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Diverse *Listeria monocytogenes* in-house clones are present in a dynamic frozen vegetable processing environment

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ABSTRACT

Listeria (*L.*) *monocytogenes* is of global concern for food safety as the listeriosis-causing pathogen is widely distributed in the food processing environments, where it can survive for a long time. Frozen vegetables contaminated with *L. monocytogenes* were recently identified as the source of two large listeriosis outbreaks in the EU and US. So far, only a few studies have investigated the occurrence and behavior of *Listeria* in frozen vegetables and the associated processing environment.

This study investigates the occurrence of *L. monocytogenes* and other *Listeria* spp. in a frozen vegetable processing environment and in frozen vegetable products. Using whole genome sequencing (WGS), the distribution of sequence types (MLST-STs) and core genome sequence types (cgMLST-CT) of *L. monocytogenes* were assessed, and in-house clones were identified. Comparative genomic analyses and phenotypical characterization of the different MLST-STs and isolates were performed, including growth ability under low temperatures, as well as survival of freeze-thaw cycles.

Listeria were widely disseminated in the processing environment and five in-house clones namely ST451-CT4117, ST20-CT3737, ST8-CT1349, ST8-CT6243, ST224-CT5623 were identified among *L. monocytogenes* isolates present in environmental swab samples. Subsequently, the identified in-house clones were also detected in product samples. Conveyor belts were a major source of contamination in the processing environment. A wide repertoire of stress resistance markers supported the colonization and survival of *L. monocytogenes* in the frozen vegetable processing facility. The presence of ArgB was significantly associated with in-house clones. Significant differences were also observed in the growth rate between different MLST-STs at low temperatures (4 °C and 10 °C), but not between in-house and non-in-house isolates. All isolates harbored major virulence genes such as full length InlA and InlB and LIPI-1, yet there were differences between MLST-STs in the genomic content.

The results of this study demonstrate that WGS is a strong tool for tracing contamination sources and transmission routes, and for identifying in-house clones. Further research targeting the co-occurring microbiota and the presence of biofilms is needed to fully understand the mechanism of colonization and persistence in a food processing environment.

1. Introduction

Listeria (*L.*) *monocytogenes* is a foodborne pathogen and the causative agent of listeriosis in humans and animals. In healthy individuals, listeriosis is restricted to self-limiting gastroenteritis. However, in susceptible populations such as the immunocompromised, the elderly, pregnant women and newborns, listeriosis can manifest as bacteremia/sepsis, an infection of the central nervous system, or can affect the fetus (Koopmans et al., 2023; Radoshevich and Cossart, 2018). Although listeriosis is a rare disease, the hospitalization and fatality rates are high.

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In 2021, >2000 cases of human listeriosis including a 13.7 % case fatality rate were reported in the EU (EFSA, 2022). Ready-to-eat (RTE) foods of various origins are associated with elevated risk, resulting in sporadic listeriosis cases or (multi)national outbreaks (EFSA, 2022; Ricci et al., 2018).

Contaminated frozen vegetables were the source of two recent listeriosis outbreaks: in the EU between 2015 and 2018 including 53 cases and 10 fatalities (EFSA, 2018; Koutsoumanis et al., 2020) and in the US during 2016, including 10 cases with a 100 % hospitalization rate (Marshall et al., 2020). These outbreaks highlighted the emerging public health risk associated with consumption of frozen vegetables contaminated with *L. monocytogenes*. As frozen vegetables are usually not intended as RTE foods, a threshold of 100 CFU/g is accepted in the EU. However, investigations of the European outbreak indicated possible improper handling of frozen vegetables by customers by omitting the cooking step before consumption e.g. by using frozen vegetables without heating in smoothies or salads (Koutsoumanis et al., 2020). Moreover, thawing and storage of contaminated frozen vegetables at refrigeration or ambient temperatures increases the risk of listeriosis infection, as *L. monocytogenes* is capable of growing under temperatures as low as −0.4 °C (Koutsoumanis et al., 2020; Walker et al., 1990).

The control of *L. monocytogenes* in food and food processing environments is challenging due to its ubiquitous nature and the ability to survive over time even under hostile environmental conditions (Belias et al., 2022; Palaiodimou et al., 2021). Therefore, a detailed monitoring plan including subtyping of *L. monocytogenes* isolates is essential for identifying contamination hotspots and in-house clones and for developing measures for *L. monocytogenes* eradication. For subtyping of *L. monocytogenes*, different approaches can be used, such as serotyping or PFGE. However, molecular subtyping methods based on whole genome sequencing (WGS) show the highest discriminatory power and are widely used for outbreak investigations (Chen et al., 2016; Lakicevic et al., 2023; Lüth et al., 2021; Pietzka et al., 2019). The determination of the core genome multi-locus sequence type (cgMLST-CT) is based on 1701 or 1748 genes (Moura et al., 2017; Ruppitsch et al., 2015), whereas multi-locus sequence typing (MLST) is based on seven housekeeping genes in *L. monocytogenes* (Cai et al., 2002; Ragon et al., 2008). The sequence types (MLST-STs), that do not vary by more than one locus allele are assigned to clonal complexes (CC) (Haase et al., 2014; Ragon et al., 2008), which are further grouped into four evolutionary lineages (Doumith et al., 2004; Nyarko and Donnelly, 2015; Seeliger and Höhne, 1979).

The different CCs are unevenly distributed among clinical and food isolates: e.g. CC1, CC2, CC6 (all lineage I) are more prevalent in human listeriosis cases, while others like CC121, CC9 and CC8 (all lineage II) are strongly associated with food origin (Maury et al., 2016). There are CC-dependent variations in the virulence gene profile which explain differences in the abundance of specific CCs among food (often hypovirulent clones) or clinical isolates (often hypervirulent clones). E.g. the *Listeria* Pathogenicity Islands LIPI-3 and LIPI-4 are detected at low frequencies in lineage II isolates and the *inlA* gene is frequently truncated in isolates of CC121 and CC9, both assigned to lineage II (Lakicevic et al., 2023; Maury et al., 2016; Muchaamba et al., 2022; Sullivan et al., 2022; Wagner et al., 2022).

A wide repertoire of stress resistance genes promotes the survival of *L. monocytogenes* and the establishment of in-house clones in food and food processing environments, where *L. monocytogenes* encounters cold temperatures, acidic or alkaline conditions, high salinity and disinfectants such as quaternary ammonium compounds (QACs) or hydrogen peroxide (Bucur et al., 2018; NicAogáin and O'Byrne, 2016). The stress resistance gene profile is source- and CC-specific. For example, the Stress-Survival-Islet (SSI)-2, involved in tolerance against alkaline and oxidative stress, was detected predominantly in CC121 strains, whereas SSI-1, which provides tolerance against acid and high osmolarity, is present in strains of CC3, CC5, CC7, CC8 and CC9 (Fagerlund et al., 2022; Harter et al., 2017; Liao et al., 2023; Malekmohammadi et al., 2017). In addition, benzalkonium chloride (BC) or cadmium resistance genes, often found on mobile genetic elements, have been identified mainly in lineage II isolates (e.g. ST121, ST8) (Naditz et al., 2019; Palma et al., 2022).

In the current study, we investigated the frequency and abundance of *L. monocytogenes* and other *Listeria* spp. in a European frozen vegetable processing facility (FVPF) in 2019–2020. The study included 3333 environmental swab and 5509 food product samples. In total 947 samples were positive for *L. monocytogenes* and 5052 samples were positive for other *Listeria* spp. We analyzed the distribution and occurrence of subtypes of *L. monocytogenes* on MLST-ST/cgMLST-CT level to identify in-house clones that frequently re-occur and survive in the processing environment as well as transmission routes and contamination sources. In addition, we studied the genomic diversity of the seven most abundant MLST-STs including the virulence gene and stress resistance gene content of 96 representative isolates. Finally, we performed growth experiments under low temperatures and determined the survival of *L. monocytogenes* after multiple freeze-thaw cycles.

2. Material and methods

2.1. Dataset and frozen vegetable processing facility structure

The presence of *L. monocytogenes* and other *Listeria* spp. was analyzed in an European frozen vegetable processing facility (FVPF) in 2019 and 2020. The dataset included environmental swab samples ($n = 3333$) and product samples ($n = 5509$). Basic operations in the FVPF included washing of raw products, optionally cutting/peeling, blanching, cooling-down and freezing of products ("production" area). In addition to frozen blanched vegetables, fried and frozen processed foods with vegetable ingredients were produced (Table S1). The production processing lines were located in three separate rooms. Subsequent to the freezing process, products could be packed in large bins for storage at the facility, mixed with other vegetables and/or coating agents and other ingredients ("mixing" area) and finally packed for retail ("packaging" area). Packaging processing lines were also present in two different rooms in the facility. Due to the different availability of vegetables over the course of the year, some processing lines were not running permanently. Processing lines in the different areas were only sampled if they were in operation. In total, the dataset contained 62 different product groups.

The environmental sampling plan comprised swab samples and 25 g product samples that were taken weekly by the quality management team of the FVPF in the production, mixing and packaging areas from in-use surfaces. Swab samples included food contact surfaces (FCS, $n = 44$), indirect food contact surfaces (iFCS, $n = 35$) and non-food-contact surfaces (nFCS, $n = 24$). The sampling plan included 48 sampling sites in the production area (only after the blanching process; main locations: blanchers, freezers, fryer, floor areas, drains), 26 sampling sites in the mixing area (main locations: conveyors, steel funnels, steel bins, drains, floor areas) and 30 sampling sites in the packaging area (main locations: conveyors, scales and filling equipment for product packaging, drains, floor areas). Products that were purchased from other food processing facilities (e.g. coating agents, other frozen vegetables) were also tested. The samples were transferred within 24 h to a laboratory for further analysis. *Listeria* detection and isolation was performed by a culture-dependent method according to ISO 11290-1:2017. Briefly, swab samples and 25 g food products were incubated for 24 h in half-Fraser broth at 30 °C (primary enrichment) and subsequently in Fraser broth at 37 °C for 24 h. Both enrichment cultures were plated out on RAPID'Lmono® selective medium agar and one colony was selected for species confirmation. *Listeria* isolation and colony selection only focused on *L. monocytogenes* and co-contamination with other *Listeria* spp. was not analyzed.

Regular enumeration of *L. monocytogenes* according to ISO 11290-2:2017 ensured the safety of the food products. All food types of the

product range were intended as non-ready-to-eat food products.

L. monocytogenes isolates were sent for WGS to the Austrian Agency for Health and Food Safety (AGES), where subtyping (MLST-ST/cgMLST-CT) was done. Briefly, DNA of *L. monocytogenes* cultures was extracted using the MagAttract high-molecular-weight (HMW) DNA kit (Qiagen, Hilden, Germany). Whole genome sequencing libraries were prepared using the Nextera XT kit (Illumina, Inc., San Diego, CA, USA) and paired-end sequencing (2 × 300 bp) was performed on a MiSeq platform (Illumina). Subtyping of *L. monocytogenes* isolates was performed using SeqSphere+ v7.2.3 (Ridom GmbH, Würzburg, Germany).

2.2. Data analysis

The spatial and temporal distribution of *L. monocytogenes* and other *Listeria* spp. were investigated using Microsoft Excel (Microsoft 365) and R v3.6.3 (R Core Team, 2020). For data analysis and illustrations, the R packages *dplyr* v1.0.10 (Wickham et al., 2022a), *tidyr* v1.2.1 (Wickham et al., 2022b), *ggplot2* v3.4.0 (Wickham, 2016), *circlize* v0.4.15 (Gu et al., 2014), and *RColorBrewer* v1.1-3 (Neuwirth, 2022) were used. First, the frequency of *L. monocytogenes* and other *Listeria* spp. in swab samples from the different main areas of the facility and on a more detailed level from different processing lines and sampling points was assessed in total for 2019 and 2020, as well as on a yearly and/or monthly basis. The same approach was applied for product samples from different areas, processing lines, and product groups. Moreover, the distribution and prevalence of different MLST-STs and cgMLST-CTs were examined. *L. monocytogenes* in-house clones were identified based on the repeated isolation of the same cgMLST-CT in environmental swab samples of the same sampling site over the course of two years (2019–2020). The spatial distribution of the identified in-house clones was further investigated. Identification of potential transmission routes was performed by visualization of interconnected environmental sampling sites by cgMLST-CT data using the R packages *dplyr* v1.1.0 (Wickham et al., 2023a), *igraph* v1.4.1 (Csárdi et al., 2023), *ggraph* v2.1.0 (Pedersen, 2022), *colormap* v0.1.4 (Karambelkar, 2016), and *viridis* v0.6.2 (Garnier et al., 2021).

2.3. Genomic characterization of selected L. monocytogenes isolates

To characterize the genomic diversity between different *L. monocytogenes* subtypes, whole genome SNP analysis followed by phylogenetic inference and an assessment of the virulence, stress resistance and plasmid gene content was performed. Therefore, 96 representative isolates from the seven most abundant MLST (ST1, $n = 10$, 8 cgMLST-CT; ST8, $n = 28$, 16 cgMLST-CT; ST20, $n = 15$, 1 cgMLST-CT; ST26, $n = 8$, 1 cgMLST-CT; ST37, $n = 13$, 13 cgMLST-CT; ST224, $n = 6$, 2 cgMLST-CT and ST451, $n = 16$, 1 cgMLST-CT) including isolates that were identified as in-house clones by cgMLST-CT (ST8-CT1349, n = 10; ST8-CT6243, $n = 4$; ST20-CT3737, n = 15; ST224-CT5623, n = 4; ST451-CT4117, n = 16) were selected. Representative isolates of the identified in-house clones included early and late occurring isolates within the observation period and isolates from different locations or product types in the processing environment. Non-in-house clones were represented by at least one isolate of each cgMLST-CT described above. For detailed analysis of ST8 isolates, the genomes of all available ST8 isolates from 2019 and 2020 ($n = 198$) were characterized.

