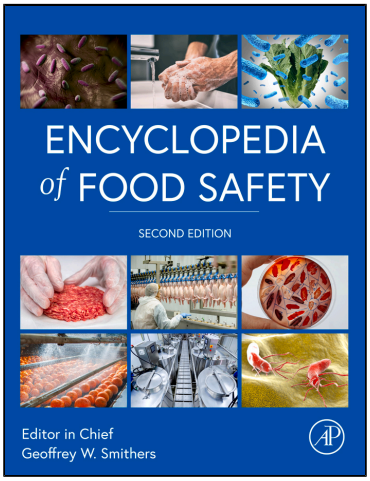

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Microbiomic Profiling of Food Processing Environments and Foods for Food Safety and Quality

Byron Brehm-Stecher[a] **and Gregory R. Siragusa**[b], [a] Department of Food Science and Human Nutrition, Iowa State University, Ames, IA, United States; and [b] Scout Microbiology LLC, Waukesha, WI, United States

Key Points

- The microbiome is defined as the population of microorganisms (bacteria, fungi, viruses, algae, protozoa) that occupy a common space
- Microbiomes of a given food or environment can be investigated using amplicon sequencing of signature genes targeting specific organisms or through shotgun sequencing-based metagenomic analysis of whole microbial communities
- Ecological investigations using microbiomics enable community profiling without prior knowledge of the sample's content
- Microbiomic profiles provide information on identity of community members, the proportion of the total population that they represent in the environment, and with some approaches, information on the functional diversity of community members
- Microbiomic profiles of foods or processing plants can provide actionable information that can be leveraged for process control and improvements in product quality and safety
- Microbiomics and culture need not be mutually exclusive. Distinct advantages may be realized through integrated use of both.

Abstract

Microbiomic profiling of foods and food processing environments provides a powerful toolset for unprecedented characterization of food and built environment microbial ecologies in support of food safety and quality activities. Several examples of food and food processing plant microbiomes are presented for the reader to understand the utility of microbiomic profiling and its place along with traditional cultural tools in the modern food microbiology laboratory.

Introduction

Traditionally, food microbiologists have used labor-intensive cultural methods to understand the microbial profile or content of foods and what influences that profile in food and food-production environments. More recently, culture-independent or genomic approaches have been gaining more traction. The whole genome sequence of a single organism is the assembled and annotated sequence of DNA (including rDNA) which defines the organism at the genomic level. Genomic characterization can be performed using differing approaches and levels of resolution. For example, the genomes of multiple isolates of the same type of organism can be evaluated in support of epidemiological investigations or for environmental tracking work. Alternatively, genomics can be applied to the study of *groups* of organisms and their interactions.

The microbiome is defined as the collective population of microorganisms (bacteria, fungi, viruses, algae, protozoa) that occupy a common space, microhabitat or niche. Microbiomics leverages the power of genomics to profile groups of microorganisms associated with a given environment. In the food realm, environments of interest for microbiomic studies include food processing surfaces, equipment or foods themselves.

Microbiomic profiles can be generated by amplicon sequencing, a targeted approach which involves PCR-amplification and sequencing of families of diagnostic genes, such as 16S ribosomal genes for bacteria or 18S ribosomal genes for fungi. Amplicon sequencing, also known as "metabarcoding" or "oligotyping", can be performed on individual isolates or on total microbial

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DNA isolated from an environment. Drawbacks of amplicon sequencing may include difficulty in resolving taxonomy beyond the genus level and limited prediction of functional traits within a microbial community. Nevertheless, amplicon sequencing of total community DNA has been used as a cost-effective means for evaluation of microbial composition or successions over time. For example, it has been used to perform quality control of starter cultures, to monitor fermentation or food spoilage processes and to examine samples taken from a food at various points during processing, from the ingredients or raw sample to the final product (Hillmann et al., 2018; Jagadeesan et al., 2019).

For a broader and richer picture, shotgun sequencing of total DNA from a mixed community—"metagenomics"—can be used to generate microbiomic profiles. Shotgun metagenomics can provide a more robust view of microbial communities, including the presence of genes associated with species, serotype, virulence, antimicrobial resistance (AMR) or fitness determinants enabling resilience to stressors such as temperature or pH extremes, desiccation, detergents, biocides, etc. (Jagadeesan et al., 2019). This information can be used for detection of pathogens and spoilage organisms within a microbial community or their emergence as the population shifts dynamically over time or in response to food processing unit operations (Jagadeesan et al., 2019).

Whole metagenome sequencing (WMS) using Next Generation Sequencing (NGS) can be done at shallow or deep levels. Deep WMS (i.e., 2.5 billion sequences per sample) is the "gold standard" for microbiomic profiling, but it can be prohibitively expensive for large-scale use (Hillmann et al., 2018, 2020). Shallow WMS (i.e., as low as 0.5 million sequences per sample) may be an attractive alternative to both amplicon sequencing and deep WMS for large-scale microbiomic studies or routine profiling. In their study of human microbiome samples, Hillmann and colleagues found that shallow WMS provided more accurate (species-level) taxonomic characterization and improved functional data than did 16S amplicon sequencing, yet at a comparable cost (Hillmann et al., 2018). It is expected that these observations will also be applicable to the study of other microbiomes, including those of foods and built environments.

Regardless of the approach used for microbiomic profiling, food microbiologists now have access to tools capable of resolving microbiologically complex environmental samples according to their individual microbial taxa and function, without prior knowledge of community composition or the need for physical separation and culture of individual cells.

For routine use in foods, microbiomic profiles provide two basic sets of information: identity and proportion. A simple way to think of this is to envision a profile displayed as a pie-chart. The **slices** of the pie represent organism identities, and the **size** of each slice represents the proportion of the whole pie (the community microbiome) that the slice comprises. Slices of this microbial pie can represent taxonomic rankings ranging from broad (i.e., phyla, class, order, family) to narrow (i.e., genus, species, sub-species).

Today's microbiomic tools can provide actionable information about food microbial communities that was once only accessible using cultural methods. These data can now be obtained rapidly and with unprecedented taxonomic precision. Microbiomic profiling liberates today's microbiologist from limitations associated with the "Great Plate Count Anomaly"—our inability to recover and analyze all microbes within an unknown sample using conventional cultural techniques. However, while genomic methods may replace culture in some applications, these two approaches may also be used together advantageously for product or process improvement. Combining the objective synthesis of the microbiome (ability to find a genotype, assign a name and determine functionality) with the subjective methodology of culture (ability to enumerate microbes and isolate pure cultures) provides a powerful toolset for characterization of food microbial ecologies by organism type ("who is there?), functional attributes ("what are they doing?") and genomic capacity ("what *can* they do").

In this combined use of molecular and cultural approaches, as each method advances, the other is fed off of the discoveries. For instance, useful or novel traits identified by genomics can be leveraged to drive the formulation of culture media and determination of growth conditions that allow selection for specific microbial groups (Jagadeesan et al., 2019). Conversely, as more microbes become culturable, microbiomic tools can be designed to detect signature sequences for those newly cultured bacterial groups. A more complete picture of community composition and access to previously unculturable microbes may enable better understanding of their functionalities in food or processing plant niches. Outputs from this molecular-cellular nexus may include improved food products and processes, or greater understanding of how organisms isolated from food processing built environments may be used as "environmental probiotics" to suppress growth of pathogens in problematic niches such as floor drains (Zhao et al., 2004).

Environmental hygiene and sanitation in food processing is paramount to production of safe (pathogen-diminished or pathogen-free) and stable (increased shelf life) foods. There are many potential applications for food microbiomics related to food preparation, packaging and storage room built environments. Several examples are presented below to illustrate the power of microbiomics as an important tool for gaining a deeper understanding of microbial food safety and quality issues with an eye toward improving their outcomes.

Microorganisms can enter food production pipelines at multiple points. The interactions of the indigenous microbiota of foods and ingredients with preservation and environmental parameters drive the microbiomes of foods. The food production environment is a major source of microbial inputs. Characterizing that microbiota and understanding how it fluctuates between sanitation, production and idle periods is made possible through use of the microbiomic lens. The impacts of resident microbiota, environmental factors, processing steps (heat, fermentation, preservatives, etc.), storage and packaging can be determined through the study of microbiomes using metagenomic tools (Yap et al., 2022).

