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Screening of *Schizophyllum commune* strains for laccase and peroxidase production

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ABSTRACT

Schizophyllum commune is an effective wood-degrading basidiomycete producing many hydrolytic, cellulolytic and lignolytic enzymes *viz*., lignin peroxidase, manganese peroxidase and laccases, which degrade complex plant biomass made of recalcitrant lignin. Three strains of *S. commune viz.*, DMRX-2156, DMRX-2157 and DMRX-2160 were used for the study. Among the *S. commune* strains, DMRX-2160 took the minimum period of 9.23 days for complete coverage on petridish with maximum radial mycelial growth (0.97 cm/day). All the three tested strains reacted positively to the qualitative plate assay for laccase and peroxidase activity. Quantitative estimation of laccase and peroxidase enzymes from mycelia and broth of *S. commune* submerged cultures was done using substrates *viz*., guaiacol and pyrogallol for laccase and peroxidase, respectively. DMRX-2156 recorded the highest extracellular laccase activity (30.55±3.66 U/g) and peroxidase activity (242.34±36.66 U/g).

Keywords: *Schizophyllum commune,* submerged fermentation, laccase, peroxidase

Mushrooms are fruiting bodies of basidiomycete/ ascomycete fungi usually produced above ground on soil or other substrates. They are potential producers of metabolites including enzymes, many of which are of great importance in various industries (Díaz-Godínez, 2016). The white rot mushrooms can break down all parts of plants, including cellulose, hemicellulose and lignin (Martínez *et al*., 2005). They can degrade lignin efficiently into low molecular weight compounds since they produce various extracellular lignolytic enzymes *viz.*, laccase (EC 1.10.3.2), lignin peroxidase (EC 1.11.1.14) and manganese peroxidase (EC 1.11.1.13) (Ardon *et al*., 1996). Laccase enzyme oxidizes phenols and catalyzes the one-electron oxidation of ortho/ para diphenols and aromatic amines, thus it breaks down lignin (Patel and Gupte, 2016). Peroxidases are extracellular glycosylated haeme proteins, which catalyze the H_2O_2-dependent one-electron oxidation of a range of aromatic compounds related to lignin. These lignolytic enzymes have potential applications in different industries like food, textile, dyes, bioremediation, cosmetics, analytical biochemistry, paper and pulp (Arabi *et al*., 2011).

S. commune, also known as split gill mushroom, belong to the category of white rot fungi and a naturally occurring medicinal mushroom that grows on rotting wood. The source of the name *Schizophyllum commune* is from the Greek words "*Schiza,"* which means "split", and "*commune,"* which means "common" (Mahajan, 2022). The fruiting body of *S. commune* is small and flagelliform (fan-shaped), with a white, hairy, stipeless cap. Due to its therapeutic characteristics, it is used as food and medicine in various countries, including Northeast India, Vietnam, Thailand, China, Korea and Malaysia (Roja *et al*., 2022). *S.*

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commune is considered as an excellent source of laccase and peroxidase enzyme, as it is a lignin-degrading fungus with an active lignocellulolytic machinery (*et al*., 2020).

The growth of *S. commune* in liquid media referred to as submerged fermentation (SmF) is also a favorable condition for obtaining fast growth of mycelia and high productivity of lignolytic enzymes under controlled conditions. Therefore, the present study was undertaken to screen different strains of *S. commune* for their laccase and peroxidase activity under submerged cultivation for their potential use in industry.

MATERIALS AND METHODS

Culturing and maintenance

Three strains of *S. commune viz.*, DMRX-2156, DMRX-2157 and DMRX-2160 were procured from All India Coordinated Research Project (AICRP) of Mushrooms, Department of Plant Pathology, College of Agriculture, Vellayani and used for the study. Pure culture of *S. commune* strains was maintained on PDA plates at 28±1°C. Hyphal characteristics of three strains were observed and mycelial diameters were measured microscopically.

Qualitative screening for laccase and peroxidase activity

Screening for laccase was done by aseptically supplementing sterilized PDA with 0.02% guaiacol in sterilized petri plates. The plates were inoculated with the mycelial bits of each *S. commune* strain and incubated at 28±1°C for 72 h. The plates were observed for the development of circular zones of reddish-brown color around the cultures due to the oxidation of guaiacol. For screening of peroxidase, 1% pyrogallol solution was mixed with 0.4% H_2O_2 in a ratio of 1:1 in a clean test tube. Using a dropper, the mixture of pyrogallol and H_2O_2 was dropped on the edge of 10-day-old cultures of *S. commune* strains in petri plates and observed for the development of yellowish-brown color in the area where reagents were dropped.

Submerged cultivation in different media

Different culture media *viz*., Stock Basal Medium (SBM), Potato Dextrose Broth (PDB), Mycological Liquid Medium (MLM) and Potato Malt Peptone Broth (PMPB) were prepared. An amount of 100 ml of each media was transferred to 250 ml flasks and autoclaved (at 121°C and 1.02 kg/cm^2 pressure) for 20 minutes. After cooling, the media were inoculated by adding mycelial plugs (5 numbers) of each *S. commune* followed by incubation at 28±1°C.

