Biofilm growth by *Listeria monocytogenes* on stainless steel and expression of biofilm-related genes under stressing conditions

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Abstract

This research was carried out to investigate the differences in adhesion and growth during biofilm formation of *L. monocytogenes* from different sources and clonal complexes. Biofilm by *L. monocytogenes* (isolates CLIST 441 and 7: both lineage I, serotype 1/2b, CC3; isolates 19 and 508: both lineage II, serotype 1/2c, CC9) was grown on stainless steel coupons under different stressing conditions (NaCl, curing salts and quaternary ammonium compounds—QAC), to determine the expression of different genes involved in biofilm formation and stress response. CLIST 441, which carries a premature stop codon (PMSC) in *agrC*, formed high-density biofilms in the presence of QAC (7.5% w/v) or curing salts (10% w/v). Reverse Transcriptase-qPCR results revealed that *L. monocytogenes* isolates presented differences in transcriptional profile of genes related to biofilm formation and adaptation to environmental conditions. Our results demonstrated how *L. monocytogenes* can survive, multiply and form biofilm under adverse conditions related to food processing environments. Differences in transcriptional expression were observed, highlighting the role of regulatory gene networks for particular serotypes under different stress responses.

Keyword *Listeria monocytogenes* · Biofilm · Stainless steel · Stressing conditions · Gene expression

Introduction

*Listeria monocytogenes* is the causative agent of listeriosis, a foodborne disease that affects mainly children, pregnant women, the elderly and immunocompromised individuals (Radoshevich and Cossart 2018). Due to its capacity to adhere and to form biofilms, *L. monocytogenes* can colonize food processing facilities and often persist in this environment for years, leading to cross contamination of foodstuffs (Møretrø and Langsrud 2004; Rodríguez-Melcón et al. 2019). *Listeria monocytogenes* can adhere on different materials, such as stainless steel, glass and polymers, favoring its environmental persistence (Carpentier and Cerf 2011). Nevertheless, *L. monocytogenes* is also known for other features that contributes for its persistence in the food processing environments, such as tolerance to disinfectants, to cold storage temperatures and high salt concentrations (Ryan et al. 2010; Belessi et al. 2011; Pieta et al. 2014; Lee et al. 2017). Thus, successful hygiene programs are required to control *L. monocytogenes* in the industry (Mazaheri et al. 2021).

*Listeria monocytogenes* can be characterized into four different lineages (I, II, III and IV), and each lineage contains specific serotypes (Haase et al. 2014; Maury et al. 2016). However, *L. monocytogenes* presents a highly heterogeneous population, that generates conflicting results among previous studies that evaluated the diversity of biofilm formation and origin of the strains (Borucki et al. 2003; Doijad et al. 2015; Lee et al. 2019). Moreover, persistent strains can reside more than decades in processing plants environments (Orsi et al. 2020).
and present different genetic traits when compared to transient strains (Verghese et al. 2011).

Several genes regulate biofilm development, allowing bacterial survival under adverse environmental conditions (Keeney et al. 2018). Despite being also probably related to L. monocytogenes pathogenicity, the molecular mechanisms responsible for the expression of biofilm related genes are complex and regulated by intrinsic and extrinsic factors (Lemon et al. 2007; Bonsaglia et al. 2014; Nowak et al. 2017; Lee et al. 2019). Currently, the increasing use of advanced molecular tools, like whole genome sequencing (WGS), is allowing deeper studies on L. monocytogenes genomics and the proper understanding of the relatedness between persistent and transient strains at sub species level (Jagadeesan et al. 2019). WGS is being increasingly used to track outbreak strains, and it provides information to identify new biomarkers in specific populations that can aid to monitor L. monocytogenes persistence in processing environments (Verghese et al. 2011; Hurley et al. 2019).

The objective of this study was to assess the adhesion and biofilm formation under stressing conditions by persistent strains of L. monocytogenes from lineages I (serotype 1/2b) and II (serotype 1/2c), using a combination of phenotypic analyses and WGS to elucidate genetic diversity among L. monocytogenes from food processing environments.

