Stress survival islets contribute to clonal and serotype-specific differences in *L. monocytogenes*

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Abstract. *Listeria monocytogenes* is an important opportunistic foodborne pathogen causing listeriosis, an often fatal infection leading to meningitis, sepsis, or infection of the fetus and abortion in susceptible individuals. Diverse ready-to-eat food (RTE) like dairy, meat, fish, vegetables, and complex foods are often linked with listeriosis outbreaks. *L. monocytogenes* is capable of surviving in stressful environmental conditions and grow in refrigerated foods. Regarding stress-related genes, SSI-1 contributes to the survival of cells under suboptimal conditions, such as high salt content and acidic environment. At the same time, SSI-2 is responsible for persistence under alkaline and oxidative stresses.

1. Introduction

*Listeria monocytogenes* is an extremely diverse species; its population structure is divided into 14 serotypes (including hypervirulent serovar 4) and four phylogenetic lineages (I, II, III, and IV) that have been classified into multiple clonal complexes (CCs) and sequence types (ST) [1,2]. *L. monocytogenes* CCs include: (i) infection-associated isolates, which belong to lineage I and are considerably connected with clinical origins and non-food contact surfaces (including CC1, CC2, CC4, and CC6), (ii) food-associated isolates, which belong to lineage II and are mainly within the production environment and associated with food contact surfaces (including CC9 and CC121), and (iii) intermediate-associated isolates that are isolated from both clinical samples and food [3-5]. Lineages III and IV are usually detected less frequently, showing exceptional biodiversity, and are primarily isolated in animals [6]. *L. monocytogenes* belongs to the *Listeria* genus encompassing 17 species, out of which 11 have been reported in the last 13 years (*L. marthii*, *L. rocourtiae*, *L. weihenstephanensis*, *L. grandensis*, *L. riparia*, *L. booriae*, *L. fleischmannii*, *L. floridensis*, *L. aquatica*, *L. newyorkensis*, and *L. cornellensis*) [7].

Listeriosis is a relatively rare disease but is responsible for high mortality rate in elderly and immunocompromised persons, pregnant women and infants. Infection usually occurs by eating food contaminated with high numbers of *L. monocytogenes* [8]. Diverse ready-to-eat food such as dairy, meat, fish, vegetables, and complex foods are often linked with listeriosis outbreaks [9].

In 2018, a total of 2,459 cases of listeriosis were reported in the European Union, with a hospitalization rate of 97.0% and a fatality rate of 15.6% [10].
2. Stress responses of *L. monocytogenes*  
Foodborne transmission of this bacterium is primarily influenced by the ability of *L. monocytogenes* to survive and replicate under a broad range of environmental stress conditions. However, there are differences among different lineages of *L. monocytogenes*. In his study, Hingston et al. [11] concluded that serotypes 1/2a and 1/2b were averagely more cold-resistant compared to serotypes 4b and 1/2c. Subsequent cold growth studies confirmed serotype 1/2a strains as more cold-resistant than serotype 4b strains [12,13]. Furthermore, lineage I strains have been proved to be more salt-tolerant than lineage II strains [14] and serotype 4b strains to be more salt-tolerant than serotype 1/2a and 1/2b [14,15]. Also, lineage I isolates tolerate acid stress conditions significantly better than lineage II isolates [11].

Implementation of cleaning-in-place procedures in food-processing facilities where excessive amounts of oxidizing agents like hydrogen peroxide, chlorine dioxide, peracetic acid, and sodium hypochlorite are used causes alkaline and oxidative stresses for *L. monocytogenes* [16,17]. Manso et al. [18] found that *L. monocytogenes* strains belonging to lineage I (ST5, ST6, ST87, and ST1) are more resistant to oxidative stress than lineage II (ST7, ST9, ST199, and ST321). According to that, this pathogen contains stress-related genes such as stress survival islets SSI-1 and SSI-2.

2.1. Stress survival islets SSI-1 and SSI-2  
SSI-1 is an islet comprising five genes that regulate the growth of *L. monocytogenes* under sub-optimal conditions [19]. These include but are not limited to tolerance to acidic, osmotic, gastric, and bile stress. The islet activity enables pathogen survival in food and enhances pathogenicity in the human host [19]. SSI-1 islet encompass following genes: *lmo0444*, *lmo0445*, *pva* (*lmo0446*), *gadD1* (*lmo0447*) and *gadT1* (*lmo0448*) [19].

