Biofilm Growth By *Listeria Monocytogenes* On Stainless Steel and Expression of Biofilm-Related Genes Under Stressing Conditions

Danilo Augusto Lopes da Silva
UFV: Universidade Federal de Vicosa
Rafaela de Melo Tavares
UFV: Universidade Federal de Vicosa
Anderson Carlos Camargo
UFV: Universidade Federal de Vicosa
Ricardo Seiti Yamatogi
UFV: Universidade Federal de Vicosa
Elaine Cristina Pereira De Martinis
USP: Universidade de Sao Paulo
Luis Augusto Nero (nero@ufv.br)
Universidade Federal de Viçosa https://orcid.org/0000-0002-4954-5824

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Abstract

This research was carried out to assess the ability of *L. monocytogenes* for adhesion and growth in biofilm on stainless steel coupons under different stressing conditions (NaCl, curing salts and quaternary ammonium compounds - QAC), besides determining the expression of different genes involved in biofilm formation and stress response. Results from crystal violet assay revealed that one isolate carrying a premature stop codon (PMSC) in *agrC* gene formed high-density biofilms in the presence of QAC or cure salts (7.5% and 10%). Reverse Transcriptase-qPCR results revealed that isolates of *L. monocytogenes* lineages I and II presented differences in transcriptional profile of genes related to biofilm formation and adaptation to environmental conditions. In conclusion, 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.

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). *L. monocytogenes* can adhere and form biofilms 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 to high salt concentrations (Ryan et al. 2010; Belessi et al. 2011; Pieta et al. 2014; Lee et al. 2017).

Several genes may 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 not completely understood (Lemon et al. 2007; Bonsaglia et al. 2014; Nowak et al. 2017). However, 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).

Here, we aimed to assess the potential for adhesion and biofilm formation under stressing conditions by persistent strains of *L. monocytogenes* from lineages I (serotype 1/2b) and II (serotype 1/2c), as well as to quantify the expression of selected genes relevant for biofilm formation and stress response.

Material And Methods

Bacterial strains

Four *L. monocytogenes* isolates were selected for this study, based on their characteristics previously described by Camargo et al. (2019) and presented in **Table 1**. These isolates presented genes enrolled in biofilm formation and stress response (*flaA, agrB, agrC, lmo0444, lmo0445* and *lmo0446*), previously identified by Whole Genome Sequencing , according to data available at GenBank (National Center for Biotechnology Information, Bethesda, MD, USA, **Supplementary Table 1**).

Table 1
Origin description and genotypes of isolates used in the present study, genotyped previously by Camargo et al., (2019).

Isolate	Year	State	Source type	Sample type		Serotype	PCR-serogroup[3]	Lineage	CC (MLST)[4]	ST (MLST)[4]	SL (cgMLST)[5]	CT (cgMLST)[5]
19	2009	MG[1]	PE[2]	Meat handlers	Before processing	1/2c	IIc	II	CC9	ST9	SL9	CT4420
508	2012	MG[1]	Food	Raw beef	Refrigerated	1/2c	IIc	II	CC9	ST9	SL9	CT4420
CLIST 441	2010	MT	Food	Raw beef	Refrigerated	1/2b	IIb	I	CC3	ST3	SL3	CT4447
7	2009	MG	Food	Raw beef	Refrigerated	1/2b	IIb	I	CC3	ST3	SL3	CT4448

MG, Minas Gerais; MT, Mato Grosso;

[1]Recovered from the same food processing facility.

[2]Production environment.

[3]According to Doumith *et al.*, (2004) and Leclercq *et al.*, (2011).

[4]Clonal complex (CC) and sequence type (ST) defined according to Ragon *et al.*, (2008).

[5]Sublineage (SL) and cgMLST type (CT) defined according to Moura *et al.*, (2016).

Adhesion on stainless steel under stressing conditions

L. 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), according to Silva et al. (2020).

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 1:1,024 (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, under constant agitation at 15 rpm. After incubation, the broths 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 as previously described by Silva et al. (2017).

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) and QAC at 1:1,024 (MIC, for lineage II). Isolates, curing salts and QAC were distributed in stainless-steel microtiter plates, and incubated at 37°C for 72 h, under constant agitation at 15 rpm. 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. Controls were prepared by growing *L. monocytogenes* in biofilms in the absence of curing salts and QAC. The results were expressed as colony forming units (CFU) per cm^2, for sessile cells. To calculate significant differences between the groups it was applied analysis of variance ($p < 0.05$) using the software XLSat 2010.2.03 (Addinsoft, New York, NY, USA).

