International Journal of Food Microbiology 453 (2026) 111734

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Listeria monocytogenes: Genomic characterization of persistent strains historically isolated from cheese processing facilities

Lena Fritsch, Remo S. Schmidt, Elisabet Marti *

Food Microbial Systems, Agroscope, Schwarzenburgstrasse 161, 3003, Bern, Switzerland

A R T I C L E I N F O

Keywords:
Listeria monocytogenes
Persistence
Cheese
cgMLST
Whole-genome sequencing

A B S T R A C T

Listeria monocytogenes remains a major challenge in the food industry. However, data on its genetic variability and the gene markers associated with persistence in food production environments are still limited. To expand current knowledge on the diversity and persistence of *L. monocytogenes* in the cheese production chain, 194 isolates collected over a 24-year period from 50 cheese processing facilities in Switzerland were characterized using WGS-based analyses. The isolates were classified into 23 different clonal complexes (CCs) with CC3, CC1 and CC101 being the most prevalent. Notably, 7 isolates were first described here for the first time as new sequence types. Seven additional isolates belonging to CC1, subtyped as ST1 and CT5518, clustered with the strain responsible for the first widely known listeriosis outbreak occurred in Switzerland during the eighties, which resulted in a high number of casualties. CC1 isolates were mostly associated with soft cheese and artisanal production, whereas CC3 isolates were more frequently isolated from hard and semi-hard cheeses, as well as from industrial and ripening facilities. Regarding persistence, some CC101 and CC3 isolates were recurrently isolated from the same facilities over periods up to 5 and 7 years, respectively. Analyses of persistence markers revealed that the mechanisms enabling survival in cheese production environments were diverse and primarily consisted of CC-specific genomic adaptations to stressors such as salinity and pH. Notably, CC3 and CC517 strains exhibited enhanced stress tolerance associated to the presence of the Stress Survival Islet 1, while other CCs showed comparable resistance through alternative genetic mechanisms. Overall, this study provides a comprehensive long-term genomic overview of *L. monocytogenes* in Swiss cheese production and demonstrates that mechanisms contributing to its persistence in cheese production are primarily CC-dependent, like the presence of SSI-1 in some CCs, while the acquisition of genomic islands under selective pressures may also contribute.

1. Introduction

Listeria monocytogenes is a gram-positive bacterium first described in 1926, recognized in the 1970s as the causative agent of listeriosis and classified in the 1980s as a food-borne pathogen (Radoshevich and Cossart, 2018). Listeriosis in adults is often manifested as a mild gastroenteritis but it can lead to more severe symptoms such as meningitis and/or septicemia in children, elderly and immunocompromised individuals and can also cause miscarriage in pregnant women (Hilliard et al., 2018). Although listeriosis is less frequent than other zoonoses in humans, such as campylobacteriosis and salmonellosis, the mortality rate among infected people is very high (such as 18.1% in the European Union (EU) in 2022), which makes it one of the most serious food-borne diseases (European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC), 2023). In Switzerland, where this study is focused, 78 confirmed cases were reported in 2022, following the same increasing tendency as in the EU during the last years (Federal Food Safety and Veterinary Office (FSVO) and Federal Office of Public Health (FOPH), 2023). Overall, Switzerland had a notification rate of listeriosis of 0.9 cases per 100,000 population in 2022, while in the EU the same index was 0.62 (EFSA and ECDC, 2023; FSVO and FOPH, 2023). Although this data evidence that listeriosis is a public health concern, many efforts have been done to strengthen the hygiene norms in the food industry, especially since the large outbreak of listeriosis which occurred in Switzerland between 1983 and 1987. During this 5-year period, a total of 122 human cases occurred (leading to 31 deaths) due to the contamination of a locally produced soft cheese *Vacherin Mont d'Or* (Bille, 1990; Büla et al., 1995). This epidemic affected the local dairy industry, resulting in the closing of 12 cheese producing companies. Following this outbreak, Swiss cheesemakers

---

* Corresponding author.
E-mail address: elisabet.martiserrano@agroscope.admin.ch (E. Marti).

https://doi.org/10.1016/j.ijfoodmicro.2026.111734
Received 17 September 2025; Received in revised form 6 March 2026; Accepted 12 March 2026

introduced key changes to reduce the risk of contamination with *L. monocytogenes* at every stage of food production and distribution (WHO, 1991). A thermization step (heating milk to sub-pasteurization levels) was implemented to lower microbial risk while preserving cheese quality (Griffiths, 1989). Moreover, the separation of production batches was included to prevent cross-contamination and to improve traceability. Routine testing of raw materials and finished products was also adopted to enable early detection and intervention. These measures, along with stricter hygiene and environmental monitoring, became essential lessons from the outbreak and have significantly reduced the risk of listeriosis linked to cheese production (Berger et al., 2019).

L. monocytogenes is a highly heterogeneous species comprising 4 evolutionary lineages and 14 serotypes. Molecular epidemiological evidence shows the variable distribution of the different genetic and serological subtypes among food products and the processing environment as well as among human and animal clinical listeriosis cases. Serotypes 1/2a, 1/2b, 1/2c, and 4b are mainly isolated in food and clinical isolates, with serotype 4b being the main responsible of human listeriosis cases (Muchaamba et al., 2022). Genotypic techniques, such as multilocus sequence typing (MLST), classifies the strains into clonal complexes (CCs) based on 7 housekeeping genes (Ragon et al., 2008). Each CC is as a group of sequence types (STs) differing by no more than one allele from at least one other ST in the group (Chen et al., 2016). The combination of whole genome sequencing (WGS) with core-genome MLST (cgMLST) or the analysis of single nucleotide polymorphisms (SNPs) has increased the discriminatory power of subtyping methods. A common measure of genetic relatedness in outbreak investigations and to identify origins of contamination in food processing environments (FPE) is the number of SNP differences between isolates, with fewer than 21 SNPs typically used as the threshold to indicate that isolates are closely related (Pightling et al., 2018; Wang et al., 2018).

The vast majority of the human listeriosis cases are linked to the consumption of contaminated food, especially ready-to-eat (RTE) foods such as dairy, meat and fish products (Ricci et al., 2018). In fact, *L. monocytogenes* is a problem for the food industry as it is resistant to a number of environmental conditions, such as refrigeration temperatures, high salt concentrations, wide pH range, as well as low humidity or low oxygen in food processing environments (Buchanan et al., 2017). Consequently, it is very difficult to eliminate it from there and it can persist for a long time. Persistence was reported to be up to 4 years in a meat processing plant (Stoller et al., 2019) and up to 17 years in a fish and seafood processing plant (Harrand et al., 2020). Indeed, *L. monocytogenes* has been identified by the EFSA as one of the most relevant microbiological safety hazards associated with persistence in the meat, fish, dairy and vegetables processing sectors (EFSA, 2024). More specifically, the subtypes CC121, CC8, CC9 from lineage II and CC5, CC6, CC2 from lineage I are commonly reported as persistent (EFSA, 2024). Although there is still no consensus on a time cut-off to define persistence, repeated isolation of the same subtype in a single facility over a minimum time interval of 6 months or 1 year has been proposed as an indicator of persistence (Leong et al., 2017; Palma et al., 2020; Harrand et al., 2020). Overall, persistence of *L. monocytogenes* in FPE has been recognized as an important source of product contamination.

Some authors suggested that the ability to form biofilms (Rodriguez-Campos et al., 2019) and the tolerance to sanitizers could facilitate the persistence of *L. monocytogenes* in food contact surfaces (Martinez-Suarez et al., 2016). Several efflux pump genes acquired by horizontal gene transfer conferring tolerance to disinfectants have been described. For instance, the gene *qacH*, located in the transposon Tn*6188*, the gene cassette *bcrABC* or the gene *ermB* mediate the efflux of quaternary ammonium compounds (QACs) (Buchanan et al., 2017). Resistance to heavy metals, specifically to cadmium, is conferred by distinct variants of *cadA* genes such as *cadA1*, found in transposon Tn*5422*, or *cadA2*, harbored by several large plasmids (Korsak et al., 2019). Stress Survival Islets (SSI) play a role in the growth of *L. monocytogenes* under salt and acidic stress conditions and also promote biofilm formation. So far, 2 SSI have been described, SSI-1 distributed among human clinical isolates and strains isolated from FPE, whereas SSI-2 predominate in FPE (Harter et al., 2017). Besides, recent investigations proposed that the evolution of *L. monocytogenes* genome by gaining and losing prophages could support the persistence of isolates in the food industry (Harrand et al., 2020; Palma et al., 2020). In brief, all the mentioned genes linked to increased environmental robustness, tolerance to disinfection and/or biofilm formation have been recently proposed as markers for persistence in *L. monocytogenes* (EFSA, 2024).

