Current Trends in Marine Biology

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Edited by

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CHAPTER 1

FUNGI IN ANTARCTIC MARINE SEDIMENTS

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Abstract

Antarctica offers a combination of extreme conditions for life, as it harbors trophic chains of animals, plants, algae, and microorganisms. The marine environment of Antarctica is characterized by high salinity and hydrostatic pressure, combined with extremely low temperatures. These abiotic factors exert strong selective pressure on the microbiota, which are mainly comprised of bacteria, archaea, and fungi. Antarctic marine habitats harbor many species of fungi associated with a variety of substrata distributed in the water column or associated with sediments. Sediment provides stability, protection, detrital particles, and other suitable conditions for fungi. The fungal propagules present in sediments could be active in nutrient cycling by interacting with the inorganic environment, by oxidizing and reducing molecules, or, in a dormant-resistant form, by being occasionally dispersed. Fungi, such as *Ascomycota*, *Basidiomycota*, *Zygomycota*, and other basal taxa, occur in sediments of Antarctica. Due to the challenge of obtaining fungi in culture-dependent methods, the detection of fungal taxa in these extreme habitats is primarily conducted by molecular and uncultured methods. However, these methods underestimate the richness of species in the marine microbial community. In view of the high selectivity, marine fungi are notable for their metabolic plasticity, which favors the production of different enzymes and biomolecules. Bioprospecting studies focusing on microorganisms that are poorly characterized and able to survive under adverse conditions are interesting. Therefore, despite the difficulty in obtaining cultivable fungi, they are still the target of interest in bioprospecting. In addition to contributing to knowledge about the diversity

and ecology of marine communities, marine fungal research has attractive biotechnological applications.

Keywords: Antarctica, Bioprospecting, Fungi, Marine

1. Introduction

Antarctica offers a combination of extreme conditions for life, harboring simple trophic chains of animals, plants, algae, and microorganisms. The marine environment, characterized by high salinity and hydrostatic pressure in combination with the extremely low temperatures of Antarctica, exerts strong selective pressure on microbiota.

Antarctic marine habitats harbor many species of fungi distributed in the water column or associated with sediments. In turn, sediments provide stability, protection, detrital particles, and other suitable conditions for fungal growth. The fungal propagules that are present in sediments could either be active in nutrient cycling and interacting with the inorganic environment, as well as oxidizing and reducing molecules, or they could be in a dormant-resistant form, while occasionally being dispersed.

Bioprospecting studies focusing on cultivable microorganisms that are poorly characterized and able to survive in near adverse conditions are of interest; therefore, despite the difficulty in obtaining cultivable fungi, they are still targets of interest in bioprospecting research. Due to the challenge of obtaining fungi in culture-dependent methods, the detection of fungal taxa in extreme habitats of the Antarctica is primarily conducted by molecular and culture-independent methods. However, this often underestimates the species richness of the marine microbial community. Phyla of fungi, such as *Ascomycota*, *Basidiomycota*, *Zygomycota*, and other basal taxa are known to be present in sediment samples from Antarctica. In view of the high selective pressure, marine fungi are targeted for their metabolic plasticity which favors the production of different enzymes and biomolecules. Apart from contributing to the diversity and ecology of marine communities, marine fungal research has potential applications in the field of biotechnology.

Antarctica

Antarctica, a continent of about 14 million km^2, is covered by a massive ice sheet of approximately 12 million km^2 (Wynn-Williams 1990; Wilkins et al. 2013). With one of the harshest and coldest environments on Earth, this

continent is considered an extreme habitat because it offers a combination of extreme climatic conditions which include extremely low temperatures, wide temperature fluctuations, low water availability, long periods of darkness, and high periodic incident solar radiation.

The lowest temperature ever recorded on this continent is -89.4°C and the highest is 15°C. The average winter temperature is -34.4°C, and the temperature inside the continent is much lower than the coast (NASA 2018). This climate anomaly occurs due to the thermal isolation of the continent by katabatic winds, which are dense air masses that accumulate on the polar plateaus and flow toward the sea that tend to keep Antarctica distant from the thermal influence of other continents (Wynn-Williams 1990).

Antarctica is geographically isolated by the Antarctic Ocean and the Antarctic circumpolar current (ACC) (Smith 1991; Wynn-Williams 1991). The ACC is the largest ocean current in the world that surrounds the continent and connects the other ocean basins. Therefore, the water circulation promoted by the ACC and the formation of sea ice influence the marine regions of Antarctica that have marine environments with distinct physical, chemical, and ecological characteristics.

