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Optimization of Lignocellulose Degrading Enzyme Laccase from Basidiomycetes Using One Variable at a Time Approach.

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ABSTRACT

Present study deals with the screening of few species of basidiomycetes for the production of *laccase*. Ten samples of basidiomycetes were collected from different lignocellulosic sources and best four fungal species viz., *Schizophyllum sp.*, *Polyporus sp.*, *Phanerochaete sp.* & *Trametes sp.* were screened for production of *laccase*. One Variable at a Time approach (OVAT) was used to study different parameters namely, pH, carbon and nitrogen sources, solid to liquid ratio and surfactants on the activity of laccase enzyme. *Schizophyllum sp.* showed maximum laccase activity (1060.5 IU ml⁻¹) at pH 6.5 amongst all other parameters investigated. It showed maximum laccase activity of 10.5 IU ml⁻¹ with NaNO₃ and Peptone.

Keywords: Basidiomycetes; *Laccase*; One variable at a time approach (OVAT)

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INTRODUCTION

Basidiomycetes are known to produce considerable amount of industrially important enzymes such as cellulase (Singh *et al.* 2009; Deswal *et al.* 2011), xylanase, lignin peroxidase (Pazarlioglu *et al.*, 2005; Jang *et al.*, 2001), manganese peroxidase, Laccase (Koroleva *et al.*, 2001; Nyanhongo *et al.*, 2002), amylase (Anto *et al.*, 2006). Laccases (EC 1.10.3.2) have been widely accepted for the degradation of lignin (Garzillo *et al.*, 1998) and found to involve in delignification of white rot of wood. Laccases are also known to oxidize highly recalcitrant environmental pollutants, detoxification of industrial effluents which are mostly generated from paper, pulp and textile industries. Also, implicated in bioremediation of contaminated soils and are extensively acceptable for the manufacture of anti-cancer drugs, (Monteiro and Carvalho, 1998). Solid state fermentation (SSF) holds tremendous potential for the production of enzymes where the crude fermented products are directly used as enzyme sources, as studied by Pandey *et al.*, (1999). Therefore, present study was aimed to screen and optimize solid substrate fermentation for the cost effective production of laccase from basidiomycetes group of fungal species using one variable at a time approach .

MATERIALS AND METHODS

Collection and isolation of fungus

Fungal samples were collected from Himalayan region and fungus isolation was carried out from the basidiocarp as described by Pong *et al.*, 2002. Basidiocarps were immersed for two to three minutes into five percent hypochlorite solution to remove contaminants and rinsed three times with sterile distilled water. Pieces of basidiocarps were cut aseptically with the help of sterile blade and placed on potato dextrose agar (Himedia) and incubated at optimum temperature of $27\pm 2^{\circ}\text{C}$ for four days.

Substrate

Wheat straw and sugarcane bagasse were used as lignocellulosic substrates, procured from the local markets of Jalandhar, Punjab, India.

Pure Cultures

Pure culture was obtained by culturing and successive sub culturing of isolated fungal species on potato dextrose agar media (Himedia).

Spawn preparation for large scale production

For spawn preparation, wheat grains were taken and mixed with sterile distilled water and boiled for 15 minutes. The water was drained off and grains were surface dried with subsequent addition of CaSO_4 and CaCO_3 . Thereafter, autoclavable bags were packed with 100gms of wheat grains each for the different strains and autoclaved at 121°C for 15 min at 15 psi. The one-eighth portion of the pure colonies obtained was used as an inoculum for spawn preparation and incubated at 30°C for 15 days.

Parameter optimization:

Screening of enzyme activity using one variable at a time approach method (OVAT) was done. Substrate was washed and sun dried. Five grams of the material was weighed and packed in the autoclavable bags followed by the addition of 15ml of Czapek Dox media into each substrate bag. The various parameters taken into account were solid to liquid ratio, pH, carbon source, nitrogen source and surfactants.