Globally, dairy products, and particularly cheeses such as surface-ripened soft cheeses, fresh cheeses and smear-ripened cheeses, have been frequently associated with human outbreaks of listeriosis (Bille et al., 2006; Fretz et al., 2010; Gaulin et al., 2012). The contamination of these products with *L. monocytogenes* has been associated to cross-contamination within FPE or to the use of contaminated raw milk (Beno et al., 2016; Gonzales-Barron et al., 2023). In Switzerland, the majority of matured cheeses are smear-ripened cheeses, meaning that they are brushed regularly with a brine solution, often from older to younger cheeses in order to propagate the surface microbiome (Ritschard and Schuppler, 2024). Thus, cheese ripening in Switzerland poses a possibility for dispersal of *L. monocytogenes*, although smearing the rind also exerts a pressure on the ecosystem by repeated application of salt and beneficial bacteria. There are still limited data about the genetic variability of *L. monocytogenes* and the gene markers that could explain their persistence in cheese producing facilities. Thus, the aim of this study was to retrospectively subtype and genotypically characterize *L. monocytogenes* strains isolated in 50 cheese processing facilities in Switzerland over 24 years to gain knowledge about the persistence of this foodborne pathogen in the cheese industry.

2. Materials and methods

2.1. *L. monocytogenes* isolates

A collection of 194 isolates previously identified as *L. monocytogenes* by traditional microbiological methods and serotyping were selected for further genotypic characterization in this study (Table S1). These isolates originated from samples collected between 1987 and 2011 across 50 cheese-processing facilities in Switzerland, in the framework of the *Listeria* Monitoring Program. This program was applied by Agroscope specialists after the *Listeria* outbreak in the 1980s and consisted of sampling cheese processing facilities (on a voluntary basis) that experienced problems with *Listeria*. These facilities included both artisanal- and industrial-scale cheese manufacturers, as well as ripening centers, processing several types of cheeses such as hard, semi-hard and soft cheeses. From more than 1000 isolates collected during the program, a selection was made targeting those isolates that had the same serotype and were isolated from the same company over time. This resulted in a total of 194 isolates that were included in this study. The isolates were recovered from different parts of cheese such as rind ($n = 64$) and smear ($n = 85$), the latter referring to the surface smear layer of smear-ripened cheeses, consisting of a removable microbial biomass distinct from the solid cheese rind. Samples from smear water ($n = 29$), equipment surfaces ($n = 14$), milk ($n = 1$) and butter ($n = 1$) were also included. To minimize redundancy among strains, only one isolate per sampling date and source was selected from the initial collection. However, isolates from companies where *Listeria* was repeatedly detected over time were specifically included to enhance the likelihood of identifying persistent strains.

2.2. DNA extraction and WGS

L. monocytogenes strains were cultured overnight at 37 °C in 5 ml of trypticase soy broth with 5 g/l yeast extract (BD, Franklin Lakes, NJ, USA) under aerobic conditions. Then, 1.4 ml were centrifuged for 5 min at 5000 rpm and the DNA was extracted from the pellet using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA) following the recommendations of the manufacturer for gram-positive bacteria. Finally, the concentration of DNA was determined using a Qubit® 3.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) and the purity was assessed determining the ratios A260/A280 and A260/A280 in a NanoDrop device (Thermo Fisher Scientific). Standard genomic libraries were sequenced using an Illumina NovaSeq 6000 (Illumina Inc., San Diego, CA, USA) platform for a paired-end reads (2 × 150 bp) by Eurofins GATC Biotech, GmbH (Konstanz, Germany). The genomes obtained in this study were deposited in NCBI/EMBL/DDBJ databases under the Bioproject accession number PRJNA1329014.

2.3. Typing analyses

MLST and cgMLST typing were performed using the MBioSEQ Ridom Typer software, version 11.1.0 (Ridom, Münster, Germany). Reads were filtered using a sliding-window approach requiring an average Phred quality score of ≥30 over a window of 20 bases. Quality metrics relevant for downstream analyses, such as read quality and sufficient coverage for reliable genome assembly and allele calling, were evaluated prior to cgMLST analysis. Briefly, trimmed sequences were *de novo* assembled by Velvet (version 11.1.04) and the ST of each isolate was determined *in silico* applying a MLST scheme previously defined (Ragon et al., 2008). Another 5-locus scheme described by Doumith et al. (2004) was applied to the genomes to classify the strains into serogroups: IIa (1/2a and 3a), IIb (1/2b), IIc (1/2c and 3c), and IVb (4b, 4d, and 4e). For cgMLST subtyping, the assembled genome was screened against 1701 genes of the seed genome from *L. monocytogenes* EGD-e, following the Ruppitsch scheme (Ruppitsch et al., 2015). Draft genomes were sorted in the same Complex Type (CT) when they had a maximum of 7 different alleles, as this cutoff has been identified as the most appropriate to determine strain clonality for *L. monocytogenes*. The assemblies of the isolates that clustered together after the cgMLST analysis were submitted to the Snippy pipeline (Seemann, 2015), to identify the SNP differences between them and increase discriminatory resolution. Isolates collected 2 or more times from the same company over a period of more than one year (Harrand et al., 2020) and with fewer than 21 SNPs differences were considered persistent. Isolates typed as ST1 were further compared with the genome of the ST1 *L. monocytogenes* strain PF49 (Accession number GCF_021312595.1) to assess their potential relatedness to the strain associated with the Swiss listeriosis outbreak in the eighties. Fisher's Exact Test from R, version 4.2.3 (R Core Team, 2022) was applied to assess the significance of associations in regard to CC and cheese types or CC and production environment.

2.4. Screening of gene markers for persistence

A total of 80 genes previously associated with tolerance to QACs, heavy metals, extreme pH and temperature (Pasquali et al., 2018, Table S3) were screened in the *Listeria* genomes. Allele sequences for these genes were downloaded from the Institut Pasteur database (https://bigsdb.pasteur.fr/cgi-bin/bigsdb/bigsdb.pl?db=pubmlst_listeria_seqdef&l=1&page=downloadAlleles) and were used to build 80 individual ABRicate databases (version 1.0.1, available at https://github.com/tseemann/abricate) each containing between 1 and 1579 sequences (median 117). Database construction was performed using ABRicate's –setupdb command on a folder containing the corresponding allele sequences. Subsequently, array jobs were launched on an on-premise computing cluster, to screen the 194 genome assemblies for the presence of the 80 gene collections using default parameters (except for the use of the custom databases). Output tsv files were concatenated and imported into Microsoft Excel for data aggregation, using the maximum value of sequence coverage as the basis for heatmap generation.

3. Results

3.1. Subtyping and phylogeny

A total of 194 strains of *L. monocytogenes* were sequenced in this study (Table S1). All samples showed high-quality whole-genome sequencing and assembly metrics (Table S2). Average sequencing coverage was consistently high (approximately 98–119×) and assembled genome sizes were uniform and consistent with *L. monocytogenes* (∼2.9–3.1 Mb). Species identification showed high confidence, with top species match identities of 0.99–1.00 for all isolates. Assemblies generated using Velvet (v1.1.04) were of good quality, with generally low contig counts, high N50 values, and robust average contig lengths. Importantly, cgMLST analysis demonstrated excellent performance across samples, with the percentage of good targets typically exceeding 98.5%, confirming that all genomes met quality thresholds for reliable downstream comparative genomic analyses. The isolates belonged to lineage I ($n = 138$, 71%) and lineage II ($n = 56$; 29%). Within lineage I, 81 isolates were assigned to serogroup IIb and 57 to serogroup IVb; within lineage 2, 53 isolates belonged to serogroup IIa and 3 to IIc. Based on 7-loci MLST, the isolates were classified into 23 different CCs (Fig. 1), with CC3 ($n = 70$) being the most prevalent, followed by CC1 ($n = 47$) and CC101 ($n = 25$). Among all the isolates, 187 matched previously described STs, while 7 displayed novel MLST profiles. Accordingly, these genomes were submitted to the BIGSdb-Lm database from the Institute Pasteur (https://bigsdb.pasteur.fr/listeria/, Moura et al., 2016), where the following new ST numbers were assigned to these isolates: 9859 (ST 2338, CC3), 9923 (ST 2333, CC199), 10112 (ST 2334, CC3), 10116 (ST 2336, CC3), 10117 (ST 2335, CC3), 23358 (ST 2337, CC193) and 23,405 (ST 2337, CC193). CgMLST analysis was performed using the 1701 target loci defined in the Ruppitsch scheme, which were nearly complete in all assembled genomes, allowing assignment to 78 distinct CTs. Phylogenetic distances between isolates were calculated by pairwise comparison, excluding missing values, resulting in the comparison of 1577 shared loci. Among the 78 CTs, 25 included two or more isolates differing by a maximum of 7 alleles, suggesting a potential epidemiologic link between them (Fig. 2). As shown in the minimum spanning tree, isolates corresponding to CC1 and CC3 were not only the most prevalent but also the most genetically diverse. CC1 isolates were mainly distributed across 5 clusters (numbered as 3, 6, 8, 12 and 14 in Fig. 2) while CC3 isolates were spread among 8 clusters (numbered as 2, 4, 5, 7, 9, 11, 13, 15 in Fig. 2). In contrast, CC101, despite being the third most prevalent CC, was largely concentrated in 2 major clusters (numbered as 1 and 19 in Fig. 2), one of them including 19 isolates. Isolates from other CCs were mostly sporadic or formed small clusters of two to three strains. It is noteworthy that six CC1 isolates identified as ST1 and CT5518 clustered with the reference genome GCA_021312595, which corresponds to the strain responsible for the *Vacherin Mont D'Or* outbreak that occurred in Switzerland between 1983 and 1987 (Fig. S1).

