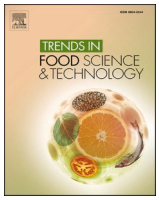
Trends in Food Science & Technology 120 (2022) 236–247

Contents lists available at ScienceDirect

Trends in Food Science & Technology

journal homepage: www.elsevier.com/locate/tifs



The evolution of knowledge on seafood spoilage microbiota from the 20th to the 21st century: Have we finished or just begun?

Dimitrios A. Anagnostopoulos, Foteini F. Parlapani, Ioannis S. Boziaris *

Laboratory of Marketing and Technology of Aquatic Products and Foods, Department of Ichthyology and Aquatic Environment, School of Agricultural Sciences, University of Thessaly, Volos, Greece

ARTICLE INFO

Keywords:
Seafood
Microbial communities
Spoilage
SSOs
HTS
Metagenomics
Novel strategies

ABSTRACT

Backround: 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 seafood sensorial degradation, resulting in high food losses along the production and distribution chain and thus, noteworthy economic losses for seafood producingcountries. In the past, the low technological capability permitted a limited and non-representative study of microbial community and thus, the results of spoilage-related microbiota present in seafood, were led to both insufficient and disputed conclusions.
Scope and approach: The scope of the present review is to evaluate how method development has improved our understanding on seafood spoilage microbiota during the past decades, discussing in parallel the current/emerging trends, as well as what could be recommended for future research efforts.
Key findings and conclusions: The advent of novel molecular technologies, mainly high throughput sequencing (HTS) set of techniques, has changed our approach regarding the study of seafood microbiota, enriching our knowledge in this field. For improving and/or ensuring seafood quality along seafood value chain, the scientific community has now the option of using such modern tools to explore and understand the complex plenomena taking place during seafood spoilage.The study of seafood microbiota changes during processing, storage and distribution, in combination with the "meta-omics" approaches, is the key to unveil the functionalities in such complicated food matrix. In the current decade, the scientific community faces the challenge to establish novel and intelligent strategies that could prevent seafood spoilage as well as to extend or even predict the shelf-life of seafood. The contribution of multi-omics is expected to enhance this attempt. Those strategies will lead to the production of high quality added value seafood, in order to meet consumers' demands.

1. Introduction

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 (Lund, 2013). In Western culture, their consumption has been proposed in relatively high amounts per week (Dietary Guidelines for Americans, 2010). According to Food and Agriculture Organization of the United Nations (Food and Agriculture Organization (FAO), 2020; Li et al., 2020), seafood industry is one of the most booming food sectors, especially in developed countries, since both global production and consumption are increasing exponentially year after year.

However, seafood are among the highest perishable foods. Seafood spoilage is the result of biochemical reactions (enzymatic activity, oxidation, etc.), and/or metabolic activity of a fraction of the seafood microbiota the so-called specific spoilage organisms (SSOs), which are responsible for the degradation of sensory characteristics during storage, making the product unacceptable and unfit for consumption (Boziaris & Parlapani, 2017; Gram & Dalgaard, 2002; Nychas & Panagou, 2011). Due to its chemical composition such as high content of nutrients and especially non-protein nitrogen compounds (NPN), high water activity and pH, this type of food is characterized as an ideal host for microbial colonization and activity by many spoilage microorganisms (Leroi & Joffraud, 2011). As these microorganisms grow, they utilize nutrients and produce a plethora of metabolites that deteriorate sensory

* Corresponding author. Dept of Ichthyology and Aquatic Environment, School of Agricultural Sciences, Fytokou street, 38446, Volos, Greece.
E-mail address: boziaris@uth.gr (I.S. Boziaris).

https://doi.org/10.1016/j.tifs.2022.01.004
Received 31 March 2021; Received in revised form 16 November 2021; Accepted 1 January 2022
Available online 6 January 2022

attributes. The selection of SSOs, as well as the matrix of their metabolic products, depend on many factors such as storage conditions (e.g., temperature and atmosphere), initial microbiota composition, microbial interactions, water activity, pH, etc. (Boziaris & Parlapani, 2017; Gram & Huss, 1996; Ioannidis et al., 2018). Poor hygiene or sanitary practices and improper conditions (e.g., abuse temperature) in harvesting, handling, storage, processing, and distribution, favor the growth of such microorganisms and thus the product shelf-life is shortened. Especially, such improper practices can individually or en masse strongly influence both the rate and course of spoilage, as they strongly affect the microbiota profile and activity in seafood.

Spoilage is a global problem regarding sustainability, since it is the most important cause of fish losses around the world. More than 30% of the total fish production is lost every year due to such problems in the food supply chain (Food and Agriculture Organization (FAO), 2020; Li et al., 2020; Gustavsson, Cederberg, & Sonesson, 2011). Fish losses can be created during icing, packaging, storage and transportation after catch (post-harvest handling and storage), processing, and distribution including losses in markets and retailers (Parlapani, 2021). Thus, to reduce the high levels of losses, stakeholders should be more vigilant during several critical points along the food value chain. By taking into account the enormous economic losses, as the result of seafood losses, spoilage constitutes a multidimensional problem, which requires effective solutions, at a global scale.

Unlike foods with low water content, seafood are characterized by high water activity and can be easily contaminated by several bacteria species (Odeyemi, Burke, Bolch, & Stanley, 2018). However, the temperament of seafood spoilage functions are quite complex, while the correlation between factors contributing to this phenomenon is unresolved. An in-depth studying and understanding of the mechanisms that result in seafood spoilage, is the key to establish novel and well documented preventive and control measurements. For instance, the monitoring of microbiota evolution during storage using novel technologies and methodologies, constitutes a rational approach for seafood quality evaluation to meet both industrial needs and consumer's demands for high-quality products (Parlapani, 2021). Revealing all the conditions under which the growth and dominance of SSOs is favored, play a fundamental role in understanding spoilage mechanisms, in order to apply effective strategies for preservation of seafoods. Through the years of extensive research, the attention of the scientific community has been focused on the study of microbial communities present in food/seafood during processing, storage and distribution in the altar of saying "who is there" (Cocolin et al., 2018). The latter could be achieved through the use of each time available technology, aiming to obtain a satisfying and representable microbiota snapshot.

In the past, the limited capabilities of the available technology allowed a limited, incomplete and thus, unrepresentative display of seafood microbiota, leading to unanswered questions, or even to wrong conclusions. With the passage of time, novel and modernized molecular techniques have been developed to cover the lack of this discriminatory power. In the last two decades, a set of novel molecular culture-independent techniques has been established, allowing a better recording of microbial communities. Subsequently, the advent of High Throughput Sequencing (HTS) technology in the last decade, brought a revolution in the field of food microbiology, since this set of methods can uncover the majority; if not all; of both cultivable and non-cultivable microbial groups directly from the sample, at high discriminant levels, making possible a suitable study of the microbiota existing in a seafood ecosystem. Undoubtedly, Next Generation Sequencing (NGS) set of techniques represents a step forward regarding the way food microbiologists determine microbial community and its complexity in several foods, enriching the current knowledge on seafood spoilage, the key role of SSOs and the factors that affect the microbiota formation along food production chain. Thus, the scientific community possesses a set of high-tech tools, the use of which makes it feasible to obtain the "real picture" about what is taking place during processing and/or storage, in such a complex matrix.

For all the aforementioned reasons, the aim of the present article is to provide an overview on how method development has improved the exploration of seafood spoilage microbiota during the past decades, highlighting in parallel the current/emerging trends, as well as what could be recommended as future prospects.

