Journal Homepage: - www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI: 10.21474/IJAR01/4337
DOI URL: http://dx.doi.org/10.21474/IJAR01/4337
ISSN NO. 2320-5407

RESEARCH ARTICLE

GANODERMA LUCIDUM: A POTENT SOURCE OF LIGNINOLYTIC ENZYME PRODUCTION.

Nikki Agrawal, Preeti Verma, Ravi Shankar Singh and Sushil Kumar Shahi*
Department of Botany, Guru Ghasidas Vishwavidyalaya, Bilaspur, Chhattisgarh 495009, India

Manuscript Info

Manuscript History

Received: 16 March 2017
Final Accepted: 18 April 2017
Published: May 2017

Key words:-
Ganoderma lucidum, Laccase, Lignin peroxidase, Manganese peroxidase.

Abstract

White rot fungi secrete extracellular ligninolytic enzymes, laccases, Lignin peroxidase (LiP), Manganese peroxidase (MnP), which is involved in the process of ligninolysis and organic pollutant degradation. The aim of the present study to investigate the ligninolytic enzyme production by white rot fungi *Ganoderma lucidum* strain CCG1, isolated from the from the Janjgir Champa district of Chhattisgarh, India. The ligninolytic enzyme production by *G. lucidum* was investigated in mineral salt broth, 1154 U/L laccase, 1175 U/L LiP and 15035 U/L MnP production was investigated. So we can use the enzymatic property of *G. lucidum* in the various applications like degradation, dye decolourization, delignification etc.

Introduction:-

Laccase, LiP and MnP are the major enzymes of the ligninolytic systems of white rot fungi, which are involved in the lignin and organic pollutant degradation (Arora and Gill, 2001; Casas et al., 2009), dye decolourization (Dominguez et al., 2005), food processing (Couto and Herrera, 2006), biopulping and biobleaching (Bermek et al., 2002), stabilization of juice and beverages (Minussi et al., 2002) oxidation of phenolic and non-phenolic compounds (Cantarelli et al., 1989; Tien and Kirt, 1983; Hammel et al., 1993; Wong, 2009).

Laccase is a multicopper containing enzyme (EC 1.10.3.2: benzenediol, oxygen oxidoreductase or p-diphenol oxidase), and LiP (EC 1.11.1.14, 1, 2-bis (3, 4-dimethoxyphenyl) propane-1, 3-diol: hydrogen-peroxide oxidoreductase), catalyze the oxidation of aromatic and non-aromatic substrates (Claus, 2004). MnP (EC 1.11.1.13 Mn (II): hydrogen-peroxide oxidoreductase) catalyze the Mn dependent reaction (Orth and Tien, 1995). Ligninolytic enzyme production occurs during the secondary metabolism and its production triggered by the carbon, sulfur and nitrogen limiting condition (Heinzkill et al., 1998).

In this study the production of ligninolytic enzymes was investigated in the isolated *G. lucidum* strain CCG1, for their further application in the degradation process.

Materials and Methods:-

Chemicals:-

Guaiacol, Azure B, 2, 6 - dimethoxy phenol (2, 6 - DMP) was purchased from Himedia, India.

Corresponding Author:- Sushil Kumar Shahi.
Address:- Department of Botany, Guru Ghasidas Vishwavidyalaya, Bilaspur, Chhattisgarh 495009, India.

Microorganism and growth Condition:-
A white rot fungi strain CCG1, was found on the decayed wood surface, was collected from the Janjgir Champa district of (Latitude 21º 58' 13.9908", Longitude 82º 28' 30.9936" and Elevation 258.7 meters) Chhattisgarh, India, fungi were isolated from the fruiting body by the spore drop method according to Choi et al. (1999). A piece of cap tissue was cut from the fruiting body and transferred in the top of a petri dish containing Sabouraud Dextrose Agar (SDA) media composition (g/L): Dextrose (40), Peptone (10), Agar (15) and streptomycin (500 mg/L) antibiotic to inhibit the bacterial growth). After the pure culture was obtained, culture was maintained in SDA media and stored at 4ºC. On the basis of morphological identification and molecular characterization through 18S rRNA, it was found that strain CCG1 was identified as *Ganoderma lucidum* (Accession no. KY464926).