3.2. Distribution of the isolates according to the cheese type

The Sankey diagram illustrates the distribution of 106 *L. monocytogenes* isolates from cheese type to CC and manufacturing setting (Fig. 3). CC1 and CC3 were selected for this analysis because they represented the majority of isolates in this study (60% of all isolates). CC3 represented the largest proportion (60/106) and was mainly associated with hard and semi-hard cheeses (25/60 and 21/60, respectively) as also with cheese ripening and pre-packaging plants (29/60), whereas CC1 (46/106) was most frequently recovered from soft cheese (20/46) and artisanal cheese dairies (23/46). The distribution of CCs differed significantly across cheese types and manufacturing settings (Fisher's exact test, both $p < 0.001$; isolates with missing metadata excluded).

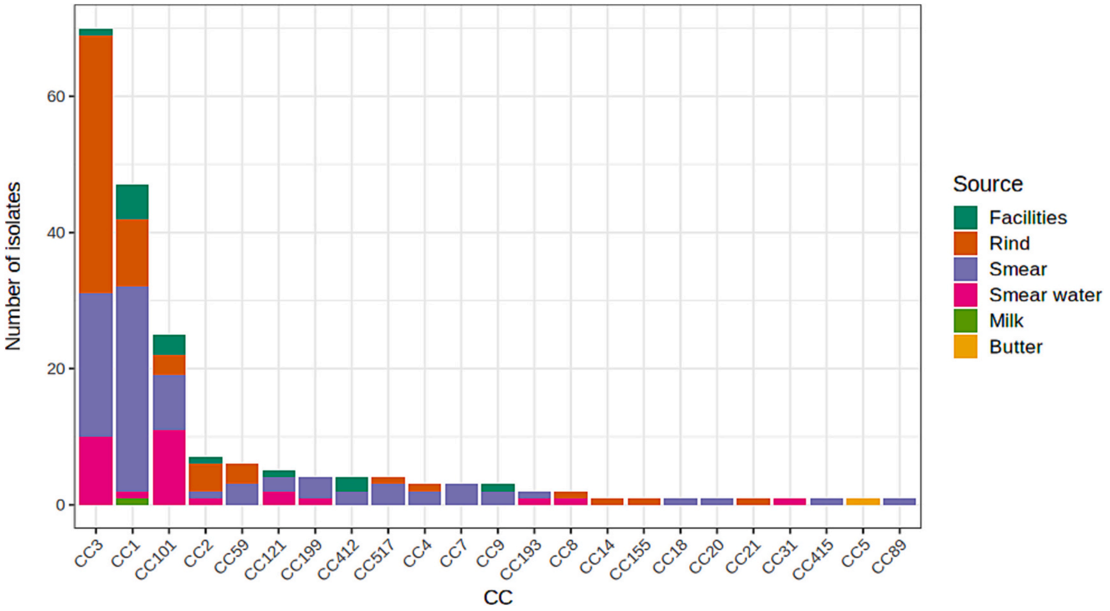


Fig. 1. Distribution of *L. monocytogenes* isolates across clonal complexes (CCs), shown as a stacked bar plot by source. The x-axis indicates clonal complexes, and the y-axis shows the number of isolates. Bar segments are color-coded by source of isolation, including facilities, rind, smear, smear water, milk, and butter.

3.3. Persistence

According to the cgMLST analyses, 12 of the 25 cgMLST clusters shown in Fig. 2 contained two or more persistent isolates, defined as isolates recovered repeatedly from the same company over a period exceeding one year and differing by a maximum of 7 alleles. Using this criterion, multiple isolates belonging to CC101, CC1, CC3, and CC517 were classified as persistent. Within CC101 (Fig. 4A), cluster 1 comprised 17 isolates repeatedly recovered from company 1 over a 3-year period ($n = 9$) and from company 2 over 5 years ($n = 8$). Similarly, several persistence events were identified among CC1 strains (Fig. 4B). In particular, 11 isolates of CT24 showed persistence (Cluster 3) being collected from company 21 over 2 years ($n = 3$), company 13 over 3 years ($n = 5$), and company 9 over 2 years (n = 3). Additionally, three CT8043 isolates (Cluster 6) were recovered from company 14 over 3 years, and 2 isolates were collected in company number 20 seven years apart. Within the same CC1, several isolates of CT5518 (cluster 8) were obtained from company 7 over 2 years ($n = 2$), from company 15 over 3 years (n = 3) and 2 isolates were recovered from company 7 over a 2-year period. Strains belonging to CC3 (Fig. 4C) accounted for 11 persistence events, 2 of which spanned up to 7 years. Particularly, four isolates of CT8082 (Cluster 2) were recovered over 7 years from company 3 and over 4, 3 and 1 year in companies 5,1 and 40, respectively. Two isolates of CT8457 (Cluster 5) persisted in company 10 for 7 years. Moreover, two isolates of CT8861 (Cluster 15) persisted in company 18 for 4 years and two isolates of CT8865 (Cluster 9) persisted in company 16 for 2 years. Finally, although the CC517 was not very prevalent, 3 isolates from this CC typed as CT8835 (Cluster 10, Fig. 4D), persisted in company 19 for 2 years. The genetic relatedness of strains within the same cgMLST cluster was further evaluated by checking for the SNP differences between them. This analysis confirmed that all strains categorized as persistent by cgMLST, thus differing by fewer than seven alleles, also exhibited fewer than 21 SNP differences (Fig. S2). These results are consistent with and further support the clustering obtained by cgMLST, reinforcing the robustness of the inferred genetic relationships.

3.4. Genes associated with persistence

A total of 80 genes associated with *L. monocytogenes* adaptation and persistence in harsh conditions were screened in the isolate's assemblies of the CC1, CC101, CC3 and CC517 strains, as these CCs included isolates that were identified as persistent. After the initial screening, the analysis was focused on the genes that were not present or absent in all strains, in order to investigate the significance of these different gene profiles (Fig. 5, Table S4). Notably, all the isolates from the CC3 and CC517 harbored the genes *lmo0444*, *lmo0445*, *lmo0446*, *lmo0447*, *lmo0448* and corresponding to the SSI-1, while they were not present in any of the CC1 and CC101 isolates. Within each CC, there were only few isolates displaying a different genetic profile. For instance, the genes corresponding to the LGI-2 (Listeria Genomic Island 2) including the arsenic operon (*arsA2*, *arsV2*, *arsD1*, *arsD2*, *arsR2*) were only found in some isolates from CC1. The genes corresponding to the LGI-3 (Listeria Genomic Island 3), also associated with stress tolerance, were present in subsets of CC3 and CC101. It is noteworthy that the *bcrABC*-cassette and *qacH* were absent in all the isolates.

4. Discussion

Despite many achievements in food safety, *L. monocytogenes* remains a major challenge in the food industry. To gain knowledge about the genomic diversity and persistence of *L. monocytogenes* in the cheese production chain in Switzerland, genomes from isolates collected over 24 years from 50 cheese processing facilities were characterized using WGS-based analyses. It is important to highlight that the isolation of these strains started on 1987, shortly after the severe listeriosis outbreak occurred in Switzerland and caused by *Vacherin Mont-d'Or* soft cheese (Weinmaier et al., 2013). In fact, most of the strains targeted in this study were collected in the frame of the “Listeria monitoring program”, that was implemented to surveil the presence of *Listeria* in cheese production facilities after the outbreak. After implementing this program and tightening hygienic regulations, *Listeria* detections gradually decreased over time. Therefore, while this retrospective study does not reflect the current situation in Switzerland, the use of sequencing-based techniques provides significantly more information than what was available at the time of the collection of these *L. monocytogenes* isolates.

The results of this study are consistent with previous research on the widespread distribution and prevalence of specific *L. monocytogenes* CCs in dairy environments (Chenal-Francisque et al., 2011; Maury et al., 2016). One of the most notable findings was the high prevalence of CC3 among the 194 typed *Listeria* strains, as they belong to the lineage I,