2. Seafood spoilage microbiota

2.1. Culture-dependent methods

Culture-dependent methods (classical/conventional approach) have been used for several decades to study seafood initial and spoilage microbiota. It involves a variety of techniques, based on plate microbial culture, through the use of culture media (selective, elective and general purpose) to enumerate and isolate targeted or non-targeted microbial groups from seafood, followed by a set of *in vitro* biochemical assays (e.g. Gram-reaction, catalase and oxidase tests, Hugh and Leifson reaction, production of gas using glucose as a unique source of carbon, growth at several temperatures, resistance to various NaCl levels, resistance to acidic and basic environment, sensitivity to various compounds etc.), and morphological or immunological tests, aiming to identify the isolated microbes, up to the genus level (Tryfinopoulou, Tsakalidou, & Nychas, 2002).

Based on such phenotypic approaches, the spoilage microbiota of several fish species, originated from temperate waters, has been found to be dominated mainly by the psychrotrophic Gram-negative bacteria *Pseudomonas* and *Shewanella* (Gennari, Tomaselli, & Cotrona, 1999; Gram & Dalgaard, 2002; Koutsoumanis & Nychas, 2000; Leisner and Gram, 1999). Of these, it has been found that primarily *Shewanella* and secondarily *Pseudomonas* spoil fish from the cold temperate waters (Dalgaard, 2003; Gram, 1992, 2009; Gram, Trolle, & Huss, 1987), while *Pseudomonas* and secondarily *Shewanella* spoil fish from the warmer temperate waters (Koutsoumanis & Nychas, 1999, 2000; Tryfinopoulou et al., 2002) stored aerobically at chilled temperatures. The domination of lactic acid bacteria (LAB), *Photobacterium* and *Brochothrix thermosphacta* has been considered also important for fish stored under vacuum or reduced oxygen and elevated carbon dioxide packaging conditions such as Modified Atmosphere Packaging (MAP) (Dalgaard, Gram, & Huss, 1993; Drosinos & Nychas, 1996; 1997a; Gram & Huss, 1996; Koutsoumanis, Taoukis, Drosinos, & Nychas, 2000). Additionally, in tropical fish, the microbiota profile was more or less the same (Emborg, Laursen, Rathjen, & Dalgaard, 2002), although it has been usually noted higher presence levels of some Gram-positive and Gram-negative bacteria such as LAB and Enterobacteriaceae, respectively (Gram, 2009). In line to fish microbiota profile, the microbiota of other seafoods such as bivalve mollusks, crustaceans and cephalopods seems to be similar, using the classical microbiological approaches, despite that those different aquatic organisms have quite a different lifestyle as well as different composition gross (Martino & Da Cruz, 2004; Seibel, Goffredi, Thuesen, Childress, & Robison, 2004), a fact that should affect somehow the spoilage patterns (Gram, 2009). However, the classical approach reveals some indicates, regarding the formation of microbial dominance in seafoods from different geographical zones. For instance, it has been noted that the spoiled shrimp from temperate waters, is dominated by *P. fragi*, while *S. putrefaciens* is the predominant bacterial species in the tropical shrimp (Chinivasagam, Bremner, Thrower, & Nottingham, 1996). *Acinetobacter* is commonly found in brown shrimp from Georgia, USA (Heinsz, Harrison, & Leiting, 1988), both at low and high temperature, indicating that this bacteria species had a strong survival capability and could interact with other spoilage bacteria, during storage. Continuously, another study deals with shrimps from central America coastal, indicated that *Shewanella* predominated, while the presence of other bacteria such as *Pseudomonas*, Coryneforms, LAB and *Acinetobacter* was limited (Benner, Staruszkiewicz, & Otwell, 2004). Furthermore, the effect of fishing zone in microbiota formation was

indicated by Chinivasagam et al. (1996), who reported that the most abundant isolated bacteria were Gram-positive bacteria regarding Australian shrimps caught at low depth zone. On the contrary, the dominance of *Pseudomonas* was profound in shrimps caught in deeper fishing zones. However, the findings by Jeyasekaran, Ganesan, Anandaraj, Jeya Shakila, and Sukumar (2006) are not fully in line with the above study, as the dominance of *Pseudomonas*, is mainly storage-dependent, indicating the significant effect of storage temperature, as well.

In the first decade of the 21st century, the classical identification of the isolates was almost replaced by the molecular identification e.g. full-lenght or partial 16 S rRNA gene sequencing analysis, genotyping using several fingerprint methods such as Random Amplified Polymorphic DNA (RAPD), Repetitive Sequence-based PCR (rep-PCR) etc., in order to reach identification at higher taxonomy levels (species or strain). The use of genes as targets significantly expanded the field of food microbiology, since the reading of the sequences revealed a great number of microbial species and strains. Consequently, the knowledge on microorganisms existing in a foodsystem like seafood, started to change. Researchers have now identified, at genus, species or strain level, spoilage associated bacteria isolated from various fish stored under air (Parlapani & Boziaris, 2016; Parlapani, Kormas, & Boziaris, 2015; Parlapani, Verdos, Haroutounian, & Boziaris, 2015; Tryfinopoulou et al., 2007), MAP (Alfaro & Hernandez, 2013; Hovda, Lunestad, Sivertsvik, & Rosnes, 2007; Hovda, Sivertsvik, Lunestad, Lorentzen, & Rosnes, 2007b; Macé et al., 2012; Parlapani, Kormas, & Boziaris, 2015; Rudi, Maugesten, Hannevik, & Nissen, 2004) or vacuum conditions at low temperatures (Macé et al., 2012; Olofsson, Ahrné, & Molin, 2007), underlining the discriminatory power of the molecular methods compared to the phenotypic tests. Moreover, apart from the known spoilage associated bacteria such as *Pseudomonas*, *Shewanella*, *Photobacterium*, etc., other bacteria such as *Psychrobacter* spp., *Pseudoalteromonas* spp., *Aeromonas* spp., *Carnobacterium* spp. and *Vagococcus* spp. have been also found to compose the spoilage cultivable microbiota of finfish and shellfish from the cold (Alfaro & Hernandez, 2013; Broekaert, Heyndrickx, Herman, Devlieghere, & Vlaemynck, 2013; Rudi et al., 2004) and/or the warmer (Hozbor, Saiz, Yeannes, & Fritz, 2006; Parlapani et al., 2020a, 2015a; Syropoulou, Parlapani, Bosmali, Madesis, & Boziaris, 2020) temperate sea waters. The knowledge of the sequences from the seafood isolated bacteria, gives us the advantage to further study their spoilage potential and activity, which is the qualitative and quantitative ability, respectively, of isolates to produce spoilage metabolites (Dalgaard, 2003), in order to elucidate their role in seafood spoilage. Due to the fact that a large number of isolates have to be sequenced, researchers have applied fingerprinting protocols e.g. denaturing gradient gel electrophoresis (DGGE), thermal gradient gel electrophoresis (TGGE), and terminal restriction fragment length polymorphism (TRFLP) analysis. Additionally, matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) has been used for differentiation and identification of seafood spoilage bacteria (Böhme et al., 2010; 2011; 2013). Furthermore, the usefulness of another culture-dependent method, High Resolution Melting (HRM), has recently been noted (Parlapani, 2021). This is a rapid and reliable molecular technique, able to group and differentiate potential SSOs at a high-resolution taxonomy level and thereafter it is possible to sequence and identify representative DNA from each group with high levels of accuracy (Parlapani, Ferrocino, et al., 2020; Syropoulou et al., 2020). Shortly, it is expected that this rapid method will contribute not only to the better understanding of the dominant microbiota present in seafood, but also to the fast and more effective development of intelligent strategies to provide high-quality seafood with extended shelf-life.

