UNIVERSIDADE FEDERAL DO RIO DE JANEIRO

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Unraveling the diversity of fungi isolated from the deep-sea coral *Desmophyllum pertusum* (Linnaeus, 1758) from the Campos Basin, Brazil

PBV - UFRJ

Master dissertation presented to the Postgraduate Research Program in Plant Biotechnology and Bioprocesses, at the Health and Sciences Center of the Federal University of Rio de Janeiro, as part of the requirements to obtain the Master's degree in Plant Biotechnology and Bioprocesses.

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Preface

This study is part of a big set of research that investigates the microbial communities of the Atlantic's deep-sea corals, sponges, sediments, and water called "Probiodeep" under the leadership of Professor Raquel Peixoto. Deep sea corals were collected from the Campos Basin offshore Brazil and specimens of *Desmophyllum pertusum* were moved to the Marine Aquarium of Rio de Janeiro (AquaRio) where they were kept alive in optimum conditions for research purposes. This dissertation sought to identify fungal communities within the coral host and identify specific genes to understand their interactions. This investigation was conducted in the Molecular Microbial Ecology Laboratory at the Federal University of Rio de Janeiro with the collaboration of The University of New South Wales and the University of São Paulo. This research was financed by CAPES, The Federal University of Rio de Janeiro and Shell Brazil.

Abstract

Despites the importance of the fungal communities associated with corals, these organisms' interactions are overlooked, which is even more accentuated in deep-sea fungal communities. In this study, we used culture-dependent methods to isolate fungi from the deep-sea coral *Desmophyllum pertusum* and culture-independent methods to do phylogenetic and functional analysis from some strains. A variety of culture media were used to isolate fungi from five coral *D. pertusum* fragments collected at 670 m depth in Campos Basin, Brazil. Morphologically distinct isolates were identified by sequencing the Internal TranscribedSpacer (ITS-1) and the subunit 18S of the ribosomal RNA gene markers. Twenty-seven strainswere obtained, including twenty-one filamentous fungi and six yeast. The isolates were morphological and phylogenetic, assigned to two divisions, eight genus, and five classes. Most of the isolates were assigned to the phylum Ascomycota (94.40%) and some of them as Basidiomycota (5.26%). Considering the Ascomycota phylum, the most common genera found were *Penicillium (*57.9%) and *Exophiala* (10.5%), followed by *Aspergillus (*(5.3%) *Pseudotaeniolina* (5.3%*) and Meyerozyma* (5.3%). Previous studies showed that strains from the *Penicillium* and *Aspergillus* genera isolated from deep-sea (benthic animals, water, sediment) were associated with denitrification processes, antimicrobial activities, and cytotoxic activities. We found tree strains (*Aspergillus versicolor*, *Penicillium griseofulvum*, and *Meyerozyma guilliermondii*) that may play important roles associated with nutrition and protection activities within this holobiont.

Keywords: Deep-sea, marine fungi, corals, *Desmophyllum pertusum*, environmental stressors, fungal diversity.

Resumo

Apesar da importância das comunidades fúngicas associadas aos corais, as interações desses organismos são negligenciadas, o que é ainda mais acentuado nas comunidades fúngicas de águas profundas. Neste estudo, utilizamos métodos dependentes de cultivo para isolar fungos do coral de águas profundas *Desmophyllum pertusum,* e métodos independentes de cultivo para realizar análises filogenéticas e funcionais de algumas cepas. Uma variedade de meios de cultura foi utilizada para isolar fungos de cinco fragmentos do coral D. pertusum coletados a 670 metros de profundidade na Bacia de Campos, Brasil. Isolados morfologicamente distintos foram identificados por sequenciamento dos marcadores do gene Espaçador Interno Transcrito (ITS-1) e da subunidade 18S do RNA ribossômico. Foram obtidas vinte e sete cepas, incluindo vinte e um fungos filamentosos e seis leveduras. Os isolados foram morfológica e filogeneticamente designados para duas divisões, oito gêneros e cinco classes. A maioria dos isolados foi atribuída ao filo *Ascomycota* (94,40%), e alguns deles ao filo *Basidiomycota* (5,26%). Considerando o filo Ascomycota, os gêneros mais comuns encontrados foram *Penicillium* (57,9%) e *Exophiala* (10,5%), seguidos por *Aspergillus* (5,3%), *Pseudotaeniolina* (5,3%) e *Meyerozyma* (5,3%). Estudos anteriores mostraram que cepas dos gêneros *Penicillium* e *Aspergillus* isoladas de águas profundas (animais bentônicos, água, sedimentos) estavam associadas a processos de desnitrificação, atividades antimicrobianas e atividades citotóxicas. Nossos achados identificaram três cepas (*Aspergillus versicolor, Penicillium griseofulvum e Meyerozyma guilliermondii*) que podem desempenhar papéis importantes associados a atividades nutricionais e de proteção dentro deste holobionte.

Key words: Fungos marinhos, águas profundas, corais, *Desmophyllum pertusum*, estressores ambientais, diversidade fúngica.

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Abbreviation's list

Adenosine 5'-phosphosulfate	APS
Average nucleotide identity	ANI
Beneficial Microorganisms for Corals	BMC
Carbon dioxide	CO2
Deoxyribonucleic acid	DNA
Dimethyl Sulfide	DMS
Dimethyl Sulfoniopropionate	DMSP
Expressed sequence tags	EST
Gamma Distributed with Invariant Sites	(G+1)
General Time Reversible model (GTR)	GTR
Genome-to-Genome Hybridization similarity	GGDH
Internal Transcribed Spacer	ITS
Potato Carrot Agar	PCA
Potato Dextrose Agar	PDA
Protein Family	PFAM
Protein Heat Shock Protein 70	HSP70
Recombinant DNA	rDNA
Recombinant RNA	rRNA
Ribonucleic acid	RNA
Sulphate Donor 3'-phosphoadenylylsulphate	PAPS
Kyoto Encyclopedia of Genes and Genomes	KEGG

Introduction

Deep-sea

Deep-sea covers approximately 65% of the Earth's surface. This environment consists of waters deeper than 200 m, where the light is scarce, pressure and salinity are high, and temperatures are low (Paulus et al., 2021). This ecosystem absorbs the excess heat and the CO_2 of the atmosphere, making it crucial in regulating Earth's climate and slowing down the increased temperature of surface waters while recycling nutrients (Levin et al., 2020). However, deep-ocean waters become warmer, more acidic, and less oxygenated during these processes, decreasing ocean productivity and biodiversity (Levin et al., 2020). Because oceans are complex ecosystems, deep-sea waters depend on shallow waters and vice versa, that is why the anthropogenic climate change, the decrease in phytoplankton, and the exhaustion of the oxygen bring long-term effects on the physicochemical characteristics of deep-sea waters (Thresher et al., 2015).

Studying the deep-sea is a big challenge because of the harsh conditions and the extended area. However, during the past 50 years, there has been an increase in global explorations and significant advances in the technology for sampling these ecosystems, changing the vision that the deep-sea basins are biologically poor (Vrijenhoek R. 2009). Despite these conditions, deep-sea harbors an unusually rich biodiversity of highly adapted animals, including some species of fish and marine mammals (Thresher et al., 2004). Almost 1000 new animal species have been described from hydrothermal vents, hydrocarbon seeps, and other chemosynthetic environments (Vrijenhoek R. 2009). Respectably a high number of microbial communities have been characterized and described from these environments, suggesting a rich biodiversity of deep-sea microorganisms (Huber et al., 2007; Vrijenhoek R. 2009). Modeling studies based on the accumulation rate of species per area in the deep-sea

concluded that these ecosystems represent a great diversity pool with high ecological importance (Dorazio et al., 2006).

The Atlantic Ocean and The Campos Basin (Brazil)

The Atlantic Ocean is the second largest ocean in the world, covering approximately one-fifth of Earth's surface with an average depth of 3,646 meters and a maximum depth of 8,380 meters (NOAA, March 2017). The Atlantic has valuable resources like fish, marine mammals, minerals, petroleum, and gas, attracting different industries for human activities (Garcia et al., 2011). During past decades, technological advances, like remotely operated vehicles (underwater robots that can be controlled from the surface to explore the deep sea), autonomous underwater vehicles (robotic vehicles that can explore the deep sea without being tethered to a surface ships), sonar technology (sound waves to map the ocean floor and locate underwater objects), and improved imaging technology (National Oceanic and Atmospheric Administration, n.d.) have enabled the exploration of the deep-sea (Bellingham et al., 2009). This has led to the increaseof information of the deep-sea habitats of the Atlantic Ocean and endemic species like cold-water coral reefs and deep-sea sponge aggregations (Kazanidis et al., 2020).

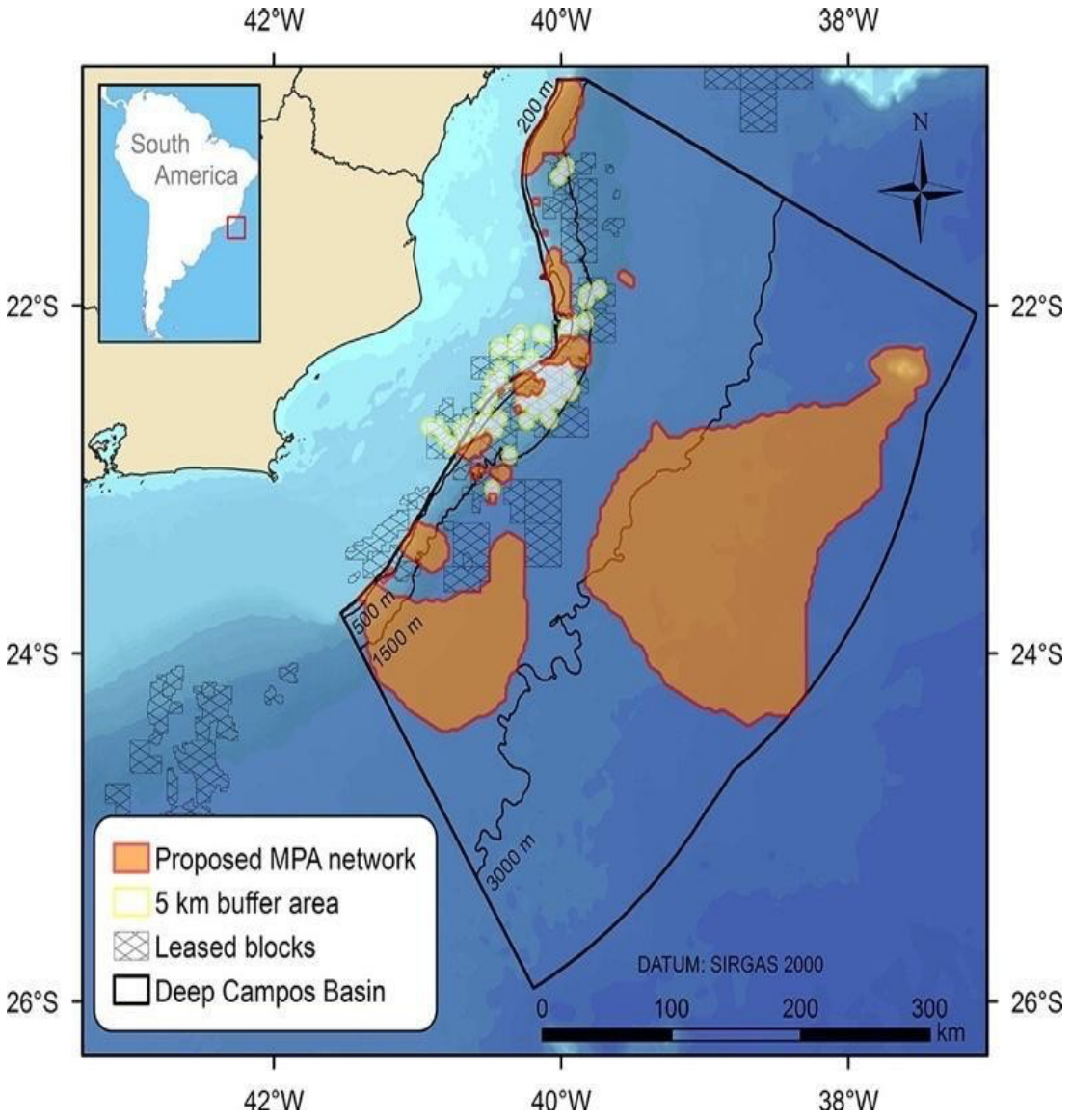

Figure 1. Location map of the explored region at the Campos Basin in Brazil.

The Campos Basin is in the South Atlantic Ocean and on the continental shelf of southeastern Brazil (Figure 1). This is Brazil's second-largest oil production site, with an area of 100,000 km^2 (Appolinario et al., 2020). This region hosts particularly high biodiversity due to its environmental gradients, which include temperatures and particulate organic influx, as well as the upwelling of cold and nutrient-rich waters (Almada and Bernardino, 2017; Appolinario et al., 2020). Consequently, the Campos Basin supports a large diversity of pelagic and benthic species, including deep-sea corals and sponges (Appolinario et al., 2020).

However, these deep-sea ecosystems are constantly threatened by human activities, including bottom trawling, oil and gas exploitation, and deep-sea mining (Kazanidis et al., 2020). These activities are catastrophic to benthic species due to their slow growth, low recovery, and little reproduction rates (Almada et al., 2017). That is why studying these ecosystems and species is crucial to understand how they have been affected by these anthropogenic stresses, and what actions can be taken to mitigate them.

Deep-sea reefs

Deep-sea reefs are mainly found on elevated zones such as undersea mountains or seamounts (Gammon et al., 2018). These are advantageous positions for deep-sea corals and sponges to feed in high currents of particulate organic matter (Gammon et al., 2018; Williams et al., 2020). The most common groups of deep-sea corals observed are Octocorallia (soft corals), Scleractinia (stony corals), and Antipatharia (black corals) (Auscavitch et al., 2020; Williams et al., 2020). Scleractinia corals are often classified as "habitat-forming" because they can form three-dimensional calcium carbonate structures, providing habitat and refuge to an abundant number of deep-sea invertebrates, fish, and sharks (Gammon et al., 2018; Williams et al., 2020).

Deep-sea reefs are mainly formed by the widely distributed stony corals *Desmophyllum pertusum* and *Solenosmillia variabilidis*. These two coral species are involved in biomass and carbon-cycling processes. Because of their branching forms, they can harbor a great variety of crypt animals (Williams et al., 2020). Studies have shown the differences in fauna assemblages between reefs with healthy and abundant corals and reefs with disease or dead corals (Chen et al., 2020). The reefs with healthy stony corals represent a "hot spot" for biodiversity with a greater abundance of species, these corals can create unique symbioses with invertebrate fauna and demersal fish communities (Auscavitch et al., 2020).

Desmophyllum pertusum

Desmophyllum pertusum (Figure 2) (former *Lophelia pertusa* due to taxonomic revisions and updates) was first described from the northeast Atlantic by Linnaeus et al. in 1758. This azooxanthellate Scleractinia coral has been found in the Atlantic, Pacific, Indian Ocean, and Mediterranean Sea (Roberts et al., 2003). They are often found at depths between 200 and 400 m, but they can also be found between 39 and 3600 m. Their habitat temperature fluctuates between 1.11 and 25.28 ºC (Rogers A. 1999, Roberts et al., 2003). This species belongs to the polyphyletic family Caryophylliidae (Addamo et al., 2016). This is one of the few coral species that deposits calcium carbonate structures and contributes to the complex three-dimensional structures in deep-sea waters. (Addamo et al., 2016; Linley et al., 2017). Because of this, *D. pertusum* offers shelter and supports a high diversity of invertebrate fish and benthic animals (Linley et al., 2017).

Figure 2. *Desmophyllum pertusum* from the South Atlantic.

***Desmophyllum pertusum* as a meta-organism**

Cold-water corals are associated with a vast, multidomain microbial community, including fungi, bacteria, archaea, and viruses. Despite the absence of symbiodinium, cold-water corals can build three-dimensional structures like those found in tropical reefs (Wada et al., 2019). This host-microbe species association is denominated meta-organism because the microbial communities and the coral host form dynamic relationships that could be fundamental for evolutionary and adaptive processes (Bosch et al., 2011). Interactions between shallow-water coral hosts and some microbial communities like zooxanthellae and bacteria have been well studied, showing beneficial interactions in which, the zooxanthellae provide the host with essential nutrients through translocated photosynthates (Wada et al., 2019), and some bacteria could supply essential nutrients through nitrogen fixation and metabolizing dimethyl sulfoniopropionate (DMSP) (Boilard et al., 2020). However, microbial diversity and functionality associated with shallow corals remain poorly understood, which is even more accentuated for deep-sea corals. In comparison to tropical corals, the research on cold-water coral microbiomes is relatively new and scarce.

Cold-water corals produce large amounts of nitrogen-rich mucus, an important food source for pelagic microbes (Wild et al., 2008). This could explain why some species of coral have different microbial communities in comparison to those found in their surrounding (water, sediments), suggesting that cold-water corals can stimulate the growth and activity of some microbial communities by releasing nutrient-rich organic matter (Wild et al., 2008; Meistertzheim et al., 2016). Nevertheless, these microbes' role in the host's metabolism is still unknown.

Fungi associated with deep-sea and deep-sea corals

The deep-sea is formed by unique and extreme habitats characterized by unusual conditions. However, despite the exceptional circumstances, cold-water coral gardens and reefs

are formed (Marchese et al., 2021). To ensure their survival, cold-water corals have developed distinctive physiological and biochemical adaptations, also they host a microbial community that is different from their surrounding (seawater and sediments), and that differs drastically between healthy and diseased corals (Galkiewicz et al., 2012; Marchese et al., 2021). Even though microbiological research on cold-water corals is scarce, it is mainly focused on bacteria, overlooking other microorganisms like fungi. Fungi represent a large portion of the microorganisms found in the ocean, and they can grow in sediments, seawater, marine plants, animals (invertebrates and vertebrates), and rocks (Xu et al.,2018). Fungi have adapted to populate different ecological niches, including hypersaline lakes, methane cold seeps, or hot hydrothermal vents, becoming a significant part of the deep-sea microbial communities (Marchese et al., 2021).

Fungi are known to associate with shallow-water and deep-sea corals as symbionts and pathogens (Galkiewicz et al., 2012). In shallow-water corals, fungi are involved in various nutrient cycling processes, antifouling and antimicrobial activities in the host. For example, the fungal community of the coral *P. astreoides* might be involved in the transformations of nitrite and nitrate into ammonia, suggesting they play an essential role in nitrogen metabolism (Wegley et al., 2007). Also, a high percentage of fungi isolated from gorgonian corals demonstrated antimicrobial and antifouling activities against bacteria, including the genus *Vibrio*, being potentially involved in pathogenic control (Zhang et al., 2012, 2019).

In 2010, Burgaud and colleagues cultured filamentous fungi and yeast from deep-sea animals in the Pacific Ocean hydrothermal vents. This was the most extensive investigation on the matter, and they mainly found ascomycetes belonging to the order Helotiales, while basidiomycetes were significantly less frequent (Burgaud et al., 2010). In 2012, Galkiewicz and colleagues surveyed the fungal biodiversity of the deep-sea coral *Desmophyllum pertusum* in the Atlantic Ocean. They predominantly found basidiomycetes belonging to the order

Ustilaginales, while ascomycetes were primarily represented by Eurotiales (Galkiewicz et al., 2012).

In March 2021, Marchese et al. showed two different fungal communities: saprotrophic fungi, which are predominantly found in sediments, and symbiotic fungi, which are mainly found in animals in the deep North Atlantic (Marchese et al., 2021). Another study on deep-sea sediments in the Atlantic Ocean showed a predominance of Ascomycota dominated by Euromycetes, and a lower proportion of Basidiomycota (Xu et al., 2017).

All these studies used culture-dependent methods, which allowed them to investigate fungal morphological and physiological traits, enhancing the understanding of marine fungal life and the potential use of fungi in biotechnology. However, culture-independent methods are essential to reveal additional aspects of the uncultivable fungal fraction.

Fungal genetics

The fungal kingdom is extremely important for global health, agriculture, ecology, and biomedical research. These eukaryotes have a vast diversity of morphologies, reproduction variations, environmental niches, interactions with other organisms, and high plasticity (Schoch et al., 2017). This kingdom inhabits nearly all ecosystems. They can be free-living, symbiotic unicellular, and multicellular organisms (Hawksworth et al., 2001). Studying the fungal genome is essential because it encodes genes that allow them to thrive in different environments, cycle nutrients in terrestrial and aquatic ecosystems, act as pathogens for animals and plants, and symbionts (Chen et al., 2018).

Fungal geneticists study the information and mechanisms of heritable information in fungi. Fungal genomes (yeast and filamentous) are widely used as model organisms for eukaryotic gene research, including cell cycle regulation, genetic recombination, and gene regulation (Xu et al., 2017). Because of their importance, morphological and genetic studies

are required. Even though morphological identification studies are great for determining an individual identity, they are not enough, and the use of molecular phylogenetic tools has become increasingly common in fungal identification (Lücking et al., 2008)

Fungal genome databases are continuously expanding thanks to individual and large-scale efforts such as Genevese, Broad Institute's Fungal Genome Initiative, and the 1000 fungal genomes project (http://1000.fungalgenomes.org) (Schoch et al., 2014). These genetic databases are used to understand how fungal adaptations have helped fungi thrive in a specific ecosystem and ecological niche. These databases are also guidelines for utilizing novel methodologies and improving the study of fungal evolution from a molecular sequence perspective (Tedersoo et al., 2010). Combining culture-dependent methods with genome sequencing, bioinformatics, and comparative genomics allows a more detailed study of the biology and evolution of fungi.

Cultured dependent methods

Corals exist in a close symbiotic relationship with their associated microbial communities, collectively referred to as the coral holobiont (Wada et al., 2019). This intricate partnership ensures that the holobiont functions as a cohesive biological unit, working together to support the health and well-being of the coral host (Peixoto et al., 2020). This delicate relationship can be affected because of changes in the environmental conditions, compromising the coral's health. In most cases, when the stress is prolonged, it can lead to the coral's death (Guldberg et al., 2019). Microorganisms are essential in supporting the function and health of multicellular life and the ecosystems in which they develop. Even though the relationships between the coral host and its associated microbiome are not yet fully understood, there is evidence that the microorganisms are involved in processes like the fixation of nitrogen, the

degradation of polysaccharides, and the production of antimicrobial compounds (Peixoto et al., 2017; Rosado et al., 2019: Assis et al., 2020).

Evidence shows that the interactions between the coral host and its microbiota have influenced the genomic evolution of the host and its symbiotic microorganisms. Also, it has shaped the coral's development and determined the ecological success of the host (Pollock et al., 2018; Matthews et al., 2020). Researchers have used culture-dependent and culture-independent methods to understand these host-microbe associations better. Culture-dependent methods are central to understanding the microorganism's metabolic pathways interacting with the host and their response to environmental changes (Sweet et al., 2021). Even though culture-independent methods like metagenomics give essential information about the potential functionality and other cellular traits, they are insufficient to explain how environmental changes have pleiotropic effects on holobiont physiology (Sweet et al., 2021).

Culturing sometimes benefits some species' growth rate while suppressing the development of others, disordering the culturable fraction (Carraro et al., 2011). However, culture-dependent methods have several disadvantages, including high selectivity allowing the growth of only a tiny portion of microbial communities and the lack of media for many of the "known" microbial groups (Schultz et al., 2022). The microorganisms are often not "unculturable," but their nutrient requirements and the range of growing conditions are unknown (Sweet et al., 2021). Cultured-dependent approaches also fail to reproduce the ecological niches and the complex natural habitats required to assess the total magnitude of the microorganisms present in the coral host (Carraro et al., 2011). Recent studies have introduced innovative culture media to mitigate culturing flaws that mimic some biological host characteristics for cultivating the so-called "dark microbial matter" in the coral meta-organism (Schultz et al., 2022). These alternative cultured-based methods retrieve a higher amount of the microbiome of any given sample, including corals (Sweet et al., 2021).

