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DECOLOURIZATION OF TEXTILE DYES BY *Schizophyllum commune*: AN ECOFRIENDLY MYCOREMEDIATION APPROACH TO TREAT WASTEWATER CONTAINING TEXTILE DYE EFFLUENTS

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ABSTRACT

Synthetic dyes are extensively used in various industries, especially the textile industry, and their discharge causes severe environmental problems and is harmful to human health. Mushrooms, especially the white rot fungus, have incredible potential for biodegradation of a variety of industrial pollutants due to the presence of enzymes such as lignin peroxidase, manganese peroxidase, laccase, etc. In this study, the common split gill fungus, *Schizophyllum commune*, collected from the Jiribam district of Manipur, India, was employed for biodegradation/decolourization of two highly toxic and non-degradable commercially used textile dyes, Congo Red and Methylene Blue and showed positive results. The screening for the presence of ligninolytic enzymes was also performed and found to be positive. The dye decolourization study was carried out on Potato Dextrose broth medium supplemented with 0.02% of the respective dyes in 250 mL Erlenmeyer flasks. Each flask was inoculated with the mycelium plugs of each mushroom culture and incubated in stationary condition in a BOD (Biochemical Oxygen Demand) incubator at 25 ± 2 ^{o}C. For Congo Red, the dye decolourization percentage was highest on the 5th day of incubation (91.8%), while in the case of Methylene Blue, the dye decolourization percentage was highest on the 3rd day, with 77.67%. The results suggested that *S. commune* can effectively degrade or decolourize these dyes, showing more potential in the case of Congo Red. It paves the way for this mushroom to be used as an efficient mycoremediation tool for the treatment of wastewater containing textile dye effluents.

Keywords: *Schizophyllum commune*, Congo Red, Methylene Blue, Dye decolourization, mycoremediation.

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INTRODUCTION

Schizophyllum commune is a mushroom that can complete its life cycle in roughly 10 days and is one of the most commonly found fungi. The distribution of this mushroom covers all over the globe with the exception of Antarctica (Ohm et al., 2010). About 150 different genera of woody plants, as well as grass silage and softwood, were reported to be colonised by *S. commune* (Ohm et al., 2010). allowing this mushroom to colonize a wide variety of lignocellulosic substrates, increasing the range of possibilities and biotechnological products {e.g., enzymes (phytase, lipase, holocellulase, etc.) (Arboleda et al., 2011; Salmon et al., 2012; Singh et al., 2015), bioethanol (Horisawa et al., 2015), polysaccharides (Singh et al., 2017), biosurfactants (Wessels et al., 1991), industrial cleaning-in-place (CiP) agents (Boyce & Walsh, 2012), polymers (Jayakumar et al., 2010), and so on} that can be obtained using this mushroom.

Since the genome of this mushroom contains 240 genes for glycoside hydrolases (89 account for plant polysaccharides degradation), 75 for glycosyl transferases, 16 genes for polysaccharide lyases, 17 for expansin-related proteins, 30 for carbohydrate esterases, and 16 gene candidates for lignin-degrading oxidoreductases (Ohm et al., 2010), *S. commune* has got a great potential and ability to break down most of the components of the lignocellulosic biomass. Ligninolytic mushrooms, especially white rot fungi, are proven to have the special capability to degrade complex polymers of synthetic dyes along with many agricultural pollutants (Gupta et al., 2018).

Synthetic dyes are widely used in many industries, like textile industries, paper, plastics, leather, food and pharmaceutical industries, etc. (Saratale et al., 2013). Over 1,000 dyes have been categorized as textile dyes that are used to colour a variety of fabrics (Abe et al., 2019). Dyes are mostly high molecular weight complex materials that are water-soluble, degradation-resistant, possibly carcinogenic, and also mutagenic. Additionally, they might stop sunlight from penetrating, thus decreasing photosynthetic reactions (Jusoh et al., 2013). Effluents from textile industries contain colours that cause aesthetic damage and stop light from diffusing into water, which leads to a decrease in dissolved oxygen levels and affects aquatic plants' photosynthesis rate (Ajaz et al., 2020). Furthermore, dyes are highly water-soluble, which in turn makes them very difficult to decolourize or remove from water by methods used traditionally (Dong et al., 2019; Lellis et al., 2019).

Dyes can be classified into six different classes as azo dye, indigoid dye, nitro dye, triphenyl methane dye, phthalein dye, and anthraquinone dye, based on their structure (Marzec, 2014). While based on application, they can be classified as acid dye, basic dye, disperse dye, direct dye, ingrain dye, vat dye, reactive dye, and moderate dyes (Hunger, 2007; Lee et al., 2014).

