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Deepsea fungi of the eastern tropical Pacific of Costa Rica: morphological, genetic, and enzymatic characterization

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Introduction: Fungal communities have only been studied in a small portion of the vast variety of habitats that exist in deepsea environments, and studies aimed at understanding fungal diversity and function are minimal.

Objective: The aim of this study is to explore both the fungal diversity in deepsea sediments and the enzymatic activities present in them, which are related to the ecological roles of the strains and their biotechnological potential.

Methods: Eighteen sediment samples from three expeditions to deepsea areas of the Eastern Tropical Pacific (ETP) of Costa Rica were analyzed. Fungi were cultured on R2A medium, followed by physical characterization and molecular analysis (ITS and whole-genome sequencing) for the taxonomic identification of the strains. Once pure cultures were established, enzymatic tests for cellobiase, chitinase, lipase, cellulase, peroxidase, and laccase activities were performed, as well as surfactant activity.

Results: Fifty-five fungal strains were isolated, and genetic analysis was conducted on 27 strains, of which 7.41% belong to the Basidiomycota group and 92.59% to Ascomycota. These strains are distributed across 14 species. Among the identified strains are *Periconia* LEGMi281a and *Hortaea* LEGMi415c. Two strains exhibited cellobiase and chitinase activity, one strain exhibited cellulase activity, and one exhibited laccase production. None of the species exhibited lipase or peroxidase activity, and no clear surfactant activity was detected. Whole-genome sequencing revealed significant size differences compared to reference genomes.

Conclusion: The enzymatic activities of the strains suggest they may play a role in the degradation of organic matter and nutrient recycling, similar to terrestrial fungal counterparts. The differences in genome sizes, with the genomes of *Periconia* LEGMi281a and *Hortaea* LEGMi415c being larger than the reference genomes, pave the way for future research into deepsea adaptations, reflected in genetic changes. Additionally, the strains were identified as having high biotechnological potential.

KEYWORDS

marine fungi, Hortaea, Periconia, biotechnological potential, eastern tropical Pacific

1 Introduction

Fungi inhabited the oceans before they conquered terrestrial ecosystems. Considering that the deep ocean represents the largest biome on the planet, there is a scarcity of studies on the diversity and ecology of fungi in these ecosystems compared to the rest of the ocean. Furthermore, what is known about microbial ecology in deepsea sediments primarily concerns bacteria and archaea (Edgcomb et al., 2011; Nagano and Nagahama, 2012; Dekas et al., 2016; Xu et al., 2018). Therefore, detailed knowledge of deepsea fungi is required to understand their contribution to marine food webs and global biogeochemical cycles (Manohar and Raghukumar, 2013; Barone et al., 2018; Drake and Ivarsson, 2018; Grossart et al., 2019; Román et al., 2019; Hassett et al., 2020).

Fungal communities have been studied only in a small portion of the vast variety of habitats that exist in deep waters. Some of these habitats include hydrothermal vents, cold methane seeps, oxygen minimum zones, and those associated with other macronutrients (Nagahama et al., 2011; Zhang et al., 2016; Batista-García et al., 2017; Rojas-Jiménez et al., 2020). Some studies have shown that the marine sediments represents an ecosystem where micro-aerobic respiration occurs and where microbial life is present, even hundreds of meters below the seafloor (D'Hondt, 2002; D'Hondt et al., 2015; Ivarsson et al., 2016a; Nagano et al., 2016).

In general, Ascomycota and Basidiomycota are the most abundant groups in deepsea ecosystems, representing between 70-80% and 10-20%, respectively. Some of the most abundant filamentous fungal genera include *Aspergillus, Cladosporium, Fusarium, and Penicillium*, while among yeasts are *Candida*, *Cryptococcus*, *Metschnikowia*, *Rhodosporidium*, and *Rhodotorula* (Li et al., 2016, 2019; Xu et al., 2016, 2019; Zhang et al., 2016; Nagano et al., 2017; Barone et al., 2018; Wang et al., 2019; Rojas-Jiménez et al., 2020).

In the deep sea, fungi must adapt to the absence of light, low temperatures, and high hydrostatic pressure. Fungi present in deepsea sediments can survive on marine snow, which consists of organic matter derived from photosynthesis that occurs in the photic zone (Bochdansky et al., 2017). In addition to performing aerobic respiration, fungi may be capable of processes such as fermentation, sulfate reduction, methanogenesis (D'Hondt, 2002; Lenhart et al., 2012), and possibly lithoautotrophy (López-García et al., 2003; Nealson et al., 2005; Ivarsson et al., 2016b). Transcriptomic analyses also confirm fungi as active members of deepsea sediments, revealing activities related to carbon and fatty acid metabolism (Pachiadaki et al., 2016). These metabolic processes may be critical for deepsea fungi as it has been observed that with increasing depth, fungal populations exhibit more multitrophic lifestyles (Li et al., 2019).

Considering the vast area to be explored to understand fungal diversity and function in deepsea sediments, it can be said that existing research is sparse and often lacks adequate spatial and temporal resolution (Grossart and Rojas-Jimenez, 2016; Grossart et al., 2019; Morales et al., 2019). As a result, there are many underexplored geographic locations, including the Eastern Tropical Pacific (ETP).

