sciendo

*J Vet Res 67, 537-544, 2023*
DOI: 10.2478/jvetres-2023-0068

***REVIEW ARTICLE***

# Why does *Listeria monocytogenes* survive in food and food-production environments?

Jacek Osek✉, Kinga Wieczorek

Department of Hygiene of Food of Animal Origin,
National Veterinary Research Institute, 24-100 Puławy, Poland
josek@piwet.pulawy.pl

*Received: July 26, 2023* *Accepted: November 28, 2023*

**Abstract**

*Listeria monocytogenes* is one of the most dangerous food-borne pathogens and is responsible for human listeriosis, a severe disease with a high mortality rate, especially among the elderly, pregnant women and newborns. Therefore, this bacterium has an important impact on food safety and public health. It is able to survive and even grow in a temperature range from −0.4°C to 45°C, a broad pH range from 4.6 to 9.5 and at a relatively low water activity ($a_W$ < 0.90), and tolerates salt content up to 20%. It is also resistant to ultraviolet light, biocides and heavy metals and forms biofilm structures on a variety of surfaces in food-production environments. These features make it difficult to remove and allow it to persist for a long time, increasing the risk of contamination of food-production facilities and ultimately of food. In the present review, the key mechanisms of the pathogen's survival and stress adaptation have been presented. This information may grant better understanding of bacterial adaptation to food environmental conditions.

**Keywords:** *Listeria monocytogenes*, food, food environments, food safety, molecular mechanisms.

## Introduction

*Listeria monocytogenes* is considered one of the most dangerous bacterial pathogens responsible for food-borne illnesses in humans (6). The infection due to this bacterium has been recognised worldwide as a serious public health problem, especially in susceptible populations, including children, pregnant women, the elderly and individuals with impaired immune systems (33). Listeriosis has a high mortality rate (20–30%) and mainly affects the elderly and immunocompromised individuals, who may develop septicaemia, meningitis and meningoencephalitis (39).

According to the recent European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC) common report for the year 2021, a total of 2,183 confirmed cases of invasive listeriosis in humans were noted in the European Union member states, with a notification rate of 0.49 cases per 100,000 population and 96.5% hospitalisation (6). The same report showed that among 25,492 samples of different food categories tested for *L. monocytogenes*, 461 (1.8%) were positive, and that these were mainly fish products (3.1%). *Listeria monocytogenes* contaminated 0.5% of 13,893 food samples at a level above 100 colony-forming units per gram (6).

## Characteristics of *L. monocytogenes*

The bacterium is a facultatively anaerobic microorganism which is motile in a temperature range of 22–28°C but non-motile above 30°C, able to grow in a temperature range from −0.4°C to 45°C, and thrives at an optimum temperature of 37°C (33). It survives at a relatively low water activity ($a_W$ < 0.90) and in a broad pH range between 4.6 and 9.5, as well as tolerates salt content up to 20% (1). These attributes make *L. monocytogenes* able to live and multiply in adverse environmental conditions often present at food production facilities (1). It has been demonstrated that the bacteria can survive in the environment for at least eight weeks (50). The persistence of *L. monocytogenes* may be contributed to by several external factors such as poor hygiene practice or ineffective cleaning and sanitation, but also by the presence of specific genomic

*© 2023 J. Osek and K. Wieczorek. This is an open access article distributed under the Creative Commons Attribution-NonCommercial-NoDerivs license (http://creativecommons.org/licenses/by-nc-nd/3.0/)*

features in some *L. monocytogenes* strains that are responsible for their long-term survival (24).

## Classification of *L. monocytogenes*

Several studies have shown that *L. monocytogenes* isolates form a structured population composed of divergent lineages (4, 37, 43). The isolates may be classified into four major phylogenetic lineages (I–IV) that together contain 13 of the 14 known serotypes (35). It has been documented that the majority of isolates cluster into lineages I and II, which are mainly found originating from food, human outbreak cases and the environment, whereas lineages III and IV are mainly recovered from ruminant animals (35). Lineages III and IV include serotype 4a and 4c strains but also some strains of serotype 4b, which are typically associated with lineage I (47). Strains of lineage I have been associated with epidemic cases of human listeriosis, while strains of lineage II have been isolated from food and food production environments (31, 35). It seems that lineage II strains are better adapted to the saprophytic and environmental life cycle than lineage I strains (47).

Classification of *L. monocytogenes* isolates into the lineages has been performed using a range of genotypic and phenotypic approaches, including ribotyping, pulsed-field gel electrophoresis, and multi-locus sequence typing (MLST) (39). This method of sequence typing is based on the nucleotide sequences of the seven housekeeping genes *abcZ*, *bglA*, *cat*, *dapE*, *dat*, *ldh* and *lhkA*, and is characterised by high resolution, good repeatability and easy access (27). Recently however, DNA sequence-based data (*e.g.* data yielded by whole-genome sequencing (WGS)) have also begun to be used to provide accurate phylogenetic characterisation of isolates and lineage assignment (15).

Members of the species are further classified into 14 serotypes based on the variation of their somatic (O) and flagellar (H) antigens. The serotypes are 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, 4h, and 7. Only four of them (1/2a, 1/2b, 1/2c and 4b) are responsible for the vast majority (approximately 95%) of human listeriosis cases worldwide (23, 45, 46), serotype 1/2a being predominantly isolated from food samples, and serotype 4b being responsible for most human listeriosis outbreaks (28).

