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Biotechnological Potential of Marine-Derived Fungi for Textile Dye Degradation via Laccase-Like Activity

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Abstract

The complex structure of synthetic dyes has made them challenging to decolorize, despite their widespread use in a variety of industries, including textiles, cosmetics, printing, paper, and pharmaceuticals. Enzymes such as oxidoreductases and peroxidases are employed in novel biotechnological procedures. An affordable and environmentally friendly solution to the issue of decolorizing commercial dyes is the use of oxidative enzymes, such as laccases derived from fungi. This study screened marine-derived fungal strains isolated from five coastal areas in Lagos, Nigeria, in order to identify laccase-like activities and assess the isolates' ability to decolorize industrial dyes in solid and liquid media by monitoring the radial growth, percentage decolorization, biosorption, and laccase activity after seven days of incubation with the dyes. After several steps of culturing on potato dextrose agar (PDA) plates, forty pure fungal cultures were isolated from a total of 100 samples (wood, nets, plants, clothing materials, soil, and water) collected from five different marine biotopes in Lagos: Oniru Beach, Makoko Lagoon, Elegushi Beach, Adekunle Lagoon, and Unilag waterfront. Through preliminary oxidative ability screening with 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), eleven isolates were discovered. Six strains of *Meyerozyma guilliermondii*, two strains of *Rhodotorula mucilaginosa*, one strain of *Candida tropicalis*, and two strains of *Penicillium oxalicum* were identified as belonging to the following species by means of molecular-based taxonomic approaches. *Meyerozyma guilliermondi* A4B and *Penicillium oxalicum* M6A effectively decolorized four synthetic dyes from various dye families and demonstrated the highest level of oxidative capacity and grew best at 2 mM $CuSO_4$ as an inducer and 2% and 1% NaCl to mimic a marine environment, and both produced the highest laccase-like active cell-free supernatants activity.

Keywords: *Meyerozyma guilliermondii*, *Penicillium oxalicum*, Marine fungi, Laccase-like activity, Dyes, Oxidative

1. Introduction

Water pollutants are a major source of pollution and pose a serious environmental problem. Many industries discharge their wastes into rivers, lakes, ponds and streams, thereby disrupting the aquatic ecosystem (Rao et al., 2010). The conversion of microbial enzymes and the detoxification of pollutants have been the subject of numerous studies (Couto, 2007). Fungi are generally able to tolerate higher concentrations of pollutants and are therefore considered to be more robust than bacteria as they produce large quantities of extracellular enzymes with great industrial potential using economical and environmentally friendly methods (Ellouze & Sayadi, 2016). Today, most fungi studied are isolated from terrestrial habitats such as forests. However, the diversity of marine fungi has not been thoroughly studied (Richards et al., 2011). On the other hand, a wide variety of fungal species occur in highly complex marine environments (Amend et al., 2019). The majority of fungi originating from marine environments appear to be related to terrestrial counterparts such as *Aspergillus* sp., *Penicillium* sp., etc, despite the identification and characterization of several new genera of fungi occurring in marine environments. Marine-

derived fungi have been shown to occur in a variety of habitats, including the deep sea, coastal regions, and marine sediments.

Laccases (EC 1.10.3.2) are a multigenic family of multicopper oxidoreductases distributed across bacteria, fungi and plants. These enzymes catalyze the one-electron oxidation of a wide range of substrate molecules, such as aromatic thiols, substituted phenols, and arylamines, to the corresponding radicals at a mononuclear copper center T1. At the same time, they reduce molecular oxygen to water at the trinuclear copper center T2/T3 (Reiss et al., 2013). Their active copper cluster allows them to function without the addition of heterogeneous cofactors, and oxygen, their co-substrate, is typically found in their environment. Most fungal species are attractive for biotechnological applications because their laccase-encoding genes exhibit high levels of inducible expression (Couto, 2007).

For industrial applications, novel laccase sources with unique properties such as increased redox potential, increased salt and temperature tolerance or cold adaptability are required. It has been discovered that many different fungal strains isolated from samples of decaying wood, seaweed, and algae can produce laccases (Kantharaj et al., 2017). Atalla et al. (2013) isolated *Trematosphaeria mangrovei*, which produces a significant amount of laccase in a mangrove ecosystem. The marine fungus *Cerrena unicolor* produces a thermostable, metal-tolerant laccase (D'Souza-Ticlo et al., 2009).

Synthetic dyes are organic compounds made from petrochemicals. The solubilities of these substances are different and they are easily absorbed and impart color to the substrates (Jamee & Siddique, 2019). Structurally, dyes consist of three components: a chromophore, the atom interaction sites with electromagnetic (EM) rays (Benkhaya et al., 2020); an auxochrome containing functional groups that introduce the chromophore, increasing the fibres' affinity for color and reducing their solubility in water (Sharma et al., 2021); and aromatic structures such as benzene, naphthalene and anthracene rings (Ozojiofor et al., 2023).

Dyes are divided into groups according to their chemical structure and type of application. Based on their chemical structure, these compounds are classified as anthraquinone, sulphur, azo, triarylmethane or phthalocyanine (Benkhaya et al., 2020). The methods used for treatment are divided into direct, reactive, dispersion, vat dyeing or basic (Yaseen & Scholz, 2019). Synthetic dyes are widely used in several industries such as textile, cosmetics, printing, paper and pharmaceutical industries (Kumar et al., 2013). The complexity of their structure makes synthetic dyes difficult to decolorize. Decolorization of textile dye wastewater does not occur well when air is present in wastewater systems. Water-soluble, brightly colored, acidic and reactive dyes are the most recalcitrant as they are generally not affected by conventional treatment systems (Kumar et al., 2013).

The aim of this study was to isolate and screen several marine-derived fungal strains with laccase activity from marine biotopes in Lagos, Nigeria, and to evaluate the bioremediation potential of the isolate on textile dyes.

2. Materials and Methods

2.1 Study area

This study was carried out in Kaduna, Kaduna State. Kaduna is the state capital of Kaduna State in North-Western Nigeria and is located at latitude 10° 36' 33.5484" N and longitude 7° 25' 46.2144" E.

2.2 Sample collection

The environmental samples (from wooden plants immersed in seawater, seaweeds, marine plants, pieces of nets, water, clothing, and sands from sea shores) used in this study were collected from five different marine biotopes in Lagos (6° 27' 55.5192" N and 3° 24' 23.2128" E) as shown in Figure 1: the fishing ports, Lagos waterways, lagoons, beaches and polluted waterways in Adekunle, Elegushi beach, Oniru beach, Makoko and Unilag waterfront all in Lagos state. These sites were chosen because of their pollution, as fungal strains that are tolerant to polluted water were isolated, and enzymes able to work in the presence of several contaminants and aromatic compounds were produced. The samples were collected in sterile tubes using a sterile spatula and stored at 4°C until use.

Figure 1. Map of Lagos showing the sample collection points. Code A-E refers to points on the map (adapted from Olufemi et al., 2010).