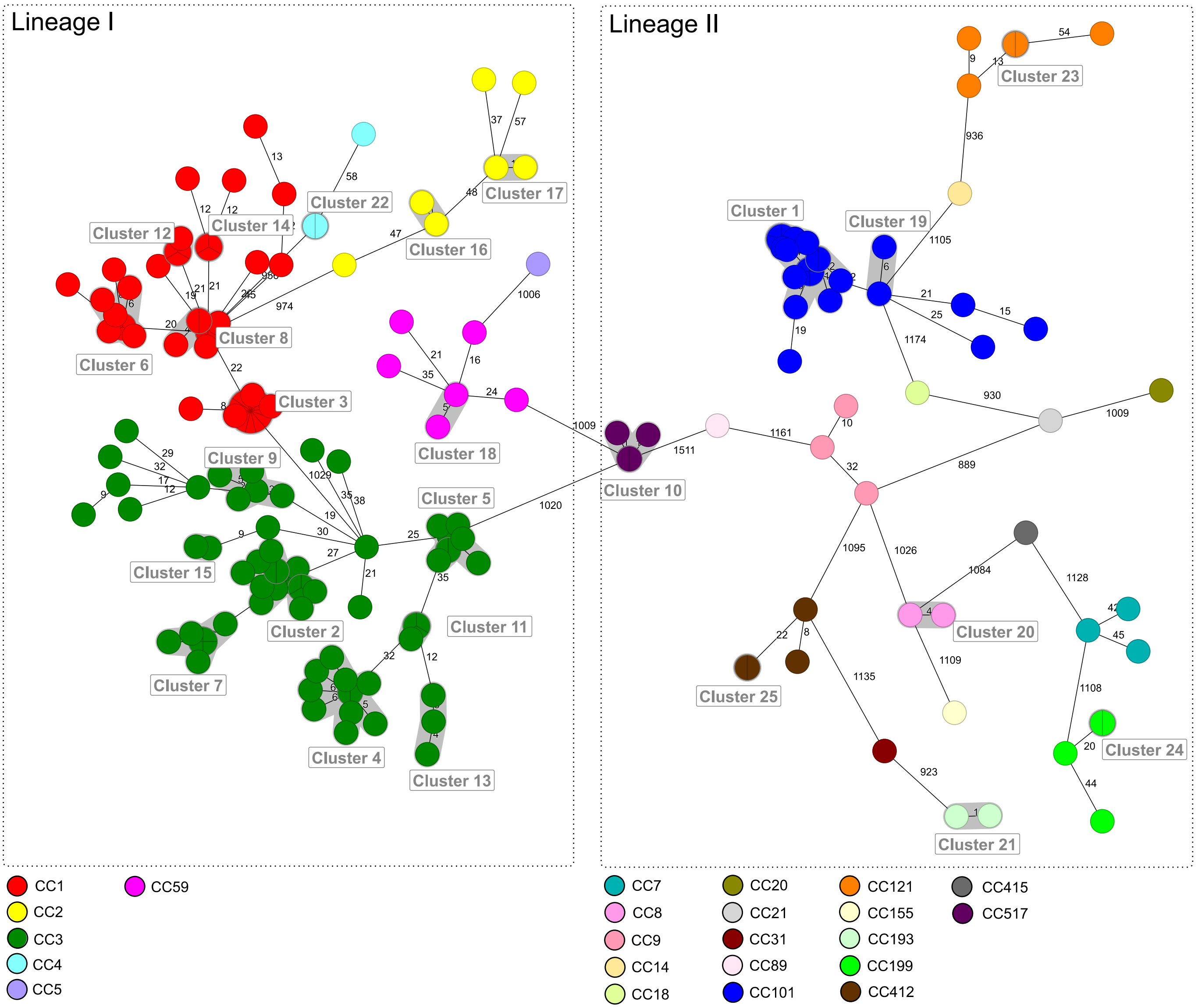


Fig. 2. Minimum spanning tree of 194 isolates based on 1570 cgMLST loci according to the Ruppitsch scheme and the 7-locus MLST scheme. No missing values were allowed in the analysis. Distances are displayed on a logarithmic scale, with a clustering threshold of a maximum of 7 allelic differences. Nodes are separated into lineage I and lineage II and colored according to clonal complex (CC), as indicated in the legend below. Numbers on the branches represent the allelic differences between neighboring nodes.

which is typically more associated with human listeriosis than with environmental isolates (Sauders et al., 2006). However, previous studies have also reported CC3 strains in meat processing environments (Félix et al., 2018), milk (Ebner et al., 2015) and cheeses (Andritsos and Mataragas, 2023; Oxaran et al., 2017). Conversely, the reported high prevalence of CC1 agrees with the literature, as this CC is well known for its strong association with dairy products (Maury et al., 2019). CC1 strains are considered hypervirulent due to the presence of pathogenicity islands and virulence factors, which enhance host cell invasion and survival, that may explain their association with human listeriosis outbreaks, including those linked to cheese products in the United States and Switzerland (Chen et al., 2016). In fact, the strain responsible for the human outbreak in Switzerland during the 1980s was typed as CC1, ST1 and CT5518. Notably, some CC1 isolates from this study differed by only 1–6 alleles from the outbreak strain, suggesting the possibility that related strains may have continued to circulate across multiple companies up to two years after the outbreak was officially declared over. The third most prevalent CC detected in our study, CC101, has been associated with listeriosis outbreaks in the U.S. and Europe, and it has been identified in cheese as well, reinforcing its relevance in the dairy sector (Chiara et al., 2014). A recent study also reported CC101 in both milk and meat products from the same company (Prieto et al., 2024). Furthermore, the LiSEQ project, that sequenced the genome of more than 1000 *Listeria* isolates from FPE, also reported that CC101 isolates were linked with milk/milk products. Indeed, the LiSEQ project showed that CC101 and CC1 were in the top10 list of the most isolated types in the FPE and also emphasized the link between CC1 and listeriosis outbreaks (Painset et al., 2019).

Before discussing persistence, it is essential to distinguish between intra-company persistence, when a single bacterial clone remains within the same facility over time, and inter-company persistence, when the same clone spreads across multiple facilities over an extended period. This distinction was already described by Palma and co-authors, to characterize intra- and inter-FPE persisting clones (Palma et al., 2020). In our study, strains belonging to CC3 accounted for 11 persistence intra-company events, 2 of which spanned 7 years, representing the longest persistence period observed in this study. Several isolates belonging to CC3 and inter-company persistence as well, as some clones clustered

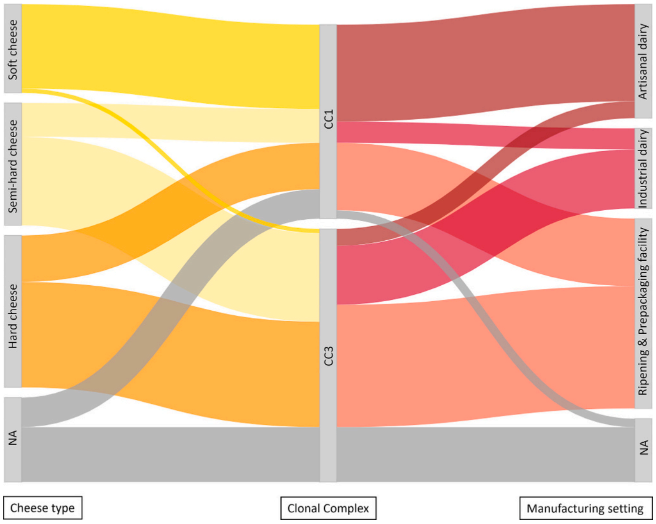
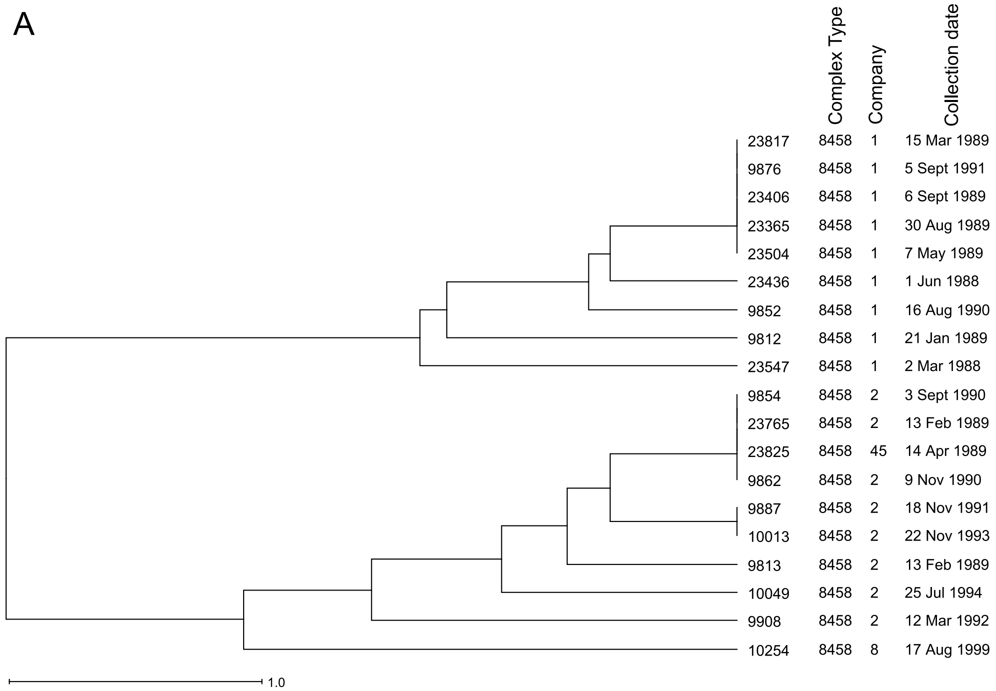


Fig. 3. Sankey diagram showing the distribution of *Listeria monocytogenes* isolates ($n = 106$) by cheese type, clonal complex (CC), and manufacturing setting. Flow width is proportional to the number of isolates. Cheese types included hard ($n = 36$), semi-hard ($n = 29$), soft ($n = 21$), and unknown (NA; $n = 20$). Isolates belonged to CC1 ($n = 46$) and CC3 ($n = 60$). Manufacturing settings included artisanal dairies ($n = 27$), industrial dairies ($n = 19$), ripening and prepackaging plants ($n = 45$), and unknown (NA; $n = 15$).

