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Decolorization of Synthetic Dyes by Marine Derived Fungi Isolated from Decaying Leaves of Rhizophora Spp

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Abstract
This study explores the potential of marine-derived fungi isolated from Rhizophora spp. as bioremediation agents for synthetic dye pollution. Four fungal strains (MF1, MF2, MF3, and MF4) were isolated from different locations (Manila Marina Baytown Access Road, Boac, Marinduque Province, and Kaingin, Cavite, Philippines), exhibited decolorization of synthetic dyes (Methylene Blue, Congo Red, and Safranin). and tested for their ability to decolorize synthetic dyes (Methylene Blue, Congo Red, and Safranin). The results showed that Methylene Blue was the easiest to decolorize, while Safranin was the most resistant. Safranin proved to be the hardest to decolorize, while methylene blue was the easiest, appearing decolorized after two days of incubation. Exoenzyme tests revealed that the fungi produced amylase, xylose isomerase, and cellulase, suggesting their potential in dye degradation.

Keywords: Marine-derived fungi, synthetic dyes, Rhizophora spp, decolorization

1. INTRODUCTION

Synthetic dyes are widely used in various industries, with an estimated 10-20% lost in industrial effluents. Due to their complex chemical structures, these dyes are often resistant to conventional treatment methods, making their removal challenging and contributing significantly to environmental contamination (Gugel et al., 2024). Bioremediation using marine-derived fungi offers a promising alternative to conventional chemical treatments (Bonugli-Santos et al., 2015). Marine microorganisms account for over 90% of ocean biomass, and their diversity is believed to result from their ability to adapt to extreme marine environmental conditions. This adaptability also makes them one of the most viable options for bioremediation. Marine-derived fungi have proven to be a source of new enzymes, including those with previously undiscovered activities.

This study investigates the ability of marine-derived fungi isolated from Rhizophora spp. to degrade synthetic dyes and evaluates their enzymatic activity in facilitating the decolorization process.

2. Objective of the Study

The research specifically aims to determine which isolated marine-derived fungi exhibit full dye decolorization, how long it takes for marine-derived fungi to decolorize synthetic dyes, and what enzyme activities are present in the marine fungal isolates. Subsequently it determined that marine-

derived fungi isolated from mangrove leaves possess the ability to fully decolorize synthetic dyes.

3. Materials and Methods

This study used an experimental research design. In an experimental study, it probes into the characteristics of marine-derived fungi isolated from mangrove forests. The independent variables can be manipulated to check their effects on the dependent variables and measure their effectivity. The study focused on the ability of marine-derived fungi to decolorize synthetic dyes. The marine-derived fungi and their characteristics were the independent variables which were analyzed to cause an effect to the following independent variables; how effective are marine-derived fungi in decolorizing synthetic dyes and how long would it take to decolorize it in a controlled environment. This study also included testing the exoenzyme activities of these marine-derived fungi

Fallen mangrove leaves were collected from Manila Marina Baytown, Boac (Marinduque), and Kaingin (Cavite). Leaves were washed in sterile seawater with antibiotics before inoculation (Jesus et al., 2019). Fungi were isolated on Peptone Yeast Glucose Seawater (PYGS) agar, sub-cultured, and characterized based on morphology (Nakagiri, 2002). Identified fungal strains were designated as MF1, MF2, MF3, and MF4. Decolorization was tested using the tube overlay method, where fungi were inoculated on PYGS agar with 0.5% and 1% synthetic dye (Torres et al., 2009), and the homogeneous dye media method, where fungi were grown in PYGS agar with 0.1% and 0.5% concentrations of Methylene Blue, Congo Red, and Safranin (Yesilada et al., 2018). Fungi were tested for the presence of cellulase, amylase, xylose isomerase, and protease using substrate incorporation in a basal medium (Fatima et al., 2016). All isolates were observed based on their ability to decolorize different concentrations of synthetic dyes. Dye decolorization was observed for 28 28-day periods. Decolorization activities were measured as no decolorization. Partial decolorization and full decolorization as changes in culture media were apparent.

4. Results and Discussion

Colony Characteristic of isolated Marine-Derived fungi

Table 1. Colony Characteristic of isolated Marine-Derived fungi

Fungal strain	Suspected Taxon	Form	Elevation	Margin	Texture	Surface Color
MF1	Halophytophthora sp.	Rosette	Flat	Filamentous	Fine	White
MF2	Halophytophthora sp.	Rosette	Flat	Filamentous	Fine	White
MF3	NI	Circular	Convex	Entire	Velvety	Green
MF4	NI	Filamentous	Raised	Filamentous	Fine	Brownish

Table 1 shows that among the four marine-derived fungi that were isolated from the senescent leaves of Rhizophora spp., MF1 and MF2 showed similar patterns of a rosette formed colony, flat elevation, filamentous margin, fine texture and white surface color. MF3 shows a fungal colony that is circular in form, has a convex elevation, that is velvety in texture and green in color while MF 4 shows filamentous form, raised elevation, filamentous with fine texture eh brownish color.