2.3 Isolation of fungi

Samples (woods immersed in seawater, seaweeds, marine plants, pieces of nets, and sands from sea shores) were inoculated on 3.9% (w/v) potato dextrose agar (PDA) and 1.8% (w/v) malt extract with 3.4% (w/v) NaCl and 0.1% (w/v) chloramphenicol to prevent bacterial growth and incubated at 30°C for 3 days until fungal growth was observed (Zouari-Mechichi et al., 2006).

The resulting isolates were subcultured on new agar plates to produce monomorphic cultures, which were subsequently cultured at 4°C. The isolates were morphologically identified using light microscopy and spore testing, and their identity and purity were confirmed using molecular methods for additional authentication.

2.4 Laccase screening activity of the isolates

Preliminary screening for oxidative activity was performed on PDA plates enriched with 2 ml of 200 µM 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) as a substrate. After three days of incubation at 30°C, the presence of purple or blue–green halos surrounding the mycelium was regarded as a good indicator of substrate oxidation (Ben Ali et al., 2020).

2.5 Molecular identification

2.5.1 DNA extraction

The mycelia of the chosen strains were cultivated in 50 mL flasks with malt extract medium for three days, after which the mycelia were collected. Using an AccuPrep® Genomic DNA Purification Kit and according to the manufacturer's instructions, genomic DNA was extracted from 40-80 mg of mycelia, and Nanodrop 2000 equipment was used to determine the concentration of DNA at 260 nm.

2.5.2 Polymerase chain reaction (PCR)

The collected DNA was utilized as a template for PCR to amplify the partial sequences of three distinct DNA loci: the translation elongation factor 1α region (TEF-1α), the internal transcribed spacer region (ITS), and D1/D2 in 28S nuclear ribosomal DNA (Table 1). The following primers were used in the amplification process:

Table 1. Primers used for DNA amplification.

S/N	Primer name	Primer sequence	References
1	NL-1 F	5'-GCATATCAATAAGCGGAGGAAAAG-3	White et al., 1990
	NL-4 R	5'-GGTCCGTGTTTCAAGACGG-3	
2	ITS5 F	5′-GGAAGTAAAAGTCGTAACAAGG-3'	Thompson et al.,1994
	ITS4 R	5′-TCCTCCGCTTATTGATATGC-3′	
3	TEF1α-983-F-CF2	5′-GCYCCYGGHCAYCGTGAYTTYAT-3′	Stielow et al., 2015
	TEF1α-2218-R-CR2	5′ATGACACCRACRGCRACRGTYTG-3′)	

F: forward primer; R: reverse primer

Using an AccuPrep® Genomic DNA Kit, PCR was carried out in a final volume of 50 µL using 5 µL of buffer (100 mM Tris HCl, 150 mM $MgCl_2$, and 500 mM KCl) with 1.5 mM $MgCl_2$, 0.25 µM of each primer, 1 µL of deoxynucleoside triphosphate (200 µM of each dNTP), 1 µL of DNA (approximately 100 ng), and Taq DNA polymerase (25 mU. μL^{-1}). The cycling conditions were as follows: 94°C for 2 minutes; 40 cycles of 94°C for 15 seconds, 51°C for 30 seconds, and 72°C for 1 minute; and a final extension lasting 10 minutes at 72°C. Parallel reactions were carried out on negative control reactions lacking any template DNA.

2.5.3 Gene sequencing

The amplified fragments were sequenced using the three PCR primers from Inqaba Biotech and visualized on a 1% agarose gel in 1× TAE (40 mM Tris-acetate in 1 mM EDTA, pH 8) buffer.

To establish the phylogeny of the fungal isolates, the D1/D2, ITS and TEF-1α sequences were compared with data available from the public database GenBank by using the BLASTn sequence match tool (Altschul et al.,1997). Phylogenetic trees were constructed using the best hits for each species that were found during the BLAST search. The sequences were aligned using the CLUSTAL W program (Thompson et al., 1994), and phylogenetic and molecular evolutionary analyses were performed using MEGA X (Kumar et al., 2018). Using bootstrapping, the maximum likelihood algorithm technique (Gascuel, 1997) was used to construct the phylogenetic tree.

2.6 Fungal cultures

The fungal isolates were cultured on MEA (30 g of malt extract with 20 g of agar) for solid-state culture. Where appropriate, the selected marine fungal strains were cultivated in submerged cultures in 50 mL of M7 medium. The ABTS-oxidizing laccase-like activity was determined from the culture supernatant as previously reported (Zouari-Mechichi et al., 2016).

Glass beads (0.6 mm) were used to vortex 50 ml of fungal mycelia precultures that lasted three days for one minute. Then, 50 mL of M7 media was placed in 250 mL Erlenmeyer flasks, which were subsequently seeded with the homogenized mycelial pieces. Glucose 5, peptone 5, yeast extract 1, ammonium tartrate 2, KH_2PO_4 1, $MgSO_4.7H_2O$ 0.5, KCl 0.5, and trace element solution 1 mL will be present in the medium (M7) in terms of grams per liter. $B_4O_7Na_2.10H_2O$ 0.1, $CuSO_4.5H_2O$ 0.01, $FeSO_4.7H_2O$ 0.05, $MnSO_4.7H_2O$ 0.01, $ZnSO_4.7H_2O$ 0.07, and $(NH4)_6Mo_7O_{24}.4H_2O$ 0.01 were the trace element solution compositions (g L^{-1}). The final pH was adjusted to 5.5 (Ben Ali et al., 2020). The cultures were incubated at 160 rpm and 30°C, after which daily aliquots were taken. Next, 2 mM $CuSO_4$ was added to M7 medium to facilitate Cu^{2+} induction.

2.7 Laccase-like activity assay

Laccase activity was determined by the method of Shin and Lee (2000) using ABTS as substrate. The reaction mixture contained 2.8 ml of sodium acetate buffer (0.1 M, pH 4.5), 0.1 ml of 1 mM ABTS and 0.1 ml of the culture supernatant. The oxidation of ABTS was observed at 420 nm ($\varepsilon 420 = 36\ mM^{-1}\ cm^{-1}$) by measuring the change in absorbance for 1 min. A single unit of ABTS-oxidizing activity is the quantity of enzyme required to oxidize one microgram of ABTS at room temperature every minute. To ensure that heme-containing peroxidases were not the cause of any activity, measurements were carried out in the presence of either H_2O_2 (0.5 mM) or catalase (280 units per milliliter of test).

2.8 Effects of NaCl and $CuSO_4$ on laccase-like activity

To compare the effects of NaCl and sea salt on the production of active cell-free supernatants, standard M7 medium was supplemented with increasing concentrations of either NaCl or sea salt (1–5% w/v). Fifty millilitre cultures were grown in 250 mL Erlenmeyer flasks for 7 days at 30°C, after which samples were withdrawn periodically. $CuSO_4$ was supplemented in culture to induce laccase-like activity when laccases were involved. To determine the suitable concentration of $CuSO_4$ for optimal laccase-like activity, the following concentrations of $CuSO_4$ were tested: 1500 µM, 2000 µM, 2500 µM and 3000 µM.

