis has been used to identify *σB*-dependent genes in *L. monocytogenes* EGD-e, which include genes involved in osmoregulation, carbon metabolism, ribosome- and envelope-function, as well as virulence and niche-specific survival genes such as those involved in bile resistance and exclusion [12].

Overall, transcriptomic analysis has deepened our understanding of *L. monocytogenes* biology, enabling the design of more effective mitigation measures to ensure food safety and public health. Moreover, RNA-seq was used to determine the transcriptional response of *L. monocytogenes* to stress induced by high pressure processing (HPP), a preservation method in the food industry, and to elucidate the gene regulatory network (GRN) activated in response to this stress [13].

Transcriptomic data alone may not fully capture the complexity of the bacterium's stress responses, as gene expression does not always correlate directly with protein levels. Therefore, integrating transcriptomic and proteomic analyses offers a more comprehensive understanding of the molecular responses of *L. monocytogenes* under various stress conditions.

This approach was employed to understand the physiological processes, identifying changes in gene and protein expression profiles, which occur during the lag phase of *L. monocytogenes* enrichment, a phase where the pathogen adapts to its new environment before resuming growth [14].

Transcriptomic and proteomic analyses were used to investigate the antibacterial mechanism of linalool against *L. monocytogenes* as well as to evaluate its application in preserving chicken breast [15].

Proteomics provides valuable information about the actual protein products synthesized in response to specific environmental conditions or during host-pathogen interactions. Proteomic analysis of *L. monocytogenes* FBUNT during infection identified genetic determinants and protein expression associated with the pathogen [16].

In the previous study D'Onofrio et al., 2022 [17], an immunoproteomic approach combined with bioinformatics pipeline was employed to characterize proteins differentially encoded by the *L. monocytogenes* ST7 strain associated with a listeriosis outbreak in Central Italy in 2016 [18].

L. monocytogenes was exposed to optimal conditions and a stress environment representative of a pork product matrix (pH 5.5; NaCl 7 %) and thermal abuse before its consumption (12 °C).

The immunoblot allowed the identification of putative immunogenic proteins for both optimal and stressing conditions. MS/MS analysis revealed a total of 226 proteins, among which 58 (28.3 %) exhibited potential antigenicity.

Interestingly, 30 of these proteins were exclusively detected in the reference control, grown at 37 °C, NaCl 0.5 %, pH 7.0 (C-opt) while 3 proteins were unique for stress condition, cultivated at 12 °C, pH 5.5, NaCl 7 % (C-stress).

Functional categorization of these latter proteins, based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, highlighted their involvement in "general stress response" (TrxB), "Carbohydrate transport, metabolism, and energy production" (PdhB), "DNA repair and maintenance" (Lmo0132).

In this study, the intent is to correlate the proteomic results with genes mapped by RNA-seq analysis performed on *L. monocytogenes* ST7 strain grown at C-opt and C-stress, as representative of pork product matrices and thermal abuse before consumption (12 °C).

By combining transcriptomic (RNA-seq) and immunoproteomic data, the intent is to elucidate the stress response mechanisms of this outbreak-associated strain. These findings will contribute to a better understanding of the molecular mechanisms underlying *L. monocytogenes* pathogenicity, stress adaptation, and virulence, which are essential for improving food safety practices.

2. Materials and methods

2.1. Bacterial strain and cultivation

L. monocytogenes ST7 strain NRG-1749-2016 was provided by the Italian National Reference Laboratory for (It NRL *Lm*) of the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise (IZSAM). The strain was cultured under C-opt and C-stress conditions that mimicked food production and storage thermal abuse, with varying cardinal growth parameters: C-opt and C-stress. The strain was grown in Brain Heart Infusion (BHI) broth (Oxoid Thermo Fisher Scientific, Rodano, Italy) and modified one (pH 5.5, NaCl 7 %) until reaching exponential growth phase.

