



Biofilm capacity of the psychrophilic bacteria triggers their persistence in the equipment and their spread to beef products throughout processing

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ABSTRACT

Microbial contamination in food processing remains a persistent and complex challenge. Understanding the sources, contributing factors, and control measures is essential for effective mitigation. In this study we employed a combination of metagenomic sequencing, targeted culturomics, and whole-genome sequencing of key isolates to gain a comprehensive view of bacterial dynamics and functional capabilities throughout a working shift in a beef slaughter and cutting facility. This allowed us to identify which bacteria are i) most prevalent in the clean facility before the start of the work, ii) able to establish themselves over time, and iii) detectable in the final product. We further generated a functional profile of the microbial community within the facility, with a particular focus on antimicrobial resistance and biofilm formation genes, and the presence of specific pathogens and spoilage organisms. Both culture-based and sequencing data showed that *Psychrobacter* and *Pseudomonas* strains present in the final product were also detected on the membrane skinner, a machine used to remove all the excess tissues from meat, and in the drains even after cleaning. We found a high number of genes involved in biofilm formation in *Psychrobacter immobilis*, a characteristic that may explain their biofilm capacity and the survival of this species during the cleaning process and persistence throughout the facility. Taken together, our findings suggest potential sources of contamination and highlight the advantages of integrating culture-dependent methods with high-throughput sequencing technologies to enhance microbial monitoring and control strategies in food production environments.

1. Introduction

Food waste is an increasing global concern. Meat, with its impact on human health and environmental sustainability, represents a particularly urgent problem, as 20% of globally produced meat is wasted yearly (Food and Agriculture Organization of the United Nations, 2021). Microbial contamination is one of the primary causes of meat spoilage

(Dave & Ghaly, 2011), contributing substantially to global meat waste across the entire supply chain (Meybeck et al., 2011; Buzby et al., 2014). Additionally, it was recently revealed that, while most studies focus solely on ESKAPEE bacteria (i.e., *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp., and *Escherichia coli*) the non-ESKAPEE fraction (e.g., *Staphylococcus equorum*, *Acinetobacter johnsonii*) are the

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main carriers of antimicrobial resistance genes (AMRG) in food production, thus pointing out the necessity to report the neglected fraction of the microbiome in the equation in order to ensure the safety of the products (Quijada et al., 2025). Therefore, it is of critical importance for both public health and sustainability to identify the primary sources of contamination.

Microbial cultivation represents the gold standard method for hygiene evaluation in the internal evaluation of meat production (European Food Safety Authority, 2016). While this is still a very relevant method for assessing overall hygiene, it usually targets specific key pathogens, and thus offers limited insight into the broader microbial diversity and dynamics of the production environment. Recent advances in microbial ecology, particularly through 16S rRNA gene amplicon sequencing, have provided a deeper insight into the composition of bacterial communities in meat-production facilities, including slaughterhouses, meat cutting facilities within slaughterhouses and retail butcheries. However, this approach only allows the detection of the microbial taxonomy occurring in a certain environment (i.e., “microbiota”), leaving their genomic functions and realm of activity (i.e., “microbiome”) aside. Despite the overall microbial diversity, studies have shown that only a limited number of species (primarily belonging to *Pseudomonas*, *Brochothrix*, *Psychrobacter*, *Streptococcus*, and *Acinetobacter*) tend to become established within these environments (De Filippis et al., 2013; Sequino et al., 2024; Stellato et al., 2016; Zwirzitz et al., 2020). Members of *Pseudomonas*, *Brochothrix*, and *Psychrobacter* have also been reported to form biofilms, which pose a major challenge in the food-processing settings, as they allow microbes to survive under cold temperature, nutrient limitation, and cleaning procedures (Wagner et al., 2020). Within biofilms, microbes can withstand many routine disinfection methods and are difficult to eradicate using standard antimicrobial and antiseptic treatments (Flemming et al., 2016; Flemming et al., 2023; Parrilli et al., 2022). Pathogens such as *Listeria monocytogenes* and *Pseudomonas aeruginosa* are known to form biofilms independently and to represent major challenges in food processing and healthcare environments (Donlan, 2002). Recent evidence suggests that these organisms can also integrate into multi-species, non-pathogenic biofilms without altering their structure or transcriptomics profiles, thereby enhancing their survival (Voglauer et al., 2025).

Previous research identified various critical points as the main contributors to microbial contamination in slaughterhouses. However, a substantial portion of the contamination detected on the final product has been shown to originate from the processing environment itself, rather than from the animal (Sequino et al., 2024; X. Yang et al., 2023; Zwirzitz et al., 2020). Therefore, we restricted our investigation to a very specific production area of a slaughterhouse (containing cutting and packing areas) located in Austria, with the goal to assess potential individual surfaces or equipment that might act as spillovers of contamination. The selection of the slaughterhouse and the sampling areas and equipment was motivated by prior literature and supported by our own observations during on-site assessment. As previous studies relied mainly on culture-based and/or amplicon sequencing surveys, in this study we performed a longitudinal metagenome approach to gain a deeper overview of the bacterial dynamics throughout a beef slaughter facility, while accounting for potential pathogens and spoilage bacteria. The addition of shotgun metagenomics provides a deep characterization of the microbial taxa occurring in an environment and the possibility to infer their functional profiles, features that are not possible by solely using 16S rRNA amplicon sequencing. Numerous previous studies have provided a broad overview of facility microbiota by sampling multiple slaughterhouses. However, they did not offer a detailed functional and taxonomic profile based on an in-depth analysis of a single facility, or specific areas within a facility, and its bacterial dynamics. In our analysis, microbial functions were annotated across the cutting and packaging facility with direct relevance to the final product quality and safety, including antimicrobial resistance, biofilm formation, and the capability to produce undesired metabolites. We evaluated the

microbial diversity considering individual equipment, including those present in the cutting and packaging areas, to identify key hotspots of microbial contamination that can ultimately affect the final meat products.

2. Results

2.1. Psychrophilic bacteria dominate the beef slaughterhouse

In this study, we aimed to trace the dissemination of bacteria within the cutting and packaging areas of a beef slaughterhouse, over a 10 h production period (a summary scheme is shown in Fig. 1.). Sampling time points were strategically selected to assess whether specific bacterial species survived the cleaning process (pre-shift), and if they increased in abundance over the working shift (mid-shift and shift-end).

Analysis of microbial alpha-diversity revealed greater species richness and evenness in the pre-shift samples, collected after surface cleaning (Fig. S1) than in samples from subsequent time points. As production progressed, overall bacterial diversity decreased, and certain species became dominant on specific surfaces. Beta-diversity analysis based on Bray-Curtis dissimilarity showed a significant difference between the microbiota in the pre-shift and the end-shift samples (PERMANOVA, $p = 0.001$, Fig. S2). No significant differences in beta dispersion were observed between time points (ANOVA, $p = 0.19$). Bacterial species including *Psychrobacter immobilis* (mean relative abundance: 6.7%, prevalence: 85.7%), *Pseudoclavibacter helvolus* (6.7%, 78.6%), *Micrococcus luteus* (6.0%, 64.3%) and *Galactobacter caseinivorans* (5.4%, 85.7%), were abundant and prevalent throughout the sampling process, while others showed substantial differences in abundance between the pre-shift and the shift-end. This was the case of *Dietzia cercidiphylli*, *Microbacterium liquefaciens* and *Micrococcus endophyticus*, which dominated the surfaces in the pre-shift period (~5.7% mean relative abundance, 87.5% prevalence for each species). In contrast, they decreased both in abundance and prevalence during the mid-shift (6%, 77.8%; 4.5%, 77.8%; 2.7%, 22.2%; respectively) and shift-end period (0.8%, 54.5%; 1.4%, 63.6%; 1.6%, 18.2%; respectively). On the other hand, *Psychrobacter maritimus*, *Moraxella porci*, and *Pseudomonas fragi*, although present at low relative abundances during the pre-shift period (0.2%, 87.5%; 0.2%, 50%; 0.7%, 87.5%; respectively) prevailed throughout the working shift, showing increased levels at shift-end (8.6%, 91%; 8.3%, 54.5%; 5%, 81.8%; respectively at shift end) (Fig. 2a). Additionally, targeted screening was performed for potential pathogens belonging to the ESKAPEE group, but no representatives were detected.

Absolute abundances were calculated by normalizing the relative abundances by BCEs obtained from qPCR. This approach provided a more accurate estimate of the actual bacterial quantities present in the facility (Fig. 2a.). To evaluate which species increased in abundance over time, we compared the surfaces sampled at pre-shift and the shift-end, by calculating the $\log_2$ fold change (FC) of species absolute abundance between the two time points (Fig. 2b.). Among the bacteria which increased in abundance at the shift-end in comparison to the pre-shift samples, the most frequently observed ones were different species of *Pseudomonas* (up to 6.3 $\log_2$ FC), *Psychrobacter* (up to 8.7 $\log_2$ FC), *Galactobacter caseinivorans* (up to 4.6 $\log_2$ FC) and *Brochothrix thermosphacta* (up to 5.6 $\log_2$ FC).

2.2. Pseudomonas and Psychrobacter increase in abundance in a short period of time

Different species of *Pseudomonas* and *Psychrobacter* increased in abundance on most of the surfaces including the conveyor belt (15.7 $\log_2$ FC and 17.5 $\log_2$ FC, respectively), cutting tables (4.4 $\log_2$ FC and 11.9 $\log_2$ FC, respectively), membrane-skinner (14.2 $\log_2$ FC and 3.9 $\log_2$ FC), non-food contact surfaces (21.9 $\log_2$ FC, *Psychrobacter*), drains (11.1 $\log_2$ FC and 13.9 $\log_2$ FC) and sorting tables (10.5 $\log_2$ FC,

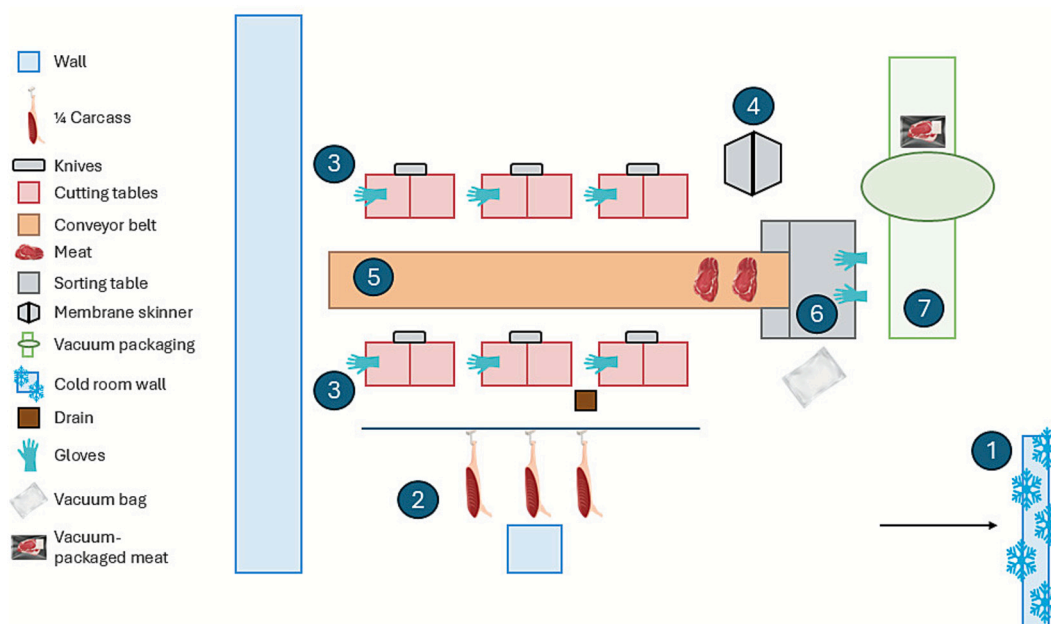


Fig. 1. Schematic layout of the sampled cutting and packing facility within the beef the slaughterhouse. Sampling locations included surfaces in direct contact with the product (e.g. knives, cutting tables, conveyor belt, sorting tables, membrane skinner, vacuum packaging machine, gloves and vacuum bags), as well as non-contact surfaces (e.g. floors, walls and drains), carcasses and meat. The numbers represent the sequence of operations in the facility. The facility was sampled at three different timepoints over the course of a single working shift. The figure was created using Freepik.