Estimation of laccase and peroxidase activity from mycelia and broth

Preparation of crude enzyme extract from mycelia and broth

Five, ten and fifteen-day-old cultures of *S. commune* strains in various broths were taken from which the biomass was filtered, and the mycelial mat was separated. 1g of the mycelial mat was homogenized with ice-cold 5ml of 0.1M sodium phosphate buffer (pH 6.5) using prechilled mortar and pestle, followed by centrifugation at 10000 rpm for 15 minutes at 4°C.

The supernatant was filtered out using Whatman No.1 filter paper and was used as a crude enzyme extract to estimate the intracellular enzyme activities (expressed in U/g). After the separation of mycelium, the remaining broth was subjected to centrifugation at 10000 rpm for 15 mins at 4°C. The supernatant obtained was filtered using a Whatman No.1 filter paper and used as crude enzyme extract for the evaluation of extracellular enzyme activities (expressed in U/ml).

Laccase assay

Laccase activity from the culture filtrate was assayed following the method of Nasreen *et al.* (2015). A 5ml reaction mixture for the laccase assay was prepared in a test tube, which includes 3ml 10 mM sodium acetate buffer, 1 ml 2mM guaiacol solution and 1 ml enzyme source.

The reaction mixture was incubated at 30°C for 15 min and observed for the development of reddish-brown color due to the oxidation of guaiacol resulting in the production of quinone. The absorbance was read at 450 nm against the blank prepared by adding distilled water instead of enzyme source, using a UV spectrophotometer. The enzyme activity was expressed in International Units (IU), where 1 IU is the amount of enzyme required to oxidize 1 ìmol of guaiacol per minute. The laccase activity (U/ml) was calculated using the formula: E.A = A x V/t x e x v

Where, E. A = Enzyme activity A =Absorbance

V =Total mixture volume (ml) v = Enzyme volume (ml)

t =incubation time

e = extinction coefficient for guaiacol (6740 $M^{-1}cm^{-1}$)

Peroxidase assay

The peroxidase activity was estimated following the method of Kang *et al.* (1993). A 3.3 ml reaction mixture for peroxidase assay was prepared in a test tube which includes, 2 ml 0.1 M phosphate buffer, 0.2 ml 0.1 M pyrogallol, 0.1 M H_2O_2 and 1 ml enzyme source.

The enzyme activity was determined from the development of yellowish-brown colored product purpurogallin due to the oxidation of pyrogallol, at 30 s intervals for 5 min at 430 nm against blank. The peroxidase activity in U/ml was calculated using the formula:

E.A = A x V/t x e x v

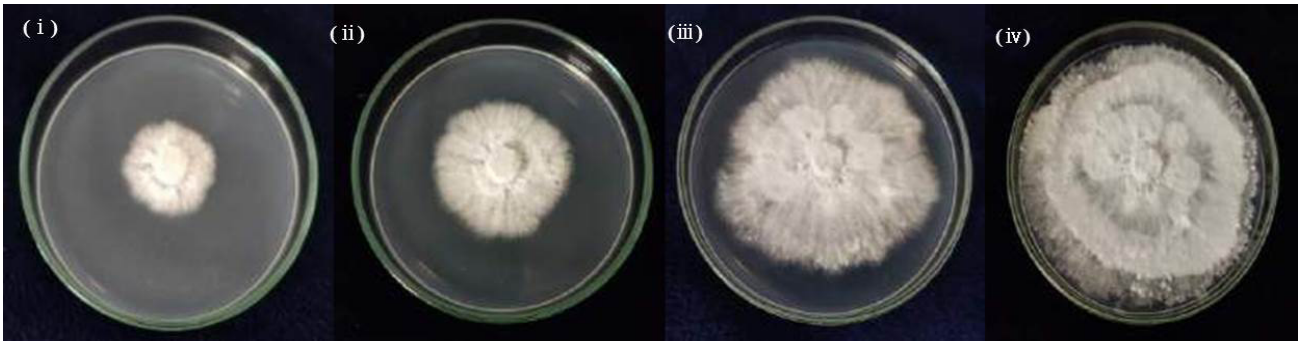
e = molar extinction coefficient for pyrogallol (2470 $M^{-1}cm^{-1}$).