The deep waters of the ETP are particularly important ecosystems in Costa Rica, as they represent over 90% of the national territory (Cortés, 2016, 2019). This area, along the subduction zone of the Cocos and Caribbean plates, features a high diversity of microhabitats (Lizano, 2001; Protti et al., 2012; Rojas and Alvarado, 2012; Cortés, 2016), including cold seeps of methane (Sahling et al., 2008; Levin et al., 2012, 2015), with a high diversity (Seid et al., in prep.), seamounts (Auscavitch et al, in prep.), the Coco Submarine Volcanic Range (Cortés, 2016; Alvarado-Induni, 2021). Previous studies have demonstrated high endemism in the diversity of macro and microorganisms in this region (Rusch et al., 2007; Cortés, 2008, 2019; Levin et al., 2015; Rojas-Jiménez, 2018). Additionally, the Costa Rican ETP is part of the Eastern Tropical Pacific Marine Corridor, that also includes Panamá, Colombia and Ecuador, which represents a crucial site for the conservation and marine regeneration of ETP species (Cortés, 2012). This study analyzed sediment samples from various points in the Costa Rican Exclusive Economic Zone to understand both the fungal diversity in deepsea sediments and the enzymatic activities present, which relate to the ecological role of the strains and their biotechnological potential.

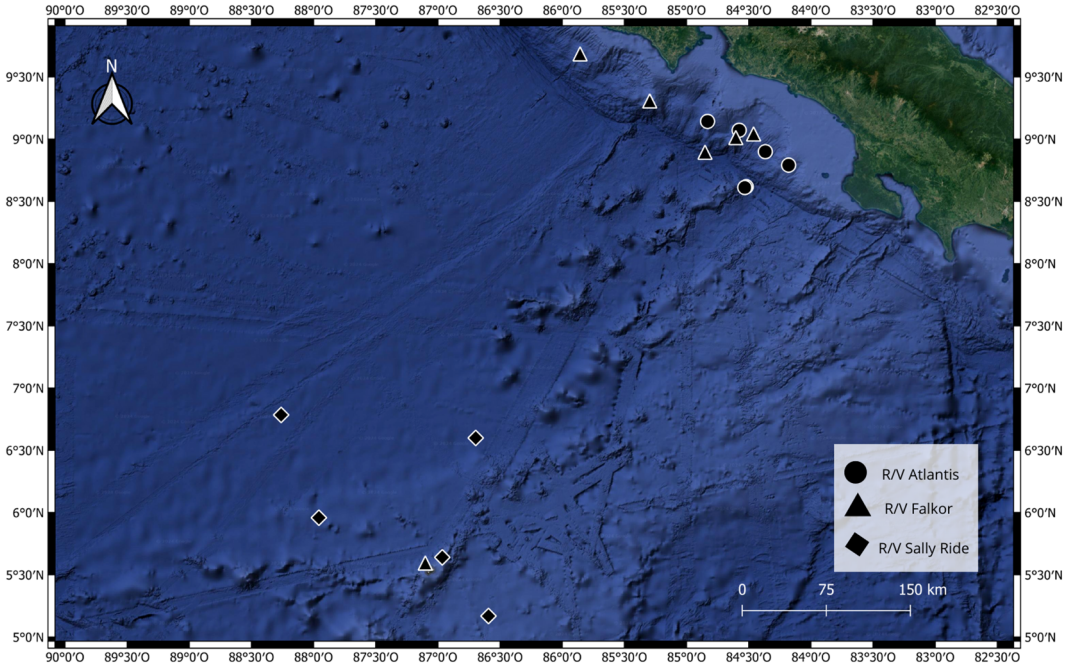

FIGURE 1
Sampling points of the three expeditions from which sediment samples were collected for this research.

2 Materials and methods

2.1 Sample collection

Eighteen sediment samples from three deepsea expeditions in the Eastern Tropical Pacific (ETP) of Costa Rica were analyzed (Figure 1). The first expedition took place from October 24 to November 5, 2018, aboard the R/V Atlantis. It was conducted along the continental shelf edge of Costa Rica, and all samples from this expedition were collected using the manned submersible Alvin. The second expedition occurred aboard the Schmidt Ocean Institute's R/V Falkor in January 2019, covering seamounts from the continental coast to Isla del Coco, with samples collected by the remotely-operated vehicle SuBastian. The third expedition was conducted in December 2021 aboard the R/V Sally Ride. Six samples were processed from each expedition, distributed along a bathymetric gradient of 250 m to 3474 m depth. Each sample consisted of a sediment core approximately 30 cm long. Immediately after collecting the core from the seafloor, a 2 ml aliquot of sediment from the surface of the core, specifically from the first centimeter of the sediment column, was taken. Onboard, the samples were preserved at -40°C, and upon arrival at the onshore laboratory, they were stored at -80°C. The samples were collected with permission from MINAE (SINAC-CUSBSE-PIR-032-2018; R-070-2018-OT-CONAGEBIO) and are housed at the Laboratory of Microbial Ecology and Genetics (LEGMi) of the School of Biology of the University of Costa Rica (UCR).