Qualitative screening for ligninolytic enzyme:-
For the screening of ligninolytic enzyme production in *G. lucidum*, different indicator compounds were used, and the method of enzyme production is as follows:

Laccase enzyme Activity:-
For the investigation of laccase assay 6 mm diameter one fungal disc of strain CCG1 was taken from the periphery of the seven days old culture grown in SDA media, then transferred on the petri dish containing 25 mL of Potato dextrose agar media (PDA g/L: Potato infusion - 200, Dextrose - 20, Agar - 20) with 0.01% Guaiacol (D'Souza et al., 2006). After that Plates were incubated at 27°C for 7 days in a static incubator and the colour change in the media was investigated.

Lignin peroxidase enzyme Activity:-
Lignin Peroxidase screening medium (g/L: Glucose - 4.0, Glycerol - 0.7, L histidine - 0.05, $CuSO_4$ - 0.01, $NaNO_3$ - 0.18, NaCl - 0.18, KCl - 0.05, $CaCl_2.H_2O$ - 0.05, KH_2PO_4 - 0.1, $FeSO_4.H_2O$ - 0.005, $MgSO_4.7H_2O$ - 0.05, Guaiacol – 10 mm (v/v), H_2O_2 – 10 mm, Agar - 2.0) was used for the lignin peroxidase enzyme assay. 6 mm diameter of one fungal disc was transferred in the LiP screening medium, then incubated for 7 days and the colour change in the screening medium was analyzed (Atalla et al., 2010; Sivakami et al., 2012).

Manganese peroxidase enzyme Activity:-
To investigate MnP enzyme activity Czapek-Dox agar medium containing 0.0025% phenol red (w/v) was employed (Kuwahara et al., 1984; Ali et al., 2012). Fungal strain CCG1 (6 mm diameter one fungal disc) was inoculated in the Czapek-Dox agar medium at 27°C for 7 days and colour zone produced in the screening medium was analyzed.

Quantitative estimation of ligninolytic Enzyme Production:-
To estimate the enzyme production in *P. elegans*, 8 mm fungal mycelium disc was transferred in the 20 ml mineral salt broth (composition g/L: Glucose – 10, KH_2PO_4 - 2, $MgSO_4.7H_2O$ – 0.5, $CaCl_2.2H_2O$ – 0.1, Ammonium tartrate – 0.2 and Trace element solution – 10 (mL). A trace element solution comprised of (in mg/L) $FeSO_4.7H_2O$ (12), $MnSO_4.7H_2O$ (3), $ZnSO_4.7H_2O$ (3), $CoSO_4.7H_2O$ (1), $(NH4)_6Mo_7O_{24}.4H_2O$ (1) (Hadibarata and Kristanti, 2012). Then incubate the culture at 27ºC in a rotatory shaker incubator for 2, 5, 10, 15, 20, 25 and 30 days, after that enzyme production was analyzed. All the experiments were performed in triplicates. The production of ligninolytic enzyme was investigated according to the following procedure:

Laccase Assay:-
The laccase enzyme production was investigated according to Sandhu and Arora (1985), take 3 mL of reaction mixture containing 0.5 mL of the enzyme extract, 1.5 mL sodium acetate buffer (10 mM, pH 5.0) and 1 mL guaiacol (2 mM), then incubated for 2 h and absorbance read at 450 nm. The laccase enzyme activity has been expressed in international units per litre of enzyme extract (U/L).

Lignin Peroxidase Assay:-
To investigate the LiP enzyme production, take 0.5 mL of the culture filtrate, 1 mL of 125 mM sodium tartrate buffer (pH 3.0), 0.5 mL of 0.16 mM azure B, then add 0.5 mL of 2 mM hydrogen peroxide, after addition of hydrogen peroxide, the reaction was initiated. One unit of enzyme activity was expressed as an O.D. decrease at 651 nm of 0.1 units per minute per litre of the culture filtrate (Archibald, 1992).