Psychrobacter). *P. immobilis* and *P. maritimus* were the most abundant *Psychrobacter* species detected in the facility. *P. immobilis* reached high relative abundances on surfaces at the shift-end, making up 24.6% on the conveyor belt, 34.5% on the cutting tables and 9.5% on the membrane skinner (Fig. 2a.). The population of *P. immobilis* increased by 4.1 log₂FC on the conveyor belt, by 8.8 log₂FC on the cutting tables, by 4.8 log₂FC on sorting tables, and by 2.3 log₂FC on non-food contact surfaces. *P. maritimus* increased by 5.3 log₂FC on the conveyor belt, 5.7 log₂FC on sorting tables, by 3.5 log₂FC on non-food contact surfaces, and 4 log₂FC on the membrane skinner (Fig. 2b. and Fig. S3).

The most commonly known spoilage-associated *Pseudomonas* species, and the one with the highest relative abundance in the facility, *P. fragi*, was observed to increase in abundance by 4.0 log₂FC on the conveyor belt, 1.3 log₂FC on the membrane skinner and by 1.2 log₂FC on non-food contact surfaces. *P. fragi* reached a relative abundance of 16.7% on the membrane skinner, a tool used for removing excess tissues, that comes into direct contact with meat. Other detected species of *Pseudomonas* have previously been observed on meat at different stages of storage, however, their potential roles in meat spoilage remain poorly characterized. Here it is important to point out that cutting tables were replaced after mid-shift sampling, and so the tables sampled at pre-shift and mid-shift were different from those sampled at shift-end.

Pseudomonas and *Psychrobacter* were also detected in high relative abundance on carcasses (*Pseudomonas* at 30.2%, with *P. fragi* at 16.4% and *Psychrobacter* at 34.7% with *P. immobilis* at 5.9% and *P. maritimus* at 20.3%) and on the meat (*Pseudomonas* at 16.2% with *P. fragi* at 6.2% and *Psychrobacter* at 34.6% with *P. immobilis* at 12.5% and *P. maritimus* at 20.9%) at the shift-end (Fig. 2a.). Importantly, their abundance increased markedly over a relatively short period, approximately 10 h, between the pre-shift and the shift-end sampling. This rapid proliferation was observed across most sampled sites within the facility, underscoring their capacity for swift colonization and potential relevance in meat spoilage dynamics.

2.3. Tetracycline, aminoglycoside and glycopeptide AMRGs were detected throughout the working shift

While the highest abundance and richness of the antimicrobial resistance genes (AMRGs, Supplementary Table S4) were detected using assembly-free approaches in the pre-shift samples, some of them, such as tetracycline, aminoglycoside and glycopeptide AMRGs, were detected in all the three time points (Fig. S4). Tetracycline AMRGs with the highest abundances were *tet(39)* and *tmexD1*. Both of them were detected in drains and non-food contact surfaces pre-shift. Additionally, *tet(39)* was detected in drains mid-shift, however its abundance reduced significantly at shift-end. On the other hand, *tmexD1* was also detected on the membrane skinner pre-shift and in the drains at shift-end. The aminoglycoside AMRG *aph(3'')-Ib* was detected over all three time points. It was detected in drains at pre-shift and mid-shift, and on carcasses, cutting tables and on the gloves at the packing station at the shift-end. Another aminoglycoside AMRG *aph(6)-Id* was detected in drains over all three sampling time points, and on carcasses and cutting tables at shift-end. The vancomycin AMRG *vanM* was also detected in the drains over all three sampling time points, and was additionally detected on the membrane skinner pre-shift and on non-food contact surfaces mid-shift.

Cooler-room walls, drains, and membrane-skinner sampled pre-shift, along with carcasses sampled at the shift-end carried the highest counts of AMRGs. However, cooler-room walls mostly contained macrolide/lincosamide/streptogramin B AMRG *erm(46)* and amphenicol AMRG *cmrA* which were not detected on other surfaces at abundance >1CPM. Many of the detected AMR families were classified by the WHO as highly important (tetracycline, beta-lactam, amphenicol, lincosamide, streptogramin A and B), and some even are considered critically important (aminoglycoside and macrolide) (World Health Organization, 2024).

2.4. Biofilm-related genes are ubiquitous in the facility metagenome but particularly abundant in the pre-shift samples

While biofilm-associated genes were found throughout the facility, certain surfaces showed a higher proportion of them. From those, the membrane-skinner and drain samples collected in the pre-shift period

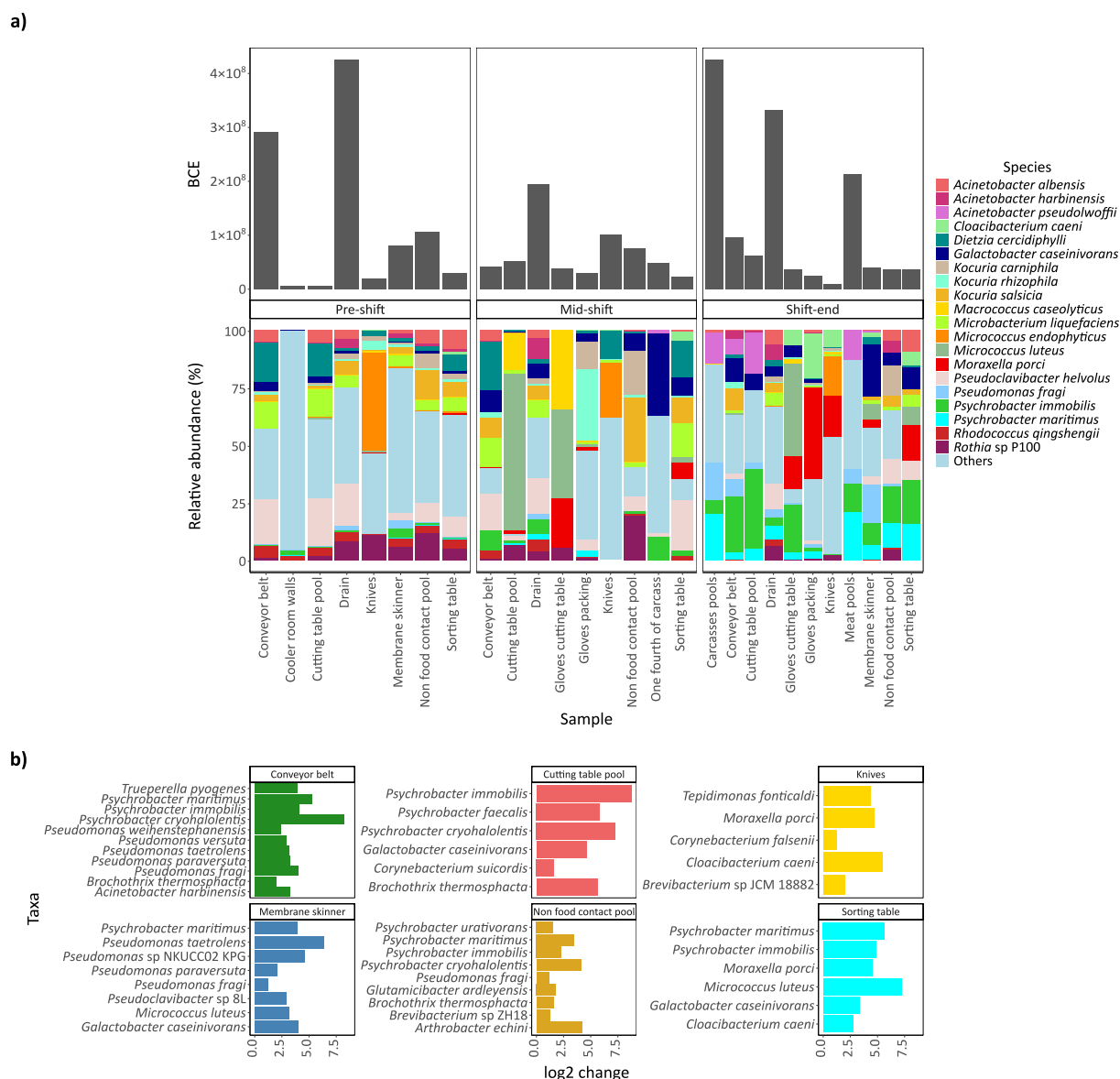


Fig. 2. Change in bacterial community composition over time. Analysis of metagenome data obtained from the swab samples collected from contact and non-contact surfaces in the cutting and packaging area of the slaughterhouse, as well as from meat and carcasses processed in the facility (as depicted in Fig. 1.). The sampling was conducted at three time points: i) pre-shift, when the facility was clean, ii) mid-shift and iii) at the shift-end. a) Relative abundance of the 20 most abundant species across three time points. The gray bars above of the relative abundance plot represent BCE values of the samples, obtained from 16S rRNA qPCR per sample. b) log2 fold change of species absolute abundance from pre-shift to the shift-end on surfaces sampled at both time points. The taxa were filtered for those with higher than 10,000 total metagenomic reads at both time points.

were the ones with the highest abundance of these genes overall. Generally, the samples collected pre-shift contained the highest abundance (calculated as counts per million, CPM) of biofilm-related genes (Fig. 3.). The most prevalent genes over all the samples were *cybA* (encoding adenylate cyclase) and *crp* (encoding cyclic AMP receptor protein), both part of the cAMP/Vfr signalling pathway and essential for flagellar assembly; *trpE* (anthranilate synthase component I), that plays a role in quorum sensing; *mucR*, that encodes a diguanylate cyclase which is a part of c-di-GMP signalling pathway; and *cpdA* (3',5'-cyclic-AMP phosphodiesterase) (Fig. 4). On the membrane skinner pre-shift, the most abundant genes were *impL* (also known as *vasK* or *icmF*) encoding for a type VI secretion system protein; *trpE*; *barA* (also known as *gacS* or *varS*), which encodes a part of the two-component signalling system; *mucR*; *chpA* encoding for chemosensory pili system protein ChpA; and *cpdA*. In the drains over all three time points, *mucR*, *crp*, *trpE* and *cpdA* were most abundant. Cutting tables and the conveyor belt also

harboured high counts of specific biofilm-related genes. On both of these surfaces the most abundant were adenylate cyclase *CyaB* encoding gene and *mucR*.