Based on PCR, further molecular typing of *L. monocytogenes* resulted in four major serogroups being identified, which include more than half of the serotypes shown above: IIa of serotypes 1/2a and 3a, IIb of serotypes 1/2b and 3b, IIc of 1/2c and 3c, and IVb of 4b, 4d and 4e (35). It is important to identify and track these serotypes in order to establish effective surveillance systems for *L. monocytogenes*.

The recent development and application of molecular typing methods using WGS and bioinformatics has resulted in further classification of *L. monocytogenes* isolates (23). Based on the MLST and core genome MLST (cgMLST) approaches, the strains may be grouped into multiple clonal complexes (CCs) and sequence types (STs) (35, 41). Core genome MLST relies on defining alleles for thousands of gene loci and translating sequence variation into numerical profiles, which are computationally easier and faster to handle and analyse than genome-based sequence alignments (25, 41). Distinct cgMLST-based schemes have been used for high-resolution typing of *L. monocytogenes* ranging from 1,013 to 1,827 loci, including the most common open-data reference cgMLST scheme of 1,748 gene loci that is used worldwide (29, 41).

## Food and food-production environment presence of *L. monocytogenes*

Some characteristics of *L. monocytogenes*, such as the abilities to grow at low temperatures, survive at low water activity and persist on food-processing surfaces under adverse conditions, increase the risk of contamination of food, especially of pre-packed and ready-to-eat food (1, 31, 50).

Several *L. monocytogenes* strains have been shown by MLST or cgMLST to share a close genetic relationship and to be the strains more often recovered from food or food production environments, whereas other isolates have been noted to be more connected with human listeriosis (4, 28, 31, 35, 41, 46).

Many studies have demonstrated that *L. monocytogenes* of sequence types ST9, ST2, ST3, ST4, ST6, ST155, ST8, ST619, ST299 and ST121 were frequently isolated from different food products and food-production environments (28, 31, 43, 49). We have also previously shown that some of these sequence types (ST2, ST6, ST8 and ST121) were identified among *L. monocytogenes* isolated from such sources in Poland (21, 22). These isolates are frequently identified in the same food production plants for several years, despite the application of sanitation measures, which makes them serious problems for the food industry and poses a potential threat to consumers (29). The bacteria can colonise niches within facilities such as cracked surfaces, drains and areas that are hard to clean (26). Several other studies also support that *L. monocytogenes* of certain sequence types have persistent properties and are identified in food-production–associated niches for a long time and even over many years (14, 36). The recovery of such persistent *L. monocytogenes* strains from the food-production environment and facility equipment after cleaning and disinfection emphasises the risk of the growth of the bacterium, particularly in sites difficult to access, and the consequent risk of food product contamination (7, 14, 23). It was shown that persistent strains can also be transferred between various food-production facilities through a contaminated environment at a point on the production chain or by contaminated equipment (23, 28, 29).

**Characteristics of persistent *L. monocytogenes***

Persistent *L. monocytogenes* strains have been defined as isolates (clones) that are repeatedly isolated from the same source or niche at intervals of 6 or more months (40, 48). Such isolates are molecularly indistinguishable when tested by genome-based methods, *e.g.* WGS (48). The ability of *L. monocytogenes* to survive and persist in food production environments or even food depends on the bacteria's various properties and the surfaces to which they attach (38). One of the important characteristics of persistent strains is directly related to *L. monocytogenes*' ability to form biofilms that protect bacteria embedded in its polymeric matrix, making the cells more resistant to environmental stress conditions encountered in food-production facilities such as cleaning and disinfection (1, 24, 50). It has been shown that the quality of *L. monocytogenes* biofilms varies greatly according to the strains which form the film, the medium in which the strains are growing, the temperature, the incubation time and the nature of the adhesion surface (24, 28). Strong biofilm-forming isolates develop biofilm structures within 24 h on surfaces typical for food-industry facilities such as stainless steel, ceramic tiles, high-density polyethylene plastics, polyvinyl chloride pipes and glass (24). Several studies have demonstrated that flagella-mediated motility plays a key role in both initial surface attachment and subsequent biofilm formation by *L. monocytogenes*, and that strains lacking the flagella-encoding *flaA* gene have reduced colonisation ability (10, 38). One of these studies has also shown a correlation between the serotype or clone of *L. monocytogenes* and biofilm formation, although this dependence has not been clearly proved (10). Using a WGS approach and analysis of data from 166 environmental and food-related *L. monocytogenes* biofilm-forming isolates, it has been suggested that serotype-specific differences in biofilm development are associated with the presence of the stress-survival islet 1 (SSI-1) sequence (18, 48). Strains which were SSI-1-positive, serotype 1/2a, and isolated mainly from food production environments formed the strongest biofilms, while isolates of serotype 4b, mainly clinical in origin were usually SSI-1-negative and were the weakest biofilms producers even if they possessed the SSI-1 sequence (18). Further investigations have also revealed a significant correlation between lineage II affiliation (serotypes l/2b and 4b originating from food) and strong biofilm formation (48). A similar observation has been described by Lee *et al.* (25), who noted that *L. monocytogenes* lineage II strains were statistically significantly more efficient than lineage I strains at biofilm production.