Besides, *L. monocytogenes* biofilms were grown on stainless steel coupons (AISI 304, 3.8 cm^2) in the presence of curing salts (7.5%) and QAC (MIC, 1:1,024) at 37°C for 72 h, under constant agitation of 15 rpm. Prior to inoculation, the coupons were cleaned and prepared according to Winkelströter et al. (2011) and placed in 24 wells polystyrene microtiter plates (TPP, Trasadingen, Switzerland). To enumerate the *L. monocytogenes* cells attached to the coupons, each coupon was rinsed three times with PBS to eliminate non-adhered cells, transferred to test tubes containing 2 mL of PBS added with Tween 80 at 1% (v/v), treated for 2 min in ultrasound bath (50–60 kHz) and vortexed for 1 min according to Leriche and Carpentier (1995), with modifications. 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). 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 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 7.5% curing salts, and isolates of lineage II (508 and 19) in the presence of the QAC at 1:1,024 (MIC). Biofilms were grown at 37°C or 72 h.

RNA extraction was performed using RNeasy 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(Autret et al. 2003; Ryan et al. 2010; Pieta et al. 2014; Miranda et al. 2018). All qPCR amplifications were performed with final volume of 20 µL, using10 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.

Table 2
Panel of genes related to the biofilm formation and environmental stress resistance evaluated by qPCR assays.

Gene	Reference	Sequence	Product lenght	Function
rplD1	Miranda *et al.*, (2017)	F: 5-GTCCCTTGACGTAGGGATGC-3 R: 5-GGAACAAACGCTGGCGAAAT-3	113 bp	Normalizer
flaA	Pieta et al., (2014)	F: 5-GTAAGCATCCAAGCGTCTGA-3 R: 5-AAGAATCAGCATCAGCAACG-3	148 bp	Influences biofilm formation
agrB	Autret et al., (2003)	F: 5-AGGTACATTTGGATTTATACTGCTCAAC-3 R: 5-TCTTCACCGATTAAAGGCAAACT-3	81 bp	Adaptation to environmental conditions
agrC	Autret et al., (2003)	F: 5-ATTGACAAGATTTCGATGGATAGTATAGATT-3 R: 5-CACAAGTTAACGCCGCTTCA-3	88 bp	Adaptation to environmental conditions
SSI-1 *lmo 0444*	Ryan et al., (2010)	F: 5-CATCTGCTCTTGTCGGTTCA-3 R: 5-CCGACACCATTCTCAAGGTT-3	85 bp	Adaptation to low pH and high salt concentration
SSI-1 *lmo 0445*	Ryan et al., (2010)	F: 5-TAGACGAGCTTTGGAACCTC-3 R: 5-GGTATCGGGGCCATTTCTTC-3	99 bp	Adaptation to low pH and high salt concentration
SSI-1 *lmo 0446* (*pva*)	Ryan et al., (2010)	F: 5-TTGCGCAACGATTAAAGATG-3 R: 5-TCACTACACAACGCCCACTC-3	131 bp	Adaptation to low pH and high salt concentration

Results

Absorbance values obtained for 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. 1). 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. 1).

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 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. 2 and 3), being QAC treatment more efficient.

To examine the transcriptional correlation between the isolates studied, gene expression was quantified by RT-qPCR. The results showed that long term persistent isolates from lineage II (508 and 19) presented transcripts for all genes evaluated (*flaA, agrB*,*agrC*, *lmo0444, lmo0445*, and *lmo0446*), in the presence or in the absence of QAC (Table 3). On the other hand, isolates 441 and 7 (lineage I) selected for biofilm formation on stainless steel coupons in the presence of 7.5% cure salts, did not present transcripts for *flaA* and *lmo0446* (Table 3).

