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Research Article

Mapping of the world scientific production on bacterial and fungal microbiota in mollusks

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ABSTRACT. This study aimed to map the world's scientific production of bacterial and fungal microbiota in mollusks. The research was carried out in the Web of Science database, using "Fungi AND Mollusk" and "Bacteria AND Mollusk" descriptors and publication time between 2001 and 2021. Indexes were built by years, countries, research areas, and two maps of the co-occurrence of terms. There was a progressive increase in the production of microbiota in mollusks, with more research on bacteria during the investigated period. China and USA were the countries with the highest percentage of publications. Research with fungi and bacteria accounted for 28.41 and 17.66% in China, respectively. While the USA, the percentage was 21.05% for fungi and 16.02% for bacteria. Areas such as marine and freshwater biology (24.77%), fisheries (23.86%), biochemistry and molecular biology (18.60%), and pharmacy (17.83%) showed a higher percentage of indexed articles. There was also a high concentration of deposits of publications in the immunology area for both themes. The groups formed for bacteria and fungi indicated that studies had described the immune responses of mollusks, the genetic aspects associated with their defense mechanisms, pathogen virulence factors, and symbiotic and pathogenic microbiota, highlighting genera *Vibrio*, *Aspergillus*, and *Escherichia coli* pathogenic pathotypes. The bacterial and fungal microbiota in mollusks have been studied in several areas of knowledge and have given rise to research that can support the proper management of mollusks, application in the pharmaceutical industry, and implementation of measures in public health.

Keywords: *Escherichia coli*; aquaculture; microbiology; public health; food security; fungal pathologies

INTRODUCTION

Aquaculture, one of the fastest-growing zootechnical activities in the world, is considered an efficient alternative to supply the world's demand for fish due to its important nutritional properties for human consumption and for functioning as a regulator of the direct demand on natural fish stocks. This growing demand makes aquaculture a promising investment, regardless of its production scale (FAO 2020a).

Considering aquaculture's rapid growth, ensuring farmed organisms' health is essential for production and marketing (Mau & Jha 2018, Wijsman et al. 2019). Mollusks have drawn the attention of competent bodies, researchers, and producers due to their biological characteristics, their role in the environment and because they are affected by diseases and mortality events associated with microorganisms (Wijsman et al. 2019, Cano et al. 2020, Carranza & Zu Ermgassen 2020, Lattos et al. 2020).

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Studies have indicated the susceptibility of mollusks to accumulate microorganisms in their tissues (bacteria, fungi, viruses, and protozoa), as they are filtering organisms (Pruzzo et al. 2005, Iwamoto et al. 2010, Mendes 2018) and, for this reason, they function as indicators of habitat quality, signaling pollution risks (Feng et al. 2017).

Mollusks have specific physiological characteristics related to their microbiota. The symbiotic relationship between microbiota and mollusks can stimulate the modulation of the immune system, resulting in mature and balanced immune responses (Ringo et al. 2016).

Therefore, it is important to highlight that the microbiome of mollusks plays an important role in several aspects of their nutritional, digestive, metabolic, immunological, reproductive, behavioral physiology, predator-prey relationships, and survival in challenging environmental conditions (Dubilier et al. 2008, King et al. 2019), which guarantees harmonious life between parasite and host.

Studies on the microbiota of aquatic animals have indicated their important role in many key functions, such as disease resistance, adequate nutrient assimilation through proper gut function, and stimulation of biological processes (Prado et al. 2010, Ringo et al. 2016, King et al. 2019, Kubick et al. 2021, Ono 2021).

However, environmental impacts on aquatic ecosystems and the lack of adequate sanitary management in farming systems can deregulate this microbiome, favoring the entry and action of pathogenic microorganisms (Chen et al. 2016). Shellfish are often consumed in fresh form, becoming vehicles for transmitting these pathogenic agents to humans, consequently representing a potential public health problem (Lattos et al. 2021).

Due to the great concern in the process of production and global trade, the World Organization for Animal Health (WOAH 2021) listed causal agents of compulsory notifiable diseases in mollusks, such as viruses (*Abalone herpesvirus*), parasites (*Bonamia exitiosa*, *B. ostreae*, *Perkisus marinus*, *P. olseni*, *Marteilia refringens*) and bacterial (*Xenohaliotis californiensis*). Other bacteria and fungi, although not listed, are described in mollusks mainly due to the risks they can cause to production and human health. Among microorganisms of interest to public health, there are the bacterial species genera *Vibrio*, *Pseudomonas*, *Aeromonas* and *Salmonella*, diarrheagenic pathotypes of *Escherichia coli*, and fungi of the genus *Aspergillus* (Garnier et al. 2008, Ottaviani et al. 2011, Borzykh & Zvereva 2014b, Kwan & Bolch 2015, Silveira et al. 2016, Santos et al. 2017, Eliseikina et al. 2021).