2.4. Quality check of raw reads and genome assembly

Whole genome sequencing Illumina paired-end raw reads were quality checked using FastQC v0.11.9 (Andrews, 2010). Removal of adapter content and trimming was performed using trimmomatic v0.39 (Bolger et al., 2014). Genomes were assembled using SPAdes (min contig size: 500 bp, min. coverage: 10) implemented in the comprehensive genome analysis pipeline provided by "The Bacterial and Viral Bioinformatics Resource Center (BV-BRC)" (Olson et al., 2023). The pipeline additionally included an assessment of the quality of all assemblies by QUAST.

2.5. SNP analysis

A reference-based whole genome SNP analysis was performed for each MLST-ST group containing in-house clones (ST8, ST20, ST224 and ST451) using the CFSAN SNP Pipeline v2.2.1 (Davis et al., 2015) with default settings. As reference genome for each MLST-ST group, an internal draft genome was selected based on metrics such as highest total length, high average coverage, and low number of contigs. SNP distances were read from the pairwise filtered SNP distance matrix, where abnormal SNPs from the end of contigs and from SNP dense regions have been removed. The resulting SNP matrix, containing the consensus base for each of the samples where SNPs were identified, was further used for phylogenetic tree building using maximum likelihood (Figure S5). Invariant sites were removed with snp-sites implemented in Snippy v4.6.0 (Seemann, 2015). Phylogenetic trees were constructed with IQ-TREE v2.0.3 (Minh et al., 2020) using the GTR + ASC substitution model and with 1000 bootstrap replicates. For visualization of phylogenetic trees iTOL v6.6 (Interactive Tree of Life) (Letunic and Bork, 2021) was used.

2.6. Phylogeny of selected 96 isolates

Trimmed reads were mapped against an internal draft reference genome (ST8-CT1348) and a reference-based alignment was generated using Snippy v4.6.0 (Seemann, 2015). Gaps and Ns were removed from the resulting multiple sequence SNP alignment file using trimAL v1.4 (Capella-Gutiérrez et al., 2009). An initial phylogeny was inferred from the resulting SNP alignment (without gaps and Ns) using IQ-TREE v2.0.3 (Kalyaanamoorthy et al., 2017; Minh et al., 2020) with automated model selection (Fig. 3A: GTR + F + R2; Fig. 3B: GTR + F + R2, Fig. S6: GTR + F + R2). Recombination removal was performed with ClonalFrameML v1.12 (Didelot and Wilson, 2015) and the recombination filtered SNP alignment was used to generate the phylogenetic tree with IQ-TREE v2.0.3 (Kalyaanamoorthy et al., 2017; Minh et al., 2020) with automated model selection (Fig. 3A: GTR + F + R5; Fig. 3B: TVM + F + R4, Fig. S6: GTR + F + R4).

2.7. Determination of virulence, stress resistance and plasmid genes

Local BLAST databases of virulence genes ($n = 99$), stress resistance genes ($n = 104$), plasmid sequences and plasmid genes ($n = 23$) were used to investigate the presence of these genes in the 96 selected *L. monocytogenes* genomes by applying a custom-made python-script (Tables S2–4). In a first step, a BlastN (BLAST version 2.13.0) search with the genomes against the databases of genes in nucleotide format was performed. An e-value of 0.01 and a culling limit of 1 was used to remove redundant hits. Plasmid sequences were identified as present, if the nucleotide identity was ≥99 % and the ratio of the largest alignment fragment length was at least 40 % compared to the reference in the database. In addition, the databases of virulence and stress-resistance genes and the single BLAST hits from the previous BlastN analysis were translated into amino acid sequences using transeq (EMBOSS v6.6.0.0) (Rice et al., 2000). Non-coding genes were excluded from this step. A custom-made python-script was used for selecting the appropriate reading frame for each gene sequence. The translated single query sequences were compared against the local database of virulence and stress-resistance genes in amino acid format using BlastP. Genes were classified as present in a genome if the amino acid sequence identity was ≥92 % and if the length ratio of the BLAST hits (amino acid) relative to the length of the gene in the database (amino acid) was ≥0.75. Double hits and genes with <0.75 length-ratio were manually investigated. A Fisher's exact test using R v4.2.2 (R Core Team, 2022) was performed to test for any significant associations of the presence or absence of stress

resistance genes with in-house or non-in-house clones. Figures showing gene presence/absence patterns were created with the R packages *ggtree* v3.6.2 (Yu et al., 2017), *tidyr* v1.3.0 (Wickham et al., 2023b), *dplyr* 1.1.0 (Wickham et al., 2023a), and *ggplot2* v3.4.1 (Wickham, 2016).

2.8. Growth and survival under low temperatures and multiple freeze-thaw cycles

We investigated the growth ability and the survival after three freeze-thaw cycles of 34 representative *L. monocytogenes* isolates from the seven most abundant MLST-STs (ST1, $n = 6$; 6 cgMLST-CT; ST8, $n = 8$, 6 cgMLST-CT; ST20, $n = 4$, 1 cgMLST-CT; ST26, $n = 3$, 1 cgMLST-CT; ST37, $n = 5$, 5 cgMLST-CT; ST224, n = 3, 2 cgMLST-CT; ST451, n = 5, 1 cgMLST-CT), including in-house and non-in-house isolates. Growth at 10 °C and 30 °C (control) was analyzed in 96 well plates with a starting OD_{600} of 0.05 in a total volume of 200 µL TSB-Y in triplicates. Growth at 4 °C was determined in 10 mL TSB-Y with a starting OD_{600} of 0.1. OD_{600} values were measured using a microplate reader (Biotek Synergy H1, Agilent). All experiments were conducted three times with three technical replicates per experiment.

The survival ability of three freeze-thaw cycles of MLST-ST specific isolate cocktails (ST1, ST8, ST20, ST26, ST37, ST224 and ST451) was assessed. Briefly, isolate cocktails were adjusted to OD_{600} 0.01 in 5 mL TSB-Y and the initial number of colony forming units (CFU) was determined by plating serial dilutions of the bacterial suspensions on TSA-Y agar in triplicates. The isolate cocktails were stored at −20 °C for 24 h and thawed at room temperature for 1 h. This procedure was repeated three times, then the CFU was determined again. Statistical analysis of the survival rate (%) between different MLST-ST isolate cocktails was performed using a Kruskal Wallis rank sum test ($p < 0.05$) using the R package *stats* v4.2.2 and *misty* v0.4.8. For detailed protocol see supplementary material.

3. Results

3.1. Spatial and temporal distribution of Listeria spp.

The abundance of *L. monocytogenes* and other *Listeria* spp. (not further characterized) in swab and product samples of the FVPF was calculated. In total, 947 samples out of 9270 total samples were positive for *L. monocytogenes* including 454 swab samples and 493 product samples. Other *Listeria* spp. were detected in 5052 samples, of which 1803 were swab samples and 3249 product samples. The relative abundance of *L. monocytogenes* decreased from 17.69 % to 8.52 % in swab samples and from 10.87 % to 6.94 % in product samples, between 2019 and 2020. In contrast, the relative abundance of other *Listeria* spp. slightly increased from 51.08 % to 57.88 % in swab samples and from 57.69 % to 60.32 % in product samples from 2019 to 2020.

We further investigated *Listeria* prevalence in swab and product samples from the three main areas of the FVPF ("production", "mixing", "packaging") and in products purchased from other food processing facilities ("purchase"; Table 1, Figs. S1A + B, S2A + B).

L. monocytogenes were only present at low levels in swab and product samples from the production area. However, other *Listeria* spp. were highly abundant: occurring in up to 73 % of swab samples and 81 % of product samples. In the mixing and packaging area, *L. monocytogenes* was more prevalent than in the production area. In the mixing area, *L. monocytogenes* was present in 37.69 % of swab samples and 18.20 % of product samples in 2019. The proportion of *L. monocytogenes* positive samples from the mixing area decreased in 2020, mainly due to the shutdown of a processing line (processing line A). The occurrence of other *Listeria* spp. was high in swab and products samples in the mixing area and even increased in 2020 (swabs: 46.32 %, products: 69.15 %). In the packaging area, up to 17.47 % (2019) and 11.38 % (2020) of swab samples and 10.48 % (2019) and 2.71 % (2020) of product samples were positive for *L. monocytogenes*. The level of other *Listeria* spp. was also high (>38 %) in the packaging area. Notably, *Listeria* prevalence in incoming processed frozen products and ingredients ("purchase") was high, since up to 12.50 % purchase products tested positive for *L. monocytogenes* and 49.33 % were positive for other *Listeria* spp. (Table 1).

3.2. Occurrence of L. monocytogenes in product groups

L. monocytogenes was detected at least once in 18 of 62 different product groups processed in the factory. Over the course of 2019–2020, the most affected product groups were broccoli (26.94 % *L. monocytogenes* positive), cauliflower (37.29 % *L. monocytogenes* positive), and mixed frozen vegetables with or without coating agents (24.42 % *L. monocytogenes* positive). All three product groups contained products or ingredients that were introduced into the local processing environment as purchase products from other processing facilities. Moreover, in 2019, 27.57 % of products and product residues of processing line A, located in the mixing area, were found to be positive for *L. monocytogenes*. Processing line A was shut down in 2020 by the FVPF. Contamination levels of *L. monocytogenes* in products of the FVPF were ≤10 CFU/g food.

3.3. Distribution of subtypes of L. monocytogenes

We further investigated the distribution and occurrence of MLST-STs and cgMLST-CTs of 947 *L. monocytogenes* isolates from swab and product samples. The isolates could be assigned to 34 different MLST-STs, of which the seven most abundant types were ST451 (26.90 %), ST8 (21.20 %), ST20 (17.83 %), ST1 (4.96 %), ST224 (4.54 %), ST37 (4.22 %) and ST26 (2.85 %, Fig. 1A, Table 2). ST1 and ST224 were assigned to

Table 1
Frequency of *L. monocytogenes* and other *Listeria* spp. in environmental swab and product samples from different areas in 2019 and 2020.

Area	Group	Swab	Swab	Swab	Product	Product	Product
		2019 numbers [%]	2020 numbers [%]	Change 2020 vs. 2019 [%]	2019 numbers [%]	2020 numbers [%]	Change 2020 vs. 2019 [%]
Production	Samples	628	639		565	715	
Production	*L. monocytogenes*	21 [3.34 %]	23 [3.60 %]	+7.64 %	4 [0.71 %]	2 [0.28 %]	−50.00 %
Production	Other *Listeria* spp.	454 [72.29 %]	467 [73.08 %]	+1.09 %	455 [80.53 %]	537 [75.10 %]	−6.74 %
Mixing	Samples	459	190		555	470	
Mixing	*L. monocytogenes*	173 [37.69 %]	29 [15.26 %]	−59.50 %	101 [18.20 %]	76 [16.17 %]	−11.14 %
Mixing	Other *Listeria* spp.	157 [34.20 %]	88 [46.32 %]	+35.41 %	307 [55.32 %]	325 [69.15 %]	+25.01 %
Packaging	Samples	767	650		1508	1107	
Packaging	*L. monocytogenes*	134 [17.47 %]	74 [11.38 %]	−34.84 %	158 [10.48 %]	30 [2.71 %]	−74.13 %
Packaging	Other *Listeria* spp.	336 [43.81 %]	301 [46.31 %]	+5.71 %	687 [45.56 %]	431 [38.93 %]	−14.54 %
Purchase	Samples				344	673	
Purchase	*L. monocytogenes*				43 [12.50 %]	79 [11.74 %]	−6.09 %
Purchase	Other *Listeria* spp.				148 [43.02 %]	332 [49.33 %]	+14.66 %

lineage I, whereas ST8, ST20, ST26, ST37 and ST451 belong to lineage II. In swab samples, 15 different MLST-STs were present and in product samples 31 different MLST-STs (Fig. 1B + C, Table S6). In the production area, ST8 was highly abundant in swab samples (68.18 %), but contamination of product samples rarely occurred (only two ST8 positive samples). In swab samples from the mixing area, mainly ST451 (44.06 %; sources: conveyor belts, stainless steel bins, scales and in drains), ST20 (28.22 %; sources: drain, steel funnel, steel bin, conveyor belt, floor areas) and ST8 (15.85 %; sources: conveyor belts, steel funnels, screw conveyor, steel bins, floor areas, slides, drains) were detected. In product samples from the mixing area, ST451 (38.42 %) and ST20 (21.47 %) were the most abundant MLST-STs, whereas ST8 was detected at a lower rate (3.39 %). However, 11.30 % of products in the mixing area were positive for ST1, which was not present in swab samples. The most abundant MLST-STs in swab samples from the packaging area were ST8 (28.37 %; sources: conveyor belts, drains, floor areas, scales for product filling), ST20 (26.44 %; sources: scales for product filling, floor areas, drains, conveyor belts), ST224 (17.79 %; sources: steel tray, conveyor belt, scale for product filling, filling funnels, drains) and ST451 (12.50 %; sources: scale for product filling, floor areas, conveyor belts, slide). *L. monocytogenes* positive product samples from the packaging area were predominantly contaminated with ST451 (33.51 %), ST8 (32.98 %) and ST20 (6.91 %). Many different MLST-STs such as ST37 (18.85 %), ST1 (14.75 %) and ST26 (11.48 %) were detected in purchase products incoming from other processing facilities and could therefore be detected in product samples in downstream processing areas such as the mixing and packaging area, but were not present or were detected in very low numbers (<0.5 %) in swab samples of the respective areas. A temporal analysis of MLST-STs distribution in the processing environment revealed strong monthly alterations in the occurrence and prevalence of different MLST-STs over the course of 2019 and 2020 underlining the dynamic process structures and operations in the facility (Fig. S3). MLST-STs were detected in different frequencies in the different months of the observation period.

For analyzing transmission dynamics of *L. monocytogenes* in the processing environment, subtyping results on cgMLST-CT level were used. The analysis demonstrated that *L. monocytogenes* were broadly disseminated and almost all sampling sites in the FVPF were highly interconnected, as demonstrated by the presence of the same subtypes on cgMLST-CT level. This further emphasizes the dynamic nature of the processing environment (Fig. 1D). Therefore, no clear transmission routes or transmission patterns within the processing environment could be defined and the distribution of cgMLST-CTs was in most cases not limited to specific areas, rooms, or processing lines.