RESULTS AND DISCUSSION

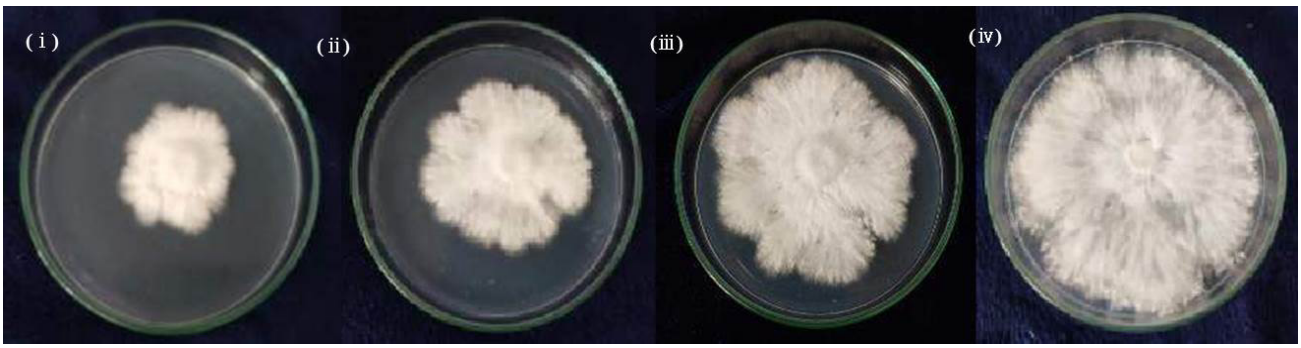
Culturing and maintenance

There was a significant difference in the radial mycelial growth and hyphal size of three strains of *S. commune* in PDA medium. Mycelial growth of *Schiophyllum* strains increased from 2 days after inoculation (DAI) and completely covered the Petri dish by 10 DAI. Among the *S. commune* strains, DMRX-2157 and DMRX-2160 showed white, thick, fluffy and leathery mycelial growth with smooth margins (Fig 1b and 1c), whereas the nature of mycelial growth of DMRX-2156 was white, thin and sparse with smooth margins (Fig 1a). DMRX-2160 took the minimum time for complete coverage on Petri dish (9.23 days) followed by DMRX-2157 (10.43 days) and DMRX-2156 (11.10 days). The radial mycelial growth was recorded the highest for DMRX- 2160 (0.97 cm/day) followed by DMRX-2157 (0.86 cm/day) and DMRX-2156 (0.81 cm/day).

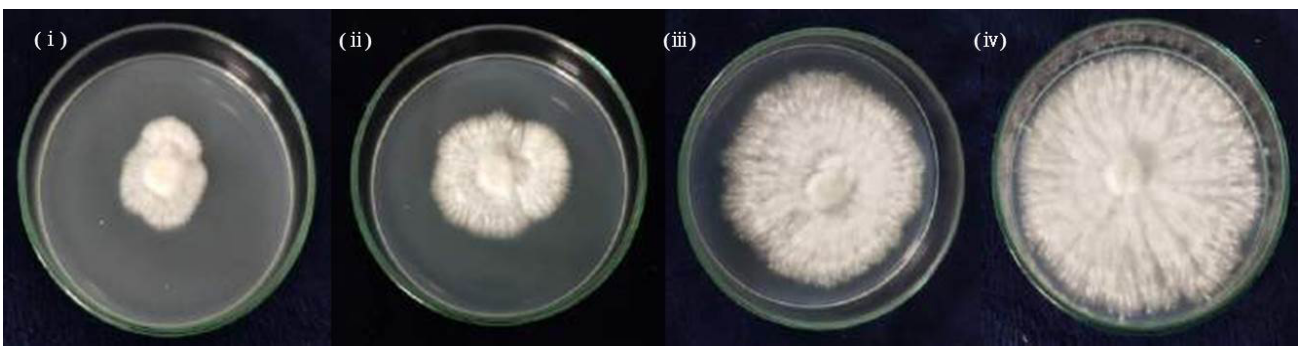
This result is in accordance with the findings of Reeja, (2002) who reported that the white leathery mycelium of *S. commune* completely covered the Petri dish at 10 DAI. Investigation by Rosnan *et al.* (2019) also showed that the mycelium of *S. commune* took 10 days to fill the Petri dish completely with a mycelial diameter of 8 cm and moderate thick density in PDA. Kurnia *et al.* (2020) reported that the growth of *S. commune* mycelium increased at 2 DAI, which was initially thin and later thickened at 4 DAI due to the branching of the mycelium which was suspected as a process of adaptation to the media aided through the formation of extracellular enzymes. Singh *et al.* (2021) also observed dense white woolly mycelial growth of *S. commune* on PDA plates with the complete growth of culture seen after seven days of



a. DMRX-2156 at (i) 3 DAI (ii) 5 DAI (iii) 7 DAI (iv) 10 DAI



b. DMRX-2157 at (i) 3 DAI (ii) 5 DAI (iii) 7 DAI (iv) 10 DAI



c. DMRX-2160 at (i) 3 DAI (ii) 5 DAI (iii) 7 DAI (iv) 10 DAI

Fig. 1. Mycelial growth of *Schizophyllum* strains in PDA at different DAI

incubation with characteristic bump formation in the centre of the plate indicated as the initial stage for emergence of basidiocarp.

The mushroom hyphae were odourless, reverse of the plate was darkened with yellow to brown coloured pigment and droplets of yellowish exudation on the surface at 12 DAI. Similarly, Buzina *et al.* (2001) described yellowish exudation on the surface and yellowish-brown pigmentation in the medium of old cultures of *S. commune*. Hyphae of *Schizophyllum* strains were septate, branched, hyaline with distinct clamp connection and spicules. Hyphae of DMRX-2156, DMRX-2157 and DMRX-2160 measured sizes of 1.43, 2.75 and 2.14 µm respectively. Similarly, Dasanayaka and Wijeyaratne (2017) microscopically confirmed septate mycelium with clamp connections in *S. commune*. Amee *et al.* (2009) and Kumar *et al.* (2018) prepared slide culture of *S. commune* isolates on Malt Extract Agar (MEA)