Such studies led us to assume that microbial spoilage of fish might be a result of the activity of different microbial consortia each time, depending on various factors in pre- and post -fishing or -farm gate practices such as; intrinsic (e.g., a_w, pH, redox potential), extrinsic (e.g., temperature and storage atmosphere), implicit (e.g. microbial interaction), and processing (e.g., heating, cooling, drying) factors. Despite their significant contribution in studying seafood microbial diversity, culture-dependent methods consist of several crucial limitations (Cocolin, Alessandria, Dolci, Gorra, & Rantsiou, 2013). Indeed, such methods are referred to a small microbial group (culturable), while a larger group of other microbes (unculturable) escapes the identification (Noh et al., 2020; Parlapani et al., 2018a). It is estimated that the latter microbial group represents an amount of 90–99.9% of the total microbiota present in a foodstuff ecosystem (Amann, Ludwig, & Schleifer, 1995; Cocolin et al., 2013). Such knowledge loss leads to limited or even useless recording of microbial communities. The "escaping" microbes could be responsible for many producing metabolites, which may cause several sensorial degradations of seafood and thus, spoilage, leading to product rejection and so the losses increase. It has been reported that it is very difficult to obtain reliable and representative results, regarding microbial communities present in a sample and thus, it is impossible to understand spoilage course. Among other noteworthy aspects, the classical approach a) is time consuming, b) might not allow significant culturable bacteria isolated from chill-stored seafood (e.g. potential spoilage microorganisms) to grow on some general growth media frequently used in seafood research, c) might not allow stressed or sublethally injured cells to recover and grow on selective media, while other populations are inhibited by microorganisms present in higher numbers (Boziaris & Parlapani, 2014, 2017; Broekaert et al., 2013; Hugenholtz, Goebel, & Pace, 1998; Odeyemi et al., 2018; Svanevik & Lunestad, 2011; Zhuang, Hong, Zhang, & Luo, 2021). Therefore, the need to explore seafood spoilage microbiota timely and more deeply has been emerged, especially in the last two decades.

2.2. Culture-independent techniques

Culture-independent methods do not depend on the cultivation of microbiota in culture media, but study and compare the microbial diversity directly from seafood samples, by extracting and sequencing either DNA or RNA molecules (Mayo et al., 2014). Commonly, a hypervariable region of the 16 S rRNA gene (e.g. V1–V4) is targeted using universal primers, aiming to identify the majority not only of culturable but also of non-culturable bacteria (Table 1).

Such methods have been widely used in the field of seafood microbiology. Among others, the most widespread culture-independent methods applied in seafood studies are 16 S rRNA gene amplification, cloning and sequencing, DGGE, TGGE, and TRFLP (Nisiotou, Parlapani, Kormas, & Boziaris, 2014). For instance, Svanevik and Lunestad (2011), Bekaert, Devriese, Maes, and Robbens (2015) and Zhao et al. (2016), studied the spoilage microbiota of mackerel, lobster and shrimps, respectively, using DGGE analysis, indicated the dominance of some bacterial groups, which were not detected by conventional approaches. Similar conclusions were highlighted on the characterization of the predominant microbiota of spoiled sea bream using cloning sequencing of 16 S rRNA gene, directly from fish tissue (Parlapani et al., 2013), indicated that *Pseudomonas* and *Shewanella* were the most abundant bacteria. The presence of other microorganisms that escaped from the traditional approaches, such as *Aeromonas*, was also at noteworthy levels. Hovda, Sivertsvik, Tore Lunestad, Lorentzen, and Rosnes (2007) have also studied the spoilage microbiota of halibut using DGGE, indicating the dominance of. *P. phosphoreum*, followed by *Pseudomonas* spp. and *B. thermosphacta*. Similar findings were observed in spoiled cod (Hovda, Lunestad, Sivertsvik, & Rosnes, 2007), as well. The dominance of *Pseudomonas* was also observed in tilapia fillets during storage at 4 °C using DGGE analysis, while *Shewanella* and *Psychrobacter* were also always present, but their increase started the third day of storage (Duan, Zhou, Miao, & Duan, 2018). Other genera detected to lower levels at the end of the storage period, were *Dietzia* and *Janthinobacterium*, which are not usually referred to as a part of spoilage microbiota in any type of seafood. In cooked and peeled tropical shrimp stored under MAP, several species belonging to the genus *Carnobacterium* (mainly

Table 1
Molecular methods in monitoring the most abundant and dominant microbiota of different seafood types, stored and preserved at several conditions, worldwide.

