



Research Paper

Listeria is Frequently Present in Biofilms in Albanian Dairies[☆]

Nadja Pracser^{1,2}, Rozeta Hasalliu³, Arbnora Durmishaj³, Sarah Thalgut^{2,†}, Felix Spiegel^{1,2}, Lauren V. Alteio², Kathrin Rychli^{1,*}

¹ Centre for Food Science, University of Veterinary Medicine Vienna, Vienna, Austria

² FFoQSI GmbH – Austrian Competence Centre for Feed and Food Quality, Safety and Innovation, Tulln, Austria

³ Department of Food Science and Biotechnology, Agricultural University of Tirana, Kamëz Tirana, Albania



ARTICLE INFO

Keywords:

Biofilm matrix
Contamination hotspots
Foodborne pathogen
Gene profiling
Whole genome sequencing

ABSTRACT

Biofilms in food processing environments are a major concern for hygiene and food safety as they can harbor spoilage bacteria and pathogens. The aim of this study was to determine the prevalence of biofilms and *Listeria* (*L.*) species (*spp.*) in five Albanian dairies. We analyzed the presence of biofilms on 80 visibly clean sites by enumeration of the bacterial load (>100 bacterial cell equivalents /cm²) and quantification of extracellular DNA, carbohydrates, and proteins of the biofilm matrix. We furthermore isolated and characterized *Listeria spp.* from 200 samples, including 80 biofilm and 120 additional samples.

The biofilm prevalence (50% of 80 biofilm samples) and *Listeria* contamination (20% of 200 samples, including biofilm and additional samples), were very high, indicating a low hygiene status of the five companies. We detected 40 biofilms in total, including food contact surfaces such as storage tanks. Furthermore, 40 of the 200 sampling sites were positive for *Listeria*. We isolated 46 strains, including 38 *L. innocua*, 7 *L. monocytogenes*, and 1 *L. grayi* isolates, resulting in a prevalence of 19% for *L. innocua*, 3.5% for *L. monocytogenes*, and 0.5% for *L. grayi*. *Listeria* were frequently present in biofilms ($n = 11$), 27.5% of 40 biofilms harbored *Listeria*. The genetic diversity of the *L. innocua* strains was low, as the isolates mainly belonged to sequence type (ST) 1489 ($n = 21$) and 1085 ($n = 6$). The *L. monocytogenes* strains, assigned to ST3, ST5, and ST619, harbored the full set of essential virulence genes, indicating a virulent genotype. Gene profiling confirmed the presence of many stress resistance genes in all *Listeria spp.* strains and the presence of plasmids in 54.3% strains.

In conclusion, we successfully identified biofilm and *Listeria spp.* contamination hotspots in Albanian dairies, which should intensify cleaning and disinfection to improve food safety.

Biofilms in food processing environments are a major concern for hygiene and food safety. The biofilm architecture includes different microbial species embedded in a self-produced extracellular matrix consisting of major biofilm components such as proteins, carbohydrates, and extracellular DNA (eDNA) (Flemming et al., 2016, 2023; Sharan et al., 2022). The biofilm matrix protects the microorganisms from diverse environmental stressors such as desiccation, nutrient scarcity, cleaning and disinfection, or osmotic stress (Flemming et al., 2023). Environmental factors in the food processing environment, such as nutrients, humidity, or hard-to-clean niches, e.g. pipes, support the establishment and persistence of biofilms. Biofilms can further lead to surface material damage in the processing environment (Goetz et al., 2025; LaPointe et al., 2025). Resistance against cleaning procedures and sanitizers contributes to the persistence of biofilms in

the food processing environment (Møretro & Langsrud, 2004). Importantly, biofilms are a potential harborage site for food spoilage bacteria and pathogens (Wagner et al., 2020; Sharan et al., 2022). Previous research identified bacterial taxa with biofilm formation and spoilage potential, such as *Pseudomonas*, *Microbacterium*, *Streptococcus*, or *Staphylococcus* in the dairy environment (Maes et al., 2019; Sadiq et al., 2024). Detachment of bacterial cells from the biofilm and dispersal in the processing environment can result in contamination of food products during processing stages or in the final product (Chmielewski & Frank, 2003; Dass & Wang, 2022).

Listeria (L.) monocytogenes is a significant food pathogen in the dairy environment, causing the disease listeriosis, which has a high mortality rate. In the European Union, the overall occurrence of *L. monocytogenes* in dairy products was 0.47%, including milk and

[☆] This article is part of a special issue entitled: 'ISOPOL' published in Journal of Food Protection.

* Corresponding author.

E-mail address: Kathrin.Rychli@vetmeduni.ac.at (K. Rychli).

[†] Current affiliation: Clinical Centre for Population Medicine in Fish, Pig and Poultry, University of Veterinary Medicine Vienna, Vienna, Austria.

different types of cheeses in 2023 (EFSA, 2024). *Listeria* are well-described for their ability to survive and grow under hostile environmental conditions and to persist despite cleaning measures (Bucur et al., 2018; Matereke & Okoh, 2020; Muchaamba et al., 2022). Biofilms are suggested to contribute to the persistence of *Listeria* in the food processing environment (Sterniša et al., 2023; Tuytschaeve et al., 2023; Rolon et al., 2024); however, there is limited knowledge on the prevalence of *Listeria* in biofilms present in food processing environments (Pracser et al., 2024), because many studies only focus on lab-grown biofilms. Additionally, there is a lack of studies identifying biofilms in the dairy processing environment.

In the current study, we investigated the occurrence of biofilms and *Listeria* species (*spp.*) in five Albanian dairies. We additionally characterized the detected *Listeria* isolates by whole genome sequencing, focusing on stress resistance and virulence genes, and plasmids.

Methods

Sampling. The processing environments of five different Albanian dairies (companies A – E) that process milk and produce different types of cheese, yogurt, and dairy drinks were sampled between 2023 and 2024, including food-contact and non-food-contact surfaces. Companies were selected based on their availability and accessibility. One sampling event was conducted for each company. In total, 80 sites without visible food residues were tested for the presence of biofilms and *Listeria* spp. (Table S1). Further, 120 additional samples were only used for *Listeria* spp. isolation as food residues were visible. Negative controls were included in each sampling event (Table S2). The number of sampled sites depended on the size of the processing environments and accessibility in the respective facilities. In detail, the number of samples included 50 sites in company A (12 sites testing for biofilms and *Listeria* spp. (BF+L), 38 sites testing for *Listeria* spp. only (L)), 49 sites in company B (11 BF+L, 38 L), 30 sites in company C (22 BF+L, 8 L), 34 sites in company D (20 BF+L, 14 L), and 37 sites in company E (15 BF+L, 22 L). For biofilm detection, each sampling site was horizontally and vertically wiped with a cell scraper (length: 225 mm, blade width: 20 mm; Carl Roth) and Nylon® flocked swabs (552C, FLOQSwabs, COPAN) (Maes et al., 2017; Wagner et al., 2022). The size of the sampled surface area ranged from 1 to 1600 cm². Samples taken with scrapers and flocked swabs were transported in 10 ml of 0.25 × Ringer solution (B. Braun Austria GmbH). For detection of *Listeria* spp., sampling sites (surface areas from 31 to 13,500 cm²) were horizontally and vertically wiped with a hydrated polyurethane (PU) sponge swab (HiCap Neutralizing swabs, Nasco Whirlpak). All samples were transported under cool conditions. Samples for biofilm detection were stored at –20 °C until further processing.

Detection and confirmation of *Listeria* spp.. Ten ml of 1 × cooled phosphate-buffered saline (PBS) was added to each sample from company A and company B, followed by a homogenization step for 1 min using a lab blender. From each sample, 8 ml of the suspension was removed. No PBS was added to samples from companies C, D, and E. Cultural enrichment and detection of *Listeria* spp. were performed according to ISO 11290-1:2017 with modifications. Briefly, 70 ml of Half-Fraser broth (Noack) was added to each bag containing a PU swab, followed by incubation at 30 °C for 48 h. From the primary enrichment, 1 ml was transferred to 10 ml of Fraser broth (Noack) and incubated at 37 °C for 48 h for secondary enrichment. For cultural detection of *Listeria* spp., 1 ml of the primary and secondary enrichment was centrifuged at 8,000 × g for 5 min. The resulting pellets were resuspended in 50 µl of 1 × PBS and plated on RAPID[®]L.mono (Bio-Rad) agar plates, which were incubated for 48 h at 37 °C. A single colony of each presumptive *Listeria* spp. was selected for further characterization based on the colony morphology on RAPID[®]L.mono agar. Due to the high background flora, putative colonies were additionally

streaked out on ALOA (Noack) agar plates and incubated at 37 °C for 48 h. Putative *Listeria* spp. isolates were confirmed, and species were differentiated by a PCR approach (Border et al., 1990; Bubert et al., 1999). DNA was extracted from putative *Listeria* spp. colonies with a Chelex-based DNA extraction method (Chelex 100, Sigma-Aldrich) (Walsh et al., 1991). Briefly, *Listeria* colonies were transferred to a solution of 50 µl 0.01 M Tris-HCl (pH 7.0) and 200 µl of Chelex solution (stock: 2.5 g Chelex 100 in 2.5 ml 0.01 M Tris-HCl (pH 7.0) and 95 ml autoclaved ddH₂O). The putative *Listeria* isolates in the Chelex solution were incubated at 95 °C for 10 min, shaking at 550 rpm. Then, the samples were centrifuged at 15,000 × g for 30 s, and 150 µl of the supernatant containing the bacterial DNA was transferred to a new 1.5 ml tube.