**Materials and methods**

**Bacterial strains**

Four L. monocytogenes isolates were selected for this study, two from lineage I (CLIST 441 and 7, both from serotype 1/2b, and clonal complex CC3) and two from lineage II (19 and 508, both from serotype 1/2c, and clonal complex CC9), described in our previous study by Camargo et al. (2019) and presented in Table 1. These isolates possess genes involved in biofilm formation and stress response (flaA, agrB, agrC, lmo0444, lmo0445 and lmo0446), identified by WGS, according to data available at GenBank (National Center for Biotechnology Information, Bethesda, MD, USA, Supplementary Table 1. Visual comparison of genome homology was performed using Blast Ring Image Generator (BRIG), the software uses BLASTALL v 2.2.25 and the comparisons were performed with default settings (Alikhan et al. 2011). L. monocytogenes EGD-e genome (NC_003210.1) was used as reference strain. Phage search tool (PHAST) was used to verify the presence of phages related to persistence and resistance to stressing conditions (Zhou et al. 2011).

| Isolate | Year | State | Source type | Sample type | Serotype | PCR-serogroup | Lineage | CC (MLST) | ST (MLST) | SL (cgMLST) | CT (cgMLST) | Accession number |
|---------|------|-------|-------------|-------------|----------|--------------|---------|-----------|-----------|-------------|-------------|-----------------|
| 441     | 2010 | MT    | Food        | Raw beef    | 1/2b     | IIb          | CC3     | I         | ST3       | SL3         | CT4447       | ERR3199905      |
| 7       | 2009 | MG    | Food        | Refrigerated | 1/2b     | I            | CC3     | I         | ST3       | SL3         | CT4448       | ERR3199904      |
| 19      | 2009 | MG    | PE          | Meat handlers Before processing | 1/2c     | IIc          | CC9     | C         | ST9       | SL9         | CT4420        | ERR3199909      |
| 508     | 2012 | MG    | Food        | Raw beef    | 1/2c     | IIc          | CC9     | C         | ST9       | SL9         | CT4420        | ERR3199912      |
Adhesion on stainless steel under stressing conditions

Listeria monocytogenes isolates (Table 1) were evaluated for adhesion and biofilm formation under stressing conditions on stainless steel microtiter plates. The stressing conditions selected for this study were: (i) curing salts (5, 7.5 and 10%, w/v); (ii) NaCl (5, 7.5 and 10%, w/v), and (iii) quaternary ammonium compounds (QAC) at the concentration of 1:1,024, which corresponded to the Minimum Inhibitory Concentration (MIC), as previously established by our group (Silva et al. 2020). The composition of curing salts used in this study was NaCl (90% w/w), sodium nitrite (6% w/w), and sodium nitrate (4% w/w).

To test the effect of QAC on adhesion of L. monocytogenes, aliquots of 20 µL (10^9 cells/mL) of the cultures were transferred to wells of a stainless-steel microtiter plate containing 130 µL of Brain Heart Infusion broth (BHI, Oxoid Ltd., Basingstoke, UK) and 30 µL of a QAC based sanitizer at 1:1024 (Kalyclean S 370, Kalykim, Alvorada, RS, Brazil). Also, 20 µL aliquots of each L. monocytogenes culture were transferred to wells containing 160 µL of BHI (Oxoid) supplemented with curing salts (Exato, São Paulo, SP, Brazil) at three different concentrations (5, 7.5 and 10%, w/v) and sodium chloride (Vetec, Rio de Janeiro, RJ, Brazil), also with varying concentrations (5, 7.5 and 10%, w/v). Plates were incubated at 37 °C for 72 h. After incubation, the obtained cultures were discarded and the wells were washed three times with Phosphate Buffered Saline (PBS, pH 7.2) to remove non-adhered cells. To predict the biofilm production, the crystal violet method was employed described by Silva et al. (2017), in our previous study.

Additionally, assays were carried out to enumerate viable cells of L. monocytogenes isolates grown on stainless steel microtiter plates, either in the presence of 7.5% curing salts (for lineage I isolates: CLIST 441 and 7) and QAC at 1:1024 (MIC, for lineage II isolates: 19 and 508). Isolates, curing salts and QAC were distributed in stainless-steel microtiter plates, and incubated at 37 °C for 72 h. Then, non-adhered cells were removed by washing with PBS, and the adhered cells were removed with 200 µL of PBS added with Tween 80 at 1% (v/v, Dinâmica, Indaiatuba, SP, Brazil). Tenfold dilutions of the biofilm suspensions were done in PBS, drop plated (10 µL) on BHI agar (Oxoid) and incubated at 37 °C for 24 h, according to Herigstad et al. (2001), with modifications. The assays were performed as biological triplicates and included the appropriate controls (wells without curing salts and QAC). To consider a biofilm was formed, the minimum of 10^3 adhered cells per cm^2 was required (Minei et al. 2008; Winkelströter and De Martinis 2015). Results were expressed as CFU per cm^2, for sessile cells, with 2 mL of the homogenate corresponding 3.8 cm^2. Analysis of variance (p < 0.05) was applied to calculate the differences between groups using the program XLSat 2010.2.03 (Addinsoft).

