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Analysis of the survival capacity and transcriptional response of *Salmonella enterica* serovar Infantis under stress conditions of clinical and food safety relevance

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Abstract

Aims: Analyze the survival and transcriptional response of *Salmonella* Infantis (*S.* Infantis) against acid, oxidative, increased osmolarity, and thermal stresses.

Methods and results: 25 strains isolated from human, food, and veterinary sources in Brazil between 2013 and 2018 were analyzed. 8 $\log_{10}$ CFU ml^{-1} inoculums were exposed for 10 min, 1 h, or 24 h to acid stress (HCl pH 2.6); oxidative stress (15 mmol l^{-1} H_2O_2); NaCl 9%; refrigeration (4°C); freezing (−20°C); and heating (63/74°C). Most strains (n = 22) survived the acid stress after 10 min and 1 h. Strains maintained high populations under oxidative stress (>87.7%) and NaCl 9% (111.4%). No strains survived heating, while refrigeration prevented multiplication (∼101%), and freezing reduced populations (<92.6%). Strain 1143/14 had its transcriptome sequenced under acid and oxidative stresses and NaCl 9%. 14 up-regulated and 38 down-regulated transcripts under acid stress, and 407 up-regulated and 143 down-regulated under oxidative stress were expressed, associated with biological processes of response and adaptation. No transcripts were differentially expressed with NaCl 9%.

Conclusions: *Salmonella* Infantis survival against most stresses represents a challenge for its control. The efficacy of heating reinforced its importance to control *Salmonella* in food. While acid survival involved the expression of virulence and broad stress response genes, oxidative stress survival showed the expression of genes specific to the adaptation to this condition.

Impact Statement

Although *Salmonella* Infantis is globally relevant, few studies analyzed its stress response, which could improve its control, pathogenicity understanding, and infection prevention.

Keywords: salmonellosis; acid stress; oxidative stress; NaCl; transcriptome

Introduction

Infections caused by non-typhoid *Salmonella* serovars are among the world's most common diarrheic foodborne diseases, with an estimation of 150 million gastroenteritis cases and 600 thousand deaths annually (CDC 2023). *Salmonella enterica* subsp. *enterica* serovar Infantis (*S.* Infantis) is a ubiquitous, zoonotic, and foodborne serovar. It has been reported worldwide as one of the most frequently isolated *Salmonella* serovars (Thai et al. 2012, Crim et al. 2015, Marzel et al. 2016, EFSA and ECDC 2017, Gelaw et al. 2018), demonstrating a potential to become a public health and food safety concern due to its capacity to contaminate multiple sources. The infection of *S.* Infantis, as well as most *Salmonella* serovars, initiates with the consumption of contaminated food or water. Poultry meat, but also pork and cattle, are common *S.* Infantis reservoirs (Thai et al. 2012, Crim et al. 2015, Marzel et al. 2016, EFSA and ECDC 2017, Gelaw et al. 2018).

To prevent and reduce the contamination of food-producing plants by *Salmonella*, disinfectants such as hydrogen peroxide (H_2O_2) and acids have been employed (Møretrø et al. 2012). To avoid the multiplication of non-typhoid *Salmonella* in food, the U.S. Centers Disease Control and Prevention (CDC) recommends the storage of meat products below 4°C, while to promote their elimination and avoid transmission, it is recommended for meat products to be cooked at minimum temperatures of 63 up to 74°C, depending on the animal of origin (CDC 2024). Other strategies, such as increased osmolarity achieved by additional salt (NaCl) levels, are also employed to prevent the multiplication and dissemination of bacteria in food (Kim et al. 2017).

After the ingestion of contaminated food, *Salmonella* must survive over different barriers established by the host organism in order to colonize and disseminate. The first barrier is the acid gastric juice, with a pH ranging from 1.0 to 3.0 (Álvarez-

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Table 1. Isolation data of the 25 *Salmonella* Infantis strains studied isolated between 2013 and 2018 from food items ($n = 9$), humans ($n = 6$), food-producing environments ($n = 6$), animals ($n = 3$), and animal feed ($n = 1$) in Brazil.

Strain	Year	Source	Material	State
2385/13	2013	Food	Soy	PR
2950/13	2013	Human	Human feces	AL
3156/13	2013	Environmental	Disposable shoe cover	SC
124/14	2014	Animal	Swine feces	RS
210/14	2014	Environmental	Dragging swab	SC
583/14	2014	Food	Chicken carcass	SC
1143/14	2014	Animal	Chicken feces	RS
1409/14	2014	Human	Human feces	RS
1441/14	2014	Food	Mayonnaise	RS
4882/14	2014	Food	Chicken carcass	MG
342/15	2015	Food	Swine heart	SC
1809/15	2015	Animal feed	Meat animal meal	SC
3056/15	2015	Food	Chicken carcass	MG
5391/15	2015	Environmental	Disposable shoe cover	SC
5912/15	2015	Environmental	Cleaning wipe	SC
4447/16	2016	Food	Pork sausage	SC
5946/16	2016	Food	Pork rib	SC
11/17	2017	Environmental	Dragging swab	PR
3380/17	2017	Human	Human fecal swab	GO
3877/17	2017	Food	Chicken wings	MG
3906/17	2017	Environmental	Sieve residue	SP
4065/17	2017	Human	Human feces	PR
4069/17	2017	Human	Human blood	PR
942/18	2018	Human	Human fecal swab	RS
2676/18	2018	Animal	Avian reproductive matrix	GO

AL, Alagoas; GO, Goiás; MG, Minas Gerais; PR, Paraná; RS, Rio Grande do Sul; SC, Santa Catarina; SP, São Paulo.

Ordóñez et al. 2011, Campioni et al. 2020). After this step, bacteria must reach the intestine, move through the intestinal mucus, and invade the intestinal epithelium cells through M cells. After the entrance, *Salmonella* reaches the basal membrane of these cells, and is phagocytized by macrophages, which produce reactive oxygen species (ROS) to kill these bacteria. However, this microorganism may survive these defenses and disseminate to the liver and spleen, where they can multiply and establish systemic infections (Álvarez-Ordóñez et al. 2011, Imre et al. 2013, Campioni et al. 2020).

With the notable evolution and broader access worldwide to new generation sequencing, the study of foodborne and zoonotic bacteria, such as *S.* Infantis, was positively impacted. In Brazil, studies have addressed the genomic diversity of this serovar among different sources in the country (Monte et al. 2019, Vilela et al. 2022, Bertani et al. 2022). However, despite the numerous reports worldwide analyzing genomic aspects of this serovar, the same has not been observed for transcriptomic data. Transcriptomic analyses have been successfully employed to evaluate the gene expression of several foodborne bacteria, and are powerful tools for studies evaluating the role of probiotic microorganisms, biofilm formation, survival strategies in food, and the pathogenicity and virulence of bacteria in humans and animals (Lamas et al. 2019, Vieira et al. 2022). Therefore, studies evaluating the gene expression of *S.* Infantis in experimental conditions of relevance for human health and food protection are needed, especially when considering their great lack in the field of transcriptomics for this serovar.

Currently, Brazil is a leading global meat exporter (ABPA et al. 2024). Despite the economic impact and relevance of *S.* Infantis for food production and clinical sectors worldwide, there is a lack of studies analyzing the survival capacity and gene expression through transcriptomics of this serovar under challenging survival conditions. In this way, this study aimed to analyze the survival of *S.* Infantis under stress conditions relevant to human health and food safety and sequenced the transcriptome of a *S.* Infantis strain to verify possible patterns of gene expression occurring under these conditions.

Materials and methods

Bacterial strains

A total of 25 *S.* Infantis strains isolated between 2013 and 2018 from food items ($n = 9$), humans ($n = 6$), food-producing environments ($n = 6$), animals ($n = 3$), and animal feed ($n = 1$) were studied (Table 1). These strains were provided by the *Enterobacteriaceae* Laboratory of Oswaldo Cruz Foundation (LABENT/IOC/FIOCRUZ/RJ) and were isolated from some states of the Southeast, South, Northeast, and Midwest regions in Brazil. These 25 strains were selected among 80 previously analyzed *S.* Infantis strains to be representatives of the diverse years and sources of isolation and the genotypic profiles obtained by pulsed-field gel electrophoresis and single-nucleotide polymorphism analyses (Vilela et al. 2022). *Salmonella* Typhi (*S.* Typhi) ATCC 19430 and *Salmonella* Typhimurium (*S.* Typhimurium) ATCC 14028 reference strains were included in the survival assays for comparison.

Survival under stress conditions

Inoculum preparation

The survival of the 25 *S.* Infantis strains analyzed was evaluated as previously described (Gomes et al. 2018, Campioni et al. 2020) with minor modifications. Initially, strains were cultivated in 5 ml of Luria Bertani (LB) broth for 16–18 h at 37°C under agitation. After incubation, the bacterial growth was adjusted to the optical density (OD) of 0.2 with an absorbance of 600 nm ($OD_{600} = 0.2$). A 1 ml aliquot of the growth of each strain, containing estimated 1×10^8 CFU ml^{-1}, was cen-

trifuged at 8000 × *g* for 5 min, and after the supernatant was discarded, the resulting pellet was suspended in one of the conditions described below to promote the exposure to diverse types of stress. All assays were conducted in triplicate for each strain studied.