PCR was performed to detect *Listeria* spp. targeting the respective 16S rRNA fragment (primer sequences: 5'-CAG CAG CCG CGG TAA TAC-3', 5'-CTC CAT AAA GGT GAC CCT-3'), and the *hly* gene for detection of *L. monocytogenes* (primer sequences: 5'-CCT AAG ACG CCA ATC GAA-3', 5'-AAG CAC TTG CAA CTG CTC-3'). The final concentration of reagents in the PCR reaction was 1.08 µM for the primers, 1.5 mM MgCl₂, 200 µM dNTPs (Thermo Fisher Scientific), 1 × PCR buffer (Invitrogen), and 1.25 U Platinum[™] Taq DNA polymerase (Invitrogen). The final volume of the PCR reaction was 25 µl and contained 2 µl DNA template. PCR settings included an initial denaturation at 94 °C for 2 min, 30 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, extension at 72 °C for 1 min, and a final extension step at 72 °C for 5 min. A second PCR targeted the *iap* gene, aiming for the differentiation and identification of different *Listeria* spp.. Upstream primer sequences targeted *L. monocytogenes* (5'-CAA ACT GCT AAC ACA GCT ACT-3'), *L. innocua* (5'-ACT AGC ACT CCA GTT AAA C-3'), *L. grayi* (5'-CCA GCA GTT TCT AAA CCT GCT-3'), and the *L. ivanovi*/*L. welshimeri*/*L. seeligeri* group (5'-TAA CTG AGG TAG CGA GCG AA-3'). The downstream primer sequence was 5'-TTA TAC GCG ACC GAA GCC AAC-3'. The final concentration of reagents in the PCR reaction was 128 nM for the primers, 1.5 mM MgCl₂, 200 µM dNTPs (Thermo Fisher Scientific), 1 × PCR buffer (Invitrogen), and 1.5 U Platinum[™] Taq DNA polymerase (Invitrogen). The final volume of the PCR reaction was 25 µl and contained 2 µl DNA template. PCR settings included an initial denaturation at 94 °C for 2 min, 30 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s, extension at 72 °C for 30 s, and a final extension step at 72 °C for 5 min. Confirmed *Listeria* spp. were grown in liquid cultures in BHI with yeast extract (BHI + Y; Biokar Diagnostics) overnight, and cryo-cultures were made for long-term storage.

Whole genome sequencing of *Listeria* spp. Isolates. *Listeria* spp. isolates were recovered from cryo-cultures and streaked out on tryptic soy agar with yeast extract (TSA + Y; Biokar Diagnostics) agar plates. For DNA extraction, a bacterial pellet was recovered from 1 ml of overnight culture by centrifugation at 12,000 × g for 2 min. The pellet was resuspended in 1 ml of 1 × PBS, and the centrifugation step was repeated. DNA was extracted using the Monarch[®] Spin gDNA extraction kit (New England Biolabs) according to the manufacturer's instructions for DNA extraction for gram-positive bacteria. The final elution step was carried out with 20 µl of preheated H₂O for molecular biology. DNA extraction of samples with low DNA-yield was repeated with 2 ml or 3 ml overnight culture volume.

Sequencing was performed on an Oxford Nanopore MinION sequencing platform using R10.4.1 (FLO-MIN114) flow cells. For library preparation, 200 ng of genomic DNA per sample was used. Unique barcodes were added to each sample using the Rapid Barcoding kit V14 (SQK-RBK114.24 / SQK-RBK114.96) to run the pooled sample on the same flow cell. AMPure XP Beads (AXP) were added to the entire pooled barcoded sample for library cleanup. Quantification of the DNA library was done with a Qubit fluorometer using the Qubit dsDNA BR assay. Sequencing adapters (Rapid Adapter) were added to the library. The sequencing run was performed for 72 h. Base-

calling was carried out with *dorado* v0.7.2+9ac85c6 using the *dna_r10.4.1_e8.2_400bps_sup@v5.0.0* model. Demultiplexing was done with *dorado* v0.9.1+c8c2c9f. *Samtools* v1.20 (Danecek et al., 2021) was used to convert file formats. Quality filtering of raw reads for a minimum read length of 200 bp and a q-score of greater than 9 was done with *chopper* v0.8.0 (De Coster and Rademakers, 2023). Assembly was carried out using *flye* v2.9.4-b1799 (Kolmogorov et al., 2019), and polishing of sequences was done with *medaka* v1.12.0 using the *r1041_e82_400bps_sup@v5.0.0* model. The assemblies were assessed using *busco* v5.8.2 (Simão et al., 2015) and evaluated with *QUAST* v5.0.2 (Gurevich et al., 2013) (Table S3). The raw sequencing reads can be found online in the NCBI data repository under the BioProject number PRJNA1312463 <https://www.ncbi.nlm.nih.gov/>.

Characterization of *Listeria* spp.. Multilocus sequence typing (MLST) of *L. monocytogenes* and *L. innocua* was done using the MLST scheme from Institut Pasteur (Moura et al., 2016) and *mlst* v2.16.1 (Seemann, n.d.). Further, phylogenetic trees were generated using a reference-based alignment produced with *Snippy* v4.6.0 (Seemann, 2015). Using *IQ-TREE* v2.0.3 with automated model selection (model for Fig. S4: TVM+F+I+G4; model for Fig. 2B: TVM+F+I; model for Fig. 2C: TVM+F+I) (Kalyaanamoorthy et al., 2017; Minh et al., 2020) and 1000 ultrafast bootstrap replicates (Hoang et al., 2017), phylogenetic trees were constructed. Phylogenies in combination with heatmaps for displaying the genetic repertoire of *Listeria* were visualized with R packages *ggtree* v3.12.0 (Yu et al., 2017) and *ggplot2* v3.5.1 (Wickham, 2016) in R v4.4.1.

Local in-house BLAST databases comprising virulence genes ($n = 100$) (Table S4), stress resistance genes ($n = 107$) (Table S5), and plasmid sequences ($n = 10$) and plasmid genes ($n = 13$) (Table S6) were used to investigate the genetic repertoire of the detected *Listeria* (Pracser et al., 2024). The presence of genes and plasmid sequences was first determined using *blastN* (ncbi-blast v2.16.0) and a custom-made Python script. An e-value of 0.01 and a culling limit of 1 were set. A minimum alignment ratio of 70% relative to the reference sequence length and a minimum nucleotide identity of 99% were set to determine the presence of plasmids. Coding sequences from the stress resistance and virulence gene search were translated into amino acid sequences using *transeq* (EMBOSS v 6.6.0.0). The appropriate reading frame was selected using a custom-made Python script. Using *blastP* (ncbi-blast v2.16.0), the translated amino acid sequences were compared against the local virulence and stress resistance gene databases in protein format. For *L. monocytogenes*, genes were classified as present if the amino acid identity of translated sequences was $\geq 92\%$ and if the alignment ratio relative to the reference sequence length was $\geq 70\%$. Sequence alignments of genes below these thresholds were classified as “absent”. An alignment ratio (relative to the reference sequence length) of $\geq 70\%$ but $< 100\%$ or $> 100\%$ was further investigated, and the type of length deviation (either “shorter variant” for length deviations $< 100\%$, or “longer variant” for length deviations $> 100\%$) was determined in Supplementary Tables S7, S9, and S10. For other *Listeria* spp., the threshold for the amino acid identity was lowered to $\geq 80\%$.

Detection of biofilms. Samples taken with scraper and flocked swabs were processed as described in previous studies (Wagner et al., 2020; Pracser et al., 2024). Two grams of hydrated cation exchange resin (CER, Amberlite® HPR110, 20–50 mesh, Sigma-Aldrich) were added to each sample taken with scraper and flocked swabs. Samples were incubated on a shaker at room temperature at 500 rpm for 15 min, followed by centrifugation at $3,220 \times g$ at 20°C for 15 min. The supernatant was collected, sterile filtered using a $0.22 \mu\text{m}$ filter membrane (Filtropur S.02, Sarstedt AG & Co KG), and stored at -20°C for later analysis of extracellular matrix components. The remaining cell pellet, including CER, was kept and stored at -20°C for analysis of the bacterial cell content.

Detection of bacterial cells in biofilms. Pellets and CER were thawed, and 5 ml $1 \times \text{PBS}$ (Thermo Fisher Scientific) was added to wash and to separate the bacterial cells from the CER. Samples were mixed by vortex agitation, and after the CER settled for 2 min, the supernatant was transferred to a new tube. The washing step was repeated a second time. The bacterial cell pellet was recovered by centrifugation for 5 min at $3,220 \times g$. DNA was extracted from bacterial cell pellets using the DNeasy® PowerSoil® ProKit (Qiagen) according to the manufacturer's instructions. The elution step was carried out twice with 25 μl of preheated (70°C) water for molecular biology. DNA concentrations were measured using a Qubit fluorometer (Invitrogen) with a Qubit high-sensitivity dsDNA kit (Invitrogen).

Enumeration of bacterial cell equivalents was performed with qPCR targeting the 16S rRNA gene (primers: 5'-CCT ACG GGA GGC AGC AG-3', 5'-ATT ACC GCG GCT GCT GG-3'), similar to previous studies (Dixon et al., 2019; Wagner et al., 2020; Pracser et al., 2024). The final concentration of primers was 250 nM, and a final concentration of $1 \times$ Brilliant III Ultra-Fast SYBR® Green qPCR master mix with low ROX (Agilent) was used. A single qPCR reaction was performed with 1 μl template DNA. The qPCR run settings were as follows: 1 cycle of denaturation at 95°C for 3 min, 40 cycles of amplification at 95°C for 5 s and 60°C for 20 s, and 1 cycle for generation of the melting curve at 95°C for 1 min, 60°C for 30 s, and 95°C for 30 s. To calculate total BCE, we used an average of 5.5 16S rRNA gene copy numbers, estimated from *rrnDB* (Větrovský & Baldrian, 2013; Stoddard et al., 2015). Background controls from the DNA extraction kit were subtracted from the copy numbers of samples to account for bacterial contamination of the used reagents.