Culture-independent methods

Before the development of molecular techniques, ecology studies and bioprospecting of microbial metabolites were based on cultured-dependent methods. However, estimates indicate that only 1-5% of microorganisms are cultivated under laboratory tests, vastly underestimating the microbial diversity (Amann et al., 1995). The coral-associated microbes have been mainly recovered from multi-omics analyses. Most recent research on the importance of coral-associated microbes used cultured-independent methods based on 16s rDNA, 18S rDNA, mitochondria, and internal transcribed spacer (ITS) region gene amplicon sequencing and metagenomics. These methods are essential for taxonomic identification and what potential functionalities they could have within the coral holobiont regarding environmental conditions (Sweet et al., 2021). Molecular techniques have several advantages, including a broader range of identification. This is especially relevant when distinguishing species with similar phenotypic characteristics and strains from the same species (Carraro et al., 2011).

While these approaches provide an essential fraction of the coral microbes, they do not assess the total microorganisms present in the coral host. That is why exploring and combining the cultured-dependent and culture-independent methods is a step closer to identifying the total amount of coral-associated microbes, exploring their taxonomy, their role in shaping the coral's health, and ultimately their potential biotechnological use (Schultz et al., 2022).

Human activities and anthropogenic stresses

In recent decades the exploration and industrialization of the deep-sea have been expanding worldwide. As oil and gas resources become scarce on land, states and private companies, have expanded their exploration into deeper waters (Cordes et al., 2016; Ragnarsson et al., 2017). The exploration and exploitation of resources from the seafloor crusts

significantly impact the benthic fauna, becoming an increasing concern, especially in the absence of sufficient knowledge on deep-sea biodiversity (Smith et al., 2008; Miller et al., 2018). Trawling, mining, and oil and gas exploration are significant threats to deep-sea ecosystems at a broad spatial scale (Smith et al., 2008). Physical disturbances tremendously impact some species because of their slow growth rates; at such low temperatures, the metabolisms and cellular/tissue activity become much slower, making the recovery process more extensive and difficult (Miller et al. 2018). For example, deep-sea coral Desmophyllum pertusum growth rate is around 4-25 mm per year, and deep-sea sponge *Rossella racovitzae* is about 2.9 mm per year (Ragnarsson et al., 2017).

Out of the above anthropogenic stresses, bottom trawling represents the harshest to cold-water coral reefs and deep-sea sponge grounds worldwide (Ragnarsson et al., 2017). The use of otter trawls and other fishing devices severally affects these biodiversity hotspots in different ways, including depletion of populations, alteration of seafloor morphology, resuspension of sediments, increased bottom water turbidity, mortality of fauna, alterednutrient cycles, and reduced benthic biodiversity (Ragnarsson et al., 2017).

Justification

The coral meta organism hosts a large and diverse number of microbial communities, including bacteria, fungi, archaea, and viruses (Peixoto et al., 2017). These interactions assure the coral host's health and survival. Fungal communities associated with shallow corals remain poorly understood, which is even more accentuated for deep-sea corals. However, they have been found in almost all marine habitats, from hydrothermal vents, subsurface deep-sea sediments, arctic ice, surface waters, sediments, and in association with animals, including corals (Wada et al., 2019). Past studies have shown that marine fungi could present beneficial characteristics for the corals, for example antimicrobial and antifouling activity against coral pathogens, antinematodal activity, oil mitigation (Silva and Villela et al., 2021), and some are involved in pathways for nitrate/nitrite ammonification and ammonia assimilation (Wegley et al., 2007). This very diverse and widespread group has been overlooked in comparison with other communities present in the coral meta-organism, creating this big gap in knowledge about the diversity of the fungal community on corals and their role in its maintenance.

An extensive bibliographic review on marine fungi and their interaction with corals is being carried out for this study. While searching for information, it was clear that there are few studies on the fungi communities in the deep-sea coral *D. pertusum*. To fill up this gap, five fragments of the deep-sea coral *D. pertusum* were used to culture fungi using five different culture media. The isolates had their Internal Transcribed Spacer (ITS-1) and the subunit 18S of the ribosomal RNA gene markers sequenced, and a phylogenetic analysis was carried out to identify each strain. Using this information, a bioprospection of marine fungal communities was done to identify genes that encode possible beneficial characteristics for the coral.

Looking into the future, this research could bring critical insights and understanding of this unique and important partnership and how it contributes to the coral's health. One of the main goals is to gather baseline data to support our further research on incorporating fungi into

the Beneficial Microorganisms for Corals (BMCs) consortia as an innovative, alternative and/or improved tool for mitigating coral death against different anthropogenic stressors.

Objectives

General Objective

Identify and characterize the fungal diversity associated with the deep-sea coral *Desmophyllum pertusum* using culture-dependent and genomic methods.

Specific objectives

- Isolated fungal strains of the coral *Desmophyllum pertusum*
- Caracterize macro and micro-morphologically the fungal stains obtained from the coral D. pertusum, as well as identify them by sequencing the ITS-1 region and the 18S rRNA subunit.
- Annotate three NCBI genomes of representative species that were identified.
- Infer function diversity and biotechnology potential of deep- sea coral fungi identifying protein families (PFAM) and enzymes involve in metabolic pathways (KEGG).

Graphical Methodology

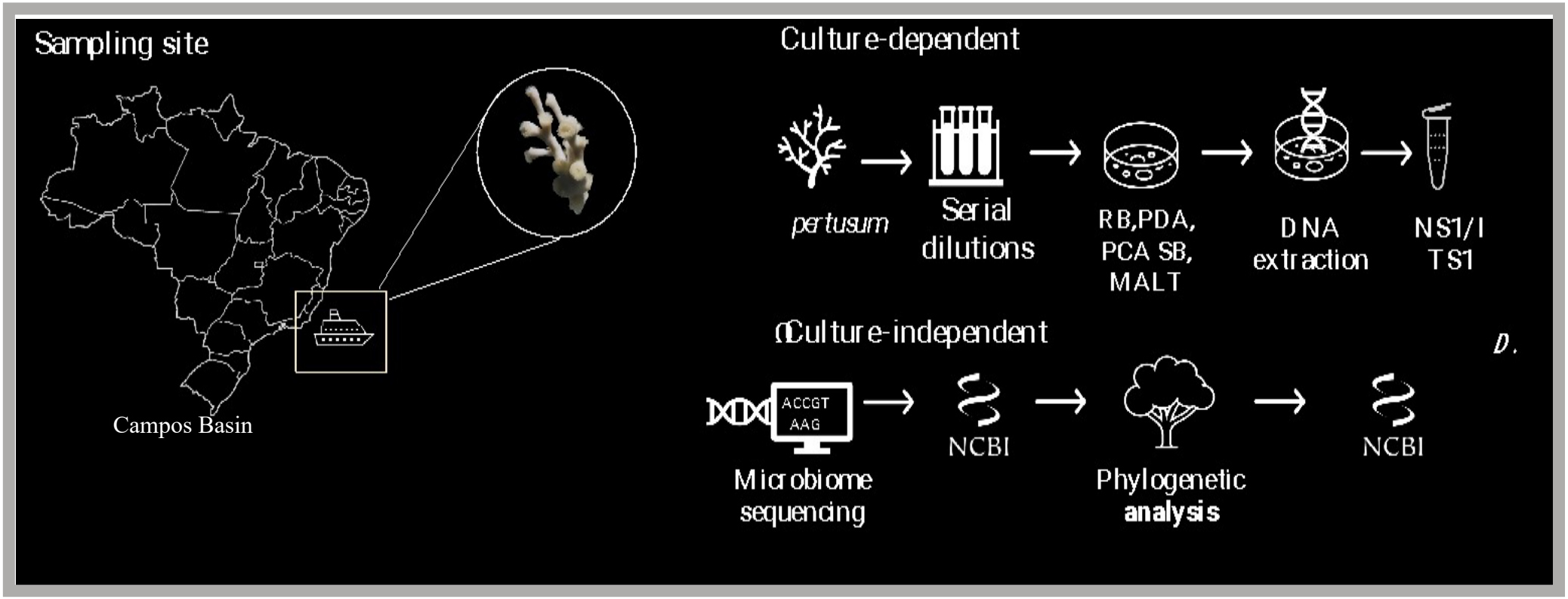

Figure 3. Workflow of the methodology showing both the culture-dependent and independent analysis.

Methodology

Coral sampling and maintenance

In conjunction with The Federal University of Rio de Janeiro, Shell Brazil, the University of São Paulo, and the University of New South Wales, coral samples were collected during one cruise (Fugro Aquarius) designed to explore the benthic ecology of the Atlantic deep-sea ocean. The samples were collected from the Campos Basin in Brazil at a depth of 670 m using a DP2 ROV. This samples were selected depending on location, then thy were placed in specific aquariums at the deep-sea simulator of Rio de Janeiro's aquarium. They have been in optimal conditions since August of 2021 and they have been used for scientific purposes.

Culture dependent analysis

Fungal isolation

We first selected 5 deep-sea coral *Desmophyllum pertusum* fragments (A,B,C,D,E) based on their healthy appearance. The fragments ((A,B,C,D,E) were weighed and macerated using a sterile mortar and pestle. The homogenate of the coral tissue, mucus, and the skeleton was transferred into a sterile Erlenmeyer flask with glass beads and kept in constant agitation at 120 rpm overnight at 4ºC. Also we collected water samples from the surrounding environment.

Each sample was then subjected to serial dilutions in saline solution (0.85% NaCl) using a 1:9 ratio in 2 ml Eppendorf® microtubes. No dilution, 10^{-1} and 10^{-2} dilutions were plated using 100 µL of sample for the spreading on a plate technique with the aid of a Drigalski loop, in a sterile culture medium. The medias used were (i) potato dextrose agar (PDA) + chloramphenicol, (ii) malt extract + chloramphenicol, (iii) potato carrot CaCo3 agar + chloramphenicol, (iv) Sabouraud + chloramphenicol, and (v) Rose Bengal + chloramphenicol

(Table 1). All the media were made with sterile marine water from the quarantine tanks in AquaRio. The plates were then incubated for 35 days at 6ºC. Morphologically differentiated colonies were isolated from the depletion technique using the same culture media until pure isolates were obtained. However, we did not replicate the pressure which is much higher than at the surface (1 atmosphere (ATM) for every 10 meters).

Table 1: Culture media used to isolate the fungi, their description, and the quantity of components.

Culture medium	**Components of the medium**
PDA + chloramphenicol	· Potato infusion 200g · Dextrose 20g · Agar 20g · Chloramphenicol 0.2g · Final pH 5.6 · SSW 1000 mL
Malt extract + chloramphenicol	· Malt extract 30g · Mycological peptone 5g · Agar 20g · Chloramphenicol 0.2g · Final pH 5.4 · SSW 1000 mL
Potato carrot CaCo3 + chloramphenicol	· Potatoes cooked and smashed 20g · Carrots cooked and smashed 20g · CaCo3 3g

	· Agar 20g · Chloramphenicol 0.2g · SSW1000 mL
Sabouraud + chloramphenicol	· Pancreatic digested casein 5g · Animal tissue peptic digest 5g · Dextrose 40g · Agar 20g · Chloramphenicol 0.2g · SSW1000 mL
Rose Bengal + chloramphenicol	· Mycological peptone 5g · Glucose 10g · Dipotassium phosphate 1g · Magnesium sulphate 0.5g · Rose-Bengal 0.05g · Agar 20g · Chloramphenicol 0.2g · SSW1000 mL

*SSW: Sterile Sea water

Preserving the fungal strains

Each strain was inoculated on Petri dishes with PDA media. When the cultures had grown to no more than three-fourths the diameter of the dish, ½ cm agar cubes with hyphae were cut from the colony margin. For most strains, 5 or 7 cubes were placed in 10 ml sterile distilled water in screw-cap test tubes, and the caps were firmly seated to prevent water loss.

The tubes were then stored at 25 ºC, and used for downstream analysis when necessary (Ellis J. 1979).

Morphological characterization of the isolates

The isolates were macroscopically characterized based on morphology and considering aspects like shape, size, texture, pigmentation, and optical property. Additionally, the isolates were smeared onto a coverslip and stained with lactophenol blue for later observation of structures using light microscopy; the characterization was based on *The Identification of Fungi: An Illustrated Introduction with Keys, Glossary, and Guide to Literature*, 2017.

Molecular identification of the isolates

DNA extraction and amplification

To identify the fungi isolated from deep-sea coral *D. pertusum* the DNA regions encoding 18S ribosomal RNA gene and the ITS-1 region were sequenced.

After the pure isolates were obtained, the DNA from each isolate was extracted using the commercial kit Powersoil DNA Isolation Kit (MoBio Laboratories, INC., Solana Beach, CA). The manufacturer protocol was followed with some modifications described by Galkiewicz and collaborators. The modifications were the following: the addition of an alternative lysis method involving two 5-minute incubations at 70 °C separated by a 3–4 s vortex, and the 4 °C incubation was increased from 5 min to 8–10 min. After the extraction, the DNA was quantified using the Qubit® 2.0 Fluorometer dsDNA high sensitivity kit (InvitrogenTM, Carlsbad, CA, USA). For amplifying the 18S rRNA subunit we used primers NS1 forward 3'- CATATGGGTTGCGTTATCAACTGGAAAGG and reverse 5'- GTAGTCATATGCTTGTCTC and to amplify the ITS-1 region we used primers 5'- TCCGTAGGTGAACCTGCGG and 3' GCTGCGTTCTTCATCGATGC. The PCR reaction

was carried out in a final volume of 12 µL, containing 3µL of BSA (1:20), 0,2 µL of each primer, DNA at a final concentration of 10 ng.µL-1 (1.5 µL) 7 µL of MyTaq® (Bioline) that contained buffer, dNTPs, MgCl2 and DNA polymerase enzyme, and ultrapure water to complete the reaction. The amplification was performed in thermocycler, in which the conditions were 15 min at 95 °C; 35 cycles of 1 min at 95 °C, 1 min at 50 °C and 2 min at 72 °C; and 10 min at 72 °C for a final extension, ending with a 4 °C hold. PCR products were purified with the SureClean Plus kit (Bioline GmbH), following the manufacturer's protocol, later quantified with Qubit 4.0 and Qubit® dsDNA HS Assay Kit (Life Technologies, USA), and the integrity of the amplicon verified on 1.5% agarose gel in 0.5X TBE buffer and stained with ethidium bromide and visualized under UV light. After purification, approximately 25 ng of PCR product was obtained from each isolate and was sent to be sequenced using Sanger platform using the primers NS1 and ITS-1 at the University of New South Wales.

Phylogenetic analysis

After sequencing, trimming, and evaluating the quality of the obtained sequences, we compared them against reference genomes manually obtained from the NCBI Blastn database. We selected 15 reference species based on their higher z-score (statistical significance of sequence alignments) values and verified and corrected the valid nomenclature when necessary.

For the phylogenetic analyses, we used MEGA X software to align the target sequence with the 15 reference sequences obtained from NCBI Blastn and an outgroup genus of the same family as the studied specimens. We performed a neighbor-joining tree as a test and obtained the measures of bootstrap support for internal branches from 500 replicates using the Maximum Composite Likelihood model. We then proceeded with the maximum-likelihood phylogenetic analyses, selecting the best model and obtaining the measures of bootstrap support for internal

branches from 1000 replicates using the General Time Reversible model (GTR) with Gamma Distributed with Invariant Sites (G+I).

Genome analyses

The fungal genomes were selected based on previous reports in the literature. We selected three strains (*Aspergillus versicolor* (M17), *Penicillium griseofulvum* (M27), and *Meyerozyma guilliermondii* (M2) that showed:1) interesting biotechnological properties, 2) are no human or animal pathogens, and 3) are found in the coral host and not in the surrounding environment. The complete genomes of these three strains were collected from the NCBI database. For this, we visit the NCBI website (https://www.ncbi.nlm.nih.gov/) and in the main navigation menu we selected “Genomes” and entered the organism’s name in the search bar. Then we click on the desired genome from the search results to access the detailed information page. We located the option to download the genome sequence in FASTA format and click on "Complete genome FASTA."

Genome Quality

To verify the NCBI genomes' quality and accuracy, we evaluated the assembly statistics to know the contiguity and completeness using QUAST (Quality Assessment Tool for Genome Assemblies). We downloaded QUAST from https://github.com/ablab/quast and the assembly stats from https://github.com/sanger-pathogens/assembly-stats. Then, we opened the terminal and run the command: quast.py aspergillus_versicolor.fasta (for QUAST) and the command: assembly-stats aspergillus_versicolor.fasta (for assembly-stats) for the three genomes. We obtained different information, including N50 and L50. N50 is a measure of assembly contiguity, the larger the N50 value, the more contiguous the assembly. L50 is the number of

contigs (or scaffolds) required to reach or surpass 50% of the total assembly size. A smaller L50 value corresponds to a more contiguous assembly.

Fungal Genome Annotation

The fungal genome annotation was performed to annotate and identify the functional elements within the three chosen fungal strains. We search into structural and functional characterization of protein-coding genes. The annotation pipeline includes gene prediction, functional annotation, and comparative analysis.

Functional prediction analysis of the isolates

Using the PFAM database

We look into PFAM domains to obtain information about conserved proteins. For this we use the software HMMER and follow the methodology described in the article *HSP-HMMER: a tool for protein domain identification on a large scale* (Bhanu et al., 2019). This software analyzes protein sequences using profile H idden Markov M odels (HMMs). We installed HMMER from http://hmmer.org/. Then we download the PFAM database from the PFAM website https://pfam.xfam.org/ and search the file title Pfam-A.hmm.gz. Then, in the terminal we extracted the compress file using the command: gzip -d Pfam-A.hmm.gz creating a file named Pfam-A.hmm in the same directory. Later we run the command hmmpress Pfam-A.hmm to build an index HMM database that HHMER will use for searching PFAM domains. Then, we execute the command: hmmsearch –cpu <2> Pfam-A.hmm aspergillus_versicolor.fasta > output.txt to search for PFAM domain in the complete genome sequence. We obtained a text file with the PFAM domain search in the complete genome with the identified domains, their locations within the genome, and their statistical score.

KEGG

KEGG (Kyoto Encyclopedia of Genes and Genomes) is a bioinformatic resource for analyzing and interpreting metabolic pathways, networks, and genome functional annotation. First, we accessed our account on the KEGG website (https://www.kegg.jp/). Then we submitted the genomes in FASTA format by clicking o n the button “Submission”. We filledin the required information, including the organism's name, NCBI ID, and other relevant data. Then we submitted the genomes.

Connected scatterplot with R and ggplot2

After obtaining the protein families' information for the three genomes, we did a connected scatterplot using R and ggplot2. First we downloaded the libraries ggplot2 and dplr from GitHub.

```
# Libraries
library(ggplot2)
library(dplyr)

# Load dataset from github
data <-
read.table("https://raw.githubusercontent.com/holtzy/data_to_viz/master/Example_dataset/3
_TwoNumOrdered.csv", header=T)
data$date <- as.Date(data$date)

# Plot
data %>%
  tail(10) %>%
  ggplot( aes(x=date, y=value)) +
  geom_line() +
  geom_point()
```

Histograms using R

After obtaining the information of the KEGG models for the three genomes we did histograms with the help of R. First, we did a data set with the metabolic processes (e.g., Carbon

fixation), the enzymes (e.g., ribose- 5 - photophate isomerase), and the quantity of models. We opened a Jupyter notebook, we uploaded our dataset e.g.:

models <- c(4,4,2,2)

hirst(data, breaks = 4)

bin_labels < -c("ribose-5-phosphate isomerase", "triose-phosphate isomerase", "ribulose-phosphate 3-epimerase", "fructose-bisphosphate aldolase")

Then we add the distribution of the data e.g.:

hist(Ixos, breaks=4, xlim=c(0,16), col=rgb(0,0,255), xlab="Kegg models in *Aspergillus Versicolor*", ylab=" Carbon fixation ")

Results

Fungal isolation

After incubating for thirty-five days, twenty-seven strains were obtained from the deep-sea coral *Desmophyllum pertusum* and surrounding water samples (figure 4). Twenty-one of the twenty-seven isolates were filamentous fungi; the remaining six were yeast, and one strain was isolated from the surrounding water samples. A complete morphological descriptionwas made based on color, size, and texture of the colonies. Most strains presented a dark greencolor with a "cottony" surface.

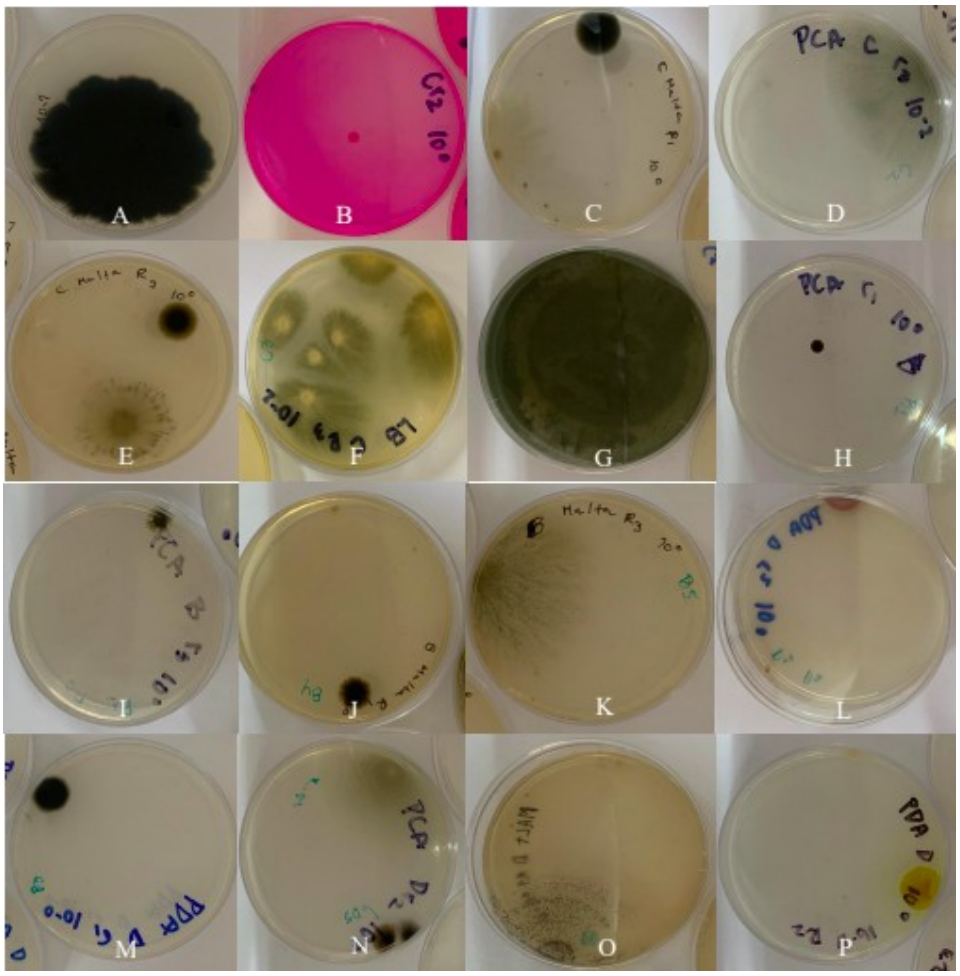

Figure 4. Fungi isolated from *Desmophyllum pertusum.* **A)** Sample M1. **B)** Sample M2. **C)** Samples M3, M4. **D)** Sample M6. **E)** Sample M7. **F)** Samples M9, M10, M11. **G)** Sample M13. **H)** Sample M14**. I)** Sample M15**. J)** Sample M16. **K)** Sample M17. **L)** Sample M18. **M)** Sample M20. **N)** Samples M21, M22. **O)** Sample M23. **P)** Sample M27.