2.2 Fungal isolation

To isolate the strains present in the sediment, the methodology of Agrawal et al. (2018) was utilized using distilled water. This was because the water collected at the sampling sites where the sediment was gathered was used in other research. For the sediment culture, 100 mg of frozen sediment was diluted in 1 ml of 1X PBS buffer to obtain a homogeneous mixture. Then, a 100 µl aliquot was placed on a Petri dish with culture medium. The medium was prepared by dissolving 4.55 g of R2A agar (Sigma-Aldrich, USA) in 250 ml of distilled water. These cultures were incubated at 28°C until they showed mycelial or yeast-like growth, for 3 weeks. The fungi from each sample were separated by morphospecies for further taxonomic and enzymatic characterization. For liquid cultures, 50 ml of MA+ culture medium (1 g/L yeast extract, 10 g/L malt extract, 1 g/L peptone, 2 g/L $KH_2PO_4$and 1 g/L $MgSO_4$) was inoculated with 4 plugs of solid cultures (5 mm diameter), which were incubated for 7 days at 100 rpm, at room temperature (25°C).

2.3 Taxonomic characterization

Each sample was observed under a light microscope at 100X to identify reproductive or taxonomically significant structures. For observations, semi-permanent mounts of the species were made using polyvinyl, which were stored in the LEGMi laboratory at UCR. In addition to physical characterization, molecular analysis was performed for taxonomic identification of the species. DNA

was extracted from 200 mg of tissue from the isolates using the Power Soil extraction Kit according to the manufacturer's instructions (Qiagen, Carlsbad, CA, United States). The quality and quantity of DNA samples were assessed using the NanoDrop 2000 spectrophotometer.

PCR tests were then performed amplifying the ITS1 and ITS4 regions, with the following reaction conditions: 94°C for 2 minutes for initial denaturation, followed by 32 cycles of 94°C for 15 seconds, 53°C for 15 seconds, 72°C for 30 seconds, and a final extension at 72°C for 5 minutes (Rojas-Jimenez et al., 2017). The generated amplicons were sequenced by Macrogen Inc. Taxonomic assignment was done using BLAST with the GeneBank database from NCBI (Benson et al., 2012). Once the strains were identified, a phylogenetic tree was generated, aligning the sequences using the MAFFT v7 tool (Katoh et al., 2019). A maximum likelihood phylogenetic tree was created using FastTree version 2.1.9 (Price et al., 2010) with a GTR evolution model and a Bootstrap value of 1000. The resulting phylogenetic tree was visualized and edited in MEGA7 (Kumar et al., 2018).

2.4 Genomic characterization of isolates

Based on the sequencing results with the ITS markers and a literature review, two strains identified as *Periconia* sp. LEGMi281a and *Hortaea* sp. LEGMi415c were selected for genome sequencing. This selection was further supported by the intriguing characteristics of their closest known relatives. *Periconia epilithographicola* was only recently described (Coronado-Ruiz et al., 2018), and little is known about its metabolic and enzymatic activities, while *Hortaea werneckii* is recognized for its ability to survive the most extreme salinity ranges (Czachura et al., 2021). DNA was extracted from the selected strains using the same method mentioned earlier and sent to Novogen for sequencing using both Illumina and PacBio technologies. Raw PacBio reads were processed with Samtools v. 1.9 (Heng et al., 2018). The presence of repeats in the genome and unique or low-complexity sequences was identified using the Jellyfish v.2.3.0 tool (Marçais and Kingsford, 2011) and GenomeScope v.1.0 (Vurture et al., 2017) was used to determine the genome size, heterozygosity level, and repeat sequences level. Assemblies were carried out with Canu v.2.2 (Koren et al., 2017) using genome sizes of 46.7 Mbp and 38.2 Mbp for *Periconia* sp. LEGMi281a and *Hortaea* sp. LEGMi415c, respectively. The quality of the genomes was evaluated with Quast v. 5.2.0 (Gurevich et al., 2013), using *P. macrospinosa* (GenBank ID: GCA_003073855.1) and *H. werneckii* (GenBank ID GCA_037044905.1) as references. Additionally, the completeness of each assembly was assessed with BUSCO v.5.4.3 (Simão et al., 2015). Subsequently, the draft assemblies were optimized with Medusa v1.6-2 (Coelho et al., 2021), using different genomes associated with the species as references (Table 1). The same analyses with Quast and BUSCO were carried out with the optimized assemblies to show the improvements. Finally, a genomic alignment of the post-medusa assemblies and their respective references was performed using minimap2 v.2.17-r941 (Li, 2018) and visualized in D-Genies (Cabanettes and Klopp, 2018). The fungal genomes of this project have been deposited at GenBank under the accession number PRJNA1162099.

TABLE 1 Reference genomes used in the assemblies.

