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Advances in Bioresearch

ORIGINAL ARTICLE

To study decolorization and detoxification of synthetic dye by microorganism producing Lignin peroxidase

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ABSTRACT
Today with growing textile industry, the major problem which India is facing is to remove dye from effluent before discharge in the water bodies. In the present study, Lignin peroxidase (Lip) enzyme producing microbial strain FSV 3was studied for decolorization and detoxification of various synthetic dye like Congo red, Methyl orange, Methylene blue and Remazol brillent blue R. From the study, it was found that FSV 3 strain is able to decolorize and detoxify methylene blue and Remazol brillent blue R upto 100mg/L by 44 and 77% respectively on 144 hours of study, while for congo red and methylene orange show bioabsorption of dye.
Key words: *Bioabsorption, Decolorization, Lignin peroxidase, Methylene blue, Remazol brillent blueR*

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INTRODUCTION

Today, with fast growing industrialization, use of synthetic dyes are majorly increase and problem faced by is its effluent discharge as it contains about 10-15% of synthetic dyes [1]. Synthetic dyes are not only used in textile industries, but also used in paper, pharmaceuticals and cosmetic industries and It has been noticed that majorly used synthetic dye are non biodegradabable due to its complex aromatic nature and it cause significant environment pollution [2, 3, 4]. Presently, physio-chemical methods are used to treat textile effluent, but method is of high expense and by-products are toxic. Moreover, after treatment about 95% of dyes remain untreated and merge in river water [5,6].

Now-a-days, scientific research is approaching biological degradation of dyes using ligninolytic enzyme producing micro-organism to overcome limitation of physical and chemical method [7,8]. Several ligninolytic enzyme producing micro-organism are studied such as *Bjerkandera adusta, Trametes versicolor, Phanerochaete chrysosporium* produce ligninolytic enzyme of which White rot fungi show complete degradation of dye[9].

Ligninolytic group of enzymes include Lignin peroxidase belong to oxidoreductase family and has several applications in the field of chemistry, food, agriculture, environment and they are used to for degradation of xenobiotic compounds and dyes [10,11].The major role of lignin peroxidase in our environment is to degrade lignin from soil [12]. It has been reported that lignin peroxidase is capable of degrading synthetic dye more efficiently [13].

The objective of this study is to determined colorization and detoxification capacity of lignin peroxidase producing FSV3 culture and to resolve problem of discharge of dye effluent in environment.

MATERIAL AND METHODS

Fugal culture: Lignin peroxidase producing culture (FSV 3) was isolated using lignin phenol model for primary screening. Secondary screening was done using Veratryl alcohol as a substrate for lignin peroxidase assay [14].

Inoculum preparation: Spore suspension (5 X 10^6 cell/ml) of culture grown on potato dextrose agar for 144 hours was inoculated in 50 ml potato dextrose broth (PDB) containing 0.1% Kraft lignin. After 24 hours of incubation at 30^0C on rotatory shaker, culture was used as an inoculum.

Preparation of media for dye decolorization experiment: 200 mg/L congo red (λmax-498nm), methylene orange (λmax-464nm), methylene blue (λmax-665nm) and Remazol brillent blue R (RBBR) dye (λmax-592nm) was prepared in production medium containing Ammonium nitrite-9g/l, potassium dihydrogen phospahe-0.5g/l and magnesium sulphate-0.5g/L.

Study of microbial decolorization of different synthetic dyes:2% inoculum was used in production flask containing different synthetic dye. Flasks were incubated at 30^0C on rotatory shaker until significant dye decolorization was observed. After interval of every 24 hours, % decolorization was calculated using following formula[15]:

$$\% \text{ dye decolorization} = \frac{(A-B) \times 100}{A}$$

Where, A= Absorbance of dye before inoculation (control)
B= Absorbance of dye after inoculation (test)

Study of optimization of different concentration of dye for decolorization: 2% Seed culture was inoculated in production medium containing various concentration of dye (50, 100, 150, 200 and 250 mg/L). Flask were incubated on rotatory shaker at 30^0C and after every 24 hours of incubation, % dye decolorization was analysed. Assay was carried out till 144 hours to observe the decolorization.