Optimization of pH

Various pH value ranges from 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5 and 8 was studied. Separate bags containing 5 gm substrate and 15 ml of Czapek dox media adjusted with different ranges of pH were autoclaved and inoculated with the different fungal species followed by incubation at 30°C for 15 days.

Optimization of surfactants

Optimization of surfactants supply was monitored in order to minimize the irreversible binding, the surfactants taken were tween20, tween80, SDS and triton x . The bags containing 5 gm substrate and 15 ml Czapek dox media were prepared and autoclaved. Solutions of different surfactant (0.1% v/v) were added to different bags and inoculate with the different fungal species and incubated at 30°C for 15 days.

Optimization of carbon source

For optimization of carbon source, various carbon sources such as glucose, fructose, sucrose, lactose and starch were tested. Sugar solution of 0.2%w/v was prepared and added to bags containing sterile autoclaved 5 gm substrate and 15 ml Czapek dox media inoculated with the different fungal species and incubated at 30°C for 15 days.

Optimization of nitrogen source

Fermentation was supplemented with NaNO₃, NH₄Cl, urea and peptone. Autoclaved sterile solutions of different nitrogen sources (0.5% w/v) were added to different bags containing sterile 5 gm substrate and 15 ml Czapek dox media inoculated with the different fungal species and incubated at 30°C for 15 days.

Enzyme assay:

Laccase assay: Laccase enzyme was assayed by referring the method by irshad *et al.*, 2011.

RESULT AND DISCUSSION

In present study, screening of fungal species which were collected from various locations of Himalayan region was done for laccase production and optimization of the parameters for maximum production of laccase from the screened fungal strain. One variable at a time approach was used to study the optimization parameters selected.



Figure 1: Spawn of the fungal strains

Effect of Ph

The four selected fungus showed variable results when tested for laccase production at different parameters. Laccase activity was found maximum for *Schizophyllum sp.* (1060.5 IU ml⁻¹) at pH 6.5. *Polyporus sp.* and *Phanerochaete sp.* showed maximum laccase activity at pH 4.5 (3.5 IU ml⁻¹) and *Trametes sp* showed maximum laccase (7.0 IU ml⁻¹) activity at pH 7.5- 8.0 (Figure 2). Dhakar and Panday (2013) observed optimum pH range from 5.5 to 7.5 for laccase production from fungal Strain of *Trametes hirsute*. The enzyme production was carried out by enzymatic assay considering that enzyme production is directly proportional to the enzyme activity.