Genus of Analyzed Strain	Genbank ID
Hortaea sp.	GCA_037044905.1
	GCA_022701445.1
	GCA_014843535.1
	GCA_014843615.1
Periconia sp.	GCA_003073855.1
	GCA_030378425.1
	GCA_948474695.1
	GCA_023627715.1

2.5 Enzymatic activities

Six different enzymatic activities were evaluated: cellobiase, chitinase, lipase, cellulase, peroxidase, and laccase. For the chitinase, cellobiase, and lipase assays, the substrates used were 4-nitrophenyl N-acetyl-β-D-glucosaminide (Sigma), 4-nitrophenyl acetate (Sigma), and 4-nitrophenyl palmitate (Sigma), respectively. To determine enzymatic activity, 10 mM of each substrate was dissolved in 50 mM of ammonium acetate buffer pH 5, with 0.7% agar, at a temperature of 55°C. A drop of the substrate solution was placed on the mycelium, and a positive result was indicated by a color change from clear to yellow (Rojas-Jiménez and Hernández, 2015; Sandi et al., 2020). Peroxidase, laccase and cellulase activities were determined using degradation assays with Remazol Brilliant Blue (RBBR), 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and Carboxymethyl cellulose (CMC) respectively. These were conducted in a medium consisting of 0.94 g/L KH_2PO_4, 1.9 g/L K_2HPO_4, 1.6 g/L KCl, 1.43 g/L NaCl, 0.15 g/L NH_4Cl, 0.037 g/L $MgSO_4$, 0.1 g/L yeast extract, 10 g/L malt extract, 15 g/L agar, pH 7.0. The substrates were added to the medium as follows: 2% RBBR, 1% ABTS and 0.75% CMC according to (Rojas-Jimenez et al., 2017).

2.6 Surfactant activity

Surfactant activity was assessed using the oil displacement assay, the emulsification index E24 and the blood hemolysis test. The oil displacement assay (ODA) was implemented following the protocol of Morikawa et al. (2000) with slight modifications, as this assay is semi-quantitative, sensitive, reliable, rapid, and simple (Da Silva et al., 2021). Sterile 90 mm diameter Petri dishes were used, where 40 mL of distilled water was placed. On top of the water, 10 µL of commercial canola oil was added in a thin layer using a

micropipette. Subsequently, 10 µL of liquid culture filtrate was placed in the center of the oil layer, and a ruler was used to measure the displacement. Milli-Q water and Tween 20 (v/v) were used as negative and positive controls, respectively. Three technical replicates were evaluated for each sample.

The emulsification index E24 (E24%) was determined using the Cooper and Goldenberg (1987) protocol. Conventional 10 x 100 mm test tubes were rinsed with 70% ethanol and Milli-Q water, and 2 mL of commercial canola oil and liquid culture filtrate were added to each tube. Each tube was vigorously shaken for two minutes and incubated at room temperature for 24 hours undisturbed. After this time, the E24% was calculated by dividing the height of the emulsified layer.

Finally, the blood hemolysis test (BHT) was performed to evaluate hemolytic activity. YPG plates supplemented with 5% (v/v) blood (donated by the School of Veterinary Medicine at UNA) were used; each plate was inoculated in the center with a 6 mm plug of mycelium grown in R2A growth medium and incubated at 37°C for five days. After this time, the diameter of the hemolysis halo was measured according to Thavasi et al. (2011). At least six repetitions were performed for each isolate, with an uninoculated plate serving as a negative control.

3 Results

3.1 Taxonomic assignment of isolates

In this study, 55 fungal strains were isolated from sediment samples from the deepsea floors of the Eastern Tropical Pacific off Costa Rica. Molecular analysis was performed on 27 of these isolates, of which 7.41% belong to the Basidiomycota group and 92.59% to Ascomycota. These strains are distributed among 14 species covering 8 orders: Xylariales (the most abundant with 7 isolates), Amphisphaeriales, Eurotiales, Cladosporiales, Capnodiales, Pleosporales, Polyporales, and Serinales (Figure 2). One of the most noteworthy isolates was a melanized (black) yeast identified as *Hortaea* sp. LEGMi415c, which represents a new record for marine fungal diversity in Costa Rica. Similarly, the presence of *Periconia* sp. LEGMi281a was noted for the first time in deepsea.

3.2 Morphological description

Hortaea sp. LEGMi415c exhibited yeast-like and filamentous growth. Macroscopically, the colonies grow slowly (2 weeks), in a