with isolates from other companies, demonstrating their ability to survive and spread across different cheese manufacturing facilities. Within each cluster, a less than seven allelic or 21 SNP differences were observed between isolates, suggesting a high degree of genetic similarity and supporting the hypothesis of long-term persistence of the same clone. Similarly, CT8458 isolates belonging to CC101, provided another example of long-term persistence both intra- and inter-company, as they were parallelly isolated in 2 different companies over a 5-year period. Isolates belonging to CC1 were also involved in eight persistence events, with one case indicating potential persistence for up to seven years. Our findings contrast with previous studies that identified CC121, CC8, CC9 from lineage II, as well as CC5, CC6, CC2 from lineage I, as the most persistent in FPE (EFSA, 2024). The observed inter-company persistence may be attributed to the fact that cheese producers, particularly small-scale or artisanal, usually share smearing machines or other equipment. This practice introduces a risk of cross-contamination and adds complexity to the challenge of controlling *Listeria* in the cheese industry. Similarly, ripening facilities, where cheeses from multiple producers are aged and smeared, provide another potential route for cross-contamination and inter-company persistence (Stessl et al., 2014). However, since most of the characterized strains were collected during the 80's and 90's, it is difficult to retrospectively determine which kind of exchange occurred between those cheese manufacturers that may have contributed to cross-contamination between them.

A deeper look at the production practices for soft and hard cheeses may help explain the observed prevalence of CC1 and CC3 in these cheese types, respectively. Soft cheeses typically undergo a short ripening period of only a few weeks and were traditionally produced using raw milk. Additionally, these cheeses were smeared with water rather than saltwater or with a low concentration of NaCl (FOAG, 2014). As a result, soft cheeses were more susceptible to contamination by pathogens present in the raw milk, since the short ripening time was insufficient to naturally inactivate them (Bachmann and Spahr, 1995). As mentioned before, CC1 strains are often associated with milk (Maury et al., 2019) and may be introduced into soft cheeses through contaminated raw milk. Given the minimal processing and short maturation time, CC1 could persist in the final product until consumption, which may explain its high prevalence in soft cheese samples observed in this study. Additionally, the cooking temperature for soft cheeses such as



	Complex Type	Company	Collection date
23817	8458	1	15 Mar 1989
9876	8458	1	5 Sept 1991
23406	8458	1	6 Sept 1989
23365	8458	1	30 Aug 1989
23504	8458	1	7 May 1989
23436	8458	1	1 Jun 1988
9852	8458	1	16 Aug 1990
9812	8458	1	21 Jan 1989
23547	8458	1	2 Mar 1988
9854	8458	2	3 Sept 1990
23765	8458	2	13 Feb 1989
23825	8458	45	14 Apr 1989
9862	8458	2	9 Nov 1990
9887	8458	2	18 Nov 1991
10013	8458	2	22 Nov 1993
9813	8458	2	13 Feb 1989
10049	8458	2	25 Jul 1994
9908	8458	2	12 Mar 1992
10254	8458	8	17 Aug 1999

Fig. 4. UPGMA (Unweighted Pair Group Method with Arithmetic Mean) phylogenetic trees of *L. monocytogenes* isolates based on cgMLST and MLST loci, with no missing values. Each tree displays the isolates belonging to the CC1, CC3, CC101, CC517, focusing on clusters containing persistent isolates, defined as the ones recovered from the same company over a period exceeding one year and differing by a maximum of 7 alleles. Tip labels correspond to isolate identifiers, with associated metadata shown to the right, including cgMLST complex type, company code and collection date. Branch lengths represent the number of allelic differences. A: Tree including isolates from the cluster number 1 of CC101. B: Tree including CC1 isolates from clusters number 3, 6, 8 and 14. C: Tree including CC3 isolates from clusters number 2, 4, 5, 7, 9 and 15. D: Tree including CC517 isolates from the cluster number 10.

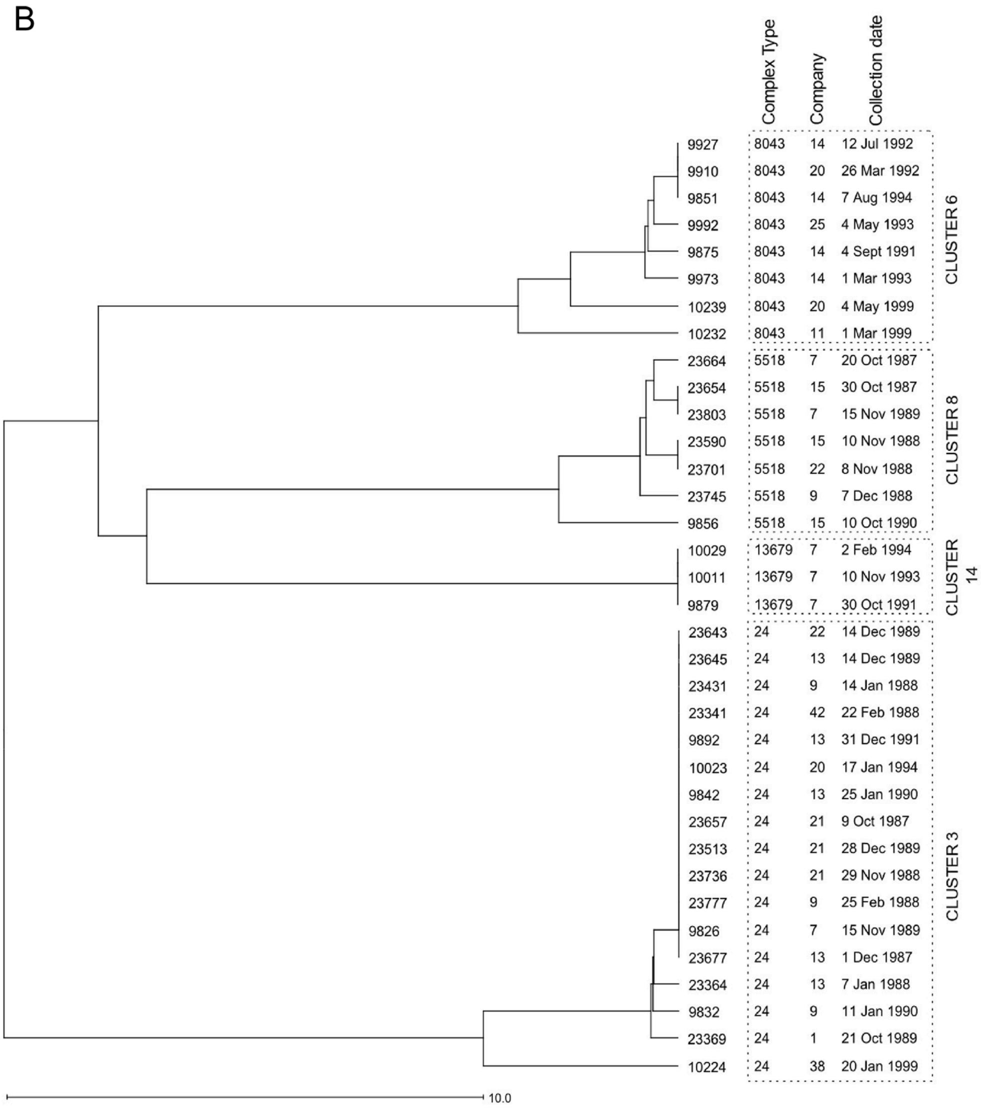


Fig. 4. (*continued*).

Vacherin Mont d'Or was typically between 32 and 38 °C. However, due to lessons learned from past outbreaks, the current product specification for *Vacherin Mont d'Or* requires that the milk must be heated to a temperature between 57 °C and 68 °C for a maximum of 15 s, or subjected to an equivalent treatment (FOAG, 2014). On the other hand, hard cheeses are exposed to a much longer ripening period, lasting from several months to years, and the smearing step typically involves salt solutions containing 1–2% salt. These conditions make contamination from environmental sources more likely than contamination originating from the milk itself. Even if contamination does occur through the milk, studies such as Bachmann and Spahr (1995) showed that the number of pathogenic bacteria decreases over time in hard cheeses, reducing the risk of contamination as the cheese matures. The repertoire of persistence-associated genes identified in the CC3 isolates could be the reason why these strains were more commonly found in hard cheese environments.

The difference of the prevalence of different *L. monocytogenes* CCs between industrial and artisanal cheese dairies were also significant. The frequent occurrence of CC1 strains in artisanal cheese factories during the 80s could be also attributed to the use raw milk without any heat treatment, coupled with the above-mentioned association between CC1 and milk (Maury et al., 2019). Additionally, in artisanal dairies, the proximity between farms and cheese processing facilities facilitates direct contamination of cheese directly from the farm environment or the milk (Pyz-Łukasik et al., 2022). In contrast, industrial facilities typically use pasteurized milk under more aseptic conditions, which likely reduces the prevalence of CC1. Furthermore, cheese ripening and pre-packaging facilities represent additional potential points of contamination. Cheese produced at one facility is often transported to a separate ripening site, where it is smeared and aged until it reaches maturity. These ripening environments introduce further risks, as they often use smearing robots, brushes, and smear water that can harbor persistent *Listeria* strains (Aziza et al., 2006). This may explain the observed association between CC3 isolates and these facilities in this study. Cross-contamination between different batches of cheese or even different products within the same facility elevates the risk of *Listeria* dissemination in these settings (Barría et al., 2020).