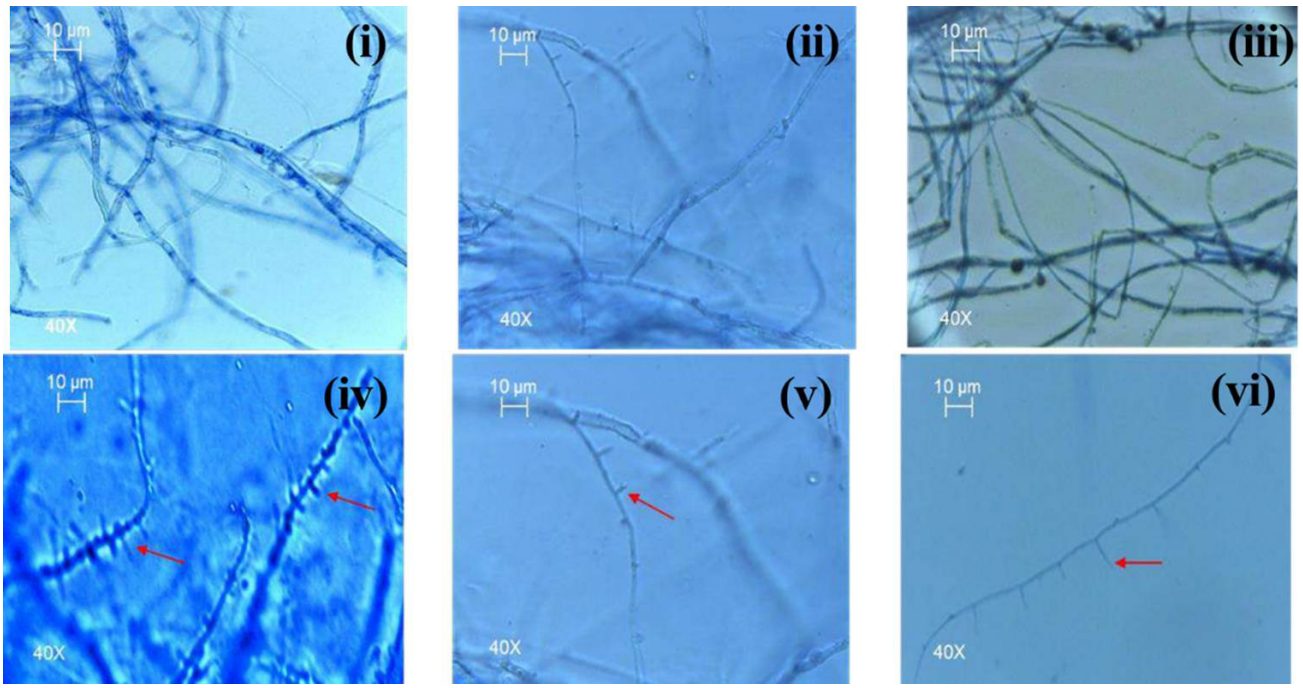


Fig. 2. Hyphal characters of *Schizophyllum* strains (i) DMRX- 2156 (ii) DMRX- 2157 (iii) DMRX- 2160. Red arrow indicates spicules on the hyphae

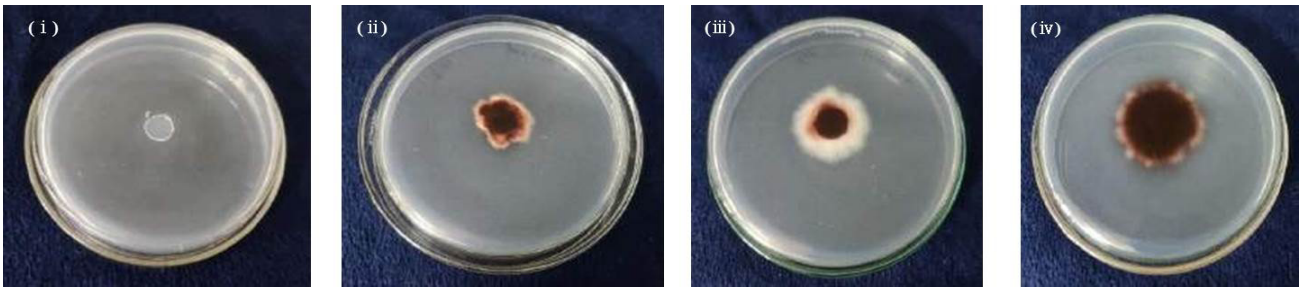
which showed hyaline, septate, thin-walled hyphae with clamp connections and spicules. Itoh *et al*. (2021) prepared slide cultures of *S. commune* showing hyaline hyphae with clamp connections, spicules and absence of conidia.

Qualitative screening for laccase and peroxidase activity

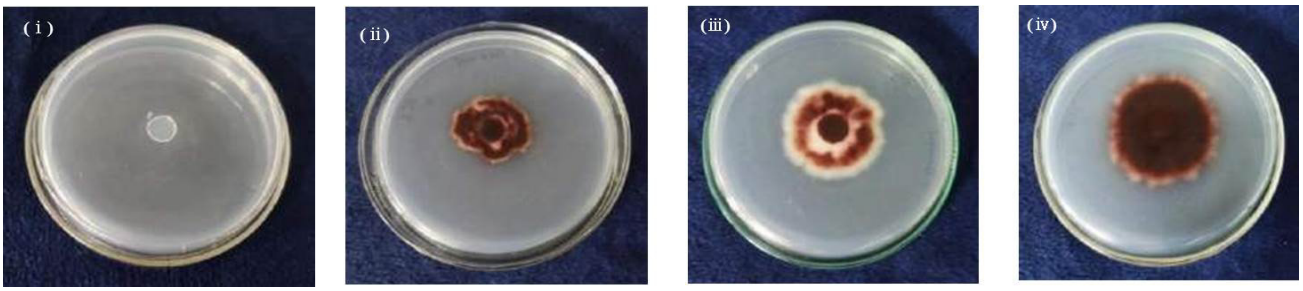
The three strains reacted positively to qualitative plate assay for laccase indicated by the development of reddish-brown oxidation zones around the fungal cultures. To determine the production potential of laccase, the ratio of zone diameter (Z) to colony diameter (C) was determined.