Given the relevance of malacoculture with an emphasis on food safety, and the need to understand the dimension of mollusk-microbiota interactions, this study aimed to contribute to the visualization of the current scientific panorama by mapping the world scientific production dealing with fungal and bacterial microbiota of mollusks, showing the different study subjects.

MATERIALS AND METHODS

This research is documentary and exploratory-descriptive with a quantitative approach in which worldwide scientific mapping was carried out-creating a representation of the research structure on the fungal and bacterial microbiota in mollusks. It provides a view of the development of scientific production on this topic by dividing elements into different groups (years, countries, publication area, and words) (Zupic & Čater 2015).

For the construction of production indexes, the following keywords were used: "Fungi AND Mollusk" and "Bacteria AND Mollusk", using the Web of Science-WoS (referencing scientific citation platform designed to support scientific and academic research with coverage in the sciences, social sciences, arts, and humanities) database with a time interval between 2001 and 2021. The search was performed in January 2022 to include articles deposited until December 2021. The filters and dynamics of the database in the analyses presented in this work considered articles published until 2021.

Data were analyzed in the WoS platform by applying filters of the following categories: year, countries, and publication area. Subsequently, they were exported and treated, highlighting the main items of each list generated since these presented a greater amount of published works. There was no exclusion of articles for analysis of production rates. However, unpublished articles referring to bacteria were exported to construct bibliographic network maps, not including the other categories.

The construction of two bibliographic network maps (Lecari 2021) was carried out based on articles selected from the VOSviewer 1.6.17 software, producing a network of terms co-occurrence arranged in clusters, constituting clusters of words with a large number of relationships (links), links to each other (co-occurrence) and highlights relevant terms.

From 546 exported records, the VOSviewer software generated a co-occurrence network with 214 similar words from the "Bacteria AND Mollusk" descriptor. For the "Fungi AND Mollusk" descriptor, 129 records were obtained with 1194 similar terms.

RESULTS

With descriptors selected for the WoS search, the evolution of publications was observed from 2001 to 2021, with the threshold between years 2019 and 2021, with 22.01% (n = 120) of articles published in this period (Fig. 1). For fungi, 129 records were found, with 73.32% (n = 92) unpublished articles and 28.68% (n = 37) literature review articles. The annual scientific production resulted in 546 records on bacteria in mollusks, whose documents presented different categories, with 87.36% (n = 477) unpublished articles, 10.45% (n = 57) literature review articles and 3.67% (n = 12) conference articles. Literary evolution was observed between the selected years, with a greater number of deposits of publications between 2014 and 2020, representing 10.07% (n = 10) of articles indexed in the WoS database (Fig. 1).

The platform identified publications made by 69 countries related to bacteria in mollusks, among which 17 countries stand out, whose publications are more quantitative and relevant. The USA and China stand out with 17.66% (n = 97) and 16.02% (n = 88), respectively, followed by France (9.65%; n = 53), Russia (8.56%; n = 47), and Italy (6.92%; n = 38). Brazil ranks sixth in the world with 6.55% (n = 36) of scientific production (Fig. 2).

WoS registered articles from 90 countries related to fungi in mollusks, among which 10 countries stand out. China has the highest number of records (28.41%; n = 32), followed by the USA with 21.05% (n = 28) of publications. Brazil occupies the sixth position with 6.97% (n = 9) of publications indexed in the WoS database (Fig. 3).

Regarding categories of publication deposits, the platform generated a list of research areas, highlighting the area of marine and freshwater biology, followed by the area of fisheries and veterinary sciences and immunology (Table 1) for bacteria in mollusks. Few articles indexed in journals focused on food safety.

The four highlighted research areas present publications in the Fish and Shellfish Immunology Journal, whose main objective is to develop studies for applicability in aquaculture. Studies on the immune defense systems of mollusks stand out in the journal, which proves to be a sub-area with a great tendency for the growth of investigations in recent years.

The main areas of interest in research related to fungi and mollusks are biochemistry and molecular biology (Table 2), followed by pharmacy, fisheries, immunology, freshwater, and marine biology. These areas are correlated since they are mostly seeking new bioactive molecules that can be used in the production of new substances for the therapeutic area or to explain defense mechanisms against the action of pathogens.

The VOSviewer software generated a co-occurrence map on bacteria in mollusks, composed of seven (7) clusters distributed into 147 items, in which three clusters stood out for the occurrence of terms, co-occurrence of words and thematic proximities between clusters and within clusters (Fig. 4).

