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Review

Omics-based monitoring of microbial dynamics across the food chain for the improvement of food safety and quality

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ABSTRACT

The diffusion of high-throughput sequencing has dramatically changed the study of food microbial ecology. Amplicon-based description of the microbial community may be routinary implemented in the food industry to understand how the processing parameters and the raw material quality may affect the microbial community of the final product, as well as how the community changes during the shelf-life. In addition, application of shotgun metagenomics may represent an invaluable resource to understand the functional potential of the microbial community, identifying the presence of spoilage-associated activities or genes related to pathogenesis. Finally, retrieving Metagenome-Assembled Genomes (MAGs) of relevant species may be useful for strain-tracking along the food chain and in case of food poisoning outbreaks.

This review gives an overview of the possible applications of sequencing-based approaches in the study of food microbial ecology, highlighting limitations that still prevent the spreading of these techniques to the food industry.

1. Introduction

The relationship between foods and their microbiome is fundamental to ensure food quality and safety and an early detection of food pathogens and spoilage microorganisms is an important step that can help to control a foodborne outbreak or limiting food losses (De Filippis, Parente, & Ercolini, 2018; Pinu, 2016).

Current methods for monitoring the microbial contamination along the food chain rely on culture-dependent analyses. However, innovative approaches have been tested as an alternative to culture-dependent procedures to track foodborne pathogens or spoilers in foods and food-handling environments with high precision and sensitivity, as well as in shorter times (de Koster & Brul, 2016; Parlapani, 2021; Wei & Zhao, 2021).

High-throughput sequencing (HTS) technologies, are revolutionizing food microbiology: they present higher sensitivity compared with culture-dependent and other culture-independent approaches, allowing the detection of subdominant communities that may play an important role in the studied ecosystem (De Filippis, Parente, et al., 2018). Two approaches using NGS technologies are commonly used (Fig. 1): amplicon sequencing or metabarcoding, which involves the amplification and sequencing of specific marker genes and shotgun metagenomics of the whole genomic content of the microbial communities. Metabarcoding is the most used in food microbial ecology and is useful to provide an overall taxonomic picture of the microbial community. On the contrary, in the shotgun-based approach, the whole DNA or RNA (after the synthesis of complementary DNA) is fragmented by enzymatic or mechanical methods and sequenced. Besides providing the taxonomic composition of the whole microbial community (including Bacteria, Fungi, Archaea, Protozoa, etc.), this analysis allows to describe its functional capability, identifying the presence and abundance of specific genes of interest (e.g., genes involved in spoilage activities or virulence) and reconstructing the related metabolic pathways (De Filippis, Parente, et al., 2018; Jagadeesan et al., 2019; Fig. 1). In addition, reconstruction of microbial genomes directly from metagenomics reads (Metagenome-Assembled Genomes, MAGs) is a promising approach to track specific microbial strains across the food chain, as well as for epidemiological purposes (Fig. 1). In this review, we aim to provide a picture of the possibilities of implementation of HTS-based approaches withing food industry and for the official control bodies, to support product quality

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and safety management plans, as well as discussing some limitations that still limit the actual application.

2. Metagenomics-based tracking of food-borne pathogens

A promising implementation of metagenomics in food safety issues is the possibility of strain typing and monitoring (De Filippis, Parente, et al., 2018). Official methods for epidemiological tracking of strains in case of an outbreak are based on culture-dependent strategies. Although such strategies have been widely validated throughout the past decades, they are time-consuming, and their effectiveness depends on the ability of the pathogen to grow in/on different media with specific environmental conditions, as well as on their growth-rate (Law, Ad Mutalib, Chan, & Lee, 2015). Indeed, it has been reported that most of food recalls in the US occur after the outbreak conclusion (Qiu et al., 2021), highlighting the limits of existing pathogen detection procedures. When a putative outbreak occurs, it is necessary to communicate quickly with stakeholders and consumers, in order to prevent the consumption of the contaminated food and limit the spread of the outbreak. Besides being time-consuming and unable to provide a quick response in case of a foodborne outbreak, these methods often fail to isolate the strain when the loads are low (Buytaers et al., 2021).

There's a strong need for novel procedures to track pathogenic bacteria (Law et al., 2015). In this context, omics-based monitoring of microbial contamination can be usefully applied for epidemiological tracking of foodborne pathogens, allowing a rapid identification of food products responsible for an outbreak. Reconstructing microbial Metagenome-Assembled Genomes (MAGs) from the metagenomes of food products and biological samples of the subjects implicated in the outbreak, it may be possible to identify the pathogenic strain responsible, as well as the specific food where it originated. In addition, foods contaminated by pathogens may benefit from strain-specific investigation of the microbiota that may reveal the occurrence of more than one pathotype in the case of correlated episodes of infection/intoxication.

Some studies have tested the ability of shotgun-metagenomic approaches to track pathogens efficiently and accurately from spiked or naturally contaminated food. For instance, Leonard, Mammel, Lacher, and Elkins (2015) showed that a Shiga Toxin-producing *Escherichia coli* strain can be easily retrieved and virulence-profiled from spiked minimally-processed spinach after an 8-h enrichment, whereas Ottesen et al. (2016) applied a similar approach to reconstruct the genomes of *Listeria monocytogenes* strains from a naturally contaminated ice-cream. In addition, Hyeon et al. (2018) successfully serotyped *Salmonella enterica* strains from both spiked and unspiked foods after a pre-enrichment step. Since foodborne pathogens may be present at low or very low levels in the products (Grützke et al., 2019), a cultural pre-enrichment step is often necessary to obtain a suitable number of genome copies from the target pathogen, which are needed to correctly characterize the pathogenic strains and its virulence-associated genes. Indeed, this approach – which has been named "quasi-metagenomic" (Hyeon et al., 2018; Ottesen et al., 2020) – is faster and more efficient than a traditional method for food surveillance purposes, since it allows to depict a pathogenic strain genome saving at minimum 2 days (Leonard et al., 2015), and it might be used soon by food industry to detect pathogens in food, recall contaminated batches of products from the market and save lives (Ottesen et al., 2020). When using a traditional approach, a foodborne pathogen can be characterized using Whole Genome Sequencing (WGS), which is widely used to identify virulence or antimicrobial resistance-related genes, as well as mobile elements (EFSA, 2019). However, WGS relies on the availability of the microbial isolate (Huang et al., 2017) and can only occur after the completion of cultural-dependent procedures, thus with no advantage in terms of analysis speed.