3.4. Identification of in-house clones

The temporal and spatial distribution of cgMLST-CTs from the seven most abundant MLST-STs was further analyzed to identify in-house clones (Table 2, Figs. 2A–D, S4A–C). An in-house clone was defined as a clone of the same cgMLST-CT, which was detected in swab samples from the same sampling site at least two times and at the same processing line at least five times over the course of 2019 and 2020. Additionally, the presence of in-house clones in product samples was investigated. In total, five different in-house clones were identified, namely ST451-CT4117 (main location: mixing area), ST20-CT3737 (main location: mixing and packaging area), ST8-CT1349 (main location: packaging area), ST8-CT6243 (main location: production area) and ST224-CT5623 (main location: subarea of packaging area). All in-house clones were also detected in product samples of the FVPF, even though ST8-CT6243 was only present once in product samples (Fig. 2A–D, Table 2). Other MLST-STs and/or cgMLST-CTs such as subtypes among ST26, ST37 and ST1 were only sporadically present in swab samples of the local processing environment and/or were mainly detected in products samples purchased from other processing facilities (Fig. S4A–C).

To investigate genomic differences with higher discriminatory power, whole genome SNP analysis was applied on isolates of MLST-ST groups containing representative isolates of previously identified in-house clones and other cgMLST-CTs (ST8, ST20, ST224, ST451). The minimum and maximum pairwise SNP number was determined between all isolates within a MLST-ST group (Tables S8–10). Isolates with ≤20 SNPs were considered as closely related (Jagadeesan et al., 2019; Wang et al., 2018) and were interpreted as single clone. For previously identified in-house clones, pairwise SNP distances ranged from 0 to 7 SNPs between ST451-CT4117 isolates, 0–18 SNPs for ST20-CT3737 isolates, 0–21 SNPs for ST8-CT1349, 2–17 SNPs for ST8-CT6243 and 3–5 SNPs for ST224-CT5623 isolates. As the maximum number of pairwise SNP for ST8-CT1349 exceeded 20 SNPs, indicating a loose genomic relatedness of some isolates, we aimed to identify the potential presence of an additional in-house clone among ST8-CT1349 isolates on whole genome SNP level. Therefore, we investigated the pairwise SNP distances of all ST8-CT1349 isolates (n = 119) from 2019 and 2020. Pairwise SNP numbers ranged from 0 and 37 among the ST8-CT1349 isolates (Table S11). Additionally, the ST8-CT1349 isolates clustered in two major clades in the phylogenetic tree, which included all available ST8 isolates from this study (n = 198, 17 different cgMLST-CTs) (Fig. S5), emphasizing the genomic diversity among the ST8-CT1349 isolates. In addition, 6 % of the ST8-CT1349 isolates clustered with ST8-CT1348 isolates. The isolates in this cluster shared <10 SNPs, indicating a high genomic relatedness and the presence of an additional in-house clone.

3.5. Presence of plasmids, and stress and virulence gene profiling

The presence of 23 plasmid associated genes, 103 stress and 97 virulence genes was investigated for 96 selected *L. monocytogenes* isolates of the seven most abundant MLST-STs, including in-house clones (Tables S12–14). We observed differences between various MLST-ST groups (Fig. 3A + B). The gene presence/absence profile within each MLST-ST group was mainly uniform.

All ST8 isolates except the isolate ST8-CT1247 harbored the plasmid pLMR479a (nucleotide identity to reference: 99.99–100 %) including the cadmium resistance genes encoding CadA1CadC1 (Mullapudi et al., 2010). Plasmids were absent in all other isolates (Fig. 3A).

SSI-1, involved in acid stress (Ryan et al., 2010), was present in isolates of ST8, ST26 and ST224 and absent in isolates of the other MLST-ST groups (ST451, ST20, ST1, ST37). SSI-2, essential in alkaline and oxidative stress (Harter et al., 2017), was absent in all 96 isolates. A gene encoding for a protein with unknown function, LMOf2365_0481 (Hein et al., 2011), located in the same hypervariable genetic hotspot, was present in ST451, ST20, ST1 and ST37.

Further, ArgB, which is involved in acid stress (Ryan et al., 2009), was absent in ST1 and ST224, but present in all other isolates. ArgB presence was further significantly (p < 0.05) associated with in-house clones (Fisher's exact test, Table S15). MdrL, a benzalkonium chloride tolerance marker (Jiang et al., 2019), was present in all isolates except for ST1-CT6739. PerR, involved in oxidative stress (Rea et al., 2005) and RsbW, which is part of the stressosome (Chaturongakul and Boor, 2004) and the general stress response, were absent in ST8-CT14031. Genes encoding for disinfectant resistance markers such as BcrABC (Elhanafi et al., 2010), EmrC (Kremer et al., 2017), EmrE (Kovacevic et al., 2016) and QacH (Müller et al., 2013) were absent in the 96 genomes.

Major virulence genes such as InlA, InlB and LIPI-1 (encoding for PrfA, PlcA, LLO, Mpl, ActA, PlcB), essential for host infection, cell invasion, escape from the vacuole and cell-to-cell spread (Bierne et al., 2007; Vázquez-Boland et al., 2001), were present in all 96 *L. monocytogenes* genomes (Fig. 4B). InlC, capable of reducing the host's innate immune response (Gouin et al., 2010), was detected in all 96 genomes. InlC2 and InlD were present in ST1 and ST224 isolates. InlE, InlF, InlG, InlH, InlJ, and InlK, which contribute to virulence and pathogenicity (Bierne et al., 2007), were present in ST451, ST8 (InlK absent in ST8-CT14340), ST20, ST37 and ST26. InlP1 and InlP3,

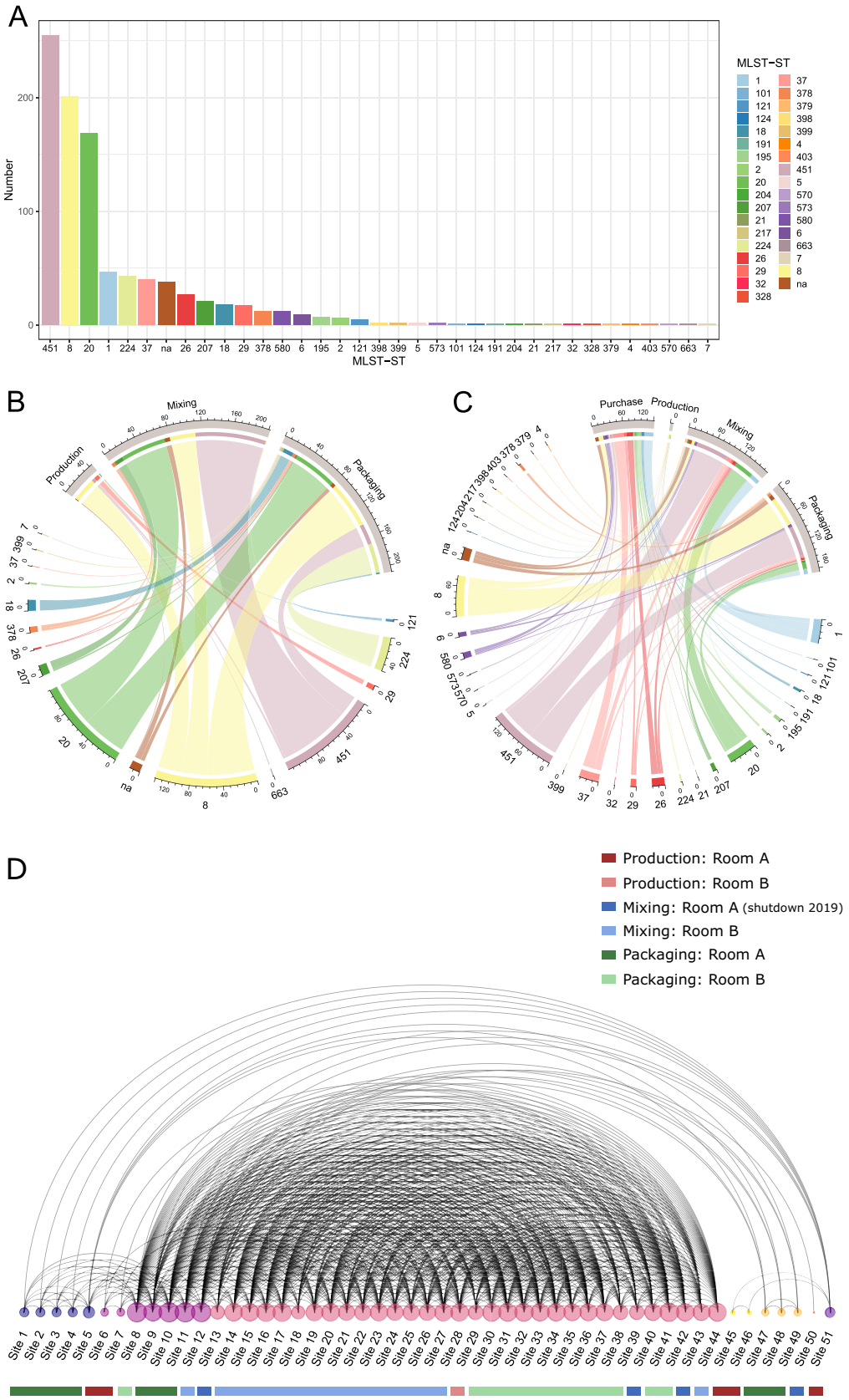

Fig. 1. (A) Frequency of different *L. monocytogenes* MLST-STs in environmental swab and product samples. Distribution of the *L. monocytogenes* MLTS-STs in environmental swab samples (B) and product samples (C) taken from the production, mixing and packaging areas, and from purchase articles from other processing facilities (only food samples); na: isolates with unassigned MLST-ST.
(D) Potential transmission routes of *L. monocytogenes* cgMLST-CTs in the processing environment. If the same cgMLST-CT was detected at least once at two different

sampling sites, the sampling sites were connected by a black line. The size of the nodes reflects the number of connections to other sampling sites. The color of nodes represents groups of sampling sites that are especially interconnected. Colored bars represent different rooms in the processing environment.

Table 2
Prevalence of *L. monocytogenes* in-house clones in swab and product samples from the main areas of the processing environment (production, mixing and packaging) and from products purchased from other processing facilities.

L. monocytogenes ST-CT	Occurrence	Main isolation sources
ST451	*n* = 255, 26.90 % of total L. *monocytogenes*	
ST451-CT4117	*n* = 237, 25.00 % of total *L. monocytogenes*	Mixing and packaging area
Swab samples	*n* = 111, 11.71 % of total *L. monocytogenes*	Conveyor belts, drains, floor areas, scales for product packaging
Product samples	*n* = 126, 13.29 % of total *L. monocytogenes*	Beans, product residues, vegetable mix, salsify, spinach
ST8	*n* = 201, 21.20 % of total *L. monocytogenes*	
ST8-CT1349	n = 119, 12.55 % of total *L. monocytogenes*	Packaging area
Swab samples	*n* = 73, 7.70 % of total *L. monocytogenes*	Conveyor belts, drains, scales for product packaging, floor areas, tunnel surface at the freezer entry
Product samples	*n* = 46, 4.85 % of total *L. monocytogenes*	Beans, product residues, fried products, vegetable mix, peas, salsify, spinach
ST8-CT6243	*n* = 42, 4.43 % of total *L. monocytogenes*	Predominantly production area
Swab samples	n = 35, 3.69 % of total *L. monocytogenes*	Blancher, stainless steel bins, plastic deflectors, drains, floor areas
Product samples	n = 7, 0.74 % of total *L. monocytogenes*	Broccoli, cauliflower, carrots
ST20	*n* = 169, 17.83 % of total *L. monocytogenes*	
ST20-3737	*n* = 158, 16.67 % of total *L. monocytogenes*	Mixing and packaging area
Swab samples	*n* = 112, 11.81 % of total *L. monocytogenes*	Drains, floor areas, conveyor belts, scales for product packaging
Product samples	*n* = 45, 4.75 % of total *L. monocytogenes*	Beans, broccoli, carrots, product residues, vegetable mix, salsify, spinach
ST224	*n* = 43, 4.54 % of total *L. monocytogenes*	
ST224-CT5623	n = 35, 3.70 % of total *L. monocytogenes*	Sub-area of packaging
Swab samples	*n* = 34, 3.59 % of total *L. monocytogenes*	Conveyor belts, steel tray, scale for product filling, filling funnels, plastic deflector, drains
Product samples	n = 1, 0.11 % of total *L. monocytogenes*	Fried products

influencing invasion in human intestinal epithelial cells (Harter et al., 2019), were absent in all 96 isolates.

LIPI-3, associated with a subset of lineage I isolates and comprising eight genes encoding a hemolytic and cytotoxic factor (Cotter et al., 2008), was partially present in ST1 and ST224 isolates: LlsA was present in four ST1, in one ST224-CT4656 and two ST224-CT5623 isolates. LlsB, LlsD, LlsG, LlsH, LlsP, LlsX and LlsY were detected in the genomes of investigated ST1 and ST224 isolates except for one ST224-CT4656 and one ST1-CT7949 isolate where LlsP was absent. LIPI-4, involved in the infection of the CNS and placenta (Maury et al., 2016), was absent from all 96 isolates.

Ami, which strongly supports the adhesion of *L. monocytogenes* to eukaryotic cells (Milohanic et al., 2001), was absent in ST1. Furthermore, Aut, essential for entry into eukaryotic cells (Cabanes et al., 2004), and GtcA, described to be involved in the pathogenicity in the gastrointestinal tract in mice (Faith et al., 2009; Promadej et al., 1999), were absent in ST1. Lmo2026/InlL, promoting binding of *L. monocytogenes* to intestinal mucin (Popowska et al., 2017), was only present in ST20, ST37 and ST26 and Vip, involved in the entry in some mammalian cells (Cabanes et al., 2005), was detected in ST451, ST20 and ST26.