The red cluster (1) presents a grouping of co-occurring terms, in which the expression "innate immunity" stands out, presenting 318 relationships with the other clusters and 61 occurrences. The green cluster (2) highlights the terms "bivalve mollusk" and "pathogenic bacteria," presenting 255 relationships with the other terms and 75 occurrences. In this cluster, there were also nine occurrences of the term "brown ring disease" and 56 relationships with the other clusters of the bibliographic network, highlighting the term "virulence," which is related to the purple cluster (3), which highlights the terms "*E. coli*", "*Salmonella*", "oysters" and "seafood".

For fungi in mollusks, the VOSviewer software generated a map with 24 relevant terms distributed in four clusters. The red cluster (1) grouped seven items: diversity, evolution, fungi, identification, mollusks, protein, and sequence; the green cluster (2) gathered seven items: algae, discovery drug, fungi, *in-vitro*, *Elysia rufescens*, sponge, and antral products; the blue cluster (3) generated five items: antibacterial activity, invertebrates, metabolites, mollusks and purification; and finally, the yellow cluster (4) gathered five terms: c-type lection, expression, innate immunity, molecular cloning, and pattern recognition receptor.

DISCUSSION

The scientific production of microorganisms in mollusks in recent years may have been influenced by the increase in world production and consumption (Fig. 1). In 2019, there was a global increase in demand, and high prices were recorded. In the previous year, shellfish (17.3 million t) accounted for 56.2% of marine and coastal aquaculture production (FAO 2020b); in addition, *per capita* consumption of fish and fishery products doubled from 10 kg in 1960 to over 20 kg in 2016 (FAO 2016).

Furthermore, some episodes of mortality and bacterial infections in economically and ecologically

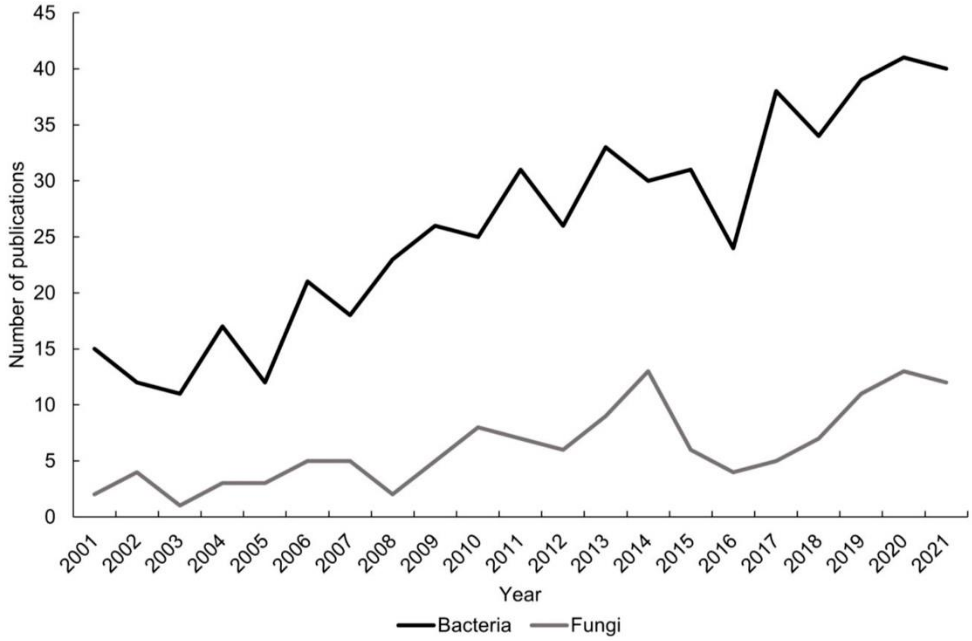

Figure 1. The number of publications of articles indexed in the Web of Science database between 2001 to 2021, using the "Mollusk AND bacteria" and "Mollusk AND fungi" descriptors.