As noted above, answers to three fundamental questions, "who is there?", "what are they doing?", and "what *can* they do?" are now available using the culture-independent tools of microbiomics and metagenomics (Gray, 2022). The use of microbiomic tools and bioinformatic analyses is perhaps the only approach to fully assess and understand the impacts of the major variables contributing to processing environment and food microbiomes. Key variables include building layout and integrity, seasonality,

temperatures, sanitation practices, waste handling, raw materials, organic residues, air handling/recycling, and employees (De Filippis et al., 2021). Additional, more sporadic microbial inputs could result from construction activities or pest management issues. For example, construction may allow ingress of air, dust or moisture that could carry microbial contaminants or aid their establishment and growth. Food plant landscaping features such as grass and water could attract migrating birds such as wild geese, whose droppings could draw other pests to the area or be tracked into the plant by employees. A full accounting of both fixed and intermittent microbial inputs to food production processes is needed to provide the holistic context in which to apply microbiomics and interpret their results.

Meat and Poultry Systems

Belk et al. (2022) demonstrated the power of microbiomic analysis in their characterization of the bacterial community of a beef processing plant from its start into 18 months of use. Swab samples were taken from select environmental fixtures (drains, doors) and potential microbial sources (employee skin and animal skins or hides) and DNA was extracted and sequenced using standardized protocols described for the Earth Microbiome Project (Thompson et al., 2017). Because they are permanent fixtures, drains and doors were chosen as representative elements of the facility, enabling reliable and repeated sampling throughout the study. Drains are recognized as potential harborage points for *Listeria* spp. and doors are direct and frequent contact points for plant employees. Doors are cleaned less frequently than floors and vary according to their mode of operation and type of employee contact (i.e., levers, push bars or push plates on swinging doors).

Key takeaways were that a core microbiome is established rapidly in meat processing facilities once work begins and that the distribution of microbial communities in different spaces is driven and shaped by organism source and environmental conditions, especially temperature. Populations of *Listeria* spp. were different depending on the room location and room function. *L. innocua* (Li) were associated more with the live animal stages of processing while *L. monocytogenes* (Lm) were found more frequently in the breakdown-fabrication rooms and areas of the process chain. The floor drains were frequently populated by both Li and Lm, yet whether these served as reservoirs or simply represented coincidental harborage was unclear. Conditions more conducive to low temperature life fostered Lm, vs. higher temperatures thought to be more conducive to Li. *Listeria* spp. were detected more frequently in association with certain bacterial groups including *Chryseobacterium* spp., *Acinetobacter* spp., and *Flavobacterium* spp., suggesting that niches containing these genera might also harbor *Listeria* spp. as members of the community.

The microbiomes of built environments are typically reflective of their primary occupants. Most built environments (i.e., homes, offices, schools, dormitories, athletic facilities, hospitals, buses, subways, etc.) are constructed expressly for human occupancy or use. Therefore, a reasonable expectation for these environments is that their observed microbiomic "cloud" or "fingerprint" will be heavily influenced by the collective bodily microbiome of their human inhabitants. Belk and colleagues argue that, in the case of food processing facilities, the true "occupants" and majority microbiomic contributors are not humans, but the food products themselves.

Although the microorganisms found on freshly-cut pork are known to influence downstream quality and safety outcomes, little is known about the overall microbial ecology of pork production environments. In an effort to understand how microbial variation between production lines and across time may impact product cross-contamination, Shedleur-Bourguignon and colleagues used 16S amplicon sequencing of conveyor belt samples to explore the microbial diversity of different product lines and how this diversity varied over the course of six plant visits. These authors were able to determine sets of bacteria that were associated with specific production lines, including the main line and those for belly, loin, Boston, picnic and ham products (Shedleur-Bourguignon et al., 2023).

The bacterial sets describing processing surface microbiota included representatives from the genera *Fusobacterium*, *Trueperella*, *Pseudomonas*, *Acinetobacter*, *Peptoniphilus*, *Rothia*, *Enhydrobacter*, *Staphylococcus*, *Bacteroides*, *Aerococcaceae*, *Psychrobacter*, *Porphyromonas*, *Parvimonas*, *Clostridium*, *Peptostreptococcus*, *Macrococcus*, *Chryseobacterium*, *Epilithonimonas* and *Prevotella*. The authors used their observations of these organisms to create models that enabled them to predict which visit a sample came from (to 94% accuracy) and from which product line it was taken (to 88% accuracy). Use of this type of approach may ultimately lead to improved prevention, surveillance and control of product contamination, which could have important quality and safety impacts.

Another 16S amplicon sequencing study of swine processing plant surfaces (Cherifi et al., 2022) revealed interesting negative in silico associations between Lm and *Sphingomonas*, *Paracoccus* and *Lactococcus lactis*. While bacteriocin-producing lactococci are well known to have anti-listerial potential, the gram-negative bacteria *Sphingomonas* and *Paracoccus* have recently been shown to inhibit *Listeria* spp. through production of the carotenoid astaxanthin, which has been posited to interact with bacterial cell membranes or with enzymes responsible for DNA synthesis. The use of microbiomics to study complex interactions among processing plant microbiota may uncover new approaches to prevent the growth and persistence of Lm or other pathogens in these environments.

Chicken is one of the most popular food animals across the globe. Unfortunately, it is also a major reservoir for foodborne pathogens (Feye et al., 2020). Poultry processing utilizes a multi-step process to convert the live animal into specific pieces for further processing and consumption and two operations, scalding and defeathering, have been identified as key points for potential carcass contamination. In a study combining indicator microbiology (aerobic plate and *Enterobacteriaceae* counts), with bacterial microbiome profiling, whole-bird carcass rinses were analyzed from small, medium and large broiler chicken processing plants, at post-scalder and post-picker locations (Wages et al., 2019). Cultural data did not indicate consistent drops in counts from either

sampling point among the three plant types. However, while culture provides quantitative data (plate counts) and some information on functionality (ability to grow on the two types of media used), it does not provide results on microbial taxonomy—the "who is there?" question noted earlier. Microbiome analysis revealed shifts not detectable by culture in populations of *Campylobacter*-like (*Arcobacter*) and *Salmonella*-like (*Erwinia* and *Serratia*) organisms at the two sampling points. *Chryseobacterium* and *Pseudomonas* were also found at each site across all three plants. *Chryseobacterium* is associated with feces and soil and *Pseudomonas* is known to enhance biofilm formation in *Salmonella*. Together, these data suggest the use of these organisms as possible process efficacy indicators for pathogens such as *Campylobacter* and *Salmonella*. This example illustrates the potential for microbiomic data to be used for process monitoring vs. as an overt means for pathogen detection. Industry may be more inclined to use microbiomics in this way, as wielding such a capable tool to look for pathogens would open companies to the legal liabilities and moral obligations associated with their detection in a processing plant.

While the examples so far demonstrate the unique capabilities of microbiomic tools, it is important to recognize that culture and microbiomics each have their own relative merits as tools for monitoring or understanding microbial communities. Culture is generally less expensive, more widely accessible and delivers more easily-interpreted results in a shorter period of time than do microbiome studies. Microbiomics can provide deep insights into microbial ecology, including predictive descriptions of how complex populations shift with time and treatment. Feye and colleagues suggest the value of integrating both culture-based and microbiomic approaches as a strategy for gaining a practical understanding of the ecological dynamics of processing plant microbiota (Feye et al., 2020).

Microbial food spoilage poses serious concerns for both food quality and safety, but it can be difficult to control, given the numerous opportunities available to contaminants along the harvest to production pipeline. A comprehensive study of meat plant bacterial biogeography and transmission mapping detailed the sharing and transfer of bacterial groups from the environment to and from meat and subsequent products (Zwirzitz et al., 2020). Prior work had established the presence of bacteria in pig meat that was not animal in origin, suggesting various routes for contamination involving personnel, equipment or other elements of the slaughter environment. To investigate further, the group took samples from 12 pigs at various points during processing (i.e., sticking, singeing, polishing, evisceration, etc.) and from various sites throughout the plant (**Fig. 1**). The group then used full-length 16S gene sequencing to identify point sources for contamination and to create a transmission map. The resulting map of bacterial flow enabled the prediction of previously unknown contamination sources (Zwirzitz et al., 2020). Using this approach, the authors traced contamination by the meat spoilage organism *Moraxella* to employee gloves, a handrail and the polishing tunnel whips used for carcass cleaning. This level of detail can enable targeted disinfection efforts aimed at contamination intervention. Microbiome analysis has been used successfully to examine other meat processing microbial issues including the flow of antibiotic resistance genes and resistant bacteria through meat processing plants (Li et al., 2022; Cobo-Díaz et al., 2021), sanitizer efficacy (Botta et al., 2020; Campos Calero et al., 2020) and meat product spoilage (Hultman et al., 2015).

Dairy

Microbiomes have proven invaluable in dissecting relationships between dairy facilities and the resulting microbial communities on or within cheeses as well as communities of bacteria in fluid milk. The concept of a facility- or "house"-specific microbiome was introduced by Bokulich and Mills (2013) in which they found fungal and bacterial communities differing between stages of cheesemaking (**Figs. 2** and **3**).

