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Fungi associated to pollution sources in Mandinga Lagoon, Veracruz, Mexico and their bioactive potential

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ABSTRACT

Introduction: The Mandinga Lagoon System, located in the state of Veracruz, Mexico, is under significant demographic pressure due to urban expansion around the municipalities of Boca del Río, Medellín and Alvarado. Aquatic fungi are known to respond to pollution, maintaining and adapting their life cycles to obtain their carbon source by decomposing organic matter.

Objectives: To isolate and identify fungi from sampling sites associated with punctual sources of contamination and explore their antiproliferative activity against solid tumor cell lines.

Methods: Two liters of water were collected in four sampling sites. The isolation of filamentous fungi was made on Potato Dextrose Agar by plate streaking method. Monosporic isolates were identified using taxonomic keys, and molecular identification was made using the primers ITS1F/ITS4. Fungal extracts were obtained from biomass and culture medium, and their antiproliferative activity was evaluated against two human solid tumor cell lines.

Results: Seven fungal strains belonging to five different genera of Ascomycetes were isolated: *Neopestalotiopsis, Cladosporium*, *Fusarium*, *Purpureocillium* and *Penicillium*. Hexane and chloroform broth-extracts from *Purpureocillium lavendulum* showed the highest activity against A549 and HeLa cell lines (GI_{50} = 3 µg/ml and GI_{50} = 12 µg/ml, respectively). Meanwhile, the chloroform biomass-extract from *Penicillium* sp. and ethyl acetate broth-extract from *Fusarium oxysporum* showed GI_{50} values < 50 µg/ml.

Conclusions: The ability of fungi to produce secondary metabolites as response to environmental stress represents an opportunity to perform bioprospecting studies. The results in this study indicate that *P. lavendulum* is a promising fungus to continue the bioguided separation of bioactive compounds with antiproliferative activity.

Key words: aquatic fungi; ITS sequencing; NCI protocol; fungal extracts; bioactive fungi; fungal bioprospecting.

RESUMEN

Hongos asociados a fuentes de contaminación en la Laguna de Mandinga, Veracruz, México y su potencial bioactivo

Introducción: El Sistema Lagunar de Mandinga, en el estado de Veracruz, México, está bajo presión demográfica significativa debido a la expansión urbana en los municipios de Boca el Río, Medellín y Alvarado. Los hongos acuáticos son conocidos por su respuesta a la contaminación, manteniendo y adaptando sus ciclos de vida para obtener su fuente de carbono descomponiendo materia orgánica.

Objetivos: Aislar e identificar hongos cultivables de sitios de muestreo asociados a fuentes puntuales de contaminación y explorar su actividad antiproliferativa contra líneas celulares de tumores sólidos.

Métodos: Dos litros de agua fueron recolectados en cuatro sitios de muestreo. El aislamiento de hongos filamentosos fue realizado en Agar Papa Dextrosa por el método de estriado en placa. Los cultivos monospóricos fueron identificados usando claves taxonómicas y la identificación molecular se realizó utilizando los cebadores ITS1F/ITS4. Los extractos fúngicos se obtuvieron de biomasa y caldos de cultivo y su actividad antiproliferativa se evaluó contra dos líneas celulares de tumores sólidos.

Resultados: Fueron aisladas siete cepas fúngicas pertenecientes a cinco géneros de Ascomicetos: *Neopestalotiopsis, Cladosporium*, *Fusarium*, *Purpureocillium* y *Penicillium*. Los extractos hexánicos y clorofórmicos del caldo de *Purpureocillium lavendulum* mostraron la actividad más alta contra las líneas celulares A549 y HeLa (GI_{50} = 3 µg/ml, GI_{50} = 12 µg/ml respectivamente). Mientras que, el extracto clorofórmico de biomasa de *Penicillium* sp. y el extracto de acetato de etilo del caldo de *Fusarium oxysporum* mostraron valores de GI_{50} < 50 µg/ml.

Conclusiones: La capacidad de los hongos de producir metabolitos secundarios en respuesta a estrés ambiental representa una oportunidad para realizar estudios de bioprospección, por lo que, *P. lavendulum* es considerado un hongo prometedor para continuar la separación biodirigida de compuestos bioactivos.

Palabras clave: hongos acuáticos; secuenciación ITS; protocolo del NCI; extractos fúngicos; hongos bioactivos; bioprospección fúngica.

