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Dye Decolorization Activities of Marine-Derived Fungi Isolated from Manila Bay and Calatagan Bay, Philippines

Jeremy Martin O. Torres[1], **Christine V. Cardenas**[1], **Llewelyn S. Moron**[1], **Ana Patricia A. Guzman**[2], and **Thomas Edison E. dela Cruz**[1,2*]

[1]Department of Biological Sciences, College of Science and
[2]Fungal Biodiversity and Systematics Group,
Research Cluster for the National and Applied Sciences,
University of Santo Tomas, España, Manila, Philippines

Synthetic dyes used in textile industries, if not treated prior to its disposal, can enter our water systems and cause pollution. Thus, our research study explores the potential of locally isolated marine-derived fungi as bioremediation agents of dye pollution. Marine-derived fungi that were initially isolated from seawater and marine sediments (MF) and from living seagrasses (EMF) were tested for their ability to decolorize synthetic dyes using the tube agar overlay method. Of the 26 isolates tested, 21 strains showed partial to full dye decolorization of 0.01% crystal violet (CV). Selected strains were then further tested for dye decolorization on different culture media, e.g. Potato Dextrose Agar (PDAS), Malt Extract Agar (MEAS), and Czapek Dox Agar (CDAS), all supplemented with 33g/L marine salts and 0.01% CV. Results showed that only EMF14 and MF6 decolorized the dye with a maximum decolorization depth of 9mm and 7mm, respectively. Three strains were further tested for the decolorization of different dyes, e.g. crystal violet, Congo red and brilliant green, in liquid media (PDBS) under agitated and stationary conditions. All tested strains (EMF14, MF6, and MF49) completely decolorized Congo red regardless of the culture condition while only EMF14 and MF49 exhibited 87 – 91% decolorization of crystal violet. No growth and/or decolorization were observed on brilliant green. Gene sequence analysis confirmed the identities of these dye-decolorizing marine-derived fungi as *Phialophora* sp. (MF 6), *Penicillium sp*. (MF 49), and *Cladosporium* sp. (EMF 14).

Key Words: bioremediation, biosorption, marine-derived fungi, synthetic dyes

INTRODUCTION

Over 7 x 10^5 metric tons of synthetic dyes are annually produced worldwide (Venkata-Mohan et al. 2008). Of these, approximately 10 – 15 % of unused dyes enter the wastewater after dyeing and after the subsequent washing processes (Rajamohan & Karthikeyan 2006). Dyes in wastewater often lead to the contamination of surface and groundwater in neighboring locales. Thus, the consumption of these waters may pose a great health risk. For instance, the incidence of bladder tumors has been reported to be particularly higher in dye industry workers than in the general population (Suryavathi et al. 2005). Numerous physicochemical wastewater treatment methods are currently employed to rid wastewater of dyes. Although physicochemical treatment techniques can be effective in decolorizing dyes in wastewater,

*Corresponding author: tedelacruz@mnl.ust.edu.ph

these methods are either chemical-dependent, expensive or of limited applicability. It is therefore imperative to find a more efficient and cost-effective alternative in the treatment of dye-contaminated waters.

Bioremediation offers an effective alternative method for degrading dyes present in wastewater. Fungi, in particular, are known to decolorize and metabolize dyes (Raghukumar 2000; McMullan et al. 2001), and in some cases, have the potential to decolorize these dyes within a comparatively shorter time (Abd El-Rahim & Moawad 2003). Thus, this research study aimed to screen locally isolated marine-derived fungi for their potential as bioremediation agents. Marine fungi is a small, ecologically-defined group of primarily filamentous Ascomycetes and their anamorphs which are major decomposers of woody and herbaceous substrates in marine ecosystems (Hyde et al. 1998). They are known to be more resistant to toxic levels of pollutants than many marine bacteria (Hyde et al. 1998), and thus, makes them ideal organisms for the bioremediation of wastewaters.

MATERIALS AND METHODS

Source of marine-derived fungal strains

The marine-derived fungal strains were obtained from the University of Santo Tomas - Collection of Microbial Strains (USTCMS) housed at the Research Center for the Natural Sciences, Thomas Aquinas Research Complex, University of Santo Tomas in Manila, Philippines. All 26 strains were maintained on Potato Carrot Agar (PCAS: 500g chopped potato, 500g carrots, 1L distilled water, boiled for 1h and macerated, 15g/L agar) supplemented with 33g/L marine salts (Bioresearch). Eleven of these marine-derived fungal strains were previously isolated from living seagrasses (EMF) collected in Brgy. Dos, Calatagan, Batangas. Fifteen strains were isolated from seawater (MF) collected in Manila Bay and nearby coastal provinces of Cavite and Batangas. Identification of these marine fungal strains was done previously based on their morphocultural characters (Yao et al. 2009).

