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Optimization of *Sclerotium rolfsii* for laccase enzyme production

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Abstract
In the present investigation optimization of *Sclerotium rolfsii* was carried out for laccase enzyme production. Different media and media amended with different nutrient sources *Viz*. carbon, nitrogen and sulphur and phosphorous were tested along with different physical parameters like pH, temperature and incubation period to standardized proper cultural condition for maximum laccase production. The results revealed that among the media tested malt extract broth significantly induced the laccase production as compare to other media. 6.5 pH and 30 ^{0}C temperature were optimum for laccase production where as laccase production is optimum after 8 days of incubation. Among the carbon sources tested lactose showed maximum laccase production where as nitrogen sources tested sodium nitrate showed maximum laccase production. Calcium sulphate and sodium phosphate showed maximum laccase enzyme production among the sulphur and phosphorus sources tested.

Keywords: Guaiacol, *Sclerotium rolfsii*, laccase, optimization

Introduction
Laccases (benzenediol: oxygen oxidoreductase EC 1.10.3.2) are blue copper-containing enzymes that catalyze the oxidation of various substrates and use molecular oxygen as an electron acceptor (Revanker and Lele, 2006) [8]. Fungal laccase have higher redox potential and are involved in the degradation of lignin and toxic phenols. Fungal laccase have wide industrial applications in paper, textile, food and pharmaceutical sectors and in the degradation of aromatic pollutants causing serious environmental problems (Sette *et al.*, 2008) [10]. *Sclerotium rolfsii* is the efficient laccase producing fungi reported by various workers (Campos *et al.*, (2001) [4]; Ryan *et al.*, (2003) [9]. In the present investigation an attempt has been made for standardization of optimum cultural condition for laccase production from *Sclerotium rolfsii*.

Materials and Methods
Sclerotium rolfsii isolated from soil were used for laccase production and optimum cultural conditions were standardized for efficient laccase production. (Mhaske and Wadikar 2017) [7]
Guaiacol was used as an indicator compound and chemical used were of analytical grade.
Enzyme activity was recorded with the help of UV-spectrophotometer.

Production of laccase
The production of laccase was made by growing the fungus in potato dextrose broth (PDB). 100 ml of PDB media was poured in 250 ml Erlenmeyer conical flask and autoclaved at 15 lbs pressure for 20 minutes. The flasks on cooling were inoculated with 1 ml of suspension culture freshly prepared 7 days culture. The flasks were incubated at 30 ^{0}C on a rotary shaker for 7 days. After the incubation period, the contents of the each flask were filtered through Whatman filter paper No.1 and the filtrate was centrifuged at 10000 rpm for 10 min at 4 ^{0}C. The clear supernatant thus obtained was treated as the enzyme extract for the study.

Enzyme assay
The Laccase activity was assayed at room temperature by using 10 mM Guaiacol in 100 mM sodium acetate buffer (pH 5.0). The reaction mixture contained 3 ml sodium acetate buffer, 1ml Guaiacol and 1ml enzyme source. The change in the absorbance of the reaction mixture

containing guaiacol was monitored at 470 nm for 10 mins of incubation using UV Spectrophotometer. Enzyme activity is measured in U/ml which is defined as the amount of enzyme catalyzing the production of one micromole of colored product per min per ml (Jadhav *et al.,* 2009) [5]. All the experiments were carried out in triplicates.

Calculation

$$\text{Laccase activity (U/ml)} = \frac{\Delta \text{ A470/min x 4 x Vt x dilution factor}}{€ \text{ X Vs}}$$

Where,
Vt = final volume of reaction mixture
Vs = sample volume
€ = extinction coefficient of guaiacol = 6740 $M^{-1}cm^{-1}$
4 = derived from unit definition and principle

Effect of different culture media on laccase production
Effect of different cultural media for laccase production were investigated by 6 different media like Potato Dextrose Broth, Malt Extract Broth, Glucose Peptone Broth, Glucose Nitrate Broth, Yeast Extract Peptone Dextrose Broth and Czapek Dox Broth. (Prasher and Chauhan (2015) [11].