Gene expression prediction by qPCR

Taking into consideration L. monocytogenes lineages I and II can present different tolerance to various stresses (Van Der Veen et al. 2008) and the phenotypic results from preliminary assays, selected biofilms grown on stainless steel coupons were recovered and RT-qPCR was performed to examine the transcriptional profile of different genes, important for biofilm growth and adaptation to environmental conditions (flaA, agrB and agrC) as well as stress-related genes (lmo0444, lmo0445 and lmo0446).

Two independent biological replicates were performed for the gene expression assays, using the protocol described above for biofilm formation by L. monocytogenes on stainless-steel coupons for isolates of lineage I (CLIST 441 and 7) in the presence of curing salts at 7.5% (w/v), and isolates of lineage II (508 and 19) in the presence of the QAC at 1:1024 (MIC). Biofilms were grown at 37 °C or 72 h.

RNA extraction was performed using RNasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocols. The extracted RNA was treated with RQ1 RNase-Free DNase (Promega, Madison, USA) and cDNA synthesis was performed by the GO Script Reverse Transcription System (Promega) according to the manufacturer’s instructions. The cDNA was used to perform the predictive expression of the genes selected (flaA, agrB, agrC, lmo0444, lmo0445 and lmo0446). The primers used in this study are shown...
in Table 2. All qPCR amplifications were performed with final volume of 20 µL, using 10 µL of GoTaq® qPCR Master (Promega, Madison, USA), 200 nMol of each primer, 2 µL of cDNA and ultrapure RNase free water to complete the final volume. The conditions for PCR reaction comprised an initial denaturation step at 95 °C for 2 min and 45 cycles of 95 °C for 15 s and 60 °C for 60 s. The dissociation curve program was followed to obtain the melting curve of the target analyzed.

**Results**

Whole genome comparison of the four isolates (7, 441, 19 and 508) was visualized by BRIG, revealing conservation and variation in gene content; *L. monocytogenes* EGD-e was used as reference strain (Fig. 1). Lineage II isolates (19 and 508) presented a higher conservation of genomic material between isolates than lineage I isolates (7 and 441). It is noteworthy that isolates 19 and 508 are persistent strains recovered four years apart from the same processing plant in Minas Gerais state, although these isolates did not express full length *comK* gene transcripts, related to persistence and carried a premature stop codon in *inlA*. All isolates presented the Stress survival islet 1 (SSI-1) in their genome.

Two different prophage regions were identified in the persistent isolates 19 and 508 (lineage II), while isolate 19 harbored an intact phage like region (position 160478 to 202258) associated to phage A118, isolate 508 harbored an intact different phage (position 673 to 51573), associated to LP_HM00113468 (Fig. 2). Despite the high genetic relatedness between these two isolates (19 and 508), highlighted by low number of genetic modifications within only 12 genes, the two distinct prophages found constitute major difference between their genomes. No intact prophage regions were found in the genome of isolates 7 and 441 (lineage I).

Absorbance values obtained on adhesion assays on stainless steel microtiter plates showed that in the presence of QAC or curing salts (7.5% and 10%) the isolate CLIST 441 (serotype 1/2b) formed higher-density biofilms, when compared to the other isolates tested (Fig. 3); interestingly, CLIST 441 harbors a PMSC in *agrC* (Suppl. Table). Data also revealed curing salts and sodium chloride treatments did not cause major changes in adherence pattern of isolates 7 (serotype 1/2b) and 508 (serotype 1/2c), while these treatments reduced biofilm production by isolate 19 (serotype 1/2c) (Fig. 3).