The continent's isolation and extreme conditions distinguish Antarctica from other ecosystems, making it a natural habitat for small invertebrates, as well as a few plants, birds, mammals, and microorganisms (Shivaji and Prasad 2009). From a terrestrial and aquatic perspective, isolation makes the continent a place where the species richness is low. Some of its ecosystems comprise the simplest ones on Earth, i.e., some endolithic communities are restricted to algae, fungi and bacteria (Chown 2007).

The process of freezing in winter and thawing in summer creates different transient microhabitats on Antarctica's ice surfaces seasonally and causes a frequent transition from anoxic to aerated environments. Some species of microorganisms found in these environments respond to a lack of oxygen as well as low nutrient availability (Wynn-Williams 1990). In addition, the formation of saline super-cooled regions applies a selective pressure that favors psychrotolerant and halotolerant species (Wynn-Williams 1990). Other factors, such as cold temperatures, low humidity, and high periodic incident radiation with long periods of complete darkness, trigger multiple adaptation mechanisms in different Antarctic species (Cowan and Tow 2004), which makes this environment an interesting target for microbial studies.

2. Fungi in Marine Ecosystems

Marine ecosystems represent a virtually unknown environment in relation to their microbial communities, where about one million microorganisms (bacteria, archaea, viruses, and fungi) are found per milliliter of seawater (Glöckner et al. 2012). These microorganisms are responsible for the biogeochemical cycling of carbon, nitrogen, phosphorus, silica, iron, and other trace elements in marine environments (Rosa 2019).

Fungi, in particular, are able to colonize a wide variety of substrates, from inert matter, such as sediment, sludge, soil, and sand, to vegetation and animals present in marine ecosystems (Rosa et al. 2019). When these materials fall into the water column, they can carry dormant propagules of terrestrial or freshwater fungi that are passively washed into the marine environment (Jones et al. 2015; Raghkumar 2017).

The distribution of these fungi in the marine environment is not uniform. Coastal areas support several fungal communities including filamentous and yeast forms (Raghkumar 2017). Kohlmeyer and Kohlmeyer (1979) consider the open sea to be a fungal desert, as it functions like an oligotrophic substrate, where nutrients are scarce and the enzymes secreted by fungi are probably lost due to their rapid diffusion into the water column (Kohlmeyer et al. 2004).

According to their biogeographical distribution, species of marine fungi can generally be classified as tropical, subtropical, temperate, polar (from the Arctic region and Antarctica), and cosmopolitan (Jones 1993). Some examples of marine fungi species from global regions are described in Table 1. Several studies on fungi have been carried out in coastal waters (Grasso et al. 1997), seawater and deep-sea (Vaz et al. 2011; Lopez-Garcia et al. 2001; Gonçalves et al. 2017), marine sediment (Vaz et al. 2011; Gonçalves et al. 2013; Gonçalves et al. 2015; Wentzel et al. 2019), driftwood (Pugh and Jones 1986), algae (Loque et al. 2010; Godinho et al. 2013; Furbino et al. 2014, 2018; Duarte et al. 2016; Poveda et al. 2018), and marine animals (Henríquez et al. 2014; Cui et al. 2016; Godinho et al. 2019). However, there are scarce reports on marine fungi from the Polar Regions, particularly Antarctic marine ecosystems.

Table 1. Examples of marine derived fungi based on their geographic distribution.

Geographic distribution	Fungal species	Source	References
Subtropical and tropical	*Aigialus* spp. *Bathyascus* sp. *Corollospora maritirna* *Halorosellinia oceânica* *Lulworthiagrandispora* *Sablecola chinensis* *Sedecimiella taiwanensis* *Tolpedospora radiata*	Driftwood, seawater foam, mangrove driftwood	Kohlmeyer and Kohlmeyer (1987); Jones (1993); Pang et al. 2004; Pang et al. (2010); Jones and Pang (2012)
Temperate	*Crinigera maritima* *Halosphaera appendiculata* *Lautosporopsis circumvestita* *Marinospora* sp. *Toriella tubulifera*	Driftwood, sand and stone	Koch and Jones (1989); Koch and Petersen (1996)
Polar (Artic)	*Alternaria* sp. *Amylocarpusen cephaloides* *Aspergillus* sp. *Ceriosporopsis tubulifera* *Cladosporium* sp. *Fusarium* sp. *Havispora longyearbyenensis* *Lautosporopsis circumvestita* *Lulworthia* sp. *Mortierella* sp. *Phaeosphaeria* sp.	Driftwood, sediment	Pang et al. (2008); Pang et al. (2011); Zhang et al. (2015)

