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ORIGINAL RESEARCH PAPER (Short Communication)

Ganoderma lucidum isozymes and basidiocarp formation using rice straw: Commercial prospects with environmental applications

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Abstract

Technology Area

This technology falls under Functional Foods & Nutraceuticals specifically focusing on enhanced enzyme and basidiocarp production.

Technology

The present technology relates to the optimized cultivation of Ganoderma lucidum using agro-residues for enhanced basidiocarp production and laccase enzyme yield. The process involves: Utilization of agricultural waste substrates (e.g., sawdust and wheat bran in 4:1 ratio, w/w) Optimization of physicochemical parameters such as pH and temperature (optimal at ~30°C) Supplementation with additives such as gypsum and calcium carbonate ($CaCO_3$) to improve yield and reduce contamination

Potential Market / Customers

Nutraceutical and pharmaceutical industries (immune-boosting mushroom products), Functional food manufacturers, Ayurvedic and herbal product companies, Enzyme production industries (laccase for: Bioremediation, Textile processing, Paper & pulp industries)

Commercial Potential

High demand for medicinal mushrooms like Ganoderma in global health markets, ncreasing market for industrial enzymes, especially laccases, Scalable and suitable for small-scale to industrial-level production Utilizes low-cost agro-waste, reducing production cost significantly

Keywords Basidiocarp; Laccase isozymes; Ganoderma; Enzyme

Introduction

Ganoderma belongs to an order polporales mushrooms that grow on wood. This include about more than 80 species and mostly tropical regions. They can be differentiated from other polypores because they are double walled basidiospore, referred as bracket fungi (Karunarathna et. al. 2025).

The name *Ganoderma* is derived from the Greek "ganos" 'brightness sheen' "derma" 'skin' erected as genus in 1881 by Karsten and this included in only one species that was *Ganoderma lucidum* (Curtis) Karst.

Ganoderma basidiocarps are characterized as perennial, large, woody brackets and also called as "conks". They are either leathery and lignicolous with or without a stem. These fruiting bodies typically grow in a hoof like or fan like form on the trunks of living or dead trees. They have double walled, spores with yellow color inner layer.

In China and Korea, it is known as *Ling-zhi* and it grows on woody substrates. Whereas, the Japanese call this mushroom as *Reishi* based on their color. Till now six different coloured varieties of *G. lucidum* are known (Table1). These are characterized as; non-poisonous and edible, which are beneficial to health. These are neutral in taste and warm also (Chang and Buswell, 1999)

G. lucidum has been recognized as a medicinal mushroom for over 2000 years and its powerful effects was documented in ancient scripts Its unique properties is to strengthened the immune system (Wasser, 2002). It is a very important and popular remedy which helps to treat in different conditions like chronic hepatitis, hypertension, cancer, low blood pressure, high blood pressure, diabetes, heart problems, paralysis, ulcers, arthritis, tiredness, hepatitis A, B, and C, sterility, psoriasis, epilepsy and alcoholism (Zang, 1999). It refreshes the body and mind, delays ageing and this is effective for diabetic patients because in case of diabetic patient's blood pressure was reduced after two months of treatment. In India the extract of this fungus was used for the treatment of joint pain (Harsh et al., 1993).

Ganoderma lucidum is used in higher amount as a medicinal herb. A different type of *Ganoderma* species needs different conditions for their proper growth and cultivation (Mizuno et al., 1997). Different types of *Ganoderma* species are favoured in different geographical region for example black *G.* is popular in south China. *G. lucidum* becomes a major source of mushroom and this has been achieved by using different types of substrates such as sawdust (wood logs, wheat brand, grains, rice straw) (Chang and Buswell, 1999; Wasser, 2014; Boh et al.,2007).It takes several months for cultivation and production of the fruiting body. In whole process different processes and different parameters are used for *Ganoderma* production and different type of culture conditions and medium compositions are used for mycelia growth and biopolymers production (Yang and Liau,1998) reported that *Ganoderma lucidium* grown at optimum temperature at 30ºC-35ºC and pH is 4-4.5. Recently, in different industries like herbal industry and consumer industries genuinely demand for