Among the predominant and persistent CCs, distinct genetic profiles and ecological niches within cheese production systems were observed. CC3 and CC517 isolates, commonly found in hard cheese environments, harbored the SSI-1, a five-gene locus involved in tolerance to acidic and high-salt conditions (Lakicevic et al., 2022; Ryan et al., 2010). This pattern is consistent with previous studies demonstrating that SSI-1 distribution is CC-dependent rather than strictly lineage-specific (Chen et al., 2020). The presence of SSI-1 likely contributes to enhanced survival under cheese-associated conditions, such as high salt

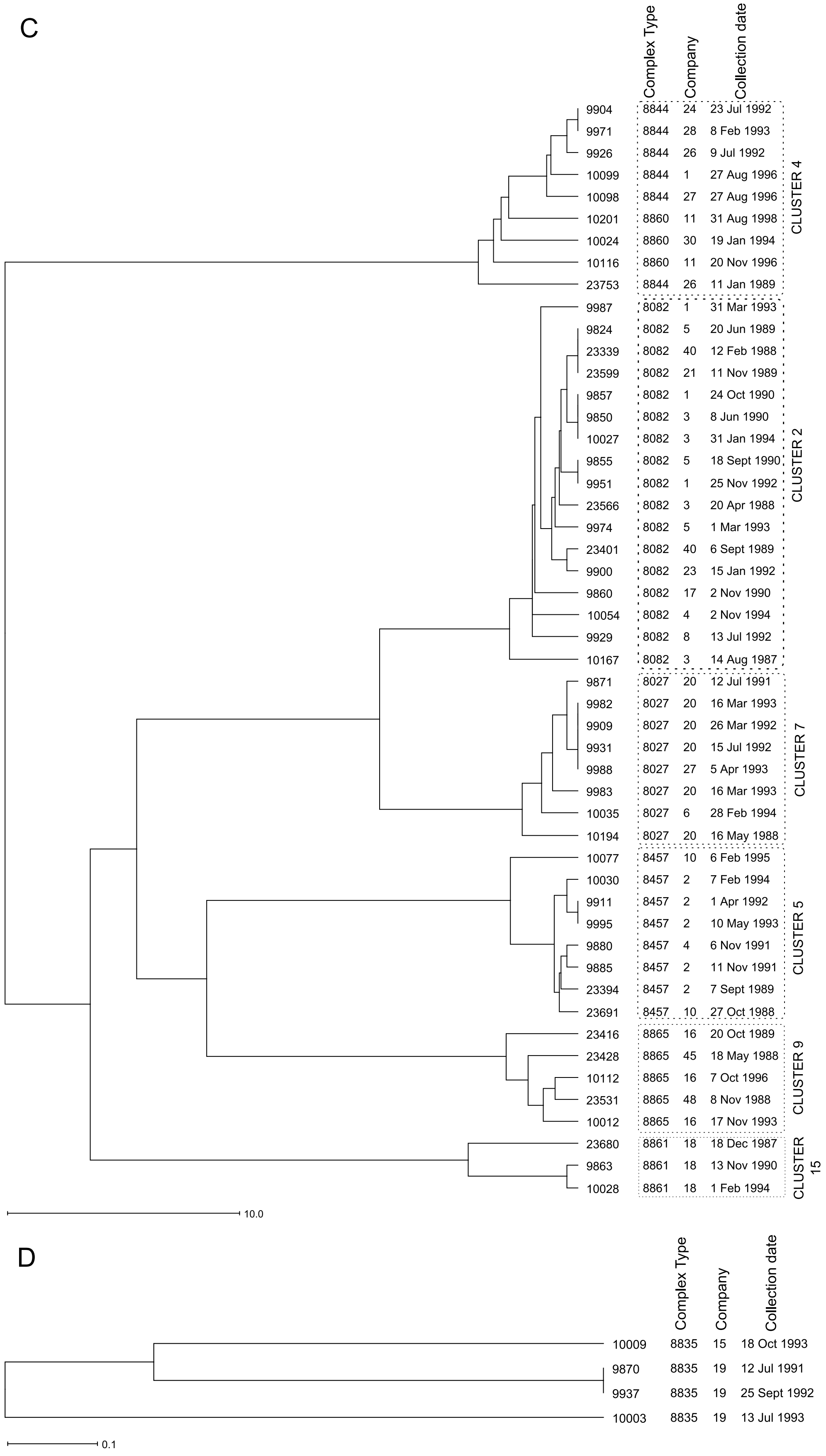


Fig. 4. (*continued*).

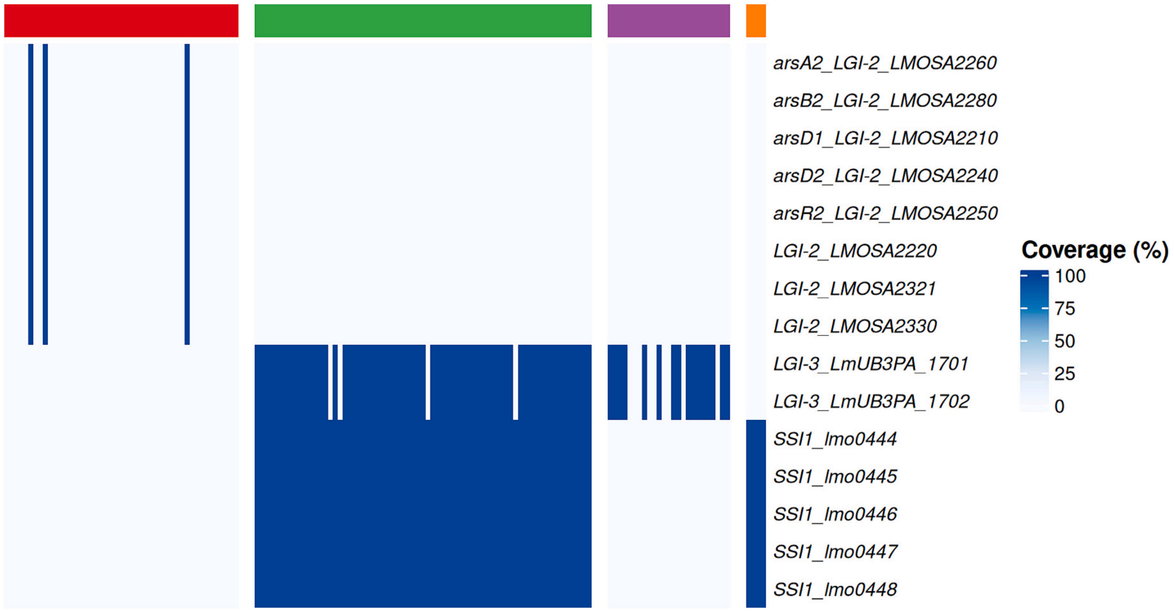


Fig. 5. Heatmap of gene coverage (%) across *L. monocytogenes* isolates from clonal complexes CC1 (red), CC3 (green), CC101 (purple), and CC517 (orange). Only genes showing variable presence across isolates are displayed.

concentrations and organic acids, which is in line with the predominance of CC3 isolates on cheese rinds and in smear water observed in this study. Moreover, SSI-1 has been linked to biofilm formation, adherence capacity, and increased tolerance to quaternary ammonium compounds (Keeney et al., 2018; Bland et al., 2021), suggesting a competitive advantage under cleaning regimes commonly applied in dairy processing environments. In addition, mobile genetic elements (MGE) potentially relevant for persistence were observed in this study. In particular, some CC3 and some CC101 isolates harbored the Listeria Genomic Island 3 (LGI-3), a mobile genomic island that has been associated with chromosomally integrated cadmium resistance determinants, as well as genes associated with environmental and processing-related niches (Palma et al., 2020). The occurrence of LGI-3 in only a subset of CC3 and CC101 isolates can be explained by the fact that MGE are typically acquired and maintained through horizontal gene transfer (HGT) under specific environmental selection pressures (Palma et al., 2020; Gelbicova et al., 2021), such the ones applied in industrial cheese production environments. In the same line, the LGI-2 was detected in only a subgroup of CC1 isolates, which could also indicate sporadic acquisition by HGT (Carvalho et al., 2020). LGI-2 harbors the arsRDAB gene cluster, which confers resistance to arsenic and other heavy metals and has been linked to bacterial persistence (Lee et al., 2017; Pasquali et al., 2018). This localized distribution suggests that acquisition of LGI-2 is driven by specific environmental selective pressures rather than representing a universal adaptation across the entire CC (Lee et al., 2017). The variable occurrence further implies that other CC1 strains may either not have been exposed to such selective pressures or rely on alternative stress survival mechanisms (Ratani et al., 2012; Gelbicova et al., 2021). Overall, our findings supported that the adaptation of *L. monocytogenes* to the cheese production environment is driven by multifactorial mechanisms that can be acquired as a response to various stressors, such as fluctuations in temperature, high salt concentrations, and repeated exposure to disinfectants (Sibanda and Buys, 2022). These stressors influence the genomic features of persistent CCs, as revealed by our results, that showed how a variation on the presence and absence of stress-associated genes may play a role in this adaptive success.