Another genomic important marker involved in biofilm formation in food production environments is the *inlA* gene responsible for the expression of internalin A. Mutations in this gene reduce the length of the InlA protein. It has been shown that *L. monocytogenes* strains which have such mutations possess strong biofilm formation properties but a lower virulence potential and are mainly isolated from food and food-processing facilities (32).

The persistence of *L. monocytogenes* in food production environments also strongly depends on resitance to disinfectants (23, 40). The relationship between the resistance to various biocides of certain subtypes of *L. monocytogenes* and the persistence of those subtypes in different food-processing niches has been studied (7). It has been shown that there is a link between resistance to benzalkonium chloride (BAC), one of the biocidal quaternary ammonium compounds (QACs), and the persistence of some strains (38). It seems that the effectiveness of QACs against *L. monocytogenes* is similar against persistent and non-persistent strains isolated from food production environments; however, there are also studies showing that persistent isolates exhibited higher resistance to QACs than non-persistent ones, especially strains of serotype 1/2c (26). Furthermore, studies by Haubert *et al.* (11) demonstrated that all 50 tested *L. monocytogenes* isolates from food were resistant to BAC, and more than 50% of these tolerant strains also displayed resistance to cadmium.

Resistance to QACs in *L. monocytogenes* depends on several genomic efflux pump systems, including the *bcrABC* three-gene cassette associated with BAC tolerance (7, 14) (Table 1). It has been demonstrated that in BAC-tolerant *L. monocytogenes* from various sources, the *bcrABC* cassette was present in almost all isolates tested (5). Another study has shown that the *bcrABC* sequence was significantly associated with *L. monocytogenes* isolates belonging to clonal complexes strongly associated with isolates from food and food-production environments, *e.g.* CC321, CC155, CC204 and CC199 (2).

As mentioned above, *L. monocytogenes* is able to tolerate a wide range of temperatures present in food-production environments during both production and storage, surviving also in frozen food (1, 38, 50). Under these adverse conditions the bacterial cells are able to decrease their metabolism and enzyme activity, change their membrane composition and express cold-shock proteins (1). As a reaction to low temperatures, *L. monocytogenes* increases the concentration of unsaturated fatty acids in the cell membrane, preventing cytoplasmic content leakage (30). Several genetic factors play a role in the adaptation of *L. monocytogenes* to these cold-temperature stress conditions (Table 1). The main role seems to be played by two operons: *gbu*, encoding the glycine betaine transporter, and *opuC*, responsible for the OpuC ABC transporter of carnitine (20). Both transporters are the means for the bacteria to accumulate glycine betaine and carnitine that prevent low temperatures disrupting bacteria cells. Sigma factor protein $\sigma^B$ (SigB) is also involved in this process, and a *sigB*-deleted *L. monocytogenes* mutant was unable to accumulate betaine or carnitine (34).

*Listeria monocytogenes* also expresses other heat-shock proteins in response to low temperatures, such as

cold-shock-domain family proteins, ferritin-like protein, or two-component-system histidine kinases. Their roles must be further investigated (38).

When the adversity is temperature at the other extreme, *L. monocytogenes* has also developed different mechanisms to survive. High temperatures that may be applied during food production may not be bactericidal in the case of *L. monocytogenes*. The efficacy of such thermal conditions in killing these bacteria may be limited because the bacteria have natural resistance to thermal conditions above 45°C (50). In response to higher temperature, *L. monocytogenes* increases production of three classes of heat-shock proteins (1). They are able to stabilise and repair partially denatured cellular proteins and prevent their intracellular aggregation under heat-stress conditions (1).

Another stress factor that may prevent the survival of some *L. monocytogenes* strains in food and food-producing facilities is adverse pH. A low pH may be generated during acid sanitation of the environment or when food is preserved, whereas a high pH is mainly associated with the use of detergents and disinfectants (3). *Listeria monocytogenes* is able to adapt to adverse pH environments by means of different mechanisms. A mildly acidic pH induces in the bacteria the acid tolerance response, the process by which the cells better resist lethal acidic conditions. The acid tolerance response is mainly the increasing of cytoplasmic buffer capacity *via* the glutamate decarboxylase (GAD) system, the main mechanisms responsible for the maintenance of intracellular homeostasis (42). The GAD-encoding genes are located on the SSI-1 (Table 1). It has been shown that the glutamate decarboxylase enzyme promotes the irreversible conversion of cytosolic glutamate to a neutral compound, γ-aminobutyrate (42).

A further important cell system that protects *L. monocytogenes* from acid pH is the arginine deiminase (ADI) pathway encoded by the *arcABC* operon, which is SigB-dependent (1, 50). Both low pH adaptation systems, *i.e.* GAD and ADI, act at the same time and ensure the survival and adaptation to acid stress conditions of *L. monocytogenes* (50).