Effect of incubation period on laccase production
The effect of incubation period on laccase production was investigated by checking the enzyme activity 4, 6, 8, 10 and 12 days of incubation period. The tested fungi were grown in 100 ml malt extract broth on rotatory shaker. 5 ml culture filtrate was taken at 2 days of interval for 12 days and centrifuge 10000 rpm for 10 minutes. The enzyme assay was done as mention above.

Effects of temperature on laccase production
Temperature effect on laccase production was investigated by incubating the production medium at various temperature ranges (25 ^{0}C to 50 ^{0}C). The enzyme assay was done as mention above.

Effect of pH on laccase production
Production of laccase was investigated by using the malt extract broth medium with various pH ranges from pH 4 to pH 8. The pH of the medium was adjusted from pH 4 to pH 8 with 0.1 NaOH and 0.1 N HCl with the pH meter.

Effect of carbon sources on laccase production
So as to study effect of carbon sources on the production of laccase five different carbon sources were used like glucose, sucrose, fructose, maltose and lactose. 1% of these sources were added in Malt extract broth medium. A flask containing malt extract broth without carbon source served as control. Enzyme assay was done as mention above.

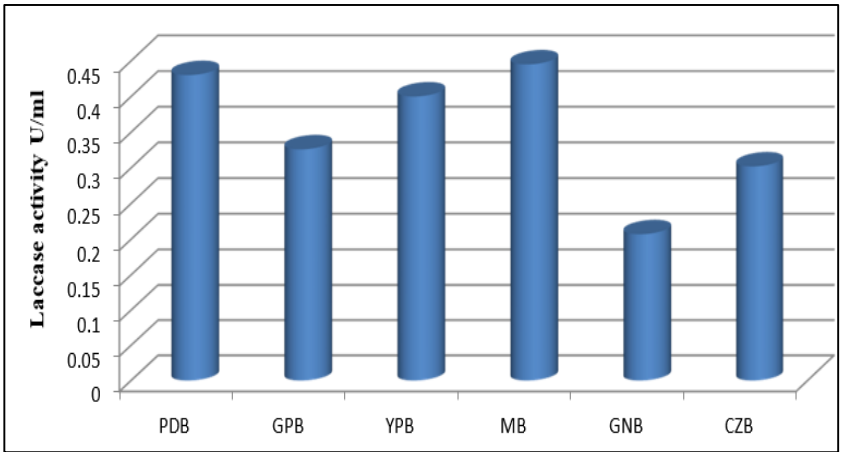
Effect of nitrogen sources on laccase production
Nitrogen sources were tested for laccase production, like Calcium Nitrate, Sodium Nitrate, Ammonium Nitrate, Urea and Casein. 1% of these sources were added in basal medium. A flask containing malt extract broth without nitrogen source served as control.
Enzyme assay was done as mention above.

Effect of sulphur sources on laccase production
To study the effect of sulphur sources on laccase production five different Sulphur sources were used like Ferrous Sulphate, Calcium Sulphate, Copper sulphate, Zinc Sulphate and Sodium Sulphate. 0.05% of these sources added in malt extract broth. A conical flask containing malt extract broth without any sulphur source served as control. Enzyme assay were carried out as mention above.

Effect of phosphorus sources on laccase production
Various phosphorus sources were tested for laccase production, like Sodium phosphate, Ammonium Phosphate, Sodium dihydrogen orthophosphate, Potassium dihydrogen orthophosphate. 0.05% of these sources added in malt extract broth. A flask containing malt extract broth without phosphorus source served as control.

Result and Discussion
Optimization of proper cultural condition was carried out for laccase production from *Sclerotium rolfsii* and results were summarized and tabulated in the following fashion.