Among the three strains DMRX-2160 recorded the highest laccase activity (brown zone diameter of 3.5 cm) followed by DMRX-2156 (3 cm) and DMRX-2157 (2.75 cm). The ratio of Z/C which indicates the efficiency of laccase production in relation to colony growth was found the maximum for DMRX-2160 (Z/C- 0.86) followed by DMRX-2156 (Z/C- 0.80) and DMRX- 2157 (Z/C- 0.75) (Fig 3).

Similar observation was made by Vijya and Reddy (2012) who screened for the ability of *S. commune* to produce ligninases on malt extract agar plates supplemented with 0.02% guaiacol. They observed circular zones reddish brown colour around the culture with diameter of 52mm due to the oxidation of guaiacol at 7 DAI. Illuri *et al.* (2021) also visualized and measured the reddish-brown zone of 6.7 cm diameter developed on guaiacol amended Crawford's modified medium at 7 DAI, showing the laccase activity of *S. commune.* Perera *et al*. (2021) described the development of reddish-brown colour on PDA plates containing guaiacol as the oxidation-indicator substrate for rapid screening of laccase producers. The oxidizing reactions resulted in the production of quinone which gave the reddish-brown colour ring formation around *Schizophyllum commune.*



a. Five-day old culture of *Schizophyllum* strains (i) Control (ii) DMRX-2156 (iii) DMRX-2157 (iv) DMRX-2160



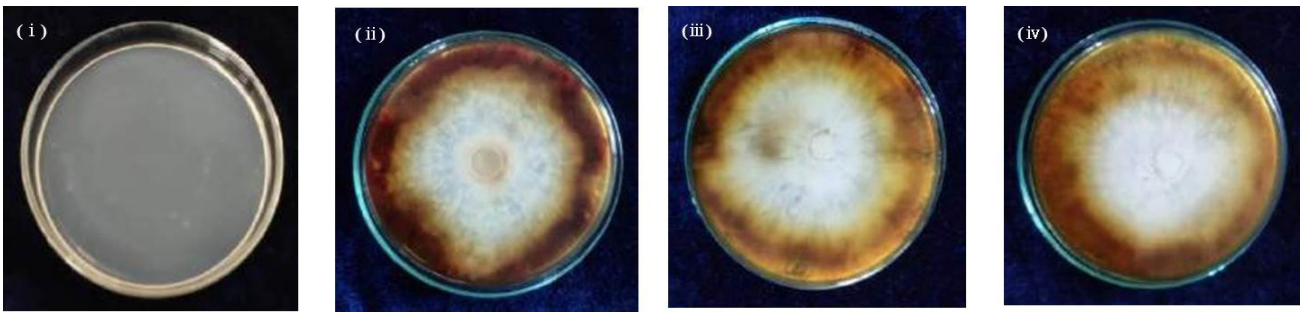
b. Seven-day old culture of *Schizophyllum* strains (i) Control (ii) DMRX-2156 (iii) DMRX-2157 (iv) DMRX-2160

Fig. 3. Development of reddish-brown zones around the *Schizophyllum* strains on PDA supplemented with 0.04% guaiacol

Qualitative plate assay for peroxidase

The three *Schizophyllum* strains showed peroxidase activity on PDA plates, indicated by development of yellowish-brown colouration around the fungal colony after its reaction with 0.4% hydrogen peroxide and 1% pyrogallol at 24 h of incubation (Fig 4) showing production of peroxidase enzymes by the fungus.

Falade (2018) reported a similar result when he qualitatively assessed ligninolytic *Bacillus* species for peroxidase activity. In the nutrient agar plates, they showed the presence of yellowish-brown colouration around the bacterial colony after its reaction with 0.4% (v/v) hydrogen peroxide and 1% pyrogallol after 48 h of incubation. Dube *et al.* (2023) added hydrogen peroxide (0.4%; 30 µl) and pyrogallol (1%) mixture to the colonies of bacterial isolates *viz.*, *Pseudomonas* spp., *Enterobacter* spp. and *Escherichia coli* separately grown on nutrient agar plates. The subsequent incubation at 30°C for 48 hours resulted in development of yellow-brown colour around bacterial colonies which indicated a positive result for peroxidase activity.