The morphological identification of fungi communities is a common practice in mycology in which the observation of macroscopic and microscopic structures helps to

describe the identity of the fungi (Wang et al., 2022). This traditional method is a great first step for identifying isolates because of its ease and low price (Wang et al., 2022). However, morphological identification has several limitations, including the difficulty of distinguishing between closely related species with similar morphology. It is a time-consuming process, requiring extensive training and expertise to identify (Will et al., 2004) accurately. he molecular identification approach is highly accurate and can distinguish between closely related species that cannot be differentiated by morphology alone. It is also less time-consuming than morphological identification, and the results can be obtained quickly and easily (Will wt al., 2004). However, it can be expensive and requires specialized equipment. We used both techniques in our investigation to achieve a more accurate and comprehensive identification.

Description of the strains

>M3
ITS sequence:
CACCTCCCACCCGTGTTTATTCTACCTTGTTGCTTCGGCGGGCCCGCCTCACGGCCGCCGGGG
GGCATCCGCCCCCGGGCCCGCGCCCGCCGAAGACACCATTGAACTCTGTCTGAAGATTGCAG
TCTGAGCAGATTAGCTAAATCAGTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGA
TGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTC
TTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGC
CCTCAAGCACGGCTTGTGTGTTGGGCTTCGCCCCCCGGCTCCCGGGGGGCGGGCCCGAAAGG
CAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTCGTCACCCGCTCTGTAGGCCCG
GCCGGCGCCCGCCGGCGACCCCAATCAATCTTTTCAGGTTGACCTCGGATCAGGTAGGGATA
CCCGCTGAACTTAAGCATATCATAAGCGGGGAAGAAAA
538bp

Morphological description:

Back: White colony, snowflake shape, dark white center.

Front: White colony, snowflake shape, dark white center, cottony / spores

Media used: Malt

Source: Fragment C of a *Desmophyllum pertusum's* colony.

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
JN585939.1	***Penicillium simplicissimum*** strain Cs/6/1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	976	976	99%	0.0	99.63%

Description:

In 1965, the American mycologist Charles Thorn first described *Penicillium simplicissimum* after isolating it from Philippine soils (Thorn et al., 1965). This particular fungus is known for its remarkable ability to break down a diverse range of organic materials, including cellulose, hemicellulose, and lignin (Andlar et al., 2018). Moreover, *Penicillium simplicissimum* has been extensively utilized in biotechnology and pharmaceuticals for the production of valuable enzymes and secondary metabolites, including renowned antibiotics like penicillin (Toghueo et al., 2020). Additionally, this species of fungus has been found to possess bioremediation capabilities, specifically in heavy metal accumulation such as cadmium, copper, and zinc (Andlar et al., 2018). *Penicillium simplicissimum* can effectively absorb these metals from the environment and store them within its biomass (Andlar et al., 2018).

Previous isolation environments:

Penicillium simplicissimum can be found in various environments, including soil, air, water, decaying organic matter, and foods such as fruits, vegetables, and grains (Awuchi et al., 2021). While it is not typically associated with marine environments, it has been reported in some marine environments, including sediments, the rhizosphere of the mangrove plant *Bruguiera sexangula* var. *rhynchopetala* (Xu et al., 2016), and marine organisms such as the

marine sponge *Chelonaplysilla* sp. (Handayani et al., 2020). To our knowledge *Penicillium simplicissimum* has not been reported to be isolated from deep-sea environments.

Human Pathogenicity:

Penicillium simplicissimum is not considered to be a human pathogen. It is classified as a saprophytic fungus, which typically feeds on decaying organic matter and is not known to cause disease in healthy humans (Awuchi et al., 2021).

>M4

ITS sequence:
GTCCACCTCCCACCCGTGTTTATTCTACCTTGTTGCTTCGGCGGGCCCGCCTCACGGCCGCCGG
GGGGCATCCGCCCCCGGGCCCGCGCCCGCCGAAGACACCATTGAACTCTGTCTGAAGATTGC
AGTCTGAGCAGATTAGCTAAATCAGTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATC
GATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAG
TCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCT
GCCCTCAAGCACGGCTTGTGTGTTGGGCTTCGCCCCCCGGCTCCCGGGGGGCGGGCCCGAAA
GGCAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTCGTCACCCGCTCTGTAGGC
CCGGCCGGCGCCCGCCGGCGACCCCAATCAATCTTTTCAGGTTGACCTCGGATCAGGTAGGG
ATACCCGCTGAACTTAAGCATATCTAAACG
530bp

Morphological description:

Back: Dark green colonies (almost black), white borders

Front: Dark gray colonies, cottony, white borders

Media used: Malt

Source: Water

Accession	Description	Max score	Total score	Query coverage	E value	Max ident

OM372818.1	***Penicillium subrubescens*** strain PesuHN18Z01 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequencesequence.	963	963	98%	0.0	100%

Description:

Penicillium subrubescens is a species of filamentous fungus that belongs to the genus Penicillium (Visagie et al., 2014). This fungus has been widely studied because of its ability to produce multiple secondary metabolites and bioactive compounds such as antibiotics, enzymes, and pigments (Saleem et al., 2015). Also, it is used in beverage fermentation, is the source of bioactive compounds for pharmaceuticals and is used as a bioremediation tool against environmental pollutants (Visagie et al., 2014).

Previous isolation environments:

Even though *Penicillium* is generally found in terrestrial habitats, there have been reports of *Penicillium subrubescens* isolated from marine environments (Mansouri et al., 2013). In 2016 Li and collaborators isolated *P. subrubescens* from the southern and northern Yellow Sea and the Bohai Sea sediments. Also, in 2014 Soo Park and collaborators isolated *P. subrubescens* strains from seawater and sediments in Korea. These studies show its enzyme potential and antifungal and antibacterial activities (Soo Park et al., 2014; Li et al., 2016).

Human Pathogenicity:

Penicillium subrubescens is generally considered to be a saprophytic fungus. However, under certain circumstances, *P. subrubescens* may potentially cause health issues in humans, particularly in individuals with compromised immune systems. There have been rare reports of *P. subrubescens* associated with allergic reactions and respiratory infections such as asthma, allergic rhinitis, or hypersensitivity (Mansouri et al., 2013).

>M6, M9, M10, M13

ITS Sequence M6:

GTCCACCTCCCACCCGTGTTTACCGTACCCTGTTGCTTCGGCGCGCCCGCCCTCGCGGCCGCC
GGGGGGCCTCCGCCCCCGGGCCCGTGCCCGCCGAAGACACCCCTGAACGCTGTCTGAAGATT
GTCGTCTGAGCGAATAGCGAAAAATAAGTTAAAACTTTCAACAACGGATCTCTTGGTTCCGG
CATCGATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAATTCAGTGAATCAT
CGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCAT
TGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGCCGCCGTCCCCCCCCTTCCCGGGGGGGACGGGCC
CGAAAGGCAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTCTGTCACCCGCTCTG
CAGGCCCGGCCGGCGCCAGCCGACCCCCATCATCCTTTTTTTCAGGTTGACCTCGGATCAGGT
AGGGATACCCGCTGAACTTAAGCATATCTAAACCGGGGAA

540bp

ITS Sequence M9:

ACCTCCCACCCGTGTTTACCGTACCCTGTTGCTTCGGCGCGCCCGCCCTCGCGGCCGCCGGGG
GGCCTCCGCCCCCGGGCCCGTGCCCGCCGAAGACACCCCTGAACGCTGTCTGAAGATTGTCGT
CTGAGCGAATAGCGAAAAATAAGTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCG
ATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAATTCAGTGAATCATCGAGT
CTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTG
CCCTCAAGCCCGGCTTGTGTGTTGGGCCGCCGTCCCCCCCTTCCCGGGGGGGACGGGCCCGAAA
GGCAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTCTGTCACCCGCTCTGCAGGC
CCGGCCGGCGCCAGCCGACCCCCATCATCCTTTTTTTCAGGTTGACCTCGGATCAGGTAGGGA
TACCCGCTGAACTTAAGCATATCTAAACGGGGAAAAAAAAAATACTGAGTGAGGGCCCTCTGG
GTCCACCCCCCCGTGTT

580bp

ITS Sequence M10:

GTCCACCTCCCACCCGTGTTTTACCGTACCCTGTTGCTTCCGGCGCGCCCGCCCTCGCGGCCGC
CGGGGGGCCTCCGCCCCCGGGCCCGTGCCCGCCGAAGACACCCCTGAACGCTGTCTGAAGAT
TGTCGTCTGAGCGAATAGCGAAAAATAAGTTAAAACTTTCAACAACGGATCTCTTGGTTCCGG
CATCGATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAATTCAGTGAATCAT
CGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCAT
TGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGCCGCCGTCCCCCCCTTCCCGGGGGGGACGGGCC
CGAAAGGCAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTCTGTCACCCGCTCTG
CAGGCCCGGCCGGCGCCAGCCGACCCCCATCATCCTTTTTTTCAGGTTGACCTCGGATCAGGT
AGGGATACCCGCTGAACTTAAGCATTCTAAACCCGCGAAG

526bp

ITS Sequence M13:

TGGGTCCACCTCCCACCCGTGTTTACCGTACCCTGTTGCTTCGGCGCGCCCGCCCTCGCGGCC
GCCGGGGGGCCTCCGCCCCCGGGCCCGTGCCCGCCGAAGACACCCCTGAACGCTGTCTGAAG
ATTGTCGTCTGAGCGAATAGCGAAAAATAAGTTAAAACTTTCAACAACGGATCTCTTGGTTCC
GGCATCGATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAATTCAGTGAATC
ATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTC
ATTGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGCCGCCGTCCCCCCCTTCCCGGGGGGGACGGG
CCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTCTGTCACCCGCTC

TGCAGGCCCGGCCGGCGCCAGCCGACCCCCATCATCCTTTTTTTCAGGTTGACCTCGGATCAG
GTAGGGATACCCGCTGAACTTAAGCATATCTAGACGGGG
540bp

Morphological description:

Back: Large colony, white, clearer edges, darker center

Front: Large colony, cottony, military green center, edges blurred to white

Media used: PCA

Source: Fragment C of a *Desmophyllum pertusum's* colony.

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
KF513181.1	**M6: *Penicillium brocae*** isolate 2-16 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	972	972	100%	0.0	99.08%
KF513181.1	**M9: *Penicillium brocae*** strain PSKV02 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	974	974	93%	0.0	100%
MH475444.1	**M10: *Penicillium brocae*** strain MA192 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	955	955	93%	0.0	99.62%
MH475444.1	**M13: *Penicillium brocae*** strain MA192 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	968	968	97%	0.0	100%

Description:

Penicillium brocae is known for its ability to produce spores in the shape of a brush, forming characteristic brush-like structures known as conidiophores, which are important for the dispersal and reproduction of the fungus (Sainz et al., 2018). *Penicillium brocae* is known to be a moderate cellulose and hemicellulose degrader, contributing to its ecological role in the decomposition of plant materials in nature (Lopez et al., 2016).

Previous isolation environments:

As mentioned before, there are limited reports on Penicillium isolated from marine environments, however, a study conducted by Jones et al. (2015) reported the isolation of Penicillium species, including *Penicillium brocae*, from marine sediments collected from the North Atlantic Ocean. Another study by Dhanji et al. (2018) reported the isolation of Penicillium species, including *Penicillium brocae*, from marine-derived samples of marine sponges collected in the Pacific Ocean. There is limited information available on the isolation of *Penicillium brocae* in deep-sea environments, and to our knowledge, there has not been reported to be isolated from deep-sea environments.

Human Pathogenicity:

There is no evidence to suggest that *Penicillium brocae* is a human pathogen.

>M7

ITS Sequence:
ACCTCCCACCCGTGTTTATCGTACCTTGTTGCTTCGGCGGGCCCGCCGCAAGGCCCCAACCTC
CCACCCGTGTTTATTTTACCTTGTTGCTTCGGCGGGCCCGCCTTAACTGGCCGCCGGGGGGCT
CACGCCCCCGGGCCCGCGCCCGCCGAAGACACCCTCGAACTCTGTCTGAAGATTGAAGTCTG
AGTGAAAATATAAATTATTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAG
AACGCAGCGAAATGCGATACGTAATGTGAATTGCAAATTCAGTGAATCATCGAGTCTTTGAA
CGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAA
GCCCGGCTTGTGTGTTGGGCCCCGTCCTCCGATCTCCGGGGGACGGGCCCGAAAGGCAGCGG
CGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTTGTCACCCGCTCTGTAGGCCCGGCCGGC
GCTTGCCGATCAACCCAAATTTTTATCCAGGTTGACCTCGGATCATGACCTCGGATCAGGTAG
GGATACCCGCTGAACTTAAGCATATC
590bp

Morphological description:

Back: White colonies, dark Center, dark gray edges

Front: Filamentous, cottony colony, white with green colony, dark center

White halo

Media used: MALT

Source: Fragment A of a *Desmophyllum pertusum's* colony.

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
MT558930.1	***Penicillium commune*** isolate 2010F15 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	907	907	83%	0.0	100%

Description:

The colonies of *Penicillium commune* are typically fast-growing, fluffy, and powdery in texture (Sainz et al., 2018). It is known to be a saprophytic fungus that feeds on decaying organic matter (Sainz et al., 2018). It can produce various bioactive compounds, including

enzymes, secondary metabolites, and mycotoxins. *P. commune* have been used in processes such as food and beverage fermentation, the production of enzymes, and as primary sources for pharmaceutical and nutraceutical applications (Shankar et al., 2022).

Previous isolation environments:

Penicillium commune is primarily known as a terrestrial fungus. However, there have been reports of it being found in marine environments. One study by Tannous et al. (2014) reported the isolation of *P. commune* from marine sediments collected from the Red Sea in Saudi Arabia. Another study by Yin and collaborators in 2015 reported the isolation of *Penicillium commune* from marine sediment samples collected from the South China Sea. Additionally, in 2014 *P. commune* was isolated from a deep-sea sediment sample from the South China Sea, these fungi had antibacterial activity against Staphylococcus aureus and Escherichia coli, and significant cytotoxicity against MCF-7, HepG2, H460, Hela, Du145, and MDA-MB-231 cell lines (Shang et al., 2014).

Human Pathogenicity:

It is important to note that *Penicillium commune* is generally considered to have low pathogenicity towards humans and is not commonly associated with causing disease in healthy individuals (Shankar et al., 2022).

>M21, M23

ITS sequence M21:

CACCTCCCACCCGTGTTGCACGAACCTGTGTTGCTTCGGCGGGCCCGCCGCCAGGCCGCCGGG
GGGCATCCGCCCCCGGGCCCGCGCCCGCCGAAGCCCCCCTCTGAACGCTGTCTGAAGTTGCA
GTCTGAGACAACTAGCTAAATTAGTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCG
ATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAATTCAGTGAATCATCGAGT
CTTTGAACGCACATTGCGCCCTCTGGTATTCCGGAGGGCATGCCTGTCCGAGCGTCATTGCTG
CCCTCAAGCACGGCTTGTGTGTTGGGCCCCGTCCCCCCCGCTCCGGGGGGGGACGGGCCCGAA
AGGCAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTCGTCACCCGCTCTTGTAG
GCCCGGCCGGCGCCAGCCGACCCCCAACCTTTTATTTTTTCTCAGGTTGACCTCGGATCAGGT
AGGGATACCCGCTGAACTTAAGCATATCATA

531bp

ITS sequence M23:

TCCACCTCCCACCCGTGTTGCACGAACCTGTGTTGCTTCGGCGGGCCCGCCGCCAGGCCGCCG
GGGGGCATCCGCCCCCGGGCCCGCGCCCGCCGAAGCCCCCCTCTGAACGCTGTCTGAAGTTG
CAGTCTGAGACAACTAGCTAAATTAGTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCAT
CGATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAATTCAGTGAATCATCGA
GTCTTTGAACGCACATTGCGCCCTCTGGTATTCCGGAGGGCATGCCTGTCCGAGCGTCATTGC
TGCCCTCAAGCACGGCTTGTGTGTTGGGCCCCGTCCCCCCCCGCTCCGGGGGGGGACGGGCCCG
AAAGGCAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTCGTCACCCGCTCTTGT
AGGCCCGGCCGGCGCCAGCCGACCCCCAACCTTTTATTTTTTCTCAGGTTGACCTCGGATCAG
GTAGGGATACCCGCTGAACTTAAGCATATCATAAACGGGGAAGAAA

546bp

Morphological description:

Back: Greenish white colonies, white center, green stretch

Front: Dusty colony, gray, greenish colonies, dark gray center, white borders, white halo

Media used M21: PCA

Media used M23: Malt

Source M21 & M23: Fragment D of a *Desmophyllum pertusum's* colony.

Accession	Description	Max score	Total score	Query coverage	E value	Max ident

MN187973.1	**M21: *Penicillium steckii*** isolate KUASN12 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	976	976	99%	0.0	100%
KX302053.1	**M23: *Penicillium steckii*** isolate D22 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	981	981	97%	0.0	99.81%

Description:

Penicillium steckii is a filamentous fungus found in diverse environments like soil, decaying plant material, water-damaged buildings, lakes, and the ocean (Yao et al., 2021). Like other species of Penicillium, *P. steckii* produce important bioactive compounds for pharmaceutical and agricultural applications (Shankar et al., 2022). Recently, it has been proven that *P. steckii* can produce antibacterial compounds against human- and aquatic-pathogenic bacteria and plant-related pathogenic fungi (Hu et al., 2022).

Previous isolation environments:

In 2016 Shin and collaborators isolated the fungal strain 108YD142, later identified as *P. steckii,* from a sponge sample collected at Wangdolcho in the Republic of Korea's Eastern reef. This strain showed the anti-inflammatory activity of Tanzawaic Acid Derivatives (Shin et al. 2016). In 2022 Hu and collaborators reported the isolation of s *P. steckii* strain from the deep-sea coral *Acanthogorgiidae sp.* tissue collected from the Magellan Seamount at a depth of 1458 m. For the first time, they reported four unusual Tanzawaic acids E–H, possessing a rarely described acrylic acid unit at C-4 isolated from the strain. They tested the compounds' antimicrobial activities against human and aquatic pathogenic bacteria and plant-related pathogenic fungi (Hu et al., 2022).

Human Pathogenicity:

To our knowledge, *Penicillium steckii* has not been reported as a human pathogen.

>M27

ITS Sequence :

ACCTCCCACCCGTGTTGCCCGAACCTATGTTGCCTCGGCGGGCCCCGCGCCCGCCGACGGCCC
CCCTGAACGCTGTCTGAAGTTGCAGTCTGAGACCTATAACGAAATTAGTTAAAACTTTCAACA
ACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATT
GCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCTCTGGTATTCCGGAGGGC
ATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGCCCCGTCCCCCCC
GCCGGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGG
CTTCGTCACCCGCTCTAGTAGGCCCGGCCGGCGCCAGCCGACCCCCAACCTTTAATTATCTCA
GGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCATAACGCGGAGGGAA

500bp

Morphological description:

Back: Yellow colony with white edges

Front: Dark mint green cologne with white border

Media used: PDA

Source: Fragment D of a *Desmophyllum pertusum's* colony.

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
OP214767.1	***Penicillium griseofulvum*** isolate TJ-403 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence.	913	913	100%	0.0	99.60%

Description:

Penicillium griseofulvum was first isolated and described by scientists in the mid-20th century. *P. griseofulvum* has the ability to produce the bioactive compound griseofulvin, which is a well-known antifungal agent used in the treatment of fungal infections. Griseofulvin is a secondary metabolite (Hu et al., 2022). It has been widely studied for its bioactive properties, particularly its antifungal activity. It has been used in griseofulvin production for clinical use (Hu et al., 2022).

Previous isolation:

In 2020 Shu et al. described the anti-allergic properties of viridicatol from the deep-sea fungus *Penicillium griseofulvum.* The deep-sea viridicatol represents a novel therapeutic for allergic diseases and can also alleviate intestinal villi injury and inhibited the degranulation of intestinal MCs to promote intestinal barrier repair (Shu et al., 2020). A similar study was conducted in 2021 by Xing et al. using a *P. griseofulvum,* isolated from a sediment sample of the Indian Ocean at a depth of 1420 m. They described novel bioactive secondary metabolisms that act as anti-food allergic compounds, they obtained five carotenes, four naphthalenes, and three viridicatol derivates (Xing et al., 2021).

Human Pathogenicity:

There are no reports on *Penicillium griseofulvum* being a human pathogen.

>M1

ITS sequence:
CCGGGATGTTCACAACCCTTTGTTGTCCGACTCTGTTGCCTCCGGGGCGACCCTCCTCCGGGC
GGGGGCCCCGGGTGGACATTTCAAACTCTTGCGTAACTTTGCAGTCTGAGTAAATTTAATTAA
TGAATTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAAT
GCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCC
CCCTGGTATTCCGGGGGGCATGCCTGTTCGAGCGTCATTTCACCACTCAAGCCTCGCTTGGTA
TTGGGCGACGCGGTCCGCCGCGCGCCTCAAATCGACCGGCTGGGTCTTTCGTCCCCTCAGCGT
TGTGGAAACTATTCGCTAAAGGGTGCCGCGGGAGGCCACGCCGTAAAACAACCCCATTTCTA
AGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCTA
489bp

Morphological description:

Back: Large colony, black, white borders

Front: Filamentous colony, dark army green, cottony, white border

Media used: PDA

Source: Fragment A of a *Desmophyllum pertusum's* colony.

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
MT635287.1	***Cladosporium halotolerans*** small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence.	889	889	99%	0.0	99.59%

Description:

Cladosporium halotolerans is a fungal species belonging to the genus Cladosporium. It was first described in 2013 by Liang et al. in the journal "International Journal of Systematic and Evolutionary Microbiology." The authors described *C. halotolerans* as a novel species of the genus Cladosporium. *C. halotolerans* can grow in environments with high salt concentrations, such as saline soils, salt marshes, and coastal areas (Ameen et al., 2021). It also

tolerates other stressful conditions, such as high temperatures and low nutrient availability (Ameen et al., 2021). Because of its unique nature of thriving in "difficult" environments *C. halotolerans* has a vast potential for biotechnical applications such as the ability to degrade and detoxify pollutants in saline environments, the production of extracellular amylase, peptidase, and lipase enzymes, used in food processing (Segal et al., 2022), detergent and biofuel production, and the production of secondary metabolites with antimicrobial, antioxidant, and anticancer activities (Segal et al., 2022).

Previous isolation:

In 2021 a study conducted at the red sea examined the fungal communities in mangrove sediments with different salinity levels. They identified *C. halotolerans* as one of the dominant fungi found in the samples. They highlighted their adaptability of *C. halotolerans* to saline conditions in mangrove ecosystems (Zhang et al., 2021). In 2013 Evans and collaborators isolated and characterized Halotolerant fungi from the Great Salt Plains of Oklahoma. They identified *C. halotolerans* and highlighted their halotolerant nature and ability to thrive in saline environments (Evans et al., 2013). In 2023 Li et al. discovered two Cytotoxic pyronederivatives of the deep-sea fungi *Cladosporium halotolerans* FS702 isolated from sediment samples of the Guangdong Provincial in China.

Human Pathogenicity:

In a study done with clinical samples in the United States, they discovered that *Cladosporium halotolerans* is the most common species of Cladosporium from clinical origins. However, *C. halotolerans* has never been associated with human infections (Sandoval- Denis et al., 2015).

>M2

Morphological description:

Back: Small, pink colony (probably from the media coloring)

Front: Yeast, small colony, pink (probably from the coloring in the middle), creamy

Media used: Bengal Rose

Source: Fragment A of a *Desmophyllum pertusum's* colony.