Screening of marine-derived fungi for dye decolorization activities

Twenty six marine-derived fungal strains were screened for their ability to degrade synthetic dyes using the tube overlay method. Initially, the fungal strains were grown on culture plates pre-filled with PCAS and incubated at room temperature for 14 days. Following incubation, mycelial agar plugs (~5 mm^2) were cut approximately 5 mm from the colony margin and inoculated on test tubes (in triplicates) containing 5 mL of Potato Dextrose Agar supplemented with 33 g/L of marine salts (PDAS) overlaid with 1 mL of PDAS with 0.01% (w/v) crystal violet dye. All culture tubes were incubated at room temperature (~25^oC) and observed weekly for up to four weeks. Clearing of the overlaid dye indicates full decolorization (++). Partial dye decolorization (+) was indicated by less dye intensity in comparison with the control (uninoculated PDAS overlaid with PDAS + 0.01% crystal violet).

Assay for the dye decolorization activities of marine-derived fungi in solid media

Twelve selected marine-derived fungal strains exhibiting either full (EMF 4, EMF 7, EMF 10, EMF 11, MF 6, MF 22, MF 25, MF 31, MF 42, and MF 49) or partial (EMF 14, MF 53) dye decolorization activities were further tested for their ability to decolorize crystal violet on different culture media. Initially, the fungal strains were grown as previously described. Following incubation, mycelial agar plugs (~5 mm^2) were cut approximately 5 mm from the colony margin and inoculated on test tubes (in triplicates) each pre-filled with 2 mL of either one of the following culture media: Potato Dextrose Agar (PDAS), Malt Extract Agar (MEAS) and Czapek Dox Agar (CDAS), all supplemented with 33 g/L of marine salts and 0.01 % (w/v) crystal violet. The culture tubes were then incubated at room temperature (~ 25^oC). The growth of the fungi and its ability to decolorize the dye were observed weekly up to four weeks. The depth of dye decolorization (in mm) indicated by clearing of the dye was then measured.

Assay for the dye decolorization activities of marine-derived fungi in liquid media

Three selected marine-derived fungal strains (MF 6, MF 49, and EMF 14) were further tested for their ability to decolorize different dyes in liquid medium. Initially, the marine fungal strains were grown on PCAS slants and incubated for two weeks at room temperature. Following incubation, 5 mL of sterile artificial seawater (ASW) was added onto the culture slants. The spores and mycelia were then dislodged using a flame-sterilized inoculating loop. Then, 10 μL of the inoculum were added on culture vials (in triplicates) pre-filled with 25 mL Potato Dextrose Broth (PDBS) supplemented with 33 g/L marine salts and 0.01% of either one of the following dyes: brilliant green (BG), Congo red (CR), and crystal violet (CV). Two sets were prepared and were incubated either under constant agitation (100 rpm, Forma Scientific Orbital Shaker) or under stationary condition. All culture vials were incubated at room temperature for 3 to 4 weeks. Growth and dye decolorization were noted every week. Following culture for 3 to 4 weeks, the culture filtrates were decanted

and subjected to spectrophotometric analysis. For the crystal violet, the absorbance reading (Abs) was done at 590 nm wavelength. Congo red was read at 497 nm while brilliant green was read at 625 nm. The extent of dye decolorization by the marine fungal strains on liquid media was calculated using the formula below:

$$\% \text{ dye decolorization} = \frac{Abs_{control} - Abs_{inoculated}}{Abs_{control}} \times 100$$

Finally, the mycelial biomass were harvested on clean petri plates and observed directly. Fungal hyphae were also mounted on clean glass slides and observed under a compound light microscope for the biosorption of dyes.

Characterization and identification of selected marine-derived fungi using modern molecular methods

Five marine-derived fungal strains previously identified by morphocultural methods (Yao et al. 2009) and exhibiting dye decolorization activities (EMF 4, EMF 14, MF 6, MF 25, and MF 49) were further characterized and identified using molecular methods. The fungal strains were initially cultured on sterile cellophane-covered Potato Dextrose Agar supplemented with 33 g/L marine salts at room temperature for 3 to 5 days. Mycelial growth from colony margin, approximately 150-200mg fresh weight, were then scraped-off, ground in liquid nitrogen, and the genomic DNA extracted with DNeasy® Plant MiniKit (Qiagen GmbH, Germany) according to the manufacturer's instructions. Extracted DNA was then stored at -20^{o}C until used in the gene sequence analysis.

PCR amplification of the nuclear DNA was done as previously described (Turner et al. 1997; Kullnig-Gradinger et al. 2002). The extracted genomic DNA was PCR-amplified with the fungal specific primer combinations for the internal transcribed spacers 1 and 2 (ITS 1 and 2) regions including the flanking 5.8S rRNA gene: SR6R (5'-AAG TAG AAG TCG TAA CAA GG-3') and LR1 (5' - GGT TGG TTT CTT TTC CT - 3') (White et al. 1990). The PCR products were then purified with the commercially available QIAquick PCR purification Kit (Qiagen GmbH, Germany). The PCR products (10 µL) were loaded on 1% agarose gel (300 mL) mixed with 6 µL of 1 µg/mL ethidium bromide in TAE buffer. Gel electrophoresis was set up at 80 V for 80 min and the DNA bands were visualized with UV. The PCR products were then sent for gene sequencing at Macrogen, Inc. (Seoul, South Korea).