Screening for the dye decolorization activities of marine-derived fungi using tube over lay in solid media with 0.5% Methylene Blue

Table 2. Dye Decolorization Activity based on Tube-Overlay Method

Strain Number	Dye Decolorization activity after 3 days	Dye Decolorization activity after 4 days
MF1	+	++
MF2	++	++
MF3	++	++
MF4	++	++

Legend: Dye Decolorization Activity based on Tube-Overlay Method (-) No Decolorization (+) Partial Decolorization (++) Full Decolorization

Table 2 shows that all marine-derived fungi exhibited full decolorization after 4 days of incubation at room temperature. 3 isolates (MF2, MF3 and MF4) exhibits full decolorization after 3 days while MF1 exhibited full decolorization after 4 days.

Screening for the dye decolorization activities of marine-derived fungi using tube over lay in solid media with 1% Methylene Blue

Table 3. Dye Decolorization Activity based on Tube-Overlay Method

Strain Number	Dye Decolorization activity after 3 days	Dye Decolorization activity after 8 days
MF1	+	++
MF2	+	++
MF3	+	++
MF4	+	++

Legend: Dye Decolorization Activity based on Tube-Overlay Method (-) No Decolorization (+) Partial Decolorization (++) Full Decolorization

All marine-derived fungi exhibited full decolorization after 8 days of incubation at room temperature as shown in table 3. All marine-derived isolates (MF1, MF2, MF3 and MF4) showed partial decolorization after 3 days while MF3 exhibited partial to full decolorization .

Assay for the dye decolorization activities of marine-derived fungi using homogenous dye media mixture with different concentration of Synthetic Dyes:

Table 4: Dye Decolorization Activity based on Homogenous Dye - Media

Strain	Dye Decolorization after 28 days	Dye Decolorization after 28 days	Dye Decolorization after 28 days

number	MB .1%	MB .5%	CR .1 %	CR .5%	S .1%	S .5%
MF1	++	++	+ to ++	+	+	- to +
MF2	++	++	+ to ++	+	+	- to +
MF3	++	++	++	+	+	- to +
MF4	++	++	+ to ++	+	+	- to +

Legend: Dye Decolorization Activity based on Homogenous Dye – Media (-) No Decolorization (+) Partial Decolorization (++) Full Decolorization

Methylene Blue (MB)

After 28 days of incubation of marine fungal strains on homogenous dye media all fungal isolates exhibited full decolorization of 0.1% and 0.5% methylene blue at room temperature. Regardless of concentration (both 0.1% and 0.5% Methylene blue), all fungal isolates (MF1, MF2, MF3 and MF4) showed full decolorization after 2 days of incubation.

Congo Red (CR)

Marine fungal strains MF1, MF2 and MF4 showed partial to full decolorization of 0.1% concentration of Congo Red on Homogenous dye media and MF3 showed full decolorization. At 0.5% concentration of Congo Red, all isolates showed partial decolorization. Fungal growth on the culture media was apparent.

Safranin (S)

All marine fungal isolates exhibited partial decolorization of 0.1% concentration of Safranin showed partial decolorization and exhibited almost no decolorization on 0.5% concentration of Safranin.

Table 5. Testing for exoenzyme activities

Substrate	Enzyme being tested	MF1	MF2	MF3	MF4
Cellulose	Cellulase	+	+	+	+
Milk	Protease	-	-	-	-
Xylose	Xylose Isomerase	+	+	-	+
Matlose	Amylase	+	+	+	+

In Table 5 MF1 and MF2 showed enzyme activities for xylose, maltose and cellulose. MF3 showed enzyme activities for maltose and cellulose, while MF4 confirmed the presence of xylose, cellulose and maltose activity. All were tested negative for protease activity

Colony Characteristic of Marine-Derived Fungi

Different types of fungi would produce different morphology of colonies. The shapes of fungal colonies exhibit diversity depending on the substrate conditions as well as on the fungal species. Even though the shapes and the surface textures of colonies provide useful evidence to determine the species or to monitor the state of growth, colony patterning looks to be highly sensitive to environmental factors. The nutrient level in the substrate is the main factor for the hyphal production. In addition, nutrient diffusion was a significant factor, since it affects the nutrient flux into the colonized area and the distribution of the location in which uptake of nutrient occurs intensely. As for the internal parameter of mycelium, the

growth rate of hyphae, or the rate of nutrient utilization, will largely contribute to determining where in the colony the hyphal production occurs (Roper & Seminara, 2019).