Study of detoxification of decolorized dye:

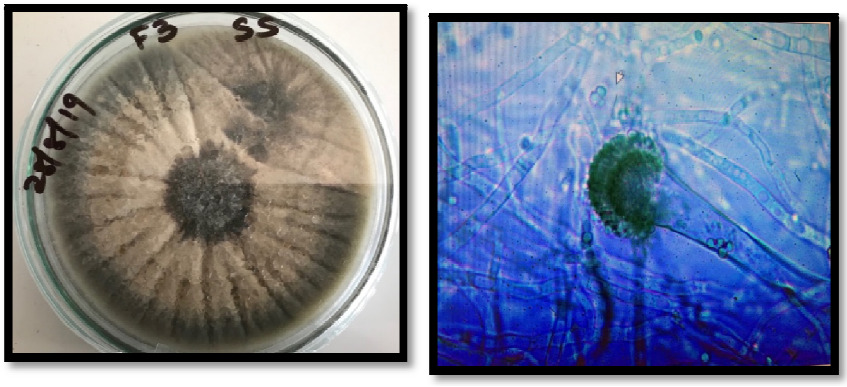
Phytotoxicity test: The test was perform using *Vigna radiata* and *Zea mays* to compare the toxic effect of dye on germination before and after treatment. Seeds were incubated after treating it with 10 ml of dye (50 mg/L) without treatment (positive control) and 10 ml of dye after treatment (test). The control set was prepared using production medium without dye. Seeds were incubated for germination and percentage germination was checked after 24 hours [16].

Microbial toxicity test: Standard Culture of *Bacillus subtilus* was used to check the toxicity effect of dye on growth. With and without treated Dye (50 mg/L) samples were compared to control (only production medium). Zone of inhibition of growth was measured after 24 hours of incubation [16].

Statistical analysis: it was carried out using one-way ANOVA and Tukey-Kramer multiple comparison test using graph pad prism to find significance of study.

RESULT AND DISCUSSION

Culture selection: FSV 3 culture (Fig. 1a & b) isolated from Andaman and Nicobar island soil sample have given positive result when screened on lignin model such as Pyrogallol and Guaiacol and decolorization of methylene blue and RBBR dye on solid medium, while in production medium maximum enzyme activity given by FSV 3 was 131 U/ml at 72 hours of incubation [14].

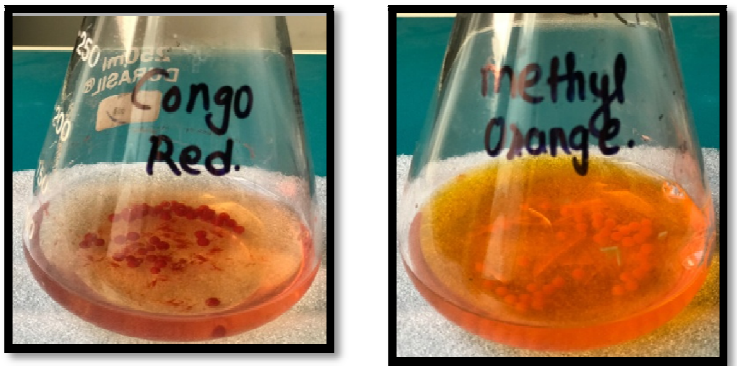


1a 1b

Figure 1: Growth of FSV 3 strain on potato dextrose agar (1a) and microscopic observation (1b)

Study of microbial decolorization of different synthetic dyes: Synthetic textile dye such as congo red, methyl orange, methylene blue and Remazol brillent blue R dye were used for dye decolorization study by lignin peroxidase producing FSV 3 culture, similar studies to check decolorization and detoxification of azo dye used in textile industries by fungal strain *Phanerochaete simplicissimum* was carried out [17]. F Bosco, 2017 have reported *Phanerochaete chrysosporium* in support of hazelnut shell show absorption of

congo red by 43%[18]. In present experiment, culture have shown bioabsorption of congo red dye and methyl orange dye by 62% and 19% respectively after 24 hours of incubation. On prolong incubation no further bioabsorbtion or decolorization of dye was observed (Fig.2a & 2b). While, FSV 3 culture shown maximum decolorization of methylene blue (12%) and Remazol brillent blue R (40%) after 72 hours of incubation. Alam *et al* [19] have used sewage water as substrate which contain methylene blue as dye and found that lignin peroxidase producing *Phanerocheate chrysosporium* after optimization is able to decolorized dye by 90% under agitation[19]. RBBR dye decolorization by lignin peroxidase producing culture Trametes versicolor was noted by Viral et al [20].