Following sequencing of the PCR products, the forward and reverse DNA sequences of the five fungal isolates were then aligned using BIOEDIT and compared with other sequences found in the NCBI GenBank. The related sequences were then sent to Dr. Irina S. Druzhinina, Institute of Chemical Engineering, Vienna University of Technology, Vienna, Austria for alignment and construction of a dendogram. The relationship of the marine-derived fungi with the sequences found in NCBI GenBank was determined based on the dendogram.

RESULTS

Dye decolorization activities of marine-derived fungi in solid media

Tube Overlay Method. Of the 26 marine-derived fungal strains, 21 strains exhibited either partial or full dye decolorization of crystal violet using tube overlay method (Table 1, Figure 1). Ten isolates (4 EMF, 6 MF) exhibited full dye decolorization within 22 days of incubation at room temperature. Eleven strains (6 EMF, 5 MF), however, were only able to partially decolorize the dye even after 28 days of incubation.

In Three Different Solid Media. Of the twelve marine-derived fungal strains grown in three different culture media with 0.01% crystal violet, 11 strains exhibited growth in at least one of the test culture media (Table 2). Growth mostly began on the third day of incubation for all marine-derived fungal strains. However, CDAS supported growth

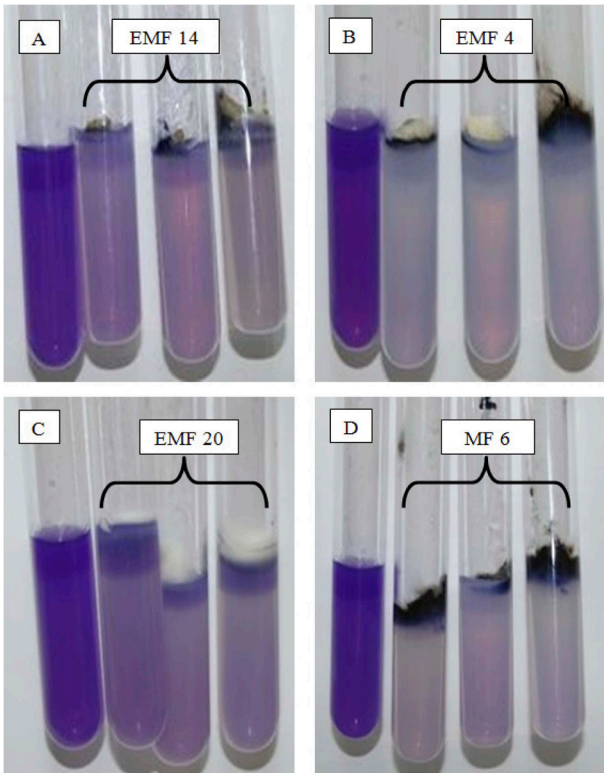

Figure 1. Marine-derived fungal strains exhibiting partial (A, C) and full (B, D) decolorization of 0.01% crystal violet using tube overlay method. Control tubes are at the leftmost part of each figure.

Table 1. The marine-derived fungal strains used in the study and their dye decolorization activities using the tube overlay method.

Collection Site	Strain Number[a]	Taxon[b]	Dye decolorization activity after 28 days of incubation[c]
Metro Manila			
Baywalk, Manila	MF 2	NI	+
	MF 6	***Phialophora verrucosa***	++
Coastal Road, Pasay City	MF 12	*Acremonium* sp.	-
Navotas Fish Port	MF 22	NI	+ to ++
	MF 23	*Acremonium* sp.	-
	MF 25	***Myrothecium* sp.**	- to ++
	MF 28	*Acremonium* sp.	-
Cavite Province			
Brgy.Tamalan, Concepcion	MF 31	*Tolypocladium inflatum*	++
	MF 33	*Acremonium terricola*	-
Brgy. Calampit, Ternate	MF 40	*Phoma* sp.	+
	MF 42	*Acremonium* sp.	+ to ++
Batangas Province			
Nasugbu	MF 49	***Penicillium* sp.**	+ to ++
Brgy. Uno, Calatagan	MF 53	*Alternaria* sp.	+
	MF 55	NI	- to +
Carpuran, Calatagan	MF 67	*Coniothyrium* sp.	- to +
Brgy. Dos, Calatagan	EMF 4	***Paecilomyces* sp.**	++
	EMF 5	NI	-
	EMF 7	NI	+ to ++
	EMF 10	NI	+ to ++
	EMF 11	NI	+ to ++
	EMF 12	NI	+
	EMF 14	***Cladosporium* sp.**	+
	EMF 16	NI	+
	EMF 17	NI	+
	EMF 18	NI	+
	EMF 20	NI	+

[a] MF strains were derived from seawater while EMF strains were isolated from seagrasses.
[b] NI = not identified. Preliminary identification was based on morphocultural characterization. Names in bold were confirmed by gene sequence analysis (see Figure 5).
[c] Dye Decolorization Activity based on Tube-Overlay Method (see also Figure 1):
++ full dye decolorization
+ partial dye decolorization
- no dye decolorization

Table 2. Growth and decolorization of 0.01% crystal violet in different solid media by 12 selected marine-derived fungal strains.