By releasing powerful degradative enzymes, mushrooms play a significant role in the ecosystem's ability to recycle organic materials. The ability of mushrooms to degrade and remove numerous harmful, toxic and polluting organic compounds of anthropogenic origin has been investigated by several researchers. Synthetic dyes in textile effluent are among the organic substances that are known to harm aquatic ecosystems by impeding photosynthesis in aquatic plants and causing harmful effects on aquatic life. *S. commune*, a common split-gill mushroom, was employed in this work to decolourize and hence degrade two commonly used textile dyes, Congo red and Methylene blue.

Although Congo red, an azo dye is soluble in water, its solubility is higher in organic solvents. The use of this dye has long been abandoned, majorly due to its carcinogenic properties (Klaus et al., 2005).

MATERIALS AND METHODS

Sample collection

Kanglayen (split gill mushroom) samples were randomly collected from different areas of the Jiribam district in Manipur during May 2021. Fruiting bodies were collected whenever

possible, and specific care was taken to avoid contamination by other fungi. For this purpose, just after plucking the mushroom, it was directly transferred to a sterile zip lock bag and brought to the lab for further processing. Figure 1 depicts the location of the sample collection site.

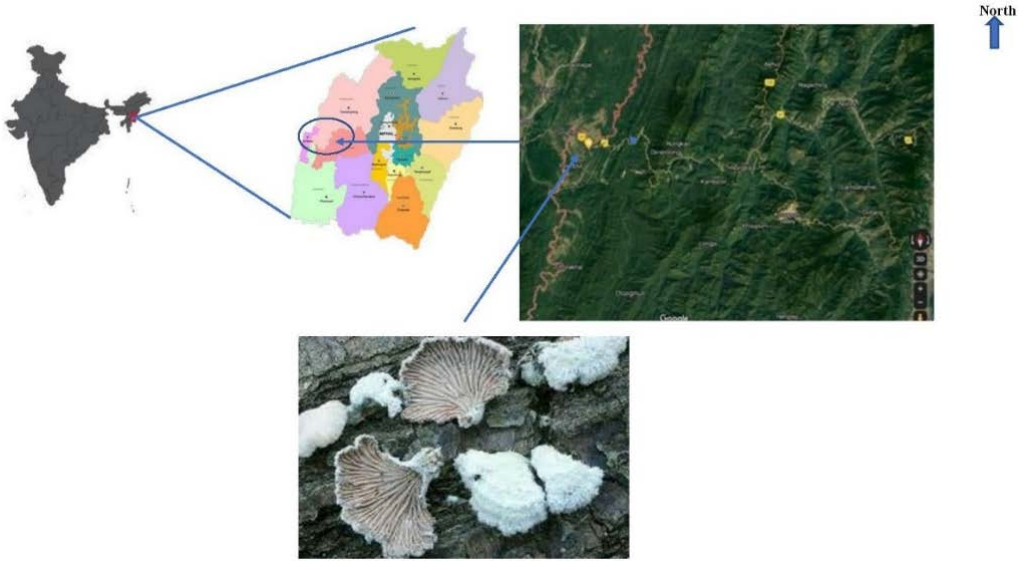

Figure 1. Map showing the site of sample collection

Identification based on morphology and other perceivable descriptions

The identification of the collected mushroom samples was ensured by consulting the book "How to identify mushrooms to genus I: macroscopic features" (Largent, 1986) and some other recognized standard keys (Singer, 1986; Kirk et al., 2008). Further confirmation was done using the information available at http://www.mushroomexpert.com (accessed on 03/05/2019) and http://www.mycobank.org (accessed on 05/05/2019).

Chemical characterization

A piece of mushroom fruiting body was executed by taking the tissue aseptically for chemical characterization using 3% phenol, 10% KOH and 10% $FeSO_4$ and colour changes were observed.

Preservation

Initially, all specimens were checked for dirt and other contaminants, and if present they were removed. They were then washed properly with tap water and dried either wholly or partly, depending on their size. Drying was performed in a sterile drying chamber at room temperature. The completely dried mushroom specimens were packed in suitable plastic bags together with silica gel. The specimens were stored in an airtight cupboard for future use.

Isolation and maintenance of pure cultures

A piece of tissue was removed, preferably from the unexposed area of the mushroom, and surface-sterilised with 70% ethanol and 2% NaOCl (2% sodium hypochlorite). The surface sterilization protocol followed was 70% ethanol (2 minutes), 2% NaOCl (2 minutes). The tissue was subsequently washed with sterile distilled water, dried and inoculated on culture media supplemented with streptomycin (100 µg/ml). Two different types of culture media were used for the isolation, namely, malt extract agar (MEA) media (for better sporulation) and potato dextrose agar (PDA) media (for better mycelium growth). Both PDA and MEA media were prepared at a pH of 5.5 at 27 °C. All the experiments were done in triplicates.

Once pure culture was obtained, they were maintained and stored at 4 °C on PDA slants for future use after incubating for a period of seven days at 27 °C. To preserve for a longer period of time, the cultures were kept at 20 ^{o}C in 20% glycerol and also in sterile distilled water (Kumla et al., 2013).