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

Treatment	Isolate	UFC/cm²	*rlpD1*	*flaA*	*agrB*	*agrC*	*Lmo0444*	*lmo0445*	*lmo0446*
BHI	CLIST 441	2.6 x 10^5							
BHI	CLIST 441	9.7 x 10^5							
BHI + Curing salt	CLIST 441	2.2 x 10^3							
BHI + Curing salt	CLIST 441	1.1 x 10^4							
BHI	7	1.1 x 10^5							
BHI	7	8.6 x 10^4							
BHI + Curing salt	7	5.2 x 10^3							
BHI + Curing salt	7	2.8 x 10^3							
BHI	508	3.6 x 10^6							
BHI	508	3.4 x 10^6							
BHI + QAC	508	2.6 x 10^2							
BHI + QAC	508	4.2 x 10^2							
BHI	19	3.6 x 10^5							
BHI	19	1.4 x 10^6							
BHI + QAC	19	7.1 x 10^2							
BHI + QAC	19	1 x 10^2							

■ Positive gene expression ■ gene expression not detected, *rlpD1*: normalizer.

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 physiochemical 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; Møretrø et al. 2017).

The results of crystal violet assays from this research (Fig. 1) revealed *L. monocytogenes* CLIST 441 (serotype 1/2b) formed high-density biofilms in the presence of QAC or curing salts (7.5%, and 10%). This isolate carries a PMSC mutation in *agrC* gene, but it remains unclear if this characteristic influenced biofilm development, since the results of crystal violet assays refer to the quantification of total biomass, and not only microbial cells. Some studies have already reported increased tolerance to QAC among 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 a 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. 3).

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). Here, the transcriptional profile of six different genes (*agrB*, and*agrC lmo0444, lmo0445*, and *lmo0446*) enrolled in stress responses, adaptation to environmental conditions and biofilm formation were quantified by analyzing bacterial cells recovered from the biofilms grown on stainless steel coupons. Transcriptional expression of the *agr* operon (*agrBDCA*) 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) 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 posttranscriptional processes, such as cleavage and degradation of *agrC* transcripts (Rieu et al. 2007).

lmo0446 is part of a five-gene island (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***).

L. 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 37°C. Gene expression patterns associated to biofilm formation can vary when different stresses are applied, however further studies need to examine the correlation between serotype and biofilm formation.

To conclude, our results highlight the ability of the selected *L. monocytogenes* isolates to survive in food processing facilities under stress conditions and persist in such environments, leading to food contamination. The difference of genetic expression between groups, allowed to identify which genes may assist survival and biofilm formation under stress conditions commonly encountered by *L. monocytogenes* in food processing environments and foodstuff.

Declarations

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Conflict of Interest

The authors declare that they have no conflict of interest.

References

Autret N, Raynaud C, Dubail I, et al (2003) Identification of the *agr* locus of *Listeria monocytogenes*: role in bacterial virulence. Infect Immun 71:4463–4471. https://doi.org/10.1128/IAI.71.8.4463-4471.2003

Begley M, Sleator RD, Gahan CGM, Hill C (2005) Contribution of three bile-associated loci, *bsh*, *pva*, and *btlB*, to gastrointestinal persistence and bile tolerance of *Listeria monocytogenes*. Infect Immun 73:894–904. https://doi.org/10.1128/IAI.73.2.894-904.2005

Belessi CEA, Gounadaki AS, Psomas AN, Skandamis PN (2011) Efficiency of different sanitation methods on *Listeria monocytogenes* biofilms formed under various environmental conditions. Int J Food Microbiol 145:. https://doi.org/10.1016/j.ijfoodmicro.2010.10.020

Bonsaglia ECR, Silva NCC, Fernades Júnior A, et al (2014) Production of biofilm by *Listeria monocytogenes* in different materials and temperatures. Food Control 35:386–391. https://doi.org/10.1016/j.foodcont.2013.07.023

Camargo AC, Moura A, Avillan J, et al (2019) Whole-genome sequencing reveals Listeria monocytogenes diversity and allows identification of long-term persistent strains in Brazil. Environ Microbiol 21:. https://doi.org/10.1111/1462-2920.14726

Carpentier B, Cerf O (2011) Review - Persistence of *Listeria monocytogenes* in food industry equipment and premises. Int. J. Food Microbiol. 145:1–8

Dons L, Rasmussen OF, Olsen JE (1992) Cloning and characterization of a gene encoding flagellin of *Listeria monocytogenes*. Mol Microbiol 6:2919–2929. https://doi.org/10.1111/j.1365-2958.1992.tb01751.x

Ferreira V, Wiedmann M, Teixeira P, Stasiewicz MJ (2014) *Listeria monocytogenes* persistence in food-associated environments: Epidemiology, strain characteristics, and implications for public health. J. Food Prot. 77:150–170

Herigstad B, Hamilton M, Heersink J (2001) How to optimize the drop plate method for enumerating bacteria. J Microbiol Methods 44:121–129. https://doi.org/10.1016/S0167-7012(00)00241-4

