

Colonization of Native Dairy Facility Biofilms by Persistent *Listeria monocytogenes* Strains

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Abstract

Listeria (L.) monocytogenes, the causative agent of listeriosis, can persist in food processing environments for months to years and poses a significant food safety risk. Persistence is often linked to integration into multispecies biofilms, yet the influence of community composition and timing of *L. monocytogenes* integration remain poorly understood. This study investigates the integration of a long-term persistent *L. monocytogenes* strain into multispecies biofilms derived from two sites in an Austrian dairy facility: one where *L. monocytogenes* was detected, and one where it was absent. Biofilms were cultivated on stainless steel slides under laboratory conditions and *L. monocytogenes* was introduced at different stages of biofilm development. After 24 h and 48 h, biofilms were analysed using qPCR targeting the *prfA* gene and the bacterial 16S rRNA gene. Community composition was characterized using full-length 16S rRNA gene sequencing. Both communities formed reproducible multispecies biofilms and were successfully colonized by *L. monocytogenes* regardless of inoculation timing. Community composition differed markedly between the biofilms. Community background, sequential inoculation and timing influenced community development. Several taxa were associated with *L. monocytogenes* integration, with *Serratia liquefaciens* showing co-occurrence and *Lactococcus lactis* displaying patterns consistent with competitive exclusion. These findings demonstrate that *L. monocytogenes* can integrate into diverse multispecies biofilms and highlight specific microbial taxa that may influence pathogen persistence. The results provide potential targets for reducing *L. monocytogenes* persistence and contamination in dairy processing environments.

Importance

Listeria monocytogenes is a foodborne pathogen that can persist for years in food processing environments, despite cleaning and disinfection, and repeatedly contaminates products. Most research on persistence focuses on the genetics of the bacterium itself. However, integration into multispecies biofilms may support long-term persistence, and how the microbial community of these biofilms affects this integration is poorly understood. This study shows that a strain that persisted for three years in an Austrian dairy facility integrated into two different biofilm communities taken from the same facility. The resident community mattered more than timing of introduction in shaping biofilm composition. Several bacteria emerged as possible promoters or inhibitors of pathogen integration. These findings suggest that monitoring based only on bacterial genomes may miss important factors, and that considering the surrounding microbial community could improve strategies to control this pathogen in food production.

1. Introduction

Listeria (L.) monocytogenes is a foodborne pathogen and causative agent of listeriosis, a potentially severe infection particularly affecting pregnant women, neonates, older adults, and immunocompromised individuals (1). Currently, listeriosis remains a major challenge for food industry

(2) and one of the most serious foodborne illnesses under EU surveillance (3). *L. monocytogenes* is an opportunistic pathogen (4, 5) and is widely distributed in natural, agricultural, and urban environments, reflecting its strong adaptation to diverse ecological niches. It exhibits resilience to environmental stressors (6–8) and can, once introduced into food processing environments, persist for months or years despite routine cleaning and disinfection procedures (9). These strains defined as “persistent” represent a continuous source of product contamination (10, 11) and have been associated with listeriosis outbreaks (12, 13).

Persistence of *L. monocytogenes* in food processing environments has been associated with several genetic determinants, including the substantial accessory genome of *L. monocytogenes* (14), the exchange of mobile genetic elements harboring resistance genes (11, 15, 16) and the presence or absence of environmental resistance genes in the genome (17, 18). Disinfectant resistance genes, such as benzalkonium chloride efflux pumps (*bcrABC*, *emrE*, *mdrL*) have been located on mobile genetic elements such as plasmids (19–22), making them candidates for horizontal gene transfer. Other stress resistance determinants are chromosomally encoded, including dedicated gene clusters such as *Listeria* Genomic Islands (LGI) and Stress Survival Islets (SSI), as well as individual genes mediating osmotic, cold, heat, acid, and oxidative stress responses (23–25).

L. monocytogenes can adhere to surfaces (e.g., stainless steel), form biofilms (26, 27), and integrate into established biofilms formed by co-occurring bacteria (26, 28, 29). In addition to harboring environmental stress tolerance genes, this likely contributes to its long-term persistence in food-processing environments. Biofilms provide structural and physiological protection against environmental stressors, such as cleaning and disinfection procedures, as well as changes in salinity and temperature (30, 31), which can occur in food processing facilities (27, 28). In addition, biofilms serve as a hotspot for interactions via intra- or interspecies horizontal gene transfer (32, 33). Through this process, stress tolerance genes can be exchanged between the same or different bacterial species and new resistances can be acquired that potentially support the persistence phenotype and the evolution of persistence associated communities (31–33).

Moreover, several studies have shown that *L. monocytogenes* can integrate into established multispecies biofilms (26), potentially benefiting from the protective matrix and interspecies interactions. Ultimately, if not effectively eliminated, biofilms can serve as sources of continual contamination of the environment, equipment, and potentially the finished products (34). As biofilms reduce efficacy of cleaning and disinfection regimes (29, 35), understanding the mechanisms that govern colonization and integration of *L. monocytogenes* into these complex microbial communities is therefore essential for the development of effective biofilm-targeted control strategies in food production systems.

Integration of *L. monocytogenes* into multispecies biofilms can occur without significantly altering community composition and overall gene expression of the resident species (26). However, broader microbial ecology studies demonstrate that specific interspecies interactions can substantially influence population dynamics, community structure and stability (36, 37). These community dynamics have also

been associated with shifts in biofilm matrix composition (38), highlighting the importance of interspecies dynamics in shaping both structural and functional properties in multispecies biofilms.

Previous studies have identified distinct microbial taxa that are consistently associated with the presence or absence of *L. monocytogenes* in food processing environments. Taxa like *Veillonella spp.*, *Pseudomonas spp.*, and other *Proteobacteria* have been reported to co-occur with *L. monocytogenes* in food processing environments (4, 39–41). In contrast, taxa negatively associated with *L. monocytogenes* in food processing environments include lactic acid bacteria, such as *Enterococcus faecium* and *Enterococcus lactis*, as well as other members of *Firmicutes* and *Enterobacteriaceae*, indicating potential competitive or inhibitory relationships (29, 42). Specific taxa such as *Ralstonia insidiosa* have been shown to facilitate integration of foodborne pathogens into multispecies biofilms (31, 43–45), suggesting that certain community members may play an important role in shaping colonization outcomes. Environmental factors such as facility design, temperature, nutrient availability, and sanitation practices further modulate microbial community assembly and persistence, with seasonal and facility-specific differences influencing microbiota composition (4, 27, 39, 46).

In this study, we aim to address these gaps by investigating the integration of a long-term persistent *L. monocytogenes* strain, which was repeatedly isolated from an Austrian dairy processing facility over a three-year period and harbored the biofilm-associated genetic determinants *vip*, *srnB*, *cycB*, and *uvrB* (47), into two multispecies biofilm communities derived from the same facility. We hypothesize that (I) *L. monocytogenes* will successfully integrate into two different pre-established multispecies biofilm communities, (II) the order of introduction to the multispecies biofilm communities influences the colonization success (total *prfA* copy numbers), (III) the integration and colonization outcomes will differ between the two multispecies biofilm communities, and (IV) specific taxa can be identified as being associated with the co-occurrence or co-exclusion of *L. monocytogenes*.

2. Results

Highly Related Persistent Isolates Harbor Biofilm-Associated Genetic Traits

To investigate the long-term persistence of the *L. monocytogenes* strain used in this study, genomes of 40 lineage I isolates belonging to ST 4 and core cgMLST 4605, sampled in a single dairy processing facility between 2020 and 2023, were analysed using the CFSAN SNP pipeline (Fig. 2A). The dataset comprised three isolates recovered during sampling in this study, supplemented with 37 historical isolates. Pairwise SNP distance analysis revealed a high degree of genomic similarity among these isolates. The pairwise SNP differences ranged from 0 to 8 SNPs across the whole genome. Several closely related subclusters were identified, including the isolate used in this study (MRL-23-01322; highlighted in red).

To further characterise the biofilm formation potential at the gene level, all isolates were screened against a custom biofilm-associated gene database based on biomarkers previously proposed by (47), including the genes *vip*, *srnB*, *cycB*, and *uvrB* (Fig. 2B). All isolates, including the isolate used in this

study (MRL-23-01322; highlighted in red), lacked the *vip* gene while carrying *srnB*, *cycB*, and *uvrB*. Phenotypically, MRL-23-01322 was characterised as a moderate biofilm former in RPMI medium (low-nutrient conditions), but only a weak biofilm former in 1:10 BHI and 1:10 TSB under monoculture conditions (Table S2). Analysis of environmental stress tolerance genes revealed minor differences in multidrug efflux, copper resistance and general stress response, as well as antimicrobial resistances and harbouring plasmids (Figure S1). The high degree of similarity across the gene profiles indicates conservation of these categories across the genomes.

qPCR Reveals Successful Colonization of *L. monocytogenes* in *Listeria* Positive and *Listeria* Negative Community

Building on the strain-level characterization above, it was next investigated how this strain (MRL-23-01322; highlighted in red) behaves within a biofilm context by sequentially introducing the pathogen to the *Listeria* positive and *Listeria* negative community and detect biofilm formation via safranin staining. All slides were safranin positive (see Supplementary File_2) and therefore harboured surface-attached communities. The successful colonization of *L. monocytogenes* across both native communities and all sequential inoculation conditions was demonstrated by qPCR (Fig. 3). The quantification of the 16S rRNA gene copy numbers revealed about a 10-fold increase in bacterial load from 24 h to 48 h in both communities (C.A and C.B, Fig. 3A). This trend can be observed in the sequential inoculation treatments where the community (*Com_Start*) or *L. monocytogenes* (*Lm_Start*) was inoculated first, as well as the simultaneous inoculation (*LmCom_Start*; Fig. 3A). Notably, when *L. monocytogenes* was inoculated first (*Lm_Start*) the biofilm exhibited the highest variability among all three biological replicates in 16S rRNA gene copy numbers, indicated by the larger standard deviations. After introduction of the communities, the variability decreased. This trend was also observed across all treatments containing the community. Furthermore, the average 16S rRNA gene copy numbers from the three technical replicates in the sequential inoculation treatments were at or above the overall bacterial load in the swab sample (used as a reference with 10^6 copy numbers), ranging from 10^6 to 10^8 copy numbers.