Characterization of the cultures

Colony morphology was observed regularly, preferably after 7 days of incubation at 27 ^{o}C, and the characteristics of the colony and other features were recorded and photographed.

Microscopic characterization of the cultures

Slides were prepared by taking a little part of the mycelium with the help of a needle and staining with lactophenol cotton blue stain (HiMedia). Then the slides were examined under a binocular compound microscope (Lynx) at 40X magnification.

Screening of the mushroom cultures for the presence of lignin-degrading enzymes

The mushroom cultures were screened on solid media (Potato Dextrose Agar) containing specific dyes and chemicals to check production of lignin-degrading enzymes or ligninolytic enzymes. The method of the Guaiacol oxidizing activity test as developed by Vijya & Reddy was followed for screening (Vijya & Reddy, 2012).

Rapid detection of ligninolytic enzymes was also performed following Bhatnagar et al. (2021) with slight modifications (Bhatnagar et al., 2021). Presence of both laccase and manganese peroxidase (MnP) can be detected on the media containing guaiacol and presence of lignin peroxidase by using media containing remazole brilliant blue R (RBBR) as a substrate.

Screening for dye decolorization potential

***On solid media*:** Two synthetic dyes, Congo Red (CR) and Methylene Blue (MB), at a concentration of 0.02% are individually added to 2% PDA media. Plates were inoculated with 8−10 days old mycelial culture plugs (5 mm in diameter) taken from the colony margin of an actively growing culture, into the centre and incubated at 25 °C± 2 °C. Each treatment was repeated in triplicate. After that, decolourization, if any was observed visually (Pointing et al., 2000).

***On liquid media*:** The mycodecolourization experiments were done in 100 ml of PDB (potato dextrose broth) medium supplemented with 0.02% dyes, i.e., Congo red and Methylene Blue, in two separate conical flasks. Each flask was inoculated with the mycelium plugs of each mushroom culture and incubated in stationary condition in a BOD (Biochemical Oxygen Demand) incubator at 25 ± 2 °C for 14 days. Results were reported as the mean value of percent dye decolorization (% DD) for triplicates following the method of Sani & Banerjee (1999).

The following formula was used to determine the percentage of dye decolourization:

$$\text{Decolourization percentage } (\%) = \frac{\text{Initial Aborbance} - \text{Final Absorbance}}{\text{Initial Aborbance}} \times 100$$

Molecular Characterization

Genomic DNA extraction

Genomic DNA was isolated by a slight modification of the conventional CTAB method (Doyle & Doyle, 1990). Sample was prepared by carefully taking out the mycelium (3 mm × 3 mm) and grinding it with a micropestle in the case of pure cultures. A fragment of the fruiting body (~10 mg) was homogenized using a high-speed homogenizer (Remi) in DNA extraction buffer (CTAB buffer) in the case of fruiting bodies.

Quantification of DNA

Quantification of the isolated DNA was done in two ways: by using UV absorbance and by agarose gel electrophoresis. Quantification

by the UV absorbance method involves measuring the absorbance of light through the DNA sample to determine the concentration of substances in the sample using a spectrophotometer. The concentration of the DNA sample and the presence of any other contaminants are estimated by these absorbance measurements. Agarose gel electrophoresis quantifies the DNA sample by comparing its band intensities to the ladder's intensity.

PCR amplification and sequencing

The PCR amplification was done for the ITS (Internal Transcribed Spacer) region by using the primers ITS1 and ITS4 as forward and reverse primers, respectively. The PCR program followed was 95 ^{o}C for 3 minutes, 35 cycles of 94 ^{o}C for 1 minute, 56 ^{o}C for 2 minutes and 72 ^{o}C for 10 minutes. Afterwards, Bidirectional sequencing was done at Eurofins Genomics India Private Limited, Bangalore, India.

RESULTS

Preliminary investigation records

During the time of sampling, some preliminary investigations were carried out to ensure proper identification of the mushrooms. The observations being recorded are shown in Table 1.

Table1. Preliminary investigation records of the collected mushrooms

Sample ID	Location	Height × Width	Single or Multiple	Colour	Smell	Habitat/ substrate	Soft or woody
DJKB1	24^{o}46'29"N 93^{o}08'16"E	(3–5) × (2–10) mm	Single	Whitish grey	Not distinct but somewhat woody smell	rotting wood log	Soft
DJKB2	24^{o}47'11.1"N 93^{o}09'14.6"E	(4–7) × (2–9) mm	Multiple	Whitish grey	Not distinct but somewhat woody smell	Old wood log	woody
DJKB3	24^{o}48'36.0"N 93^{o}06'47.8"E	(2–4) × (2–7) mm	Multiple	Whitish grey	Not distinct but somewhat woody smell	Fallen wood log	woody