ITS sequence:
GCGCGGCGAAAACCTTACACACAGTGTCTTTTTGATACAGAACTCTTGCTTTGGTTTGGCCTA
GAGATAGGTTGGGCCAGAGGTTTAACAAAACACAATTTAATTATTTTTACAGTTAGTCAAATT
TTGAATTAATCTTCAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAACGCA
GCGAAATGCGATAAGTAATATGAATTGCAGATTTTCGTGAATCATCGAATCTTTGAACGCACA
TTGCGCCCTCTGGTATTCCAGAGGGCATGCCTGTTTGAGCGTCATTTCTCTCTCAAACCCCCGG
GTTTGGTATTGAGTGATACTCTTAGTCGGACTAGGCGTTTGCTTGAAAAGTATTGGCATGGGT
AGTACTAGATAGTGCTGTCGACCTCTCAATGTATTAGGTTTATCCAACTCGTTGAATGGTGTG
GCGGGATATTTCTGGTATTGTTGGCCCGGCCTTACAACAACCAAACAAGTTTGACCTCAAATC
AGGTAGGAATACCCGCTGAACTTAAGCATAT
536bp

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
MT033088.1	***Meyerozyma guilliermondii*** isolate BoCF2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	990	990	100%	0.0	100.0%

Description:

Meyerozyma guilliermondii, is a yeast species that belongs to the phylum Ascomycota and the class Saccharomycetes. It can assimilate many carbon sources, including glucose, fructose, galactose, maltose, lactose, and sucrose (Yan et al., 2021). It can also grow at a wide range of temperatures, typically between 4-40°C, with an optimum temperature for growth around 30°C. It is used to produce enzymes, bioethanol, and biocontrol agents against plant

pathogens (Yan et al., 2021). *M. guilliermondii* is amenable to genetic manipulation, which makes it a useful model organism for studying various molecular and cellular processes (Yan et al., 2021).

Previous isolation:

Meyerozyma guilliermondii has been isolated in different marine environments, such as seawater, sea sediment, sea stars, sea snails, sea sponges, and mangroves. In 2020 Kaewkrajay et al. isolated *M. guilliermondii* from corals and zoanthids in the Gulf of Thailand.However, they did not describe its role in the coral holobiont. In 2021 Marchese and collaborators surveyed the fungal biodiversity in the deep-sea corals and sediments from the Irish Atlantic Ocean. They demonstrated that M. *guilliermondii* was detected with highfrequency and at different depths in the Atlantic, showing the remarkable preference to colonize this habitat in the marine environment. This yeast was mainly isolated from deep-seasediments.

Human Pathogenicity:

Meyerozyma guilliermondii can also cause opportunistic infections in humans, particularly in immunocompromised individuals, and has been associated with bloodstream infections, urinary tract infections, and other types of infections.

>M14

ITS sequence:
TGGGCCGACCTCCCAACCCTTTGTCTATTGAACCTTTTGTTGCTTCGGCGGGCCCGTTGTCTCT
CGCGAGACGGCCGCCGGAGGACCGTCGTTTTTGGCGTCCTCTGGCCCGCGCCCGCCGGTGGC
CCCAAAACTCAAAACTCTTGCATGAATCGTGTCTACATTCTAAGTCTTGATTAAATCAAAAAC
AAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATA
AGTAATGCGAATTGCAGAATTCCAGTGAGTCATCGAATCTTTGAACGCACATTGCGCCCTTTG
GTATTCCGAAGGGCATGCCTGTTCGAGCGTCATTATCACCCCTCGAGCCCCCGGGCTTGGTGT
TGGACGGCCTGGTCGCGCCCCGCGCGTGACCCCTCCTAAAGACAATGACGGCGGCCTCGTGG
AGCCCCCGGTACACTGAGCTTCTTAACTGAGCACGTATCGGATCTAGGGCGCCCGGGTCCCG
GTCCTCCTCCTCTCTCGTCCTCCCGGGCGGGAGACGAAACTTCTAAGGTTGACCTCGGATCAG
GTAGGGATACCCGCTGAACTTAAGCATATCTAAACG
600bp

Morphological description:

Back: Dark gray cologne, cottony/yeast

Front: Dark gray cologne, small cottony

Media used: PCA

Source: Fragment B of a *Desmophyllum pertusum's* colony.

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
KY315571.1	***Exophiala alcalophila*** isolate eab1 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	1080	1080	99%	0.0	99.66%

Description:

Exophiala alcalophila is a black yeast specie belonging to the phylum Ascomycota and the class Eurotiomycetes. It is a thermotolerant fungus commonly found in various environmental niches, such as soil, decaying plant material, and water sources (de Hong et al.,

2011). *Exophiala alcalophila* tolerates extreme environmental conditions, including high salinity, alkalinity, and temperature. It can also grow in environments with high concentrations of heavy metals, making it a highly adaptable fungus (Segal et al., 2022). Its genome contains several genes involved in stress response, metabolism, and other cellular functions (Segal et al., 2022).

Previous isolation:

Exophiala alcalophila is commonly known as a marine pathogen causing cutaneous infections in cold-blooded water animals (de Hong et al., 2011). In 1966 Carmichael introduced the genus Exophiala from cerebral lesions in fish with up to 40 % mortality in fishhatcheries in Calgary, Canada. This species has been isolated from fish, frogs, toads, turtles, and crabs (de Hong et al., 2011). Infections by black yeast-like fungi in cold-blooded animalsthus appear relatively frequent, at least in captive and farmed fish and amphibians (de Hong etal., 2011). To our knowledge, no specific reports of Exophiala alcalophila being isolated from deep-sea environments exist.

Human Pathogenicity:

Exophiala alcalophila has been associated with various human infections, particularly in immunocompromised individuals. It has been implicated in cases of cutaneous and subcutaneous infections and systemic infections like bloodstream infections and pneumonia. In 2011 de Hong et al. described how *Exophiala alcalophila* might be dispersed via municipal drinking water leading to visceral invasión of users in Scotland.

>M16

ITS Sequence:
CCAACCCATTTGTTTTATGATACCTAGTGTTGCTTCGGTAGGCCTGGTCTATCTGTTATAGACC
TGCCGGGGGGCCGTAAGACGCCCGCCGGAGAGTGCCTACCGACAGCCTCAACTCCAAAATTC
TTTAACCAAACGTGTCTTTGTCTGAGTAACGTCTTTTAAAATAAAGCAAAACTTTCAACAACG
GATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGCGAATTGCA
GAATTCTCGTGAGTCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCGAAGGGCAT
GCCTGTTCGAGCGTCATTTTCACCCCTCAAGCCCCCGGCTTGGTGTTGGACGGTCTGGTCCGG
GGACCTCAAACCCCCTGGACCCCTCCCAAAGACAATGACGGCGGGCTGTTGAACCCCCGGTA
CACTGAGCATCTTCACGGAGCACGTACCGGTCTCAAGGGTCGACGGCACCCGGTCTATACCT
ATATTTTTCAAGGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTT
550bp

Morphological description:

Back: Dark green almost black colony, thin white edges

Front: Cottony colonies, dark Army Green, white embossed center

Media used: Malt

Source: Fragment B of a *Desmophyllum pertusum's* colony.

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
KP017891.1	***Exophiala oligosperma*** strain IR-4Pa-3-1-2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	992	992	97%	0.0	100.0%

Description:

Exophiala oligosperma is a species of melanized fungus that belongs to the genus Exophiala. *E. oligosperma* is a slow-growing fungus that can grow at a wide range of temperatures, typically between 10-37°C. It can grow on various agar media, including Sabouraud agar, malt extract agar, and potato dextrose agar. Also, because it is a melanized fungus, it produces dark-pigmented cells called melanin, which may help protect it from environmental stresses, such as UV radiation and extreme temperatures (Hovhannisyan et al., 2018).

Previous isolation:

In 2022 Hong and collaborators described a new compound, exophilone, and nine known compounds isolated from the deep-sea-derived fungus, *Exophiala oligosperma*. This fungus was obtained from Marine Culture Collection of China. It was originally isolated from seawater collected at a depth of 3300 m in the northern basin of the South China Sea. This new exophilone was identified as a promising anti-pulmonary fibrosis agent (Hong et a., 2022).

Human Pathogenicity:

Exophiala oligosperma has been reported to cause opportunistic infections in humans such as soft-tissue infection, particularly in individuals with compromised immune systems. It can cause localized skin infections, respiratory tract infections, and systemic infections.

>M15

ITS sequence:
CCCTCCACCCCATGTCGAAACGACTCTGTTGCCTCGGGGGCGACCCGGCCTTCGGGCGTCGGG
GCCCCCGGCGGACACCTTCATAACTCTTGCATCTCTTGCGTCTGAGTGATACATATAATCAAT
CAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGAT
AAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCCTG
GCATTCCGGGGGGCATGCCTGTTCGAGCGTCATTACACCACTCAAGCCTGGCTTGGTATTGAG
CGCCGCGGCCTGCCCGCGCGCTCCAATGTCTCCGGCTGAGCCGTCCGTCTCTAAGCGTTGTGA
ATAGCGATCGCTTGCGAGGCCCGGGCGGTTCGACGCCGTTAAACCCCCCATTTTCTATGGTTG
ACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATATAAACCCGGGAAAGAAAA
500bp

Morphological description:

Back: Black colony, small colony

Front: Yeast, black colony, small colony, creamy

Media used: PCA

Source: Fragment B of a *Desmophyllum pertusum's* colony.

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
MH748181.1	***Hortaea werneckii*** isolate CA44 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	883	883	96%	0.0	99.79%

Description:

Hortaea werneckii is a black-pigmented yeast-like fungus that belongs to the family Dothioraceae. *H. werneckii* is found in various environments, such as soil, marine habitats, hypersaline environments (such as salt flats and salterns), and human skin (Zalar et al., 2019). One of the unique characteristics of *Hortaea werneckii* is its ability to tolerate high salt concentrations. It is considered a halophilic fungus, capable of growing in environments with salt concentrations ranging from 2-30% (w/v) (Plemenita et al., 2014). *H. werneckii* has also

been studied for its unique melanin production capabilities. The melanin extracted from this fungus has shown potential antioxidant, antitumor, and antiviral properties (Zalar et al., 2019).

Previous isolation:

Hortaea werneckii has been found in deep-sea environments, such as deep-sea hydrothermal ecosystems in the Pacific Ocean and sediments at 5000 m depths in the Central Indian Basin (Romeo et al., 2020). In 2020 Romeo et al., isolated *H. werneckii* from different stations and depths in the Mediterranean Sea, where it was shown to be the dominant fungal species. This study's findings support the hypothesis that many strains of *Hortaea werneckii* may have arisen through intraspecific hybridization. Moreover, the observed phylogenomic differences among strains from different sources suggest that marine strains may be undergoing evolutionary changes in their well-adapted marine environments (Romeo et al., 2020).

Human Pathogenicity:

Hortaea werneckii has been associated with various human skin conditions, particularly in tropical and subtropical regions, including tinea nigra and chromoblastomycosis. Tinea nigra is a superficial infection that affects the palms and soles, presenting as asymptomatic dark brown to black patches (Liao et al., 2022). Chromoblastomycosis, on the other hand, is a chronic and progressive skin and subcutaneous tissue infection caused by traumatic inoculation of the fungus, leading to nodules, ulcers, and abscesses ((Liao et al., 2022).

>M17

ITS sequence:
CACCCGTGAATACCTAACACTGTTGCTTCGGCGGGGAACCCCCTCGGGGGCGAGCCGCCGGG
GACTACTGAACTTCATGCCTGAGAGTGATGCAGTCTGAGTCTGAATATAAAATCAGTCAAAA
CTTTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAACTGCGATAAGTA
ATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGCATT
CCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCATCAAGCCCGGCTTGTGTGTTGGGTCG
TCGTCCCCCCCCGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGTGTCCGGTCCTCGAGCG
TATGGGGCTTTGTCACCCGCTCGACTAGGGCCGGCCGGGCGCCAGCCGACGTCTCCAACCATT
TTTCTTCAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATA
490bp

Morphological description:

Back: White colony with green streaks

Front: Filamentous colony, dusty, dark green, green stretch, white borders

Media used: Malt

Source: Fragment B of a *Desmophyllum pertusum's* colony.

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
MN547369.1	***Aspergillus versicolor*** isolate RS1-S1-06 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	905	905	100%	0.0	100.0%

Description:

Aspergillus versicolor is a filamentous fungus belonging to the genus Aspergillus. It is a ubiquitous fungus commonly found in diverse environments, including indoor and outdoor environments, soil, decaying plant material, and air (Piecková et al., 2017). *Aspergillus versicolor* has been studied for its ability to degrade cellulose and other plant materials, which makes it a potential candidate for bioremediation of cellulose-rich waste materials, such as agricultural residues and paper waste. It can also degrade certain toxic compounds, such as pentachlorophenol and other pollutants, which makes it a potential candidate for

bioremediation of contaminated soils and water (Mäkelä et al., 2020). It has also been evaluated as a potential biocontrol agent against plant pathogens. Some strains of *A. versicolor* produce secondary metabolites that have antimicrobial properties, which can inhibit the growth of other fungi or bacteria that cause plant diseases (Mäkelä et al., 2020).

Previous isolation:

In 2020 Yang et al. isolated *Aspergillus Versicolor* from a deep-sea sediment sample collected at a depth of 5455 m from the Marina Trench. They found fifteen polyketides, including four new compounds. These compounds were evaluated for their antimicrobial activities against four human pathogenic microbes and five fouling bacterial strains. Inaddition, the antioxidant assays of the isolated compounds revealed that aspermutarubrol/violaceol-I exhibited significant 1,1-diphenyl-2-picrylhydrazyl (DPPH)radical scavenging activity with the IC50 value of 34.1 μM. In addition, it displayed strong reduction of Fe^{3+} with the ferric reducing antioxidant power (FRAP) value of 9.0 mM under the concentration of 3.1 μg/mL, which were more potent than ascorbic acid (Yang et al., 2020).In 2021 Dong Li et al. isolated from deep-sea sediment a strain of *Aspergillus Versicolor*, thisstrain showed one new aromatic bisabolene-type sesquiterpenoid, along with four known analogues. All the compounds were evaluated against human and aquatic pathogenic bacteria for antimicrobial activities. Some compounds exhibited selective inhibitory activities against zoonotic pathogenic bacteria such as *Aeromonas hydrophilia*, *Escherichia coli*, *Edwardsiella tarda* and *Vibrio harveyi* (Dong Li et al., 2021).

Human Pathogenicity:

Aspergillus versicolor is not a common cause of human disease, however, inhalation of *Aspergillus versicolor* spores can trigger allergic reactions and respiratory symptoms in some

people, particularly those with pre-existing respiratory conditions or weakened immune systems.

>M18

ITS sequence:
AATTGGATCCCAATCGATCCAAATCCTCATATATCCATCCTCACTCCACACTGTGAAATACAA
ACTATTCGGGCGGCCTTACAGGGGCCGGTCGAGGTCTAGAGATGGGACTGAGTAAAAAAAAA
AAAATTGTTGGGGAGGGCCTCCCCTTTTCAAGGGGAGGGGACGATCTACAGTTTTACTTCTGT
TCTCTGATCTAGCCAAATTACCCAATTTTTAGAGACAATGTTAAATTTGAATGTGTTTTTTTTT
TATTAAACAAATTAAAACTTTCAGTGACGGATCTTTTGGCTCTCGCATCGATAAAAAACGCAT
TAAATCGCGATACGTAATGTGAATTGCAAAAATATGTGAATCATCGAATCTTTGAACGCATCT
GGCGCTCTGGGGTACTCCTCAAAGCAGGCCTGTTTGAGTGTCTTTTAATTCTCATCTCAAAATT
TTTATTAATTTAAAATAATTATAGGTGGATCGTGGCTGTTTTGACAACTAACCTCGTCTCACCT
GAAATATAGAAAGCGACGTCTAAAATTCAGAGTAATAAGATGTTAACGTCGGCGTGATAAGT
AAATTACGCAGTCAGTTTTCTGTCTATCTCTCTGGTGTTCGCTACGAATTAC
620bp

Morphological description:

Back: Gray-brown cologne, thin white border

Front: Filamentous colony, sporulate, light pink color, thin white border

Media used: PDA

Source: Fragment D of a *Desmophyllum pertusum's* colony.

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
NR_155894.1	***Tritirachium roseum*** CBS 183.42 ITS region; from TYPE material.	390	390	61%	2e-103	85.23%

* The strain M18 had the greatest percentage of identity with *Tritirachium oryzae* (94.21%), however, the ITS region was unavailable from a TYPE strain.

Description:

Tritirachium roseum is a species of filamentous fungus belonging to the family Monascaceae. The fungus produces a pinkish-red to orange-red colored mycelium that is thin

and filamentous. *T. roseum* has several potential applications in biotechnology and the production of enzymes. It produces a variety of enzymes, including cellulases, xylanases, and peptidases, which are used in various industrial processes such as biofuel production and waste management (Lübeck et al., 2022).

Previous isolation:

Wang et al. 2015 evaluated the antimicrobial activities of the secondary metabolites produced by deep-sea *Tritirachium roseum* isolated from sediments of the South China Sea. The ethyl acetate extract of the fungi specie shows strong antibacterial activity against two larval-settlement-inducing bacteria *Loktanella hongkongensis* and *Micrococcus luteus,* and one marine pathogenic bacterium (Wang et al., 2015).

Human Pathogenicity:

Tritirachium roseum is not typically considered a primary human pathogen. While individuals with weakened immune systems or respiratory conditions can experience respiratory symptoms upon exposure to the fungus, such cases are rare.

>M20

ITS sequence:
GACCTCCCAACCCTTTGTTTACTGAACCTTTGTTGCTTCGGCGGGCCCGTCTTCTCGCGAGGAC
CGCCGGAGGACCGCCCTCACGGGGGTCCTCTGGCCCGCGCCCGCCGGTGGCCCCCCTCCAAA
AACTCTTGTCTCAACCGTGTCGTCTGTCTGAATGTCATAAAAAGCAAATAAATGCAAAACTTT
CAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGC
GAATTGCAGAATTCCAGTGAGTCATCGAATCTTTGAACGCACATTGCGCCCTTTGGCATTCCG
AAGGGCATGCCTGTTCGAGCGTCATTATCACCCCTCAAGCCCTGCGCTTGGTGTTGGACGGTT
TGGTCGATGACACCCGACCCCTCCCAAAGACAATGACGGCGGCCTGTGGCTCCCCCGGCACA
CTGAGCTTTTCACCGAGCACGTGTCGGATTGCGGGCGTCCAGGTCCCGGTCGGCCTCCCTGTT
GCGACAGGGAACGTTTTTTTCAAGGTTGACCTCGGATCAGGTAGGAATACGCGCTGAACTTA
AGCATA
570bp

Morphological description:

Back: Dark gray cologne, cottony

Front: Cottony colonie, dark Army Green

Media used: PDA

Source: Fragment D of a *Desmophyllum pertusum's* colony.

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
LC192112.1	***Cladophialophora mycetomatis*** genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2 and 28S rRNA, partial and complete sequence, strain: CBS 454.82	990	990	99%	0.0	98.07%

Description:

Cladophialophora mycetomatis is a species of black fungus (dematiaceous fungus) that can cause mycetoma. This chronic and debilitating disease primarily affects the skin and subcutaneous tissues. The fungus is found in soil and decaying plant material in tropical and subtropical regions worldwide, particularly in arid and semi-arid areas (Reis et al., 2018). Cladophialophora mycetomatis is not typically considered a species with significant

biotechnological applications. This is because the fungus is primarily known for causing mycetoma (Reis et al., 2018). However, some research has been conducted on the use of *Cladophialophora mycetomatis* and related species in the production of melanin. This pigment has a range of potential applications in biotechnology, including as a natural dye, a sunscreen ingredient, and in the development of bioelectronics and biocompatible materials (Reis et al., 2018).

Previous isolation:

Cladophialophora mycetomatis is part of a collection of 163 strains of black yeast-like fungi from the CBS Fungal Biodiversity Center (Utrecht, The Netherlands). The strain was isolated from a polluted soil sample. In 2016 Blasi and collaborators described the ability of the strain to grow on toluene as the sole carbon and energy source. *C. mycetomatis* have a high potential to grow in polluted environments and metabolize hydrocarbons as the sole carbon and energy source (Blasi et al., 2016). *Cladophialophora mycetomatis* has been mainly isolated from human adults between 16 – 50 years old. This fungus produces mycetoma a frequent chronic subcutaneous infection in tropical and subtropical regions. *C. mycetomatis* may enter the body through a break in the skin, often on a person's foot. The resulting infection causes firm, usually painless but debilitating masses under the skin that can eventually affect the underlying bone (López et al., 2013).

Human Pathogenicity:

Cladophialophora mycetomatis is a human pathogen that can cause mycetoma, a chronic and debilitating disease primarily affecting the skin and subcutaneous tissues. The fungus can also cause infections in other body parts, such as the lungs. It can be particularly problematic in individuals with weakened immune systems.

Culture dependent analysis

The fungal strains obtained from the coral *D. pertusum* were isolated using PDA, rose Bengal agar (RB), M alt Extract A gar (MEA), P otato C arrot A gar (PCA) and S abouraud A gar (SB) all enriched with chloramphenicol to avoid bacterial growth. Most of the isolates were obtained using M alt A xtract A gar (32%) and PDA (26%) media,respectively (Figure 5). MEA is optimal for fungal growth; it uses Dextrin and Glycerin ascarbon sources and Peptone as a nitrogen source, also, its acidic pH allows the growth of yeastand filamentous fungi while restricting bacterial growth (Tudzynski et al., 2014). Additionally,, PDA is also an excellent alternative for fungal isolation. It is composed of potato infusion anddextrose that encourages fungal growth; also, its low pH inhibits bacterial growth (Aryal et al.,2022). Rose Bengal was the least effective media for isolating fungI (5%). This media was expected as it is mainly used to isolate yeast because the medium contains Rose Bengal, whichinhibits the growth of filamentous fungi while allowing the growth of yeasts.

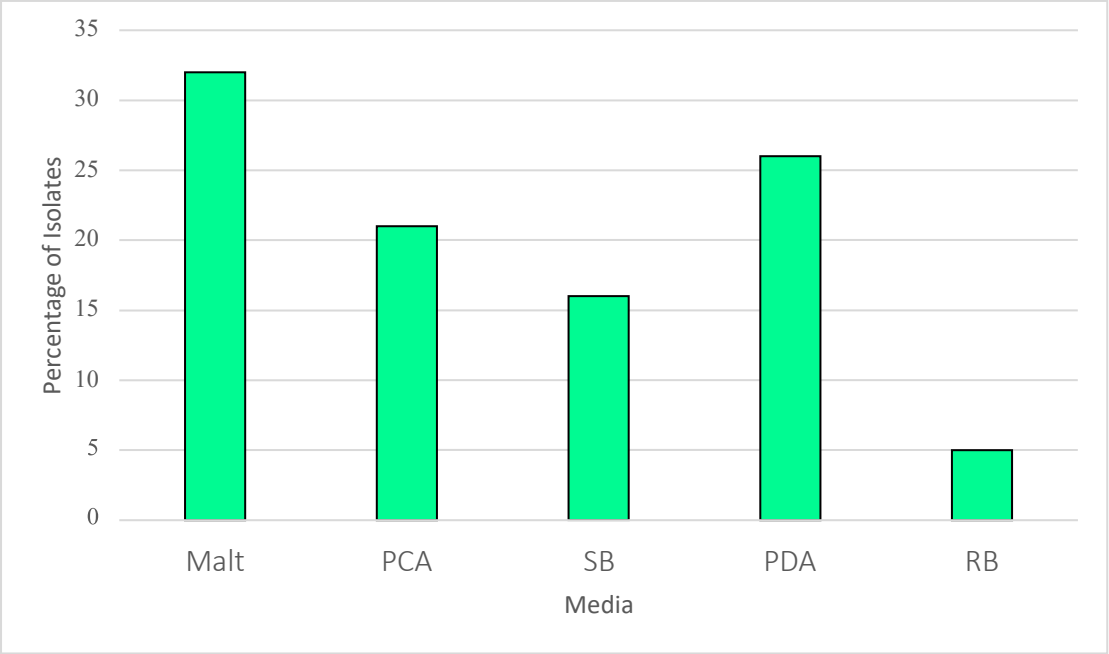

Figure 5. Percentage of fungal strains isolated in 5 different types of media (MALT, PCA SB, PDA, and RB).