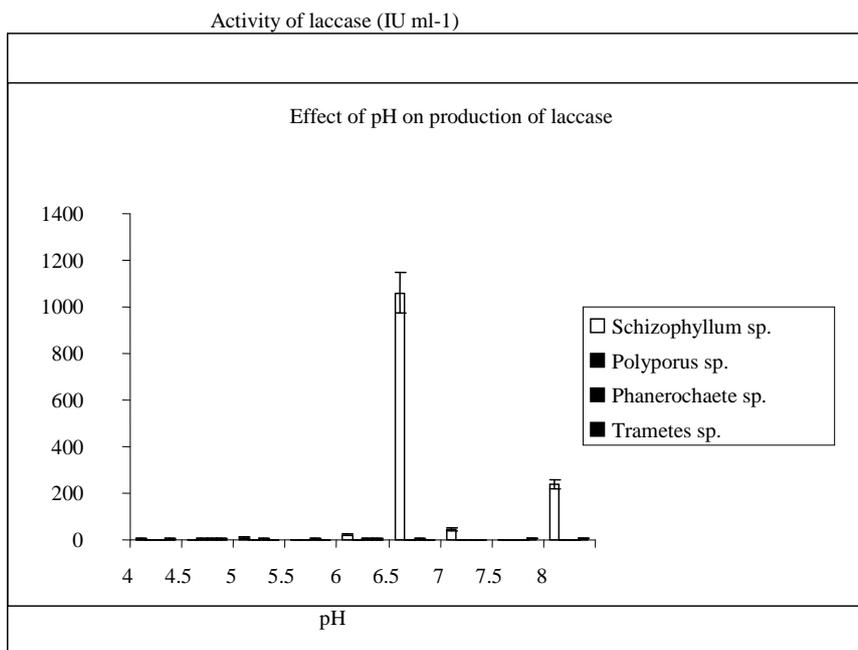


Figure 2: Graph depicting the effect of pH on Laccase activity

Effect of Solid to Liquid Ratio

At different conditions of solid to liquid ratio, no *Schizophyllum sp.* was observed whereas *Polyporus sp.* showed maximum production at 1:3, 1:2 (3.5 IU ml⁻¹) respectively, *Phanerochaete sp.* also showed maximum yield at 1:1, 1:2, and 1:3 (3.5 IU ml⁻¹) respectively and same was observed with *Trametes sp.* at 2:1 (3.5 IU ml⁻¹) which was not found significantly high.

Effect of Surfactants

Addition of the surfactants increases the membrane permeability and enhances the bioleaching of the enzymes as per literature available. Laccase production was studied by using different surfactants. However, *Schizophyllum sp.* failed to produce laccase with any of the surfactant, maximum laccase activity was observed for *Polyporus sp.* with Tween 80, SDS i.e., 7.0 IU ml⁻¹ for both the surfactants whereas *Phanerochaete sp.* maximum Laccase activity of 10.5 IU ml⁻¹ with Triton-X was noted. Maximum Laccase activity was also observed for *Trametes sp.* with Tween 20 i.e., 7.0 IU ml⁻¹. It indicated that laccase production by fungal species is not significantly affected by surfactants (Figure 3).

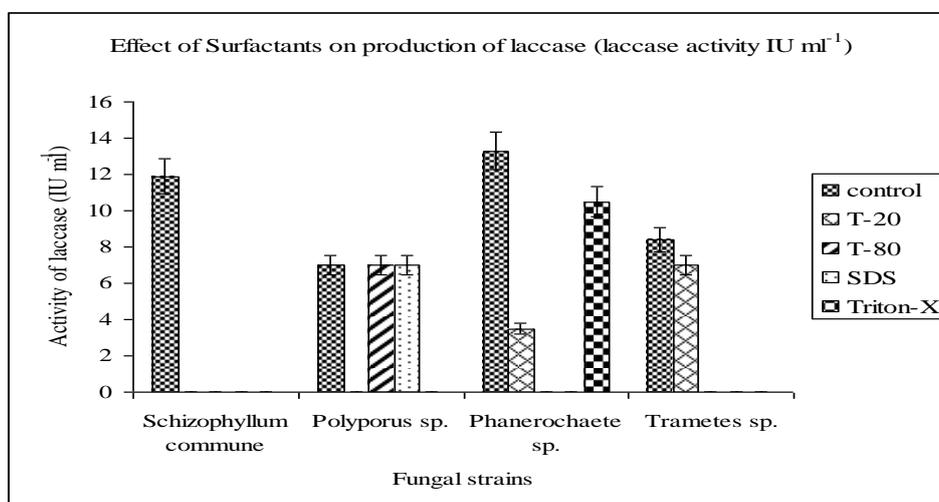


Figure 3: Graph depicting the effect of surfactants on laccase activity

Effect of additional carbon source

The fungal strains were subjected to different carbon sources and it was found that *Schizophyllum sp.* did not show laccase activity with any of the carbon source, *Polyporus sp.* showed maximum laccase activity with galactose & maltose which was recorded approximately the same (3.5 IU ml⁻¹). *Phanerochaete sp.* showed maximum laccase activity with galactose (3.5 IU ml⁻¹). *Trametes sp.* showed maximum laccase activity with sucrose (3.5 IU ml⁻¹) (Figure 4). Dhakar and Panday (2013) found fructose as an effective carbon source for the production of laccase from *Trametes hirsute*.