Morphological characterization

The fruiting bodies of the mushroom were observed growing scattered or clustered on wood logs and branches. The fruiting body was found to be gymnocarpous which means the spore containing hymenium was exposed and visible only to the underside of the fruiting body. Only the lower side had the hymenophore which was gilled, distinct on the underside, also folded and split down the middle, which again looked like a groove. The fruiting body was 2−10 mm wide and laterally attached to the substratum, stemless, and the shape was almost shell shaped. The upper surface was covered with small whitish to greyish hairs. The colour of the spore print was found to be white when the spore print was taken on black paper. Based on these features, the specimen may be identified as *S. commune.*

Chemical characterization

The pieces of mushroom fruiting body took on a brown colour when 3% phenol was added, but no change in colour was observed when 10% KOH was added. The tissue becomes a little bit darker when we add 10% $FeSO_4$. Changing the colour when 3% phenol and 10% $FeSO_4$ can be used as an identifying character for this mushroom by future researchers.

Cultural characterization

The outline of the colony as grown on PDA and MEA media was white in colour, without any specific odour. Starting very close to the inoculum and then gradually spreading throughout the surface of the culture medium, the mycelial mat looked perfectly white. The appearance of the surface seems to be folded. The key points observed

were as follows: **form:** circular; **surface:** veined; **colour:** whitish or pale greyish, reverse white**; margin:** undulate. The 3 days old and 5 days old pure cultures on PDA media, as representative images are shown in Figure 2.

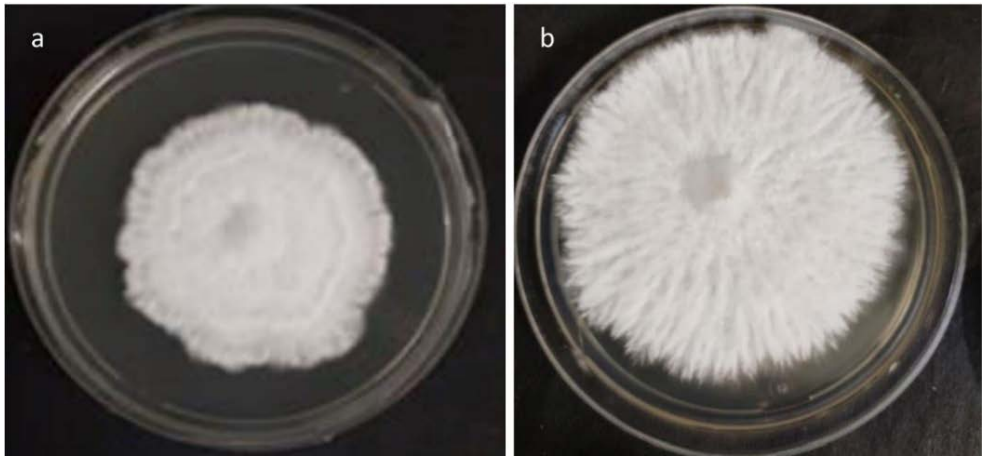

Figure 2. Pure culture of *Schizophyllum commune* on Potato dextrose Agar media. (a) 3 days old culture and (b) 5 days old culture

Microscopic characterization

The microscopic examination of the mycelia stained with Lactophenol cotton blue (HiMedia) showed absence of conidia or sporangiospores. The hyphae were thin-walled, rough, and septate. Branching was also observed. Chlamydospores are present in the terminal position, as shown in Figure 3. Two chlamydospores were visible.

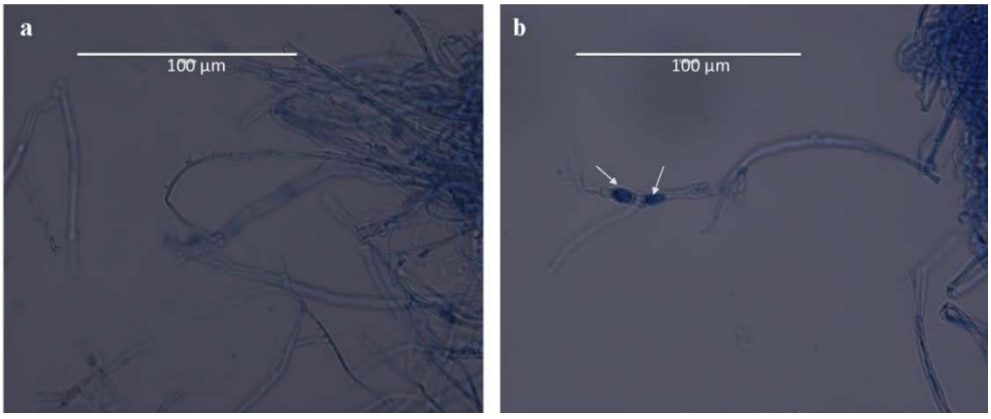

Figure 3. Microphotographs of mycelium obtained from a pure culture of *Schizophyllum commune*. (a) mycelia showing wide and narrow hyphae and (b) a single hypha containing chlamydospores (indicated by the arrows)

Molecular characterization

Isolation of genomic DNA

After isolation of genomic DNA, the quality was ensured and visualized on agarose gel. The genomic DNA extracted was greater than 10 kb, indicating high molecular weight. The quantification and purity checking using a spectrophotometer reveal that the DNA had concentrations in the range of 124–250 ng/µL

and purity in the range of 1.93–2.01 in terms of the absorbance ratio at 260/280 nm.