(i) Control (ii) DMRX-2156 (iii) DMRX-2157 (iv) DMRX-2160

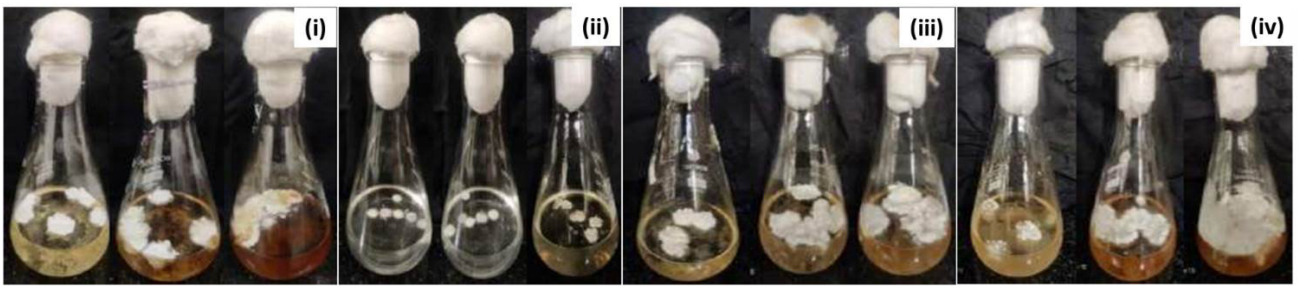
Fig. 4. Development of brownish yellow colour around the *Schizophyllum* strains after adding the mixture of 1% pyrogallol and 4% hydrogen peroxide

Different from the above method, Koyani *et al.* (2014) and Thiribhuvanamala *et al.* (2020) investigated the peroxidase enzyme production of *S. commune* by plate decolorization assay on solid media supplemented with substrates *viz*., magenta textile dyes and Azure B, respectively. Absolute decolorization of the textile dye and faint decolourisation of Azure B after 12 days of fungal inoculation indicated the peroxidase activity of *S. commune*.

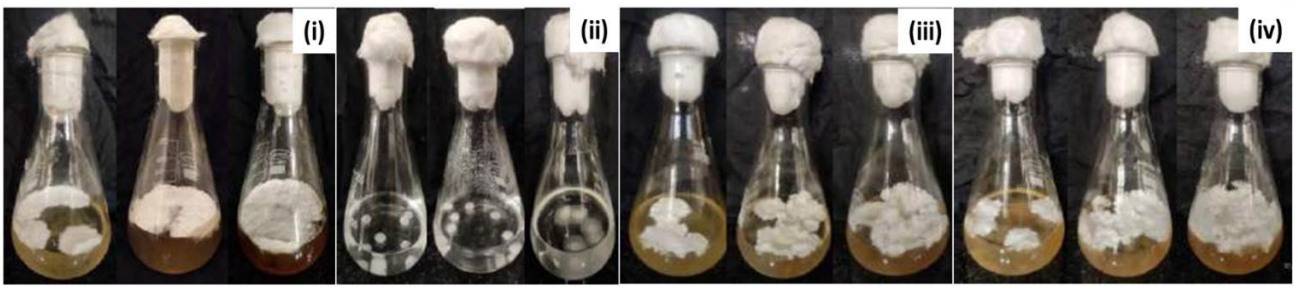
Submerged cultivation in different media

Submerged cultivation of *Schizophyllum* strains was studied in four different liquid media since the production rates of hydrolytic enzymes varies with composition of different media (Fig 5). Among the media tested, MVM could not support significant mycelial growth of *Schizophyllum* strains due to the presence of high levels of citrate which acted as a chelator and made the concentration of calcium and trace elements uncertain. Moreover, the high coefficient for the pKa values of citrate made the pH of media sensitive to ionic strength. In the other media, mycelial biomass rapidly increased from 3 to 13 DAI with dark coloration in the broth. The mycelial growth decreased at 15 DAI followed by pinhead emergence and fruiting body formation.

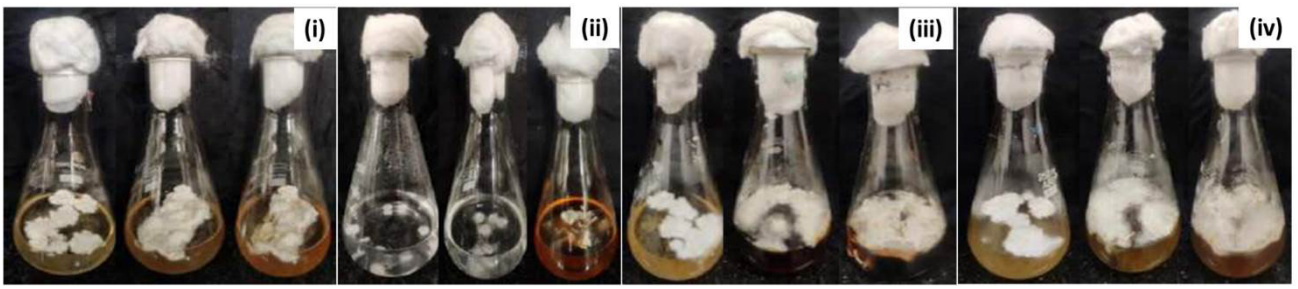
The result of present study was in accordance with the findings of Bolla *et al*. (2008) who reported



a. DMRX- 2156 in (i) SBM at 5, 10, 15 DAI (ii) MVM at 5, 10, 15 DAI (iii) PDB at 5, 10, 15 DAI (iv) PMPB at 5, 10, 15 DAI



b. DMRX- 2157 in (i) SBM at 5, 10, 15 DAI (ii) MVM at 5, 10, 15 DAI (iii) PDB at 5, 10, 15 DAI (iv) PMPB at 5, 10, 15 DAI



c. DMRX- 2160 in (i) SBM at 5, 10, 15 DAI (ii) MVM at 5, 10, 15 DAI (iii) PDB at 5, 10, 15 DAI (iv) PMPB at 5, 10, 15 DAI