Detection of extracellular matrix components. eDNA was precipitated using an ethanol precipitation method according to Zetzmann et al. (2015). Briefly, $0.1 \times$ (of sample volume) 3 M Na-acetate pH 5.2, $0.1 \times 0.1 \text{ M}$ MgCl_2 , and $2.5 \times$ ice-cold ethanol absolute were added to each sample. Subsequently, the samples were incubated at -20°C for 24 h. Recovery of precipitated DNA was done by centrifugation at $20,817 \times g$ for 15 min at 4°C . After discarding the supernatant, the recovered pellet was washed with 70% ethanol at a 1:1 ratio to the initial sample volume. A repetition of the centrifugation step followed, and the resulting pellets were allowed to air-dry for 1 h at room temperature. The pellets were resuspended in 21 μl sterile H_2O . The concentration of eDNA was measured with a Qubit fluorometer (Invitrogen). Negative precipitation controls were used to assess the limit of detection.

Samples were concentrated using a vacuum concentrator to evaporate all the liquid for the detection of carbohydrates in the extracellular matrix. The pellet was resuspended in 200 μl sterile H_2O . Carbohydrates were detected using a phenol-sulfuric acid method (Masuko et al., 2005). A standard curve was created with glucose standards for the calculation of glucose equivalents in each measured sample. The limit of quantification (LOQ) was 1.52 mg/l glucose equivalents.

Proteins were precipitated overnight with $0.1 \times$ (of sample volume) TCA/Acetone (1 g/ml) and $0.01 \times 2\%$ sodium deoxycholate at 4°C (Rychli et al., 2016). A pellet of precipitated proteins was recovered by centrifugation at $20,817 \times g$ at 4°C for 30 min. Subsequently, the pellet was washed with ice-cold acetone, and a repetition of the centrifugation step was done. The supernatant was discarded, and the pellet was allowed to air-dry for 1 h at room temperature. The pellet was dissolved in 20 μl 50 mM Tris-HCl for 1 h at room temperature. Measurement of the protein content was done using a Quant-iT™ protein assay kit (Invitrogen) according to the manufacturer's instructions with modifications. Due to the low biomass in biofilm samples, we spiked the Quant-iT protein buffer with 250 ng bovine serum albumin per sample to overcome the detection limit of the kit. The fluorescence of the spiked solution was subtracted from the real samples.

The threshold for matrix component detection was set at 2 ng/cm² for biofilm identification (Wagner et al., 2020).

Results and discussion

Bacterial load and the presence of biofilms. We detected bacterial cells in all 80 samples, which were evaluated for the presence of biofilms. The average density of bacterial cell equivalents ranged from 9×10^{-2} to 4.2×10^7 bacterial cell equivalents (BCE)/cm² (Fig. 1A, Table S1). The lowest bacterial load was detected on the walls of a freezer (# 62) in company D, and the highest load was in a water hose used for cleaning (# 11) in company A. Because biofilms are densely populated structures (Flemming et al., 2016), only sites with >100 BCE/cm² were defined as potential biofilm-harboring sites, which was the case for 51 biofilm samples (Fig. 1B). Since biofilms are characterized by the presence of extracellular matrix components, at least two of the three investigated matrix components (eDNA, carbohydrates and proteins) had to be detectable for a sample to be defined as a biofilm.

eDNA exceeding the threshold of 2 ng/cm² (Wagner et al., 2020) was detected in 13 samples (16.25%), ranging from 2.32 to 241.08 ng/cm² (Fig. 1B, Table S1, Fig. S1). Carbohydrates were detected in all 80 samples, ranging from 4.90 ng/cm² at a table for cheese salting in company D (# 54) to 120,875 ng/cm² at a whey hose joint in company B (# 15) (Fig. 1B, Table S1, Fig. S2). Proteins exceeding the threshold of 2 ng/cm² were present in 75% of biofilm samples ranging from 2.09 (drain company C, # 42) to 3,902.42 ng/cm² (whey output, # 75) (Fig. 1B, Table S1, Fig. S3).

Although only samples from visibly clean surfaces (i.e. no visible food residues with the naked eye) were included, we cannot fully exclude that non-visible food residues like lactose, casein, or whey proteins likely increased the number of carbohydrate- and protein-positive samples.

Overall, we detected 40 biofilms among 80 samples (50%; Fig. 1, Table S1): four of 12 samples in company A (33.3%), six of 11 samples in company B (54.6%), 13 of 22 samples in company C (59.1%), eight of 20 samples in company D (40%), and nine of 15 samples in company E (60%) were biofilm positive. Biofilms were detected on food contact surfaces, for example a milk storage tank (# 9, #25), a cheese cutting machine (# 18, 19), a milk hose (# 21), a working table (# 35, # 53), a yogurt hoses for filling (# 45), cans (# 51), a table for cheese salting (# 54), a brine tank (# 78), and a brine can (# 79).

In comparison to other studies, the prevalence of biofilms was high in the Albanian dairies. Data on biofilm prevalence in food processing environments are rare. This is the second study targeting biofilms in the dairy processing environments and the first analyzing biofilms in Albanian food-producing environments. Previous studies found biofilms at a prevalence of 12.7% in a frozen vegetable facility (Pracser et al., 2024), 9.3% in a meat processing facility (Wagner et al., 2020), or 17% in diverse types of food processing facilities, including two dairies from Belgium with a limited number of samples (Maes et al., 2017). One reason for the high biofilm occurrence in the Albanian dairies could be insufficient or incomplete cleaning. As we used sensitive methods for the detection of biofilm matrix components, we cannot fully exclude that carbohydrates and proteins from non-visible food residues interfered with matrix component detection, leading to false positive results. Future research in dairies is necessary to gain knowledge about baseline concentrations of proteins and carbohydrates in dairy processing environments, enabling the definition of cut-off values for biofilm detection. Biofilms on food contact surfaces, such as working tables or storage tanks, which were contaminated in the Albanian dairies, pose a potential threat for product safety, as pathogens could reside in the biofilms. Moreover, contamination of products with spoilage bacteria could occur (Voglauer et al., 2022; Zarei et al., 2023; Alves et al., 2024). In our study, all water hoses except one harbored a biofilm. Water hoses have been

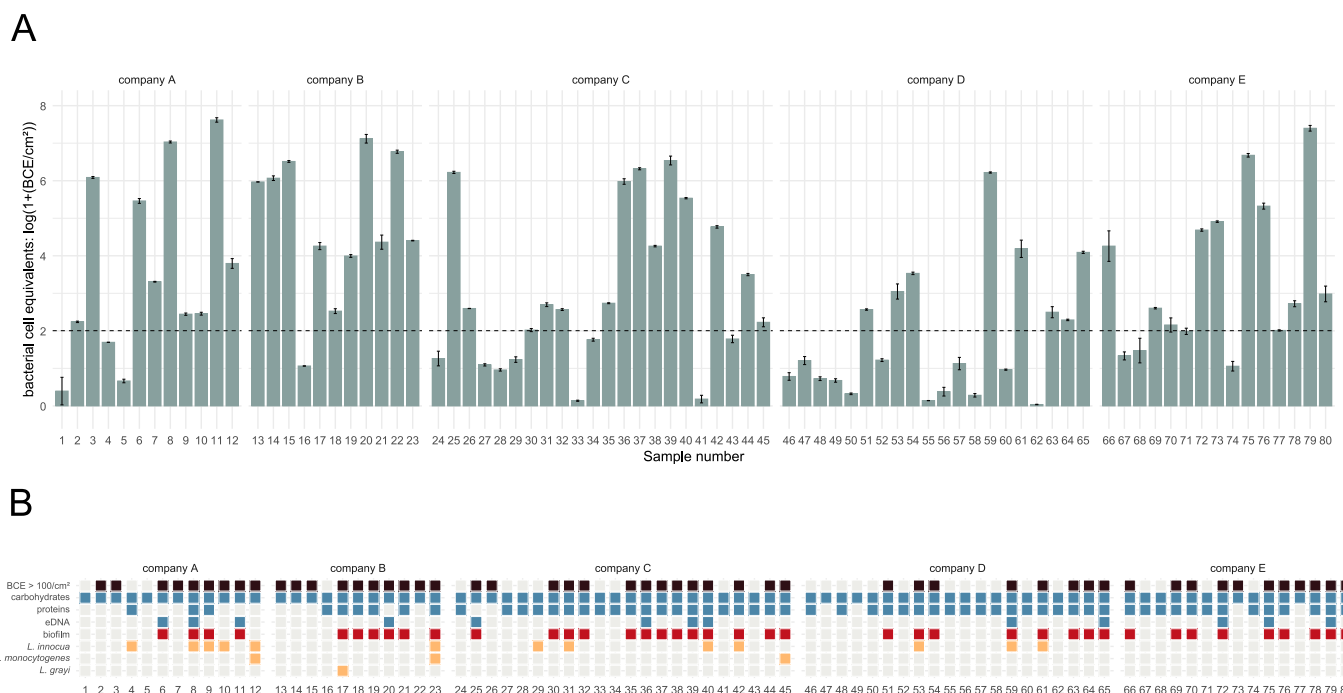


Figure 1. (A) Bacterial cell equivalents per cm². Mean values $\pm$ standard deviations are shown. A dashed line indicates the cut-off for BCE > 100/cm², which was defined as the minimum bacterial density for detection of biofilms in this study. **(B) Presence of biofilms.** The presence of biofilms (red squares) is defined as sites harboring >100 BCE/cm² (black squares) and at least two extracellular matrix components (eDNA, proteins, carbohydrates; blue squares). Overview of the presence of *L. innocua*, *L. monocytogenes*, and *L. grayi* (orange squares). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

identified as a hidden harborage site for biofilms in the food-producing environment (Liu et al., 2016; Soto-Giron et al., 2016; Voglauer et al., 2022; Moreno et al., 2024). The dispersal of biofilms in water hoses used for cleaning or directly used for food processing may contribute to the dispersal of bacteria onto the food and in the facilities.