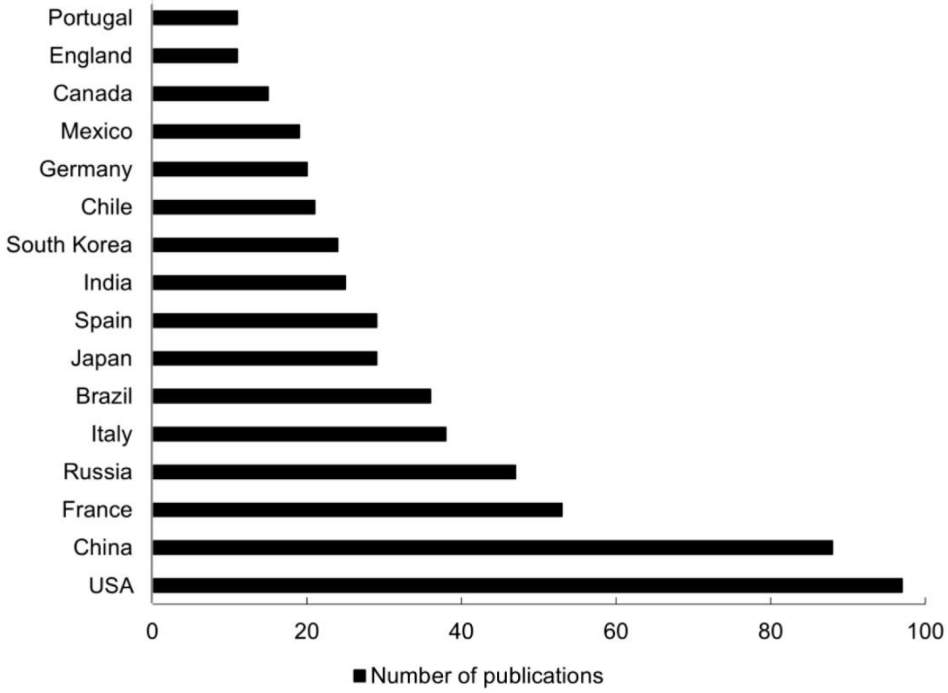

Figure 2. World ranking of publications of articles indexed in the Web of Science database for the "Mollusk AND bacteria" descriptor from 2001 to 2021.

important mollusks have been recorded since 2012 in countries such as China (Bai et al. 2017, Wei et al. 2019), USA (Henley et al. 2019), Italy (Cano et al. 2020), Spain (Ruiz et al. 2013, 2015), Chile (Lohrmann

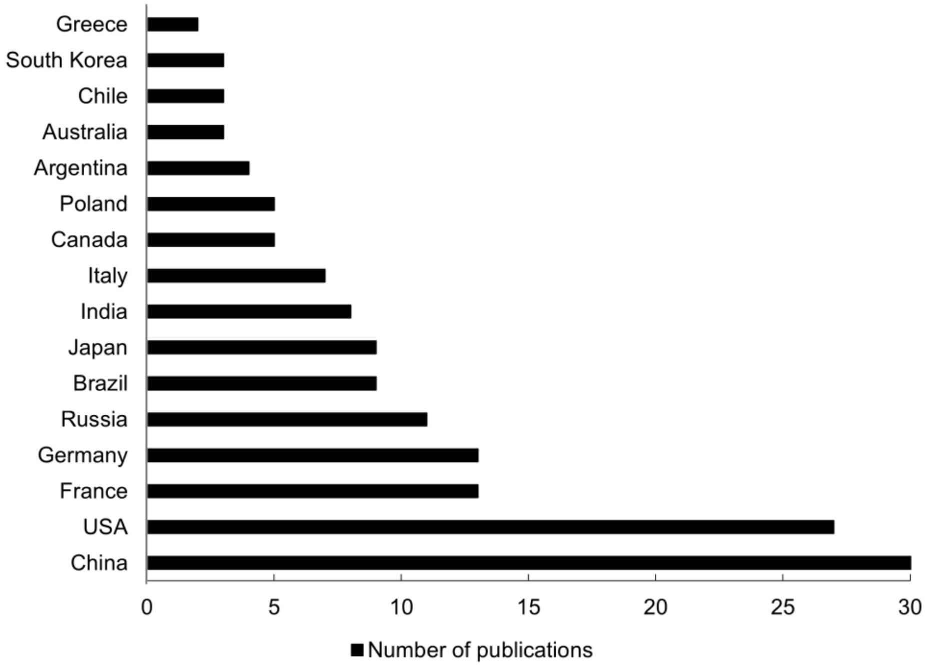

Figure 3. World ranking of publications of articles indexed in the Web of Science database for the "Mollusk AND fungi" descriptor from 2001 to 2021.

Table 1. Categories of research areas listed on the Web of Science database for bacteria in mollusks.

Research areas on the Web of Science	%
Marine and freshwater biology	24.77
Fisheries	23.86
Veterinary sciences	18.94
Immunology	17.85
Microbiology	12.93
Biochemistry and molecular biology	11.65
Zoology	10.38
Microbiology applied to biotechnology	9.83
Food science technology	6.74
Environmental sciences	6.37

et al. 2019) and Greece (Lattos et al. 2020). Thus, epidemiological investigations suggest a causal link between these mortalities and infections.