The bacterium has also developed various mechanisms that allow it to survive in the alkaline conditions often created in food-processing environments by the application of alkaline-based detergents and disinfectants (1, 29). One of them is the increased synthesis of intracellular acids through deamination of amino acids and fermentation of sugars (40). Another protection mechanism against high pH is the expression by *L. monocytogenes* of transporters and enzymes directly responsible for proton retention and cell surface modifications (38). Furthermore, the acidic cell-wall polymers, *e.g.* teichuronic acid and teichuronopeptides, contribute to pH homeostasis, making a passive bacterial barrier to ion flux and increasing the cytoplasmic buffering capacity (38). All these protection mechanisms may act independently or simultaneously depending on the duration of exposure to alkalinity, concentration of the alkaline and temperature of the environment.

**Table 1.** The gene markers associated with resistance of *Listeria monocytogenes* to stress conditions

| Stress factor | Gene marker | Gene product | Phenotypic effect | References |
|---|---|---|---|---|
| Biocides | *bcrABC* cassette | resistance protein transporters and transcriptional factor | resistance to benzalkonium chloride | 7, 14 |
| | *mdrL* and *lde* | endogenous efflux pumps | | 2, 5 |
| Low temperature | *gbu* | glycine betaine transporter | survival and growth at low temperatures | 20 |
| | *opuC* | transport of carnitine | | 20 |
| | *sigB* | sigma factor protein $\sigma^B$ | response to temperature downshift | 34 |
| | *cspA* | cold-shock domain family proteins | chaperone that facilitates growth at low temperatures | 38 |
| High temperature | *grpE*, *dnaK*, *dnaJ*, *groEL*, *clpP*, *clpE* | heat-shock proteins | stabilisation and repair partially denatured proteins | 1, 50 |
| Adverse pH | *gadA*, *gadT* | glutamate decarboxylase system | conversion of glutamate to neutral γ-aminobutyrate | 3, 42 |
| | *arcABCD* | arginine deiminase | conversion of arginine to ornithine | 1, 50 |
| Osmolarity | *kdpABC*, *lmo0993* | transporter system | restoration turgor pressure and cell volume | 12, 16, 48 |
| High pressure | *sigB* | sigma factor protein $\sigma^B$ | inhibition of cell membrane permeability and protein disruption | 13, 48 |
| Ultraviolet light | *sigB* | sigma factor protein $\sigma^B$ | increased ultraviolet light tolerance | 8 |
| | *uvrX*, *uvrA* | Y-family DNA polymerase | | 9, 19 |
| Heavy metals | *cadABC*, *arsABCD*, *lgi-2* | efflux P-type ATPase pumps and membrane transporters | increased resistance to cadmium and arsenic | 25, 41 |

ATPase – adenosine 5′-triphosphatase

### Resistance to other stress conditions

*Listeria monocytogenes* can survive and even grow in higher osmolarity resulting, for example, from the addition of NaCl during food production. In response to this adverse condition, the bacteria undergo the cellular process called osmoadaptation (12). This is a biphasic response, in the first step of which bacteria increase the uptake of potassium ions ($K^+$) and glutamate, and then, in the second stage, the bacteria replace part of the accumulated $K^+$ with low-molecular-weight molecules termed compatible solutes or osmolytes (12). The bacterium possesses two kinds of $K^+$ transporters: a high-affinity KdpABC transporter system and a low-affinity system encoded by the *lmo0993* gene (16) (Table 1). The uptake of relevant osmolytes helps the bacterial cell restore physiological turgor pressure and cell volume and stabilise the cell protein structure and functions. Several osmolytes that are involved in the reduction of osmotic stress have been identified, *e.g.* betaine, carnitine, proline, proline betaine, acetylcarnitine and γ-butyrobetaine, and the first of these seems to play a key role in the survival of *L. monocytogenes* (12, 48). The expression of genes encoding betaine, carnitine and proline transporters is regulated by the SigB general stress protein (12, 16). It has been shown that the Ctc general stress protein produced by *L. monocytogenes* is also involved in osmotolerance in the absence of the osmolytes named above in the bacterial environment (16). The activation of most genes involved in the osmoadaptation of *L. monocytogenes* usually takes place when other environmental stressors besides high osmolarity affect the bacterium, such as the low temperature and low pH to which *L. monocytogenes* is often subjected during food production and processing (3, 12, 30, 42) (Table 1).

Survival at the high hydrostatic pressure (HPP) that is used for food preservation is also possible for *L. monocytogenes* (13). The applied pressure in some studied preservation processes ranged from 250 to 700 MPa over durations from a second to a minute, depending on the food category (1). During these processes which raise hydrostatic pressure, the permeability of bacterial cell membranes increases and intracellular proteins are disrupted, resulting in cell death (13). Like other bacterial species, *L. monocytogenes* is also sensitive to high pressure, but it has developed several mechanisms that make it become more resistant and even survive under this adverse condition. It has been shown that the resistance of *L. monocytogenes* to HPP depends on the strain and the kind of food which the bacteria is colonising (13, 48). As mentioned above, the microorganism is able to survive in the high salt concentration which may be present in some foods. This higher salt content may induce the uptake of compatible solutes, which stabilise cells during application of high pressure (13). Even damaged, *L. monocytogenes* cells may still be viable and are able to recover during food storage subsequent to the high-pressure preservation process (13). It has been shown that this survival and recovery of the cells is due to the activation of several genes responsible for the DNA repair, transcription and translation of the intracellular proteins involved in cell division, protein secretion systems, flagella assemblage and motility (1). Additional genetic mechanisms triggered by HPP are the suppression of genes connected with energy production and carbohydrate metabolism, and more importantly, the activation of SigB, which itself induces several protective genes (1). Furthermore, HPP also switches on other genes, *e.g.* those responsible for the expression of the PfrA listeriolysin regulatory protein and the CspL cold-shock protein, as well as induces mutations in the *ctsR* stress gene – mutations which are connected to spontaneous resistance of *L. monocytogenes* to HPP (17). It has also been shown that these processes stimulate higher expression of the *clpB*, *clpC*, *clpE* and *clpP* genes encoding the Clp caseinolytic protein that is able to degrade adverse proteins forming in the bacterial cells during HPP treatment (1).