INTRODUCTION

The coastal lagoons of the Gulf of Mexico are dynamic and highly productive ecosystems with significant biodiversity, providing essential environmental services to humans. For example, they help mitigate flooding, control erosion, retain sediments, nutrients and toxic substances, store carbon, provide food, transportation and recreational services. They also contribute to ecosystem stability and integrity, as well as carbon dioxide retention. The Sustainable Development Goals (SDGs) highlight coastal ecosystems as some of the most valuable globally due to their ecological functions and role as habitats for high biodiversity (Ortiz-Lozano et al., 2010; Rivera Arriaga et al., 2010).

In recent years, the Mandinga Lagoon System (MLS) has been under intense demographic pressure due to urban expansion in the municipalities of Boca del Río, Medellín and Alvarado. These municipalities surround the ecosystem with urban and tourism development activities, resulting in a land-use change from mangrove areas to residential and commercial zones. The latter could cause alterations in the water quality of MLS by wastewater and negative impact activities such as aquaculture, fishing and tourism in the region. In addition, the biological risk associated with wastewater is related to pathogens that can be transported by these waters as bacteria, viruses and fungi (Farkas et al., 2020; Numberger et al., 2022; Ezeonuegbu et al., 2022), that could lead to serious public health issues. Although aquatic fungal communities respond to pollution, only a few studies have quantitatively assessed the effect of freshwater pollution on fungal diversity and composition in tropical systems (Ortiz-Vera et al., 2018).

Aquatic fungi can remain their whole life cycle or part of it on an aquatic ecosystem.

Fungal communities provide ecological services as organic matter decomposers, since they have the ability of obtain the carbon source from several biomolecules as carbohydrates, lipids, proteins and aromatic hydrocarbons (Grossart et al., 2019). Moreover, aquatic fungi represent a valuable source for the discovery of novelty chemical structures with potential biomedical application (Grossart & Rojas-Jimenez, 2016; Patridge et al., 2016). In this context, the antiproliferative and cytotoxic activities of crude extracts from aquatic fungi such as *Acremonium* sp., *Aspergillus* sp., *Cladosporium* sp., *Pestalotiopsis* sp., *Nigrospora* sp., and *Ochroconis* sp. have been reported against cancer cell lines, including Hep G2 (liver), HeLa (cervix), SW 1573, A-549 (lung), A-431 (skin), MCF 7, HBL-100, and T-47D (breast cancer) (Espinoza et al., 2021; Ameen et al., 2022). Moreover, compounds such as trichodermamide B, DC1149B, and nafuredin A have been isolated from *Trichoderma lixii*, a marine-derived fungus, through bioassay-guided fractionation, exhibiting strong antiproliferative activity against cancer cell lines (Sirimangkalakitti et al., 2024).Therefore, this study was aimed to identify cultivable aquatic fungi from sampling sites associated to punctual sources of contamination and to explore the antiproliferative activity of the isolated fungi against solid tumor cell lines in order to determine their bioactive potential to continue the bioprospecting study.

MATERIALS AND METHODS

Study area: The MLS is located in the central region of the state of Veracruz, Mexico. It comprises Laguna Larga, also known as the estuary, Laguna Chica, and Laguna Grande. The system is located between coordinates 18°58' and 19°06' N and 96°01' and 96°08' W, in Boca del Río, Veracruz, and covers an approximate area of 3 250 hectares.

Four sampling sites were selected (Table 1) in adjacent areas to the punctual sources of contamination identified within the MLS (Fig. 1).