Exposure to acid and oxidative stress

In order to mimic the survival in the acid pH of the human gastric juice, pellets were suspended in 1 ml of LB broth acidified with HCl in pH 2.6. After that, bacterial suspensions were incubated for 10 min and 1 h at 37°C under agitation.

To simulate the ROSs produced by human macrophages and evaluate the action of H_2O_2, pellets were suspended in 1 ml of a NaCl 0.8% solution with additional 15 mmol l^{-1} H_2O_2. After that, bacterial suspensions were incubated for 10 min and 1 h at 37°C under agitation.

Exposure to increased osmolarity

In order to expose the samples to increased osmolarity with salt, as employed for the conservation of food items, pellets were suspended in 1 ml of LB broth with additional 9% NaCl. After that, bacterial suspensions were incubated for 10 min and 1 h at 37°C under agitation.

Exposure to minimum cooking temperatures

The survival of the strains under recommended minimum cooking temperatures was evaluated at 63°C (employed for beef, veal, lamb, pork, fresh ham, and fish meat) and 74°C (for chicken and turkey meat and leftovers). For that, pellets were suspended in 1 ml of LB broth pre-heated in the temperatures mentioned above and were incubated for 10 min and 1 h in a water bath under the same described temperatures. After the incubations, samples were chilled until reaching room temperature prior to the dilution.

Exposure to refrigeration and freezing

To evaluate the survival of the strains under domestic refrigeration temperature (4°C), pellets were suspended in 1 ml of LB broth pre-chilled at 4°C, and then aliquots were refrigerated at 4°C for 1 and 24 h. After the incubations, samples were chilled until reaching room temperature prior to the dilution.

To evaluate the survival of the strains under domestic freezing temperature (−20°C), pellets were suspended in 1 ml of LB broth pre-chilled at 4°C, and then aliquots were frozen at −20°C for 1 and 24 h. Frozen samples were thawed at room temperature prior to dilution.

Assessment of *Salmonella* population following stress exposure

After the incubations on each specific stress condition described above, serial dilutions of the bacterial suspensions were performed in phosphate buffered saline (PBS) 1 ×. CFU ml^{-1} counting was performed through the drop plate method by inoculating 10 µl of each dilution in LB agar as a drop, followed by 24 h of incubation at 37°C (Gomes et al. 2018, Campioni et al. 2020).

CFU ml^{-1} counts obtained were converted to $\log_{10}$. The survival values of the *S.* Infantis strains tested were compared to the survival of *S.* Typhimurium ATCC 14028 using an analysis of variance (ANOVA) with Tukey's post-hoc test. Significance was set to 0.05. Survival percentages were calculated by Sp = S ÷ 8 × 100, where *S* is the survival of each strain studied in CFU ml^{-1} ($\log_{10}$), 8 corresponds to the initial inoculum (1×10^8 CFU ml^{-1}) in $\log_{10}$, and Sp is the resulting survival percentage. All calculations and statistical analyses were performed with GraphPad Prism 8 (GraphPad Software) and Microsoft Excel.

RNA extraction and sequencing

S. Infantis strain 1143/14 was selected for the transcriptome sequencing under the acid, oxidative, and increased osmolarity stresses. These three stresses were selected for being more relevant in clinical and food safety contexts, usually more harmful for bacteria, and due to the capacity of most strains analyzed to survive, maintain populations, or even multiply under these conditions. Strain 1143/14 was selected because of its overall high capacity of survival under the three selected stresses and exposure times, which would favor the conduction of the assays and, possibly, the identification of upregulated genes associated with its survival.

The strain was initially cultivated in 5 ml of LB broth for 16–18 h at 37°C under agitation. Then, the bacterial growth was adjusted to $OD_{600} = 0.2$, and a 4 ml aliquot was centrifuged at 8000 × *g* for 5 min. The supernatant was discarded, and the resulting pellet was suspended in 4 ml of the following solutions: (1) control, with LB broth; (2) acidified LB broth with HCl in pH 2.6; (3) NaCl 0.8% solution with additional 15 mmol l^{-1} H_2O_2; (4) LB broth with additional 9% NaCl. After that, bacterial suspensions were incubated for 10 min (for acid stress) or 1 h (for the control, oxidative stress, and increased osmolarity) at 37°C and under agitation. After incubation, bacterial suspensions were immediately used for RNA extraction. RNA extractions were performed in triplicate for each of the four conditions tested.

The total RNA of the samples was extracted using the PureLink RNA Mini Kit (Thermo Fischer Scientific) according to the kit's protocol. Quantification of the RNA extracts obtained was performed with Qubit RNA Broad-Range Kit (Invitrogen). Quality control was performed with High Sensitivity RNA ScreenTape (Agilent Technologies). Depletion of ribosomal RNAs and RNA library preparations were carried out with Illumina Stranded Total RNA with Ribo Zero Plus kit (Illumina). Clusterization and sequencing were performed in Illumina NovaSeq 6000 using NovaSeq 6000 SP Reagent Kit (200 cycles).

Mapping of RNA-seq libraries and differential gene expression analysis

Raw sequence files (fastq) were deposited at the Sequence Read Archive (SRA; BioProject accession no. PRJNA1117524). Quality control of raw reads was performed with FastQC (Babraham Institute, Cambridge, UK). Raw reads were mapped with Kallisto version 0.48.0 (Bray et al. 2016) to *S.* Infantis reference transcriptome str. SARB 27 (GCA_000230875.1), downloaded through Ensembl Bacteria (https://bacteria.ensembl.org/index.html).

The subsequent analyses were conducted using the statistical computing environment R v.4.3.2 (https://www.r-project.org/) in RStudio v.2023.12.0 Build 369 and Bioconductor version 3.19 (Huber et al. 2015). *TxImport* package was used to read Kallisto outputs into the R environment and to quantify transcript data (Soneson et al. 2015). Normalization was performed using the trimmed mean of M values (TMM) method in *edgeR* (Robinson et al. 2010). Filtering was carried out to remove lowly expressed genes with <1 count per million

(CPM) in at least 5 or more samples. To identify differentially expressed transcripts (DETs), precision weights were first applied to each gene based on its mean-variance relationship using the voom function in *limma* (Ritchie et al. 2015), and then data was normalized with the TMM method in *edgeR*. Linear modeling and Bayesian stats were employed via *limma* to find DETs that were up- or down-regulated by 4-fold or more, with a false-discovery rate (FDR) of 0.01, that were represented as a volcano plot. Pearson correlation was used to cluster differentially expressed genes, which were then represented as heatmap with the data scaled by *Z*-score for each row.

The genes and encoded proteins of the DETs detected were identified by searching their accession numbers in the genome annotation of *S.* Infantis str. SARB 27 (GCA_000230875.1) in Ensembl Bacteria or at UniProt (https://www.uniprot.org/). Gene ontology (GO) of the biological process related to the identified genes was carried out using PantherDB 18.0 (https://www.pantherdb.org/).

Results

Acid, oxidative, and increased osmolarity stresses

The survival of the 25 *S.* Infantis strains studied under acid and oxidative stress and increased osmolarity is displayed in Fig. 1, and the survival percentages and survival decays in relation to the initial inoculum (1×10^8 CFU ml^{-1}) are displayed in Table 2.

After 10 min under acid stress (Fig. 1a, Table 2), the 25 *S.* Infantis strains analyzed showed an average survival of 82.0% (average decay of 1.4 $\log_{10}$). After 1 h, three strains did not survive, and an average survival of 34.8% (average decay of 5.2 $\log_{10}$) was obtained. Respectively, *S.* Typhimurium ATCC 14028 survived 105.0% and 46.3% (decays of −0.4 and 4.3 $\log_{10}$) after 10 min and 1 h, while *S.* Typhi ATCC 19430 survived 25% (decay of 6.0 $\log_{10}$) only after 10 min. In comparison to *S.* Typhimurium ATCC 14028, 20 *S.* Infantis strains statistically had lower survival after 10 min, and 13 after 1 h ($P < .05$).

After 10 min of exposure to oxidative stress (Fig. 1b, Table 2), the 25 *S.* Infantis strains showed an average survival of 100.3%, without an average decay. After 1 h, one strain did not survive, and an average survival of 87.7% (average decay of 1.0 $\log_{10}$) was obtained. Respectively, *S.* Typhimurium ATCC 14028 survived 98.8% and 97.5% (decays of 0.1 and 0.2 $\log_{10}$) after 10 min and 1 h, while *S.* Typhi ATCC 19430 survived 72.1% (decay of 2.2 log10) only after 10 min. In comparison to *S.* Typhimurium ATCC 14028, two *S.* Infantis strains statistically showed higher survival after 10 min, while 14 showed lower survival after 1 h ($P < .05$).

