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In vitro optimization of cultural condition for laccase biosynthesis by using *Ganoderma lucidum* author citation

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Abstract

Ganoderma lucidum, (Curtis) P. Karst is a white rot fungus belongs to basidiomycetes group, distributed throughout world. In present study, *G. lucidum* was used to assess laccase production under varying culture conditions like inoculum size, temperature, pH and incubation period. Maximum laccase enzyme activity was obtained at 6 pH, 25 °C at 7th day of incubation with 5 disc of inoculums.
Keywords: Biotechnology, Fungi, Lignin, *Ganoderma lucidum.*

Introduction

Recently, there has been an increasing utilization of organic wastes such as residues from the forestry, agricultural and food industries to produce ligninolytic enzymes (Kalogeris *et al*., 2003). These wastes act as cheap and environment friendly source of substrates besides acting as inducers of the ligninolytic enzymes. Many efforts have been made to make use of enzymes for the degradation of lignin in the pulp and paper industry (Call and Mücke, 1997). In this connection, laccase (benzenediol:oxygen oxidoreductase E.C. 1.10.3.2) is oxidase exhibited a major role in natural delignification process and widely distributed among plants, insects and fungi (Call and Mücke, 1997). This enzyme contributes in major physiological functions in plant, fungi and animals. Laccases are mostly glycoproteins, extracellular, multinuclear enzymes with molecular weight between 60-80 KDa. There is an increasing demand for laccase in the market for various applications such as bio-bleaching (Sigoillot *et al*., 2004), organic synthesis (Aktas *et al*., 2003), cosmetic, dermatological preparations, biofuel cells etc. (Alcalde, 2007).

White rot fungi are good source of laccase, and this enzyme can be induced during fungal growth or secreted constitutively. White-rot fungi i.e. *Ganoderma lucidum* (Elisashvili *et al.,* 2010), *Coriolus versicolor* (Arockiasamy *et al*., 2008), *Phanerochaete chrysosporium* (Srinivasan *et al*., 1995) and *Pycnoporus cinnabarinus* (Meza *et al*., 2005) are well-known for the production of laccase. Use of laccase in biotechnological processes needs its production in maximum amounts at low cost and hence recently laccase research is focused towards the optimization of medium components by various statistical methods.

Ganoderma lucidum (named as Lingzhi in China), a species of basidiomycetes which belongs to Ganodermataceae (Polyporales) is one of the medicinal mushrooms in Japan, Korea, China, and other Asian countries. Laccase synthesis from different white rot fungi is known to be affected by the culture conditions, including variations in the types and concentration of nutrients available (Elisashvili *et al.,* 2010). Present work was conducted to optimize parameters like temperature, pH, inoculum size and incubation period for the production of laccase from *G. lucidum*.

Materials and Methods

A pure culture of *Ganoderma lucidum* used in this experiment was procured from Environment and Mushroom Biotechnology laboratory, Institute of Agricultural Sciences (IAGS), University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan. Fungal culture was maintained and re-cultured on Tien and Kirk agar medium. The media plates were inoculated with a disc from the parent culture and were incubated at 25 ± 2 °C for 7 days.

To find the optimal culture requirements for high yield of laccase, experiments were conducted by inoculating Tien and Kirk agar medium with fungus inoculam and kept on electrical shakers at 150 rpm. The effect of temperature in laccase production was determined at different

temperatures i.e. 15-35 °C with 5 °C difference. For pH optimization, 5 different levels of pH viz., 4, 5, 6, 8 and 9 were taken. The inoculum effect in enzyme production was examined by inoculating different inoculum sizes ranging from 2 discs, 3 discs, 4 discs, 5 discs and 6 discs of 14 mm in diameter from a pure culture of *G. lucidum.* Role of incubation period in enzyme production was determined by examining different periods for incubation from 5-9 days.

The enzyme activity was assessed through spectrophotometric method at 470 nm using sodium acetate buffer. Fungal biomass was dried in oven at 80 °C for 2 hours after incubation period (7 days) of each experiment. Each experiment was conducted in triplicate and means were compared by Duncan's multiple range tests (Steel *et al*., 1997).

Results and Discussion

Laccases are the oldest and most studied enzymatic systems (Williamson, 1994) and many wood rotting fungi are known for its production (Murugesan *et al.,* 2006; Wang, 2006; Quaratino *et al*., 2007; Litthaueer *et al*., 2007). This enzyme is constitutively produced in basidiomycetes, and many fermentation factors like as temperature, pH, medium composition etc. could affect its production. In current work, it was observed that laccase activity was highest at 25 to 30 °C (Fig. 1A) with maximum enzyme activity (11.85 U mL^{-1}) (Fig. 1B). The same trend has been demonstrated by Zadrazil *et al*. (1999) when *Pleurotus* sp. and *Dichomitus squalens* were cultivated at temperatures higher than 30 ºC.

The optimum pH for laccase activity was 6 (Fig. 2A) with the highest laccase production was 12.07 U mL^{-1}(Fig. 2B). Likewise, the optimum pH for laccase production from *G. lucidium* and *G. raminis* var. *tritici* was reported within range of 3.5-6 (Chefetz *et al*., 1998; Edens *et al*., 1999; Ko *et al*., 2001). The maximum biomass production was obtained at 5 discs with activity 12.41 U mL^{-1}, while the minimum was obtained at 2 discs with activity 10.48 U mL^{-1} (Fig. 3A and B). It could be attributed to insufficiency of inoculum to initiate growth and higher level may cause competitive inhibition (Sabu *et al*., 2005). Sharma *et al*. (1996) reported that smaller inoculum size increased the lag phase. The optimum incubation period for the high biomass production was observed at 7th day with enzyme activity of 12.64 U mL^{-1}(Fig. 4A and B). Similarly maximum laccase production was reported at the 7th and 10th day of incubation in case of *Lentinus edodes* and *Ganoderma* sp., respectively (Sivakumar *et al*., 2010).