In addition, several foodborne pathogens such as *Escherichia coli* O157:H7 and *Listeria monocytogenes* may be unculturable in some circumstances, as they enter in a "viable but non-culturable" (VBNC) state after being exposed to extreme conditions (e.g., several food sanitization treatments; Ferro, Amorico, & Deo, 2018), although being metabolically active and able to cause illness upon ingestion (Li, Mendis, Trigui, Oliver, & Faucher, 2014; Zhao, Zhong, Wei, Lin, & Ding, 2017). Once

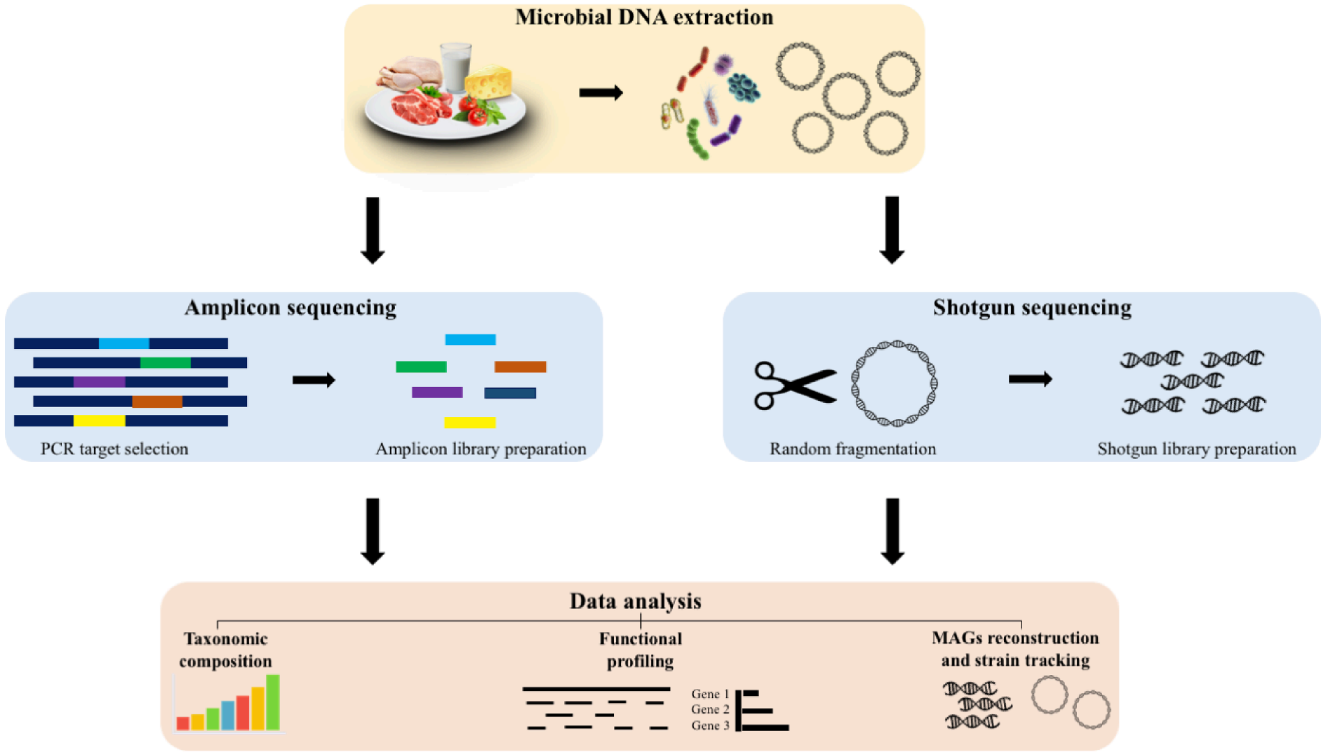

Fig. 1. Graphic representation of high-throughput sequencing applications for the study of food microbiome. Abbreviations: **MAGs**: Metagenome-Assembled Genomes.

bacteria enter in the VBNC state, they are unable to grow on media, leading to an underestimation of their concentration or to false-negative results (EFSA, 2019). Also in this case, a metagenomic-based approach may be useful to detect these strains.

3. Next-generation, omics'-based risk assessment

Risk assessment models allow to predict the behaviour and the growth rate of a microorganism in response to a specific environmental condition. In order to develop a reliable model, several parameters should be taken into account, such as the food matrix intrinsic factors (e.g., pH, a_w, composition) and the extrinsic factors related to the storage conditions (e.g., temperature, type of packaging) and to the treatment applied (e.g., thermal treatment, high pressure processing). In addition, the interaction of the microorganism studied with the microbial community should be also considered. Indeed, the specific food microbiota may contrast the growth of the pathogenic taxon, through the production of antimicrobials (e.g., organic acids, bacteriocins). On the contrary, mutualism or synergistic interactions may also occur, where some taxa may promote the growth of the pathogen (den Besten et al., 2018). In this context, combining data on presence/absence of a pathogen with the total microbial community description obtained by 'omics techniques, may help to develop more realistic models for risk assessment (Cocolin et al., 2018). In addition, the use of genomics and transcriptomics data may help in fine tuning the models to take into account the different metabolic responses of the pathogen to external conditions, as well as the strain-level diversity that may affect this response. Indeed, integration of 'omics might allow the development of quantitative models predicting the pathogen abundance and its development across the food chain (den Besten et al., 2018).

Finally, implementation of 'omics data in Quantitative Microbiological Risk Assessment (QMRA) models would promote the application of such models to food spoilage dynamics. Indeed, although the use of QMRA to food spoilage is still limited, this approach has great potential to support the food quality management decisions, to select the most appropriate combinations of treatment, additives and storage conditions and to define the effective expiration date of the product (Cocolin et al., 2018; Koutsoumanis, Tsaloumi, Aspridou, Tassou, & Gougouli, 2021).

Although the potential of 'omics-based predictive models, this approach is still in its infancy. One of the most important steps towards its development is the collection and storage of environmental variables and metadata on the process that can constitute a database to inform the model development.

4. Use of metagenomics for monitoring food spoilage dynamics

'Omics may be successfully implemented within the food industry, for microbiome mapping in the processing plant environment, microbial source tracking investigation or for monitoring the product shelf-life, identifying the presence of microbial spoilers and how processing/storage conditions may affect microbial dynamics (Forbes, Knox, Ronholm, Pagotto, & Reimer, 2017; Jagadeesan et al., 2019; Miller, Montoya, Gardy, Patrick, & Tang, 2013; Zhou et al., 2016).

In food processing facilities, a resident microbiome adapted to the specific food industry environment can colonize the surfaces of equipment and tools and be transferred to the food product during handling, manufacturing, processing and storage. It is recognized that routinary cleaning and disinfection procedures are not always effective in eliminating the resident microbial consortia specific for each food plant, since they often develop as a biofilm on surfaces that are particularly difficult to clean and may represent a potential risk for food safety and quality (De Filippis, Valentino, Alvarez-Ordóñez, Cotter, & Ercolini, 2020). In this context, mapping the environmental microbiome using sequencing-based approaches may help identifying the presence of potentially spoilage agents even when their abundance is low, recognizing possible contamination routes and critical process points, and support the development of more appropriate cleaning procedures.

In addition, variations in the baseline microbiome of raw materials, in the product intrinsic factors (e.g., pH, salt concentration, water activity, antimicrobials), or in processing conditions (e.g., heat treatments) may lead to changes in the microbial community, thus affecting the product shelf-life. These data may be implemented in statistical and predictive models, helping to predict the product shelf-life, anticipate microbial spoilage and avoid food loss (Ercolini, 2013; Jagadeesan et al., 2019).