The complexity of the cheesemaking process is only partially represented by the number of steps (**Fig. 2**) and although there are similarities, facilities may differ not only in design and possibly starter cultures, but also according to milk source and dairy plant layout. Here again the power and utility of microbiomics is made clear, as such a detailed study based only on culture-dependent methods would have been technically infeasible or prohibitively expensive.

Even though these cheeses were made by inoculation of milk with starter cultures, environmental bacteria and fungi populated the cheese surfaces thus reflecting the dominant role of the facility or environmental microbiome in shaping the microbial community and ultimately, the sensory characteristics of the cheese (**Fig. 3**).

Milk comes in contact with multiple elements of the built environment during the journey from raw milk to finished dairy product. This contact has the potential to introduce microorganisms and impact their viability and growth. More information is needed on microbial population dynamics occurring in milk during processing and how they may contribute to dairy spoilage and safety outcomes. Kable and colleagues examined samples from various contact points for milk with the built environment, from raw milk to pasteurization, concentration, separation, blending and storage (Kable et al., 2019). The diversity of bacterial taxa was analyzed using 16S microbiome analysis and viable counts by qPCR (quantitative polymerase chain reaction), using propidium monoazide (PMA) treatment to distinguish viable from nonviable cells. Shifts in populations were demonstrated at various taxonomic levels during each stage of processing. While results roughly approximated those determined by culture, microbiomic analysis provided highly detailed and diverse systematics (**Fig. 4**).

Other examples of dairy facility, milk and cheese microbiome applications include: determining the sources of heat-resistant, flavor-altering bacterial enzymes and the effects of processing steps on their bacterial originators (Machado et al., 2017), the sources of virulent dairy bacteriophages (Paillet et al., 2022), and investigating the microbial communities occurring on cheese-ripening boards (Wadhawan et al., 2021).

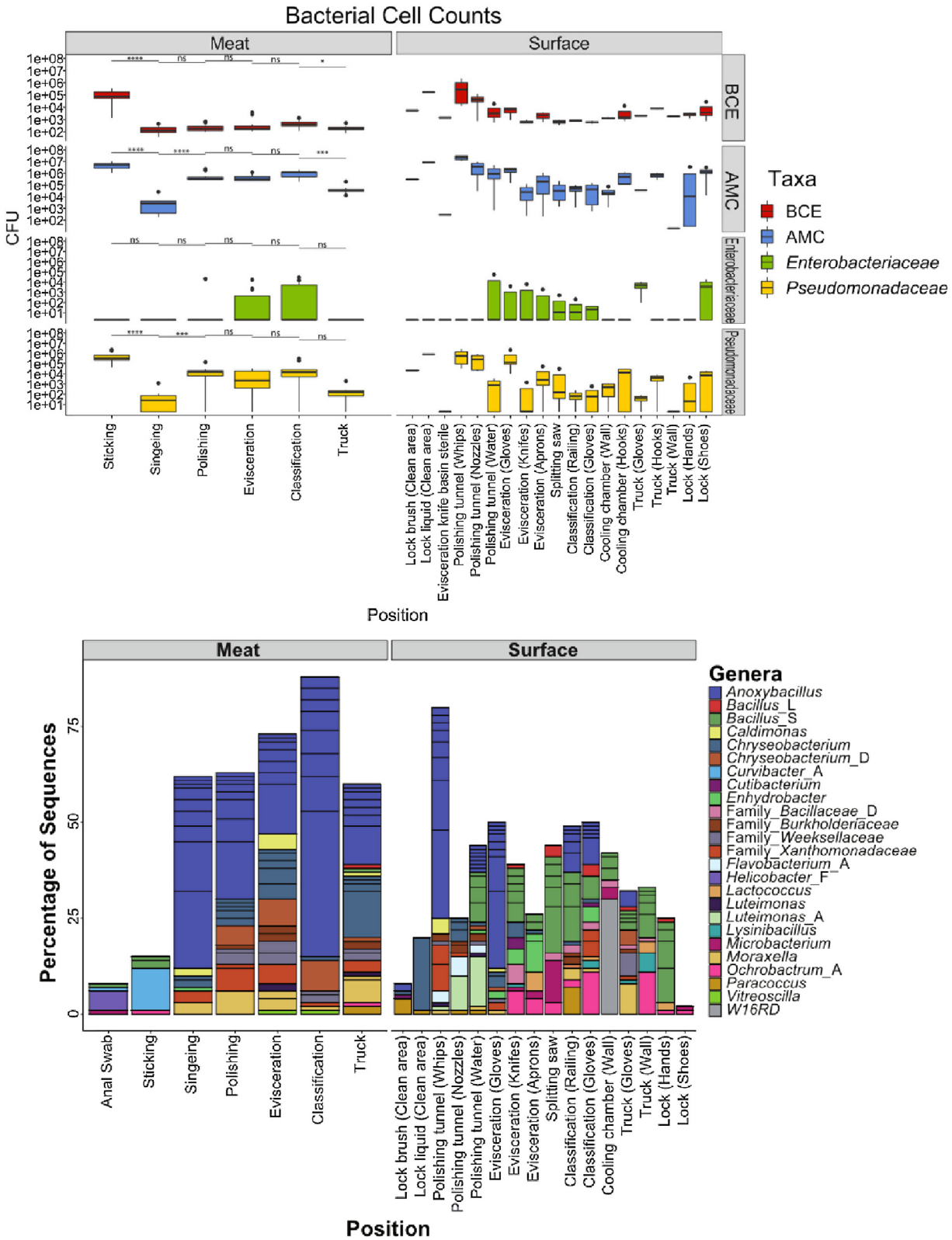

Fig. 1 Microbial counts and microbiome analysis of a meat plant environmental sampling used to identify the sources and transmission routes of microbial populations throughout a meat processing facility. BCE: bacterial cell equivalents, AMC: aerobic mesophilic counts. From Zwirzitz et al. (2020).

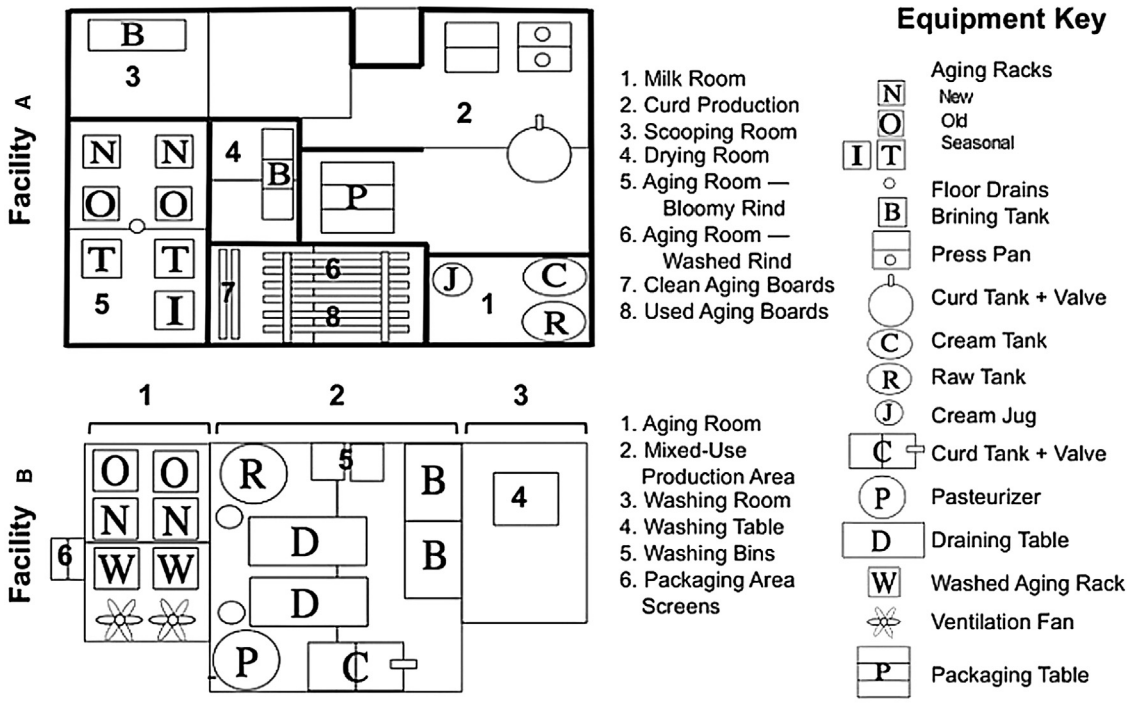

Fig. 2 Sampling plan from two separate surface ripened cheese facilities. From Bokulich and Mills (2013).