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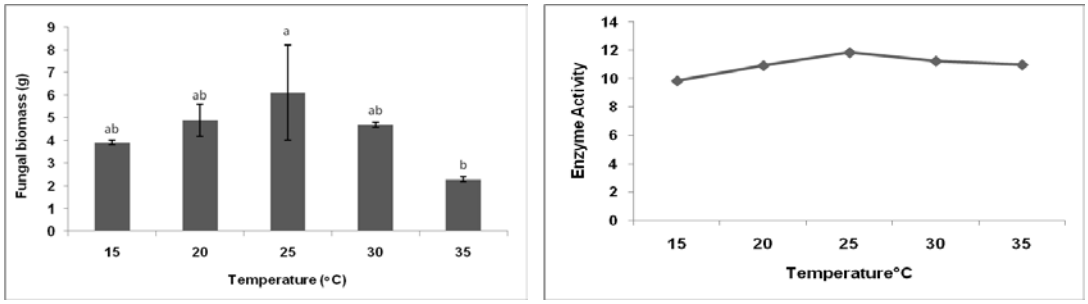

Fig. 1A and B: Mycelial Biomass production (A) and laccase activity (B) by *Ganoderma lucidum* with varying temperatures. Vertical lines show the standard error while letters show significant difference.

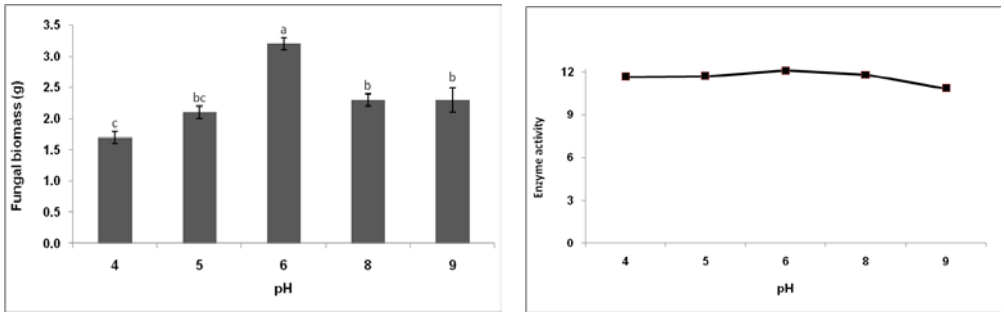

Fig. 2A and B: Mycelial Biomass production (A) and laccase activity (B) by *Ganoderma lucidum* with varying pH. Vertical lines show the standard error while letters show significant difference.

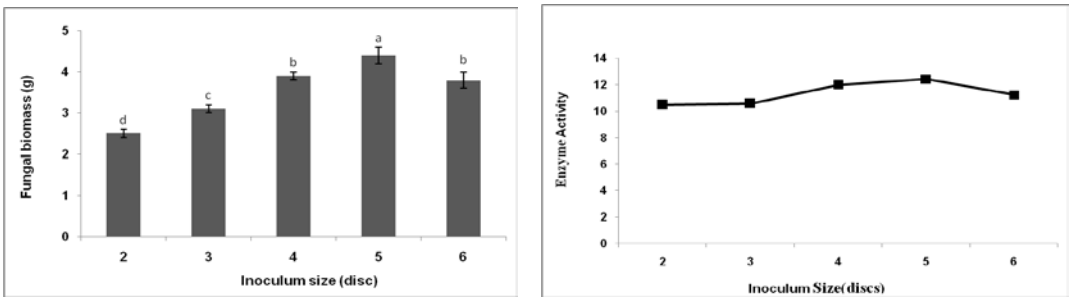

Fig. 3A and B: Mycelial Biomass production (A) and laccase activity (B) by *Ganoderma lucidum* with varying inoculam levels. Vertical lines show the standard error while letters show significant difference.

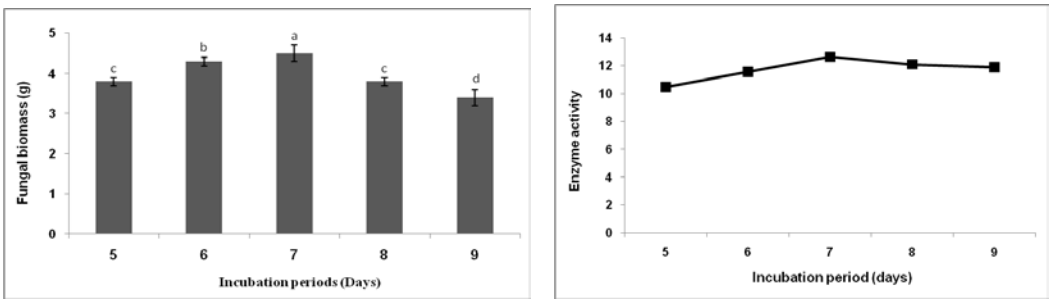

Fig. 4A and B: Mycelial Biomass production (A) and laccase activity (B) by *Ganoderma lucidum* with varying inoculam levels. Vertical lines show the standard error while letters show significant difference.

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