While shotgun metagenomics is still underexploited in food microbiome studies, amplicon sequencing data on food-associated microbiota accumulated in public repositories (Table 1). These data potentially represent a useful resource to be exploited in *meta*-studies, to improve our knowledge of food microbial consortia involved in food spoilage and on the influence of processing parameters on their development. With this aim, some years ago a repository of food-associated microbiota based on public data was developed, namely FoodMicrobionet (Parente et al., 2016), that collect taxonomic data on the microbial community of foods and food-related environment, together with available metadata retrieved from the original study.

However, several issues limit the use of publicly available data by the stakeholders of the food system. Firstly, bioinformatics skills are necessary for data analysis and specifically trained personnel is rarely present in food industry, regulatory agencies, and control bodies. Another important point is the still limited number of studies that make sequencing data publicly available. Indeed, although this practice is becoming common in most recent years since several journal consider it mandatory for publications, there are a consistent number of studies where sequences are not available on public repositories or where the scant metadata present make them useless for *meta*-studies. Indeed, the standardization and quality of metadata released with sequencing data is another important issue. In order to create a database of microbial communities in foods and related environment, metadata about product composition, processing and storage conditions should be considered as the minimum requirements. Besides this, the standardization of protocols and the development of Standard Operating Procedures (SOPs) for food microbiota analysis would be necessary, to make results of different studies more comparable. Indeed, all steps including sample collection, DNA extraction, the choice of target gene and primers to be used in PCR, as well as the data analysis pipeline may affect the final results (De Filippis, Parente, Zotta, & Ercolini, 2018; Keisam, Romi, Ahmed, & Jeyaram, 2016; Maillet et al., 2021; Witte et al., 2018).

To provide some examples on how amplicon sequencing data may be used to understand microbial dynamics during food spoilage, we carried out a *meta*-analysis of publicly available data on three different food chains (raw meat, milk and fishery products). We retrieved sequences and related metadata from 18 16S rRNA sequencing studies (Table 1), for a total of 981 samples (601 from meat and meat-processing environment, 148 from milk, 232 from fish and seafood products). Data were analyzed using a common pipeline, as implemented in QIIME2 (Bolyen et al., 2019; https://qiime2.org).

4.1. Case 1. The raw meat chain

Meat and meat products are highly perishable, with colonization and development of a variety of microorganisms, especially bacteria. This is due to complex nutrient-rich environment with chemical and physical conditions favorable to bacterial development (Cauchie et al., 2020). Raw meat is characterized by a complex microbiota, that may originate from the animals themselves or from the processing environments, such as slaughterhouses or butcher shop, as well as from the ingredients used (Van Reckem, De Vuyst, Weckx, & Leroy, 2021). Microbial spoilage of meat is a complex process affected by competition among different microbial groups and their biotic and abiotic interactions (Yang, Zhu, Zhang, Liang, & Luo, 2018). The different storage conditions, such as gaseous atmosphere, storage temperature and the application of

Table 1
HTS studies of food microbiota used for the *meta*-analysis. Studies are grouped according to the type of food.

Food Category	Environment sampling	16S V region	Study Aim	Primer used	Sequence Read Archive Accession Number	Reference
Meat	no	VI-V3	Investigate the influence of pathogen contamination and storage temperature on the beef microbiota, analyze microbial interaction in the beef microbiota under different storage conditions over time after contamination and understand the effects of contamination and temperature on the spoilage of meat.	340F and 518R	PRJEB35020	Choi, Hwang, Kim, & Choi, 2020
Meat	no	V1-V3	Study the development of microbial communities in beef during chilled storage in high oxygen MAP and VP.	8F and 518R	PRJNA293921	Jääskeläinen, Hultman, Parshintsev, Riekkola, & Björkroth, 2016
Meat	yes	VI-V3	Investigate the bacterial community composition in various processing stages of a man-made food manufacture niche to characterize the processing plant and product microbiomes and to address the persistent hygiene problem.	8F and 518R	PRJNA293141	Hultman, Rahkila, Ali, Rousu, & Björkroth, 2015
meat	no	VI-V3	Assess the role of sodium chloride reduction in meat products, both at the level of spoilage development and at the level of bacterial diversity, using 16S rRNA amplicon sequencing and raw pork sausage as a meat model.	27F and 534R	PRJEB9493	Fougy et al., 2016
meat	yes	V1-V3	Provide an in-depth description of the microbiota of meat and meat processing environments, highlight the importance of the environment as a contamination source of spoilage bacteria and show that the size of the retail facility does not affect the level and type of contamination.	28F and 519R	PRJNA314853	Stellato et al., 2015
meat	no	VI-V3	Assess the viable bacterial communities in beef burgers stored in nisin-based antimicrobial packaging and follow the changes in bacterial counts and diversity during storage at 4 °C.	28F and 519R	PRJNA272131	Ferrocino et al., 2016
meat	no	V1-V3	Use of metagenomics to compare the composition, diversity and metabolic potential of the microbial communities marinated and unmarinted broiler fillet strips.	pA' and pD'	PRJEB2763	Nieminen et al., 2012
meat	no	V3-V4	Investigate the effect of AO and EW treatments on the complexity and dynamics of the potential active microbiota of beefsteaks and their associated volatilome, during storage at 4 °C and in vacuum packaging conditions.	341F and 785R	PRJNA358161	Botta et al., 2018
meat	yes	V1-V3	Investigate the microbiota encompassing putative bacteria of whole broiler meat, packaged in modified atmosphere, during and exceeding shelf-life.	27F and 534R	PRJNA523688	Lauritsen, Kjeldgaard, Ingmer, Bisgaard, & Christensen, 2019
meat/fish	no	VI-V3	Provide a more integrated view of the bacterial communities associated with meat and seafood spoilage.	27F and 534R	PRJEB4975	Chaillou et al., 2015
fish	no	VI-V3	Investigate the microbiota of small octopuses, collected from different sampling sites and compare the differences of microbiota, based on the regions and the sampling times. Assess the influence of pathogens on the composition and potential functions of the indigenous microbiota through artificial infection experiments.	340F and 518R	PRJEB21616	Choi et al., 2020
fish	no	V3-V4	Characterize the spoilage microbiota of hake fillets stored under MAP at different temperatures using high-throughput 16S rRNA gene sequencing and to compare these results with those obtained using traditional microbiology techniques.	341F and 805R	PRJNA574112	Antunes-Rohling et al., 2019
dairy	no	V1-V3	Investigate the dynamics of bacterial populations during Fontina PDO cheese manufacture and ripening, and to evaluate possible correlations between microbiota and different lactation stages.	28F and 519R	PRJNA230456	Dolci, De Filippis, La Storia, Ercolini, & Cocolin, 2014
dairy	no	V1-V3	Longitudinal characterization of the microbial community structure and gene expression during ripening of the traditional Italian Caciocavallo Silano cheese and evaluation of the effect of temperature on microbial metabolism.	28F and 519R	PRJNA290349	De Filippis, Genovese, Ferranti, Gilbert, & Ercolini, 2016
dairy	no	V3-V4	Investigate the bacterial communities of raw milk from ten dairy farms in Shanghai (China) with high-throughput sequencing technology and the variation in the bacterial communities throughout one whole year.	338F and 806R	PRJNA389757	Li et al., 2018
dairy	no	V3-V4	Determine the dynamic changes in flavor compounds and microbiota profiles in HTST pasteurized milk products, provide new insights into exploring the variances and similarities of the safety of pasteurization, and analyze the core functional microbiota related to the formation of flavor compounds and safety of pasteurized milk.	338F and 806R	PRJNA627305	Ding et al., 2020
dairy	no	V1-V3	Study the microbial dynamics of the active fraction of the microbiota during the manufacturing and ripening of a raw-milk, long-ripened, hard-cooked, Grana-type cheese, with particular emphasis on the contribution of milk and whey starter, by coupling reverse transcriptase PCR (RT-PCR)-	28F and 519R	PRJNA255096	Alessandria et al., 2016