2.5. *Pseudomonas* and *Psychrobacter* isolates from beef harbor genes linked to biofilm formation and spoilage

Meat samples were taken during the working day and subjected to targeted-culturomics accounting for the information provided by the metagenomic profiling. Based on their abundance on meat samples and the widespread of their genera throughout the surfaces, we selected two isolates, belonging to *Pseudomonas bubulae* (strain 0015) and *Psychrobacter immobilis* (strain 1601) for further WGS and in vitro biofilm formation assays. The genome sizes of these two strains were 5,231,483 bp and 3,456,519 bp, respectively (Supplementary Table S5). Even though isolates belonging to other species were also captured (e.g.,

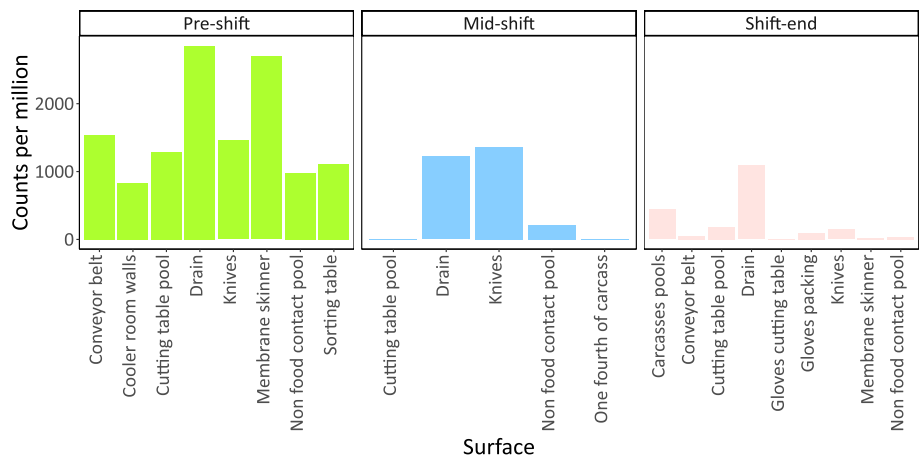


Fig. 3. Quantification of biofilm related genes grouped by three different KEGG biofilm formation pathways. The profile of biofilm formation genes was evaluated by aligning the metagenomic sequencing reads against the KEGG database. The bars represent the gene counts per million and colors indicate the three pathways.

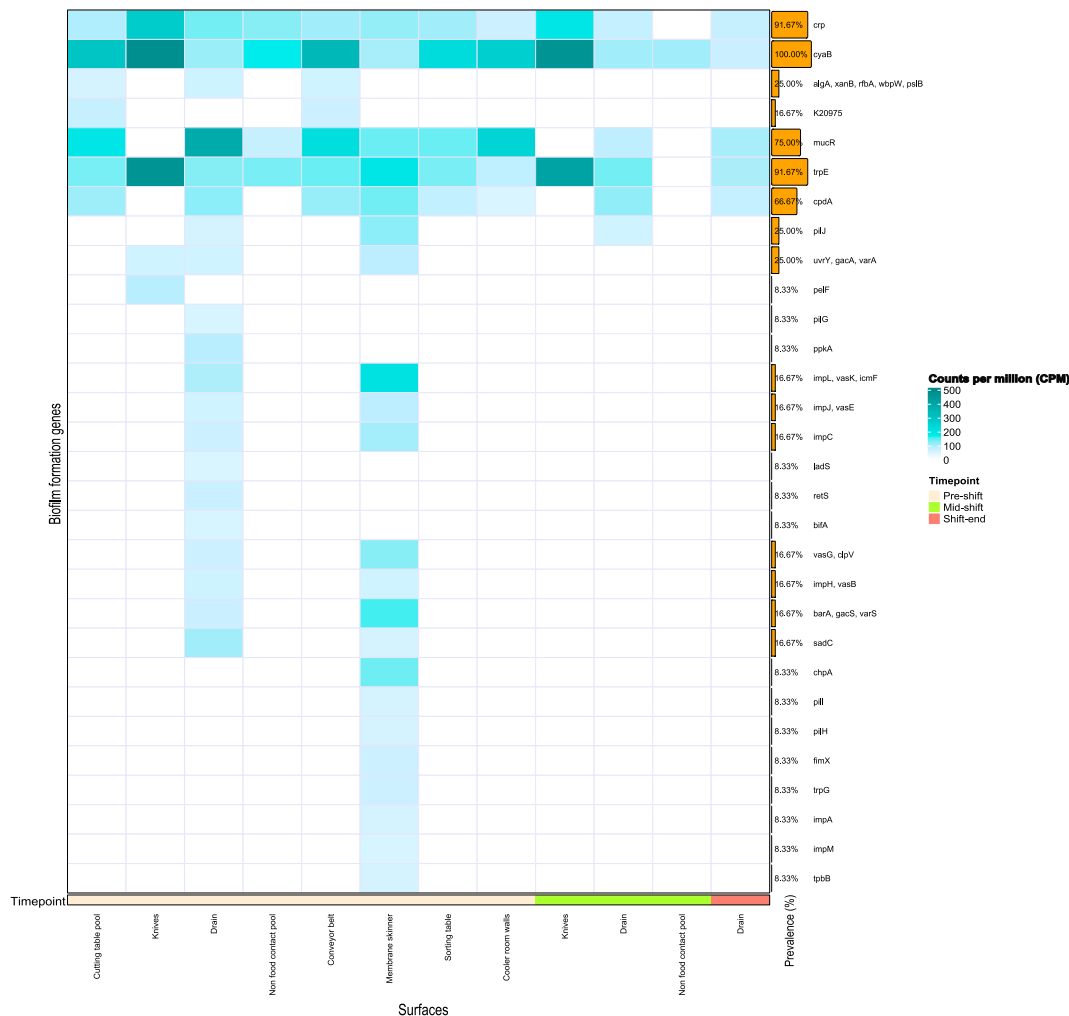


Fig. 4. The prevalence of biofilm related genes detected in the metagenome. KEGG gene annotations, classified as part of the biofilm formation pathway, were filtered for and used to determine the prevalence of biofilm related genes in the metagenomes. If multiple gene names were available for a single KEGG annotation, all are listed on the right-hand Y-axis, separated by commas. For annotations without associated gene names, the KEGG ID is provided (e.g., K20975). The heat map represents the normalized abundance of genes, expressed as counts per million (CPM) of total reads mapped to each detected gene across facility surface samples, with a minimum cut off of 50 CPM per sample. The overall prevalence of each gene across all the samples are represented by the orange bars on the left side of the heatmap.

G. caseinivorans and *Micrococcus luteus*), the rationale of focusing on *Pseudomonas* and *Psychrobacter* was based on their ubiquitousness in food-producing environments, their role in food spoilage, their capability to grow in cold condition, and their ability to form biofilms which might provide them with a resistance mechanism to the cleaning practices undertaken in the facility.

The *Pseudomonas bubulae* 0015 isolate showed a high genetic similarity (average nucleotide identity, ANI, of ~99.5%) to contigs assigned to *Pseudomonas* spp. in several surfaces, which is within the range of genomovars, and thus potentially suggesting it may represent of a single intraspecies ecological unit within the facility (Conrad et al., 2024). In contrast, the *Psychrobacter* genus displayed higher within-species diversity (ANI of ~98.5%, Fig. S5 (Jain et al., 2018)). Comparison of the metagenomic data with the isolated genomes showed that *P. bubulae* 0015 was present in the drain samples at all three time points and on the membrane skinner during the pre-shift. *P. immobilis* 1601 was detected in drain samples during mid-shift and shift-end, on the membrane skinner in pre-shift, and on the cutting tables both at the pre-shift and at shift-end.

Genomic annotation of the strains showed multiple survival and spoilage-related traits, including cold shock proteins, and enzymes involved in amino acid metabolism leading to off-products (Fig. 5 and Supplementary Table S6). Both organisms encoded the cold shock protein CspA, which might have a role in their persistence in the cold temperatures present in the facility (Giuliodori et al., 2023). From pathways leading to production of spoilage related metabolites, both strains encoded genes involved in ammonia production through nitrate/nitrite reduction (*NRT*, *narG*, *narI*, *narH*, *nirB*, *nirD*, *nasA* and *nasB*), urea hydrolysis (*ureA*, *ureB*, *ureC* and *ureE*), and oxidative deamination of L-glutamate (*GDH2*). Of particular interest was the arginine metabolism pathway, as both strains contained arginine decarboxylase (*adiA*, *speA*), agmatine deiminase (*aguA*), N-carbamoylputrescine amidase (*aguB*) and ornithine decarboxylase (*speC*) that are required for the classical arginine-derived putrescine biosynthesis pathway. Putrescine is a biogenic-amine commonly produced by microorganisms during food spoilage and associated with health issues after consumption (Majer-Baranyi et al., 2023). Additionally, both organisms contained genes required for acetoin production (*ilvB*, *ilvH* and *budC*), highlighting the potential role as meat spoilers for these two strains.

We assessed the occurrence of motility- and biofilm-related genes in the genomes of the two isolates (Supplementary Table S3). *P. bubulae* 0015 encoded for a complete pathway necessary for flagellar assembly

and contained *pilG*, *pilH*, *pilI*, *pilJ* and *mqsR* genes, required for twitching motility. *P. immobilis* 1601, however lacked a complete flagellar assembly pathway, but did encode *pilT*, *pilU*, *pilG*, *pilH*, *pilI* and *pilJ*, indicating partial motility potential in its genome.

Regarding biofilm formation, *P. bubulae* 0015 possessed key regulatory pathways, including the cAMP/Vfr signalling pathway necessary for flagellar biosynthesis and assembly, the Gac/Rsm pathway necessary for assembly of the type VI secretion system regulation, as well as the c-di-GMP signalling pathway essential for biofilm formation. It also encoded for BarA-UvrY/CsrA system, known in *E. coli* to regulate biofilm synthesis, along with additional systems regulating biofilm formation under exposure to various environmental stressors such as envelope stress, amino acid starvation, osmolarity, and carbohydrate limitations. Additionally, it contained the VarS/VarA, two component system required for quorum sensing and partially encoded pathways for polysaccharide synthesis and the type II secretion system which play a role in biofilm formation.