Quantification of the conserved *prfA* gene, used as a marker for *L. monocytogenes*, demonstrated successful integration of the pathogen into both communities across all sequential inoculation treatments (Fig. 3B). Particularly, the *Listeria* positive community, was negative for *prfA* or below the detection limit in the original swab sample. After the enrichment procedure for 24 h and 48 h, however, *L. monocytogenes* became detectable with *prfA* copy numbers ranging from approximately 10^2 to 10^4 . The sequential inoculation treatments showed substantially higher *prfA* copy numbers ranging from 10^5 to 10^8 .

Within the *Listeria* positive community, the sequential inoculation treatment where *L. monocytogenes* was introduced first (*Lm_Start*) showed a slight reduction of *prfA* copy numbers at 48 h compared to 24 h following the community inoculation. This decrease was not observed in the *Listeria* negative community. Similarly, in the simultaneous inoculation, *Listeria* positive community displayed an increase in *prfA* abundance between 24 h and 48 h. The *Listeria* negative community showed relatively stable

copy number across both timepoints. When the community was introduced first (*Com_Start*), *prfA* copy numbers reached levels after 48 h that were comparable to those after 48 h in the simultaneous inoculation (*LmCom_Start*).

Native Community Structure Dominates Beta Diversity Despite Effects of Sequential Inoculation, Timepoint and *L. monocytogenes* Presence

The effects of community background, sequential inoculations and timepoint on beta diversity were assessed using PERMANOVA models, while controlling for the remaining variables. For downstream analysis, the original swab samples and the *Lm_Start* samples at timepoint 24 h were excluded to avoid biasing the analysis. The swab samples represent a planktonic community rather than a surface-attached community and the *Lm_Start* 24 h samples consisted exclusively of an *L. monocytogenes* monoculture without background community.

Overall, the community type, i.e. community the biofilms derived from, explained the largest proportion of variation in community composition across the complete dataset (PERMANOVA: $R^2 = 0.508$, $F = 70.26$, $p = 0.0001$; Figure S2A and B). In addition to the main effects, significant interactions between community type and order of sequential inoculation ($R^2 = 0.040$, $F = 3.24$, $p = 0.004$), community type and harvest timepoint ($R^2 = 0.036$, $F = 8.75$, $p = 0.0005$), and sequential inoculation and harvest timepoint ($R^2 = 0.034$, $F = 8.25$, $p = 0.0008$) were observed. Dispersion homogeneity was confirmed for community background and timepoint (PERMDISP, $p > 0.05$), whereas sequential inoculation showed slight differences in dispersion ($p = 0.022$).

Because community background showed the strongest effect, the following analyses were performed for communities separately. In the *Listeria* positive community, both order of inoculation and timepoint significantly affected beta diversity (PERMANOVA: sequential inoculation $R^2 = 0.591$, $F = 10.72$, $p = 0.0001$; timepoint $R^2 = 0.370$, $F = 20.11$, $p = 0.0001$; Fig. 4A). The samples clustered according to inoculation order and harvest timepoint, particularly in *Com_Start* and *LmCom_Start* at 24 h and 48 h. One overlap was observed with *Lm_Start* and *Com_Start*. The homogeneity of beta dispersion was maintained for inoculation order and harvest timepoint (PERMDISP: $p = 0.062$ and $p = 0.077$, respectively). In the *Listeria* negative community the alternative community, order of inoculation and harvest timepoint also significantly influenced community composition (PERMANOVA: sequential inoculation $R^2 = 0.428$, $F = 5.96$, $p = 0.0005$; timepoint $R^2 = 0.242$, $F = 10.13$, $p = 0.0005$; Fig. 4B). Both microbial communities in the treatments *LmCom_Start* and *Com_Start* separated between timepoint 24 h and 48 h. In the *Listeria* negative community, a partial overlap between *Com_Alone* and *Com_Start* samples was observed. Beta dispersion was stable like in the other community (PERMDISP, $p > 0.05$).

Furthermore, the effect of *L. monocytogenes* presence on beta diversity was evaluated using a model controlling for community background, sequential inoculation and timepoint. Because homogeneity of beta dispersion was not given in *Listeria* positive community (PERMDISP: $p = 0.031$), the analysis was performed across the complete dataset. Across both communities, *L. monocytogenes* presence had a statistically significant but relatively small effect on beta diversity compared to the other factors

(PERMANOVA: $R^2 = 0.028$, $F = 4.32$, $p = 0.009$; Figure S2B). Although samples primarily clustered according to community background and timepoint, a secondary separation associated with *L. monocytogenes* presence was also observed.

Differences in Alpha Diversity Between Communities Under Co-incubation and Sequential Inoculation Conditions

To assess whether the presence of *L. monocytogenes* was associated with changes in within-sample microbial diversity, alpha diversity was compared between samples with and without *L. monocytogenes*. Across the full dataset, Shannon and Simpson diversity indices were significantly lower in samples where *L. monocytogenes* was present (Shannon: $p = 0.0009$; Simpson: $p = 0.0010$; Figure S3A). When analysed separately at the community level, significant differences in diversity indices were detected within the *Listeria* negative community (Shannon: $p < 0.0001$; Simpson: $p < 0.001$). In contrast the same trend was not statistically significant within the *Listeria* positive community (both $p > 0.05$). This may be due to the limited number of samples in the *L. monocytogenes*-absent category within this community ($n = 3$) and the resulting variability among samples. Overall, comparison of both baseline swab communities revealed higher alpha diversity in the *Listeria* positive community compared to the *Listeria* negative community (Fig. 5A–B). Furthermore, the diversity in the multispecies biofilms incubated for 48 h appeared more stable in the *Listeria* negative community than in the *Listeria* positive community when comparing *Swab* with *Com_Alone*. In the sequential inoculations, alpha diversity significantly reduced in both communities when *L. monocytogenes* was co-incubated with the communities from the start for 48 h (all $p < 0.01$). Analysis after removing *L. monocytogenes* reads and recalculating alpha diversity showed a similarly significant reduction in alpha diversity (Figure S4A–B).

In the setups, where the community received a 24 h head start prior to *L. monocytogenes* inoculation (*Com_Start*; Fig. 5C – D), contrasting dynamics were observed between the two communities. In the *Listeria* positive community, alpha diversity increased from 24 h to 48 h (Shannon: $p = 0.029$; Simpson: $p = 0.085$), whereas in the *Listeria* negative community, it decreased over time (Shannon: $p = 0.014$; Simpson: $p = 0.022$). Following the removal of *L. monocytogenes* reads (Figure S4C–D), the same directional trends appeared with both indexes reaching significance in the *Listeria* positive community.

Simultaneous inoculation of the communities and *L. monocytogenes* (*LmCom_Start*; Fig. 5E–F) exhibited opposing responses in alpha diversity. The *Listeria* positive community showed a reduction in alpha diversity, from 24 h to 48 h (Shannon: $p = 0.29$; Simpson: $p = 0.027$), whereas the *Listeria* negative community showed significant increases for both indices (both $p < 0.001$). After removal of *L. monocytogenes* reads (Figure S4E–F), the decrease in the *Listeria* positive community was significant for both indices, while the increases observed in the *Listeria* negative community were no longer significant.

Community Composition of the Top 25 Taxa Remained Stable Across Experimental Conditions, Whereas *Listeria monocytogenes* Increased After Introduction

The community composition of the top 25 taxa in absolute abundance (log₁₀ 16S rRNA gene copies) was assessed for both communities across all sequential inoculation conditions (Fig. 6). The technical replicates showed a high similarity in community composition and were therefore summarised as means (Figure S5). The two communities derived from the original swab showed markedly different compositions. Only *Hafnia-Obesumbacterium paralvei* and individual Enterobacteriaceae ASVs were shared among the dominant taxa of both communities. Most highly abundant taxa were community-specific, confirming that the *Listeria* positive and *Listeria* negative community represent distinct microbial communities. The *Listeria* positive community harboured a greater number of highly abundant taxa compared to the *Listeria* negative community (Fig. 6), consistent with the higher alpha diversity observed in the *Listeria* positive community (Fig. 5A-B).

In the *Listeria* positive community (Fig. 6A), the dominant taxa in the swab were *Hafnia-Obesumbacterium alvei*, *Serratia liquefaciens*, *Kluyvera intermedia*, *Acinetobacter johnsonii*, *Citrobacter werkmanii*, *Stenotrophomonas maltophilia*, and *Hafnia-Obesumbacterium paralvei*. During the enrichments and sequential inoculations, *Hafnia-Obesumbacterium alvei*, *Serratia liquefaciens*, and *Kluyvera intermedia* showed a stable absolute abundance across conditions, while *Pseudomonas fragi*, *Acinetobacter johnsonii*, *Serratia marcescens*, and *Lactococcus lactis* increased in absolute abundance. The overall taxonomic profile remained relatively stable across sequential inoculation conditions, suggesting no major compositional shifts during enrichments.

In the *Listeria* negative community (Fig. 6B), the dominant taxa in the swab were *Citrobacter werkmanii*, *Morganella morganii*, *Lelliottia jeotgali*, *Raoultella* spp., *Lactiplantibacillus* spp., *Staphylococcus xylosus*, and Enterobacteriaceae ASVs. *Citrobacter werkmanii*, *Morganella morganii*, and *Lelliottia jeotgali* maintained stable abundances during enrichment, while *Lactiplantibacillus* spp., *Staphylococcus xylosus*, and several Enterobacteriaceae ASVs decreased substantially. In contrast, *Raoultella* spp., *Lelliottia* spp., and *Hafnia-Obesumbacterium paralvei* increased with enrichment. In comparison with the *Listeria* positive community, the *Listeria* negative community showed a more pronounced compositional shift during enrichments, with some swab-dominant taxa declining in abundance.