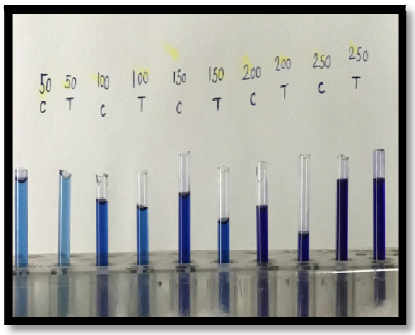


2a 2b

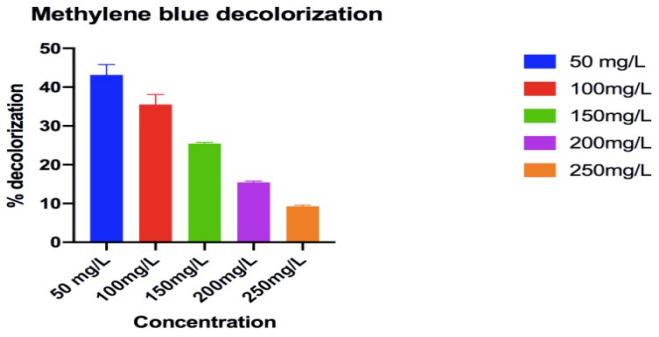
Figure 2 Bioabsorption of congo red (2a) and methyl orange (2b) by FSV 3 strain

Optimization of different concentration of dye decolorization:

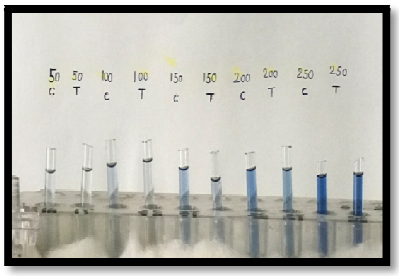
Out of 4 synthetic dye used, FSV 3 strain was able to decolorize methylene blue and Remazole brillent blue R. Further optimization of methylene blue and Remazole brillent blue R for various concentration was carried out. Culture have shown highest decolorization of 44.15±2.67% and 76.92±1.40% of methylene blue and RBBR dye respectively with 50 mg/L concentration after 144 hours(Fig. 3a-3d), while at 250 mg/L concentration 9.26±0.31 and 30.46±1.54% dye decolorization of methylene blue and RBBR respectively was also observed. One-way analysis of variance (ANOVA) with Tukey-Kramer comparison test shows significant effect ($p < 0.0001$).



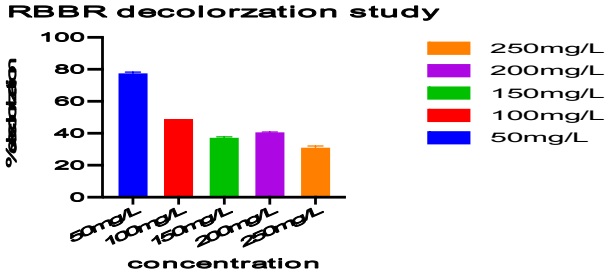
3a



3b



3c



3d

Figure 3: % decolorization of methylene blue (3a), Graph showing methylene blue decolorization at various concentration (3b), % decolorization of RBBR (3c), graph showing maximum % decolorization at different concentration of RBBR dye (3d)

Phytotoxicity study:

Detoxification Study of treated dye samples is required as treated dye will be discharge in environment. Jing Si, 2012 have studied effect of phytotoxicity from degraded azo dye by Lip producing white rot using *Phaseolus mungo, Sorghum vulgare* and *Triticum aestivum* seeds and checked % germination of seeds [21]. In the given study, *Vigna radiata* and *Zea mays* seed were treated with decolorized sample (50 mg/L methylene blue and RBBR) and response was observed in terms of % germination after 24 hours. In methylene blue treated experiment, control setof *Vigna radiata* and *zea maize* have shown 100% and 53.33± 5.16% germination respectively. In compare to control, Positive control have shown 20 % decreased germination while test sample have shown same germination (Table 1, fig. 4a). while in RBBR treated set, control have shown 96.67± 5.16% germination of *Vigna radiata* and 100% germination of *zea maize* seeds. RBBR treated sample have shown 96± 5.16 % and 40 % germination of *Vigna radiata* and *zea maiz*e seeds respectively, positive control has shown low percentage of germination around 4% compare to test (Table 2, fig. 4b).