PCR amplification and sequencing of ITS gene

The primers ITS1 and ITS4 led to successful PCR amplification which were then visualized with the help of Gel Doc where clear bands were visible in the range of 600 bp to 700 bp, as depicted in Figure 4. After purification of the PCR products, no smearing was observed in the gel, indicating non-degradation of the PCR amplicons. Additionally, there were no signs of primers' traces on the purified PCR products, which are essential for getting good sequencing results. The best PCR product was sequenced bidirectionally at Eurofins Genomics India Pvt. Ltd., Bangalore. The generated sequence's length was in the range of 700 to 1,200 nucleotides.

After annotation and preparation of the contig, the sequence was submitted to NCBI, and an accession number was obtained as PP470591. The annotated sequence was then blasted in the NCBI BLASTn programme, and the percent similarity check with those sequences already uploaded to the GenBank database was performed. The sequence matched well with the organism *S. commune*, with a percent similarity of 99.64% with a 100% query coverage, which clearly indicated that our mushroom is *S. commune*.

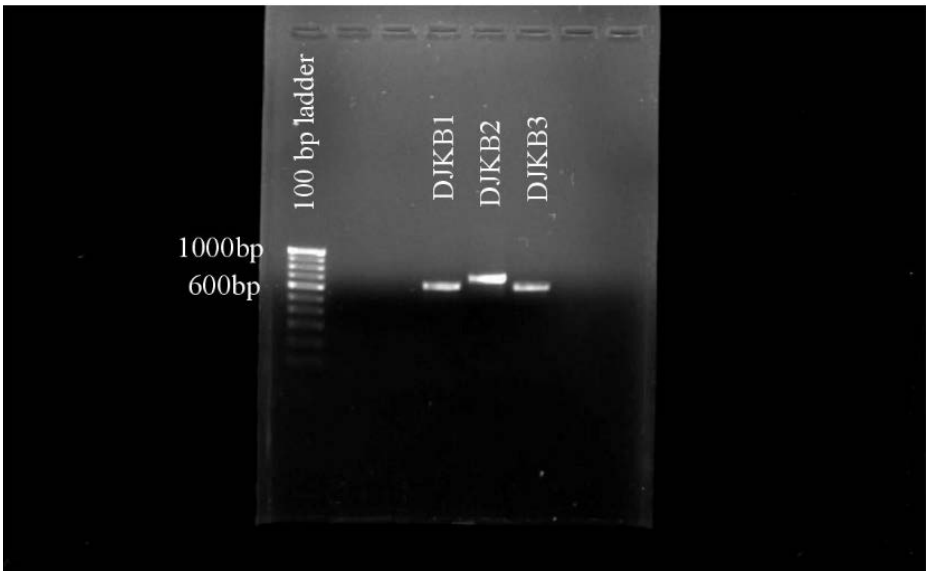

Figure 4. Agarose gel showing amplified products of ITS gene of the mushroom *Schizophyllum commune*. 1st lane is 100 bp ladder, and lanes 4 and 6 denotes amplified ITS gene of *Schizophyllum commune.*

When phylogenetic analysis was performed using MEGA11, the Neighbour-joining tree constructed using five other related sequences downloaded from NCBI also clearly indicates that our sequence, PP470591, clustered together with other *S. commune* sequences as can be seen in Figure 5. The Neighbour-joining method was used to determine the evolutionary history, and the optimal tree is displayed. Above the branches, the percentage of the replicate trees where the related taxa clustered together in the bootstrap test (500 repetitions) is displayed. The tree was drawn with branch lengths in the same units as the evolutionary distances, used to generate the phylogenetic tree. The Kimura 2-parameter method was used for the calculation of evolutionary distances. The analysis was carried out using MEGA11(Tamura et al., 2021).