At food-production establishments, ultraviolet light (UV) may be used at 200–280 nm for decontamination of water and air. This approach has high bactericidal potential against bacterial microorganisms, including *L. monocytogenes*. This effect is mainly caused by DNA damage but also by the formation of several photoproducts and destruction of the bacterial cells resulting from the absorption of the high-energy light pulses (9). It seems that *L. monocytogenes* is more resistant to UV light than other bacterial pathogens that may be present in food-production environments (9). This phenomenon is mainly due to the low penetration of UV light through the environment where these bacteria are present, *i.e.* in higher concentrations of salt, materials with low pH or food debris on stainless steel surfaces of food-producing equipment. At the molecular level, higher resistance of *L. monocytogenes* to UV light may be due to a reduction in gene expression in the bacterial cells, including those encoding the SigB stress protein (8). However, some genes of *L. monocytogenes* exposed to UV may be upregulated, *e.g.* the putative stress-response *uvrX* plasmid gene or chromosomally located *uvrA* sequence. Both are necessary for bacterial UV stress survival (19) (Table 1), their role in UV-stress tolerance nevertheless still needing further investigation.

The resistance shown by *L. monocytogenes* to certain heavy metals, especially to cadmium and arsenic, is higher than that shown by other bacteria, and is sufficient for strains of the bacterium to survive in food-production environments after they are cleaned with chemicals containing compounds of these metals. It has been shown that several *L. monocytogenes* isolates from foods and food-processing plants were resistant to cadmium (44). This resistance depends on several genes, which are mainly located on plasmids or other mobile genetic sequences but also on chromosomes, such as *cadA1*, *cadA2*, *cadA3*, *cadA4* and *cadA5* (Table 1). The *cadA2* sequence has been shown to be located at the

transposon that also harbours genes for resistance to BAC (44). Four of these genes bestow high levels of resistance to cadmium (*cadA1–cadA4*), while the fifth has lower resistance levels as its effect (*cadA5*) (25, 38). It seems that some cadmium-resistance determinants (*e.g. cadA1*) were more common among strains isolated from food and food-production environments and classified into serotypes 1/2a and 1/2b (44). However, there are also *L. monocytogenes* isolates lacking any known cadmium-resistance sequences that are still cadmium resistant, which suggests other mechanisms of decreased sensitivity to this heavy metal.

Resistance to arsenic is usually encoded by the genes carried on the *Listeria* genomic island 2 (LGI2) (25). Such resistance is mainly associated with clinical *L. monocytogenes* isolates of serotype 4b, and strains identified in food or food-production environments of serotypes 1/2a, 1/2b, and 1/2c rarely possess LGI2 (41). Several *ars* genes and their products encode resistance to arsenic, one being the ATP-dependent anion pump that releases arsenite (the most common form of inorganic arsenic) out of the bacterial cells (25).

## Conclusions

The *L. monocytogenes* which colonise food and food-production environments are often characterised by an ability to develop biofilms and tolerance to several stress conditions such as sanitation procedures, ultraviolet light, low and high temperatures and the presence of heavy metals (40). Since they are difficult or impossible to eliminate from food-production environments, they pose serious problems to food-business operators and threaten final consumers. Persistent strains are often isolated from food-processing environments after cleaning and disinfection because they show increased biofilm formation in relation to non-persistent strains. Interestingly, persistent *L. monocytogenes* are often associated with higher survival and stronger biofilm formation in the presence of sublethal concentrations of BAC, a component of several disinfectants. It has been also shown that persistent *L. monocytogenes* usually possess a lower pathogenic potential compared to clinical isolates because their *inlA* internalin gene is truncated, they frequently lack LIPI-3 and LIPI-4 virulence factors, and their *prfA* regulon encoding expression of listeriolysin regulatory protein PfrA is mutated. Additional knowledge is needed to more thoroughly understand the mechanisms that allow *L. monocytogenes* to adapt to various environmental stress conditions in food-production establishments and during food storage.

**Conflict of Interests Statement:** The authors declare that there is no conflict of interests regarding the publication of this article.

**Financial Disclosure Statement:** This article was financed within the reference activity of the National Veterinary Research Institute, Puławy, Poland.