Overall, *L. monocytogenes* abundance increased over time in both communities in all experimental setups. In *Com_Alone*, an increase in absolute abundance of *L. monocytogenes* was observed in the *Listeria* positive community, where it was present from the beginning, whereas it remained absent in the *Listeria* negative community, where no inoculation occurred. In the sequential inoculation *Lm_Start*, a strong establishment was observed after 48 h, even with the inoculation of the community at 24 h. In *Com_Start*, the addition of *L. monocytogenes* after 24 h also resulted in a strong establishment with 48 h. In *LmCom_Start* the simultaneous inoculation also led to an increase in absolute abundance from 24 h to 48 h in both communities.

Listeria Negative Communities Better Preserve Taxonomic Evenness During Co-incubation with *L. monocytogenes*

Across the sequential inoculations (*LmCom_Start* and *Com_Start*) of *L. monocytogenes* with the *Listeria* negative and the *Listeria* positive community, *L. monocytogenes* increased in abundance between 24 h and 48 h and constituted a substantial proportion of total 16S rRNA gene copy numbers (Fig. 7A and B). In the *Listeria* positive community *L. monocytogenes* reached higher absolute abundance compared to the *Listeria* negative community.

Comparison of the most abundant taxa in both the *Listeria* positive and *Listeria* negative communities revealed a distinct difference in community structure. In the *Listeria* positive community, *Serratia liquefaciens* was the most abundant co-occurring taxon, while *Lactococcus lactis*, *Pseudomonas fragi*, and *Kluyvera intermedia* occurred at substantially lower abundance. Surprisingly, *Hafnia-Obesumbacterium alvei* was among the four most abundant taxa under simultaneous inoculation of *L. monocytogenes* and the *Listeria* positive community (*LmCom_Start*). Under the sequential inoculation *Com_Start*, *Lactococcus lactis* ranked among the four most abundant taxa, while *Hafnia-Obesumbacterium alvei* was no longer among them. In the *Listeria* negative community, a more even distribution among the dominant taxa was observed in both sequential inoculations. *Raoultella* spp. and *Lelliottia* spp., each represented by multiple highly abundant ASVs lacking species level assignment, occurred at abundances comparable to *Citrobacter werkmanii*. *Morganella morganii* was present at markedly lower abundance.

A more detailed inspection of the *Listeria* positive community composition (Fig. 7C) showed that *Serratia liquefaciens* absolute abundance remained relatively stable between 24 h and 48 h in the sequential inoculation (*LmCom_Start*), where *L. monocytogenes* and the community were co-inoculated. When the community was given a head start inoculation (*Com_Start*), *Serratia liquefaciens* increased in absolute abundance over time. In *LmCom_Start*, *Pseudomonas fragi*, *Hafnia-Obesumbacterium alvei* and *Kluyvera intermedia* increased in absolute abundance between 24 h and 48 h. A similar pattern was observed under *Com_Start* for *Pseudomonas fragi* and *Kluyvera intermedia*. In the *Listeria* negative community (Fig. 7D), a similar pattern was observed between the sequential inoculations, where *Raoultella* spp., *Lelliottia* spp., *Citrobacter werkmanii*, and *Morganella morganii* increased between timepoints 24 h and 48 h.

Differential abundance analysis (Fig. 8) identified key taxa that show log fold changes when *L. monocytogenes* was inoculated across the whole dataset. Strongest negative log-fold changes were shown by *Pseudomonas poae*, followed by *Citrobacter werkmanii* and the family of Enterobacteriaceae where *L. monocytogenes* was absent. When *L. monocytogenes* was present, only for *Acinetobacter johnsonii* a positive log-fold change was observed. Besides the family of Enterobacteriaceae, which was found in the top 25 taxa of both communities, *Pseudomonas poae* and *Citrobacter werkmanii* were only found in the top 25 taxa in the *Listeria* negative community, whereas *Acinetobacter johnsonii* was only found in top 25 of the *Listeria* positive community.

3. Discussion

This study aimed to integrate the genomic analysis of a long-term persistent strain with community level biofilm ecology. Previous work has associated and observed genotypic traits with enhanced biofilm formation potential (47). However, the genomic analysis alone cannot predict how these strains behave in multispecies biofilm communities. The long-term persistent *L. monocytogenes* strain (Lineage I, CC4, ST4, cgMLST 4605) used in this study did carry biofilm-associated genetic signatures but was also capable of successfully integrating in complex multispecies biofilms. This highlights the need to integrate ecological interactions alongside genomic investigations to fully understand the persistence phenomenon of *L. monocytogenes*, as it can be viewed as a result from both strain-specific adaptations and community-level processes.

The genomic characterization confirmed that the isolate met the operational definition previously used for long-term persistence (48, 49). Whole-genome sequencing showed an overall high genetic similarity across the 40 isolates recovered from the Austrian dairy facility, 0–8 pairwise SNPs across three years (2020–2023) (Fig. 2). The conserved genomes carried numerous stress-resistance determinants (Figure S1), supporting long-term establishment under facility-specific selective pressures.

Moreover, the persistent phenotype is potentially supported by the strain's predicted biofilm-associated genotype. The genetic profile described by Wang et al. (47), including the presence of *srnB*, *cycB* and *uvrB* and absence of the *vip*, was described to be associated with enhanced biofilm formation and was harboured by all isolates collected from this facility (Fig. 2), including the one used in this study. It must be noted that this strain was characterised as a moderate biofilm former, the behaviour observed in this study also consistent with a multispecies biofilm adapted phenotype.

qPCR of *prfA* showed that *L. monocytogenes* integrated into both multispecies biofilm communities, regardless of community background and sequential inoculation order, supporting hypothesis I. This observation is consistent with previous studies showing that *L. monocytogenes* can integrate into multispecies biofilms (26, 28, 29). While these studies used defined multispecies communities, the findings of this study broaden these observations to naturally occurring microbial communities from dairy-food processing environments. This finding is particularly relevant from a food safety perspective, as it suggests that once introduced into a facility, *L. monocytogenes* might exploit existing biofilm structures and gain protection from environmental stressors and sanitation procedures supporting persistence (31, 32).

Successful establishment in a multispecies biofilm alone might not be sufficient to explain *L. monocytogenes* persistence. Interactions with surrounding microbiota in mature biofilms, such as horizontal gene transfer with adaptive traits like resistance genes (11, 15, 33), may therefore modulate the pathogen's abundance and survival (30–32). Plasmids in food processing environments are known to carry adaptive traits like benzalkonium chloride resistances (e.g, *bcrABC* cassette or *qacH*) and are frequently encountered in food processing environments (40, 50, 51).

Overall, beta diversity analyses showed that the samples primarily clustered according to community background. This indicates that the resident microbial community from the initial Swab sample was the

dominant factor shaping community composition. Community, sequential inoculation and harvesting timepoint all contributed to differences in community composition, which the clearest separation observed between 24 and 48 h in the sequential inoculation treatments *Com_Start* and *LmCom_Start*. Similar concepts have been proposed for biofilms in food processing environments, where specific taxa shape the biofilm community through interspecies interactions (4, 40). The order of introduction also influenced community composition, although its effect was smaller than that of the initial community. In contrast, colonization success (*prfA* copy numbers) was not clearly affected by inoculation order, so hypothesis II was only partly supported.

Despite the successful integration of *L. monocytogenes* into both communities, the community responses were different. The *Listeria* positive community showed a stronger effect of sequential inoculation on community composition, reflected by the higher variance in beta diversity explained by sequential inoculation and timepoint compared to the *Listeria* negative community (Fig. 4). This difference was also reflected on the alpha diversity analysis, although the direction of the response was more dependent on the inoculation condition. After the exclusion of *L. monocytogenes* reads prior to diversity calculation, alpha diversity increased in the *Listeria* positive community under *Com_Start*, whereas the opposite pattern was observed under *LmCom_Start* (Figs. 5, S3). Similar effects have been reported where specific members can alter the community structure through competitive interactions (36, 37). Whether this pattern results from the influence of a specific community member altering the overall community structure or from the existing community structure shaping the response to the introduced strain remains unclear. Nevertheless, although *L. monocytogenes* integrated into both communities, it reached higher abundance in the *Listeria* positive community and the community responses differed, supporting hypothesis III.

Across the dataset, several taxa emerged as being associated with *L. monocytogenes* integration, especially under conditions where *L. monocytogenes* was co-inoculated with the community (*LmCom_Start*) or the community was given a head start (*Com_Start*) (Fig. 7). In the *Listeria* positive community, *Serratia liquefaciens* was the most abundant taxon co-occurring with *L. monocytogenes*, alongside *Lactococcus lactis* and other taxa at lower abundance (Fig. 7). Lactic acid bacteria, including *Lactococcus* spp., can influence microbial community dynamics through the production of organic acids and subsequent pH reduction, which may contribute to competitive interactions involving *L. monocytogenes* (46, 52).

Serratia liquefaciens maintained high absolute abundance across conditions despite *L. monocytogenes* presence, potentially indicating a neutral or positive association. These dynamics might be supported through extracellular matrix production, metabolic cross feeding, or modification of the local biofilm environment (36, 38).