Based on absorbance values recorded by crystal violet assay, specific treatments were employed in further testing, where the isolates 7 and CLIST 441 (lineage I, serotype

### Table 2

Panel of genes related to the biofilm formation and environmental stress resistance evaluated by qPCR assays

| Gene     | References       | Sequence                                                                 | Product length | Function                                      |
|----------|------------------|--------------------------------------------------------------------------|----------------|-----------------------------------------------|
| *rplD1* | Miranda et al. (2018) | F: 5- GTCCCTTGACGTAGGATGC-3  
R: 5- GGAAACAAAACGCTGGCAAAAT-3 | 113 bp         | Normalizer                                  |
| *flaA*  | Pieta et al. (2014)       | F: 5- GTAAAGCATCAAACGTCTGA-3  
R: 5- AAGAATTCAGCATCACGCAA CG-3 | 148 bp         | Influence on biofilm growth                  |
| *agrB*  | Autret et al. (2003)       | F: 5- AGGTACATTGGATTTATATCTGCTCAAC-3  
R: 5- TCTTCACCGATAAAGGC AAACCT-3 | 81 bp          | Adaptation to environmental conditions       |
| *agrC*  | Autret et al. (2003)       | F: 5- ATTGCAAGATTTGCATGGATAGTATAGTT-3  
R: 5- CACAAGTTAACGCGCCTCA-3 | 88 bp          | Adaptation to environmental conditions       |
| SSI-1 *lmo 0444* | Ryan et al. (2010) | F: 5- CATCTGCTTTGTCGCTTTCA-3  
R: 5- CCGACACCATTCTCAAGG TT-3 | 85 bp          | Adaptation to low pH and high salt concentration |
| SSI-1 *lmo 0445* | Ryan et al. (2010) | F: 5- TAGACGACCTTTGGAACC TC-3  
R: 5- GGTATCGGGGCGATTTC TT-3 | 99 bp          | Adaptation to low pH and high salt concentration |
| SSI-1 *lmo 0446 (pva)* | Ryan et al. (2010) | F: 5- TGTCGCAACAGATTAAGAGA TG-3  
R: 5- TCACACTACAACGCGCCAC TC-3 | 131 bp         | Adaptation to low pH and high salt concentration |
1/2b) were exposed to cure salts while isolates 19 and 508 (lineage II, serotype 1/2c) were exposed to QAC treatment, on stainless steel microtiter plates and coupons. The results revealed a similar behavior, where the treatments reduced the viable attached cells significantly (Figs. 4 and 5), with QAC treatment showing more efficiency.

**Discussion**

In addition to strain specific properties, initial cell attachment and biofilm formation can be influenced by environmental conditions (Katsikogianni et al. 2004) and
extrinsic factors, including the physicochemical characteristics of the surface material (Van Houdt and Michiels 2010). It is well known that *L. monocytogenes* persistence in food processing facilities is usually associated with biofilm formation and the capacity to resist sanitization procedures (Poimenidou et al. 2016; Martínez-Suárez et al. 2016; Møretrø et al. 2017). However, it is difficult to establish a clear relationship between biofilm formation and persistent genotypes (Lee et al. 2019). Although some studies reported more efficient biofilm production to certain genotypes due to fitness advantage under specific environmental conditions, Maury et al. (2019) found that CC9 and CC121 harbor additional stress resistance genes, including BC tolerance and BC efflux pumps (such as *qac* [Tn6188], *bcrABC*, and *emrE*) which are mainly present in lineage II isolates and that are not present in host-associated clones and Hingston et al. (2017) associated the clonal complex to predict stress tolerance of different *L. monocytogenes*.

Acquisition of novel genes is critical to the exploitation of novel niches by lineages of *Listeria* (Nightingale et al. 2005). In this scenario, phages are important vehicles for horizontal gene transfer, affecting bacterial genome structure (Canchaya et al. 2003; Brüssow et al. 2004). Certain
prophage regions like A118, found in isolate 19 in this study, have been described in *L. monocytogenes* 1/2 serotype that are more often implicated in food-borne outbreaks of listeriosis, and presents the specific site integration occurring into *comK* homologue of *L. monocytogenes* host strains (Loessler et al. 2000). Furthermore, it is noteworthy the *comK* prophage presence in persistent strains of 4b serotype in food processing plants (Verghese et al. 2011). Short term evolution of *L. monocytogenes* can involve limited diversification of genomic backbone, although can alter considerably phage-mediated diversification (Orsi et al. 2008). The identification of prophage diversification between *L. monocytogenes* strains is important and supported by the fact that it can occur much faster than Single Nucleotide Polymorphism (SNP) for nearly identical isolates (Harrand et al. 2020).