**Animal Rights Statement:** None required.

## References

1. Bucur F.I., Grigore-Gurgu L., Crauwels P., Riedel C.U., Nicolau A.I.: Resistance of *Listeria monocytogenes* to stress conditions encountered in food and food processing environments. Front Microbiol 2018, 9, 2700, doi: 10.3389/fmicb.2018.02700.
2. Cooper A.L., Carrillo C.D., Deschênes M., Blais B.W.: Genomic markers for quaternary ammonium compound resistance as a persistence indicator for *Listeria monocytogenes* contamination in food manufacturing environments. J Food Prot 2021, 84, 389–398, doi: 10.4315/JFP-20-328.
3. Cotter P.D., Hill C.: Surviving the acid test: responses of Gram-positive bacteria to low pH. Microbiol Mol Biol Rev 2003, 67, 429–453, doi: 10.1128/MMBR.67.3.429-453.2003.
4. den Bakker H.C., Didelot X., Fortes E.D., Nightingale K.K., Wiedmann M.: Lineage specific recombination rates and microevolution in *Listeria monocytogenes*. BMC Evol Biol 2008, 8, 277, doi: 10.1186/1471-2148-8-277.
5. Dutta V., Elhanaf D., Kathariou S.: Conservation and distribution of the benzalkonium chloride resistance cassette *bcrABC* in *Listeria monocytogenes*. Appl Environ Microbiol 2013, 79, 6067–6074, doi: 10.1128/AEM.01751-13.
6. European Food Safety Authority and European Centre for Disease Prevention and Control: The European Union One Health 2021 zoonoses report. EFSA J 2022, 12, 7666, doi: 10.2903/j.efsa.2022.7666.
7. Ferreira V., Wiedmann M., Teixeira P., Stasiewicz M.J.: *Listeria monocytogenes* persistence in food-associated environments: epidemiology, strain characteristics, and implications for public health. J Food Prot 2014, 77, 150–170, doi: 10.4315/0362-028X.JFP-13-150.
8. Gayán E., Serrano M.J., Pagán R., Álvarez I., Condón S.: Environmental and biological factors influencing the UV-C resistance of *Listeria monocytogenes*. Food Microbiol 2015, 46, 246–253, doi: 10.1016/j.fm.2014.08.011.
9. Gómez-López V.M., Ragaert P., Debevere J., Devlieghere F.: Pulsed light for food decontamination: a review. Trends Food Sci Technol 2007, 18, 464–473, doi: 10.1016/j.tifs.2007.03.010.
10. Harvey J., Keenan K.P., Gilmour A.: Assessing biofilm formation by *Listeria monocytogenes* strains. Food Microbiol 2007, 24, 380–392, doi: 10.1016/j.fm.2006.06.006.
11. Haubert L., Zehetmeyr M.L., da Silva W.P.: Resistance to benzalkonium chloride and cadmium chloride in *Listeria monocytogenes* isolates from food and food-processing environments in southern Brazil. Can J Microbiol 2019, 65, 429–435, doi: 10.1139/cjm-2018-0618.
12. Hill C., Cotter P.D., Sleator R.D., Gahan C.G.M.: Bacterial stress response in *Listeria monocytogenes*: jumping the hurdles imposed by minimal processing. Intern Dairy J 2002, 12, 273–283, doi: 10.1016/S0958-6946(01)00125-X.
13. Huang H.W., Lung H.M., Yang B.B., Wang, C.Y.: Responses of microorganisms to high hydrostatic pressure processing. Food Control 2014, 40, 250–259, doi: 10.1016/j.foodcont.2013.12.007.
14. Hurley D., Luque-Sastre L., Parker C.T., Huynh S., Eshwar A.K., Nguyen S.V., Andrews N., Moura A., Fox E.M., Jordan K., Lehner A., Stephan R., Fanning S.: Whole-genome sequencing-based characterization of 100 *Listeria monocytogenes* isolates collected from food processing environments over a four-year period. mSphere 2019, 4, e00252-19, doi: 10.1128/msphere.00252-19.