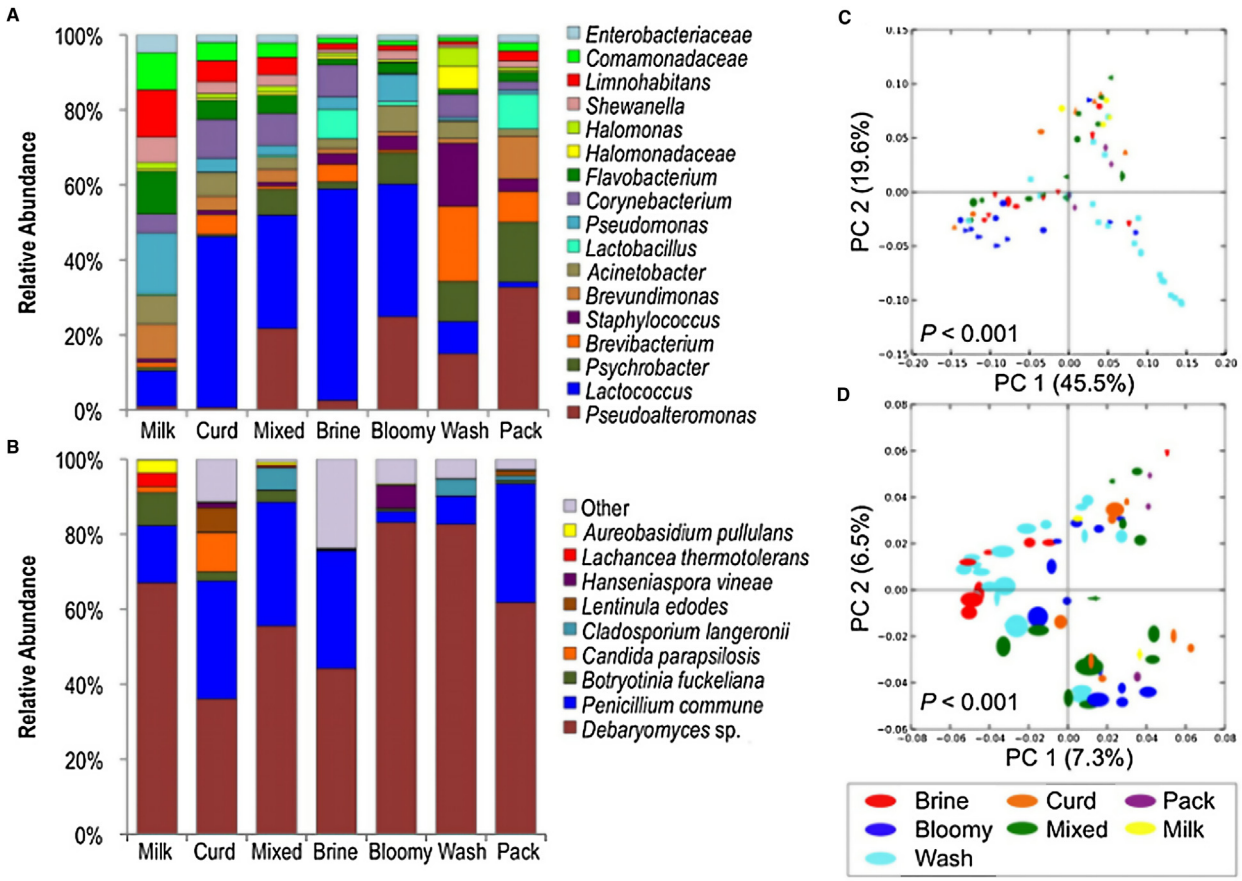

Fig. 3 Bacterial (A) and fungal (B) microbiomes from surface ripened cheese samples. Similarity comparisons by Principal Component Analysis of bacterial (C) and fungal (D) communities tended to group by process step. From Bokulich and Mills (2013).

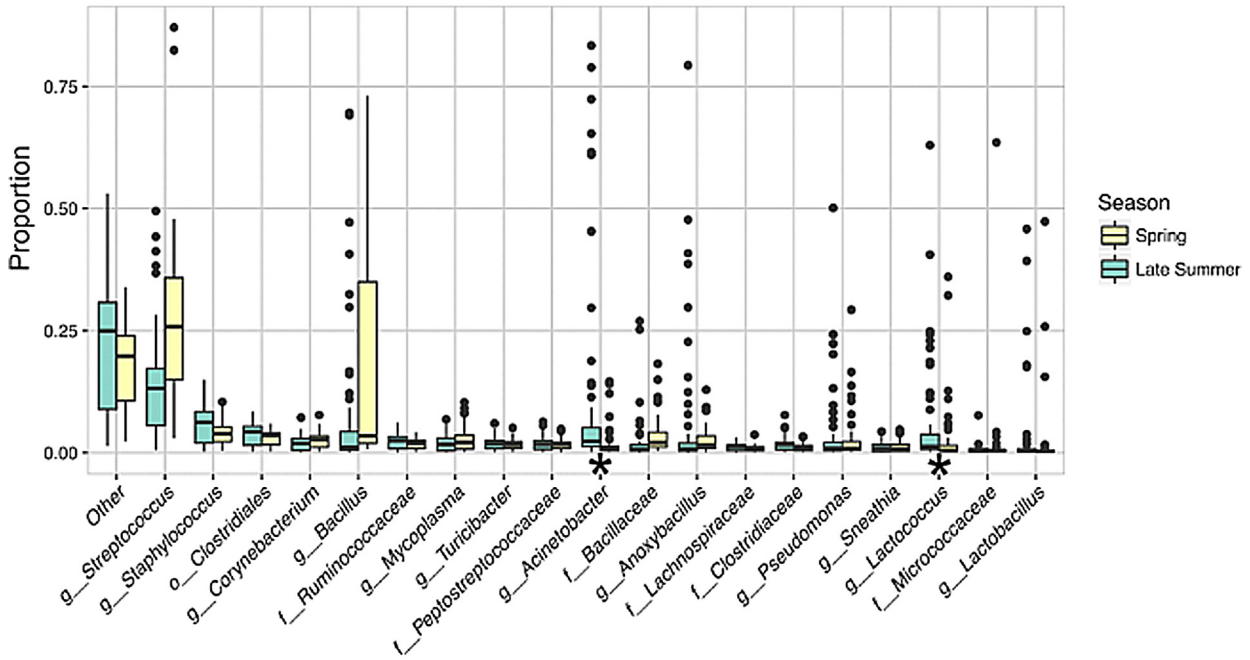

Fig. 4 Bacterial Genus (g) and Family (f) diversity of cheese-milk processing. From Kable et al. (2019).

Built Environment Harborages in Biofilms and Sanitation

The built environment refers to mostly man-made structures and surfaces which harbor microbial communities. In the context of food safety and quality, this includes not only buildings and holding containers but any implements which might constitute zones 1–4 of food proximity and contact. Zone 1 includes all cleaning utensils and food contact surfaces, such as sponges or rubber seals and gaskets. These must be regularly cleaned and sanitized in order to reduce the risk of cross contamination. For example, kitchen sponge microbiomes are highly diverse and have been the subject of considerable study using cultural and culture-independent methods. Microwave heating is common practice at home and in the food industry for reducing sponge microbial loads. Although it is counterintuitive, some evidence suggests that regular microwave treatment of sponges may actually select for malodorous or pathogenic microbiota. Jacksch and colleagues used metagenomic and bioinformatics tools to examine how microwave sanitization impacted the community structure (**Fig. 5**) and functional diversity (**Fig. 6**) of sponge microbiomes (Jacksch et al., 2020). The sponges were from 20 households of similar sizes and were used for dishwashing then treated with a standard sanitation regimen of soapy water rinse followed by microwave heating. Metagenomics results indicated colonization of sponges by bacteria, archaea, viruses (mostly bacteriophage) and eukaryotes, similar to observations made in a different study on household sponges that found archaea, viruses, and insects (Brandau et al., 2022). In the present study, non-microbial eukaryotic signatures from human, food or environmental sources considered to be of minimal significance to hygiene were not evaluated further. The authors found that microwave treatment promoted the increase of *Gammaproteobacteria*, a class containing a number of organisms of food safety or medical concern. Genera increased by microwave heating of sponges included *Acinetobacter*, *Klebsiella*, *Enterobacter* and *Pseudomonas*, all of which contain species known to be human pathogens. Further analysis is needed to determine the pathogenic potential within these sponge-associated genera.