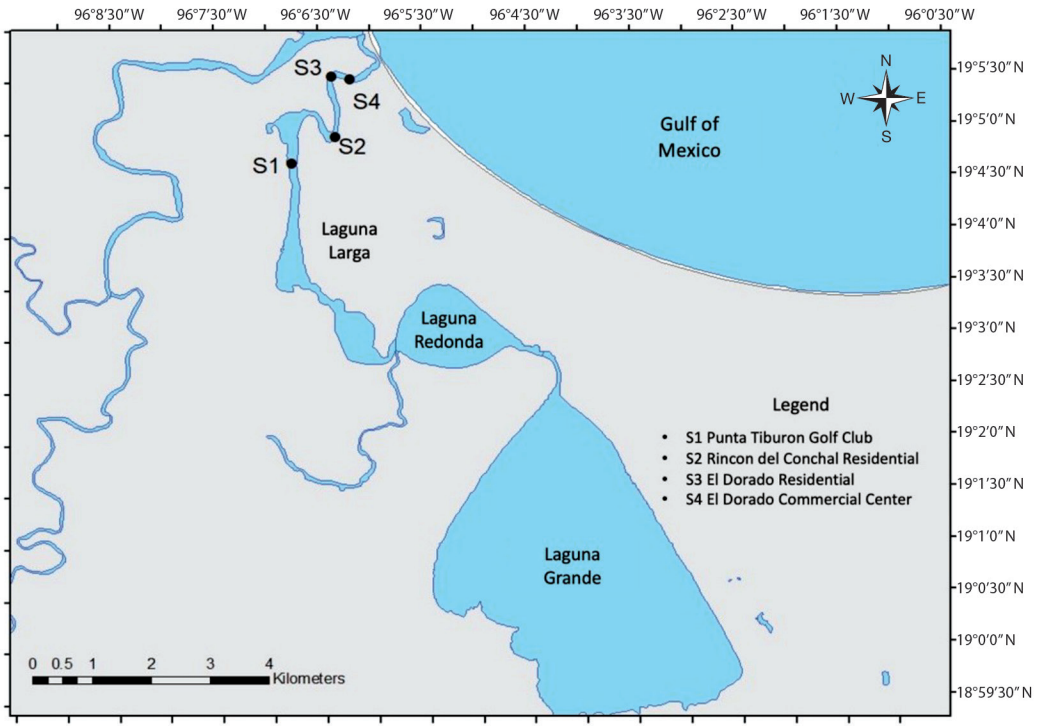

Fig. 1. Location of sampling sites in the Mandinga Lagoon System, Veracruz, Mexico.

Table 1
Geographic location of sampling sites in the Mandinga Lagoon System, Veracruz, Mexico.

Site	Description	Latitude (N)	Longitude (W)
S1	Punta Tiburón Golf Club	19°04'41"	96°06'44"
S2	Rincón del Conchal Residential	19°04'57"	96°06'21"
S3	El Dorado Residential	19°05'31"	96°06'22"
S4	El Dorado Commercial Center	19°05'30"	96°06'12"

These sites were strategically chosen to assess the impact of anthropogenic activities on water quality and ecosystem health. Two liters of water were collected from each sampling site at a depth of 10 to 15 cm below the surface. The samples were preserved at 4 °C until analysis in the laboratories of Ecotechnologies at the Instituto Tecnológico de Boca del Río and the Centro de Investigación en Micología Aplicada at the Universidad Veracruzana.

Fungal isolation: The isolation of filamentous fungi was performed using the plate streaking method. A volume of 250 µL of the sample was inoculated onto the surface of Petri dishes containing Potato Dextrose Agar (PDA) (MCD LAB) supplemented with gentamicin (0.2 g/L) and ampicillin (0.2 g/L). For samples with abundant growth, serial dilutions ranging from 10^{-5} to 10^{-6} were prepared. From each dilution, 100 µL was taken and plated onto PDA, followed by incubation at 25 ± 2 °C for 7 days.

Monosporic culture: To ensure the purity of fungal isolates, monosporic cultures were prepared from fresh mycelium. Hyphal tips were excised using a scalpel under a stereomicroscope (Leica EZ4) and transferred to Petri dishes containing PDA. These were incubated at 25 ± 2 °C for 7 days. The resulting pure isolates were stored at 4 °C until identification.

Morphological identification: Monosporic isolates were identified to the genus level by comparing reproductive structures with those described in taxonomic keys by Barnett and Hunter (1998). Samples were prepared using the imprint technique, which involved placing a drop of lactophenol blue on a glass slide and attaching a mycelial sample. The samples were observed under a Carl Zeiss optical microscope (GmbH, Oberkochen, Germany) using 40X and 100X objectives to analyze their reproductive structures (Lumbreras-Martínez et al., 2018).