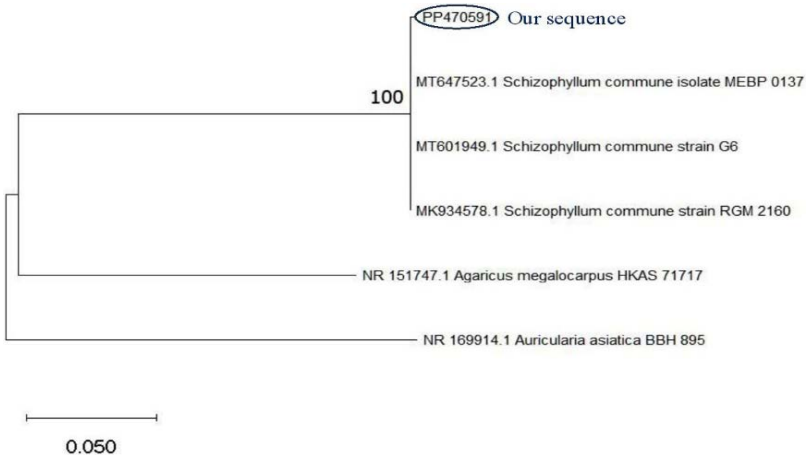

Figure 5. Neighbour-joining tree constructed using the generated sequence and other 5 sequences from GenBank. Our sequence (PP470591) is seen clustered with other *Schizophyllum commune* sequences

Screening for the presence of lignin-degrading enzymes (ligninolytic enzymes)

Mushrooms that produce lignin-degrading enzymes, especially laccases, have been screened on PDA (Potato dextrose agar) media containing specific dyes and chemicals, which are indicator compounds that enable visual detection of enzyme production. The following two tests were performed.

Guaiacol oxidizing activity test

The mushrooms having ligninolytic activity developed coloured zones of reddish-brown colour which was due to the oxidation of guaiacol, and they are noted as ligninolytic enzyme-positive. The results are depicted in Figure 6, where brown halo formation is clearly visible in (a), whereas (b) is a negative control without inoculation and (c) is a negative control without substrate which is guaiacol.

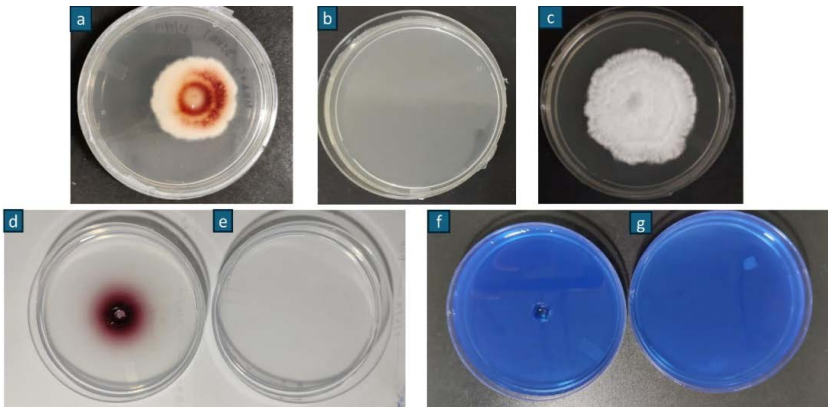

Figure 6. Screening of ligninolytic activity of *Schizophyllum commune* by guaiacol oxidation activity test. The brown halo zone is clearly seen on the culture. (a) is inoculated with *Schizophyllum commune*, (b) is control without inoculation , and (c) is without guaiacol. Second line indicates Rapid detection test for ligninolytic enzymes. (d) for laccase and manganese peroxidase and (f) for lignin peroxidase. Brown colouration in (d) indicates presence of laccase and (or) manganese peroxidase and (f) clearly indicates no change, proving absence of lignin peroxidase. (e) and (g) are respective controls.

Rapid detection test for ligninolytic enzymes

From the rapid detection test of ligninolytic enzymes (Fig. 6), it is seen that only the media containing guaiacol show brown colouration (d), while the media containing RBBR does not show any decolourization pattern or new colouration (f). These results indicate that *S. commune* produces laccase and manganese peroxidase since brown colouration was observed, or it produces only laccase or only manganese peroxidase. On the other hand, there is no sign of lignin peroxidase production.

Screening for textile dye decolourization potential

Potato dextrose agar plates supplemented with 0.02% Congo red and Methylene blue, and then inoculated with mushroom culture were examined for the ligninolytic activity after 7 days of incubation. The mushroom cultures having ligninolytic activity developed decolourized zones. Dye decolourization experiment was again performed on liquid media using the dyes Methylene Blue (MB) and Congo Red (CR) with the mushroom culture. Table 2 & Figure 7 show the respective dye decolourization percentages of the two dyes, and Figure 8 depicts the photographs visually confirming the dye decolourization ability of the mushroom *S. commune* tested in liquid culture.

For Congo Red, the dye decolourization percentage rises slowly from 90.7% on the 3rd day to 91.8% on the 5th day and decreases to 86.2% on the 7th day and then to 81.5% on the 10th day of incubation. In the case of Methylene Blue, the dye decolourization percentage was noted as 77.67% on the 3rd day, 76.9% on the 5th day, 74.8% on the 7th day, and 71.5% on the 10th day of incubation, respectively. Thus, confirming that *S. commune* can decolourize Congo red more effectively with a dye decolourization percentage of 91.8% on the 5th day of incubation, while it can decolourize Methylene blue too, but with lesser efficiency.