Rubber gaskets and seals are a particular concern in food processing plants as robust sources of microbial harborage. Rubber dishwasher gaskets were also shown to be a reservoir for bacteria in the home environment (Zupančič et al., 2019). Bacterial diversity was observed on gaskets of varying ages and in houses with differing levels of water hardness (**Fig. 7**). The study indicated that the primary input for bacteria to this rubber gasket community was food from dirty dishes. Mechanical, oxidative, thermal and water activity stressors or inputs from dishwasher operation were found to select for and enrich biofilm-forming bacterial species, some of which may be of concern due to their pathogenic potential and antibiotic resistance. This study indicates that domestic dishwashers should not be ignored as a potential source of infectious bacteria.

Microbiomic analysis of household dust has demonstrated a relationship between antibiotic resistance genes and use of the common antimicrobial compounds such as triclosan and methylparaben. Although the presence of these antimicrobials in the household environment do not appear to shape microbial communities, they may influence the promotion and retention of antibiotic resistance genes. Due to importance of the indoor microbiome on human health, the relationship between household disinfectants and the prevalence of antibiotic-resistant genes in household dust microbiota deserves additional attention (Hartmann et al., 2016).

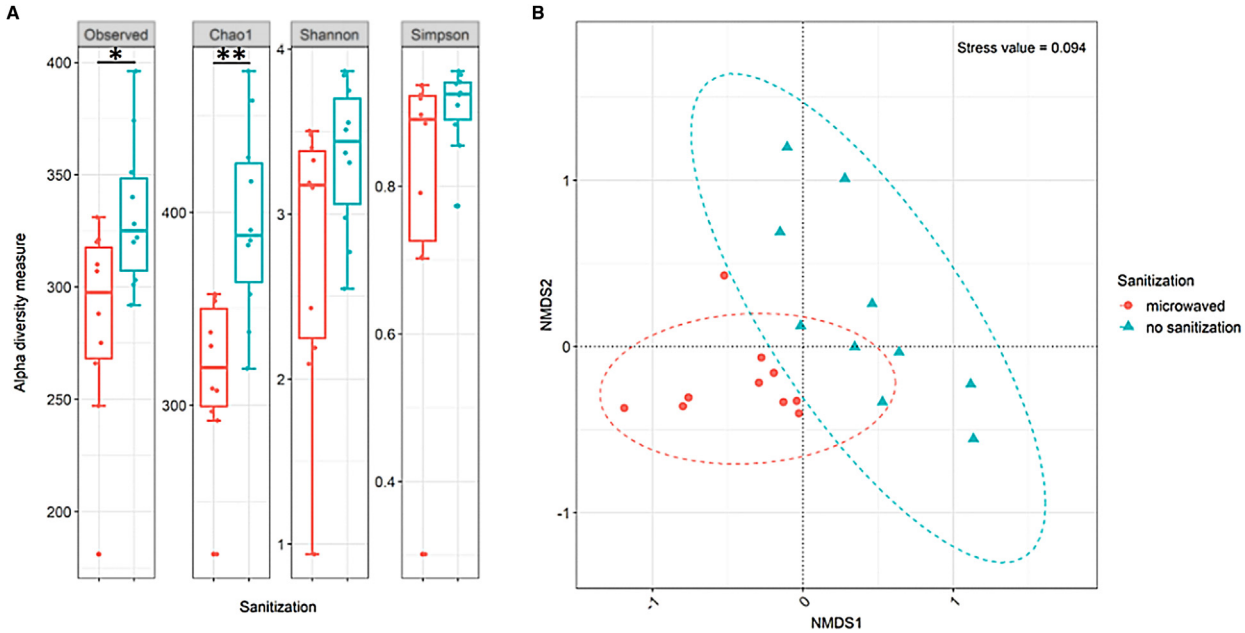

Fig. 5 Diversity indices of bacterial microbiomes of microwaved and unsanitized sponges (A) and separation based on community similarities (B). From Jacksch et al. (2020).

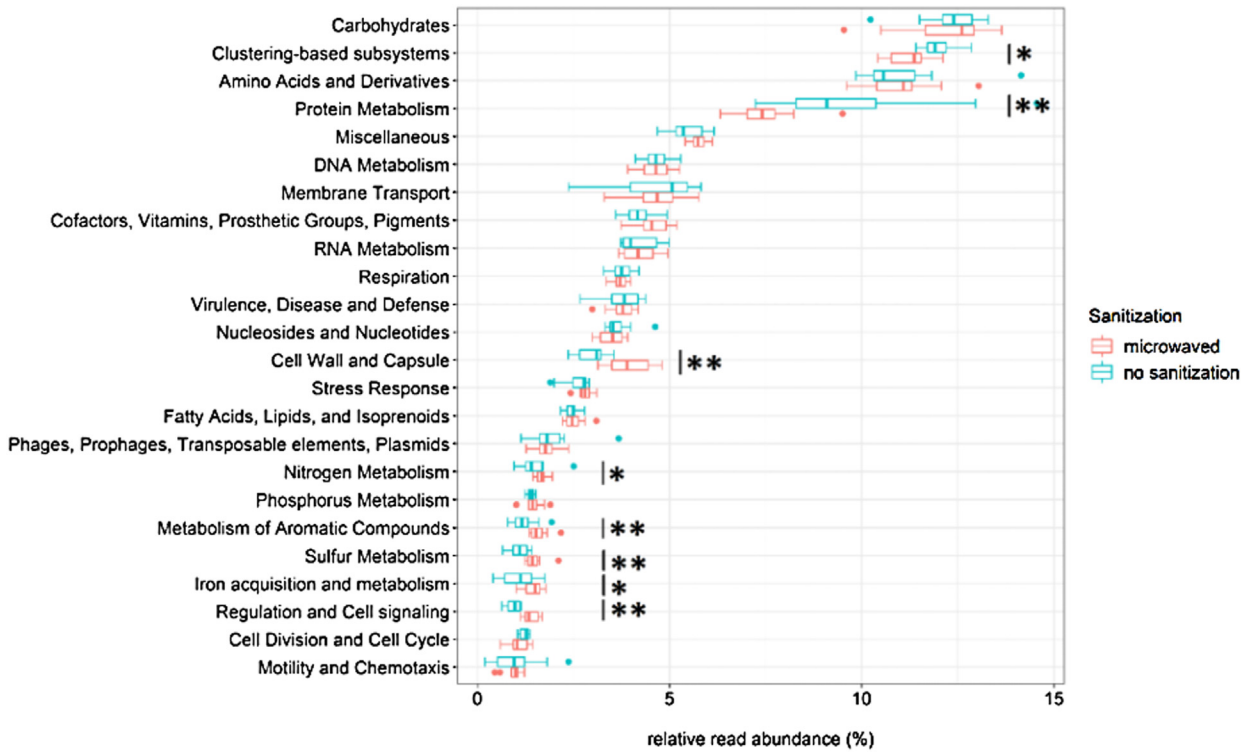

Fig. 6 Gene function distribution found in household sponges sanitized and not sanitized. From Jacksch et al. (2020).

Biofilms, considered to be the primary microbial phenotype in both natural and built environments, are complex assemblages of microbes that form on surfaces in food processing plants and can be comprised of members from multiple domains of life (Bacteria, Archaea, Eukaryota) and viruses. Because of their physically crowded and diverse nature, biofilms can be "hot spots" for horizontal gene transfer. Exchange of plasmids encoding genes important for biofilm formation may lead to biofilm-affirming feedback loop-s.In the food industry, cleaning and subsequent sanitation are heavily impacted by the extent of biofilm formation and recalcitrance