2.9 Dye decolorization

2.9.1 Dye decolorization on solid media

PDA plates supplemented with chloramphenicol (30 μg/ml) and 0.2 g/L dye (i.e., Direct Red 75, Indigo carmine, Remazol Brilliant Blue R, and Malachite Green, all supplied by Glentham Life Science) were inoculated with mycelia placed in the center of each plate (Fernández-Remacha et al., 2022). The plates were incubated at room temperature for 5 days and then examined daily for the presence of halos corresponding to the areas in which the dyes were degraded and in which the radial growth per day was measured.

2.9.2 Dye decolorization in liquid systems

For further investigation on liquid media, the fungal strains that displayed a decolorized zone on the solid media were chosen. The concentration of each dye was increased to 50 mg/L in liquid medium that had already undergone autoclaving. A plug made from the edge of the chosen fungal strains on PDA was injected at 25°C for one week after being transferred to 10 mL of liquid medium containing the appropriate dye. After centrifuging the liquid medium containing the fungal mycelia for 10 minutes at 5000 rpm, aliquots of the supernatants were transferred to fresh tubes and diluted as needed to obtain the absorbance. An untreated liquid medium was used as a control for every dye.

The standard curve of each dye was generated at its maximum absorbance wavelength, with a concentration ranging from 0 to 50 mg/L. The percentage of dye decolorization in the samples was calculated using the formula $D = (A_C - A_T)/A_C \times 100$, where D represents the decolorization rate (%) and A_C and A_T represent the absorbance measured for the control sample and the treated sample, respectively (Rosales, 2011).

2.10 Dye biosorption

The dye biosorption of fungal mycelia was evaluated as follows: after the centrifugation process described above, the fungal mycelia in each centrifuge tube were put on filter paper (Whatman) to remove the additional liquid; then, the mycelia were transferred to a new centrifuge tube that contained 10 mL of ethanol and 10 mL of fresh liquid medium. These tubes were centrifuged for 10 minutes at 5000 rpm after being shaken at 160 rpm for 20 minutes at room temperature. To execute the control for each dye, 10 mL of ethanol was added to 10 mL of liquid medium containing 50 mg/L dye. The dye biosorption rate was expressed as a percentage as follows: $A = A_T/A_C \times 100$, where A is the dye adsorption rate of each fungal mycelia (%), and A_T and A_C are the concentrations measured in the treatment and control, respectively.

2.11 Statistical analysis

All data generated were analysed using the SPSS statistics 23.0 software. The results were presented as mean ± standard deviation of three determinations and were analysed by one-way analysis of variance (ANOVA) and the significant differences between treatments were compared by Duncan test at $P < 0.05$ considered statistically significant.

3. Results

3.1 Fungal isolation and laccase production assay

A total of 100 samples (wood, nets, plants, clothing materials, soil, water) were collected from five different marine biotopes in Lagos, namely, Oniru beach, Makoko Lagoon, Elegushi beach, Adekunle Lagoon and the Unilag waterfront.

Table 2 shows the number of fungi isolated from the samples collected from the marine habitats in Lagos, Nigeria. A total of forty (40) pure fungal cultures were isolated after several steps of culture and subculturing on potato dextrose agar (PDA) plates.

Table 3 shows the preliminary results for the presence of the laccase enzyme in the forty pure fungal isolates. Eleven isolates showed blue–green or purplish coloration when grown on plates supplemented with the laccase substrate, 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). Oxidative activity was determined on the 7th day of cultivation after the addition of the specific substrate (ABTS) for the enzyme. A positive or negative sign indicates the presence or absence of the enzyme laccase, respectively, in the pure isolates. Eleven isolates showed positive signs for the presence of laccase.

Table 2. Samples collected for isolation of fungi and number of isolates from different locations.

Locations	Number of samples	Number of Fungi isolates (%)
ONIRU BEACH	30	11(11)
UNILAG WATERFRONT	10	2 (2)
ELEGUSHI BEACH	20	9 (9)
ADEKUNLE LAGOON	15	5 (5)
MAKOKO LAGOON	25	13 (13)
TOTAL	100	40 (40)

Table 3. Qualitative assay for lignin-degrading enzyme (Laccase) detection on potato dextrose agar (PDA) plates.

S/N	Isolates	Outcome	S/N	Isolates	Outcome	S/N	Isolates	Outcome
1	M6	+	18	A4B	+	35	M1A	_
2	M4A	_	19	M2A	_	36	O8A	_
3	A1A	_	20	M4	+	37	O9	_
4	M6A	+	21	O8B	_	38	O4	_
5	E7A	+	22	O9B	_	39	U2A	_
6	E7	+	23	O6C	+	40	M7A	_
7	E7C	+	24	A3	_			
8	A3A	_	25	E1	_			
9	A1B	_	26	M6D	_			
10	E6C	_	27	O5	_			
11	MIB	+	28	O5A	_			
12	M2C	_	29	O8B	_			
13	M5BI	+	30	O7B	_			
14	M2	_	31	E3A	_			
15	O6A	+	32	E5A	_			
16	E6B	_	33	E7B	_			
17	U2	_	34	M5B	_			

O-Oniru, A-Adekunle, M-Makoko, E-Elegushi, U-Unilag

3.2 Molecular identification of the isolates

The results of molecular identification of the eleven (11) laccase-positive isolates are presented as Plates 1 a-c. The electropherograms of the isolates revealed the presence of amplicons of 500-600 bp. Table 4 shows the nBlast results of the fungi isolates DNA sequence with the NCBI database. It shows the species name, percentage identity, Accession number and that of the organism with the highest identity in the GenBank.

Phylogenetic analysis of the DNA sequences of the fungal isolates

Phylogenetic analysis of the eleven fungal isolates with laccase activity revealed that some of the fungal strains clustered with *Meyerozyma guilliermondii*, *Rhodotorula mucilaginosa, Candida tropicalis* or *Penicillium oxalicum,* as shown in Figures 2a-g.

3.3 Laccase-like activity assay of the isolates

Figure 3a shows the time course study of the laccase-like activity of the eleven (11) isolates. Isolate A4B had the highest laccase activity of all the eleven isolates after 24 hours incubation. At 96 hours, the laccase activity was 609.2±31.7 UL^{-1}. Figure 3b shows the determination of a suitable concentration of $CuSO_4$ for optimal production of laccase-like activity by the eleven (11) isolates at different concentrations of $CuSO_4$. Isolates A4B, M5B1, M6, M1B, O6C and M6A exhibited high laccase-like activity above 600 UL^{-1} at 2.0 mM $CuSO_4$. For the determination of a suitable concentration of NaCl for optimal production of laccase-like activity by the eleven (11) isolates. Isolates A4B and M5B1 exhibited high laccase-like activity above 147 UL^{-1} at different concentrations (1-5%) of NaCl as shown in Figure 3c.