P. immobilis 1601 isolate had a partial gene set for flagellar biosynthesis and assembly, as well as components of the Gac/Rsm pathway, BarA-UvrY/CsrA system and the VarS/VarA two component system. This genetic capacity to form biofilm supports the hypothesis that both strains, *Pseudomonas* and *Psychrobacter*, are capable of forming biofilms (Fig. S6).

2.6. *P. immobilis* 1601 strain forms biofilms in nutrient poor environments

To confirm the biofilm-forming capacity of the isolated strains, a safranin biofilm staining assay was performed. The strains were incubated for 22 h at 5 °C, 10 °C, 14 °C and 25 °C, both in monoculture and in coculture. The temperatures were chosen to fit the range of temperatures recorded in different parts of the slaughterhouse, whereas the incubation length was chosen to fit the amount of time between the facility cleaning procedures. *P. fragi* BF1 strain was used as a positive control due to their previously reported biofilm forming capabilities (Wagner et al., 2020). The bacteria were inoculated into three different media that challenged nutrient availability, i.e., 1:10 BHI, 1:10 TSB, and RPMI. Under the tested conditions, *P. bubulae* 0015 did not form detectable biofilms. *P. fragi* BF1 was able to build a lower amount of biofilm at 14 °C and 25 °C. *P. immobilis* 1601 produced a low amount of biofilm in 1:10 TSB at 5 °C, and a larger biofilm in RPMI media at all tested temperatures.

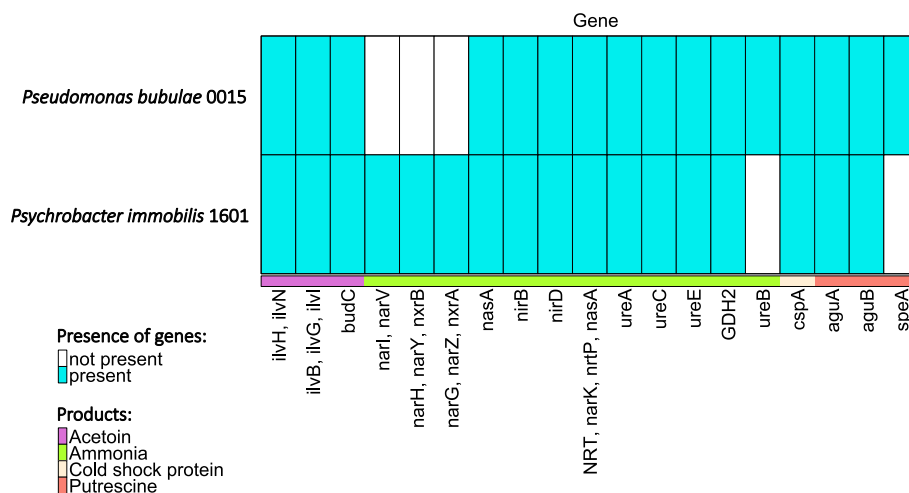


Fig. 5. Presence of metabolism related genes in the genomes of *Pseudomonas bubulae* 0015 and *Psychrobacter immobilis* 1601. Heat map depicting the presence or absence of cold shock-related genes, genes required for production of acetoin, ammonia and putrescine, based on KEGG Pathway annotations in the two strains. Genes were grouped according to metabolic pathways that produce end products relevant to meat spoilage. If multiple gene names were available for a single KEGG annotation, all are listed on the bottom X-axis, separated by a comma.

Biofilm formation in coculture was detectable only in RPMI media at all tested temperatures, although the coculture biofilms were smaller than those formed by *P. immobilis* 1601 alone (Fig. 6). These results indicate that *Psychrobacter immobilis* 1601 was the best biofilm former out of those tested, particularly under nutrient-limited conditions.

3. Discussion

The results presented here offer a comprehensive overview of how a certain fraction of the bacterial community might be able to survive cleaning procedures and spread in a beef slaughterhouse during production. Additionally, it includes a detailed characterization of two bacterial strains isolated directly from meat processed during the same production shift. By integrating a longitudinal metagenomic sequencing through a working shift and by sampling individual equipment (instead of common practices pooling food-contact and non-food-contact equipment all together), followed by targeted culturing, whole-genome sequencing and in vitro validation, we present a novel perspective on contamination risks in these facilities. Additionally, we highlight specific equipment (i.e., membrane skinner) that can act as key sources of microbial contamination to the meat product and that must undergo a deeper and dedicated cleaning procedure.

The microbial diversity directly after cleaning, pre-shift, was found to be significantly higher than at later time points. This can indicate that

the microbes present at the beginning of the shift tend to establish themselves, with certain taxa dominating. If such a scenario is taking place, the functional profile of the pre-shift microbiome would be dominated by those features harboured by the dominant taxa, which might explain the decrease in certain genomic features belonging to other members of the microbiome. However, any conclusions should be drawn carefully, as several technological limitations occur. First, our attempts to perform high-quality metagenomic assemblies failed, with a big portion of the sequencing data (>70%) being excluded from the final assemblies in some cases. In such a scenario, the vast majority of the biological information is not considered and thus the results would be misleading. Therefore, we opted for an assembly-free approach, where all the high-quality sequencing reads are aligned against taxonomic and functional references. While the approach is solid, the short length of the reads (150 bp, Illumina sequencing) enable their alignment against reference genes but hinders the investigation of the genomic surrounding, restricting the capacity to associate multiple features and taxonomy for the same sequencing read. Additionally, our metagenomic results must be considered semiquantitative (and referenced as relative abundances and/or counts per million, CPM). In order to infer absolute abundances, we calculated BCEs as a solid proxy for bacterial quantification. However, this approach can be biased by the DNA presumably coming from lysed or dead microbes, which might be inherently purified together with the other nucleic acids and sequenced. While fragmented

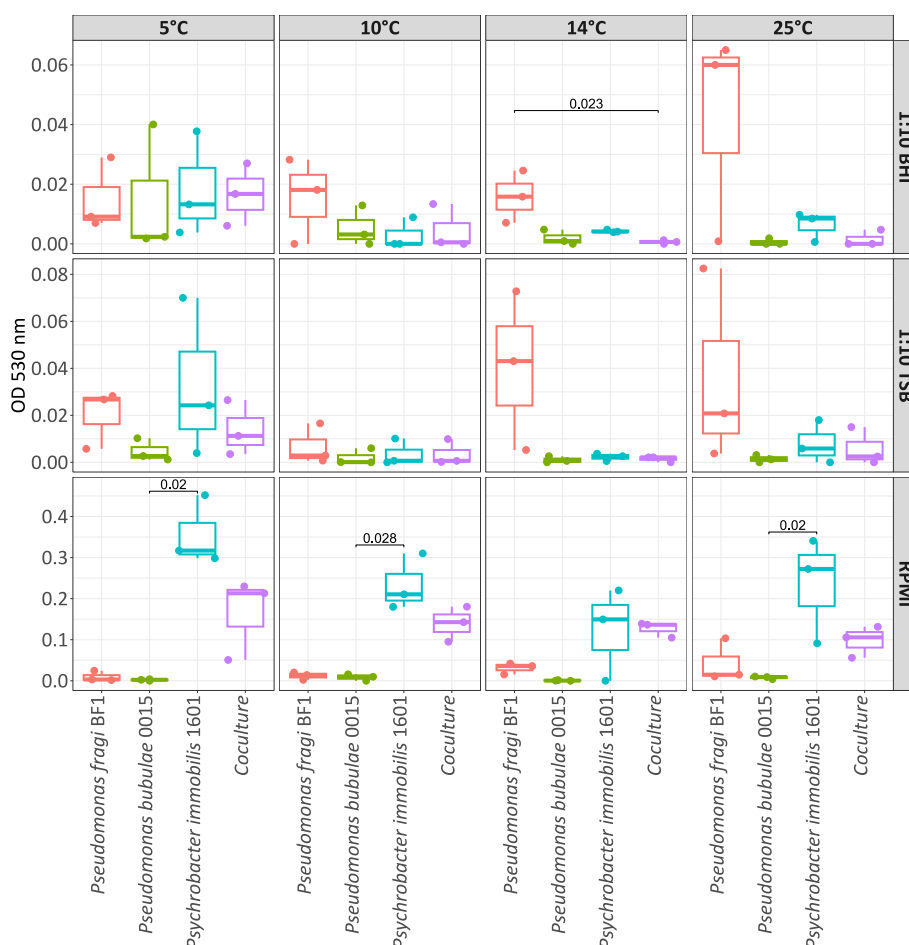


Fig. 6. Comparison of biofilm formation phenotype between *Pseudomonas bubulae* 0015 and *Psychrobacter immobilis* 1601. a) The box plots show the optical densities at 530 nm of the safranin-stained biofilms resuspended in 33% acetic acid. Biofilm formation was tested for *Pseudomonas bubulae* 0015, *Psychrobacter immobilis* 1601 and the coculture of those two strains. *Pseudomonas fragi* BF1 was used as a positive control. The bacteria were incubated in three media types: 1:10 BHI, 1:10 TSB and RPMI ($n = 3$). Statistically significant differences between the cultures were determined using Kruskal–Wallis test, followed by Dunn's post-hoc test. Box plots show the median with hinges that correspond to the 25th and 75th percentiles, and whiskers extending from the hinge to the highest and lowest values within 1.5 * interquartile range. Points indicate the means of four technical replicates.

or low quality DNA might have been removed during library preparation and sequencing quality control, further studies collating complementary techniques such as RNA sequencing or the use of DNA-binding dyes (e. g., propidium monoazide, PMA) might be needed to assess the viability of the cells occurring on presumably clean surfaces. These limitations are also inherent to most microbiome studies, which might be benefited by the evolution of the state-of-art techniques in the field, including a combination of longer read sequencing, multiomics integration (i.e., transcriptomics, proteomics and metabolomics) and high-throughput culturing and phenotyping.

AMRG showed a higher abundance in pre-shift samples, collected from the cleaned facility, with some AMRGs reappearing by end of the production shift. The drop of AMRGs from pre-shift to mid-shift might point towards a good cleaning efficiency. While the higher count in the pre-shift samples can be argued to derive from relic DNA, the increase of AMRGs at the shift-end compared to mid-shift that can be observed on some surfaces (such as the conveyor belt, gloves at the packing station and the knives) points towards the establishment of certain taxa that overcome production and cleaning practices. The emergence of AMRGs at the shift-end could be correlated to that on the carcasses, where the AMRG load appeared to be higher than on the rest of the surfaces (Fig. S4). However, from our data we cannot point or rule out the carcasses as the source of AMRG spread through the facility, which might require further investigation. The same trend was observed also for the biofilm related genes annotated in the metagenome. While most of the genes were detected after cleaning, there was an increase in the abundance of biofilm related genes on different surfaces at shift-end compared to mid-shift, such as the conveyor belt, cutting table pool and gloves used for packing. These genes were also detected on the carcasses at shift-end.