Among the fungal isolates, MF1 and MF2 showed the most distinct colony characteristics that resembles the rosette colonies of the Halophytophthora species. Halophytophthora sp. is considered one of the first decomposers of mangrove litter among marine fungi (Ho & Jong 1990). Zoosporangia was identified to belong to that of Halophytophthora sp.. Fungal isolates from MF1 and MF2 were later identified under the microscope and observed a suspected a zoosporangium of Halophytophthora sp.

Assay for the dye decolorization activities of marine-derived fungi using homogenous dye media mixture with different concentration of Synthetic Dyes

The elimination of colored substances in wastewater is based mainly on physical or chemical methods. 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 (Torres et. al, 2009). Thus, it is essential to assess the dye decolorization activity of marine-derived fungi in an homogenous dye mixture than in a tube overlay method. Tube overlay method seems to be a much more effective method in decolorization of higher concentrations of dye, though using it for screening of fungal extracellular enzyme production is highly effective, it is not conclusive in confirming if a particular fungal species cannot produce a particular enzyme (Torres et al., 2009).

Marine mycelial decomposers are highly adapted for capture of solid substrate by pervasion and digestion from within. In mangroves, submerged fallen leaves appear to support minor fungal occupancy, but ubiquitous and rapid occupancy by oomycotes (Raghukumar, 2017). The chief problem with digesting plant material is that the more nutritious cell contents are surrounded by relatively indigestible cell walls of lignin and cellulose.

Since all marine fungi (MF1, MF2, MF3 and MF4) (table 4) tested positive for enzyme activities for cellulose, it shows that all marine-derived fungi strains that were isolated contains ligninolytic enzyme, as cellulose was only found on the deepest layer of leaf tissue beneath a thick epicuticular wax (Jetter & Riederer, 2016). Lignin is a nitrogen-free co-polymer of various phenyl propenyl alcohols that is abundant in vascular plants. It is present in cell walls where it imposes rigidity and minimizes water permeation (Emonet & Hay, 2022). Species of Halophytophthora may be particularly well suited as first invaders of fallen mangrove leaves due to their ability to readily utilize a rather wide variety of sugars and amino acids leaching from them (Maia et al., 2022).

Marine-derived fungi produce ligninolytic enzymes and play an important role in the degradation of lignocellulose in marine ecosystems. Marine fungi not only exhibit digestion of lignocellulose under natural-decay circumstances, but also dissolved organic material from outside of the decaying shoots, or from living portions of shoots (Raghukumar, 2017). Ligninolytic enzymes are essential for lignin degradation, however for lignin mineralization they often combine with other processes involving additional enzymes. Because of their lack of substrate specificity, ligninolytic enzymes are also capable of degrading a wide range of xenobiotics and recalcitrant substances such as chlorophenols, polycyclic aromatic hydrocarbons (PAHs), organophosphorus compounds, and phenols (Yesilada et al., 2018).

Aromatic compounds are environmental contaminants associated with the production and use of dyes, explosives, pesticides and pharmaceuticals. Lignin is an aromatic polymer, in which extracellular lignin-degrading enzymes can non-specifically attack aromatic compounds such as synthetic dyes (Congo red, Methylene blue and safranin) which bears little similarity with lignin (Li et al., 2023). Synthetic dye consists of aromatic structures having little resemblance to lignin but since the lignin-degrading system

is a generalized and non-specific degradative system, it can still degrade other aromatic compounds (Durruty et al., 2017).

Among the three synthetic dyes, methylene blue and Congo red show partial to full decolorization of synthetic dyes. As safranin homozygous dye mixture exhibited no to partial decolorization. Methylene blue is a hetero-polyaromatic compound. The decolorization of MB originated either from an aromatic S+-link, yet less reactive increased the oxidation state of the dye (Santana et al., 2019).

The small molecular weight favors Congo Red. On the opposite end, the weaker adsorption constant concerns CR. This can be explained by the large steric hindrance due to large aromatic ensembles, including one central biphenyl group and two symmetric naphtalenic groups. But since azo dyes such as Congo Red generally do not act as carbon sources as they are electron deficient owing to the presence of their azo linkage (–N==N–) (Solís et al., 2012) microbial decolorization and degradation of azo dyes generally depends on the presence and type of a carbon source used, because they act as an electron donor for azo-dye reduction.