The exposure to NaCl 9% (Fig. 1c, Table 2) resulted for the 25 *S.* Infantis strains on an average survival of 111.4% and average decay of −0.9 $\log_{10}$, either after 10 min or 1 h. After 10 min and 1 h, respectively, *S.* Typhimurium ATCC 14028 survived 98.8% and 101.3% (decays of 0.1 and −0.1 $\log_{10}$), and *S.* Typhi ATCC 19430 survived 100% and 97.5% (decay of 0.2, only after 1 h). In comparison to *S.* Typhimurium ATCC 14028, 18 *S.* Infantis strains statistically showed higher survival after 10 min, and 14 after 1 h ($P < .05$).

Thermal stresses

After 10 min and 1 h of exposure to 63°C and 74°C, none of the 25 *S.* Infantis strains analyzed, *S.* Typhi ATCC 19430, or *S.* Typhimurium ATCC 14028 survived the heating stresses. Their survival under refrigeration and freezing is displayed in Fig. 1, and the survival percentages and survival decays in relation to the initial inoculum (1×10^8 CFU ml^{-1}) are displayed in Table 2.

After 1 h and 24 h, respectively, under 4°C, the 25 *S.* Infantis strains showed average survivals of 101.3% and 100.7%, both with an average decay of −0.1 $\log_{10}$. Respectively after 1 h and 24 h, *S.* Typhimurium ATCC 14028 survived 101.3% and 100.0% (decay of −0.1 $\log_{10}$ only after 1 h), and *S.* Typhi ATCC 19430 survived 106.3% and 107.5% (decays of −0.5 and −0.6 $\log_{10}$). In comparison to *S.* Typhimurium ATCC 14028, one *S.* Infantis strain statistically showed lower survival after 1 h, while one showed lower and two showed higher survival after 24 h ($P < .05$).

Under −20°C, the 25 *S.* Infantis strains survived, respectively, 92.6% after 1 h (decay of 0.6 $\log_{10}$) and 82.2% after 24 h (decay of 1.4 $\log_{10}$). Respectively after 1 and 24 h, *S.* Typhimurium ATCC 14028 survived 105.0% and 100.0% (decay of −0.4 $\log_{10}$ only after 1 h), and *S.* Typhi ATCC 19430 survived 103.8% and 76.3% (decays of −0.3 and 1.9 $\log_{10}$). In comparison to *S.* Typhimurium ATCC 14028, 21 *S.* Infantis strains after 1 h and all 25 after 24 h showed statistically lower survival ($P < .05$).

Transcriptomic analyses

After quality checking, alignment, normalization, and filtering of the transcriptomes, lowly expressed genes were removed (Fig. 2), reducing the number of transcripts from 4530 to 4028. Principal component analyses (PCAs) showed that the major variation in expressed transcripts among the four conditions (control, NaCl, acid, and H_2O_2) was observed in the transcriptome of oxidative stress, followed by the acid stress (Fig. 3).

In comparison to the control condition, acid stress transcriptome showed 14 up-regulated and 38 down-regulated DETs (Fig. 4a; Fig. 5a; Supplementary Table 1). 5 of the 14 up-regulated DETs were not identified to a gene level (Table 3; Supplementary Table 1). The identified DEGs under the acid stress were demonstrated by GO enrichment to be associated with biological processes of response to stimulus and cellular, metabolic, and homeostatic processes (Fig. 6).

The oxidative stress transcriptomes showed 407 up-regulated and 143 down-regulated genes (Fig. 4b; Fig. 5b; Supplementary Table 2), and 23 and 9 DETs, respectively, were not identified to a gene level (Table 3; Supplementary Table 2). The 10 most up-regulated genes in the oxidative stress were *acs* (acetyl-coenzyme A synthetase), *argI* (ornithine carbamoyltransferase subunit I), *artJ* (ABC transporter arginine-binding protein 1), *cysD* (sulfate adenylyltransferase subunit 2), *fadA* (3-ketoacyl-CoA thiolase), *fadB* (fatty acid oxidation complex subunit alpha), *glaH* (glutarate 2-hydroxylase), *gltI* (glutamate/aspartate import solute-binding protein), *kdpA* (potassium-transporting ATPase potassium-binding subunit), and *kdpB* (potassium-transporting ATPase ATP-binding subunit). The identified DEGs under the oxidative stress were demonstrated by GO enrichment to be associated with biological processes of regulation, cellular, metabolic, and homeostatic processes, localization, locomotion, and response to stimulus (Fig. 6).

According to the parameters established for the classification of DETs, no genes were up- or down-regulated in the

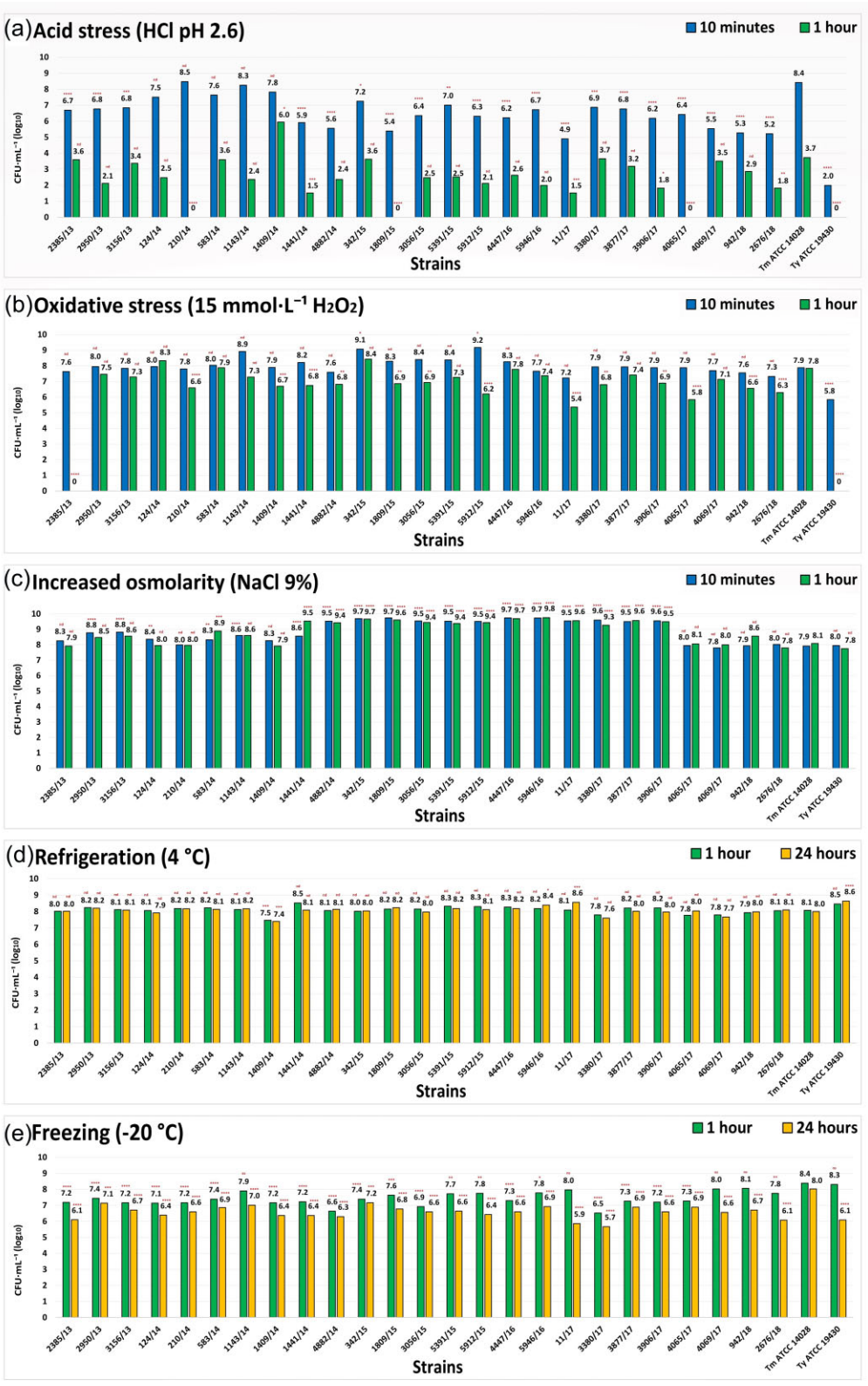

Figure 1. Survival rates of 25 *Salmonella* Infantis (*S.* Infantis) strains after exposure to acid stress—HCl pH 2.6 (a), oxidative stress—15 mmol l^{-1} H_2O_2 (b), increased osmolarity—NaCl 9% (c), refrigeration at 4 °C (d), and freezing at −20 °C (e) for 10 min (blue), 1 h (green), or 24 h (yellow). Survival values of the 25 *S.* Infantis strains and *Salmonella* Typhi (Ty) ATCC 19430 were compared to the values of *Salmonella* Typhimurium (Tm) ATCC 14028 using ANOVA with Tukey's post-hoc test ($P < .05$). In red, each "∗" indicates the level of statistical difference, while "nd" means no statistical difference.

Table 2. Survival percentage and decay of 25 *Salmonella* Infantis strains isolated from diverse sources after 10 min and 1 h of exposure to acid stress (pH 2.6), oxidative stress (15 mmol l^{-1} H_2O_2), increased osmolarity (NaCl 9%), and after 1 h and 24 h at 4°C and −20°C.