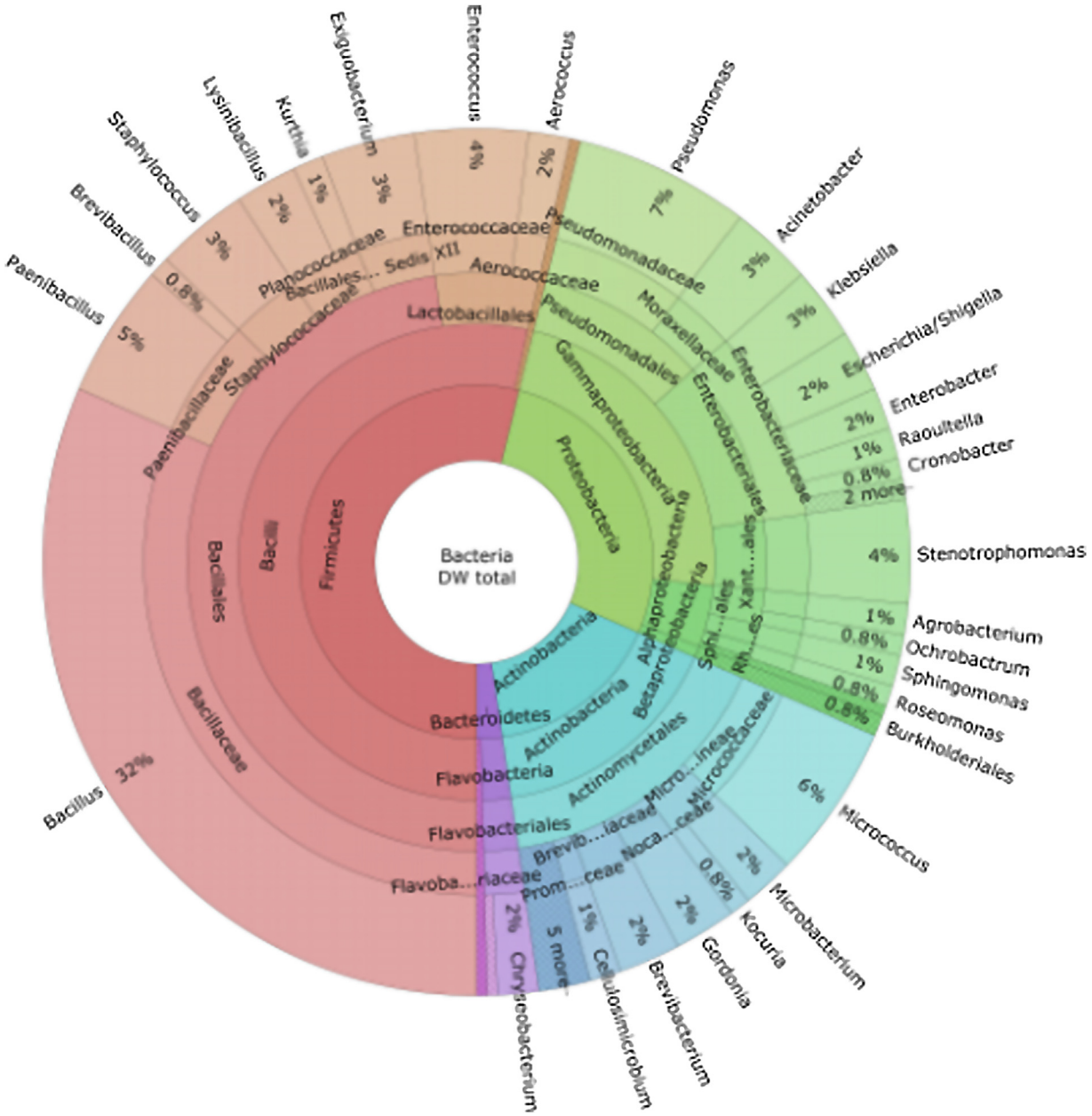

Fig. 7 Bacterial diversity of rubber home dishwashing machine gasket. The graphic illustrator used by these authors is a kinetic (i.e., expandable between taxonomic levels) circular display public-domain software "Krona" (https://bio.tools/krona). From Zupančič et al. (2019).

to removal (Fagerlund et al., 2021). Microbiome analysis of biofilms in four different food plants (Fig. 8) exemplifies the biodiversity of biofilms in food plants and how this may vary according to the types of foods processed.

Assorted Food Plant Microbiome Applications

Interspecies interactions within a microbiome can alter the ability of pathogens to survive and colonize a surface or facility. Microbiome compositions (bacterial and fungal) associated with positive and negative *Listeria monocytogenes* tests were studied in apple processing plants for samples taken at major washing and drying stages from the conveyor belt brushes over time. Results indicated that specific community population makeup was associated with either Lm-positive or Lm-negative tests of fruit (Tan et al., 2019). As illustrated (Fig. 9), plant 2 had lower diversity both in bacteria and fungi and was consistently populated with members of the bacterial family *Pseudomonadaceae* and fungal family *Dipodascaceae*. Reduced processing plant diversity was indicative of Lm

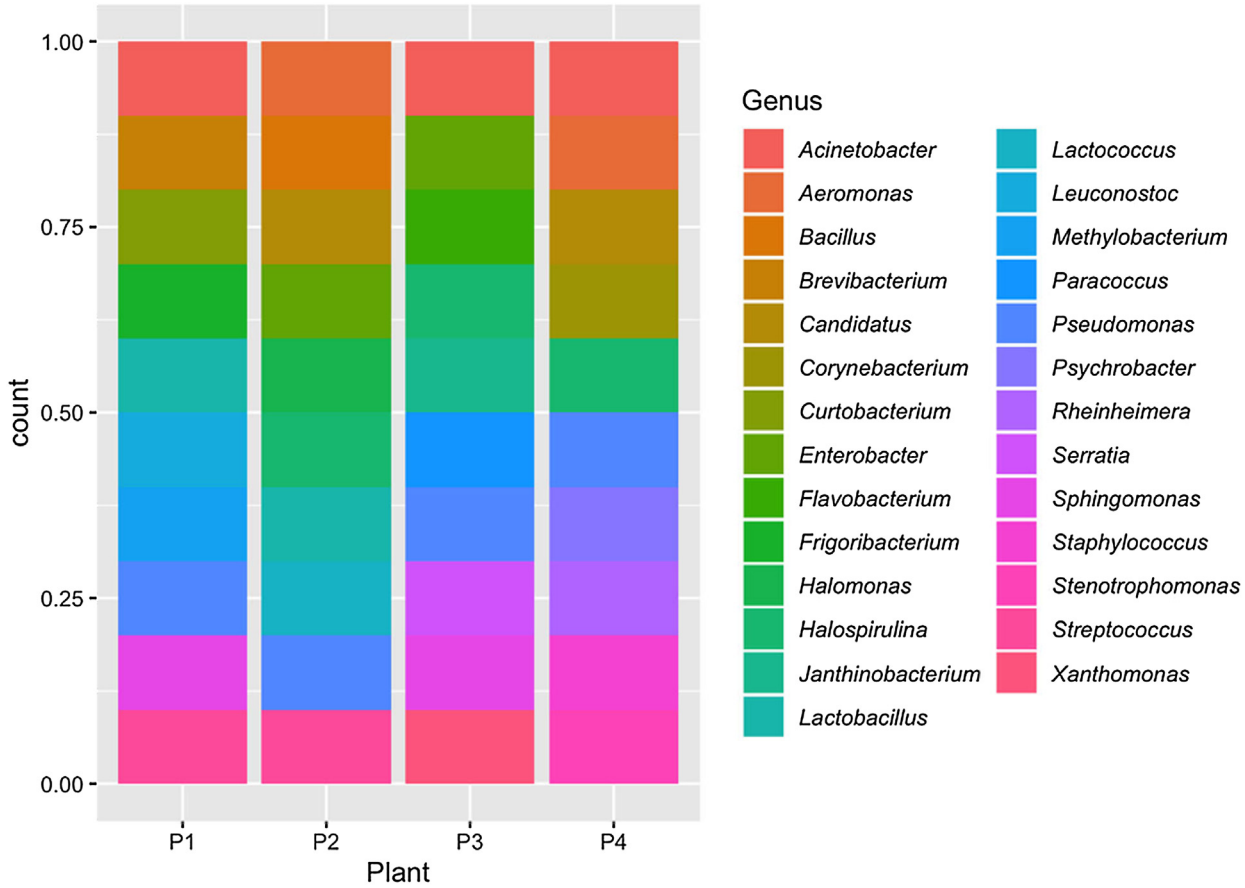

Fig. 8 Bacterial microbiomes of biofilms in four different food plants. P1, P2 produced ready-to-eat meals containing pastas and pizzas, P3,P4 processed meat plants producing hams, sausage, meatballs and mortadella. Temperatures averaged ~13 °C and humidity 67%. Post-cleaning sanitation was accomplished with quaternary ammonium compounds. From Caraballo Guzmán et al. (2020).

persistence, although causation in this relationship was not clear. *Pseudomonadaceae* and *Dipodascaceae* are associated with biofilm formation and unsanitary conditions, respectively.

Subsequently, the same laboratory used microbiome analysis to evaluate the biocontrol capacity of putative anti-*Listeria* lactic acid bacteria (LAB) in either simple dual-culture mixtures (LAB + Lm), or in the presence of a complex natural microbial community (LAB + Lm + apple processing plant biofilm) (Sinclair et al., 2022). This study indicated that the anti-listerial activity of the two added LAB was significantly reduced when tested in the biofilm matrix vs. the more defined inhibition assay. This work highlights the importance of the microbial landscape on pathogen persistence or pathogen mitigation strategies, such as sanitation or competitive exclusion with pathogen-antagonistic cultures. The relationships between microbial community composition or diversity and the ability of food pathogens to establish themselves and persist within food plant environments bears further attention.