Marine-derived fungal strain		PDAS[a]		MEAS		CDAS	
		Growth	Depth of decolorization[b] (in mm)	Growth	Depth of decolorization (in mm)	Growth	Depth of decolorization (in mm)
EMF	4	+	0	+	0	-	0
	7	-	0	+	0	-	0
	10	+	0	+	0	-	0
	11	+	0	+	0	+	0
	14	+	8-9	+	6-7	+	5-6
MF	6	+	7	+	5-6	+	6
	22	+	0	+	0	-	0
	25	+	0	+	0	-	0
	31	-	0	-	0	-	0
	42	+	0	+	0	+	0
	49	+	0	+	0	-	0
	53	+	0	+	0	+	0

[a] All culture media used in the assay were supplemented with 33 g/L marine salts:
PDA: Potato Dextrose Agar
MEA: Malt Extract Agar
CDA: Czapek-Dox Agar
[b] Depth of decolorization refers to the length of the tubed medium with clearing of the CV dye.

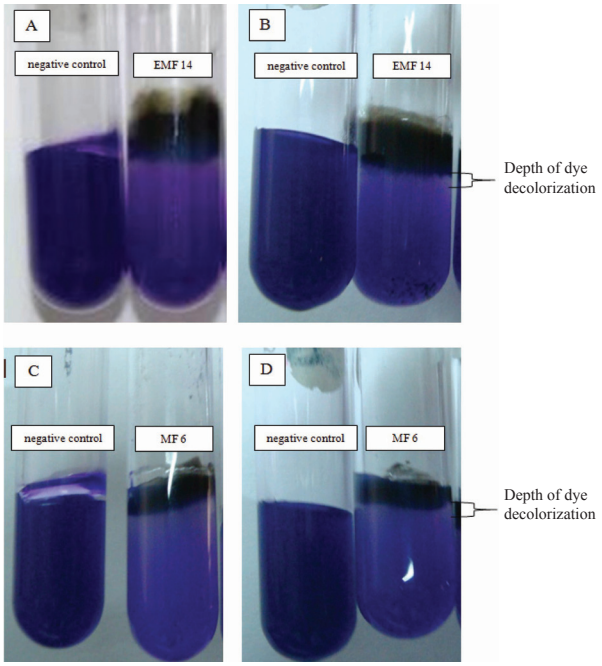

Figure 2. Two marine-derived fungal strains exhibiting depth of dye decolorization in two solid media: EMF 14 in PDAS (A) and MEAS (B) and MF 6 in PDAS (C) and MEAS (D). PDAS and MEAS were supplemented with 0.01% crystal violet.

of only five test fungi in contrast to the growth of 10 to 11 test fungi on MEAS or PDAS (Table 2). Moreover, only two strains (EMF 14 and MF 6) exhibited depths of dye decolorization of 5-6 mm after 4 weeks of incubation on CDAS (Table 2). The two strains also showed a maximum decolorization depth of 9 mm and 7 mm in PDAS and MEAS, respectively (Table 2, Figure 2).

Dye decolorization activities of isolated marine-derived fungi in liquid media

Three marine-derived fungal strains, MF 6, EMF 14, and MF 49, were then selected as test strains for the assay in liquid medium. MF 6 and EMF 14 showed depth of dye decolorization of at least 5 mm in the three media tested. MF 49 did not show any visible depth of decolorization but exhibited good growth on PDAS and MEAS with crystal violet. Our results showed that all three strains completely decolorized Congo red regardless of the culture condition used (Figure 3). Dye decolorization took place the quickest (within 1 wk) in PDBS with Congo red. Spectrophotometric analysis at 590 nm also showed 91 % and 89 % dye decolorization of crystal violet by MF 49 under constant agitation