Strains	Acid stress (HCl pH 2.6)				Oxidative stress (15 mmol l^{-1} H_2O_2)				Increased osmolarity (NaCl 9%)				Refrigeration (4 °C)				Freezing (−20 °C)			
	Survival (%)		Survival decay (log_{10})		Survival (%)		Survival decay (log_{10})		Survival (%)		Survival decay (log_{10})		Survival (%)		Survival decay (log_{10})		Survival (%)		Survival decay (log_{10})	
	10 min	1 h	10 min	1 h	10 min	1 h	10 min	1 h	10 min	1 h	10 min	1 h	1 h	24 h	1 h	24 h	1 h	24 h	1 h	24 h
2385/13	83.8	45.0	1.3	4.4	95.0	–	0.4	–	103.8	98.8	−0.3	0.1	100.0	100.0	0.0	0.0	90.0	76.3	0.8	1.9
2950/13	85.0	26.3	1.2	5.9	100.0	93.8	0.0	0.5	110.0	106.3	−0.8	−0.5	103.8	102.5	−0.2	−0.2	92.5	88.8	0.6	0.9
3156/13	85.0	42.5	1.2	4.6	97.5	91.3	0.2	0.7	110.0	107.5	−0.8	−0.6	101.3	101.3	−0.1	−0.1	90.0	83.8	0.8	1.3
124/14	93.8	31.3	0.5	5.5	100.0	103.8	0.0	−0.3	105.0	100.0	−0.4	0.0	101.3	98.8	−0.1	0.1	88.8	80.0	0.9	1.6
210/14	106.3	–	−0.5	–	97.5	82.5	0.2	1.4	100.0	100.0	0.0	0.0	102.5	102.5	−0.2	−0.2	90.0	82.5	0.8	1.4
583/14	95.0	45.0	0.4	4.4	100.0	98.8	0.0	0.1	103.8	111.3	−0.3	−0.9	102.5	101.3	−0.2	−0.1	92.5	86.3	0.6	1.1
1143/14	103.8	30.0	−0.3	5.6	111.3	91.3	−0.9	0.7	107.5	107.5	−0.6	−0.6	101.3	102.5	−0.1	−0.2	98.8	87.5	0.1	1.0
1409/14	97.5	75.0	0.2	2.0	98.8	83.8	0.1	1.3	103.8	98.8	−0.3	0.1	93.8	92.5	0.5	0.6	90.0	80.0	0.8	1.6
1441/14	73.8	18.8	2.1	6.5	102.5	85.0	−0.2	1.2	107.5	118.8	−0.6	−1.5	106.3	101.3	−0.5	−0.1	90.0	80.0	0.8	1.6
4882/14	70.0	30.0	2.4	5.6	95.0	85.0	0.4	1.2	118.8	117.5	−1.5	−1.4	101.3	101.3	−0.1	−0.1	82.5	78.8	1.4	1.7
342/15	90.0	45.0	0.8	4.4	113.8	105.0	−1.1	−0.4	121.3	121.3	−1.7	−1.7	100.0	100.0	0.0	0.0	92.5	90.0	0.6	0.8
1809/15	67.5	–	2.6	–	103.8	86.3	−0.3	1.1	121.3	120.0	−1.7	−1.6	102.5	102.5	−0.2	−0.2	95.0	85.0	0.4	1.2
3056/15	80.0	31.3	1.6	5.5	105.0	86.3	−0.4	1.1	118.8	117.5	−1.5	−1.4	102.5	100.0	−0.2	0.0	86.3	82.5	1.1	1.4
5391/15	87.5	31.3	1.0	5.5	105.0	91.3	−0.4	0.7	118.8	117.5	−1.5	−1.4	103.8	102.5	−0.3	−0.2	96.3	82.5	0.3	1.4
5912/15	78.8	26.3	1.7	5.9	115.0	77.5	−1.2	1.8	118.8	117.5	−1.5	−1.4	103.8	101.3	−0.3	−0.1	97.5	80.0	0.2	1.6
4447/16	77.5	32.5	1.8	5.4	103.8	97.5	−0.3	0.2	121.3	121.3	−1.7	−1.7	103.8	102.5	−0.3	−0.2	91.3	82.5	0.7	1.4
5946/16	83.8	25.0	1.3	6.0	96.3	92.5	0.3	0.6	121.3	122.5	−1.7	−1.8	102.5	105.0	−0.2	−0.4	97.5	86.3	0.2	1.1
11/17	61.3	18.8	3.1	6.5	90.0	67.5	0.8	2.6	118.8	120.0	−1.5	−1.6	101.3	107.5	−0.1	−0.6	100.0	73.8	0.0	2.1
3380/17	86.3	46.3	1.1	4.3	98.8	85.0	0.1	1.2	120.0	116.3	−1.6	−1.3	97.5	95.0	0.2	0.4	81.3	71.3	1.5	2.3
3877/17	85.0	40.0	1.2	4.8	98.8	92.5	0.1	0.6	118.8	120.0	−1.5	−1.6	102.5	100.0	−0.2	0.0	91.3	86.3	0.7	1.1
3906/17	77.5	22.5	1.8	6.2	98.8	86.3	0.1	1.1	120.0	118.8	−1.6	−1.5	102.5	100.0	−0.2	0.0	90.0	82.5	0.8	1.4
4065/17	80.0	–	1.6	–	98.8	72.5	0.1	2.2	100.0	101.3	0.0	−0.1	97.5	100.0	0.2	0.0	91.3	86.3	0.7	1.1
4069/17	68.8	43.8	2.5	4.5	96.3	88.8	0.3	0.9	97.5	100.0	0.2	0.0	97.5	96.3	0.2	0.3	100.0	82.5	0.0	1.4
942/18	66.3	36.3	2.7	5.1	95.0	82.5	0.4	1.4	98.8	107.5	0.1	−0.6	98.8	100.0	0.1	0.0	101.3	83.8	−0.1	1.3
2676/18	65.0	22.5	2.8	6.2	91.3	78.8	0.7	1.7	100.0	97.5	0.0	0.2	101.3	101.3	−0.1	−0.1	97.5	76.3	0.2	1.9
Average (*S.* Infantis)	82.0	34.8	1.4	5.2	100.3	87.7	0.0	1.0	111.4	111.4	−0.9	−0.9	101.3	100.7	−0.1	−0.1	92.6	82.2	0.6	1.4
***S.* Typhimurium ATCC 14028**	105.0	46.3	−0.4	4.3	98.8	97.5	0.1	0.2	98.8	101.3	0.1	−0.1	101.3	100.0	−0.1	0.0	105.0	100.0	−0.4	0.0
***S.* Typhi ATCC 19430**	25.0	–	6.0	–	72.5	–	2.2	–	100.0	97.5	0.0	0.2	106.3	107.5	−0.5	−0.6	103.8	76.3	−0.3	1.9

Survival decay (log_{10}) was calculated considering the initial inoculum at 1×10^8 CFU ml^{-1}. Negative values indicate that the strain tested multiplied in relation to the initial inoculum tested.
Fields with "-" indicate that the strain did not survive the respective stress tested.