ANCOM-BC analyses identified *Pseudomonas poae*, *Citrobacter werkmanii*, and members of the family Enterobacteriaceae as having reduced abundance following inoculation with *L. monocytogenes* (Fig. 8). *Acinetobacter johnsonii* increased in abundance. Previous work has shown that interactions with gram-

negative bacteria such as *Pseudomonas* spp., can reduce growth of *L. monocytogenes* but not prevent establishment in biofilm communities (53). Members of the family Enterobacteriaceae have been reported to interact competitively with *L. monocytogenes*. For example, *Enterobacter agglomerans* and *Rahnella aquatilis*, which belong to the same family as *Citrobacter werkmanii*, have been shown to reduce the maximum growth of *L. monocytogenes* in co-culture (54). Together, these findings partially support our hypothesis that specific taxa influence *L. monocytogenes* integration. While several candidate taxa associated with antagonistic or facilitative associations were identified, the present study cannot establish causal relationships.

Although the multispecies biofilm communities used in this study originated from the same dairy facility as the persistent *L. monocytogenes* strain, the laboratory model cannot fully reproduce the environmental complexity in food processing environments. Furthermore, the enrichment procedure likely favoured fast-growing species, potentially influencing the community composition of the surface-attached communities. Moreover, full-length 16S rRNA gene sequencing provides a high taxonomic resolution but does not capture microbial interactions and activity in the biofilm. Despite these limitations, the experimental model enabled a controlled investigation into multispecies biofilms while disentangling the effects of community composition and inoculation sequence. Targeted co-culture experiments and community-level interaction networks (36, 52, 55) are needed to validate these associations. This could help identify ecological drivers of persistence and provide new intervention targets for food safety professionals to manage and eliminate persistent *L. monocytogenes* in food processing environments. In addition to examining pairwise species interactions, future studies should investigate community level interaction networks and dynamics to better understand the mechanisms underlying this community specific control of *L. monocytogenes* (36, 55, 56). Integrating microbial ecology and biofilm dynamics into surveillance may therefore improve the management and control of persistent *L. monocytogenes* in food processing environments.

4. Materials and Methods

Facility Sampling

Facility-wide surface swab sampling was performed at an Austrian dairy processing facility and samples were processed as previously described (49). Briefly, microbial communities were obtained from surface swab samples collected from previously identified hotspots for persistent *L. monocytogenes*. Following homogenization of the swabs via a stomacher lab blender (BagMixer, Interscience) for 1 min at medium speed, 500 µL of the homogenate from each sampling location was transferred into sterile glycerol tubes containing 500 µL of 25% glycerol and stored at – 70°C for downstream culture-based analyses. DNA was extracted from the remaining swab sample material using the DNeasy PowerSoil Kit (QIAGEN, Hilden, Germany). An aliquot of each DNA extract was subjected to qPCR targeting the bacterial 16S rRNA gene to assess total bacterial load (57). Samples with fewer than 400 copies of the 16S rRNA gene were excluded from downstream analyses, as quantification below this threshold was considered unreliable. The remaining DNA extracts were submitted to the Vienna Biocenter Core Facility (VBCF, Next

Generation Sequencing, Vienna, Austria) for full-length 16S rRNA gene sequencing using the PacBio Sequel IIe System.

Isolation of *L. monocytogenes* and PCR Confirmation

Isolation and PCR confirmation of *L. monocytogenes* were performed following the workflow described previously (49). Briefly, remaining homogenates from swab samples were subjected to a two-step enrichment procedure, followed by selective plating, colony confirmation by multiplex PCR targeting *Listeria* spp. and the *hly* gene of *L. monocytogenes*, and preservation of confirmed isolates. Confirmed *L. monocytogenes* colonies were grown overnight in TSB-Y, and 500 µL of the culture was transferred into sterile tubes containing 500 µL of 25% glycerol and stored at – 70°C for downstream analyses.

Whole Genome Sequencing and Comparative Genomics of *L. monocytogenes* Isolates

Following PCR confirmation, three *L. monocytogenes* isolate, one of which was isolated from the *L. monocytogenes*-positive community hotspot, were submitted to the National Reference Laboratory for Listeria (Austrian Agency for Health and Food Safety, AGES) for whole-genome sequencing. Whole-genome sequencing, genome assembly, quality assessment, sequence typing (ST), cgMLST, SNP analysis, and environmental resistance gene characterization were performed as previously described (49). Based on the cgMLST and SNP analyses, the Austrian Agency for Health and Food Safety provided 37 closely related genomes (≤ 8 SNP differences) isolated from the same facility between 2020 and 2023 to assess long-term persistence. Comparative genomic analyses were performed as previously described by Spiegel et al. (49), with the additional screening of biofilm-associated biomarker genes (*vip*, *srmB*, *cycB*, and *uvrB*), identified as potential genetic markers by Wang et al. (47), were analyzed at both the nucleotide and protein levels.

Selection of Microbial Communities and Experimental design

This study focuses on two microbial communities recovered from two *L. monocytogenes* hotspots identified during the facility-wide surface swab sampling. Both sampling locations were situated on the floor of two different cheese ripening room. The two sampling locations are known hotspots for the repeated detection of closely related *L. monocytogenes* strains within the facility. One microbial community tested positive for *L. monocytogenes* following the enrichment described above (referred to as *Listeria* positive community) and one negative (referred to as *Listeria* negative community). The *L. monocytogenes* isolated from the *Listeria* positive community was later identified as a persistent strain (Lineage I, CC4, ST4, cgMLST 4605) based on SNP analysis showing ≤ 8 SNP differences across the genome compared with 37 previously isolated strains from different sampling dates, as well as two additional isolates recovered during the same sampling event. The *Listeria* positive and negative community as well as the isolate obtained from the *Listeria* positive community were used for the experiment.

For each microbiome community (*Listeria* positive and *Listeria* negative community) one independent experimental setup was conducted (Fig. 1). Each setup consisted of four sequential inoculation conditions: (I) community culture (*Com_Alone*), (II) *L. monocytogenes* followed by community culture (*Lm_Start*), (III) community culture followed by *L. monocytogenes* (*Com_Start*), and (IV) community culture and *L. monocytogenes* at the same time (*LmCom_Start*). For each condition, three biological replicates were prepared. Biofilms were harvested at 24 h and 48 h post inoculation.

Biofilm Cultivation

Stainless steel surfaces used for the biofilm cultivation experiment were prepared using stainless steel band (1 cm × 3.5 cm; thickness: 0.4 mm, sanded with 240-grit sandpaper to improve the surface for biofilm formation). The bands were sonicated and wiped down as part of the cleaning procedure, followed by autoclaving before the experiment. Each sterile stainless-steel slide was placed into one sterile 5 mL Greiner tube per replicate. Additionally, one replicate per incubation condition and sampling timepoint contained two slides positioned facing each other (smooth sides inward) for biofilm staining. TSB-Y (Tryptic Soy Broth + 0.6% yeast extract; Merck, Darmstadt, Germany) was diluted 1:2 and 1:10 with sterile deionised water to promote biofilm formation. After preparation, sterility tests were conducted via incubation of an aliquot at 37°C and plating the incubated media on TSB-Y agar plates. For overnight cultures of *L. monocytogenes* and the community samples, 1:2 TSB-Y media was inoculated using a 1 µl inoculation loop from the corresponding glycerol stocks (*Listeria* positive community, *Listeria* negative community, and *L. monocytogenes* isolate) and incubated at room temperature overnight while shaking at 120 rpm. Overnight cultures were adjusted to OD₆₀₀ 0.1 by centrifugation and resuspension in 1:10 TSB-Y. 3.5ml of the inoculum was added to the prepared sterile 5ml Greiner tubes containing the two stainless steel slides. The tubes were incubated at 20°C while shaking at 120 rpm. After 24 h, the stainless-steel slides were rinsed with 0.9% NaCl solution to remove unattached cells remaining in suspension. The slides were either transferred into a fresh 3.5ml 1:10 TSB-Y containing a second inoculum, depending on the succession scenario, or harvested. More information on the experimental design is depicted in Fig. 1.

Biofilm Harvesting, DNA Extraction, and Quantification of *prfA* and 16S rRNA Gene Copy Numbers

Per experimental condition, one stainless-steel slide was used for to confirm biofilm formation visually, by Safranin staining. The remaining stainless-steel slides were used for molecular analysis and were transferred into sterile 5ml Greiner tubes containing 1 mL 1X PBS and six glass beads (4 mm diameter). Surface-attached cells were detached from the stainless-steel strips by vortexing for 3 min and DNA was extracted using the DNeasy PowerSoil Kit (Qiagen, Germany) following the manufacturer's instructions. An aliquot of each DNA extract was subjected to two qPCR assays: one targeting the *L. monocytogenes* *prfA* gene following Rossmanith et al. (58) to assess successful integration of the pathogen, and a second targeting the 16S rRNA gene to assess total bacterial colonization (57). For the *prfA* gene, values ≤ 50 gene copies were considered negative and were excluded from downstream quantitative analyses as they were considered unreliable. The corresponding samples were retained as *L.*

monocytogenes negative in the dataset. The *prfA* qPCR assay was also performed on DNA extracted from the original swab samples from which the *Listeria* positive and *Listeria* negative communities were derived to determine whether *L. monocytogenes* could be detected via molecular analysis in the source material.

The standards for both assays were prepared from overnight cultures (TSB-Y, 37°C) of either the persistent *L. monocytogenes* strain used in this experiment or a mixture (ratio 1:1) of overnight cultures (TSB-Y, 37°C) of *Listeria* positive and *Listeria* negative community, which were pelleted and extracted using the NucleoSpin Tissue (Macherey-Nagel, Germany) DNA Extraction Kit following the manufacturer's instructions. Target fragments of 16S rRNA genes were amplified using the same primer sets and qPCR master mix as used for the qPCR assay. The PCR products were run on a 1% agarose gel, bands of expected size were cut and purified using the innuPREP DOUBLEpure (Innuscreen GmbH, Germany) DNA purification kit according to the manufacturer's instructions. Purified 16S rRNA amplicon DNA and extracted genomic DNA of *L. monocytogenes* was quantified using the Qubit™ dsDNA Broad Range (BR) Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA), diluted to the required concentrations for standards, and subsequently quantified using the Qubit™ dsDNA High Sensitivity (HS) Assay Kit. The copy numbers for the qPCR standard curves were calculated based on the DNA concentration and amplicon length using the Avogadro's constant and the average molecular mass of the double-stranded DNA. qPCR standards were analysed in duplicate, whereas samples were analysed in single reactions due to limited sample volume. The remaining DNA extract was sent to the Vienna Biocenter Core Facility (VBCF Next Generation Sequencing, Vienna, Austria) for full-length 16S rRNA gene sequencing using the PacBio SMRTbell system.

