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Original Research Paper

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TEXTILE DYE DEGRADATION POTENTIAL OF FUNGI ISOLATED FROM WESTERN GHATS OF KODAGU DISTRICT IN KARNATAKA STATE, INDIA

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ABSTRACT In textile industries, a dye is an integral element used to impart color to textile materials. The extensive use of synthetic dyes resulting in increased generation of wastes during the processing and treatment of the dye containing inorganic and organic compounds which are hazardous has led to several environmental issues, thereby posing a serious threat to the ecosystem. It is therefore essential to use cost-efficient and ecofriendly methods against these effluents to protect our natural resources. Mycoremediation using natural fungi inhabiting the litter is an effective, economical and appropriate approach for degradation of these dye rich effluents. In this context, the present work was undertaken to study the efficiency of fungi isolated from litter samples of Kodagu district in the Western Ghats of Karnataka for the degradation of few common textile dyes.

KEYWORDS : Dyes, fungi, Mycoremediation, Enzymes

INTRODUCTION

The fungi inhabiting the soil-litter layers, co-exist and compete with other micro-organisms. Due to the production of extra-cellular, non-specific and non-stereo selective enzyme systems these fungi are well known for their dye-decolorizing ability (1).

The extra-cellular enzyme system involved in the degradation of dye, mainly composes of laccase (Lac), manganese peroxidase (MnP) and lignin peroxidase (LiP) and have the potential applications in several industries (2).

To degrade the vast amount of xenobiotics which are recalcitrant such as synthetic dyes, there is a need for screening of large number of fungi (3).

Unfortunately, the wastewater treatment plants are often unable to remove the commercial textile synthetic dyes from contaminated waste water and contribute to water pollution (4). Now a days, severe and intense studies and research pertaining to environmental concerns mainly on the degradation of synthetic dyes has become an area of interest.

Therefore, there are possibilities of utilization of the Mycoremediation, which is promising, eco-friendly and cost-effective alternative to the physical and chemical purification methods used for the removal of these toxic textile dyes (5). Interestingly, synthetic dyes are being biodegraded through conversion and co- oxidation by some potential fungi (6).

METHODOLOGY

The litter samples were collected from forest areas of Kodagu district in the Western Ghats of Karnataka, India.

Isolation of fungi

The litter sample was inoculated onto the sterile malt-extract agar (MEA) medium supplemented with 1% tannic acid, incubated at 28±2° C. The plates were regularly observed for the formation of complete browning around fungal growth (7).

The positive fungal colonies were isolated, purified and based on the mycelial growth, the diameter of the complete browning zone and sporulation, the potent fungal strains were selected and used for further studies.

Fungal Decolorization of dyes on a solid media

Dye-decolorizing ability of fungal strains was done by qualitative plate assays using 2% MEA medium amended with 100 mg l^{-1} of each dye using commercially available polymeric dyes namely Remazol Brilliant Blue R, phenol red, methyl green and methylene blue (8). The efficiency of dye-decolourization was analyzed by visual disappearance of dye on the plates by the production of enzymes by the fungal strains.

Fungal Decolorization of dyes on a liquid media

Ability to decolorize the dyes on **a liquid media** was done by quantitative assays with four dyes as the protocol by inoculating 7-days-old fungal culture actively growing on 2% MEA broth supplemented with the dyes at 100 mg l^{-1} of concentration each(9). The extent of dye-decolourization was determined by measuring the decrease in the absorbance of each dye by using the UV- visible spectrophotometer. The efficiency of dye-decolourization was calculated using the following formula and expressed in terms of decolourization percentage:

RESULTS AND DISCUSSION

In the present study, a total of 12 strains of fungi isolated from different litter samples were found to form the complete browning on MEA medium supplemented with 1% tannic acid **(Fig. 1)**.

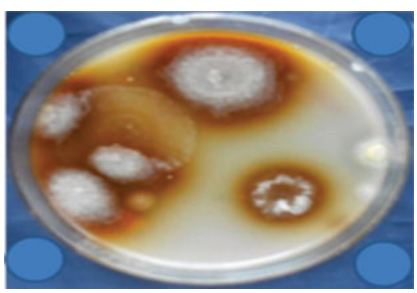

Fig. 1 Screening of fungi on MEA medium

Dye decolorization ability of fungi on a solid and liquid media

Of the 12 strains tested for dye decolorization ability, only 4 strains were significantly ($p \leq 0.05$) found to decolorize the dyes used **(Table 1 and 2)** compared to respective controls. All the strains were showed significantly ($p \leq 0.05$) varied results of decolourization with increasing the incubation time on solid media with respect to different dyes used. The strains showing significantly ($p \leq 0.05$) highest diameter of decolourization zone were designated as CR1, CR 2, CR 3, CR 4 respectively.