Of note, not all biofilms are undesired. Microorganisms and biofilms on wooden tools are commonly described and even mentioned as beneficial and desirable for cheese maturing and ripening in dairy processing (Lortal et al., 2014; Gaglio et al., 2019; Ruta et al., 2025). Since no wooden boards for cheese maturing and ripening were sampled in the current study, none of the biofilms can be categorized as beneficial.

Presence of *Listeria*. The presence of *Listeria* was analyzed in 200 samples (composed of 80 biofilm and 120 additional samples). In total, we isolated 46 *Listeria* spp. isolates from 40 sites, including 38 *L. innocua*, 7 *L. monocytogenes*, and 1 *L. grayi* isolates, resulting in a prevalence of 19% for *L. innocua*, 3.5% for *L. monocytogenes*, and 0.5% for *L. grayi*. (Fig. 2A, Fig. S4).

In company A, *Listeria* spp. was detected in 26 of 50 samples (52.00%, Fig. 2A, Table S1, Table S2), of which we recovered 26 *L. innocua* and 5 *L. monocytogenes* isolates. *L. monocytogenes* was present on a plastic pallet (# 12), on a table (# S6), on the wheels of a trolley (# S11), on a cart (# S21), and on the floor near a pallet (# S22). Considering company A (as well as the other facilities) had insufficient hygiene barriers and hygiene zoning, *Listeria* on the wheels of carts and trolleys can easily be transmitted in the processing environment. Two *L. innocua* isolates were present in biofilms (# 8, 9; floor under milk tank and milk storage tank).

In company B, the *Listeria* spp. prevalence was lower (8.16%) with *Listeria* detected on four of 49 sampling points. *L. monocytogenes* was present in a biofilm on a storage tank mesh for curd (# 23), *L. innocua* was detected on the same storage tank (# 23) as well as in drains (# S61, S62), and *L. grayi* was found in a biofilm of a plastic shape for cheese packaging (# 17).

In company C, *Listeria* spp. were found on five of 30 sampling sites (16.67%). *L. monocytogenes* was present in a biofilm in a yogurt hose for filling (# 45) and *L. innocua* on the floor (# 29), in a biofilm on a plastic pallet (# 31), and in drain biofilms (# 40, 42). The presence of *L. monocytogenes* on food contact surfaces, especially at points such as hoses for filling, poses a high risk for contamination of final products.

In company D, five of 34 sampling points harbored *Listeria* spp. (14.71%, Fig. 2A, Table S1, Table S2). All isolates in company D were identified as *L. innocua* and were found on biofilms of working tables (# 53, S97), in a drain biofilm (# 59), in a biofilm on a curd table (# 61), and on a weigher (# S96). We could not detect *Listeria* spp. in company E. All negative controls were tested negative for *Listeria* spp..

Overall, we detected *Listeria* spp. in 11 of 40 biofilms (27.5%). Among the 80 biofilm sampling sites, 15 tested positive for *Listeria* spp. (18.75%), including 11 biofilms. This clearly indicates that biofilms are a reservoir for *Listeria*, potentially providing protection from environmental stress and cleaning, and contributing to persistence (Mazaheri et al., 2021; Sterniša et al., 2023; Tuytschaever et al., 2023; Zarei et al., 2023), similar to a study in a frozen vegetable processing environment (Pracser et al., 2024). In line with previous research, drains were one niche for *Listeria* (Pracser et al., 2024) and biofilms (Dzieciol et al., 2016; Wagner et al., 2020).

Characterization of *Listeria* isolates. We determined the MLST of the *L. monocytogenes* and *L. innocua* isolates (Table S1, Table S2).

The seven *L. monocytogenes* isolates were assigned to three MLSTs: ST3 ($n = 1$), ST5 ($n = 2$), and ST619 ($n = 3$, all lineage I). One isolate could not be assigned to any ST. The ST5 isolates were found on a plastic pallet (# 12) and on a table (# S6) in company A, while ST619 was found on the wheels of a trolley (# S11), on a cart (# S21), and on the floor (# S22), also in company A. The ST3 strain was isolated from a

yogurt hose used for filling (# 45) in company C and the unassigned strain from a storage tank (# 23) in company B.

In company A, 20 *L. innocua* isolates belong to ST1489 and two isolates to ST1480. No MLST could be assigned to four *L. innocua* isolates in company A. In company B, one *L. innocua* isolate was assigned to ST1489. No MLST could be assigned to the other two *L. innocua* isolates in company B. In company C, all four *L. innocua* isolates belonged to ST1085. In company D, two subtypes of *L. innocua* were detected, namely ST1085 ($n = 2$) and ST474 ($n = 3$) (Fig. S4).

The presence of dominant subtypes of *L. innocua* indicated that ST1489 is a potential in-house subtype in companies A and B, whereas ST1085 was detected only in companies C and D. Previous research identified *L. monocytogenes* ST5 as prevalent in diverse types of food processing facilities, including dairy (Maury et al., 2019). ST619 has so far only been isolated from meat products (Chen et al., 2019; Wang et al., 2021), in fishery products (Chen et al., 2018), in a vegetable dish (Chen et al., 2020), and in clinical cases in China (Lu et al., 2021). Which STs frequently occur in Albania is so far unknown, as this is the first study subtyping *Listeria* in Albania.

In addition, we analyzed the plasmid (Table S7 and S8), stress resistance (Table S9), and virulence gene (Table S10) content in the *Listeria* strains. A total of 25 *Listeria* isolates (54.35%) harbored a plasmid. We detected the plasmid pLM33 in four *L. monocytogenes* (ST5, ST3, and unknown ST) and 11 *L. innocua* (ST1489, ST1085, and unknown ST) isolates from companies A, B, and C. The plasmid harbors, among other genes, the cadmium resistance gene *cadA1* (Mullapudi et al., 2010). A recent study showed that pLM33 was crucial for resisting diverse environmental stressors in food processing environments in persistent *L. monocytogenes* ST5 isolates (Liu et al., 2025). Further, pLM1-2bUG1 was found in 10 *L. innocua* isolates (ST1489, ST1085, and unknown ST) from companies A and C.

The gene profiling of the *L. monocytogenes* isolates (ST3, ST5, ST619, and unknown ST) confirmed the presence of many stress resistance genes (Fig. 2B, Table S9). The stress survival islet SSI-1 (Ryan et al., 2010) was present in ST5, ST3 isolates, and in the unassigned ST isolate, whereas the ST619 isolates harbored *LMOF2365_0481*, a gene from the same hypervariable genetic hotspot with unknown function (Hein et al., 2011). SSI-2, involved in alkaline and oxidative stress response (Harter et al., 2017), was not detected in the five *L. monocytogenes* isolates. All *L. monocytogenes* isolates harbored *arcA* and *arcC*, and *argC*, *argD*, *argF*, *argG*, *argH*, and *argR*, which are genes involved in acid stress response (Ryan et al., 2009). In addition, osmotic stress genes *betL* (Sleator et al., 1999), *gbuA*, *gbuB*, *gbuC* (Ko & Smith, 1999), *opuCA*, *opuCB*, *opuCC*, and *opuCD* (Fraser et al., 2000) were also present in all *L. monocytogenes* isolates. Further, all *L. monocytogenes* were equipped with genes involved in heat stress – *clpP* (Nair et al., 2000), *clpX* (Balogh et al., 2017), *ctsR* (Nair et al., 2000), *dnaK* (Hanawa et al., 1999), *groEL*, *groES* (Gahan et al., 2001), *grpE* (Hanawa et al., 1999), and *hrcA* (Hanawa et al., 1999). *DnaJ*, involved in heat stress (Hanawa et al., 1999), was only detected in a ST5 isolate from company A. Genes involved in cold stress, *cspD* (Schmid et al., 2009), *oppA* (Borezee et al., 2000), and *sigL* (Raimann et al., 2009) were present in all *L. monocytogenes* isolates. Quaternary ammonium compound (QAC) resistance genes 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 *L. monocytogenes* isolates, except *mdrL* (Jiang et al., 2019) and *mepA* (Haubert et al., 2018). Further, genes associated with oxidative stress, *kat* (Rea et al., 2005), *lmo1433* (Hain et al., 2008), *lmo2460* (Chaturongakul et al., 2008), *pdhB* (Chaturongakul et al., 2008), and *perR* (Rea et al., 2005), were found in all *L. monocytogenes* isolates. Genes associated with the stressosome (*rsbR*, *rsbS*, *rsbT*, *rsbU*, *rsbV*, *rsbW*, and *rsbX*) (Chaturongakul and Boor, 2004; Ferreira et al., 2004) and genes involved in the general stress response (*lisk*, *lisR*, *lmo1470*, *lmo1601*, *lmo1602*, and *sigB*) (Cotter et al., 1999; Kazmierczak et al., 2003; Hu et al., 2007;

