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Research Article

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PRODUCTION OF LACCASE ENZYME BY WHITE ROT FUNGI *CORIOLUS VERSICOLOR*

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Abstract

White rot fungi such as *Coriolus versicolor* is known producer of lignolytic enzymes that are involved in the natural delignification of wood. Thirteen fungal samples were isolated from soil samples by serial dilution of 1 gm soil in 10 mL water. Dilutions of sample ($10^{-5}$and 10^{-6}) were prepared and transferred 0.1 mL of each dilution into malt extract agar medium plates supplemented with streptomycin and incubated at 35^0C for 3-5 days and named as F_2-F_{14}. Out of 13 samples one strain F_3 from soil samples compared with F_1 from rotted wood was selected for further studies. After three days selected fungal strains for ligninolytic activity were observed for the appearance of brown, yellow and green colours respectively. Ligninolytic activities of the selected fungal strains were observed positive in F_2, F_3, F_5, F_6, F_7 and F_{14}, whereas culture no. F_4 and F_{10} showed negative results. The maximum and minimum diameter of fungal colony was recorded as 9.4 cm and 2.3 cm respectively, in F1 strain. The study revealed that maximum laccase activity was observed after 16 days of incubation period in $CuSO_4$ whereas in veratryl alcohol that was recorded after 10 days of incubation periods, whereas minimum laccase activity was recorded in $CuSO_4$ after 4 days of incubation period whereas in veratryl alcohol that was recorded after 16 day of incubation period.

Keywords: *Coriolus versicolor*, laccase enzyme, ligninolytic enzymes, malt extract agar medium, $CuSO_4$ and veratrly alcohol.

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Introduction

Laccase is a multicopper blue oxidase which have been found to be widely distributed among plants where they are involved in the synthesis of lignin and in the wounding response. It was thought that only the ligninolytic system of some white rot fungi capable of degrading this recalcitrant polymer to a major extinct involved lignin peroxide and manganese peroxidase (Rogalski *et al*, 1991). Laccase polymerize lignin by coupling of the phenoxy redicle produced from oxidation of lignin phenolic groups. Recently, most of the laccase studied are of fungal origin, especially from white-rot fungi, *Anthracophyllum discolor*, *Coriolus versicolor*, *Pycnoporus sanguineus*, *Trichoderma harzianum*etc, *Phlebia radiata*, *Pleurotus ostreatus* and *Trametes versicolor* (Bourbonnais *et al*, 1995; Muñoz, 1997; Palmieri *et al*, 2000; Arora and Gill, 2000; Acevedo, 2011). Laccases have also been found in various basidiomycetous and ascomycetous fungi (Singh *et al*, 2015a) and thus far fungal laccases have accounted for the most important group of multicopper oxidases (MCOs) with respect to number and extent of characterization (Giardin *et at*, 2010). Laccase was also produced earlier when the fungus was cultivated in a substrate with a high N_2 concentration and these changes did not reflect differences in biomass. (Singh and Charaya, 2010).

Laccase activity was detected in the cultures of a wide range of fungi, from Ascomycetes to Basidiomycetes (Pela´ez *et al*, 1995). One of the major limitations for large scale application of fungal laccases is the low production rates by both wild type and recombinant fungal strains. In this research the production of laccase is determined in the white rot fungi *Coriolus versicolor*.

MATERIAL AND METHODS

Isolation of ligninolytic strains from soil samples:

Thirteen fungal samples were isolated from soil by serial dilution of 1 gm soil in 10 mL water. 10^{-5} and 10^{-6} dilutions were prepared and transferred 0.1 mL of each dilution into malt extract agar medium plates supplemented with streptomycin and incubated at 35^0C for 3-5 days in incubator and named as F_2-F_{14}.

Isolation of ligninolytic strains from rotted woods:

Isolation of fungi from rotted wood was done by removing upper surface and picking the pieces of rotted wood. These were then placed on Potato Dextrose Agar (PDA) plates and further incubated at 35^0C in incubator for 2-3 days and named as F_1.

Screening for ligninolytic enzyme production:

Malt Extract Agar (MEA) was supplemented with Gallic acid (0.01% w/v) to test for polyphenol oxidase enzyme; phenol red (0.01% w/v) for manganese peroxidase enzyme and about 2 µL, A.B.T.S. {2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)} for laccase enzyme. A piece of about 5 mm diameter agar disc of 3-4 days old fungus culture was placed on each selective medium and incubated at 35^0C in dark for three days. The ligninolytic activity was selected by development of colour around the mycelium.

Determination of maximum temperature for maximum fungal growth:

After 24 and 48 hours of incubation period, 5 fresh fungal discs were cut with the help of 5 mm cork borer and inoculated on 5 fresh MEA plates and incubated at 28^0C and 34^0C .Ten fungal hyphal discs were inoculated into 100 mL flasks of malt extract broth medium and pH was adjusted from 4.0 to 6.0 and incubated at optimum temperature for 8 and 10 days. After 10 days, the cultures were harvested and growth was determined by mycelia dry weight method and then laccase assay was estimated.