3.6. Cold stress

The intrinsic growth rate (r in hours^{-1}), describing the growth rate per hour assuming no limitations regarding the total population size, was assessed for the different MLST-ST groups (Fig. 4A–C) and for in-house versus non-in-house clones (Fig. S7A–C) under cold conditions at 4 °C and 10 °C, and at 30 °C as growth control. Intrinsic growth rate values ranged from 0.024 to 0.154 at 4 °C, 0.110 to 0.877 at 10 °C and 1.149 to 1.690 at 30 °C. The intrinsic growth rate was significantly increased for ST37 compared to ST1, ST20 and ST224 and for ST8 versus ST224 at 4 °C. At 10 °C, the growth rate was significantly higher for ST37 compared to ST1 and ST8. Interestingly, intrinsic growth rates were significantly higher for ST20 and ST26 in comparison to ST37 as well as ST1, ST8, and ST224 at 30 °C.

When comparing in-house versus non-in-house clones, no significant differences in their intrinsic growth rates was detected at all three temperatures.

3.7. Survival of freeze-thaw cycles

ST1, ST8, ST20, ST26, ST37, ST224 and ST451 isolate cocktails were subjected to three freeze-thaw cycles. The resulting survival rate of the MLST-ST groups was assessed under freeze-thaw stress, as this condition is frequently found in frozen vegetable processing environments. Average survival rates ranged from 75.51 % to 90.9 %. No significant differences between the MLST-ST groups regarding their survival rates under freeze-thaw stress were observed (Fig. 4D).

4. Discussion

The current study investigated the occurrence and distribution of *L. monocytogenes* and other *Listeria* spp. in a European FVPF and identified in-house clones, which were successfully able to colonize the processing environment.

The prevalence of *L. monocytogenes* was dependent on the isolation area, product type and isolation time point, which indicates a highly dynamic environment. We detected a *L. monocytogenes* prevalence of 3.34 % to 37.69 % in environmental swab samples, which is in line with other studies reporting 12.5 % (Truchado et al., 2022) to 41.3 % (Pappelbaum et al., 2008) *L. monocytogenes* positive environmental samples. The prevalence of *L. monocytogenes* was especially low in the production area after the blancher (swab max. 3.60 %, product max. 0.71 %). However, recontamination of products occurred in the mixing area and packaging area resulting in up to 18 % *L. monocytogenes* positive product samples. In parallel, the prevalence of *L. monocytogenes* in environmental swab samples was up to 38 % in the mixing and up to 17 % in the packaging area. In addition, products purchased from other processing facilities were frequently contaminated with *L. monocytogenes* (up to 13 %). Thus, introduction of these products to the process chain is one aspect further increasing the prevalence of *L. monocytogenes* in the post-blanching areas of the processing environment. On average, other studies found *L. monocytogenes* in 0.56 % in fresh and frozen vegetables (Maćkiw et al., 2021), or in 5.5 % (Skowron et al., 2019) to 46.8 % (Pappelbaum et al., 2008) of frozen vegetables with variation in prevalence numbers across different years or vegetable types. Among different types of vegetables in our study, *L. monocytogenes* was highly abundant in broccoli (26.94 %), cauliflower (37.29 %), and vegetable mix products (24.42 %), which is in line with a study of Pappelbaum

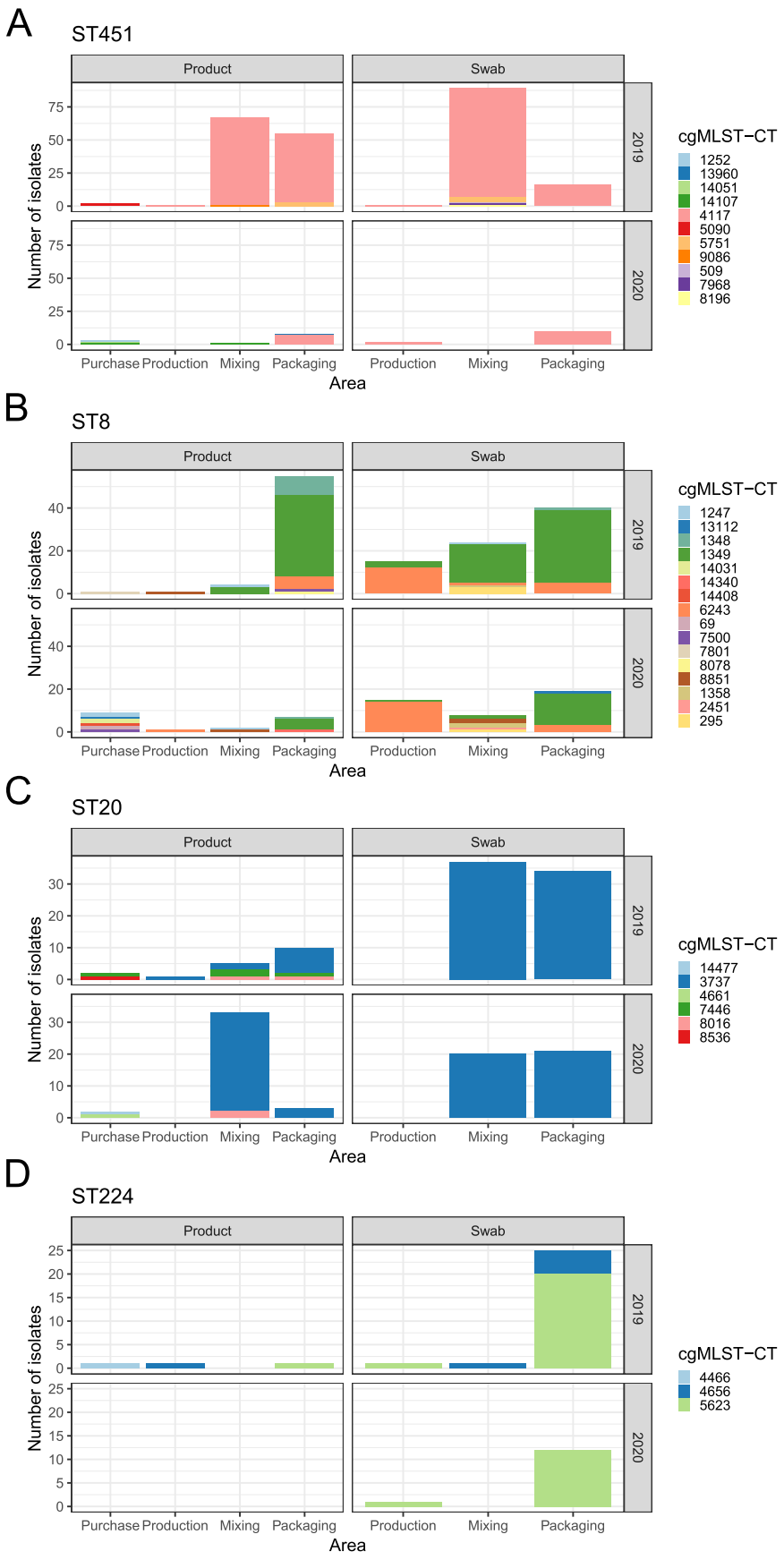

Fig. 2. Distribution and frequency of *L. monocytogenes* cgMLST-CTs belonging to ST451 (A), ST8 (B), ST20 (C) and ST224 (D) in environmental swab samples and product samples taken in the production, mixing and packaging areas, and from purchase articles from other processing facilities (only food samples) in 2019 and 2020.

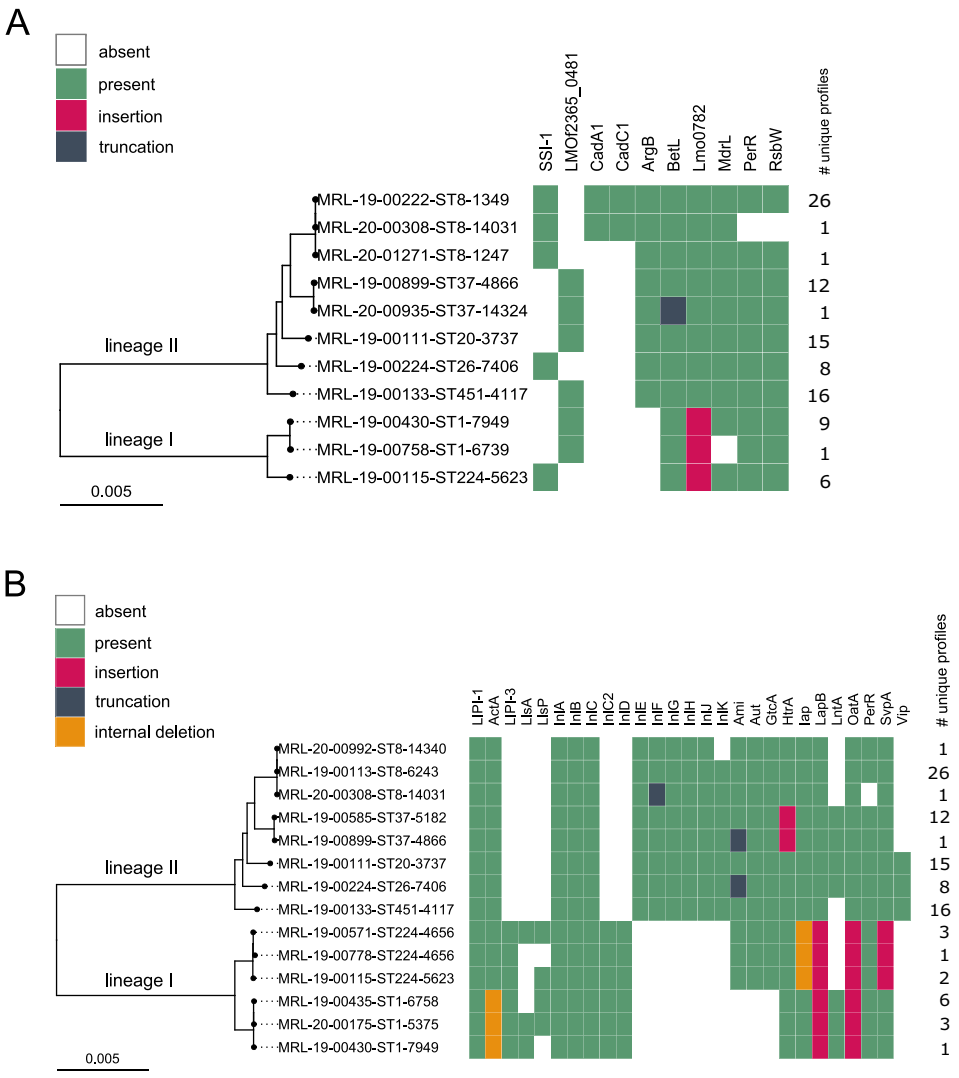

Fig. 3. The stress (A) and virulence (B) gene profile of *L. monocytogenes* isolates of the seven most abundant MLST-STs. Selected isolates representing unique gene patterns of each MLST-ST group are shown. The number of genomes harboring the same unique gene profile is indicated in the right column. Midpoint rooted maximum likelihood phylogeny based on a multiple sequence alignment shows one arbitrarily selected genome from each of the groups of genomes containing the same unique gene combination.

et al. (2008), where *Listeria* were also prevalent in frozen cauliflower, mixed vegetables and broccoli samples. We hypothesize that the rough and complex structure of broccoli and cauliflower provides a favorable niche for survival of *Listeria* compared to other vegetable types. According to a report from the European Food Safety Authority (EFSA), the occurrence of *L. monocytogenes* in frozen vegetables (estimated average prevalence 11.4 %) is high compared to the average occurrence in other food types (RTE) such as fish and fishery products (4.7 %), meat and meat products (2.3 %) and milk and milk products (0.51 %) (EFSA, 2022; Koutsoumanis et al., 2020). Yet, the risk of listeriosis is lower for blanched frozen vegetables compared to most other food types (Koutsoumanis et al., 2020). Using a risk assessment tool, the median estimated listeriosis risk for frozen vegetables per serving was reported to be $<1.0 \times 10^{-16}$, if a minimum of 50 % servings were cooked (Zoellner et al., 2019). Contamination levels of frozen vegetables did not exceed 10 CFU/g in the current study, compliant with the recommended limit of 100 CFU/g at the moment of consumption, as frozen vegetables do not support growth of *Listeria* if not thawed for an extended time period (Koutsoumanis et al., 2020). Therefore, the risk of listeriosis would be extremely low for consumers, if food preparation instructions are followed. Previous listeriosis outbreaks caused by frozen vegetables occurred in the EU in 2015–2018 (EFSA, 2018; McLauchlin et al., 2021) and 2016 in the US (Madad et al., 2023). Outbreak sources and responsible manufacturers could be identified by WGS. In both cases, the outbreaks clones persisted in the processing environment (Madad et al., 2023; McLauchlin et al., 2021).

In the current study, 34 different MLST-STs were identified. ST451, ST8, ST20, ST1, ST224, ST37 and ST26 were the most abundant MLST-STs. Surprisingly, ST121 and ST9 were nearly absent in the facility, although they are frequently isolated from food and food processing environment. Moreover, ST121 has also been reported to be the most prevalent MLST-ST among fruits and vegetables (Maury et al., 2019).