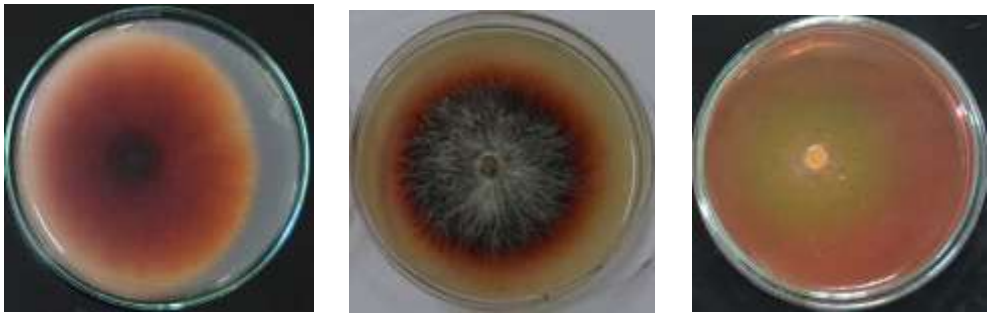
Manganese peroxidase Assay:-
According to de Jong et al. (1992), MnP activity was accessed on the basis of oxidation of 2, 6 - DMP at 468 nm. Take 3 mL of reaction mixture contained 0.5 mL culture filtrate, 1 mL of sodium tartrate buffer (50 mM, pH 4.0) and 1 mL of 2 mM 2, 6-DMP. The reaction was started by the addition of 0.5 mL of 0.4 mM hydrogen peroxide.

Results and Discussion:-

Qualitative screening for the ligninolytic enzyme Activity:-
Laccase assay – After the qualitative screening it was investigated that guaiacol oxidize by the produced laccase enzyme by *G. lucidum*, and 80.00 mm diameter intense red colour zone was appeared (Fig. 1 a). According to Kiiskinen et al., 2004, guaiacol is a chromogenic compound, on the basis of their oxidation reaction it is used for the screening of the production of laccase enzyme. Similar experiment was performed by Atalla et al., (2010), and found that fungi *Pleurotus ostreatus* and *Trematosphaeria mangrovei* showed 32.00 mm reddish brown colour zone due to production of laccase.

LiP assay – LiP screening medium used for the LiP enzyme assay, in the LiP screening medium, guaiacol oxidized by the LiP enzyme in the presence of hydrogen peroxide, and 70.00 mm diameter brick red colour zone (Fig. 1 b) appeared around the mycelium.

MnP assay –Due to the oxidation of phenol red by MnP enzyme, the formation of 40.00 mm yellow colour zone was investigated during the MnP enzyme assay (Fig. 1 c).



a b c

Fig. 1:- Qualitative screening for ligninolytic enzyme in *G. lucidum*, a - Laccase (red colour zone), b - Lignin peroxidase (reddish brown zone), and c - Manganese peroxidase (yellow colour zone) production by *G. lucidum*.

Production of ligninolytic Enzyme:-
The ligninolytic enzyme production was investigated in the fungal strain CCG1 after 2, 5, 10, 15, 20, 25 and 30 days of incubation in mineral salt broth, the ligninolytic enzyme production increases in the fungal strain CCG1 with the incubation period.

Laccase enzyme production:-
The production of the maximum laccase enzyme was found after 15 days of incubation, at 15th day 1154 U/L laccase enzyme production was investigated (Fig. 2), and then the production of enzyme decreased and after 30 days 184.9 U/L laccase enzyme production was found. The maximum 11.72 U/mg Specific laccase activity was investigated after 25 days of incubation (Fig. 2).