Microbiomic analyses have been leveraged to help understand issues affecting a wide variety of food production settings, including salmon and oyster seafood processing plants (Thomassen et al., 2023; Hines et al., 2023), breweries (Bokulich et al., 2012), mushroom processing environments (Lake et al., 2023), vegan kimchi production (Zabat et al., 2018) and many others.

Conclusions and Future Perspectives

Microbial interactions drive many important and dynamic interactions in nature as well as in built environments such as food processing facilities. A greater understanding of "who is there?", "what are they doing?" and "what *can* they do?" may help us decipher the complex and sometimes counterintuitive roles that microbial communities play in food quality and food safety outcomes. Microbiomic tools and subsequent bioinformatic analyses have been instrumental in understanding aspects of microbial presence and behavior that cannot be answered effectively or affordably using traditional cultural tools. However, while microbiomics and culture offer different insights regarding assessment of microbial communities, they are not mutually exclusive. Culture may be faster, more convenient or less expensive than microbiomics for some aspects of food environment monitoring. Microbiomics may unlock our ability to identify and culture organisms that may have previously evaded our efforts to grow (Epstein, 2013).

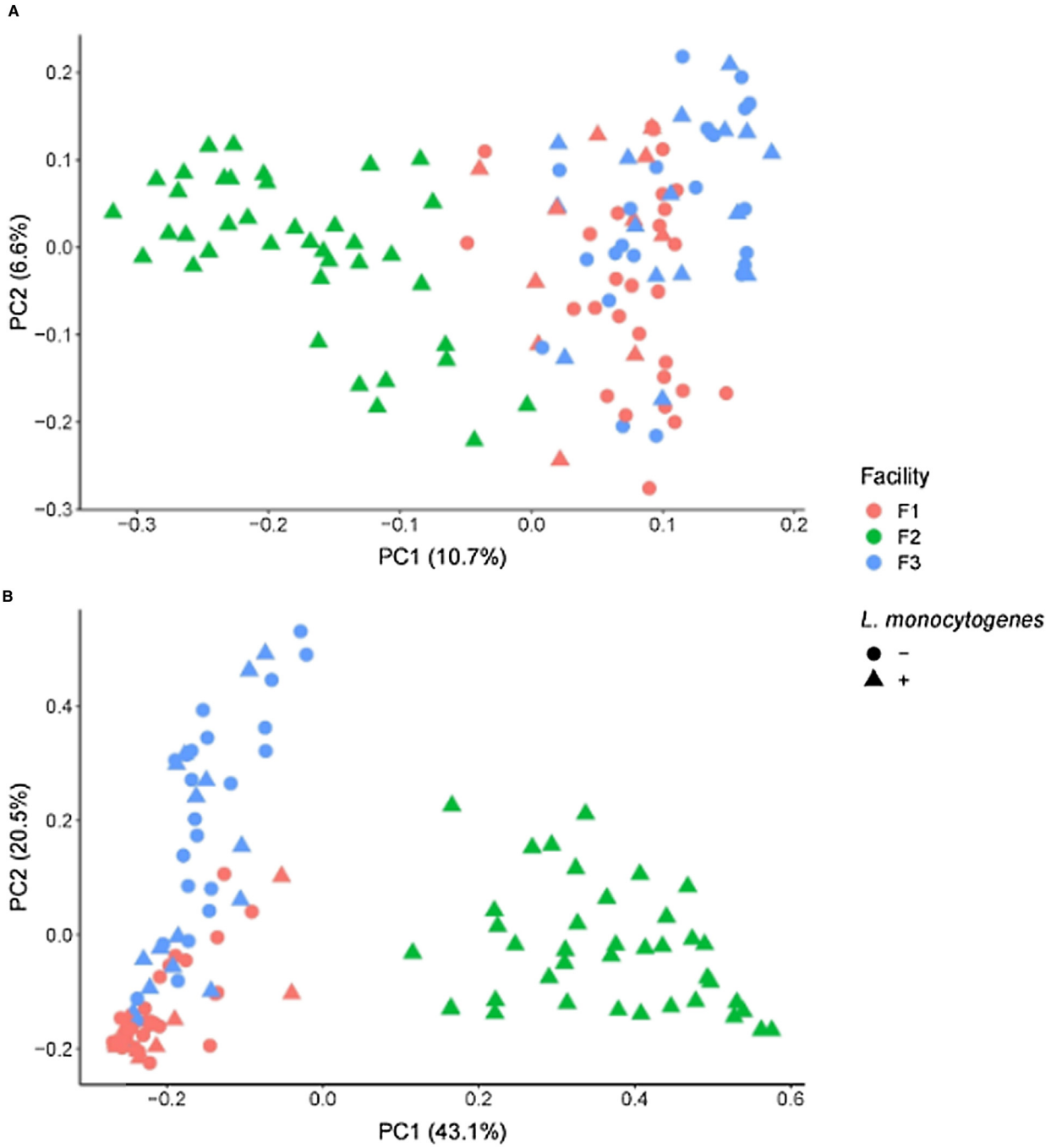

Fig. 9 Bacterial (A) and fungal (B) microbiomes in apple processing facilities and the overlay of *L. monocytogenes* cultural test results of conveyor belt brushes located underneath the belt during washing, drying and waxing stages. From Tan et al. (2019).

Therefore, integrated use of both culture and microbiomics may represent a powerful and flexible strategy for gaining a practical understanding of the ecological dynamics of processing plant or related microbiota. As sequencing technologies are developed to include handheld devices (Pugh, 2023) and advanced analytical software including artificial intelligence (Ricke et al., 2018) and machine learning are implemented, microbiomics will become increasingly used as a reliable and necessary component of the food microbiologist's toolbox.

References

Belk, A.D., Frazier, A.N., Fuerniss, L.K., Delmore, R., Keith, B., Borlee, B., Geornaras, I., Martin, J.N., Metcalf, J.L., 2022. A Pilot Study: the development of a facility-associated microbiome and its association with the presence of *Listeria* spp. in one small meat processing facility. Microbiol. Spectr. 10 (5), e02045-22. https://doi.org/10.1128/spectrum.02045-22.

Bokulich, N.A., Mills, D.A., 2013. Facility-specific "house" microbiome drives microbial landscapes of Artisan cheesemaking plants. Appl. Environ. Microbiol. 79 (17), 5214–5223. https://doi.org/10.1128/AEM.00934-13.

Bokulich, N.A., Bamforth, C.W., Mills, D.A., 2012. Brewhouse-resident microbiota are responsible for multi-stage fermentation of American coolship ale. PloS One 7 (4), e35507. https://doi.org/10.1371/journal.pone.0035507.

Botta, C., Ferrocino, I., Pessione, A., Cocolin, L., Rantsiou, K., 2020. Spatiotemporal distribution of the environmental microbiota in food processing plants as impacted by cleaning and sanitizing procedures: the case of slaughterhouses and gaseous ozone. Appl. Environ. Microbiol. 86 (23), e01861-20. https://doi.org/10.1128/AEM.01861-20.

Brandau, L., Jacksch, S., Weis, S., Schnell, S., Egert, M., 2022. Minority report: small-scale metagenomic analysis of the non-bacterial kitchen sponge microbiota. Arch. Microbiol. 204 (7), 363. https://doi.org/10.1007/s00203-022-02969-9.

Calero, G.C., Gómez, N.C., Lerma, L.L., Benomar, N., Knapp, C.W., Abriouel, H., 2020. In silico mapping of microbial communities and stress responses in a porcine slaughterhouse and pork products through its production chain, and the efficacy of HLE disinfectant. Food Res. Int. 136, 109486. https://doi.org/10.1016/j.foodres.2020.109486.

Caraballo Guzmán, A., et al., 2020. Metagenomic characterization of bacterial biofilm in four food processing plants in Colombia. Braz. J. Microbiol. 51 (3), 1259–1267. https://doi.org/10.1007/s42770-020-00260-x.

Cherifi, T., Arsenault, J., Quessy, S., Fravalo, P., 2022. Co-occurrence of *L. Monocytogenes* with other bacterial genera and bacterial diversity on cleaned conveyor surfaces in a swine slaughterhouse. Microorganisms 10 (3), 613. https://doi.org/10.3390/microorganisms10030613.

Cobo-Díaz, J.F., Alvarez-Molina, A., Alexa, E.A., Walsh, C.J., Mencía-Ares, O., Puente-Gómez, P., Likotrafiti, E., et al., 2021. Microbial colonization and resistome dynamics in food processing environments of a newly opened pork cutting industry during 1.5 years of activity. Microbiome 9 (1), 204. https://doi.org/10.1186/s40168-021-01131-9.