15. Hyden P., Pietzka A., Lennkh A., Murer A., Springer B., Blaschitz M., Indra A., Huhulescu S., Allerberger F., Ruppitsch W., Sensen C.W.: Whole genome sequence-based serogrouping of *Listeria monocytogenes* isolates. J Biotechnol 2016, 235, 181–186, doi: 10.1016/j.jbiotec.2016.06.005.
16. Kallipolitis B.H., Ingmer H.: *Listeria monocytogenes* response regulators important for stress tolerance and pathogenesis. FEMS Microbiol Lett 2001, 204, 111–115, doi: 10.1111/j.1574-6968.2001.tb10872.x.
17. Karatzas K.A.G., Wouters J.A., Gahan C.G.M., Hill C., Abee T., Bennik M.H.J.: The CtsR regulator of *Listeria monocytogenes* contains a variant glycine repeat region that affects piezotolerance, stress resistance, motility and virulence. Mol Microbiol 2003, 49, 1227–1238, doi: 10.1046/j.1365-2958.2003.03636.x.
18. Keeney K., Trmcic A., Zhu Z., Delaquis P., Wang S.: Stress survival islet 1 contributes to serotype-specific differences in biofilm formation in *Listeria monocytogenes*. Lett Appl Microbiol 2018, 67, 530–536, doi: 10.1111/lam.13072.
19. Kim S.H., Gorski L., Reynolds J., Orozco E., Fielding S., Park Y.H., Borucki M.K.: Role of *uvrA* in the growth and survival of *Listeria monocytogenes* under UV radiation and acid and bile stress. J Food Prot 2006, 69, 3031–3036, doi: 10.4315/0362-028x-69.12.3031.
20. Ko R., Smith L.T., Smith G.M.: Glycine betaine confers enhanced osmotolerance and cryotolerance of *L. monocytogenes*. J Bacteriol 1994, 176, 426–431, doi: 10.1128/jb.176.2.426-431.1994.
21. Lachtara B., Osek J., Wieczorek K.: Molecular typing of *Listeria monocytogenes* IVb serogroup isolated from food and food production environments in Poland. Pathogens 2021, 10, 482, doi: 10.3390/pathogens10040482.
22. Lachtara B., Wieczorek K., Osek J.: Genetic diversity and relationships of *Listeria monocytogenes* serogroup IIa isolated in Poland. Microorganisms 2022, 10, 532, doi: 10.3390/microorganisms10030532.
23. Lakicevic B., Jankovic V., Pietzka A., Ruppitsch W.: Wholegenome sequencing as the gold standard approach for control of *Listeria monocytogenes* in the food chain. J Food Prot 2023, 86, 100003, doi: 10.1016/j.jfp.2022.10.002.
24. Lee B.H., Cole S., Badel-Berchoux S., Guillier L., Felix B., Krezdorn N., Hébraud M., Bernardi T., Sultan I., Piveteau P.: Biofilm formation of *Listeria monocytogenes* strains under food processing environments and pan-genome-wide association study. Front Microbiol 2019, 10, 2698, doi: 10.3389/fmicb.2019.02698.
25. Lee S., Ward T.J., Jima D.D., Parsons C., Kathariou S.: The arsenic resistance-associated *Listeria* genomic island LGI2 exhibits sequence and integration site diversity and a propensity for three *Listeria monocytogenes* clones with enhanced virulence. Appl Environ Microbiol 2017, 83, e01189-17, doi: 10.1128/AEM.01189-17.
26. Magalhães R., Ferreira V., Brandão T.R., Casquete Palencia R.C., Almeida G., Teixeira P.: Persistent and non-persistent strains of *Listeria monocytogenes*: a focus on growth kinetics under different temperature, salt, and pH conditions and their sensitivity to sanitizers. Food Microbiol 2016, 57, 103–108, doi: 10.1016/j.fm.2016.02.005.
27. Maiden M.C.J., van Rensburg M.J.J., Bray J.E., Earle S.G., Ford S.A., Jolley K.A., McCarthy N.D.: MLST revisited: the gene-by-gene approach to bacterial genomics. Nat Rev Microbiol 2013, 11, 728–736, doi: 10.1038/nrmicro3093.
28. Maury M.M., Tsai Y.-H., Charlier C., Touchon M., Chenal-Francisque V., Leclercq A., Criscuolo A., Gaultier C., Roussel S., Brisabois A., Disson O., Rocha E.P.C., Brisse S., Lecuit M.: Uncovering *Listeria monocytogenes* hypervirulence by harnessing its biodiversity. Nat Genet 2016, 48, 308–313, doi: 10.1038/ng.3501.
29. Moura A., Criscuolo A., Pouseele H., Maury M.M., Leclercq A., Tarr C., Björkman J.T., Dallman T., Reimer A., Enouf V., Larsonneur E., Carleton H., Bracq-Dieye H., Katz L.S., Jones L., Touchon M., Tourdjman M., Walker M., Stroika S., Cantinelli T., Chenal-Francisque V., Kucerova Z., Nadon C., Rocha E.P.C., Grant K., Nielsen E.M., Pot B., Gerner-Smidt P., Lecuit M., Brisse S.: Whole genome-based population biology and epidemiological surveillance of *Listeria monocytogenes*. Nat Microbiol 2016, 2, 16185, doi: 10.1038/nmicrobiol.2016.185.
30. NicAogáin K., O'Byrne C.P.: The role of stress and stress adaptations in determining the fate of the bacterial pathogen *Listeria monocytogenes* in the food chain. Front Microbiol 2016, 7, 1865, doi: 10.3389/fmicb.2016.01865.
31. Nightingale K.K., Lyles K., Ayodele M., Jalan P., Nielsen R., Wiedmann M.: Novel method to identify source-associated phylogenetic clustering shows that *Listeria monocytogenes* includes niche-adapted clonal groups with distinct ecological preferences. J Clin Microbiol 2006, 44, 3742–3751, doi: 10.1128/JCM.00618-06.
32. Nightingale K.K., Windham K., Martin K.E., Yeung M., Wiedmann M.: Select *Listeria monocytogenes* subtypes commonly found in foods carry distinct nonsense mutations in *inlA*, leading to expression of truncated and secreted internalin A, and are associated with a reduced invasion phenotype for human intestinal epithelial cells. Appl Environ Microbiol 2005, 71, 8764–8772, doi: 10.1128/AEM.71.12.8764-8772.2005.
33. Noordhout de C.M., Devleesschauwer B., Angulo F.J., Verbeke G., Haagsma J., Kirk M., Havelaar A., Speybroeck N.: The global burden of listeriosis: a systematic review and meta-analysis. Lancet Infect Dis 2014, 14, 1073–1082, doi: 10.1016/S1473-3099(14)70870-9.
34. O'Byrne C.P., Karatzas K.A.: The role of Sigma B (sB) in the stress adaptations of *Listeria monocytogenes*: overlaps between stress adaptation and virulence. Adv Appl Microbiol 2008, 65, 115–140, doi: 10.1016/S0065-2164(08)00605-9.
35. Orsi R.H., den Bakker H.C., Wiedmann M.: *Listeria monocytogenes* lineages: genomics, evolution, ecology, and phenotypic characteristics. Int J Med Microbiol 2011, 301, 79–96, doi: 10.1016/j.ijmm.2010.05.002.
36. Orsi R.H., Jagadeesan B., Baert L., Wiedmann M.: Identification of closely related *Listeria monocytogenes* isolates with no apparent evidence for a common source or location: A retrospective whole genome sequencing analysis. J Food Prot 2021, 84, 1104–1113, doi: 10.4315/jfp-20-417.
37. Orsi R.H., Maron S.B., Nightingale K.K., Jerome M., Tabor H., Wiedmann M.: Lineage specific recombination and positive selection in coding and intragenic regions contributed to evolution of the main *Listeria monocytogenes* virulence gene cluster. Infect Genet Evol 2008, 8, 566–576, doi: 10.1016/j.meegid.2008.04.006.
38. Osek J., Lachtara B., Wieczorek K.: *Listeria monocytogenes* – how this pathogen survives in food-production environments? Front Microbiol 2022, 13, 866462, doi: 10.3389/fmicb.2022.866462.
39. Osek J., Lachtara B., Wieczorek K.: *Listeria monocytogenes* in foods – from culture identification to whole-genome characteristics. Food Sci Nutr 2022, 10, 2825–2854, doi: 10.1002/fsn3.2910.
40. Palaiodimou L., Fanning S., Fox E.M.: Genomic insights into persistence of *Listeria* species in the food processing environment. J Appl Microbiol 2021, 131, 2082–2094, doi: 10.1111/jam.15089.
41. Palma F., Mangone I., Janowicz A., Moura A., Chiaverini A., Torresi M., Garofolo G., Criscuolo A., Brisse S., Di Pasquale A., Cammà C., Radomski N.: *In vitro* and *in silico* parameters for precise cgMLST typing of *Listeria monocytogenes*. BMC Genomics 2022, 23, 235, doi: 10.1186/s12864-022-08437-4.
42. Phan-Thanh L., Mahouin F., Aligé S.: Acid responses of *Listeria monocytogenes*. Intern J Food Microbiol 2000, 55, 121–126, doi: 10.1016/s0168-1605(00)00167-7.
43. Ragon M., Wirth T., Hollandt F., Lavenir R., Lecuit M., Le Monnier A., Brisse S.: A new perspective on *Listeria monocytogenes* evolution. PLoS Pathog 2008, 4, e1000146, doi: 10.1371/journal.ppat.1000146.
44. Ratani S.S., Siletzky R.M., Dutta V., Yildirim S., Osborne J., Lin W., Hitchins A.D., Ward T.J., Kathariou S.: Heavy metal and disinfectant resistance of *Listeria monocytogenes* from foods and