Each value is the mean of three replicates (n=3) with the standard error (SE±). Mean value is followed by the same letter(s) written within the same column are not different significantly (at $p \leq 0.05$) according to the Tukey's HSD test.

A wide variety of fungi have been fungi have been remarkably

degraded the wide range of genotoxic textile dyes, and their ability has been associated with the non-specific and non-stereo selective nature of the lignin-degrading enzymes (10). The study site selected in the present study was that Kodagu district of Karnataka (India) which was considered as one of the world's greatest hotspots of biodiversity and ecologically sensitive area due to discharge of the pollutants after pulping the coffee berries by the coffee planters into the nearby rivers, reservoirs and tanks (11).

Table 1 Dye decolourization ability of lignolytic fungi on solid agar media supplemented with dyes against different incubation time intervals (days).

	Isolate code	Diameter of decolourization zone (in mm)				
		2 day	4 day	6 day	8 day	10 day
	Remazol Brilliant Blue R					
1	CR1	12±1.15^{a}	23±1.73^{a}	25±1.15^{bcd}	32±1.15^{bcd}	45±2.88^{ab}
2	CR2	11±0.57^{a}	23±0.57^{a}	31±0.57^{ab}	34±1.15^{abc}	41±0.66^{abc}
3	CR3	10±0.66^{a}	20±2.30^{ab}	27±0.88^{abc}	32±1.15^{bcd}	41±1.20^{abc}
4	CR4	11±1.33^{a}	25±0.88^{a}	34±0.88^{a}	41±0.57^{a}	46±0.57^{a}
5.	Control	0	0	0	0	0
	Phenol red					
1	CR1	10±1.20^{ab}	20±2.30^{abc}	28±2.30^{bcd}	35±2.30^{ab}	40±1.73^{ab}
2	CR2	7±1.15^{bc}	20±1.73^{abc}	36±1.33^{a}	40±0.33^{a}	43±1.73^{abc}
3	CR3	7±0.57^{bc}	19±1.73^{abcd}	28±1.73^{bcd}	32±1.76^{b}	41±2.30^{ab}
4	CR4	5±0.66^{cd}	11±0.88^{e}	23±0.88^{cd}	32±1.20^{b}	44±0.66^{bc}
5	Control	0	0	0	0	0
	Methyl green					
1	CR1	15±2.30^{a}	20±1.15^{ab}	17±7.42^{c}	37±1.15^{abc}	41±4.04^{a}
2	CR2	13±1.52^{ab}	22±0.57^{a}	27±1.73^{abc}	32±1.20^{bc}	43±1.20^{ab}
3	CR3	10±2.30^{abc}	20±1.15^{ab}	32±1.00^{ab}	38±0.66^{ab}	40±0.33^{abcd}
4	CR4	6±0.66^{cd}	16±1.73^{bc}	20±2.88^{abc}	31±0.57^{c}	44±0.33^{de}
5	Control	0	0	0	0	0
	Methylene blue					
1	CR1	10±0.33^{a}	22±1.45^{a}	25±1.20^{b}	32±1.20^{bcd}	40±2.88^{bcd}
2	CR2	11±1.20^{a}	20±0.88^{a}	26±2.02^{b}	35±0.66^{bc}	42±0.66^{abc}
3	CR3	10±2.88^{a}	22±1.15^{a}	25±2.02^{b}	31±1.00^{bcde}	36±0.33^{cdef}
4	CR4	12±1.00^{a}	21±0.66^{a}	35±2.66^{a}	42±1.20^{a}	49±0.66^{a}
5	Control	0	0	0	0	0

Table 2 Dye decolourization ability of fungi in liquid media supplemented with dyes against different incubation time intervals (days).