Safranin is a representative example of an organic dye, which belongs to the quinone–imine class and is widely used for counterstaining purposes, for example, as a metachromatic method for cartilages, which is stained yellow. Since the dye is known to be carcinogenic in nature, any presence of this dye in wastewater would have detrimental effects on the marine environment. Safranin exhibited no to partial decolorization as it is as the optimum conditions for the degradation of the dye have been found as 5.0×10−5 M dye concentration, pH 5.7, and 12 mg catalyst dose was not achieved.

Exoenzyme activities

Xylose is the main building block for hemi-cellulose, which comprises about 30% of plant matter. Xylose is otherwise pervasive, being found in the embryos of most edible plants. Clearing indicates the presence of enzymes that are biological catalysts which accelerate the various biological reactions without participating in them (Fatima. al. 2016), which would aid the lininolytic enzymes in degrading mangrove leaves. Marine fungal isolates MF1, MF2 and MF3 show clearing of the xylose substrate.

A positive result for the maltose test indicates the presence of amylase, which breaks the covalent bonds between glucose molecules in starch and other polysaccharides to produce the disaccharides maltose and isomaltose (VanPutte et al., 2024). All marine-derived fungal isolates exhibited amylase production. Li et al. (2023) indicated that some marine-derived shows the presence of amylase.

Approximately 30–50% of the organic matter of the leaves is leachable in this way, and much of what remains is insoluble structural carbohydrate such as cellulose. This is subsequently attacked by extracellular enzymes secreted by bacteria or fungi. Lignocellulose, which makes up much of the bulk of woody material, is highly refractory to decay, which indicates the presence of ligninolytic enzymes. Some crabs secrete enzymes such as cellulase: this has not been demonstrated in sesarmids, but would certainly be useful in breaking down plant material (Raghukumar, 2017) as was observed when all marine fungal isolates exhibited positive result.

All marine-derived fungal isolates show negative result in protease test due to the structure of the mangrove tree in the habitat.

In summary the results indicated that Methylene Blue was fully decolorized within two days at all concentrations (Durruty et al., 2017). Congo Red exhibited partial to full decolorization within 28 days, depending on the fungal strain (Kumar et al., 2020). Safranin showed partial or no decolorization at higher concentrations . All fungal strains exhibited cellulase activity MF1, MF2, and MF4 showed xylose isomerase activity (Jetter & Riederer, 2016). MF1, MF2, MF3, and MF4 tested positive for

amylase production, but no fungal strain showed protease activity.

The findings indicate that marine-derived fungi exhibit potential in synthetic dye bioremediation. Methylene Blue was the most readily decolorized dye, suggesting that its molecular structure is more susceptible to fungal enzymatic degradation (Almeida & Corso, 2019). Congo Red required a longer incubation period, while Safranin exhibited the least decolorization, likely due to its quinone-imine structure (Choo et al., 2022). Enzymatic assays suggest that cellulase, amylase, and xylose isomerase play roles in dye degradation. The absence of protease activity indicates that protein degradation is not a primary mechanism in fungal dye decolorization (Hyde & Pointing, 2013).

5. Conclusion and Recommendations

Marine-derived fungi isolated from Rhizophora spp. demonstrated significant decolorization capabilities, particularly for Methylene Blue and Congo Red (Eichlerová et al., 2006) All the isolates proved to have the same effects in decolorizing the three synthetic dyes, as all marine derived fungi shows full decolorization on Methylene Blue, partial to full decolorization on Congo Red and no to partial decolorization to Safranin. . It also proved that marine-derived fungi show significant decolorization as well as indicating that they serve as decomposers of organic matter. Exoenzyme test also showed positive results which indicate more enzymes could be used in decolorizing synthetic dye but also served as the primary decomposers of our marine environment. Subsequently, marine-derived fungal isolates (MF1, MF2, MF3, and MF4) exhibit varying capacities for dye decolorization, with the highest efficiency observed for Methylene Blue, followed by moderate effectiveness for Congo Red and minimal impact on Safranin. MF3 showed the most promising decolorization potential, particularly for Congo Red at lower concentrations. Additionally, enzyme activity analysis revealed that MF1 and MF2 utilized xylose, maltose, and cellulose, MF3 exhibited activity for maltose and cellulose, and MF4 demonstrated activity for all three. These findings suggest that marine fungi possess significant bioremediation potential, with enzymatic capabilities that may contribute to their dye degradation efficiency.

Enzymes such as cellulase, amylase, and xylose isomerase contribute to the degradation process. Safranin proved resistant to fungal degradation, suggesting the need for alternative bioremediation approaches (Hassaan & El Nemr, 2017). Future studies should identify the specific fungal species involved and use different mangrove leaves found in different locations.

Liquid media decolorization assays should be performed to simulate real-world wastewater treatment conditions. Additional environmental parameters, such as pH and salinity, should be considered for optimizing fungal decolorization efficiency

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