(*continued on next page*)

Table 1 (*continued*)

Food Category	Environment sampling	16S V region	Study Aim	Primer used	Sequence Read Archive Accession Number	Reference
			denaturing gradient gel electrophoresis (DGGE), quantitative PCR (qPCR), and rRNA pyrosequencing.			
dairy	no	V3-V4	Study of the bacterial communities of Liqvan cheese during its manufacturing through reverse transcriptase PCR (RT-PCR)-DGGE. Furthermore, the total counts and diversity of the viable bacterial populations in the raw milk, curd and ripened cheese have been investigated using RT quantitative PCR (RT-qPCR) and 16S rRNA gene amplicon sequencing, respectively.	341F and 805R	PRJNA319425	Ramezani, Hosseini, Ferrocino, Amoozegar, & Cocolin, 2017

antibacterial compounds, may have a major effect on microbial growth and dynamics of different populations (Wang et al., 2017). Thus, food spoilage is problematic for two main reasons: first, it renders food unfit for human consumption and, secondly, it results in significant economic losses. In particular meat accounts for approximately 4% of total food waste, with meat losses being severest at the end of the food chain in industrialized regions. Here, almost half of meat waste occurs at the consumption level (Cauchie et al., 2020; Duthoo et al., 2021).

Furthermore, meat and poultry, especially their processed products, often prone to adulteration practice in the food supply for economic gain. Their impacts are related to food security and safety, consumers' acceptability and lifestyle. Therefore, it is necessary to develop reliable methods for identifying the species of origin of meat and poultry products (Xing et al., 2019).

For example, Lactic Acid Bacteria (LAB) are considered the main players in spoilage of meat stored under vacuum or in carbon dioxide-rich atmosphere. *Leuconostoc*, *Carnobacterium*, *Lactobacillus* and *Lactococcus* are among the most frequently involved genera, since psychotropic species can easily outcompete the aerobic microbiota (Doulgeraki, Paramithiotis, Kagkli, & Nychas, 2010; Pothakos, Devlieghere, Villani, Björkroth, & Ercolini, 2015) (Fig. 2). Chaillou et al. (2015) highlighted that the microbiota of aerobically stored beef was dominated by *Pseudomonas* and led to more rapid spoilage. On the contrary, LAB were associated with vacuum packaging, and both *Carnobacterium* and *Pseudomonas* were identified as SSOs in modified-atmosphere packaging, leading to different spoilage-associated

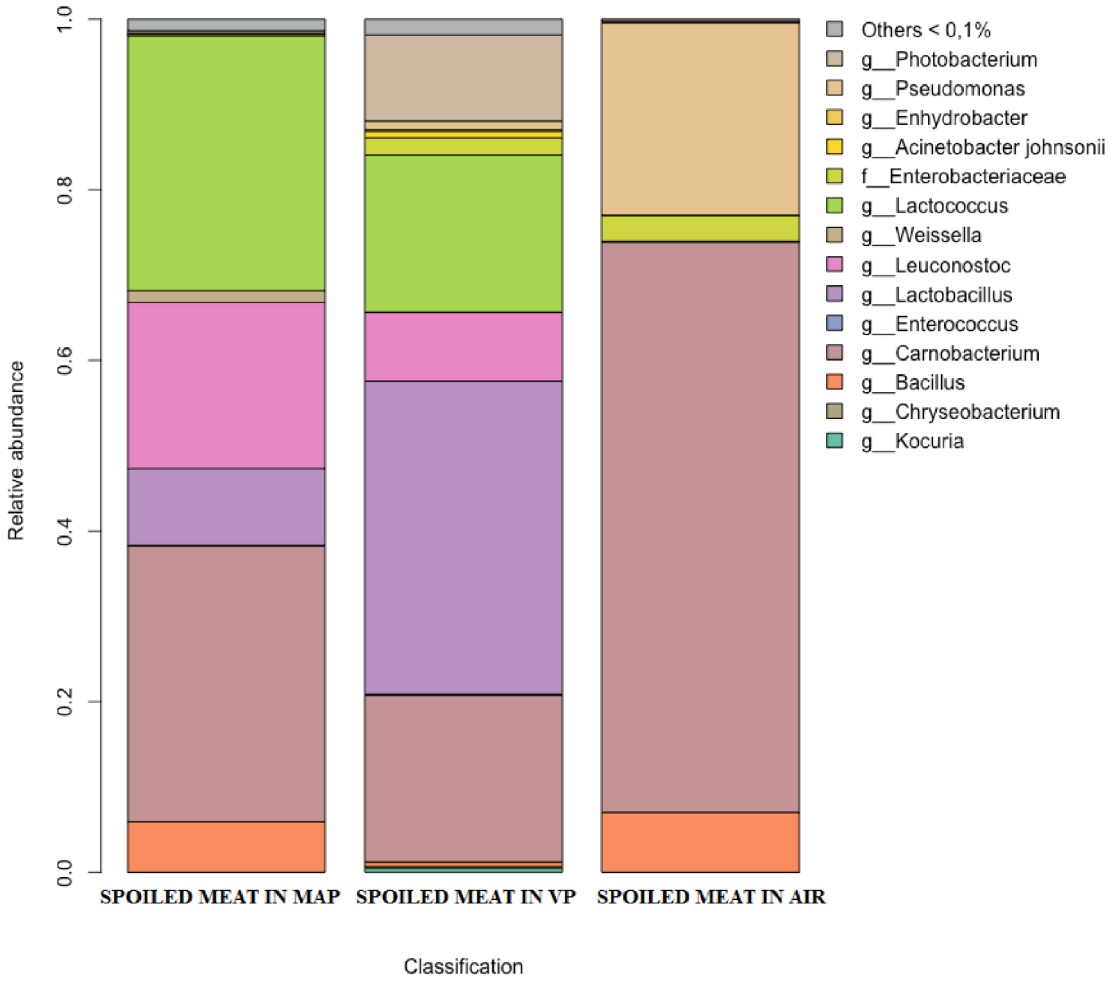

Fig. 2. Histogram showing the relative abundance of bacterial genera (>0.1%) in spoiled meat under various packaging conditions. **MAP**: Modified Atmosphere Packaging; **VP**: Vacuum packaging; AIR: Aerobic packaging.

metabolomes. However, gaseous atmosphere may also exert a selection at strain level. For example, it was demonstrated that some strains of *Pseudomonas fragi*, usually considered as a spoiler of meat stored aerobically, may select during storage under vacuum (De Filippis, La Storia, Villani, & Ercolini, 2019). In this case, the use of shotgun metagenomics would be more informative. As reported above (see paragraph 2), reconstruction of MAGs may identify different strains and track their dynamics during storage.