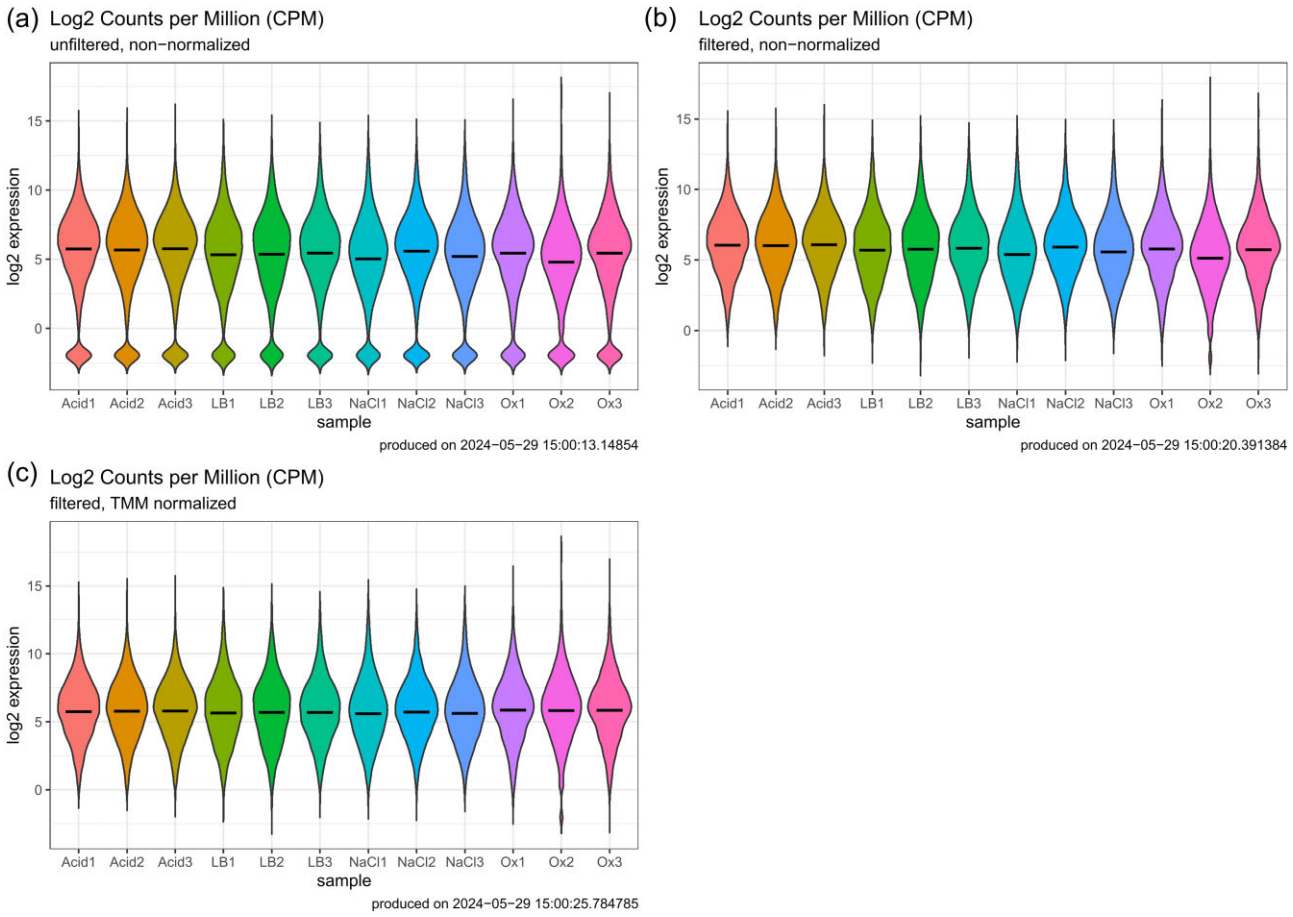

Figure 2. Effect of normalization and filtering on the 12 transcriptomes of *Salmonella* Infantis strain 1143/14 under the control condition (LB1, LB2, LB3), acid stress (Acid1, Acid2, Acid3), oxidative stress (Ox1, Ox2, Ox3), and increased osmolarity (NaCl1, NaCl2, NaCl3). (a) unfiltered and non-normalized transcriptomes. (b) non-normalized transcriptomes after filtering lowly expressed transcripts, with less than 1 count per million in at least 5 or more samples. (c) filtered transcriptomes after normalization using the trimmed mean of *M* values method.

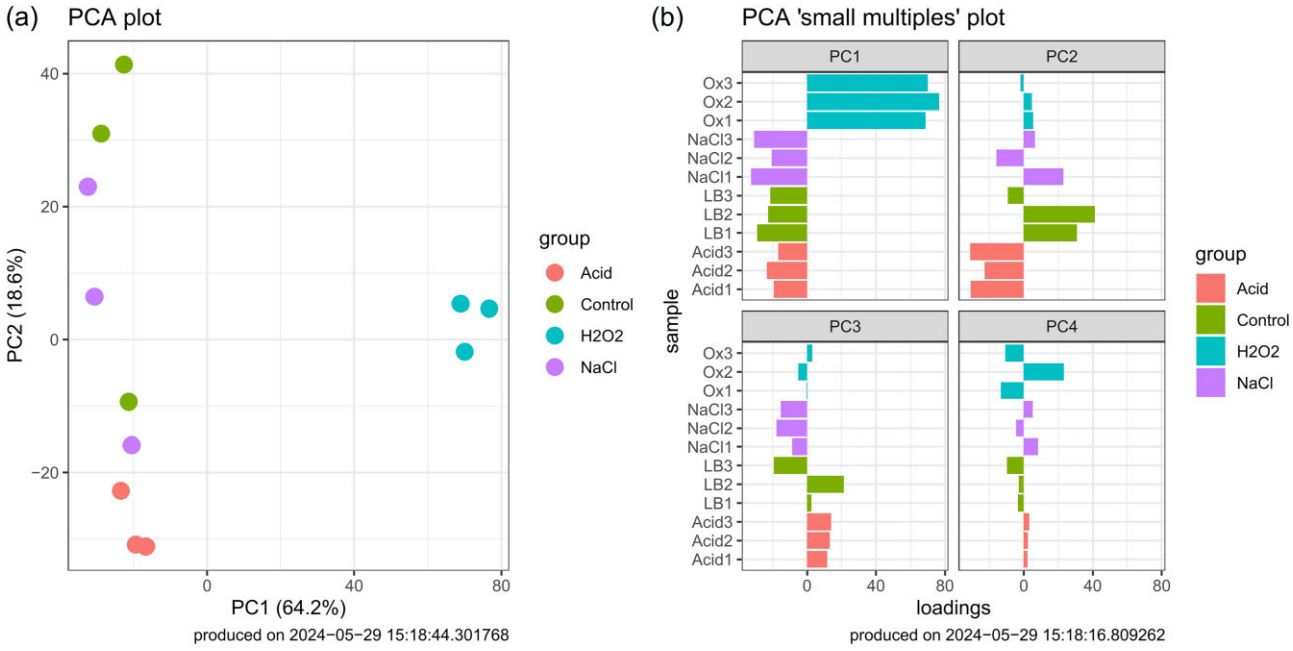

Figure 3. PCA of the 12 transcriptomes of *Salmonella* Infantis strain 1143/14 under the control condition (LB1, LB2, LB3), acid stress (Acid1, Acid2, Acid3), oxidative stress (Ox1, Ox2, Ox3), and increased osmolarity (NaCl1, NaCl2, NaCl3). (a) Principal Component (PC) 1 versus PC2 describes 82.8% of the total variance in the dataset. (b): PC1 to PC4 plots based on the four transcriptome groups analyzed, showing the higher variances in PC1 (oxidative stress) and PC2 (acid stress).

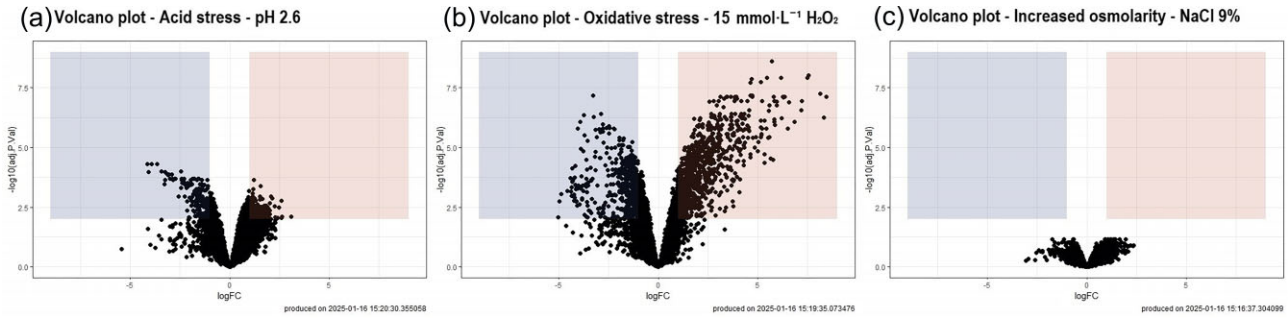

Figure 4. Volcano plots of the transcripts (black dots) of the transcriptomes of *Salmonella* Infantis strain 1143/14 under acid stress (a), oxidative stress (b), and increased osmolarity (c). DETs by 4-fold or more, with a FDR of 0.01, are contained in red or blue sectors, which represent up- and down-regulated genes, respectively. DETs were found exclusively in the acid (a) and oxidative (b) stresses.