Molecular identification: Genomic DNA was extracted from fresh mycelium of each isolate following the protocol described by Liu et al. (2000). The Internal Transcribed Spacer (ITS) region from nuclear ribosomal DNA was amplified by PCR (BioRad T100, Thermal Cycler) using the primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990; Gardes & Bruns, 1993). PCR conditions consisted of an initial denaturation at 94 °C for 3 min, followed by 34 cycles of 94 °C for 45 sec, 53 °C for 45 sec, and 72 °C for 1 min, with a final extension step at 72 °C for 10 min. The amplicons (604 bp for CIMA-IL006 and 560 bp for the other isolates) were purified using the Wizard® SV Gel and PCR System Clean Up kit (Progema) and sequenced by Sanger sequencing (Labsergen Langebio, Irapuato, Gto. Mexico). The obtained sequences were edited using BioEdit software (Hall, 1999) and subsequently compared against the NCBI GenBank database (Benson et al., 2013) using the Basic Local Alignment Search Tool (BLAST) (Zhang et al., 2000) for taxonomic assignment and determination of percentage identity.

Phylogenetic analysis: The ITS sequence of isolate with the highest bioactivity (CIMA-IL006) was aligned with eight related sequences from GenBank using MAFFT (Katoh et al., 2019), with *Drechmeria zeospora* CBS:335.80

Table 2
Fungal species, voucher specimen codes, GenBank accession numbers, and countries of origin of the sequences included in the phylogenetic analysis of CIMA-IL006 strain.

Species	Voucher	GenBank Number	Country
Purpureocillium lavendulum	CBS:128677	MH864976	Spain
Purpureocillium lilacinum	CBS:284.36	MH855800	USA
Purpureocillium roseum	IOM 325363.2	MT560196	N/D
Purpureocillium sodanum	IBRC-M 30175	KX668542	N/D
Purpureocillium takamizusanense	BCC49261	MK632046	Thailand
Purpureocillium lavendulum	Dak Lak	OQ402318	Vietnam
Purpureocillium lavendulum	HF2S15	OP179025	France
Purpureocillium lavendulum	CaFI1	MZ545387	Panama
Purpureocillium lavendulum	CIMA-IL006	PV804120	Mexico
Drechmeria zeospora	CBS:335.80	MH861269	Iraq

N/D: No data.

designated as the outgroup (Table 2). The dataset was analyzed using MEGA v7.0.26 (Kumar et al., 2016) and Mesquite v3.61 (Maddison & Maddison, 2019). The TPM3uf+G nucleotide substitution model was selected with jModelTest 2 (Darriba et al., 2012) under the Akaike Information Criterion (AIC). Maximum likelihood (ML) analysis was performed using raxmlGUI v2.0 (Edler et al., 2020), while Bayesian inference was conducted with MrBayes v3.2 (Ronquist et al., 2012). Bootstrap support was estimated with 1 000 replicates, and posterior probabilities were calculated with 5 million generations. The resulting phylogenetic trees were visualized in FigTree v1.4.4 (Rambaut, 2016), with nodes supported by bootstrap values (BS) ≥ 70 % and posterior probabilities (BPP) ≥ 0.9 considered strongly supported. The ITS sequence of *Purpureocillium lavendulum* CIMA-IL006 was deposited in the GenBank database.

Fungal extracts: A liquid culture (600 ml) was prepared from the pure strains using Potato Dextrose Broth (PDB) (20 %) enriched with dextrose (2 %). The medium was inoculated with 3 ml of a preinoculum from a 14-day-old fresh mycelial culture. The culture was incubated in the dark at 25 ± 2 °C for 14 days with continuous shaking at 150 rpm, followed by 7 days in a static environment. After the incubation period, the biomass was separated from the culture medium by vacuum filtration. The culture medium (broth) was subjected to liquid-liquid extraction with ethyl acetate. On the other hand, the biomass was lyophilized and subjected to multiple maceration extractions in an ascending polarity gradient using hexane, chloroform, ethyl acetate and methanol as solvents.

Antiproliferative activity assay: The NCI protocol (National Cancer Institute, United States of America) with modifications was employed to evaluate the antiproliferative activity of fungal extracts from biomass and culture medium against two human solid tumor cell lines: A549 (lung) and HeLa (cervix). Cells grown in RPMI-1640 medium supplemented with 5 % FBS, 2 mM L-glutamine, 100 U/ml of penicillin G and 0.1 mg/ml of streptomycin (37 °C, 95 % humidity, 5 % CO_2) were seeded in 96-well microplates (5 000 cells per well) 24 h before the addition of fungal extracts. A stock solution of fungal extracts was prepared in DMSO at 100 mg/ml. Control cells (negative control) were exposed to an equivalent concentration of DMSO (0.25 % v/v). Cell lines were exposed to fungal extracts during 48 h, afterward cells were fixed with ice cold trichloroacetic acid (50 % w/v) for 1 h at 4 °C. Following that, the Sulforhodamine B (SRB) assay