Fig. 5. Submerged culture of *Schizophyllum* strains in different media at different DAI

the highest mycelial growth of *S. commune* at 14 DAI in the basal medium. Rangkhawong *et al.* (2014) reported that during the submerged culture of *S. commune* in PDB the mycelial growth and biomass increased rapidly during 3 to 9 days of fermentation, and it slightly decreased after 15 days which was the end of fermentation. Dulay *et al.* (2016) reported that rice bran broth supported the efficient mycelial growth of *S. commune* with the maximum yield at 12 DAI. On the contrary, Pandee *et al*. (2008) reported the maximum growth of *S. commune* BL23 mycelia during the submerged culture in peptone yeast extract glucose medium (PYGM) after 7 days of cultivation and thereafter, the fungal biomass remained nearly constant.

Estimation of laccase and peroxidase activity from mycelia and broth

Observations of the present study correlates with the finding of Bose *et al.* (2007) who detected laccase enzyme in the culture filtrate of white-rot fungus *Termitomyces clypeatus* at the late phase of submerged growth. They reported that laccase produced by *T. clypeatus* is an intracellular enzyme and is released in the medium due to cell lysis at the end of the growing phase. The specific activity of intracellular laccase decreased from 10 DAI along with the appearance of extracellular laccase activity in the culture filtrate. Similar observation was recorded by Vanitha and Umamaheswari, (2016) when they evaluated the intracellular and extracellular laccase activity of *S. commune* under liquid-state fermentation. The extracellular laccase showed a maximum activity of 33 U/ml at 10 DAI, when compared to that of the intracellular laccase which showed a maximum activity of 40 U/ml at 8 DAI and then lowered.

Kaur *et al*. (2023) studied the effect of incubation period on intra-extracellular enzyme activities based on mycelium and liquid broth of *Pleurotus florida*. They observed the highest intracellular laccase (10.23 IU/mg) and manganese peroxidase (9.98 IU/mg) activity at 14 DAI. Similar to the current study, the intracellular enzyme activities decreased with storage period due to the oxidative enzymes produced by the fungi during secondary metabolism, induced by nutritional deprivation (Sousa *et al.*, 2019). They reported the highest extracellular enzyme activity of laccase (15.43 IU/ml), lignin peroxidase (11.21 IU/ml) and manganese peroxidase (5.24 IU/ml), was found on the 14th day of submerged cultivation similar to the findings of the present study.

Different from the results of present study, Adejoye and Fasidi, (2010) obtained an optimal higher laccase activity of 51.5±2.12 U/min with highest mycelial biomass yield from 7 days old culture of *S. commune* in a chemically defined medium at a temperature of 28^{0}C; however, the laccase activity in the media decreased at 14 DAI. Kumar *et al.* (2018) observed that extracellular laccase activity of *S. commune* in liquid basal media increased from 2 DAI and recorded the highest activity at 7 DAI (738 UmL^{-1}). However, the laccase activity significantly decreased from 8 DAI and neither LiP nor MnP activities were detected during the entire ten days of submerged cultivation. Thiribhuvanamala *et al.* (2020) reported that liquid culture of *S. commune* secreted maximum laccase (0.59 U/ml) at 12 DAI while the maximum LiP (0.054 U/ml) and MnP (0.204 U/ml) activities were recorded at 7 and 8 DAI, respectively.

Lu *et al*. (2023) reported an increase in laccase activity of *S. commune* with increase of culture time in liquid media and the enzyme activity reached a maximum of 33.89 U/ml at 7 DAI. After seventh day, the enzyme activity began to decrease which is substantiated as the depletion of nutrients in the culture medium that reduced the enzyme producing ability of hyphae. Contrary to the findings of present study, they reported that during the fermentation process, neither

Table 1. Intracellular laccase activity of *Schizophyllum* strains in different liquid media at different DAI

Media	Day after inoculation	DMRX-2156	DMRX-2157	DMRX-2160	CD (0.05)	SEm±
SBM	5 DAI	11.37±2.27^{c}	10.39±0.93^{c}	15.21±2.32^{c}	6.02	2.03
	10 DAI	4.45±1.48^{c}	5.44±0.72^{cd}	7.79±1.11^{bc}	2.99	1.01
	15 DAI	2.72±1.13^{c}	2.66±0.46^{c}	4.08±1.7^{abc}	2.16	0.73
PDB	5 DAI	21.32±4.34^{b}	27.68 ±3.14^{a}	13.12±6.16^{c}	6.02	2.03
	10 DAI	6.92±1.71^{bcd}	11.99±2.04^{a}	9.73±2.62^{ab}	2.99	1.01
	15 DAI	2.63±0.86^{c}	4.97±2.73^{ab}	4.42±0.00^{abc}	2.16	0.73
PMPB	5 DAI	30.55±3.66^{a}	9.89±1.34^{c}	25.63±4.21^{ab}	6.02	2.03
	10 DAI	12.67±2.09^{a}	5.15±1.26^{cd}	5.63±1.88^{cd}	2.99	1.01
	15 DAI	6.12±0.61^{a}	3.35±0.10^{bc}	3.02±1.16^{bc}	2.16	0.73

*Values are mean ± SD of three replications. Means of treatments (AxB) followed by similar superscripts are not significantly different at 5% level.