	Phoma sp. *Phoma* sp. *Remispora spitsbergenensis* *Sablecola chinensis* *Sebacina* sp. *Zalerionvarium*		
Polar (Antarctica)	*Antarctomyces psychrotrophicus* *G. antarctica* *Havispora longyearbyenensis,* *Metschnikowia australis* *L. circumvestita* *Mortierella antarctica* *Phoma* sp. *Phenoliferia glacialis* *Purpureocillium lilacinum* *Spathulospora antarcticum* *T. tubulifera* *Thelebolus globosus* *Yamadazyma mexicana*	Driftwood, algae, seawater, shallow sediment	Pugh and Jones (1986); Pang et al. (2011); Vaz et al. (2011); Godinho et al. (2013); Furbino et al. (2014); Gonçalves et al. (2017)
Cosmopolitan	*Aspergillus* sp. *Cladosporium* sp. *Fusarium solani* *Penicillium solitum* *Penicillium* sp.	Algae, corals, sponges, sediments	Li and Wang (2009); Barrero-Canosa et al. (2013); Godinho et al. (2013); Furbino et al. (2014); Gonçalves et al. (2013, 2015); Wentzel et al. (2019)

In addition to the water column and open sea, marine habitats colonized by fungi also include sediments present on the ocean floor. The ocean floor corresponds to two-thirds of the Earth's surface and is characterized by anoxic, cold, and dark environments with absence of sunlight, high hydrostatic pressure, low temperatures, and limited nutrient availability (Rosa 2019).

Despite the adverse conditions, the ocean floor harbors microbial communities accounting for a total cellular carbon content of approximately 3 x 10^{17} g (Whitman et al. 1998). The communities include bacteria, archaea, protists, and fungi (Snelgrove et al. 1997). However, most studies have focused on characterizing prokaryotes in deep-sea samples (Delong and Pace 2001; Sogin et al. 2006; Hongxiang et al. 2008; Luna et al. 2009; Sass and Parkes 2011).

Fungal diversity remains relatively unexplored amongst deep-sea mycobiota, with some studies showing a low diversity of individuals, most of which use non-cultivable methods (Raghukumar et al. 2004; Nagano et al. 2010; Edgcomb et al. 2011; Singh et al. 2012; Rédou et al. 2015). Roth et al. (1964) reported the isolation of deep-sea fungi in samples collected from the Atlantic Ocean at a depth of 4,450 m. Isolation of some genera of *Aspergillus*, *Candida*, *Exophiala*, *Fusarium*, *Penicillium*, and *Rhodotorula* using culture-dependent and culture-independent methods have been reported (Singh et al. 2012; Rédou et al. 2015).

Certain factors need to be considered for isolating microorganisms from extreme marine environments (especially in order to simulate the limitations of the environment); these include hydrostatic pressure, salinity, nutrient profile, incubation temperature, and oxygen levels (Rosa 2019). The difficulty in obtaining cultivable forms explains the preponderance of sequence-based studies, where ascomycetic and basidiomycetic yeast structures are reported as being most abundant in the deep-sea (Basset al. 2007; Kohlmeyer and Kohlmeyer 1979).

When it comes to bioprospecting studies, these organisms are still of potential interest even with the challenges in simulating the marine environment for culture-dependent methods. Research involving cultivable fungi of extreme environments have been conducted to investigate the diversity of fungal assemblages and their molecular and regulatory mechanisms, as well as the products of their metabolism, such as proteins, enzymes, and other substances, which are of great importance and interest for biotechnological processes. Several marine fungi have been chemically

evaluated and many bioactive metabolites have been detected for these species, including alkaloids, macrolides, quinones, cyclic peptides, xanthones, terpenoids, isoprenoids, and other aromatics (Zhang and Kim 2012; Liang et al. 2014; Hasan et al. 2015).

In marine environments, studies have prospected fungi associated with algae, invertebrates (Henríquez et al. 2014; Cui et al. 2016), soil (Godinho et al. 2013; Gomes et al. 2018), and sediments (Gonçalves et al. 2015); however, few species have been chemically investigated to date in Antarctica (Furbino 2014). The imposition of the environment (e.g., the limiting and oligotrophic substrates available in the Antarctic region) apparently generates a great metabolic diversity in these extremophilic fungi. Consequently, these microorganisms have become interesting to scientific researchers as potential producers of prototype biomolecules and new drugs.