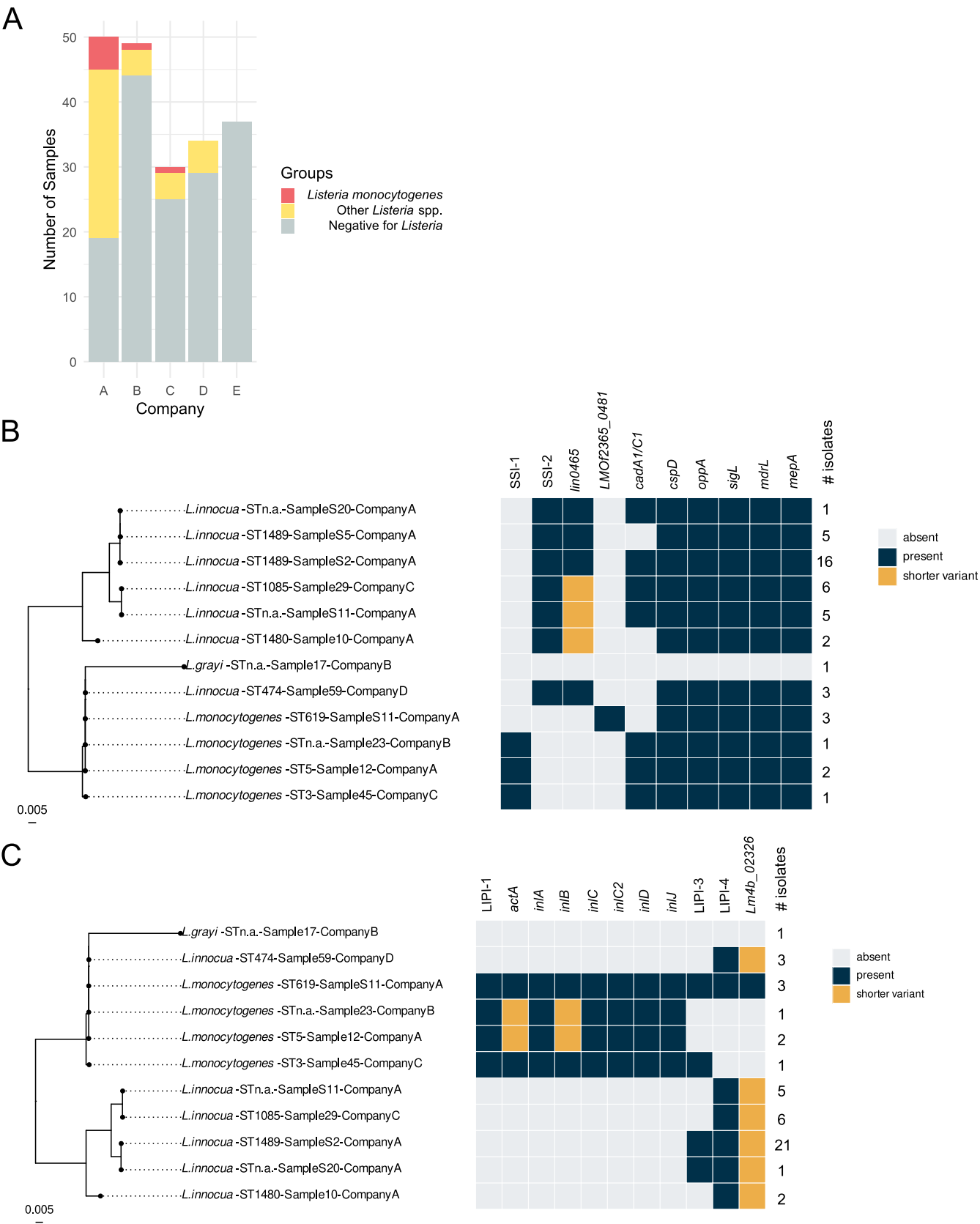


Figure 2. (A) Prevalence of *Listeria* spp.. The prevalence of *L. monocytogenes* and other *Listeria* spp. in Albanian dairies A – E. **(B) Presence of major stress resistance and (C) virulence genes.** Midpoint rooted maximum likelihood phylogenies show one representative isolate for each combination of *Listeria* species, ST, and genetic profile of selected genes. # isolates: the number of isolates from the same species/ST harboring the same genetic set.

Chaturongakul et al., 2008; Seifart Gomes et al., 2011) were detected in all *L. monocytogenes*.

We further investigated the presence of homologous stress-related genes in the *L. innocua* and *L. grayi* isolates (Table S9). SSI-2 was detected in all *L. innocua* isolates. The genomes of all *L. innocua* isolates harbored genes associated with cold stress, such as *cspD* (Schmid et al., 2009), *oppA* (Borezee et al., 2000), and *sigL* (Raimann et al., 2009). Similar to *L. monocytogenes* isolates, only the QAC resistance genes *mdrI* (Jiang et al., 2019) and *mepA* (Haubert et al., 2018) were present. Further, several genes involved in drug resistance were present in all isolates, such as *fepA* (Guérin et al., 2014), *fosX* (Fillgrove et al., 2007), *norB* (Haubert et al., 2018), *penA* (Srinivasan et al., 2005), and *tetR* (Ramos et al., 2005). *SigB*, a general stress response factor regulating the expression of many stress response genes (Kazmierczak et al., 2003), was detected in all *L. innocua* isolates. Five genes showed different length variants compared to their homologues in *L. monocytogenes*, among them *arcA*, *lmo0782*, *lmo0783*, *penA*, in all 38 *L. innocua* isolates, and *lin0465* only in 13 strains. The wide repertoire of stress resistance genes associated with various types of stressors, both in *L. monocytogenes* and *L. innocua*, likely supports the survival in the dairy processing environment where *Listeria* encounters various types of stress e.g. cold stress, salt stress, and disinfectants.

In contrast, we detected only a few hits of stress-related genes (that had been previously characterized in *L. monocytogenes*) in the single *L. grayi* isolate. We detected the heat stress-associated genes *dnaK* (Hanawa et al., 1999), *clpP* (Nair et al., 2000), and *groEL* (Gahan et al., 2001). Further, *pdhB*, involved in oxidative stress (Chaturongakul et al., 2008), *ldh*, essential in energy stress and cellular metabolism (Chaturongakul et al., 2008), and *rsbW*, which is part of the stressosome (Chaturongakul and Boor, 2004), were detected. The low number of hits reflects the higher gene variability between *L. monocytogenes* and *L. grayi* compared to *L. innocua*.

We identified major virulence genes in *L. monocytogenes* (Fig. 2C). *InlA* and *InlB*, essential in host cell invasion (Bierne et al., 2007), as well as LIPI-1, playing a major role in invasion, vacuolar escape, and intracellular survival and spread (Vázquez-Boland et al., 2001), were present in all *L. monocytogenes*. LIPI-3, contributing to virulence by hemolytic activity and microbial competition (Cotter et al., 2008), was detected in the ST3 and ST619 isolates. Additionally, LIPI-4, which promotes infection of the CNS and placenta, was present in ST619. A study from China described the presence of LIPI-3 and LIPI-4 in all isolates of ST619, underlining the potential hypervirulence of this ST (Anwar et al., 2022). The genetic background (including major virulence genes and accessory virulence genes, Table S10) of the isolates in this study suggests the full virulence potential (Bierne et al., 2007; Cotter et al., 2008; Maury et al., 2016).

The *L. innocua* isolates in the present study did not harbor any close homologs of virulence genes such as *inlA*, *inlB*, and LIPI-1. However, LIPI-3 was partly detected among *L. innocua* isolates, namely in all ST1489 and in one isolate with an unassigned ST. Moreover, LIPI-4 was detected in all *L. innocua* isolates. These results are in accordance with a previous study investigating the presence of LIPI-3 and LIPI-4 in apathogenic *L. innocua* (Lee et al., 2023). Essential virulence genes as well as LIPI-3 and LIPI-4 were not detected in the *L. grayi* isolate.

In conclusion, biofilms were highly prevalent in all five Albanian dairies with an overall frequency of 50%. *Listeria* spp., mainly *L. innocua*, was detected in four out of five companies with a prevalence ranging from 8 to 52%. The repertoire of stress-related genes in *L. monocytogenes* and *L. innocua* may contribute to survival in the dairy processing environments. *L. monocytogenes* was present on food contact surfaces such as a table and a hose for filling yogurt. We detected *Listeria* spp. in 11 out of 40 biofilms, confirming that biofilms are one factor promoting the survival of *Listeria* in the food processing environment. Analysis of the virulence gene content showed that all *L. monocytogenes* harbored the genetic repertoire for virulence or even for hypervirulence, such as in ST619. Based on the results of the study,

cleaning and disinfection, particularly using methods (enzymatic or mechanic) to target biofilms, should be intensified. The establishment of hygiene barriers or hygiene zoning could further improve hygiene.

CRedit authorship contribution statement

Nadja Pracser: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Rozeta Hasalliu:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization. **Arbnora Durmishaj:** Writing – review & editing, Investigation, Data curation. **Sarah Thalguter:** Writing – review & editing, Investigation, Data curation. **Felix Spiegel:** Writing – review & editing, Conceptualization. **Lauren V. Alteio:** Writing – review & editing, Investigation. **Kathrin Rychli:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization.

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.

Acknowledgment

This study was mainly funded by the Albania/Austria Scientific & Technological cooperation WTZ grant (project No. AL07/2022), financed by the Federal Ministry of Women, Science and Research led by the National Agency for Scientific Research and Innovation (NASRI) in Albania and OeAD in Austria.