This islet is a characteristic of both ST-7 (CC7) and ST-8 (CC8) strains linked with persistence in a study of *L. monocytogenes* strains isolated over twenty years ago from food-processing plants. However, this islet can occasionally be found in some sporadic strains isolated from the food establishments [20]. Besides these two CCs, literature data indicate that SSI-1 is also present in *L. monocytogenes* ST3, ST5, ST7, ST9, ST14, ST36, ST199, ST204, ST226, ST296, ST321, ST375, ST379, ST489, ST739, and ST1041 [21,22,23,24]. Zang et al [25] found that SSI-1 was present in both lineages (Ic, Id, Ile, IIg, III, IIj, and IIk). The common predecessor of subgroups Ic and Id may have acquired SSI-1. The same acquisition occurred in subgroups IIi, IIj, and IIk. On the contrary, the predecessors of subgroups Ile and IIg obtained SSI-1 independently. Genome sequencing revealed that upstream of SSI-1 islet, there is a respective gene encoding transcriptional regulator protein [25]. Further research studies proved that isolates belonging to serotype 1/2b, the majority of which carried SSI-1 (such as CC3 and CC5), were found to create the strongest biofilms. In contrast, isolates belonging to serotype 4b, most of which did not harbor SSI-1 (such as CC2 and CC6), created the weakest biofilms [26,27]. In the study conducted by Arguedas-Villa et al. [28], the growth of *L. monocytogenes* strains isolated in Switzerland and Canada that harboured SSI-1 was not enhanced in cold environment stress conditions.

SSI-1 offers a wider spectrum of adaption than SSI-2. The SSI-2 islet involves two genes: the transcription factor gene *lin0464* and the PpiP protease gene *lin0465*. On the contrary to SSI-1, the SSI-2 islet confers increased survival during alkaline, and oxidative stress conditions frequently met in food processing environments [17]. SSI-2 is a feature of most *L. monocytogenes* ST121 strains (lineage II), which are the most prevalent clones isolated from food or food processing environments. Additionally, the mutation rate of the SSI-2 islet is extremely low, resulting in almost 100% nucleotide identity shared among various ST121 strains [29,30]. Interestingly, SSI-2 positive *L. monocytogenes* strains are detected in lineage I and III and in *L. innocua* but with slightly shorter islets [17,31]. Although *L. monocytogenes* and *L. innocua* have the highest phylogenetic similarity compared to other members of *Listeria* genus and share the same ecological niches, it has been hypothesized that *L. monocytogenes* obtained SSI-2 islet through the horizontal gene transfer event from *L. innocua* [32].

Most *L. monocytogenes* isolates contain one of the two known stress survival islets, SSI-1 or SSI-2, and/or plasmids carrying genes associated with resistance to stress conditions, heavy metals, or biocides.
These islets and resistance-associated plasmids could be responsible for the survival and development of L. monocytogenes under the harsh conditions prevailing in food processing plants [3].

Recent investigations highlight whole-genome sequencing (WGS) to be an affordable, fast, and powerful tool for identifying diverse genetic markers associated with stress (SSI-1 and SSI-2), virulence, antimicrobial resistance, and heavy metal resistance. Also, WGS has been used in a few national studies for Listeria outbreak detection and investigations, e.g., in Austria [34,35], Australia [36], the United States [37], Denmark [38], and France [39]. Interestingly, the presence of SSI-1 could provoke a prolonged outbreak associated with the L. monocytogenes. RTE salmon products were the likely source of this multi-country outbreak affecting five EU countries: Denmark, Estonia, Finland, France, and Sweden [40].

3. Conclusion

L. monocytogenes is considered to be one of the several important foodborne pathogens transmitted to humans via contaminated food. It can survive under suboptimal conditions (high salt, low pH, and alkaline and oxidative stress) commonly present in food processing environments due to recently identified stress resistance markers SSI-1 and SSI-2. When it comes to the prevalence of SSI’s in isolates, there is clear evidence that SSI-1 is equally circulating among infection-associated isolates and food-associated isolates. At the same time, SSI-2 islet is mainly found in food-associated ST121 strains (CC121, lineage II), indicating genetic adaptation and resistance to alkaline and oxidative stresses.

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