3. Fungi in the Marine Sediments of Antarctica

Sediment comprises aggregates of rock and soil particles carried from land areas to the ocean through the wind, ice, rivers and currents, underwater volcanic products, seawater chemical precipitates, and space materials (Augustyn 2018), as well as organic matter from the remains of marine and plant organisms that accumulate on sediments (Oni et al. 2015). The role of fungi in sediments is not easily observable, and hence it has been often neglected (Rhaghukumar 2017). However, due to the production of acids and other compounds that modify minerals, fungi have an important role in mineralization, as they create a chemical environment that characterizes their substrate. Fungal hyphae attach to substrates, such as sediments and detritus, and draw nutrients from their polymers by secreting extracellular depolymerizing enzymes (Rhaghukumar 2017). In turn, marine sediments provide stability, protection, detrital particles, and other suitable conditions for fungal assemblages which interact with the inorganic environment, thereby oxidizing and reducing molecules on detrital organic matter to obtain nutrients and energy. Thereby, the decomposition of the organic matter also depends on the physicochemical interactions mediated by the characteristics of the water column and sediment (Rhaghukumar 2017).

The most important dispersal route for fungi is air transport. As millions of tons of dust containing viable microorganisms, trace metals, and organic materials are transported between continents each year, the microbial communities of Antarctica often come from propagules carried by atmospheric air from other regions (Choi et al. 1997; Garrison et al. 2003).

In the South Pole, there are low levels of airborne particles (Sattler and Storrie-Lombardi 2009); hence, dispersion could occur locally by birds, fish, mammals, meltwater, and ocean circulation (Vincent 2000). Ocean circulation that carries the particles from terrestrial habitats represent a primary front for fungal propagules dispersion in marine sediments. During sediment deposition in ocean environments, these detritus and particles can drag fungal propagules (which compose communities in sediments) in a dormant or active form.

Among the scarce reports on fungi from the Antarctic seafloor, López-Garcia et al. (2001) evaluated the deep-sea next to the Antarctic Ocean and studied the diversity of eukaryotes in Antarctic frontal boundary sediment samples (along the Drake Passage); they reported only one fungal taxon that was found > 3,000 m deep, whose genus was not identified. Using culture-independent techniques, Bass et al. (2007) evaluated water samples from the Drake Passage at different depths (250–500 m and 200–3,000 m) and concluded that fungi are relatively rare in these deep-water habitats, and mainly occur in the form of recovering yeast. Recently, at different sites across Gerlache and Bransfield Straits, Antarctica, Gonçalves et al. (2017) identified fungi in seawater at different depths (1.3–1,352 m). The authors obtained 12 taxa belonging to the genera: *Acremonium*, *Aspergillus*, *Cladosporium*, *Cystobasidium*, *Exophiala*, *Glaciozyma*, *Graphium*, *Lecanicillium*, *Metschnikowia*, *Purpureocillium*, *Penicillium*, and *Simplicillium*. The majority of genera comprise cosmopolitan species and the endemic species of the Antarctic marine environment, i.e., *Metschnikowia australis*.

According to Rosa et al. (2019), the majority of taxa reported for Antarctic marine sediments are similar to those found in marine sediments from the Atlantic and other oceans. Table 2 summarizes a few reports on fungi in marine sediments of Antarctica; essentially, only a few studies have identified cultivable fungi, indicating that this region is underexplored in this context.

Table 2. Marine fungi in sediments of Antarctica.