Effect of inducers on the growth and laccase production by selected fungal strains:

Two inducers, $CuSO_4$ suspension (500 µL) and veratryl alcohol (15mM) were used. Three sets of 100 mL MEB flasks were prepared. First set of MEB flasks was supplemented with $CuSO_4$ and second with veratryl alcohol, whereas third was kept as control. The fungal discs were placed onto first and second set of flasks. All the flasks were incubated at optimum temperature and screened after 4, 10, and 16 days.

RESULTS AND DISCUSSION:-

Screening for ligninolytic enzyme production:

After three days selected fungal strains for ligninolytic activity were observed for the appearance of brown, yellow and green colours respectively. Ligninolytic activities of the selected fungal strains were observed positive in F_2, F_3, F_5, F_6, F_7 and F_{14} for all three ligninolytic enzymes (poly-phenol peroxidase, manganese peroxidase and laccase), whereas culture no. F_4 and F_{10} showed negative results for all (Table 1).

Determination of maximum temperature for maximum fungal growth:

After 48 hours of incubation, the maximum diameter of fungal colony was recorded as 9.4 cm in F_1 strain, whereas 2.3 cm and 2.9 cm in F_3 strain at 28ºC and 34º C respectively. After 24 hours of incubation period, the minimum diameter of fungal colony was observed 1.0 cm and 1.25 cm at 28ºC and 34ºC temperature respectively, in F_3 strain; whereas 3.8 cm and 4.5 cm fungal diameter was observed after 48 hours of incubation period, at 28ºC and 34º C respectively in F_1 strain (Table 2).

Table 1: **Screening of isolated fungal strains for lignin degrading enzyme production.**

No. of culture	MEA+ Gallic acid	MEA+ Phenol red	MEA+ A.B.T.S.
F_1	-	+	+
F_2	+	+	+
F_3	+	+	+
F_4	-	-	-
F_5	+	+	+
F_6	+	+	+
F_7	+	+	+
F_8	-	-	+
F_9	-	+	+
F_{10}	-	-	-
F_{11}	-	+	+
F_{12}	+	-	+
F_{13}	-	+	+
F_{14}	+	+	+

Table 2: Effect of temperature on growth of selected fungal strains:

Temperature (^{0}C)	Incubation period (in hours)	Diameter of colony (in cm)	
		F_1	F_3
28	24	3.8	1.0
	48	9.4	2.3
34	24	4.5	1.25
	48	9.4	2.9

Maximum biomass was recorded as 0.370 gm and 0.397 after 16 days of incubation period in $CuSO_4$ and veratryl alcohol respectively, whereas minimum biomass as 0.108 gm and 0.106 gm after 4 days of incubation period in $CuSO_4$ and veratryl alcohol respectively. Maximum laccase activity (176.66 IU/mL) was observed after 16 days of incubation period in $CuSO_4$, whereas in veratryl alcohol it was recorded (46.23 IU/mL) after 10 days of incubation period. Minimum laccase activity was observed as 42.8 IU/mL in $CuSO_4$ after 4 days of incubation period, whereas in veratryl alcohol it was observed as 19.83 IU/mL after 16 day of incubation period (Table 3).

Table 3: Comparative study of effect of inducers on biomass production and Laccase production by selected fungal strains:

Inducers used	Incubation period (in days)	Final pH	Biomass (in gm)	Laccase activity (IU/mL)
Control	4	4.32	0.117	28.58
	10	4.80	0.273	70.15
	16	4.68	0.366	15.22
$CuSO_4$	4	4.14	0.108	42.8
	10	4.87	0.334	175
	16	4.67	0.370	176.66
Veratryl Alcohol	4	4.22	0.106	40.12
	10	4.85	0.306	46.23
	16	4.49	0.397	19.83

Laccase enzyme is known to play an important role in delignification of wood and this enzyme is produced by white rot fungi (Nagadesi *et al*, 2013; Singh *et al*, 2015a and Singh *et al*, 2015b). Laccase may be applied to degrade various substances like undesirable contaminants, byproducts or discarded materials and plastic wastes (Raaman *et al*., 2012). Laccase may be used as O_2 scavenger for improving food quality and packaging (Johansson *et al*., 2012).

Conclusion

Laccase enzyme can be used in food and related industries to improve quality of the products. In present study white rot fungi *Coriolus versicolor* was used to test its ability to produce laccase enzyme. The study revealed that the fungi showed maximum and efficient laccase enzyme activity in media supplemented with $CuSO_4$. This approach may be applied to maximize laccase production commercially.

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