Table 2. Dye decolourization percentages of the two dyes, Congo red and Methylene blue, at different days of incubation

		Dye decolourization %		
Dye	3 days old	5 days old	7 days old	10 days old
Congo red	90.7	91.8	86.2	81.5
Methylene blue	77.67	76.9	74.8	71.5

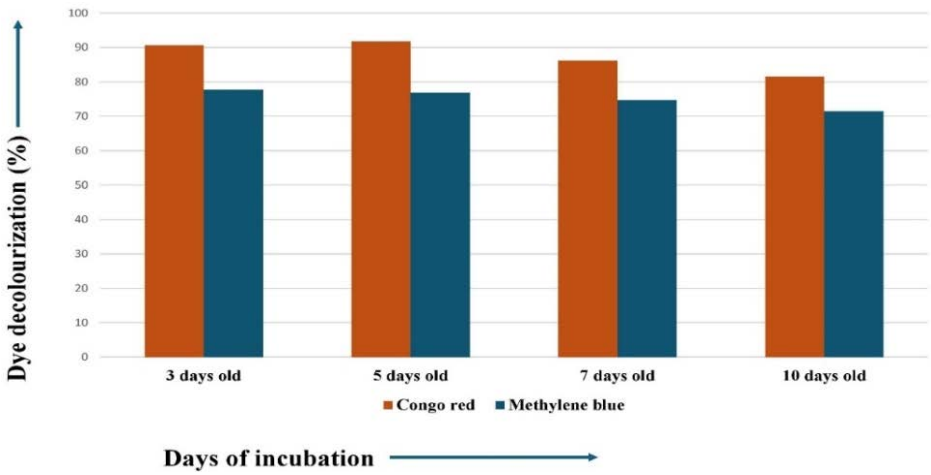

Figure 7. Dye decolourization percentages of Congo red and methylene blue using cell free culture extract of *Shizophyllum commune*

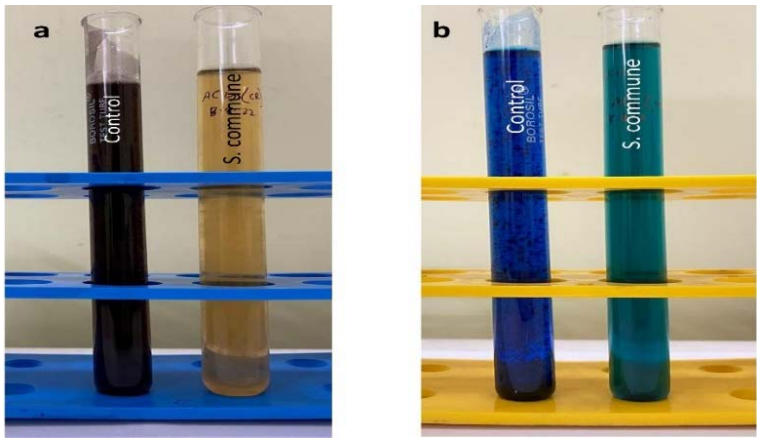

Figure 8. Dye decolourization of (a) Congo red and (b) Methylene blue dye in liquid culture by *Schizophyllum commune*.

DISCUSSION

The azo structure in Congo red produces its unique hue of colour in an aqueous solution. Due to the fact that it is a di-azo dye, it appears red in basic medium and blue in acidic one. On cleavage of its azo groups, Congo red can form an amine constituent such as benzidine, which is a common carcinogen; therefore, Congo red is regarded as one of the banned azo dyes (NCBI Pubchem). Despite the fact that Congo red has useful practical applications in a variety of sectors, including the textile, pigment, cosmetics, leather, pharmaceutical, food, pulp, and paper industries, the extensive use of this dye also contributes to industrial pollution (Siddiqui et al., 2023).

Methylene blue is a cationic azo dye which has benzene rings, also toxic and very persistent in the environment (Sedigheh et al., 2022). It is one of the most common pollutants found in textile wastewater. The cationic dyes are more toxic than the anionic dyes. They produce toxic compounds through the metal complexes which are harmful for aquatic organisms. Extreme exposure to methylene blue led to increased heart rate, vomiting, gout and paralysis in human beings (Getu et al., 2021).

Mycoremediation employs fungi that exhibit a high ability to produce extracellular enzymes that can degrade a variety of tough to degrade compounds (Mohamadhasani et al., 2022). Mushrooms, especially the white rot fungi are the most extensively and well-studied dye decolourizing microorganisms. (Radhika et al., 2014).