The most abundant bacteria at the shift-end were *Psychrobacter immobilis*, *Psychrobacter maritimus*, *Moraxella porci*, *Galactobacter caseinivorans*, *Micrococcus luteus*, *Pseudomonas fragi* and *Acinetobacter pseudolwoffii*, all of which have frequently been reported as part of bacterial communities in food processing environments (Barcenilla, Cobo-Díaz, Puente, et al., 2024; Sequino et al., 2024; Xu et al., 2023; Zhang et al., 2019; Zwirzitz et al., 2020). Sadly, despite our efforts to improve DNA purification, the swabs taken from the meat at mid-shift and from packed meat at shift-end had to be removed from analysis due to very low sequencing depth, thus limiting our vision to the events occurring at the slaughterhouse (a complementary study following the same meat after packaging was performed in Roch et al., 2025). *P. fragi*, *P. immobilis* and *P. maritimus* were detected before the working started, and their abundance increased throughout the working shift. These three species were detected on most surfaces at the shift-end, with *Psychrobacter* species present on conveyor belt, cutting tables, drain, gloves, membrane skinner, non-food contact surfaces, and on sorting tables. In the pre-shift samples, the same species were detected, albeit at lower abundances, on conveyor belts, cutting tables, non-food contact surfaces and drain, with *P. maritimus* and *P. fragi* additionally observed on the membrane skinner. In this study, *Pseudomonas* and *Psychrobacter* were selected as focal taxa due to their increasing abundance in the facility over time and their established roles as spoilage organisms. Both genera exhibited a higher overall prevalence than *Brochothrix thermosphacta*, another common food spoiler that also increased in abundance during the sampling period. In contrast, *Galactobacter caseinivorans*, although detected and observed to increase in abundance, is not currently associated with food spoilage.

The *P. fragi* and *P. immobilis* isolates were observed to grow at 5 °C and 10 °C in both nutrient-poor and nutrient-rich media within 24 h, confirming their ability to proliferate under conditions present in the facility. *P. fragi* and *Psychrobacter* have also been shown to increase in abundance through duplication within 10 h (Hebraud et al., 1994; Juni & Heym, 1986), and their biofilm forming ability could allow them to persist on some surfaces, particularly on those where the complexity of the equipment might lead to inefficient cleaning procedures, such as the

membrane skinner. Additionally, our results suggest that the contamination might also originate from beef carcasses. *P. fragi*, *P. immobilis* and *P. maritimus* were detected on meat and carcasses sampled at the shift-end. Based on their ability to grow on cold surfaces, we consider that *Psychrobacter*, *Pseudomonas*, and other psychrotrophic bacteria might also proliferate on carcasses during the cooling period, from where they subsequently contaminate contact surfaces within the facility. This hypothesis would need to be confirmed by a more detailed focus on carcasses over the storage period. Furthermore, *Psychrobacter*, *Acinetobacter*, *Pseudomonas*, and *Moraxella* were also detected on the surface of meat processed and packed in the same facility on the day of sampling (Roch et al., 2025), indicating that the bacteria found in high abundance at the shift-end might also end up on the final product. Therefore, we consider that the initial contamination in the producing surfaces might originate from carcasses, while the biofilm-forming capability of the psychrophilic fraction of the bacterial community causes their persistence in the equipment despite the cleaning procedures.

The persistence of *Pseudomonas* in the beef-producing facility can promote meat loss, as they have been reported to cause off-odours and slime in air stored and even modified atmosphere packaged meat (MAP) (Doulgeraki et al., 2012; Ercolini et al., 2009; European Food Safety Authority, 2016). In meat, *Psychrobacter* is usually detected at the beginning of shelf life, however it is later replaced mainly by lactic acid bacteria in vacuum packaged meat (Roch et al., 2025) or by *Pseudomonas* and *Brochothrix* in air-stored meat and MAP-meat (Doulgeraki et al., 2012; J. Yang et al., 2025).

Both *P. bubulae* 0015 and *P. immobilis* 1601 isolates harboured genes associated with biofilm formation. Interestingly, even though *P. bubulae* 0015 encoded for more biofilm related genes and more complete biofilm formation pathways, *P. immobilis* 1601 was a better biofilm former under experimental conditions, even exceeding the capacities of the *P. fragi* BF1 strain used as control based on its previous strong biofilm forming capacities (Wagner et al., 2020). Our hypothesis as to why *P. bubulae* did not form biofilms in the tested conditions was that the incubation time of 24 h was not a long enough time for *P. bubulae* to form biofilms.

P. immobilis 1601, might be more efficient in biofilm formation due to lack of *bifA* gene (Fig. S6). Previous studies have showed that *bifA* deletion mutants of *Pseudomonas aeruginosa* accumulate c-di-GMP, and subsequently have increased biofilm production in comparison to the wild type (Kuchma et al., 2007). Interestingly, combining *P. bubulae* 0015 and *P. immobilis* 1601 reduced the biofilm capacity of the latter, highlighting the potential competition of the two species.

Psychrobacter has typically received little attention as a potential biofilm former in food processing environments and neglected in its role as potential spoiler. However, the results generated here unravel that *P. immobilis* is able to form biofilms within 24 h at low temperatures in nutritionally poor environments, imitating food-producing conditions. The biofilm producing capacity of *P. immobilis* might explain their survival in pre-shift samples on cutting tables and membrane skinner, from where they can contaminate the meat processed in the facility—based on the evidence that we found metagenomic regions in meat with high ANI similarity to the *P. immobilis* genome. This finding implicated the cutting tables and the membrane skinner as potential hot spots harbouring spoilage bacteria. Even though the cutting tables were exchanged after mid-shift sampling, the tables used at shift-end were in the facility the entire time of the sampling and thus were exposed to the facility's environment. In the case of the cutting tables, it is worth considering whether the material facilitates better bacterial adhesion and biofilm formation, as the tables in the sampled facility were made of plastic. However, while our results provide novel insights into biofilm forming abilities of *P. immobilis*, our isolate-based biofilm assays do not provide a complete overview of the biofilm forming capacity of the entire microbiome occurring in the facility. Additionally, it only provides the confirmation that the tested strain is able to build a biofilm by its own, without considering multispecies biofilms that might happen in

the producing environment.

It is important to mention that *P. bubulae* was not annotated by MetaPhlAn as this species is included in the same SGB with *P. fragi*. However, tracing *P. fragi*, we reported them on the membrane skinner in the pre-shift sample and on the meat, indicating that *P. bubulae* 0015 also survives cleaning and is sufficiently established and present enough to be transferred on the meat. The fact that it was detected only on the membrane skinner and not on other surfaces could be attributed to differences in cleaning frequency and thoroughness. Cutting tables, conveyer tables, gloves and knives are exchanged and cleaned more frequently and thoroughly than the membrane skinner. Due to its complexity, the membrane skinner needs to be disassembled for cleaning and cannot therefore be cleaned while the facility is operational. This limitation increases the likelihood of psychrophilic bacteria growing on its surfaces. Additionally, the membrane skinner is covered in excess fat, membranes and other tissues throughout the day, making it a relatively nutrient-rich zone compared to other surfaces in the facility.

Our research provides a small window into the microbial colonization of a cutting and packing area in an abattoir after cleaning and brings a new perspective on the factors triggering food waste. However, focusing on one facility can only give us a snapshot of the bacterial community for this specific facility at a specific time. In future research it would be worth looking into multiple different beef abattoirs and comparing the functional profiles of their in-house microbiota. While most food-safety surveys focus solely on pathogens, it is pivotal to include potential spoilage bacteria in the equation, due to their role in food waste and their subsequent economic and climatic impact.

4. Methods

4.1. Sampling plan

The sampling was conducted in the cutting and packing area of an International Featured Standard-Food certified beef slaughterhouse located in Austria. Surface samples from meat-contact surfaces, non-contact surfaces, and the surfaces of meat and carcasses were sampled using Whirl-Pak B01592WA Hydrated PolyProbe™ swabs (Fig. 1). The samples were obtained at three distinct time points during a single work shift: i) pre-shift: at the start of the day, when the cleaning of the facility was finished; ii) mid-shift, three hours after the start of the shift; and iii) shift-end: six hours and 30 min after the second sampling. Same surfaces were sampled over the three time points, with certain exceptions. The gloves were exchanged during the shift and represent snapshots of bacterial diversity present at the cutting and packing stations at the specific time points. Different knives were sampled each time point, however all the knives were kept together and were in contact with one another throughout the entire shift. The cutting tables were exchanged after the mid-shift sampling. The cutting tables used to make the switch were stored in the facility the entire time of the sampling. The new tables were used for cutting for ~3 h before they were sampled. Samples were selected after a thorough inspection of the meat processing facility to cover a wide variety of surfaces (Fig. 1, Supplementary Table S1 and S2). All the surfaces along the cutting and packing line were observed to come into direct contact with the product were sampled, including gloves and knives used at time of the sampling. Non-contact surface samples included the swabs taken from the floor where the carcasses pass on the rail and the walls where traces of blood from the carcasses were observed. The meat samples taken mid-shift, and the samples of vacuum bags and the packed meat taken at shift-end were omitted from further analysis due to a low number of reads. The temperature of the cutting area varied between 10 °C and 15 °C during the sampling period. In contrast, the cold room used for cooling freshly slaughtered halved carcasses varied between 0 °C and 8 °C over a 24 h-period, while the cold room for storing primal cuts remained stable at around 2 °C.

4.2. DNA extraction and shotgun metagenome sequencing

Swabs samples collected from the meat processing facility were processed following the protocol described by Barcenilla et al. (Barcenilla, Cobo-Díaz, De Filippis, et al., 2024). Three positive controls were prepared by adding 10 ml of ZymoBiomics Microbial Community Standard D6300 (Zymo Research) at three different concentrations (1.4×10^6 , 1.4×10^4 and 1.4×10^2 cells/ml) into five clean pooled swabs per control. Additionally, three negative controls were included, i) a facility control consisting of five swabs exposed to ambient air in the processing area for one minute, ii) a laboratory control consisting of five swabs exposed to ambient air in the lab for one minute and iii) a DNA extraction negative control. DNA extraction was performed using the DNeasy Power Food Kit (QIAGEN GmbH, 2017) according to the manufacturer's protocol, with an additional step of involving heating the bacterial suspension in lysis buffer at 70 °C for 10 min. DNA was eluted in 50 µl of DEPC-treated water. All controls were processed identically to the experimental samples. Deep shotgun metagenome sequencing was conducted on Illumina NovaSeq 6000 platform, with a 2×150 bp paired-end run, and with a throughput of 60 million paired-end reads per sample.