Jagadeesan B, Baert L, Wiedmann M, Orsi RH (2019) Comparative analysis of tools and approaches for source tracking *Listeria monocytogenes* in a food facility using whole-genome sequence data. Front Microbiol 10:. https://doi.org/10.3389/fmicb.2019.00947

Katsikogianni M, Missirlis YF, Harris L, Douglas J (2004) Concise review of mechanisms of bacterial adhesion to biomaterials and of techniques used in estimating bacteria-material interactions. Eur. Cells Mater. 8:37–57

Keeney K, Trmcic A, Zhu Z, et al (2018) Stress survival islet 1 contributes to serotype-specific differences in biofilm formation in *Listeria monocytogenes* . Lett Appl Microbiol 67:530–536. https://doi.org/10.1111/lam.13072

Lee BH, Hébraud M, Bernardi T (2017) Increased adhesion of *Listeria monocytogenes* strains to abiotic surfaces under cold stress. Front Microbiol 8:. https://doi.org/10.3389/fmicb.2017.02221

Lemon KP, Higgins DE, Kolter R (2007) Flagellar motility is critical for *Listeria monocytogenes* biofilm formation. J Bacteriol 189:4418–4424. https://doi.org/10.1128/JB.01967-06

Leriche V, Carpentier B (1995) Viable but nonculturable *Salmonella* Typhimurium in single- and binary-species biofilms in response to chlorine treatment. J Food Prot 58:1186–1191. https://doi.org/10.4315/0362-028x-58.11.1186

Liu S, Graham JE, Bigelow L, et al (2002) Identification of *Listeria monocytogenes* genes expressed in response to growth at low temperature. Appl Environ Microbiol 68:1697–1705. https://doi.org/10.1128/AEM.68.4.1697-1705.2002

Mereghetti L, Quentin R, Marquet-Van Der Mee N, Audurier A (2000) Low sensitivity of *Listeria monocytogenes* to quaternary ammonium compounds. Appl Environ Microbiol 66:5083–5086. https://doi.org/10.1128/AEM.66.11.5083-5086.2000

Minei CC, Gomes BC, Ratti RP, et al (2008) Influence of peroxyacetic acid and nisin and coculture with *Enterococcus faecium* on *Listeria monocytogenes* biofilm formation. J Food Prot 71:634–638. https://doi.org/10.4315/0362-028X-71.3.634

Miranda RO, Campos-Galvão MEM, Nero LA (2018) Expression of genes associated with stress conditions by *Listeria monocytogenes* in interaction with nisin producer *Lactococcus lactis*. Food Res Int 105:897–904. https://doi.org/10.1016/j.foodres.2017.12.030

Møretrø T, Langsrud S (2004) *Listeria monocytogenes*: biofilm formation and persistence in food-processing environments. Biofilms 1:107–121. https://doi.org/10.1017/s1479050504001322

Møretrø T, Schirmer BCT, Heir E, et al (2017) Tolerance to quaternary ammonium compound disinfectants may enhance growth of *Listeria monocytogenes* in the food industry. Int J Food Microbiol 241:215–224. https://doi.org/10.1016/j.ijfoodmicro.2016.10.025

Nowak J, Cruz CD, Tempelaars M, et al (2017) Persistent *Listeria monocytogenes* strains isolated from mussel production facilities form more biofilm but are not linked to specific genetic markers. Int J Food Microbiol 256:45–53. https://doi.org/10.1016/j.ijfoodmicro.2017.05.024

Orsi RH, Bakker HC de., Wiedmann M (2011) *Listeria monocytogenes* lineages: Genomics, evolution, ecology, and phenotypic characteristics. Int. J. Med. Microbiol. 301:79–96

Ortiz S, López V, Martínez-Suárez J V. (2014) Control of *Listeria monocytogenes* contamination in an Iberian pork processing plant and selection of benzalkonium chloride-resistant strains. Food Microbiol 39:81–88. https://doi.org/10.1016/j.fm.2013.11.007

Ouyang Y, Li J, Dong Y, et al (2012) Genome-wide screening of genes required for Listeria monocytogenes biofilm formation. J Biotech Res 4:13–25

Pieta L, Garcia FB, Riboldi GP, et al (2014) Transcriptional analysis of genes related to biofilm formation, stress-response, and virulence in *Listeria monocytogenes* strains grown at different temperatures. Ann Microbiol 64:1707–1714. https://doi.org/10.1007/s13213-014-0814-2