was performed (Orellana & Kasinski, 2016) and the unbonded SRB was solubilized with Tris Base (10 mM). The Optical Density (OD) was measured at dual wavelength (540/620 nm) using a microplate reader. The antiproliferative activity was expressed as GI_{50} (50 % growth inhibition) and was calculated using the NCI formulae (Monks et al., 1991). Triplicates were performed with extracts from the fungal strain that exhibited GI_{50} values ≤ 50 µg/ml.

RESULTS

Morphological description of fungal strains: The isolated fungal strains were morphologically identified as belonging to five different genera of Ascomycetes. At site S2, the origin of the punctual source of contamination (PSC) was domestic water and two isolates with morphological characteristics of the genera *Neopestalotiopsis* sp. (CIMA-IL001) and *Cladosporium* sp. (CIMA-IL002) were identified.

***Neopestalotiopsis* sp.:** White, rugose mycelium with rapid growth. Conidia are fusiform with three pigmented central cells and hyaline basal cells, featuring three apical setulae and a basal pedicel (Fig. 2).

***Cladosporium* sp.:** Velvety mycelium with olive green, gray, brown, or dark brown coloration and radial folds. Thin, septate, branched brown hyphae were observed. Hyphae support branched chains of unicellular, ellipsoidal, or cylindrical conidia, some lemon-shaped due to attachment scars (Fig. 2).

At site S4, the origin of the PSC was domestic and industrial wastewater, and five isolates were identified belonging to the genera *Fusarium* sp. (CIMA-IL003), *Cladosporium* sp. (CIMA-IL004/CIMA-IL005), *Purpureocillium* sp. (CIMA-IL006) and *Penicillium* sp. (CIMA-IL008).

***Fusarium* sp.:** White, cottony mycelium on the front, with pale yellow-orange coloration on the reverse. Reproductive structures included ellipsoidal microconidia and wedge-shaped, septate macroconidia (Fig. 2).

***Purpureocillium* sp.:** Bulky, cottony mycelium, white in color, which develops a purple pigment as it ages. Reproductive structures resemble those of the genus *Penicillium* (Fig. 2).

***Penicillium* sp.:** White mycelium that later turns grayish green, retaining a white, velvety, powdery edge. Branched biverticillate conidiophores, phialides, and smooth, ellipsoidal conidia arranged in columns (Fig. 2).

Molecular identification and phylogenetic analyses: Sequence comparison against the GenBank database, supported by morphological characterization, allowed the identification of isolates CIMA-IL002 as *Cladosporium xanthochromaticum* (MF473319), CIMA-IL003 as *Fusarium oxysporum* (MT560380), CIMA-IL004 as *Cladosporium cladosporioides* (MT466517), and CIMA-IL006 as *P. lavendulum* (OQ402318), all exhibiting 100 % sequence identity, 100 % coverage, and E-value of 0.0, indicating that these matches are highly significant and confirm their placement within the respective clade. Conversely, isolates CIMA-IL001, CIMA-IL005, and CIMA-IL008 showed identity values below 98 % (100 % coverage, E-value = 0.0), precluding species-level identification based on ITS1F/ITS4 amplification. The ML phylogenetic tree showed that *P. lavendulum* CIMA-IL006 clustered with robust support (BS = 100 %; BPP = 1) alongside other *P. lavendulum* sequences, further confirming its taxonomic placement (Fig. 3).

Antiproliferative activity: The antiproliferative activity of four fungi was evaluated from which, were tested a total of 20 fungal extracts, four extracts from culture medium (broth) and 16 extracts from biomass. A GI_{50} = ≤ 50 µg/ml was established as a cut-off value to determine whether the fungal extracts were bioactive. In this sense, ethyl acetate (broth), hexane and chloroform biomass-extracts from *P. lavendulum* (CIMA-IL-006) exhibited the