Table 1: % Germination of seeds grown on treatment set of methylene blue dye

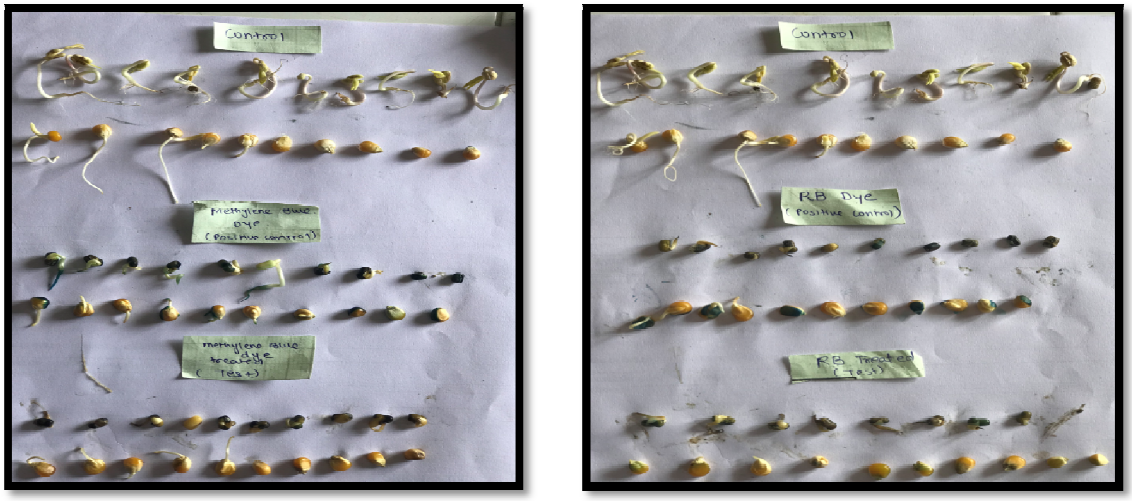
Experiment	Seed	Treatment	% Germination
Methylene blue treatment	*Vigna radiata*	Control	100.0±0.00
		Positive control	83.33±5.16
		Test	93.33±5.16
	Zea mays	Control	53.33±5.16
		Positive control	33.33±5.16
		Test	46.67±5.16

Values are mean of three experiments ± SE

Table 2: % Germination of seeds grown on treatment set of RBBR dye

Experiment	Seed	Treatment	% Germination
RBBR treatment	Vigna radiata	Control	96.67±5.16
		Positive control	90.00±8.94
		Test	96.67±5.16
	Zea mays	Control	100.0±0.00
		Positive control	36.67±5.16
		Test	40.00±0.00

Values are mean of three experiments ± SE



4a 4b

Figure 4: % germination of Vigna radiate and zea maize seeds form ethylene blue (4a) and RBBR (4b) treatment

Microtoxicity study:

In microtoxicity study, treated dye sample in comparison to positive control show lesser zone of inhibition, while treated autoclave treated sample does not give zone of inhibition which shows that after dye decolorization treatment toxic effect of dye can be reduced which were shown in table 3 and figure 5.Nedra *et al*, 2018 have shown similar results, where congo red dye decolorized using *Aspergillus niger* culture have studied and shown no toxicity towards *B. cereus* ATCC 11778 and *E. coli* ATCC 10536 [22].

Table 3: zone of inhibition for microtoxicity study

Methylene Blue dye		RBBR	
Set	Zone of inhibition	**Set**	Zone of inhibition
Control	0±0.0	**Control**	0±0.0
Positive control	2.41±0.09	**Positive control**	2.73±0.21
treatment	2.00±0.15	**Treatment**	1.87±0.15
treatment (autoclave)	0±0.0	**treatment (autoclave)**	0±0.0

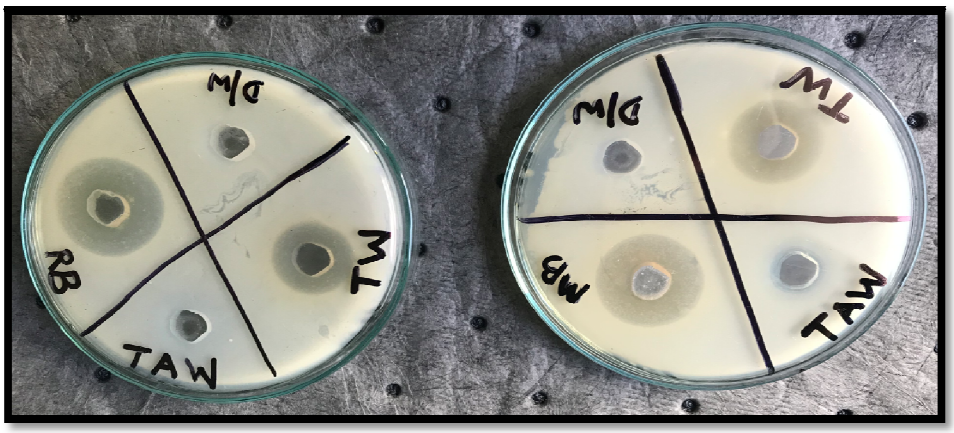


Figure 5 : Result of microtoxicity study (D/W= control, MB= methylene blue(positive control), RB=RBBR dye (positive dye), Tw= treated sample, TAW=treated autoclave sample

CONCLUSION

Lignin peroxidase producing FSV 3 strain is capable of decolorizing and detoxifying Methylene blue and Remazole brillent blue R dye more efficiently at 50 mg/L. The significant dye decolorization was observed up to 150 mg/L. Thus, the culture FSV 3 is efficient for biodegradation and detoxification of water effluent containing methylene blue and RBBR dye.

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