Using traditional media, we isolated 8 genus of fungi, including *Penicillium, Cladosporium, Meyerozyma, Exophiala, Hortaea, Aspergillus, Tritirachium,* and *Cladophialophora.* Figure 6 demonstrates that the genus *Penicillium* was able to grow in most culture media used, whether they are generalist or selective for specific microbial groups.Still, the genera *Exophiala* was able to grow in various culture media (Malt, PCA), indicating genotypic plasticity. This plasticity indicates how the fungi can modify their growth in response to nutrient availability, considering that the other parameters like light and temperature were the same for all the strains. Nevertheless, this does not mean that other fungal strains that did not grow in several media lack plasticity.

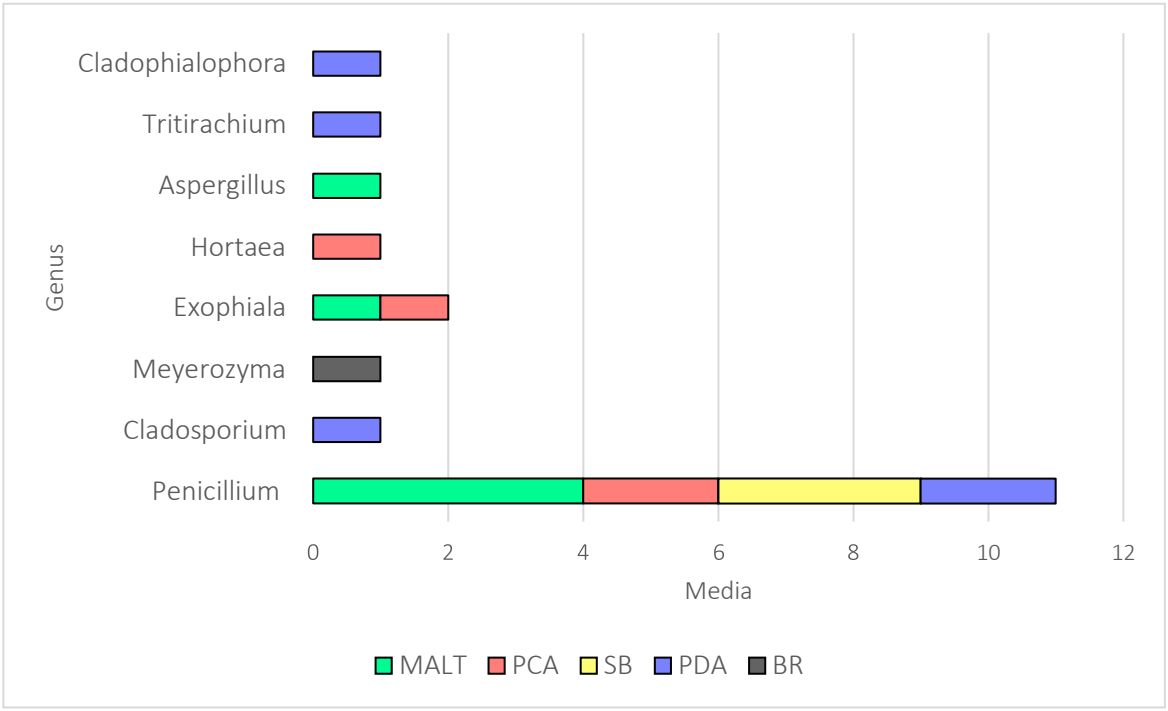

Figure 6. Ratio of fungal diversity obtained in all culture media used for the strain's isolation.

Phylogenetic analysis

A multilocus sequence analysis was performed where partial 18S rRNA gene and ITS-1 region sequences were analyzed. Phylogenetic trees based on the 18S rDNA phylogenies were used to determine more distant phylogenetic relationships between isolates (Galkiewicz et al., 2012), while the ITS-1 region of fungal rDNA was used to determine close relationships between strains (Galkiewicz et al., 2012),. However, even though both markers have advantages and limitations,

the ITS-1 region is generally considered more accurate than 18S rDNA for the identification of fungi. This is because the ITS-1 region is more variable than the 18S rRNA gene, meaning that there are more differences in this region between different species of fungi. This makes ITS-1 a more reliable marker for distinguishing between closely related species. Also, The ITS-1 region has been widely used for fungal identification. As a result, many reference databases are available to compare ITS-1 sequences.

A total of 94.47% of the strains isolated from the deep-sea coral D. pertusum were classified as Ascomycota and 5.26% as Basidiomycota. A total 80% of the five fragments of *D. Pertusum* samples yielded culturable fungi (we could not isolate fungi from fragment E), giving 8 isolated fungal taxa (19 isolates). This low frequency of isolation in corals was previously described by Marchese and collaborators in 2021. They noticed that the isolation range of corals is between 0.9 isolates per sample, while in sponges was 1.5 isolates per sample. Also, they concluded that in comparison with sediment samples, animals yielded a significantly lower number of fungal communities.

D. pertusum-associated fungal isolates belong mainly to the Ascomycota phylum , inwhich Eurotiomycetes were the most abundant class (63% - 12 strains), followed by Dothideomycetes (11% - 2 strains), Hemiascomycetes (5% - 1 strain), and Chaetothyriomycetes (16% - 3 strains). A strain classified as belonging to Basidiomycetes phylum were also isolated, in which was assigned to Tritirachiomycetes class (5% - 1 strain) (Figure 7).

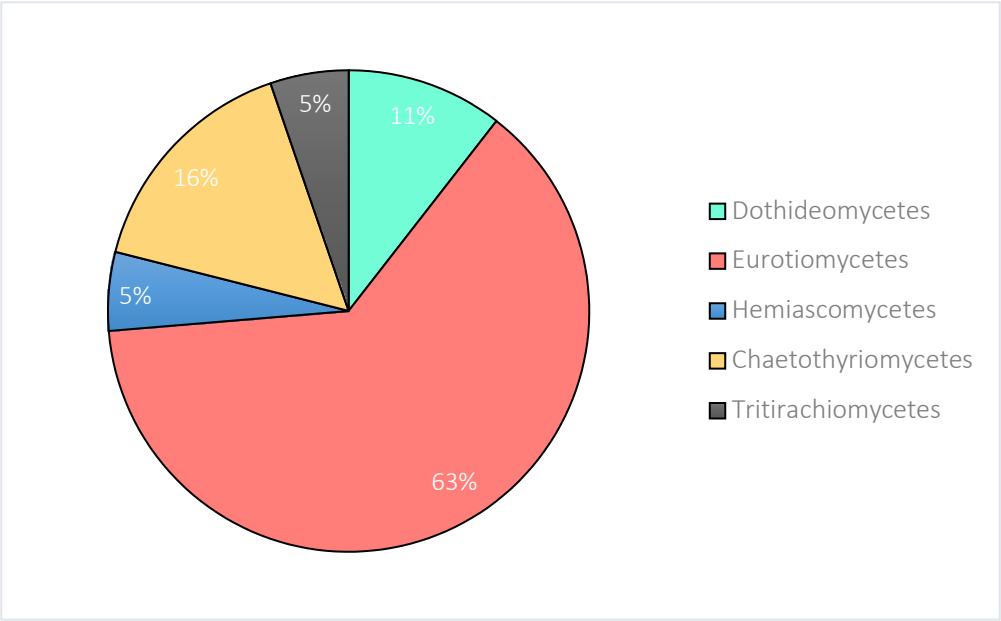

Figure 7. Fungal class biodiversity detected in/on *Desmophyllum pertusum.*

The top GenBank matches for the ITS-1 and 18S rRNA gene sequences from each isolate were compared to determine phylogenetic assignments (Table 4). Most isolates had top GenBank matches of the same genus for 18S rDNA and ITS-1 sequences except for sample M17. The strain M17 showed identification of 98.61% with *Thermoascus crustaceus* in the 18S rD NA region and 100% with *Aspergillus versicolor* in the ITS-1 region—both *Thermoascus* sp. and *Aspergillus*sp. belong to the Eurotiomycetes class and the Trichocomaceae family, demonstrating they share a common ancestor. Also, in 2022 Wenjing and collaborators used the term "highly connected genera" to describe *Aspergillus* and *Thermoascus*. In this study, both genera have significant biotechnological potential in the fermentation of Chinese liquor because of the secretion of various enzymes, such as those related to saccharification, liquefaction enzymes, and peptidases (Wenjing et al., 2022).

Most of the isolates (17 out of 19) showed sequence similarity higher than 98% with previous records of the GenBank, with the exceptions of M4: 18S rDNA region - *Penicillium limosum* (94.75%) and M18: 18S rDNA region - *Tritirachium egenum* (94.11%) and the ITS region - *Tritirachium roseum* (85.23%). However, in the case on strain M18 at the 18S rDNA

region it showed a larger similarity with *Tritirachium oryzae* (99.16%), the 18S rDNA sequencewas not available from a TYPE strain at the NCBI data base. The low levels of similarities between our strains and the top GenBank matches, could indicate that the sequence could have suffered from genetic variations or mutations that have occurred naturally within the strain's genome over time, the strains have been poorly studied, or it could be a newly discovered organism(Van Rossum et al., 2020).

Table 4. Comparison of the top GenBank matches for each isolate, based on BLAST searches of partial 18S rRNA genes and ITS-1 sequences.

Isolate	***GenBank matches***	
	18S rDNA	**ITS-1 region**
M1	*Cladosporium halotolerans* (98.88%)	*Cladosporium halotolerans* (99.59%)
M2	*Meyerozyma guilliermondii* (100%)	*Meyerozyma guilliermondii* (100%)
M3	*Penicillium limosum* (98.54%)	*Penicillium subrubescens* (99.63%)
M4	*Penicillium limosum* (94.75%)	*Penicillium subrubescens* (100%)
M6	*Penicillium limosum* (99.70%)	*Penicillium brocae* (99.08%)
M7	*Penicillium macrosclerotiorum* (99.48%)	*Penicillium commune* (100%)
M9	*Penicillium limosum* (98.66%)	*Penicillium brocae* (100%)

M10	*Penicillium limosum* (97.99%)	*Penicillium brocae* (99.62%)
M11	*Penicillium limosum* (99.80%)	*Penicillium senticosum* (99.08%)
M13	*Penicillium limosum* (98.81%)	*Penicillium brocae* (100%)
M14	*Exophiala halophila* (98.84%)	*Exophiala alcalophila* (99.66%)
M15	*Hortaea werneckii* (99.50%)	*Hortaea werneckii* (99.79%)
M16	*Exophiala exophialae* (98.99%)	*Exophiala oligosperma* (100%)
M17	*Thermoascus crustaceus* (98.61%)	*Aspergillus versicolor* (100%)
M18	*Tritirachium egenum* (94.11%)	*Tritirachium roseum* (85.23%)
M20	*Cladophialophora mycetomatis* (98.89%)	*Cladophialophora mycetomatis* (98.07%)
M21	*Penicillium limosum* (99.27%)	*Penicillium steckii* (100%)
M23	*Penicillium limosum* (98.98%)	*Penicillium steckii* (99.81%)
M27	*Penicillium chrysogenum* (98.46%)	*Penicillium griseofulvum* (100%)

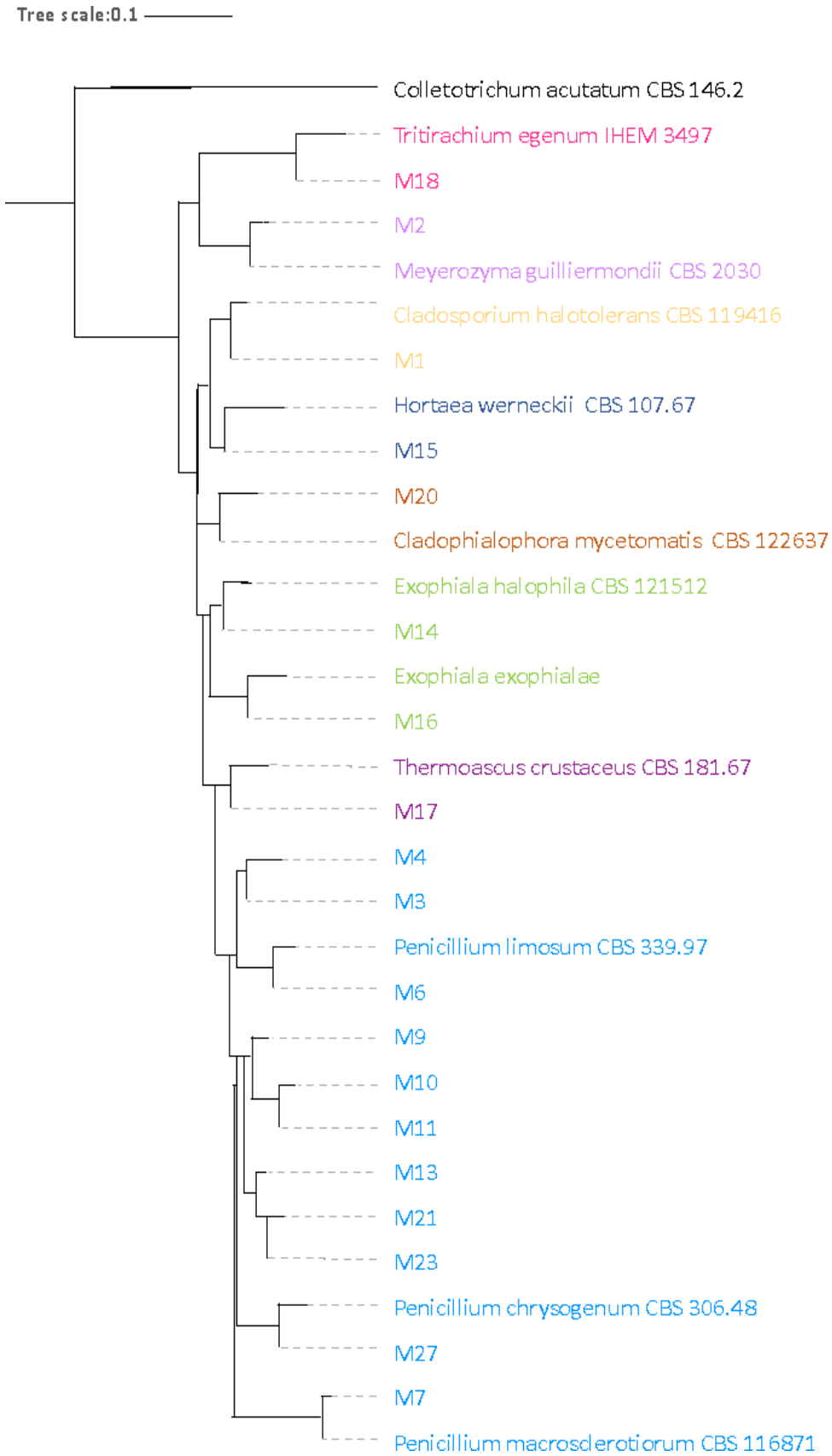
Tree scale:0.1
Colletotrichum acutatum CBS 146.2
Tritirachium egenum IHEM 3497
M18
M2
Meyerozyma guilliermondii CBS 2030
Cladosporium halotolerans CBS 119416
M1
Hortaea werneckii CBS 107.67
M15
M20
Cladophialophora mycetomatis CBS 122637
Exophiala halophila CBS 121512
M14
Exophiala exophialae
M16
Thermoascus crustaceus CBS 181.67
M17
M4
M3
Penicillium limosum CBS 339.97
M6
M9
M10
M11
M13
M21
M23
Penicillium chrysogenum CBS 306.48
M27
M7
Penicillium macrosclerotiorum CBS 116871

Figure 8. Phylogenetic tree of 18S rDNA from fungal isolates constructed using MEGA X and the neighbor-joining method, with bootstrap values less than 50% not shown, using 1,000 bootstrap iterations, based on alignments with Clustal W using sequences 4500 bp.

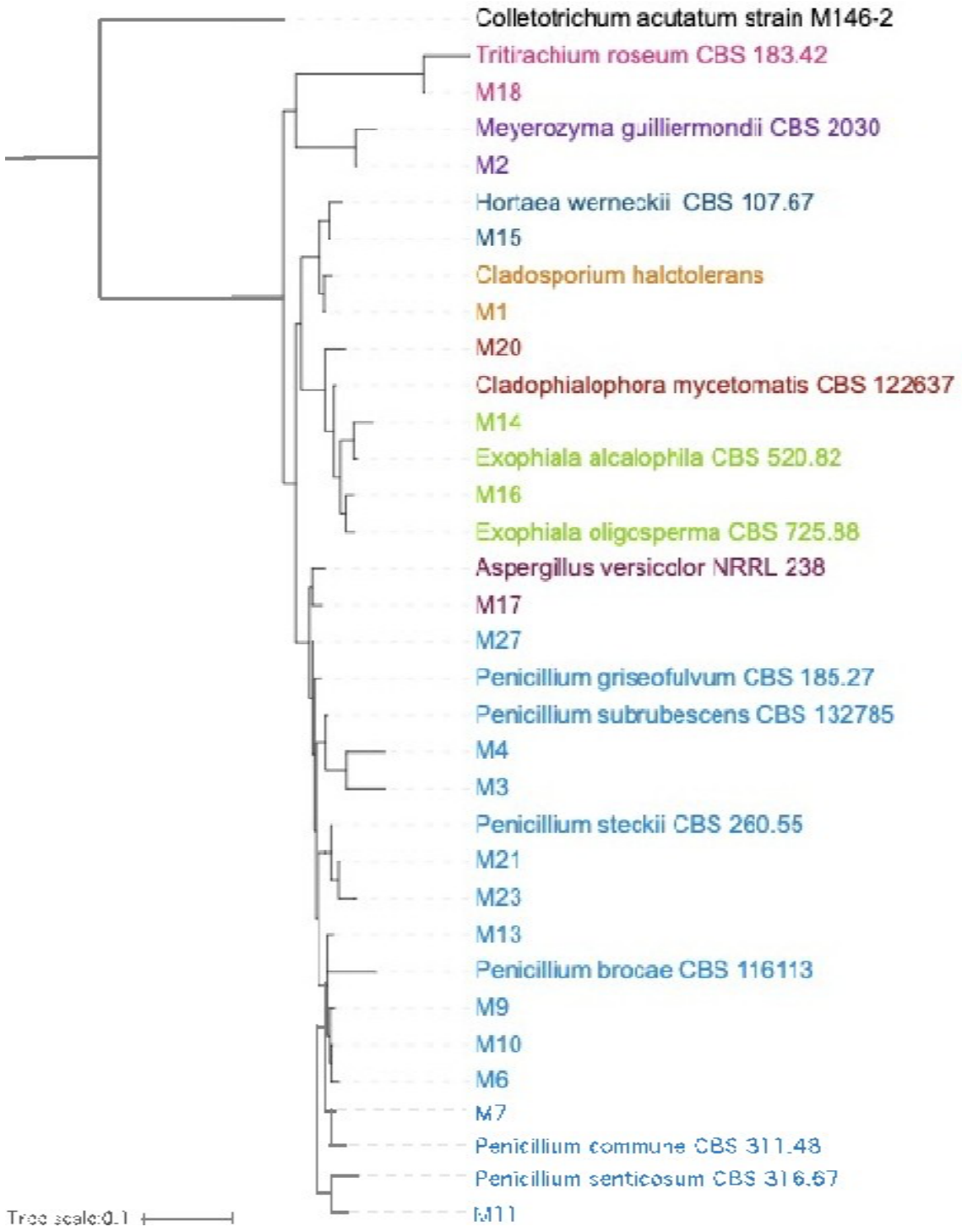

Figure 9. Phylogenetic tree of the ITS-1 region sequences from fungal isolates constructed using MEGA X and the neighbor-joining method, with bootstrap values less than 50% not shown, using 1,000 bootstrap iterations, based on alignments with Clustal W using sequences 4500 bp.

Penicillium was the most frequent fungal genus observed in both 18S rD NA and ITSregions (figure 8, 9). The 18S rRNA region showed three species isolated of *Penicillium*: *Penicillium limosum, Penicillium macrosclerotiorum,* and *Penicillium chrysogenum* (table 4). ThePenicillium species most frequently isolated was *Penicillium limosum* (M3, M4, M6, M9, M10, M11, M13, M21, M23). *P. limosum*, like other species in the genus Penicillium, produces a varietyof secondary metabolisms, some of which have shown antibiotic, antifungal, antioxidant, and anticancer properties (Grijseels et al., 2017). However, it is most known for producing penicillin, widely used as an antibiotic. *P. limosum* has been isolated from damp soil and fresh water in Korea(Pangging et al., 2021), the fynbos biome in South Africa (Visagie et al., 2015), and the Lake Magadi in Kenya (Orwa et al., 2020) among many others. Still, to our knowledge, it is the first timeisolated from a marine environment and a benthic animal. In 2019 Niu and collaborators isolated *Penicillium chrysogenum* (M27) from Deep-sea sediment of the South Atlantic Ocean at a depth of2076 meters; they were able to isolate three new version-type analogs, peniciversiols A–C, and twonovel lactone derivatives, penicilactones A and B (Niu et al., 2019). On the other hand, the third specie of *Penicillium*, *Penicillium macrosclerotiorum* (M7), was first isolated from a soil sample insouth China in 2007 and has not been isolated from marine environments or benthic animals (Wanget al., 2007).

The second most frequent genus observed in the 18S rD NA and ITS regions was Exophiala with two isolated species. In the 18s rRNA region, we isolated *Exophiala halophila* (M14) and *Exophiala exophialae* (M16). *Exophiala halophila* has been isolated as a pathogen from cold-blooded animals (frogs, fish, toads, turtles, and crabs) (Hoog et al., 2011).*Exophiala exophialae* has been isolated from human skin samples. It is a human pathogen causing infections

(Hoog et al., 2011). Other genera observed in the 18s rRNA region results were *Cladosporium* sp*, Meyerozyma* sp*., Hortaea* sp*., Thermoascus* sp*., Tritirachium* sp., and *Cladophialophora* sp*.*

The ITS region showed seven species isolated of *Penicillium*: *Penicillium simplicissimum, Penicillium subrubescens, Penicillium brocae, Penicillium commune, Penicillium senticosum, Penicillium steckii,* and *Penicillium griseofulvum.* The *Penicillium* species most frequently isolated was *Penicillium brocae* (M6, M9, M10, M13). Even though there are previous reports on *Penicillium brocae* isolated from marine animals and sediment to our knowledge is the first time it has been isolated from the deep-sea coral *D. Pertusum.* The second most common *Penicillium* species was *Penicillium steckii* (M21, M23). It has been previously isolated from a sponge sample and the deep-sea coral *Acanthogorgiidae sp.* tissue. These strains have shown anti-inflammatory and antimicrobial activity against plant and human pathogens (Shin et al., 2016; Hu et al., 2022). Other genera observed in the ITS region results were *Cladosporium sp., Meyerozyma sp., Exophiala* sp*., Hortaea* s*p., Aspergillus* sp.*, Tritirachium* sp.*,* and *Cladophialophora* sp.

Fungal Genome Annotation

The fungal genome annotation was performed with the reference sequences from the NCBI database that had the highest percentage of completeness and showed high phylogenetic similarities with our selected strains: *Aspergillus versicolor* (M17), *Penicillium griseofulvum* (M27), and *Meyerozyma guilliermondii* (M2). The three strains selected in this study have been recognized for providing beneficial functions to the host. These marine fungi can produce extracellular polysaccharides with novel structures and diverse activities due to their marine environment. For this study, we looked deeper into the genomes of *Penicillium griseofulvum, Aspergillus versicolor,* and *Meyerozyma guilliermondii*.

Microorganisms present in the coral holobiont have sources of bioactive secondary metabolites that are indispensable in pharmaceuticals and biotechnological industries and can

protect the coral host. *Aspergillus v ersicolor* has shown antioxidant activity from an extracellular polysaccharide (Chen et al., 2012), antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter aerogenes*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Candida albicans,* and cytotoxicity against P388 and Hela cell lines (Zhuang et al., 2011). Another interesting strain is *Penicillium griseofulvum.* It has been described as a promising source of new secondary metabolisms. In 2019 Heydari et al. described that the fungal extract of *P. griseofulvum* has high antioxidant, antimicrobial activities, and significant cytotoxic activity against HCT-116 cells.