Surprisingly, ST451 was the most prevalent type in the current study and the subtype ST451-CT4117 was identified as a highly abundant in-house clone, first located at processing line A in the mixing area. ST451 is rarely isolated from food and food processing environment. One study detected ST451 in low numbers in mixed frozen vegetables (Willis et al., 2020) and another the persistence of ST451 in a Czech rabbit meat processing facility (Gelbíčová et al., 2019). Moreover, ST451 isolates were detected among clinical and food isolates, mainly associated with dairy products, in an Austrian study, but they were assigned to different cgMLST-CTs than found in this study (Cabal et al., 2019). With the shutdown of processing line A in 2019, numbers of ST451-CT4117 were strongly reduced, which further supports the identification of a

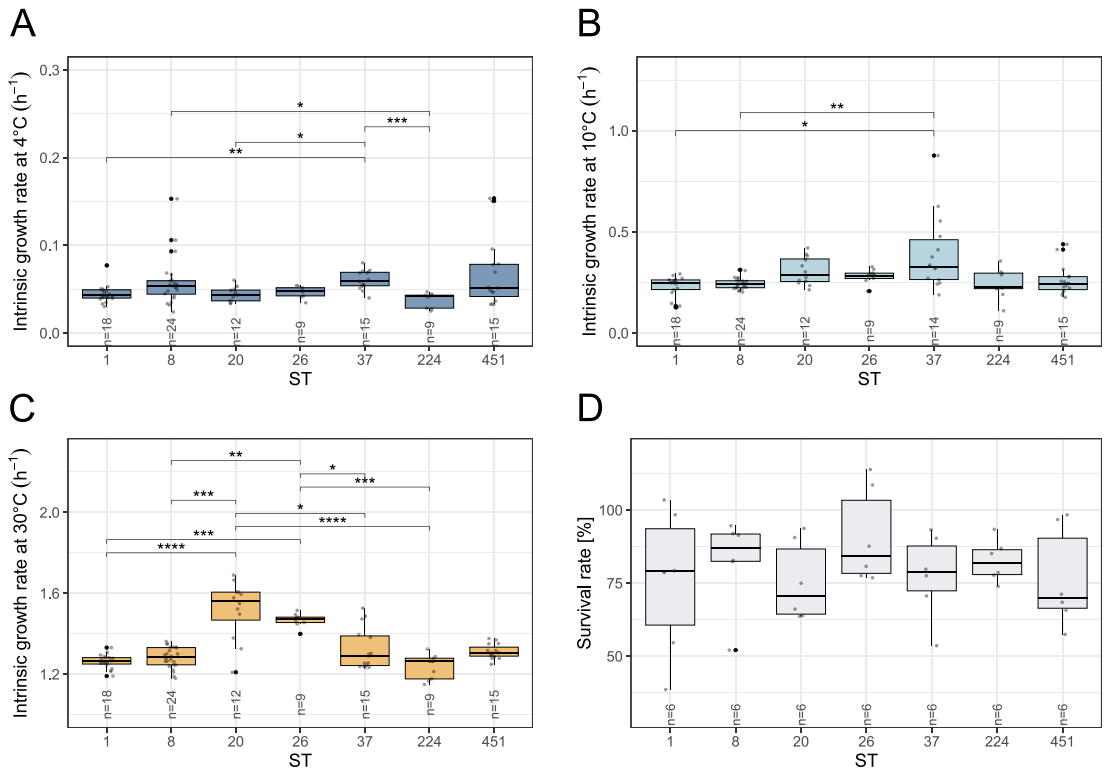

Fig. 4. The intrinsic growth rate (r in hours^{-1}) of isolates grouped per MLST-ST depictured at 4 °C (A), 10 °C (B) and 30 °C (C) shown as boxplots. (D) The survival rate (%) of isolates grouped per MLST-ST after three freeze-thaw cycles shown as boxplots. Significant differences between the MLST-ST groups are labeled with an asterisk (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

contamination hotspot. Transmission of ST451-CT4117 via contaminated products from the mixing area to the packaging area was likely as these clones were also detected in environmental swab samples of the packaging area. ST451-CT4117 harbored different genes contributing to survival under stress conditions found in food processing environments, such as genes associated with the stressosome (Chaturongakul and Boor, 2004; Ferreira et al., 2004), the general stress response (Kazmierczak et al., 2003; Seifart Gomes et al., 2011), further osmotic, acid stress and heat stress. In addition, genes supporting resistance against biocides such as *mepA* against QAC (Haubert et al., 2018), *mdrL* against benzalkonium chloride (Jiang et al., 2019) and *perR* against hydrogen peroxide (Rea et al., 2005) and genes associated with resistance against cold stress, *cspD* (Schmid et al., 2009), *oppA* (Borezee et al., 2000) and *sigL* (Raimann et al., 2009), were present in ST451-CT4117.

ST8 was the second highly prevalent subtype in this study, which is known to be widely disseminated in different types of food and food processing environments (Cabal et al., 2019). ST8 isolates were also previously detected in vegetables (Maćkiw et al., 2021), in frozen fruit and frozen vegetables (Willis et al., 2020) and in a frozen vegetable producing environment (Truchado et al., 2022). Moreover, ST8 was identified as the second top MLST-ST of foodborne *L. monocytogenes* in China (Ji et al., 2023). The set of stress resistance genes was similar to ST451, but ST8 isolates additionally contained SSI-1 (Ryan et al., 2010) and the plasmid pLMR479a (except for ST8-CT1247), carrying genes involved in stress response such as cadmium resistance genes (Schmitz-Esser et al., 2015), probably supporting survival and colonization of the processing environment by the two in-house clones (ST8-CT1349, ST8-CT6243). However, the prevalence and source of these two in-house clones were different. ST8-CT1349 frequently contaminated food products in the packing area, whereas ST8-CT6243 was mainly found in environmental samples from the production area. ST8 showed elevated growth rate under 4 °C, significantly different to ST224, potentially further supporting the wide dissemination and survival under low temperatures found in a frozen vegetable processing environment.

In the ST20 group, the predominant type ST20-CT3737 was identified as in-house clone, which originated in the mixing area, where products were already contaminated with this type. ST20-CT3737 was further detected in environmental swab samples and in product samples in the packaging area, which indicates further colonization of the packaging area by ST20-CT3737. Survival of ST20 was supported by a large set of stress resistance genes, which is very similar to the stress resistance gene content of ST451. ST20 was also present in frozen vegetables in a previous study (Willis et al., 2020) as well as in animals (Jennison et al., 2017) and other animal derived food products such as fish (Ciolacu et al., 2015) and artisanal cheese (Pyz-Łukasik et al., 2022).

ST224 was almost exclusively present at a specific area in the packaging area, which was also the location of the in-house clone ST224-CT5623. ST224 isolates were previously isolated from clinical as well as food sources (Maury et al., 2016) such as RTE foods, meat and the meat processing environment (Cheng et al., 2022; De Cesare et al., 2017; Palma et al., 2017) or edible mushrooms (Chen et al., 2018). The stress resistance gene set of ST224 comprised a wide set of genes similar to ST451, likely contributing to the successful colonization of the processing environment, including biocide tolerance genes *mdrL* (Jiang et al., 2019), *perR* (Rea et al., 2005), and *mepA* (Haubert et al., 2018).

In addition to in-house clones, we detected transient isolates mainly belonging to ST1, ST37 and ST26. These isolates regularly entered the facility via purchase products, but were not able to colonize the processing environment. The repeated finding of specific cgMLST-CTs in purchase article indicated the presence of in-house clones in the supplier's facility.

The findings of this study emphasize the significance of using WGS data for identification of in-house clones and transient clones, which were (repeatedly) introduced in the processing environment but were apparently not able to colonize the processing environment. Moreover,

contamination sources and transmission routes within the local processing environment could be characterized. Previous studies have identified several food contact and non-food contact points as potential niches for *L. monocytogenes* in a frozen vegetable processing environment such as conveyor belts, the entry/exit of the freezing tunnel, drains, exit of the blancher, filling equipment, scales, floor areas and drains (EFSA et al., 2018; Pappelbaum et al., 2008; Truchado et al., 2022), which were similar to sites where in-house clones were present in the local processing environment. Conveyor belts covering large areas of the processing facility were one of the main harborage sites for *L. monocytogenes* in this study, probably due to the hard-to-clean and rough surface, and modular structure. An insufficient blanching process or a (re)contamination right after the blancher was indicated by the presence of in-house clones and other *Listeria* spp., and further may contribute to the colonization of the FVPF (Pappelbaum et al., 2008). Potential cross-transmission in the processing environment was investigated in detail on cgMLST-CT level using WGS data to identify concrete transmission sources. Although one would expect distinct clusters of sampling sites with few connections to sampling sites in other areas/rooms, almost all sampling sites were connected to each other by at least one common cgMLST-CT found on each sampling site. The centralized building structure and undirected employee movement along the main corridor connecting all areas and rooms, movement of vehicles and insufficient disinfection of working shoes are some possible explanations for the vast transmission of *L. monocytogenes* MLST-STs/cgMLST-CTs in the processing environment, as these factors were also found to promote transmission and colonization of other potential niches in other studies (Alvarez-Molina et al., 2021; Stessl et al., 2022; Zhang et al., 2021).

We further applied whole genome SNP analysis to verify the identification of in-house clones by cgMLST-CTs, considering isolates with $\leq$20 SNPs as closely related with respect to the (meta)data (Jagadeesan et al., 2019; Wang et al., 2018). The maximum number of SNPs exceeded 20 SNPs only for ST8-CT1349 and some isolates of ST8-CT1349 clustered with ST8-CT1348 in the phylogenetic tree, indicating the existence of two different in-house clones in the ST8-CT1349 group. Since the identification of in-house clones based on cgMLST-CTs was sufficient for most of the isolates and whole genome SNP analysis requires extensive bioinformatics training, we concluded that in-house clone identification at the cgMLST-CT level is adequate for food companies.

Explaining the colonization potential of some isolates based solely on their genomic content is challenging. Many different genomic features were associated with the ability of *L. monocytogenes* to survive long-term and grow in a food processing environment despite cleaning and disinfection measures. Survival and/or growth have been linked to the presence of stress resistance genes in the genome or on mobile genetic elements such as plasmids or biofilm formation (Gray et al., 2021; Liu et al., 2022; Maggio et al., 2021; NicAogáin and O'Byrne, 2016): e.g. QAC resistance genes *bcrABC* (Elhanafi et al., 2010), *qacH* (Müller et al., 2013), and *emrC* (Kremer et al., 2017), and *emrE* (Kovacevic et al., 2016) were identified in previous studies (Palaiodimou et al., 2021; Palma et al., 2022). However, the isolates of the FVPF, either in-house clones or not, harbored only *mdrL* and *mepA*, and lacked biocide tolerance genes such as *bcrABC*, *qacH*, *emrC* and *emrE*. Yet, presence of *argB*, which is involved in the arginine biosynthesis pathway, was significantly ($p < 0.05$) associated with in-house clones. Availability of arginine is essential for stress response via the arginine deiminase pathway under acidic conditions (Ryan et al., 2009) supporting the survival and colonization of the food processing environment. Presence of *argB* was MLST-ST and lineage dependent in the current study, as only lineage II isolates (ST451, ST8, ST20, ST37, ST26) but not lineage I isolates (ST1, ST224) harbored *argB*. Although there were differences between MLST-STs regarding their growth ability under cold stress, genes correlated with tolerance to low temperatures such as *cspD*, *oppA* or *sigL* were present in all tested isolates. Moreover, all MLST-STs were able to survive multiple freeze-thaw cycles promoting the survival in a frozen vegetable processing facility. We hypothesize that additional factors may play a role, such as a high number of incoming bacterial cells of one subtype of *L. monocytogenes*, or the interplay with the microbiota present in a certain niche are essential factors for the establishment and colonization ability of in-house clones in the frozen vegetable processing environment.

The virulence gene content and length polymorphisms of virulence genes varied between MLST-STs and lineage I and lineage II isolates, as for example LIPI-3, was partially present in lineage I isolates (ST1 and ST224), which is in line with previous findings (Chen et al., 2022; Quereda et al., 2016; Tavares et al., 2020). Full length InlA and LIPI-1, which are required for virulence and pathogenicity, were present in all isolates of this study. Further, ActA, responsible for actin polymerization and supporting movement in the host cell, harbored an internal deletion in isolates of ST1 in the current study. Different ActA variants including internal truncations in ST1 were also noted in a previous study (Rychli et al., 2018), but the impact on virulence of *actA* variants is not fully understood yet (Pistor et al., 1995; Roberts and Wiedmann, 2006; Travier et al., 2013). Interestingly, accessory virulence factors *inlEFGHJK* were not present in lineage I isolates (ST1 and ST224) but were detected in lineage II isolates (ST451, ST8, ST20, ST37, ST26), which is in line with a previous study (Wagner et al., 2022). While lineage II isolates have been previously associated with hypovirulence and persistence in food and food processing environments (Maury et al., 2016; Nightingale et al., 2006; Orsi et al., 2011), the content of accessory virulence genes indicates a virulent phenotype (Bierne et al., 2007; Gou et al., 2022; Ling et al., 2022; Sabet et al., 2008; Schiavano et al., 2021).

In conclusion, we identified five different *L. monocytogenes* in-house clones in a European frozen vegetable processing facility. By using whole genome SNP data, the in-house clone ST8-CT1349 was discriminated into two different in-house clones, which is supported by their location in the processing facility. The results demonstrate that WGS data is a powerful tool for improving food safety, since WGS allows the identification and tracing of sources and transmission of clones on cgMLST-CT or SNP level. Conveyor belts covering large distances in the processing environment were a main niche for in-house clones. Strong indicators for transmission in the whole processing environment underline the importance of an appropriate structure of the building minimizing movements across processing areas. Insufficient sanitation procedures were identified by the FVPF and actions were set to improve the situation. We concluded, that reducing the introduction of contaminated products and ingredients into post-blanching areas and replacing the type of conveyor belt used in the processing environment would reduce the prevalence of *Listeria*. The FVPF is regularly monitoring the safety of products, which drastically minimizes the risk for consumers, if cooking instructions are followed. Analysis of the genomic content of *L. monocytogenes* suggested that the colonization of the processing environment was supported by a wide repertoire of stress resistance genes and the ability of different MLST-STs to grow under low temperatures and survive multiple freeze-thaw cycles. All analyzed isolates were considered as potentially virulent and are important to be eliminated from the processing environment by the frozen vegetable producer. Further investigations of mechanisms and factors supporting the establishment of in-house clones is necessary such as analyzing the co-occurring microbiota at niches that were colonized by *Listeria*, since the diversity in the genomic content was not sufficiently able to explain differences between in-house and transient clones.

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Declaration of competing interest

None of the authors declare to have financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Alvarez-Molina, A., Cobo-Díaz, J.F., López, M., Prieto, M., de Toro, M., Alvarez-Ordóñez, A., 2021. Unraveling the emergence and population diversity of *Listeria monocytogenes* in a newly built meat facility through whole genome sequencing. Int. J. Food Microbiol. 340, 109043 https://doi.org/10.1016/j.ijfoodmicro.2021.109043.