Poimenidou S V., Chrysadakou M, Tzakoniati A, et al (2016) Variability of *Listeria monocytogenes* strains in biofilm formation on stainless steel and polystyrene materials and resistance to peracetic acid and quaternary ammonium compounds. Int J Food Microbiol 237:164–171. https://doi.org/10.1016/j.ijfoodmicro.2016.08.029

Radoshevich L, Cossart P (2018) *Listeria monocytogenes*: Towards a complete picture of its physiology and pathogenesis. Nat Rev Microbiol 16:32–46. https://doi.org/10.1038/nrmicro.2017.126

Riedel CU, Monk IR, Casey PG, et al (2009) AgrD-dependent quorum sensing affects biofilm formation, invasion, virulence and global gene expression profiles in *Listeria monocytogenes*. Mol Microbiol 71:1177–1189. https://doi.org/10.1111/j.1365-2958.2008.06589.x

Rieu A, Weidmann S, Garmyn D, et al (2007) *agr* system of *Listeria monocytogenes* EGD-e: Role in adherence and differential expression pattern. Appl Environ Microbiol 73:6125–6133. https://doi.org/10.1128/AEM.00608-07

Rodríguez-Melcón C, Capita R, Rodríguez-Jerez JJ, et al (2019) Effect of low doses of disinfectants on the biofilm-forming ability of *Listeria monocytogenes*. Foodborne Pathog Dis 16:262–268. https://doi.org/10.1089/fpd.2018.2472

Ryan S, Begley M, Hill C, Gahan CGM (2010) A five-gene stress survival islet (SSI-1) that contributes to the growth of *Listeria monocytogenes* in suboptimal conditions. J Appl Microbiol 109:984–995. https://doi.org/10.1111/j.1365-2672.2010.04726.x

Silva DAL, Camargo AC, Todorov SD, Nero LA (2017) *Listeria* spp. contamination in a butcher shop environment and *Listeria monocytogenes* adhesion ability and sensitivity to food-contact surface sanitizers. J Food Saf 37:e12313. https://doi.org/10.1111/jfs.12313

Silva DAL, Tavares RM, Nero LA (2020) Interference of sanitizers, NaCl and curing salts on *Listeria monocytogenes* adhesion and subsequent biofilm formation. Lett Appl Microbiol 71:438–443. https://doi.org/10.1111/lam.13374

Van Der Veen S, Moezelaar R, Abee T, Wells-Bennik MHJ (2008) The growth limits of a large number of *Listeria monocytogenes* strains at combinations of stresses show serotype- and niche-specific traits. J Appl Microbiol 105:1246–1258. https://doi.org/10.1111/j.1365-2672.2008.03873.x

Van Houdt R, Michiels CW (2010) Biofilm formation and the food industry, a focus on the bacterial outer surface. J. Appl. Microbiol. 109:1117–1131

Winkelströter LK, De Martinis ECP (2015) Different methods to quantify *Listeria monocytogenes* biofilms cells showed different profile in their viability. Brazilian J Microbiol 46:231–235. https://doi.org/10.1590/S1517-838220131071

Winkelströter LK, Gomes BC, Thomaz MRS, et al (2011) *Lactobacillus sakei* 1 and its bacteriocin influence adhesion of *Listeria monocytogenes* on stainless steel surface. Food Control 22:1404–1407. https://doi.org/10.1016/j.foodcont.2011.02.021