Part of the study was funded within a research project of the Austrian Competence Centre for Feed and Food Quality, Safety and Innovation (FFoQSI). The COMET-K1 competence centre FFoQSI is funded by the Austrian federal ministries BMK, BMDW, and the Austrian provinces Lower Austria, Upper Austria, and Vienna within the scope of COMET - Competence Centers for Excellent Technologies. The program COMET is handled by the Austrian Research Promotion Agency FFG. LA and FS are currently funded by the European Union's Horizon 2020 research and innovation program under grant agreement no. 101060698.

Appendix A. Supplementary material

Supplementary material to this article can be found online at <https://doi.org/10.1016/j.jfp.2026.100757>.

References

- Alves, V. F., Tadielo, L. E., da S Pires, A. C. M., Pereira, M. G., dos Bersot, L. S., & De Martinis, E. C. P. (2024). Hidden places for foodborne bacterial pathogens and novel approaches to control biofilms in the meat industry. *Foods*, 13, 3994. <https://doi.org/10.3390/foods13243994>.
- Anwar, T. M., Pan, H., Chai, W., Ed-Dra, A., Fang, W., Li, Y., et al. (2022). Genetic diversity, virulence factors, and antimicrobial resistance of *Listeria monocytogenes* from food, livestock, and clinical samples between 2002 and 2019 in China. *International Journal of Food Microbiology*, 366, 109572. <https://doi.org/10.1016/j.ijfoodmicro.2022.109572>.
- Balogh, D., Dahmen, M., Stahl, M., Poreba, M., Gersch, M., Drag, M., et al. (2017). Insights into ClpXP proteolysis: Heterooligomerization and partial deactivation enhance chaperone affinity and substrate turnover in *Listeria monocytogenes*. *Chemical Science*, 8, 1592–1600. <https://doi.org/10.1039/c6sc03438a>.
- Bierne, H., Sabet, C., Personnic, N., & Cossart, P. (2007). Internalins: A complex family of leucine-rich repeat-containing proteins in *Listeria monocytogenes*. *Microbes and Infection*, 9, 1156–1166. <https://doi.org/10.1016/j.micinf.2007.05.003>.
- Border, P. M., Howard, J. J., Plastow, G. S., & Siggins, K. W. (1990). Detection of *Listeria* species and *Listeria monocytogenes* using polymerase chain reaction. *Letters in Applied Microbiology*, 11, 158–162. <https://doi.org/10.1111/j.1472-765X.1990.tb00149.x>.
- 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. *Infection and Immunity*, 68, 7069–7077. <https://doi.org/10.1128/IAI.68.12.7069-7077.2000>.

- Bubert, A., Hein, I., Rauch, M., Lehner, A., Yoon, B., Goebel, W., et al. (1999). Detection and differentiation of *Listeria* spp. by a single reaction based on multiplex PCR. *Applied and Environmental Microbiology*, 65, 4688–4692. <https://doi.org/10.1128/AEM.65.10.4688-4692.1999>.
- 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. *Frontiers in Microbiology*, 9, 1–18. <https://doi.org/10.3389/fmicb.2018.02700>.
- Chaturongakul, S., & Boor, K. J. (2004). RsbT and RsbV contribute to σ^B -dependent survival under environmental, energy, and intracellular stress conditions in *Listeria monocytogenes*. *Applied and Environmental Microbiology*, 70, 5349–5356. <https://doi.org/10.1128/AEM.70.9.5349-5356.2004>.
- Chaturongakul, S., Raengpradub, S., Wiedmann, M., & Boor, K. J. (2008). Modulation of stress and virulence in *Listeria monocytogenes*. *Trends in Microbiology*, 16, 388–396. <https://doi.org/10.1016/j.tim.2008.05.006>.
- Chen, Y., Chen, M., Wang, J., Wu, Q., Cheng, J., Zhang, J., et al. (2020). Heterogeneity, characteristics, and public health implications of *Listeria monocytogenes* in ready-to-eat foods and pasteurized milk in China. *Frontiers in Microbiology*, 11, 1–10. <https://doi.org/10.3389/fmicb.2020.00642>.
- Chen, M., Cheng, J., Wu, Q., Zhang, J., Chen, Y., Xue, L., et al. (2018). Occurrence, antibiotic resistance, and population diversity of *Listeria monocytogenes* isolated from fresh aquatic products in China. *Frontiers in Microbiology*, 9, 1–11. <https://doi.org/10.3389/fmicb.2018.02215>.
- Chen, M., Cheng, J., Zhang, J., Chen, Y., Zeng, H., Xue, L., et al. (2019). Isolation, potential virulence, and population diversity of *Listeria monocytogenes* from meat and meat products in China. *Frontiers in Microbiology*, 10, 1–10. <https://doi.org/10.3389/fmicb.2019.00946>.
- Chmielewski, R. A. N., & Frank, J. F. (2003). Biofilm formation and control in food processing facilities. *Comprehensive Reviews in Food Science and Food Safety*, 2, 22–32. <https://doi.org/10.1111/j.1541-4337.2003.tb00012.x>.
- Cotter, P. D., Draper, L. A., Lawton, E. M., Daly, K. M., Groeger, D. S., Casey, P. G., et al. (2008). Listeriolysin S, a novel peptide haemolysin associated with a subset of lineage I *Listeria monocytogenes*. *PLoS Pathogens*, 4, e1000144. <https://doi.org/10.1371/journal.ppat.1000144>.
- Cotter, P. D., Emerson, N., Gahan, C. G. M., & Hill, C. (1999). Identification and disruption of *lisRK*, a genetic locus encoding a two-component signal transduction system involved in stress tolerance and virulence in *Listeria monocytogenes*. *Journal of Bacteriology*, 181, 6840–6843. <https://doi.org/10.1128/jb.181.21.6840-6843.1999>.
- Danecek, P., Bonfield, J. K., Liddle, J., Marshall, J., Ohan, V., Pollard, M. O., et al. (2021). Twelve years of SAMtools and BCFtools. *GigaScience*, 10, 1–4. <https://doi.org/10.1093/gigascience/giab008>.
- Dass, S. C., & Wang, R. (2022). Biofilm through the looking glass: A microbial food safety perspective. *Pathogens*, 11. <https://doi.org/10.3390/pathogens11030346>.
- De Coster, W., & Rademakers, R. (2023). NanoPack2: Population-scale evaluation of long-read sequencing data. *Bioinformatics*, 39, 2. <https://doi.org/10.1093/bioinformatics/btad311>.
- Dixon, E., Rabanser, I., Dzieciol, M., Zwirzitz, B., Wagner, M., Mann, E., et al. (2019). Reduction potential of steam vacuum and high-pressure water treatment on microbes during beef meat processing. *Food Control*, 106, 106728. <https://doi.org/10.1016/j.foodcont.2019.106728>.
- Dzieciol, M., Schornsteiner, E., Muhterem-Uyar, M., Stessl, B., Wagner, M., & Schmitz-Esser, S. (2016). Bacterial diversity of floor drain biofilms and drain waters in a *Listeria monocytogenes* contaminated food processing environment. *International Journal of Food Microbiology*, 223, 33–40. <https://doi.org/10.1016/j.ijfoodmicro.2016.02.004>.
- EFSA (2024). The European Union One Health 2023 Zoonoses report. *EFSA Journal*, 22. <https://doi.org/10.2903/j.efsa.2024.9106>.
- 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. *Applied and Environmental Microbiology*, 76, 8231–8238. <https://doi.org/10.1128/AEM.02056-10>.
- 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. *Current Microbiology*, 48, 39–46. <https://doi.org/10.1007/s00284-003-4020-x>.