Andrews, S., 2010. FastQC. A quality control tool for high throughput sequence data. https://www.bioinformatics.babraham.ac.uk/projects/fastqc/.

Belias, A., Sullivan, G., Wiedmann, M., Ivanek, R., 2022. Factors that contribute to persistent *Listeria* in food processing facilities and relevant interventions: a rapid review. Food Control 133, 108579. https://doi.org/10.1016/j.foodcont.2021.108579.

Bierne, H., Sabet, C., Personnic, N., Cossart, P., 2007. Internalins: a complex family of leucine-rich repeat-containing proteins in *Listeria monocytogenes*. Microbes Infect. 9, 1156–1166. https://doi.org/10.1016/j.micinf.2007.05.003.

Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114–2120. https://doi.org/10.1093/bioinformatics/btu170.

Borezee, E., Pellegrini, E., Berche, P., 2000. OppA of *Listeria monocytogenes*, an oligopeptide-binding protein required for bacterial growth at low temperature and involved in intracellular survival. Infect. Immun. 68, 7069–7077. https://doi.org/10.1128/IAI.68.12.7069-7077.2000.

Bucur, F.I., Grigore-Gurgu, L., Crauwels, P., Riedel, C.U., Nicolau, A.I., 2018. Resistance of *Listeria monocytogenes* to stress conditions encountered in food and food processing environments. Front. Microbiol. 9, 1–18. https://doi.org/10.3389/fmicb.2018.02700.

Cabal, A., Pietzka, A., Huhulescu, S., Allerberger, F., Ruppitsch, W., Schmid, D., 2019. Isolate-based surveillance of *Listeria monocytogenes* by whole genome sequencing in Austria. Front. Microbiol. 10, 1–8. https://doi.org/10.3389/fmicb.2019.02282.

Cabanes, D., Dussurget, O., Dehoux, P., Cossart, P., 2004. Auto, a surface associated autolysin of *Listeria monocytogenes* required for entry into eukaryotic cells and virulence. Mol. Microbiol. 51, 1601–1614. https://doi.org/10.1111/j.1365-2958.2003.03945.x.

Cabanes, D., Sousa, S., Cebriá, A., Lecuit, M., García-del Portillo, F., Cossart, P., 2005. Gp96 is a receptor for a novel *Listeria monocytogenes* virulence factor, Vip, a surface protein. EMBO J. 24, 2827–2838. https://doi.org/10.1038/sj.emboj.7600750.

Cai, S., Kabuki, D.Y., Kuaye, A.Y., Cargioli, T.G., Chung, M.S., Nielsen, R., Wiedmann, M., 2002. Rational design of DNA sequence-based strategies for subtyping *Listeria monocytogenes*. J. Clin. Microbiol. 40, 3319–3325. https://doi.org/10.1128/JCM.40.9.3319-3325.2002.

Capella-Gutiérrez, S., Silla-Martínez, J.M., Gabaldón, T., 2009. trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics 25, 1972–1973. https://doi.org/10.1093/bioinformatics/btp348.

Chaturongakul, S., Boor, K.J., 2004. RsbT and RsbV contribute to σB -dependent survival under environmental, energy, and intracellular stress conditions in *Listeria monocytogenes*. Appl. Environ. Microbiol. 70, 5349–5356. https://doi.org/10.1128/AEM.70.9.5349-5356.2004.

Chen, Y., Gonzalez-Escalona, N., Hammack, T.S., Allard, M.W., Strain, E.A., Brown, E.W., 2016. Core genome multilocus sequence typing for identification of globally distributed clonal groups and differentiation of outbreak strains of *Listeria monocytogenes*. Appl. Environ. Microbiol. 82, 6258–6272. https://doi.org/10.1128/AEM.01532-16.

Chen, M., Cheng, J., Wu, Q., Zhang, J., Chen, Y., Zeng, H., Ye, Q., Wu, S., Cai, S., Wang, J., Ding, Y., 2018. Prevalence, potential virulence, and genetic diversity of *Listeria monocytogenes* isolates from edible mushrooms in Chinese markets. Front. Microbiol. 9, 1–12. https://doi.org/10.3389/fmicb.2018.01711.

Chen, Y., Simonetti, T., Peter, K., Jin, Q., Brown, E., LaBorde, L.F., Macarisin, D., 2022. Genetic diversity of *Listeria monocytogenes* isolated from three commercial tree fruit packinghouses and evidence of persistent and transient contamination. Front. Microbiol. 12, 1–15. https://doi.org/10.3389/fmicb.2021.756688.

Cheng, Y., Dong, Q., Liu, Y., Liu, H., Zhang, H., Wang, X., 2022. Systematic review of *Listeria monocytogenes* from food and clinical samples in Chinese mainland from 2010 to 2019. Food Qual. Saf. 6, 753–758. https://doi.org/10.1093/fqsafe/fyac021.

Ciolacu, L., Nicolau, A.I., Wagner, M., Rychli, K., 2015. *Listeria monocytogenes* isolated from food samples from a Romanian black market show distinct virulence profiles. Int. J. Food Microbiol. 209, 44–51. https://doi.org/10.1016/j.ijfoodmicro.2014.08.035.

Cotter, P.D., Draper, L.A., Lawton, E.M., Daly, K.M., Groeger, D.S., Casey, P.G., Ross, R. P., Hill, C., 2008. Listeriolysin S, a novel peptide haemolysin associated with a subset of lineage I *Listeria monocytogenes*. PLoS Pathog. 4, e1000144 https://doi.org/10.1371/journal.ppat.1000144.

Csárdi, G., Nepusz, T., Traag, V., Horvát, S., Zanini, F., Noom, D., Müller, K., 2023. {Igraph}: Network Analysis and Visualization in R. https://doi.org/10.5281/zenodo.7682609.

Davis, S., Pettengill, J.B., Luo, Y., Payne, J., Shpuntoff, A., Rand, H., Strain, E., 2015. CFSAN SNP pipeline: an automated method for constructing snp matrices fromnext-generation sequence data. PeerJ Comput. Sci. 2015, 1–11. https://doi.org/10.7717/peerj-cs.20.

De Cesare, A., Parisi, A., Mioni, R., Comin, D., Lucchi, A., Manfreda, G., 2017. *Listeria monocytogenes* circulating in rabbit meat products and slaughterhouses in Italy: prevalence data and comparison among typing results. Foodborne Pathog. Dis. 14, 167–176. https://doi.org/10.1089/fpd.2016.2211.

Didelot, X., Wilson, D.J., 2015. ClonalFrameML: efficient inference of recombination in whole bacterial genomes. PLoS Comput. Biol. 11, 1–18. https://doi.org/10.1371/journal.pcbi.1004041.

Doumith, M., Buchrieser, C., Glaser, P., Jacquet, C., Martin, P., 2004. Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. J. Clin. Microbiol. 42, 3819–3822. https://doi.org/10.1128/JCM.42.8.3819-3822.2004.

EFSA, 2018. Multi-country outbreak of *Listeria monocytogenes* serogroup IVb, multi-locus sequence type 6, infections linked to frozen corn and possibly to other frozen vegetables – first update. EFSA Support. Publ. 15 https://doi.org/10.2903/sp.efsa.2018.EN-1448.

EFSA, 2022. The European Union One Health 2021 zoonoses report. EFSA J. 20 https://doi.org/10.2903/j.efsa.2022.7666.

EFSA, Allende, A., Barre, L., Jacxsens, L., Liebana, E., Messens, W., Sarno, E., da Silva Felicio, M.T., 2018. Urgent scientific and technical assistance to provide recommendations for sampling and testing in the processing plants of frozen vegetables aiming at detecting *Listeria monocytogenes*. EFSA Support. Publ. 15 https://doi.org/10.2903/sp.efsa.2018.EN-1445.

Elhanafi, D., Utta, V., Kathariou, S., 2010. Genetic characterization of plasmid-associated benzalkonium chloride resistance determinants in a *Listeria monocytogenes* Strain from the 1998–1999 outbreak. Appl. Environ. Microbiol. 76, 8231–8238. https://doi.org/10.1128/AEM.02056-10.

Fagerlund, A., Wagner, E., Møretrø, T., Heir, E., Moen, B., Rychli, K., Langsrud, S., 2022. Pervasive *Listeria monocytogenes* is common in the Norwegian food system and is associated with increased prevalence of stress survival and resistance determinants. Appl. Environ. Microbiol. 88 https://doi.org/10.1128/aem.00861-22.

Faith, N., Kathariou, S., Cheng, Y., Promadej, N., Neudeck, B.L., Zhang, Q., Luchansky, J., Czuprynski, C., 2009. The role of *L. monocytogenes* serotype 4b *gtcA* in gastrointestinal listeriosis in A/J mice. Foodborne Pathog. Dis. 6, 39–48. https://doi.org/10.1089/fpd.2008.0154.

Ferreira, A., Gray, M., Wiedmann, M., Boor, K.J., 2004. Comparative genomic analysis of the *sigB* operon in *Listeria monocytogenes* and in other gram-positive bacteria. Curr. Microbiol. 48, 39–46. https://doi.org/10.1007/s00284-003-4020-x.

Garnier, Simon, Ross, Noam, Rudis, Robert, Camargo, Pedro A., Sciaini, Marco, Scherer, Cédric, 2021. {Viridis} - Colorblind-Friendly Color Maps for R. https://doi.org/10.5281/zenodo.4679424.

Gelbíčová, T., Florianová, M., Tomáštíková, Z., Pospíšilová, L., Koláčková, I., Karpíšková, R., 2019. Prediction of persistence of *Listeria monocytogenes* ST451 in a rabbit meat processing plant in the Czech Republic. J. Food Prot. 82, 1350–1356. https://doi.org/10.4315/0362-028X.JFP-19-030.

Gou, H., Liu, Y., Shi, W., Nan, J., Wang, C., Sun, Y., Cao, Q., Wei, H., Song, C., Tian, C., Wei, Y., Xue, H., 2022. The characteristics and function of Internalin G in *Listeria monocytogenes*. Polish J. Microbiol. 71, 63–71. https://doi.org/10.33073/pjm-2022-009.

Gouin, E., Adib-Conquy, M., Balestrino, D., Nahori, M.-A., Villiers, V., Colland, F., Dramsi, S., Dussurget, O., Cossart, P., 2010. The *Listeria monocytogenes* InlC protein interferes with innate immune responses by targeting the IκB kinase subunit IKKα. Proc. Natl. Acad. Sci. 107, 17333–17338. https://doi.org/10.1073/pnas.1007765107.

Gray, J.A., Chandry, P.S., Kaur, M., Kocharunchitt, C., Bowman, J.P., Fox, E.M., 2021. Characterisation of *Listeria monocytogenes* food-associated isolates to assess environmental fitness and virulence potential. Int. J. Food Microbiol. 350, 109247 https://doi.org/10.1016/j.ijfoodmicro.2021.109247.

Gu, Z., Gu, L., Eils, R., Schlesner, M., Brors, B., 2014. Circlize implements and enhances circular visualization in R. Bioinformatics 30, 2811–2812. https://doi.org/10.1093/bioinformatics/btu393.

Haase, J.K., Didelot, X., Lecuit, M., Korkeala, H., Achtman, M., Leclercq, A., Grant, K., Wiedmann, M., Apfalter, P., 2014. The ubiquitous nature of *Listeria monocytogenes* clones: a large-scale Multilocus Sequence Typing study. Environ. Microbiol. 16, 405–416. https://doi.org/10.1111/1462-2920.12342.

Harter, E., Wagner, E.M., Zaiser, A., Halecker, S., Wagner, M., Rychli, K., 2017. Stress survival islet 2, predominantly present in *Listeria monocytogenes* strains of sequence type 121, is involved in the alkaline and oxidative stress responses. Appl. Environ. Microbiol. 83 https://doi.org/10.1128/AEM.00827-17.

Harter, E., Lassnig, C., Wagner, E.M., Zaiser, A., Wagner, M., Rychli, K., 2019. The novel internalins InlP1 and InlP4 and the internalin-like protein InlP3 enhance the pathogenicity of *Listeria monocytogenes*. Front. Microbiol. 10, 1–11. https://doi.org/10.3389/fmicb.2019.01644.

Haubert, L., Kremer, F.S., da Silva, W.P., 2018. Whole-genome sequencing identification of a multidrug-resistant *Listeria monocytogenes* serotype 1/2a isolated from fresh mixed sausage in southern Brazil. Infect. Genet. Evol. 65, 127–130. https://doi.org/10.1016/j.meegid.2018.07.028.

Hein, I., Klinger, S., Dooms, M., Flekna, G., Stessl, B., Leclercq, A., Hill, C., Allerberger, F., Wagner, M., 2011. Stress survival islet 1 (SSI-1) survey in *Listeria monocytogenes* reveals an insert common to *Listeria innocua* in sequence type 121 *L. monocytogenes* strains. Appl. Environ. Microbiol. 77, 2169–2173. https://doi.org/10.1128/AEM.02159-10.

Jagadeesan, B., Baert, L., Wiedmann, M., Orsi, R.H., 2019. Comparative analysis of tools and approaches for source tracking *Listeria monocytogenes* in a food facility using whole-genome sequence data. Front. Microbiol. 10, 1–18. https://doi.org/10.3389/fmicb.2019.00947.

Jennison, A.V., Masson, J.J., Fang, N.X., Graham, R.M., Bradbury, M.I., Fegan, N., Gobius, K.S., Graham, T.M., Guglielmino, C.J., Brown, J.L., Fox, E.M., 2017. Analysis of the *Listeria monocytogenes* population structure among isolates from 1931 to 2015 in Australia. Front. Microbiol. 8, 1–13. https://doi.org/10.3389/fmicb.2017.00603.