Seafood	Area	Method	Preservation/ Storage	Top Identified Bacteria	Dominant Bacteria	Reference
Atlantic Cod	Atlantic Ocean	16 S rRNA amplicon sequencing (V3–V4)	Air & MAP, Chilled/4 & 8 °C	*Photobacterium, Acinetobacter, Psychrobacter, Pseudomonas, Flavobacterium*	*Photobacterium*	Kuuliala et al. (2018)
Cod fillets	French market	16 S & *gyr*B rRNA amplicon sequencing (V3–V4)	MAP/8 °C	*Photobacterium, Aeromonas, Acinetobacter, Psychrobacter, Serratia*	*Photobacterium, Aeromonas*	Poirier et al. (2018)
Salmon fillets		16 S & *gyr*B rRNA amplicon sequencing		*Photobacterium, Aeromonas, Acinetobacter, Psychrobacter, Serratia*	*Photobacterium, Serratia*	
Gilt-head seabream	Ionian Sea	16 S rRNA amplicon sequencing (V3–V4)	0 °C, 4 °C, 8 °C	*Pseudomonas, Psychrobacter, Carnobacterium, Acinetobacter, Comamonas, Rhodococcus, Shewanella, Sphingomonas, Aeromonas, Blastococcus, Brevundimonas, Brochothrix, Arthrobacter, Lactobacillus*	*Pseudomonas*	Parlapani, Michailidou, Anagnostopoulos, et al. (2018)
	Aegean Sea	16 S rRNA amplicon sequencing (V3–V4)		*Pseudomonas, Psychrobacter, Bacillus, Acinetobacter, Exiguobacterium, Stenotrophomonas, Brevundimonas*	*Psychrobacter*	
Largemouth bass fillets	Guangzhou	16 S rRNA amplicon sequencing (V3–V4)	0.1% LAE solution/4 °C	*Aeromonas, Pseudomonas*	*Aeromonas*	Zhuang et al. (2020)
		16 S rRNA amplicon sequencing (V3–V4)	4 °C	*Aeromonas, Pseudomonas*	*Pseudomonas*	
Cod fillets	Greenland	16 S rRNA amplicon sequencing (V2–V3)	Iced or superchilled in air	*Pseudomonas, Photobacterium, Shewanella, Acinetobacter, Psychrobacter, Janthinobacterium*	*Pseudomonas*	Sørensen, Bøknæs, Mejlholm, and Dalgaard (2020)
		16 S rRNA amplicon sequencing (V1–V3)	Iced or superchilled in MAP	*Pseudomonas, Photobacterium, Shewanella, Acinetobacter, Psychrobacter*	*Photobacterium*	
Hake fillets	Bay of Biscay	16 S rRNA amplicon sequencing (V3–V4)	MAP/1 °C, 4 °C, 7 °C	*Pseudoalteromonas, Carnobacterium, Shewanella, Psychrobacter Photobacterium, Phychromonas*	*Photobacterium, Psychrobacter*	Antunes-rohling et al. (2019)
Grass carp fillets	Chinese market	16 S rRNA amplicon sequencing (V3–V4)	0.1% Cinnamon bark oil/4 °C	*Pseudomonas, Acinetobacter, Shewanella, Aeromonas*	*Pseudomonas*	Huang, Liu, Jia, and Luo (2017)
Farmed Common carp	Beijing market	16 S rRNA amplicon sequencing (V3–V4)	Chilled/Freeze chilled	*Aeromonas Pseudomonas Acinetobacter Shewanella, Lactococcus,*	*Aeromonas Pseudomonas Lactococcus*	Li et al. (2018)
Grass carp fillets	Beijing market	16 S rRNA amplicon sequencing (V3–V4)	0.1% (v/v) essential oil emulsions (oregano)/4 °C	*Pseudomonas, Aeromonas, Acinetobacter, Shewanella, Lactococcus*	*Pseudomonas*	Huang et al. (2018)
			0.1% (v/v) essential oil emulsions (thyme, and star anise)/4 °C		*Aeromonas*	
Groupers	Shanghai market	16 S rRNA amplicon sequencing (V3–V4)	4 °C	*Pseudomonas, Psychrobacter, Carnobacteium, Shewanella*	*Pseudomonas (P. azotoformans), Psychrobacter (P. faecalis)*	Huang and Xie (2020)
Pacific Saury whole & gutted	Pacific Ocean	16 S rRNA amplicon sequencing (V3–V4)	2 °C	Pseudomonadaceae, Unknown/Others	Pseudomonadaceae	Cao, Lin, et al. (2020)
Bighead Carp fillets	Chinese market	16 S rRNA amplicon sequencing (V3–V4)	4 °C	*Aeromonas, Pseudomonas, Shewanella, Acinetobacter*	*Aeromonas, Pseudomonas*	Liu, Li, Li, and Luo (2018)
	Chinese market	16 S rRNA amplicon sequencing (V3–V4)	0.25% ε-Polylysine/ 4 °C	*Aeromonas, Janthinobacterium, Flavobacterium, Shewanella, Comamonas,*	*Janthinobacterium*	
Grass Carp fillets	Chinese market	16 S rRNA amplicon sequencing (V3–V4)	4 °C	*Acinetobacter, Pseudomonas Aeromonas, Shewanella, Lactococcus, Psychrobacter*	*Pseudomonas*	Zhang et al. (2019)
	Chinese market	16 S rRNA amplicon sequencing (V3–V4)	VP or MAP/4 °C	*Iodobacter, Pseudomonas Aeromonas, Shewanella, Lactococcus, Carnobacterium*	*Lactococcus*	
Peeled tilapia fillets	Haikou	16 S rRNA amplicon sequencing (V3–V4)	EGCG-gelatin (EGT)/4 °C	*Enterobacter, Aeromonas, Lactococcus, Pseudomonas, Gluconacetobacter, Citrobacter*	*Enterobacter*	Cao, Lin, et al. (2020)
Atlantic salmon fillet	Norway	16 S rRNA amplicon sequencing (V3–V4)	VP/3 °C	*Photobacterium, Flavobacterium Pseudomonas, Fusobacteriales, Acinetobacter*	*Photobacterium*	Jääskeläinen et al. (2019)
Yellowfin tuna fillet	Maldives	16 S rRNA amplicon sequencing (V3–V4)	VP/3 °C	*Pseudomonas, Shewanella, Flavobacterium, Pseudoalteromonas, Chryseobacterium, Acinetobacter*	*Pseudomonas*	
	North Sea		0 °C		*Pseudomonas*	Zotta et al. (2019)

(*continued on next page*)

Table 1 (*continued*)

Seafood	Area	Method	Preservation/ Storage	Top Identified Bacteria	Dominant Bacteria	Reference
Thawed European plaice fillet		16 S rRNA amplicon sequencing (V3–V4)		*Psychrobacter, Pseudomonas, Acinetobacter, Janthinobacterium, Carnobacterium, Brochothrix, Chryseobacterium, Arthrobacter*		
Hake fillet	South Africa	16 S rRNA amplicon sequencing (V3–V4)	0 °C	*Psychrobacter, Carnobacterium, Acinetobacter, Arthrobacter, Chryseobacterium, Vagococcus, Janthinobacterium*	*Pseudomonas Psychrobacter*	
	Red drum fillet	Atlantic coast	16 S rRNA amplicon sequencing (V3–V4)	VP &MAP/4 °C	*Carnobacterium (C. maltaromaticum, C. inhibens and C. gallinarum) Vagococcus (V. teuberi and V. fluvialis) Lactococcus, Leuconostoc (L. gelidum), Enterococcus (*E. sulfureus), *Serratia, Hafnia*	*Carnobacterium*
Silbande et al. (2018)						
Rose shrimp	Aegean Sea	16 S rRNA amplicon sequencing (V3–V4)	0 °C	*Photobacterium, Candidatus, Psychrobacter, Acinetobacter Delftia, Brevundimonas, Stenotrophomonas, Bacillus, Enterococcus, Enterobacter, Carnobacterium*	*Psychrobacter*	Parlapani, Ferrocino, et al. (2020)
Blue crab	Aegean Sea	16 S rRNA amplicon sequencing (V3–V4)	4 °C	*Psychrobacter, Pseudomonas, Acinetobacter, Photobacterium,* Unknown Bacteria	Unknown Bacteria	Parlapani, Michailidou, et al. (2019)
Blue crab	Aegean Sea	16 S rRNA amplicon sequencing (V3–V4)	10 °C	Unknown bacteria, *Pseudoalteromonas Pseudahrensia, Psychrobacter, Shewanella, Photobacterium*	Unknown bacteria, *Pseudoalteromonas*	
Maryland Blue crab	Chesapeake Bay	16 S rRNA amplicon sequencing	Uncultured, Fresh	*Psychrobacter, Propionibacterium, Shewanella, Exiguobacterium, Pseudoalteromonas, Lysinibacillus, Enterococcus*	*Shewanella, Exiguobacterium*	Ramachandran et al. (2018)
			Cultured, Fresh	*Psychrobacter, Propionibacterium, Shewanella, Exiguobacterium, Pseudoalteromonas, Lysinibacillus, Enterococcus*	*Exiguobacterium, Lysinibacillus, Shewanella, and Enterococcus*	
Sardine	Santa Catarina	16 S rRNA amplicon sequencing (V4)	Fresh	*Macrococcus, Acinetobacter, Pseudomonas, Psychrobacter, Aeromonas, Vagococcus*	*Macrococcus*	de Lira et al., 2020
	Rio de Janeiro		Frozen (−18 °C)	*Phyllobacterium, Pseudomonas, Acinetobacter, Psychrobacter*	*Phyllobacterium*	
White shrimp	Beijing market	16 S rRNA amplicon sequencing (V3–V4)	0.1% ε-Polylysine/ 0 °C	*Candindatus Bacilloplasma, Pseudoalteromonas, Phychromonas, Phychrobacter, Shewanella,* Others	*Candindatus Bacilloplasma*	Jia et al. (2019)
Thawed common cuttlefish	Greece	16 S rRNA amplicon sequencing (V3–V4)	2 °C	*Psychrobacter, Pseudomona, Shewanella, Comamonas, Carnobacterium,*	*Psychrobacter*	Parlapani, Michailidou, Anagnostopoulos, et al. (2018)
Pacific oysters	British Columbia	16 S rRNA amplicon sequencing (V4–V5)	4 °C	Unknown, *Spirochaeta, Psychrobacter, Oceanisphaera, Pseudoalteromonas, Arcobacter, Fusobacterium,*	Unknown, *Arcobacter*	Chen et al. (2019)
Eastern oysters	New Brunswick			*Spirochaeta, Photobacterium, Marinomonas, Psychromonas, Pseudoalteromonas, Pseudomonas, Arcobacter, Marinifilum*	*Spirochaeta*	
	Prince Edward Island			Unknown, *Spirochaeta, Psychrobacter,* Oceanisphaera, *Psychromonas, Arcobacter, Fusobacterium,*	*Psychrobacter*	
Farmed seabream	Greece	16 S rRNA (V3–V4) 454-pyrosequencing	0 °C	*Gammaprobacteria, Betaproteobacteria, Alphaproteobacteria, Actinobacteria*	*Pseudomonas, Stenotrophomonas, Shewanella, Staphylococcus, Arthrobacter, Sphingobacterium*	Parlapani, Michailidou, et al. (2019)
Mussels	Greece	16 S rRNA HRM (V3–V4)	4 °C	*Ps. pulmonis, Ps. celer, Ps. sp., O. smirnovii, Ps. alimentarius*	*Ps. alimentarius*	Parlapani, Ferrocino, et al. (2020)
Farmed Sea bass	Greece	16 S rRNA HRM (V3–V4)	0 °C	*Ps. fozii, Ps. maritimus, Ps. cryohalolentis, Pseudomonas* sp., *Carnobacterium* sp., *Paeniglutamicibacter* sp.	*Ps. glacincola*	Syropoulou et al. (2020)
Atlantic mackerel	Norwegian Sea	16 S rRNA DGGE (V3)	–	*Psychrobacter* sp., *P. immobilis, P. marincola, P. cibarius, P. faecalis, Proteus* sp., *P. vulgaris, Photobacterium* sp., *P. phosphoreum, Vibrio* sp., *V. kanaloae, V. splendidus, V. pomeroyi, Shewanella* sp., *S. putrefaciens, Oceanisphaera* sp., Flavobacteriaceae, *Bizonia* sp., *B. paragorgiae, Pseudoalteromonas* sp., *P. tetradonis, Synechococcus* sp.	*Psychrobacter* sp.,	Svanevik and Lunestad (2011)