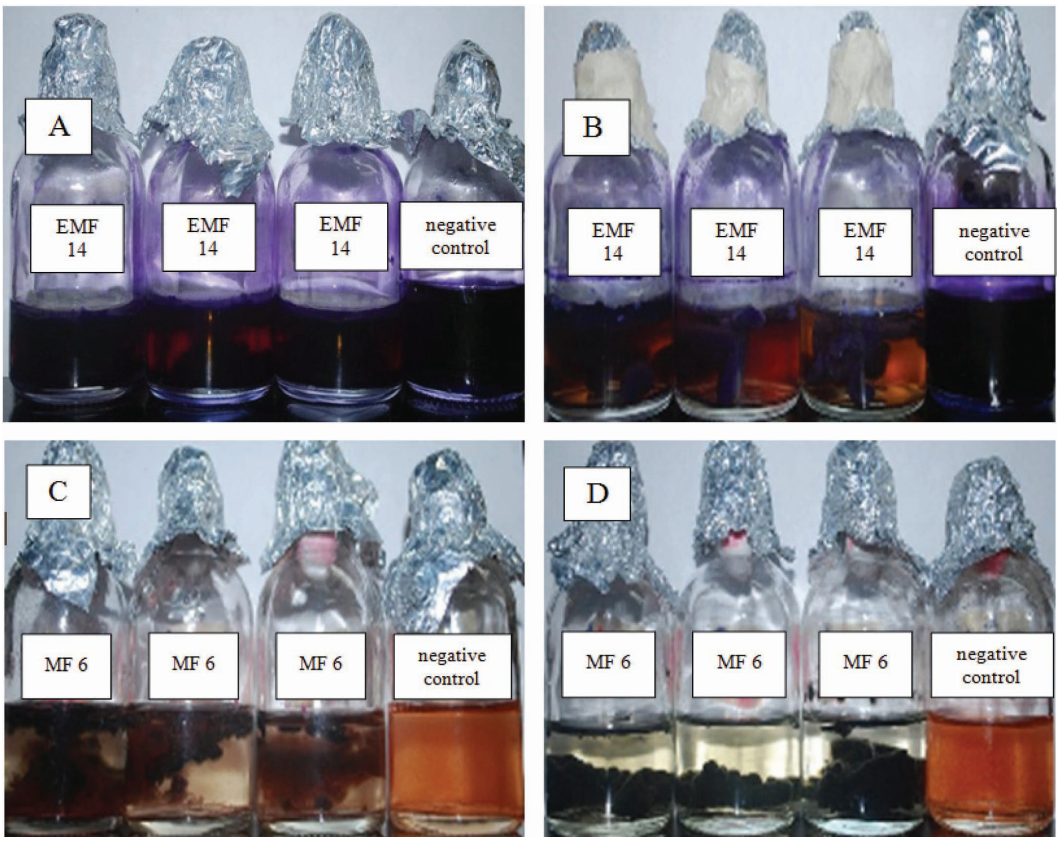

Figure 3. Dye decolorization activities of marine-derived fungi in PDBS. Strain EMF 14 exhibiting decolorization of crystal violet under stationary (A) and agitated (B) conditions after 4 weeks of incubation. Strain MF 6 exhibiting complete decolorization of Congo red under stationary (C) and agitated (D) condition after 3 weeks of incubation. Vials on the far right are negative controls (uninoculated PDBS with CV/CR).

Table 3. Percent decolorization of crystal violet by the three marine-derived fungal strains in PDBS cultivated under agitated and stationary conditions after 4 weeks of incubation.

Marine-derived Fungal Strains	% Dye Decolorization of Crystal Violet	
	Under Agitated Condition	Under Stationary Condition
MF 6[a]	0 %	0 %
MF 49	91.27 ± 3.22 %	89.04 ± 0.77 %
EMF 14	87.44 ± 0.84 %	87.73 ± 1.04 %

[a] No fungal growth was observed on PDBS with crystal violet under both culture conditions.