Scientific production of bacterial microbiota in mollusks was greater than that of fungi biota in the last 20 years. It is assumed that this increase in scientific production is related to the pathogenicity mechanism of bacteria since fungi have high phenotypic variability, slow growth, good compatibility with the host, and extrinsic factors that make their identification difficult (Trabulsi & Alterthum 2015, Tortora et al. 2017).

Table 2. Main research areas of articles indexed in the Web of Science for fungi in mollusks.

Research areas on the Web of Science	%
Biochemistry and molecular biology	18.60
Pharmacy	17.83
Fisheries	12.40
Immunology	12.40
Marine and freshwater biology	12.40
Veterinary sciences	11.63
Zoology	9.30
Plant sciences	8.53
Ecology and environmental sciences	7.75
Microbiology	6.97
Microbiology applied to biotechnology	5.43

Although the literature on fungi in mollusks is scarce (Fig. 3), several genera of filamentous fungi have been described in Brazil and Japan (Borzykh & Zvereva 2012, 2014a, Santos et al. 2017, 2020). Santos et al. (2017) identified *Penicillium* sp., *Trichoderma* sp., *Cladosporium* sp., *Mycelia sterilia*, *Aspergillus* sp., unidentified dematiaceous fungi, *Fusarium* sp. and made the first report of *Pestalotiopsis* in *Nodipecten nodosus*, demonstrating the need for monitoring in the context of public health.

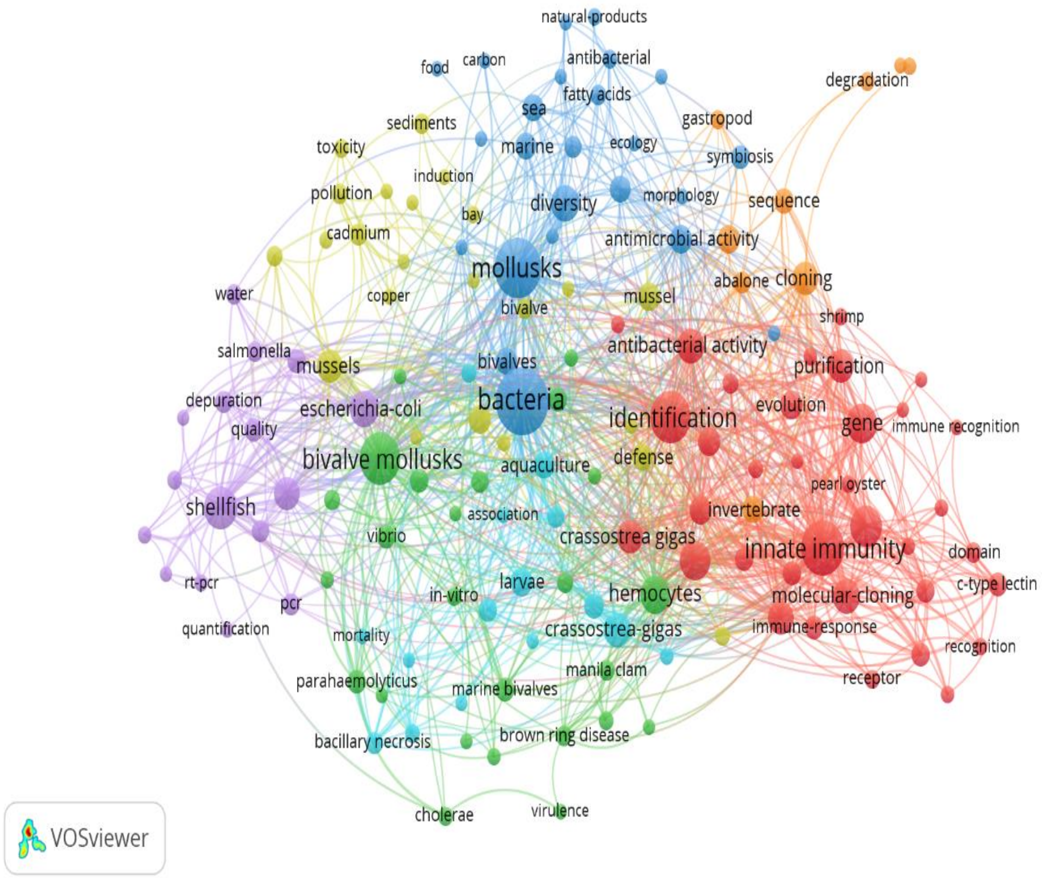

Figure 4. Map of co-occurrences of terms and clusters from the "Mollusk AND bacteria" descriptor from 2001 to 2021. Cluster 1: red, cluster 2: green, cluster 3: purple, cluster 4: orange, cluster 5: yellow, cluster 6: light blue, and cluster 7: dark blue.