Fungi in sediments[a]	Marine substrates[b]	References
Ascomycota		
Acremonium sp.	Macroalgae, seawater, deep sediment	Godinho et al. (2013); Gonçalves et al. (2017); Poveda et al. (2018)
*Cadophora*sp.	Macroalgae and shallow sediment	Furbino et al. (2014); Wentzel et al. (2019)
Cladosporium sp.	Macroalgae, mollusc, aquatic worms and shallow sediment	Godinho et al. (2013); Furbino et al. (2014); Furbino et al. (2018); Godinho et al. (2019); Wentzel et al. (2019)
Metschnikowia australis	Macroalgae, aquatic worm, mollusc, stalked jellyfish, oligo-polychaete, brittle star, seawater, shallow sediment	Loque et al. (2010); Vaz et al. (2010) Godinho et al. (2013); Furbino et al. (2014); Duarte et al. (2016); Furbino et al. (2018); Godinho et al. (2019); Wentzel et al. (2019)
Metschnikowia sp.	Shallow sediment	Wentzel et al. 2019
Meyerozyma sp.	Mollusc and shallow sediment	Godinho et al. (2019); Wentzel et al. 2019
Paraconiothyrium sp.	Shallow sediment	Wentzel et al. 2019
Penicillium solitum	Deep sediment	Gonçalves et al. (2013); Ogaki et al. 2019
Penicillium chrysogenum	Macroalgae and seawater	Gonçalves et al. (2017); Furbino et al. (2018)
*Penicillium*sp.	Macroalgae, aquatic worm, copepod, oligochaete, seawater, shallow and deep sediment	Loque et al. (2010); Godinho et al. (2013); Furbino et al. (2014); Duarte et al. (2016); Furbino et al. (2018); Gonçalves et al. (2017); Godinho et al. (2019); Ogaki et al. 2019; Wentzel et al. (2019)
Pestalotiopsis sp.	Ascidian (Urochordata), oligochaete and shallow sediment	Godinho et al. (2019); Wentzel et al. 2019
Pseudocercosporella sp.	Shallow sediment	Wentzel et al. 2019

Pseudogymnoascus sp.	Macroalgae, mollusc, copepod, oligochaete, shallow and deep sediment	Furbino et al. (2014); Godinho et al. (2013); Gonçalves et al. (2015); Godinho et al. (2019); Ogaki et al. 2019
Simplicillium lamellicola	Deep sediment	Gonçalves et al. (2015)
Toxicocladosporium sp.	Shallow sediment	Wentzel et al. 2019
Basidiomycota		
Cryptococcus sp.	Macroalgae and shallow sediment	Duarte et al. 2016; Wentzel et al. 2019
Cystobasidium sp.	Shallow sediment	Wentzel et al. 2019
Glaciozyma sp.	Mollusks and shallow sediment	Vaz et al. (2011); Godinho et al. (2019); Wentzel et al. (2019)
Holtermanniella sp.	Macroalgae and shallow sediment	Duarte et al. (2016); Wentzel et al. (2019)
Leucosporidium muscorum	Macroalgae and shallow sediment	Furbino et al. (2018); Duarte et al. (2016); Vaz et al. (2011)
Mrakia sp.	Macroalgae, marine worm and shallow sediment	Duarte et al. 2016; Godinho et al. (2019); Wentzel et al. 2019
Phenoliferia sp.	Shallow sediment	Wentzel et al. (2019)
Phenolipheria glacialis	Macroalgae and shallow sediment	Duarte et al. (2016); Vaz et al. (2011)
Rhodotorula sp.	Macroalgae, seawater, and shallow sediment	Loque et al. (2010); Vaz et al. (2011); Vaca et al. 2013; Furbino et al. (2014; 2018); Duarte et al. (2016); Wentzel et al. (2019)
Vishniacozyma victoriae	Macroalgae, mollusks, and shallow sediment	Furbino et al. (2014); Duarte et al. (2016); Godinho et al. (2013); Vaz et al. (2011); Godinho et al. (2019)

[a] Examples of fungi reported in shallow and deep marine sediments in Antarctica, according to Vaz et al. 2011; Gonçalves et al. 2013, 2015; Ogaki et al. 2019 and Wentzel et al. 2019.[b] Other marine substrates where sediment taxa were obtained in Antarctica.

Vaz et al. (2011) carried out cultivable and bioprospecting studies on shallow coastal samples and identified few yeast species. These include *Glaciozyma antarctica*, *Vishniacozyma victoriae*, *Dioszegia hungarica*, and *Leucosporidium scottii*, which are psychrophilic yeasts of Antarctica. Some genera such as *Exophiala*, *Rhodotorula*, and *Metschnikowia* have also been cited for marine environments of Antarctica (Furbino et al. 2014, 2018; Vaca et al. 2013; Gonçalves et al. 2017).

Sampling in coastal areas is simpler; however, in the case of ocean floor samples, some collection tools are required to achieve collection from greater depths. Different marine geological equipment is commonly used to obtain sediment samples. Samples are obtained aboard a research vessel, where equipment anchored to a wire is launched with minimal disturbance to the sediment surface. Specialized equipment is used for higher a volume that reaches shallower depths, e.g., a box corer. However, for lower volumes and greater depths, a gravity corer is preferred.