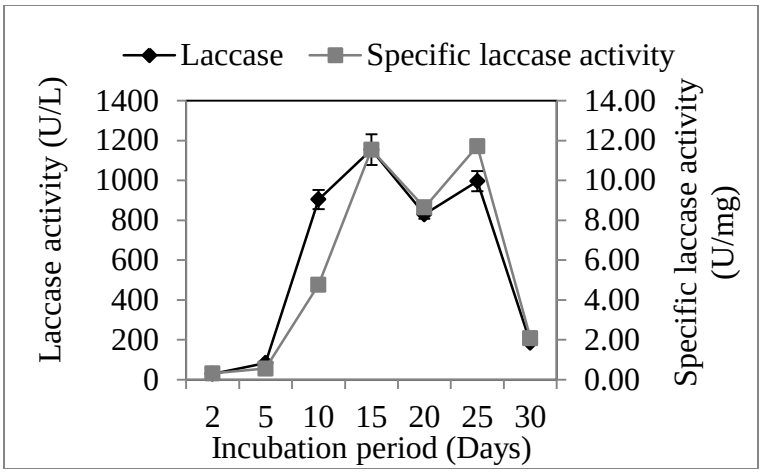


Fig. 2. Laccase enzyme production by *G. lucidum*.

LiP enzyme production:-

LiP enzyme production was investigated on the basis of the oxidation of Azure B by the LiP enzyme in the presence of hydrogen peroxidase, it was found that after 15th day of incubation maximum 1175 U/L LiP enzyme activity was found, and 11.75 U/mg Specific LiP activity was investigated (Fig. 3).

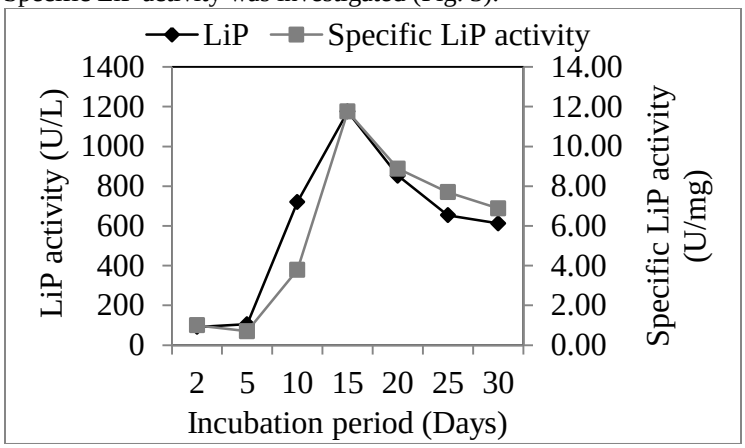


Fig. 3:- LiP enzyme production by *G. lucidum*.

MnP enzyme production:-

The MnP enzyme activity was determined by the oxidation of 2, 6 DMP by MnP. The maximum 15035 U/L MnP, 150.35 U/mg Specific MnP activity was determined after 15th day of incubation (Fig. 4).

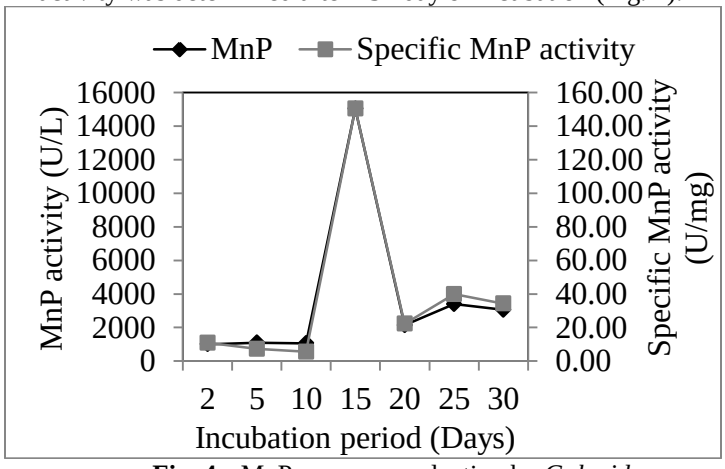


Fig. 4:- MnP enzyme production by *G. lucidum*.

Conclusions:-

In this study it was found that *G. lucidum* produced effective amount of ligninolytic enzyme, therefore, it can be used for the organic pollutant degradation study after successful investigation.

Acknowledgements:-

The authors are thankful to Mr. Anand Barapatre, SRF (Department of Occupational Health) JNARDDC Campus, Nagpur to improve my research work. The authors are also thankful to
Head, Department of Botany, Guru Ghasidas Vishwavidyalaya, providing infrastructural facilities. Also, thank to Guru GhasidasVishwavidyalaya, Bilaspur (C.G.) for providing financial assistance.

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