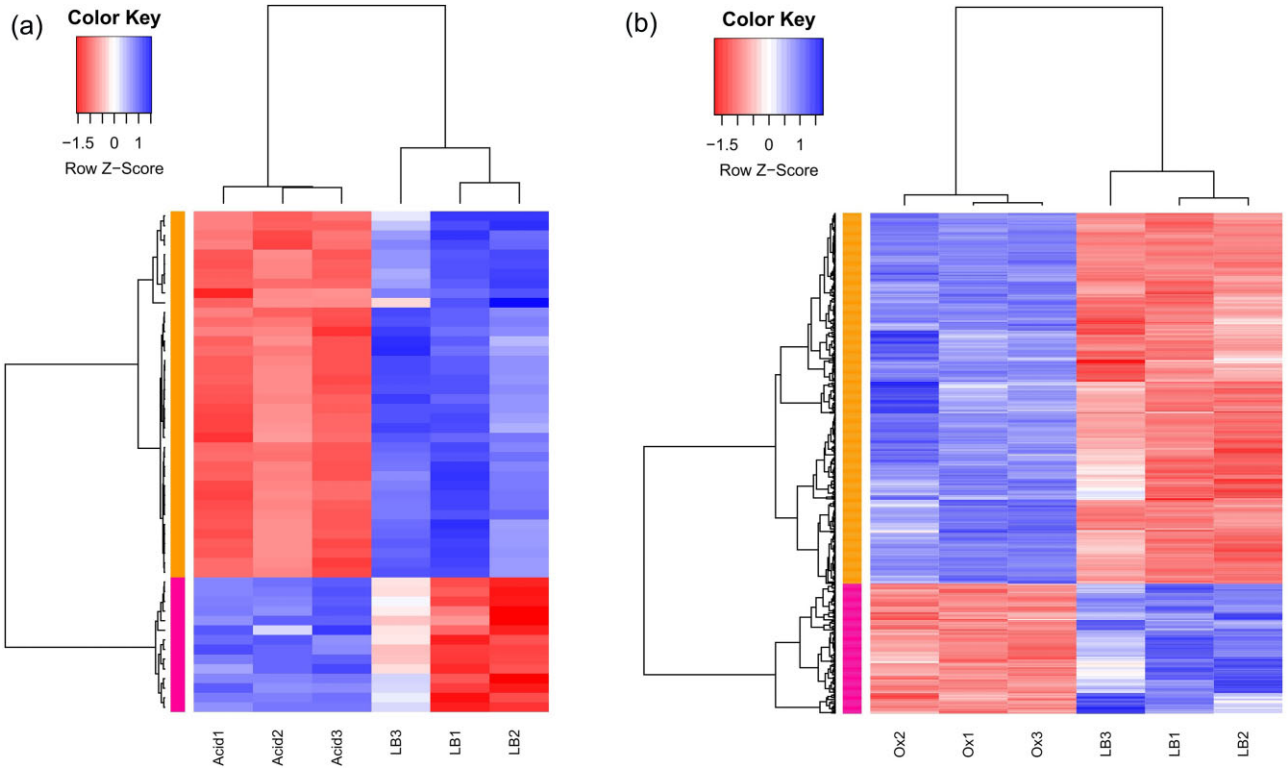

Figure 5. Heatmaps of the DETs of the transcriptomes of *Salmonella* Infantis strain 1143/14 under acid stress (Acid1, Acid2, Acid3; Figure a) and oxidative stress (Ox1, Ox2, Ox3; Figure b) in comparison to the control condition (LB1, LB2, LB3).

NaCl 9% transcriptome in comparison to the control condition (Fig. 3).

Discussion

Adaptation to stresses is essential for *Salmonella* to survive and multiply in harsh conditions in food and the environment, and to endure moderate variations in pH, temperature, humidity, and osmolarity in the human host. The conduction of studies evaluating this capacity of survival against stresses is pivotal to monitor the effectiveness of these measures against non-typhoidal *Salmonella*, especially in the context of the prevention of food contamination and human infections. Previous studies, mostly using *S.* Typhimurium as a non-typhoid serovar model, have demonstrated that mild stress exposures can co-protect bacteria from more than one type of stress (Gruzdev et al. 2011, Kim et al. 2021), influence their virulence to the host (by favoring biofilm formation, invasion in host tissues, survival within cells of the immune system) (Hosseini et al. 2024), and even stimulate the development of antimicrobial resistance (Fanous et al. 2025). The genetic response to these stresses can be mediated by constantly expressed innate mechanisms and by adaptive mechanisms expressed by exposure that promote the adaptation to broad stress types (Guillén et al. 2021, Hosseini et al. 2024).

Although *S.* Infantis is among the most isolated and well-studied non-typhoid serovars worldwide due to its zoonotic nature and high prevalence (Thai et al. 2012, Crim et al. 2015, Marzel et al. 2016, EFSA and ECDC 2017, Gelaw et al. 2018), little is known about its current capacity to survive against mild stresses and the genetic traits behind this response. Therefore, the present study analyzed the survival

Table 3. Identified DETs and corresponding genes of the transcriptomes of *Salmonella* Infantis strain 1143/14 under acid and oxidative stress.

Stress	Regulation	DETs (*n*)	Identified genes (*n*)	Genes
Acid	Up	14	9	*grxA, iraM, lpxD, napF, narK, nasD, osmB, sifA, spiC*
	Down	38	38	*aceE, aceF, alaS, atpC, atpD, atpG, clpB, dnaK, folK, fusA, gcvP, glnA, glyS, groL, infB, lysS, priB, ptsO, rplB, rplB, rplD, rplF, rplO, rplP, rplQ, rplV, rplW, rpmD, rpoA, rpoB, rpoC, rpsC, rpsD, rpsE, rpsH, rpsK, rpsS, typA*
Oxidative	Up	407	384	*aceA, aceB, aceK, acs, actP, adhP, agp, aidB, ail*(∗), *aldB, araC, argA, argB, argC, argD, argG, argH, argI, argT, arnA, arnB, arnC, arnD, aroP, aroQ, artI, artJ, artP, asnA, astA, astB, astC, astD, astE, basR, bdm, betB, bolA, bsmA, carA, carB, cdh, citB, citE, clpS, crp, csiE, cspD, cstA, cutC, cycA, cydA, cysA, cysC, cysD, cysH, cysI, cysJ, cysK, cysN, cysP, cysT, cysW, dadA, dadX, dctA, deoR, dinI, dppA, dppB, dppC, dppD, dps, elaB, elmGT, entA, entB, entC, entE, entF, entH, eptA, exbB, exuT*(*), *fadA, fadB, fadD, fadE, fadH, fadI, fadJ, fadL, fbaB, fepA, fepB, fes, fes, fhuE, fliY, fumA, fumC*(*), *gabD, gabD1, gabP, gabT, gatB, gatB, gatC, gbpR, gdhA, glaH, glaR, glgS, glnQ, gltA, gltI, gltJ, gltK, gnsA, golB, gpmA, gsiB, gstB, higB-2, hisJ, hisP, hisQ, hmp, hmrR, hpcB, hpcD, hpcE, hupB, hutH, hutI, hutU, iaaA, ibpA, icd, ifcA, ilvB, ilvG, ilvM, ilvN, iolD, iraP, iroC, iroE, ivbL, katE, kbp* (*), *kdgM, kdpA, kdpB, kdpC, kdpD, lacG, lamB, lhgD, lldD, lldP, lldR, lpxO, lrp, lsrA, lsrC, lsrF, ltnD, maeB, malE, malK, malT, mdh, mglA, mglB, mglC, mgtS, mhbT, mngR, mntB*(*), *mntH, mntS, mscL, msrB, nagI, nagK, nagL, nanT, narI, ndk, nlpD, nrdE, nrdF, nrdH, nrdI, ompC, oppA, oppB, oppF, orf245, osmC, osmE, osmX, osmY*(*), *otnC, otnI, otnK, otsA, otsB, pagD, pagN, patA, pckA*(*), *pfeA, pgdA, phnO, phoH, phoN, pipB2, pipD, pla, pmrD, pmrR, por, potF, poxB, ppsA, pqaA*(*), *proV, proX, prpB, prpC, prpD, prpR, psiF, psp, pspA, pspB, pspD, pspG, putA, putP, qseC, queA, raiA, ramA, RBKS, rbsB, rcnB, rhaR*(*), *rhtC, rof, rpe, rpoS, rsd, rspA, rspB, sbmC, sbp, sdhA, sdhB, sdhC, sdhD, sgrR, sitA, slyB, sodA, soxS, spy, sra, srlA, sstT, steA, sucA, sucB, sucC, sucD, sufA, sufB, sufC, sufD, sufE, sufS, tabA, tal, tctA, tctD, tcyP, thlA, tisB, torD, treA, ucpA, ugd, ugpB, ugtL, ulaC, ulaC, uspB, uspF, uspG, uxaC, uxuA, vanX, virK, wrbA, yaeH, yaeP, yahO, yaiY, yaiZ, ybaY, ybdD, ybdZ, ybeL, ybgS, ybhQ, yccJ, ychH, yciE, yciF, yciG, yciW, ydfK, ydiE, yeaG, yeaH, yeaR, yebF, yebG, yebV, yeeA, yehZ, yejG, ygaM, ygbA, ygbE, ygdR, yghA, ygiW, yhaL, yhbO, yhcO, yibT, yicS, yidQ, yigI, yihV, yjbE, yjbJ, yjcH, yjfN, yjhB, yjiJ, yjiS, ykgO, yliH, ymdF, ymgE, yncE, ynhG, yoaG, yodD, yohO, yqjC, yqjD, yqjH, ysdC, ysgA, ytfE, ytfF, ytfJ, ytjA, yurO, zntA*
	Down	143	134	*aceE, aceF, ackA, acpP, btsT, btuD, cadA, cadB, ccmB*(*), *ccmC*(*), *ccmE*(*), *ccmF, ccmH, cntE, csdA, cydA, cydB, cydX, deaD, dmsA*(*), *dmsB*(*), *dmsC, eamB, emrA, emrB, fdnG, fdnH, flgJ, flhA, fliH, fliI, fliJ, fliK, focA, fruB, gloA, glpA, glpB, glpC, glpD, gpmI, grcA, guaB, hilA, hybB, hybC, hybD, hybE, hybF, hybG, hybO, iagB, invA, invF, invH, lpxD, lpxP, menD, mntP, mprA, mxiC, nemA, nemR, nrdD, orgA, orgB, orgC, pdhR, pflB, prfA, prgH, prgI, prgJ, prgK, prmC, prsE, pta, pykF, pyrG, rarD, rhaR*(**), *rhlE, rimP, rnt, rplD, rplW, sctC, sicA, sicP, sipA, sipB, sipC, sipD, sodB, sopA, sopE2, spaK, spaL, spaM, spaN, spaO, spaP, spaQ, spaR, spaS*(*), *sprB, sptP, suhB, tolC, treB, treC, tsgA, ubiV, ybgE, ycaD, ycaO, ydfO, ydiY, yecH, yhbU, yhcN, yjiE, yjiH, ynfF, yohJ, yohK*

*DETs/genes detected twice.
**DETs/genes detected three times.

of *S.* Infantis strains under several stress conditions for human health and food protection, as well as the transcriptional response of a strain with high survival under some of these conditions.