Bioinformatic Processing of 16S rRNA Gene Sequences

Following PacBio full-length 16S rRNA gene sequencing, Bam files were transformed to fastq files using SAMtools version 1.23 (59). Raw full-length 16S rRNA reads were processed using the dada2 version 1.38.0 (60), including quality filtering, dereplication, denoising, and inference of ASVs. Sequence handling was done by ShortRead version 1.68.0 (61) and Biostrings version 2.78.0 (62). Chimeric sequences were removed, before generating an ASV table. Taxonomic classification was performed using SILVA database version 138.2 (63). The sequencing depth was assessed via visualisation of rarefaction curves. Reagent and laboratory contaminants were identified using decontam version 1.30.0 (64) using five negative controls and contaminant ASVs were removed. The phyloseq object was constructed by combining ASV table, taxonomy table, and sample metadata using phyloseq version 1.54.2 (65). Differences in sequencing depth were accounted for by normalising ASV read counts to relative abundance (ASV reads / total reads per sample). The relative abundance was then multiplied by the total 16S rRNA gene copy numbers per sample obtained by qPCR, which yielded absolute abundance of each taxon. For visualisation of microbial community composition, absolute abundances were log₁₀-transformed (log₁₀(x + 1)) to reduce the dynamic range and improve visual contrast between low- and high-abundance taxa. Heatmaps and ordination plots were generated using ampvis2 version 2.8.11 (66). Heatmaps displaying SNP distances and gene presence, and absence derived from the genomic

analysis were generated using Complex Heatmap version 2.26.1 (67). The R code used for these steps can be found in Supplement File1. All analysis was performed in R studio version 2023.9.1.494 (68).

Statistical Analysis of Community Composition

Beta diversity was assessed based on Bray-Curtis dissimilarity calculated using the vegan package version 2.7.2 (69). Beta diversity was visualized by principal coordinates analysis (PCoA). Differences in beta diversity between groups were tested using PERMANOVA (adonis2, 9999 permutations), with community identity, sequential inoculation condition, timepoint, and *L. monocytogenes* presence as predictors. Homogeneity of dispersions was tested using PERMDISP (betadisper, 9999 permutations) to verify significant PERMANOVA results reflect true diversity effects rather than differences in dispersion. Analysis was performed on the full dataset and repeated for each community separately.

Alpha diversity indices (Shannon and Simpson) were calculated using the vegan package. Three sets of comparisons were performed using linear models: (i) to evaluate the effect of *L. monocytogenes* presence across and within microbial communities; (ii) to compare alpha diversity within each community across the sequential inoculations *Com_Alone* and *Lm_Start*; (iii) to assess temporal dynamics of alpha diversity between 24 h and 48 h in the sequential inoculation conditions *Com_Start* and *LmCom_Start*. For full dataset models, community identity, sequential inoculation condition, and timepoint were included as predictors. For within-community models, community identity was omitted. P-values were obtained from the corresponding regression coefficients of the fitted linear models, with the corresponding reference categories specified for each categorical predictor. Alpha diversity analyses were additionally repeated excluding *L. monocytogenes* reads to confirm results were not driven by compositional effects of the pathogen.

The temporal dynamics of *L. monocytogenes* and the five most abundant community taxa per community were visualized by comparing mean absolute abundance at 24 h and 48 h across sequential inoculation conditions. For visualization, ASVs were aggregated at species level, where species annotation was available, and at genus level where species annotation was absent.

The differential abundance of ASVs with *L. monocytogenes* inoculation was assessed using ANCOM-BC version 2.12.1 (70). *L. monocytogenes* ASVs were excluded prior to this analysis to avoid introducing a compositional bias. Furthermore, *Swab* samples and *L. monocytogenes* cultures from the *Lm_Start* samples at 24 h were excluded to investigate sequential inoculations only and remove *L. monocytogenes* monoculture. Taxa present in fewer than 15% of samples were excluded (prevalence cutoff = 0.15), and samples with fewer than 500 reads were removed. Inoculation of *L. monocytogenes* was modelled as the primary predictor, with sequential inoculation condition, timepoint, and community identity included as covariates. A conservative bias correction was applied (conserve = TRUE). All statistical analysis and visualisation were performed in R studio version 2023.9.1.494 (68) and can be found in Supplementary File 1.

Declarations

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7. Figures and Legends

Author Contributions

Felix Spiegel: Conceptualization, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review and editing. **Alvaro Cañete Reyes:** Conceptualization, Investigation, Data curation, Writing – original draft, Writing – review and editing. **Rina Bachmann:** Investigation, Writing – review and editing. **Benjamin Zwirzitz:** Formal analysis, Writing – review and editing. **Ariane Pietzka:** Formal analysis, Data curation, Writing – review and editing. **Martin Wagner:** Funding acquisition, Supervision, Writing – original draft, Writing – review and editing, Project administration. **Lauren V. Alteio:** Conceptualization, Investigation, Data curation, Formal analysis, Visualization, Supervision, Writing – original draft, Writing – review and editing, Project administration.

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5. Data Availability

The data for this study have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under accession number PRJEB127362 (<https://www.ebi.ac.uk/ena/browser/view/PRJEB127362>).

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Figures

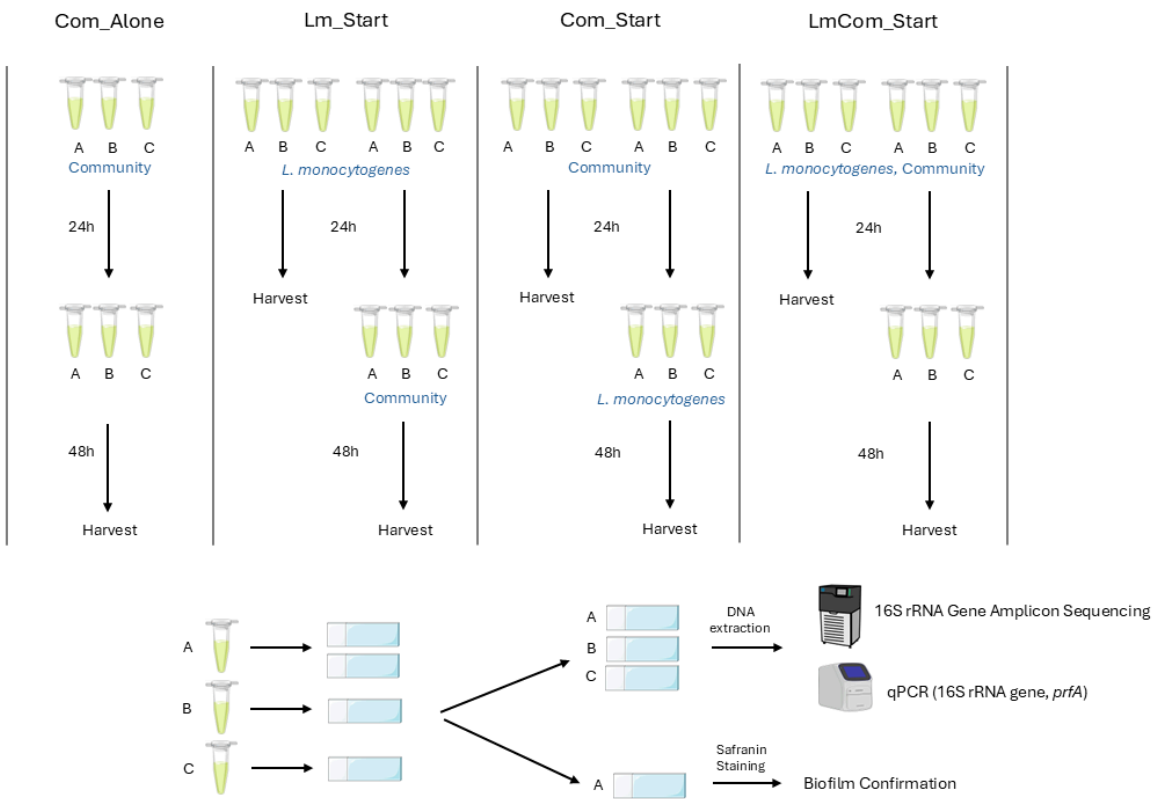


Figure 1

Experimental study design for biofilm formation in communities C.A and C.B. Two independent experimental setups were performed, one for each microbiome community (C.A. and C.B.). Each setup included four conditions: (I) community culture (*Com_Alone*), (II) *L. monocytogenes* followed by community (*Lm_Start*), (III) community followed by *L. monocytogenes* (*Com_Start*), and (IV) community and *L. monocytogenes* at the same time (*LmCom_Start*). For each condition, three biological replicates were prepared. Biofilms were harvested at 24 h and 48 h post inoculation.