(*continued on next page*)

Table 1 (*continued*)

Seafood	Area	Method	Preservation/ Storage	Top Identified Bacteria	Dominant Bacteria	Reference
Norway Lobster	North Sea	16 S rRNA DGGE (V3)	Melting ice/ 2 °C	*Pseudomonas* spp, *Psychrobacter* spp.	*Pseudomonas* spp, *Psychrobacter* spp.	Bekaert et al. (2015)
Farmed shrimp	Shanghai	16 S rRNA DGGE	4 °C	*Acinetobacter, Aeromonas, Lactococcus Exiguobacterium, Kurthia*	*Acinetobacter*	Zhao et al. (2016)
Farmed Sea bream	Greece	16 S rRNA gene amplification, cloning and sequencing	Melting ice/ 4 °C	*A. salmonicida, Pseudomonas* sp., *S. putrefaciens*	*P. fluorescens, S. putrefaciens*	(Parlapani, Meziti, Kormas, & Boziaris, 2013)
Farmed Atlantic halibut	Hjelmeland	16 S rRNA DGGE (V3)	MAP/4 °C	*Pseudomonas putida, Pseudomonas* spp., *B. thermosphacta, Serratia* sp., *P. phosphoreum,*	*Pseudomonas putida, Pseudomonas* spp.	Hovda, Lunestad, Sivertsvik, and Rosnes (2007)
Farmed Atlantic cod	Brønnøysund	16 S rRNA DGGE (V3)	MAP (CO_2:O_2)/ 0 °C	*Pseudomonas* sp. *Photobacterium* spp., *S. putrefaciens and*	*Pseudomonas*	Hovda, Sivertsvik, et al. (2007)
			MAP (CO_2:N_2)/ 0 °C	*Pseudomonas* spp	*Photobacterium*	
Salmon fillets	Norway	16 S rRNA T-RFLP	MAP/1 °C	*C. piscicola, C. divergens, B. thermosphacta*	*C. piscicola, C. divergens*	Rudi et al. (2004)
Cold-smoked salmon	Norway	16 S rRNA gene amplification, cloning and sequencing	7 °C	*Lactobacillus, Photobacterium, Photobacterium, Brochothrix*	*Lactobacillus, Photobacterium*	Olofsson et al. (2007)
Tilapia fillets	China	16 S rRNA DGGE	4 °C	*Shewanella, Psychrobacter, Pseudomonas, Acinetobacter, Brevibacterium, Flavobacterium, Dietzia, Janthinobacterium.*	*Pseudomonas*	Duan et al. (2018)
Cod fillets	France	16 S rRNA (V1–V3) 454-pyrosequencing	–	-	*Shewanella, Psychrobacter, Arthrobacter*	Chaillou et al. (2015)
White shrimp	hina	16 S rRNA amplicon sequencing (V3–V4)	4 °C	*Acinetobacter, Psychrobacter, Shewanella, Carnobacterium, Pseudomonas, Vibrio*	*Acinetobacter*	Yang, Xie, and Qian (2017)
			25 °C	*Vibrio, Acinetobacter, Lactococcus, Flavobacterium, Myroides, Vagococcus*	*Vibrio*	

C. maltaromaticum, and *C. divergens*), followed by *Enterococcus* spp. and *Vagococcus* spp. were found to dominate using TGGE, cloning and sequencing (Jaffrès et al., 2009). In another study, Rudi et al. (2004) used TRFLP to highlight the different microbiota developed in salmon and coalfish, stored under MAP conditions, indicating the dominance of *Carnobacterium* spp. and *P. phosphoreum,* respectively. Another work deals with the study of the microbiota present in vacuum-packed cold-smoked salmon, directly from tissue, using 16 S rRNA gene sequencing of cloned DNA, indicating the dominance of *Lactobacillus* spp., followed by *Photobacterium* spp., while a remarkable percentage of detected bacteria were unknown or unidentified (Olofsson et al., 2007). The use of such methodologies gave us the advantage to get a deeper and clearer picture about the microorganisms existing or dominating in seafoods, compared to the classical ones, however none of them have allowed a full description of the microbiota present in a sample (e.g. finfish, shellfish).