Another fungus associated with corals is yeast; they can play a crucial role in the coral's health by contributing to its nutrition, defense, immunity, and development (Yang et al., 2020). *Meyerozyma guilliermondii* is a yeast found in corals that expresses heat shock protein 70 (HSP70). It is a main part of the cell's machinery for protein folding and contributes to protecting cells against stress. It also inhibits apoptosis (Yang et al., 2020). Zhang and collaborators in 2018 studied the mechanisms of HSP70 in *Pocillopora damicornis* through stress. They concluded that HSP70 was essentially a stress regulatory protein; therefore, different environmental stresses could induce HSP70 mRNA expression and maintain stability during the anthropogenic stresses (Zhang et al., 2018). The downregulation of HSP70 can weaken the adaptability ability and immune response to the external environment (Zhang et al., 2018; Yang et al., 2020). *Meyerozyma guilliermondii* has also exhibited antagonistic activity against gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacteria as well as a fungus *(Penicillium* sp.) (Sadeghiet al., 2019).

These three strains have various genes related to the assimilation of chemical elements such as carbon fixation and nitrogen metabolisms, secondary metabolites, DNA repair mechanisms, and increasing resistance to toxic metals. However, although these three strains (M2, M17, M27) present remarkable abilities for the coral hots, our study has other valuable species. Some strains, such as *Cladosporium halotolerans*, have significant cytotoxicity activity against two prostatic cancer cell

lines (C4-2B and 22RV1) (Chao-Nan et al., 2021), antifouling activity, high resistance to copper (Dobretsov et al., 2021), and antimicrobial activity against five marine pathogenic bacteria: *Staphylococcus aureus, Photobacterium damselae, Vibrio parahaemolyticus, Vibrio harveyi,* and *Vibrio alginolyticus* (Xu et al., 2018), could be a potential target for this study. However, because its complete genome is unavailable in the NCBI databases, we couldn't study it. Another good example is *Penicillium commune* which has antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. However, because of the lack of data on its genome in NCBI we couldn't analyze it.

The whole genomes of *Aspergillus v ersicolor* (M17), *Penicillium griseofulvum* (M27), and *Meyerozyma guilliermondii* (M2) were taken from the NCBI database. We used the KEGG (Kyoto Encyclopedia of Genes and Genomes) and PFAM (Protein Family Database) databases. These are two widely used resources for genome annotation and functional analysis. While KEGGprovides a comprehensive collection of databases and tools for analyzing the functional properties and biological pathways of genes, PFAM database provides protein families and domains.

***Aspergillus v ersicolor* (M17)**

The genome from *Aspergillus v ersicolor* was sequenced by Vries et al., in 2017 in the publication "**Comparative genomics reveals high biological diversity and specific adaptations in the industrially and medically important fungal genus *Aspergillus*".** This genome is available in the NCBI database under the ID: GCA_020284045.1. This genome has a size of 22.13 Mbp.

***Penicillium griseofulvum* (M27)**

The genome from *Penicillium griseofulvum* was sequenced by Banani et al., in 2016 in the publication "**Genome sequencing and secondary metabolism of the postharvest pathogen *Penicillium griseofulvum*".** This genome is available in the NCBI database under the ID: GCA_020284045.1. This genome has a size of 29.14 Mbp. We analyzed: Energy Metabolism, Metabolism of Other Amino Acids, and Biosynthesis of Secondary Metabolites (figure 11).

***Meyerozyma guilliermondii* (M2)**

The genome from *Meyerozyma guilliermondii* was sequenced by Butler et al., 2009 in the publication "**Evolution of pathogenicity and sexual reproduction in eight *Candida* genomes".** This genome is available in the NCBI database under the taxonomy ID: 4929 This genome has a size of 10.61 Mbp.

PFAM results

We conducted a protein family analysis using the PFAM database to gain insights into the functional properties of the three genomes chosen (*Aspergillus v ersicolor* (M17), *Penicillium griseofulvum* (M27), and *Meyerozyma guilliermondii* (M2)). PFAM is a widely used database that provides information about protein families, domains, and functional motifs. By comparing our genome sequences against the PFAM database, we identified significant matches to specific protein domains. This analysis allows us to infer our fungal strains' potential functions and characteristics within the coral host. We mainly focus on 1) Sulfate assimilation, 2) Nitrate assimilation, 3) Carbohydrate metabolic process, 4) Phosphorylation, 5) Denitrification, and 6) Secondary metabolisms (figure 10).

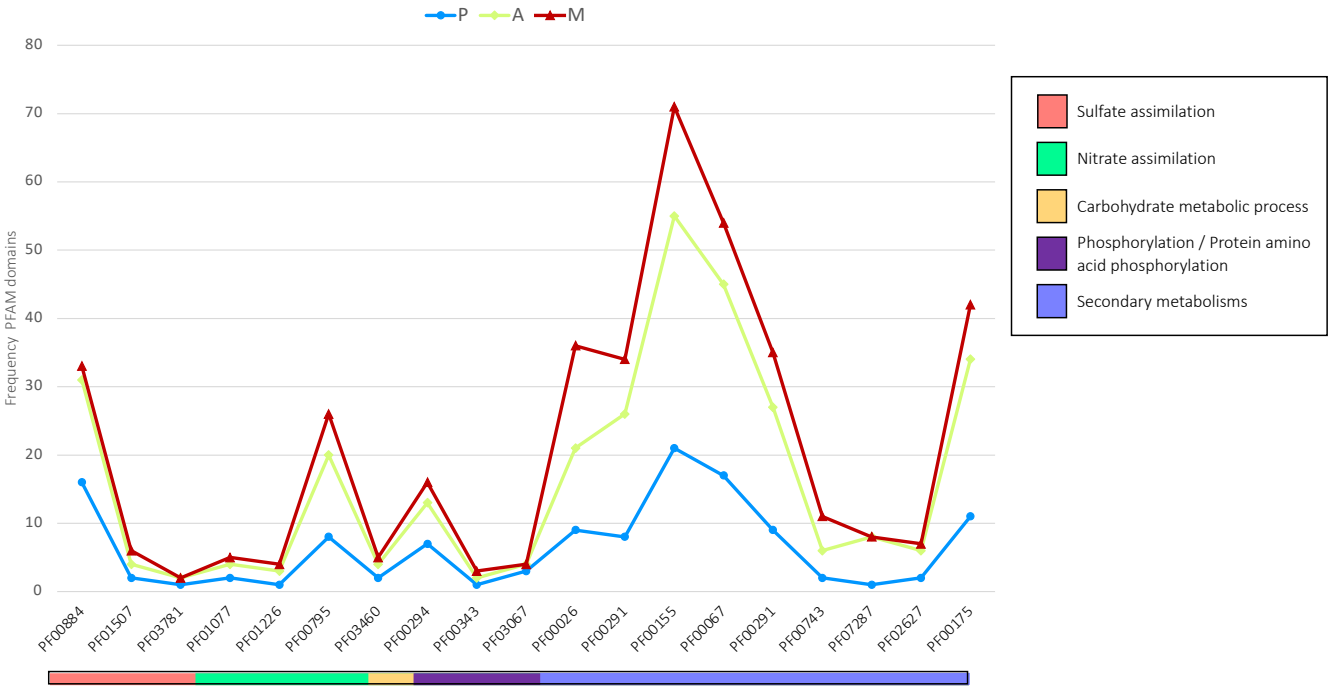

Figure 10. PFAM Analysis of Selected Fungal Strains: *Aspergillus versicolor* (A), *Penicillium griseofulvum* (P), and *Meyerozyma guilliermondii* (M).

Figure 10 displays a line plot with markers depicting the PFAM analysis results of three selected fungal strains. The Y-axis represents the abundance of specific domains in the genome of each strain. The plot provides a comparative view of the domain distribution among the selected strains. Each data point represents the abundance of a particular domain in the respective strain's genome. We observed a relationship in the PFAM domains across the different strains, which means that the protein domain is conserved and found in the three strains. This conservation implies that the protein domain performs a similar or related function in those organisms, despite differences in their genus. When a relationship in PFAM is across different organisms, it suggests that the protein domain plays a fundamental role in biological processes, such as protein structure, enzyme activity, or interaction with other molecules, that is crucial for the survival or function of the fungi.

KEGG Results

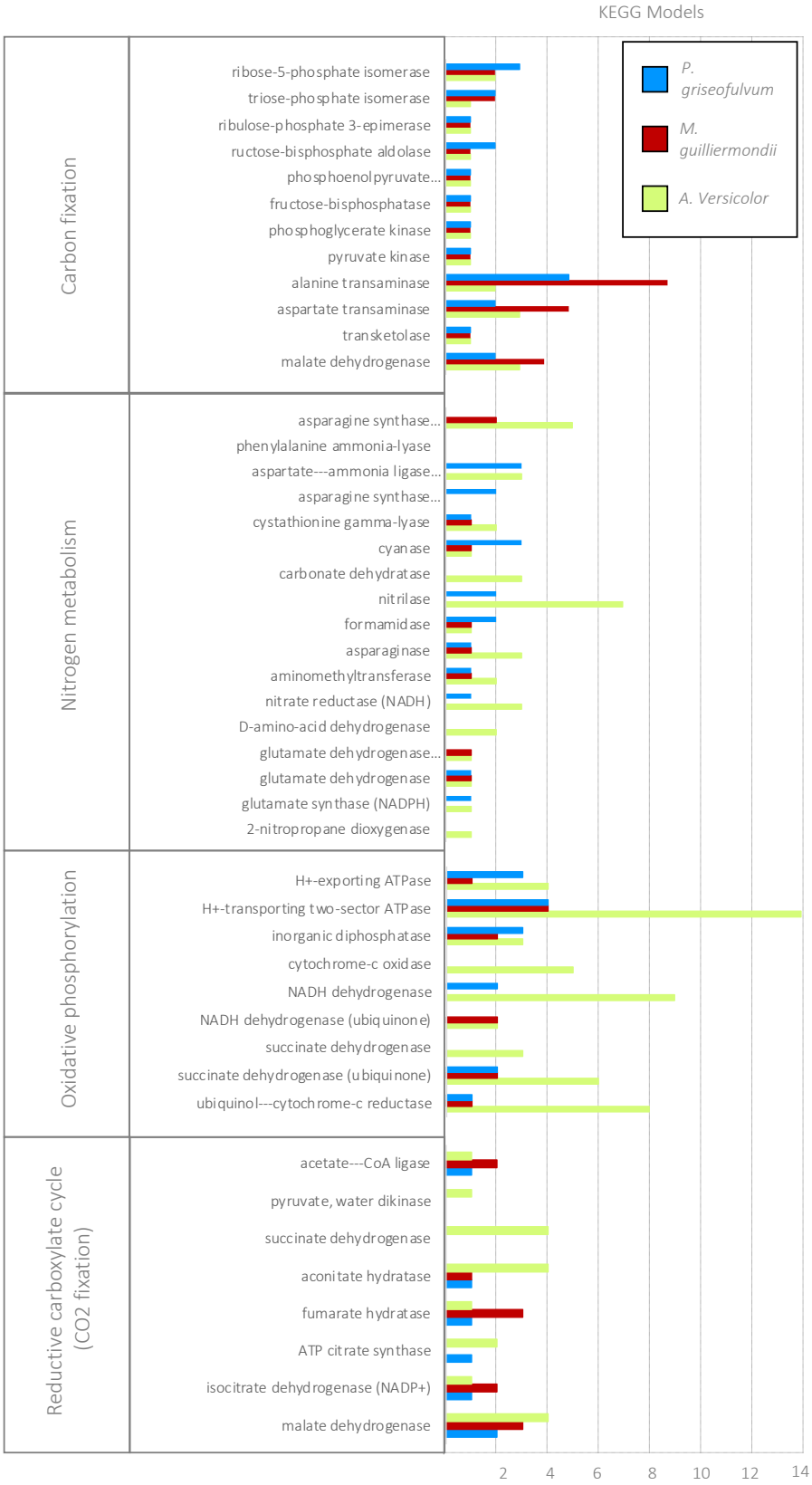

Figure 11. Comparative Analysis of Energy Metabolism Pathways in *Aspergillus versicolor* (green), *Penicillium griseofulvum* (blue), and *Meyerozyma guilliermondi* (red).

The results of the KEGG analysis for the strains *Aspergillus v ersicolor, Penicillium griseofulvum*, and *Meyerozyma guilliermondii* are presented in Figure 11. The graph depicts the abundance of specific metabolic enzymes related to carbon fixation, nitrogen metabolism, oxidative phosphorylation, and the reductive carbohydrate cycle.

Regarding carbon fixation, all three strains exhibited a similar pattern, with a notable abundance of enzymes involved in the Calvin-Benson-Bassham (CBB) cycle (Malate dehydrogenase, Transketolase, Aspartate transaminase, and alanine transaminase, etc.). These findings suggest their ability to use carbon dioxide as a carbon source and convert it into organic compounds through photosynthesis or carbon assimilation pathways (Roth et al., 2014). This fact is especially important for marine animals such as corals. Corals are sessile marine organisms that rely on energy metabolism to carry out essential physiological processes and maintain health (Miaoet al., 2012). The energy metabolism in corals involves the acquisition and utilization of energy sources, primarily through the process of autotrophic and heterotrophic feeding. The genomes of the three strains showed carbon fixation enzymes. These enzymes convert inorganic carbon(primarily carbon dioxide) into organic carbon compounds through photosynthesis (Panich et al., 2021). The coral host utilizes the organic carbon compounds produced through carbon fixation forvarious metabolic processes. These compounds serve as a source of energy and carbon for growth,tissue maintenance, and reproduction. The fixed carbon is also utilized to synthesize structural components such as proteins, lipids, and carbohydrates, essential for coral growth and development(Roth et al., 2014).

Regarding nitrogen metabolism, the three strains exhibited variations in the abundance of specific pathways. *Aspergillus Versicolor* and *Penicillium griseofulvum* displayed a higher abundance of enzymes related to nitrogen assimilation, specifically those associated with the glutamate synthase pathway. Probably this suggests their capacity to efficiently incorporate inorganic nitrogen sources, such as ammonium, into organic molecules (Tanaka et al., 2006).

Additionally, all three strains showed an abundance of enzymes associated with nitrogen recycling, indicating their ability to utilize and recycle nitrogenous compounds effectively.

Nitrogen is an essential nutrient for coral growth, protein synthesis, and overall metabolic functions. Nitrogen metabolism in corals involves the uptake, assimilation, recycling, and excretion of nitrogen compounds (Tanaka et al., 2006). Corals obtain nitrogen from various environmental sources, including inorganic nitrogen compounds such as nitrate (NO_3-) and ammonia, which are present in the surrounding seawater (Yap et al., 2020). However, high nitrite concentrations (NO_2-) can be toxic to corals, negatively affecting their health and survival (Yap et al., 2020). To maintain low nitrite levels, nitrification occurs, converting nitrite into nitrate, typically facilitated by fungi (Aczel et al., 2019). Fungi play a crucial role in the nitrogen cycle, participating in processes such as nitrification and denitrification (Aczel et al., 2019). Some fungi can convert ammonium to nitrate through nitrification. In contrast, others can convert nitrate to nitrogen gas through denitrification, contributing to the cycling and transformation of nitrogen in ecosystems (Aczel et al., 2019).

Figure 11 displays enzymes involved in nitrification, such as Nitrite reductase [NAD(P)H], Cyanase, Ammonia monooxygenase, and Hydroxylamine oxidoreductase. The presence of these enzymes suggests that the selected strains may participate in the denitrification cycle in the ocean, reducing nitrite levels in the environment and making nitrate available for coral uptake.

The strains also displayed enzymes involved in oxidative phosphorylation, all three strains displayed a high abundance of pathways associated with the electron transport chain and ATP synthesis. This indicates their efficient utilization of respiratory pathways to generate energy through the transfer of electrons and the production of ATP. *Aspergillus v ersicolor* (green) showa larger amount of H+-transporting two-sector ATPasein KEGG models in comparison to the other two strains. This may be due to the environmental adaptations faced by *Aspergillus Versicolor*. This is a wide distributed fungi found in diverse environments such as soil, decay matter, lakes, and the ocean. The ocean present different challenges such as pH variations and nutrient availability.

Having more H+-transporting two-sector ATPase could provide Aspergillus with a competitive advantage by allowing them to maintain pH homeostasis and efficiently utilize available nutrients through ATP synthesis.

Also, we found reductive carboxylate cycle (CO_2 fixation) enzymes in the studied genomes. Fungi do not typically utilize the reductive carboxylate cycle for CO_2 fixation. However, it has been described that fungi engage in symbiotic and mutualistic associations can provide their host essential nutrients, including carbon, in exchange for organic compounds such as sugars (Vigneron et al., 2018). Corals typically rely on the symbiotic relationship with zooxanthellae for fixing carbon dioxide. However, deep-sea corals do not possess zooxanthellae, so the coral must have other mechanisms to fix carbon. A reasonable hypothesis is that fungi could help with this process. The strains analyzed in our study can take up dissolved CO_2 from the surrounding seawater through specialized transporters in the reductive TCA cycle or reverse TCA cycle. This metabolic pathway found in our strains enables them to fix carbon dioxide in the absence of light (Xu et al., 2022).

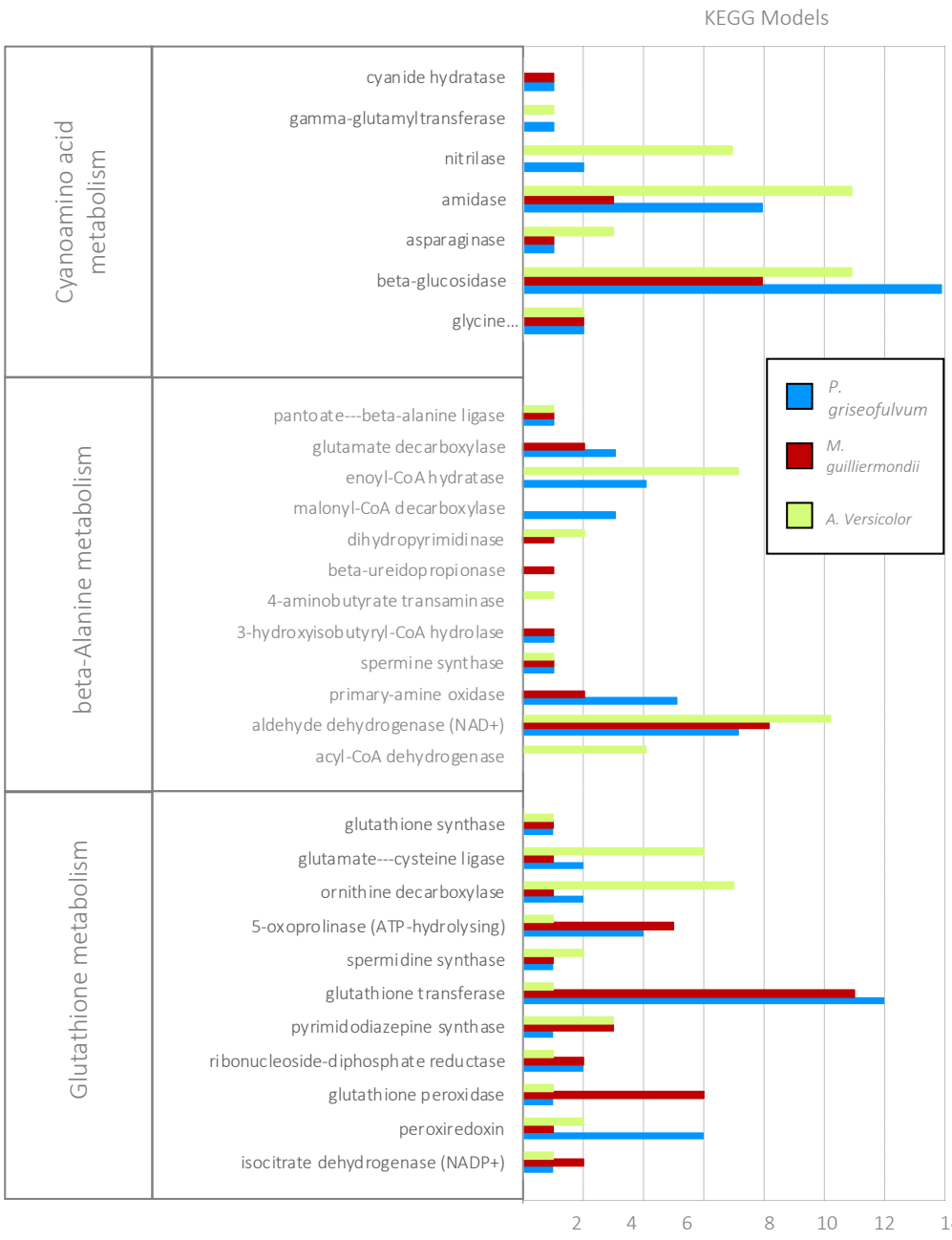

Figure 12. Comparative Analysis of Metabolism of Other Amino Acids in *Aspergillus Versicolor*, *Penicillium griseofulvum,* and *Meyerozyma guilliermondi.*

Some species of fungi, including the three strains analyzed in our study, have been shown to produce cyanide compounds. These compounds are considered secondary metabolisms that have several ecological functions, including antimicrobial properties, defense against pathogens, and can

also be involved in fungal-fungal interactions (Keller et al., 2018). In table 12 we observed a higher abundance of beta-glucosidase in *Penicillium griseofulvum* (blue) compared to *Aspergillus Versicolor* (green) and *Meyerozyma guilliermondii* (red), suggesting that *P. griseofulvum* may have a greater capacity to metabolize beta-glucosides indicating that this specie has a stronger capability to hydrolyze beta-glucosides and release glucose. *P. griseofulvum* may more efficiently utilize these compounds as a carbon source.

Aspergillus Versicolor, *Penicillium griseofulvum*, and *Meyerozyma guilliermondii* showed beta-Alanine metabolisms. Beta-alanine can undergo several metabolic processes in fungi. They can incorporate beta-alanine into proteins during protein synthesis, which serves as a building block of polypeptide chains (Nicholson et al., 2009). Also, some fungi produce secondary metabolites that contain beta-alanine or related compounds. These metabolites can have various biological activities, such as antimicrobial or signaling properties, and contribute to fungal physiology and environmental interactions (Bills et al., 2017).

The strains in the study showed glutathione metabolism which is important for maintaining cellular homeostasis and protecting cells from oxidative damage (Perrone et al., 2005). It plays a crucial role in cellular redox balance, detoxification processes, and the regulation of oxidative stress (Sharma et al., 2021). Glutathione is an antioxidant by scavenging reactive oxygen species (ROS) and neutralizing harmful free radicals generated during normal cellular metabolism or under stress conditions. Glutathione helps to protect corals by maintaining the redox balance and reducing oxidative damage (Hatem et al., 2014). Glutathione is involved in modulating the immune response in corals. It plays a role in regulating immune cells, such as phagocytes, and producing immune signaling molecules (Hatem et al., 2014).

Discussion

Isolation of fungi

Our results (Table 2) show the uneven distribution of the fungi isolated from the five fragments (A, B, C, D, E) of *D. pertusum* used in this study. This uneven distribution may be because only one or two polyps from each sample were crushed. A better sampling technique would involve homogenizing a more significant portion of coral and then plating on various media types. This technique has proven useful in previous studies of coral microbiomes because microbial communities' spatial distribution varies along the entire coral branch (including skeleton and tissue) (Hansson et al., 2009). However, considering that the five fragments came from the same individual, we were able to have a broader view of the fungi communities present in the whole coral. The narrow phylotypes of fungi isolated from *D. pertusum* could be attributed to the limited nature of culturing and the lack of information on culturing deep-sea coral fungi. An alternative to traditional media could be custom-made culture media like coral juice and other techniques such as winogradsky columns, coculture, culturomics, cultivation chambers, isolation chips, and multiwell microbial culture chips (Schultz et al., 2022). Other factors that could have interfered with the growth of the isolates were the lack of pressure and the incubation time. Because these organisms inhabit such cold environments, their metabolism is much slower than the fungi thriving on the ocean's surface or terrestrial environments (Galkiewicz et al., 2012). That is why 35 days was probably not enough time for all phylotypes to grow.