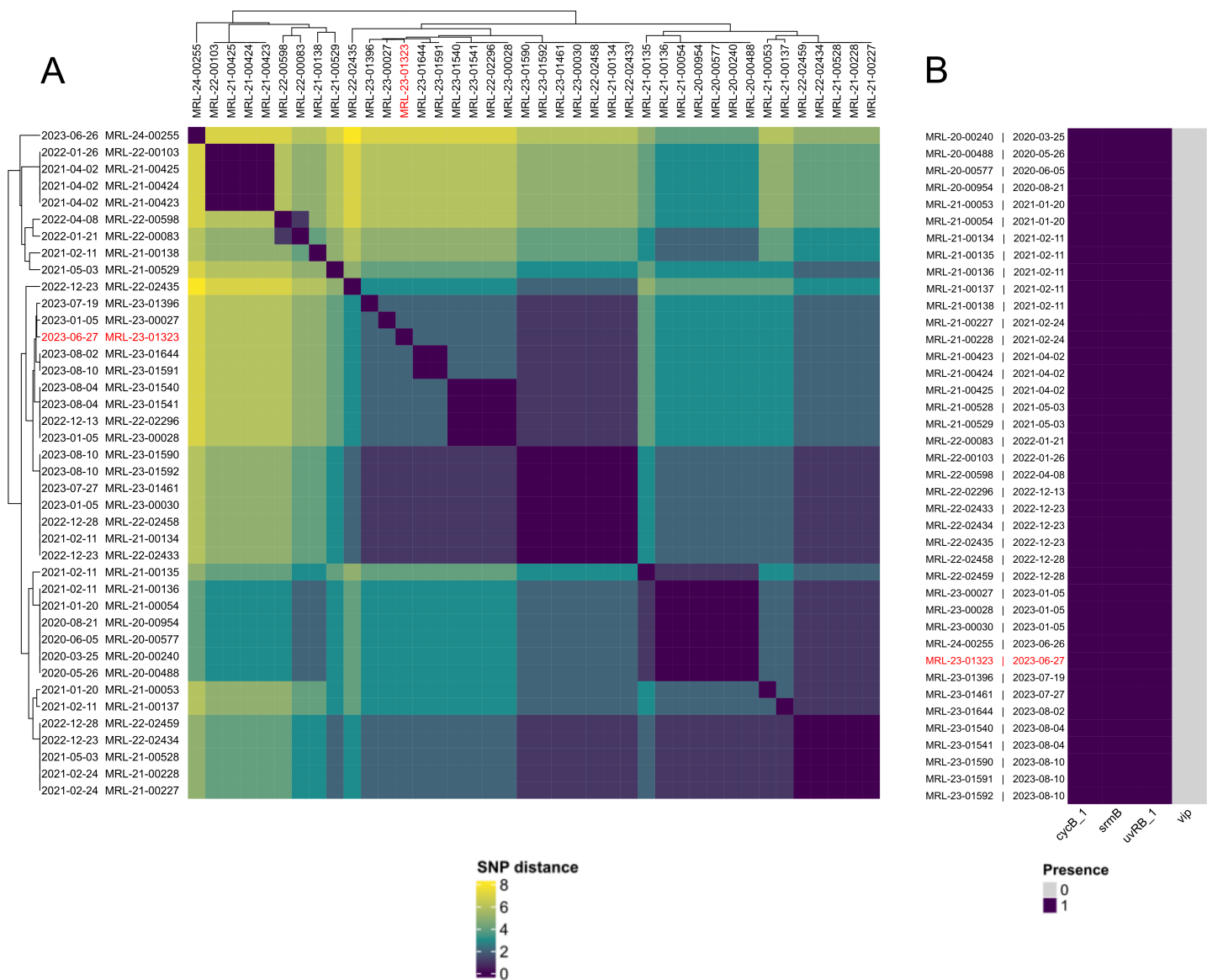


Figure 2

Genomic relatedness and biofilm-associated gene profiles of persistent *L. monocytogenes* isolates recovered from sampled facility between 2020 and 20203. (A) Pairwise SNP-distance matrix of 40 lineage I ST4 and cgmLST4605 isolates generated using the CFSAN SNP pipeline. The isolate used in this study (MRL-23-01322) is highlighted in red. (B) Presence and absence profile of biofilm-associated genes (*vip*, *srmB*, *cycB*, and *uvrB*) screened using a custom biofilm-associated gene databank.

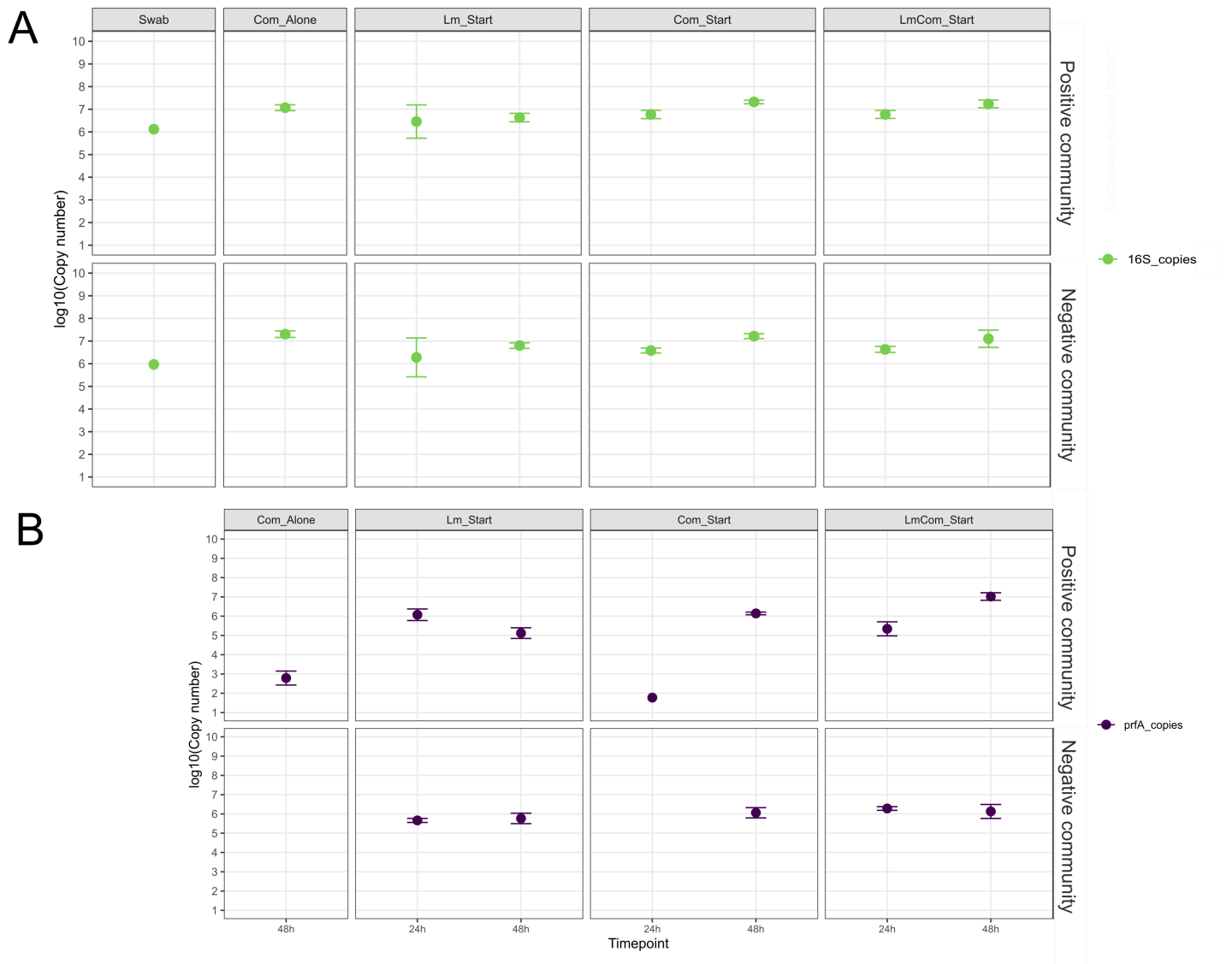


Figure 3

qPCR analysis of *L. monocytogenes* colonization in native multispecies biofilm communities. (A) Quantification of total bacterial abundance based on 16S rRNA gene copy numbers across communities (C.A and C.B), sequential inoculations treatments and timepoints, including the original swab sample. (B) Quantification of *L. monocytogenes* abundance based on *prfA* gene copy numbers across sequential inoculation treatments and timepoints and communities. Data represents mean $\pm$ SD of three biological replicates except for the *swab* sample ($n = 1$) and *Com_Start* in community A, where only one replicate remained after filtering.