4.3. Quantitative qPCR for swab samples

DNA (1 µl) from all samples was pooled, and a conventional 16S rRNA gene PCR was performed using the primer set based on Muyzer et al. (Muyzer et al., 1993), 341F (5'-CCTACGGGNGGCWGCAG-3') and 534R (5'-ATTACCGGGCTGTGG-3') to generate the required amount of DNA (194 bp) for the qPCR standard. The resulting DNA was purified using the innuPREP PCRpure Kit (IST Innuscreen GmbH, Germany) and quantified with the Qubit™ dsDNA BR Assay Kit (Thermo Fisher Scientific), using a Qubit™ 2.0 Fluorometer (Life Technologies Corporation, Carlsbad, California, United States). Briefly, DNA samples were assayed in duplicate in a 20-µl reaction mixture, which contained 10 µl Brilliant III Ultra-Fast SYBR MM (Agilent), 5 µl DEPC-treated water, 2 µl of each primer (stock concentration 10 µM), and 1 µl of the diluted template (gDNA). The quantification of DNA was performed in an Mx3000P qPCR instrument (v.4.10; Stratagene, La Jolla, CA, United States) with an initial denaturation at 95 °C for 3 min, followed by 45 cycles of 95 °C for 5 s, 60 °C for 20 s.

To determine the specificity of the amplifications, dissociation curves after each reaction were recorded and carried out at 95 °C for 1 min, followed by complete annealing at 50 °C for 30 s, and a gradually increasing temperature up to 95 °C. Post-run melting curves were checked for the presence of multiple peaks due to primer-dimers or non-specific amplification. Additionally, negative controls without templates were included in each qPCR reaction. The copy number of the 16S rRNA gene was determined by assuming that based on the entered amount of DNA (1 ng), weight and 194 bp length of the template, 1 ng of DNA equals 4.78×10^9 number of copies.

4.4. Metagenomic sequencing data analysis

Raw sequence reads shorter than 75 bp and with a Phred quality score below = 25 were discarded by using fastp (Chen, 2023), which also eliminated residual adapter sequences. Reads originating from the host, human, and PhiX reads were removed using Bowtie2 (Langmead & Salzberg, 2012).

Using an assembly-free approach, we performed a semi-quantitative assessment of microbial taxonomy and the abundance of key functional genes. MetaPhlAn4 (Blanco-Míguez et al., 2023) was used for microbiome profiling and identification of the relative abundance (in percentage, RA_i) of the different taxa within the samples.

The number of bacterial cell equivalents (BCE) was calculated from the total 16S rRNA copy numbers per sample obtained from qPCR (tCN) using the following formula:

$$BCE = \frac{tCN \times \text{dilution factor} \times \text{elution volume}}{MCN} \quad (1)$$

Here, MCN represents the mean 16S rRNA gene copy number with a value of six, estimated by the rrnDB database (Stoddard et al., 2015). BCEs were then used to calculate the absolute abundance (AA_i) of the different taxa within the samples using the following formula:

$$AA_i = \frac{RA_i}{100} \times BCE \quad (2)$$

This was done in order to estimate and compare the biomass of individual samples.

Antimicrobial resistance (AMR) quantitative profile was evaluated by aligning the sequencing reads using Bowtie2 (Langmead & Salzberg, 2012) against the ResFinder (Bortolaia et al., 2020) database. To minimize spurious or multi-alignments caused by short-length reads, we clustered AMR gene sequences from ResFinder over 90% identity and coverage similarity by using CD-HIT (Li et al., 2001, 2002; Li & Godzik, 2006). For AMR and biofilm relative abundance estimation, alignments were calculated by using featureCounts (Liao et al., 2014) and gene counts were normalized to counts per million (CPM) based on the proportion of reads assigned to bacteria. Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa & Goto, 2000) annotations for each sample were filtered for genes assigned to the KEGG biofilm-formation pathways of *Pseudomonas aeruginosa* (map02025), *Escherichia coli* (map02026), and *Vibrio cholerae* (map05111).

For the assembly-based approach, the QC-controlled sequencing reads were assembled using Spades (Prjibelski et al., 2020). The quality of the assemblies was evaluated with Quast (Mikheenko et al., 2018), and contigs shorter than 1000 bp were excluded from further analysis. Taxonomy classification of the contigs was performed using Kraken2 (Wood et al., 2019) and the PlusPFP database. Bacterial coding sequences (CDS) and protein predictions were generated using Prodigal (Hyatt et al., 2010). Functional protein annotation of predicted proteins was performed using Diamond (Buchfink et al., 2021) against the KEGG database. AMRGs were screened by aligning the predicted CDS to the ResFinder (Bortolaia et al., 2020) database using BLASTN (Altschul et al., 1990).

4.5. *Psychrobacter* and *Pseudomonas* isolates

Psychrobacter immobilis 1601 and *Pseudomonas bubulae* 0015 strains were isolated and cultured as part of a thorough culturomic assay performed in a complementary study with a different scope that was conducted in the same slaughterhouse and on the same day and working shifts of the present study (Roch et al., 2025). These two strains were isolated from the final meat products (during separate sampling not subjected to whole DNA extraction to avoid cross-contamination). *Pseudomonas* 0015 was isolated using a Plate Count Agar (PCA, Biokar Diagnostics, Allonne, France), incubated aerobically at 30 °C for 48 h. In order to isolate *Psychrobacter* spp., a targeted cultivation on Marine Agar (marine broth (Roth, Karlsruhe, Germany), supplemented with 1.5% bacteriological agar type E (Biokar Diagnostics)) was performed. For that, frozen cell pellets, obtained from the meat samples with the highest relative abundances of *Psychrobacter* spp., were thawed, serially diluted, and plated. Plates were incubated aerobically at 20 °C for 72 h.

4.6. Whole genome sequencing

DNA extraction, library preparation, sequencing and sequence analysis were performed following the protocol outlined by Roch et al. (Roch et al., 2025). DNA extraction was performed according to Nanopore-only Microbial Isolate Sequencing Solution (NO-MISS) Rapid Barcoding Kit, as recommended by Nanopore (Oxford Nanopore Technologies, 2024a, 2024b). Bacterial pellets were resuspended in 100 µl lysozyme (25 mg ml⁻¹) from chicken egg white (Sigma-Aldrich,

dissolved in TE-buffer ((pH 8.0; 10 mM TRIS Pufferan®, Carl Roth GmbH + Co. KG, Karlsruhe, Germany), 1 mM EDTA (Sigma-Aldrich)). The DNA was eluted in 40 µl of preheated (70 °C) Monarch™ gDNA elution buffer.

Library preparation was performed using the Rapid Barcoding Kit V14 (SQK-RBK114.24, Oxford Nanopore Technologies, Oxford, UK) according to protocol ISO_9205_v114_revC_30Sept2024 for the MinION (Oxford Nanopore Technologies, 2024a, 2024b). The sequencing was carried out for 72 h using the MinKNOW™ software (v. 24.06.16, Oxford Nanopore Technologies) on a MinION Mk1B device (Oxford Nanopore Technologies) using the MinION Flow Cells (FLO-MIN106D, Oxford Nanopore Technologies).

4.7. Whole genome sequencing analysis of the isolated species

The basecalling, using model dna_r10.4.1_e8.2400bps_sup@v.5.0.0 and the demultiplexing was done with dorado (v. 0.8.3) (Oxford Nanopore Technologies, 2024b). The reads were then filtered for a min length of 1000 bp and a min mean quality score of 9 using filtlong (v. 0.2.1) (Wick, 2021). The genome assembly was done using 3 iterations of flye (v. 2.9.5) (Kolmogorov et al., 2019), followed by a polishing using medaka (v. 2.0.1) (Oxford Nanopore Technologies, 2022). The quality of the assemblies was checked using checkm (v. 1.2.3) (Parks et al., 2015). The bacterial isolates were classified taxonomically by using GTDB-tk (v. 2.4.0) (Chaumeil et al., 2020, 2022) and Kraken2 against the PlusPFP database (downloaded on 13.12.2024). Protein-coding sequences were predicted using Prodigal (Hyatt et al., 2010), whereas protein annotation was performed using Diamond (Buchfink et al., 2021) against the KEGG database. InStrain (Olm et al., 2021) was used to compare the metagenomic data to the whole genomes of the isolates and to determine the average nucleotide identity (ANI) between the isolates and bacterial strains present in the metagenomes. A coverage threshold of >1 was applied to determine which samples aligned best to the isolate genomes. Metabolic pathways, biofilm formation associated genes and flagellar assembly associated genes were subsequently mapped, using the KEGGREST (Tenenbaum & Maintainer, 2025) R package (Supplementary Table S3). Genomes were further screened for genes associated with bacterial motility, as listed in Supplementary Table S3.

4.8. Statistics and visualisation

All statistical analysis and visualisation was performed in the R (R Core Team, 2024) environment (v. 4.3.3). Alpha diversity was estimated using Shannon diversity indices, calculated with the vegan (Oksanen et al., 2022) package. The Shapiro–Wilk test was applied to assess normality. Group comparisons were conducted using ANOVA tests, followed by Tukey's post-hoc tests. For beta diversity analyses, Bray–Curtis dissimilarities distance matrices were generated, tested for homogeneity of multivariate dispersion using the ANOVA test, and subsequently used for graphical (PCoA) and statistical (PERMANOVA) analysis also using the vegan (Oksanen et al., 2022) package. The Differences in biofilm formation between cultures were evaluated for statistical significance using the Kruskal–Wallis test, followed by Dunn's post-hoc test using the dunn.test (Dinno, 2024) R package. Plots were generated using ggplot2 (Wickham, 2016) and ComplexHeatmap (Gu, 2022; Gu et al., 2016) packages, whereas amino-acid metabolic pathways and biofilm formation pathways were mapped using the pathview (Luo & Brouwer, 2013) and KEGGREST (Tenenbaum & Maintainer, 2025) packages in the R (R Core Team, 2024) environment.