Activity of laccase (IU/ ml)

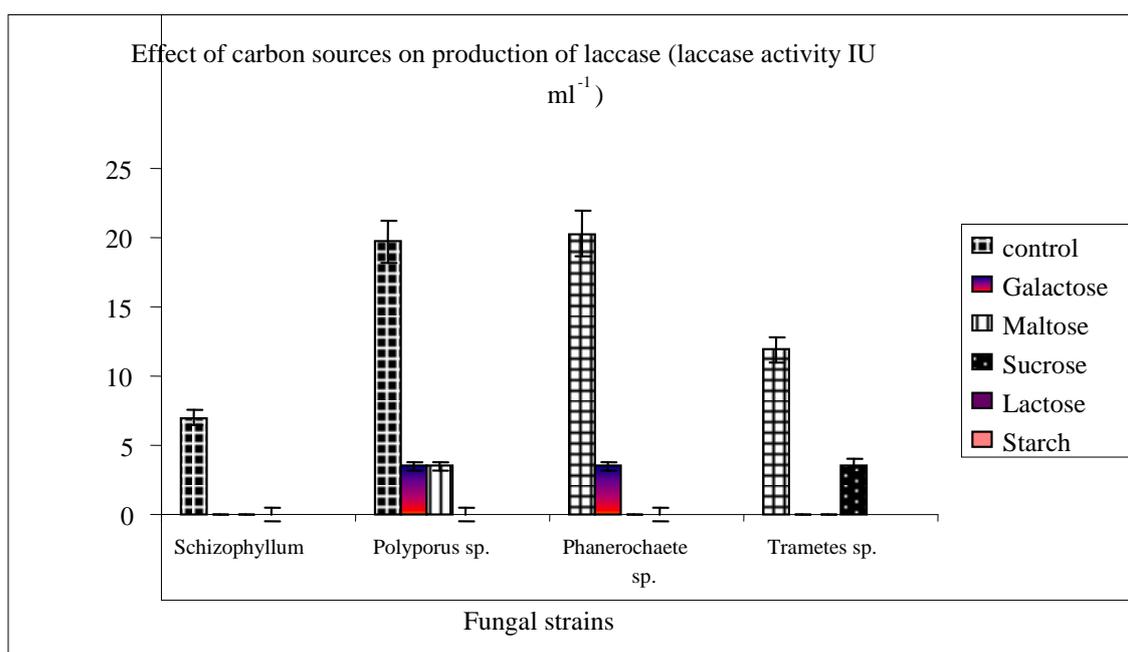


Figure 4: Graph depicting the effect of carbon source on laccase activity

Effect of additional nitrogen source

Nitrogen is a major element occurs in all living organisms. It plays a crucial role in the living organisms. However, many different sources are there but cost benefit analysis is employed for the maximization of the yield of enzyme. NH₄Cl, NaNO₃, urea and peptone were selected as nitrogen for the fungal cultivation for the production of laccase enzyme. *Schizophyllum sp.* showed maximum laccase activity of 10.5 IU ml⁻¹ with NaNO₃ and Peptone. *Polyporus sp.* showed maximum laccase activity of 3.5 and 7.0 IU ml⁻¹ with NH₄Cl and peptone respectively. *Phanerochaete sp.* showed maximum laccase activity of 3.5 IU ml⁻¹ with NH₄Cl. Enzyme activity of 3.5 IU ml⁻¹ was observed for *Trametes sp.* when urea was used as a nitrogen source (Figure 5). Dhakar and Panday (2013) indicated ammonium sulfate as the best nitrogen source for the production of laccase from *Trametes hirsute*.