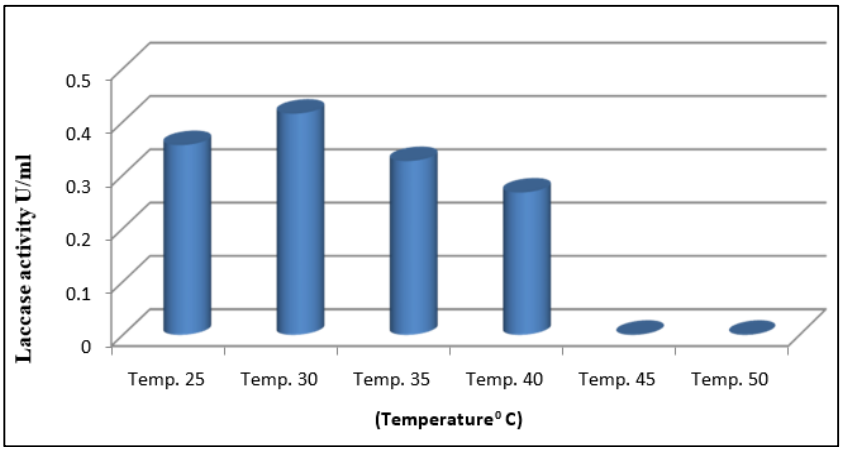
Effect of different culture media on laccase production
For optimization of suitable culture media six different culture media were tested out of that malt extract broth media found to be optimum with 0.445 U/ml followed by potato dextrose broth with 0.430 U/ml and glucose nitrate broth were significantly reduced laccase production and results were expressed in the form graphical representation. (Graph no.1) more or less similar results were recorded by Manimozhi and Kaviyarasan (2012) [6] for *Agaricus heterocystis.*



Graph 1: Effect of different media on laccase activity

Effect of temperature on laccase production

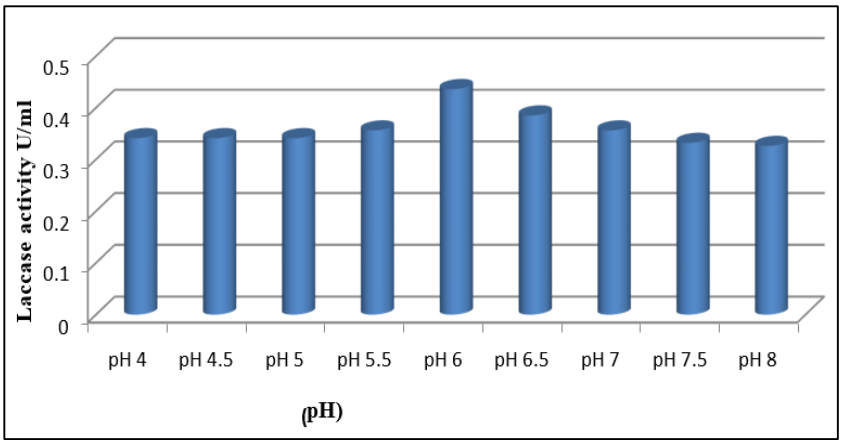
In order to study effect of different temperature range on laccase production of *Sclerotium rolfsii* six different temperature ranges were tested from 25-50 ^{0}C and results were recorded in the graphical form results revealed that 25-35 ^{0}C temperature range is the optimum for laccase production where as at 50 ^{0}C laccase activity was completely reduced. (Graph No. 2) Banerjee and Vohra (1991) [3] found more or less similar findings for *Curvularia* and stated that 30 ^{0}C was optimum for laccase production.