Moreover, the addition of other ingredients (e.g., salt, spices) that may have an antimicrobial activity also impact the selection of Spoilage Specific Organisms (SSOs), thus influencing the spoilage rate. Indeed, Fougy et al. (2016) demonstrated that the salt concentration in pork sausages stored under vacuum may influence SSOs diversity and the spoilage speed.

However, regardless the packaging conditions, a common spoilage-associated core microbiome can be observed (Fig. 3). These taxa are always present in fresh and spoiled meat, as well as in meat-handling environment, highlighting that they are well-adapted and able to become resident in this type of environment and can be transferred to the meat (De Filippis, La Storia, Villani, & Ercolini, 2013; Stellato, De Filippis, La Storia, & Ercolini, 2015). During storage, extrinsic factors, mainly related to temperature and type of packaging, may exert a selective pressure, boosting the development of some of them (Fig. 3). Therefore, using HTS for microbiota mapping in meat processing and handling environments is an interesting opportunity for food industry to identify possible contamination routes.

4.2. Case 2. Milk chain

Milk is a highly nutritious food that can be obtained from a variety of animal sources such as cows, goats, sheep and buffalo. However, the high nutrient content, coupled to a near neutral pH and a high-water activity, makes it the ideal environment for the growth of many microorganisms (Quigley et al., 2013). The microbiota of raw milk is highly complex, including LAB, such as *Lactobacillus*, *Streptococcus*, *Enterococcus*, *Lactococcus* and *Leuconostoc,* besides several other Gram-positive bacteria (e.g., *Bacillus*, *Macrococcus* and *Corynebacterium*). However, also Gram-negative, such as *Enterobacteriaceae* (e.g., *Enterobacter*, *Hafnia*, *Klebsiella*) and *Pseudomonas* spp. are frequently found in raw milk (Fig. 4).

Psychrotrophic populations usually develop during cold storage, including *Pseudomonas*, *Aeromonas* and *Acinetobacter* spp. (Quigley et al., 2013), producing extracellular lipases and proteases and leading to spoilage (Rasolofo, St-Gelais, LaPointe, & Roy, 2010). In addition, some of these enzymes are heat resistant and can endure to pasteurization or even to ultra-high temperature (UHT) treatment (Rasolofo et al., 2010).

One of the most important milk-spoiler is Pseudomonas spp. (von Neubeck et al., 2015). In particular, *Pseudomonas fluorescens, Pseudomonas gessardii, Pseudomonas fragi* and *Pseudomonas lundensis* are the species found more frequently. *Pseudomonas* spp. can become predominant in raw milk stored at low temperatures, constituting up to 70–90% of the microbial population. Many other psychrotolerant microorganisms are present in milk and may participate to spoilage, such as

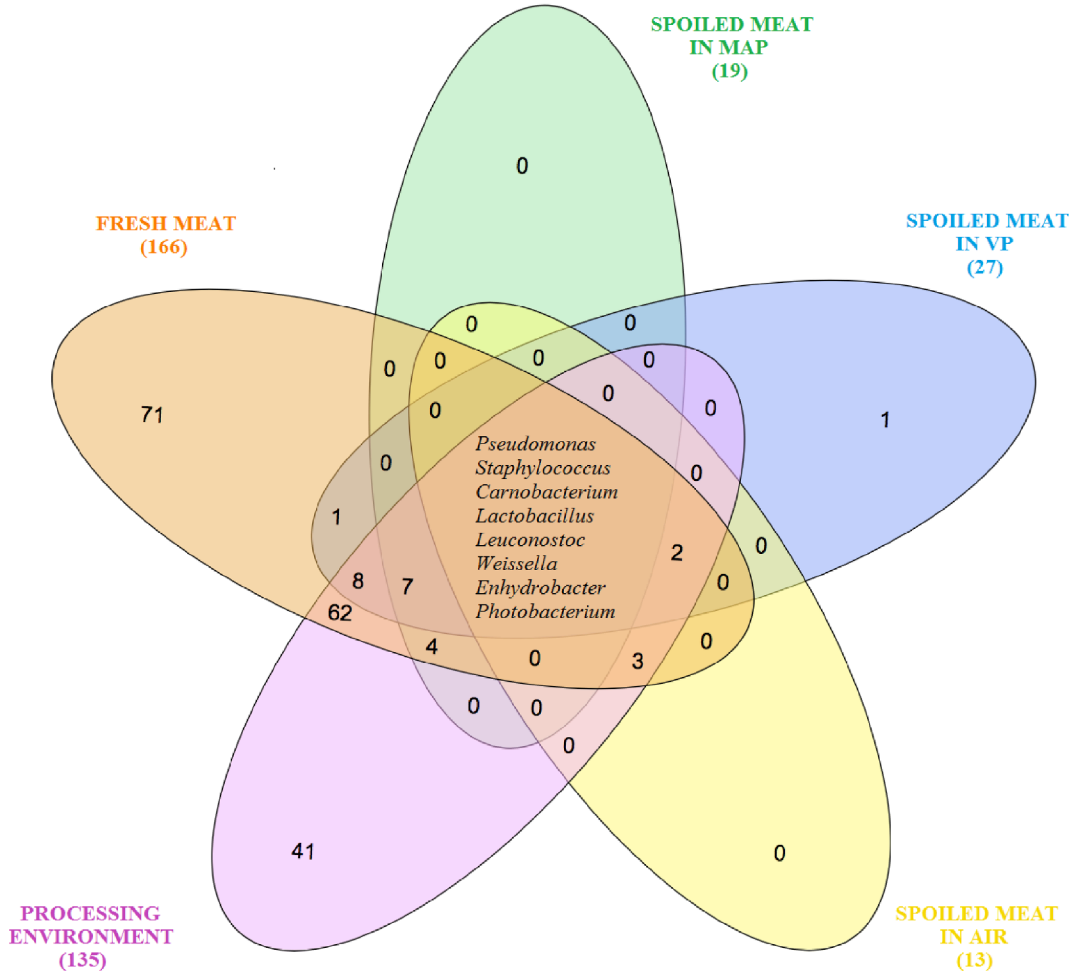

Fig. 3. Venn diagram reveals shared microbial genera among fresh or spoiled meat and related processing environments. Only genera with abundance greater than 0.1% are included. Numbers indicate the number of shared taxa. MAP: Modified Atmosphere Packaging; VP: Vacuum packaging; AIR: Aerobic packaging.