those type of *Ganoderma* which are free of contaminated microbes and other harmful chemicals. Due to commercial medicinal importance, its cultivation is very high and the globe rate is approx $ 4.0 billion. Currently, approximately 110,000tonnes has been produced in China, followed by South Korea (4,500 tons) and Japan (1,500 tons) (Wu et. al.,2024). The price of *Ganoderma* is very high in international market price which was approximately 22-30 US$/kg. Its fruiting body sold in market approximately @Rs 600-700/kg (Perumal, 2009). Trading of *Ganoderma* is growing fastly in various countries including India. *Ganoderma* poses some contamination so to overcome those problems, the various biochemical and molecular methods are used. Availability of *Ganoderma* takes place in various type of regions like tropical, temperate and subtropical region (Hapuarachchi et. al. 2018). Here, we had reported the Production of basidiocarp from *Ganoderma lucidum* MDU-7 using different agricultural waste. Also, comparison of laccase isozymes from basidiocarp and mycelium was done.

Materials and Methods

Chemicals and Reagents

Various buffers were used during the course of this study, which are described as follows:

Citrate phosphate buffer

Stock solutions

A: 0.1 M Citric acid solution (prepared by dissolving 19.21 g of citric acid in 1000ml of DDW).

B: 0.2 M dibasic sodium phosphate solution (prepared by dissolving 53.65 g of $Na_2HPO_4.2H_2O$ or 71.7g of $Na_2HPO_4.12H_2O$ in 1000 ml of DDW).

X ml of A + Y ml of B and diluted to 100 ml using DDW.

Preparation of Media

All the media used in present study were prepared in double distilled water and autoclaved at 15 psi pressure for 15 min.

Microbial culture

Laccase producing basidiomycetous fungus, *G. lucidum* MDU-7 (submitted to national fungal culture collection of India (NFCCI) (NFCCI accession no. 3873). MDU-7 was obtained from a culture collection of laboratory of Enzymology and Recombinant DNA Technology, Department of Microbiology, Maharshi Dayanand University, India. *G. lucidum* MDU-7 was maintained on malt extract agar at 30°C, as described earlier (Kumar *et al*., 2017).

Different Substrate compositions

Wheat bran (40 gm), Saw dust (20 gm) and Rice straw (40gm)

For the production of *G. lucidum* instead of the rice straw, saw dust and wheat bran are also used for the fungal culture prepared, wheat bran, saw dust, rice straw are used as a substrate in different quantity.

The rice straw is collected from local rice mill and saw dust collected from a local shop, wheat bran collected from a factory of a village. Wash all the substrate with tap water and drained completely after dried filled all the substrate into a polybag and autoclaved at 15psi for 20 min. the substrate was allowed to cool after sterilization process and cool overnight then the disc culture of the *Ganoderma* culture is inoculated and incubated at 28ºC. Addition of distilled water after every 4thday, and the growth of this culture was recorded.

Rice straw (50 gm) and saw dust (50 gm)

Substrate mixed in a composition of rice straw and saw dust (1:1). After this, straw was put this into a polybag and tighten it properly. Autoclaved at 15 psi for 20 min. Substrate was allowed to cool after sterilization process. Thereafter, it was inoculated by using *Ganoderma* spawn culture and incubated at (25± 3ºC). Distilled water was added and after every 4th day, and the fungal culture production was recorded.

Wheat bran (10gm), saw dust (5gm) and rice straw (5gm)

Instead of using rice straw, wheat bran and saw dust used as a substrate. Firstly, mixed all the substrate gently and added 40 ml of autoclaved mineral salt on it. Mineral salt helps to reduce the other fungal contamination. Inoculated into a flask and incubated in laboratory at (25 ±3ºC), distilled water added after every 3rd or 4th day.

Rice straw, 20 gm

In this treatment only rice straw was used. Firstly, washed the 20 gm rice straw with the help of the chemical formalin. This chemical removed the growth of other fungal growth. After washing, filled the substrate into a polybag and autoclaved it at 15 psi for 20 min. Cool it after sterilization process and inoculated the fugal culture (disc) and incubated at (25±3ºC). Provide all the conditions which was needed for the growth of *Ganoderma* culture.