food processing plants. Appl Environ Microbiol 2012, 78, 6938–6945, doi: 10.1128/AEM.01553-12.

45. Seeliger H.P., Hohne K.: Serotyping of *Listeria monocytogenes* and related species. Meth Microbiol 1979, 13, 31, doi: 10.1016/S0580-9517(08)70372-6.
46. Swaminathan B., Gerner-Smidt P.: The epidemiology of human listeriosis. Microb Infect 2007, 9, 1236–1243, doi: 10.1016/j.micinf.2007.05.011.
47. Tsai Y.-H.L., Maron S.B., McGann P., Nightingale K.K., Wiedmann M., Orsi R.H.: Recombination and positive selection contributed to the evolution of *Listeria monocytogenes* lineages III and I V, two distinct and well supported uncommon *L. monocytogenes* lineages. Infect Genet Evol 2011, 11, 1881–1890, doi: 10.1016/j.meegid.2011.08.001.
48. Unrath N., McCabe E., Macori G., Fanning S.: Application of whole genome sequencing to aid in deciphering the persistence potential of *Listeria monocytogenes* in food production environments. Microorganisms 2021, 9, 1856, doi: 10.3390/microorganisms9091856.
49. Wang H., Luo L.J., Zhang Z.D., Deng J., Wang Y., Miao Y., Zhang L., Chen X., Liu X., Sun S., Xiao B., Li Q., Ye C.: Prevalence and molecular characteristics of *Listeria monocytogenes* in cooked products and its comparison with isolates from listeriosis cases. Front Med 2018, 12, 104–112, doi: 10.1007/s11684-017-0593-9.
50. Wiktorczyk-Kapischke N., Skowron K., Grudlewska-Buda K., Wałecka-Zacharska E., Korkus J., Gospodarek-Komkowska E.: Adaptive response of *Listeria monocytogenes* to the stress factors in the food processing environment. Front Microbiol 2021, 12, 710085, doi: 10.3389/fmicb.2021.710085.