The trials were performed in biological and technical triplicates. Bacteria were cultured overnight (o.n.), diluted 1:100 in fresh BHI and modified (pH 5.5, NaCl 7 %) BHI broths, and incubated with constant shaking at 140–150 rpm. Collection of bacteria occurred during the exponential growth phase (OD_{600}): C-opt and C-stress were incubated for 3.5 h and 10 days, respectively (Supplementary material 1). Bacterial cells were washed three times with sterile ice-cold 0.01 M phosphate-buffered saline (PBS) at pH 7, harvested by

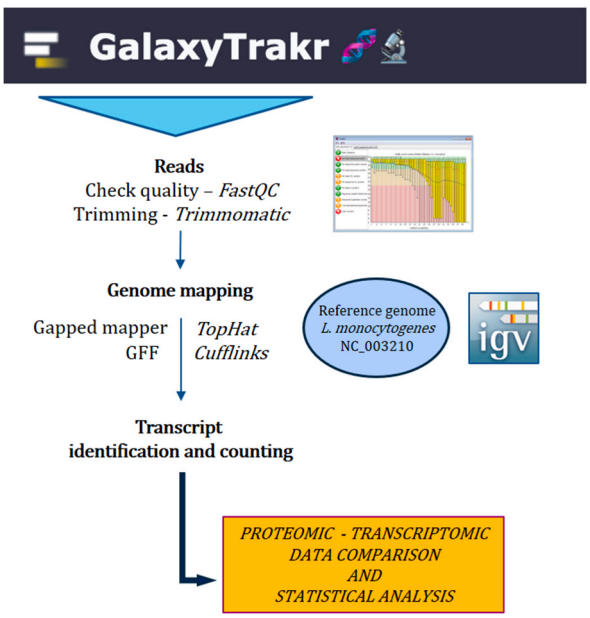

Fig. 1. Genome mapping analysis workflow and proteomic-transcriptomic data integration.

centrifugation (Eppendorf, Hamburg, Germany) at 5600×*g* at 4 °C for 10 min and stored at −80 °C until further use.

2.2. RNA isolation, ribosomal RNA depletion, library construction, and sequencing

The samples were treated with RNA protect reagent (Qiagen, Hilden, Germany) prior to extraction. Total RNA was extracted using the RNeasy Mini Kit according to the manufacturer's instructions (Qiagen, Hilden, Germany), treated with DNase and then quantified by Qubit® RNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA).

Bacterial RNA samples were subjected to rRNA depletion using the Ribo-Zero™ Magnetic Kit for Bacteria (Illumina, San Diego, California), followed by purification with RNeasy MinElute Cleanup Kit columns (Qiagen, Hilden, Germany). Enzymatic RNA fragmentation, cDNA reverse transcription, adapter addition, cDNA purification, library amplification, and indexing barcode insertion were performed using the ScriptSeq™ v2 RNA-Seq Library Preparation Kit (Epicentre, Madison, Wisconsin, USA) according to the manufacturer's instructions. Sequencing was conducted on the NextSeq 500 platform (Illumina Inc., San Diego, CA, USA) using the NextSeq 500/550 Mid Output Reagent Cartridge v2 150 cycles, generating paired-end reads of 75 bp.

2.3. Data analysis

Quality control and trimming of the raw reads were performed using FastQC and Trimmomatic, respectively (Fig. 1). RNA-seq analysis was conducted by genome mapping approach on GalaxyTrakr platform [19]. The reads mapping step was performed with TopHat, using *L. monocytogenes* NC_003210 (NCBI) as the reference genome [20]. The counting of the mapped reads was carried out using Cufflinks, considering the annotation file of the reference genome described in GFF format. The expression level of each gene was reported in "fragments per kilobase of transcript per million fragments mapped reads" (FPKM) [21]. Enrichment analysis was performed by Shiny GO v.0.80 and STRING v.12 [22].

2.4. Statistical analyses

The FPKM mean value obtained from Cufflinks was used to evaluate significant differences between C-opt and C-stress by T-test ($p < 0.05$) (Supplementary material 2). Moreover, the proteins that were considered unique to each growth condition were further analyzed to determine whether their presence under C-opt and C-stress could also be confirmed at the transcriptomic level (Supplementary material 3).