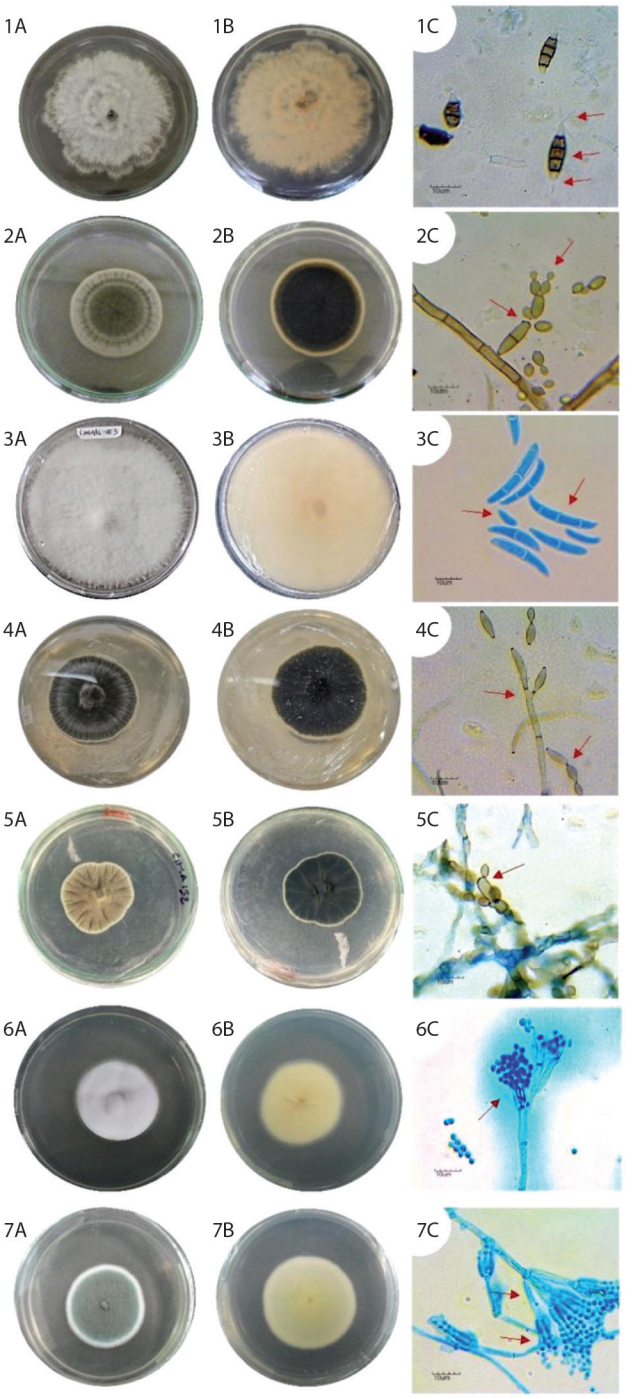

Fig. 2. Colonies on PDA at 25 ± 2 °C after 7 days of growth of [1] *Neopestalotiopsis* sp., [2] *Cladosporium* sp., [3] *Fusarium* sp., [4] *Cladosporium* sp., [5] *Cladosporium* sp., [6] *Purpureocillium* sp., [7] *Penicillium* sp. **A.** Front view. **B.** Reverse view. **C.** Reproductive structures (100X) marked with red arrows, scale bars represent 10 µm.

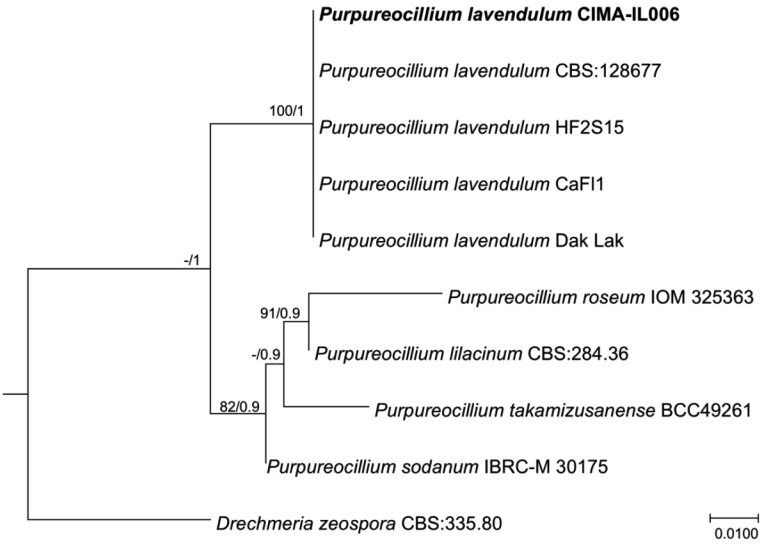

Fig. 3. Maximum likelihood phylogenetic tree of *Purpureocillium lavendulum* CIMA-IL006 and related taxa. Bootstrap support values (BS ≥ 70 %; 1 000 replicates) and Bayesian posterior probabilities (BPP ≥ 0.9) are shown at the corresponding nodes. *Drechmeria zeospora* CBS:335.80 was used as the outgroup.