4.9. Biofilm assay

Single colonies of *Pseudomonas bubulae* 0015 and *Pseudomonas fragi* BF1 grown on TSA + Y plates (TSA and Yeast, Biokar Diagnostics) for 24 h at 30 °C, and *Psychrobacter immobilis* 1601, grown for 48 h at 25 °C on Marine Agar (Marine Broth (Roth, Karlsruhe, Germany) plates, were

inoculated using a sterile loop into 8 ml tryptone soy broth with yeast extract (TSB and Yeast, Biokar Diagnostics) and incubated at room temperature with shaking at 320 rpm. A *P. fragi* BF1 isolate was included as a positive control, as it has been previously described as a strong biofilm former (Wagner et al., 2021). The following day, the optical density at 600 nm (OD₆₀₀) was measured and adjusted to 0.1 in three different media: 1:10 diluted brain-heart infusion broth (BHI, Biokar Diagnostics), 1:10 diluted TSB (Biokar Diagnostics) and Roswell Park Memorial Institute medium (RPMI 1640 Media, Cytiva HyClone™, Wilmington DE, United States). RPMI media was selected for its low concentration of amino acids and lack of peptides and lipids. Aliquots of each diluted culture (150 µL per well) were dispensed into a 96-well flat-bottom CELLSTAR® microtiter plate (Greiner Bio-One, Kremsmünster, Austria), with four technical replicates per culture–medium combination. For co-culture conditions, 75 µL of each isolate (*P. bubulae* 0015 and *P. immobilis* 1601) were combined per well. Plates were subsequently incubated at 5 °C, 10 °C, 14 °C, and 25 °C. After 22 h, the supernatants were gently removed, and the biofilms were washed three times with 200 µL of 0.9% NaCl solution. The plates were air-dried for 30 min and subsequently stained with 200 µL of Gram's safranin solution (solution for microscopy, Sigma-Aldrich). Safranin staining has been successfully used recently to investigate biofilm formation (Lembke et al., 2006; Ommen et al., 2017; Standar et al., 2010) and reduces worker exposure to carcinogen or mutagenic chemicals. After 10 min the staining solution was removed, and wells were again washed three times with 200 µL of 0.9% NaCl solution. Following a second drying step (30 min at room temperature), biofilms were solubilized in 200 µL of 33% glacial acetic acid per well and incubated for 30 min. Absorbance was measured at 530 nm to quantify biomass. Biofilm assays were performed in three biological replicates, each with four technical replicates. All media used in the experiments were routinely checked for potential contamination. Biofilm formation was assessed comparatively, without application of a quantitative scale.

CRedit authorship contribution statement

Tea Movsesijan: Conceptualization, Investigation, Formal analysis, Writing – Original Draft, Writing – Review & Editing, Data curation. **Alejandro J. Alcañiz:** Formal analysis, Data curation. **Franz-Ferdinand Roch:** Conceptualization, Investigation, Formal analysis, Writing – review & editing. **Muhammad Sharjeel Chaughtai:** Investigation. **Monika Dzieciol:** Investigation, Methodology, Writing – Review & Editing. **Beatrix Stessl:** Conceptualization. **Sarah Thalgueter:** Conceptualization, Investigation. **Cameron R. Strachan:** Formal analysis, Data curation. **Markus Raindl:** Conceptualization. **Martin Wagner:** Conceptualization, Funding acquisition. **Evelyn Selberherr:** Conceptualization, Writing – Original Draft, Writing – Review & Editing, Supervision. **Narciso M. Quijada:** Formal analysis, Writing – Original Draft, Writing – Review & Editing, Data curation, Supervision. All authors provided critical feedback on the research, analysis, and manuscript and approved the final version of the manuscript.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

M.R. is employed by Rudolf Großfurtner GmbH, who's facilities and products we sampled. This, however, did not influence sampling, analyses, or data interpretation.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodres.2026.118808>.

Data availability

All sequencing data supporting the findings of this study are openly available in European Nucleotide Archive (ENA) under the study accession numbers PRJEB102793.

References

- Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, 215(3), 403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)
- Barcenilla, C., Cobo-Díaz, J. F., De Filippis, F., Valentino, V., Cabrera Rubio, R., O'Neil, D., ... Alvarez-Ordóñez, A. (2024). Improved sampling and DNA extraction procedures for microbiome analysis in food-processing environments. *Nature Protocols*, 19(5), 1291–1310. <https://doi.org/10.1038/s41596-023-00949-x>
- Barcenilla, C., Cobo-Díaz, J. F., Puente, A., Valentino, V., De Filippis, F., Ercolini, D., ... Alvarez-Ordóñez, A. (2024). In-depth characterization of food and environmental microbiomes across different meat processing plants. *Microbiome*, 12(1), 199. <https://doi.org/10.1186/s40168-024-01856-3>
- Blanco-Míguez, A., Beghini, F., Cumbo, F., McIver, L. J., Thompson, K. N., Zolfo, M., ... Segata, N. (2023). Extending and improving metagenomic taxonomic profiling with uncharacterized species using MetaPhlAn 4. *Nature Biotechnology*, 41(11), 1633–1644. <https://doi.org/10.1038/s41587-023-01688-w>
- Bortolaia, V., Kaas, R. S., Ruppe, E., Roberts, M. C., Schwarz, S., Cattoir, V., ... Aarestrup, F. M. (2020). ResFinder 4.0 for predictions of phenotypes from genotypes. *Journal of Antimicrobial Chemotherapy*, 75(12), 3491–3500. <https://doi.org/10.1093/jac/dkaa345>
- Buchfink, B., Reuter, K., & Drost, H. G. (2021). Sensitive protein alignments at tree-of-life scale using DIAMOND. *Nature Methods*, 18(4), 366–368. <https://doi.org/10.1038/s41592-021-01101-x>
- Buzby, J. C., Wells, H. F., & Hyman, J. (2014). The estimated amount, value, and calories of postharvest food losses at the retail and consumer levels in the United States. *Food Loss in the United States: Selected Analyses*, 1–42. <https://doi.org/10.2139/ssrn.2501659>
- Chaumeil, P.-A., Mussig, A. J., Hugenholtz, P., & Parks, D. H. (2020). GTDB-Tk: A toolkit to classify genomes with the genome taxonomy database. *Bioinformatics*, 36(6), 1925–1927. <https://doi.org/10.1093/bioinformatics/btz848>
- Chaumeil, P.-A., Mussig, A. J., Hugenholtz, P., & Parks, D. H. (2022). GTDB-Tk v2: Memory friendly classification with the genome taxonomy database. *Bioinformatics*, 38(23), 5315–5316. <https://doi.org/10.1093/bioinformatics/btac672>
- Chen, S. (2023). Ultrafast one-pass FASTQ data preprocessing quality control and deduplication using fastp. *IMeta*, 2. <https://doi.org/10.1002/imt2.107>
- Conrad, R. E., Brink, C. E., Viver, T., Rodríguez-R, L. M., Aldegue-Riquelme, B., Hatt, J. K., ... Konstantinidis, K. T. (2024). Microbial species and intraspecies units exist and are maintained by ecological cohesiveness coupled to high homologous recombination. *Nature Communications*, 15(1), 1–12. <https://doi.org/10.1038/s41467-024-53787-0>
- Dave, D., & Ghaly, A. E. (2011). Meat spoilage mechanisms and preservation techniques: A critical review. *American Journal of Agricultural and Biological Science*, 6(4), 486–510. <https://doi.org/10.3844/ajabssp.2011.486.510>
- De Filippis, F., La Stora, A., Villani, F., & Ercolini, D. (2013). Exploring the sources of bacterial spoilers in beefsteaks by culture-independent high-throughput sequencing. *PLoS One*, 8(7), Article e70222. <https://doi.org/10.1371/journal.pon.0070222>
- Dinno, A. (2024). *Dunn Test: Dunn's test of multiple comparisons using rank sums (version 1.3.6) [Computer software]*. CRAN. <https://doi.org/10.32614/CRAN.package.dunn.test>
- Donlan, R. M. (2002). Biofilms: Microbial life on surfaces. *Emerging Infectious Diseases*, 8(9), 881–890. <https://doi.org/10.3201/eid0809.020063>