The USA and China (Figs. 2-3) are countries with references in studies related to bacterial and fungal microbiota. Although China has shown a decline in production, the country has significantly contributed to the growth of aquaculture and with greater technological apparatus for the development of shellfish production (FAO 2020a).

The main line of research investigated by these countries is focused on the immune system of mollusks since the importance of the innate defense mechanisms of mollusks against pathogens developed by the host-pathogen interaction throughout the evolutionary process was observed (Song et al. 2015, Wang et al. 2017).

Although developing countries present few studies on this topic, Brazil has advanced in investigations on bacterial infections (Pereira et al. 2007, Ramos et al. 2014, Silva et al. 2018) due to the increase in aquaculture production in recent years, which in 2018 was 445,000 t, with seafood production corresponding to 4.7% (FAO 2018). Consequently, there has been an increase in diseases caused by consuming contaminated seafood, especially in developing countries (Barbosa et al. 2019, Silva et al. 2020). Due to the damage caused by pathogenic *Escherichia coli* strains in these countries, there has been great concern regarding microorganisms in mollusks, mainly due to a lack of basic sanitation and high consumption (Barbosa et al. 2019).

It is worth mentioning that epidemiological data in Brazil on diseases transmitted by shellfish consumption are insufficient and have resulted in the lack of knowledge of the real number of food poisoning outbreaks involving shellfish consumption, impairing the identification of pathogens that cause diseases (Souza et al. 2014, MS/SVS 2022).

Despite their importance, bacteria and fungi associated with a lack of sanitary management in mollusk farming stations can generate losses in production, marketing, and consumption, becoming a risk to human health. The presence of clinical signs in mollusks varies according to the degree of infection, being mostly absent, which may be related to symbiosis associations between microbiota and mollusk and the quality of the environment (Santos et al. 2020, Liu et al. 2021).

The USA, European Union, and Brazil use the group of thermotolerant coliforms and *E. coli* as bacterial indicators of sanitary quality to classify mollusk collection areas (NRC 2004, Rees et al. 2010, MPA 2014). The severity of pathogenic *E. coli* justifies the existence of international and national surveillance programs that monitor and track outbreaks caused by this microorganism to protect aquaculture and bivalve fisheries in natural and farmed environments (Lightner 2012, MPA 2014, NSSP 2019, MAPA 2020).

In this study, biochemistry, molecular biology, and microbiology research areas applied to biotechnology appear strongly related since research has shown that mollusks have antimicrobial molecules as an essential line of defense to survival (He et al. 2020) and important bioactive compounds that could be used in the pharmaceutical industry (Ordaz et al. 2010, An et al. 2019, Liu et al. 2021).

The other journals show greater interest in diseases and pathogens, aquatic microbial ecology, and toxicology, whose themes are related to veterinary sciences, microbiology, and ecology. Zanella et al. (2017) highlighted concepts such as "holobiont" and "hologenome" in their study to include the vast ecological and genomic complexity involving mollusks and their total microbiota and updated the conventional view of host-microbiota symbioses.

These studies' publications on food safety are scarce since a complementary approach on the subject was observed in worldwide investigations. This scenario can be understood as a reflection of efforts applied to control environmental quality through the application of regulatory legislation, leading to improvement of the microbiological quality of water and mollusks (NRC 2004, MPA 2014, MS 2019, NSSP 2019), with positive effects on farming systems and natural environments, reducing economic losses and infections caused by shellfish ingestion.

However, studies on food safety and quality control highlight the potential risks to public health from the ingestion of bivalve mollusks contaminated by bacteria and fungi (Santos et al. 2017, 2020), mainly due to their special edible form, raw or partially cooked (Su et al. 2010). Investigations are also justified by indicators that show outbreaks caused by contaminated food, which has been causing 420,000 deaths each year worldwide (FAO 2020a).

The co-occurrence network for bacterial microbiota highlighted terms related to the innate immunity of mollusks, important bacteria that infect these organisms, species of bivalve mollusks susceptible to infections, and virulence aspects of bacteria.

The expression "innate immunity" stood out in the co-occurrence network's red cluster (1). This term is related to studies investigating the antimicrobial activity expressed through proteins produced by mollusks, which function as pattern recognition receptors (PRRs) (Yang et al. 2010).

At least seven groups of PRRs have been identified in mollusk species, including peptidoglycan recognition proteins (Wei et al. 2012, Priyathilaka et al. 2020), Toll-like receptor (Wang et al. 2015), calreticulin (Wang et al. 2017), galectin (Song et al. 2011), lipopolysaccharide and β-1, 3-glucan binding protein (Yang et al. 2010), type-i lysozyme (Wang et al. 2018a), a-d-galactose binding lectin (MytiLec) (Hasan et al. 2016).