Ji, S., Song, Z., Luo, L., Wang, Yiqian, Li, L., Mao, P., Ye, C., Wang, Yan, 2023. Whole-genome sequencing reveals genomic characterization of *Listeria monocytogenes* from food in China. Front. Microbiol. 13, 1–12. https://doi.org/10.3389/fmicb.2022.1049843.

Jiang, X., Yu, T., Xu, Y., Wang, H., Korkeala, H., Shi, L., 2019. MdrL, a major facilitator superfamily efflux pump of *Listeria monocytogenes* involved in tolerance to benzalkonium chloride. Appl. Microbiol. Biotechnol. 103, 1339–1350. https://doi.org/10.1007/s00253-018-9551-y.

Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., von Haeseler, A., Jermiin, L.S., 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat. Methods 14, 587–589. https://doi.org/10.1038/nmeth.4285.

Karambelkar, B., 2016. Colormap: color palettes using colormaps node module. https://cran.r-project.org/package=colormap.

Kazmierczak, M.J., Mithoe, S.C., Boor, K.J., Wiedmann, M., 2003. *Listeria monocytogenes* σB regulates stress response and virulence functions. J. Bacteriol. 185, 5722–5734. https://doi.org/10.1128/JB.185.19.5722-5734.2003.

Koopmans, M.M., Brouwer, M.C., Vázquez-Boland, J.A., van de Beek, D., 2023. Human listeriosis. Clin. Microbiol. Rev. 36, 120–121. https://doi.org/10.1128/cmr.00060-19.

Koutsoumanis, K., Alvarez-Ordóñez, A., Bolton, D., Bover-Cid, S., Chemaly, M., Davies, R., De Cesare, A., Herman, L., Hilbert, F., Lindqvist, R., Nauta, M., Peixe, L., Ru, G., Simmons, M., Skandamis, P., Suffredini, E., Jordan, K., Sampers, I., Wagner, M., Da Silva Felicio, M.T., Georgiadis, M., Messens, W., Mosbach-Schulz, O., Allende, A., 2020. The public health risk posed by *Listeria monocytogenes* in frozen fruit and vegetables including herbs, blanched during processing. EFSA J. 18, 1–102. https://doi.org/10.2903/j.efsa.2020.6092.

Kovacevic, J., Ziegler, J., Wałecka-Zacharska, E., Reimer, A., Kitts, D.D., Gilmour, M.W., 2016. Tolerance of *Listeria monocytogenes* to quaternary ammonium sanitizers is mediated by a novel efflux pump encoded by *emrE*. Appl. Environ. Microbiol. 82, 939–953. https://doi.org/10.1128/AEM.03741-15.

Kremer, P.H.C., Lees, J.A., Koopmans, M.M., Ferwerda, B., Arends, A.W.M., Feller, M.M., Schipper, K., Valls Seron, M., van der Ende, A., Brouwer, M.C., van de Beek, D., Bentley, S.D., 2017. Benzalkonium tolerance genes and outcome in *Listeria monocytogenes* meningitis. Clin. Microbiol. Infect. 23, 265.e1–265.e7. https://doi.org/10.1016/j.cmi.2016.12.008.

Lakicevic, B., Jankovic, V., Pietzka, A., Ruppitsch, W., 2023. Wholegenome sequencing as the gold standard approach for control of *Listeria monocytogenes* in the food chain. J. Food Prot. 86, 100003 https://doi.org/10.1016/j.jfp.2022.10.002.

Letunic, I., Bork, P., 2021. Interactive tree of life (iTOL) v5: an online tool for phylogenetic tree display and annotation. Nucleic Acids Res. 49, W293–W296. https://doi.org/10.1093/nar/gkab301.

Liao, J., Guo, X., Li, S., Anupoju, S.M.B., Cheng, R.A., Weller, D.L., Sullivan, G., Zhang, H., Deng, X., Wiedmann, M., 2023. Comparative genomics unveils extensive genomic variation between populations of *Listeria* species in natural and food-associated environments. ISME Commun. 3, 85. https://doi.org/10.1038/s43705-023-00293-x.

Ling, Z., Zhao, D., Xie, X., Yao, H., Wang, Y., Kong, S., Chen, X., Pan, Z., Jiao, X., Yin, Y., 2022. *inlF* enhances *Listeria monocytogenes* early-stage infection by inhibiting the inflammatory response. Front. Cell. Infect. Microbiol. 11, 1–10. https://doi.org/10.3389/fcimb.2021.748461.

Liu, X., Chen, W., Fang, Z., Yu, Y., Bi, J., Wang, J., Dong, Q., Zhang, H., 2022. Persistence of *Listeria monocytogenes* ST5 in ready-to-eat food processing environment. Foods 11, 2561. https://doi.org/10.3390/foods11172561.

Lüth, S., Deneke, C., Kleta, S., Al Dahouk, S., 2021. Translatability of WGS typing results can simplify data exchange for surveillance and control of *Listeria monocytogenes*. Microb. Genomics 7, 1–12. https://doi.org/10.1099/mgen.0.000491.

Maćkiw, E., Korsak, D., Kowalska, J., Felix, B., Stasiak, M., Kucharek, K., Postupolski, J., 2021. Incidence and genetic variability of *Listeria monocytogenes* isolated from vegetables in Poland. Int. J. Food Microbiol. 339, 109023 https://doi.org/10.1016/j.ijfoodmicro.2020.109023.

Madad, A., Marshall, K.E., Blessington, T., Hardy, C., Salter, M., Basler, C., Conrad, A., Stroika, S., Luo, Y., Dwarka, A., Gerhardt, T., Rosa, Y., Cibulskas, K., Rosen, H.E., Adcock, B., Kiang, D., Hutton, S., Parish, M., Podoski, B., Patel, B., Viazis, S., Melius, B., Boyle, M.M., Brockmeyer, J., Chen, Y., Blickenstaff, K., Wise, M., 2023. Investigation of a multistate outbreak of *Listeria monocytogenes* infections linked to frozen vegetables produced at individually quick-frozen vegetable manufacturing facilities. J. Food Prot. 86 https://doi.org/10.1016/j.jfp.2023.100117.

Maggio, F., Rossi, C., Chiaverini, A., Ruolo, A., Orsini, M., Centorame, P., Acciari, V.A., Chaves López, C., Salini, R., Torresi, M., Serio, A., Pomilio, F., Paparella, A., 2021. Genetic relationships and biofilm formation of *Listeria monocytogenes* isolated from the smoked salmon industry. Int. J. Food Microbiol. 356, 109353 https://doi.org/10.1016/j.ijfoodmicro.2021.109353.

Malekmohammadi, S., Kodjovi, K.K., Sherwood, J., Bergholz, T.M., 2017. Genetic and environmental factors influence *Listeria monocytogenes* nisin resistance. J. Appl. Microbiol. 123, 262–270. https://doi.org/10.1111/jam.13479.

Marshall, K.E., Nguyen, T.A., Ablan, M., Nichols, M.C., Robyn, M.P., Sundararaman, P., Whitlock, L., Wise, M.E., Jhung, M.A., 2020. Investigations of possible multistate outbreaks of *Salmonella*, Shiga toxin-producing *Escherichia coli*, and *Listeria monocytogenes* infections - United States, 2016. MMWR Surveill. Summ. 69, 1–14. https://doi.org/10.15585/mmwr.ss6906a1.

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., 2016. Uncovering *Listeria monocytogenes* hypervirulence by harnessing its biodiversity. Nat. Genet. 48, 308–313. https://doi.org/10.1038/ng.3501.

Maury, M.M., Bracq-Dieye, H., Huang, L., Vales, G., Lavina, M., Thouvenot, P., Disson, O., Leclercq, A., Brisse, S., Lecuit, M., 2019. Hypervirulent *Listeria monocytogenes* clones' adaption to mammalian gut accounts for their association with dairy products. Nat. Commun. 10, 2488. https://doi.org/10.1038/s41467-019-10380-0.

McLauchlin, J., Aird, H., Amar, C., Barker, C., Dallman, T., Lai, S., Painset, A., Willis, C., 2021. An outbreak of human listeriosis associated with frozen sweet corn consumption: investigations in the UK. Int. J. Food Microbiol. 338, 108994 https://doi.org/10.1016/j.ijfoodmicro.2020.108994.

Milohanic, E., Jonquieres, R., Cossart, P., Berche, P., Gaillard, J.-L., 2001. The autolysin Ami contributes to the adhesion of *Listeria monocytogenes* to eukaryotic cells via its cell wall anchor. Mol. Microbiol. 39, 1212–1224. https://doi.org/10.1046/j.1365-2958.2001.02208.x.

Minh, B.Q., Schmidt, H.A., Chernomor, O., Schrempf, D., Woodhams, M.D., Von Haeseler, A., Lanfear, R., Teeling, E., 2020. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. Mol. Biol. Evol. 37, 1530–1534. https://doi.org/10.1093/molbev/msaa015.

Moura, A., Tourdjman, M., Leclercq, A., Hamelin, E., Laurent, E., Fredriksen, N., Van Cauteren, D., Bracq-Dieye, H., Thouvenot, P., Vales, G., Tessaud-Rita, N., Maury, M. M., Alexandru, A., Criscuolo, A., Quevillon, E., Donguy, M.-P., Enouf, V., de Valk, H., Brisse, S., Lecuit, M., 2017. Real-time whole-genome sequencing for surveillance of *Listeria monocytogenes*. France. Emerg. Infect. Dis. 23, 1462–1470. https://doi.org/10.3201/eid2309.170336.

Muchaamba, F., Eshwar, A.K., Stevens, M.J.A., Stephan, R., Tasara, T., 2022. Different shades of *Listeria monocytogenes*: strain, serotype, and lineage-based variability in virulence and stress tolerance profiles. Front. Microbiol. 12, 1–23. https://doi.org/10.3389/fmicb.2021.792162.

Mullapudi, S., Siletzky, R.M., Kathariou, S., 2010. Diverse cadmium resistance determinants in *Listeria monocytogenes* isolates from the Turkey processing plant environment. Appl. Environ. Microbiol. 76, 627–630. https://doi.org/10.1128/AEM.01751-09.

Müller, A., Rychli, K., Muhterem-Uyar, M., Zaiser, A., Stessl, B., Guinane, C.M., Cotter, P. D., Wagner, M., Schmitz-Esser, S., 2013. Tn6188 - a novel transposon in *Listeria monocytogenes* responsible for tolerance to benzalkonium chloride. PloS One 8, e76835. https://doi.org/10.1371/journal.pone.0076835.

Naditz, A.L., Dzieciol, M., Wagner, M., Schmitz-Esser, S., 2019. Plasmids contribute to food processing environment–associated stress survival in three *Listeria monocytogenes* ST121, ST8, and ST5 strains. Int. J. Food Microbiol. 299, 39–46. https://doi.org/10.1016/j.ijfoodmicro.2019.03.016.

Neuwirth, E., 2022. RColorBrewer: ColorBrewer palettes. https://cran.r-project.org/package=RColorBrewer.

NicAogáin, K., O'Byrne, C.P., 2016. The role of stress and stress adaptations in determining the fate of the bacterial pathogen *Listeria monocytogenes* in the food chain. Front. Microbiol. 7, 1–16. https://doi.org/10.3389/fmicb.2016.01865.

Nightingale, K.K., Lyles, K., Ayodele, M., Jalan, P., Nielsen, R., Wiedmann, M., 2006. Novel method to identify source-associated phylogenetic clustering shows that *Listeria monocytogenes* includes niche-adapted clonal groups with distinct ecological preferences. J. Clin. Microbiol. 44, 3742–3751. https://doi.org/10.1128/JCM.00618-06.

Nyarko, E.B., Donnelly, C.W., 2015. *Listeria monocytogenes*: strain heterogeneity, methods, and challenges of subtyping. J. Food Sci. 80, M2868–M2878. https://doi.org/10.1111/1750-3841.13133.

Olson, R.D., Assaf, R., Brettin, T., Conrad, N., Cucinell, C., Davis, J.J., Dempsey, D.M., Dickerman, A., Dietrich, E.M., Kenyon, R.W., Kuscuoglu, M., Lefkowitz, E.J., Lu, J., Machi, D., Macken, C., Mao, C., Niewiadomska, A., Nguyen, M., Olsen, G.J., Overbeek, J.C., Parrello, B., Parrello, V., Porter, J.S., Pusch, G.D., Shukla, M., Singh, I., Stewart, L., Tan, G., Thomas, C., VanOeffelen, M., Vonstein, V., Wallace, Z. S., Warren, A.S., Wattam, A.R., Xia, F., Yoo, H., Zhang, Y., Zmasek, C.M., Scheuermann, R.H., Stevens, R.L., 2023. Introducing the Bacterial and Viral Bioinformatics Resource Center (BV-BRC): a resource combining PATRIC, IRD and ViPR. Nucleic Acids Res. 51, D678–D689. https://doi.org/10.1093/nar/gkac1003.

Orsi, R.H., den Bakker, H.C., Wiedmann, M., 2011. *Listeria monocytogenes* lineages: genomics, evolution, ecology, and phenotypic characteristics. Int. J. Med. Microbiol. https://doi.org/10.1016/j.ijmm.2010.05.002.

Palaiodimou, L., Fanning, S., Fox, E.M., 2021. Genomic insights into persistence of *Listeria* species in the food processing environment. J. Appl. Microbiol. 131, 2082–2094. https://doi.org/10.1111/jam.15089.

Palma, F., Pasquali, F., Lucchi, A., de Cesare, A., Manfreda, G., 2017. Whole genome sequencing for typing and characterisation of *Listeria monocytogenes* isolated in a rabbit meat processing plant. Ital. J. Food Saf. 6, 125–130. https://doi.org/10.4081/ijfs.2017.6879.

Palma, F., Radomski, N., Guérin, A., Sévellec, Y., Félix, B., Bridier, A., Soumet, C., Roussel, S., Guillier, L., 2022. Genomic elements located in the accessory repertoire drive the adaptation to biocides in *Listeria monocytogenes* strains from different ecological niches. Food Microbiol. 106 https://doi.org/10.1016/j.fm.2021.103757.