5. Conclusions

This study confirms the widespread distribution of specific *L. monocytogenes* CCs in dairy environments. However, the distinct patterns observed, such as the high prevalence of CC3, CC1 and CC101, highlight the influence of ecological niches within cheese production on strain persistence. The complexity of dairy production, including unique practices such as the use of smear water, extended ripening periods, and varying milk types, complicates the identification of contamination routes and persistence mechanisms. However, the detection of identical strains across multiple settings suggests historical supply chain dissemination, revealing potential weaknesses in controlling cross-contamination between production sites. Our results indicate that persistence is primarily CC-dependent, with some CCs relying on elements like SSI-1, while the presence of genomic islands acquired by HGT under selective pressures may also contribute. Understanding these adaptations is critical for developing targeted strategies to mitigate contamination and improve food safety in dairy production systems.

CRediT authorship contribution statement

Lena Fritsch: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Remo S. Schmidt:** Writing – review & editing, Methodology. **Elisabet Marti:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used chatgpt to improve the readability and language of the manuscript. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank René Imhof that led the Listeria Monitoring Program and Dieter Weik for technical assistance with the isolation of the bacterial strains. Additionally, we thank Jörg Hummerjohann for fruitful discussions. This work was supported by Agroscope and did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2026.111734.

Data availability

The genomes obtained in this study were deposited in NCBI/EMBL/DDBJ databases under the Bioproject accession number PRJNA1329014.

References

Aziza, F., et al., 2006. Stochastic, compartmental, and dynamic modeling of cross-contamination during mechanical smearing of cheeses. Risk Anal. 26 (3), 731–745.

Bachmann, H.P., Spahr, U., 1995. The fate of potentially pathogenic bacteria in Swiss hard and semihard cheeses made from raw milk. J. Dairy Sci. 78 (3), 476–483.

Barría, C., et al., 2020. Tracing Listeria monocytogenes contamination in artisanal cheese to the processing environments in cheese producers in southern Chile. Food Microbiol. 90, 103499. https://doi.org/10.1016/j.fm.2020.103499.

Beno, S.M., Stasiewicz, M.J., Andrus, A.D., Ralyea, R.D., Kent, D.J., Martin, N.H., et al., 2016. Development and validation of pathogen environmental monitoring programs for small cheese processing facilities. J. Food Prot. 79 (12), 2095–2106.

Berger, T., Fritsch, L., Imhof, R., Jakob, E., Stanke, T., Romano, A., 2019. Experiences from Listeria consulting – summary of the Colloquium of 29 August 2019. In: Agroscope Transfer, Nr. 295. Agroscope.

Bille, J., 1990. Epidemiology of human listeriosis in Europe with special reference to the Swiss outbreak. In: Miller, A.J., Smith, J.L., Somkuti, G.A. (Eds.), Foodborne Listeriosis. Elsevier, New York, NY, pp. 71–74.

Bille, J., Blanc, D.S., Schmid, H., Boubaker, K., Baumgartner, A., Siegrist, H.H., et al., 2006. Outbreak of human listeriosis associated with tomme cheese in northwest Switzerland, 2005. Eurosurveillance 11 (11), 633.

Bland, R.N., Johnson, J.D., Waite-Cusic, J.G., Weisberg, A.J., Riutta, E.R., Chang, J.H., Kovacevic, J., 2021. Application of whole genome sequencing to understand diversity and presence of genes associated with sanitizer tolerance in Listeria monocytogenes from produce handling sources. Foods 10 (10), 2454. https://doi.org/10.3390/foods10102454.

Buchanan, R.L., Gorris, L.G.M., Hayman, M.M., Jackson, T.C., Whiting, R.C., 2017. A review of Listeria monocytogenes: an update on outbreaks, virulence, dose-response, ecology, and risk assessments. Food Control 75, 1–13.

Büla, C.J., Bille, J., Glauser, M.P., 1995. An epidemic of food-borne listeriosis in western Switzerland: description of 57 cases involving adults. Clin. Infect. Dis. 20 (1), 66–72.

Carvalho, G., Fouchet, D., Danesh, G., Godeux, A.S., Laaberki, M.H., Pontier, D., Charpentier, X., Venner, S., 2020. Bacterial transformation buffers environmental fluctuations through the reversible integration of mobile genetic elements. Mbio 11 (2), 10–1128.

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 (20), 6258–6272.

Chen, Y., Chen, Y., Pouillot, R., Dennis, S., Xian, Z., Luchansky, J.B., et al., 2020. Genetic diversity and profiles of genes associated with virulence and stress resistance among isolates from the 2010–2013 interagency *Listeria monocytogenes* market basket survey. PLoS One 15 (4), e0231393.

Chenal-Francisque, V., Lopez, J., Cantinelli, T., Caro, V., Tran, C., Leclercq, A., et al., 2011. Worldwide distribution of major clones of Listeria monocytogenes. Emerg. Infect. Dis. 17 (6), 1110–1112.

Chiara, M., D'Erchia, A.M., Manzari, C., Minotto, A., Montagna, C., Addante, N., et al., 2014. Draft genome sequences of six *Listeria monocytogenes* strains isolated from dairy products from a processing plant in southern Italy. Genome Announc. 2 (1). https://doi.org/10.1128/genomeA.01147-13.

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 (8), 3819–3822. https://doi.org/10.1128/JCM.42.8.3819-3822.2004.

Ebner, R., et al., 2015. Phenotypic and genotypic characteristics of Listeria monocytogenes strains isolated during 2011–2014 from different food matrices in Switzerland. Food Control 57, 321–326.

EFSA BIOHAZ Panel, 2024. Persistence of microbiological hazards in food and feed production and processing environments. EFSA J. 22 (1), e8521. https://doi.org/10.2903/j.efsa.2024.8521.

EFSA, ECDC, 2023. The European Union One Health 2022 zoonoses report. EFSA J. 21 (12), e8442. https://doi.org/10.2903/j.efsa.2023.8442.

Félix, B., et al., 2018. Population genetic structure of *Listeria monocytogenes* strains isolated from the pig and pork production chain in France. Front. Microbiol. 9, 684. https://doi.org/10.3389/fmicb.2018.00684.

FOAG, 2014. Product Specification: Vacherin Mont-d'Or, Registered as Protected Designation of Origin (PDO) (Originally Issued 7 May 2003; Amended 5 January 2006, 1 April 2008, 12 April 2010, and 27 May 2014). Bern, Switzerland.

Fretz, R., Sagel, U., Ruppitsch, W., Pietzka, A.T., Stöger, A., Huhulescu, S., 2010. Listeriosis outbreak caused by acid curd cheese 'Quargel', Austria and Germany 2009. Eurosurveillance 15, 19477.

FSVO & FOPH, 2023. Bericht zur Überwachung von Zoonosen und lebensmittelbedingten Krankheitsausbrüchen, Daten 2022.

Gaulin, C., Ramsay, D., Bekal, S., 2012. Widespread listeriosis outbreak attributable to pasteurized cheese, which led to extensive cross-contamination affecting cheese retailers, Quebec, Canada, 2008. J. Food Prot. 75 (1), 71–78.

Gelbicova, T., Florianova, M., Hluchanova, L., Kalova, A., Korena, K., Strakova, N., Karpiskova, R., 2021. Comparative analysis of genetic determinants encoding cadmium, arsenic, and benzalkonium chloride resistance in Listeria monocytogenes of human, food, and environmental origin. Front. Microbiol. 11, 599882.

Gonzales-Barron, U., Cadavez, V., Guillier, L., Sanaa, M., 2023. A critical review of risk assessment models for Listeria monocytogenes in dairy products. Foods 12 (24), 4436.

Griffiths, M.W., 1989. Listeria monocytogenes: its importance in the dairy industry. J. Sci. Food Agric. 47 (2), 133–158.

Harrand, A.S., Jagadeesan, B., Baert, L., Wiedmann, M., Orsi, R.H., 2020. Evolution of Listeria monocytogenes in a food processing plant involves limited single-nucleotide substitutions but considerable diversification by gain and loss of prophages. Appl. Environ. Microbiol. 86, e01942-19. https://doi.org/10.1128/AEM.01942-19.

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, e00827-17.

Hilliard, A., Leong, D., O'Callaghan, A., Culligan, E., Morgan, C., DeLappe, N., et al., 2018. Genomic characterization of *Listeria monocytogenes* isolates associated with clinical listeriosis and the food production environment in Ireland. Genes 9 (1), 361. https://doi.org/10.3390/genes9010361.

Andritsos, N.D., Mataragas, M., 2023. Characterization and antibiotic resistance of *Listeria monocytogenes* strains isolated from Greek Myzithra soft whey cheese and related food processing surfaces over two-and-a-half years of safety monitoring in a cheese processing facility. Foods 12 (6), 1200.