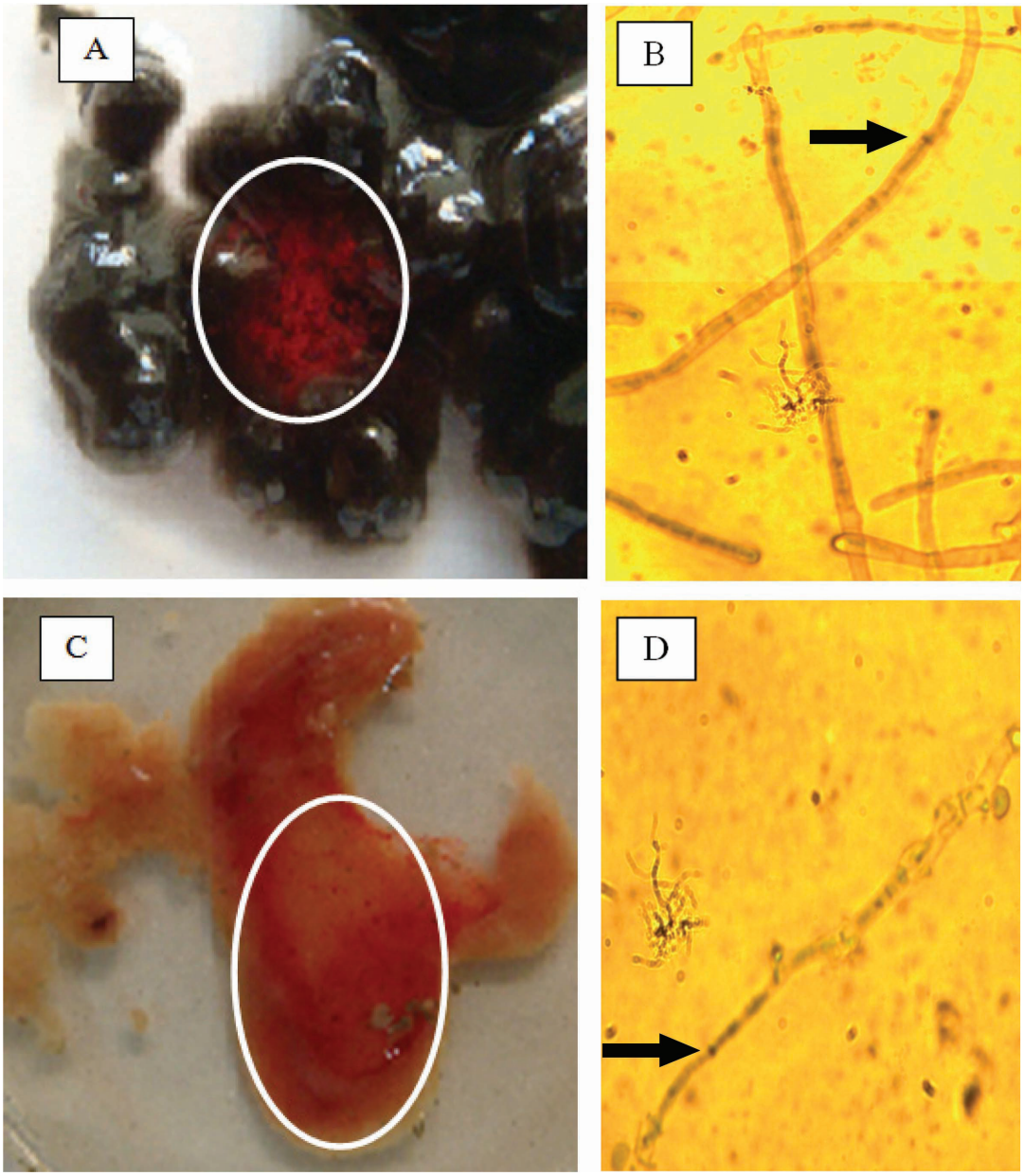

Figure 4. Biosorption of Congo red by marine-derived fungi. Congo red adsorbed to the fungal mycelia of MF 6 (A) and MF 49 (C). Dye particles absorbed within the hyphae of MF 6 (B) and MF 49 (D) viewed under compound light microscope (1000x).

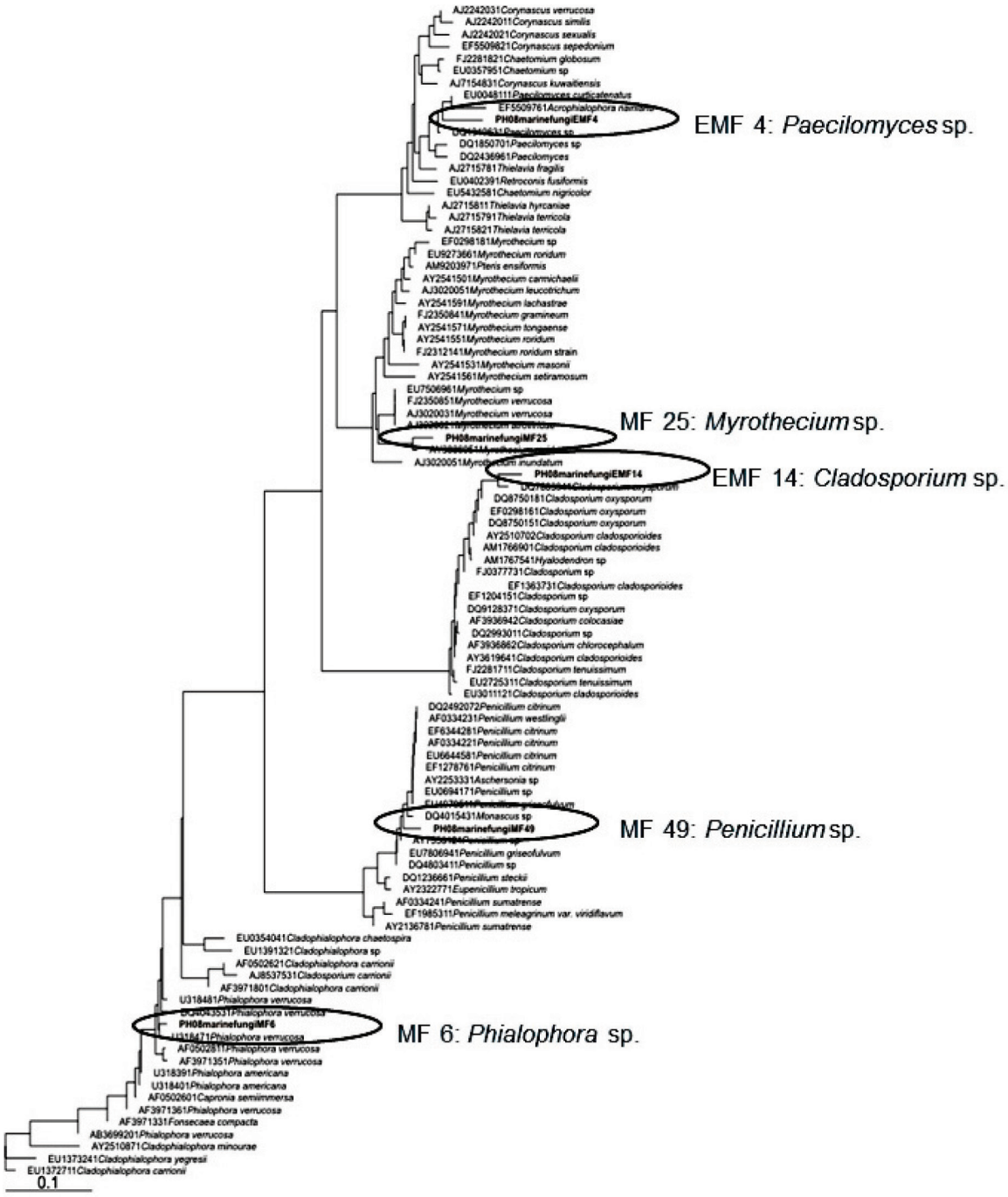

Figure 5. Dendogram showing the closely related species of the five isolated marine-derived fungal strains (EMF 4, EMF 14, MF 6, MF 25, and MF 49) exhibiting dye decolorization activities.

and stationary condition, respectively (Table 3, Figure 3). EMF 14 exhibited 87 to 88 % dye decolorization of crystal violet under the same incubation conditions. However, MF 6 showed no growth and no decolorization of crystal violet in both agitated and stationary conditions following three weeks of incubation (Table 3). No growth and decolorization was observed among the three fungi in PDBS with brilliant green under the two culture conditions tested (data not shown). Gross and microscopic examinations showed adsorption of Congo red to the fungal mycelia (Figure 4).