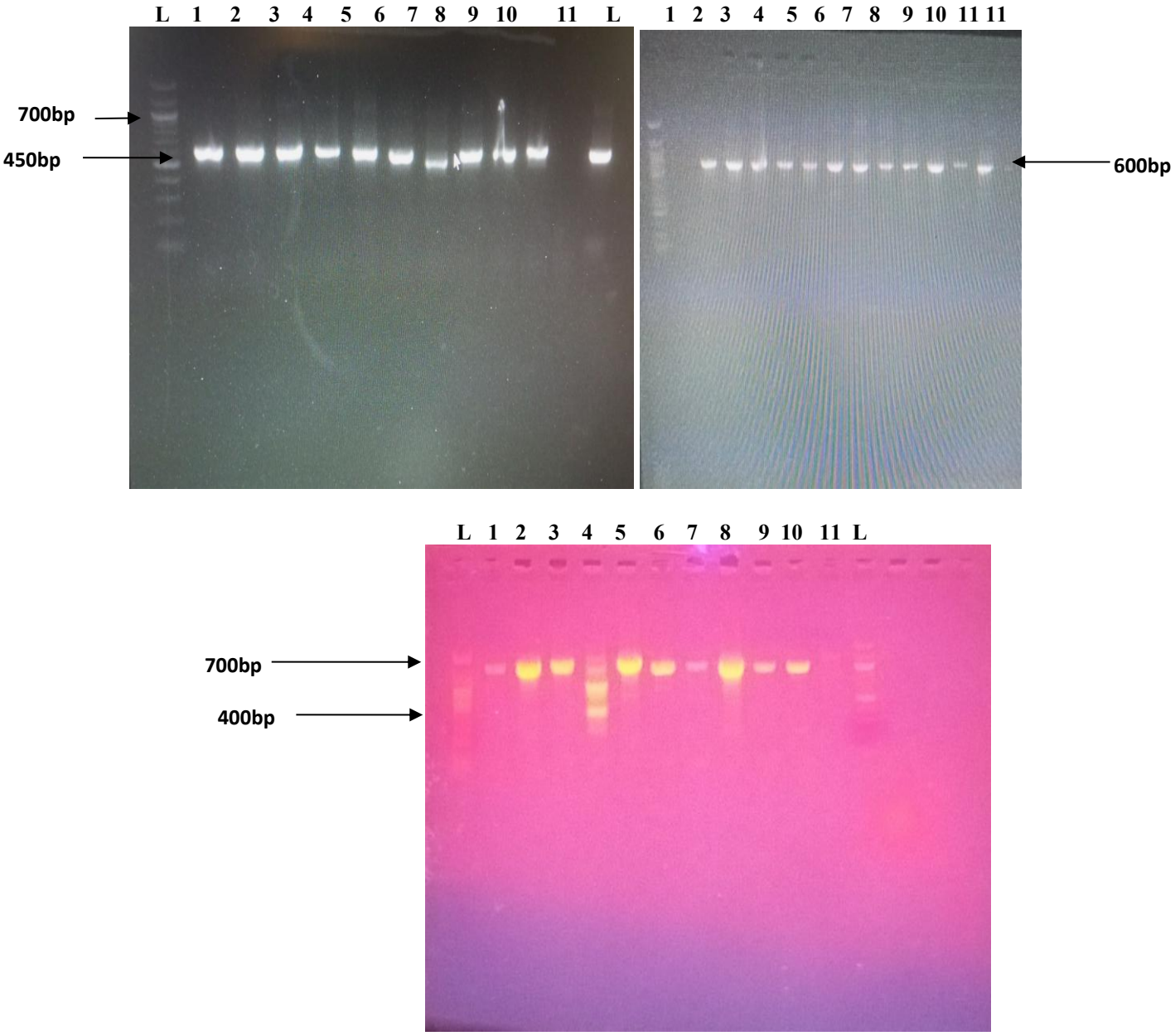
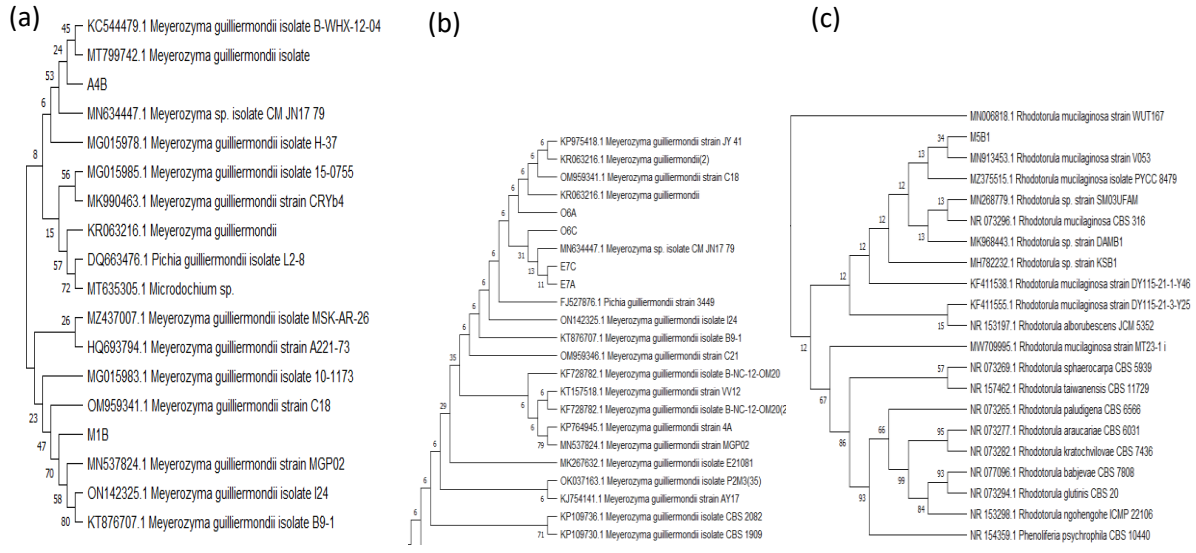


Plate 1. Agarose gel (1%) electrophoresis of the PCR product of the (a) Internal Transcribed Spacer (ITS), (b) D1/D2 domain (NL-1/NL-4) (c) Translational elongation factor 1α (TEF1 α) region of the eleven fungal isolates. L: Molecular ladder, 1: E7C, 2: 06C, 3: E7A, 4: M4, 5: M5B1, 6: M6A, 7: M6, 8: A4B, 9: O6A, 10: M1B, 11: E7.

Table 4. Identification of the fungal isolates with laccase activity from nBLAST.