After the ingestion of contaminated food, the survival of *Salmonella* to the acid environment of the human gastric juice is a key step to allow its progression to the intestine, its main site of infection (Álvarez-Ordóñez et al. 2011, Campioni et al. 2020). In the present study, the 25 *S.* Infantis strains studied showed a relatively high average survival of 82% after 10 min of exposure to pH 2.6, while after 1 h, 22 strains were still able to survive under this condition. In comparison to *S.* Typhimurium ATCC 14028, most *S.* Infantis strains and *S.* Typhi ATCC 19430 had statistically lower survival values after 10 min ($P < .05$), while this difference was not as significant after 1 h (Fig. 1a). Similarly, previous studies reported that *S.* Infantis strains showed low or non-significant reductions in cell counts after the exposure to HCl pH 3.0 (Wang et al. 2020a) or pH 3.8 (Nielsen and Knøchel 2020).

The transcriptome of strain 1143/14 after the acid stress, in comparison to the control condition, showed a total of 14 up-regulated and 38 down-regulated DETs. Although most of the genes identified were not assigned to a biological process, some were interestingly related to cellular processes and stimulus response. The acid response in *Salmonella* (considering *S.* Typhimurium as a model serovar) has been described to comprise systems of pH homeostasis, modifications of membrane components, and synthesis of acid shock proteins that can be

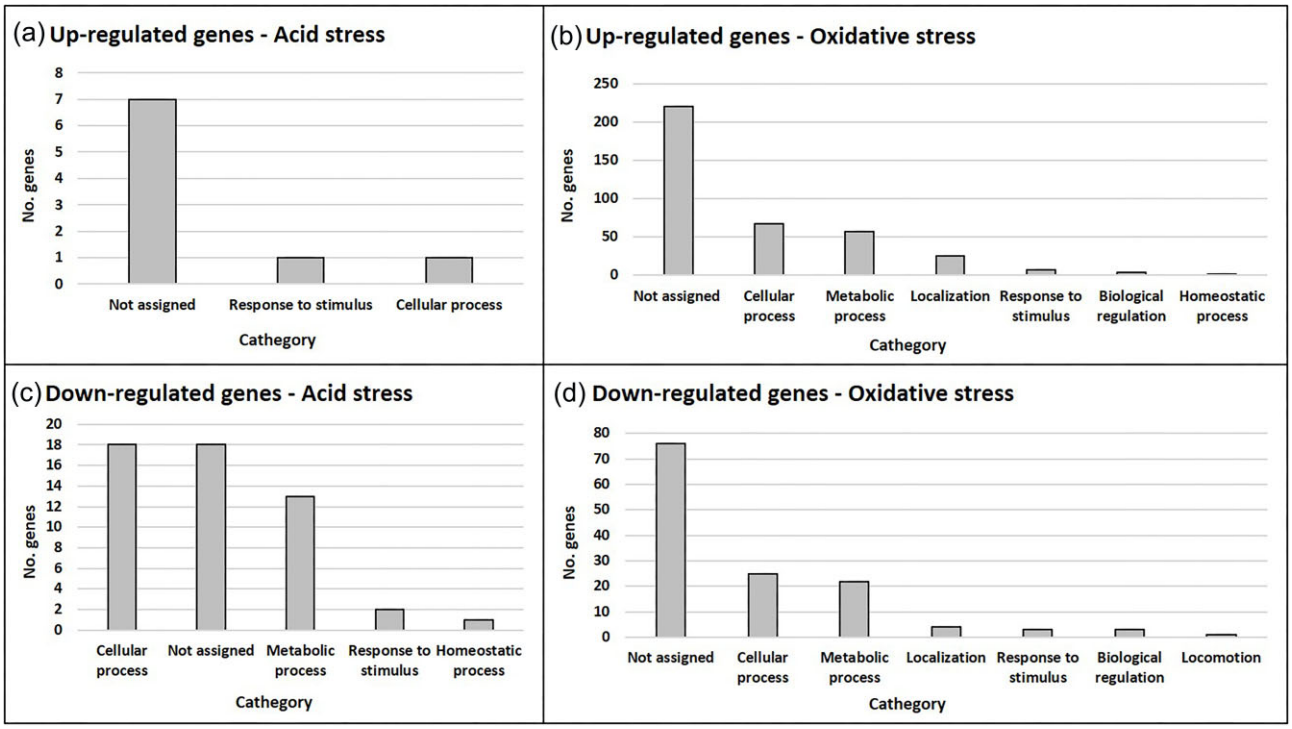

Figure 6. GO enrichment analysis showing the biological processes related to the identified differentially expressed genes found in the transcriptomes of *Salmonella* Infantis strain 1143/14 under acid stress (a and c) and oxidative stress (b and d).

induced by genes like *fur, ompR, phoP, phoQ, rpoE,* and *rpoS* (Álvarez-Ordóñez et al. 2011, Guillén et al. 2021). None of these genes were found up- or down-regulated among the *S.* Infantis transcriptome here analyzed.

There is also no data associating the identified up-regulated genes during the acid exposure to *S.* Infantis, as well as limited information about their expression and phenotypic influence in *Salmonella* during this type of stress. Previous research, however, demonstrated the expression of some of these genes in other *Salmonella* serovars in response to other stress conditions and virulence. For example, the overexpression of *grxA*, a gene related to oxidative stress, has been described in *Salmonella* Enteritidis after exposure to heat (Kobayashi et al. 2005). The activation of *napF* and *nark* under lower oxygen levels promotes nitrate utilization, which influences *S.* Typhimurium systemic infection in mice (Li et al. 2022). The expression of *osmB*, which encodes an osmotic inducible protein, can influence biofilm formation (Hermans et al. 2016). The overexpression of *sifA*, a *Salmonella* Pathogenicity Island 2 gene, was reported under oxidative and osmotic stresses (Kirthika et al. 2020). These results showed that *S.* Infantis strains can survive in low acid pH, similarly to the human stomach. This concerning finding alerts for the capacity of strains of this serovar to overcome this important barrier for the progression of infection in humans. The genes expressed during the acid exposure are related to the response to other types of stresses and/or to contribute to the virulence of *Salmonella*, which are necessary steps for the colonization and infection of the human intestine after the survival to the stomach's acid pH.

H_2O_2 is commonly used as a disinfectant in the food production field (Møretrø et al. 2012). In the present study, *S.* Infantis strains were tested in order to evaluate their survival capacity against 15 mmol l^{-1} H_2O_2. This condition was also tested to mimic the survival against ROS produced by host macrophages to kill *Salmonella* after the invasion of the intestinal epithelium and avoid the development of a systemic infection (Álvarez-Ordóñez et al. 2011, Imre et al. 2013, Campioni et al. 2020). The 25 *S.* Infantis strains studied were capable of maintaining the initial inoculum or multiplying after 10 min of exposure, with a 100.3% average survival, and also maintained a high survival average of 87.7% after 1 h (Fig. 1; Table 2). While after 10 min most *S.* Infantis strains showed no statistical difference on their survival compared to *S.* Typhimurium ATCC 14028, the populations decreased after 1 h (Fig. 1b). Wang and collaborators have reported that a 1-h exposure to 2 mmol l^{-1} to H_2O_2 did not promote reductions in *S.* Infantis populations (Wang et al. 2020a). Differently, Bezek and collaborators demonstrated 15%–50% reductions on the biofilm formation of a *S.* Infantis strain carrying the pESI endemic plasmid after a 15-min exposure to H_2O_2 1% (Bezek et al. 2023).

The transcriptomic analysis of strain 1143/14 after 1 h of exposure to 15 mmol l^{-1} H_2O_2 showed 407 up- and 143 down-regulated DETs, related to biological processes of regulation, cellular, metabolic, and homeostatic processes, localization, locomotion, and response to stimulus. To our knowledge, Wang and collaborators were the only ones reporting the expression of genes in *S.* Infantis under oxidative stress, specifically the down-regulation of *cyaR*, *micAC*, *invR*, *rybB*, and *dsrA* (Wang et al. 2020a).