Rice straw (40 gm) saw dust (40 gm) and wheat bran (20 gm)

Three different substrates are used in this treatment. Chopped the rice straw and removed the extra dust which was present inside the substrate. Added 100 ml of mineral salt and 1% $CaCO_3$ into the substrate and mixed gently. Autoclaved and cooled after sterilization process; thereafter, inoculated with *Ganoderma* culture (disc) and incubated at (25±3ºC). Added distilled water after every 3rd or 4thday for moisture.

Compost (288 gm) was autoclaved in different trays at 15 psi for 20 min. The proper amount of distilled water after every 4th day. After 10 days added 25 gm of wheat bran into it, thereafter, inoculated with 25 ml pellet and mat. On another compost pellet (25 ml) and flask mat (25 min) was used to inoculated and incubate at (25±3ºC). After 20-25 days growth of *Ganoderma* basidiocarp was recorded.

Enzyme production from G. lucidum MDU-7

Inoculum preparation

Four mycelial disks (8mm diameter each) were cut from the periphery of 6-day old fungal culture and inoculated 25ml MEB in 250ml Erlenmeyer flask. The flasks were then incubated at 30 °C for 4 days and fungal mycelial mat was transferred to autoclaved mortar and pestle. Thereafter, the mycelial mat was crushed under sterile condition in a laminar flow. Mycelial slury (1ml) of fine crushed fungal mycelial mat was then inoculated into 250 ml Erlenmeyer flask containing 50 ml MEB. The flasks were then incubated at 30 °C, 125 rpm.

Analytical procedure

Guaiacol was used as a substrate for assaying laccase activity following the method as described by Vasdev and Kuhad (1994). 200 µL of culture supernatant (enzyme) was added to 1800 µL of substrate (10mM guaiacol in citrate-phosphate buffer, pH 4.0), vortexed at room temperature and incubated at 30 °C for 30 minutes. The absorbance was measured at 470 nm against 10 mM guaiacol in citrate-phosphate buffer, pH 4.0 as blank. One unit (U) of laccase was defined as the change in absorbance of 0.01 $ml^{-1}min^{-1}$ at 470 nm.

Estimation of total proteins

The standard procedure for Lowry was used for determining the total protein in an unknown sample (Lowry et. al.1951).

Polyacryamide Gel electrophoresis (PAGE)

Acrylamide-bis-acrylamide solution (30% mix) 29% (w/v) acrylamide and 1% (w/v) N, N' methylene bis-acrylamide was dissolved in warm DDW. The pH was checked to be 7.0 and stored in amber bottles at 4 ºC. Resolving gel buffer was prepared by dissolving 91.0 g Tris in 300 ml distilled water. The pH of the solution was adjusted to 8.8 using concentrated HCl and the volume was build up to 500 ml with DDW. The solution was stored at 4 ºC. Stacking gel buffer was prepared by dissolving 6.05 g Tris in 40 ml DDW. The pH of the solution was adjusted to 6.8 using concentrated HCl and the volume was build up to 100 ml with DDW. The solution was stored at 4 ºC.

Two different gels are prepared – Resolving Gel and Stacking Gel were prepared and an appropriate amount of protein samples were mixed with loading/tracking dye in ratio of 1:4 (dye: enzyme) and then loaded in the gel. The electrophoresis was carried out at a constant voltage of 70V for stacking gel and 110V for resolving gel till tracking dye (Bromophenol blue) was released out of the gel. Gel was stained in substrate buffer (10 m guaiacol in CP buffer pH- 4.0).

Calculation of enzyme activity by performing laccase assay

Approx. 250 µl supernatant was taken in Eppendorf and approximately 250µl guaiacol (25 mM) in 100 mM citrate phosphate buffer (pH 7-7.5), and 2 mM copper chloride was added. Tubes were vortexed for 2 minutes and then incubated at 37 °C for 30 minutes. Tubes were observed for development of red brown colour. Guaiacol(500µl) was used as blank, and OD was measured at 470 nm.