De Filippis, F., Valentino, V., Alvarez-Ordóñez, A., Cotter, P.D., Ercolini, D., 2021. Environmental microbiome mapping as a strategy to improve quality and safety in the food industry. Curr. Opin. Food Sci. 38, 168–176. https://doi.org/10.1016/j.cofs.2020.11.012.

Epstein, S.S., 2013. The Phenomenon of microbial uncultivability. Curr. Opin. Microbiol. 16 (5), 636–642. https://doi.org/10.1016/j.mib.2013.08.003. Antimicrobials ● Genomics.

Fagerlund, A., Langsrud, S., Møretrø, T., 2021. Microbial diversity and ecology of biofilms in food industry environments associated with *Listeria monocytogenes* persistence. Curr. Opin. Food Sci. 37, 171–178. https://doi.org/10.1016/j.cofs.2020.10.015.

Feye, K.M., Thompson, D.R., Rothrock, M.J., Kogut, M.H., Ricke, S.C., 2020. Poultry processing and the application of microbiome mapping. Poult. Sci. 99 (2), 678–688. https://doi.org/10.1016/j.psj.2019.12.019.

Gray, J., 2022. Omic applications to understand food system microbiomes. Microbiol. Aust. 43 (2), 49–51. https://doi.org/10.1071/MA22018.

Hartmann, E.M., Hickey, R., Hsu, T., et al., 2016. Antimicrobial chemicals are associated with elevated antibiotic resistance genes in the indoor dust microbiome. Environ. Sci. Technol. 50 (18), 9807–9815. https://doi.org/10.1021/acs.est.6b00262.

Hillmann, B., Al-Ghalith, G.A., Shields-Cutler, R.R., Zhu, Q., Gohl, D.M., Beckman, K.B., Knight, R., Knights, D., 2018. Evaluating the information content of shallow shotgun metagenomics. Msystems 3 (6), e00069–18. https://doi.org/10.1128/mSystems.00069-18.

Hillmann, B., Al-Ghalith, G.A., Shields-Cutler, R.R., Zhu, Q., Knight, R., Knights, D., 2020. SHOGUN: a modular, accurate and scalable framework for microbiome quantification. Bioinformatics 36 (13), 4088–4090. https://doi.org/10.1093/bioinformatics/btaa277.

Hines, I.S., Madanick, J.M., Smith, S.A., Kuhn, D.D., Stevens, A.M., 2023. Analysis of the core bacterial community associated with consumer-ready Eastern oysters (*Crassostrea virginica*). PloS One 18 (2), e0281747. https://doi.org/10.1371/journal.pone.0281747.

Hultman, J., Rahkila, R., Ali, J., Rousu, J., Björkroth, K.J., 2015. Meat processing plant microbiome and contamination patterns of cold-tolerant bacteria causing food safety and spoilage risks in the manufacture of vacuum-packaged cooked sausages. Appl. Environ. Microbiol. 81 (20), 7088–7097. https://doi.org/10.1128/AEM.02228-15.

Jacksch, S., Thota, J., Shetty, S., Smidt, H., Schnell, S., Egert, M., 2020. Metagenomic analysis of regularly microwave-treated and untreated domestic kitchen sponges. Microorganisms 8 (5), 736. https://doi.org/10.3390/microorganisms8050736.

Jagadeesan, B., Gerner-Smidt, P., Allard, M.W., Leuillet, S., Winkler, A., Xiao, Y., Chaffron, S., Van Der Vossen, J., Tang, S., Katase, M., 2019. The use of next generation sequencing for improving food safety: translation into practice. Food Microbiol. 79, 96–115. https://doi.org/10.1016/j.fm.2018.11.005.

Kable, M.E., Srisengfa, Y., Xue, Z., Coates, L.C., Marco, M.L., 2019. Viable and total bacterial populations undergo equipment- and time-dependent shifts during milk processing. Appl. Environ. Microbiol. 85 (13), e00270-19. https://doi.org/10.1128/AEM.00270-19.

Lake, F.B., et al., 2023. Growth performance of *Listeria monocytogenes* and background microbiota from mushroom processing environments. Int. J. Food Microbiol. 395, 110183. https://doi.org/10.1016/j.ijfoodmicro.2023.110183.

Li, L., Xiao, Y., et al., 2022. Exploring the resistome, virulome, mobilome and microbiome along pork production chain using metagenomics. Int. J. Food Microbiol. 371, 109674. https://doi.org/10.1016/j.ijfoodmicro.2022.109674.

Machado, S.G., et al., 2017. The biodiversity of the microbiota producing heat-resistant enzymes responsible for spoilage in processed bovine milk and dairy products. Front. Microbiol. 8. https://www.frontiersin.org/articles/10.3389/fmicb.2017.00302.

Paillet, T., Lossouarn, J., Figueroa, C., Midoux, C., Olivier, R., Petit, M.-A., Dugat-Bony, E., 2022. Virulent phages isolated from a smear-ripened cheese are also detected in reservoirs of the cheese factory. Viruses 14 (8), 1620. https://doi.org/10.3390/v14081620.

Pugh, J., 2023. The current state of nanopore sequencing. Methods Mol. Biol. 2632, 3–14. https://doi.org/10.1007/978-1-0716-2996-3_1.

Ricke, S.C., et al., 2018. Chapter 19—unraveling food production microbiomes: concepts and future directions. In: Ricke, S.C., Atungulu, G.G., Rainwater, C.E., Park, S.H. (Eds.), Food and Feed Safety Systems and Analysis. Academic Press, pp. 347–374. https://doi.org/10.1016/B978-0-12-811835-1.00019-1.

Shedleur-Bourguignon, F., et al., 2023. Distinct microbiotas are associated with different production lines in the cutting room of a swine slaughterhouse. Microorganisms 11 (1), 133. https://doi.org/10.3390/microorganisms11010133.

Sinclair, P., et al., 2022. Ability of two strains of lactic acid bacteria to inhibit *Listeria monocytogenes* by spot inoculation and in an environmental microbiome context. Microbiol. Spectr. 10 (4), e01018-22. https://doi.org/10.1128/spectrum.01018-22.

Tan, X., et al., 2019. The occurrence of *Listeria monocytogenes* is associated with built environment microbiota in three tree fruit processing facilities. Microbiome 7 (1), 115. https://doi.org/10.1186/s40168-019-0726-2.

Thomassen, G.M.B., Krych, L., Knøchel, S., Mehli, L., 2023. Bacterial community development and diversity during the first year of production in a new salmon processing plant. Food Microbiol. 109, 104138. https://doi.org/10.1016/j.fm.2022.104138.

Thompson, L.R., et al., 2017. A communal catalogue reveals earth's multiscale microbial diversity. Nature 551 (7681), 457–463. https://doi.org/10.1038/nature24621.

Wadhawan, K., Steinberger, A.J., Rankin, S.A., Suen, G., Czuprynski, C.J., 2021. Characterizing the microbiota of wooden boards used for cheese ripening. JDS Commun. 2 (4), 171–176. https://doi.org/10.3168/jdsc.2020-0014.

Wages, J.A., et al., 2019. Comparison of 16S RDNA next sequencing of microbiome communities from post-scalder and post-picker stages in three different commercial poultry plants processing three classes of broilers. Front. Microbiol. 10, 972. https://doi.org/10.3389/fmicb.2019.00972.

Yap, M., et al., 2022. Next-generation food research: use of meta-omic approaches for characterizing microbial communities along the food chain. Annu. Rev. Food Sci. Technol. 13 (1), 361–384. https://doi.org/10.1146/annurev-food-052720-010751.

Zabat, M.A., et al., 2018. The impact of vegan production on the kimchi microbiome. Food Microbiol. 74, 171–178. https://doi.org/10.1016/j.fm.2018.04.001.

Zhao, T., Doyle, M.P., Zhao, P., 2004. Control of *Listeria monocytogenes* in a biofilm by competitive-exclusion microorganisms. Appl. Environ. Microbiol. 70 (7), 3996–4003. https://doi.org/10.1128/AEM.70.7.3996-4003.2004.

Zupančič, J., et al., 2019. The dishwasher rubber seal acts as a reservoir of bacteria in the home environment. BMC Microbiol. 19 (1), 300. https://doi.org/10.1186/s12866-019-1674-5.

Zwirzitz, B., Wetzels, S.U., Dixon, E.D., et al., 2020. The sources and transmission routes of microbial populations throughout a meat processing facility. NPJ Biofilms Microbiomes 6 (1), 26. https://doi.org/10.1038/s41522-020-0136-z.