The response to oxidative stresses in *Salmonella* is mainly mediated by genes and regulons related to broad stress response (Imlay 2009, Guillén et al. 2021). Several of these factors were found among the up-regulated genes in the *S.* Infantis transcriptome: sigma factor encoding *rpoS*; HPII catalase

encoding *katE*; *otsA* and *otsB*, related to threalose synthesis; *soxS* and *sodA*, part of the SoxRS regulon; and *dps*, *mnth*, and the *suf* operon, part of the OxyR system (Imlay 2009, Guillén et al. 2021). Previous studies exposing *S.* Typhimurium and *S.* Enteritidis strains to oxidative stress also identified up-regulated genes of gene families found in the present study, such as *bol* (Chen et al. 2023), *omp* (Li et al. 2015, Liu et al. 2020), *sly* (Cabezas et al. 2018), and *nrd* (Liu et al. 2020). Regarding the 10 most up-regulated genes of the oxidative stress transcriptome, the available information is limited on their relation to the response to this type of stress. Deletion of *acs*, encoding acetyl-coenzyme A synthetase, has been demonstrated to reduce the accumulation of *rpoS*, which could influence on the response to oxidative stress (Bergman et al. 2014). Genes *arg* and *art* are related to arginine metabolism, a component described to improve the resistance to oxidative stress (Margolis et al. 2023). The expression of *cys* regulon genes was described to be induced during oxidative stress (Turnbull and Surette 2010). The potassium-transporting encoding genes of the *kdp* operon have been reported to be activated during exposure to NaCl (Balaji et al. 2005).

Several *Salmonella* virulence genes were down-regulated under the oxidative stress, such as those related to flagella and fimbria encoding (*flg, flh, fli*) and those located in SPI-1 and SPI-2 (*inv, sic, sop, sip, spa*). This is curious, especially regarding SPI-2 genes, whose main role is the encoding of a type-three secretion system promoting the survival within macrophages after the invasion of intestinal epithelial cells (Wang et al. 2020b). Kim et al. (2022) have demonstrated that SPI-2 genes are expressed by *S.* Typhimurium during oxidative stress (Kim et al. 2022). We hypothesize that the down-regulation of these genes could be a particular strategy of *S.* Infantis to favor the expression of genes that allow its adaptation to the oxidative stress instead of expressing genes related to pathogenicity and infection.

These results demonstrated that *S.* Infantis strains can successfully survive the oxidative stress of H_2O_2, demonstrating not only a relative inefficacy of this compound for disinfection purposes but also suggesting a possible increased capacity of this serovar to survive against ROS species produced by the host defense. Survival under this condition was mediated by the expression of several genes related to the adaptation to this specific stress and other stresses. The down-regulation of known *Salmonella* virulence genes under the condition tested showed that further studies are necessary to understand specific mechanisms employed by *S.* Infantis and their phenotypic influence in the resistance to H_2O_2 exposure.

The high osmolarity achieved by increased NaCl levels is a well-known strategy used to control the bacterial proliferation in food (Kim et al. 2017). According to Brazilian guidelines for *Salmonella* isolation, brine has a limited effect on the survival of *Salmonella* strains due to their high survival capacity (Brasil 2011). This has been successfully demonstrated in the present study. The *S.* Infantis studied were able to multiply after 10 min and 1 h in contact with NaCl 9%, showing average rates of 111.4%. In comparison to *S.* Typhimurium ATCC 14028, the *S.* Infantis strains studied showed statistically higher or equivalent survival rates (Fig. 1c). Other studies have also addressed the high survival of this serovar in increased NaCl levels (Gruzdev et al. 2011, Pye et al. 2023).

It is interesting to note that no genes were differentially up- or down-regulated in the transcriptome of *S.* Infantis strain 1143/14 in this condition. To our knowledge, no data has been reported on the gene expression of *S.* Infantis during osmotic resistance, and no specific molecular pathways and mechanisms associated with this type of stress have been described. However, several studies have reported genes whose expression was increased in response to high salt levels and osmotic stresses in *S.* Typhimurium. Stress-response genes such as *proUP*, *rpoS*, *kdp*, *otsB*, and *ompC* were expressed after exposure to 0.3 M NaCl in different time points (Balaji et al. 2005). Encoding genes for virulence (*sopA*, *ssaD*), regulation and stress response (*ompR*, *phoP*, *cspC*, *uspA*), membrane transporters and fimbriae (*acrA*, *fimA*, *stcC*, *yehZ*), guanylate kinase (*gmK*), and acetyl transferase (*yhhK*) were expressed in the presence of 0.5 M NaCl (Elabed et al. 2016). Genes of *Salmonella* Pathogenicity Island 3 (*mgtBC*, *misL*, *cigR*, *slsA*, *fidL*, and *marT*), two-component system (*dcuBRS*), and sodium ion transport (*yihPO*) were expressed in response to NaCl 3% (Mandal et al. 2021).

Considering the high survival (and even multiplication) rates of the *S.* Infantis strains in contact with NaCl 9%, and the absence of differentially expressed genes in this condition when compared to the control, we may hypothesize that strains of this serovar could have a natural halotolerant ability. However, this capacity does not seem to be a particular feature of this serovar, considering that the *S.* Typhimurium and *S.* Typhi included in the analysis also displayed similar survival values. In addition, it would be interesting to evaluate in future studies if no differential gene expression would still occur with higher or lower levels than 9% of NaCl, so the hypothesis of a natural halotolerance not mediated by the overexpression of any genetic mechanisms could be confirmed. Therefore, the present results alert for a possible intrinsic capacity of *S.* Infantis to easily tolerate this stress, which would not be adequate as a strategy for the control of this serovar in food, for example.

Refrigerating and freezing food items are important strategies to prevent bacterial multiplication before their consumption, especially at home, and to extend their shelf life (Ricke et al. 2018). The effect of cold in bacterial cells leads to a drastic reduction of the biochemical reactions and metabolism, including ineffective ribosomal functioning and protein synthesis. This stress may also promote damage to bacterial membranes, leading to cell death (Ricke et al. 2018). The *csp* genes, which form the cold-shock operon, are the most well described mechanisms associated with the response to cold stress in *Salmonella* (Ricke et al. 2018).

In the present study, as expected, refrigeration at 4°C was able to maintain the initial inoculums, evidenced by average survival percentages around 100%–101% either after 1 or 24 h (Table 2). The survival rates of the vast majority of the *S.* Infantis strains analyzed were statistically similar to *S.* Typhimurium ATCC 14028, while *S.* Typhi ATCC 19430 showed higher survival values, being even statistically superior after 24 h ($P < .05$) (Fig. 1d). Pye and collaborators have also reported a high survival capacity of *S.* Infantis against long-term refrigeration (Pye et al. 2023). Freezing at −20 °C was able to reduce the initial inoculum of the *S.* Infantis strains analyzed in an average of 0.6 $\log_{10}$ after 1 h and 1.4 log10 after 24 h, resulting in survival rates of 92.6% and 82.2% after 1 h and 24 h, respectively (Table 2). *Salmonella* Typhimurium ATCC 14028 had a statistically higher survival than the majority of the *S.* Infantis strains tested, similarly to *S.* Typhi 19 430 (Fig. 1e). A factor that could explain this reduction during freezing is the cell damage caused by the formation of

ice crystals, considering that LB broth was used with no protective agent for this test. This should be taken into consideration especially because meat and other food have been hypothesized to play a protective role to maintain *Salmonella* viable during freezing (Müller et al. 2012). Therefore, although freezing and refrigeration might not be useful for the eradication of *S.* Infantis, these methods remain effective at their use to prevent bacterial multiplication prior to food preparation and consumption.

Heating remains as a key and effective method to eliminate foodborne bacteria like *Salmonella* from food, especially in a domestic context, due to its capacity to denature proteins and inactivate enzymes (Doyle and Mazzotta 2000, Jarvis et al. 2016). The gene response to heat in *Salmonella* also involves the action of the regulon of *rpo* genes (Guillén et al. 2021). In this study, heating at 63°C and 74°C, regardless of the incubation time, was the only stress test that completely eradicated all *S.* Infantis strains tested. Heating inactivation has been demonstrated to vary among *Salmonella* serovars (Doyle and Mazzotta 2000). For example, our research group previously reported the survival of *S.* Enteritidis isolated in Brazil at 74°C (Campioni et al. 2020). Low levels of water in food and in controlled assays, such as desiccation, can also enhance *Salmonella* thermal survival (Gruzdev et al. 2011). In this way, the results obtained provided favorable preliminary results for the use of heating as an effective method to eliminate *S.* Infantis in food.

In conclusion, *S.* Infantis ability to successfully survive against most of the stress conditions tested represents a food safety challenge for its control and the prevention of human infections. The efficacy of minimum cooking temperatures reinforced the importance of heating to control *Salmonella* in food. Moreover, while the survival to acid involved the expression of genes associated with virulence and broad stress response, oxidative stress survival showed the expression of genes specific to the adaptation to this condition.

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Author contributions

Felipe Pinheiro Vilela (Conceptualization, Formal Analysis, Investigation, Methodology, Visualization, Writing – original draft), Dália dos Prazeres Rodrigues (Data curation, Investigation, Resources), Daniel Beiting (Investigation, Methodology, Project administration, Resources, Writing – review & editing), Juliana Pfrimer Falcao (Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Visualization, Writing – review & editing).

Supplementary data

Supplementary data is available at *JAMBIO Journal* online.

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Data availability

Transcriptome raw sequence files were deposited at the Sequence Read Archive (BioProject accession no. PRJNA1117524). Additional information can be found throughout the manuscript or in supplementary files.

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