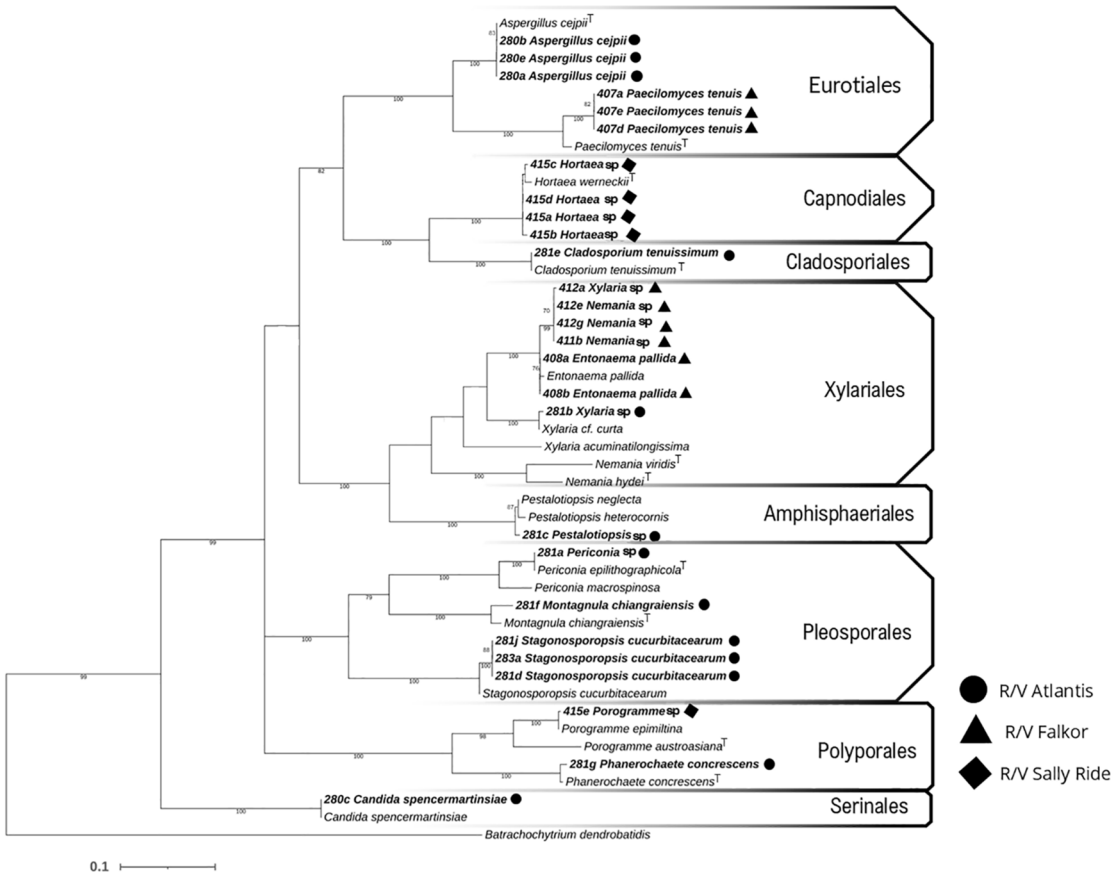

FIGURE 2
Phylogenetic relationship with maximum likelihood based on ITS markers, using the GTR model. Bootstrap values at nodes based on 1000 iterations, visualized in MEGA7.

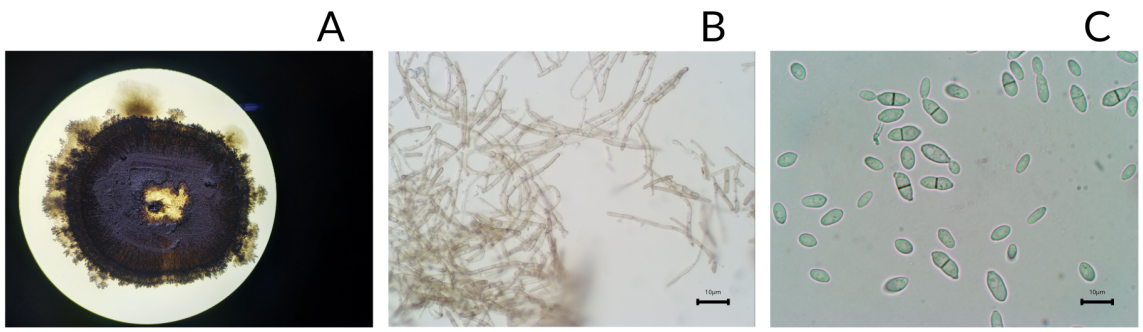

FIGURE 3
Photographs of *Hortaea* sp. LEGMi415c. Macroscopic view of the colony on a Petri dish **(A)**, microscopic view of mycelial growth **(B)**, microscopic view of yeast-like growth **(C)**. The microscopy images were taken at 100X with immersion oil.

circular arrangement, with black/greenish-brown, mucous, shiny, and moist (Figure 3A). After 3 to 4 weeks, filamentous growth with brown pigments is observed at the colony's periphery (Figure 3B). Microscopically, yeast-like cells were mostly observed as two-celled hyaline individuals, separated by a defined septum in the center of the transverse axis (Figure 3C).

Periconia sp. LEGMi281a was characterized by growing on a plate with pink pigment mycelium. In the first week of growth, the