Using a box corer of 50 cm^3, Gonçalves et al. (2013) obtained several isolates of *Penicillium solitum* from marine sediments at depths of 100, 500, 700, and 1,100 m in King George Island Admiralty Bay. This was the first study involving fungi in the deep-sea of Antarctica. Using a Van Veen grab (similar to a box corer), Gonçalves et al. (2015) conducted a bioprospecting study on fungi from different substrates, including deep-sea samples at depths not exceeding 60 m in Admiralty Bay, King George Island, and Deception Island, Maritime Antarctica; only taxa *Simplicillium lamellicola* and *Pseudogymnoascus sp.* were identified. The *Pseudogymnoascus* species has a ubiquitous distribution in Antarctica (Gonçalves et al. 2015) and were described to be associated with thalli of macroalgae (Loque et al. 2010; Godinho et al. 2013; Furbino et al. 2014) and sediment (Wentzel et al. 2019) in marine habitats. *Simplicillium lamellicola* is a plant parasite of agronomic interest (Shin et al. 2017), but the genus has also been identified as endophytic in blue-green algae. Apparently, it may be a saprophytic fungus of plant substrates and the species may play a similar role in marine sediments in Antarctica.

Wentzel et al. (2019) evaluated deep-sea sediments from King George Island Admiralty Bay using a Van Veen grab; they listed 17 genera, including some filamentous fungi and psychrophilic yeasts already cited for marine environments such as *Glaciozyma*, *Mrakia*, *Metschnikowia*, and *Phenoliferia* (Vaz et al. 2011; Godinho et al. 2013; Furbino et al. 2014, 2018; Duarte et al. 2016). Recently, Ogaki et al. (2019) obtained *Penicillium, Pseudogymnoascus*, and *Acremonium* species from deep-sea sediment

samples across the South Shetlands, Antarctica, using a gravity corer; only the base of the cores (a total of 1 m) was processed, indicating that the fungal propagules present were highly resistant to the hydrostatic pressure exerted by the water column and the compacted sediment.

The few reports of fungi in sediments from Antarctica mainly focus on cultivable studies for bioprospecting; this is due to the unique metabolic systems of these fungal communities, which is highly influenced by the selective pressure exerted by abiotic factors. Besides the difficult logistics required for obtaining sediment samples, there is difficulty in simulating cultivation conditions in studies of cultivable fungi. It is also difficult to obtain significant results in non-cultivable studies due to the highly reactive nature of the organic matter present in sediment samples, which influences the total amount of DNA that can be obtained for analysis. Therefore, studies involving fungal diversity and ecology are extremely valuable for understanding the dynamics of this substrate as it is difficult to access.

4. Conclusion

Antarctica is geographically isolated by the Antarctic Circumpolar Current, which encompasses diverse marine zones, ranging from maritime to continental Antarctica. This marine habitat harbors a considerable fungal diversity, which is responsible for nutrient cycling through the decomposition of organic matter. Once carried by the air and other extra-aquatic and marine sources, some of these fungi inhabit marine sediments in both coastal and deep-sea areas.

Even though studies describing fungi in marine ecosystems are substantial, little is known about the fungi present in Antarctic marine sediments. Based on the reports available on fungi in sediments, it could be stated that several of these are cosmopolitan in distribution; further, some fungal species identified in sediments are psychrophilic and halotolerant. From this perspective, ecological and diversity studies (involving culture-dependent studies for bioprospecting and others by metagenomic techniques), could help us to understand the fungal communities of the Antarctic marine sediments; these studies would help to clarify their ecological role regionally.

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CHAPTER 2

TRACEABILITY AND AUTHENTICATION OF SEAFOOD PRODUCTS: AN OVERVIEW OF MARKERS

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Abstract

Several types of seafood (entire organisms or processed products) are utilized worldwide for human consumption. The ever increasing requirement for seafood products coupled with globalization have compelled the expansion of the seafood market. In consequence, this has encouraged aquaculture practices phenomenally. However, this has made the fishery market vulnerable to adulteration and mislabeling. Reliable and rapid traceability tools (which provide information regarding a species and its origin) are the prime prerequisites for the substantiation of a species and its origin. Authentication determines the commercial value of a fish or seafood product in the market. The analytical tools available for the identification and evaluation of the origin of seafood (especially fish) help augment transparency and fair trade practices in the fishery market, as well as guarantee safety. Several pieces of research have been focused on developing easy and novel methods for the determination of origin. Seafood is one of the most highly traded commodities in the world, and their substantiation is very important. Several analytical tools are currently