- Fillgrove, K. L., Pakhomova, S., Schaab, M. R., Newcomer, M. E., & Armstrong, R. N. (2007). Structure and mechanism of the genomically encoded fosfomycin resistance protein, FosX, from *Listeria monocytogenes*. *Biochemistry*, 46, 8110–8120. <https://doi.org/10.1021/bi700625p>.
- Flemming, H.-C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S. A., & Kjelleberg, S. (2016). Biofilms: An emergent form of bacterial life. *Nature Reviews Microbiology*, 14, 563–575. <https://doi.org/10.1038/nrmicro.2016.94>.
- Flemming, H.-C., van Hullebusch, E. D., Neu, T. R., Nielsen, P. H., Seviour, T., Stoodley, P., et al. (2023). The biofilm matrix: Multitasking in a shared space. *Nature Reviews Microbiology*, 21, 70–86. <https://doi.org/10.1038/s41579-022-00791-0>.
- Fraser, K. R., Harvie, D., Coote, P. J., & O'Byrne, C. P. (2000). Identification and characterization of an ATP binding cassette L-carnitine transporter in *Listeria monocytogenes*. *Applied and Environmental Microbiology*, 66, 4696–4704. <https://doi.org/10.1128/AEM.66.11.4696-4704.2000>.
- Gaglio, R., Cruciata, M., Scatassa, M. L., Tolone, M., Mancuso, I., Cardamone, C., et al. (2019). Influence of the early bacterial biofilms developed on vats made with seven wood types on PDO Vastedda della valle del BelUce cheese characteristics. *International Journal of Food Microbiology*, 291, 91–103. <https://doi.org/10.1016/j.ijfoodmicro.2018.11.017>.
- Gahan, C. G. M., O'Mahony, J., & Hill, C. (2001). Characterization of the *groESL* operon in *Listeria monocytogenes*: Utilization of two reporter systems (*gfp* and *hly*) for evaluating in vivo expression. *Infection and Immunity*, 69, 3924–3932. <https://doi.org/10.1128/IAI.69.6.3924-3932.2001>.
- Goetz, C., Sanschagrin, L., Jubinville, E., Jacques, M., & Jean, J. (2025). Biofilm formation in dairy: A food safety concern—recent progress in antibiofilm strategies in the dairy industry. *Journal of Dairy Science*, 108, 8157–8175. <https://doi.org/10.3168/jds.2024-25554>.
- Guérin, F., Galimand, M., Tuambilangana, F., Courvalin, P., & Cattoir, V. (2014). Overexpression of the novel MATE fluoroquinolone efflux pump *FepA* in *Listeria monocytogenes* is driven by inactivation of its local repressor *FepR*. *PLoS One*, 9. <https://doi.org/10.1371/journal.pone.0106340>.
- Gurevich, A., Saveliev, V., Vyahhi, N., & Tesler, G. (2013). QUAST: Quality assessment tool for genome assemblies. *Bioinformatics*, 29, 1072–1075. <https://doi.org/10.1093/bioinformatics/btt086>.
- Hain, T., Hossain, H., Chatterjee, S. S., MacHata, S., Volk, U., Wagner, S., et al. (2008). Temporal transcriptomic analysis of the *Listeria monocytogenes* EGD-e σ^B regulon. *BMC Microbiology*, 8, 1–12. <https://doi.org/10.1186/1471-2180-8-20>.
- Hanawa, T., Fukuda, M., Kawakami, H., Hirano, H., Kamiya, S., & Yamamoto, T. (1999). The *Listeria monocytogenes* DnaK chaperone is required for stress tolerance and efficient phagocytosis with macrophages. *Cell Stress & Chaperones*, 4, 118–128. <https://doi.org/10.1054/csac.1998.0141>.
- 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. *Applied and Environmental Microbiology*, 83. <https://doi.org/10.1128/AEM.00827-17>.
- 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. *Infection, Genetics and Evolution*, 65, 127–130. <https://doi.org/10.1016/j.meegid.2018.07.028>.
- Hein, I., Klinger, S., Doms, M., Flekna, G., Stessl, B., Leclercq, A., et al. (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. *Applied and Environmental Microbiology*, 77, 2169–2173. <https://doi.org/10.1128/AEM.02159-10>.
- Hoang, D. T., Chernomor, O., Haeseler, A. V., Minh, B. Q., & Vinh, L. S. (2017). UFBboot2: Improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution*, 35, 518–522. <https://doi.org/10.5281/zenodo.854445>.
- Hu, Y., Oliver, H. F., Raengpradub, S., Palmer, M. E., Orsi, R. H., Wiedmann, M., et al. (2017). Transcriptomic and phenotypic analyses suggest a network between the transcriptional regulators *HrcA* and σ^B in *Listeria monocytogenes*. *Applied and Environmental Microbiology*, 73, 7981–7991. <https://doi.org/10.1128/AEM.01281-07>.
- 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. *Applied Microbiology and Biotechnology*, 103, 1339–1350. <https://doi.org/10.1007/s00253-018-9551-y>.
- Kalyanamamorthy, S., Minh, B. Q., Wong, T. K. F., von Haeseler, A., & Jermini, L. S. (2017). ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nature Methods*, 14, 587–589. <https://doi.org/10.1038/nmeth.4285>.
- Kazmierczak, M. J., Mithoe, S. C., Boor, K. J., & Wiedmann, M. (2003). *Listeria monocytogenes* σ^B regulates stress response and virulence functions. *Journal of Bacteriology*, 185, 5722–5734. <https://doi.org/10.1128/JB.185.19.5722-5734.2003>.
- Ko, R., & Smith, L. T. (1999). Identification of an ATP-driven, osmoregulated glycine betaine transport system in *Listeria monocytogenes*. *Applied and Environmental Microbiology*, 65, 4040–4048. <https://doi.org/10.1128/aem.65.9.4040-4048.1999>.
- Kolmogorov, M., Yuan, J., Lin, Y., & Pevzner, P. A. (2019). Assembly of long, error-prone reads using repeat graphs. *Nature Biotechnology*, 37, 540–546. <https://doi.org/10.1038/s41587-019-0072-8>.
- Kovacevic, J., Ziegler, J., Wałęcka-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*. *Applied and Environmental Microbiology*, 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., et al. (2017). Benzalkonium tolerance genes and outcome in *Listeria monocytogenes* meningitis. *Clinical Microbiology and Infection*, 23, 265.e1–265.e7. <https://doi.org/10.1016/j.cmi.2016.12.008>.
- LaPointe, G., Wilson, T., Tarrah, A., Gagnon, M., & Roy, D. (2025). Biofilm formation in dairy: A food safety concern—microbial community tracking from dairy farm to factory: insights on biofilm management for enhanced food safety and quality. *Journal of Dairy Science*, 108, 8101–8119. <https://doi.org/10.3168/jds.2024-25397>.
- Lee, S., Parsons, C., Chen, Y., Dungan, R. S., & Kathariou, S. (2023). Contrasting genetic diversity of *Listeria* Pathogenicity Islands 3 and 4 harbored by nonpathogenic *Listeria* spp. *Applied and Environmental Microbiology*, 89, 1–16. <https://doi.org/10.1128/aem.02097-22>.
- Liu, S., Gunawan, C., Barraud, N., Rice, S. A., Harry, E. J., & Amal, R. (2016). Understanding, monitoring, and controlling biofilm growth in drinking water distribution systems. *Environmental Science & Technology*, 50, 8954–8976. <https://doi.org/10.1021/acs.est.6b00835>.
- Liu, X., Shi, T., Li, J., Wu, H., Zhao, Q., Fang, Z., et al. (2025). pLM33 provides tolerance of persistent *Listeria monocytogenes* ST5 to various stress conditions and also enhances its virulence. *Food Microbiology*, 126, 104675. <https://doi.org/10.1016/j.fm.2024.104675>.