Identities of selected marine-derived fungi as inferred from gene sequence analysis

Following gene sequence analysis, the marine-derived fungal isolates were identified as *Paecilomyces* sp. (EMF

4), *Cladosporium* sp. (EMF 14), *Phialophora* sp. (MF 6), *Myrothecium* sp. (MF 25), and *Penicillium* sp. (MF 49) (Figure 5). Identities of these isolated marine-derived fungi were in agreement with their identities based on their morphocultural characters.

DISCUSSION

Kohlmeyer (1974) described marine fungi as those that grow and sporulate exclusively in estuarine or marine habitats and as those fungal species from terrestrial or freshwater areas able to grow in estuarine or marine environment. Marine fungi are major decomposers of woody and herbaceous substrates in marine ecosystems (Hyde et al. 1998). Obligate and facultative marine fungi occurring in coastal marine environment can be used in the bioremediation of polluted soil and wastewaters (Raghukumar 2000). The use of these fungi thus offers a much cheaper and efficient alternative treatment of wastewaters. Screening of the marine-derived fungal strains using tube overlay method showed dye decolorization activities by most of the isolates (Table 1). However, no correlation was observed between the substrates where the marine-derived fungi were isolated and the decolorization activity, though it can be noted that 10 of the 11 EMF strains whose substrates were seagrasses, were able to decolorize the dye (Table 1). PDAS and MEAS with CV both supported growth of more marine-derived fungal strains as compared to CDAS with CV (Table 2). This can be attributed to the minimal nutrients available for the growth of fungi in Czapek Dox Agar. However, it can be noted that the number of marine-derived fungi that exhibited dye decolorization in the three solid media considerably decreased from 21 as observed in the tube overlay method to two (Table 1, Table 2). The diffusion of the enzymes needed for the decolorization of the dye appeared to be better in the tube overlay method than in the homogenous dye-media mixture (Figure 1, Figure 2). This perhaps explains the less number of marine-derived fungal strains exhibiting dye decolorization in the homogenous dye-media mixture or in the three solid media. However, even though qualitative assays using the tube overlay method are powerful tools in screening fungi for extracellular enzyme production, they are not conclusive in that a negative reaction is not an absolute confirmation of a species' inability to produce a particular enzyme (Abdel-Raheem & Shearer 2002). Hence, the tube agar overlay method only provides an easier and quicker method to screen a large number of isolates for dye decolorization activity

The removal of the dye color is vital in the potential application of these organisms as bioremediation agents in wastewater treatment plants and in coastal waters. Thus, it is essential to test marine-derived fungal strains for dye decolorization in liquid medium. Our results showed that Congo red was completely decolorized by the test marine-derived fungal strains (Figure 3). This efficient decolorization maybe attributed either through the action of extracellular enzymes and/or biosorption of the fungal biomass (Figure 4). Ollikka et al. (1993) reported that Congo red was a substrate for the ligninolytic enzyme lignin peroxidase. Tatarko and Bumpus (1997) confirmed that Congo red was readily degraded in liquid cultures as also shown in this study. Furthermore, the marine fungal strains also showed promising decolorization activities against crystal violet (Figure 3, Table 3). Results of the spectrophotometric analysis were even comparable with the percent dye decolorization exhibited by the white-rot fungus *Trametes versicolor* (Yang et al. 2009), *Pleurotus ostreatus* (Yan et al. 2009), and even *Phanerochaete chrysosporium* (Bumpus & Brock 1988). In contrast, no growth and decolorization was observed on all strains in PDBS with 0.01% brilliant green. Although some bacteria can decolorize brilliant green with up to 100% rate of decolorization (Kumar Sani & Chand Banerjee 1999), it was evident that the growth of the tested marine-derived fungal strains in our experiments were inhibited, thereby, inhibiting dye decolorization ability. This could be attributed to the nature of brilliant green as a fungicide, controlling fungal attacks on fishes and other aquatic organisms at specific concentrations (Srivastava et al. 2004).

Marine fungi possess ligninolytic enzymes and play an important role in the degradation of lignocellulose in marine ecosystems (Raghukumar et al. 1999; Raghukumar 2000). These lignin-degrading enzymes are directly involved not only in the degradation of lignin in their natural lignocellulosic substrates but also in the degradation of various xenobiotic compounds, including dyes (Wesenberg et al. 2003). Moreover, ligninolytic enzymes have been reported to oxidize many recalcitrant substances such as chlorophenols, polycyclic aromatic hydrocarbons (PAHs), organophosphorus compounds, and phenols (Wesenberg et al. 2003). Our results supports the idea that ligninolytic enzymes act on decolorizing the dye since seagrasses, like leaves and wood, are good sources of lignocellulose (Benner & Hodson 1985).