available for seafood substantiation, but most of them require standard reference materials and protocols, skilled human resources, sophisticated techniques, and/or authentic databases. In this chapter, we will discuss the applicability of several markers and analytical techniques that are used for the authentication of seafood products (especially fish). This essay appraises the utility of some of these methods, as well as discusses their pros and cons, aptness, sensitivity, reliability, user-friendliness, rapidity, and practicality. As all of these methods have certain short-comings (e.g., certain methods are unable to authenticate processed products), it is indispensable to cross-evaluate authenticity and origin by using multiple methods. There is a strong need to establish a fishery-specific analytical traceability protocol comprising multiple techniques. This protocol should conform to the international guidelines, be comprised of a well-coordinated synchronized network of certified laboratories and databases, and should be easily accessible to public. This could help to corroborate the origin of fish and seafood products, as well as identify them appropriately.

Keywords: Authentication, Fish, Markers, Origin, Seafood, Traceability

1. Introduction

The globalization of the food industry, the extensive growth in technology, the consummate ease of transport of food commodities across countries and continents, as well as the consumer's preferences for eco-friendly foods and new tastes, have made it difficult to classify food products based on regions (Drivelos and Georgiou 2012).

The global trade in seafood products has increased phenomenally. Recognizing the substantial potential of seafood trade, several countries have capitalized on this opportunity, e.g., the total seafood production in South Korea has reached 3.26 million tons (Sea fish, Korea). The global seafood market is comprised of diverse species and products (wild and farmed varieties). The United States Food and Drug Administration (FDA) Seafood list constitutes 1700 species of commercial finfish and shellfish (Griffiths et al. 2014). Nevertheless, the upsurge in the global seafood demand has galvanized aquaculture practices. Figure 1 illustrates the rise in aquaculture trade from 1955 to 2012 (Global aquaculture production, FAO).

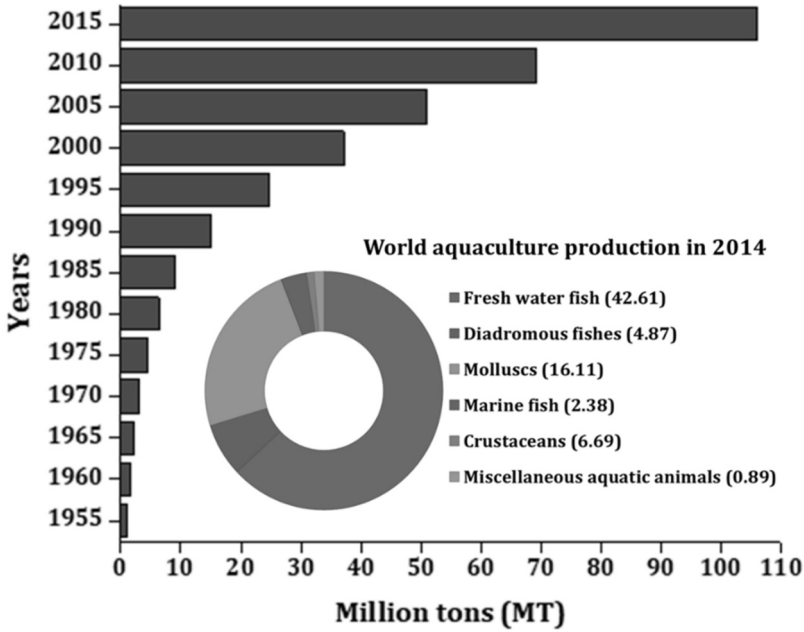

Figure 1. Increase in aquaculture production (as per FAO), including a break-up of world aquaculture production in 2012 (illustrating fishes and other miscellaneous organisms).

Fish are an affordable source of high-quality animal protein. A portion of 150g of fish provides about 50–60% of the daily protein requirements for an adult (Carrera et al. 2013a). They are commendable sources of micronutrients, including vitamins (A, D, E, B1, B6, and B12) and minerals (Fe, I, P, Na, Ca, and K). They contain a good amount of polyunsaturated omega 3 (ω–3) fatty acids, which are reported to be beneficial in the prevention and treatment of cardiovascular, neurological, and inflammatory diseases; dementia; age-related macular degeneration in the elderly; and attention-deficit hyperactive disorder and asthma in the pediatric population (Carrera et al. 2013a; Mohanty et al. 2013).