Strain number	Species name	Division	Accession number of the isolates	% identity	Accession number with closest identity in the GenBank
M6	*Candida tropicalis*	Ascomycota	PP077348	91.93	KP675553
M6A	*Penicillium oxalicum*	Ascomycota	PP077349	88.9	MG818939
E7A	*Meyerozyma guilliermondi*	Ascomycota	OR727112	99.65	KT157518
E7	*Rhodotorula mucilaginosa*	Basidiomycota	PP082041	99	MN006818
E7C	*Meyerozyma guilliermondi*	Ascomycota	OR726612	99.82	MN537824
MIB	*Meyerozyma guilliermondi*	Ascomycota	OR733332	99.82	ON142325
M5BI	*Rhodotorula mucilaginosa*	Basidiomycota	OR736286	89.20	MN006818
O6A	*Meyerozyma guilliermondi*	Ascomycota	OR733334	99.65	MG846135
O6C	*Meyerozyma guilliermondi*	Ascomycota	OR726614	98.6	MN537824
A4B	*Meyerozyma guilliermondi*	Ascomycota	OR722221	89.4	MN634447
M4	*Penicillium* sp	Ascomycota	PP151162	100	MT597864



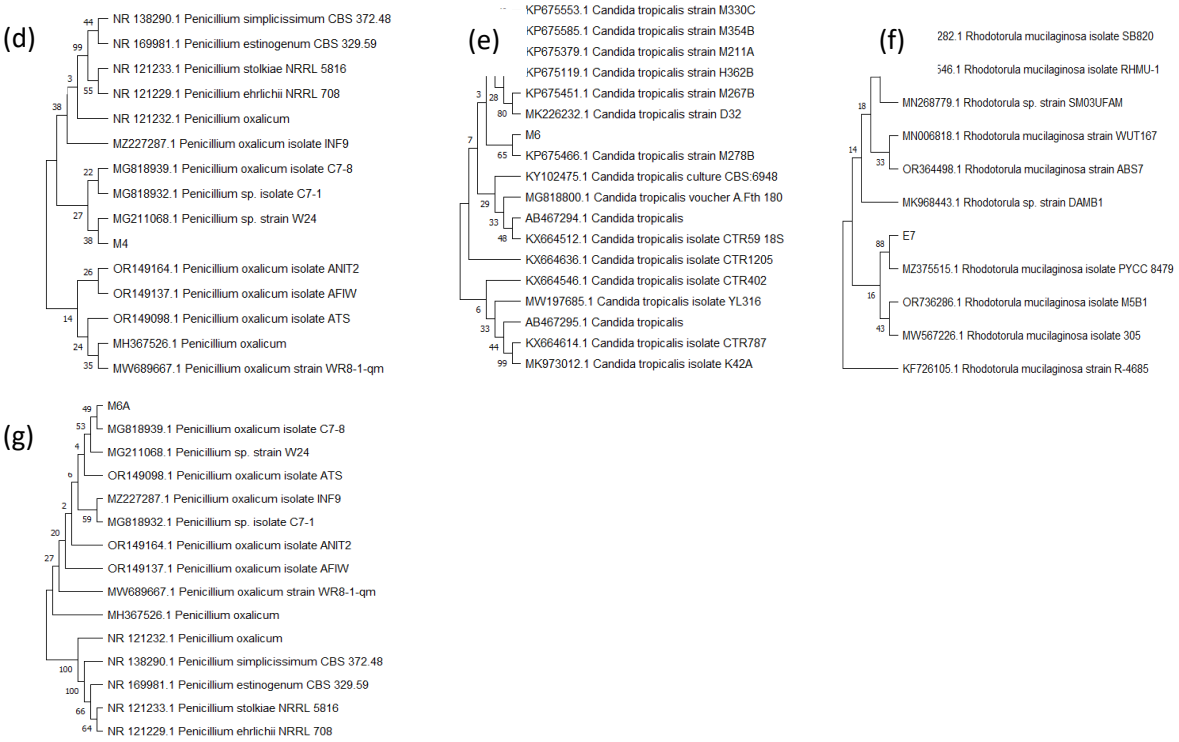
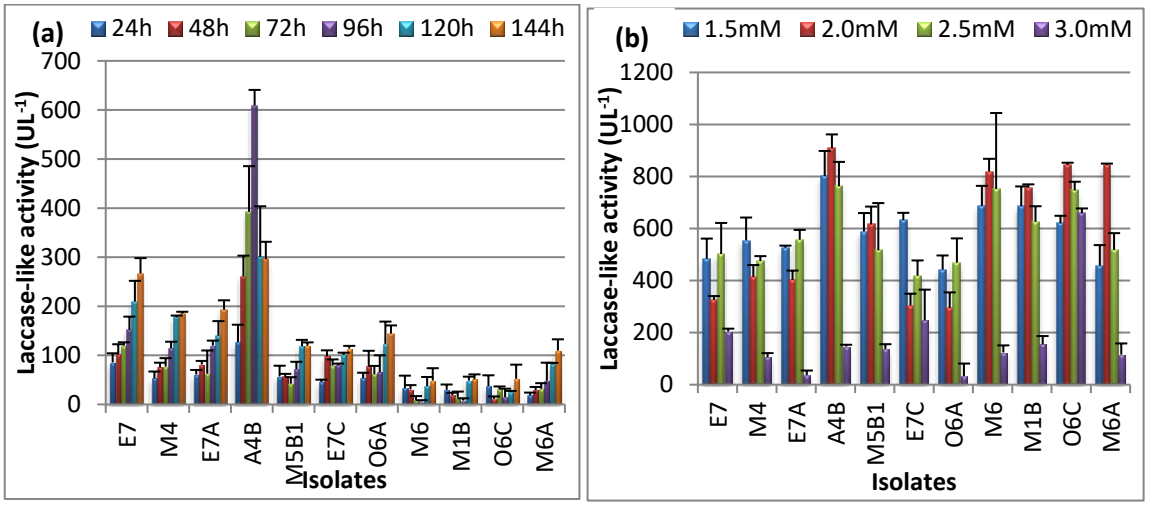


Figure 2. Phylogenetic reconstruction for the isolate (a) A4B and M1B (b) E7C, E7A, O6A and O6C (c) M5B1 (d) M6 (e) M4 (f) E7 (g) M6A based on ITS analysis using the maximum likelihood algorithm method and 1000 replicate bootstraps.

3.4 Screening of the ability of the fungal isolates to degrade textile dyes in solid media

After 7 days of incubation of the isolates on plates supplemented with the different dyes, M6 showed the greatest radial growth after 7 days of incubation with Direct red 75 (1.036±0.030 cm/day) and Indigo carmine (1.072±0.021 cm/day), while A4B and M1B showed the greatest radial growth with Malachite green (1.036±0.030 cm/day) and Remazol brilliant blue R (1.036±0.030 cm/day) respectively, as presented in Table 5. Radial growth of the isolates on plates supplemented with Malachite green was the lowest among the dyes during the days of incubation.



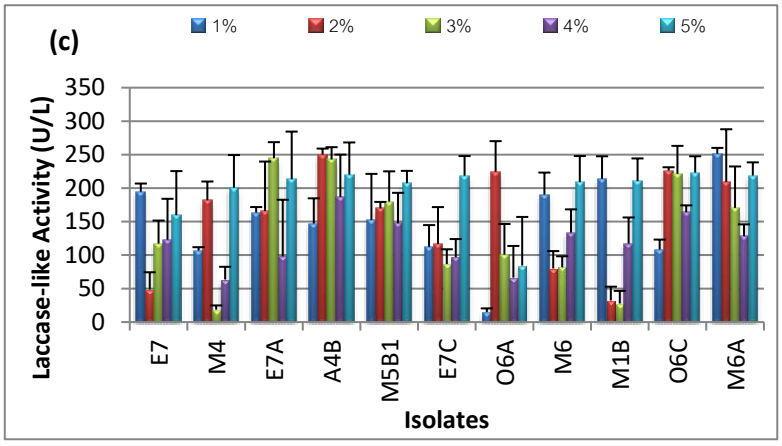


Figure 3. Laccase activity of the isolates over time (a) Laccase activity of the isolates at different concentrations of $CuSO_4$ (b) Laccase activity of the isolates at different concentrations of salt (NaCl) (c).