2.3. High throughput sequencing

In the last decade, the advent of HTS technology provided to the scientific community an alternative point of view, regarding the way of seafood microbiota evaluation (Walsh, Crispie, Claesson, & Cotter, 2017). For instance, 16 S metabarcoding sequencing analysis using Illumina technology; a fast and cost-effective high throughput DNA sequencing technology; has currently changed our knowledge about the dominant microbiota of seafood (Table 1), since this modernized set of techniques reveals; a deeper and more representative, than the previous approaches, snapshot of the microbiota present in a food ecosystem (De Filippis, Parente, & Ercolini, 2017). Whilst the use of the traditional Sanger sequencing approach is applied on a unique DNA molecule (Sanger & Coulson, 1975), NGS makes it possible to simultaneously amplify and sequence all nucleic acids from a complex ecosystem and study in-depth the microbial dynamics in a food/seafood sample (Cocolin et al., 2018). From the development of this set of technology and its use in seafood microbial communities studies, two crucial findings have been arisen. Firstly, microbial communities are richer than those estimated using conventional methods and secondly, several undiscovered microbes may significantly affect spoilage. Therefore, it is clear that NGS can contribute to our knowledge improvement, opening a new era in food/seafood microbiology. Although the use of HTS and the possibilities that can provide, are still at a relatively early stage, its use is increasing year after year, as it is being purveyable; in terms of cost and skills required; not only for researchers but also for the food industry (Ercolini, 2013). Indeed, there are several companies, which have already included this type of analysis in their services, while the cost is exponentially decreasing.

As mentioned above, for many decades using conventional methods, the genera *Pseudomonas* and *Shewanella* were considered as the most usual and important SSOs in the majority of seafood from several regions. Over the last decade, *Psychrobacter* has also been found to compose the cultivable microbiota of seafood during chilled storage (Bekaert et al., 2015; Broekaert et al., 2013; Parlapani, Ferrocino, et al., 2020; Syropoulou et al., 2020). HTS analysis confirmed the dominance of *Psychrobacter* in several seafood, characterizing these bacteria as potential players in seafood spoilage (Antunes-rohling et al., 2019; Parlapani et al., 2018a; 2018b; Parlapani, Syropoulou et al., 2020). Furthermore, it is crucial to mention that other microbial species, the presence of which was never noted in seafood, have now been arised, using metabarcoding analysis (Parlapani, Michailidou, et al., 2019). Additionally, tag-pyrosequencing, another HTS analysis, usually targeting the amplification of V1–V4 hypervariable regions of the 16 S rRNA gene, has also been proposed to study seafood microbiota profile. Studies have already highlighted the significant contribution of such a method to determine several bacterial species, that could not been detected using previous culture-independent methods (Parlapani, Michailidou, et al., 2019; Roh et al., 2010). Consequently, in line with Illumina technology, pyrosequencing is considered as a very powerful and reliable HTS tool for the determination of microbiota during seafood spoilage. However it is crucial to point out that this technology it is no longer used for amplicon sequencing.

HTS approach came to confirm the indications that had already been emerged from the conventional methods, regarding the strong linkage between seafood microbial diversity with a plethora of parameters such as type of seafood, season, habitat (e.g. geographical origin, water type, water contamination, farming conditions etc.) (Møretrø, Moen, Heir, Hansen, & Langsrud, 2016; Songré-Ouattara et al., 2008), processing approaches (e.g. hygiene practices, harvesting and handling, etc.) (Chaillou et al., 2015), as well as storage conditions (temperature, preservation type, etc.) (Parlapani et al., 2018a; 2019b; Rosado et al., 2019). Nowadays, scientists attempt to establish a hierarchy from the most to the least effective parameters that determine the microbiota profile of this complex matrix, highlighting that the most effective ones are the type of seafood, the geographical origin, as well as the handling, processing and storage conditions. More specifically, genera including *Pseudomonas*, *Psychrobacter*, *Photobacterium*, *Flavobacterium*, *Acinetobacter*, and *Chryseobacterium* are dominated in several fresh fish species (e.g. yellowfin tuna, salmon and cod) from Scandinavian area (Jääskeläinen et al., 2019; Kuuliala et al., 2018) and Italy (Zotta, Parente, Ianniello, De Filippis, & Ricciardi, 2019), while in all cases *Photobacterium* and *Pseudomonas* were found to be the dominant bacterial genera, at the end of shelf-life. However, the findings of Parlapani et al. (2020a) revealed low relative abundance of *Photobacterium* in chill-stored shrimps originated from Aegean waters, at the end of the shelf-life, although this bacterial group was the most abundant in fresh samples. In the same study, more than 160 identified bacteria, the majority of them rarely found in seafood (e.g. *Stenotrophomonas, Candidatus Hepatoplasma* and *Candidatus Bacilloplasma*) exhibited relative abundances more than 1%, while the dominant bacteria at the sensory rejection time point was *Psychrobacter*. However, the presence of *Carnobacterium* is also remarkable. The latter genus was also found in high levels in Greek farmed gilt-head seabream from different regions (both Aegean and Ionian waters), stored aerobically at 8 °C (Parlapani, Michailidou, Anagnostopoulos, et al., 2018). *Pseudomonas* dominated in seabream from Ionian waters, while *Psychrobacter* dominance was profound in samples from Aegean region (Parlapani, Michailidou, Anagnostopoulos, et al., 2018). Additional findings were highlighted by Chen et al. (2019), who studied the microbiota profile of spoiled Pacific (British Columbia area) and Eastern (New Brunswick and Prince Edward Island) oysters. Results indicated significant differences in microbial dominance between different origins, where the dominant bacteria in Pacific origin were unknown bacteria followed by *Arcobacter*, while *Spirochaeta* and *Psychrobacter* were the most abundant genera in oysters from the New Brunswick and Prince Edward Island, respectively. Furthermore, the microbiota profile of Asian freshwater fishes, like grouper, and farmed common carp (Huang & Xie, 2020; Li, Zhang, & Luo, 2018), is almost in line with this of European finfish. Finally, Cao et al., 2020 compared the bacterial profile of whole and gutted Pacific Saury, at refrigerated storage, observing no differences between them, while Pseudomonadaceae was by far the most abundant bacteria family at the time that product was spoiled.

Subsequently, different microbiota profile has been noted in American waters. For instance, apart from the common *Shewanella* and *Psychrobacter*, several other bacteria genera such as *Propionibacterium*, *Enterococcus*, *Exiguobacterium*, *Pseudoalteromonas* and *Lysinibacillus*, seem to thrive in the fresh Atlantic blue crab (Ramachandran, Reed, & Ottesen, 2018). On the contrary, other bacterial groups, such as *Enterobacter*, *Candidatus*, *Pseudahrensia*, *Comamonas* and *Filomicrobium* were found in blue crab from the Mediterranean region (Parlapani, Michailidou, et al., 2019). Furthermore, significant differences have been noted in microbial dominance between cultured (*Exiguobacterium*, *Lysinibacillus*, *Shewanella*, and *Enterococcus*) and wild blue crab (*Psychrobacter* and *Propionibacterium* spp.) (Ramachandran et al., 2018). In a recent study, significant differences in the microbiota of fresh and frozen sardines originated from Brazil, were found (de Lira et al., 2020). More specifically in the fresh sardines, the dominant genera were *Macrococcus* spp., *Acinetobacter* spp., *Pseudomonas* spp., *Psychrobacter* spp., *Aeromonas* spp., and *Vagococcus* spp, while in frozen sardines, bacteria such as *Phyllobacterium* spp., *Pseudomonas* spp., *Acinetobacter* spp., and *Psychrobacter* spp. exhibited the higher relative abundances.