Lignin is a random aromatic polymer, thus, extracellular lignin-degrading enzymes can non-specifically attack aromatic compounds bearing little similarity with lignin (Field et al. 1992). Phenolic azo dyes, e.g. Congo red, and triphenylmethane dyes, e.g. crystal violet and brilliant green, consist of aromatic structures having little resemblance to lignin or lignin model compounds. Nevertheless, since the lignin-degrading system is a generalized and non-specific degradative system, it can still degrade other aromatic compounds, e.g. azo and triphenylmethane dyes (Field et al. 1992;

Haritash & Kaushik 2009). It is thus possible that the ability of marine-derived fungi to degrade crystal violet and Congo red as shown in this study can be largely attributed to the lignin-degrading enzyme system of the organism. In addition to extracellular enzymes, it is also possible that dye decolorization activity of the marine-derived fungi could be attributed to the ability of the fungal mycelia to adsorb/absorb the dye in their mycelia (Figure 4). Biosorption of dyes occur mainly either through complexation, adsorption by physical forces, precipitation, entrapment in inner spaces, ion exchange due to surface ionization, and by formation of hydrogen bonds (Yeddou-Mezenner 2010). Due to an increased cell-to-surface ratio, fungi have a greater physical contact with the environment (Kaushik & Malik 2009). Thus, some fungi have demonstrated better dye adsorption potential exceeding that of activated charcoal (Fu & Viraraghavan 2002; O'Mahony et al. 2002). Additionally, it is not unusual for some species to demonstrate both enzyme-mediated degradation and biosorption in the decolorization of textile dyes (Park et al. 2007). It is thus possible that in addition to the production of extracellular enzymes, the ability of the marine-derived fungi to decolorize synthetic dyes is coupled also with their biosorption abilities. This may account for the more efficient Congo red decolorization as compared to crystal violet decolorization by the marine-derived fungal strains (Figure 3).

The extent of dye decolorization under shaking and stationary conditions did not appear to vary based on percent dye decolorization (Table 3). This is in contrast to the study of Rigas and Dritsa (2006) where a higher efficiency in Poly R-478 decolorization was reported in shaking conditions than in static conditions. Parshetti et al. (2007) also reported that shaking conditions were better for faster and complete adsorption and decolorization of Reactive Blue-250 dye. Higher decolorization efficiency was observed in shake cultures because of better oxygen transfer and nutrient distribution as compared to stationary cultures (Kaushik & Malik 2009). However, it has been reported that ligninolytic enzymes are better expressed in static cultures and agitation of the cultures lead to decreased enzyme activity (Kaushik & Malik 2009). A strain of the fungus *Irpex lacteus* exhibited a 380-fold increase in manganese peroxidase (MnP) levels when grown in stationary cultures as compared to shake cultures (Novotny et al. 2004). Therefore, in these previously reported studies, better oxidation and possibly biosorption can be achieved using shake cultures while better enzymatic activities can be observed in stationary cultures. This could also be true for the dye decolorization activities exhibited by the isolated marine-derived fungi under shaking and stationary conditions in this study.

Gene sequence analysis confirmed the identities of the marine-derived fungal strains exhibiting dye decolorization activities as *Cladosporium* sp. (EMF 14), *Penicillium* sp. (MF 49), and *Phialophora* sp. (MF 6) (Figure 5). These are known terrestrial fungal genera that could have been washed away through coastal wave action. Nevertheless, these marine-derived fungal isolates exhibited ability to decolorize synthetic dyes in this research study. Species of *Penicillium* have also been found to degrade triphenylmethane dye, e.g. cotton blue (Shedbalkar et al. 2008). Muthezhilan et al. (2008) reported the inability of a strain of *Penicillium oxalicum* to decolorize crystal violet, but a strain of *P. citrinum* decolorized 64% of the same dye. Similarly, other reports also showed that species of *Cladosporium*, e.g. *C. sphaerospermum* (Grover et al. 2004) and *C. cladosporioides* (Vijaykumar et al. 2006), can degrade textile dyes. To the best of our knowledge, there are no published reports that assessed the dye decolorization activities of *Phialophora* species. It has been established though that these can produce ligninolytic enzymes such as lignin peroxidase and manganese peroxidase (Lopez et al. 2007). This is the first report of dye decolorization activity by a *Phialophora* species.

CONCLUSIONS

Locally isolated marine-derived fungal strains exhibited dye decolorization activities of synthetic dyes, specifically, crystal violet and Congo red, in solid and liquid culture media. Three marine-derived fungal strains, *Phialophora* sp. (MF 6), *Penicillium* sp. (MF 49), and *Cladosporium* sp. (EMF 14) showed complete decolorization of 0.01% Congo red and up to 91 % decolorization of 0.01% crystal violet. Aeration/agitation does not seem to be a factor for the dye decolorization activities of the test fungi. Enzyme production and/or biosorption were inferred as possible mechanisms responsible for dye decolorization of these fungi.

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