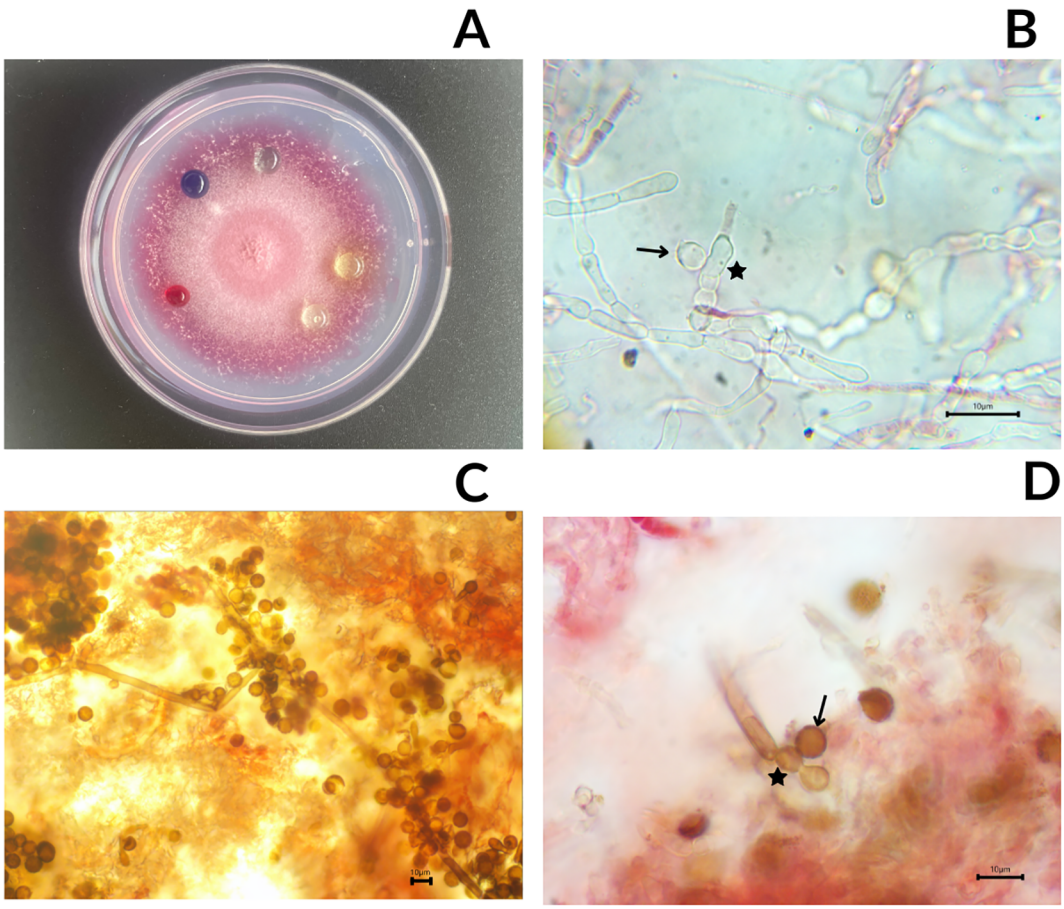

FIGURE 4
Photographs of *Periconia* sp. LEGMi281a. Macroscopic view of the colony on a Petri dish with drops from enzymatic assays **(A)**, microscopy of immature spore marked with an arrow, in a conidiogenous cell marked with a star **(B)**, microscopy of conidiophores **(C)**, microscopy of mature spore marked with an arrow, in a conidiogenous cell marked with a star **(D)**. The microscopy images were taken at 100X with immersion oil.

mycelium was white, and around day 7, the first signs of pink coloration were observed (Figure 4A). Isolates grown on R2A did not show sporulation. However, two cells were observed with signs of being immature spores (Figure 4B), but the cultures did not develop further and did not reach sporulation. However, when these isolates were transferred to PDA, the specimens sporulated with spores averaging 8.5 µm in diameter (n=12) (Figure 4C, D).

3.3 Enzymatic activities

None of the 27 analyzed strains showed lipase or peroxidase activity. The only species with laccase activity was *Hortaea* sp. LEGMi415c, which exhibited a color change in the form of a halo with purple-blue tones after two weeks of growth. Regarding cellulase activity, only *Periconia* sp. LEGMi281a exhibited it, evident by observing colorless spots after staining the medium (with CMC) using Congo Red. Both species showed chitinase and cellobiase activity, with color changes from transparent to yellow in less than 10 minutes, indicating strong enzymatic activity.

3.4 Surfactant activity

Neither in the ODA test nor in the E24% test did *Periconia* sp. LEGMi281a show any activity. In the ODA tests, *Hortaea* sp. LEGMi415c exhibited partial displacement of the oil droplet, measuring 1.5 cm, 1.5 cm, and 2 cm in each of the replicates. In the E24% tests, two out of three replicates showed an emulsification index, with values of 31.58% and 26.32%. In the BHT tests, all three replicates of *Hortaea* sp. LEGMi415c were negative for hemolytic activity.

3.5 Genomic characteristics

Initially, taxonomic classifications using only ITS markers identified the closest matches as *Periconia epilithographicola* and *Hortaea werneckii*. However, full genome analysis of the strains revealed discrepancies in the genomic assembly sizes compared to reference genomes (Table 2). The genomic size of *Periconia* sp. LEGMi281a is 68.5 Mbp, showing a difference of more than 10 Mbp compared to reference genomes, such as *Periconia macrospinosa*, which has a size of 55 Mbp. In the case of *Hortaea* sp. LEGMi415c, the size is 47.7 Mbp, which is also larger than the reference genome of *Hortaea werneckii* at 38.2 Mbp.