Activity of laccase (IU ml⁻¹)

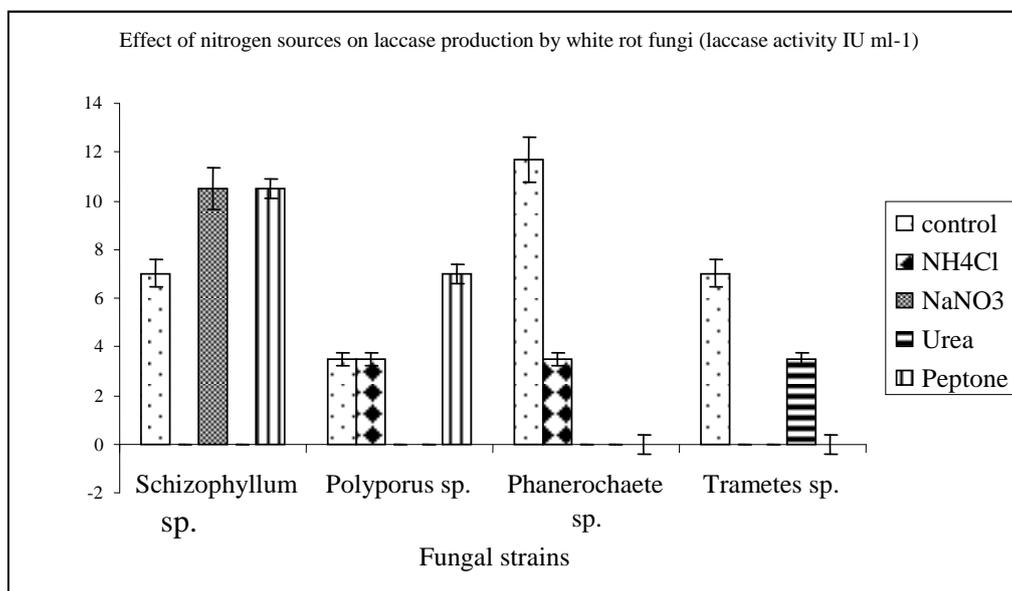


Figure 5: Graph depicting the effect of nitrogen source on Laccase activity

Kumar *et al.*, 1996 revealed that lignin-degrading enzymes laccase, manganese-peroxidase and lignin peroxidases screened from basidiomycetous fungi found to be useful in colored effluent treatment and xenobiotics compounds. White rot fungi produce extracellular phenoloxidases and can decompose lignin efficiently, as studied by Ohkuma *et al.*, (2001). Our study comparable with the study conducted by Nyanhongo *et al.*, 2002 on effects of the carbon and nitrogen sources, initial pH and incubation temperature on laccase production by *Trametes modesta* were evaluated by using the one-factor-at-a-time method. Laccase acts on both phenolic and non-phenolic lignin at wide temperature range and pH activity. Pazarlioglu *et al.*, (2005) isolated laccase from *T.versicolor* which can be employed for the washing of denim even without mediator, in textile industries and also used as a tool for bioremediation (Alcalde *et al.*, 2006). Anto *et al.*, in 2006, investigated the production of α -amylase under solid-state fermentation by *Bacillus cereus* using wheat bran and rice flake manufacturing waste as substrates. In present study we isolated white rot fungus and optimization was done at various parameters. Our study is comparable with that of Kanmani *et al.*, 2009 in concern of solid state fermentation, which is found to be the most economical method for the bioconversion of lignocellulosic coir using *Phanerochaete chrysosporium* and *Rhizopus stolonifer* whereas our study focused on isolation of *Schizophyllum commune*, *Polporus sp.*, *Phanerochaete sp.* and *Trametes sp.* and observed that optimum pH range from 6.5 to 8.0 enhanced laccase production by *Schizophyllum sp.*

CONCLUSION

Present study concluded that the effect of individual parameter can be best studied by using one variable at a time approach, to judge the effectiveness of each parameter on enzyme activity. pH has been observed the effective parameter for maximum laccase production from *Schizophyllum sp.*

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