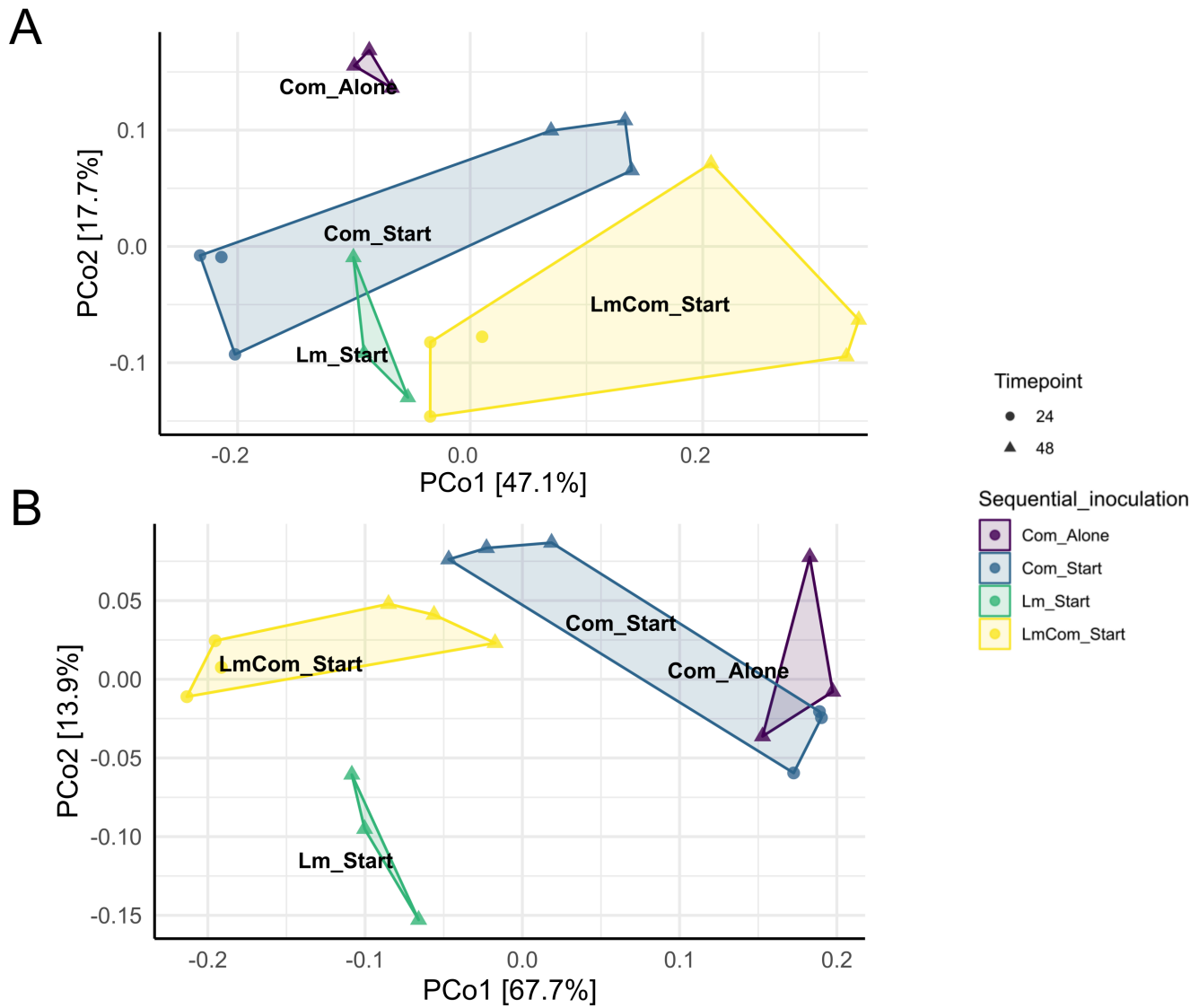


Figure 4

A–B. Principal coordinate analysis (PCoA) of Bray-Curtis dissimilarity for the *Listeria* positive community (A) and the *Listeria* negative community (B). Each point represents one sample, with shape indicating timepoint (circle: 24 h; triangle: 48 h) and colour indicating sequential inoculation (*Com_Alone*, purple; *Com_Start*, blue; *Lm_Start*, green; *LmCom_Start*, yellow). PCo1 and PCo2 explain 47.1% and 17.7% of total variation in the *Listeria* positive community, and 67.7% and 13.9% in the *Listeria* negative community , respectively.

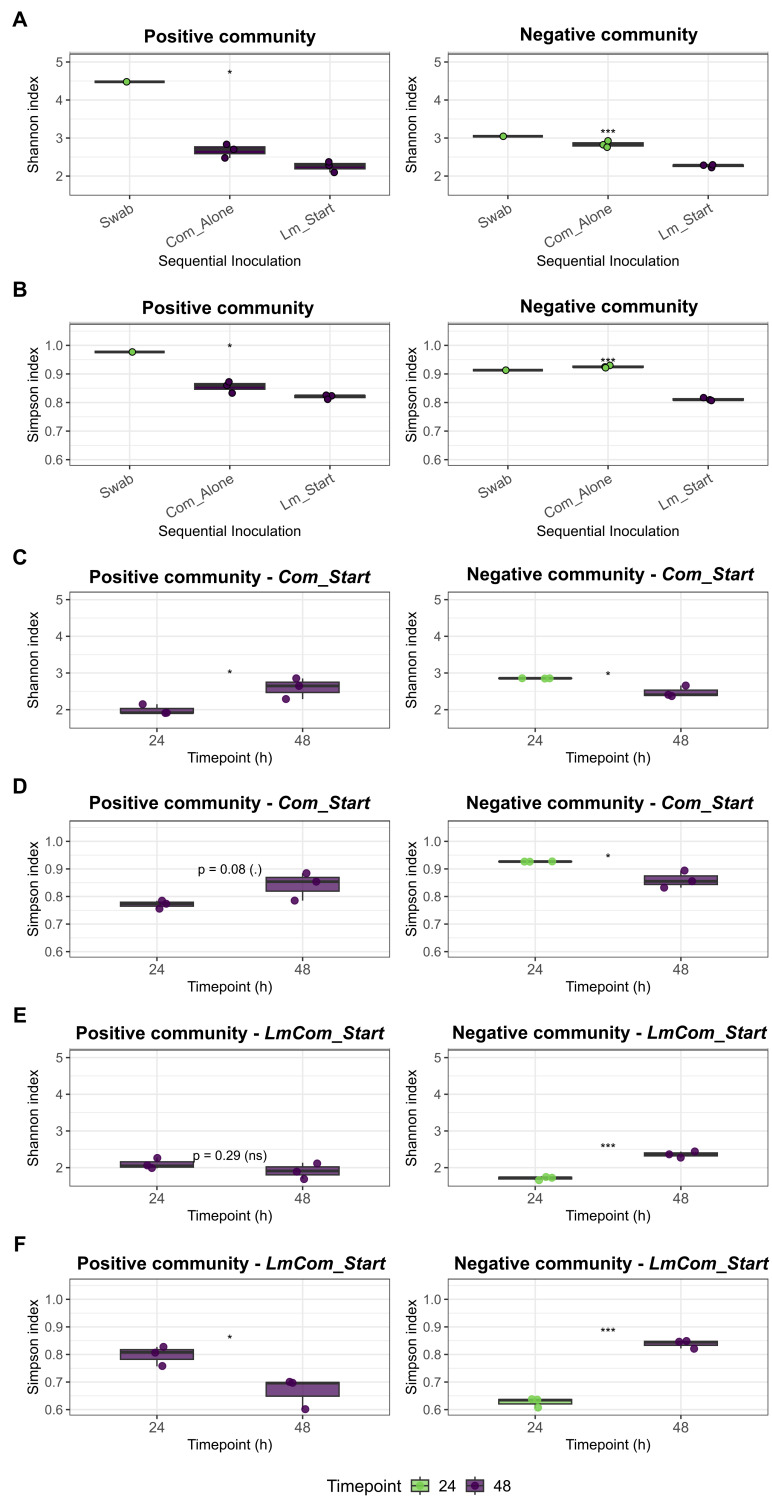


Figure 5

Alpha diversity of microbial communities under different sequential inoculation conditions and timepoints. (A) Shannon diversity indices for the *Listeria* positive and negative communities of Swab samples and multispecies biofilm communities under sequential inoculation *Com_Alone* and *Lm_Start* after at 48 h. (B) Simpson diversity indices for *Listeria* positive and negative Swab communities and multispecies biofilm communities under sequential inoculation *Com_Alone* and *Lm_Start* after at 48 h.

(C) Shannon diversity index for sequential inoculation *Com_Start* at 24 h and at 48 h for *Listeria* positive and negative communities. (D) Simpson diversity index for sequential inoculation *Com_Start* at 24 h and at 48 h for *Listeria* positive and negative communities. (E) Shannon diversity index for sequential inoculation *LmCom_Start* at 24 h and at 48 h for *Listeria* positive and negative communities. (F) Simpson diversity index for sequential inoculation *LmCom_Start* at 24 h and at 48 h for *Listeria* positive and negative communities.

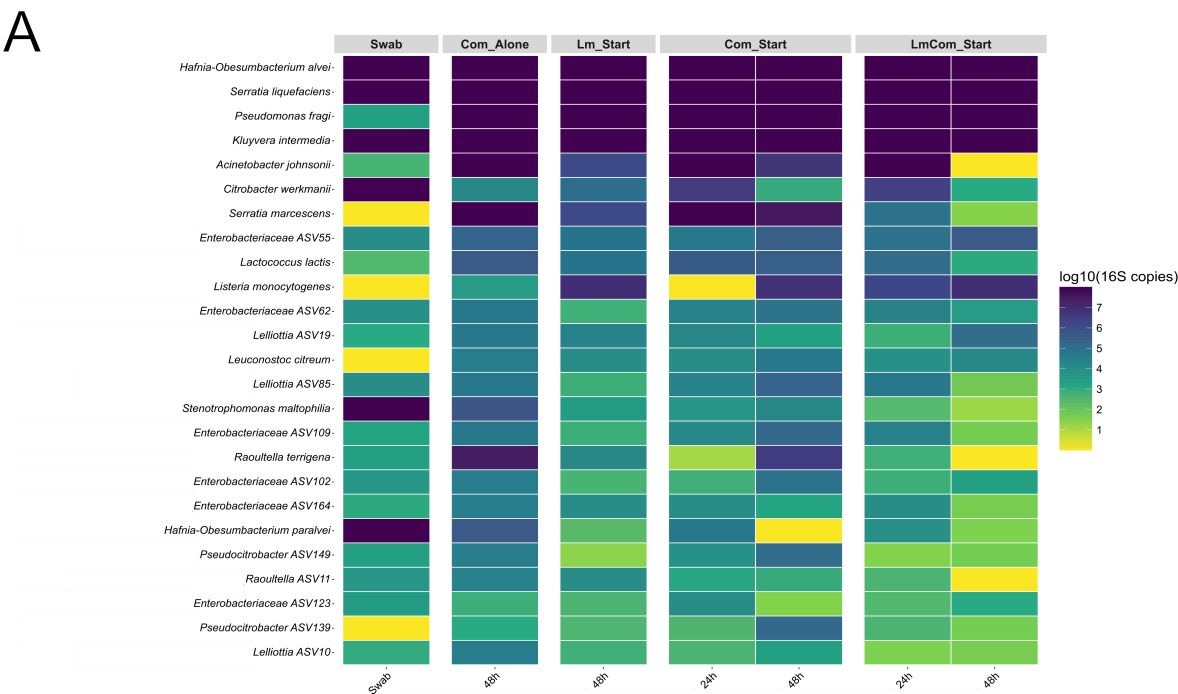


Figure 6

Community composition dynamics across sequential inoculation conditions in communities A and B. Heatmap shows the top 25 taxa based on absolute abundance of the log10 transformed 16S rRNA gene copies across the swab as well as sequential inoculation conditions for (A) *Listeria* positive community and (B) *Listeria* negative community. Columns represent the original swab communities and the different inoculation setups (*Com_Alone*, *Lm_Start*, *Com_Start*, and *LmCom_Start*) at the indicated incubation times. Rows represent individual taxa, and colours indicate absolute abundance on a log10-transformed scale.