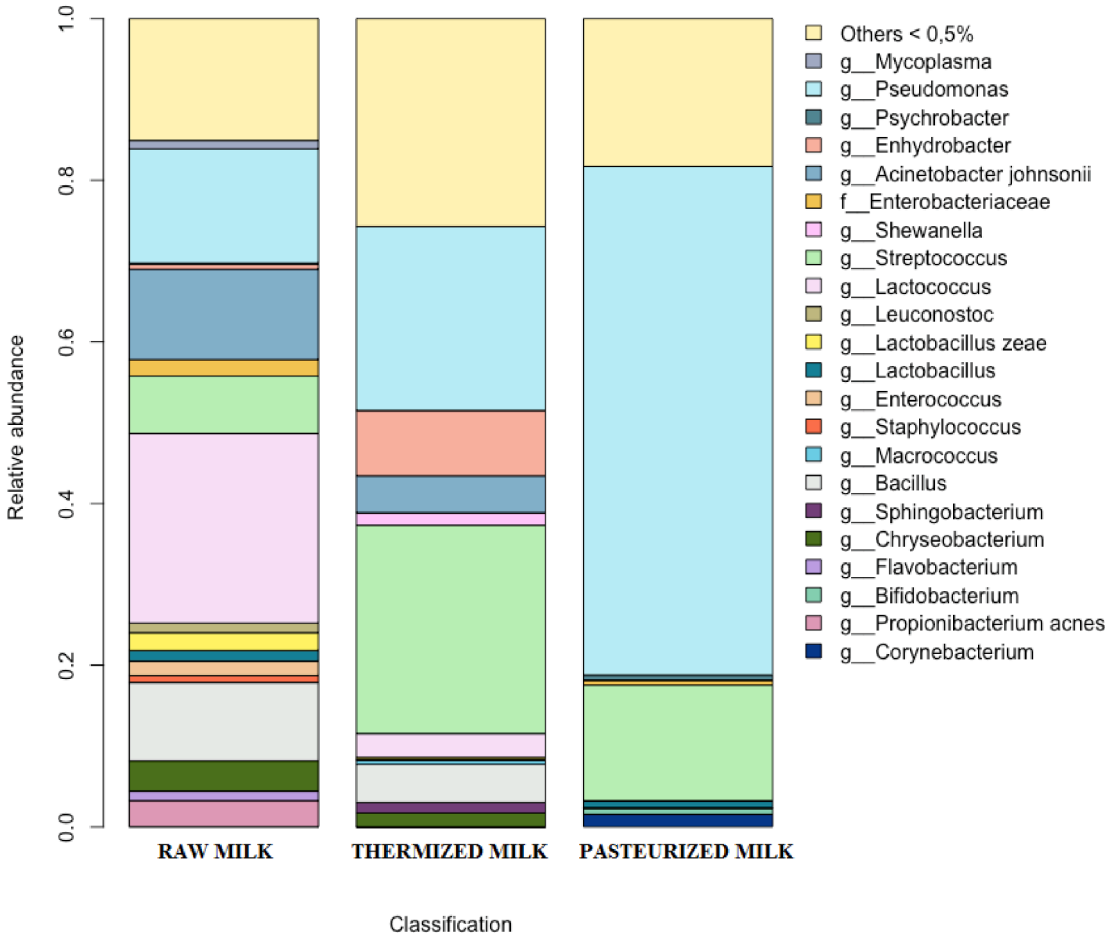

Fig. 4. Histogram showing the relative abundance of bacterial genera (>0.5%) in the milk samples analyzed.

Acinetobacter, Microbacterium, Aeromonas, Enterobacter, Flavobacterium, Corynebacterium, Clostridium, Bacillus, Staphylococcus and some LAB (Hantsis-Zacharov & Halpern, 2007).

At the same time, raw milk may be contaminated with pathogens, such as *Salmonella* spp., *Listeria monocytogenes*, *Escherichia coli*, *Campylobacter* spp., *Brucella* spp. and *Shigella* spp., that may originate from the mammary gland or associated lymph nodes of cows suffering from systemic infections, as well as from the equipment, raw milk tankers and personnel (Langer et al., 2012; McHugh et al., 2021). Indeed, milk storage silos can be considered as one of the main contamination source, since their microbiome is dominated by psychrotrophic spoilage-associated bacteria (McHugh et al., 2021).

Several treatments may be applied to increase milk shelf-life and maintain its quality over longer conservation periods. Thermization consists in heating milk at around 65 °C for 10–20 s and is effective in reducing mesophilic and psychrotrophic bacteria (Rasolofo et al., 2010). In some cases, for example when milk has to be used for cheese production, it is preferred to treatments at higher temperature because of its milder effects on beneficial milk microbiota and on the functionality of milk caseins and salts. However, thermization cannot destroy heat resistant proteolytic and lipolytic enzymes produced by psychrotrophic bacteria (Rasolofo et al., 2010; Samelis et al., 2009). The most common treatment for fresh milk destinated to human consumption is pasteurization (at least 72 °C for 15 sec), which eliminates pathogens and strongly reduce alterative bacteria, without compromising the nutritive and flavor attributes (Ding et al., 2020).

Evaluating the impact of different heat treatments on the composition of the milk microbiota is also extremely important for the subsequent production of dairy products. Indeed, several research described the microbiota of raw milk (n = 131), while only few samples of pasteurized/thermized milk are available in the literature (n = 15 and n = 2, respectively; Table 1).

Principal Coordinate Analysis (PCoA) based on Bray-Curtis distance (Fig. 5A) and box plots showing the Simpson's diversity index (Fig. 5B) of analyzed milk samples revealed that raw milk contain a high biodiversity, that may be influenced by several factors, such as farm practices, animal management and breed, milking hygiene, seasonality. On the contrary, pasteurized and thermized milk samples cluster together and have a more similar microbiota, due to a standardization of the microbial composition caused by the application of the heat treatment (Breitenwieser, Doll, Clavel, Scherer, & Wenning, 2020; Quigley et al., 2013; Fig. 5A). However, several psychrotrophic taxa (e.g. *Pseudomonas* and *Acinetobacter*) are shared by all milk samples (raw, thermized and pasteurized milk), highlighting that this core microbiota may persist after the heat treatments and develop during refrigerated storage, decreasing the product shelf-life (Fig. 6).

4.3. Case 3. Fish and seafood chain

Seafoods are among the most popular and healthiest foodstuffs worldwide, containing a variety of essential elements for human diet such as proteins, vitamins, nutrients and long-chain polyunsaturated fatty acids, including omega-3. The modern dietary trends have led to a continuously increasing demand for seafood. Both high quality and extended shelf-life of seafood is required to satisfy the nowadays dietary tendency, as well as the industrial interest to increase the added value of such products.