The PRRs have been identified in *Crassostrea gigas* (Thunberg, 1793), *Crassostrea virginica* (Gmelin, 1791), *Chlamys farreri* (KH Jones & Preston, 1904), *Mytilus galloprovincialis* (Lamarck, 1819), *Argopecten purpuratus* (Lamarck, 1819), and *Haliotis discus discus* (Reeve, 1846); economically important species in America, Asia, and Europe (Hasan et al. 2016, González et al. 2017, Wang et al. 2018b, Priyathilaka et al. 2020, Pacor et al. 2021).

An important aspect of the immune defense mechanisms in bivalve mollusks is the expression of antimicrobial peptides encoded during infection. These molecules have a broad spectrum of action against pathogenic bacteria, which explains the efficient defense mechanisms of mollusks since they are in close contact with large amounts of pathogens (Zanella et al. 2017).

With recent advances in molecular biology and bioinformatics, studies on immunology have been describing the interactions between the host's immune response and infections by pathogens (Bordenstein & Theis 2015).

Thus, terms related to molecular studies and genetic engineering techniques that have identified and characterized genes that are important in the expression patterns of proteins involved in the immune responses of mollusks are also highlighted in the red cluster (1) (González et al. 2020, Zhang et al. 2021). Among techniques are recombinant DNA and DNA sequencing (Gueguen et al. 2003, Wei et al. 2012, He et al. 2020).

The green cluster (2) highlights items related to the main bacteria responsible for infections in mollusks that present a potential risk to food safety, such as *Vibrio parahaemolyticus*, *V. cholerae*, and *V. vulnificus* (Bonnin-Jusserand et al. 2019).

Most bacterial diseases of bivalves are caused by a wide variety of gram-negative bacteria of the genus *Vibrio* (*V. aestuarianuns*, *V. alginolyticus*, *V. anguillarum*, *V. harveyi*, *V. neptunius, V. splendidus*, *V. tapetis*, and *V. tubiashi*) and *Pseudomonas*, *Aeromonas*, *Salmonella* and *E. coli* (Pereira et al. 2007, Garnier et al. 2008, Biel et al. 2014, Prado et al. 2014, Kwan & Bolch 2015, Bazzoni et al. 2019).

The green cluster (2) also highlights bivalve mollusk species *C. gigas*, *Mytilus edulis* (Linnaeus, 1758), and *Ruditapes philippinarum* (Adams & Reeve, 1850), which are more susceptible to infections by pathogens, and *M. galloprovincialis*, which has more efficient bacterial growth inhibition mechanisms (Vezzulli et al. 2018, Rahmani et al. 2021), whose term is also related to the red cluster (1), which highlights the innate immunity of mollusks.

The expression "brown ring disease" in the green cluster indicates investigations on the bacterium *V. tapetis*. According to Bidault et al. (2015), *V. tapetis* is the activating agent of an epizootic infection described in adult shellfish, called brown ring disease, in which *R. philippinarum* is the most sensitive species to the pathogen and is present in European coastal systems (Rahmani et al. 2021).

Although more studies have identified *Vibrio* in mollusks carried out in Asia, Europe, and the USA, in Brazil, the presence of *Vibrio* spp. in *Perna perna* (Linnaeus, 1758) (Pereira et al. 2007), *C. gigas* and *Vibrio* spp., virulence aspects of *V. parahaemolyticus* in oysters were observed (Ramos et al. 2014, Silva et al. 2018).

Cluster two (2) has strong relationships with cluster three (3), highlighting *E. coli* and *Salmonella*, which are related to "oysters" and "seafood" within the cluster. Enteropathogenic *E. coli* strains are widely distributed in coastal areas and are causative agents of gastro-enteritis in humans after consuming contaminated seafood (Kanayama et al. 2015).

E. coli has already been isolated and detected in several parts of the world (Şeker & Yardimci 2008, Parvez et al. 2017, Aijuka et al. 2018, Maniha & Noor 2020). Studies on the characterization of this pathogen are little explored. In 2019, researchers from Brazil and the USA performed a genotypic and phenotypic characterization of *E. coli* extracted from mollusks. It was shown in the study that oysters can bioaccumulate this pathogen to concentration four times higher than in the surrounding water. In addition, the study showed the high genetic diversity of *E. coli* and a higher frequency of pathogenic serogroups in mussels than in oysters (Miotto et al. 2019).