Pappelbaum, K., Grif, K., Heller, I., Würzner, R., Hein, I., Ellerbroek, L., Wagner, M., 2008. Monitoring hygiene on- and at-line is critical for controlling *Listeria monocytogenes* during produce processing. J. Food Prot. 71, 735–741. https://doi.org/10.4315/0362-028X-71.4.735.

Pedersen, T.L., 2022. ggraph: an implementation of grammar of graphics for graphs and networks. https://cran.r-project.org/package=ggraph.

Pietzka, A., Allerberger, F., Murer, A., Lennkh, A., Stöger, A., Cabal Rosel, A., Huhulescu, S., Maritschnik, S., Springer, B., Lepuschitz, S., Ruppitsch, W., Schmid, D., 2019. Whole genome sequencing based surveillance of *L. monocytogenes* for early detection and investigations of listeriosis outbreaks. Front. Public Heal. 7, 1–8. https://doi.org/10.3389/fpubh.2019.00139.

Pistor, S., Chakraborty, T., Walter, U., Wehland, J., 1995. The bacterial actin nucleator protein ActA of *Listeria monocytogenes* contains multiple binding sites for host microfilament proteins. Curr. Biol. 5, 517–525. https://doi.org/10.1016/S0960-9822(95)00104-7.

Popowska, M., Krawczyk-Balska, A., Ostrowski, R., Desvaux, M., 2017. InlL from *Listeria monocytogenes* is involved in biofilm formation and adhesion to mucin. Front. Microbiol. 8, 1–11. https://doi.org/10.3389/fmicb.2017.00660.

Promadej, N., Fiedler, F., Cossart, P., Dramsi, S., Kathariou, S., 1999. Cell wall teichoic acid glycosylation in *Listeria monocytogenes* serotype 4b requires *gtcA*, a novel, serogroup-specific gene. J. Bacteriol. 181, 418–425. https://doi.org/10.1128/jb.181.2.418-425.1999.

Pyz-Łukasik, R., Paszkiewicz, W., Kiełbus, M., Ziomek, M., Gondek, M., Domaradzki, P., Michalak, K., Pietras-Ożga, D., 2022. Genetic diversity and potential virulence of *Listeria monocytogenes* isolates originating from Polish artisanal cheeses. Foods 11, 2805. https://doi.org/10.3390/foods11182805.

Quereda, J.J., Dussurget, O., Nahori, M., Ghozlane, A., Volant, S., Dillies, M.-A., Regnault, B., Kennedy, S., Mondot, S., Villoing, B., Cossart, P., Pizarro-Cerda, J., 2016. Bacteriocin from epidemic *Listeria* strains alters the host intestinal microbiota to favor infection. Proc. Natl. Acad. Sci. 113, 5706–5711. https://doi.org/10.1073/pnas.1523899113.

R Core Team, 2020. R: a language and environment for statistical computing. http://www.r-project.org/.

R Core Team, 2022. R: a language and environment for statistical computing. https://www.r-project.org/.

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.

Ragon, M., Wirth, T., Hollandt, F., Lavenir, R., Lecuit, M., Le Monnier, A., Brisse, S., 2008. A new perspective on *Listeria monocytogenes* evolution. PLoS Pathog. 4, e1000146 https://doi.org/10.1371/journal.ppat.1000146.

Raimann, E., Schmid, B., Stephan, R., Tasara, T., 2009. The alternative sigma factor σL of *L. monocytogenes* promotes growth under diverse environmental stresses. Foodborne Pathog. Dis. 6, 583–591. https://doi.org/10.1089/fpd.2008.0248.

Rea, R., Hill, C., Gahan, C.G.M., 2005. *Listeria monocytogenes* PerR mutants display a small-colony phenotype, increased sensitivity to hydrogen peroxide, and significantly reduced murine virulence. Appl. Environ. Microbiol. 71, 8314–8322. https://doi.org/10.1128/AEM.71.12.8314-8322.2005.

Ricci, A., Allende, A., Bolton, D., Chemaly, M., Davies, R., Fernández Escámez, P.S., Girones, R., Herman, L., Koutsoumanis, K., Nørrung, B., Robertson, L., Ru, G., Sanaa, M., Simmons, M., Skandamis, P., Snary, E., Speybroeck, N., Ter Kuile, B., Threlfall, J., Wahlström, H., Takkinen, J., Wagner, M., Arcella, D., Da Silva Felicio, M.T., Georgiadis, M., Messens, W., Lindqvist, R., 2018. *Listeria monocytogenes* contamination of ready-to-eat foods and the risk for human health in the EU. EFSA J. 16 https://doi.org/10.2903/j.efsa.2018.5134.

Rice, P., Longden, L., Bleasby, A., 2000. EMBOSS: the European molecular biology open software suite. Trends Genet. 16, 276–277. https://doi.org/10.1016/S0168-9525(00)02024-2.

Roberts, A.J., Wiedmann, M., 2006. Allelic exchange and site-directed mutagenesis probe the contribution of ActA amino-acid variability to phosphorylation and virulence-associated phenotypes among *Listeria monocytogenes* strains. FEMS Microbiol. Lett. 254, 300–307. https://doi.org/10.1111/j.1574-6968.2005.00041.x.

Ruppitsch, W., Pietzka, A., Prior, K., Bletz, S., Fernandez, H.L., Allerberger, F., Harmsen, D., Mellmann, A., 2015. Defining and evaluating a core genome multilocus sequence typing scheme for whole-genome sequence-based typing of *Listeria monocytogenes*. J. Clin. Microbiol. 53, 2869–2876. https://doi.org/10.1128/JCM.01193-15.

Ryan, S., Begley, M., Gahan, C.G.M., Hill, C., 2009. Molecular characterization of the arginine deiminase system in *Listeria monocytogenes*: regulation and role in acid tolerance. Environ. Microbiol. 11, 432–445. https://doi.org/10.1111/j.1462-2920.2008.01782.x.

Ryan, S., Begley, M., Hill, C., Gahan, C.G.M., 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.

Rychli, K., Stessl, B., Szakmary-Brändle, K., Strauß, A., Wagner, M., Schoder, D., 2018. *Listeria monocytogenes* isolated from illegally imported food products into the European Union harbor different virulence factor variants. Genes (Basel) 9, 428. https://doi.org/10.3390/genes9090428.

Sabet, C., Toledo-Arana, A., Personnic, N., Lecuit, M., Dubrac, S., Poupel, O., Gouin, E., Nahori, M.A., Cossart, P., Bierne, H., 2008. The *Listeria monocytogenes* virulence factor InlJ is specifically expressed in vivo and behaves as an adhesin. Infect. Immun. 76, 1368–1378. https://doi.org/10.1128/IAI.01519-07.

Schiavano, G.F., Ateba, C.N., Petruzzelli, A., Mele, V., Amagliani, G., Guidi, F., De Santi, M., Pomilio, F., Blasi, G., Gattuso, A., Di Lullo, S., Rocchegiani, E., Brandi, G., 2021. Whole-genome sequencing characterization of virulence profiles of *Listeria monocytogenes* food and human isolates and in vitro adhesion/invasion assessment. Microorganisms 10, 62. https://doi.org/10.3390/microorganisms10010062.

Schmid, B., Klumpp, J., Raimann, E., Loessner, M.J., Stephan, R., Tasara, T., 2009. Role of cold shock proteins in growth of *Listeria monocytogenes* under cold and osmotic stress conditions. Appl. Environ. Microbiol. 75, 1621–1627. https://doi.org/10.1128/AEM.02154-08.

Schmitz-Esser, S., Gram, L., Wagner, M., 2015. Complete genome sequence of the persistent *Listeria monocytogenes* strain R479a. Genome Announc. 3, 10–11. https://doi.org/10.1128/genomeA.00150-15.

Seeliger, H.P.R., Höhne, K., 1979. In: Bergan, T., Norris, J.R.B.T.-M. in M (Eds.), Chapter II Serotyping of *Listeria monocytogenes* and Related Species. Academic Press, pp. 31–49. https://doi.org/10.1016/S0580-9517(08)70372-6.

Seemann, T., 2015. snippy: fast bacterial variant calling from NGS reads. https://github.com/tseemann/snippy.

Seifart Gomes, C., Izar, B., Pazan, F., Mohamed, W., Mraheil, M.A., Mukherjee, K., Billion, A., Aharonowitz, Y., Chakraborty, T., Hain, T., 2011. Universal stress proteins are important for oxidative and acid stress resistance and growth of *Listeria monocytogenes* EGD-e in vitro and in vivo. PloS One 6, e24965. https://doi.org/10.1371/journal.pone.0024965.

Skowron, K., Grudlewska, K., Lewandowski, D., Gajewski, P., Reśliński, A., Gospodarek-Komkowska, E., 2019. Antibiotic susceptibility and ability to form biofilm of *Listeria monocytogenes* strains isolated from frozen vegetables. Acta Aliment. 48, 65–75. https://doi.org/10.1556/066.2019.48.1.8.

Stessl, B., Ruppitsch, W., Wagner, M., 2022. *Listeria monocytogenes* post-outbreak management - when could a food production be considered under control again? Int. J. Food Microbiol. 379, 109844 https://doi.org/10.1016/j.ijfoodmicro.2022.109844.

Sullivan, G., Orsi, R.H., Estrada, E., Strawn, L., Wiedmann, M., 2022. Whole-genome sequencing-based characterization of *Listeria* isolates from produce packinghouses and fresh-cut facilities suggests both persistence and reintroduction of fully virulent *L. monocytogenes*. Appl. Environ. Microbiol. 88, 1–18. https://doi.org/10.1128/aem.01177-22.

Tavares, R.D.M., da Silva, D.A.L., Camargo, A.C., Yamatogi, R.S., Nero, L.A., 2020. Interference of the acid stress on the expression of *llsX* by *Listeria monocytogenes* pathogenic island 3 (LIPI-3) variants. Food Res. Int. 132, 109063 https://doi.org/10.1016/j.foodres.2020.109063.

Travier, L., Guadagnini, S., Gouin, E., Dufour, A., Chenal-Francisque, V., Cossart, P., Olivo-Marin, J.-C., Ghigo, J.-M., Disson, O., Lecuit, M., 2013. ActA promotes *Listeria monocytogenes* aggregation, intestinal colonization and carriage. PLoS Pathog. 9, e1003131 https://doi.org/10.1371/journal.ppat.1003131.

Truchado, P., Gil, M.I., Querido-Ferreira, A.P., Capón, C.L., Álvarez-Ordoñez, A., Allende, A., 2022. Frozen vegetable processing plants can harbour diverse *Listeria monocytogenes* populations: identification of critical operations by WGS. Foods 11, 1546. https://doi.org/10.3390/foods11111546.

Vázquez-Boland, J.A., Domínguez-Bernal, G., González-Zorn, B., Kreft, J., Goebel, W., 2001. Pathogenicity islands and virulence evolution in *Listeria*. Microbes Infect. 3, 571–584. https://doi.org/10.1016/S1286-4579(01)01413-7.

Wagner, E., Fagerlund, A., Thalguter, S., Jensen, M.R., Heir, E., Møretrø, T., Moen, B., Langsrud, S., Rychli, K., 2022. Deciphering the virulence potential of *Listeria monocytogenes* in the Norwegian meat and salmon processing industry by combining whole genome sequencing and in vitro data. Int. J. Food Microbiol. 383, 109962 https://doi.org/10.1016/j.ijfoodmicro.2022.109962.

Walker, S.J., Archer, P., Banks, J.G., 1990. Growth of *Listeria monocytogenes* at refrigeration temperatures. J. Appl. Bacteriol. 68, 157–162. https://doi.org/10.1111/j.1365-2672.1990.tb02561.x.

Wang, Y., Pettengill, J.B., Pightling, A., Timme, R., Allard, M., Strain, E., Rand, H., 2018. Genetic diversity of *Salmonella* and *Listeria* isolates from food facilities. J. Food Prot. 81, 2082–2089. https://doi.org/10.4315/0362-028X.JFP-18-093.

Wickham, H., . ggplot2: Elegant Graphics for Data Analysis. https://ggplot2.tidyverse.org. Springer-Verlag, New York.

Wickham, H., François, R., Henry, L., Müller, K., Vaughan, D., 2022a. dplyr: a grammar of data manipulation. https://cran.r-project.org/package=dplyr.

Wickham, H., Vaughan, D., Girlich, M., 2022b. tidyr: tidy messy data. https://cran.r-project.org/package=tidyr.

Wickham, H., François, R., Henry, L., Müller, K., Vaughan, D., 2023a. dplyr: a grammar of data manipulation. https://cran.r-project.org/package=dplyr.

Wickham, H., Vaughan, D., Girlich, M., 2023b. tidyr: tidy messy data. https://cran.r-project.org/package=tidyr.

Willis, C., McLauchlin, J., Aird, H., Amar, C., Barker, C., Dallman, T., Elviss, N., Lai, S., Sadler-Reeves, L., 2020. Occurrence of *Listeria* and *Escherichia coli* in frozen fruit and

vegetables collected from retail and catering premises in England 2018–2019. Int. J. Food Microbiol. 334, 108849 https://doi.org/10.1016/j.ijfoodmicro.2020.108849.

Yu, G., Smith, D.K., Zhu, H., Guan, Y., Lam, T.T.Y., 2017. Ggtree: an R package for visualization and annotation of phylogenetic trees with their covariates and other associated data. Methods Ecol. Evol. 8, 28–36. https://doi.org/10.1111/2041-210X.12628.

Zhang, H., Wang, J., Chang, Z., Liu, X., Chen, W., Yu, Y., Wang, X., Dong, Q., Ye, Y., Zhang, X., 2021. *Listeria monocytogenes* contamination characteristics in two ready-to-eat meat plants from 2019 to 2020 in Shanghai. Front. Microbiol. 12, 1–9. https://doi.org/10.3389/fmicb.2021.729114.

Zoellner, C., Wiedmann, M., Ivanek, R., 2019. An assessment of listeriosis risk associated with a contaminated production lot of frozen vegetables consumed under alternative consumer handling scenarios. J. Food Prot. 82, 2174–2193. https://doi.org/10.4315/0362-028X.JFP-19-092.