Figures

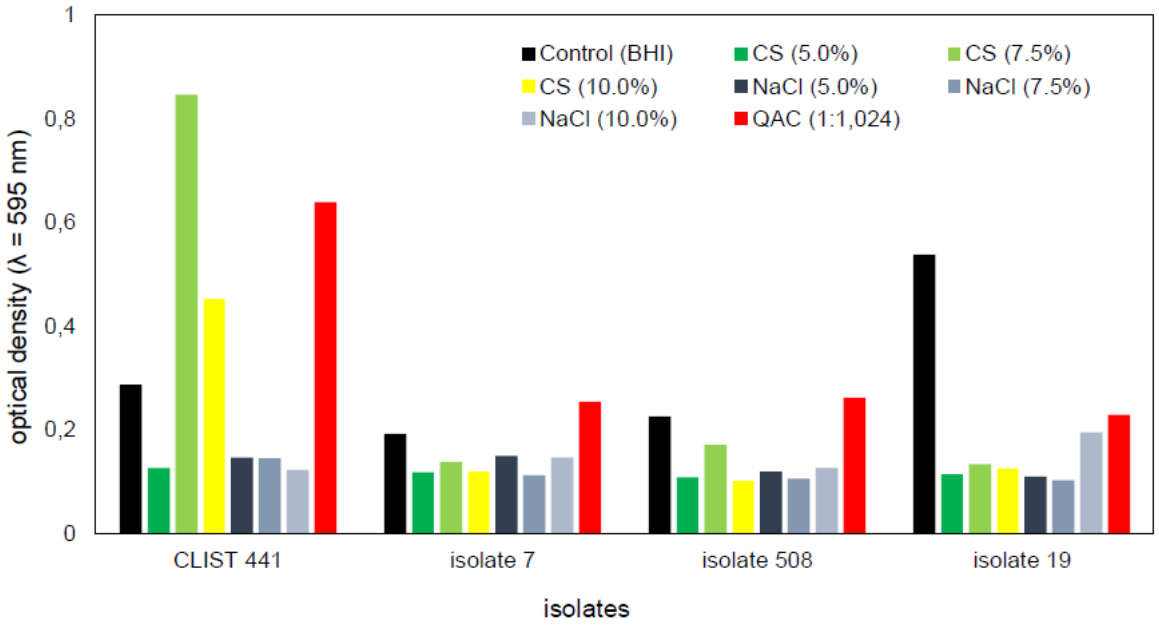

Figure 1

Absorbance values recorded for L. monocytogenes isolates subjected to adhesion assay in stainless-steel microtiter plates, after incubation at 37 °C for 72 h. Bars indicate the treatments with different stressing conditions: Control (isolate alone, with brain heart infusion, BHI), curing salts (CS), NaCl and quaternary ammonium compound (QAC) based disinfectant.