greatest activity against the cell lines tested by showing GI_{50} values ranging from 3 to 12 µg/ml. Only the chloroform biomass-extract from *Penicillium* sp. (CIMA-IL-008) showed a GI_{50} = ≤ 50 µg/ml against the two cell lines and the ethyl acetate broth-extract from *F. oxysporum* just exhibited activity against one of the cell lines tested (A549) whereas all the methanolic extracts were inactive (Table 3).

DISCUSSION

Seven filamentous fungi were isolated from the water of Mandinga Lagoon, four of them from the sampling site S4, located near the El Dorado Commercial Center, that receives discharges of domestic and industrial wastewater from the mall, marina, and adjacent residential zone. Meanwhile, at site S2 which is impacted by domestic wastewater from the Rincón del Conchal Residential, a lower number of filamentous fungi were isolated. Domestic wastewater often transports bacteria, viruses, fungi and parasites from human or animal reservoirs. The punctual contamination sources identified at sites S2 and S4 generate wastewater classified as domestic and industrial (World Health Organization [WHO], 2002), which contain fecal organic matter, food wastes, detergents, oils, and solvents. In contrast, at sites S1 and S3, direct isolation of filamentous fungi was not achieved, likely because the punctual contamination sources did not favor the presence of cultivable filamentous fungi. However, these results do not rule out the presence of yeast-like fungi, whose isolation and identification were not considered in this study but have been detected in aquatic systems impacted by urban discharges (Monapathi et al., 2017; Fotedar et al., 2022).

Considering the environmental conditions of the sampling sites, temperature is an important factor as it influences the growth of some fungi (Graça et al., 2023). According to González-Vázquez et al. (2019), the water temperature in the Mandinga Lagoon System ranges between 28 and 31 °C during the dry and rainy seasons, respectively, and drops to 21.9 °C during the winter season (Salcedo-Garduño et al., 2019). Therefore, the environmental

Table 3
Antiproliferative activity of fungal extracts expressed in GI_{50} (µg/ml).

Code	Strain	Extract	Cell lines	
			A549	HeLa
CIMA-IL002	*Cladosporium xanthochromaticum*	Broth, Ethyl Acetate	101	93
		Biomass, Hexane	50	54
		Biomass, Chloroform	104	> 250
		Biomass, Ethyl Acetate	56	59
		Biomass, Methanol	> 250	> 250
CIMA-IL003	*Fusarium oxysporum*	Broth, Ethyl Acetate	**45**	50
		Biomass, Hexane	> 250	> 250
		Biomass, Chloroform	> 250	> 250
		Biomass, Ethyl Acetate	216	176
		Biomass, Methanol	> 250	> 250
CIMA-IL006	*Purpureocillium lavendulum*	Broth, Ethyl Acetate	**3** ± 1	**3** ± 0.64
		Biomass, Hexane	**10** ± 2	**12** ± 0.50
		Biomass, Chloroform	**9** ± 1	**8** ± 0.40
		Biomass, Ethyl Acetate	91 ± 11	125 ± 25
		Biomass, Methanol	> 250	> 250
CIMA-IL008	*Penicillium* sp.	Broth, Ethyl Acetate	127	103
		Biomass, Hexane	53	50
		Biomass, Chloroform	**30**	**46**
		Biomass, Ethyl Acetate	81	68
		Biomass, Methanol	> 250	> 250

Cmax = 250 µg/ml, Bold = GI_{50} = ≤ 50 µg/ml, Standard deviation calculated from of mean of GI_{50} values of three experiments.