However, microbial spoilage is the main factor linked with the rapid

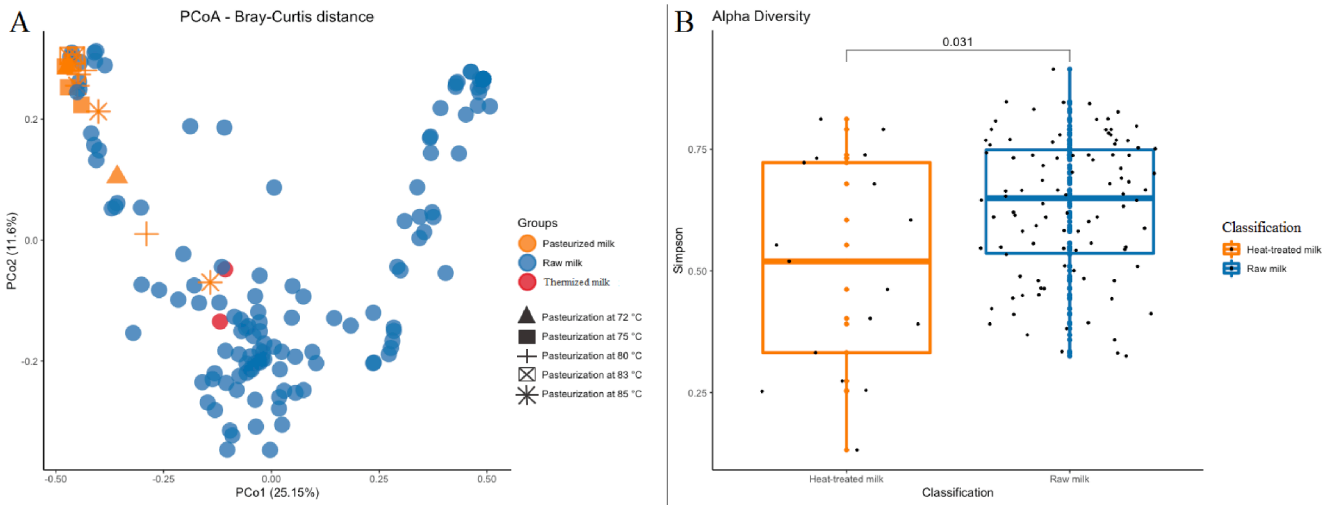

Fig. 5. Microbial diversity in milk products. **A:** Principal Coordinate Analysis (PCoA) based on Bray-Curtis distance matrices of milk samples. Sample color indicates the type of heat treatment, while shapes indicates the different temperature of the treatment (this information is available from the original studies only for pasteurized samples). **B:** Box plots showing the Simpson's diversity index of analyzed samples. Orange: Heat-treated milk; Blue: Raw milk. Boxes represent the interquartile range (IQR) between the first and third quartiles, and the line inside represents the median (2nd quartile). Whiskers denote the lowest and the highest values within 1.5 IQR from the first and third quartiles, respectively. P-value < 0.05 was considered statistically significant. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

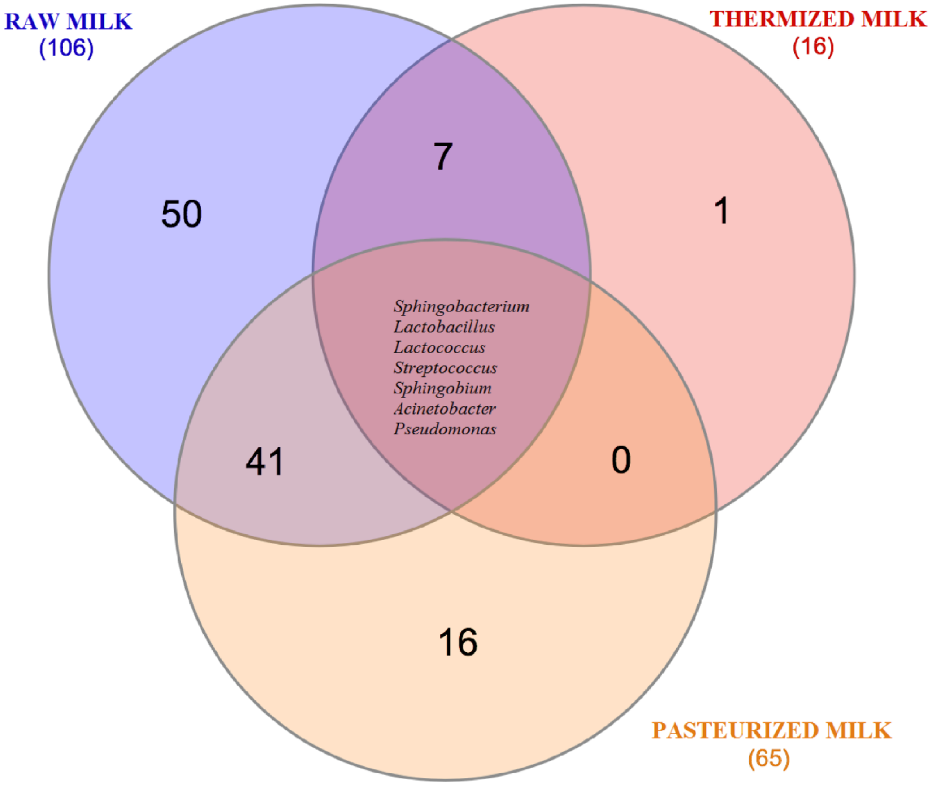

Fig. 6. Venn diagram showing shared microbial genera among among raw, thermized and pasteurized milk samples. Only genera with abundance $> 0.1\%$ are included. Numbers indicate the number of taxa.

seafood sensorial degradation, resulting in high food losses along the production and distribution chain and thus, noteworthy economic losses for seafood producing countries (Anagnostopoulos, Parlapani, & Boziaris, 2022).

Various factors such as fish feeding habits, season, geographical location, sea temperature and type of fish determine the indigenous fish microbiota (Parlapani et al., 2018).

Total fish and seafood production in the European Union reached 6.45×10^6 metric tons in 2017, 3% of the world production (Zotta, Parente, Ianniello, De Filippis, & Ricciardi, 2019).

Seafood microbiota mainly originate from the animal skin, although contamination may occur during evisceration and the following

processing steps (Chaillou et al., 2015).

The geographical location, the season and the fishing method are the main factors influencing the bacterial loads and the diversity of microbes. Indeed, the fish skin microbiota has been suggested as a method to trace its geographical origin (Liu et al., 2020).

Microbial growth and metabolism represent the major cause of spoilage of fish products. Proteins are hydrolyzed into peptides and amino acids by microbial protease, leading to changes in physico-chemical properties of fish flesh (texture, color, water-holding capability, etc.) and amino acids released by protein degradation can be converted into α-keto acids, sulfides, ammonia, trimethylamine and various kinds of biogenic amines. Some of these compounds, besides having a sensorial impact, may represent a safety risk, since may cause intoxication when ingested at high concentration (Biji, Ravishankar, Venkateswarlu, Mohan, & Srinivasa Gopal, 2016). Visible slime may also appear as a result of the production of extracellular polysaccharides (Zhuang, Hong, Zhang, & Luo, 2021).