3. Results

A total of 3059 genes were identified across the C-opt and C-stress conditions and FPKM values of 807 genes were significantly higher in C-stress. GO analysis revealed 183 genes associated to "Transport" (GO:0006810). The most enriched GO function were "Carbohydrate transmembrane transporter activity" (GO:0015144), "Phosphotransferase activity, alcohol group as acceptor" (GO:0016773), "Transmembrane transporter activity" (GO:0022857) and "Protein-N(PI)-phosphohistidine-sugar phosphotransferase activity" (GO:0008982). Additionally, the "Flagellar assembly (KEGG map02040), the "Phosphotransferase system" (PTS) (KEGG map02060) and the "Fructose and mannose metabolism" (KEGG map00051) were significantly enriched by KEGG pathway analysis (FDR <0.01) and are listed in Table 1.

C-opt and C-stress were filtered out to consider only those genes that encode proteins revealed by immunoblot in C-opt and C-stress (Supplementary materials 4 and 6).

Sixty-four out of 121 genes, which encode proteins considered unique to the optimal condition, were significantly differentially expressed. (Supplementary material 4).

In the C-stress condition, 12 out of 32 genes had a FPKM value significantly higher than optimal condition (Supplementary material 6).

The most enriched pathways for C-opt are described in Table 2 in which each row represents a different pathway, and the columns provide specific information about each pathway's enrichment significance and characteristics. Moreover, the results from Table 2 are visualized as a network in Fig. 2.

The transcript levels confirming the presence of unique proteins, identified via mass spectrometry under C-stress, with significantly

Table 1
Enrichment analysis obtained from STRING v.12. KEGG Pathways mostly enriched in C-stress.

Enrichment FDR[a]	nGenes [b]	Term ID[c]	KEGG Pathways
0.0018	26	map02040	Flagellar assembly
0.0018	65	map02060	Phosphotransferase system (PTS)
0.0076	39	map00051	Fructose and mannose metabolism

[a] **Enrichment FDR**: Assesses the significance of the enrichment, displaying p-values adjusted for multiple testing within each category using the Benjamini–Hochberg procedure.
[b] **nGenes** Total number of genes involved in the pathway.
[c] **Term ID**: Identification code for each pathway enriched.

Table 2
Enrichment analysis results for C-opt.

Enrichment FDR[a]	nGenes[b]	Pathway Genes[c]	Fold Enrichment[d]	Pathways[e]
8.2E-10	136	807	1.7	Macromolecule metabolic process
2.2E-09	79	372	2.1	Cellular macromolecule biosynthetic process
2.6E-09	79	376	2.1	Macromolecule biosynthetic process
3.6E-08	109	630	1.7	Cellular macromolecule metabolic process
7.2E-07	191	1417	1.3	Primary metabolic process
1.4E-06	177	1289	1.3	Nitrogen compound metabolic process
2.5E-06	213	1667	1.3	Organic substance metabolic process
4.4E-06	235	1918	1.2	Metabolic process
3.0E-05	135	942	1.4	Organic substance biosynthetic process
3.1E-05	136	953	1.4	Biosynthetic process

[a] **Enrichment FDR**: False Discovery Rate (FDR) associated with the enrichment of the pathway. It indicates the probability that the observed enrichment is a false positive. A lower FDR value signifies higher confidence in the enrichment.
[b] **nGenes**: the total number of genes in the dataset that are involved in the respective pathway.
[c] **Pathway Genes**: the total number of genes known to be associated with the pathway.
[d] **Fold Enrichment**: the column shows the fold enrichment, which is the ratio of the observed number of genes in the dataset (nGenes) to the expected number of genes based on the overall representation of the pathway genes. It indicates how much more frequently genes in this pathway appear in the dataset compared to what would be expected by chance.
[e] **Pathways:** name or description of the pathway being analyzed.

higher FPKM values are listed in Table 3.