conditions of the lagoon plus the constant input of organic matter from contamination sources could favor the fungi development. Among the seven fungal isolates, the genus *Cladosporium* (3) was the most common and was isolated from both sampling sites, being identified molecularly only two of them as *C. xanthochromaticum* and *C. cladosporioides*. *Cladosporium* species are isolated frequently from aquatic ecosystems due to their possessing small conidia that are easily transported by air or water (Grossart et al., 2019; Mohamed & Ibrahim, 2021). Some species of the genus *Cladosporium*, alike the genera *Penicillium* and *Fusarium* have been correlated with health issues such as allergic rhinitis and asthma (Katotomichelakis et al., 2016; Murrison et al., 2019; van Tilburg Bernardes et al., 2020). Species belonging to genus *Fusarium* are ubiquitously distributed, been isolated from water, soil and plants. *Fusarium* spp. are responsible for human infections such as onychomycosis and species as *F. oxysporum* and *Fusarium solani* may cause keratitis (Hof & Schrecker, 2024). This is particularly concerning as the Mandinga Lagoon offers tourism and recreational services. In addition, some species of *Penicillium* and *Fusarium* are capable of producing, under certain conditions, secondary metabolites as mycotoxins that, even at low concentrations, bioaccumulate in shellfish as the Eastern oyster (*Crassostrea virginica*), one of the main fishery resources in Mandinga Lagoon. The risk of contaminating fishery resources with mycotoxins represents a potential health hazard for consumers by chronic exposure, due to the mycotoxins are correlated to liver damage and certain types of cancer (Magwaza et al., 2017; Pierce & Ward, 2019).

The drug discovery considered natural products, including fungi, as a valuable source for the identification of new bioactive

compounds (Patridge et al., 2016; Newman & Cragg, 2020). In this context, fungal bioprospecting studies represents a starting point in the pursuit of compounds with biological activities. Thereby, the antiproliferative activity of extracts from four fungal strains were evaluated, being the ethyl acetate extract (broth) from *P. lavendulum* the most active. *P. lavendulum* has been isolated from soil and reported as nematophagous fungus (Liu et al., 2019), isolating compounds as fatty acids, sterols and the 5-methoxymethyl-1H-pyrrole-2-carboxaldehyde, some of them with nematocidal activity (Bao et al., 2022; Liu et al., 2022). Regarding the antiproliferative activity of *P. lavendulum*, the data is scarce. However, the antiproliferative activity of peptidic fungal metabolites identified as leucinostatins, obtained from ethyl acetate extracts of *Purpureocillium* spp. isolated from soils has been reported, exhibiting GI_{50} values ranged from 1.4–8.0 nM against breast cell lines MDA-MB-453 and SUM-185PE (Kil et al., 2020). Our findings are in line with this report; however, it is important to notice that the ethyl acetate extract from broth exhibited the strongest activity against the two cell lines tested (GI_{50} = 3 µg/ml) meanwhile the ethyl acetate extract from biomass shown GI_{50} values higher the cut-off value, therefore, was considered as inactive. Thereby, it is possible considered that the responsible compounds of the antiproliferative activity of *P. lavendulum* were secreted into the culture medium. Regarding to the leucinostatins, have been also isolated from *Purpureocillium lilacinum* and their antifungal activity against *Botrytis cinerea* (Liu et al., 2020) and antimalarial activity (Niu et al., 2024) have been reported. Lastly, this initial part of the bioprospecting study at MLS allowed us to select the isolate CIMA-IL006, identified as *P. lavendulum* as a promising fungus to continue the mycochemical study and, to the best of our knowledge, this is the first report of the presence of *P. lavendulum* in a water environment.

The demographic pressure on the MLS is driven by urban and commercial development in the area. This has led to an increase in the discharge of partially treated and untreated wastewater, which is associated with the presence of filamentous fungi. These aquatic fungi may disrupt ecological balance, degrade water quality, contaminate fishery resources and pose risks to human health. Nevertheless, the ability of fungi to produce secondary metabolites as response to environmental stress, represents an opportunity to perform bioprospecting studies in the pursuit of compounds with biological activities. The above study allowed the identification of *P. lavendulum* from water and the evaluation of their antiproliferative activity exhibited interesting values by the which, *P. lavendulum* is considered a promising fungus to continue the bioguided separation of bioactive compounds.

Ethical statement: The authors declare that they all agree with this publication and made significant contributions; that there is no conflict of interest of any kind; and that we followed all pertinent ethical and legal procedures and requirements. All financial sources are fully and clearly stated in the acknowledgments section. A signed document has been filed in the journal archives.

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