4 Discussion

In a previous metagenomic study, 787 ASVs (Amplicon Sequence Variants) were identified in 40 sediment subsamples from deep areas of the Costa Rica ETP (Rojas-Jiménez et al., 2020). These findings anticipated a high fungal diversity and potential importance of these microorganisms to the ecosystem, which was later confirmed by the isolation of 27 strains in the present study. Similar research in the Indian Ocean reported 39 species with culture-dependent methods and 91 OTUs with molecular methods (Singh et al., 2012; Zhang et al., 2014). Molecular methods consistently reveal greater diversity than culture-dependent ones. This discrepancy arises because most microorganisms, including deepsea marine fungi, are not cultivable due to differences between culture conditions and their natural habitat (Lewis et al., 2021). While it is expected that adjusting variables such as temperature, pressure, and salinity in future studies will lead to the isolation of more strains, including new species, all isolates found in this study, regardless of identification at genus or species level are of great importance as they represent 14 new records of deep marine fungi for the country. This is a significant step in understanding the diversity of the deep ocean, particularly its mycobiota, as marine fungi account for less than 1% of known species (Raghukumar et al., 2010).

Fungi are crucial for nutrient recycling and energy flow in marine ecosystems, functioning as saprotrophs, mutualists, endobionts, and parasites (Jones et al., 2019; Grossart et al., 2019). However, their specific roles in deepsea ecosystems remain unclear, underscoring the need for isolates to study their physiology and ecological functions. Some species from this study, also found in terrestrial environments, have been reported in other marine ecosystems. For example, *Aspergillus cejpii* was found in

TABLE 2 General genomic characteristics of the sssemblies.

Genomic Data	*Hortaea* sp.LEGMi415c pre- Medusa	*Hortaea* sp.LEGMi415c post- Medusa	*Hortaea werneckii**	*Periconia* sp.LEGMi281a pre- Medusa	*Periconia* sp.LEGMi281a post- Medusa	*Periconia macrospinosa***
Coverage	99.7 X	99.7 X	100.0 X	–	–	139.X
N° of contigs	1362	269	186	1703	1215	2976
Genome Size	47.6 Mbp	47.7 Mbp	38.2 Mbp	68 Mbp	68.5 Mbp	55Mbp
Contig N50	47.5 Kb	356.3 kb	651.3 kb	51.6 Kbp	156.4 Kbp	94.2 Kbp
Contig LG50	322	41	18	241	82	163
GC %	53.39	53.39	53	49.91	49.91	47.24

*Reference: GenBank Assembly: GCA_037044905.1.
**Reference: GenBank Assembly: GCA_003073855.1.

hydrothermal vents (Pacheco, 2024), and *Stagonosporopsis cucurbitacearum* was isolated from a marine sponge (Haga et al., 2013). These strains have known saprotrophic activity in terrestrial environments (Edgcomb et al., 2011), so given their adaptations to osmotic stress and polysaccharide degradation (Gonçalves et al., 2022), it is hypothesized that these fungi primarily act as saprotrophs in sediments, recycling nutrients from marine snow and deepsea fauna waste.

The genus *Hortaea*, from the order Capnodiales, is commonly isolated from soil, marine products like dried fish, dust, wood, compost, and human skin (Prieto-Granada et al., 2010). While reported in marine ecosystems such as the Mediterranean water column, deepsea stations Vector and Geostar, and the Rainbow deep hydrothermal vent (Romeo et al., 2020; Cochereau et al., 2023; De Leo et al., 2019), it has not been recorded in Latin American deepsea ecosystems, making this a new report for the region.

Hortaea includes the most halotolerant fungus known, capable of growing in salinities from 0% to 32% NaCl (Butinar et al., 2005; Czachura et al., 2021). Melanin production is crucial for this halotolerance, acting as an osmoregulatory agent and maintaining cell wall integrity (Kejžar et al., 2013). The fungus alternates between yeast-like and mycelial phases (Butinar et al., 2005; Czachura et al., 2021). At low temperatures (15 ± 5°C), yeast-like growth predominates (Elsayed et al., 2016). Given that deepsea temperatures are around 2°C, *Hortaea* sp. LEGMi415c likely exhibits yeast-like growth in these ecosystems. Conversely, at high temperatures (30 ± 5°C), mycelial growth predominates, but yeast-like growth can occur even at elevated temperatures in the absence of NaCl (Elsayed et al., 2016). This explains the predominant yeast-like growth observed at 28°C without added NaCl. However, the transition to mycelial growth over time may be due to nutrient depletion, as mycelial growth allows for better nutrient exploration and absorption. Further research is needed to understand how temperature, salinity, and nutrient interactions affect the growth forms of *Hortaea* sp. LEGMi415c.

Hortaea sp. LEGMi415c exhibited laccase, chitinase, and cellobiase activities, suggesting that this strain contributes to nutrient recycling in the ecosystem. Laccases can degrade complex aromatic compounds and the ones previously reported in marine fungi can maintain their degradative capacity in marine ecosystems (Ali et al., 2020a, b; Xu et al., 1996; Bonugli-Santo et al., 2010). On the other hand, chitinase recycles nitrogen and carbon from chitin, which is found in crustacean exoskeletons and polychaetes, common in deepsea environments (Kellner et al., 2010; Choi et al., 2022). Additionally, chitinases play a role in growth modulation and spore formation and germination (El Gueddari et al., 2002; Karlsson and Stenlid, 2008). These enzymatic activities suggest that *Hortaea* sp. LEGMi415c is metabolically active in deepsea ecosystems, participating in nutrient recycling through both surface biomass and remains of deepsea organisms.