4. Discussions

Enrichment analysis conducted on C-opt revealed that the pathways and processes activated are fundamental to the functioning of living organisms involving the synthesis, modification, and breakdown of macromolecules and other organic substances crucial for cellular activities and overall metabolism. Among these results, the "Macromolecule metabolic process" pathway exhibited the lowest False Discovery Rate (FDR) of 8.2E-10 and a fold enrichment of 1.7, indicating its high significance and enrichment in the dataset. Similarly, other metabolic processes such as "Cellular macromolecule biosynthetic process" (FDR 2.2E-09) and "Macromolecule biosynthetic process" (FDR 2.6E-09) also demonstrated significant enrichment (2.1).

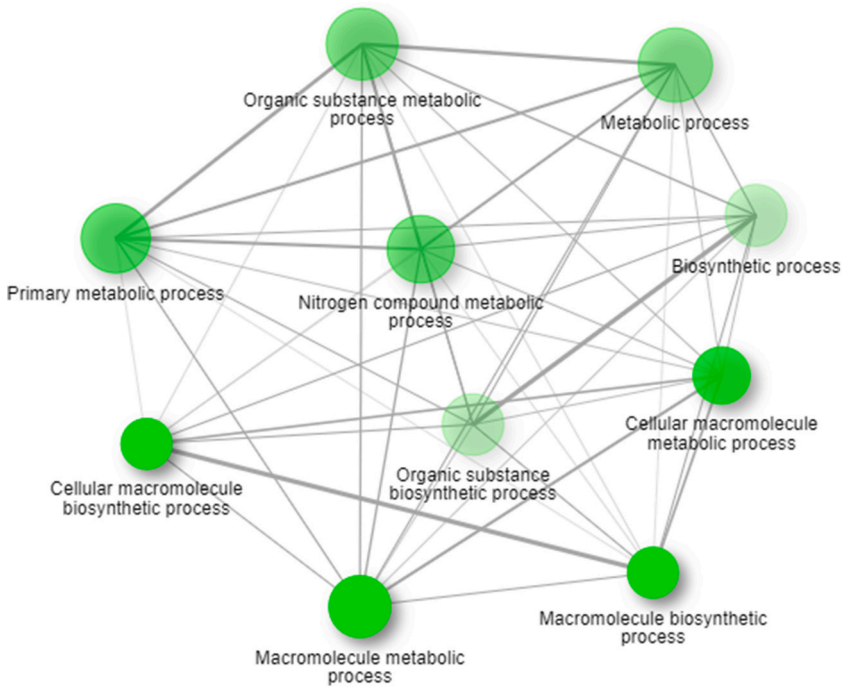

Fig. 2. The network visualizes the relationship between the enriched pathways in C-opt. Nodes (representing pathways) are connected if they share 20 % or more genes. Darker nodes indicate more significantly enriched gene sets. Thicker edges between nodes signify a higher degree of overlap in shared genes.

Table 3
Gene-set significantly upregulated (p-value <0.05) in C-stress compared to C-opt.

Locus	Gene	Functional annotation	Comments	(*)FPKM[a] C-opt	(*)FPKM[a] C-stress	P-value
lmo0823	*lmo0823*	oxidoreductase	Bacteriocins resistance; Cold tolerance	−2429	−3092	0,00052749
lmo1525	*lmo1525*	recombination protein RecS	Cold tolerance; DNA damage	−2419	−2826	0,00060162
lmo0919	*lmo0919*	antibiotic ABC transporter ATP-binding protein	Antibiotic resistance	−1577	−2329	0,00027221
lmo1910	*lmo1910*	oxidoreductase	Stress response; Dessication response	−2433	−2676	0,04317871
lmo2338	*pepC*	aminopeptidase C	Cold tolerance	−1660	−2311	0,00137432
lmo0956	*lmo0956*	putative N-acetyl-glucosamine-6 phosphate deacetylase	Amino-sugar metabolism; TA[b] biosynthesis; Antibiotic resistance	−2220	−2473	0,00166559
lmo2005	*lmo2005*	oxidoreductase	Acidic stress	−2591	−2699	0,00958239
lmo2695	*lmo2695*	dihydroxyacetone kinase subunit DhaK	PrfaA and sigB regulated; virulence; glycerol metabolism	−2042	−3249	7,9608E-05
lmo0613	*lmo0613*	oxireductase	Oxidative stress; Antibiotic resistance	−1722	−2813	0,00017482
lmo2478	*trxB*	Thioredoxin reductase	Oxidative stress	−2868	−3651	0,00307804
lmo2157	*sepA*	Hypotetical protein	Acidic stress	−2495	−2815	0,00551796
lmo1053	*PdhB*	pyruvate dehydrogenase subunit E1 beta	DNA damage	−2975	−3457	0,00448443