The dynamic demand for fish worldwide has created fraudulent practices, such as mislabeling and species substitution (intentional or unintentional for monetary benefits or due to lack of knowledge). This includes the substitution of species carrying health advisories (e.g., king mackerel sold as grouper; escolar sold as white tuna), cheaper farmed fish sold as wild (e.g., tilapia sold as red snapper), and overfished, imperiled, or vulnerable

species sold as more sustainable catch (e.g., Atlantic halibut sold as Pacific halibut) (Warner et al. 2013; El Sheikha and Montet 2016; Lewis and Boyle 2017). Then again, several different genera of fish share the same generic commercial name as the organisms previously sold in the market, but do not correspond to the same species, which could be antigenic (Barbuto et al. 2010). As many fish varieties aggravate allergic symptoms in sensitive patients, they pose potential health risks (Carrera et al. 2013a). Thereby, disclosure of the species' identity, production method, and geographical origin are mandatory, along with clear regulations and traceability systems, which also govern the international seafood trade (Chatterjee et al. 2019).

The authors recognize the insatiable demand for fishery products, their overwhelming export and import, the vulnerability of a fishery product to mislabeling with respect to species and origin (either intentional or unintentional), the customer's right to information, and the enforcement of regulations with regard to fish product labelling. With these aspects in view, this chapter showcases the various methods and techniques used for the identification of fish species and verification of its origin (i.e., geographic provenance and method of culture [i.e. aquaculture or wild]).

2. Molecular strategies

(a) DNA-based markers and technologies

DNA markers provide information on individual entities of fish, in terms of their population or species (Chauhan and Kumar 2010). For the authentication of fish, it is important to establish the extent of their polymorphisms, which are expressed as differences in the quantity and quality of alleles, genes, chromosomes, and gene arrangements on the chromosomes. These include base substitutions, insertions, or deletions of nucleotide sequences within a locus; inversion of a segment of DNA within a locus; and rearrangement of DNA segments around a locus. Compared to proteins, DNA-based markers have certain distinct advantages when it comes to the identification of species. Apart from being more informative, specific, sensitive, and reliable, they are the method of choice for processed samples. Lavilla et al. (2013) elaborate that, although DNA can be degraded, it is more resistant to heat treatments compared to proteins; hence, it can be extracted from fresh, frozen, cooked, and dried seafood products. It is possible to recover fragments of approximately 300 base pairs (bp) from processed samples (techniques that target small DNA fragments are more suitable processed samples).

Proteins vary with factors, such as tissue, age, and the status of the individual, while DNA molecules are largely independent of these factors.

DNA-based markers are classified either by i) function—as type I (markers associated with genes of known function, e.g., allozyme markers) and type II (markers associated with anonymous genomic segments, e.g. RAPD markers, microsatellites, and AFLPs) origin as nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) (Chauhan and Kumar 2010). Their usefulness can be established using their polymorphic information content (PIC). Relatively slowly evolving genes, such as cytochrome *b* (cyt *b*), Au12 target mt 16S rRNA, or COI, are more suitable for species identification (Lavilla et al. 2013). In the case of the detection of fish species, different fragments, such as target 5S rRNA, α–actin, nuclear ribosomal internal transcribed spacer, and α–tropomyosin, have been used (Lavilla et al. 2013).

Advances in DNA technology have encouraged the substantial use of DNA markers in fishery product authentication. These include restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified-fragment length polymorphism (AFLP), mini- and microsatellites, and single-nucleotide polymorphism (SNP) (Rasmussen and Morrissey 2008; Chauhan and Kumar 2010). Chauhan and Kumar (2010) have reported the use of various molecular markers, proteins, or DNA (mtDNA or nuclear DNA, such as microsatellites, SNP, or RAPD) in fisheries and aquaculture, as they assist species identification, and also help in differentiating between wild and hatchery populations. Though the nuclear DNA markers have been examined, most DNA-based methods for fish species identification encompass the amplification of mitochondrial DNA, with the most prominent being the mitochondrial gene cyt *b* (Catanese et al. 2010; Hashimoto et al. 2010).

A variety of DNA-based techniques, including multiplex polymerase chain reaction (PCR), fragment length polymorphism (PCR–RFLP), random amplified polymorphic DNA (PCR-RAPD), amplified-fragment length polymorphism (PCR–AFLP), single-strand conformation polymorphism (PCR-SSCP), and forensically informative nucleotide sequencing (FINS), which are based on genetic polymorphisms in the genetic codes of different species (Rasmussen and Morrissey 2008), could be employed for fish. Seven species of *Lophius* or angler fish are reported to be able to identified by forensically informative nucleotide sequencing (FINS). FINS is also used for the authentication of scombroid products, from fresh fish