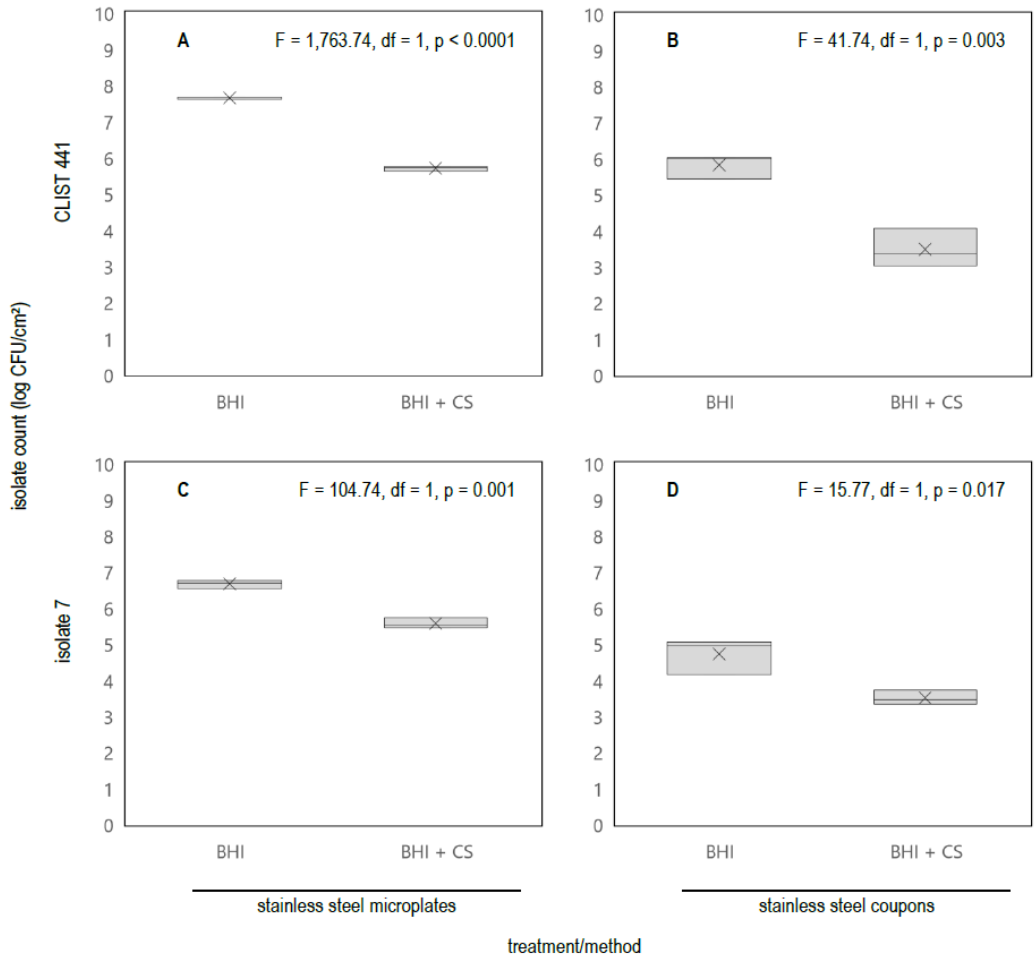

Figure 2

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).

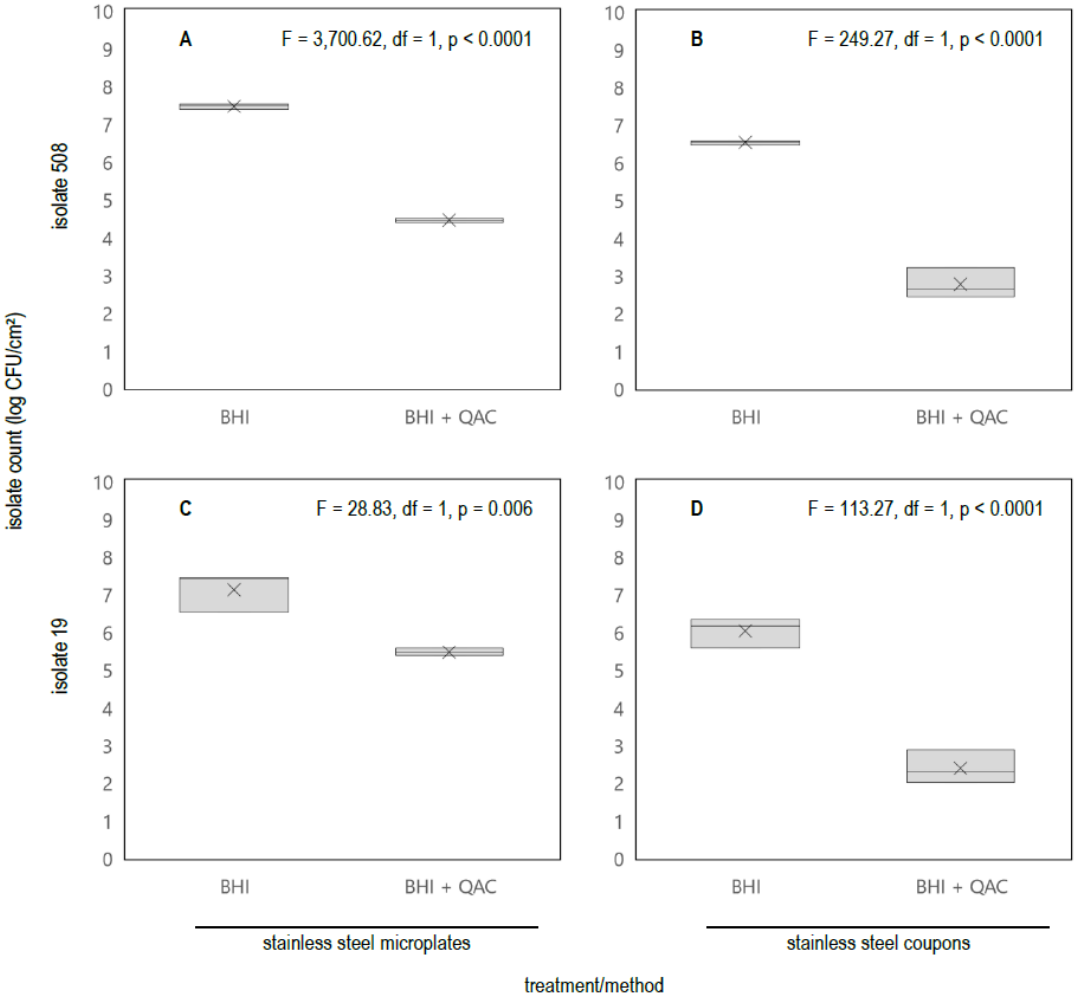

Figure 3

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 (1:1,024).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- 05SupplementaryTable20210329.docx