[*] FPKM Value converted to -Log_2.
[a] **FPKM**: Fragments Per Kilobase of Transcript per Million Mapped Reads.
[b] **TA:** Teichoic acids.

Additionally, "Primary metabolic process" and "Nitrogen compound metabolic process" exhibited moderate enrichment levels (1.3).

Enrichment analysis of C-stress identified, the "Flagellar Assembly" pathway as significantly enriched. It is essential for *L. monocytogenes* motility, survival and persistance in challenging environments [23]. The flagellum is also well known to be a strong immunogen and impacts bacterial infection [24].

Moreover, it seems that such bacteria responds to stress by modulating the transcription of genes involved in "PTS" and "fructose and mannose metabolisms", as confirmed by Quesille-Villalobos et al. This study highlights how *L. monocytogenes* activates genes involved in these pathways to adapt to low temperatures [25]. For example *lmo2000* and *lmo2001* are involved in mannose metabolism. They play a role in the adaptive response of *L. monocytogenes* during food processing or within the host and are regulated by sigma factor (σB), which is crucial for stress response and virulence [26]. Furthermore, as described by Gahan, C.G.M., & Hill, C. (2014) [27]. *lmo0914* seems to positively influence adaptation to acidic environments and infection establishment. *lmo0084* and *lmo0377* in *L. monocytogenes*, are up-regulated to face low temperature but also have roles in maintaining pH homeostasis and surviving to acidic environments [28].

Interestingly, all the unique proteins with significantly higher FPKM values in C-stress are involved in stress response and virulence pathways (Table 3). Among these proteins, Lmo0823 functions as an aldo/keto reductase. Melian et al. (2021) observed that the overexpression of Lmo0823 in sessile cells of *L. monocytogenes* treated with lactocin AL705 at low temperatures (10 °C) suggests a specific response to these environmental stressors and potentially contributes to the activation of the oxidative stress response pathway.

Additionally, Lmo1910 protein is involved in general stress and desiccation responses [29]; while Lmo0613, described by Ref. [30], shows increased transcriptional activity in cells exposed to ClO2 (chlorine dioxide), indicating a potential role in conferring resistance to oxidative stress in *L. monocytogenes*.

The gene *lmo2005* plays a role in controlling pyruvate flux and the NAD/NADH ratio within the cell. It has been demonstrated that transcriptional regulator YtoI, which influence cellular metabolism and virulence pathways, can regulate the expression of *lmo2005*, suggesting its involvement in response to environmental stresses or during infection [31].

Interestingly, thioredoxin reductase (TrxB), encoded by *lmo2478* and pyruvate dehydrogenase E1 beta-subunit (PdhB), encoded by *lmo1053,* are also significantly upregulated at transcriptomic level (−3457 and −3651 respectively) and are uniques to C-stress [17]. TrxB is associated with oxidative stress responses and is regulated by the peroxide operon PerR, showing upregulation in harsh environmental conditions, such as after 48 h of desiccation on stainless steel and under acid shock at pH 3.0 with HCl in highly acid-tolerant field strains. PdhB is involved in carbohydrate metabolism and exhibits high induction in response to redox shock, low temperatures, and lactocin exposure.