Another parameter which highly contributes to microbiota and SSOs selection of seafood, is the preservation or packaging type. In a recent work related to tilapia fillets, Cao et al. (2020a) studied the effect of EGCG-gelatin biofilm (EGB) during chilled storage and evaluate the microbial communities by 16 S rRNA metabarcoding analysis. Although the results indicated similar microbial profiles in both control and treated samples, like *Pseudomonas*, *Aeromonas* and *Enterobacter*, the abundance of *Aeromonas* was significantly lower in the treatment indicating that the EGB affected the growth of bacterial group with high spoilage potential, such as *Aeromonas*. Also, Huang, Liu, Jia, Zhang, and Luo (2018), studied the microbial composition of Grass carp fillet during chilled storage, using three different essential oils (oregano, thyme and star anise) as preservatives. The authors noted that the predominant microbiota found in all groups were *Aeromonas* (for thyme and star anise treatments) and *Pseudomonas* (oregano treatment), although the relative abundances of *Aeromonas* were significantly lower in all treated samples, compared to the control. Regarding Hake fillets under MAP conditions, stored under various temperatures, *Photobacterium* and *Psychrobacter* were the dominant bacteria at the time that product was spoiled (Antunes-rohling et al., 2019). Furthermore, Kuuliala et al. (2018), who determined the microbial communities of vacuum-packed Atlantic cod, stored at various temperatures, indicated that *Photobacterium* dominated in all cases,. Both studies indicated the effect of both MAP and vacuum package, on spoilage microbiota selection, since the microbiota was not condiderably different in respect to the tested storage temperatures.

3. Discussion, challenges & future perspectives

Studying and understanding the seafood microbiota profile changes, as well as the microbial ecology from different perspectives, researchers will be able to develop intelligent strategies to assure quality, and extend shelf-life of seafood. Although our understanding level is at the preliminary stages, HTS approach is the key to attain this challenge. As mentioned above, seafood quality has largely been studied from the perspective of targeting potential microbial spoilers, using amplicon-based DNA metabarcoding sequencing of 16 S rRNA loci. This approach has overcome the limitations of conventional and other earlier molecular approaches, providing a step forward on seafood microbiota studies (Cocolin et al., 2018). In recent years, another HTS method, the shotgun metagenomics of either DNA-seq or RNA-seq, has gained the attention of food microbiologists, since it surpasses the limitation of metabarcoding approach regarding PCR bias (Ferrocino & Cocolin, 2017). Thus, a more reliable snapshot of microbiota present in a sample, can be achieved by applying this method (De Filippis et al., 2017). Apart from performing a reliable snapshot of microbiota related to seafood microenvironment, other useful quantitative information could be elicited by such approaches, providing a deeper insight into knowledge about estimation of biodiversity within a sample (alpha diversity) or between different seafood samples/treatments (beta diversity) (Zhuang et al., 2021). Especially the latter one reflects variations between different samples, based on the distances mirrored by different microbiota profiles. Several studies have been involved in such kind of analysis, highlighting its significant contribution, regarding the better understanding of different treatments effects on microbiota evolution. As a representative example, Maillet et al. (2021), used PCoA to exemplify the impact of different DNA extractions and sampling methods on microbiota evolution of cold-smoked salmon, indicating worthnoted differences by both parameters. Similarly, Jia et al. (2018) highlighted the distance between treated with tea polyphenols and non-treated carp fillets in a PCoA plot, enhancing the above-mentioned hypothesis. Finally, Zotta et al. (2019) noted significant distance on PCoA plot, regarding microbial communities of thawed fish fillets stored in two

different temperatures (0 and 10 °C). Furthermore, there are several studies attempting to statistically combine the results of HTS with that of physicochemical and/or sensory analyses (Parlapani, Ferrocino, et al, 2020; Zhuang et al., 2020). This could be of a great scientific interest, despite that De Filippis, Parente, and Ercolini (2018) and Zhuang et al. (2021) suggested that a potential statistical relation between those data, does not necessarily mean a biological phenomenon. Thus, despite the widespread application of NGS in several seafood spoilagerelated studies, which is progressively leading to knowledge enrichment, there are not enough and/or reliable indicators to fully understand the mechanisms involved in seafood spoilage, as well as their interlinkage, that influence spoilage, and their impact on the sensorial attributes of seafood. Through such a complex matrix, the scientific community is trying to elucidate and combine the mechanisms affecting the whole process.

What is needed now, is to fully understand the correlations of seafood microbiota patterns, which can allow us to see the "big picture" of where spoilers are come from, how they interact with other microbiota in such a complex matrix and which is their specific role, in order to establish novel strategies to retard spoilage and thus, the deterioration of sensory attributes of seafood. In this regard, further steps are required to better understand those mechanisms. This could be achieved by shifting the approaching study from the "presence" (metataxonomics) to "functionality" (metagenomics) (Cocolin et al., 2018). In situ monitoring and establishing a clear relationship between microbiota changes with metabolic activity, gene expression and functional profile is fundamental to be evaluated, as the basis for the development of intelligent and novel strategies for preservation. The application of *meta*-omics in seafood quality evaluation allows the answering of questions that were not possible to be addressed so far with traditional microbiological methods or even with predictive models. Approaching this complex foodstuff matrix from different metagenomic perspectives (metatranscriptomics, metaproteomics and metabolomics) allows for a holistic/rational representation of which microorganisms are present, how they behave, how they interact, what they metabolize, which gene is responsible for metabolism and which are the phenotypic manifestations in the product (Cocolin et al., 2018). The combination of such analyses would facilitate the development of a "biological network" at metagenome-scale (Branco dos Santos, de Vos, & Teusink, 2013). Thus, a multi-omics approach could help to clarify the bacterial ecology providing an invaluable impact on seafood quality, in order to better control spoilage process, and even extend shelf-life. However, to succeed this, several difficulties and obstacles must be overcome. More specifically, the translation of such molecular data into practical applications is a pre-requisite (Jagadeesan et al., 2019), to give to the food industry specific guides and solutions on how to make seafood products of high quality and extended shelf-life. On this point, software of high advanced statistical analysis has the potential to be a turning point to bridge the gap between metagenomics and translation into practice. Therefore, by applying *meta*-omics data to statistical advanced metagenome predictive tools such as Tax4Fun; a tool that is based on the metagenome data collected in many databases, like KEGG pathway (De Filippis et al., 2018); it is possible to predict the potential functionality of the dominant microbiota present in a seafood product, opening new insights regarding spoilage strategies development (Zhuang et al., 2021). For instance, Hong et al. (2016) used KEGG pathways to predict the quality of wine rice. They found some crucial metabolic pathways (synthesis of biotin, malolactic fermentation etc.) which are closely related with the early growth of *L. brevis* during fermentation. In another study, Ferrocino et al. (2018) applied a predictive analysis in starter-driven fermented sausages using KEGG, to connect the existing microbiota with gene expression and VOCs production. They highlighted several pathways, in which the starter culture could alter the organoleptic characterisitcs of the final product. Furthermore, De Filippis, Genovese, Ferranti, Gilbert, and Ercolini (2016) used KEGG and provided strong indications of a key role of non-starter LAB enzymatic activity, in cheese maturation rate, depended on the storage temperature. Based on the aforementioned, similar studies should be applied in the field of seafood spoilage as well. Howeverit wouldn't be omitted that this approach is statistical-based and thus the predictions may not have a biological impact in many cases. Nevertheless, the increase of metagenomics related studies is the key to obtain much more data, and increase the prediction capacity of the predictive tools.