Keeney, K., Trmcic, A., Zhu, Z., Delaquis, P., Wang, S., 2018. Stress survival islet 1 contributes to serotype-specific differences in biofilm formation in Listeria monocytogenes. Lett. Appl. Microbiol. 67 (6), 530–536. https://doi.org/10.1111/lam.13174.

Korsak, D., Chmielowska, C., Szuplewska, M., Bartosik, D., 2019. Prevalence of plasmid-borne benzalkonium chloride resistance cassette bcrABC and cadmium resistance cadA genes in nonpathogenic Listeria spp. isolated from food and food-processing environments. Int. J. Food Microbiol. 290, 247–253. https://doi.org/10.1016/j.ijfoodmicro.2018.10.007.

Lakicevic, B.Z., Den Besten, H.M.W., De Biase, D., 2022. Landscape of stress response and virulence genes among Listeria monocytogenes strains. Front. Microbiol. 12, 738470. https://doi.org/10.3389/fmicb.2021.738470.

Lee, S., Ward, T.J., Jima, D.D., Parsons, C., Kathariou, S., 2017. The arsenic resistance-associated Listeria genomic island LGI2 exhibits sequence and integration site diversity and a propensity for three Listeria monocytogenes clones with enhanced virulence. Appl. Environ. Microbiol. 83 (21), e01189-17.

Leong, D., NicAogain, K., Luque-Sastre, L., McManamon, O., Hunt, K., Alvarez-Ordonez, A., et al., 2017. A 3-year multi-food study of the presence and persistence of *Listeria monocytogenes* in 54 small food businesses in Ireland. Int. J. Food Microbiol. 249, 18–26. https://doi.org/10.1016/j.ijfoodmicro.2017.03.019.

Martinez-Suarez, J.V., Ortiz, S., Lopez-Alonso, V., 2016. Potential impact of the resistance to quaternary ammonium disinfectants on the persistence of Listeria monocytogenes in food processing environments. Front. Microbiol. 7, 638. https://doi.org/10.3389/fmicb.2016.00638.

Maury, M.M., Tsai, Y.H., Charlier, C., Touchon, M., Chenal-Francisque, V., Leclercq, A., et al., 2016. Uncovering *Listeria monocytogenes* hypervirulence by harnessing its biodiversity. Nat. Genet. 48, 308–313. https://doi.org/10.1038/ng.3491.

Maury, M.M., Bracq-Dieye, H., Huang, L., et al., 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.

Moura, A., Criscuolo, A., Pouseele, H., Maury, M. M., Leclercq, A., Tarr, C., et al. (2016). Whole genome-based population biology and epidemiological surveillance of *Listeria monocytogenes*. Nat. Microbiol., 2, 16185. doi:https://doi.org/10.1038/nmicrobiol.2016.185.

Muchaamba, F., Eshwar, A.K., Stevens, M.J.A, Stephan, R. and Tasara, T. (2022). Different shades of Listeria monocytogenes: strain, serotype, and lineage-based variability in virulence and stress tolerance profiles. Front. Microbiol., 12, 792162. doi:https://doi.org/10.3389/fmicb.2021.792162.

Oxaran, V., Lee, S.H.I., Chaul, L.T., Corassin, C.H., Barancelli, G.V., Alves, V.F., et al., 2017. *Listeria monocytogenes* incidence changes and diversity in some Brazilian dairy industries and retail products. Food Microbiol. 68, 16–23. https://doi.org/10.1016/j.fm.2017.06.001.

Painset, A., Björkman, J. T., Kiil, K., Guillier, L., Mariet, J. F., Félix, B., et al. (2019). LiSEQ—whole-genome sequencing of a cross-sectional survey of *Listeria monocytogenes* in ready-to-eat foods and human clinical cases in Europe. Microb. Genomics, 5(2), e000257. doi:https://doi.org/10.1099/mgen.0.000257.

Palma, F., Brauge, T., Radomski, N., Mallet, L., Felten, A., Mistou, M.Y., et al., 2020. Dynamics of mobile genetic elements of *Listeria monocytogenes* persisting in ready-to-eat seafood processing plants in France. BMC Genomics 21, 130. https://doi.org/10.1186/s12864-020-6546-7.

Pasquali, F., Palma, F., Guillier, L., Lucchi, A., De Cesare, A., Manfreda, G., 2018. Listeria monocytogenes sequence types 121 and 14 repeatedly isolated within one year of sampling in a rabbit meat processing plant: persistence and ecophysiology. Front. Microbiol. 9, 596. https://doi.org/10.3389/fmicb.2018.00596.

Pightling, A.W., Pettengill, J.B., Luo, Y., Baugher, J.D., Rand, H., Strain, E., 2018. Interpreting whole-genome sequence analyses of foodborne bacteria for regulatory applications and outbreak investigations. Front. Microbiol. 9, 1482. https://doi.org/10.3389/fmicb.2018.01482.

Prieto, B.D., Pietzka, A., Martinovic, A., Ruppitsch, W., Bogdanovic, I.Z., 2024. Surveillance and genetic characterization *of Listeria monocytogenes* in the food chain in Montenegro during the period 2014–2022. Front. Microbiol. 15, 1418333. https://doi.org/10.3389/fmicb.2024.1418333.

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 (18), 2805. https://doi.org/10.3390/foods11182805.

R Core Team, 2022. R: a language and environment for statistical computing, R Foundation 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 (9), e1000146. https://doi.org/10.1371/journal.ppat.1000146.

Ratani, S.S., Siletzky, R.M., Dutta, V., Yildirim, S., Osborne, J.A., Lin, W., Hitchins, A.D., Ward, T.J., Kathariou, S., 2012. Heavy metal and disinfectant resistance of Listeria monocytogenes from foods and food processing plants. Appl. Environ. Microbiol. 78 (19), 6938–6945.

Ricci, A., Allende, A., Bolton, D., Chemaly, M., Davies, R., Fernández Escámez, P.S., et al., 2018. *Listeria monocytogenes* contamination of ready-to-eat foods and the risk for human health in the EU. EFSA J. 16 (1), e05134. https://doi.org/10.2903/j.efsa.2018.5134.

Ritschard, J.S., Schuppler, M., 2024. The microbial diversity on the surface of smear-ripened cheeses and its impact on cheese quality and safety. Foods 13 (2), 214. https://doi.org/10.3390/foods13020214.

Rodriguez-Campos, D., Rodriguez-Melcon, C., Alonso-Calleja, C., Capita, R., 2019. Persistent Listeria monocytogenes isolates from a poultry-processing facility form more biofilm but do not have a greater resistance to disinfectants than sporadic strains. Pathogens 8 (1), 35. https://doi.org/10.3390/pathogens8010035.

Ruppitsch, W., Pietzka, A., Prior, K., Bletz, S., Fernandez, H.L., Allerberger, F., et al., 2015. Defining and evaluating a core genome multilocus sequence typing scheme for whole-genome sequence-based typing of Listeria monocytogenes. J. Clin. Microbiol. 53 (9), 2869–2876. https://doi.org/10.1128/JCM.01193-15.

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 (3), 984–995. https://doi.org/10.1111/j.1365-2672.2010.04725.x.

Sauders, B., Durak, M., Fortes, E., Windham, K., Schukken, Y., Lembo, A., Akey, B., Nightingale, K., Wiedmann, M., 2006. Molecular characterization of Listeria monocytogenes from natural and urban environments. J. Food Prot. 69 (1), 93–105. https://doi.org/10.4315/0362-028X-69.1.93.

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

Sibanda, T., Buys, E.M., 2022. *Listeria monocytogenes* pathogenesis: the role of stress adaptation. Microorganisms 10 (8), 1522. https://doi.org/10.3390/microorganisms10081522.

Stessl, B., Fricker, M., Fox, E., Karpiskova, R., Demnerova, K., Jordan, K., Ehling-Schulz, M., Wagner, M., 2014. Collaborative survey on the colonization of different types of cheese-processing facilities with Listeria monocytogenes. Foodborne Pathog. Dis. 11 (1), 8–14. https://doi.org/10.1089/fpd.2013.1578.

Stoller, A., Stevens, M.J.A., Stephan, R., Guldimann, C., 2019. Characteristics of Listeria monocytogenes strains persisting in a meat processing facility over a 4-year period. Pathogens 8 (1), 32. https://doi.org/10.3390/pathogens8010032.

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 (12), 2082–2089. https://doi.org/10.4315/0362-028X.JFP-18-093.

Weinmaier, T., Riesing, M., Rattei, T., Bille, J., Arguedas-Villa, C., Stephan, R., Tasara, T., 2013. Complete genome sequence of Listeria monocytogenes LL195, a serotype 4b strain from the 1983–1987 listeriosis epidemic in Switzerland. Genome Announc. 1 (4), e00152-12. https://doi.org/10.1128/genomeA.00152-12.

World Health Organization, 1991. Listeriosis Situation in Switzerland Two Years After Vacherin Mont-d'Or Outbreak, WHO: World Health Organization. Switzerland. Retrieved from https://coilink.org/20.500.12592/t1hwvb on 07 Aug 2025. COI: 20.500.12592/t1hwvb.