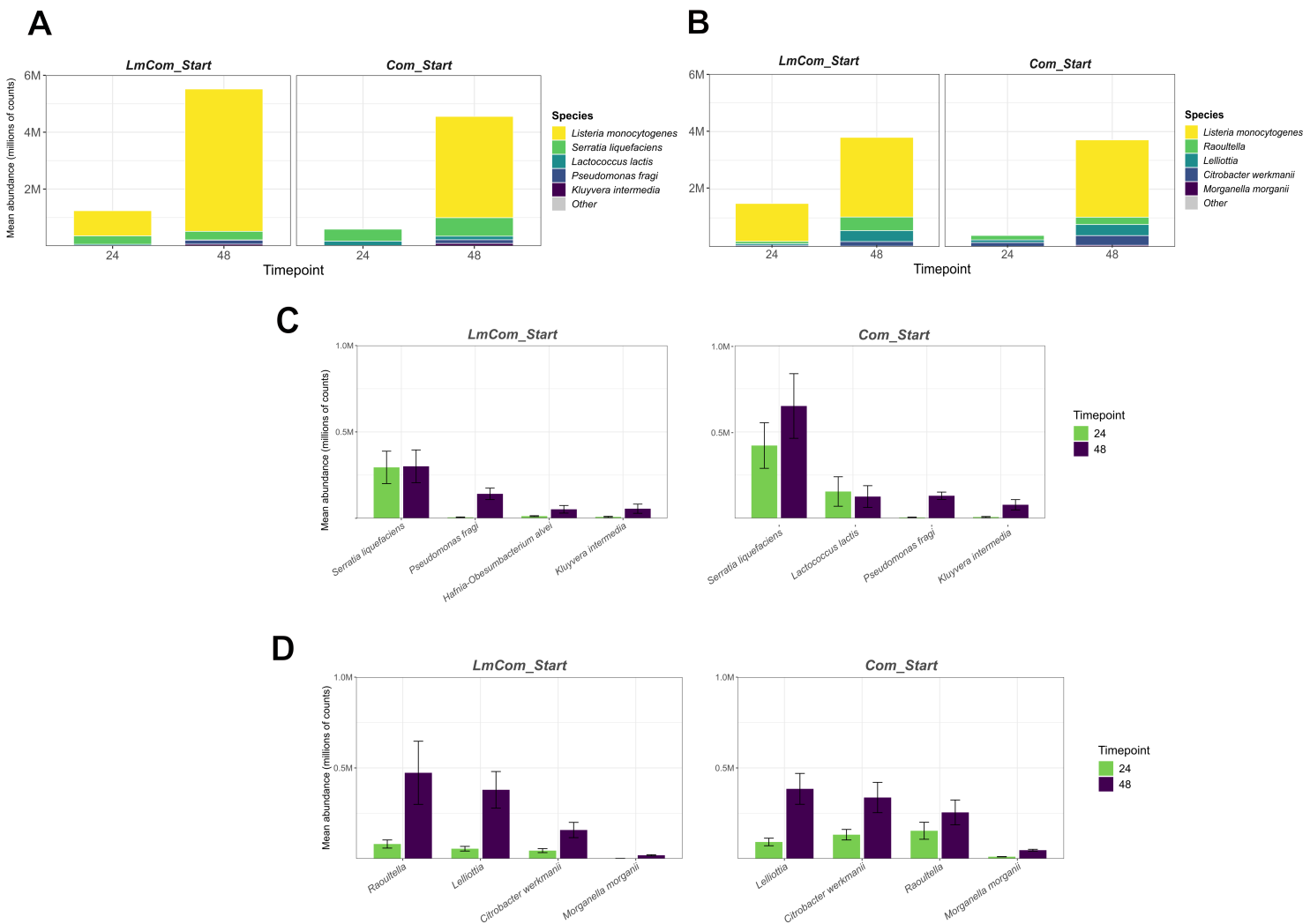


Figure 7

Microbial community composition and abundance dynamics in *LmCom_Start* and *Com_Start* conditions over two timepoints (24 h and 48 h). (A) Stacked bar charts showing total mean species abundance (counts) at 24 h and 48 h for *LmCom_Start* (left) and *Com_Start*(right) in the *Listeria* positive community.

(B) Stacked bar charts showing total mean species abundance (counts) at 24 h and 48 h for *LmCom_Start*(left) and *Com_Start* (right) in the *Listeria* negative community. (C) Grouped bar charts showing mean abundances of the most abundant taxa in the *Listeria* positive community, with error bars indicating standard deviation. (D) Grouped bar charts showing mean abundances of the most abundant taxa in the *Listeria* negative community, with error bars indicating standard deviation.

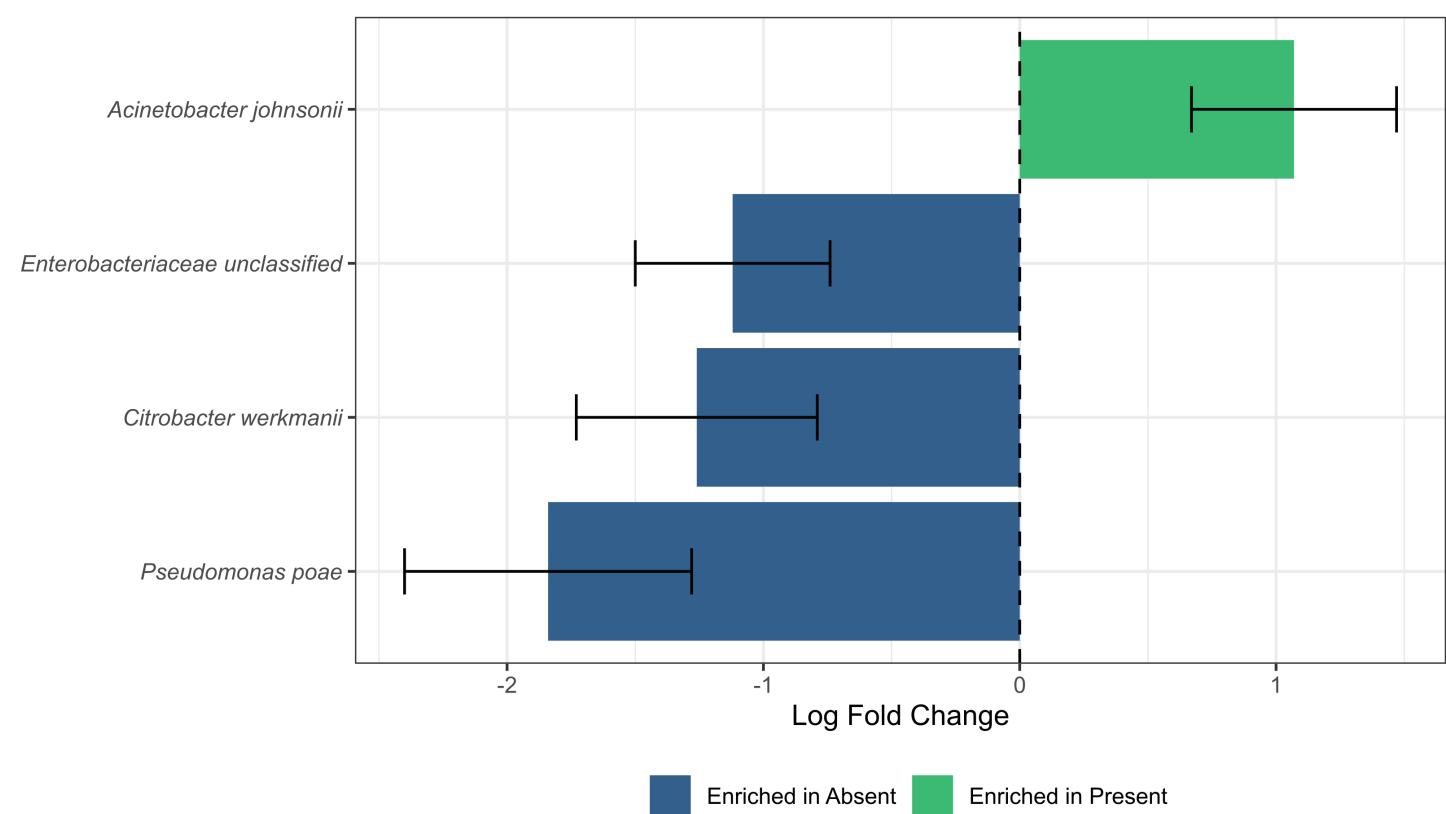


Figure 8

Differential abundance of bacterial taxa associated with *L. monocytogenes* inoculation. Horizontal bar chart displaying log fold change (LFC) values for four differentially abundant taxa identified by ANCOM-BC analysis (BH-adjusted $q < 0.05$). Only taxa present in $\geq 15\%$ of samples were included. The model controlled for sequential inoculation order, timepoint, and community composition. Bars extending to the right (green) indicate taxa enriched when *L. monocytogenes* is inoculated, while bars extending to the left (blue) indicate taxa enriched when *L. monocytogenes* is not inoculated. Error bars represent the standard error of the LFC estimate.

Supplementary Files

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