Additionally, the lack of pressure in the deep-sea simulator and culturing could affect the growth of the fungi. Previous studies have shown that pressure plays a crucial role in the growth of fungi, as most enzymes are affected by pressure (Huang et al., 2019). Pressure controls the rate of rebinding. Low pressure might positively affect enzymes and enhance

biochemical reactions (Gamage et al., 2017). By not mimicking this parameter wemay have limited the growth of potential phylotypes found in deep-sea cnidarians.

Despite the limitations of culture-dependent methods (e.g., high selectivity, lack of media for many "known" microbial groups), they provide valuable insights into the metabolic abilities of the isolates. However, complementing this knowledge with phylogenetic identification, amplicon sequences, and metagenomics is ideal for determining an essential fraction of the coral-associated microbes.

Fungal phylogeny

In this study, we used the 18S rDNA and ITS-1 region for the phylogenetic identification of each isolate. The trees based on 18S rDNA determine more distant phylogenetic relationships between isolates, while the ITS region was used to determine close relationships between the strains (Galkiewicz et al., 2012). The 18S rDNA phylogenetic tree was constructed from partial 18S rRNA gene sequences of each isolate and their top GenBank matches. Two main clades were found, corresponding to Basidiomycota and Ascomycota. In both 18S rDNA and ITS trees, the B asidiomycete s *Tritirachium* spp. formed a branch that grouped closely with the ascomycetes *Meyerozyma* spp. Bootstrap values of 43% supported these branches in the 18S rDNA tree and 98% in the ITS tree. In both cases (18S andITS), it was the most remote branch within the rest of the A scomycetes. This could be explained because, until recently, *Tritirachium* sp. was included within Ascomycota (Sumathi et al., 2014). However, additional and more precise phylogenetic analysis showed that the members of this genus formed a significantly supported clade within the Basidiomycota (Sumathi et al., 2014).

The fungal strains isolated from deep-sea coral *D. pertusum* were often closely related to terrestrial species. These phenotypes can reach to the deep sea as spores or hyphae

associated with terrestrial organic matter (Damare et al., 2006). Also, because *D. Pertusum* don't possess zooxanthellae, its heterotrophic diet consists of particulate organic matter that down-swelled from the surface and is resuspended from the benthos possibly containing fungal spores, this supports the hypothesis that some of the fungal isolates came from terrestrial environments (Davies et al., 2009). However, all fungi genus found in this study have been previously isolated from other marine environments and animals, corroborating the hypothesis that the shift of the water column due to phenomena like upwelling and downwelling episodes can transport the microbial communities from shallow waters to deep-sea environments and vice versa.

Functional role of fungi

The potential functionality of the fungal communities associated with *D. pertusum* could be classified anywhere on the symbiotic continuum: mutualism, commensalisms, or parasitism. In past studies regarding shallow water corals, some of the fungal strains isolated in this study have shown beneficial functions to the host, like quorum sensing genes, osmotic stress sequences, DNA repair mechanisms, antimicrobial activity, and increasing resistance to toxic metals (Hernandez-Agreda et al., 2016). Even though the study on deep-sea fungal biodiversity is scarce, understanding the eco-physiological role of these fungi is essential to know their roles within the host and the environment.

Our results show some saprotrophic fungi like; *Penicillium, Cladosporium, Exophiala, Aspergillus,* and *Cladophialophora*. They have been previously reported in the Indian Ocean sediments from decaying wood, dung, and undefined biomass (Xu et al., 2018). Because of vertical transportation in the water column, some wood-associated saprotrophs are found in the deep-sea. They could be part of the deep-sea mycobiota specialized in degrading lignocellulosic biomasses from sunk ships and other wooden materials (Marchese et al., 2021). Also, other saprotrophs can decompose non-recalcitrant organic matter such as animal

carcasses. Most of the phenotypes found in our study have been reported in the literature that they are capable of degrading both substrates (Marchese et al., 2021), representing the ability of fungi to thrive in nutrient-poor environments by metabolizing organic matter depending on availability. Deep-sea saprotrophic fungi could play a vital role in decomposing organic matter, essential for nutrient turnover and contributing to the marine carbon cycle (Marchese et al., 2021; Velez et al., 2022).

Other genera found in this study (*Meyerozyma, Tritirachium, Hortaea)* have been previously described as opportunistic pathogens or mutualistic symbionts in plants, animals or other fungi (Marchese et al., 2021). Probably these genera of fungi prefer to live in association with other organisms instead of expressing complex enzymatic reactions to degrade environmental organic matter. Also, the interaction between the coral and the fungi could be motivated by the microbial contribution to the nitrogen cycle, stabilizing the holobiont functioning under oligotrophic conditions and promoting the coral well-being (Wegley et al., 2007). *Meyerozyma* spp., *Tritirachium* spp., and *Hortaea* spp., have never been described as coral pathogens. However, the genera *Hortaea* is known to cause a non-invasive skin infection in humans denominated Tinea nigra, also known as *Hortaea*, is a halotolerant fungus that has been extensively studied to understand its mechanisms of salt tolerance in response to osmotic shock (Grajales et al., 2016). On the other hand, the basidiomycete *Tritirachium* is known to produce a secondary metabolite compound called 1 (2-(4-hydroxyphenyl)-4-methyl tetrahydrofuran-3-ol), which has been shown to possess antioxidant activity (Nguyen et al., 2018).

There is a tendency for fungal communities to be associated with terrestrial and shallow water organisms, this might suggest that some fungi taxa might be involved in similarsymbiosis with deep-sea benthic animals. The lack of knowledge of deep-sea fungi roles highlights the urgent need for additional studies and the development of databases. With this

we might identify the deep-sea fungal key species and potential pathogens as health indicators of deep-water reefs.

Sulfate Assimilation and Oxidation

The PFAM results (figure 10) indicate that all three fungi strains possess protein families associated with sulfate assimilation, suggesting their ability to utilize sulfate as a sulfur source and convert it into essential biomolecules (Wall et al., 2014). Also, figure 10 highlights the presence of protein families (PFAMs) involved in sulfide oxidation, such as PF00884, PF01507, PF0371, and PF01077. Recent research has provided evidence demonstrating the ability of terrestrial fungi to undergo the oxidation of elemental sulfur and other reduced forms of sulfur present in soil environments (Wall et al., 2014). Also, some thermophilic fungi have been reported to oxidize sulfur and metal sulfides to sulfates (Rawlings et al., 2005; Salcedo et al., 2023). Sulfide is a common form of sulfur in deep-sea and deep-sea hydrothermal vents. However, sulfide is a toxic compound that can harm corals at high concentrations. Oxidizing sulfides in deep-sea environments is important for various reasons, including transforming sulfide compounds into less harmful forms and more readily available for other organisms like corals to utilize (Salcedo et al., 2023). These events might hint at the potential functional role of fungi in hydrothermal vents, where they could oxidize sulfides from vent fluids, like sulfur-oxidizing bacteria. However, the exact mechanisms in how fungi can oxidize sulfur isn't well understood and more studies are required.

Sulfate ions are the main form of sulfur in the ocean. A major portion of the sulfur is assimilated by the coral holobiont in the form of cysteine and methionine. Subsequently, it is

converted into dimethylsulfoniopropionate (DMSP), a highly stable and soluble compound (Raina et al., 2013; Guibert et al., 2020). This compound is an essential metabolite involved in cellular and ecological processes. DMSP possesses antioxidant properties, serves as an osmolyte, is involved in antiviral defense mechanisms and in sulfide detoxification (Guibert et al., 2020). Also, DMSP plays an important role in climate regulation because it can be converted into dimethylsulfide, a trace gas involved in cloud formation (Guibert et al., 2020).

It has been assumed that DMSP production comes exclusively from corals that possess endosymbiotic microalgae Symbiodinium. However, evidence of the total amount of DMSP recorded in the coral is considerably higher than those recorded from the Symbiodinium alone. In 2013, Raina and collaborators refuted the hypothesis that photosynthetic organisms are the only DMSP producers in the ocean. They used *Acropora* corals on their larval stages and confirmed that the coral is capable of biosynthesizing DMSP in high concentrations in the absence of the zooxanthellae. Also, the coral holobiont is known for harboring bacteria that can catabolize DMSP (Rosado et al., 2019). However, the recent discovery of bacteria capable of producing DMSP in coastal and deep-sea environments raises the possibility of other microorganisms present in the coral doing the same (Kuek et al., 2022). In 2009, Todd and collaborators described the ability of two genera of Ascomycota, the *Aspergillus* and *Fusarium* to produce gas dimethyl sulfide (DMS) from DMSP thanks to a gene termed *ddd*P. In our results (supplementary table 1) *Aspergillus versicolor* presented this gene, nevertheless, this strain was obtained from a rodent, so their metabolic pathways may differ drastically from the strain isolated by us.

Inorganic sulfur is also essential for the coral holobiont. It plays a key role in synthesizing essential biomolecules, such as antioxidants and vitamins (Todd et al., 2009). The Sulfate adenylyl transferase participates in assimilatory sulfur reduction and dissimilatory sulfur oxidation and reduction. Also, it participates in the sulfur cycle (Li et al., 2022). In the dissimilatory sulfate reduction, the *sat* enzyme acts as the first step in converting sulfate to

Adenosine 5'-phosphosulfate (APS) from an ATP and free sulfate (Li et al., 2022).

Denitrification

Corals commonly live in nutrient-poor environments, which is particularly evident in the oligotrophic waters of the deep sea. Deep-sea corals are highly efficient in assimilating nitrogen because heterotrophic feeding can meet some of their nitrogen requirements, but this is insufficient for their growth and reproduction (Smith et al., 2019). Cold-water corals are suspected of acting as a source of dissolved inorganic nitrogen as ammonium in the deep sea, presumably due to active nitrifying microbial communities in the coral holobiont (Naumann et al., 2012). A dynamic nitrogen cycle on cold water reefs occurs due to archaea, fungi, and bacteria in the coral host (Naumann et al., 2012).

Nitrogen fixation in the ocean predominantly comprises two microbial pathways: nitrogen fixation as a source and denitrification as a sink (Gruber et al., 2008; Naumann et al., 2012). Denitrification is a distinct process in which oxidized nitrogen compounds (NO^{3-} and NO^{2-}) are utilized as electron acceptors for energy production (Braker et al., 2000). The protein families responsible for denitrification such as PF0406, PF03458, PF03937, and PF04332 were found in our results (Figure 11). The steps for denitrification are catalyzed by the enzymes nitrate reductase, nitrite reductase, nitric oxide reductase, and nitrous oxide reductase found in our results (complementary table 1) (Zumft et al., 1997). *Aspergillus versicolor* and *Meyerozyma guilliermondii* presented these enzymes and the *nir* genes, which are associated with nitrite reductase. Cold-water corals, such as *Desmophyllium pertusum*, consume up to 20 times more oxygen and release twice as much dissolved inorganic nitrogen, resulting in an unbalanced nitrogen budget in the ocean (Maier et al., 2021).

In previous studies, a metagenomic approach was utilized to investigate the holobiont associated with two shallow-water coral species, *Porites astreoides* (Wegley et al., 2007) and *Acropora hyacinthus* (Amend et al., 2012). These studies revealed that the dominant fungal groups within these coral holobionts were Ascomycota, Basidiomycota, and Chytridiomycota.

Furthermore, more comprehensive metagenomic and metatranscriptomic analyses have shed light on the potential involvement of endolithic fungi in nitrogen-cycling processes within the coral.

It is worth noting that the presence and role of endolithic fungi in deep-sea corals remain to be thoroughly investigated. Future studies could employ omics strategies to explore the presence and ecological significance of endolithic fungi in deep-sea and shallow coral ecosystems. Such investigations have the potential to provide valuable insights into the functional roles and contributions of these fungi to the nitrogen cycle and overall coral health in deep-sea environments.

Nitrate assimilation

The comparison of nitrate assimilation among the three strains *Penicillium griseofulvum*, *Aspergillus versicolor*, and *Meyerozyma guilliermondii* can provide insights into their abilities to utilize nitrate as a nitrogen source. Nitrate assimilation is an important metabolic process that allows organisms to incorporate nitrogen from nitrate into organic molecules for multiple cellular functions (Schinko et al., 2010).

In our study, we observed distinct patterns in the nitrate assimilation capabilities of these fungal genomes (figure 10). *Penicillium griseofulvum*, represented in blue, exhibited a significant abundance of protein families (PFAMs) associated with nitrate assimilation, such as PF01568, PF02975, PF01226, and PF00795. *P. griseofulvum* has a well-developed nitrate assimilation pathway, enabling efficient utilization of nitrate as a nitrogen source for growth and metabolic functions (Jonassen et al., 2009). The presence of these enzyme families indicates its potential to catalyze nitrate reduction to nitrite and then to ammonium, which can be incorporated into organic molecules.

Aspergillus versicolor, represented in green in figure 10, also displayed a moderate abundance of enzymes and protein families associated with nitrate assimilation. While not as

pronounced as *P. griseofulvum*, the presence of these enzyme families suggests that *A. versicolor* possesses the capacity for nitrate assimilation to a lesser extent. This fact implies that *A. versicolor* may be able to utilize nitrate as a nitrogen source but with potentially lower efficiency compared to *P. griseofulvum*. However, previous studies done by Garret et al., showed the capacity of *Aspergillus nidulans* to assimilate nitrate. In this study they identify the two enzymes involved in the assimilatory reduction of nitrate to ammonia, the nitrate reductase and nitrite reductase. Even though the study by Garret et al. on *Aspergillus nidulans* further supports the capacity of Aspergillus species to assimilate nitrate, it is important to note that even within the same genus, different species or strains can exhibit variations in their metabolic capabilities due to genetic diversity and ecological adaptations.

On the other hand, *Meyerozyma guilliermondii*, in red in figure xx, showed a comparatively lower abundance of enzymes and protein families associated with nitrate assimilation. This could be because yeasts often inhabit different ecological niches compared to filamentous fungi (Yurkov et al., 2018). While filamentous fungi are commonly found in soil and plant-associated environments, yeasts are frequently associated with fermentative and sugar-rich environments, such as fruit surfaces or fermentation processes. These environments may provide an abundance of organic nitrogen sources, reducing the selective pressure for efficient nitrate assimilation (Yurkov et al., 2018). However, the lower abundance of nitrate assimilation-related enzymes in *Meyerozyma guilliermondii* does not necessarily imply an inability to utilize nitrate, but rather a potentially different strategy for nitrogen acquisition.

Carbohydrate metabolic process

Deep-sea fungi act as saprotrophs, breaking down organic matter and playing an important role in oceanic carbon cycling, including the biological carbon pump (Lopez et al., 2018). They can degrade organic matter using carbohydrate-active enzymes (CAZymes). Glycoside hydrolases (GHs) are a broad group of CAZymes that are the primary mode of

degradation of some polysaccharides like cellulose (supplementary table 1) (Berlemont et al., 2013). Our three strains *Penicillium griseofulvum*, *Aspergillus versicolor*, and *Meyerozyma guilliermondii* presented GHs including GH3 β-glycans such as beta-glucosidase (3.2.1.21), beta-xylosidase (3.2.1.37), N-acetyl beta-glucosaminidase (3.2.1.52), glucan beta-1,3-glucosidase (3.2.1.58), cellodextrinase (3.2.1.74), and exo-1,3-1,4-glucanase (3.2.1); GH27 and GH36 that include alpha-galactosidases and alpha-N-acetylgalactosaminidases, which catalyze the hydrolysis of melibiose into galactose and glucose (Da Silva et al., 2020); and GH18 chitin, which includes chitinase, chitodextrinase, and the killer toxin of Kluyveromyces lactis (yeast), indicating that chitin degradation is a functional role of deep-sea fungi, as is commonly seen in fungi from freshwater (Li et al., 2019).

In 2020, Christmas and collaborators surveyed planktonic fungi Glycoside hydrolases gene activity depending on the sampling site depth. They discovered that in shallow depths, the number of unique GH7s involved in cellulose/hemicellulose degradation was more remarkable, especially in zones with high productivity, such as the Mediterranean Sea and the Indian Ocean, and there was a significant drop in unique GH7s in the mesopelagic. Overall, most GHs had a greater number of unique GHs in shallow waters than those in the mesopelagic. This suggests that the depletion of available carbon sources like cellulose limits the saprotrophic action of deep-sea fungi.

The PF00294 protein family (figure 10), which includes glycosyltransferases, is involved in various carbohydrate metabolic processes. *A. versicolor*, *P. griseofulvum*, and *M. guilliermondii* are known to be versatile fungi with diverse metabolic capacities. Glycosyltransferases are enzymes that catalyze the transfer of sugar moieties onto various molecules, such as proteins, lipids, and other carbohydrates (Da Silva et al., 2020). The presence of the PF00294 protein family in these three fungal strains suggests their capability to modify and utilize carbohydrates. These enzymes are involved in synthesizing and modifying complex carbohydrates, such as polysaccharides and glycoproteins. They are crucial

for the biosynthesis of cell wall components, extracellular matrix molecules, and other carbohydrate-based structures (Chrismas et al., 2020).

In *A. versicolor* and *P. griseofulvum*, the presence of the PF00294 protein family indicates their potential to perform various carbohydrate modifications and utilization. These fungi are known for their ability to degrade complex plant polysaccharides, such as cellulose and hemicellulose, through the secretion of a range of carbohydrate-active enzymes (Resl et al., 2022). The presence of glycosyltransferases suggests their involvement in the synthesis and remodeling of fungal cell walls, as well as the modification of carbohydrates for energy production and other metabolic processes.

On the other hand, *M. guilliermondii*, being a yeast species, may exhibit a different pattern in carbohydrate metabolism compared to filamentous fungi. While yeast species typically have a reduced set of glycosyltransferases compared to filamentous fungi, they still possess the ability to modify and utilize carbohydrates (Garcia et al., 2020). *M. guilliermondii* may utilize glycosyltransferases to synthesize cell wall components, glycoproteins, and other carbohydrate-related processes necessary for its growth and survival (Garcia et al., 2020).

Phosphorylation / Protein amino acid phosphorylation

Phosphorylation is commonly associated with protein activity and is critical in protein function regulation. It regulates the protein by conducting conformational changes by holding mechanisms to activate or inactivate some enzymes and receptors by phosphorylation and dephosphorylation events (Ardito et al., 2017). A key protein regulation element is kinases, which are responsible for cellular transduction signaling and their hyperactivity (Ardito et al., 2017).

Living organisms have developed adaptive mechanisms to survive in a constantly changing environment. Cells have complex signaling pathways to sense danger and trigger events to mitigate adverse effects (Courtial et al., 2017). Some kinases can stimulate the

expression of stress response genes and control critical cellular processes. An example is The MAPK gene family, which has three subgroups, including the JNK group (N-terminal kinase), which is evolutionarily conserved and found in all eukaryotes (complementary table 1). When activated, JNKs play a key role in response to thermal and ultraviolet radiation stresses in animals and several terrestrial and marine plants (Courtial et al., 2017).

Also, a study published in the journal "Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology" found that phosphorylation of the protein, starmaker, is involved in the formation of the aragonite skeleton in the coral *Acropora digitifera* (Mydlarz et al., 2012). The researchers showed that the protein's phosphorylation of specific serine and threonine residues is necessary for its function in biomineralization (Mydlarz et al., 2012).

Another study published in the journal "Journal of Proteome Research" identified several proteins involved in biomineralization in the coral *Stylophora pistillata* that undergo phosphorylation. These include proteins involved in calcium signaling and transport, as well as structural proteins involved in the formation of the coral skeleton (Moya et al., 2008).

Secondary metabolites biosynthesis, transport, and catabolism

Secondary metabolites are molecules produced by organisms that are not essential for basic cellular functions but instead have specific ecological functions such as defense against predators, competition for resources or symbiotic relationships (Newman et al., 2016). Marine fungi

In corals, their microbial symbionts (bacteria, fungi, viruses) are known to produce secondary metabolites that are involved in a variety of biological functions, including defense against predators, communication with other organisms, and protection against environmental stressors such as UV radiation and pathogens (Bhagwat et al., 2019; Chen et al., 2020).

Catabolism of secondary metabolites in corals is a complex process involving a variety of enzymes and pathways. The breakdown of these molecules can produce useful products that

can be recycled by the coral, such as amino acids and sugars. The catabolism of secondary metabolites may also play a role in regulating the concentration and activity of these molecules in the coral tissue (Scheffel et al., 2019).

The biosynthesis of secondary metabolites in corals involves the activity of enzymes such as polyketide synthases, non-ribosomal peptide synthases, and tAMP-dependent synthetase/ligase domain, all found in our results (supplementary table 1). These enzymes catalyze the formation of complex molecules from simple building blocks (Li et al., 2021). Also, our results show the presence of Multicopper oxidases, these are a family of enzymes that contain copper ions and are involved in a wide range of biological processes, including lignin degradation, iron oxidation, and melanin biosynthesis (Wu et al., 2020). In corals, a specific type of Multicopper oxidase known as laccase has been identified playing a role in the biomineralization of their calcium carbonate skeletons. Laccase has been shown to oxidize phenolic compounds, which are thought to be involved in the regulation of calcium carbonate precipitation and crystal morphology (Rahman et al., 2019). However, further research is needed to fully understand the function of these enzymes and domains in coral biology.

The biosynthesis, transport, and catabolism of secondary metabolites in corals are complex processes that are likely to be influenced by a variety of factors including environmental conditions, and the presence of other organisms (Grottoli et al., 2018).

Conclusion

This study has provided valuable insights into the fungal communities associated with deep-sea coral *Desmophyllum pertusum* in the South Atlantic. We observed the colonization of diverse fungal taxa, some of which have not been previously described in association with *D. pertusum* or deep-sea habitats, and other species commonly found and adapted to deep-sea corals. By employing culture-based methods, we were able to selectively isolate fungal microbial communities while excluding other microorganisms. Our findings suggest that PDA and Malt extract media are suitable for future studies. They were efficientfor isolating most of the strains (26.31% and 31.58%, respectively).

The culturomics approach adopted in this project facilitated the morphological and phylogenetic identification of the isolated strains, leading to the establishment of a valuable collection of deep-sea fungi with potential bioremediation and biotechnological applications. Furthermore, this collection enhances our understanding of marine mycology by exploring a new location, the Campos Basin in Brazil.

Deep-sea fungi, thriving in unique environments, exhibit a remarkable ability toproduce a wide range of secondary metabolites with novel bioactive properties and distinctive physiological characteristics. In this study, we delved into the genomes of three fungal species, namely *Aspergillus v ersicolor* (M17), *Penicillium griseofulvum* (M27), and *Meyerozyma guilliermondii* (M2), which demonstrated to have XX% similarity with our strains and then were used to investigate the potential roles of these fungi in the deep-sea environment. These genomic analyses revealed intriguing metabolic pathways that hold promise for obtaining novel bioactive compounds and further expanding our knowledge of the potential applications of deep-sea fungi.

In conclusion, our study sheds light on the diversity and functional potential of fungal communities associated with deep-sea coral *D. pertusum*. The obtained strains, together with their unique metabolisms, offer opportunities for future research in bioremediation, biotechnology, and the discovery of new bioactive compounds. It is our hope that this study contributes to the growing field of marine mycology and encourages further exploration of the fascinating microbial world inhabiting deep-sea environments.

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Supplementary Information

Selection of the strains for the genome annotation

Because of the extreme conditions of the deep-sea, organisms, including microbes, are especially suited with certain physical and biochemical traits that help them survive these habitats. Because of this, many microorganisms can produce specialized metabolites that can be different to those found in terrestrial and shallow water organisms (Hong et al., 2022). Recent studies have shown how fungal strains isolated from deep-sea habitats have great biotechnological, clinical, and environmental potential.