Result and Discussion

Production of laccase of basidiocarp using agricultural substrate

Laccase was extracted from fungal culture *Ganoderma lucidium* MDU-7 using agricultural wastes (rice straw, wheat straw and saw dust) as substrate under SSF condition. Laccase activity was higher (78.05U/ml) at 30°C incubation. (Table 1). Laccase protein was 206.6µg/ml from rice straw 57.43 µg/ml from wheat bran, 120.6µg/ml from saw dust. Revankar and coworker reported that laccase activity was 2,400 U at 25◦C with 70% moisture content under SSF condition (Revankar and Lele 2006).

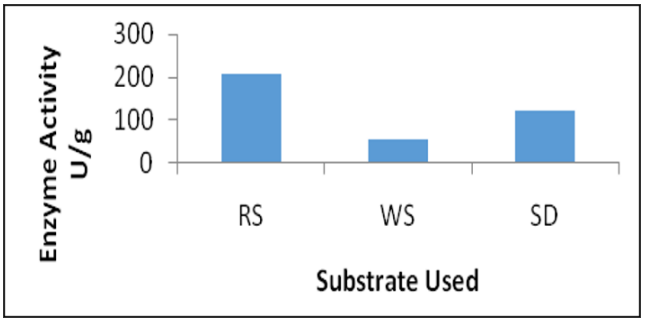

Fig 1. Production of laccases from different agricultural waste; rice straw (RS); wheat straw (WS); saw dust (SD)

Production of basidiocarp from *Ganoderma lucidum* MDU-7

Different substrates were used for production of basidiocarp of *G. lucidum* MDU-7

Basidiocarp production from rice straw at flask level

Basidiocarp production was carried out using rice straw as a substrate using rice straw floppy and cottony mycelial growth was observed within 7-8 days (Fig.2A). Formation of pinning bodies was started from 14 days of incubation at 25◦C with 80% humidity under proper conditions (Fig 2B) also growth of pinning body was measured with $CaCO_3$concentration (1% w/v). Elongated basidiocarp was formed after 28days of incubation (Fig 2C).

Table 1. Laccase production from *G. lucidum* during basidiocarp formation

Condition	Laccase activity U/ml
Flask	78.05
Tray	63.72

Fig 2. Production of basidiocarp from rice straw in flask; A) mycelia growth of *G. lucidum* MDU-7; B) pinning body and C) basidiocarp

Production of basidiocarp by using rice straw as a substrate at tray level

Basidiocarp production was carried out using consortium of rice straw and wheat bran as a substrate using rice straw and wheat bran floppy and cottony mycelial growth was observed within 6-7 days (Fig A). Formation of pinning bodies was started from 12-13 days of incubation at 25◦C with 80% humidity under proper conditions (Fig B) also growth of pinning body was measured with $CaCO_3$ concentration (1%w/100). Rounded shaped basidiocarp was formed after 25 days of incubation.

Production of laccase from basidiocarp

Laccase was extracted from basidiocarp produced from rice straw at tray and flask level (Fig A). With the higher activity 2.37mg/gm was produced from rice straw at tray level in comparison with flask level. Specific activity of laccase was higher in case of flask level, whereas protein concentration was lower. Protein concentration was calculated using BSA standard curve.

Comparative studies of laccase isozymes from *Ganoderma lucidum* MDU-7

The laccase isozyme secretion pattern has been studied for *Ganoderma lucidum* MDU-7 (Fig. 4). A comparative studies of laccase isozyme produced under different condition, i.e., different types of vessels, like tray and flask, humidity, the isozymes are shown in fig 4. Zymogram of Native page study confirmed formation of isozyme in Fig 4. It shows two isozymes bands performed in flask level (Fig 4B) and shows three laccase isozymes from basidiocarp produced in rice straw at tray level (Fig 4C). Kumar et al. (2017) reported laccase isozymes from *Ganoderma lucidum* MDU-7 under both static and shaking situations. Further conditions had been stabilized for laccase production by Cu^{2+} which is determined as an essential component in basal