Table 2. Extracellular laccase activity of *Schizophyllum* strains in different liquid media at different DAI

Media	Day after inoculation	DMRX-2156	DMRX-2157	DMRX-2160	CD (0.05)	SEm±
SBM	5 DAI	1.67±0.19	1.45±0.46	1.77±0.50	NS	0.2
	10 DAI	10.38±0.74b	8.57±0.91c	15.58±0.74a	1.09	0.37
	15 DAI	3.96±1.13	3.52±0.83	5.19±0.74	NS	0.37
PDB	5 DAI	0.96±0.22	1.07±0.38	1.08±0.23	NS	0.2
	10 DAI	1.63±0.26f	1.57±0.32f	3.09±0.21de	1.09	0.37
	15 DAI	1.16±0.04	1.54±0.11	1.67±0.49	NS	0.37
PMPB	5 DAI	1.71±0.22	1.48±0.37	1.85±0.37	NS	0.2
	10 DAI	3.85±0.96d	2.32±0.08ef	11.12±0.74b	1.09	0.37
	15 DAI	2.32±0.56	1.85±0.37	4.45±0.74	NS	0.37

*Values are mean ± SD of three replications. Means of treatments (AxB) followed by similar superscripts are not significantly different at 5% level.

Table 3. Intracellular peroxidase activity of *Schizophyllum* strains in different liquid media at different DAI

Media	Day after inoculation	DMRX-2156	DMRX-2157	DMRX-2160	CD(0.05)	SEm±
SBM	5 DAI	40.08±4.01^{de}	39.61±0.67^{de}	41.42±6.12^{de}	51.82	17.44
	10 DAI	13.36±2.67^{e}	19.30±1.45^{e}	20.04±4.01^{e}	12.16	4.09
	15 DAI	4.90±2.04^{e}	5.84±1.44^{e}	8.02±4.01^{e}	8.04	2.70
PDB	5 DAI	242.34±36.66^{a}	138.81±60.16^{bc}	72.11±4.00^{de}	51.82	17.44
	10 DAI	116.49±5.25^{a}	90.85±5.34^{b}	62.72±9.15^{c}	12.16	4.09
	15 DAI	85.85±9.39^{a}	49.31±3.15^{bc}	41.68±3.11^{c}	8.04	2.70
PMPB	5 DAI	168.45±35.21^{b}	87.60±43.80^{cd}	21.53±4.64^{e}	51.82	17.44
	10 DAI	113.13±9.11^{a}	47.01±14.25^{d}	18.06±1.12^{e}	12.16	4.09
	15 DAI	50.36±7.81^{b}	26.32±2.04^{d}	4.62±1.54^{e}	8.04	2.70

Values are mean ± SD of three replications. Means of treatments (AxB) followed by similar superscripts are not significantly different at 5% level.

Table 4. Extracellular peroxidase activity of *Schizophyllum* strains in different liquid media at different DAI

Media	Day after inoculation	DMRX-2156	DMRX-2157	DMRX-2160	CD(0.05)	SEm±
SBM	5 DAI	5.94±0.53	4.84±0.43	6.01±0.67	NS	1.17
	10 DAI	7.70±2.22 ^{de}	7.30±1.03 ^{de}	8.73±1.08 ^{de}	3.59	1.21
	15 DAI	18.70±1.34^{de}	17.61±1.25^{e}	28.95±2.04^{c}	4.28	1.44
PDB	5 DAI	6.12±1.17	6.46±2.24	7.61±0.70	NS	1.17
	10 DAI	6.68±1.16^{e}	9.04±4.21^{de}	10.73±2.35^{a}	3.59	1.21
	15 DAI	22.89±5.13^{d}	18.48±1.93^{e}	27.27±2.61^{c}	4.28	1.44
PMPB	5 DAI	11.98±1.21	9.75±4.27	12.47±3.08	NS	1.17
	10 DAI	24.05±1.33^{b}	15.41±2.00^{c}	33.40±1.34^{a}	3.59	1.21
	15 DAI	31.17±2.04^{c}	46.73±2.36^{b}	52.10±1.34^{a}	4.28	1.44

*Values are mean ± SD of three replications. Means of treatments (AxB) followed by similar superscripts are not significantly different at 5% level.

MnP nor LiP was detected indicating that the growth of *Schizophyllum* hyphae mainly relied on degradation by laccase enzyme.

CONCLUSION

DMRX-2160 was identified as the best *S. commune* strain, in terms of maximum extracellular enzyme activities during submerged cultivation. The maximum intracellular activity of *Schizophyllum* strains obtained at 5 DAI declined towards 15 DAI whereas, extracellular activities increased from 5 DAI and reached the maximum at 15 DAI. Among the media tested, SBM supported maximum secretion of laccase however, PMPB supported peroxidase.

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