Table 5. Radial growth of the isolates after seven days of incubation in media supplemented with textile dyes.

	RADIAL GROWTH AT DAY 7 (cm/day)			
ISOLATES	DIRECT RED	REMAZOL BRILLIANT BLUE	MALACHITE GREEN	INDIGO CARMINE
E7	0.279±0.011[a]	0.279±0.006[b]	0.107±0.010[c]	0.270±0.042[d]
E7A	0.380±0.014[a]	0.324±0.094[a]	0.165±0.050[c]	0.322±0.030[d]
E7C	0.664±0.071[a]	0.686±0.081[b]	0.164±0.010[c]	0.779±0.011[d]
M4	0.629±0.040[a]	0.558±0.040[b]	0.172±0.040[c]	0.786±0.101[d]
M5B1	0.307±0.010[a]	0.271±0.0[b]	0.107±0.010[c]	0.171±0.00[d]
M6	1.036±0.030[a]	0.800±0.020[a]	0.165±0.050[c]	1.072±0.021[d]
M6A	0.850±0.051[a]	0.736±0.030[b]	0.186±0.040[c]	0.879±0.011[d]
O6A	0.300±0.020[a]	0.200±0.040[b]	0.165±0.030[c]	0.228±0.020[d]
O6C	0.331±0.084[a]	0.264±0.071[b]	0.115±0.021[c]	0.272±0.021[d]
M1B	0.843±0.020[a]	0.836±0.050[b]	0.136±0.030[c]	0.872±0.162[d]
A4B	0.936±0.010[a]	0.643±0.061[b]	0.221±0.071[c]	0.872±0.040[d]

Superscript (a, b, c…) indicates significant difference across the rows at a 95% confidence interval

3.5 Screening of the ability of the fungal isolates to degrade textile dyes in liquid media

The results of the percentage degradation of the eleven isolates after incubation in liquid media supplemented with textile dyes are presented in Table 6.

The decolorization of the dyes in liquid media by the eleven isolates after 168 hours (7 days) of incubation, A4B and M6A had the highest percentage of decolorization (above 80%) on day 7 for RBBR, DR75 and IC, while M6 had the highest percentage of decolorization for MG.

3.6 Laccase activity of the isolates during degradation after seven days of incubation in liquid media supplemented with textile dyes

The laccase activity of the eleven isolates during degradation after 7 days of incubation in liquid media supplemented with textile dyes showed that A4B had the highest laccase activity followed by M6A in all the media supplemented with the four dyes as shown in Table 7.

The percentage biosorption rate of the dyes by the eleven isolates after 7 days of incubation showed that E7C had the highest percentage biosorption rate of 17% while A4B had the lowest percentage biosorption rate of 0.2% as presented in Figure 4.

Table 6. Percentage decolorization of the dyes by the isolates after 7 days.

ISOLATES	REMAZOL BRILLIANT BLUE R (%)	MALACHITE GREEN (%)	INDIGO CARMINE (%)	DIRECT RED 75 (%)
E7C	83.75±0.14^{a}	82.99±0.18^{b}	56.38±0.07^{c}	76.97±0.08^{d}
E7A	81.25±0.41^{a}	76.82±0.09^{b}	63.21±0.21^{c}	79.26±0.16^{d}
E7	78.95±0.13^{a}	82.02±0.09^{b}	57.5±0.14^{c}	81.6±0.16^{d}
M4	81.54±0.27^{a}	18.38±0.09^{b}	76.85±0.07^{c}	75.26±0.08^{d}
M5B1	86.54±0.0^{a}	81.37±0.09^{b}	62.82±0.07^{c}	79.03±0.24^{d}
M6	71.73±0.27^{a}	88.9±0.09^{b}	67.69±0.07^{c}	76.06±0.08^{d}
M6A	91.06±0.13^{a}	65.58±0.18^{b}	83.04±0.55^{c}	84.17±0.08^{d}
O6A	87.31±0.27^{a}	84.49±0.09^{b}	63.11±0.07^{c}	80.34±0.0^{d}
O6C	89.14±0.13^{a}	41.24±0.46^{b}	68.37±0.07^{c}	74.75±0.16^{d}
M1B	86.25±0.41^{a}	24.88±0.09^{b}	81.24±0.07^{c}	80.37±0.08^{d}
A4B	90.19±0.27^{a}	65.84±0.55^{b}	83.4±0.07^{b}	83.2±0.16^{c}

Superscript (a, b, c…) indicates significant difference across the rows at a 95% confidence interval.

Table 7. Laccase activity of the isolates during dye degradation after 7 days.

ISOLATES	DIRECT RED (UL^{-1})	REMAZOL BRILLIANT BLUE R (UL^{-1})	INDIGO CARMINE (UL^{-1})	MALACHITE GREEN (UL^{-1})
E7	104.4±0.48^{a}	147.5±4.3^{b}	134.4±1.3^{c}	232.2±5.3^{d}
M4	108.6±0.48^{a}	65.8±0^{b}	81.1±0.5^{c}	295±0.8^{d}
E7A	50.6±0.96^{a}	144.2±13^{b}	90.3±0.5^{c}	157.2±6.2^{d}
A4B	278.3±24.6^{a}	175.0±8.3^{b}	169.4±5.5^{c}	344.7±4.6^{d}
M5B1	130.8±0.8^{a}	99.4±1.3^{b}	118.3±34.6^{c}	78.1±3.8^{d}
E7C	168.1±1.0^{a}	85.8±0^{b}	166.7±7.2^{c}	88.1±0.5^{d}
O6A	228.1±2.4^{a}	89.2±0^{b}	142.2±0.5^{c}	92.2±1.0^{d}
M6	63.1±2.4^{a}	40.6±3.2^{b}	134.2±0.8^{c}	63.9±1.0^{d}
M1B	58.9±0.5^{a}	77.2±0.5^{b}	117.5±0.8^{c}	310.6±0.5^{d}
O6C	85.8±2.9^{a}	63.6±0.5^{b}	88.9±1.0^{c}	176.9±4.1^{d}
M6A	241.7±8.3^{a}	161.389±3.4^{b}	155.8±5.2^{c}	323.1±2.1^{d}

Superscript (a, b, c…) indicates significant difference across the rows at a 95% confidence interval

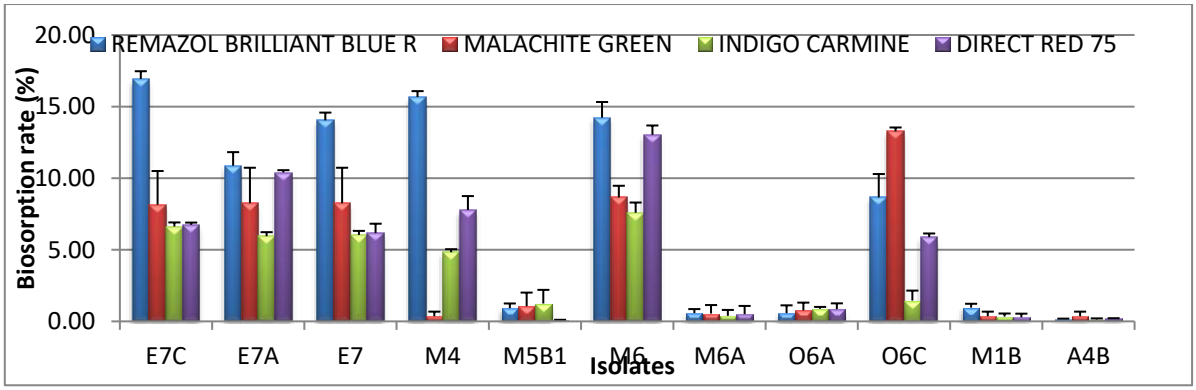


Figure 4. Percentage dye biosorption rate of the isolates after 7 days.