Graph 2: Effect of temerature on laccase production

Effects of different pH range on laccase production

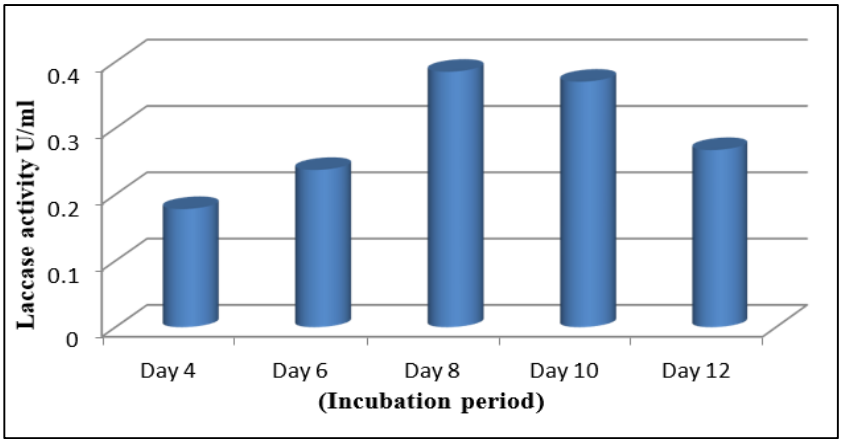
In order to study effects of different pH range on laccase production varied pH range were maintained from 4- 8 and results were recorded in graphical fashion. Results depicted that maximum laccase production was at pH 6 followed by 6.5 and 7 pH (graph No. 3) Arora and Gill (2005) [2] Carried out optimization of proper culture conditions for laccase production and reported that pH 4.5 is the optimum which is quite contrasting with present findings.



Graph 3: Effect of pH on laccase production

Effect of incubation period on laccase production

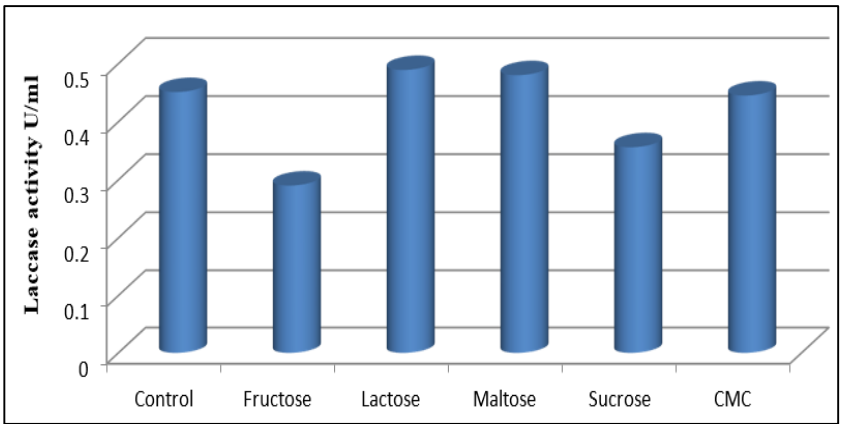
In order to study effect of incubation period on laccase production the fungus was grown in incubation period 1 to 12 days and results were recorded at interval of 2 days. The maximum production was recorded at 8th day of incubation whereas after 12 days the production was significantly reduced. (Graph No.4)



Graph 4: Effect of incubation period on laccase production

Effect of carbon sources on laccase production

Different carbon sources were tested like fructose, lactose, maltose, sucrose and CMC among the carbon sources tested, lactose significantly induced laccase production of *sclerotium rolfsii* followed by maltose as compare to control. Laccase production was significantly reduced by Sucrose and fructose respectively (Graph no.5). Jhadav *et al.,* (2009) [5] reported that glucose as carbon source has positive effects on laccase production.