But when we think about mushrooms, only one thing that comes to our mind is a tasty, healthy, delicious dish, but other than this, mushrooms also have other useful applications like medicinal, environmental protection or combating environmental pollution, etc. Various applications of edible mushrooms are also explored by many researchers, including the synthesis of biosorbent, probiotics, biochar, edible films/coating, nanoparticles and cosmetic products (Kumar et al., 2021). Devi et al. (2023) gave a detailed report on eco-friendly utilization of lignocellulosic waste using mushrooms, where how the lignocellulosic wastes could be value added was presented. Vaksmaa et al. (2023) reported various applications of fungi in bioremediation of emerging pollutants particularly in the aquatic environment.

A major reason for dye decolourization is the production of extracellular enzymes by the mushrooms during the biodegradation of the dyes to be tested. Radhika et al. (2014) compiled various research reports studied by several researchers on decolourization of dyes by using ligninolytic enzymes, especially laccase from *Trametes hirsuta*, *Sclerotium*

roysii, *Trametes modesta* (Nyanhongo et al., 2002), *Laccaria fraternal*, *Pleurotus ostreatus* (Balaraju et al., 2007), LiP from *P. ostreatus* (Shrivastava et al., 2005) and *Phanerochaete chrysosporium* (Chivukula & Renganathan, 1995). Ollikka et al. (1993) reported studies on the degradation of remazole brilliant blue R (RBBR) and methylene blue using the mushroom, *P. chrysosporium*.

Oladoye et al. (2022) carried out a detailed study on various reports on methylene blue dye toxicity and potential elimination technology from wastewater. They stated that Methylene blue is one of the most popular cationic dyes that is environmentally persistent, toxic, carcinogenic and mutagenic. They concluded that it is feasible to use microbial enzymes or microorganisms or both in conjunction with a physicochemical method, such as adsorption technology, to effectively remove and decontaminate methylene blue from water (Oladoye et al., 2022).

S. commune has been studied by different researchers for the removal and decolourization of various textile dyes like drimaren turquoise CL-B (Raees et al., 2023), basic violet 3, acid violet 17, basic red 9, acid orange 5, direct blue 71, reactive black 5, disperse red 60, acid blue 74, and reactive blue 19 (Abadulla et al., 2000). Here, we have presented mycodecolourization study using *S. commune* to decolourize and hence help in degrading Congo red and Methylene blue.

Before the dye decolorization experiment, it is crucial to screen the selected mushroom for its ability to produce ligninolytic enzymes, and for this purpose, the cultures were subjected to guaiacol oxidizing activity test. Guaiacol is one of the methoxy functional group-containing organic phenolic compounds. It is used as an indicator compound that can aid in easy visual detection of enzyme production. If the mushroom produces ligninolytic enzyme, the development or formation of a clear brown coloured halo zone will be obvious, as shown in Figure 6.

The rapid detection test of ligninolytic enzymes (Fig. 6) clearly indicated that *S. commune* produces laccase and/or manganese peroxidase since brown colouration or halo formation was observed, and absence of lignin peroxidase. As mentioned by Tovar-Herrera et al. (2018), *S. commune* produces laccase but does not produce lignin peroxidase and manganese peroxidase. It is also confirmed that our isolate produces laccase but no lignin peroxidase, and the results go as par with the previously published reports.

For, textile dye decolourization experiment, potato dextrose broth medium supplemented with 0.02% Congo red and methylene blue and then inoculated with mushroom culture was examined for the ligninolytic activity of the mushroom. From the results, it was confirmed that *S. commune* can decolourize Congo red more effectively with a dye decolourization percentage of 91.8% on the 5^{th} day of incubation, while it can decolourize Methylene blue too but with lesser efficacy. From this experiment, it is clearly observed that the efficiency of textile dye decolourization by the studied mushroom decreases over time, which may be due to decline in the mycelium's metabolic activity and enzyme production. Additionally, inactivation of enzymes through the meantime, accumulation of toxic intermediates and other factors may be the reason behind the decline in its efficiency of decolourization.

CONCLUSION

The purpose of the present study was to screen one of the most widely available wild mushrooms, *S. commune*, collected from the Jiribam district of Manipur, India, for its dye decolourization potential towards two toxic and non-degradable textile dyes, Congo red and Methylene blue. Nature is the biggest source to find organisms containing various properties, such as lignin-degrading enzymes. The results suggested that *S. commune* can efficiently degrade or decolourize these dyes, showing more potential in the case of Congo Red. It paves the way for this mushroom to be used as an efficient mycoremediation tool for

the treatment of dye containing textile industrial effluents.

This study emphasizes the need to explore more wild mushrooms to evaluate their real potential for the production of ligninolytic enzymes. The tremendously increasing application of synthetic dyes impacts the environment and human health. So, there is an utmost requirement for an effective response in terms of modern and viable treatment processes for dye containing effluents, prior to their discharge into waterways. The pathways for dye decolourization and degradation are still not totally understood; therefore, thorough research in that direction has high relevance for the development of future modern technology.

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