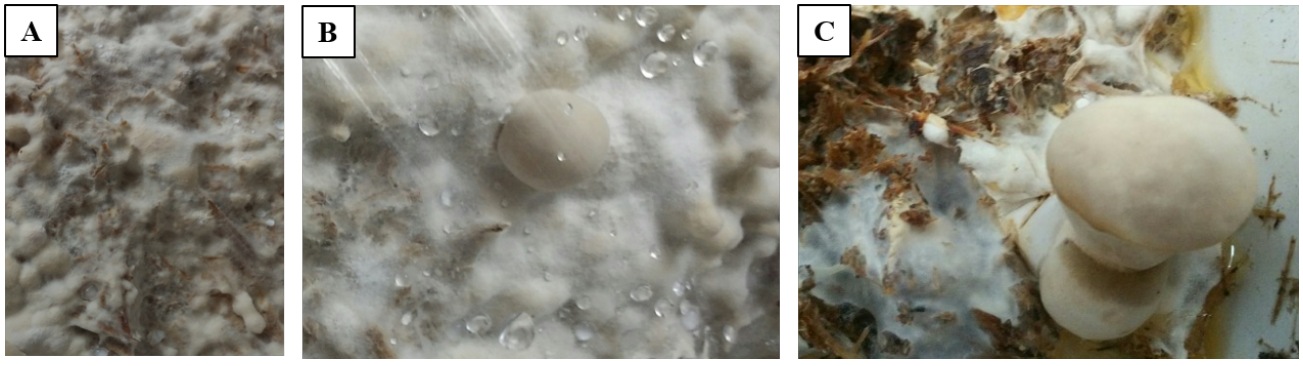

Fig 3. Production of basidiocarp from rice straw in tray; A) mycelia growth of *G. lucidum* mdu-7; B) pinning body and C) basidiocarp

media. Variation in laccase production is probably because of the unavailability or issue of Cu^{2+} ions. Addition of Cu^{2+} (0.06mm) in fermentation media has been considered to be important for the regular production of laccase in the course of fermentation. The production of laccase by fungi is preferred at acidic pH conditions (Sharma et al., 2005, Kumar et al., 2017). Earlier studies confirmed, six isozymes from *G. lucidum* MDU-07 (Kumar et al., 2015), three isozymes by way of *Pleurotus ostreatus* (Palmieri et al., 2000), and two isozymes through *Phlebia brevispora* (Fonseca et al., 2014). It has been reported that expression of laccase is regulated at the transcriptional level by copper (Palmieri et al., 2000). Laccase gene promoter place incorporate steel –responsive factor which are indirectly affected by the presence of copper inside the cultivation media, as a result regulating laccase gene expression in white rot fungi (Jain et. al. 2020) which was no longer located at other pH situations of media. The impact of various pH valves media on laccase isozymes manufacturing has been studied by using Fonseca et

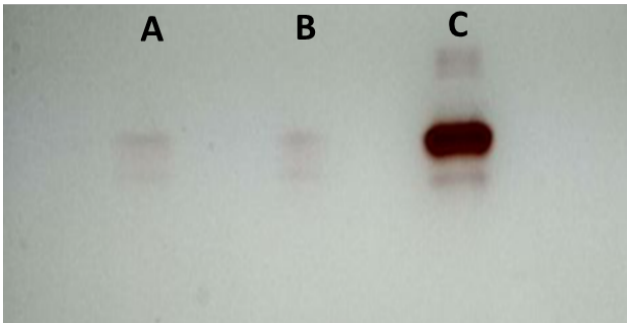

Fig. 4 Zymogram of laccase isozymes by using different substrate laccases

al., 2013. Comparative studies of laccase isozyme produced under different condition –different types of substrates were used like substrate present in tray and flask, depends upon humidity effects, the isozymes were different as shown in Fig 4.