4. Discussion

The oceans, which make up about 70% of the Earth's surface, unquestionably support enormous habitats and are abundant providers of resources for a wide range of living things. The ability of fungi to produce an extensive variety of extracellular enzymes has become well known (Dhouib et al., 2005). Despite these limitations, few studies have investigated the intricate diversity of marine fungal species. To date, the vast majority of fungi investigated have been isolated from a wide range of terrestrial habitats and ecosystems, such as grasslands, forests, and marshy terrains. Owing to the emergence of strains that are capable of thriving in adverse marine conditions such as low temperature, high pressure, oligotrophic nutrients, and high salt levels, a substantial portion of the diversity of fungi in the marine environment may have originated from their terrestrial counterparts (Rateb & Ebel, 2011). There is significant variation between the enzymes produced by microorganisms derived from marine environments and those from terrestrial environments, and this variation could be a result of the conditions of high pressure, low temperature, oligotrophic nutrients, and high salt levels in which marine microbes live (Zhang & Kim, 2010). These conditions could make microbes and enzymes from marine habitats suitable for industrial applications. However, further studies on the ability of marine microorganisms to degrade plant materials and synthesize novel natural products are needed (Ben Ali, 2020).

In this study, a total of forty marine-derived fungi were isolated from five different marine biotopes in Lagos, Nigeria. Eleven of these strains, which exhibited oxidative activity in ABTS or demonstrated laccase-like activity, were selected. These eleven fungal strains included nine identified ascomycetes and belonged to the following species: *Penicillium oxalicum* (two strains), *Meyerozyma guilliermondii* (six strains), and *Candida tropicalis. The other two strains were* identified as basiodiomycetes and belong to the species *Rhodotorula mucilaginosa*. The species phylogenetically closest to the six isolates, *Meyerozyma* sp., was *Meyerozyma guilliermondii,* with two isolates, *Penicillium* sp., clustered with *Penicillium oxalicum* and two isolates, *Rhodotorula* sp., clustered with *Rhodotorula mucilaginosa.*

The most common taxa of marine fungi primarily belong to the phyla Ascomycota and Basidiomycota, which account for more than 90% of all described marine fungi. Recent developments in high throughput sequencing technologies have revealed an increase in fungal diversity in the marine realm, and culture-dependent methods have further demonstrated this (Jones et al., 2019). Grossart et al. (2019) reported yeast-like marine fungi within the phyla Ascomycota and Basidiomycota. In this study, all eleven isolates belonged to either of these two phyla, as *Meyerozyma guilliermondi* and *Rhodotorula mucilaginosa* strains were yeasts and *Penicillium oxalicum* strains were moulds. Bankole et al. (2022) isolated two marine-derived fungi, *Aspergillus aculeatus and Mucor irregularis*, from the shores of the Lagos Atlantic Ocean.

Bu et al. (2021) isolated a fungus, *Meyerozyma guilliermondii* GXDK6, which could survive independently under high copper stress (1400 ppm) from the subtropical marine mangrove sediments in China, while Li et al. (2022) isolated marine-derived *Penicillium oxalicum* MEFC104, which possesses antibacterial activity, from the north Yellow Sea in China, and Gerritse et al. (2020) isolated a *Rhodotorula mucilaginosa* strain capable of degrading plastics from a 350 L laboratory seawater microcosm containing a variety of plastic items retrieved from the North Sea in the Netherlands. Similarly, Yan et al. (2010) isolated about forty-four strains of *Candida tropicalis* from seawater, sea sediments, mud of sea salterns, guts of marine fishes, mangrove plants, and marine algae in China. All these studies agree with our work and show the distribution and diversity of fungi strains in different marine environments.

In this study, the culture supernatants of eleven fungal isolates exhibited laccase-like activity when ABTS was used as the substrate. The isolate *Meyerozyma guilliermondii* A4B showed the highest laccase-like activity (609.2±31.7 $UL^{-1)}$ after 96 hours of incubation in the absence of NaCl and the highest activity (186.94 $UL^{-1)}$ in the presence of 4% NaCl. On the other hand, following 7 days of culture in 3% (w/v) NaCl, *Aspergillus sclerotiorum* that was obtained from marine habitats exhibited 9.26 U/L laccase-like activity (Ben Ali, 2020). They also reported that a marine-derived *Trichoderma asperellum* isolated from the Tunisian biotope produced a laccase-like activity of approximately 190 UL^{-1} in 1% NaCl.

Saravanakumar and Kathiresan (2014) also reported that optimising laccase-like activity levels from *Trichoderma* sp. grown in 0.5% NaCl yielded about 2000 UL^{-1}, but these results are not directly comparable because the activity was measured using o-tolidine as a substrate instead of ABTS.

This study showed that some fungal cultures could exhibit laccase-like activity in media supplemented with varying concentrations of NaCl. These fungi, which are able to grow in NaCl-containing media, could find immense application in biotechnological and industrial processes where high salinity prevails (Raghukumar, 2008). In this study, it was observed that when some marine-derived fungi use synthetic dyes as substrates, they can exhibit some laccase-like activity with high levels of salt tolerance. In the textile industry, the effluent contains dyes and high salt concentrations. With this in mind, the ability of some fungi to produce laccase-like activity under saline conditions could lead to the discovery of new enzymes that are secreted by these fungi that can be employed in the textile industry.

This study evaluated the effect of a known laccase inducer, $CuSO_4$, on the laccase-like activity of the isolates. Several fungal species have been reported to be significantly induced by copper (Domínguez et al., 2007). Increased laccase activity has been shown to be proportional to the concentration of copper added to the culture medium (Palmieri et al., 2000). In this study, the *Meyerozyma guilliermondiii* A4B isolate exhibited the highest laccase activity of 908 UL^{-1} at 2 mM $CuSO_4$, followed by the *Penicillium oxalicum* M6A isolate at 846 UL^{-1}, and the activity of all the isolates decreased at $CuSO_4$ concentrations above 2 mM. Ben Ali et al. (2020) reported an optimal $CuSO_4$ concentration of 1.8 mM for *T. asperellum*, and Hao et al. (2007) also observed that cultures of *Pestalotiopsis* sp. with 2.0 mM $CuSO_4$ had peak laccase activity (32.7 U mL^{-1}), with decreased activity above this concentration; both of these studies agree with this work. The optimal $CuSO_4$ concentration for *Polyporus brumalis* laccase synthesis was found to be 0.25 mM (Nakade et al., 2013).