	Isolate code	% of dye-decolourization				
		2 day	4 day	6 day	8 day	10 day
	Remazol Brilliant Blue R					
1	CR1	11.0±0.57^{d}	21.0±3.05^{cd}	36.3±1.33^{ab}	49.0±3.21^{ab}	52.6±1.85^{ab}
2	CR2	14.3±0.66^{bcd}	20.6±1.76^{cd}	30.0±3.60^{b}	35.6±4.70^{b}	48.0±3.78^{b}
3	CR3	14.3±1.20^{bcd}	26.0±2.00^{abcd}	36.0±2.64^{ab}	46.6±5.20^{ab}	52.0±6.80^{ab}
4	CR4	10.3±0.33^{d}	17.3±0.88^{d}	37.0±1.15^{ab}	53.0±1.52^{ab}	64.3±0.88^{a}
5.	Control	0	0	0	0	0
	Phenol red					
1	CR1	12.3±0.88^{c}	24.0±2.64^{cd}	37.0±4.04^{bcd}	43.6±4.84^{c}	51.3±2.18^{b}
2	CR2	14.3±0.66^{bc}	20.3±0.66^{d}	29.3±2.66^{d}	42.3±4.05^{c}	50.0±2.51^{b}
3	CR3	14.0±1.15^{bc}	23.3±1.45^{cd}	34.0±1.00^{cd}	42.6±1.76^{c}	51.6±3.52^{b}
4	CR4	12.3±0.88^{c}	26.3±1.76^{cd}	41.0±0.57^{abcd}	48.0±0.57^{bc}	55.0±0.57^{b}
5.	Control	0	0	0	0	0
	Methyl green					
1	CR1	12.6±1.76^{bc}	27.3±3.17^{b}	39.0±3.78^{ab}	46.3±4.17^{abc}	52.6±3.52^{bc}
2	CR2	15.3±2.18^{abc}	23.3±3.17^{b}	27.6±1.20^{b}	33.3±1.45^{c}	52.6±3.33^{bc}
3	CR3	14.3±0.66^{abc}	24.6±2.02^{b}	34.6±2.60^{ab}	41.6±3.84^{bc}	55.0±3.00^{bc}
4	CR4	12.3±1.45^{c}	31.0±0.57^{ab}	42.6±1.45^{ab}	50.3±0.33^{ab}	56.0±1.15^{bc}
5.	Control					
	Methylene blue					
1	CR1	18.3±2.02^{ab}	32.6±1.33^{ab}	43.3±1.20^{ab}	57.0±1.15^{ab}	68.0±1.52^{a}
2	CR2	20.3±1.20^{ab}	33.6±2.02^{ab}	47.0±4.58^{ab}	58.0±1.52^{ab}	66.6±1.66^{a}
3	CR3	19.6±1.20^{ab}	34.3±3.38^{ab}	44.3±4.25^{ab}	55.6±1.45^{ab}	64.0±2.30^{a}
4	CR4	22.0±2.08^{a}	34.3±2.60^{ab}	45.3±2.96^{ab}	59.3±2.60^{ab}	69.6±2.60^{a}
5.	Control	0	0	0	0	0

Each value is the mean of three replicates (n=3) with the standard error (SE±). Mean value is followed by the same letter(s) written within the same column are not different significantly (at $p \leq 0.05$) according to the Tukey's HSD test.

The fungal strains were found to decolorize all the four polymeric dyes (Remazol Brilliant Blue R, phenol red, methyl green, and methylene blue) used which was confirmed by the halo zone around the fungal strains. All the positive strains significantly showed varied results (halo zones) for the different dyes used.

Among the fungal strains tested for the dye decolourization in liquid media, 4 strains which decolorized the dyes on solid media were also significantly found to decolorize the dyes with the varied percentage in the liquid media. Four different dyes used in the present study were dexterously decolorized after 10 days of fungal inoculation in case of both solid and liquid decolourization experiments. Our findings were in accordance with a study which shows the decolorizing potential of *Phanerochaete chrysosporium* by removing the color of two reactive textile dyes namely (Reactive Yellow MERL and Reactive Red ME4BL**(12).**

Previous studies showed that the dye decolourization and production of lignolytic enzymes are inter-related with each other which indicated the ecological importance of litter degrading fungi in the processes of degradation of lignin and humic substances. It has been reported that the degradation of Remazol Brilliant Blue R was found to reflect the laccase activity in *Trametes versicolor* and *Pleurotus ostreatus***(13).**In our study, the degradation of synthetic dyes was reflected by the production of enzymes. Further, four litter degrading fungi (such as CR1, CR 2, CR 3, CR 4 respectively) with the varied dye decolorizing ability were used to study their utilization in the industrial applications.

Selected 4 fungal strains were identified based on their morphological characters observed and molecular characterization, the strains were identiied as 2 species of *Ceriporiopsis (*CR1 andCR 2*),* one species of *Penicillium (*CR3*),* 1 species of *Phanerochaete (*CR4*).* Of all the strains tested, *Phanerochaete (*CR4) species was found to efficiently degrade the dyes under study.

CONCLUSION

Textile industry uses a wide range of dyes to color a variety of fabrics. These dyes are extremely cytotoxic to mammalian tissues and are difficult to decompose naturally. Dyes are difficult to remove by traditional methods. But each method has its own constrains and drawbacks. Mycoremediation using fungi are found to be an efficient method for dye decolorization and degradation. This naturally occurring vast diversity of fungi has to be exploited for cleaning up of polluted environments. From the present study, it is also clear that the litter degrading fungi represent as the most promising fungi with respect to dye decolourization due to their enzyme activity.

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