From a biotechnological perspective, laccases are highly versatile and have applications in the textile, pharmaceutical, materials industries, biomedicine, and bioremediation due to their ability to degrade complex compounds (Chaudhary et al., 2022). Chitinases are used in agricultural biocontrol and in the pharmaceutical industry (Sharma et al., 2023), and cellobiase is important for ethanol production (Li et al., 2020). The present study found extracellular activities of *Hortaea* sp. LEGMi415c, such as laccase production, despite other studies on *Hortaea* isolates showing no extracellular enzymatic activity with 0% NaCl (Rizk and Magdy, 2022). This suggests enzymatic plasticity related to environmental conditions. The ability to produce enzymatic extracts without adjusting salinity is advantageous industrially (Mesbah, 2022), highlighting the industrial potential of deepsea microorganisms. Although ITS markers classified the strain as *Hortaea werneckii* and the isolated strain shows similar behaviors, whole-genome analyses reveal significant discrepancies, with genome sizes differing by 9.5 Mbp. Further analysis is needed to determine if these differences indicate adaptations to deepsea ecosystems and if the strain represents a new species.

The genus *Periconia* is polymorphic and belongs to the order Pleosporales. Species of this genus are commonly isolated from plant samples, as endophytes (Su et al., 2023), and from marine and freshwater ecosystems (Morrison-Gardiner, 2002; Cai et al., 2002). This is the first report of a *Periconia* species in deepsea marine environments. Differences in genome sizes and GC percentages indicate that the strain isolated from deepsea sediments differs from other reference species. As there is no complete genome sequencing for *P. epilithographicola* at the moment of this study, it was not included in the genomic analysis, but the morphological description of the isolated strain aligns with *P. epilithographicola*, supporting its taxonomic classification based on ITS markers.

P. epilithographicola was initially discovered on 19th-century lithographs (Coronado-Ruiz et al., 2018). The natural history of the genus explains why *Periconia* sp. LEGMi281a, despite being isolated from an environment without cellulose production, is the only strain analyzed with cellulolytic capability. Cellulose is the most abundant natural polysaccharide, and marine fungi can degrade it (Le Strat et al., 2022). Degrading this polymer is advantageous since a significant portion of terrestrial cellulose ends up in the deep sea, and algal cellulose reaches deepsea areas through marine snow (Tsudome et al., 2022). Cellulase activity may provide a competitive edge by utilizing this carbon source, unavailable to other fungi without these enzymes. Additionally, the chitinase and cellobiase activities of *Periconia* sp. LEGMi281a suggest it, like *Hortaea* sp. LEGMi415c, plays a role in degrading organic remains of deepsea organisms.

Since this species was only described six years ago, there is limited research on its biotechnological potential. However, it has been reported that its presence in a community increases in the presence of humic acids (Da Silva et al., 2022), so due its cellulase, chitinase, and cellobiase activity, this species could be a candidate for bioremediation studies. Other species within the genus are already considered for biocontrol due to the presence of chitinases (Sharma et al., 2023), suggesting that *Periconia* sp. LEGMi281a could also be used in this area in the future. Additionally, its potential to maintain these activities under low temperatures and high hydrostatic pressure highlights its significant biotechnological potential, warranting further exploration.

This research indicates that the deepsea sediments in the ETP harbor rich fungal communities that deserve more detailed investigation. Comparisons with molecular studies show common microorganisms in these communities that were not isolated. For example, the genus *Metschnikowia* was the most abundant in several samples from a previous metagenomic study (Rojas-Jiménez et al., 2020) but was not present in the isolates from this study. This underscores the high potential for discovering new species.

The fungi isolated from the sediment samples suggest important ecological roles and physiological traits with biotechnological applications. These microorganisms possess enzymes with a wide range of industrial uses and may be more resilient than other deepsea fungi (Mahmoudi et al., 2020). Moreover, their ability to grow under regular environmental conditions (1 atm and 25°C) implies that their industrial production would not require high-energy systems like cooling or pressure-increasing systems.

The results raise questions about the role of these species in deepsea ecosystems. Obligate marine fungi can only grow and sporulate in marine ecosystems, while facultative fungi can thrive in other habitats due to their physiological adaptations (Kohlmeyer and Kohlmeyer, 1979). Since *Periconia* and *Hortaea* are facultative marine fungi, further research is needed on the ecological roles and enzymatic activities of *Periconia* sp. LEGMi281a and *Hortaea* sp. LEGMi415c in marine environments. Additionally, it is essential to understand the evolutionary significance of metabolic traits thought to be selected by terrestrial pressures in a deepsea context. The industrial and biomedical relevance of these microorganisms, alongside the gaps in our knowledge about their ecology and natural history in deepsea ecosystems, supports the need for conservation of these habitats.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

IR-R: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft. SS-G: Data curation, Investigation, Methodology, Writing – review & editing. JC: Conceptualization, Data curation, Investigation, Writing – review & editing. KR-J: Data curation, Investigation, Methodology, Writing – review & editing, Conceptualization, Project administration, Supervision.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The author(s) declare that no Generative AI was used in the creation of this manuscript.

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