Fungal laccases have an active site that consists of four copper atoms arranged in three copper centers and two disulfide bonds from the amino acid cysteine. This explains why the $CuSO_4$-mediated induction of laccase via the addition of copper to the culture medium induces laccase gene transcription (Piscitelli et al., 2011). The induction of laccase concentrations by $CuSO_4$ should be carefully monitored, as copper toxicity has been reported to occur through interactions with proteins, enzymes, nucleic acids, and metabolites associated with major cellular functions (Piscitelli et al., 2011).

In this study, all eleven isolates were screened for their ability to degrade textile dyes on solid and liquid media. After 7 days, *C. tropicalis* M6 exhibited the greatest radial growth after 7 days of incubation with DR75, RBBR, or IC, while *Meyerozyma guilliermondii* A4B exhibited the greatest radial growth with MG. The radial growth measurement is a parameter used to assess the inhibition of the fungi by fungicides on solid media (Hendricks et al., 2017). It shows that after 7 days of incubation of the isolates, *C. tropicalis* M6 was the least inhibited in media supplemented with DR75 and IC, while *Meyerozyma guilliermondii* M1B *and Meyerozyma guilliermondii* A4B was the least inhibited in media supplemented with RBBR and MG respectively. Ali et al. (2015) reported the antifungal properties of Malachite green, and from this study, the growth of *Meyerozyma guilliermondii* A4B in media supplemented with MG is remarkable.

In a study on *Emmia latemarginata* strains (MAP03, MAP04, and MAP05) and *Mucor circinelloides* strains (MAP01, MAP02, and MAP06), Juárez-Hernández et al. (2021) reported radial growth of more than 0.3 cm/day after six days of incubation with six textile dyes, which agrees with our work. In this study, eight isolates showed high decolorization efficiency for multiple dyes, which should provide advantages for the treatment of effluents with complex dye compositions. Feng et al. (2023) reported the decolorization of seven different azo dyes (40.0 µmol/L) by the yeast *M. guilliermondii* A4, with decolorization percentages between 86.79–99.54% within 12 h. Gharieb et al. (2020) isolated *M. guilliermondii* S20 and *N. diffluens* S18 fungal strains that exhibited high degradation activity of 87% for DR81 dye and 70.7% for reactive red dye after incubating for 48 h. Flórez-Restrepo et al. (2018) reported the degradation of reactive black by *R. mucilaginosa* (79.31%), and all these reports were on terrestrially derived fungi. To the best of our knowledge, studies on the decolorization abilities of several marine-derived fungal species, such as *Meyerozyma guilliermondii, Penicillium oxalicum, Candida tropicalis,* and *Rhodotorula mucilaginosa,* for use in decolorizing synthetic dyes are scanty in the literatures.

Three types of mechanisms are identified for the elimination of fungal dyes: bioaccumulation, bioabsorption, and biodegradation (Kaushik & Malik, 2009). The term "bioaccumulation" describes the build-up of contaminants caused by cells that are actively developing and metabolising substances. Dye molecules are bound by amino, carboxyl, thiol, lipid, and phosphate groups found in fungal cell walls during the process of biosorption, which can take place in either living or dead biomass (Kaushiki & Malik, 2009; Kyzas et al., 2013). Since fungi can produce a variety of nonspecific extracellular and intracellular enzymes involved in the dye decolorization

process, including laccase, manganese peroxidase (MnP), manganese-independent peroxidase (MIP), lignin peroxidase (LiP), tyrosinase, and others, biodegradation is the primary mechanism of decolorization by fungi (Kaushiki & Malik, 2009; Kyzas et al., 2013).

In this work, an attempt was made to determine whether the decolorization of the dyes by the isolates was due to biosorption by determining the percentage biosorption of the dyes by the eleven isolates. Five isolates—*Rhodotorula mucilaginosa* M5B1, *Meyerozyma guilliermondii* M1B, *Penicillium oxalicum* M6A, *Meyerozyma guilliermondii* O6A, and *Meyerozyma guilliermondii* A4B—had biosorption percentages of less than 2% across the four dyes with *M. guilliermondii* A4B showing the least biosorption rate, while six had biosorption rates close to 17%. This finding showed that dye biosorption contributed less to the decolorization of the dye by the five fungal mycelia than the other methods. Yang et al. (2016) reported dye biosorption percentages for thirteen freshwater fungi on eleven synthetic dyes, with percentages ranging from 0–41%. This finding is in agreement with our work, where we observed biosorption percentages ranging from 0 to 16.93%, even though the isolates in their published work were from fresh water habitat. They also reported that a number of enzymes are involved in the decolorization process, based on their evaluation of several fungal strains' capacity to remove various colors; of which laccase is one of them. There are few studies on the biosorption percentages of marine-derived fungi. The laccase-like activity of the isolates during degradation was assayed after seven days of degradation, and the results showed that the *M. guilliermondii* A4B and *Penicillium oxalicum* M6A isolates had the highest activity across the four dyes.

This study explored the largely understudied marine-derived fungi biodiversity of five different coastal sites in Lagos, Nigeria— a region whose marine mycobiota has received little scientific attention. While most fungal bioremediation studies have been on the use of terrestrial fungi, this work isolated and characterized marine fungi from Nigeria waterways, introducing new myco-resources for environmental biotechnology. Notably, *M. guilliermondii* A4B demonstrated outstanding laccase-like activity and decolorization efficiency across multiple synthetic dyes, even under saline conditions as obtained in textile industry effluents. The study showed that dye removal by these isolates was mainly through enzymatic biodegradation rather than passive biosorption, highlighting their active metabolic capacity. These findings offer novel, salt-tolerant fungal candidates with promising potential for environmentally friendly and sustainable industrial wastewater treatment applications.

5. Conclusion

In this study, out of the forty fungi isolated from some marine habitats, eleven exhibited laccase activity, from which only two isolates (*Meyerozyma guilliermondi* A4B and *Penicillium oxalicum* M6A) degraded four textile dyes (Malachite Green (MG), Indigo Carmine (IC), Remazol Brilliant Blue R (RBBR), and Direct Red 75 (DR75)) effectively. With regard to oxidative activity, *Meyerozyma* guilliermondii A4B and *Penicillium oxalicum* M6A exhibited the greatest activity. The two fungi effectively decolorized four synthetic dyes from various dye families and demonstrated the highest level of oxidative capacity and grew best at 2 mM $CuSO_4$ as an inducer and 2% and 1% NaCl respectively, to mimic a marine environment, and both produced the highest laccase-like active cell-free supernatants activity. Further studies are needed to identify the various enzyme machinery that the fungi use to break down textile dyes. The enzymatic mechanism used by fungi derived from marine environments, as well as the degradation pathway involved in the breakdown of dyes needs to be better understood. There is also a need to further explore the untapped potential of the marine habitats especially in Nigeria, for other fungi that could have immense application in biotechnology.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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