Two proteins seems to play crucial roles in L. monocytogenes' not only in adaptation to stress but also in pathogenicity. SepA's is known to be controlled by both *PrfA* and *σB*: in fact, its deletion has been shown to decrease virulence [32]. Furthermore, it is produced in response to acid shock [33]. The Lmo2695 protein and its homologs Lmo2696 and Lmo2697 are crucial for glycerol metabolism and stress adaptation, particularly regulated by *σB* Lmo2695 is a crucial component regulated by SigB. This protein, along with its homologs, is involved in glycerol metabolism: the reduction in their expression in a Δ-*σB* mutant background correlates with impaired growth on glycerol, indicating their crucial role in stress response mechanisms [34].

The recombination protein S (RecS) and aminopeptidase C (PepC) are both involved in strategies employed by *L. monocytogenes* to face cold temperatures [35,36]. RecS is involved in DNA repair mechanisms, particularly in the presynaptic stage of DNA double-strand break (DSB) repair [37].

Both Lmo0956 and Lmo0919 play significant roles in antibiotic resistance and cell wall biosynthesis in *L. monocytogenes* [38].

Lmo0956 is involved in cell wall biosynthesis by deacetylating N-acetyl-glucosamine-6 phosphate to glucosamine-6 phosphate and acetate. This enzyme is essential for maintaining cell morphology, cell wall integrity, and sensitivity to antibiotics. *Lmo0956* is part of operon 150, along with *lmo0957* and *lmo0958*, which are involved in amino-sugar metabolism. Mutational analysis of *lmo0956* revealed its significance in bacterial physiology and virulence, suggesting it as a potential target for anti-listerial drug therapy [39].

Lmo0919, also known as Vga, plays a crucial role in mediating resistance to lincosamide antibiotics in *L. monocytogenes* by actively pumping them out of the bacterial ribosome [40].

5. Conclusions

Integrating immunoproteomics and RNA-seq data has offered insights into the molecular responses of *L. monocytogenes* to stress environments.

Through this comprehensive approach, the study highlighted the importance of proteins in response to specific environmental stressors, indicating their involvement in stress response, virulence significance and infection.

Moreover, the level of transcripts, which codify for TrxB and PdhB proteins were significantly higher when *L. monocytogenes* grows under stress conditions. These findings confirm the data obtained from the previous study in which these proteins were identified only under stress condition (C-stress).

While these findings are significant, they represent a step toward a deeper understanding of *L. monocytogenes* biology. The comparison between the partial proteome (immunoproteome) and the total transcriptome, indicates a need for further research studies to comprehensively analyze the relationship between transcript levels and whole proteome.

CRediT authorship contribution statement

Federica D'Onofrio: Writing – original draft, Methodology, Data curation, Conceptualization. **Francis Butler:** Validation, Investigation. **Ivanka Krasteva:** Writing – original draft, Methodology, Data curation. **Maria Schirone:** Visualization. **Luigi Iannetti:** Supervision, Investigation, Funding acquisition. **Marina Torresi:** Writing – review & editing, Methodology. **Chiara Di Pancrazio:** Formal analysis. **Fabrizia Perletta:** Formal analysis. **Marta Maggetti:** Writing – original draft, Formal analysis. **Maurilia Marcacci:** Methodology. **Massimo Ancora:** Data curation. **Marco Di Domenico:** Methodology. **Valeria Di Lollo:** Methodology. **Cesare Cammà:** Visualization, Resources. **Manuela Tittarelli:** Visualization, Supervision. **Flavio Sacchini:** Supervision. **Francesco Pomilio:** Validation, Resources. **Nicola D'Alterio:** Visualization, Resources. **Mirella Luciani:** Writing – review & editing, Conceptualization.

Data availability statement

The original contributions presented in the study are included in the "Supplementary material" section. For further inquiries please contact the corresponding author.*Listeria monocytogenes* genome deposited at the NCBI (Accession No: NC_003210). The raw sequencing data are avilable at the NCBI Sequence Read Archive under Bioproject No. PRJNA1111030.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e39832.

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