However, we must keep in mind that many of these "environmentally friendly" fungi can represent pathogenic activity against humans. So, can human pathogens be environmentally friendly? The answer is yes, a good example of this are black yeasts. In 2015 Blasi and collaborators demonstrated how black yeast-like fungi isolated from a patient with chronic sinusitis can grow on environmental pollutants like; hexadecane, toluene, and polychlorinated biphenyl 126 as the sole carbon source. Black yeast-like fungi have the outstanding ability to thrive in extreme environments making them ideal for the bioremediation of pollutant soils, rivers, and oceans (Blasi et al., 2015). In this study they found that *Exophiala mesophila* and *Cladophialophora immunda* showed the characteristics described above, being able to grow on toluene as their only carbon source. Both genera were found in our results

Exophiala alcalophila (M14), *Exophiala oligosperma* (M16), and *Cladophialophora mycetomatis* (M20). However, for the purpose of this study we are not going to consider human or animal pathogens.

The most abundant genera found in our results was *Penicillium spp.* This widely distributed genera have been isolated in almost all environments in which scientists have dared to search, and the deep-sea is not the exception. Previous studies have shown the versatile metabolites of penicillium isolated from deep-sea sediments. In 2021 Pang and collaborators described new sorbicillinoids isolated from cultures of penicillium. They show a variety of biological activities including cytotoxic, antioxidant, antiviral and antimicrobial activity against α-glycosidase and acetylcholinesterase (AChE) in vitro (Pang et al 2021). Also, in 2022 Dramae and collaborators demonstrated the antimicrobial and cytotoxic activity against both cancerous and non-cancerous cells of tanzawaic acids derived from *Penicillium citrinum*. The unique versatility of penicillium metabolites make them attractive candidates for developing new pharmaceutical, bioremediation tools, and agrochemical agents. We selected the strain *Penicillium griseofulvum* (M27) to analyzing its genome. *Penicillium griseofulvum* is of great biotechnological importance because of its anti-allergic and antifungal properties. The production of griseofulvin characterized these fungi. The griseofulvin is primarily used to treat fungal infections such as ringworm (tinea corporis), athlete's foot (tinea pedis), jock itch (tinea cruris), and fungal infections of the scalp, hair, and nails (Hu et al., 2022).

We excluded the strains of Penicillium found in water samples (*Penicillium subrubescens*).

Table 1. Definition of some important domains found within the genome of *Penicillium griseofulvum*, *Aspergillus versicolor* and *Meyerozyma guilliermondii*.

Sulfate Assimilation			
ID	Name	Definition	Reference

EC2.7.1.25 IPR002891	Adenylyl-sulfate kinase	The enzyme Adenylylsulphate kinase (2.7.1.25) facilitates the process of phosphorylating adenylylsulphate into 3'-phosphoadenylylsulphate. This enzyme is commonly present as a fusion protein with sulphate adenylyltransferase. The combined activity of both enzymes is essential for the synthesis of PAPS (phosphoadenosine-phosphosulphate) from inorganic sulfate.	A multifunctional Urechis caupo protein, PAPS synthetase, has both ATP sulfurylase and APS kinase activities. Rosenthal E, Leustek T. *Gene* 165, 243-8, (1995).
EC2.7.7.4 IPR002650	Sulphate adenylyltransferase	ATP-sulfurylase or Sulphate adenylyltransferase (2.7.7.4) initiates the formation of APS by combining ATP and free sulphate, which is the first step in creating the activated sulphate donor, PAPS. Sometimes, it is present as a bifunctional protein where the APS kinase domain catalyses the second and final step - the conversion of APS into PAPS. The combined ATP sulfurylase/APS kinase is known as PAPS synthase. In certain organisms, this enzyme generates APS from sulfate and ATP, while in others, it operates in the opposite direction to create ATP from APS and pyrophosphate. It belongs to a vast superfamily of nucleotidyltransferases that includes pantothenate synthetase (PanC), phosphopantetheine adenylyltransferase (PPAT), amino-acyl tRNA synthetases, and the dissimilatory sulphate adenylyltransferase (sat) found in the sulphate reducer Archaeoglobus fulgidus.	Crystal structure of a novel zinc-binding ATP sulfurylase from Thermus thermophilus HB8. Taguchi Y, Sugishima M, Fukuyama K. *Biochemistry* 43, 4111-8, (2004).
IPR011800	Phosphoadenosine phosphosulfate reductase CysH	Phosphoadenosine phosphosulfate reductase utilizes thioredoxin as an electron donor to initiate the reduction process of PAPS into sulphite and PAP.	Identification of a new class of 5'-adenylylsulfate (APS) reductases from sulfate-assimilating bacteria. Bick JA, Dennis JJ, Zylstra GJ, Nowack J, Leustek T. *J. Bacteriol.* 182, 135-42, (2000).
DddP polypeptide	M24 metallopeptidase family	Belonging to the vast family of M24 metallopeptidases, the DddP polypeptide is distinctly separate from two other enzymes, DddD and DddL, that have previously been identified as catalysts for DMS generation from dimethylsulfoniopropionate.	
Nitrate assimilation			
EC1.7.1.1. EC1.7.1.2. EC1.7.1.3. EC1.8.2.1. IPR012137 IPR008335 IPR008333 IPR005066 IPR001709 IPR001433 IPR001199 IPR000572	nitrate reductase	The NAD(P)H:nitrate reductase (NR), a multidomain redox enzyme, performs the reduction of nitrate to nitrite in a single polypeptide electron transport chain. The electron flow takes place from NAD(P)H-FAD-cytochrome b5-molybdopterin-NO(3). Three types of NR have been identified: an NADH-specific enzyme found in higher plants and algae (1.7.1.1); an NAD(P)H-bispecific enzyme found in higher plants, algae and fungi (1.7.1.2); and an NADPH-specific enzyme discovered solely in fungi (1.7.1.3). On the other hand, the mitochondrial enzyme sulfite oxidase (sulphite:ferricytochrome c oxidoreductase; 1.8.2.1) facilitates the oxidation of sulphite to sulphate, utilizing cytochrome c as the physiological electron acceptor. The enzyme comprises two structure/function domains, namely, an N-terminal haem domain, similar to cytochrome b5, and a C-terminal molybdopterin domain.	Site-directed mutagenesis of nitrate reductase from Aspergillus nidulans. Identification of some essential and some nonessential amino acids among conserved residues. Garde J, Kinghorn JR, Tomsett AB. *J. Biol. Chem.* 270, 6644-50, (1995).
IPR001834 IPR001199	NADH:cytochrome b5 reductase-like	NADH:cytochrome b5 reductase (CBR) acts as an electron donor for cytochrome b5, a widely distributed electron carrier, thereby playing a vital role in several metabolic pathways, such as steroid biosynthesis, desaturation and elongation of fatty acids, P450-dependent reactions, methemoglobin reduction, and so on.	Functional domains of assimilatory nitrate reductases and nitrite reductases. Campbell WH, Kinghorn KR. *Trends Biochem. Sci.* 15, 315-9, (1990).

IPR012744	Nitrite reductase [NAD(P)H] large subunit, NirB	NirB is the large subunit of nitrite reductase [NAD(P)H] (also known as the assimilatory nitrite reductase). NirB interacts with NirD, the small subunit, in most organisms. However, in some bacteria like Klebsiella pneumoniae and fungi, the two regions are combined into a single unit.	
EC1.8.1.12 IPR006067 IPR006066 IPR005117	Nitrite and sulphite reductase 4Fe-4S domain-like superfamily	Sulfite reductases (SiRs) and their closely related counterparts, nitrite reductases (NiRs), facilitate the six-electron reduction reactions of sulphite to sulphide and nitrite to ammonia, respectively. The transfer of electrons occurs from NADPH to FAD and then to FMN in SiR-FP. Subsequently, the electrons are transferred to the metal center of SiR-HP, which reduces the siroheme-bound sulphite. SiR-HP exhibits a two-fold symmetry that leads to a unique alpha/beta fold in three domains, which governs the assembly and reactivity of the enzyme.	
IPR011420	Nitrogen regulatory AreA, N-terminal	The nitrogen regulatory proteins AreA, which belong to the GATA type transcription factors, exhibit a highly preserved N-terminus and possess IPR000679 at the C-terminus.	
IPR000679	Zinc finger, NHR/GATA -type	Transcription factors, including nitrogen regulatory proteins and erythroid-specific transcription factors, bind to the DNA sequence (A/T)GATA(A/G) in gene regulatory regions and are known as GATA-binding transcription factors. These interactions occur through Znf domains that contain four cysteine residues coordinating the zinc ion. NMR studies have revealed that the Znf core consists of two anti-parallel β-sheets and an α-helix, followed by a long loop to the C-terminal end of the finger. The N-terminal part, including the helix, has a structure similar to that of the N-terminal zinc module of the glucocorticoid receptor DNA-binding domain, but not the same sequence. The helix and loop bind to the major groove of the DNA, while the C-terminal tail wraps around into the minor groove, which is essential for specific binding. The interaction between the Znf and DNA is mainly hydrophobic, explaining the presence of thymines in the binding site.	Activity and tissue-specific expression of the transcription factor NF-E1 multigene family. Yamamoto M, Ko LJ, Leonard MW, Beug H, Orkin SH, Engel JD. *Genes Dev.* 4, 1650-62, (1990).
Carbohydrate metabolic process			
EC3.2.1.22 EC3.2.1.49 IPR002241	Glycoside hydrolase	Enzymes known as O-glycosyl hydrolases (3.2.1.) are a diverse group that break down the glycosidic bond between carbohydrates or between a carbohydrate and another molecule. The glycoside hydrolase family 27 (GH27) includes enzymes with multiple activities such as alpha-galactosidase (3.2.1.22), alpha-N-acetylgalactosaminidase (3.2.1.49), and isomalto-dextranase (3.2.1.94).	Structures and mechanisms of glycosyl hydrolases. Davies G, Henrissat B. *Structure* 3, 853-9, (1995).
EC3.2.1.22 IPR000111	*Glyco_hydro_27/36_CS*	Alpha-galactosidase (3.2.1.22), also known as melibiase, breaks down melibiose into galactose and glucose. It is present in a wide range of organisms. Alpha-N-acetylgalactosaminidase (3.2.1.49) catalyses the hydrolysis of terminal non-reducing N-acetyl-D-galactosamine residues in N-acetyl-alpha-D-galactosaminides. This entry represents a conserved site in families 27 and 36. It includes two conserved aspartic acid residues that may be involved in the catalytic mechanism.	Conserved catalytic machinery and the prediction of a common fold for several families of glycosyl hydrolases. Henrissat B, Callebaut I, Fabrega S, Lehn P, Mornon JP, Davies G. *Proc. Natl. Acad. Sci. U.S.A.* 92, 7090-4, (1995).

EC3.2.1.21 EC3.2.1.37 EC3.2.1.52 EC3.2.1.58 IPR002772	Glycoside hydrolase family 3 C-terminal domain	Enzymes belonging to glycoside hydrolase family 3 (GH3) have diverse activities, including beta-glucosidase (3.2.1.21), beta-xylosidase (3.2.1.37), N-acetyl beta-glucosaminidase (3.2.1.52), glucan beta-1,3-glucosidase (3.2.1.58), cellodextrinase (3.2.1.74), and exo-1,3-1,4-glucanase (3.2.1).	Three-dimensional structure of a barley beta-D-glucan exohydrolase, a family 3 glycosyl hydrolase. Varghese JN, Hrmova M, Fincher GB. *Structure* 7, 179-90, (1999).
EC3.2.1.21 EC3.2.1.37 EC3.2.1.52 EC3.2.1.74 IPR001764 IPR001137	Glycoside hydrolase, family 3, N-termina	The O-Glycosyl hydrolases (3.2.1.) are a group of enzymes that commonly hydrolyze glycosidic bonds between carbohydrates or between a carbohydrate and a non-carbohydrate moiety. Among them, Glycoside hydrolase family 3 GH3 includes enzymes with diverse activities such as beta-glucosidase (3.2.1.21), beta-xylosidase (3.2.1.37), N-acetyl beta-glucosaminidase (3.2.1.52), glucan beta-1,3-glucosidase (3.2.1.58), cellodextrinase (3.2.1.74), and exo-1,3-1,4-glucanase (3.2.1). These enzymes are characterized by a two-domain globular structure and are N-glycosylated at three specific sites, with the glycosylated domain located predominantly at the N-terminus of the glycoside hydrolase family 3.	
IPR002509	NodB homology domain	The NodB homology domain is a catalytic domain consisting of approximately 200 amino acid residues, named after its similarity to the rhizobial NodB chitooligosaccharide deacetylase. It is present in members of carbohydrate esterase family 4 (CE4) and PuuE proteins. CE4 family members display metal-dependent deacetylation of O- and N-acetylated polysaccharides such as chitin, peptidoglycan, and acetylxylan. The proteins belonging to this family possess conserved residues that are crucial for metal coordination (D-H-H triad) and enzymatic activity. The PuuE proteins have an E-H-W substitution for the conserved D-H-H metal-binding triad, and they also exhibit amino acid substitutions in residues involved in catalysis, making the enzyme independent of metal. The NodB homology domain adopts a distorted (beta/alpha) barrel fold that consists of eight parallel β-strands. The solvent-exposed active site region is formed by the C-terminal ends of five of these strands, which is surrounded by eight alpha-helices.	Carbohydrate esterase family 4 enzymes: substrate specificity. Caufrier F, Martinou A, Dupont C, Bouriotis V. *Carbohydr. Res.* 338, 687-92, (2003).
EC3.2.1 EC3.2.1.23 EC3.2.1.25 EC3.2.1.31 IPR006102	Glycoside hydrolase, family 2, immunoglobulin-like beta-sandwich	Enzymes with diverse activities such as beta-galactosidase (3.2.1.23), beta-mannosidase (3.2.1.25), and beta-glucuronidase (3.2.1.31) belong to the glycoside hydrolase family 2 GH2. These enzymes possess a highly conserved glutamic acid residue that acts as the general acid/base catalyst in the active site. The domain described in this entry is the immunoglobulin-like β-sandwich domain.	
EC3.2.1 IPR011583 IPR002482 IPR001412 IPR001223 IPR001002	Chitinase II	This family is classified as glycoside hydrolase family 18 (GH18) and comprises members of the chitinase class II group, such as chitinase, chitodextrinase, and the killer toxin found in Kluyveromyces lactis (Yeast) and Candida sphaerica. These enzymes are involved in the hydrolysis of chitin oligosaccharides. In addition to chitinases, GH18 also includes chitinase-like proteins that bind to chitin but do not cleave it.	Structures and mechanisms of glycosyl hydrolases. Davies G, Henrissat B. *Structure* 3, 853-9, (1995).
EC5.3.1.26 EC5.3.1.6 IPR003500	Sugar-phosphate isomerase, RpiB/LacA/ LacB family	This entry describes the sugar isomerase enzymes RpiB, LacA, and LacB. The heteromultimeric protein Galactose-6-phosphate isomerase (5.3.1.26) consists of subunits LacA and LacB and catalyzes the conversion of D-galactose 6-phosphate to D-tagatose and 6-phosphate in the tagatose 6-phosphate pathway of lactose catabolism. Galactose-6-phosphate isomerase is induced by galactose or lactose. Ribose 5-phosphate isomerase (5.3.1.6) is a homodimeric enzyme that catalyzes the interconversion of D-ribose 5-phosphate and D-ribulose 5-phosphate in the non-oxidative branch of the pentose phosphate pathway. This reaction allows the synthesis of ribose from other sugars and the recycling of sugars from nucleotide breakdown.	The 2.2 A resolution structure of RpiB/AlsB from Escherichia coli illustrates a new approach to the ribose-5-phosphate isomerase reaction. Zhang RG, Andersson CE, Skarina T, Evdokimova E, Edwards AM, Joachimiak A, Savchenko A, Mowbray SL. *J. Mol. Biol.* 332, 1083-94, (2003).

EC2.6.1.16 IPR005855 IPR001347 IPR000583	Glucosamine-fructose-6-phosphate aminotransferase, isomerising	The hexosamine biosynthetic pathway is initiated by Glucosamine:fructose-6-phosphate aminotransferase (GFAT or GlmS, 2.6.1.16), which catalyzes the conversion of fructose-6-phosphate and glutamine to glucosamine 6-phosphate, a reaction that represents the first and rate-limiting step. The ultimate product of this pathway is UDP-N-acetylglucosamine, which serves as an important precursor for the biosynthesis of various macromolecules containing amino sugars.	Glucosamine-6-phosphate synthase--the multi-facets enzyme. Milewski S. *Biochim. Biophys. Acta* 1597, 173-92, (2002)
IPR000490 EC3.2.39 EC3.2.1.73 EC3.2.1.58	Glycoside hydrolase family 17	Enzymes in glycoside hydrolase family 17 (GH17) exhibit various activities including endo-1,3-beta-glucosidase (3.2.1.39), lichenase (3.2.1.73), and exo-1,3-glucanase (3.2.1.58). GH17 enzymes have been found exclusively in plants and fungi.	Structures and mechanisms of glycosyl hydrolases. Davies G, Henrissat B. *Structure* 3, 853-9, (1995).
Phosphorylation / Protein amino acid phosphorylation			
PR006204 EC2.7.1.6 EC2.7.1.39 EC2.7.1.36 EC2.7.4.2	GHMP kinase N-terminal domain	The N-terminal region of galactokinase (2.7.1.6), homoserine kinase (2.7.1.39), mevalonate kinase (2.7.1.36), and phosphomevalonate kinase (2.7.4.2) enzymes contains a conserved domain with a region rich in Gly/Ser residues that is likely involved in ATP binding.	Crystal structures of Trypanosoma brucei and Staphylococcus aureus mevalonate diphosphate decarboxylase inform on the determinants of specificity and reactivity. Byres E, Alphey MS, Smith TK, Hunter WN. *J. Mol. Biol.* 371, 540-53, (2007).
PR008266 EC2.7.11.- IPR002290 IPR017441 IPR008271 IPR002290 IPR000719	Protein phosphorylation Tyrosine-protein kinase, active site Serine/Threonine protein kinases active-site	Protein phosphorylation is a reversible process that plays a crucial role in most cellular activities, facilitated by protein kinases and phosphoprotein phosphatases. Protein kinases transfer the gamma phosphate from nucleotide triphosphates, typically ATP, to one or more amino acid residues on a protein substrate side chain, inducing a conformational change that alters protein function. Three classes of protein kinases are known, differentiated by substrate specificity: Serine/threonine-protein kinases, Tyrosine-protein kinases, and Dual specificity protein kinases (e.g., MEK, phosphorylating both Thr and Tyr on target proteins). Protein kinases have broad involvement in cellular processes, including division, proliferation, apoptosis, and differentiation. Tyrosine-protein kinases comprise two main groups: Receptor tyrosine kinases (RTK), transmembrane proteins involved in signal transduction, with pivotal roles in growth, differentiation, metabolism, adhesion, motility, death and oncogenesis. Cytoplasmic / non-receptor tyrosine kinases act as regulatory proteins, with key roles in cell differentiation, motility, proliferation, and survival.	
IPR000008 IPR001680 EC2.7.11.13	C2 domain	The C2 domain is a Ca2+-dependent membrane-targeting module found in many cellular proteins involved in signal transduction or membrane trafficking. C2 domains are unique among membrane targeting domains in that they show a wide range of lipid selectivity for the major components of cell membranes, including phosphatidylserine and phosphatidylcholine. The C2 domain is thought to be involved in calcium-dependent phospholipid binding and in membrane targeting processes such as subcellular localisation.	The role of C2 domains in PKC signaling. Farah CA, Sossin WS. *Adv. Exp. Med. Biol.* 740, 663-83, (2012).
Secondary metabolites biosynthesis, transport and catabolism			
IPR000873	Nonribosomal Peptide Synthetases	Several enzymes in both prokaryotes and eukaryotes seem to act by covalently binding AMP to their substrate using ATP. These enzymes include luciferase, long-chain fatty acid Co-A ligase, long-chain fatty	Structural Insights into Anthranilate Priming during Type II Polyketide Biosynthesis. Jackson DR, Tu SS, Nguyen M, Barajas JF, Schaub AJ, Krug D,

	AMP-dependent synthetase/ligase domain Type II Polyketide Biosynthesis.	acid transport proteins that also act as acyl-CoA ligases, acetyl-CoA synthetase, and other closely-related synthetases.	Pistorius D, Luo R, Muller R, Tsai SC. *ACS Chem. Biol.* 11, 95-103, (2016).
IPR000086	NUDIX hydrolase domain	Nudix hydrolases are a diverse family of proteins that catalyze the hydrolysis of nucleoside diphosphate derivatives. These enzymes serve a range of functions, from maintaining cellular cleanliness to regulating substrate levels to ensure physiological balance. The Nudix superfamily is primarily composed of pyrophosphohydrolases that target a variety of substrates, including canonical and oxidized derivatives of (d)NTPs, nucleotide sugars and alcohols, dinucleoside polyphosphates (NpnN), dinucleotide coenzymes, and capped RNAs. This family includes members capable of degrading potentially mutagenic oxidized nucleotides, as well as those involved in controlling the levels of metabolic intermediates and signaling compounds. The number of Nudix genes present in an organism varies widely, ranging from zero to over thirty in prokaryotes and simple eukaryotes. This variation reflects the metabolic complexity and adaptability of different organisms.	
IPR001117 IPR011706 IPR011707 EC1.10.3.2 EC1.7.2.1	Multicopper oxidase, second cupredoxin domain	This protein functions as a ferroxidase and is essential for the regulation of copper transport and homeostasis. Copper plays a crucial role in biological processes due to its redox properties; however, it can be toxic when present in free form, even at low concentrations. Therefore, living organisms have developed sophisticated mechanisms to maintain copper homeostasis. In eukaryotes, copper (II) ions are reduced to copper (I) before being transported into cells via high-affinity copper transporters of the CTR family. The copper (I) ion is stabilized in blue copper proteins, such as cupredoxin, by a constrained His2Cys coordination environment. Multicopper oxidases utilize a mononuclear copper center to accept electrons from their substrate and transfer them to a trinuclear copper center. The trinuclear center binds dioxygen, which is then reduced to two molecules of water after the transfer of four electrons. The following enzymes are examples of multicopper oxidases: Laccase (1.10.3.2) (urishiol oxidase), a 3-domain enzyme found in fungi and plants that oxidizes various phenols and diamines. Laccases play a role in copper resistance. Nitrite reductase (1.7.2.1), a 2-domain enzyme that contains type-1 and type-2 copper centers. Fission yeast fio1 (also known as SpAC1F7.08), a multicopper oxidase that contains three cupredoxin domains and may work together with Frp1 in iron and copper uptakes in S. pombe. In addition to these enzymes, there are several other proteins that share structural and sequence similarities with multicopper oxidases but have lost their ability to bind copper. These include copper resistance protein A (copA) from a plasmid in Pseudomonas syringae, domain A of blood coagulation factors V (Fa V) and VIII (Fa VIII), yeast FET3 required for ferrous iron uptake, and yeast FET5 (YFL041w), which is also an iron transport multicopper oxidase required for high-affinity Fe2+ uptake and targeted to vacuoles via the AP-3 pathway.	
IPR006076	FAD dependent	This entry pertains to a group of oxidoreductases that are dependent on flavin adenine dinucleotide (FAD) and includes glycerol-3-phosphate	The primary structure of the flavoprotein D-aspartate oxidase from beef kidney. Negri A, Ceciliani F, Tedeschi G, Simonic

	oxidoreductase	dehydrogenase (1.1.99.5), sarcosine oxidase beta subunit (1.5.3.1), D-amino acid dehydrogenase (1.4.99.1), and D-aspartate oxidase (1.4.3.1). D-amino acid oxidase (1.4.3.3) (DAMOX or DAO) is a flavoenzyme that uses FAD as a cofactor to facilitate the oxidation of neutral and basic D-amino acids, resulting in the formation of corresponding keto acids. DAOs have been identified and sequenced in both fungi and vertebrates, and are primarily found in the peroxisomes.	T, Ronchi S. *J. Biol. Chem.* 267, 11865-71, (1992).