- Lortal, S., Licitra, G., & Valence, F. (2014). Wooden tools: Reservoirs of microbial biodiversity in traditional cheesemaking. *Cheese and Microbes*, 167–176. <https://doi.org/10.1128/9781555818593.ch8>.
- Lu, B., Yang, J., Gao, C., Li, D., Cui, Y., Huang, L., et al. (2021). Listeriosis cases and genetic diversity of their *Listeria monocytogenes* isolates in China, 2008–2019. *Frontiers in Cellular and Infection Microbiology*, 11. <https://doi.org/10.3389/fcimb.2021.608352>.
- Maes, S., Heyndrickx, M., Vackier, T., Steenackers, H., Verplaetse, A., & De Reu, K. (2019). Identification and spoilage potential of the remaining dominant microbiota on food contact surfaces after cleaning and disinfection in different food industries. *Journal of Food Protection*, 82, 262–275. <https://doi.org/10.4315/0362-028X.JFP-18-226>.
- Maes, S., Huu, S. N., Heyndrickx, M., Van Weyenberg, S., Steenackers, H., Verplaetse, A., et al. (2017). Evaluation of two surface sampling methods for microbiological and chemical analyses to assess the presence of biofilms in food companies. *Journal of Food Protection*, 80, 2022–2028. <https://doi.org/10.4315/0362-028X.JFP-17-210>.
- Masuko, T., Minami, A., Iwasaki, N., Majima, T., Nishimura, S. I., & Lee, Y. C. (2005). Carbohydrate analysis by a phenol-sulfuric acid method in microplate format. *Analytical Biochemistry*, 339, 69–72. <https://doi.org/10.1016/j.ab.2004.12.001>.
- Matereke, L. T., & Okoh, A. I. (2020). *Listeria monocytogenes* virulence, antimicrobial resistance and environmental persistence: A review. *Pathogens*, 9, 1–12. <https://doi.org/10.3390/pathogens9070528>.
- Maury, M. M., Bracq-Dieye, H., Huang, L., Vales, G., Lavina, M., Thouvenot, P., et al. (2019). Hypervirulent *Listeria monocytogenes* clones' adaption to mammalian gut accounts for their association with dairy products. *Nature Communications*, 10, 2488. <https://doi.org/10.1038/s41467-019-10380-0>.
- 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. *Nature Genetics*, 48, 308–313. <https://doi.org/10.1038/ng.3501>.
- Mazaheri, T., Cervantes-Huamán, B. R. H., Bermúdez-Capdevila, M., Ripolles-Avila, C., & Rodríguez-Jerez, J. J. (2021). *Listeria monocytogenes* biofilms in the food industry: Is the current hygiene program sufficient to combat the persistence of the pathogen? *Microorganisms*, 9, 1–19. <https://doi.org/10.3390/microorganisms9010181>.
- Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D., Woodhams, M. D., Von Haeseler, A., et al. (2020). IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution*, 37, 1530–1534. <https://doi.org/10.1093/molbev/msaa015>.
- Moreno, Y., Moreno-Mesonero, L., Soler, P., Zornoza, A., & Soriano, A. (2024). Influence of drinking water biofilm microbiome on water quality: Insights from a real-scale distribution system. *The Science of the Total Environment*, 921. <https://doi.org/10.1016/j.scitotenv.2024.171086>.
- Mørseth, T., & Langsrud, S. (2004). *Listeria monocytogenes*: Biofilm formation and persistence in food-processing environments. *Biofilms*, 1, 107–121. <https://doi.org/10.1017/S1479050504001322>.
- 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*. *Nature Microbiology*, 2, 16185. <https://doi.org/10.1038/nmicrobiol.2016.185>.
- 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. *Frontiers in Microbiology*, 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. *Applied and Environmental Microbiology*, 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., et al. (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>.
- Nair, S., Derré, I., Msadek, T., Gaillot, O., & Berche, P. (2000). CtsR controls class III heat shock gene expression in the human pathogen *Listeria monocytogenes*. *Molecular Microbiology*, 35, 800–811. <https://doi.org/10.1046/j.1365-2958.2000.01752.x>.
- Pracser, N., Voglauer, E. M., Thalgueter, S., Pietzka, A., Selberherr, E., Wagner, M., et al. (2024). Exploring the occurrence of *Listeria* in biofilms and deciphering the bacterial community in a frozen vegetable producing environment. *Frontiers in Microbiology*, 15, 1–15. <https://doi.org/10.3389/fmicb.2024.1404002>.
- Raimann, E., Schmid, B., Stephan, R., & Tasara, T. (2009). The alternative sigma factor σ L of *L. monocytogenes* promotes growth under diverse environmental stresses. *Foodborne Pathogens and Disease*, 6, 583–591. <https://doi.org/10.1089/fpd.2008.0248>.
- Ramos, J. L., Martínez-Bueno, M., Molina-Henares, A. J., Terán, W., Watanabe, K., Zhang, X., et al. (2005). The TetR family of transcriptional repressors. *Microbiology and Molecular Biology Reviews*, 69, 326–356. <https://doi.org/10.1128/mmr.69.2.326-356.2005>.
- 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. *Applied and Environmental Microbiology*, 71, 8314–8322. <https://doi.org/10.1128/AEM.71.12.8314-8322.2005>.
- Rolon, M. L., Voloshchuk, O., Bartlett, K. V., LaBorde, L. F., & Kovac, J. (2024). Multi-species biofilms of environmental microbiota isolated from fruit packing facilities promoted tolerance of *Listeria monocytogenes* to benzalkonium chloride. *Biofilm*, 7, 100177. <https://doi.org/10.1016/j.biofilm.2024.100177>.
- Ruta, S., Belvedere, G., Licitra, G., Nero, L. A., Caggia, C., Randazzo, C. L., et al. (2025). Recent insights on the multifaceted roles of wooden tools in cheese-making: A review of their impacts on safety and final traits of traditional cheeses. *International Journal of Food Microbiology*, 435, 111179. <https://doi.org/10.1016/j.ijfoodmicro.2025.111179>.
- 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. *Environmental Microbiology*, 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. *Journal of Applied Microbiology*, 109, 984–995. <https://doi.org/10.1111/j.1365-2672.2010.04726.x>.
- Rychli, K., Grunert, T., Ciolacu, L., Zaiser, A., Razzazi-Fazeli, E., Schmitz-Esser, S., et al. (2016). Exoproteome analysis reveals higher abundance of proteins linked to alkaline stress in persistent *Listeria monocytogenes* strains. *International Journal of Food Microbiology*, 218, 17–26. <https://doi.org/10.1016/j.ijfoodmicro.2015.11.002>.
- Sadiq, F. A., De Reu, K., Yang, N., Burmelle, M., & Heyndrickx, M. (2024). Interspecies interactions in dairy biofilms drive community structure and response against cleaning and disinfection. *Biofilm*, 7. <https://doi.org/10.1016/j.biofilm.2024.100195>.
- 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. *Applied and Environmental Microbiology*, 75, 1621–1627. <https://doi.org/10.1128/AEM.02154-08>.
- Seemann, T. (2015). snippy: fast bacterial variant calling from NGS reads. <https://github.com/tseemann/snippy>. Available at: <https://github.com/tseemann/snippy>.
- Seemann, T. (n.d.). mlst. Available at: <https://github.com/tseemann/mlst>.
- Seifart Gomes, C., Izar, B., Pazan, F., Mohamed, W., Mraheil, M. A., Mukherjee, K., et al. (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>.
- Sharan, M., Vijay, D., Dhaka, P., Bedi, J. S., & Gill, J. P. S. (2022). Biofilms as a microbial hazard in the food industry: A scoping review. *Journal of Applied Microbiology*, 133, 2210–2234. <https://doi.org/10.1111/jam.15766>.
- Simão, F. A., Waterhouse, R. M., Ioannidis, P., Kriventseva, E. V., & Zdobnov, E. M. (2015). BUSCO: Assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics*, 31, 3210–3212. <https://doi.org/10.1093/bioinformatics/btv351>.
- Sleator, R. D., Gahan, C. G. M., Abbe, T., & Hill, C. (1999). Identification and disruption of BetL, a secondary glycine betaine transport system linked to the salt tolerance of *Listeria monocytogenes* L028. *Applied and Environmental Microbiology*, 65, 2078–2083. <https://doi.org/10.1128/AEM.65.5.2078-2083.1999>.
- Soto-Giron, M. J., Rodríguez-R, L. M., Luo, C., Elk, M., Ryu, H., Hoelle, J., et al. (2016). Biofilms on hospital shower hoses: Characterization and implications for nosocomial infections. *Applied and Environmental Microbiology*, 82, 2872–2883. <https://doi.org/10.1128/AEM.03529-15>.
- Srinivasan, V., Nam, H. M., Nguyen, L. T., Tamilselvam, B., Murinda, S. E., & Oliver, S. P. (2005). Prevalence of antimicrobial resistance genes in *Listeria monocytogenes* isolated from dairy farms. *Foodborne Pathogens and Disease*, 2, 201–211. <https://doi.org/10.1089/fpd.2005.2.201>.
- Sterniša, M., Gradišar Centa, U., Drnovšek, A., Remškar, M., & Smole Možina, S. (2023). *Pseudomonas fragi* biofilm on stainless steel (at low temperatures) affects the survival of *Campylobacter jejuni* and *Listeria monocytogenes* and their control by a polymer molybdenum oxide nanocomposite coating. *International Journal of Food Microbiology*, 394. <https://doi.org/10.1016/j.ijfoodmicro.2023.110159>.
- Stoddard, S. F., Smith, B. J., Hein, R., Roller, B. R. K., & Schmidt, T. M. (2015). rrnDB: Improved tools for interpreting rRNA gene abundance in bacteria and archaea and a new foundation for future development. *Nucleic Acids Research*, 43, D593–D598. <https://doi.org/10.1093/nar/gku1201>.
- Tuystchaever, T., Raes, K., & Samperis, I. (2023). *Listeria monocytogenes* in food businesses: From persistence strategies to intervention/prevention strategies—A review. *Comprehensive Reviews in Food Science and Food Safety*, 22, 3910–3950. <https://doi.org/10.1111/1541-4337.13219>.
- Vázquez-Boland, J. A., Domínguez-Bernal, G., González-Zorn, B., Kreft, J., & Goebel, W. (2001). Pathogenicity islands and virulence evolution in *Listeria*. *Microbes and Infection*, 3, 571–584. [https://doi.org/10.1016/S1286-4579\(01\)01413-7](https://doi.org/10.1016/S1286-4579(01)01413-7).
- Větrovský, T., & Baldrian, P. (2013). The variability of the 16S rRNA gene in bacterial genomes and its consequences for bacterial community analyses. *PLoS One*, 8, 1–10. <https://doi.org/10.1371/journal.pone.0057923>.
- Voglauer, E. M., Zwirzitz, B., Thalgueter, S., Selberherr, E., Wagner, M., & Rychli, K. (2022). Biofilms in water hoses of a meat processing environment harbor complex microbial communities. *Frontiers in Microbiology*, 13. <https://doi.org/10.3389/fmicb.2022.832213>.
- Wagner, E., Fagerlund, A., Thalgueter, S., Jensen, M. R., Heir, E., Mørseth, T., et al. (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. *International Journal of Food Microbiology*, 383, 109962. <https://doi.org/10.1016/j.ijfoodmicro.2022.109962>.
- Wagner, E. M., Pracser, N., Thalgueter, S., Fischel, K., Rammer, N., Pospíšilová, L., et al. (2020). Identification of biofilm hotspots in a meat processing environment: Detection of spoilage bacteria in multi-species biofilms. *International Journal of Food Microbiology*, 328, 108668. <https://doi.org/10.1016/j.ijfoodmicro.2020.108668>.
- Walsh, P. S., Metzger, D. A., & Higuchi, R. (1991). Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *BioTechniques*, 10, 506–513.
- Wang, Y., Ji, Q., Li, S., & Liu, M. (2021). Prevalence and genetic diversity of *Listeria monocytogenes* isolated from retail pork in Wuhan, China. *Front Microbiol*, 12, 1–14. <https://doi.org/10.3389/fmicb.2021.620482>.

- Wickham, H. (2016). ggplot2: Elegant graphics for data analysis. Available at: <https://ggplot2.tidyverse.org>.
- 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 in Ecology and Evolution*, 8, 28–36. <https://doi.org/10.1111/2041-210X.12628>.
- Zarei, M., Rahimi, S., Fazlara, A., & Anvari, S. E. (2023). High biofilm-forming *Pseudomonas* strains isolated from poultry slaughterhouse surfaces: Their importance in the persistence of *Salmonella enteritidis* in slaughterhouses. *International Journal of Food Microbiology*, 390, 110126. <https://doi.org/10.1016/j.ijfoodmicro.2023.110126>.
- Zetzmann, M., Okshevsky, M., Endres, J., Sedlag, A., Caccia, N., Auchter, M., et al. (2015). DNase-sensitive and -resistant modes of biofilm formation by *Listeria monocytogenes*. *Frontiers in Microbiology*, 6, 1–11. <https://doi.org/10.3389/fmicb.2015.01428>.