- Doulgeraki, A. I., Ercolini, D., Villani, F., & Nychas, G. J. E. (2012). Spoilage microbiota associated to the storage of raw meat in different conditions. *International Journal of Food Microbiology*, 157(2), 130–141. <https://doi.org/10.1016/j.ijfoodmicro.2012.05.020>
- Ercolini, D., Russo, F., Nasi, A., Ferranti, P., & Villani, F. (2009). Mesophilic and psychrotrophic bacteria from meat and their spoilage potential in vitro and in beef. *Applied and Environmental Microbiology*, 75(7), 1990–2001. <https://doi.org/10.1128/AEM.02762-08>
- European Food Safety Authority. (2016). Growth of spoilage bacteria during storage and transport of meat. *EFSA Journal*, 14(6), Article 4523. <https://doi.org/10.2903/j.efsa.2016.4523>
- Flemming, H. C., van Hullebusch, E. D., Neu, T. R., Nielsen, P. H., Seviour, T., Stoodley, P., ... Wuertz, S. (2023). The biofilm matrix: Multitasking in a shared space. *Nature Reviews Microbiology*, 21(2), 70–86. <https://doi.org/10.1038/s41579-022-00791-0>
- 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(9), 563–575. <https://doi.org/10.1038/nrmicro.2016.94>
- Food and Agriculture Organization of the United Nations. (2021). *The state of food and agriculture 2021: Making agrifood systems more resilient to shocks and stresses*. <https://doi.org/10.4060/cb4476en>
- Giuliodori, A. M., Belardinelli, R., Duval, M., Garofalo, R., Schenckbecher, E., Hauryliuk, V., ... Marzi, S. (2023). Escherichia coli CspA stimulates translation in the cold of its own mRNA by promoting ribosome progression. *Frontiers in Microbiology*, 14, 1–16. <https://doi.org/10.3389/fmicb.2023.1118329>
- Gu, Z. (2022). Complex heatmap visualization. *IMeta*, 1(3), Article e43. <https://doi.org/10.1002/imt2.43>
- Gu, Z., Ellis, R., & Schlesner, M. (2016). Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics*, 32(18), 2847–2849. <https://doi.org/10.1093/bioinformatics/btw313>
- Hebraud, M., Dubois, E., Potier, P., & Labadie, J. (1994). Effect of growth temperatures on the protein levels in a psychrotrophic bacterium, *Pseudomonas fragi*. *Journal of Bacteriology*, 176(13), 4017–4024. <https://doi.org/10.1128/jb.176.13.4017-4024.1994>
- Hyatt, D., Chen, G. L., Locascio, P. F., Land, M. L., Larimer, F. W., & Hauser, L. J. (2010). Prodigal: Prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics*, 11. <https://doi.org/10.1186/1471-2105-11-119>
- Jain, C., Rodríguez-R, L. M., Philipp, A. M., Konstantinidis, K. T., & Aluru, S. (2018). High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. *Nature Communications*, 9(1), 1–8. <https://doi.org/10.1038/s41467-018-07641-9>
- Juni, E., & Heym, G. A. (1986). *Psychrobacter immobilis* gen. Nov., sp. nov.: Genospecies composed of gram-negative, aerobic, oxidase-positive coccobacilli. *International Journal of Systematic and Evolutionary Microbiology*, 36(3), 388–391. <https://doi.org/10.1099/00207173-36-3-388>
- Kanehisa, M., & Goto, S. (2000). KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Research*, 28(1), 27–30. <https://doi.org/10.1093/nar/28.1.27>
- Kolmogorov, M., Yuan, J., Lin, Y., & Pevzner, P. A. (2019). Assembly of long, error-prone reads using repeat graphs. *Nature Biotechnology*, 37(5), 540–546. <https://doi.org/10.1038/s41587-019-0072-8>
- Kuchma, S. L., Brothers, K. M., Merritt, J. H., Liberati, N. T., Ausubel, F. M., & O'Toole, G. A. (2007). BifA, a cyclic-di-GMP phosphodiesterase, inversely regulates biofilm formation and swarming motility by *Pseudomonas aeruginosa* PA14. *Journal of Bacteriology*, 189(22), 8165–8178. <https://doi.org/10.1128/JB.00586-07>
- Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with bowtie 2. *Nature Methods*, 9(4), 357–359. <https://doi.org/10.1038/nmeth.1923>
- Lembke, C., Podbielski, A., Hidalgo-Grass, C., Jonas, L., Hanski, E., & Kreikemeyer, B. (2006). Characterization of biofilm formation by clinically relevant serotypes of group A streptococci. *Applied and Environmental Microbiology*, 72(4), 2864–2875. <https://doi.org/10.1128/AEM.72.4.2864-2875.2006>
- Li, W., & Godzik, A. (2006). Cd-hit: A fast program for clustering and comparing large sets of protein or nucleotide sequences. *Bioinformatics*, 22(13), 1658–1659. <https://doi.org/10.1093/bioinformatics/btl158>
- Li, W., Jaroszewski, L., & Godzik, A. (2001). Clustering of highly homologous sequences to reduce the size of large protein databases. *Bioinformatics (Oxford, England)*, 17(3), 282–283. <https://doi.org/10.1093/bioinformatics/17.3.282>
- Li, W., Jaroszewski, L., & Godzik, A. (2002). Tolerating some redundancy significantly speeds up clustering of large protein databases. *Bioinformatics*, 18(1), 77–82. <https://doi.org/10.1093/bioinformatics/btt177>
- Liao, Y., Smyth, G. K., & Shi, W. (2014). FeatureCounts: An efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics*, 30(7), 923–930. <https://doi.org/10.1093/bioinformatics/btt656>
- Luo, W., & Brouwer, C. (2013). Pathview: An R/Bioconductor package for pathway-based data integration and visualization. *Bioinformatics*, 29(14), 1830–1831. <https://doi.org/10.1093/bioinformatics/btt285>
- Majer-Baranyi, K., Székács, A., & Adányi, N. (2023). Application of electrochemical biosensors for determination of food spoilage. *Biosensors*, 13(4), 456. <https://doi.org/10.3390/bios13040456>
- Meybeck, A., Cederberg, C., Gustavsson, J., van Otterdijk, R., & Sonesson, U. (2011). *Global food losses and food waste*. FAO.
- Mikheenko, A., Pribelski, A., Saveliev, V., Antipov, D., & Gurevich, A. (2018). Versatile genome assembly evaluation with QUAST-LG. *Bioinformatics*, 34(13), i142–i150. <https://doi.org/10.1093/bioinformatics/bty266>
- Muyzer, G., de Waal, E. C., & Uitterlinden, A. G. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology*, 59(3), 695–700. <https://doi.org/10.1128/aem.59.3.695-700.1993>
- Oksanen, J., Simpson, G., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., ... McGinn, D. (2022). *Vegan: Community ecology package (version 2.6-4)* [computer software]. CRAN.
- Olm, M. R., Crits-Christoph, A., Bouma-Gregson, K., Firek, B. A., Morowitz, M. J., & Banfield, J. F. (2021). inStrain profiles population microdiversity from metagenomic data and sensitively detects shared microbial strains. *Nature Biotechnology*, 39(6), 727–736. <https://doi.org/10.1038/s41587-020-00797-0>
- Ommen, P., Zobek, N., & Meyer, R. L. (2017). Quantification of biofilm biomass by staining: Non-toxic safranin can replace the popular crystal violet. *Journal of Microbiological Methods*, 141, 87–89. <https://doi.org/10.1016/j.mimet.2017.08.003>
- Oxford Nanopore Technologies. (2022). *Medaka* [computer software]. <https://github.com/nanoporetech/medaka>
- Oxford Nanopore Technologies. (2024a). *Bacteria gDNA extraction for nanopore-only microbial isolate sequencing solution (NO-MISS)* [laboratory protocol]. <https://nanoporetech.com/document/extraction-method/bact-gdna-no-miss>
- Oxford Nanopore Technologies. (2024b). *Dorado* [computer software]. <https://github.com/nanoporetech/dorado>
- Parks, D. H., Imelfort, M., Skennerton, C. T., Hugenholtz, P., & Tyson, G. W. (2015). CheckM [computer software]. <https://github.com/ecogenomics/CheckM>
- Parrilli, E., Tutino, M. L., & Marino, G. (2022). Biofilm as an adaptation strategy to extreme conditions. *Rendiconti Lincei*, 33(3), 527–536. <https://doi.org/10.1007/s12210-022-01083-8>
- Pribelski, A., Antipov, D., Meleshko, D., Lapidus, A., & Korobeynikov, A. (2020). Using SPAdes de novo assembler. *Current Protocols in Bioinformatics*, 70(1), Article e102. <https://doi.org/10.1002/cpbi.102>
- QIAGEN GmbH. (2017). *DNeasy PowerFood microbial kit handbook* [Laboratory manual]. <https://www.qiagen.com/>
- Quijada, N. M., Cobo-Díaz, J. F., Valentino, V., Barcenilla, C., De Filippis, F., Cabrera-Rubio, R., ... Alvarez-Ordóñez, A. (2025). The food-associated resistome is shaped by processing and production environments. *Nature Microbiology*, 10(8), 1854–1867. <https://doi.org/10.1038/s41564-025-02059-8>
- R Core Team. (2024). *R: A language and environment for statistical computing* [computer software]. R foundation for statistical Computing. <https://www.R-project.org/>
- Roch, F. F., Dziściol, M., Strachan, C. R., Chughtai, M. S., Quijada, N. M., Movsesijan, T., & Selberherr, E. (2025). Microbial succession and interaction in vacuum-packed beef: A longitudinal study of bacterial and fungal dynamics. *npj Science of Food*, 9(1), 105. <https://doi.org/10.1038/s41538-025-00479-8>
- Sequino, G., Cobo-Díaz, J. F., Valentino, V., Tassou, C., Volpe, S., Torrieri, E., ... De Filippis, F. (2024). Microbiome mapping in beef processing reveals safety-relevant variations in microbial diversity and genomic features. *Food Research International*, 186. <https://doi.org/10.1016/j.foodres.2024.114318>
- Standar, K., Kreikemeyer, B., Redanz, S., Münter, W. L., Laue, M., & Podbielski, A. (2010). Setup of an in vitro test system for basic studies on biofilm behavior of mixed-species cultures with dental and periodontal pathogens. *PLoS One*, 5(10), Article e13135. <https://doi.org/10.1371/journal.pone.0013135>
- Stellato, G., La Stora, A., De Filippis, F., Borriello, G., Villani, F., & Ercolini, D. (2016). Overlap of spoilage-associated microbiota between meat and the meat processing environment in small-scale and large-scale retail distributions. *Applied and Environmental Microbiology*, 82(13), 4045–4054. <https://doi.org/10.1128/AEM.00793-16>
- 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(D1), D593–D598. <https://doi.org/10.1093/nar/gku1201>
- Tenenbaum, D., & Maintainer, B. (2025). *KEGGREST: Client-side REST access to the Kyoto Encyclopedia of genes and genomes (KEGG). (version 1.48.0)* [computer software]. Bioconductor. <https://doi.org/10.18129/B9.bioc.KEGGREST>
- Voglauer, E. M., Alteio, L. V., Pracer, N., Thalgueter, S., Quijada, N. M., Wagner, M., & Rychli, K. (2025). *Listeria monocytogenes* colonises established multispecies biofilms and resides within them without altering biofilm composition or gene expression. *Microbiological Research*, 292, Article 127997. <https://doi.org/10.1016/j.micres.2024.127997>
- Wagner, E. M., Fischel, K., Rammer, N., Beer, C., Palmethofer, A. L., Conrady, B., ... Rychli, K. (2021). Bacteria of eleven different species isolated from biofilms in a meat processing environment have diverse biofilm forming abilities. *International Journal of Food Microbiology*, 349, Article 109232. <https://doi.org/10.1016/j.ijfoodmicro.2021.109232>
- Wagner, E. M., Pracer, N., Thalgueter, S., Fischel, K., Rammer, N., Pospíšilová, L., ... Rychli, K. (2020). Identification of biofilm hotspots in a meat processing environment: Detection of spoilage bacteria in multi-species biofilms. *International Journal of Food Microbiology*, 328. <https://doi.org/10.1016/j.ijfoodmicro.2020.108668>
- Wick, R. R. (2021). *Filterlong* [computer software]. <https://github.com/rrwick/Filterlong>
- Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis*. Springer. <https://doi.org/10.1007/978-3-319-24277-4>
- Wood, D. E., Lu, J., & Langmead, B. (2019). Improved metagenomic analysis with kraken 2. *Genome Biology*, 20(1), 1–13. <https://doi.org/10.1186/s13059-019-1891-0>
- World Health Organization. (2024). WHO's list of medically important antimicrobials: A risk management tool for mitigating antimicrobial resistance due to non-human use (Version 2024). <https://cdn.who.int/media/docs/default-source/gcp/who-mia-list-2024-iv.pdf>
- Xu, Z. S., Ju, T., Yang, X., & Gänzle, M. (2023). A meta-analysis of bacterial communities in food processing facilities: Driving forces for assembly of core and accessory

- microbiomes across different food commodities. *Microorganisms*, 11(6), 1575. <https://doi.org/10.3390/microorganisms11061575>
- Yang, J., Yu, X., Liu, Y., Liang, R., Zhu, L., Dong, P., Zhang, Y., & Yang, X. (2025). The off-odor identification and CO₂-inhibition for high-oxygen packaged beef spoilage associated with *Brochothrix thermosphacta* growth and metabolism. *Food Research International*, 216, Article 116711. <https://doi.org/10.1016/j.foodres.2025.116711>
- Yang, X., Narvaez-Bravo, C., & Zhang, P. (2023). Driving forces shaping the microbial ecology in meat packing plants. *Frontiers in Microbiology*, 14, 1–16. <https://doi.org/10.3389/fmicb.2023.1333696>
- Zhang, Y., Wei, J., Yuan, Y., & Yue, T. (2019). Diversity and characterization of spoilage-associated psychrotrophs in food in cold chain. *International Journal of Food Microbiology*, 290, 86–95. <https://doi.org/10.1016/j.ijfoodmicro.2018.09.026>
- Zwirzitz, B., Wetzels, S. U., Dixon, E. D., Stessl, B., Zaiser, A., Rabanser, I., ... Selberherr, E. (2020). The sources and transmission routes of microbial populations throughout a meat processing facility. *npj Biofilms and Microbiomes*, 6(1), 1–12. <https://doi.org/10.1038/s41522-020-0136-z>