Psychrotolerant Gram-negative bacteria (e.g., *Pseudomonas*, *Psychrobacter*, *Arcobacter, Rubritalea, Shewanella* spp.) dominate chilled fish aerobically stored, while vacuum or CO_2 packing selects for *Photobacterium, Brochothrix* and LAB (Fig. 7A) (Zotta et al., 2019). The predominance of *Photobacterium* in MAP was reported in hake fillets (Antunes-Rohling et al., 2019) and salmon steaks (Emborg, Laursen, Rathjen, & Dalgaard, 2002). Recently, MAP was also combined with other preservation strategies such as plant extracts, ozone treatment (Goncalves & Santos, 2019), and cold plasma (Olatunde, Benjakul, & Vongkamjan, 2020), to achieve a more efficient preservation effect. However, the effect on CO_2-resistant bacteria, such as *Photobacterium,* is still unexplored (Parlapani & Boziaris, 2016; Zhuang et al., 2021).

The mean relative abundances of frequently detected genera (>1% of total microbiota) in fresh and spoiled fish stored in different packaging conditions were compared (Fig. 7A). *Carnobacterium*, *Photobacterium*, *Psychrobacter*, *Leuconostoc*, *Flavobacterium*, *Lactococcus* and families such as Moraxellaceae, *Spirochaetaceae*, *Fusobacteriaceae* were confirmed as the main players in fish spoilage (Fig. 7A). In addition, microbial diversity strongly decreases during fish storage, regardless the packaging type, highlighting the selection of a well-adapted and psychrotolerant microbiome (Fig. 7B).

5. Conclusions and future perspectives

Food spoilage leads to significant wastes and is an important economic issue in food industry. Besides this, food contamination by pathogenic microbes may represent a significant safety concern.

After more than one decade of massive usage of amplicon-based HTS for the study of food microbial ecology, a huge amount of data was accumulated in public databases. As shown in the examples above, these data may be exploited in *meta*-studies useful to understand which microbial taxa represent a potential risk for food quality and safety in different food chains and how processing parameters may select a specific microbiota. These data might potentially represent a database of information useful for the development of QMRA models. However, metadata about the samples and the technological process are often not available or quite scant, thus limiting the exploitation of this resource. The definition of a minimum quality standards for published metadata and the widespread use of common metadata fields are advisable. In addition, several published studies do not make sequence data and metadata publicly available, and this strongly limit their usefulness for the scientific community and the stakeholders.

The application of shotgun metagenomics in the food chains is still limited but is advisable to understand the functional potential of food-related microbiome, as well as for tracking of specific strains relevant for safety or spoilage-related activities. Microbiome mapping in food processing plants and along the food chains may help in identifying possible contamination routes and critical points where the operators may intervene, as well as how the microbiome respond to processing and storage conditions. In this context, the reconstruction of MAGs can be an important step to identify specific pathogenic or spoiling strains and track their dynamics across the food chain. In addition, shotgun metagenomics may help in deciphering food spoilage dynamics. Indeed, it is often difficult to establish a direct link between a given spoilage phenomenon and one microbial species, since complex interactions within the microbial community are more often implicated (Yang et al., 2018). Therefore, the integration of shotgun metagenomics or metatranscriptomics may help to identify the complex pool of genes and metabolic pathways involved in the production of off-flavors or other undesired demonstration of spoilage (e.g., slime production), highlight which microbial taxa can be responsible. Data on the metabolic potential of the microbiome might be also integrated into models able to

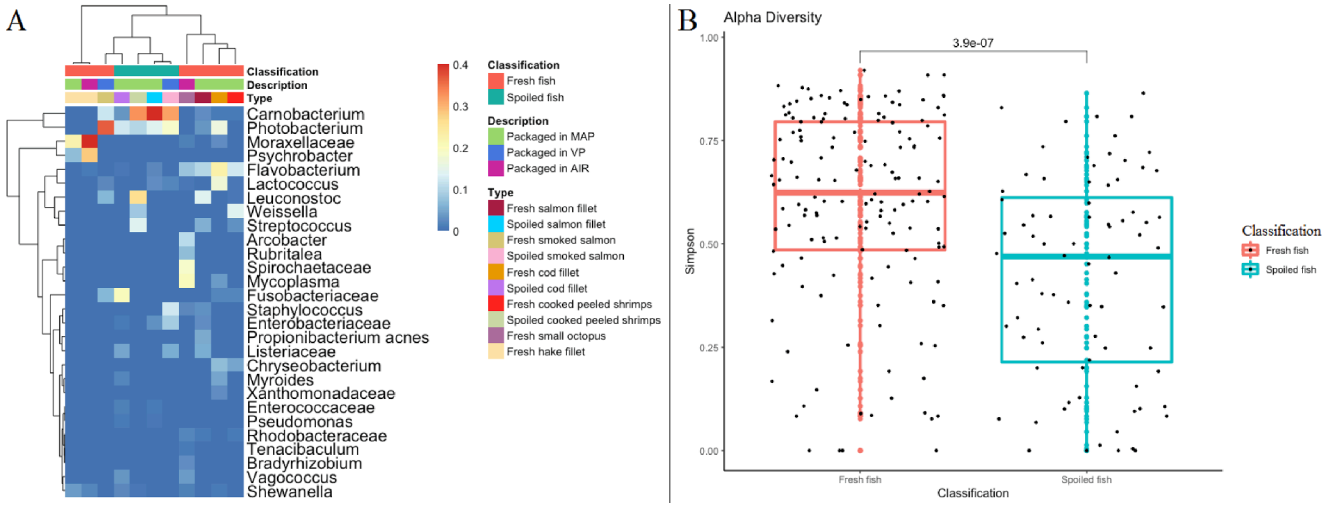

Fig. 7. Microbial diversity in fresh/spoiled fish and seafood products. **A:** Heat plot showing the abundance of the most abundant microbial taxa in fish samples analyzed (*listed on the right*). Only taxa with abundances > 1% are included. Taxa abundance was averaged for each product type. The column bars are colored according to the classification (fresh or spoiled), the different packaging and the type of product. **B**: Box plots showing the Simpson's diversity index of fresh and spoiled fish products. Red: Fresh fish; Green: Spoiled fish. Boxes represent the interquartile range (IQR) between the first and third quartiles, and the line inside represents the median (2nd quartile). Whiskers denote the lowest and the highest values within 1.5 IQR from the first and third quartiles, respectively. P-value < 0.05 was considered statistically significant. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

predict the speed and the type of dynamics occurring during spoilage, based on the microbial community description at the beginning of the shelf-life.

However, several issues should be overcome before a widespread application of 'omics in food industry: the still relatively high cost and the need for advanced bioinformatics skills for data analysis can be considered as the most important bottlenecks. In addition, a standardization of data analysis pipelines and the definition of common requirements for metadata collection would boost its inclusion in international standards contemplated and approved by legislators, as well as into the routinary quality management plans of the food industry.

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CRediT authorship contribution statement

Giuseppina Sequino: Conceptualization, Data curation, Formal analysis, Writing – original draft. **Vincenzo Valentino:** Formal analysis, Writing – original draft. **Francesco Villani:** Writing – review & editing. **Francesca De Filippis:** Conceptualization, Funding acquisition, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

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

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