Furthermore, it is noteworthy the *comK* prophage presence in persistent strains of 4b serotype in food processing plants (Verghese et al. 2011).

The results of crystal violet assays from this research (Fig. 3) revealed *L. monocytogenes* CLIST 441 (serotype 1/2b, CC3) formed high-density biofilms in the presence of QAC or curing salts (7.5%, and 10%). Some studies have already reported increased tolerance to QAC among

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**Fig. 4** Mean counts of *L. monocytogenes* isolates (lineage I) after biofilm forming assays (stainless-steel microplates and stainless-steel coupons) under specific stressing conditions. BHI: Control, using brain hearth infusion (BHI), BHI + CS: BHI added with curing salt (CS) at 7.5% (w/v)
persistent *L. monocytogenes* strains (Ortiz et al. 2014; Møretrø et al. 2017). Moreover, *L. monocytogenes* can differ in ability to grow and form biofilm in different stress conditions between lineages and serotypes (Orsi et al. 2011). Lineage II strains present a significant association with resistance to QAC and are more commonly found in food processing environments, compared to lineage I strains (Mereghetti et al. 2000; Ferreira et al. 2014; Poimenidou et al. 2016).

In agreement with our previous research (Silva et al. 2020), long term persistent strains tested in this study (isolates 19 and 508, from Lineage II) were able to survive and to form biofilms on stainless steel microtiter plates and coupons in the presence of QAC at MIC (Fig. 4). However, when submitted to the same treatment with curing salts and sodium chloride treatments they did present different growth patterns, highlighting the better adaptation to changes in a stressing environment of isolate 508 when compared to 19.

Regulatory networks ensure proper regulation of biofilm formation under different environmental condition and nutrient sources, and it can be studied to investigate the connection of different serotypes and different stress responses (Katsikogianni et al. 2004; Ouyang et al. 2012).

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**Fig. 5** Mean counts of *L. monocytogenes* isolates (lineage II) after biofilm forming assays (stainless-steel microplates and stainless-steel coupons) under specific stressing conditions. BHI: Control, using brain hearth infusion (BHI), BHI+QAC: BHI added with quaternary ammonium compound (QAC) based disinfectant (1:1024)
The transcriptional profile of six different genes (flaA, agrB, agrC lmo0444, lmo0445, and lmo0446) involved in stress responses, adaptation to environmental conditions and biofilm formation were studied by analyzing bacterial cells recovered from the biofilms grown on stainless steel coupons. Transcriptional expression of the agr operon (agrB-DCA) was assessed through RT-qPCR for two genes of the agr system (agrB and agrC) and for each gene the results showed differences even for the same treatment, where agrC was not expressed in strain 19 (serotype 1/2c, CC9) in one of the treatments (QAC). The agr system plays an important role in adaptation and biofilm formation by L. monocytogenes (Riedel et al. 2009). Although the agr system is transcribed as a single messenger, it can present differential expression of the agr genes depending on different growth phases and environmental conditions, due to post transcriptional processes, such as cleavage and degradation of agrC transcripts (Rieu et al. 2007; Gandra et al. 2019).

lmo0446 is part of a five-gene island, SSI-1 (lmo0444–lmo0448), previously associated to growth of L. monocytogenes under low pH and high salt concentrations, that favors survival of certain strains in food associated environments (Ryan et al. 2010). Also, lmo0446 is predicted to encode a bile tolerance locus (Begley et al. 2005). The absence of lmo0446 transcripts in the isolates from lineage I (7 and CLIST 441) was not expected, as the island is self-regulated by the product of lmo0445, a putative transcriptional regulator, that presented normal expression (Table 3). Although SSI-1 has been previously associated with stress response (Ronholm et al. 2017), it was not correlated in this study with the stress tolerance associated to biofilm formation. The transcriptional expression of these genes showed that only long-term persistent isolates from lineage II (19 and 508) presented transcripts for all genes evaluated (lmo0444, lmo0445, and lmo0446) in the presence or in the absence of QAC (Table 3). On the other hand, isolates CLIST 441 and 7 (lineage I) selected for biofilm formation on stainless steel coupons in the presence of curing salts at 7.5% (w/v), did not express detectable transcripts for lmo0446 nor flaA.