Cluster two (2) also highlights the term "virulence", related to the bacteria. Investigations have demonstrated the mechanisms of pathogenicity and profiles of virulence factors, mainly for the genus *Vibrio* and species *E. coli*. Silva et al. (2018) found that *V. parahaemolyticus* carry virulence markers and have high resistance to antimicrobial drugs that may be ineffective in treating their infections. Barbosa et al. (2019) isolated *E. coli* from *Mytella guyanensis* and identified the *elt* gene related to the pathogen virulence. According to Anand et al. (2016), this gene encodes enterotoxigenic *E. coli* toxins that cause diarrhea due to the release of potent enterotoxins.

The network tree of terms related to fungal pathologies (Fig. 5) in mollusks has shown interest in the search for biological responses through bioactive molecules of the immune system of mollusks, considering that mollusks can produce important molecules that can be used in the pharmaceutical industry (Liu et al. 2021).

Alkaloids, saturated asteroids, aspartate D and E, and aspertoryadins are some examples of secondary metabolites extracted from fungi in mollusks, whose antimicrobial and biochemical activity were evaluated (Ordaz et al. 2010, Dubrovskaya et al. 2018, An et al. 2021, Liu et al. 2021). *Aspergillus* and *Fusarium* filamentous fungi can produce important compounds to produce medicines and other pharmaceutical products (Liu et al. 2021) from association with mollusks (Kong et al. 2010, King et al. 2019).

The marine environment is considered a site for exploring and prospecting bioactive molecules. The search for biologically active molecules in marine invertebrates is well evidenced in this study. In addition to vertebrate and invertebrate animals, some marine microorganisms can produce compounds capable of being used in the pharmaceutical industry (Barreca et al. 2020, An et al. 2021, Liu et al. 2021).

Scientific mapping allowed visualizing investigations in several areas of knowledge, especially fisheries, immunology, and molecular biology, which have aroused the scientific community's interest since they are associated with the intrinsic and extrinsic factors of the microbiota-mollusk interaction.

Thus, it is important to expand these productions worldwide to generate subsidies for the proper management of mollusks, application in the pharma-

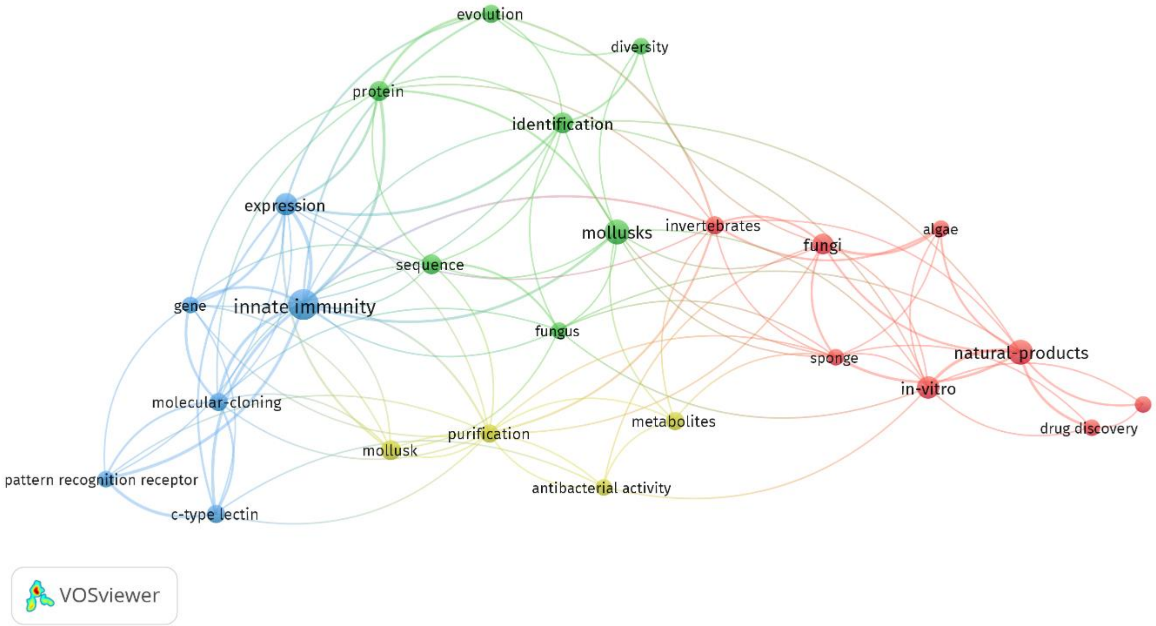

Figure 5. Map of co-occurrences of terms and clusters from the "Mollusk AND Fungi" descriptor from 2001 to 2021. Cluster 1: blue, cluster 2: green, cluster 3: red, cluster 4: yellow.

ceutical industry, and implementation of measures in public health.

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