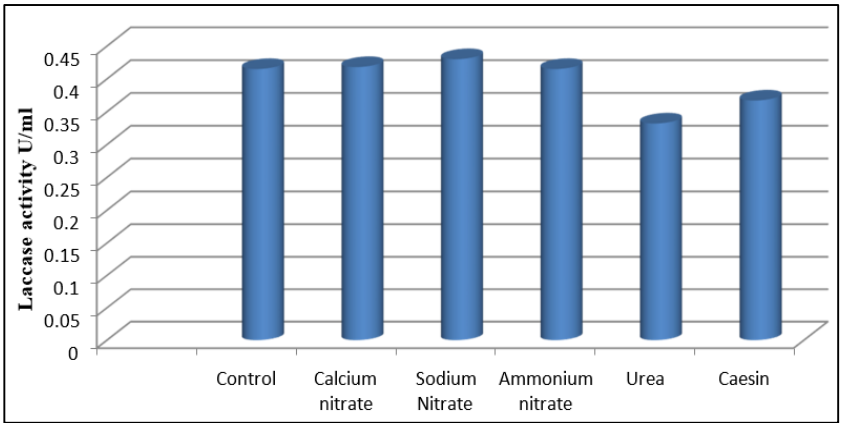


Graph 5: Effect of carbon sources on laccase production

Effect of nitrogen sources on laccase production

Various nitrogen sources were tested for laccase production out of these sources tested laccase production was significantly induced by sodium nitrate followed by calcium nitrate as compareto control where as urea and casein significantly reduced laccase production as compare to control.(graph no.6) Manimozhi and Kaviyarasan (2012) [6] carried out more or less similar experiment and found that ammonium tartarate and yeast extract significantly induced laccase production which is controversial with present findings.

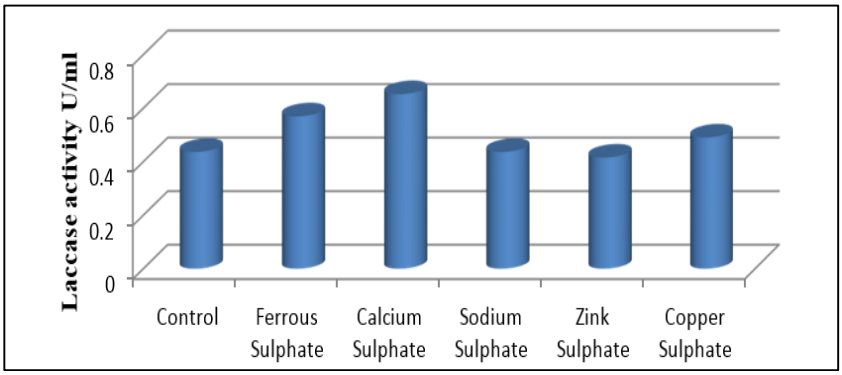
Sulphur sources like ferrous sulphate, calcium sulphate, sodium sulphate, zinc sulphate and copper sulphate were tested out of these all sulphur sources except zinc sulphate significantly.



Graph 6: Effect of nitrogen sources on laccase production

Effect of sulphur sources on laccase production

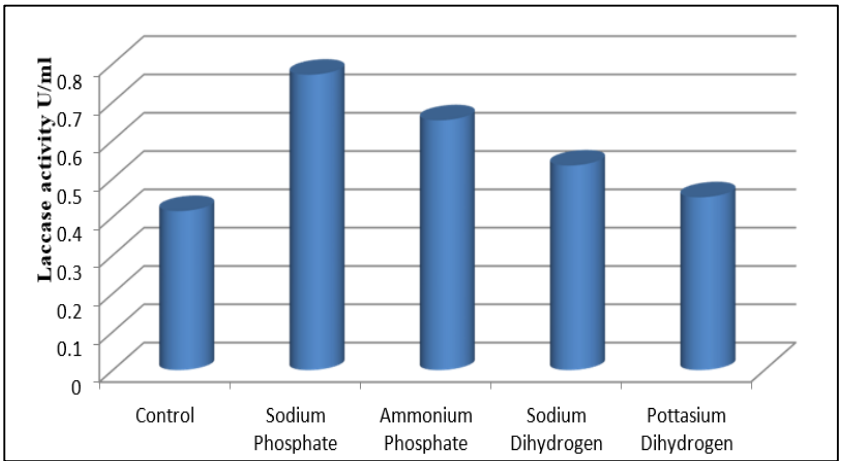
Induced laccase production as compare to control. (Graph no.7) Afreen *et al.,* (2016) [1] reported that copper sulphate was best activator for laccase production.



Graph 7: Effect of sulphur sources on laccase production

Effect of phosphorus sources on laccase production

Phosphorus sources were tested for laccase production and results were recorded. All the phosphorus sources significantly induced laccase production as compare to control. Maximum laccase production was recorded on media amended with sodium phosphate. (Graph no.8)



Graph 8: Effect of phosphorus sources on laccase production

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