Conclusion

Basidiocarp was produced using different types of agricultural waste. Laccase activity was higher (78.05U/ml) at 30°C incubation. During the production of basidiocarp complex substrate shows better growth of mycelia, and further pinning and complex structure of basidiocarp. Laccase isozymes was extracted from basidiocarp produce from rice straw and native page study confirmed formation of three laccase isozymes from basidiocarp produced tray rice straw at tray level where as only one laccase isozyme was observed in case of flask level. Agricultural waste is a cheap and cost-effective substrate. Agricultural waste contain high amount of cellulose and lignin along with other essential micronutrients that can support the growth of microorganism. Further, for the production of enzyme in this study from different agricultural waste were tested for the production of laccase and basidiocarp. Among these, rice straw is easily available, and show better mycelial and basidiocarp formation, therefore It can be used for the production of basidiocarp at large scale.

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References

Boh B, Berovic M, Zhang J, Zhi-Bin L. (2007). *Ganoderma lucidum* and its pharmaceutically active compounds. Biotechnol Annu Rev.13:265-301

Chang ST and Buswell JA. (1999). *Ganodermalucidum* - a mushrooming medicinal mushroom. International Journal of Medicinal Mushroom.1(2):139-146.

Hapuarachchi K.K., El khateeb W.A., Karunarathna S.C., Cheng C.R., Bandara A.R., Kakumyan P., Hyde K.D., Daba G.M., Wen T.C. (2018) Current status of global Ganoderma cultivation, products, industry and market. Mycosphere 9(5): 1025–1052.

Karunarathna SC, Patabendige NM, Luangharn T, Hapuarachchi KK. (2025). Ganodermataceae-current status, research, and development in Lower Mekong Basin. Front Cell Infect Microbiol.12;15:1545135. doi: 10.3389/fcimb.2025.1545135.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. (1951). Protein measurement with the Folin phenol reagent. J Biol Chem.193(1):265-75.

Jain, K.K., Kumar, A., Shankar, A., Pandey, D., Chaudhary, B. and Sharma, K.K., 2020. De novo transcriptome assembly and protein profiling of copper-induced lignocellulolytic fungus Ganoderma lucidum MDU-7 reveals genes involved in lignocellulose degradation and terpenoid biosynthetic pathways. Genomics, 112(1), 184-198.

Kumar, A., Singh, D., Sharma, K.K., Arora, S., Singh, A.K., Gill, S.S. and Singhal, B., 2017. Gel-Based Purification and Biochemical Study of Laccase Isozymes from Ganoderma sp. and Its Role in Enhanced Cotton Callogenesis. Frontiers in microbiology, 8, 674.

Revankar M.S., Lele S.S. (2006). Enhanced production of laccase using a new isolate of white rot fungus WR-1. Process Biochem. 41:581–588. doi: 10.1016/j.procbio.2005.07.019.

Sharma, K.K., Kapoor, M. and Kuhad, R.C., 2005. In vivo enzymatic digestion, in vitro xylanase digestion, metabolic analogues, surfactants and polyethylene glycol ameliorate laccase production from Ganoderma sp. kk-02. Letters in applied microbiology, 41(1), 24-31.

Vasdev, K. and Kuhad, R .C. 1994. Induction of laccase production in Cyathus bulleri under shaking and static culture conditions. Folia Microbiologica, (39), 326-330.

Wasser SP. (2014). Medicinal mushroom science: Current perspectives, advances, evidences, and challenges. Biomed J.37(6):345-356.

Wasser S. (2002). Medicinal mushrooms as a source of antitumour and immunomodulating polysaccharides. Applied Microbiology and Biotechnology 3: 258-274.

Wu P, Zhang C, Yin Y, Zhang X, Li Q, Yuan L, Sun Y, Zhou S, Ying S, Wu J. (2024) Bioactivities and industrial standardization status of Ganoderma lucidum: A comprehensive review. Heliyon. 29;10(19):e36987. doi: 10.1016/j.heliyon.2024.e36987.

Yang FC, Liau CB. (1998). The influence of environmental conditions on polysaccharide formation by Ganodermalucidum in submerged cultures. Process Biochem. 33:547–553.

Zang TL. (1999). Recent advances in research of Pharmacology and clinical applications of *Ganoderma* P. Karst. in China. International Journal of Medicinal Mushrooms 1: 63-67.