Listeria monocytogenes gene flaA encodes the flagellin protein FlaA (Dons et al. 1992), previously associated with growth at low temperatures and biofilm formation on abiotic surfaces (Liu et al. 2002; Lemon et al. 2007). Under static conditions, flagellum-mediated motility has been linked to initial surface attachment and biofilm development (Ouyang et al. 2012). Liu et al. (2002) described that regulation of flaA is increased specially under low temperatures, with low levels of transcripts detected in bacteria grown at 37ºC. Gene expression patterns associated to biofilm formation varies when different stresses are applied (Lee et al. 2019). In this study, flaA expression was different between the different stressing conditions applied during biofilm formation (curing salts at 7.5%, w/v, and QAC at 1:1,024, MIC) where it was not expressed in the presence of curing salts for lineage I isolates and it was expressed in lineage II in the presence

### Table 3 Panel of gene expression prediction by qPCR, for the different genes and two distinct treatments

| Treatmenta | Isolate   | CFU/cm² | rlpD1 | flaA | agrB | agrC | lmo0444 | lmo0445 | lmo0446 |
|------------|-----------|---------|-------|------|------|------|---------|---------|---------|
| BHI        | CLIST 441 | 2.6 x 10⁵ | +     | −    | +    | +    | +       | +       | −       |
| BHI        | CLIST 441 | 9.7 x 10⁵ | +     | −    | +    | +    | +       | +       | −       |
| BHI + curing salts | CLIST 441 | 2.2 x 10⁴ | +     | −    | +    | +    | +       | +       | −       |
| BHI + curing salts | CLIST 441 | 1.1 x 10⁴ | +     | −    | +    | +    | +       | +       | −       |
| BHI        | 7         | 1.1 x 10⁵ | +     | −    | +    | +    | +       | +       | −       |
| BHI        | 7         | 8.6 x 10⁵ | +     | −    | +    | +    | +       | +       | −       |
| BHI + curing salts | 7         | 5.2 x 10⁵ | +     | −    | +    | +    | +       | +       | −       |
| BHI + curing salts | 7         | 2.8 x 10⁵ | +     | −    | +    | +    | +       | +       | −       |
| BHI        | 508       | 3.6 x 10⁶ | +     | +    | +    | +    | +       | +       | +       |
| BHI        | 508       | 3.4 x 10⁶ | +     | +    | +    | +    | +       | +       | +       |
| BHI + QAC  | 508       | 2.6 x 10² | +     | +    | +    | +    | +       | +       | +       |
| BHI + QAC  | 508       | 4.2 x 10² | +     | +    | +    | +    | +       | +       | +       |
| BHI        | 19        | 3.6 x 10⁵ | +     | +    | +    | +    | +       | +       | +       |
| BHI        | 19        | 1.4 x 10⁶ | +     | +    | +    | +    | +       | +       | +       |
| BHI + QAC  | 19        | 7.1 x 10² | +     | +    | +    | +    | +       | +       | +       |
| BHI + QAC  | 19        | 1 x 10² | +     | +    | +    | +    | +       | +       | +       |

*a*As detailed in the “Material and methods” section; BHI brain hearth infusion (control), curing salts: composed by NaCl (90% w/w), sodium nitrite (6% w/w) and sodium nitrate (4% w/w), at 7.5% (w/v), QAC: quaternary ammonium compounds (at 1:1024, MIC)

*b*rlpD1 is the normalizer; + expression of transcripts; − no expression of transcripts
of QAC. Considering that different stressing conditions were applied to the different lineages, further investigation must be done for the same stressing condition (curing salts at 7.5%, w/v) to check if it will present the same behavior.

To conclude, our results highlight the ability of the selected *L. monocytogenes* isolates to survive in food processing facilities under stress conditions and to persist in such environments, leading to food contamination. The difference of genetic expression between groups, allows the identification of which genes may assist survival and biofilm formation under stress conditions commonly encountered by *L. monocytogenes* in food processing environments and foodstuff. Importantly, our study also contributes to diversification of data from food associated isolates, providing gene transcriptional insights in persistent *L. monocytogenes* isolates.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s11274-021-03092-5.

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**Declarations**

**Conflict of interest** The authors declare that they have no conflict of interest.

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