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69

OPTIMIZATION OF CULTURAL CONDITION FOR LACCASE PRODUCTION FORM *CURVULARIA LUNATA*

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Abstract:

In the present investigation optimization of cultural condition of *Curvularia lunata* was carried out for laccase enzyme production. Different media and media amended with different nutrient sources *viz.* carbon, nitrogen, 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 Yeast Extract Peptone Dextrose Broth significantly induced the laccase production as compare to other media. The physical factors like 6.5 pH and 30^0 C temperatures were optimum for laccase production, where as laccase production is optimum after 10 days of incubation. Among the carbon sources tested maltose showed maximum laccase production where as nitrogen sources tested casein showed maximum laccase production. Ferrous sulphate and sodium phosphate showed maximum laccase enzyme production among the sulphur and phosphorus sources tested.

Keywords: *Curvularia lunata,* Guaiacol, laccase, optimization.

Introduction:

Laccases are the oldest and most studied enzyamatic systems (benzenediol:oxygen oxidoredutases, EC 1.10.3.2). Yoshida in 1883 first described laccase from the exudates of the Japanese lacquer tree, *Rhus vernicifera* and in 1896 laccase was demonstrated to be present in fungi for the first time by Bertrand and Laborde (Thurston, 1994). Laccases carry out vital role during fungal life cycle including morphogenesis, fungal plant pathogen/ host interaction, stress defence and lignin degradation, delignification, pigmentation, fruiting body formation, pathogenesis and protection from toxic phenolic monomers of polyphenol (Thruston 1994, Fatima *et al* , 2015).

Curvularia lunata is Ascomycetes belongs to family Pleospraceae. *Curvularia lunata* is a hyphomycete mold fungus which is a facultative pathogen of many plant species and of the soil. Most *Curvularia* are found in tropical regions, though a few are found in temperate zones. *Curvularia lunata* appears as shiny velvety-black, fluffy growth on the colony surface. It is distinguished by septate, dematiaceous hyphae producing brown, geniculate conidiophores.

Materials and Methods:

Curvularia lunata isolated from soil were used for laccase production and optimum cultural conditions were standardized for efficient laccase production (Mhaske *et al.*, 2019). Guaiacol was used as an indicator compound and chemical used were of analytical grade. Laccase 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 °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° 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 minutes 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). All the experiments were carried out in triplicates.

Calculation:

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

Where,
Vt = final volume of reaction mixture
Vs = sample volume
ϵ = 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).

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°C to 50°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 sucrose, fructose, maltose, CMC 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:

Effect of different culture media on laccase production:

For optimization of suitable culture media six different culture media were tested out of that Yeast extract peptone dextrose found to be optimum with (0.267 U/ml). Potato dextrose broth, Glucose peptone broth and Malt extract broth showed laccase activity (0.237 U/ml) followed by Czapek Dox broth (0.207 U/ml). Glucose nitrate broth was significantly reduced laccase production (0.183 U/ml).

Effect of temperature on laccase production:

In order to study effect of different temperature range on laccase production of *Curvularia lunata* six different temperature ranges were tested from 25-50^{0}C and results revealed that 25-30^{0}C temperature range is the optimum for laccase production. Maximum laccase activity was found at 30^{0}C (0.237 U/ml) followed by 25^{0}C (0.237 U/ml). Laccase production reduced when temperature increase at 35^{0}C (0.089 U/ml) and at 40^{0}C (0.059 U/ml). Laccase activity was completely reduced at 45-50^{0}C. Banerjee and Vohra (1991) found more or less similar findings for *Curvularia* and stated that 30^{0}C was optimum for laccase production.

Effects of different pH range on laccase production:

The effects of different pH range on laccase production varied pH range were maintained from 4-8 and results were recorded. Results depicted that maximum laccase production was at pH 6.5 (0.237 U/ml) followed by pH 6 (0.207 U/ml) and pH 5.5 (0.178 U/ml). The range between pH 4 to 5 showed laccase activity i.e. (0.118 U/ml). Minimum laccase production was observed at pH 8 (0.089 U/ml). Mhaske and Wadikar (2017) found similar findings for *Sclerotium rolfsi* and reported that pH 6 and 6.5 was optimum pH for laccase production. Arora and Gill (2005) 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.

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 10th day (0.326 U/ml) followed by 8th day (0.296 U/ml) of incubation. The laccase production was significantly reduced at 4 to 6 days incubation.

Effect of carbon sources on laccase production:

Different carbon sources were tested like fructose, lactose, maltose, sucrose and CMC. Among the carbon sources tested, Maltose significantly induced laccase production (0.391 U/ml) of *Curvularia lunata* followed by CMC (0.385 U/ml). All tested carbon sources significantly induced laccase production as compare to control.

Effect of nitrogen sources on laccase production:

Five different nitrogen sources were tested for laccase production. Out of these sources, laccase production was significantly induced by Casein (0.563 U/ml) followed by Sodium nitrate (0.415 U/ml) followed by Ammonium nitrate (0.359 U/ml) followed by Calcium nitrate (0.281 U/ml) as compare to control (0.252 U/ml). Whereas urea (0.103 U/ml) significantly reduced laccase production as compare to control. Manimozhi and Kaviyarasan (2012) 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.

Effect of sulphur sources on laccase production:

Sulphur sources like ferrous sulphate, calcium sulphate, sodium sulphate, zinc sulphate and copper sulphate were tested. Among of these all sulphur sources except zinc sulphate (0.178 U/ml) reduced laccase production as compare to control (0.252 U/ml). Other sources showed laccase production such as Ferrous Sulphate (0.334 U/ml), Sodium Sulphate (0.326 U/ml), Copper Sulphate (0.286 U/ml) and Calcium Sulphate (0.267 U/ml). Afreen *et al.*, (2016) reported that copper sulphate was best activator for 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 (0.237 U/ml). Maximum laccase production was recorded on media amended with sodium phosphate (0.599 U/ml) followed by Ammonium Phosphate (0.474 U/ml) followed by Sodium Dihydrogen (0.302 U/ml). The minimum laccase production was found in Pottasium Dihydrogen (0.290 U/ml). Mhaske and Wadikar (2017) reported similar findings for *Sclerotium rolfsi*.

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