However, by the time of writing this paper, there are still some obstacles to be addressed. A studythat combines a series of *meta*-omics; as described above; is still of high cost, while raw data analysis requires high bioinformatic skills. Both of them have led to limited application of combined metagenomic studies, the majority of which so far is applied in dairy fermentation (De Filippis et al., 2017). Nevertheless, within the next few years, it is expected that the cost of applying NGS technology will be reduced, being easily accessible not only to academia but also to industry and even more to out-compete the cost of microbiological conventional examination (Jagadeesan et al., 2019).

As stated above, shotgun metagenomics is a very promising approach in the attempt to study food microbiota, surpassing the limitatations of short reads applied by the metabarcoding analysis of 16 S rRNA gene. According to Almeida and De Martinis (2021), the determination of metagenome-assembled genomes (MAGs) is an new alternative and modernized way a) to study the potential ecological roles of several microbial species, even at strain level, rather than study just the microbiota snapshot in a food sample and b) to reveal important biochemical pathways of microbial activity. Indeed, Walsh, Macori, Kilcawley, and Cotter (2020), have already highlighted the importance of coupling MAGs with advanced bionformatic analysis, since the possibility of reconstructing population genomes from metagenomes has the potential to open new insights in food microbial ecology studies. For instance, the authors revealed that indigenous bacteria from cheese samples are using clustered regularly interspaced short palindromic repeats (CRISPR), to protect themselves against bacteriophages, while they produce bacteriocins to eliminate each other. To our knowledge, no such studies are available in the field of seafood microbial ecology and thus, this should be one of the main challenges of scientific community in the near future.

Nowadays, scientists have already taken the advantage of the benefits provided by HTS in terms of metabarcoding in their attempt to establish novel and intelligent strategies to tackle the spoilage phenomenon. Collecting information from *meta*-omics analysis should be seriously taken into consideration by food technologists to use the most suitable seafood preservation practice. In this regard, several innovative strategies have been applied during seafood processing/storage, in order to inhibit microbial spoilers. Among others, ozone washing (Okpala, 2014), pulsed electric fields (Toepfl, Heinz, & Knorr, 2006), high hydrostatic pressure (Abdu et al., 2018), antimicrobial substrates (Jasour, Ehsani, Mehryar, & Naghibi, 2015) and natural preservatives (Baptista, Horita, & Sant'Ana, 2020; Mei, Ma, & Xie, 2019), such as plant extracts and essential oils (Hassoun & Emir Çoban, 2017; Karoui & Hassoun, 2017) are the most promising innovative strategies. The majority of studies concluded that a combination of the above technologies with packing-based technology (e.g essential oils and vacuum package) is the most appropriate way for microbial spoilage's inhibition. On this point, it must be mentioned that HTS analysis could shed more light regarding microbial interactions, functions and thus, product's sensorial impact of such applications.

Specific emphasis should be given to the so-called natural biopreservation, which is usually referred to the application of isolated microorganisms (mainly LAB) in order to prevent spoilers' growth, and extend seafood shelf-life (Matamoros, Pilet, Gigout, Prévost, & Leroi, 2009; Wiernasz et al., 2020). The use of a proper LAB strain could not only enhance the competition for nutrients, but also prevent or reduce the growth rate of spoilers, via the production of several elements with proven antimicrobial activity such as bacteriocins or bacteriocins like inhibitors substances (BLIS). BLIS includes a variety of primary and

secondary metabolites, such as organic acids (mainly lactic, succinic and acetic acids), hydrogen peroxide, etc. This field is an active scientific topic, although few studies have evaluated the effect of using LAB in the preservation of seafood products (Gómez-Sala et al., 2016; Matamoros et al., 2009; Saraoui et al., 2017), due to some concerns about the potentially undesirable effect on sensorial attributes of seafood products. The latter requires further exploitation to confirm or refute this concern. In this sense, metagenomics and more specifically whole genome sequence (WGS); another HTS approach; could contribute to identifying genes of isolated strains, which could be potentially responsible for seafood organoleptic abnormality. Furthermore, a multi-omics approach could be applied to monitor the seafood (in which LAB will be used) during storage, in order to study the impact in microbiota alteration, potential spoilers' inhibition and genes expression profile, which are responsible for the sensorial attributes of the final product. The application of HTS in such studies, is of great biotechnological interest, opening new insights in food/seafood microbiology.

Except that, obtaining the useful knowledge by summarizing all of those findings, as well as exploiting the multi-omics data to potentially highlight novel biomarkers (e.g. specific microbial group or gene or protein, or a combination of them etc.), we could be able to produce novel, intelligent and simple for the industry tools such as biosensors, for rapid and reliable detection of a potential spoilage threat (either specific microbial group or genes), at early stages of chain production. Indeed, the development of such biosensors has attracted the attention of industry, as a promising and effective tool to reduce food/seafood wastes. For instance, specific biological molecules such as enzymes or antibodies (Santana Oliveira, da Silva Junior, de Andrade, & Lima Oliveira, 2019) could be used as biosensors in combination with HTS data, for creating a statistically-based network between them. This tool could be very useful for the industry, in order to be used at any time along chain production. Moreover, the rapid detection of a spoilage threat requires both a rapid and an intelligent solution. The development of nano-technology for seafood spoilage, is another major challenging. Specific molecules exhibiting rapid and effective defensive mechanisms, such as antimicrobial or antioxidant ablility, could be useful for the industry to tackle spoilage, ensuring products freshness and quality (Mustafa & Andreescu, 2018).

All the aforementioned aspects are likely to become the key to connect scientific and industrial communities, by translating the complex scientific findings to a comprehensive language for industry and other stakeholders. To this, more studies are required in the near future. Finally, all of these challenges, could contribute to tackle the major threat our planet faces nowadays; the climate change (Parlapani, 2021). Indeed, the global warming, extreme and short weather phenomena are expected to extremely affect seafood industry (Misiou & Koutsoumanis, 2021). Those effects might favor the abundance of spoilers, leading to the capture of seafood with a burdened microbial load already from their environment which are in turn, pass through along chain production at higher populations and could provoke seafood spoilage at earlier stages. Thus, there is need to develop strategies in order to tackle the undesired effects of climate change.

4. Conclusions

Seafood is a complex matrix, the quality of which is affected by several parameters along chain production, that need to be deeply explored, in order to fully understand and tackle it. After almost 10 years of the widespread use of HTS analysis in seafood microbiota, genomic databases have collected a large amount of data from amplicon-based studies. However, it seems that we have just begun to explore seafood microbial ecology and with the advent of the new decade, it is time for a step forward. The coupling of different metagenomic approaches (metataxonomics, metabolomics, metatrancriptomics, metaproteomics), using new and powerful bioinformatic and/or statistical software, have to be employed to fully understand the mechanisms of spoilage and link the genotype of spoilage microbiota with the "phenotype" of spoiled seafood. Those types of works are now needed as never before, since the scientific community has at its disposal all necessary tools to address questions, could not before. Understanding the dynamics of spoilage microbiota, the "biological network" between microbiota presence, gene expression profile and metabolites production, as well as what is the real impact on seafood quality and sensory attributes, are mandatory to fill in the knowledge gaps and proceed to the next level.

To conclude, we stand in the "Foodomics" era. "Next generation" of rapid and effective strategies that could predict and/or extend seafood spoilage is on its way to be developed and the application of HTS is playing a key role to achieve this aim. The establishment of such strategies will lead to the production of high-quality seafood products, with extended shelf-life, harmonizing both industry needs and consumer demands, minimizing wastes and thus, economic losses.

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